



28 July 2010

John D. Rogner
Assistant Director
Illinois Department of Natural Resources

Dear John:

I have attached a summary of findings pertaining to the possible environmental history of two bighead carps recently caught in the Chicago area. These data were collected, analyzed, and interpreted by Dr. Greg Whitlege, an Assistant Professor in our Fisheries and Illinois Aquaculture Center and Department of Zoology at Southern Illinois University Carbondale. To get an objective opinion of Greg's analysis and interpretation of the results, Greg sent his summary to Dr. Stuart Ludsin in the Department of Ecology, Evolution, and Organismal Ecology at The Ohio State University. Stuart is a recognized expert in the field of microchemistry analysis of fish in the Laurentian Great Lakes. Dr. Steve Campana, a recognized leader in the field of otolith (fish ear bone) work, also chimed in. Both Greg's summary and the reviews are attached.

Greg is out of town at the moment and could not completely address Stuart's concerns about the research summary. However, the attached summary does provide error estimates and some revised information. In my view, most of Stuart's comments revolve around some assumptions that had to be made about the data to interpret them; these assumptions are clearly stated in Greg's summary. Steve's comments are mostly a call for more data. Unfortunately, we only had a sample size of two fish, an incomplete environmental history, and limited otolith material to analyze. The bottom line is that the data are interesting and merit more investigation. But we knew that when collecting them. These data must be viewed in light of the other pieces of information about fish assemblage composition and movement in the Chicago waterway.

I know this information is time sensitive. I can answer questions to the best of my ability. Greg returns on August 10, 2010 and can provide better insight into the meaning of these results.

Sincerely,

A handwritten signature in black ink, appearing to read "James E. Garvey", with a stylized flourish at the end.

James E. Garvey
Director
Fisheries and Illinois Aquaculture Center

Trace element and isotopic compositions of otoliths from two bighead carp collected in the Chicago area

Greg Whitledge
Assistant Professor
Fisheries and Illinois Aquaculture Center
Southern Illinois University
Carbondale, IL 62901-6511

Introduction

Two bighead carp (*Hypophthalmichthys nobilis*) were collected from Chicago area waterways during the past year. One fish was collected during fall 2009 downstream from the electrical barriers installed in the Chicago Sanitary and Ship Canal; the other fish was collected from Lake Calumet during June 2010. Given that these fish were collected near or (in the case of fish captured from Lake Calumet) beyond the electrical barriers intended to prevent Asian carp from entering the Great Lakes, there is much interest in the source environment of these two individual fish and how much time the fish captured from Lake Calumet spent in the area in which it was collected. Herein, I present the results of stable isotopic and trace elemental analyses of otoliths obtained from these two fish. These analyses were performed for comparison with existing otolith chemistry data from Asian carp captured in the Illinois, Mississippi, and Missouri Rivers and otolith chemistry data from other fish species collected from these three rivers, Illinois River tributaries, and Lake Michigan to determine whether stable isotopic and trace element compositions of otoliths from the two bighead carp might provide some insight into the environmental history of these two fish.

Methods

Both fish were frozen prior to otolith removal. Otoliths were removed from both fish using non-metallic forceps, cleaned with distilled water, and stored dry in polyethylene vials. One otolith from each fish was analyzed for stable oxygen and carbon isotopic compositions (expressed as $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, respectively). Sub-samples of otolith material (~200 μg each) were obtained from the outer edge of the otolith (which reflects a fish's recent environmental history) and from the otolith core (which reflects a fish's early life history) for the fish collected in Lake Calumet. For the fish captured downstream from the barrier, a broken otolith permitted stable isotope analysis of an otolith edge sample only (not the otolith core). Sub-samples of otolith material were pulverized with a mortar and pestle and analyzed for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ at the University of Arizona Environmental Isotope Laboratory. Two replicates from each sub-sample were analyzed. A second otolith from each fish was analyzed for strontium:calcium (Sr:Ca) molar ratios. Otoliths for Sr:Ca analysis were embedded in epoxy, sectioned in a transverse plane with an ISOMET low-speed saw, and polished. Otolith thin sections were prepared for analysis under Class-100 clean conditions following procedures described in Whitledge et al. (2007). Each otolith thin section was analyzed for ^{86}Sr , ^{88}Sr , and ^{46}Ca by GeoMed Analytical, LLC (University of Massachusetts-Boston) using laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS). Three spots (each 100 μm diameter) were ablated from the core of each otolith thin section (material deposited during the fish's early life history; temporal resolution is unknown but is

