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Experimental and field evaluation of otolith strontium as a marker to discriminate between river-spawning populations of walleye in Lake Erie

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Abstract

Otolith microchemistry is a commonly used tool for stock discrimination in fisheries management. Two key questions remain with respect to its effectiveness in discriminating among river-spawning populations. First, do larvae remain in their natal river long enough for their otoliths to pick up that system's characteristic chemical signature? Second, are larval otolith microchemical differences between natal rivers sufficiently large to overcome spatiotemporal variation in water chemistry? We quantified how larval age, the ratio of ambient strontium to calcium concentrations (Sr:Ca), and water temperature influence otolith Sr in both lab-reared and wild-collected Lake Erie walleye (*Sander vitreus*). Otolith microchemistry shows promise as a spawning stock discrimination tool, given that otolith Sr in larval walleye: 1) is more strongly influenced by ambient Sr:Ca than by temperature; 2) reflects Sr:Ca levels in the natal environment, even in larvae as young as 2 d; and 3) can effectively discriminate between larvae captured in two key Lake Erie spawning tributaries, even in the face of short larval river-residence times and within-year and across-year variation in ambient Sr:Ca.

Key Words: Great Lakes, natural tag, early life history, percid, LA-ICPMS, maternal effects

Introduction

Ability to understand stock structure and estimate the relative contributions of stocks to the fishery is a common goal of both freshwater and marine fisheries management. Toward this end, research aimed at identifying “natural” tags (markers) that can reliably discriminate among spawning populations or stocks has grown (Begg and Waldman 1999). Among the many genetic and biogeochemical approaches that have emerged otolith microchemistry has shown great potential to discriminate among subpopulations that spawn in geographically discrete locations, especially locations that vary in trace-element chemistry (Rieman et al. 1994, Gillanders 2002, Pangle et al. 2010, Barnett-Johnson et al. 2010).

For otolith trace-elemental composition to be a consistent, reliable spawning-site marker, otoliths of young reared in different sites must accumulate trace elements (e.g., strontium, Sr; barium, Ba; calcium, Ca) in amounts that reflect the abundance of these elements in the ambient water (Campana and Neilson 1985, Thorrold et al. 1997, Campana 1999, Campana et al. 2000). The differences between spawning sites must be robust to within-site spatiotemporal variation in ambient environmental conditions (e.g., temperature, water chemistry) and changes in diet and physiology, all of which can affect otolith trace-elemental accumulation rates (Pangle et al. 2010, Walther et al. 2010, Sturrock et al. 2014). In addition, the young must remain in their natal site long enough for their otolith chemistry to reflect that of their natal site.

While researchers have successfully discriminated among subpopulations of fish that spawn in different rivers but form mixed populations as adults (e.g., American shad *Alosa sapidissima*, Thorrold et al. 1998, Walther et al. 2008; Chinook salmon *Oncorhynchus tshawytscha*, Barnett-Johnson et al. 2008, Brennan et al. 2015), doing so can be a challenge for several reasons. For species in which individuals spend only a short time in the river before

moving downstream, otolith chemistry may not have sufficient time to come to equilibrium with the chemistry of the ambient water. While not yet tested in newly produced individuals (e.g., larvae), when juvenile or adult fish move to a new environment, otolith chemistry typically takes about 21 d to reach equilibrium with the ambient water chemistry (Milton and Chenery 2001, Elsdon and Gillanders 2005, Lowe et al. 2009). Water temperature also can affect otolith elemental uptake (Fowler et al. 1995, Bath et al. 2000, Martin et al. 2004), with previous studies showing positive (Arai et al. 1996, Martin et al. 2004), negative (Radtke et al. 1990, Townsend et al. 1992), or no correlation (Gallahar and Kingsford 1996) between otolith elemental concentration (e.g., the ratio of Sr to calcium, Sr:Ca) and ambient water temperature. In addition, because water chemistry within a river can vary, individuals hatching at different times during a single year or individuals hatching in different years might experience different water chemistry. To most effectively use otolith microchemistry for stock discrimination, we must understand the time course and magnitude of otolith trace element deposition during early life stages, as well as whether inter-site differences in trace-elemental chemistry are large enough and consistent enough to overcome variability associated with environmental variation, development, and natal river residence time.

Toward this end, we combined a laboratory experiment with field collections to better identify how water temperature, water chemistry, and the exposure time of larvae to water in their natal site influences otolith micro-elemental composition. Ultimately, this understanding could help management agencies assess the potential use of otolith microchemistry as a tool to discriminate between river-spawning subpopulations or other locations with a short natal-site residence time. In a controlled laboratory experiment, we reared the eggs and larvae from wild-caught walleye (*Sander vitreus*) in each of three levels of Sr:Ca crossed by two water

temperatures. The ranges of both variables were chosen to span levels found in two important western Lake Erie (USA-Canada) spawning locations (i.e., Maumee and Sandusky rivers, Ohio, USA) during the larval production period. To provide field verification and to assess whether between-site variability in otolith Sr concentration [Sr] is robust to within-site variability, we measured otolith [Sr] of larvae collected from the Maumee and Sandusky rivers during three years (1993-1995) and from two collection sites (river proper and bay sites) within each river-year combination.

We tested two primary hypotheses. First, we hypothesized that, despite a long (~21 d) lag time for otolith elemental concentrations of juveniles and adults to equilibrate with the ambient water after transplantation (Milton and Chenery 2001, Elsdon and Gillanders 2005, Lowe et al. 2009), the equilibrium time would be shorter in river-spawned walleye larvae because the eggs hydrate and hatch in the same water as the larvae, increasing the total residence time by about 14 d (in addition to the post-hatch residence time). Second, we hypothesized that underlying differences in ambient Sr:Ca between the Maumee and Sandusky rivers would have a stronger influence on otolith [Sr] than variation in water temperatures or flow rates, given observations made with larval yellow perch (*Perca flavescens*) in the laboratory (Collingsworth et al. 2010) and from Lake Erie (Ludsin et al. 2006, Pangle et al. 2010) studies.

Materials and methods

Study species and system

Lake Erie walleye, which support important recreational and commercial fisheries (Roseman et al. 2013, Vandergoot 2014, Walleye Task Group Report [WTG] 2015) are supported by numerous local spawning subpopulations (Mion et al. 1998, DuFour et al. 2015).

Mature individuals from Lake Erie's two largest river-spawning stocks (Maumee and Sandusky) deposit small eggs (~ 1.6 mm in diameter) during early spring (late February through April), which hatch into larvae that reside in their natal river for just a few days to a couple of weeks, depending on flow conditions (Mion et al. 1998, DuFour 2012), before entering a mixed population in Lake Erie proper. Because female walleye also spend a short time in these natal rivers (on the order of days; Pritt et al. 2013), maternal effects on otolith chemistry are likely lesser than semelparous salmon species.

Previous work has shown differences in water Sr:Ca and otolith [Sr] of walleye collected from the Maumee and Sandusky rivers. Both in absolute [Sr] and in Sr:Ca ratio, water in the Sandusky River has higher levels (mean $\pm$ SD: [Sr] = 1506 ± 745 ppb; Sr:Ca = 8.97 ± 2.78 mmol:mol) than water in the Maumee River (mean $\pm$ SD: [Sr] = 531 ± 180 ppb; Sr:Ca = 3.95 ± 0.92 mmol:mol) during the spring (1983-2001, Pangle et al. 2010). Likewise, Hedges (2002) found otolith [Sr] to be significantly higher in Sandusky River walleye larvae than in Maumee River walleye larvae collected in the rivers during 2001, using a small sample of larvae collected on a single date in both systems. Even so, walleye otoliths [Sr] and water Sr:Ca have been shown to vary considerably within and among years in both systems (Hedges 2002, Pangle et al. 2010), which might reduce the effectiveness of otolith microchemistry as a stock discrimination tool.

