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FINAL REPORT

EFFECTS OF TEMPERATURE AND ELEMENTAL CONCENTRATION ON THE CHEMICAL COMPOSITION OF PERCID OTOLITHS

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INTRODUCTION:

Understanding the life history of animals has become a central focus of resource managers interested in exploitation as well as conservation of these animals. Understanding movement patterns is instrumental to the understanding of a species’ life history because movement patterns are relevant to determining spatial structure of populations and metapopulations, ontogenetic and seasonal changes in habitat use, and ontogenetic and seasonal migration patterns (Rubenstein and Hobson 2004). Animal movements historically have been estimated using mark-recapture techniques or, more directly, using telemetry. However, mark-recapture techniques are difficult with early life stages of fish because of low survival and with fish in large lakes because of low recapture rates. Telemetry is difficult to carry out on small fish (i.e., early life stages) due to tag size and is quite expensive when large sample sizes are needed.

Biogeochemical markers (e.g., trace elements and stable isotopes) provide an alternative technique being used more frequently in determining movement patterns of animals (Campana 1999, Kelly 2000, Webster et al. 2002). This method allows for focus on any life stage, inclusion of large sample sizes, and a broad geographic coverage. Further, previously archived samples can be analyzed for biogeochemicals, allowing the possibility of investigating long term changes in populations.

Biogeochemical techniques have been used to study a broad range of animals and ecological questions (Rubenstein and Hobson 2004). Identification of overwintering habitat of Monarch butterflies (Danaus plexippus) (Wassenaar and Hobson 1998), essential foraging habitat before migration to nesting location of loggerhead females (Caretta caretta) (Hatase et al. 2002), long-distance migration patterns of baleen whales (Hobson 1999), and habitat use and diet of bird populations (Bearhop et al. 2003) are only a few examples. Fishery ecologists, in particular, have used biogeochemical markers to identify natal origins (Atlantic salmon, Salmo salar,

Fishery ecologists use the concentration of trace elements in the otoliths, or otolith microchemistry, as the biogeochemical marker. Otoliths are the earbones of fish and grow outward chronologically, similar to the rings of trees. As otoliths are growing, they incorporate trace elements from the water as substitutes for Ca in the matrix (CaCO) of the otolith, making a permanent record of the elemental character of the water where a fish has been. Otoliths are conservative structures and the elements are not resorbed or released once incorporated into the otolith (Campana and Neilson 1985). Using the correlation between elemental concentration of the water and that of the otolith, we can potentially identify where an individual lived as it was forming different sections of its otolith. Using laser ablation technology, we can pinpoint the area of the otolith we want to analyze and then we can choose a precise age (e.g., the larval period) and associate elemental composition of the otolith with that age. In this way, we can sample older fish, determine where they spent their larval period, and determine which larval habitats are contributing most to the lakewide population. Trace elements typically studied in fish otoliths are Sr, Ba, Mg, and Mn, with the first two elements being the primary focus.

Walleye (*Sander vitreus*) and yellow perch (*Perca flavescens*) are the two most important sport and commercial fisheries of Lake Erie. The primary goal of the Ohio Department of Natural Resources (ODNR) as well as all members of the Lake Erie Committee for these two fish species is to maintain self-sustaining and healthy populations. Currently, our laboratory has two new, multi-year research projects funded by the ODNR that will use otolith microchemistry to
better understand walleye and yellow perch life history. This research will determine habitat use during both spawning and feeding periods, whether distinct spawning locations exist, whether individuals use the same spawning location yearly, which spawning groups (and thus which spawning habitats) contribute the most recruits to the population, and the location and extent of seasonal migratory movements.

Although otolith microchemistry is becoming a popular tool of freshwater fishery ecologists, we are recognizing some limits in our understanding of the elemental uptake process. For this technique to be successful, an uptake relationship must exist between elemental concentration in the environment and resultant elemental concentration in the otolith. Although this relationship has been shown for marine and estuarine fish in both field and laboratory studies with a variety of species (Campana 1999, Martin et al. 2004), the relationship is not well documented for freshwater fish species (Wells et al. 2003). Abiotic factors, such as salinity and temperature, are known to influence elemental uptake rates, particularly for Sr (Fowler et al. 1995, Bath et al. 2000, Martin et al. 2004). While it is likely that such relationships exist with temperature in freshwater, the exact influence of temperature has yet to be established in freshwater. Because environmental concentrations are lower in freshwater systems than in marine systems and because physiology of freshwater fish differs substantially from marine fish, we cannot rely on previously established findings from marine studies (Wells et al. 2003, Hedges et al. 2004). Characterization of how environmental concentration of trace elements and temperature influence uptake rates by freshwater fish is necessary if this technique is to be applied successfully in Lake Erie and other freshwater environments.

