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GREAT LAKES FISHERY COMMISSION

2008 Project Completion Report¹

ASSESSMENT OF OTOLITH CHEMISTRY AS AN INDICATOR OF FISH MOVEMENT OR TRANSFER BETWEEN THE ILLINOIS RIVER SYSTEM AND LAKE MICHIGAN

by:

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ABSTRACT:

Naturally occurring chemical markers in otoliths offer a potential means to identify source environment for fishes in the upper Illinois River system and Lake Michigan, including individuals that may breach electrical barriers in the Chicago Sanitary and Ship Canal or be transferred via bait buckets between these formerly isolated drainages. The objectives of this study were to determine whether water and fish otolith stable isotopic and elemental compositions differ among Lake Michigan, the upper Illinois River, and three tributaries of the upper Illinois River (Fox, Des Plaines and DuPage Rivers) and to determine whether otolith isotopic and elemental signatures could be used to identify the water body from which individual fish were collected. Water and fish otolith samples were obtained from each site during summer 2007 and analyzed for $\delta^{18}\text{O}$ and a suite of trace element concentrations; otoliths were also analyzed for $\delta^{13}\text{C}$. Otolith $\delta^{13}\text{C}$ values for Lake Michigan fish were distinct from individuals collected in the Illinois River and tributaries. Fish collected in the Fox and Des Plaines Rivers could be distinguished from one another and from fish captured in the Illinois and DuPage Rivers using otolith Sr:Ca and Ba:Ca ratios. Otoliths reflected differences in water Sr:Ca and Ba:Ca among environments. Otolith isotopic and elemental compositions may enable determination of source environment for any Asian carp discovered in Lake Michigan and could also be useful as indicators of environmental history for fishes in the upper Illinois River and its tributaries.

INTRODUCTION:

Nonnative bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*H. molitrix*), commonly known as Asian carps, have rapidly expanded their range in the Mississippi River basin and have been observed in the upper Illinois River within about 50 miles of Lake Michigan (Kolar et al. 2005). Predictions that Asian carps could become established in the Great Lakes (Kolar and Lodge 2002), with probable deleterious effects on native fauna and economically important fisheries, have aroused great concern from anglers, commercial fishers, and government agencies in the Great Lakes region (Kolar et al. 2005). The likelihood that Asian carps would enter the Great Lakes prompted construction of a demonstration electrical barrier in the Chicago Sanitary and Ship Canal, which connects the upper Illinois River with Lake Michigan; a second, permanent electrical barrier is under construction. Although assessments have indicated that the demonstration barrier acts as a fish deterrent, it is unlikely that the electrical barriers will completely eliminate the potential for fish movement between the Illinois River and Lake Michigan. In addition to the possibility of Asian carps breaching the electrical barrier, unintentional release of wild-caught baitfish contaminated with young bighead or silver carp is another probable mechanism by which these species could be introduced to waters outside their current range (Kolar et al. 2005), including the Great Lakes. Young Asian carps closely resemble some commonly collected baitfish, such as gizzard shad (Kolar et al. 2005); anglers sometimes catch young Asian carps in Illinois and use them as live bait, although not always in the waters where they were collected (M. Pegg, Illinois Natural History Survey, personal communication, cited in Kolar et al. 2005). Bait bucket introductions have been a major pathway for the spread of fishes outside their native ranges in the U.S. (Fuller et al. 1999). Tributaries of the upper Illinois River (such as the Des Plaines and Fox Rivers in northeastern Illinois) may be among the most likely pathways for bait bucket transfer of young Asian carps into Lake Michigan because they are accessible to Asian carps (their confluences with the Illinois River are downstream of the electrical barriers), their headwaters flow within 5-15 miles (overland) of Lake Michigan, and they support substantial recreational fisheries.

Knowledge of the source and transfer pathway of any Asian carps that may be discovered in Lake Michigan (especially during the early stages of an invasion or introduction before a viable population becomes established and eradication is still feasible) would be essential for developing strategies to prevent further introductions or invasions. Questions regarding sources and date of invasion or introduction of exotic fish species have been difficult to answer with confidence (Radtke 1995; McMahon and Bennett 1996; Fuller et al. 1999). However, microchemical and stable isotopic analyses of otoliths represent a potential technique that could be used to quickly gain insight into the source and transfer pathway of any Asian carp that appear in Lake Michigan. Assays of otolith trace element and isotopic compositions have emerged as effective techniques for addressing questions regarding fish environmental history that have been difficult to resolve with other approaches (Campana 1999). Otolith aragonite accretes daily in concentric increments that are permanently retained after formation, providing a record of growth over a fish's lifetime (Campana and Thorrold 2001). Trace element and stable isotopic compositions of otoliths often reflect those of environments occupied by a fish (e.g., Gao and Beamish 1999; Kennedy et al. 2002; Wells et al. 2003; Whitley et al. 2006) and are unaltered metabolically following

deposition (Campana and Thorrold 2001). Thus, association of otolith biochronology with isotopic and elemental composition enables retrospective description of fish environmental history when an individual has resided in chemically distinct locations for a period of time sufficient to incorporate the signature of those sites (Kennedy et al. 2002). The vast majority of published studies employing otolith isotopic and elemental analyses have been conducted with marine or anadromous species. However, these techniques are rapidly emerging as effective means for reconstructing environmental history of freshwater resident fishes, including those that live in the Great Lakes and adjacent waters (e.g., Bronte et al. 1996; Joukhadar et al. 2002; Brazner et al. 2004; Dufour et al. 2005), and as a method for determining sources and date of invasion or transfer of exotic fish species (Munro et al. 2005; Whitley et al. 2007).