Laboratory experiment

To understand the effects of water Sr:Ca and temperature on otolith [Sr] for walleye larvae, we conducted a controlled laboratory experiment in which walleye larvae were exposed to different combinations of water Sr:Ca (three levels) and temperature (two levels) in a full-factorial design. Complete details of our experimental procedures, including the artificial

fertilization of walleye eggs and rearing of larvae can be found in Rinchard et al. (2005) and (Nims 2009), respectively. Below, we report details that are specific to our study.

The larvae used in our study emanated from eggs and milt collected from spawning walleye sampled from the Maumee River during 2008. After fertilization in the laboratory (Rinchard et al. 2005), the eggs were reared in three water Sr:Ca treatments (2.5 ± 0.35 , 8 ± 1.98 , 13 ± 1.85 Sr:Ca, see supplementary material Table S1) until hatching. These elemental treatments were chosen to represent a range of conditions found in Lake Erie tributaries, with the low water Sr:Ca ratio representing conditions found in the Maumee River and the two higher water Sr:Ca ratios representing conditions found in the Sandusky River. All treatments were maintained at the same temperature (average 13°C) while the eggs were incubating in their treated water.

After hatching, the larvae from each Sr:Ca treatment were immediately transferred into replicated experimental tanks with temperatures of either 8°C or 13°C , but with the same water Sr:Ca as the original rearing tank. In this way, larvae were exposed to one of six treatments (3 Sr:Ca levels x 2 temperature levels x 4 replicates/treatment = 24 total tanks). To understand how otolith [Sr] varied with exposure time to the ambient water, we sampled ~10 larvae from each treatment every other day, from 4 to 24 d post-hatch. We preserved all larvae in 95% ethanol, which has been shown to have no effect on otolith [Sr] (Hedges et al. 2004).

Field collections & Processing

To provide field verification for our experimental results, we measured otolith [Sr] of archived samples of larval walleye collected from the Maumee and Sandusky River systems in a previous study (Mion et al. 1998). During spring 1993-1995, walleye larvae were collected from the rivers proper and near the river mouths, where they enter the bays adjoining Lake Erie.

Larvae were collected weekly during April through May with either a 1-m diameter ichthyoplankton net or 1×2 m neuston nets (500 µm mesh on both net types), both of which were towed at the water surface (Mion et al. 1998). All larvae were stored in 95% ethanol.

Otoliths from approximately 10 fish per year from each location were processed. We removed both sagittal otoliths from each individual, using one for aging and the other for [Sr] measurement. The otoliths prepared for aging were mounted with crystal bond on microscope slides and then aged using an image analysis system consisting of a compound microscope and Nikon's NIS-Elements software (ver. 4.00.03 Basic Research edition, 2013, Melville, NY). Daily rings from the hatch check to the outermost ring of each otolith were counted by two people. If two readings were not consistent, a third person provided a reading.

The second otolith in each pair was prepared for micro-elemental analysis following the procedures of Ludsin et al. (2006). In brief, under a dissecting microscope located inside a Class 100 clean hood, we removed otoliths from larvae on a microscope slide, rinsed them in Millipore deionized water three times, and then mounted them on a clean petrographic slide that was stored in Class 100 clean conditions until micro-elemental analysis. All glass materials that came in contact with otoliths (e.g., glass probes, petrographic slides) were pre-washed in 13% nitric acid and rinsed in deionized water. The only deviation from the Ludsin et al. (2006) cleaning protocol was that we replaced sonication with a low-intensity laser pulse from a laser-ablation system as the final cleaning procedure on otoliths, which has been shown to be equally as effective in cleaning otoliths for [Sr] measurement as sonication, without risk of otolith damage or loss (Gover et al. 2014).

The [Sr] in all otoliths was measured using a laser-ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS) system located in The Ohio State University's Trace Elemental

Research Laboratory. The system consisted of a New Wave Research UP-193HE 193 nm excimer laser with beam homogenizing optics and a Thermo Finnigan Element 2 ICP Sector Field Mass Spectrometer. To determine otolith [Sr], we first cleaned the surface of each otolith with a short-duration, low-intensity laser pulse (Gover et al. 2014) and then ablated each otolith near its edge, using three 15 μm diameter laser spots (laser pulse energy of 13 J/cm^2 , raw power of 23 μJ , and 40 laser pulses at 10 Hz). Glass reference standards from the National Institute of Standards and Technology (NIST 610 and 612) also were measured once every five otolith samples to assess precision and account for any changes in sensitivity.

Statistical analyses

To test for the effects of water Sr:Ca, temperature, and their interaction on otolith [Sr] in our laboratory experiment, we first used two-way analysis of variance (ANOVA), with final (day 18-24) otolith [Sr] as the response variable. We used *post-hoc* Tukey honestly significant difference (hsd) tests to do pairwise comparisons of mean otolith [Sr] for all treatments. To test for the effect of water Sr:Ca on otolith [Sr] over the entire course of the experiment, we used analysis of covariance (ANCOVA), with age as the covariate. We had extremely low sample sizes for young ages in the 13°C treatments because their otoliths were difficult to extract and handle, and hence were lost (K.-Y. Chen, pers. comm.). Thus, we conducted the ANCOVA using data only from the 8°C treatments. We also conducted linear regression analyses to quantify the relationship between otolith [Sr] and fish age within each level of water Sr:Ca at 8°C.

We conducted multiple analyses to test for effects of year, river, and collection site within a river on otolith [Sr], and the relationship between age and otolith [Sr] in field-collected walleye. First, to determine if otolith [Sr] varied between rivers, collection sites within a river, and years, we used a three-way ANOVA followed by *post-hoc* Tukey hsd tests. Factors included Year,

Location (Maumee vs. Sandusky), Site (river vs. bay near the mouth), Location $\times$ Site interaction, and the Location $\times$ Year interaction. Second, similar to the laboratory experiment, we used an ANCOVA to quantify how the relationship between otolith [Sr] and larval walleye age varied between the two larval production locations, using age as the covariate. Finally, we conducted linear regression analyses to quantify the relationship between otolith [Sr] and fish age in each river.

Although our results demonstrated significant differences in otolith [Sr] between rivers, the data were variable enough that some overlap existed in the ranges of otolith [Sr] between rivers in different years. To quantify the extent to which this overlap would affect our ability to assign walleye to natal origins based on larval otolith [Sr], we used logistic regression on data from our field-collected larvae, with all years pooled to create a predictive model of natal origins (Maumee or Sandusky River). This analysis quantified the probability of individuals originating from the Sandusky River ($P(SR)$) as

$$P(SR) = \frac{1}{1 + e^{-\alpha([Sr]_o - \beta)}}$$

where $[Sr]_o$ is the otolith [Sr] (ppm), and the probability of individuals originating from the Maumee River as $1 - P(SR)$.

Results

Laboratory experiment

Otolith [Sr] varied with ambient conditions and fish age in our experiment (Table 1, Figures 1-2). Water Sr:Ca had a strong positive effect on otolith [Sr] in 18-24 d old fish in our experiment (Two-way ANOVA: $F = 775.5$, $P < 0.001$; Figure 1). The effect of temperature also was significant, but weaker than that of water Sr:Ca (Two-way ANOVA: $F = 4.42$, $P < 0.05$;

Figure 1). An interaction between water Sr:Ca and temperature was found (Two-way ANOVA: $F = 8.28$, $P < 0.01$), with pairwise comparisons revealing that the temperature effect was significant only at the highest (13 ± 1.85) water Sr:Ca treatment (Table 1, Figure 1). The positive effect of water Sr:Ca on otolith [Sr] was still significant after accounting for fish age in the 8°C treatments (ANCOVA: $F = 425.2$, $P < 0.001$); however, we also found that otolith [Sr] varied with fish age (ANCOVA: $F = 105.8$, $P < 0.001$, Figure 2). In the two higher water Sr:Ca treatments, otolith [Sr] increased with age of fish (linear regression: P of slope < 0.001), whereas otolith [Sr] did not vary with fish age in the lowest water Sr:Ca treatment (linear regression: P of slope < 0.05). Although otolith [Sr] may not have reached equilibrium by the end of the experiment in the two higher Sr:Ca treatments, differences among the treatments were already evident in fish at 4 d of age. Specifically, we found that otolith [Sr] levels at 4-d post hatch were 669 ± 59 ppm, 1145 ± 145 ppm, and 1522 ± 130 ppm (mean $\pm$ 95% confidence intervals, CIs) in low, medium, and high Sr:Ca treatments, respectively (see otolith chemistry data of experimental fish in supplemental material Table S2 and Figure S1, upper panel).

