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Otolith trace element and stable isotopic compositions differentiate fishes from the Middle Mississippi River, its tributaries, and floodplain lakes.

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Abstract

Naturally occurring stable isotope and trace elemental markers in otoliths have emerged as powerful tools for determining natal origins and environmental history of fishes in a variety of marine and freshwater environments. However, few studies have examined the applicability of this technique in large river-floodplain ecosystems. This study evaluated otolith microchemistry and stable isotopic composition as tools for determining environmental history of fishes in the Middle Mississippi River, its tributaries, and floodplain lakes in Illinois and Missouri, U.S.A. Fishes were collected from 14 sites and water samples obtained from 16 sites during summer and fall 2006 and spring 2007. Otolith and water samples were analyzed for stable oxygen isotopic composition ($\delta^{18}O$) and concentrations of a suite of trace elements; otoliths were also analyzed for carbon isotopic composition ($\delta^{13}C$). Tributaries, floodplain lakes, and the Mississippi and Lower Missouri Rivers possessed distinct isotopic and elemental signatures that were reflected in fish otoliths. Fish from tributaries on the Missouri and Illinois sides of the middle Mississippi River could also be distinguished from one another by their elemental and isotopic fingerprints. Linear discriminant function analysis of otolith chemical signatures indicated that fish could be classified back to their environment of capture (Mississippi River, floodplain lake, tributary on the Illinois or Missouri side of the Mississippi River, or lower Missouri River) with 71-100% accuracy. This study demonstrates the potential applicability of otolith microchemistry and stable isotope analyses to determine natal origins and describe environmental history of fishes in the Middle Mississippi River, its tributaries, and floodplain lakes. The ability to reconstruct environmental history of individual fish using naturally occurring isotopic markers in otoliths may also facilitate efforts to quantify nutrient and energy subsidies to the Mississippi River provided by fishes that emigrate from floodplain lakes or tributaries.

Introduction

Understanding fish use of large rivers, their tributaries, and floodplain lakes throughout their life history is important for the management of riverine fish populations and the habitats they depend upon. Many riverine species are believed to require connectivity of large rivers and floodplain habitats for spawning and larval nursery (Bayley & Li, 1992; Gozlan et al., 1998; Nunn et al., 2007a), foraging (Nunn et al., 2007b), and winter refuge (Knights et al., 1995; Raibley et al., 1997). However, there are currently several hypotheses regarding the primary source habitat (floodplain lakes, tributaries, and river channel) for fishes in large, regulated rivers. Larval fishes are common in many floodplain lakes, with rheophilic taxa comprising an increasing proportion of fish assemblages in lakes as river-floodplain connectivity increases (Scheimer, 2000); larval fishes are also known to frequently drift into and out of connected floodplain lakes (Grift et al., 2003; Csoboth & Garvey, 2008). Tributary streams may also contribute to fish assemblages in large rivers, at least near their confluences (Brown & Coon, 1994; Robinson et al., 1998; Kiffney et al., 2006). Larval fishes are sometimes abundant in the main channel of large rivers, suggesting that main channels may be more hospitable as larval nursery areas than previously thought; species composition of larval fishes in the main channel is often distinct from that of off-channel habitats (Holland, 1986; Dettmers et al., 2001). Nearshore, slack-water habitats may provide the primary nursery area for rheophilic fishes in some rivers (i.e., the "inshore retention concept", Scheimer et al., 2001; Keckeis & Scheimer, 2002). Additionally, spawning of some species of riverine fishes does not appear to coincide with the annual flood pulse, but occurs primarily under non-flood conditions within the river's main channel (Humphries et al., 1999). Uncertainty regarding the relative importance of source habitats (floodplain lakes, tributaries, and river channel) for fishes in large, regulated rivers such as the Mississippi River and the presence of fishes of commercial, recreational, and conservation importance in these highly modified ecosystems demonstrates a need for techniques to facilitate identification of recruitment sources for large river fishes.