The goal of this study was to evaluate the potential utility of otolith microchemistry and stable isotopic composition for distinguishing among fish that inhabit southwestern Lake Michigan, the upper Illinois River, and three tributaries of the upper Illinois River. Characterization of trace elemental and stable isotopic signatures for these water bodies would provide a new technique to quickly determine the source and likely transfer pathway of Asian carps to Lake Michigan in the event that any Asian carps are discovered in the lake.

OBJECTIVES:

Objectives of this project were to: 1) Determine whether stable hydrogen and oxygen isotopic and trace element compositions of water differ significantly among Lake Michigan, the upper Illinois River, and three tributaries of the upper Illinois River (Fox, DuPage, and Des Plaines Rivers), 2) Determine whether differences in water chemistry among Lake Michigan, the upper Illinois River, and the three tributaries of the upper Illinois River are reflected in the chemical composition of the otoliths of fishes from these different water bodies, and 3) Determine the accuracy with which individual fish can be reclassified to their collection locations based on otolith elemental and stable isotopic compositions.

METHODS:

Water and fish otolith samples were collected from Lake Michigan, the upper Illinois River, and each of three tributaries of the upper Illinois River (Fox, DuPage, and Des Plaines Rivers) during June 2007. Triplicate water samples for stable hydrogen and oxygen isotope analysis were collected from each water body in 20-mL scintillation vials containing minimal air space and sealed with Parafilm® to curtail evaporative loss and fractionation (Kendall and McDonnell 1998). Three to four additional water samples for analysis of trace element (Sr, Ba, Pb, Mn), Mg and Ca concentrations by high-resolution, inductively coupled plasma mass spectrometry (HR-ICPMS) were collected from each water body using protocols described in Shiller (2003). Lake Michigan water samples were obtained from four locations along the Illinois shoreline. Twelve to thirteen fishes were also collected from each water body for subsequent otolith extraction and analysis. Species collected included green sunfish (*Lepomis cyanellus*), bluegill (*L. macrochirus*), pumpkinseed sunfish (*L. gibbosus*), orangespotted sunfish (*L. humilis*), black crappie (*Pomoxis nigromaculatus*), smallmouth bass (*Micropterus dolomieu*), largemouth bass (*M. salmoides*), freshwater drum (*Aplodinotus grunniens*), and common carp (*Cyprinus carpio*). No Asian carps were encountered during sampling for this study. Fish were identified to species, measured for total length (nearest mm), euthanized with MS-222, placed on ice and stored frozen until otolith removal.

Sagittal otoliths for stable isotope analysis were removed from fish using forceps, cleaned with distilled water, and stored dry in polyethylene vials. One set of otoliths (one per fish) from each location was analyzed for stable oxygen and carbon isotopic composition using a ThermoFinnigan Delta plus XP® isotope ratio mass spectrometer interfaced with a Gas Bench II® carbonate analyzer. Subsamples of otolith material for stable isotope analysis (Gao 1999) were taken from near the outer edge of each otolith, which reflects a fish's most recent environmental history. Stable hydrogen, oxygen, and carbon isotope ratios of water and otolith samples were expressed in standard δ notation, defined as the parts per thousand deviation between isotope ratios of a sample and a standard material: $\delta^2\text{H}$, $\delta^{18}\text{O}$, or $\delta^{13}\text{C}$ (‰) = $[(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$, where R represents $^2\text{H}/^1\text{H}$, $^{18}\text{O}/^{16}\text{O}$, or $^{13}\text{C}/^{12}\text{C}$. Standards are PDB for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in otolith carbonate and VSMOW for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in water. A second set of otoliths (one per fish) from each location were extracted for trace element analysis using non-metallic, acid washed tools, embedded in Epo-fix® epoxy, sectioned in a transverse plane using an ISOMET low-speed saw, and polished to reveal annuli. Otolith thin sections were prepared for analysis under Class-100 clean conditions following procedures described in Whitley et al. (2007). Each otolith thin section was analyzed for ^{86}Sr , ^{137}Ba , ^{55}Mn , ^{208}Pb , and ^{24}Mg using a Perkin-Elmer DRC II® ICPMS coupled with a

CETAC Technologies LSX-500[®] laser ablation system. A series of three spots (100 μm diameter) were ablated with the laser (213 nm wavelength, energy level 70%, pulse rate 20 Hz) along the outer edge of each otolith thin section. Data from the three ablation spots were averaged for each otolith. A standard developed by the U.S. Geological Survey (MACS-1, CaCO_3 matrix) was analyzed prior to every sample; each sample analysis was also preceded by a gas blank measurement. Isotopic counts were converted to elemental concentrations ($\mu\text{g/g}$) after correction for gas blank, matrix, and drift effects. Elemental concentrations were normalized to ^{46}Ca concentration based on the consideration of calcium as a pseudointernal standard (Bickford and Hannigan 2005; Ludsins et al. 2006; Whitledge et al. 2007); data are reported as element:calcium ratios (mmol/mol). Mean limits of detection (ng/g) were 12.32 for Sr, 12.13 for Ba, 0.57 for Mn, 1.07 for Pb, and 7.57 for Mg.