likely an integration over the first few weeks to months of the fish's life). Data from the three ablation spots were averaged for each otolith.

Results and Discussion

Table 1. Otolith oxygen ($\delta^{18}\text{O}$) and carbon ($\delta^{13}\text{C}$) isotope data for the two bighead carp. SD is standard deviation of two replicates for each sample.

Fish (collection location)	Sub-sample	$\delta^{13}\text{C}$ (‰, VPDB)	SD	$\delta^{18}\text{O}$ (‰, VPDB)	SD
Chicago Ship Canal (below barrier)	edge	-11.60	0.025	-7.18	0.009
Lake Calumet	core	-6.89	0.028	-6.56	0.078
	edge	-7.47	0.016	-6.55	0.009

Table 2. Otolith Sr:Ca data for the two bighead carp (means from three spots (each 100 μm diameter) at otolith core \pm SD).

Fish (collection location)	Sr:Ca ($\mu\text{mol/mol}$)	SD
Chicago Ship Canal (below barrier)	441.5	17.8
Lake Calumet	791.0	32.0

Comparison with existing otolith chemistry data for Asian carp and other fish species

Otolith edge $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values for the bighead carp collected downstream from the electric barrier were within the ranges of values observed for Asian carp collected from the Illinois River in 2009 (see Figure 1) and within the ranges of otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values for other fish species collected from the Illinois River during 2006-2007 (Whitledge 2009; Zeigler and Whitledge 2010). Otolith core Sr:Ca for this fish was also within the range of otolith Sr:Ca values (388-800 $\mu\text{mol/mol}$) observed for other fish species collected from the Illinois River during 2006-2007 (Whitledge 2009; Zeigler and Whitledge 2010).

Otolith core and edge $\delta^{13}\text{C}$ values for the bighead carp collected from Lake Calumet were within the range of otolith $\delta^{13}\text{C}$ values for centrarchids and common carp collected from Lake Michigan harbors along the Illinois shoreline during 2007 (see Fig. 3 in Whitley 2009). Otolith $\delta^{13}\text{C}$ values from these fish are more positive (enriched in ^{13}C) compared to those of fish sampled from the Illinois River, its tributaries and floodplain lakes, and the Mississippi River (Whitley 2009; Zeigler 2010; Zeigler and Whitley 2010). However, otolith $\delta^{13}\text{C}$ is influenced by fish age and metabolic rate in addition to $\delta^{13}\text{C}$ of dissolved inorganic carbon. Thus, otolith $\delta^{13}\text{C}$ data for the two bighead carp and other fishes previously sampled from these water bodies may not be directly comparable. Otolith core and edge $\delta^{18}\text{O}$ values for the bighead carp captured from Lake Calumet were slightly more positive than otolith $\delta^{18}\text{O}$ values for centrarchids and common carp collected from Lake Michigan harbors in 2007 (range -6.68 to -7.96‰). However, otolith $\delta^{18}\text{O}$ has previously been found to be indistinguishable between fish collected from the Illinois River and Lake Michigan (Whitley 2009). Otolith core Sr:Ca for the bighead carp collected from Lake Calumet was greater than otolith Sr:Ca observed for other fish species collected from Lake Michigan harbors during 2007 (range 112-588 $\mu\text{mol/mol}$; Whitley 2009), but was within the range of otolith Sr:Ca values (388-800 $\mu\text{mol/mol}$) observed for other fish species collected from the Illinois River during 2006-2007 (Whitley 2009; Zeigler and Whitley 2010).

