

**Otolith Chemistry of Asian Carps in Lake Erie  
Final Report**

Lake Erie Protection Fund Project SG 464 – 2014

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## **ABSTRACT**

The ability to rapidly identify spawning habitat being successfully utilized by invasive Asian carp will be critical in efforts to eradicate/suppress them should an invasion occur. We employed total digestion and laser ablation analysis of otoliths from common carp (*Cyprinus carpio*), the well-established Asian carp species, collected from impounded reservoirs in NW Ohio. We found a strong linear relationship between Sr:Ca ratios in both the aragonitic lapillus and vateritic asteriscus otoliths over a broad range of Sr:Ca water chemistries that encompasses the Grand, Maumee, and Sandusky Rivers, which have been identified as providing suitable reaches for spawning of invasive Asian carp. In addition, we analyzed otoliths from grass, bighead, and silver carp that were reared in the same water mass. The lapillus Sr:Ca otolith-water partition coefficient for common carp is similar to the bighead and silver carp, which are all significantly less than the grass carp. Hence, the Sr:Ca otolith-water relationship established for the common carp can be directly employed to evaluate natal tributary utilization and subsequent migrations by the highly destructive Asian carp should they invade Lake Erie based on their otolith microchemistry.

## **ACTIVITIES, TIMELINE, AND HURDLES**

Late Fall-early Winter 2013 preliminary water samples and common carp were collected.

Winter 2013-2014 preliminary samples were analyzed to decide which reservoirs were most appropriate for further study.

Summer and Fall 2014 additional water and common carp samples were collected and analyzed.

Our primary hurdle in conducting this study was identifying sources of invasive Asian carp that were reared together in stable water masses. Our early attempts using fish from hatcheries in Arkansas demonstrated too much variability across the otoliths due either to seasonal changes in water chemistry or fish migration. We finally were able to get fish from the USGS-UMESC facility in Fall 2014 which prompted us to request a no-cost extension to March, 2015.

Late Fall-early Winter 2014-2015 preparation and analysis of the water and invasive Asian carp otoliths was completed.

February, 2015 the graduate student working on the projected presented the common carp data at the 75<sup>th</sup> Midwest Fish and Wildlife Conference in Indianapolis, IN.

## **DELIVERABLES**

Final project deliverables include a final report, a presentation at the 75<sup>th</sup> Midwest Fish and Wildlife Conference, and a manuscript that is in preparation for submission to a peer-reviewed journal. Also, the invasive Asian carp data will provide the preliminary data for a MS Thesis project that is to be done in collaboration with the USGS-UMESC. These data will also be used to request additional funding from various federal agencies.

## TECHNICAL REPORT

### Introduction

#### *Invasive Asian carp*

Invasive Asian carp have been introduced into North American waters primarily through aquaculture escapes and intentional introduction. Common carp (*Cyprinus carpio*) were first introduced in the mid-1800s and were intentionally stocked into many watersheds in the Mississippi and Great Lakes drainages (Mills et al. 2005). Grass carp (*Ctenopharyngodon idella*) were introduced to North America in the late 1960s as a means of improving water quality in aquaculture hatchery ponds (Guillory and Gasaway 1978). The silver carp (*Hypophthalmichthys molitrix*) and the bighead carp (*Hypophthalmichthys nobilis*) were imported in the early 1970s for food aquaculture. Escape from hatchery ponds to the Mississippi drainage happened quickly after importation (Kolar et al. 2005). Currently the common carp is the only established species in the Great Lakes.

There is great concern regarding the invasion of Asian carps into the Great Lakes and the resulting impact they will have on native fish and the ecosystem (Kolar et al. 2005), especially in the highly productive western basin of Lake Erie. Invasive Asian carps, including bighead carp (*Hypophthalmichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), and black carp (*Mylopharyngodon piceus*), have been shown to have negative impacts on aquatic ecosystems including the alteration of the plankton and fish communities, competition with benthic macroinvertebrates and fishes, and disease transmission. Kocovsky et al. (2012) have shown the Sandusky, Maumee, and Grand Rivers provide suitable reaches to support spawning Asian carps in Lake Erie. Recent environmental DNA evidence from Lake Michigan and Lake Erie suggests that Asian carps are making inroads into the Great Lakes drainage (MDNR 2012, ODNR 2013). This evidence may indicate the presence of Asian carp; however it does not provide any information regarding their natal origins. Our ability to eliminate or suppress an Asian carp invasion in its early growth phase requires the rapid identification of where these individuals were spawned (i.e., their natal tributary). Previous research on yellow perch (Pangle et al. 2010), white bass (Hayden et al. 2011), and steelhead trout (Boehler et al. 2012) have demonstrated that otolith microchemistry can be employed to discriminate adult fish collected in Lake Erie tributaries to their natal hatchery/tributary with very high confidence. Most recently, Chapman et al. (2014), based largely on otolith microchemistry, showed four grass carp caught in the Sandusky River in October, 2012 were the results of successful natural reproduction.

#### *Otolith Micro-Chemistry*

Teleost fishes contain three pairs of otoliths, the sagittae, asterisci, and lapilli. Otoliths are hearing and balance structures composed of a calcium carbonate ( $\text{CaCO}_3$ ) matrix which is typically the aragonite polymorph of  $\text{CaCO}_3$ , and less commonly, the vaterite or calcite polymorphs (Gauldie 1993). In addition to  $\text{CaCO}_3$ , the matrix can also incorporate certain trace elements into the crystalline lattice in proportion to their abundance in the environment (Campana 1999). The most robust being Sr, Ba, and Mg ions that, due to their similarities in size

and charge, substitute readily into the Ca-lattice sites (Melancon et al. 2005). The trace element uptake is known to be dependent on both the species and the polymorphic form of the otolith. The calcium carbonate otoliths, unlike the calcium phosphate bones and teeth, are not resorbed or reworked by physiological processes (Campana and Neilson, 1990). Hence they act as natural tags and as recorders of a fish's lifelong environmental history.

Most otolith microchemical data have been collected using laser ablation methods where the high spatial resolution of the laser beam (often <25 microns diameter) translates to a very high temporal resolution (days to weeks) in the life of the fish. As such, the laser ablation method has proven invaluable in evaluating fish migratory behaviors (see Pracheil et al. 2014 and reference therein). However, for fish that do not migrate between different water chemistries, total otolith digestion provides a much less time consuming and less expensive means to analyze the otoliths (see Methods section). Previous workers have shown an excellent correlation between laser ablation and total digestion data for otoliths from yellow perch (Ludsin et al. 2006). As such, we employed total digestion for the majority of our samples and cross-correlated with a sub-set analyzed using laser ablation.