Both univariate and multivariate approaches were used to statistically analyze and describe differences in water and otolith trace element and stable isotopic signatures among sampling sites. Analysis of variance (ANOVA) followed by Tukey's HSD test for multiple comparisons were used to test for significant differences in individual water chemistry parameters among sampling locations. Water chemistry parameters that were significantly different among sampling locations were entered into a multivariate analysis of variance (MANOVA) and a discriminant analysis (CANDISC procedure in SAS[®]) to characterize the multivariate water chemistry signatures of sampling locations. Pillai's trace statistic was used to assess significance of differences in multivariate water chemistry signatures. Spearman's rank correlations were used to assess significance of relationships between each parameter that was measured in both water and otoliths. Statistical analyses of otolith chemistry data were equivalent to those used for water chemistry data. Additionally, linear discriminant function analysis with a leave-one-out jackknife procedure was used to determine the accuracy with which individual fish could be classified back to their collection locations based on their otolith elemental and stable isotopic signatures. P-values ≤ 0.05 were considered significant for all statistical tests.

RESULTS:

Five of the seven water chemistry parameters measured were significantly different among sites (ANOVA, $p < 0.0001$ for all significant tests). Water Sr:Ca was highest in the Fox River, intermediate in the Des Plaines River, and lowest in the DuPage and Illinois Rivers and southwestern Lake Michigan (Figure 1). Water Ba:Ca was higher in the Fox River compared to all other water bodies sampled (Figure 1). Water Mg:Ca was significantly different among all water bodies sampled except for the Des Plaines and DuPage Rivers; the Fox River had the highest mean Mg:Ca, followed by the Des Plaines and DuPage Rivers, the Illinois River, and Lake Michigan. Lead (Pb) concentration was significantly different among all locations sampled except for the Illinois and Fox Rivers; Pb was highest in the DuPage River, followed by the Des Plaines River, the Illinois and Fox Rivers, and Lake Michigan. Water Mn concentration was higher in the Des Plaines and DuPage Rivers compared to all other sites. Water $\delta^2\text{H}$ and $\delta^{18}\text{O}$ were not significantly different among the five environments sampled ($p > 0.1$).

Three of the five trace elemental markers that were significantly different among water samples from Lake Michigan and the Fox, DuPage, Des Plaines, and Illinois Rivers also exhibited significant differences among otoliths from fish collected from these water bodies (ANOVA, $p < 0.0002$ for all significant tests). Mean otolith Sr:Ca was highest in the Fox River, intermediate in the Des Plaines River, and lowest in the DuPage and Illinois Rivers and southwestern Lake Michigan (Figure 2), consistent with the pattern observed for water Sr:Ca. Mean otolith Ba:Ca was higher for fish from the Fox River compared to mean otolith Ba:Ca for fish from all other sites, which was also consistent with water chemistry data (Figure 2). Otolith Mg:Ca was higher in fish from the Des Plaines and Fox Rivers compared to fish collected from the other three sites; this pattern was not consistent with that observed for water Mg:Ca. Mean otolith Pb:Ca, Mn:Ca, and $\delta^{18}\text{O}$ values were not significantly different among fish from the five water bodies sampled ($p > 0.1$ for all tests). Otolith $\delta^2\text{H}$ was not measured because no significant differences in water $\delta^2\text{H}$ among sites were detected. However, mean otolith $\delta^{13}\text{C}$ (which was analyzed in conjunction with $\delta^{18}\text{O}$ of otolith carbonate) was significantly different for Lake Michigan fish compared to fish collected in the Illinois River and each of the three Illinois River tributaries ($p < 0.0001$); mean otolith $\delta^{13}\text{C}$ was also significantly different between fish from the Illinois and Des Plaines Rivers (Figure 3).

Three markers ($\delta^{13}\text{C}$, Sr:Ca, and Ba:Ca) in otoliths were retained for multivariate and discriminant analyses based on univariate statistical analysis of water and otolith chemistry data. Otolith Mg:Ca was not included because it was weakly, though significantly correlated with water Mg:Ca ($r = 0.36$, $p = 0.006$) and did not enable differentiation of fishes from among the five collection sites beyond those that could already be distinguished based on otolith Sr:Ca. Otolith Sr:Ca and Ba:Ca were strongly correlated with water Sr:Ca and Ba:Ca,

respectively ($p < 0.0001$, $r = 0.82$ for Ba:Ca, $r = 0.88$ for Sr:Ca). Inter-site differences in otolith chemistry were highly significant when analyzed as a multivariate fingerprint including $\delta^{13}\text{C}$, Sr:Ca, and Ba:Ca (MANOVA, Pillai's trace statistic, $p < 0.0001$). A plot of the first two canonical variables (Figure 4), which accounted for 98% of the total dispersion in the otolith chemistry dataset, indicated that the first canonical variable discriminated among fish from three groups of sites: Lake Michigan, the Illinois and DuPage Rivers, and the Fox and Des Plaines Rivers. The second canonical variable primarily distinguished fish from the Fox River and Lake Michigan from fish collected at the remaining sites. Multivariate analysis of water chemistry data (including Sr:Ca and Ba:Ca only) also indicated significant differences among sites (Pillai's trace statistic, $p < 0.0001$).