Field collections

Using a three-way ANOVA, we learned that spawning locations, collection site within spawning locations, and year influenced otolith [Sr] in our wild-caught larvae (Figure 3). Both of the interactions explored (Location $\times$ Site and Location $\times$ Year) were significant (Tukey hsd test: both $F > 11.6$, both $P < 0.001$). For example, during 1994, otolith [Sr] did not vary between collection sites in the Maumee River system, whereas river-collected larvae had higher otolith [Sr] than bay-collected larvae in the Sandusky River (Figure 3). By contrast, during 1995, no differences were found between collection sites in the Sandusky River system, whereas river-collected larvae had higher otolith [Sr] than bay-collected larvae in the Maumee River system

(Figure 3). Similarly, while a Year effect was not detected (Three-way ANOVA: $F = 0.84$, $P = 0.44$), otolith [Sr] in larvae varied consistently within locations among the three years (i.e., significant inter-annual variation was detected). Thus, while the larvae always had higher otolith [Sr] in the Sandusky River than Maumee River system in all Location $\times$ Year contrasts (all $P < 0.001$), otolith [Sr] did not differ between 1994 and 1995 within either the Sandusky River or the Maumee River system (Tukey hsd test: $P = 0.96$). Importantly, despite these interactions, and a significant Collection Site effect (Three-way ANOVA: $F = 35.42$, $P < 0.001$), otolith [Sr] always was higher, on average in the Sandusky River system relative to the Maumee River system, as indicated by a significant Location effect (Three-way ANOVA: $F = 873.20$, $P < 0.001$; Figure 3).

The slope of the relationship between larval otolith [Sr] and age was inconsistent between the two river systems (ANCOVA, Location $\times$ Age interaction, $P < 0.05$). Thus, we conducted separate linear regressions for each river system. Similar to the results from our laboratory experiment, no relationship was found between otolith [Sr] and larval age in water with low Sr:Ca (i.e., Maumee River system, 95% CI of the slope = $[-3.89, 36.72]$, $P = 0.11$), whereas a positive relationship between otolith [Sr] and age was found in water with higher Sr:Ca (i.e., Sandusky River system; 95% CI of the slope = $[4.87, 72.88]$, $P = 0.03$) (Figure 4). The intercept of this relationship in the Sandusky River system (1438 ppm) was higher than that in the Maumee River system (685 ppm) (ANCOVA: $P < 0.001$), suggesting that otolith [Sr] can be distinguished between the two natal sites at a very young age. Otolith microchemistry data for field fish can be found in supplemental material Table S3 and Figure S1, lower panel.

Discriminatory power of otolith [Sr]

While differences in otolith [Sr] between the Maumee and Sandusky River larvae were robust to collection-site differences within a year, collection-year differences, and larval age,

otolith [Sr] could not predict river origin with 100% certainty without prior knowledge of year, collection site, and age. A binary logistic regression on field data pooled across years, collection sites, and ages gave the probability that an individual originated from the Sandusky River ($P(SR)$) as a function of otolith [Sr]

$$P(SR) = \frac{1}{1 + e^{-0.012([Sr]_o - 1180)}}$$

(Figure 5). A trade-off exists, however, between the percent certainty in assignments to natal rivers, based on otolith [Sr], and the percent of fish that can be assigned. For example, with a 95% certainty in assignment, fish with otolith [Sr] between 936 and 1423 ppm could not be reliably assigned to either natal river. In turn, we would be unable to assign 16% of our field-caught larvae to either river as their otolith [Sr] fell within this range (Table 2). If we reduced our level of certainty in assignment to 80%, for example, we could reliably assign more fish to a river (all but 8% in this case; Table 2).

Discussion

The ultimate goal of this study was to assess the potential of otolith microchemistry as a stock discrimination tool in freshwater fish populations that are composed of multiple river-spawning subpopulations. For otolith microchemistry to be a successful stock discrimination tool, larvae would have to spend sufficient time in their natal rivers to register a site-specific signal in their otoliths, and the differences in otolith microchemistry between natal sites would have to be robust to spatiotemporal within-site variation in water chemistry and temperature. Our findings indicate that larvae collected from two Lake Erie spawning tributaries that vary in water Sr:Ca (Maumee and Sandusky rivers) differed in otolith [Sr] at sufficiently young ages (2-4 d) to allow discrimination between the rivers. Differences in otolith [Sr] between sites were strong and

consistent, though temperature and year effects added some level of variation in otolith [Sr]. While this variation limited our ability to assign a small percentage of larvae to their natal location, collectively our results indicate that otolith [Sr] could be a powerful tool to discriminate between Lak Erie's two most important spawning tributaries.

Otolith [Sr] response to water Sr:Ca and temperature. Most previous laboratory studies that have linked otolith [Sr] to fish location histories in the wild have focused on marine species or species that spend part of their life cycles in marine systems. These studies tend to use the effects of salinity and water temperature on otolith Sr uptake rate to assign individuals to locations (Fowler et al. 1995, Elsdon and Gillanders 2002, Martin et al. 2004, Zimmerman 2005), rather than using differences in water Sr:Ca among locations, as was our goal here (also see Walther et al. 2008, Muhlfeld et al. 2012). In those few controlled laboratory studies that quantified the response of otolith [Sr] to water Sr:Ca, both marine (Bath et al. 2000, Elsdon and Gillanders 2005) and freshwater (Collingsworth et al. 2010), a consistent positive relationship was found, just as we observed in our experiment.

The effect of temperature on otolith [Sr] was not as consistent as that of water Sr:Ca either in our experiment or in other studies. Otolith [Sr] in our study showed no effect of temperature, except at high water Sr:Ca, when otolith [Sr] was positively related to water temperature. Results from other controlled laboratory experiments on marine fish in which both temperature and water Sr:Ca were varied differed from our results. For example, Radtke et al. (1990) found a negative relationship between temperature and otolith Sr:Ca in Atlantic herring (*Clupea harengus*) larvae across all water Sr:Ca treatments, and Martin et al. (2004) observed temperature and otolith Sr:Ca to have a positive correlation across all Sr treatments in larval spot (*Leiostomus xanthurus*). Our results, however, are consistent with patterns in another freshwater

fish, yellow perch, for which otolith Sr:Ca in juveniles was unaffected by water temperature at low water Sr:Ca, but as water Sr:Ca increased, temperature had an increasingly positive effect on otolith Sr:Ca (Collingsworth et al. 2010).

The difference between the temperature patterns in marine experiments and freshwater experiments may be due partly to the large difference in background Sr:Ca between the two systems, with marine systems having consistently higher Sr:Ca than their freshwater ones (Brown and Severin 2009). In addition, although we did not directly test the effect of larval growth on otolith chemistry in our study, previous studies have shown that larval walleye have faster growth rates in warmer waters than colder waters (Santucci Jr. and Wahl 1993). Thus, while we cannot rule out that growth rate cannot offset or swamp the effects of ambient Sr:Ca on otolith Sr accumulation in other settings, its effect was less than that of water Sr:Ca in conditions representative of Lake Erie during the spring.