There are significant differences in water chemistry between the Sandusky, Maumee, and Grand Rivers (e.g., mean Sr:Ca values are 8.97, 3.95, and 1.29 mmol/mol, respectively, Pangle et al. 2010). As such, we anticipate readily resolvable differences in otolith chemistry for carp spawned and reared in these rivers and associated watersheds. The chemistries of waters originating from the Sandusky and Maumee rivers are unique because both drainages have areas of celestine (SrSO<sub>4</sub>)-bearing upper Silurian-age dolostone bedrocks of the Salina Group (Carlson 1987). This unique geological occurrence of celestine influences the ground water chemistry by enrichment in Sr. The Sandusky River consistently has a higher Sr concentration than the Maumee River, which in turn is higher than the Grand River and open Lake Erie waters (see Pangle et al. 2010 for a thorough review of Lake Erie water chemistry).

As noted above, while most otoliths are the aragonite form of CaCO<sub>3</sub>, some have the vaterite form. In common carp (*Cyprinus carpio*) MacDonald et al. (2012) have shown the lapillus otoliths are aragonite whilst the asteriscus otoliths are vaterite. Previous studies have shown that aragonite and vaterite do not incorporate trace elements at the same concentrations (e.g., Gaudie 1996, Melancon et al. 2005, Veinott et al. 2009). Specifically, aragonite preferentially incorporates the larger Sr and Ba ions whereas vaterite preferentially incorporates the smaller Mg and Mn ions. Consistent with these previous studies, MacDonald et al. (2012) reported that asteriscus otoliths from common carp were enriched in Mg and Mn and depleted in Sr and Ba relative to the lapillus otoliths from the same fish.

The most useful trace element in otolith studies has been Sr due to its relatively high abundance/substitution in the CaCO<sub>3</sub> matrix and its range in concentrations in different waters. Chowdury and Blust (2001) observed that due to their similarity in size and charge, Sr and Ca ions compete for the uptake paths in fish and act as competitive inhibitors. Calcium is a major element in most aquatic systems while Sr is a trace element. As such, Sr is unlikely to inhibit Ca uptake, and hence, it is the concentration of Sr relative to Ca in the water and not the absolute concentration of Sr that should determine the Sr uptake into the otolith. Similar arguments can be

made for Ba. Hence, the Sr and Ba concentrations in water as well as the otoliths are typically ratioed to the Ca concentrations and are reported as element:Ca (mmol/mol).

The relative uptake of Sr and Ba in otoliths is dependent upon both the polymorphic form of the otolith and the species of fish. The relative uptake is typically reported as the element:Ca partition coefficient which is defined as (see also Kraus and Secor 2004, Melancon et al. 2005):

Element:Ca (mmol/mol) in otolith / Element:Ca (mmol/mol) in water, or

Element:Ca (mmol/mol) in aragonite / Element:Ca (mmol/mol) in vaterite

While Kocovsky et al. (2012) identified the Sandusky, Maumee, and Grand Rivers as providing suitable reaches for invasive Asian carp spawning, gladly, invasive grass, bighead, and silver carps have yet to establish a stable population in Lake Erie and its tributaries. However, common carp represent an Asian carp species that is well established throughout North America and the Great Lakes region (Figure 1). As such, it is logical to first employ common carp to establish partition coefficients for trace elements, especially Sr, as a function of water chemistry and polymorphic structure. Having established the otolith trace element concentration as a function of water chemistry in common carp, the data can be used to compare otolith microchemical signatures of invasive Asian carps that are hatched and reared in similar water chemistries to establish a natal signature without actually introducing these invasive fish into Lake Erie. Once natal signatures are established, the natal site and migration history for any Asian carp caught in Lake Erie could be determined by microchemical analysis of its otoliths.

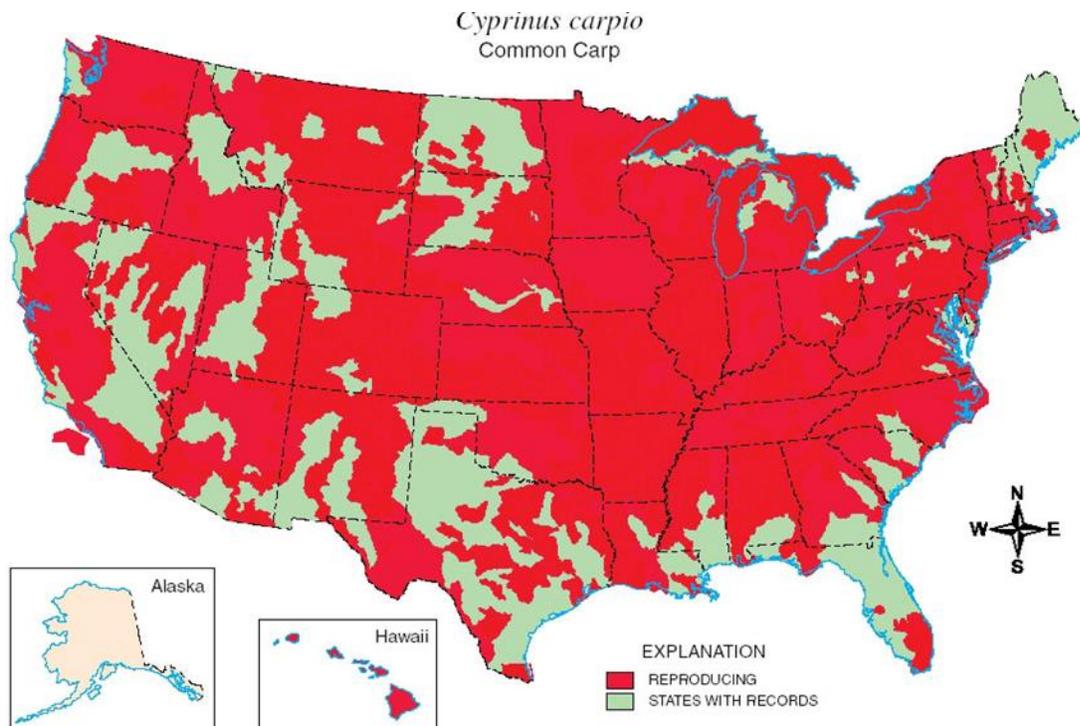


Figure 1. 2014 distribution of common carp (*Cyprinus carpio*) in the US. **Green** areas represent recorded sightings. **Red** areas indicate reproducing populations. From [http://fl.biology.usgs.gov/Carp\\_ID/html/cyprinus\\_carpio.html](http://fl.biology.usgs.gov/Carp_ID/html/cyprinus_carpio.html).

## OBJECTIVES

The primary objective of this project is to preemptively establish a multi-variate algorithm using both lapillus and asteriscus otolith chemistries for discriminating Asian carps that may eventually utilize the Sandusky, Maumee, and Grand Rivers for spawning and nursery habitat. Once these microchemical tags are established, we can rapidly identify the nursery habitats for any Asian carps found in Lake Erie waters, thus allowing managers to focus rapid eradication efforts in these areas. To accomplish this objective we will: [1] Determine trace element (Sr, Ba, Mg) otolith:water uptake ratios for common carp over a range of water chemistries inclusive of the Sandusky, Maumee and Grand Rivers for both lapillus and asteriscus otoliths if possible, and [2] Determine the common carp and Asian carps (grass, bighead and silver) element uptake ratios (partition coefficients) for lapillus and, if possible, asteriscus otoliths.