Assumptions

Otolith and environmental “signatures” are consistent over time. Temporal stability of environmental and otolith chemical “signatures” is critical to reconstruction of fish environmental history using otolith chemistry (e.g., see Schaffler and Winkelmann 2008).

Relationships between environmental and otolith chemical “signatures” for Asian carp are equivalent to those of other fish species sampled.

Given these significant assumptions, any inferences regarding environmental history of the two bighead carp based on their otolith isotopic and trace elemental signatures should be viewed as preliminary. No conclusive statements regarding the environmental history of these two fish are currently possible given data limitations for addressing these key assumptions.

If these assumptions were correct, otolith isotopic and Sr:Ca data would suggest that the bighead carp collected below the electric barrier in fall 2009 spent both the early and latter portions of its life in the Illinois River. This fish was estimated to be age 3 or 4 by other researchers at SIU based on otolith and fin ray annuli counts, although these aging techniques have not been validated for Asian carp. Asian carp are known to be reproducing in the Illinois River. Thus, otolith chemical signatures suggestive of Illinois River origin and residency for this fish would not be surprising.

Again, **if** the above assumptions were correct, otolith core and edge stable isotopic data for the bighead carp collected from Lake Calumet in 2010 would suggest that this fish was present at or near its collection site (in Lake Calumet and/or Lake Michigan) during both the majority of its early life (approximately the first year given the large amount of otolith

material subsampled from the core for stable isotope analysis and the small mass of Asian carp otoliths in the fall of the fish's year of life) and latter portions of its life. This fish was estimated to be age 6 by other researchers at SIU. The fact that otolith core Sr:Ca (obtained using LA-ICPMS, a higher-resolution, more precise technique than the procedure used for stable isotope analysis) for this fish was within the range of otolith Sr:Ca values previously observed for other fish species collected from the Illinois River would suggest that the bighead carp collected from Lake Calumet may have originated in the Illinois River (or another water body with a Sr:Ca signature equivalent to that of the Illinois River) and then moved or was transported to Lake Calumet/Lake Michigan during the early portion of its life.

Literature Cited

- Schaffler, J.J. and D.L. Winkelman. 2008. Temporal and spatial variability in otolith trace-element signatures of juvenile striped bass from spawning locations in Lake Texoma, Oklahoma-Texas. *Transactions of the American Fisheries Society* 137: 818-829.
- Whitledge, G.W. 2009. Otolith microchemistry and isotopic composition as potential indicators of fish movement between the Illinois River drainage and Lake Michigan. *Journal of Great Lakes Research* 35:100-105.
- Whitledge, G.W., B.M. Johnson, P.J. Martinez, and A.M. Martinez. 2007. Sources of non-native centrarchids in the upper Colorado River revealed by stable isotope and microchemical analyses of otoliths. *Transactions of the American Fisheries Society* 136:1263-1275.
- Zeigler, J.M. 2009. Utility of otolith microchemistry and stable isotopic composition for determining fish environmental history in the Mississippi and Illinois Rivers. M.S. Thesis, Southern Illinois University-Carbondale: 72 pp.
- Zeigler, J.M. and G.W. Whitledge. 2010. Assessment of otolith chemistry for identifying source environment of fishes in the lower Illinois River, Illinois. *Hydrobiologia* 638:109-119.