We have completed an experiment that will aid our understanding of the elemental uptake process in otoliths of walleye and yellow perch. Our experiment, conducted during the summer
of 2005 and 2006, measures the influence of temperature and elemental concentration of the water on otolith microchemistry. Our ultimate goal is to be able to use the results generated from this study in studies in which we take otoliths from fish that are captured at a given location in Lake Erie and at a given time and determine where those fish had lived previously. Because of our incomplete understanding of the interaction between environmental elemental concentration and temperature on the ultimate elemental concentration of the otolith, we risk ambiguity in assigning sources to fish in these studies. For example, suppose we capture a yellow perch and determine from its otoliths that it is 20 days old and has otolith strontium:calcium ratio of \( x \). Given our sampling of elemental concentrations in the water at different sites, we determine that it came either from a high Sr site with low temperatures or a medium Sr site with high temperatures. Because different elements will have different uptake relationships with temperature and occur at different concentrations in the environment, each element will produce a different set of possible sources. So now if we add to the Sr:Ca information the data from another element, say Ba, that has a different uptake relationship with temperature, we can begin to narrow down the number of possible sources for this fish. Because Lake Erie spawning and larval-rearing habitats experience a range of temperatures between locations at a given time and within locations over a season (Figure 1, Van Tassell, unpublished data), we expect the problem of temperature to be important in these studies.

**METHODS:**

During the summer of 2005 and 2006, we conducted experiments to examine the influence of water temperature and elemental concentration on the elemental uptake of percid otoliths. These experiments were conducted using funding from NOAA and the Lake Erie Protection Fund.
Juvenile walleye and yellow perch were obtained from the St. Marys State Fish Hatchery in May 2005 and 2006. Fish were hatched and reared at the hatchery using water from a common initial source prior to the experiment. For the experiment, fish were transported to the wetlab facilities of the Aquatic Ecology Laboratory (AEL) at The Ohio State University. Yellow perch and walleye were maintained on a 12-h light : 12-h dark cycle. Yellow perch were fed a diet of freeze-dried zooplankton for the duration of the experiment. Walleye were fed live guppies.

**Experimental Design:**

We used a factorial design (Figure 2) with two replicates of each of three levels of elemental concentrations (native laboratory water served as a control and laboratory water spiked with low and high concentrations of strontium (Sr) 300 and 1500 μg/L, barium (Ba) 35 and 70 μg/L, magnesium (Mg) 20 and 40 mg/L, and manganese (Mn) 10 and 40 μg/L served as the treatments) crossed with three temperatures (10, 15, and 20°C), representing a range of expected conditions in Lake Erie and its tributaries. (2 reps \( \times \) 3 temps \( \times \) 3 concentrations = 18 experimental units). Elemental levels were established by diluting water spiked with a known elemental concentration. Water spikes were prepared by adding appropriate amounts of SrCl₂, BaCl₂, MgCl₂ \( \cdot \) 6H₂O, and MnCl₂ \( \cdot \) 4H₂O to dechlorinated water (Bath et al. 2000). Water was changed three times per week at a 50% volume to maintain water quality and elemental concentrations within the tanks. Treatments were randomly assigned to 100-L tanks. In 2005, only a control and high elemental concentration with all three temperatures for yellow perch were run. In 2006, a control and middle elemental concentration with all three temperatures for yellow perch and the entire experimental design for walleye were run. At the onset of the experiment, juvenile yellow perch and walleye were randomly distributed in equal numbers among experimental tanks at an initial density between 10 to 15 fish per tank. To maximize survival, we acclimated fish in experimental tanks for at least one week while temperature treatments were established. Temperature treatments were established by bringing
environmental chambers to 10°, 15°, and 20° at a rate of 1° C per day. After acclimation, treatments were maintained for 45 days. Water temperature and dissolved oxygen were monitored daily and pH and ammonia and nitrate levels were monitored every three days.