Ultrasonic and radio telemetry have provided valuable insights into movement and habitat use of fishes in the Mississippi River and tributaries (Zigler et al., 2003; Vokoun & Rabeni, 2005; DeGrandchamp et al., 2008). However, this technique is

limited primarily to large bodied species and older individuals. Tracking tagged fish can also be labor intensive and sample sizes are often limited due to cost or large study areas and rapid movement of tagged fish (Winter, 1996). Much uncertainty remains regarding the principle recruitment sources and environments used during early life stages for several fishes that inhabit the Mississippi River, including species of recreational and commercial interest (e.g., ictalurids, centrarchids, *Ictiobus* spp.), conservation concern (e.g., shovelnose sturgeon *Scaphirhynchus platorynchus*, paddlefish *Polyodon spathula*, blue sucker *Cycleptus elongatus*, and chubs *Macrhybopsis* spp.) and invasive species (e.g., bighead carp *Hypophthalmichthys nobilis* and silver carp *H. molitrix*). Improved knowledge of natal habitats and environmental history of early life stages of Mississippi River fishes would be valuable for their conservation and management.

Microchemical and stable isotopic analyses of fish otoliths offer the potential to provide new insights into the relative importance of river channel, floodplain lake, and tributary habitats as source environments for fishes large, regulated rivers. Application of otolith trace element and isotopic compositions as natural tags has emerged as an effective technique for addressing questions regarding environmental history of freshwater fishes (e.g., Wells et al., 2003; Brazner et al., 2004; Dufour et al., 2005; Munro et al., 2005; Feyrer et al., 2007; Whitledge et al., 2007; Schaffler & Winkelman, 2008). Trace element and stable isotopic compositions of otoliths often reflect those of environments occupied by a fish (e.g., Kennedy et al., 2002; Wells et al., 2003; Dufour et al., 2005; Whitledge et al., 2006) and are unaltered metabolically following deposition (Campana & 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). Crook & Gillanders (2006) demonstrated the applicability of otolith microchemistry for identifying common carp recruitment sources in the Murray River, Australia, and Zeigler & Whitledge (2010) confirmed that stable isotopic signatures in otoliths can be used to reliably identify environment of origin (river, floodplain lake, or tributary) for fishes in the Illinois River, Illinois. However, the applicability of otolith trace element and stable isotopic signatures as natural markers of fish environmental history in the Mississippi River and associated water bodies has not been assessed.

The goal of this study was to assess whether otolith microchemistry and stable isotopic analyses may be useful tools for determining environmental history of fishes in the Middle Mississippi River in Illinois and Missouri, U.S.A. Specific objectives were to determine whether water trace elemental and stable oxygen isotopic compositions differed among floodplain lakes, tributaries, and the Middle Mississippi River, to determine whether fish otolith microchemistry and isotopic compositions reflected those of environments (floodplain lakes, tributaries, and the Mississippi River) in which they were captured, and to determine the accuracy with which individual fish could be classified back to their collection locations based on otolith elemental and stable isotopic signatures.

Study Area

The Middle Mississippi River is defined as the unimpounded section of the river extending from the mouth of the Missouri River to the Ohio River confluence. Our study area included the entire Middle Mississippi River and extended upstream into lower portion of the Upper Mississippi River (pool 25). Fish and water samples were collected from 14 Mississippi River main stem, tributary, and floodplain lake locations (Fig. 1). These sites included the Upper Mississippi River at Batchtown, Illinois, Middle Mississippi River at Thebes, Illinois, five tributaries of the Mississippi River on the Missouri side (Apple Creek, Cuivre River, Headwater Diversion Channel, Meramec River, and Missouri River), four tributaries of the Mississippi River on the Illinois side (Big Muddy River, Clear Creek, Illinois River, and Kaskaskia River), and four backwater/floodplain lakes (Grand Tower Chute, Middle Swan Lake, Prairie Pond, and a pond at the Middle Mississippi River Wetlands Field Station). One lake (Middle Swan Lake) is adjacent to the Illinois River, but is close (about 7 km) to the Illinois River-Mississippi River confluence. Water samples were also collected from two additional tributaries of the Mississippi River, including one on the Missouri side (River Aux Vases) and one on the Illinois side (Mary's River). Sample collection locations included all major tributaries and floodplain lakes along the Middle Mississippi River.