Linear discriminant function analysis indicated classification accuracies of 64-100% for assigning individual fish to the body of water in which they were collected using otolith $\delta^{13}\text{C}$, Sr:Ca, and Ba:Ca (Table 1). All twelve fish obtained from Lake Michigan were correctly classified to their collection location; no fish collected in the Illinois River or its tributaries were misidentified as Lake Michigan residents. Most misclassifications occurred among fish from the Illinois and DuPage Rivers; these two rivers did not significantly differ with respect to any of the trace elemental or isotopic markers measured in this study. One fish from the Fox River was incorrectly classified as having been collected in the Des Plaines River. Two fish captured in the Des Plaines River were misidentified as Illinois River residents and one individual from the Des Plaines River was incorrectly characterized as being from the DuPage River.

DISCUSSION:

Results indicated that fishes from the upper Illinois River and three of its principle tributaries could be distinguished from fishes that reside in southwestern Lake Michigan based on otolith isotopic and trace elemental compositions. A combination of otolith $\delta^{13}\text{C}$, Sr:Ca and Ba:Ca was also demonstrated to be capable of distinguishing fish captured in the upper Illinois River from fish that reside in the Fox and Des Plaines Rivers with a high degree of accuracy. The three naturally-occurring markers in otoliths that best discriminated among fishes from the five water bodies sampled in this study ($\delta^{13}\text{C}$, Sr:Ca and Ba:Ca) have frequently been among the most useful indicators of fish environmental history in other geographic locations (e.g., Wells et al. 2003; Brazner et al. 2004; Bickford and Hannigan 2005; Dufour et al. 2005; Munro et al. 2005; Wurster et al. 2005; Whitledge et al. 2007). Classification success rates for individual fish to collection sites in this study were comparable to or greater than those of published studies using otolith microchemistry and stable isotopic composition as indicators of source environment or habitat for freshwater fishes, including studies involving Great Lakes fish (Bronte et al. 1996; Wells et al. 2003; Brazner et al. 2004; Clarke et al. 2007). Some of the misclassifications of fish to environment of capture within the upper Illinois River system (e.g., three of the fish captured in the Des Plaines River) may have been due to recent inter-river movement by some individuals. The inability to collect individuals of known environmental history in interconnected rivers or to subsample otolith material for isotopic or trace element analysis precisely at the otolith edge allows recent immigrants to hinder precise characterization of an otolith chemistry fingerprint for a collection location.

Otoliths reflected differences in water Sr:Ca and Ba:Ca among the upper Illinois River, three of its tributaries and southwestern Lake Michigan. Strong correlations between water and otolith Sr:Ca and Ba:Ca are expected given that water, rather than food, contributes the majority of Sr and Ba to fish otoliths (Walther and Thorrold 2006). Mechanisms responsible for observed differences in water Sr:Ca and Ba:Ca and otolith $\delta^{13}\text{C}$ among environments sampled in this study are unknown. Otoliths incorporate both dissolved inorganic carbon (DIC) and metabolically-derived carbon (Kalish 1991). Thus, observed differences in otolith $\delta^{13}\text{C}$ may reflect differences in $\delta^{13}\text{C}$ of DIC between Lake Michigan and the upper Illinois River and its tributaries. Lake Michigan DIC $\delta^{13}\text{C}$ averages -7.0‰ (Budai et al. 2002); $\delta^{13}\text{C}$ of DIC in the Illinois River and tributaries is unknown, but is likely influenced by isotopically lighter respired carbon (Hoefs 2004). $\delta^{13}\text{C}$ values of DIC in Lake Ontario tributaries are lower than $\delta^{13}\text{C}$ of DIC in Lake Ontario (Leggett et al. 1999). Higher otolith $\delta^{13}\text{C}$ values for Lake Michigan fish compared to those captured in the Illinois River system may also be due lower metabolic rates resulting from lower water temperatures in Lake Michigan; lower metabolic rates reduce contribution of respiratory carbon to otoliths and increase otolith $\delta^{13}\text{C}$ values (Wurster et al. 2005). Regardless of the mechanisms responsible for differences in water and otolith elemental and isotopic compositions among sites, multi-parameter chemical "fingerprints" in otoliths established in this study will be useful for distinguishing among fish from Lake Michigan and the Fox, Des Plaines, and upper Illinois Rivers if environmental signatures are stable over time. Other studies have reported interannual stability in Sr:Ca and Ba:Ca signatures in some freshwater environments (Zimmerman and Reeves 2002; Wells et al. 2003; Munro et al. 2005; Ludsing et al. 2006;

Whitledge et al. 2007). Otolith $\delta^{13}\text{C}$ may change seasonally and with fish age in association with changes in metabolic rate (Wurster et al. 2005). Future research involving otolith isotopic and elemental compositions as indicators of fish environmental history in the upper Illinois River system and southwestern Lake Michigan should determine the extent of temporal variation in environmental signatures reported in this study. Substantial inter-annual variation in environmental signatures may preclude the use of otolith chemistry for distinguishing among fish in the environments sampled in this study during some years or may require that multiple source-environment classification models be developed and applied to fish from different year classes.

Analysis of water and otolith Sr:Ca and Ba:Ca data from 27 locations in Illinois and Missouri (including sites sampled in this study) indicated no significant differences in otolith Sr:Ca among seven of the nine species collected in this study (ANCOVA with water Sr:Ca as the covariate, $p>0.05$; less than five smallmouth bass and common carp were collected, so they were excluded from this analysis). Otolith Ba:Ca was significantly higher for freshwater drum compared to all centrarchids except bluegill (ANCOVA with water Ba:Ca as the covariate); no significant differences in otolith Ba:Ca were detected among the six species of centrarchids tested ($p>0.05$).