Water samples were collected three times per week to monitor elemental conditions within the tanks. Water samples were filtered through 0.45μm cellulose nitrate membrane filters, preserved and acidified with 2% (volume : volume) trace metal grade nitric acid, and stored for elemental analyses. On day 45, all fish were removed from the tanks and euthanized with an overdose of MS-222, measured for total length, and weighed. To avoid contamination, otoliths were processed according to methods described in Fowler et al. (1995). Otoliths were handled using only non-metallic instruments. We used glass probes to remove sagittal otoliths from juvenile fish under a dissecting microscope. Otoliths were prepared for elemental analyses by sectioning near the otolith primordium using an Isomet low-speed saw and polished using 3-μm Imperial lapping film to expose the primordium. Sectioned otoliths were cleaned in an ultrasonic water bath, triple rinsed in Super-Q water and air-dried in the class-100 hood. Dry otoliths were measured, weighed and placed in acid-washed polypropylene vials.

Chemical Analyses:
All elemental analyses were performed by the Microscopic and Chemical Analysis Research Center (MARC) located at The Ohio State University. The concentrations of Ca, Ba, Mg, Mn and Sr in the water from the tanks that the fish were held in were measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) using a PerkinElmer Optima 4300 DV instrument. The instrument was calibrated using a set of standard solutions made by appropriate dilution and mixing of single element standards (CPI International). Details on the type of equipment and the procedures used to analyze these particular elements in otoliths can be found in Appendix I.
Next, a series of experiments were carried out to determine where and how to sample the otoliths to obtain accurate measurements of the uptake of elements from the water in the tanks (treated water versus control) into the otoliths. LA-ICP-MS measurements were made across the otolith along the longest axis using line scan mode and a series of spots spaced 75 \( \mu \text{m} \) apart. While the line scan provides continuous measurement of elemental concentrations across the otolith, there are several advantages of making measurements with a series of spots. The effective time constant of the ablation cell was determined to be approximately 10 s, which can limit spatial resolution. In the case of single spots, any surface contamination would be obvious in the signal versus time data and can be discarded from the signal. The lateral spatial location responsible for the signal well defined without the need to account for the cell wash out time constant. Ablation particles are smaller, ensuring more complete vaporization in the ICP.

Based on the experimental results from the line scan and series of spots, it was clear that uptake elements in the tank water occurred mainly in the outermost 50 to 75 \( \mu \text{m} \) of the otolith. Based on experience gained from optical observation of otoliths, it was decided that the best region to sample was likely the region to the lower right of the sulcus. Others have reported that growth lines are most distinct in this region. For comparison, we also sampled near the end of the otolith to the right of the sulcus near the longest axis, where growth should be most rapid.

A 30 \( \mu \text{m} \) laser spot size was used to provide resolution so that the area of the otolith being sampled should be relatively homogeneous while also providing sufficient sensitivity. The laser energy per pulse at the sample was approximately 0.11 mJ, which corresponds to approximately 15 J/cm\(^2\). Data were acquired for each of the five isotopes each second for approximately 40 s at each ablation spot. A delay of 20 seconds was used between measurements at successive spots. All other isotope signals were ratioed to the \(^{43}\text{Ca}^+\) signal to
compensate for any changes in ablation rate over time or from sampling location to sampling location.

Calibration was based on a blank measurement (shutter closed so that no laser radiation hit the sample) and a NIST 610 glass standard reference material. Preferred concentrations listed by Pearce et al (N. J. G. Pearce, W. T. Perkins, J. A. Westgate, M. P. Gorton, S. E. Jackson, C. R. Neal and S. P. Chenery, “A Compilation of New and Published Major and Trace Element Data for NIST SRM 610 and NIST SRM 612 Glass Reference Materials”, Geostandards Newsletter, 21, 115-144 (1998). As a check for instrumental drift, NIST 610 was measured before and after each set of measurements of otoliths from fish in the treated and control water.