Early differences in otolith [Sr]. We observed differences in otolith [Sr] in young larval walleye. In our laboratory experiment, otolith [Sr] of 4-d post-hatch larvae was higher in the high water Sr:Ca treatments than in the low treatment. Further, our field data showed that the Sandusky River walleye larvae had higher otolith [Sr] than the Maumee River larvae at the youngest ages that we collected (2-3 d post-hatch).

The divergence in otolith [Sr] in such young larvae (2-4 d post-hatch) suggests that eggs and embryos are taking up trace elements that are ending up in the developing otoliths. Previous studies have shown maternal effects, with gravid spawners passing trace elements from the ambient water to their eggs (Ruttenberg et al. 2005, Thorrold et al. 2006). A similar effect could contribute to the early differences in otolith [Sr] in young larvae collected from the Sandusky and Maumee rivers in our field samples. The fish in our laboratory experiments, however, all

resulted from spawners collected from the Maumee River, so we had removed the possibility of a maternal effect driving differences in otolith [Sr] in young larvae. Previous physiological studies have found that trace-metal ions in water can enter the eggs after fertilization, particularly during the egg-swelling phase, and accumulate in the eggs (Cerdà et al. 2007, Jezierska et al. 2008, Lubzens et al. 2010). In our experiments, the 10-14 d between fertilization and hatching apparently was sufficient time for trace metals to accumulate in the eggs and begin to become incorporated into the otoliths. In the field, this process may have been enhanced further by maternal effects. Overall, our results suggest that, even though larvae may reside in their natal rivers for only a short time, otolith [Sr] can be an effective marker for discriminating between the populations when the water Sr:Ca difference between the natal rivers is large, as in this study.

Spatial and annual variability within a natal river. If water Sr:Ca varies spatially in the natal river or varies across years, then the otolith [Sr] signal from that site may not be consistent enough to be useful as a stock discrimination marker. In this system, walleye larvae may move quickly out of the natal rivers and reside for some time in the bays at the mouths of the rivers. If the waters of the lake heavily influence the water chemistry of the bays, then the bays may be similar enough that walleye otoliths do not pick up distinguishing signals from their natal rivers (Gillanders 2002, Pangle et al. 2010). Our results show that otolith [Sr] of larval walleye collected from the bays of the river did indeed differ, in some years, from those collected in the river itself; however, the variation was not great enough to affect the strong difference in otolith [Sr] between the two river systems.

Water Sr:Ca has been shown to vary across years within a river, often due to precipitation and river discharge levels (Skougstad and Albert 1963; Pangle et al. 2010). If this variation is great enough, it may result in otolith [Sr] being a useful discrimination marker within, but not

across, years. (Gillanders 2002) found overlap of elemental fingerprints of juvenile snapper (*Pargus auratus*) otoliths collected from different estuaries in southeast Australia when she included fish from different year-classes. We observed inter-annual variability in otolith [Sr] within each natal river system, but not enough to confound the spatial differences between the two natal river systems. In both the spatial (bay versus river) and temporal (inter-annual) variation that we observed in otolith [Sr] in the field, only rarely did it cause a larval walleye collected in one river system to have otolith [Sr] that made it appear similar to larvae collected from the other river system. We recommend continued collections of larvae across years to better document otolith [Sr] variation that may confound our ability to discriminate between natal rivers.

Stock discrimination. When we pooled all of the larval walleye sampled across bays, rivers, and years, otolith [Sr] proved to be an effective marker for stock discrimination. Because of the within-river system spatial and temporal variability, we could not discriminate with 100% certainty. However, our data allowed us to assign nearly 90% of the individuals with at least 90% certainty. Although assignment accuracies in other studies are variable, ranging from 100% to less than 50%, depending on study systems and assignment methods, few studies have reported their desired assignment certainty along with the assignment accuracies (Brennan et al. 2015). Geffen et al. (2011) used multiple trace elements in otoliths to discriminate among several spawning groups in a west British Isles herring (*Clupea harengus*) population, and they considered 67% certainty across all fish, with 30%-100% correct assignment rate, as providing reasonable confidence in assignments. Miller et al. (2010) adopted conservative criteria, with 90% certainty, to determine the source of individual Chinook salmon, using genetic microsatellite DNA markers, otolith Sr isotopic composition ($^{87}\text{Sr}/^{86}\text{Sr}$), and artificial tags

combined. Given these comparisons with previous studies, our results showed that otolith Sr is a reliable marker to discriminate between the Sandusky and Maumee River stocks.

Our study focused on two of the principal spawning sites for walleye in Lake Erie, the Maumee and Sandusky rivers, but many other spawning sites may contribute fish to the lakewide population. Previous work has shown that the Sandusky River has trace Sr levels higher than other walleye spawning sites in Lake Erie, but that the Maumee River has Sr levels similar to other spawning sites, including the nearby Ohio reef complex (Bigrigg 2008, Pangle et al. 2010). While we anticipate that otolith microchemistry will be effective for discriminating between the Sandusky River stock and other stocks in the lake, we do not expect it to be effective in discriminating among all other spawning stocks. To be able to discriminate among other spawning stocks, we will have to combine otolith microchemistry with other types of markers, such as genetic markers.

Recommendations

Our results demonstrate that otolith [Sr] can be used to discriminate between the Maumee River and Sandusky River spawning stocks, two of the major walleye stocks in Lake Erie. Despite some spatial and annual variation, we found consistent differences in otolith [Sr] between these rivers across the years of our study. With our current results, we are capable of discriminating between the two spawning stocks with high certainty on a high percent of individuals. Most of the remaining uncertainty is due to annual variation in water Sr:Ca within rivers. To reduce this uncertainty and to increase the percent of individuals that can be reliably assigned back to a specific natal river, we recommend that annual larval walleye collections be made in these rivers to establish an annual library of otolith [Sr]. A similar recommendation was made for Lake Erie yellow perch by Pangle et al. (2010), given inter-annual variability observed

in that study. Such a library would allow individuals in the mixed (open-lake) population to be classified back to their natal origins using both age (year-class) and otolith [Sr] information. In addition, discrimination of spawning stocks on a lakewide population level, beyond these two rivers, will require the development of discrimination markers beyond otolith [Sr]. Such ability to discriminate among spawning stocks would better position management agencies to understand stock structure and identify the relative contributions of stocks to the fishery, both of which will help keep Lake Erie's walleye fishery sustainable.

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Table 1. Average larval walleye otolith [Sr] (ppm, below diagonal) and pairwise *P* values (above diagonal) between all treatments from a controlled laboratory experiment in which three levels of water strontium:calcium (Sr:Ca) were crossed by two temperatures. Analysis consisted of two-way ANOVA with Tukey hsd test.

Water Sr:Ca	2.5 ± 0.35	8 ± 1.98	13 ± 1.85
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	Temp.	8°C	13°C	8°C	13°C	8°C	13°C
2.5 ± 0.35	8°C		0.087	0.000*	0.000*	0.000*	0.000*
	13°C	131		0.000*	0.000*	0.000*	0.000*
8 ± 1.98	8°C	1081	950		0.013*	0.000*	0.000*
	13°C	1301	1170	220		0.898	0.000*
13 ± 1.85	8°C	1372	1240	291	70		0.000*
	13°C	1972	1840	890	670	600	

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623 Table 2. Otolith [Sr] (ppm) limits for larval walleye assignment to the Maumee and Sandusky
624 rivers at different probability thresholds (i.e., certainty of assignment), the percentage (number)
625 of larvae in our data assigned to each river, and percentage (number) of larvae that could not be
626 assigned to either river. Estimates were based on binary logistic regression of all wild-caught
627 larvae.