Potential applications of otolith microchemistry and stable isotopic composition for fishes in the upper Illinois River system and southwestern Lake Michigan include determining source environment for any Asian carps that may be discovered in Lake Michigan. Intra-otolith isotopic and elemental analyses could potentially be used to determine whether a fish had an otolith signature immediately prior to entering Lake Michigan that was characteristic of the Illinois River (which would suggest that the fish may have breached the electrical barriers), a tributary to the upper Illinois River (which would suggest that the fish was transferred to Lake Michigan via bait bucket or other means), or an otolith signature that did not fall within expected ranges of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, Sr:Ca or Ba:Ca values for fish from the upper Illinois River system (which would suggest that the fish was introduced into Lake Michigan from another source). Application of otolith chemistry for retrospectively describing environmental history of Asian carps will require characterization of relationships between water and otolith trace element and stable isotopic values for both bighead and silver carp.

Results of this study suggest that otolith microchemistry and isotopic analysis may also be applicable for determining environmental history of fishes within the upper Illinois River system. Detection of fish use of particular tributary habitats (e.g., the Fox or Des Plaines Rivers) may be possible using otolith Sr:Ca and Ba:Ca. However, elemental and stable isotopic signatures of other upper Illinois River tributaries (e.g., the Kankakee and Vermilion Rivers) should be determined. It may also be possible to identify fish that immigrated to the upper Illinois River system from Lake Michigan; this could be valuable for assessing the risk of viral hemorrhagic septicemia spreading from the Great Lakes into inland Illinois waters via movement of affected fishes.

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DELIVERABLES:

Deliverables resulting from this project include this final report to the GLFC and abstracts submitted for poster presentations at 2008 annual meetings of the American Fisheries Society and the Illinois Chapter of the American Fisheries Society. Submission of a manuscript for a peer-reviewed journal is planned for the near future.

PRESS RELEASE:

STABLE ISOTOPES AND TRACE ELEMENTS IN OTOLITHS DISTINGUISH FISH FROM THE UPPER ILLINOIS RIVER DRAINAGE AND LAKE MICHIGAN

ANN ARBOR, MI—Researchers at Southern Illinois University-Carbondale have completed a study evaluating otolith microchemistry and stable isotopic composition as tools for distinguishing among fish from the upper Illinois River and three of its principle tributaries and southwestern Lake Michigan. The pilot project, supported by the Great Lakes Fishery Commission, may provide a new technique to rapidly determine the source environment and likely transfer pathway of any Asian carp that may be discovered in Lake Michigan.

Asian carp have rapidly expanded their range in the Mississippi River basin and have been observed in the upper Illinois River within 50 miles of Lake Michigan. Electrical barriers have been constructed in the Chicago Sanitary and Ship Canal to try to prevent Asian carp from entering the Great Lakes. In addition to the possibility of Asian carp breaching the electrical barriers, unintentional release of wild-caught baitfish contaminated with young bighead or silver carp is another probable mechanism by which these species could be introduced into the Great Lakes. Tributaries of the upper Illinois River may be among the most likely pathways for unintentional bait bucket transfer of young Asian carp into Lake Michigan because their confluences with the Illinois River are downstream of the electrical barriers, their headwaters flow within 5-15 miles of Lake Michigan, and they support substantial recreational fisheries. Knowledge of the source and transfer pathway of any Asian carps that may be discovered in Lake Michigan (especially during the early stages of an invasion or introduction before a viable population becomes established and eradication is still feasible) would be essential for developing strategies to prevent further introductions or invasions.

Greg Whitley, the lead researcher on this project, conducted stable isotope and trace element analysis of water and fish otolith samples from the Illinois, Fox, Des Plaines, and DuPage Rivers and Lake Michigan. Trace element and stable isotopic compositions of otoliths reflect those of environments occupied by fish and can be used to reconstruct an individual fish's environmental history. "The results of this research show that we can distinguish fish from Lake Michigan and the Illinois, Fox, and Des Plaines Rivers with a high degree of accuracy using stable isotope and trace element analysis of otoliths," said Whitley. "This opens up the possibility of being able to identify the source environment for Asian carp in the unfortunate event that any are discovered in Lake Michigan."

APPENDICES:

Table 1. Results of linear discriminant function analysis showing classification accuracy (determined by jackknife procedure) for individual fish to collection locations (DES=Des Plaines River, DUP=DuPage River, FOX=Fox River, ILR=Illinois River, LMI=Lake Michigan) based on otolith Sr:Ca, Ba:Ca, and $\delta^{13}\text{C}$. n=number of fish per site.