Data Analysis:
The ICPMS results for all elements were standardized to calcium and converted to molar ratios for statistical analysis. In addition, we calculated partition coefficients ($D_{Me}$ from Bath et al. 2000) for each element to calcium ratio, where

$$\left(\frac{Me}{Ca}\right)_{\text{Otolith}} = D_{Me} \left(\frac{Me}{Ca}\right)_{\text{Water}}$$

Diagnostic tests (Shapiro-Wilk Test) revealed that the residuals of the Mn:Ca ratios were not normally distributed so this variable was log transformed prior to analysis. We used a repeated measures analysis of variance (ANOVA) to monitor the elemental composition of the water in the experimental tanks and to validate the separation between elemental conditions in the treatment versus control tanks through the duration of the experiment. A two-way ANOVA was used to compare the elemental composition of otoliths among treatments, with temperature and water elemental concentration as independent variables in the model and the element to calcium ratio and $D_{Me}$ for each element as dependent variables. All statistical tests were
performed using JMPIN software (SAS Institute, Inc. 2001) at a significance level of 0.05, and, when significant differences were found, Tukey’s HSD test was used to indicate where differences occurred.

RESULTS:

Water:
Mean element to calcium ratios differed between treatment and control tanks for the elements Ba, Mg, and Sr through the duration of the experiment (Table 1). We were unable to accurately measure Mn:Ca in the tanks due to concentrations being below the detection limits of our instruments, therefore, Mn was excluded from the partition coefficient analysis.

Juvenile percid growth:
The juvenile walleye grew during the 45 days of the experiment. During the experiment, we tried to maintain similar growth rates among chemical treatments. At the end of the experiment, walleye length was similar among elemental treatments within a given temperature ($F = 0.761$, $df = 2$, $P < 0.469$) but differed among temperature treatments (Figure 3, $F = 53.199$, $df = 2$, $P < 0.0001$). Similarly with yellow perch, length did not differ among elemental treatments within a temperature (2005, $F = 0.204$, $df = 1$, $P < 0.652$, 2006, $F = 102.687$, $df = 2$, $P < 0.0001$) but differed among temperature treatments (Figure 4, 2005, $F = 0.649$, $df = 1$, $P < 0.495$, 2006, $F = 9.323$, $df = 2$, $P = 0.0002$). Otolith diameter positively related to total length for juvenile percids (Figure 3 and 4).

Walleye otoliths:
Rather than finding a single relationship for all elements, we found that uptake of individual elements into the otolith was uniquely influenced by water elemental concentration and temperature (Figure 5). The Mg:Ca in otoliths was influenced neither by water Mg elemental
concentrations ($F = 0.720, df = 2, P = 0.49$) nor by temperature ($F = 0.030, df = 2, P = 0.97$).
Mn:Ca in otoliths did not vary with Mn concentration in the water ($F = 0.609, df = 2, P = 0.545$)
nor with temperature ($F = 0.574, df = 2, P = 0.564$). Sr:Ca did vary with water concentrations of
Sr ($F = 4.916, df = 2, P = 0.0086$), with fish otoliths from the low treatment having lower Sr:Ca
ratios than fish otoliths from both control and high treatments (Figure 6). Otolith Sr:Ca
increased with increasing temperature (Figure 5, $F = 114.202, df = 2, P < 0.0001$). Ba:Ca in the
otolith increased with increasing Ba concentration in the water ($F = 43.577, df = 2, P < 0.0001$),
but decreased with temperature in control otoliths (Figure 7, treatment x temperature: $F = 3.213,
df = 4, P = 0.015$). Otolith Ba:Ca varied with temperature ($F = 5.984, df = 2, P = 0.0032$) with
concentrations greater in 15°C than 10°C, and 20°C similar to both of these temperatures
(Figure 5).

We calculated partition coefficients for Sr and Ba to more directly compare elemental
concentrations in specific rearing tanks to elemental concentrations in otoliths. Partition
coefficients for individual elements were uniquely influenced by water elemental concentration
and temperature. Ba partitioning generally increased with Ba concentration in the water, while
increasing with temperature in the low and high treatments but declining with temperature in the
control treatment (Figure 8). For Sr, partition coefficients increased with increasing
temperatures, with the control treatment being much higher than with the other two treatments.
Sr partition coefficient in the control treatment was near one or above for all temperatures
(Figure 8). By the end of the experiment, walleye otoliths had equal or more Sr than was
present in the water.