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Probability Threshold	otolith [Sr] (ppm)			Larvae	Larvae	Larvae
	Max [Sr]	Min [Sr]	Could not	assigned to	assigned to	unable to be
	Maumee	Sandusky	assign to	Maumee	Sandusky	assigned
			either river	% (N)	% (N)	% (N)
95%	936	1423	936-1423	43.8% (53)	40.5% (49)	15.7% (19)
90%	998	1362	998-1362	46.3% (56)	42.1% (51)	11.6% (14)
80%	1065	1294	1065-1294	47.1% (57)	44.6% (54)	8.3% (10)
70%	1110	1250	1110-1250	47.1% (57)	46.3% (56)	6.6% (8)

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Figure 1. Otolith strontium concentrations [Sr] of 18-24 d walleye larvae reared in a controlled laboratory experiment with three levels of water Sr:Ca and two temperatures, 8°C (gray boxes) and 13°C (black boxes). Sample sizes are reported below each box. Box plot details: the line within the box is the median; top and bottom edges of the box are the 25th and 75th percentiles; the ends of whiskers are the minimum and maximum of non-outliers, and outliers are shown as black circles. Boxes with a shared letter do not differ statistically based on Tukey hsd post hoc comparisons ($\alpha = 0.05$).

Figure 2. Otolith [Sr] of walleye larvae reared in the laboratory in 8°C water at three ambient Sr:Ca (square: 2.5 ± 0.35 ; triangle: 8 ± 1.98 ; circle: 13 ± 1.85 mmol:mol) for 24 d. Each symbol represents an individual, with the symbols for fish sampled on the same day slightly offset horizontally. Linear regressions were conducted on data from each water Sr:Ca treatment with the shaded region along each regression line portraying its 95% confidence interval.

Figure 3. Spatial and temporal variability of otolith [Sr] of walleye larvae collected from four sites (from dark gray to white boxes: Maumee Bay, Maumee River, Sandusky Bay, and Sandusky River, respectively) during spring 1993, 1994, and 1995. Sample sizes for each box range 9-11. See Figure 1 for box-plot description details. Boxes with a shared letter do not differ statistically based on Tukey hsd post hoc comparisons ($\alpha = 0.05$).

Figure 4. Relationships between otolith strontium concentration [Sr] and age in larval walleye collected from the Maumee River system (black) and Sandusky River system (gray) during spring 1993 (circles), 1994 (triangles), and 1995 (squares). The otolith [Sr] and age of the Sandusky River fish had a significant positive relationship (P value of slope < 0.05), whereas Maumee River fish did not (P value of slope = 0.11).

Figure 5. Probability of assignment (from binary logistic regression analysis) of larvae to the Maumee River (black circles) and Sandusky River (gray squares), based on otolith strontium concentrations [Sr] in walleye larvae collected in both systems during 1993-1995.

Experiment water chemistry data

Tank	Treatment	Ca (ppm)	Sr (ppm)	Sr:Ca (mmol:mol)	Treatment water	Sr:Ca
A1	Medium	42.44271	0.721097	7.768467	High	13.25936268
A2	High	42.72688	1.213355	12.98469	Medium	7.949106089
A3	Medium	43.5831	0.756063	7.932035	Low	2.516360052
A4	High	35.49253	1.011811	13.03488		
A5	High	35.391	1.002292	12.9493		
A6	Medium	36.27697	0.620522	7.821152		
A7	Low	36.04652	0.266855	3.384987		
A8	High	35.70313	1.01288	12.97169		
A9	Low	36.71632	0.193179	2.405717		
A10	Medium	38.035	0.64223	7.72062		
A11	Low	37.69252	0.196749	2.386724		
A12	Low	38.36812	0.199345	2.375631		
B1	High	34.69567	1.036804	13.66363		
B2	Low	34.71047	0.184593	2.431636		
B3	Low	34.90281	0.183282	2.401069		
B4	High	36.8408	1.085804	13.47619		
B5	Medium	40.13238	0.703311	8.013044		
B6	High	40.66436	1.202067	13.51635		
B7	Low	32.93147	0.172045	2.388774		
B8	Medium	33.73873	0.606815	8.2238		
B9	Medium	33.42198	0.588768	8.054835		
B10	Low	32.52375	0.167608	2.356341		
B11	Medium	33.95553	0.598468	8.058896		
B12	High	33.51171	0.987831	13.47817		

Standard deviation	Standard error
1.849903924	0.654039805
1.978684589	0.699570645
0.351673537	0.124335371

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Otolith microchemistry data of experimental fish (walleye larvae)

day	temp	waterSr	id	otoSr.ppm	otoCa.ppm	Sr:Ca (mmol:mol)
4	8	300	M5-2	637.52	390780.8	0.745943
4	8	300	M5-3	854.74	416421.2	0.938525
4	8	300	M5-4	693.3	426493.7	0.743282
4	8	300	M5-6	609.65	431054.3	0.646686
4	8	300	M5-7	595.1	388306.3	0.700746
4	8	300	M5-11	595.18	392162.5	0.693948
4	8	300	M5-9	637.22	402813.8	0.723319
4	8	300	M5-14	590.88	390515.4	0.69184
4	8	300	M5-15	545.17	393075.2	0.634163
4	8	300	M5-18	633.33	362053.2	0.799839
4	8	300	M5-21	687.53	368769.3	0.852475
4	8	900	M5-1	1209.03	421132.3	1.312693
4	8	900	M5-13	1312.44	410457.6	1.462029
4	8	900	M5-17	1019.39	458040.2	1.01761
4	8	900	M5-19	1123.48	409188.4	1.255414
4	8	900	M5-20	1063.07	430519.4	1.129052
4	8	1500	M6-4	1574.05	407598.6	1.765756
4	8	1500	M6-5	1525.57	416527.3	1.674686
4	8	1500	M6-7	1469.21	376319.8	1.785137
4	13	300	M6-9	837.86	371231.5	1.03198
4	13	300	M6-12	785.9	432260.3	0.831317
6	8	300	2B-12	680.14	434355	0.715976
6	8	300	2B-14	648.02	388613.8	0.762456
6	8	300	2B-15	477.04	430814.4	0.506302
6	8	300	2B-16	601.37	397024.3	0.692579
6	8	300	2B-17	439.97	419117.7	0.47999
6	8	300	2B-18	709.75	339858.5	0.954887
6	8	300	2B-19	805.26	524281.3	0.70229
6	8	300	2B-21	390.07	362296.6	0.492292
6	8	300	2B-10	614.9	424903.1	0.661697
6	8	300	N21-6	621.41	402751.4	0.705482
6	8	300	N21-7	376.78	390577.2	0.441088
6	8	300	N21-4	630.66	413102.5	0.698043
6	8	900	2B-1	1559.02	406676.7	1.75286
6	8	900	2B-21	1562.06	406015.2	1.759139
6	8	900	2B-4	1680.83	395034.2	1.945512
6	8	900	2B-5	1328.39	403904.7	1.503805
6	8	900	2B-6	1424.32	389347.7	1.672687
6	8	900	2B-7	1648.38	411887.1	1.829886
6	8	900	2B-8	1564.4	386460	1.850922