## METHODS

### *Sampling*

Use of the total digestion method requires a constant otolith microchemistry throughout, meaning constant water chemistry. As such, nine impounded reservoirs located in NW Ohio were selected for this study (Table 1). The reservoirs were distributed in different bedrock units (Figure 2) and preliminary water samples collected from each of the reservoirs showed a broad range of Sr:Ca values that extended beyond the range for the Grand, Maumee, and Sandusky Rivers (Table 1). The common carp were collected by ODNR Division 2 personal during annual fall assessment surveys in 2013 and 2014. The samples were kept frozen until otolith extraction.

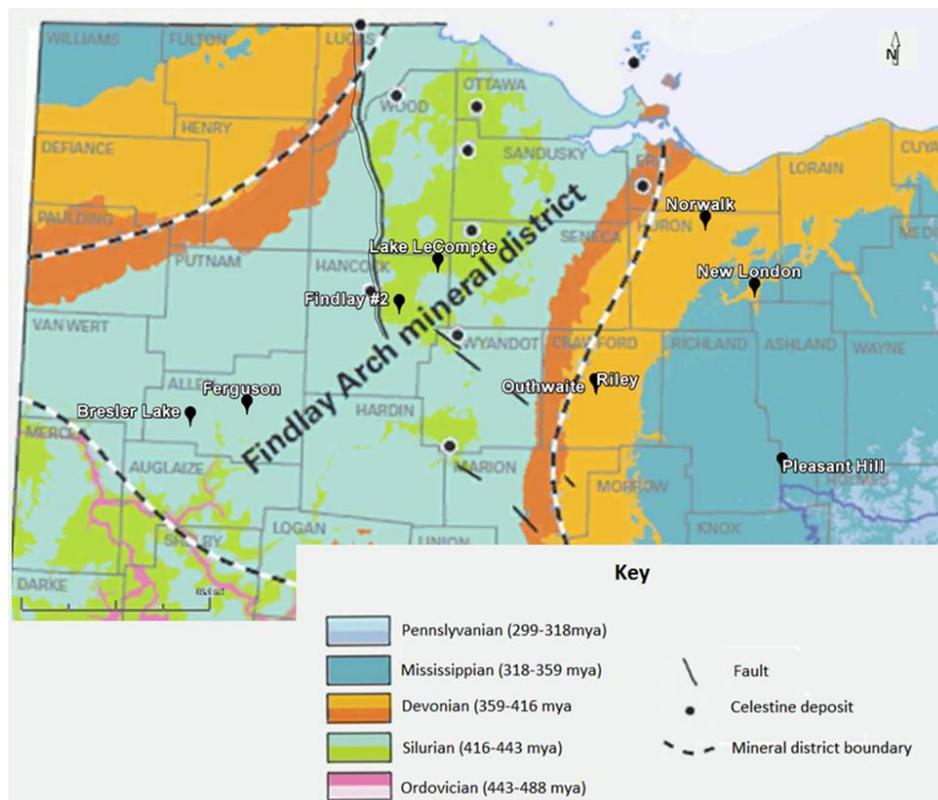


Figure 2. Sample locations for common carp. Note the reservoirs reside in different bedrock units and also note proximity to Celestine ( $\text{SrSO}_4$ ) deposits.

In order to directly compare element:Ca partition coefficients for different carp species, Grass, silver, and bighead carps that were raised in the same water chemistry and with the same food source were obtained (40 of each species) through the USGS Upper Midwest Environmental Science Center (UMESC). Water samples also were collected and sent along with the fish.

All water samples (50 mL) were collected using a separate Luer-Lok Tip sterile syringe and filtered through a 0.45  $\mu\text{m}$  nylon syringe filter into an acid washed, polypropylene Nalgene bottle. The filtered water samples were acidified (2% vol/vol) with metals grade nitric acid following US EPA method 3015. Water samples were transported on ice and stored at 4°C prior to element analysis.

### ***Otolith Preparation and Micro-Chemical Analyses***

The majority of the otoliths were analyzed by total digestion with a sub-set analyzed using laser ablation at the University of Windsor to confirm constant microchemistry across the otoliths and to cross-correlate the methods.

Otoliths were removed with acid-washed glass probes by careful dissection under 8-16x magnification. Each otolith was placed into an acid-washed beaker containing a 20 mL solution of ultrapure Milli-Q water (MQW) and 3% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). The beaker containing the otolith was then sonicated for 5 minutes. After sonication, the otolith was removed from the solution, rinsed with MQW, placed into a clean petri dish, and left to dry overnight under a hood. One lapillus and one asterici otolith were used for total digestion and a subset of the other lapillus was used for laser ablation.

### ***Total Digestion***

The dried otoliths were weighed and the weight recorded. Each otolith was digested in a 20% metals grade  $\text{HNO}_3$  acid solution overnight. After total digestion, the solution was taken-up to 25 ml volume using MQW (Ludsin et. al 2006). Each sample was then analyzed by the ICP-OES following EPA protocols (EPA Method 6010C).

Precision of the ICP-OES analysis was calculated (minimum detection limits) based on blanks and standards prepared using SPEX CertiPrep AA/ICP-AES Plasma Grade standard solutions. Reliability of the otolith digestion method was evaluated using method (acid) blanks and standard reference material (SRM 88b Dolomitic Limestone). Potential instrument drift during analysis was monitored by running a quality control sample after every ten unknown samples. The accuracy of the ICP-OES analysis was checked using SPEX CertiPrep Multi-element Solution 2 certified standard which was randomly included in each set of thirty unknowns. The minimum detection limits for Ba, Ca, Mg, and Sr in solution were routinely less than 0.045, 5.0, 4.5, and 0.022 ppb, respectively, and acid blanks were below the detection limits.

### ***Laser Ablation***

Otoliths were prepared for laser ablation micro-chemical analysis following standard methods (e.g., Secor et al. 1991; Hayden et al. 2011). Otoliths were mounted in West System epoxy resin (#105 resin and #206 slowhardener) and sectioned to a thickness of 1.0 mm in the transverse plane, using a low speed diamond blade saw. Otoliths were then polished to a thickness of approximately 100  $\mu\text{m}$  using progressively finer polishing media including 3 M silicon carbide sandpaper, 3 M lapping paper, and aluminum oxide powder (20  $\mu\text{m}$ , 10  $\mu\text{m}$ , 6

µm, and 0.3 µm) to expose the primordial core. Polished sections were mounted on petrographic slides using a thin layer of West System epoxy resin. The slides were then cleaned by triple rinsing in ultrapure water (18.2 MΩ cm @25 °C) and sonicated for 10 min. All samples were stored in acid washed Petri dishes while awaiting analyses.