Yellow perch otoliths:
As with walleye, uptake of individual elements into the otolith was uniquely influenced by water
elemental concentration and temperature (Figure 9). The concentration of Mg in otoliths was
not influenced by water elemental concentrations ($F = 2.190$, $df = 2$, $P = 0.114$) nor by temperature ($F = 0.5790$, $df = 2$, $P = 0.561$). Otolith Mn:Ca concentration was not influenced by Mn concentration in the water ($F = 0.540$, $df = 2$, $P = 0.478$). Mn:Ca concentration in the otolith declined from 10°C to 15 and 20°C for the control and high treatments ($F = 8.268$, $df = 2$, $P = 0.0003$), with the low treatment not varying by temperature (Figure 9, treatment x temperature: $F = 2.903$, $df = 2$, $P = 0.0226$). Sr:Ca increased with increasing water Sr (Figure 10, $F = 143.011$, $df = 2$, $P < 0.0001$) and temperature ($F = 30.415$, $df = 2$, $P < 0.0001$) in treatment otoliths, but decreased with temperature in control otoliths (Figure 9, temperature x treatment: $F = 20.35$, $df = 2$, $P < 0.0001$). Ba:Ca in otoliths increased as Ba increased in the water (Figure 11, $F = 98.128$, $df = 2$, $P < 0.0001$), with control otoliths declining and low otoliths increasing with temperature and high otoliths not varying with temperature (Figure 9, treatment x temperature: $F = 15.5139$, $df = 1$, $P = 0.018$).

As with walleye, we calculated Ba and Sr partition coefficients. Again, partition coefficients differed for individual elements as influenced by water elemental concentration and temperature (Figure 12). Ba partition coefficients declined in the control treatment, increased in the low treatment, and remained constant in the high treatment as temperature increased. In the Sr control treatment, temperature did not influence the partition coefficient. For the low and high treatments, Sr partitioning increased as temperature increased and were similar in value.

**DISCUSSION:**

Previous studies involving marine fish have demonstrated that fish reared in high salinity environments have elevated levels of trace elements in their otoliths (Kalish 1990, Secor 1992). However, when considered, water temperature effects on otolith elemental concentrations often exceed those of salinity (Fowler et al. 1995). In our experiments, we found that water elemental concentrations and temperature had different, often interacting, effects for individual elements.
In most cases, we found a positive relationship between elemental to calcium ratios in otoliths and in rearing water for barium and strontium. Otolith Ba:Ca and Sr:Ca increased with increasing water elemental concentrations for yellow perch. For walleye, otolith Ba:Ca, but not Sr:Ca increased with increasing water elemental concentrations. However, for both species, the magnitude of differences between Ba:Ca and Sr:Ca ratios was significantly influenced by temperature. This is consistent with previous studies that suggest the rate of incorporation of elements, particularly strontium, into otoliths is controlled by water temperature (Radkte 1989). In our study, otolith Ba:Ca and Sr:Ca levels from fish reared in control conditions were often higher than those from fish reared under low elemental concentrations at 10°C, but this pattern was reversed at higher temperatures. To our knowledge, this experiment is the first attempt to characterize elemental ratios in otoliths from fish reared at temperatures below 20°C, but such temperatures are characteristic of environmental conditions experienced by early life stages of percids in the Great Lakes. Our results suggest that water temperature should be considered during any attempts to characterize fish based on otolith chemistry in the Great Lakes region.

Contrary to previous studies, we did not find a positive relationship between ambient water and otolith Sr:Ca for walleye. Instead, Sr:Ca ratios in walleye otoliths increased with temperature across all ambient water conditions. The unusually high Sr:Ca ratios in walleye otoliths (> 5 mmol:mol in most cases) and the lack of a relationship between ambient water and otolith concentrations suggests that the walleye otoliths may have been contaminated during the course of the study. At present, we are reanalyzing the walleye otoliths using different mounting methods to identify possible sources of contamination.

Trace elements, such as barium and strontium, are believed to be substituted into the otolith by substituting for calcium ions (Campana 1999) Therefore, differences in uptake rate may occur due to competition for limited spaces in the otolith matrix. In the current study, we found that Sr...
is incorporated into the otolith more efficiently (higher DSr values) than Ba, suggesting different uptake rates for these elements. For both walleye and yellow perch, strontium partition coefficients were higher for fish reared in control water than those reared in water spiked with strontium, suggesting that strontium is incorporated into otoliths more efficiently at lower ambient levels. On the other hand, barium partitioning was higher in otoliths from fish reared in both spiked treatments than from fish reared under control conditions at 15 and 20°C. These results are consistent with a recent study by de Vries et al. (2005), who found that, rather than competing with strontium for space in the otolith, barium uptake into otoliths can be facilitated by high levels of strontium. Our results illustrate the importance of measuring multiple elements in water samples because the uptake of one element may alter the relationship between otolith and ambient water conditions in other elements.