Methods

Two water samples were collected from each of the 16 sites (Fig. 1), including one for stable oxygen isotope analysis and one for analysis of concentrations of a suite of twenty major, minor, and trace elements (including Ca, Sr, Ba, Mg, and Mn). Both water samples were collected at all sites during summer and fall 2006 and again in spring 2007 to assess seasonal changes in stable isotopic and elemental compositions within the Mississippi River, tributaries, and floodplain lakes. Water samples for stable oxygen isotope analysis were collected in scintillation vials containing minimal air space and sealed with Parafilm to curtail evaporative loss and fractionation (Kendall & Caldwell, 1998). Water samples were analyzed for stable oxygen isotopic composition using a Thermo Finnigan Delta Plus XL® isotope ratio mass spectrometer. Stable oxygen isotope ratios were expressed in standard δ notation, defined as parts per thousand deviation between the isotope ratios of a sample and standard material (Vienna Standard Mean Ocean Water):

$$\delta^{18}O\ (‰) = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R represents $^{18}O/^{16}O$. Mean standard deviation of replicate measurements of water $\delta^{18}O$ was 0.23‰ (n = 3 replicates per sample). Water samples for elemental analysis were collected using a syringe filtration technique described in Shiller (2003). Samples for analysis of elemental concentrations were stored on ice or refrigerated until overnight shipment and analysis by high-resolution, inductively coupled plasma mass spectrometry (HR-ICPMS) at the Center for Trace Analysis, University of Southern Mississippi. Elemental concentration data were converted to molar element:calcium ratios (mmol/mol). Mean standard deviations of replicate measurements of water Sr:Ca, Ba:Ca, Mn:Ca, and Mg:Ca were 0.03 mmol/mol, 0.02 mmol/mol, 0.02 mmol/mol, and 0.02 mol/mol, respectively (n = 2 replicates per sample).

Fishes were collected from each of 14 sites during summer or early fall 2006. Up to thirty individuals were collected from each site. Centrarchids (largemouth bass *Micropterus salmoides*, spotted bass *M. punctulatus*, green sunfish *Lepomis cyanellus*, bluegill *L. macrochirus*, orangespotted sunfish *L. humilis*, longear sunfish *L. megalotis*, and black crappie *Pomoxis nigromaculatus*) were collected where possible due to their recreational importance and widespread availability, however temperate basses (yellow bass *Morone mississippiensis* and white bass *M. chrysops*) and freshwater drum

(*Aplodinotus grunniens*) were collected where sufficient numbers of centrarchids could not be found since they are also common in riverine, tributary, and floodplain lake habitats. A recent analysis of otolith and water chemistry data from the Mississippi and Illinois Rivers found that there were no significant differences in relationships between water and otolith stable oxygen isotopic compositions ($\delta^{18}O$) or Sr:Ca ratios among the fish species collected in this study (Zeigler and Whitledge 2010). Fishes were captured by alternating current (AC) or direct current (DC) electrofishing or trap netting at sites where a boat could be launched. At sites without boat access, fish were captured by seining and angling. Fishes were euthanized with MS-222, placed on ice for transport to the laboratory, and stored frozen until otolith removal.

Sagittal otoliths were removed from each fish using non-metallic forceps, rinsed with distilled water, and stored dry in polyethylene microcentrifuge tubes until preparation for analysis. From each fish one otolith was analyzed for stable isotopic composition. One otolith from each fish was analyzed for stable oxygen and carbon isotopic composition. Material from the outer edge of otoliths > 1 mg was subsampled and pulverized, as this portion of the otolith reflects a fish's most recent environmental history. Otoliths < 1 mg were pulverized whole with a mortar and pestle due to the difficulty of obtaining a sufficient edge subsample. Otoliths were 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. Stable oxygen and carbon isotope ratios for otolith samples were expressed in standard δ notation ($\delta^{18}O$ or $\delta^{13}C$, ‰); mean standard deviation for replicate measurements (n=2 replicates per sample) was 0.8‰ for $\delta^{18}O$ and 0.4‰ for $\delta^{13}C$.

The second sagittal otolith from each fish was used for analysis of trace element:calcium ratios (e.g., Sr:Ca, Ba:Ca, Mg:Ca, Mn:Ca). Otoliths for trace element analysis were embedded in Epo-fix epoxy, sectioned in the transverse plane using an ISOMET low-speed saw, and then sanded and polished to reveal annuli. Otolith thin sections were prepared for analysis under a class 100 laminar flow hood and handled only with nonmetallic acid-washed forceps. Thin sections were mounted on acid-washed glass slides using double-sided tape, ultrasonically cleaned for 5 min in ultrapure water, and dried for 24 h under the laminar flow hood. Mounted and cleaned thin sections were stored in acid-washed polypropylene Petri dishes in a sealed container until analysis.