Transverse sections of otoliths were assayed for trace metals using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) at the Great Lakes Institute for Environmental Research, University of Windsor, ON, Canada. The LA-ICP-MS system consisted of a Quantronix Integra C femtosecond laser operated at a 100 Hz pulse rate producing a 24.8-25.9 mJ/pulse at the 2.5 mm pinhole. The laser was linked to a Thermo-Elemental X7 quadrupole ICPMS operating in low resolution peak-jumping mode (isotope dwell time: 10 ms, carrier gas: Ar) (Shaheen et al. 2008). Instrument drift was controlled by analyzing a National Institute of Standards and Technology 610 (NIST 610) glass standard before and after each slide of 15 otoliths. A gas blank was analyzed before and after each sample to obtain background readings. To account for variability in sample mass, internal standards were set to the stoichiometric concentration of calcium in aragonite (400432 µg Ca • g<sup>-1</sup> CaCO<sub>3</sub>). This system produced a laser crater ≈23 µm in diameter, that traversed the otolith from the edge (corresponding to time of capture) to the core (corresponding to time of hatch), at speeds of 4.6 to 5.8 µm/s.

Raw chemistry data (elemental counts per second) were analyzed using Thermo Plasma Lab software (Thermo Scientific™). The data-processing protocol provides background- subtracted and drift-corrected elemental concentrations. Each otolith transect was integrated from edge to core (≈ 200 µm). The edge of the otolith was determined by observing a Sn spike that was produced when the laser ablated the epoxy mounting material. Data were then processed using an Excel macro developed at the University of Windsor (Yang 2003).

## **RESULTS**

### ***Water Chemistry***

The water chemistry results are presented in Table 1. The mean Sr:Ca ratios in the reservoir waters ranged from 0.870 to 23.747 mmol/mol. This range is inclusive of waters from the Grand (1.29 mmol/mol), Maumee (3.95 mmol/mol), and Sandusky (8.97 mmol/mol) Rivers (Pangle et al. 2010). In addition, the Sr:Ca ratios in some of these fresh water sources significantly exceed the value for typical seawater of ~9 mmol/mol. The Ba:Ca and Mg:Ca ratios in the reservoir waters are also presented in Table 1. However, the range in these values is much more limited than the Sr:Ca values.

### ***Otolith Chemistry***

#### ***Laser Ablation Results***

To confirm that the microchemistry of otoliths from the common carp that were collected from the different reservoirs was constant over time, reflecting the essentially constant water chemistries in the respective reservoirs, 24 lapillus otoliths were analyzed using laser ablation. A representative laser ablation transect for a common carp from the Findlay #2 reservoir is presented in Figure 3. All of the common carp otoliths analyzed by laser ablation showed constant Sr:Ca ratios across the entire otolith.

**Table 1. Mean Water Chemistries for Reservoirs and USGS-UMESC Well.**

	Ba (ppm)	Ca (ppm)	Mg (ppm)	Sr (ppm)	Sr:Ca (mmol/mol)	Ba:Ca (mmol/mol)	Mg:Ca (mmol/mol)
Bresler Lake Reservoir	0.027	39.21	23.02	1.623	18.93	3.03	968.1
Ferguson Reservoir	0.023	35.97	17.09	0.565	7.18	2.89	783.1
Findlay Reservoir #2	0.042	48.60	20.56	2.523	23.75	3.81	697.3
Lake Lecomte	0.032	40.38	12.37	0.457	5.17	3.50	505.1
New London Reservoir	0.026	40.08	13.81	0.189	2.16	2.90	568.1
Norwalk Reservoir	0.024	52.91	11.27	0.101	0.87	2.01	351.1
Outhwaite Reservoir	0.037	41.92	17.46	0.643	7.01	3.89	686.6
Pleasant Hill Reservoir	0.046	42.02	14.53	0.165	1.80	4.90	570.3
Riley Reservoir	0.030	33.42	14.06	0.356	4.87	4.05	693.5
USGS-UMESC Well	0.065	48.33	14.17	0.049	0.46	5.96	483.3

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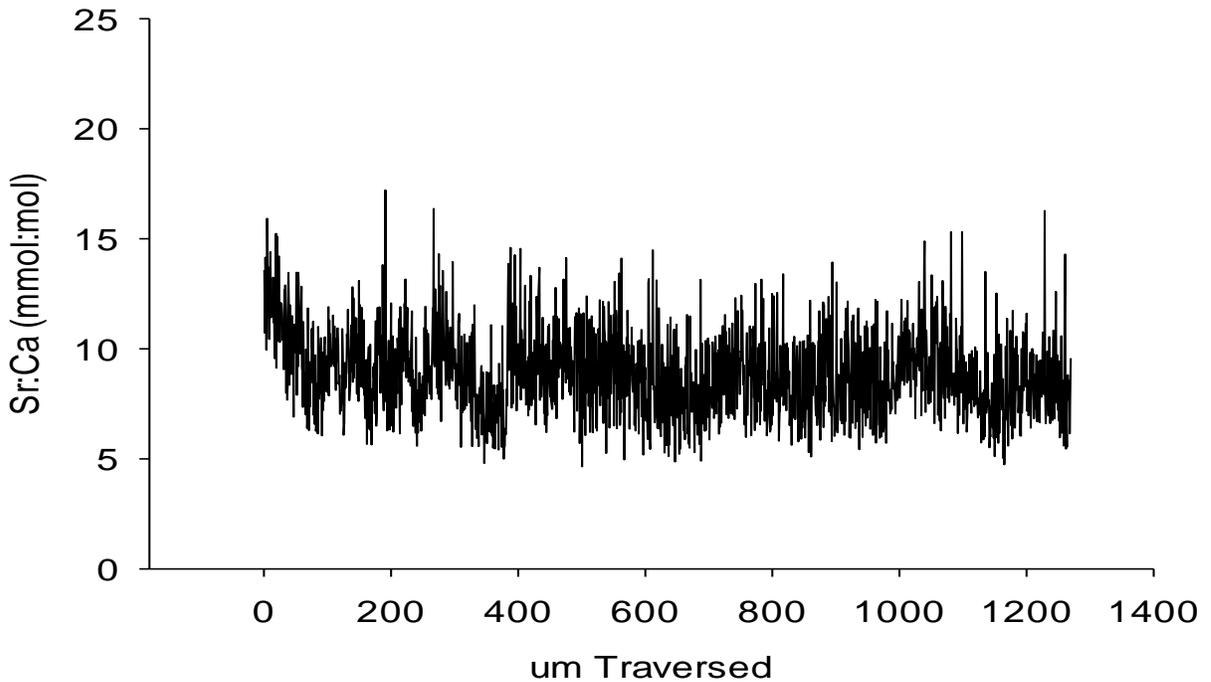


Figure 3. Representative laser ablation traverse across common carp otolith from Findlay Reservoir #2. Note the Sr:Ca value is constant across the entire otolith.