6	8	900 M7-15	1539.87	396536	1.775605
6	8	900 2B-9	1453.1	408342.8	1.627104
6	8	900 2B-11	1365.65	402223.2	1.552448
6	8	1500 2B-23	1303.37	393465.8	1.514627
6	8	1500 2B-24	1424.49	409845.3	1.589221
6	8	1500 2B-25	1354.75	395511.9	1.56619
6	8	1500 2B-26	1790.47	407427.9	2.009375
6	8	1500 2B-27	1579.76	390226.2	1.851056
6	8	1500 2B-28	1202.7	395405.1	1.390784
6	8	1500 M7-14	1740	411354.2	1.934096
6	8	1500 2B-29	1686	401579	1.919691
6	8	1500 N22-2	2359.14	364097.5	2.962653
6	8	1500 2B-30	1258.95	412616.5	1.395104
6	13	300 2B-31	1154.77	387594.7	1.362268
6	13	300 2B-32	1374.71	479553.7	1.310746
6	13	300 2B-33	1377.3	386921	1.627612
6	13	300 N18-1	742.48	396183.9	0.856905
6	13	300 N18-3	1007.86	377354.2	1.221225
6	13	300 N22-3	959.3	358453.5	1.223676
6	13	300 N18-4	1289.03	415178.5	1.419623
8	8	1500 M9-1	1894.73	401073	2.160074
8	8	1500 M9-3	1527.31	381213.9	1.831906
8	8	1500 M9-4	1769.85	413941.9	1.954978
8	8	1500 M9-5	1736.17	379178.3	2.093599
8	8	1500 M9-6	1659.52	404863	1.874214
8	8	1500 M9-7	1980.72	385523.3	2.349185
8	8	1500 M9-8	2009.12	417910.1	2.198203
8	8	1500 M9-9	2154.41	435916.7	2.259798
8	8	1500 M9-10	1734.37	389425.6	2.036395
10	8	300 M1-1	665.72	407516	0.74695
10	8	300 N22-4	646.93	405097.1	0.730202
10	8	300 N22-5	572.13	376930.3	0.69403
10	8	300 N22-6	628.46	398117.2	0.721791
10	8	300 N22-7	678.44	401484.5	0.772658
10	8	300 M7-16	612.97	391350.5	0.716173
10	8	300 M7-17	697	416496.8	0.765184
10	8	300 M7-18	602.37	384849.5	0.715677
10	8	300 M7-19	822.96	390729.9	0.963046
10	8	300 M7-20	752.1	399815.2	0.860124
10	8	900 N22-8	1409.29	384839.9	1.674423
10	8	900 N22-9	1498.87	381551.7	1.796203
10	8	900 N22-10	1454.63	394741.1	1.684942
10	8	900 N22-11	2028.11	388845.1	2.384842

10	8	900 N23-1	1979.58	402936.6	2.246369
10	8	900 N23-2	1602.32	408307.2	1.794349
10	8	900 N23-3	1952.85	407621	2.19057
10	8	900 N23-4	1881.06	408964.5	2.103109
10	8	900 N23-5	1919.62	410764.5	2.136816
10	8	900 N23-6	1806.05	409218.8	2.01799
14	8	300 M2-3	638.32	405349.4	0.720035
14	8	300 M2-4	646.78	383535.1	0.771074
14	8	300 M2-5	406.71	438182.6	0.424399
14	8	300 M2-6	655.03	421966.5	0.709787
14	8	300 M2-7	677.02	409389.3	0.756153
14	8	300 M2-8	555.13	402413.8	0.630764
14	8	300 M2-9	336.47	365714.3	0.420677
14	8	300 M3-1	497.51	363694.2	0.625475
14	8	300 M3-2	617.53	389460.1	0.725003
14	8	300 M3-8	629.57	407900.2	0.705724
14	8	900 M2-1	1591.1	396440.2	1.83512
14	8	900 M3-4	1910.44	423793.2	2.061219
14	8	900 M3-5	1743.59	389156.5	2.048636
14	8	900 M3-6	2091.9	403150.2	2.372569
14	8	900 M3-7	1789.21	375991.2	2.175848
14	13	300 M1-5	677.6	408518.3	0.758415
14	13	300 M1-6	596.52	337594.8	0.807931
14	13	300 N23-8	620.83	413790.5	0.68602
14	13	300 N23-9	884.92	410572.7	0.985505
14	13	300 N23-10	789.04	443124.7	0.814175
14	13	300 N23-11	1059.79	457687.9	1.058754
14	13	300 N24-1	708.64	407116.6	0.795887
14	13	300 N24-2	1115.68	447909.5	1.138922
14	13	900 N24-3	2101.61	358757.3	2.678528
14	13	900 N24-4	2353.49	419711.9	2.563928
14	13	900 N24-5	1805.06	339837.8	2.428649
16	8	1500 M7-21	2460.75	399385.9	2.817212
16	8	1500 M7-22	2067.73	390318.6	2.422252
16	8	1500 M7-24	2311.58	402084.4	2.628672
16	8	1500 M7-25	2502.42	388830.6	2.94269
16	13	300 N5-1	530.85	387694.5	0.626076
16	13	300 N5-2	493.58	417098.3	0.541083
16	13	300 N5-3	606.65	398579.7	0.695934
16	13	300 N5-4	627.26	399553.8	0.717823
16	13	300 N5-5	628.04	411837.8	0.697278
16	13	300 N5-6	578.27	403067.9	0.65599
16	13	300 N5-7	508.96	405568.1	0.573805

16	13	300 N5-8	547.1	397378.2	0.629517
16	13	300 N5-9	561.33	405071.4	0.633624
16	13	300 N5-10	441.03	392429.3	0.513868
16	13	900 N7-1	2106.48	399764	2.409342
16	13	900 N7-2	1865.23	404213.9	2.10992
16	13	900 N7-3	1911.41	415949.9	2.101153
16	13	900 N7-4	1915.07	405827.8	2.157683
16	13	900 N7-5	2145.61	414331.4	2.367815
18	8	300 9c3	1130.02	412777.2	1.251743
18	8	300 9d3	901.33	414829.3	0.99348
18	8	300 10b1	779.59	400303.3	0.890475
18	8	300 10b2	664.87	406524.5	0.747816
18	8	300 10b3	608.48	398210.5	0.69868
18	8	300 10c3	811.16	423034.1	0.87675
18	8	300 10c2	718.36	406426.9	0.808173
18	8	300 10c1	978.85	418686.5	1.068986
18	8	300 11c1	507.57	394964.7	0.587601
18	8	300 11b1	443.17	394027.7	0.514267
18	8	300 N11-22	1070.95	417952.9	1.17162
18	8	900 11c2	1931.1	397408.8	2.221837
18	8	900 11c3	1708.06	394702.6	1.978691
18	8	900 11b2	1582.63	380902.8	1.899809
18	8	900 13a3	1875.6	354037.8	2.422341
18	8	900 13a2	1902.68	393188.5	2.212635
18	8	900 13a1	2016.78	398492	2.314109
18	8	900 13b1	1894.97	393790.4	2.2003
18	8	900 N11-21	2198.19	408552.5	2.460153
18	8	1500 9c1	2769.2	410443.6	3.084933
18	8	1500 9c2	2695.17	437524.1	2.816625
18	8	1500 9d1	2867.43	399772.4	3.27963
18	8	1500 9d2	2317.04	406417.6	2.606788
18	8	1500 9a1	1990.66	397998.2	2.286971
18	8	1500 9a2	2390.44	402408.1	2.716164
18	8	1500 9a3	2339.49	394214.7	2.713521
18	8	1500 M7-1	2390.62	373059	2.930069
18	13	300 N1-1	645.59	414451.8	0.712242
18	13	300 N1-2	721.91	398363.6	0.828606
18	13	900 N1-13	2083.17	410097.6	2.322642
18	13	900 N1-14	1925.08	409360.3	2.150245
18	13	900 N1-15	2209.3	397513.8	2.541249
18	13	900 N1-16	2178.37	389370.4	2.558077
18	13	900 N1-17	2481.49	448392.1	2.530459
18	13	1500 N1-3	2395.01	400538.3	2.73406