Source location	n	Assigned location					% Correct
		DES	DUP	FOX	ILR	LMI	
DES	12	9	1	0	2	0	75
DUP	11	0	8	0	3	0	73
FOX	13	1	0	12	0	0	92
ILR	11	0	4	0	7	0	64
LMI	12	0	0	0	0	12	100

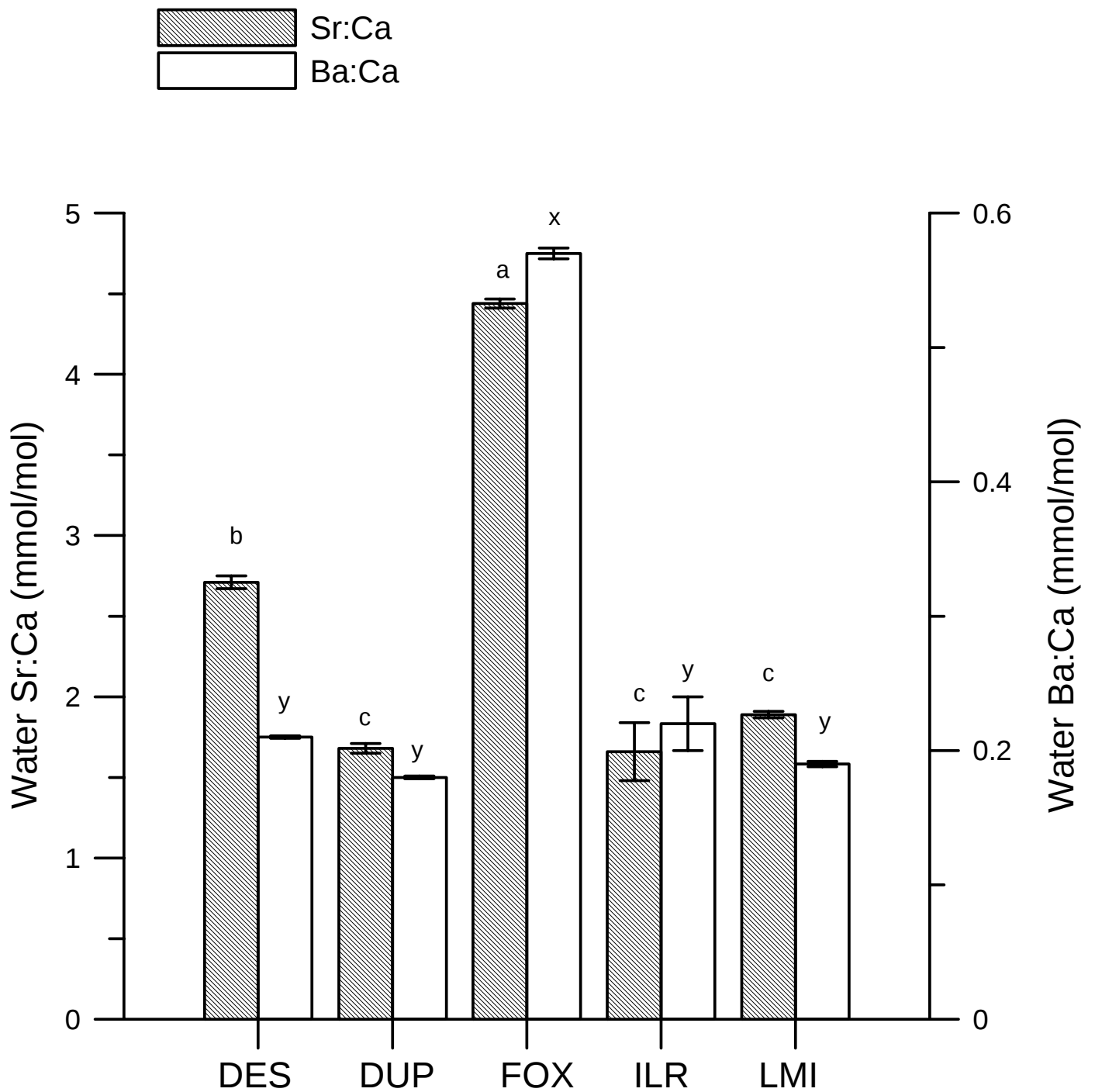


Figure 1. Mean water Sr:Ca and Ba:Ca ($\pm$ SE) for the Des Plaines River (DES), DuPage River (DUP), Fox River (FOX), upper Illinois River (ILR), and southwestern Lake Michigan (LMI). Site means for each variable that are marked with the same letter are not significantly different (ANOVA followed by Tukey's HSD test, $p < 0.05$). $n=4$ for ILR and LMI; $n=3$ for each Illinois River tributary.