## Total Digestion Results

### Lapillus Otoliths

The results from total digestion of the aragonitic lapillus otoliths from the common carp are presented in Table 2. Their mean Sr:Ca ratios range from 0.574 to 8.099 mmol/mol and mean Ba:Ca ratios range from 0.0066 to 0.0158 mmol/mol. The mean Sr:Ca ratios for the lapillus otoliths are plotted against the Sr:Ca ratios of the respective reservoirs in which they resided in Figure 4. The plot shows a strong linear relationship ( $R^2 = 0.968$ ) over the entire range of water chemistries. The mean Ba:Ca and Mg:Ca ratios for the lapillus otoliths are plotted against the Ba:Ca and Mg:Ca ratios of the respective reservoir waters in which they resided in Figures 5 and 6, respectively. The plots show weak positive trends but have large uncertainties that limit their usefulness.

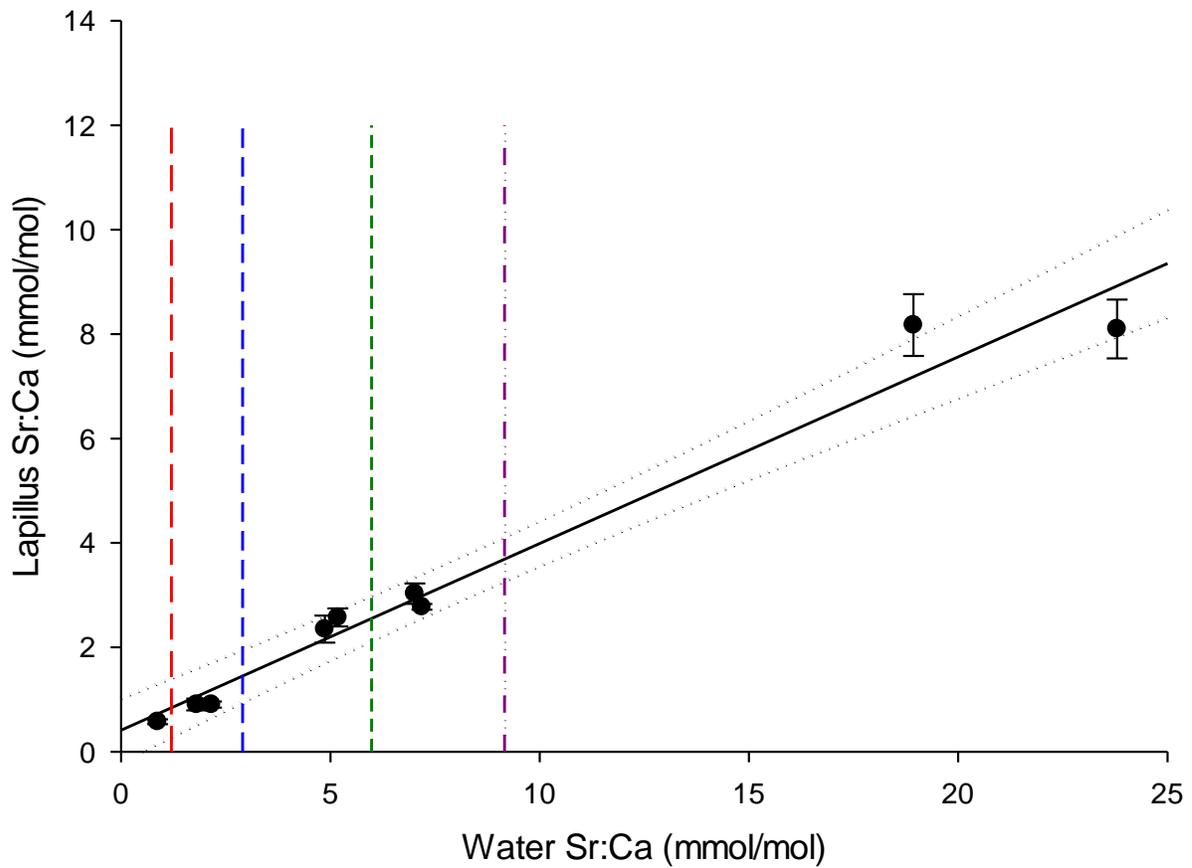


Figure 4. Plot of Sr:Ca in common carp lapillus otoliths against Sr:Ca in resident waters ( $n = 79$  otoliths and  $n = 9$  reservoirs). Linear regression yields  $y=0.359x+0.409$ ,  $R^2=0.968$ . Dashed lines indicate 95% confidence interval. Vertical lines indicate Sr:Ca in Grand River (red), open Lake Erie (blue), Maumee River (green), and Sandusky River (purple).

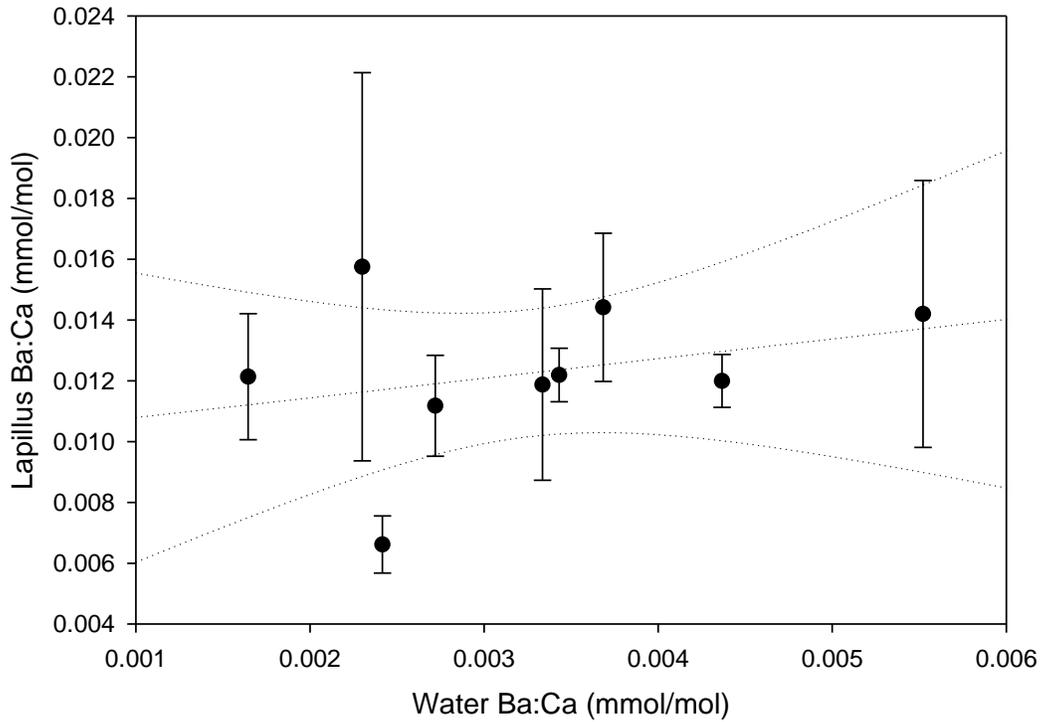


Figure 5. Plot of Ba:Ca in common carp lapillus otoliths against Ba:Ca in resident waters (n = 72 otoliths and n = 9 reservoirs). Dashed lines indicate 95% confidence interval.