18	13	1500 N1-4	2901.67	403062.4	3.291702
18	13	1500 N1-5	2468.85	395813.1	2.851999
18	13	1500 N1-6	2534.45	411931.8	2.813217
18	13	1500 N1-7	2607.69	409438.5	2.912139
18	13	1500 N1-8	2607.12	409610.5	2.910279
18	13	1500 N1-9	2235.89	386896.4	2.642412
18	13	1500 N1-10	2696.94	411904.5	2.993778
18	13	1500 N1-11	2472.54	397522.5	2.843979
18	13	1500 N1-12	2530.89	410456.8	2.819361
20	13	300 N3-1	726.3467	389267.6	0.85318
20	13	300 N3-2	748.61	400004.2	0.855728
20	13	300 N3-4	691.7533	415193.8	0.761807
20	13	300 N3-5	588.2067	407978.4	0.659231
20	13	300 N3-6	701.44	396930.4	0.808018
20	13	300 N3-7	539.9067	402899.3	0.612727
20	13	300 N3-8	940.39	446412.1	0.963201
20	13	300 N3-10	654.16	392108.5	0.762821
20	13	300 N3-3	897.765	377618.2	1.087062
20	13	300 N3-9	1025.64	387921.8	1.208914
24	8	300 17a1	609.91	413171.9	0.674963
24	8	300 17b3	728.32	428344.6	0.777452
24	8	300 17d1	487.45	404721.3	0.550705
24	8	300 N11-16	655.75	393248.9	0.762457
24	8	300 N11-17	798.25	401111.6	0.909952
24	8	300 N11-18	573.1	407233.7	0.643475
24	8	300 M10-16	540.5	389364.9	0.634722
24	8	300 M10-17	804.95	422171.2	0.871817
24	8	300 N11-19	672.03	398359.8	0.771361
24	8	300 N11-20	638.13	414311.6	0.70425
24	8	900 N3-12	2106.39	419890.9	2.293756
24	8	900 N3-13	1665.32	397097.4	1.917544
24	8	900 N3-14	1600.36	382603.4	1.912554
24	8	900 N3-15	1908.54	410125.6	2.127792
24	8	900 N3-16	2544.3	449809.5	2.586333
24	8	900 N3-17	2105.49	436711.4	2.204466
24	8	900 N3-18	2005.51	417992.1	2.193823
24	8	900 N3-19	1756.44	363739.6	2.207941
24	8	900 N3-20	2240.02	407472.8	2.513611
24	8	1500 17b2	2151.53	430662.1	2.284312
24	8	1500 N11-7	2207.53	405642.1	2.488332
24	8	1500 N11-8	2317.03	402106.3	2.634726
24	8	1500 N11-9	2158	392868.3	2.511593
24	8	1500 N11-10	2115.66	399728	2.42006

24	8	1500 N11-11	2221.93	404920.6	2.509027
24	8	1500 N11-12	2302.67	409564.6	2.570716
24	8	1500 M10-14	2639.55	397460.4	3.036552
24	8	1500 M10-15	2035.69	407578.2	2.283734
24	8	1500 N11-14	2006.63	421518.8	2.176683
24	8	1500 N11-15	2096.74	412438.5	2.324503
24	13	300 N11-3	590.08	410163.9	0.657807
24	13	300 N11-4	571.5	397659.7	0.657127
24	13	300 M10-8	928.95	385773.6	1.101044
24	13	300 M10-9	699.4	409169.8	0.781568
24	13	300 M10-10	713.33	404179.7	0.806976
24	13	300 M10-11	767.71	408151.4	0.860044
24	13	300 M10-12	726.32	391343.2	0.848623
24	13	300 M10-13	588.91	381174.5	0.706431
24	13	300 N11-5	565.16	405446	0.637358
24	13	300 N11-6	641	414174	0.707652
24	13	900 12a2	1722.14	419705.8	1.876153
24	13	900 12a1	1974.6	416647.9	2.166979
24	13	900 12b1	1752.4	412150.5	1.944116
24	13	900 12b2	1887.45	389392.6	2.21632
24	13	900 12c3	1682.77	372661.8	2.064689
24	13	900 12d1	1784.32	417950.9	1.952056
24	13	900 12d2	1861.56	379701.5	2.24171
24	13	900 12d3	1726.81	380933.5	2.072718
24	13	900 N11-1	1777.83	395365.1	2.056064
24	13	900 N11-2	1654.8	416835.6	1.815204
24	13	1500 27b1	2553.97	395529.3	2.952446
24	13	1500 27b2	2808.19	391327.8	3.281184
24	13	1500 27c3	2725.45	409304.7	3.044642
24	13	1500 27c1	3120.1	411222.6	3.469256
24	13	1500 27d2	2637.87	406159.4	2.969625
24	13	1500 27d3	2583.15	402060.3	2.937672
24	13	1500 M10-6	2657.7	380748.6	3.191629
24	13	1500 M10-7	2759.98	401627.1	3.142156
24	13	1500 27 e2	2643.8	395405.5	3.057248
24	13	1500 27 e1	2652.37	406037.6	2.986844

Otolith microchemistry data of field fish (1993-1995 walleye larvae)

sampleID	year	location	site	age.d	otoSr.ppm	otoCa.ppm
93SR1	1993	Sandusky	wriver	3	1649.59	397936.5
93SR2	1993	Sandusky	wriver	2	1273.915	397204.5533
93SR3	1993	Sandusky	wriver	2	1616.993333	403533.0433
93SR4	1993	Sandusky	wriver	2	1579.083333	381034.95
93SR5	1993	Sandusky	wriver	2	1556.35	404073.5
93SR6	1993	Sandusky	wriver	4	1510.593333	395690.92
93SR7	1993	Sandusky	wriver	7	1264.386667	387093.75
93SR8	1993	Sandusky	wriver	3	1504.88	393692.2
93SR9	1993	Sandusky	wriver	3	1531.566667	430897.6333
93SR10	1993	Sandusky	wriver	4	1367.696667	374134.8967
93SB1	1993	Sandusky	bay	7	1445.683333	395326.9033
93SB2	1993	Sandusky	bay	9	1502.396667	391924.75
93SB3	1993	Sandusky	bay	8	1537.546667	385656.1067
93SB4	1993	Sandusky	bay	5	1712.273333	384764.9767
93SB5	1993	Sandusky	bay	7	1628.823333	411818.79
93SB6	1993	Sandusky	bay	8	1718.23	399413.4367
93SB7	1993	Sandusky	bay	8	1699.396667	390565.72
93SB8	1993	Sandusky	bay	4	1215.375	401462.765
93SB9	1993	Sandusky	bay	9	1619.413333	376334.4167
93SB11	1993	Sandusky	bay	6	1689.09	424734.77
93MR2	1993	Maumee	wriver	5	1381.026667	389295.9367
93MR6	1993	Maumee	wriver	4	1196.67	364659.97
93MR7	1993	Maumee	wriver	5	852.065	404976.815
93MR8	1993	Maumee	wriver	6	1052.283333	390465.3233
93MR9	1993	Maumee	wriver	4	705.16	398811.84
93MR10	1993	Maumee	wriver	3	773.1	389450.03
93MR11	1993	Maumee	wriver	4	868.9	395743.28
93MR12	1993	Maumee	wriver	4	805.32	399837.19
93MR13	1993	Maumee	wriver	4	1337.73	376334.4167
93MR14	1993	Maumee	wriver	7	1139.43	399646.9367
93MB1	1993	Maumee	bay	3	842.15	397520.3333
93MB2	1993	Maumee	bay	8	930.0566667	399844.8433
93MB3	1993	Maumee	bay	3	897.9833333	392934.76
93MB4	1993	Maumee	bay	4	986.34	392473.9267
93MB5	1993	Maumee	bay	5	956.0766667	411666.8433
93MB6	1993	Maumee	bay	3	687.5033333	391128.5767
93MB7	1993	Maumee	bay	4	797.8666667	389305.5767
93MB8	1993	Maumee	bay	6	975.0233333	382385.1433
93MB9	1993	Maumee	bay	3	899.9733333	378919.7933
93MB10	1993	Maumee	bay	4	858.625	394571.345