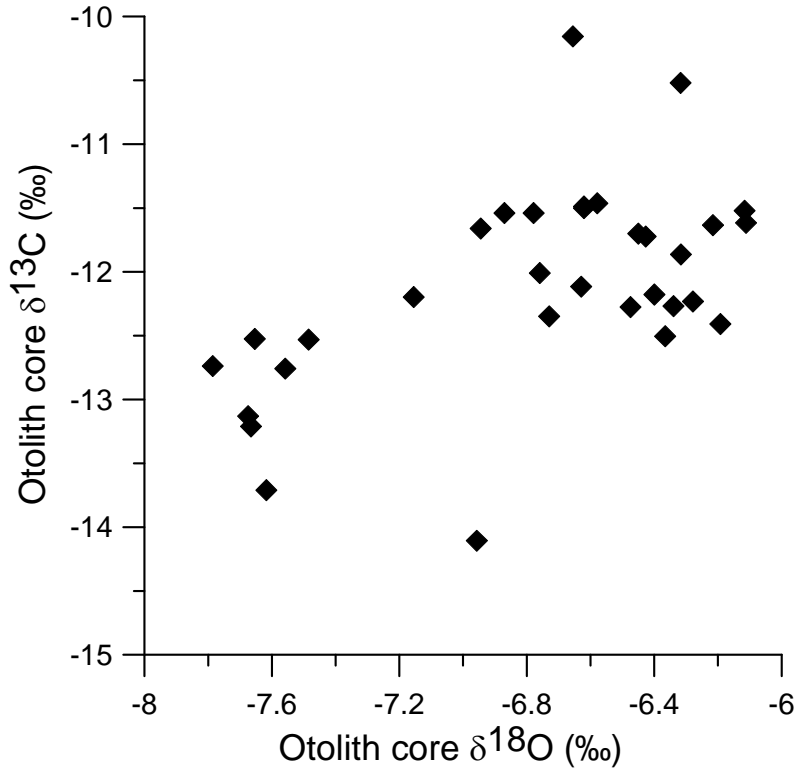


Figure 1. Otolith core $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of Asian carp collected from the Illinois River between Starved Rock Dam and Morris, IL during 2009. Points represent data from individual fish.

Review by Dr. Stuart Ludsin, The Ohio State University; 28 July 2010

I feel the analysis is rigorous and your interpretation is sound as the data allow it to be, with one exception (see below). However, if I played Devil's advocate, I could fully discount your interpretation (or any other that you provided), owing to the two untested assumptions that you fully signify (i.e., limited data). In my opinion, you would have no choice but to back off, if someone pressed you hard about the stability of these chemical signatures through time or the assumption of using one species to represent the other.

The one interpretation that I am uncertain about is the origin of the Lake Calumet fish. The $\delta^{13}\text{C}$ value is highly enriched and nowhere close to the Illinois River signature (in Fig. 3 of Whitley 2009). To me, this enriched $\delta^{13}\text{C}$ value in the core points directly at Lake Michigan as the source. I fully understand your rationale for suggesting the Illinois River as the source origin (because Sr is higher in the core, LA-ICPMS integrates over less of the life-history), but cannot simply ignore the highly enriched $\delta^{13}\text{C}$ in the region that you call the core. This then raises a key question about where exactly your Sr core ablations lie relative to material removed for isotope work. Are the three ablations for Sr:Ca only in the innermost portion of the core (i.e., with in the first few weeks of life only), or are they spread out a bit such that they encompass a larger portion of early life (similar to the isotope portion)? Is there a way to quantify (via aging) the period of life that the LA-ICPMS ablation is integrating over such that you can definitively say that this Sr:Ca estimate better represents source waters than the isotope data? I think something along these lines would help a lot, as the data (as presented now) lead the reader to believe that both the isotope and Sr:Ca data in the core are representing the same portion of life (and only someone knowledgeable about these techniques can appreciate your arguments).

I also am hesitant to buy your argument that Sr:Ca in the core or the Lake Calumet fish better reflects the Illinois River than Lake Michigan simply because no SD (or more importantly, confidence intervals) are provided for the Sr:Ca core data. Do the baseline confidence intervals for the Sr:Ca signatures that represent Lake Michigan (Fig. 3 in Whitley 2009) overlap with the confidence intervals for the Sr:Ca data from the core region of this Lake Calumet fish? If so, then this would make it even more difficult to rule out Lake Michigan as the origin. You clearly have the data to test overlap of these confidence limits, and I suggest that you look into it.