Otolith thin sections were analyzed for ^{88}Sr, ^{137}Ba, ^{24}Mg, ^{55}Mn, and ^{44}Ca using a Perkin-Elmer ELAN 6000 inductively coupled plasma mass spectrometer (ICPMS) coupled with a CETAC Technologies LSX-500 laser ablation system. The laser ablated a transect along the long axis of the otolith section from one side of the otolith core to the edge of the opposite side of the otolith (beam diameter = 25 µm, scan rate = 10 µm/s, laser pulse rate = 10 Hz, laser energy level = 9mJ, wavelength = 266 nm). A standard developed by the U. S. Geological Survey (MACS-1, $CaCO_3$ matrix) was analyzed every 12-15 samples to adjust for possible instrument drift. Each sample analysis was preceded by a gas blank measurement. Isotopic counts were converted to elemental concentrations (µg/g) after correction for gas blank, matrix, and drift effects. Mean limits of detection for ^{88}Sr, ^{137}Ba, ^{24}Mg, and ^{55}Mn were 0.06, 0.35, 0.66, and 0.75 µg/g, respectively; concentrations of these elements in all otoliths were well above detection limits. Otolith elemental concentrations were calculated from integrations over the final 10 s of laser ablation transects, as the outer portion of the otolith reflects a fish's most recent environmental history. Trace element concentrations were normalized to calcium (Ca) concentration based on the consideration of Ca as a pseudointernal standard (Bickford & Hannigan, 2005; Ludsin et al., 2006); data are reported as element:Ca ratios (mmol/mol).

Both univariate and multivariate approaches were used to assess differences in water and otolith trace element and stable isotopic signatures among sites and site types (floodplain lakes, Mississippi River tributaries on the Illinois side of the river, Mississippi River tributaries on the Missouri side of the river, the Upper Mississippi River, the Middle Mississippi River, and the lower Missouri River). One way analyses of variance (ANOVAs) followed by Tukey's HSD test for multiple comparisons were used to assess differences in individual water and otolith chemistry parameters among sites and site types. Individual otolith chemistry parameters that differed significantly among sampling locations were entered into a multivariate analysis of variance (MANOVA) and a canonical discriminant analysis (CANDISC procedure in SAS®) to characterize the multivariate otolith chemistry signatures of the Mississippi River, its floodplain lakes, tributaries on the Missouri and Illinois sides of the Mississippi River, and the lower Missouri River; a plot of the first two canonical variates was used to visually depict differences among site types. Pillai's trace statistic was used to assess significance of differences in multivariate otolith chemistry signatures among site types. 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 environment of capture (the Mississippi River, its floodplain lakes, tributaries on the Missouri and Illinois sides of the Mississippi River, or the lower Missouri River) using multivariate otolith chemistry signatures (Brazner et al., 2004). P-values ≤ 0.05 were considered significant for all analyses.

Results

Mean water $\delta^{18}O$ differed among sites (ANOVA, F=2.11, df=15,32, p=0.0376) and site types (ANOVA, F=4.15, df=5,42, p=0.0062) with floodplain lakes generally being more enriched in ^{18}O than the Missouri and Mississippi rivers (Fig. 2a). Water Sr:Ca exhibited differences among individual sites (ANOVA, F=13.17, df=15,32, p<0.0001, Fig. 2b) and site types (ANOVA, F=8.81, df=5,42, p<0.0001), with one floodplain lake (Grand Tower Chute) and the Missouri River having the highest mean water Sr:Ca values. Water Ba:Ca also differed significantly among individual sites (ANOVA, F=16.94, df=15,32, p<0.0001, Fig. 2c), with Grand Tower Chute and the Meramec River possessing the highest mean water Ba:Ca values and Middle Swan Lake, the Big Muddy River, and the Illinois River having the lowest mean water Ba:Ca values. Mean water Mn:Ca and Mg:Ca differed among individual sites (ANOVAs, F=2.96, df=15,32, p=0.006 and F=17.37, df=15,32, p<0.0001, respectively) but neither water Mn:Ca nor Mg:Ca differed among site types (ANOVAs, F=0.65, df=5,42, p=0.66 and F=0.66, df=5,42, p=0.66, respectively)

Mean otolith $\delta^{18}O$ values were significantly different among sites (ANOVA, F=27.21, df=13,128, p<0.0001) and site types (ANOVA, F=32.62, df=5,136, p<0.0001), with otoliths of fish from floodplain lakes enriched in ^{18}O compared to those of individuals collected from the Mississippi and Missouri rivers (Fig. 3a). Fish collected from smaller tributaries to the Mississippi River displayed intermediate otolith $\delta^{18}O$ values. Mean otolith $\delta^{13}C$ also exhibited differences among sites (F=52.88, df=13,128, p<0.0001) and site types (F=87.03, df=5,136, p<0.0001), with otoliths from fish collected in most floodplain lakes and the Missouri River being more enriched in ^{13}C than those from fish captured in the Mississippi River and its smaller tributaries (Fig. 3b).