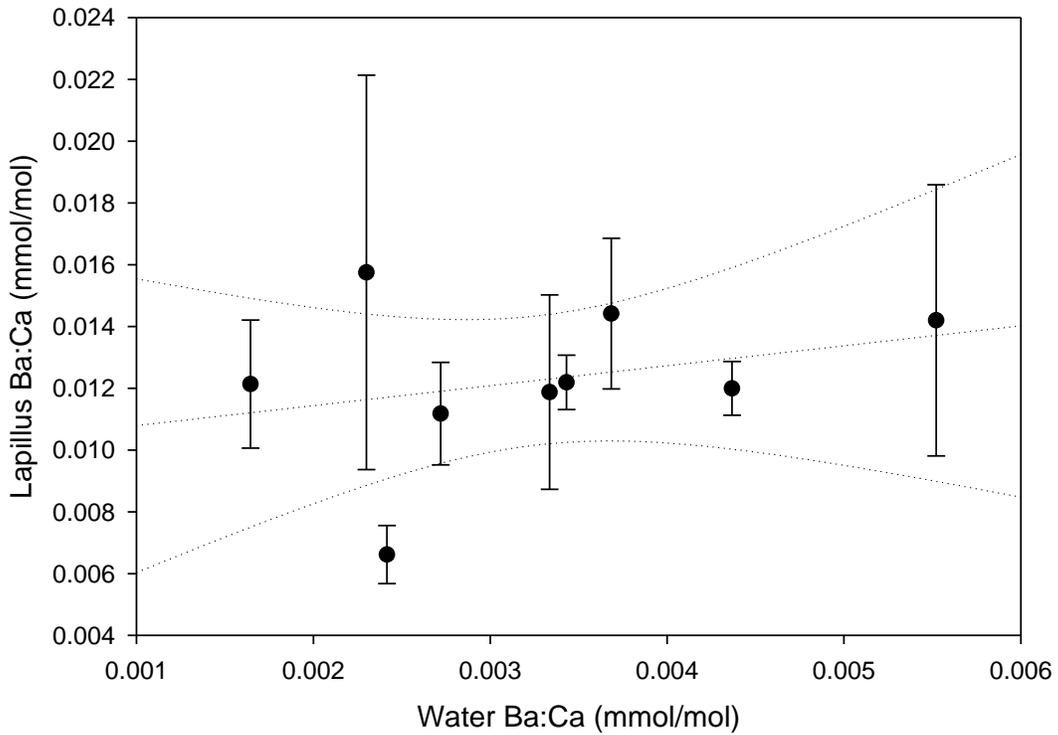


Figure 6. Plot of Mg:Ca in common carp lapillus otoliths against Mg:Ca in resident waters (n = 74 otoliths and n = 9 reservoirs). Dashed lines indicate 95% confidence interval.

### Asteriscus Otoliths

The microchemistry of the asteriscus otoliths from the common carp samples are also presented in Table 2. As anticipated, the vateritic asteriscus otoliths show a marked decrease in Sr and Ba concentrations to the point that several of the digested otoliths had Ba concentrations in the solution that were below detection limits. In similar fashion, the Mg concentrations were significantly greater in the vateritic asteriscus otoliths compared to the aragonitic lapillus otoliths.

**Table 2. Mean Chemistries for Common Carp Otoliths by Reservoir.**

	Lapillus			Asteriscus		
	Sr:Ca (mmol/mol)	Ba:Ca (mmol/mol)	Mg:Ca (mol/mol)	Sr:Ca (mmol/mol)	Ba:Ca (mmol/mol)	Mg:Ca (mol/mol)
Bresler Lake Reservoir	8.074	0.007	0.146	0.790	0.00014	3.001
Ferguson Reservoir	2.774	0.012	0.059	n/a	0.00046	2.753
Findlay Reservoir #2	8.099	0.011	0.061	0.634	0.00061	2.830
Lake Lecomte	2.571	0.012	0.044	0.223	0.00059	2.679
New London Reservoir	0.904	.012	0.042	0.0776	0.00170	2.742
Norwalk Reservoir	0.574	0.014	0.096	0.0637	0.00062	2.706
Outhwaite Reservoir	3.029	0.014	0.057	0.308	0.00047	2.763
Pleasant Hill Reservoir	0.942	0.016	0.114	0.1009	n/a	3.011
Riley Reservoir	2.35	0.012	0.327	0.0490	0.00014	3.001

Despite the decrease in Sr concentration (and Sr:Ca ratio) in the asteriscus otoliths, a plot of Sr:Ca in asteriscus otoliths versus Sr:Ca in the respective reservoir waters yields a strong linear relationship ( $R^2 = 0.894$ ) over the broad range of water chemistries observed in the study (Figure 7). In addition, a plot of Sr:Ca in the lapillus otoliths versus the asteriscus otoliths yields a strong linear relationship with  $R^2 = 0.972$  (Figure 8).

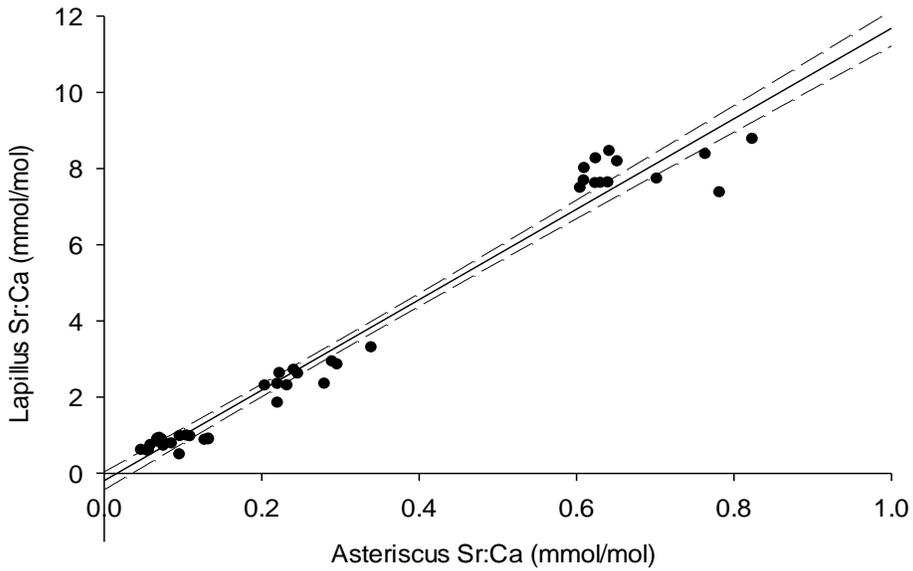


Figure 7. Plot of Sr:Ca in common carp asteriscus otoliths against Sr:Ca in resident waters (n = 46 otoliths and n = 8 reservoirs). Linear regression yields  $y=0.0299x+0.0636$ ,  $R^2=0.894$ . Dashed lines indicate 95% confidence interval.