Have any studies been conducted elsewhere in the Great Lakes to show that isotopic signatures are stable through time? If you could draw upon the literature to show that isotopic signatures (δC and δO) are either completely stable or only fluctuate with a small range (such that there is little possibility of overlap with other rivers), this would be helpful. You then could be in a position to put on worst-case confidence bounds (based on the literature) to your source populations, to show that there is no overlap in $\delta^{13}\text{C}$ among rivers. In turn, you better identify potential source origins.

I have a few other questions that could cause issues, or that you might want to be prepared for (in case the other side pulls out an otolith chemistry/isotope expert).

1. When were your fish collected relative to when your baseline signatures that represent your potential source streams were determined? What years and seasons were baseline data collected, how does this differ from the two carp collections? If you could gin up studies to show strong stability of signatures (for isotopes and chemistry) both within and across years, this would help your cause a lot. However, my guess is that the stability that you desire for the Sr:Ca data will not be there, but the isotope stability might (although δO should vary with temperature and δC with diet and/or carbon inputs related to runoff).

2. How were the two carp preserved after collection? Frozen? Stored in ethanol? Formalin? You just need to be sure that your data are not tainted by preservation technique, as isotopes can be affected by preservation technique (freezing vs. ethanol), whereas Sr is robust to ethanol and freezing.
3. What is the precision of the isotope instrument? I would think that the instrument precision is much better than your SD, but it might be worthwhile to report instrument precision for both isotopes.
4. In the document, I feel that you should report SD and confidence limits for Sr:Ca and $\delta^{18}O/C$ for your fish, as well as the means, SD, and confidence limits of your source waters (from your previous work) for isotopes and . This would allow everyone to easily access data and visually see how everything stacks up relative to one another.

Comments by Steve Campana, Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, Nova Scotia, Canada B2Y 4A2

I've taken a quick look at the report you sent. I was at a bit of a disadvantage, since I'm not familiar with the locations and watersheds you discuss, and no map was provided. Therefore, it's difficult for me to comment on your conclusions. However, I can make a few comments on your assumptions and results. I like the fact that you used both trace elements and stable isotopes to examine the otoliths. I was a bit surprised that you didn't look at other key elements, such as Ba, since you were already doing the ICPMS assays and thus would get the results with no additional effort. And if you really want to get (potentially) better spatial discrimination, stable strontium isotopes would be a useful assay. The big advantage of stable Sr and stable oxygen is that their composition in the otolith is the same as that in the water (albeit with minor modification by temperature for oxygen). I would not suggest focusing on the stable carbon assay results. C-13 is known to be under significant metabolic influence, and thus changes with the size and individual history of the fish. It is a poor reflector of environmental conditions. Depending on how rigorous you wanted to make your results, you might want to use a Micromill to remove otolith material from only the annuli which correspond to a year common to all of your samples.

Your second assumption - that the chemical signatures of the Asian carp are similar to that of other species - is very reasonable for stable oxygen. However, as I'm sure you're aware, otolith Sr:Ca is proportional to, but not the same as, the Sr:Ca of the water. It is affected by other variables, including the relative growth rate of the fish. Therefore, it's not unusual to have species which grow up together in the same water, but have different Sr:Ca ratios. So it may or may not be informative that other species have the same (or different) otolith Sr:Ca ratios. The fact that your carp values fell within the fairly broad range of 388-800 $\mu\text{mol/mol}$ is good, but does it exclude any other values from the area? In other words, does it give you any discriminatory power? I think if I were going to defend this in court, I'd want to know the elemental and isotopic values characteristic of each of the potential source areas. I don't want to appear negative - I think you've got some interesting results, and your conclusions may well be correct. But if you do indeed end up going to court, you may find it difficult to defend your conclusions against alternative scenarios.