Mean otolith Sr:Ca displayed significant differences among individual sites (ANOVA, $F=23.86$, $df=13,124$, $p<0.0001$), such as fish from Grand Tower Chute having a higher mean otolith Sr:Ca value than fish from all other floodplain lakes (Fig. 3c). Otolith Sr:Ca also exhibited significant differences among site types (ANOVA, $F=10.10$, $df=5,132$, $p<0.0001$) with fish from the Missouri River having higher otolith Sr:Ca compared to fish collected in the Mississippi River and smaller tributaries that enter the Mississippi River in Missouri. Fish from floodplain lakes and tributaries entering the Mississippi River in Illinois had intermediate otolith Sr:Ca values that varied among individual water bodies. Mean otolith Ba:Ca was significantly different among fish from individual sites (ANOVA, $F=9.00$, $df=13,124$, $p<0.0001$, Fig. 3d) and among site types (ANOVA, $F=7.93$, $df=5,132$, $p<0.0001$). Fish from one floodplain lake (Grand Tower Chute) had the highest mean otolith Ba:Ca value, while otolith Ba:Ca values were lowest for fish from the Big Muddy River and the Illinois River. Mean otolith Mg:Ca differed among sites (ANOVA, $F=7.90$, $df=13,124$, $p<0.0001$) and site types (ANOVA, $F=11.39$, $df=5,132$, $p<0.0001$). Mean otolith Mn:Ca differed among individual sites (ANOVA, $F=4.81$, $df=13,124$, $p<0.0001$), but did not differ among site types (ANOVA, $F=2.09$, $df=5,132$, $p=0.08$). Otolith Mg:Ca and Mn:Ca were not included in subsequent multivariate and discriminant function analyses because relationships between otolith and water Mg:Ca and Mn:Ca values for individual fish were non-significant (Mg:Ca: $r^2 = 0.0455$, $p = 0.3285$; Mn:Ca: $r^2 = 0.0334$, $p = 0.4037$). In contrast, otolith $\delta^{18}O$, Sr:Ca, and to a lesser degree, Ba:Ca of individual fish were correlated with corresponding water values for each of these markers ($\delta^{18}O$: $r^2 = 0.62$, $p<0.0001$; Sr:Ca: $r^2 = 0.73$, $p<0.0001$; Ba:Ca: $r^2 = 0.21$, $p<0.0001$).

Multivariate analysis of otolith data incorporating $\delta^{18}O$, $\delta^{13}C$, Sr:Ca, and Ba:Ca indicated that the Mississippi and Missouri Rivers, Mississippi River tributaries, and floodplain lakes possessed significantly different otolith chemistry signatures (Pillai's Trace Statistic=1.67, $F=14.52$, $df=16, 324$, $p<0.0001$). A plot of the first two canonical variates from the CANDISC procedure in SAS depicts distinct otolith chemistry signatures among site types (Fig. 4). The first discriminant function (CAN1) and second discriminant function (CAN2) from this model accounted for 61% and 28% of the total dispersion in the dataset, respectively. Otolith carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopic compositions, and Sr:Ca and Ba:Ca compositions were correlated with CAN1

(correlation coefficients of 0.99, 0.41, 0.31, and 0.26 respectively; $p<0.05$). Otolith $\delta^{18}O$ and Sr:Ca were also correlated with CAN2 (correlation coefficients of 0.72 and -0.43 respectively; $p<0.05$), however $\delta^{13}C$ and Ba:Ca were not associated with CAN2 ($p>0.10$). Thus, $\delta^{13}C$ and $\delta^{18}O$ appeared to be the most important markers in defining otolith chemical fingerprints in multivariate discriminant space; however, otolith Sr:Ca and, to a lesser extent, Ba:Ca also contributed to differences in multivariate otolith chemistry signatures among site types.