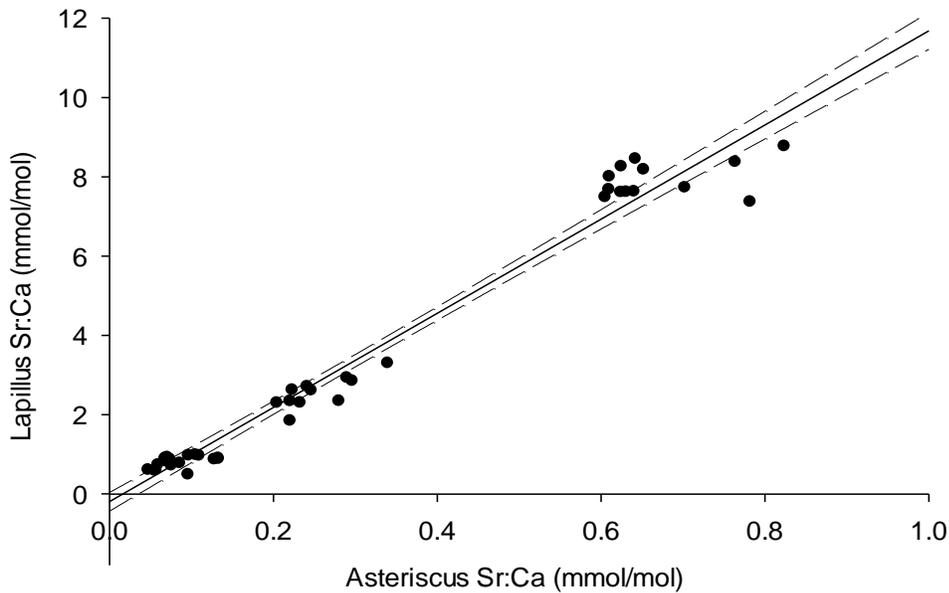


Figure 8. Plot of Sr:Ca in common carp lapillus otoliths against Sr:Ca in corresponding asteriscus otoliths (n = 45 pairs of otoliths, n = 8 reservoirs). Linear regression yields  $y=11.88x-0.195$ ,  $R^2=0.972$ . Dashed lines indicate 95% confidence interval.

## Asian Carp Results

The water chemistry data for USGS-UMESC are included in Table 1. The value for Sr:Ca in the water is 0.46 mmol/mol. The lapillus otolith Sr:Ca, Ba:Ca, and Mg:Ca for the grass, bighead, and silver carp from USGS-UMESC are presented in Table 3. Comparison of the Sr:Ca, Ba:Ca, and Mg:Ca values in the lapillus otoliths of the four different carp species investigated relative to the Sr:Ca, Ba:Ca, and Mg:Ca values in the water (i.e., the partition coefficients) are presented in Figures 9, 10, and 11, respectively. The common, bighead, and silver carp have statistically similar Sr:Ca partition coefficients whereas the grass carp have a statistically greater partition coefficient than the common carp and bighead carp. The Ba:Ca and Mg:Ca partition coefficients are similar for all four species with uncertainties.

**Table 3. Mean Chemistries for Grass, Bighead, and Silver Carp Lapillus Otoliths from USGS-UMESC.**

	<b>Sr:Ca</b> (mmol/mol)	<b>Ba:Ca</b> (mmol/mol)	<b>Mg:Ca</b> (mol/mol)
Grass Carp	.540	0.011	0.165
Bighead Carp	0.299	0.011	0.122
Silver Carp	0.469	0.0297	0.177

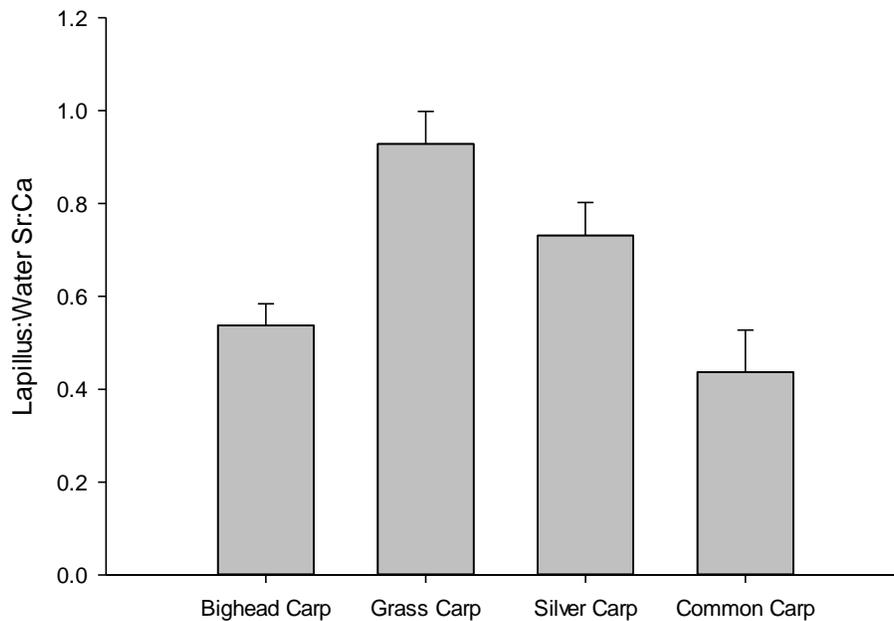


Figure 9. Comparison of lapillus otolith – water Sr:Ca partition coefficients for four different Asian carp species (BH, n = 15; SV, n = 17; GS, n = 14; CC, n = 79).

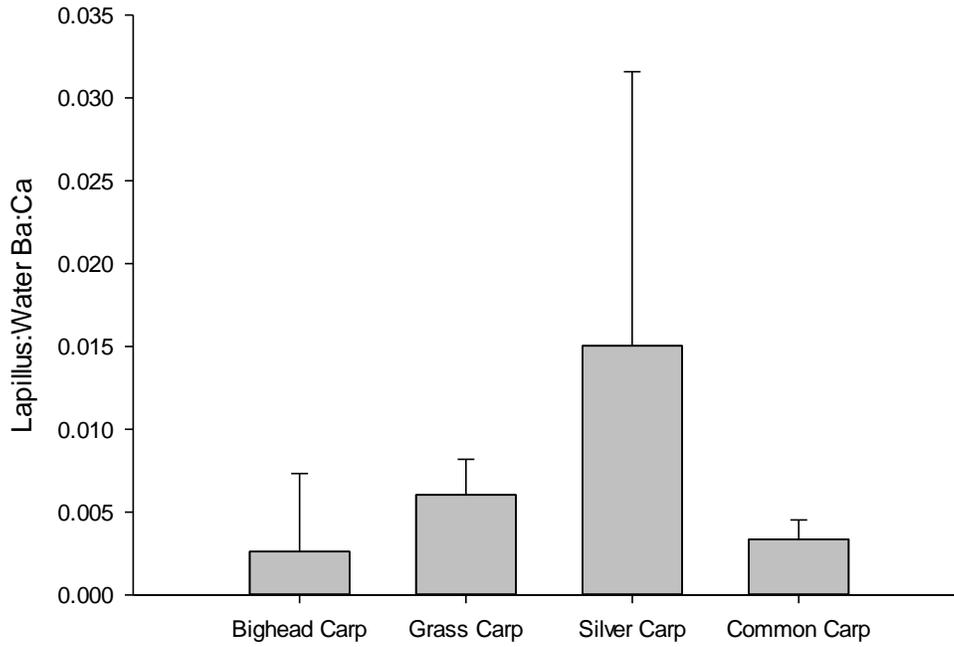


Figure 10. Comparison of lapillus otolith – water Ba:Ca partition coefficients for four different Asian carp species (BH, n = 13; SV, n = 3; GS, n = 14; CC, n = 72).