LITERATURE CITED:


Campana, S.E. and Neilson, J.D., 1985, “Microstructure of fish otoliths,” Canadian Journal of Fisheries and Aquatic Sciences, 42, 5, 1014-1032.


Hatase, H., Takai, N., Matsuzawa, Y., Sakamoto, W., Omuta, K., Goto, K., Arai, N., Fujiwara, T.,


In order to assess and ensure high reliability of the data results from three different emission lines (listed in Table A1) were used for each element. If a spectral overlap occurred, results from the different lines would not agree. If the matrix for the calibration standards did not adequately match the samples, then lines due to emission from ions (Emission lines 1 and 2 in Table 1) would produce different results than emission from atoms (I) or ions (II) with low excitation energies (Emission line 3 in Table 1). Emission was measured from the end of the plasma “axially” for all emission lines. In addition, emission was viewed and separately measured both from the side of the plasma (“radially”) for Sr and Ca which had high intensities. Axial and radial results were compared to be sure that self-absorption did not occur. Results were averaged from all of the emission lines.

Laser ablation inductively-coupled mass spectrometry (LA-ICPMS) can provide quantitative elemental analysis directly from solid samples with spatial resolution on the tens of micrometer spatial scale. A New Wave Research UP-193HE 193 nm excimer laser with two 9x9 arrays of lenses to provide a fully homogenized laser beam was used for ablation of the sample. The use of a beam homogenized, 193 nm laser and flowing helium through the ablation cell ensures controlled ablation and generation of small particles that are transported and then effectively vaporized in the ICPMS. In this system, the laser spot size at the sample is controlled by computer controlled wheel with different apertures. The selected aperture is imaged on the sample surface with a magnification of approximately 16. Aerosol generated by the ablation process was transported to a ThermoFinnigan Element 2 Inductively Coupled Plasma Sector Field Mass Spectrometer.

The appropriate isotopes and mass spectral resolution to measure ICP-MS signals rapidly
enough to follow the transient laser ablation signals were determined experimentally. Spectral overlaps can be one of the main limitations of ICP-MS. Quadrupole mass spectrometers, that are most commonly used for ICP-MS provide resolution of 0.7 to 1 amu. This is insufficient to resolve most molecular ions from the elemental ion of interest. The mass spectral resolution provided by the double focusing, sector based mass spectrometer used in the ThermoFinnigan Element 2 ICP-MS can provide sufficient mass spectral resolution to overcome many of these spectral overlaps. We acquired medium (R=\text{m/m}=4,000) and high (R=\text{m/m}=10,000) spectra within one amu of the elemental ions of interest using LA-ICP-MS of otoliths to determine the severity of potential spectral overlaps and what resolution is required to avoid the overlaps. We determined that while \textsuperscript{12}C\textsubscript{2}\textsuperscript{+} signals were small compared to \textsuperscript{24}Mg\textsuperscript{+} signals, \textsuperscript{48}Ca\textsuperscript{2+} signals were significant and sometimes large compared to the \textsuperscript{24}Mg\textsuperscript{+}. \textsuperscript{ArN}\textsuperscript{+} or \textsuperscript{ArNH}\textsuperscript{+} signals were significant compared to \textsuperscript{55}Mn\textsuperscript{+}. Therefore, medium resolution was used. In the past, it would have been difficult to measure LA-ICP-MS signals with medium resolution exclusively at the peak maxima due to instability and slow magnet response. The fast magnet option on our instrument, together with the new AutoLock mass feature that measures and compensates for mass drift by measuring \textsuperscript{Ar}\textsuperscript{2+} spectra immediately before each sample allowed us to develop a method with short settling times while reliably sampling with 5\% of the peak mass signal.