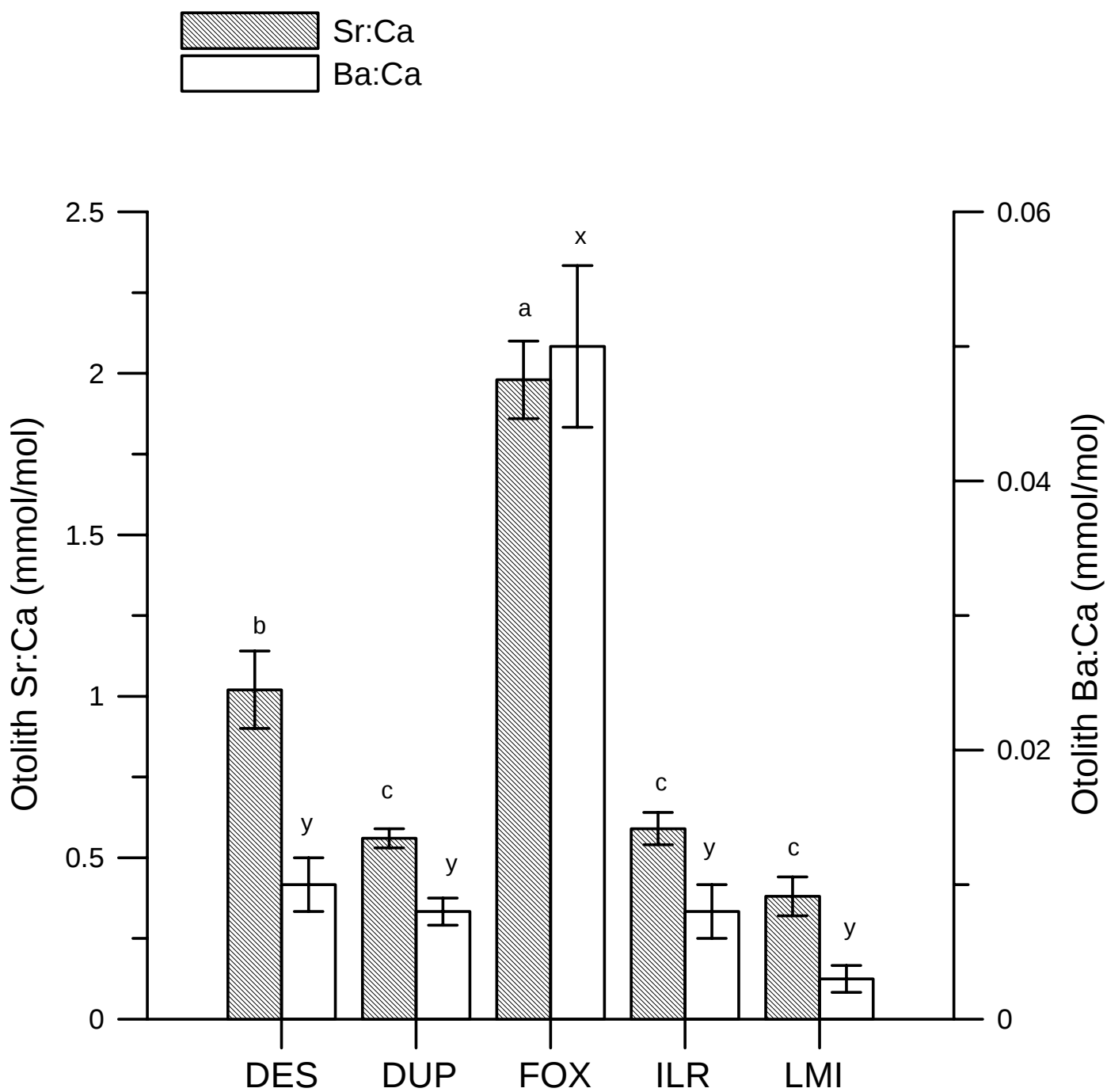


Figure 2. Mean otolith Sr:Ca and Ba:Ca ($\pm$ SE) for fish collected in the Des Plaines River (DES), DuPage River (DUP), Fox River (FOX), upper Illinois River (ILR), and southwestern Lake Michigan (LMI). Site means for each variable that are marked with the same letter are not significantly different (ANOVA followed by Tukey's HSD test, $p < 0.05$). $n = 12-13$ fish per location.