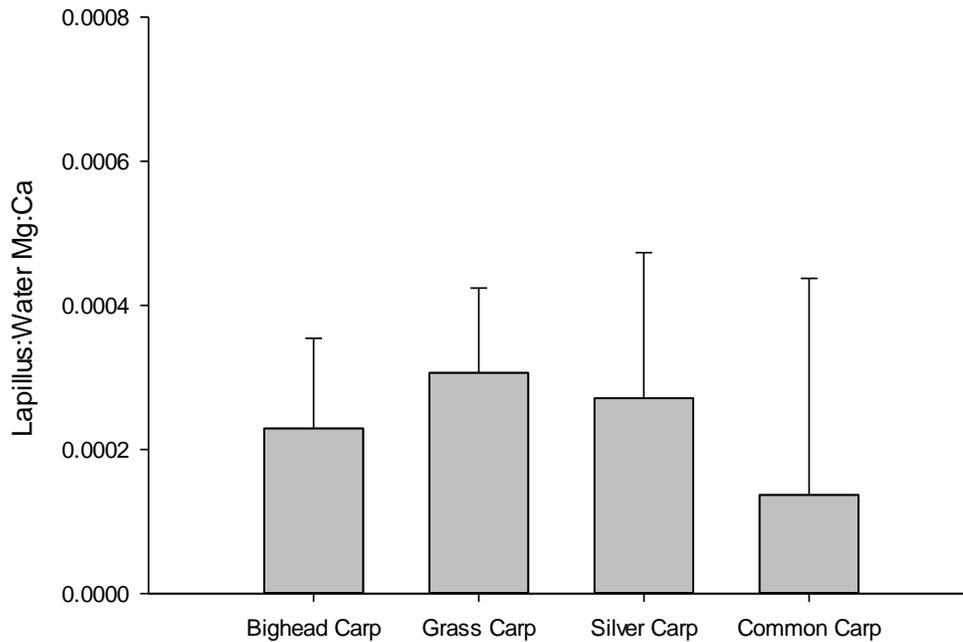


Figure 11. Comparison of lapillus otolith – water Mg:Ca partition coefficients for four different Asian carp species (BH, n = 15; SV, n = 14; GS, n = 14; CC, n = 74).

## **DISCUSSION**

The strong linear relationship between Sr:Ca in lapillus otoliths of common carp relative to their resident water Sr:Ca provides an exceptional means to evaluate the water masses in which common carp reside throughout their life histories as well as any migratory behavior. The otolith-water Sr:Ca relationship is especially useful as it spans essentially the entire range of Sr:Ca found in natural waters, both freshwater and seawater.

The strong linear relationship in Sr:Ca between asteriscus otoliths from common carp compared to their respective water Sr:Ca values also provides an excellent independent means to evaluate fish residence and migrations. Previous workers have questioned using vateritic otoliths for fish residence and migration studies (e.g., Melancon et al. 2005, Tzeng et al. 2007) however, the results of this study, as well as MacDonald et al. (2012), indicate vateritic otoliths may also prove useful even though they have significantly lower Sr:Ca ratios than coexisting aragonitic otoliths.

The Ba:Ca and Mg:Ca otolith to water relationships determined in this study did not prove to be robust enough to be of use for determining natal water chemistries or migratory behavior in common carp. In the case of Ba:Ca this is largely due to the very low and narrow range of Ba concentrations in the otoliths. Analysis by laser ablation – mass spectrometry is likely necessary to resolve the Ba:Ca otolith to water relationships.

The comparison of lapillus otolith to water Sr:Ca partition coefficients for the four different Asian carp species suggests that the Sr:Ca otolith to water relationships determined for the common carp can be directly applied (within statistical uncertainty limits) to bighead and silver carp. However, analysis of the grass carp yielded a significantly greater Sr:Ca partition coefficient than the other three carp species.

At first glance, it is tempting to ascribe the difference in Sr:Ca partition coefficients between the grass carp and the other three carp species to differences in their respective feeding habits. Common carp are benthivores and bighead and silver carp are filter feeders while grass carp consume aquatic macrophytes (Kolar et al. 2005). However, the grass, bighead, and silver carp obtained from the USGS-UMESC were fed the same diet. Clearly, additional research is necessary to accurately quantify the Sr:Ca otolith to water relationship for grass carp.

## **SUMMARY/SIGNIFICANCE**

If invasive Asian carps are to be extirpated from Lake Erie when they arrive, standard conservation biology and management strongly indicates that we need to act rapidly when the invading population is very small. By employing a total digestion method we were able to analyze a relatively large number of otoliths ( $n = 171$ ) in a relatively short period of time at minimal expense. Clearly, evaluation of natal sites and migratory patterns of any invasive carp captured in Lake Erie tributaries would require the spatial/temporal resolution afforded by laser ablation analysis. However, we now have a very robust relationship established between Sr:Ca in otoliths (both aragonitic lapillus and vateritic asteriscus) in common carp relative to residence waters over a broad range of water Sr:Ca values inclusive of the Grand, Maumee, and Sandusky Rivers, and have shown that Sr:Ca otolith-water partition coefficients are similar between common carp and the invasive bighead and silver carps. As such, we have established the tributary chemical signatures of invasive Asian carps without introducing these fish into Lake Erie (or other at-risk locations).

## **FUTURE RESEARCH**

The results of this study provide sound analysis of Sr:Ca otolith-water relationships in common carp. However, data for Ba:Ca and Mg:Ca otolith-water relationships need to be investigated further using laser ablation methods. One of the most intriguing findings of this study is while the Sr:Ca otolith-water partition coefficients are similar between the common, bighead, and silver carp, the grass carp appears to be significantly different. A better understanding of the Sr:Ca otolith-water relationship for the grass carp would have both a very practical application to identifying natal waters for any fish capture in Lake Erie waters and any subsequent migration based on otolith analysis and also may provide valuable insights into how different species regulate Sr:Ca in their bodies and the role of water versus food in influencing Sr:Ca uptake.

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