94SR1	1994	Sandusky	wriver	9	2444.786667	408665.6867
94SR2	1994	Sandusky	wriver	8	2133.993333	375971.49
94SR3	1994	Sandusky	wriver	7	2389.943333	392316.4167
94SR6	1994	Sandusky	wriver	9	2459.173333	393813.8467
94SR7	1994	Sandusky	wriver	7	2374.683333	410863.91
94SR8	1994	Sandusky	wriver	6	2643.113333	437140.57
94SR9	1994	Sandusky	wriver	7	2073.81	401914.8533
94SR10	1994	Sandusky	wriver	4	1697.47	399871.3233
94SR12	1994	Sandusky	wriver	6	2310.736667	406086.77
94SR13	1994	Sandusky	wriver		2886.35	423081.4933
94SB2	1994	Sandusky	bay	4	1393.583333	405283.9267
94SB3	1994	Sandusky	bay	3	1331.443333	387252.7933
94SB4	1994	Sandusky	bay	11	1643.71	403227.24
94SB5	1994	Sandusky	bay	4	1430.9	396150.5433
94SB6	1994	Sandusky	bay	3	952.1033333	400636.76
94SB7	1994	Sandusky	bay	6	1309.193333	387703.56
94SB8	1994	Sandusky	bay	8	1441.45	376503.0833
94SB9	1994	Sandusky	bay	5	1214.84	392508.2633
94SB10	1994	Sandusky	bay	5	1354.04	396048.1467
94SB11	1994	Sandusky	bay	NA	1445.593333	394562.2367
94MR1	1994	Maumee	wriver	NA	750.305	390947.89
94MR2	1994	Maumee	wriver	NA	522.08	393312.545
94MR4	1994	Maumee	wriver	3	837.57	396873.81
94MR5	1994	Maumee	wriver	4	499.025	390345.925
94MR6	1994	Maumee	wriver	4	645.025	396576.11
94MR7	1994	Maumee	wriver	3	550.8066667	397625.51
94MR8	1994	Maumee	wriver	4	733.56	387882.39
94MR9	1994	Maumee	wriver	4	704.92	397288.425
94MR10	1994	Maumee	wriver	NA	621.0433333	385179.5933
94MR11	1994	Maumee	wriver	3	782.43	429200.82
94MR12	1994	Maumee	wriver	4	481.035	405771.455
94MB1	1994	Maumee	bay	7	628.6766667	398205.8233
94MB2	1994	Maumee	bay	5	699.9766667	396356.77
94MB3	1994	Maumee	bay	7	794.98	385402.27
94MB4	1994	Maumee	bay	8	660.1133333	384679.8433
94MB5	1994	Maumee	bay	9	729.6033333	404770.7067
94MB6	1994	Maumee	bay	6	894.5533333	401698.2267
94MB7	1994	Maumee	bay	6	792.5933333	399218.73
94MB8	1994	Maumee	bay	5	773.56	390398.03
94MB9	1994	Maumee	bay	5	869.8933333	396326.1267
94MB10	1994	Maumee	bay	8	744.7333333	398465.3
95SR1	1995	Sandusky	wriver	11	1699.886667	400561.9033
95SR2	1995	Sandusky	wriver	10	1775.196667	418484.8433

95SR3	1995	Sandusky	wriver	4	1886.726667	406951.31
95SR5	1995	Sandusky	wriver	14	1548.703333	391601.3133
95SR6	1995	Sandusky	wriver	9	2148.1025	414611.3333
95SR7	1995	Sandusky	wriver	10	1752.556667	423396.6367
95SR8	1995	Sandusky	wriver	13	1734.253333	434863.17
95SR9	1995	Sandusky	wriver	12	1607.906667	411717.83
95SR10	1995	Sandusky	wriver	10	1870.626667	427994.0633
95SR11	1995	Sandusky	wriver	7	2010.22	438493.68
95SB	1995	Sandusky	bay	NA	1840.456667	438335.22
95SB	1995	Sandusky	bay	NA	1818.455	412999.4167
95SB	1995	Sandusky	bay	NA	1667.636667	439556.3233
95SB	1995	Sandusky	bay	NA	1466.52	430491.55
95SB	1995	Sandusky	bay	NA	2092.656667	422097.6367
95SB	1995	Sandusky	bay	NA	1674.553333	413481.25
95SB	1995	Sandusky	bay	NA	1651.806667	419389.73
95SB	1995	Sandusky	bay	NA	2031.886667	418901.3333
95SB	1995	Sandusky	bay	NA	1737.096667	417623.6567
95SB	1995	Sandusky	bay	NA	1195.893333	409088.1333
95MR2	1995	Maumee	wriver	12	903.2033333	409941.1767
95MR4	1995	Maumee	wriver	14	844.39	396458.8833
95MR5	1995	Maumee	wriver	7	840.37	378590
95MR6	1995	Maumee	wriver	NA	727.9833333	373858.2167
95MR7	1995	Maumee	wriver	7	719.8	381444.74
95MR8	1995	Maumee	wriver	11	815.6933333	382578.7933
95MR9	1995	Maumee	wriver	8	802.1633333	371659.26
95MR10	1995	Maumee	wriver	10	788.9766667	401833.2733
95MR11	1995	Maumee	wriver	6	856.4966667	425720.42
95MR12	1995	Maumee	wriver	10	799.5966667	378442.97
95MR13	1995	Maumee	wriver	13	775.7033333	387113.2
95MB1	1995	Maumee	bay	4	522.06	416975.8467
95MB2	1995	Maumee	bay	3	562.88	391035.065
95MB3	1995	Maumee	bay	3	418.3	390941.92
95MB5	1995	Maumee	bay	4	452.465	409610.39
95MB7	1995	Maumee	bay	3	477.38	405729.53
95MB9	1995	Maumee	bay	3	529.96	405240.9467
95MB10	1995	Maumee	bay	3	473.515	395388.595
95MB11	1995	Maumee	bay	3	445.39	413726
95MB12	1995	Maumee	bay	4	395.855	388020.065

Sr:Ca (mmol:mol)

1.895426807
1.46646267
1.832204333
1.894894417
1.761131073
1.745565936
1.49351135
1.747792371
1.625199112
1.67149988
1.672097443
1.752777141
1.822942091
2.034802858
1.808475389
1.966995679
1.989506759
1.384234938
1.967562571
1.818359521
1.622060218
1.50048304
0.962027186
1.232239142
0.808470923
0.907671524
1.003924441
0.92093733
1.625321606
1.303634786
0.968667847
1.063561701
1.044943012
1.149107382
1.061919533
0.803711236
0.937096845
1.165893223
1.085993257
0.99499928

2.735379411
2.595272256
2.785453425
2.855242096
2.642728136
2.764645495
2.359281092
1.941004878
2.601814887
3.119390487
1.572238195
1.572073719
1.863889292
1.651557913
1.08662094
1.54400526
1.750555259
1.415191001
1.563249463
1.675233693
0.877533584
0.606937591
0.964969201
0.584544304
0.743694857
0.63338785
0.864729665
0.811294814
0.737230702
0.833546304
0.542050768
0.721879177
0.807499128
0.94316291
0.784628093
0.824180839
1.018242226
0.907787682
0.906006193
1.003594052
0.854584605
1.940417152
1.939597071

Draft

2.119880189
1.808293298
2.368964816
1.892646233
1.823495474
1.78568948
1.998453997
2.096162902
1.919835458
2.013250888
1.734729176
1.557643755
2.266886834
1.851773832
1.80088592
2.217851772
1.901882466
1.336658124
1.007415822
0.973844705
1.014953583
0.890346971
0.862829512
0.974879188
0.986876092
0.897765772
0.919911344
0.96608494
0.916225673
0.572472065
0.658180322
0.489238248
0.505078411
0.537987743
0.597963327
0.54758858
0.492234928
0.466473235

Draft