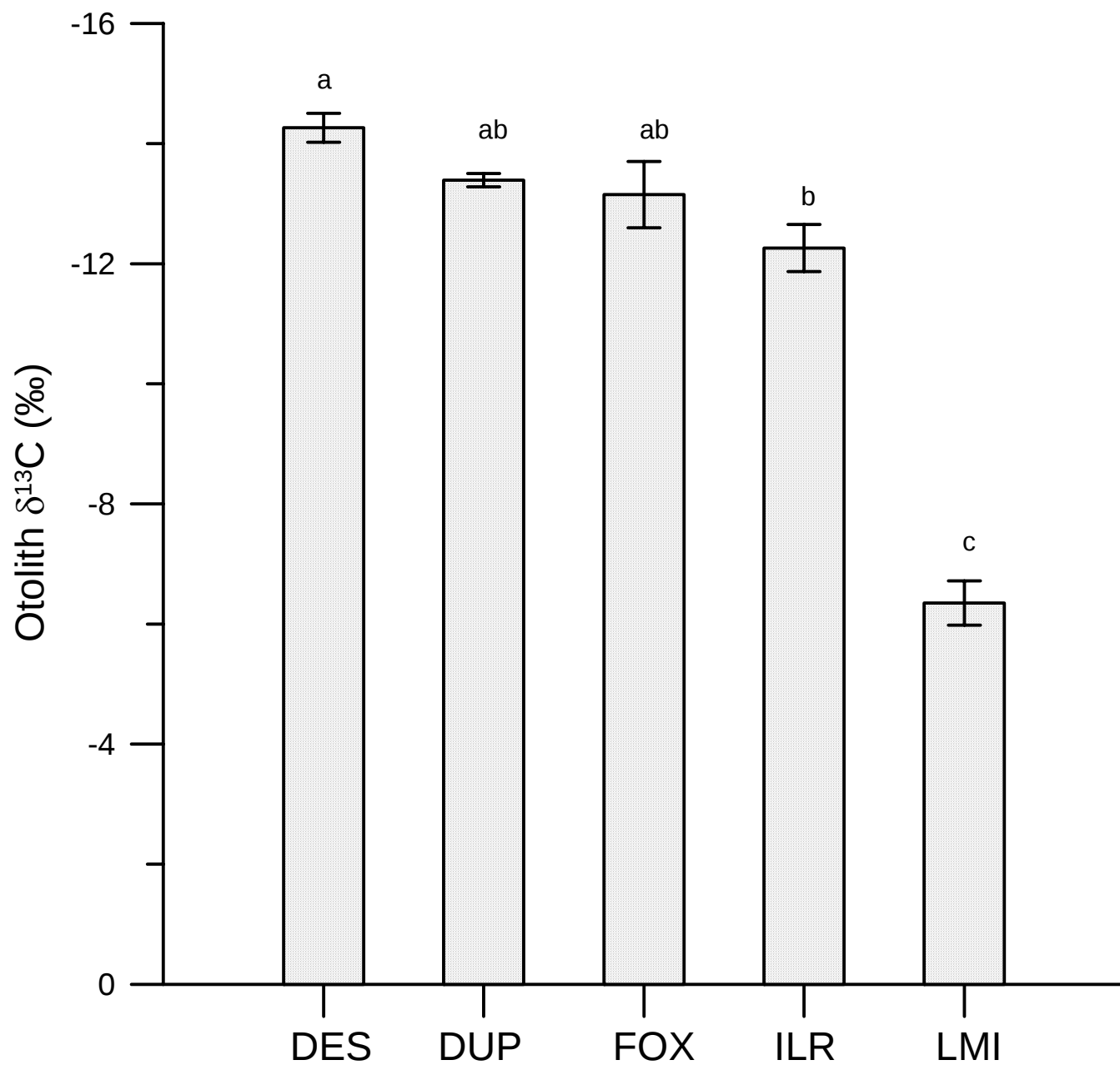


Figure 3. Mean otolith $\delta^{13}\text{C}$ ($\pm$ SE) for fish collected in the Des Plaines River (DES), DuPage River (DUP), Fox River (FOX), upper Illinois River (ILR), and southwestern Lake Michigan (LMI). Site means that are marked with the same letter are not significantly different (ANOVA followed by Tukey's HSD test, $p < 0.05$). $n=12-13$ fish per location.

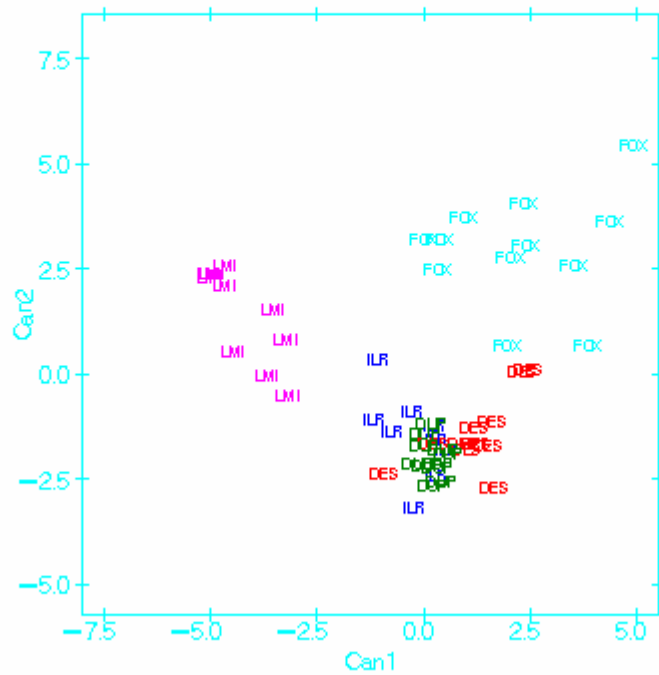


Figure 4. Elemental and isotopic fingerprints for fish otoliths from the Des Plaines River (DES), DuPage River (DUP), Fox River (FOX), upper Illinois River (ILR), and southwestern Lake Michigan (LMI) based on the first two canonical variables obtained through linear discriminant function analysis including $\delta^{13}\text{C}$, Sr:Ca and Ba:Ca.

Abstract for a poster presentation submitted for the 2008 annual meeting of the Illinois Chapter of the American Fisheries Society:

Assessment of Otolith Chemistry as an Indicator of Fish Environmental History in the Upper Illinois River System and Lake Michigan.

Whitledge, Gregory. Fisheries and Illinois Aquaculture Center, Southern Illinois University, Carbondale, IL 62901-6511; 618-453-6089; gwhit@siu.edu

Naturally occurring chemical markers in otoliths offer a potential means to identify source environment for fish within the upper Illinois River system and in Lake Michigan, including individuals that may breach the electrical barriers or be transferred via bait buckets between these formerly isolated drainages. The objectives of this study were to determine whether water and fish otolith stable isotopic and elemental compositions differ among Lake Michigan, the upper Illinois River, and three tributaries of the upper Illinois River and to determine whether otolith isotopic and elemental signatures could be used to identify the water body from which individual fish were collected. Water and fish otolith samples were obtained from each site during summer 2007 and analyzed for $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and a suite of trace element concentrations. Otolith $\delta^{13}\text{C}$ values for Lake Michigan fish were distinct from individuals collected in the Illinois River and tributaries. Fish collected in the Fox and Des Plaines Rivers could be distinguished from one another and from fish captured in the Illinois and DuPage Rivers using otolith Sr:Ca and Ba:Ca ratios; otoliths reflected differences in water chemistry among environments. Otolith isotopic and elemental compositions may enable determination of source environment for any Asian carp discovered in Lake Michigan and could also be used as indicator of environmental history for fishes in the upper Illinois River and its tributaries.

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