In order to maximize the percentage of time during which signal is being acquired, short settling times were used following magnet jumps, electric field based jumps were used whenever possible and only masses near with 5\% of the peak mass for all isotopes except \textsuperscript{24}Mg\textsuperscript{+} which was sampled within 12.5\% of the peak mass. In order to make all measurements in pulse counting mode so that noise due to switching of the detection circuit between pulse counting and analogy modes could be avoided, a minor isotope of Ca (\textsuperscript{43}Ca\textsuperscript{+}, 0.135\%) was measured. Other isotope measured included: \textsuperscript{24}Mg\textsuperscript{+}, \textsuperscript{55}Mn\textsuperscript{+}, \textsuperscript{88}Sr\textsuperscript{+} and \textsuperscript{138}Ba\textsuperscript{+}. 
<table>
<thead>
<tr>
<th>Element</th>
<th>Emission line 1</th>
<th>Emission line 2</th>
<th>Emission line 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>317.933 nm (II)</td>
<td>315.887 nm (II)</td>
<td>422.673 nm (I)</td>
</tr>
<tr>
<td>Ba</td>
<td>233.527 nm (II)</td>
<td>493.408 nm (II)</td>
<td>455.403 nm (II*)</td>
</tr>
<tr>
<td>Mg</td>
<td>279.077 nm (II)</td>
<td>280.271 nm (II)</td>
<td>285.213 nm (I)</td>
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<td>Mn</td>
<td>257.610 nm (II)</td>
<td>259.372 nm (II)</td>
<td>260.568 nm (I)</td>
</tr>
<tr>
<td>Sr</td>
<td>407.771 nm (II)</td>
<td>421.552 nm (II)</td>
<td>460.733 nm (I)</td>
</tr>
</tbody>
</table>

Table A1. Summary of the different emission lines used for each element during the LA-ICPMS analyses.
Table 1. Water element to calcium ratios (mmol:mmol) measured in the experimental treatments. Means of all days averaged over replicate tanks are reported with standard errors in brackets. All elemental concentration treatments used for both walleye and yellow perch differed (Repeated-measures ANOVA, $P < 0.05$).

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Year</th>
<th>Element</th>
<th>Ba:Ca</th>
<th>Mg:Ca</th>
<th>Sr:Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walleye</td>
<td>Control</td>
<td>2006</td>
<td></td>
<td>4.81 (0.09)</td>
<td>527.04 (1.79)</td>
<td>3.89 (0.03)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>2006</td>
<td></td>
<td>5.93 (0.08)</td>
<td>601.69 (2.10)</td>
<td>10.85 (0.08)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>2006</td>
<td></td>
<td>7.03 (0.09)</td>
<td>673.30 (2.71)</td>
<td>17.41 (0.11)</td>
</tr>
<tr>
<td>Yellow perch</td>
<td>Control</td>
<td>2005</td>
<td></td>
<td>4.61 (0.06)</td>
<td>499.04 (3.33)</td>
<td>3.06 (0.16)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2006</td>
<td></td>
<td>5.19 (0.10)</td>
<td>539.44 (4.53)</td>
<td>3.63 (0.37)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>2006</td>
<td></td>
<td>6.03 (0.14)</td>
<td>605.69 (2.94)</td>
<td>9.73 (0.09)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>2005</td>
<td></td>
<td>6.32 (0.15)</td>
<td>667.65 (5.03)</td>
<td>15.87 (0.07)</td>
</tr>
</tbody>
</table>
Figure 1. Surface temperatures for three walleye spawning locations in Lake Erie measured during the 2004 spawning run (Van Tassell, unpublished data).
Figure 2. Schematic of the 3 x 3 experimental design of the walleye and yellow perch experiment.
Figure 3. Total length versus otolith diameter for juvenile walleye. Fish from each temperature treatment were combined in the regression.
Figure 4. Total length versus otolith diameter for juvenile yellow perch. Fish from each temperature treatment were combined in the regression.
Figure 5. Mean element to calcium ratios for Mg, Mn, Sr, and Ba in otoliths taken from walleye during the temperature experiment. Error bars are standard errors.
Figure 6. Mean element to calcium ratios for Sr in otoliths versus water for walleye. Error bars are standard errors.
Figure 7. Mean element to calcium ratios for Ba in otoliths versus water for walleye. Error bars are standard errors.
Figure 8. Mean partition coefficients calculated for Ba and Sr at the three experimental temperatures and elemental concentrations for walleye. Error bars are standard errors.
Figure 9. Mean element to calcium ratios for Mg, Mn, Sr, and Ba in otoliths taken from yellow perch during the temperature experiment. Error bars are standard errors.
Figure 10. Mean element to calcium ratios for Sr in otoliths versus water for yellow perch. Error bars are standard errors.
Figure 11. Mean element to calcium ratios for Ba in otoliths versus water for yellow perch. Error bars are standard errors.
Figure 12. Mean partition coefficients calculated for Ba and Sr at the three experimental temperatures and elemental concentrations for yellow perch. Error bars are standard errors.