Linear discriminant function analysis of otolith chemistry data incorporating $\delta^{18}O$, $\delta^{13}C$, Sr:Ca, and Ba:Ca with a leave-one-out jackknife procedure indicated that fish could be classified back to their environment of capture (the Middle Mississippi River, Upper Mississippi River, floodplain lakes, tributaries on the Missouri and Illinois sides of the Mississippi River, or the Lower Missouri River) with 71-100 % accuracy (Table 1). Most classification errors occurred among fish collected from small tributaries of the Mississippi River, although some of these individuals were misidentified as having been collected in small tributaries on the opposite side of the Mississippi River (in Illinois or Missouri) from their location of capture. Two fish from the Upper Mississippi River were erroneously assigned to tributaries that enter the Mississippi River on its eastern side (tributaries that enter the Mississippi River from the state of the Illinois). All fish captured in the Lower Missouri and Middle Mississippi Rivers were correctly assigned to the environment in which they were collected. Neither the addition of otolith Mg:Ca nor otolith Mn:Ca data to the linear discriminant function analysis improved classification accuracy.

Discussion

Results indicated that fishes from the Mississippi River, floodplain lakes, and tributaries in both Illinois and Missouri, including the lower Missouri River, could be distinguished with a high degree of accuracy based on multivariate otolith chemical signatures. The naturally-occurring markers that best discriminated among environments and fish from the water bodies sampled in this study ($\delta^{18}O$, $\delta^{13}C$, Sr:Ca and Ba:Ca) have frequently been among the most useful indicators of fish origin in other geographic locations (Gao et al., 2001; Wells et al., 2003; Brazner et al., 2004; Bickford and Hannigan, 2005; Dufour et al., 2005; Whitledge et al., 2007; Whitledge, 2009).

Classification success rates for individual fish to environment of capture in this study were comparable to or greater than those of published studies that used microchemistry and stable isotopic composition as indicators of source location for fishes in freshwater (Bronte et al., 1996; Wells et al., 2003; Brazner et al., 2004; Clarke et al., 2007; Schaffler & Winkelman, 2008; Whitledge, 2009; Zeigler & Whitledge, 2010), marine (Campana et al., 1995), and estuarine (Thorrold et al., 1998; Gillanders & Kingsford, 2000) environments. Most classification errors occurred among fish collected from small tributaries of the Mississippi River, although some of these individuals were misidentified as having been collected in small tributaries on the opposite side of the Mississippi River (in Illinois or Missouri) from their location of capture. These classification errors are potentially less serious given that they are confined to a similar habitat type (small tributaries). Only one fish from a floodplain lake was inaccurately classified to a tributary of the Mississippi River in Illinois and no fish collected from the Middle Mississippi and Missouri Rivers were misclassified as having come from other environments, reflecting the distinct otolith chemistry signatures of the environments sampled during this study.

Differences in water $\delta^{18}O$ among rivers and streams sampled in this study can be attributed principally to regional and continental-scale variations in the $\delta^{18}O$ values of precipitation, with the Missouri and Mississippi Rivers having more negative water $\delta^{18}O$ values compared to local tributaries because these large rivers convey isotopically depleted waters from the distant upstream portions of their watersheds (Coplen & Kendall, 2000; Winston & Criss, 2003; Hoefs, 2004). Distinct water $\delta^{18}O$ signatures of floodplain lakes and the Mississippi River are likely due primarily to the greater opportunity for evaporative fractionation (Hoefs, 2004) to be expressed in floodplain lakes as a result of their longer water residence times compared to the river. Whitledge et al. (2007) observed similar differences in water hydrogen isotopic composition ($\delta^{2}H$, which undergoes similar fractionation processes in the hydrologic cycle) between floodplain ponds and the upper Colorado River due to higher evaporation rates of pond water. Persistent differences in water $\delta^{18}O$ between the Illinois River and its floodplain lakes have also been documented (Zeigler & Whitledge, 2010). Otolith $\delta^{18}O$ is influenced by water $\delta^{18}O$ and temperature (Kalish, 1991); geographic differences in water $\delta^{18}O$ can thus potentially lead to corresponding differences in otolith $\delta^{18}O$ that can be

used to identify natal origins of fishes (Dufour et al., 2005). Otolith $\delta^{13}C$ also differed between fish from the middle Mississippi River and its smaller tributaries and fish collected in floodplain lakes, with otolith $\delta^{13}C$ values for fish from floodplain lakes enriched in ^{13}C compared to fish collected in riverine environments; this pattern has previously been observed in the Illinois River and its floodplain lakes (Zeigler & Whitledge, 2010). Mechanisms responsible for observed differences in otolith $\delta^{13}C$ among environments sampled in this study are unknown. Otoliths incorporate both dissolved inorganic carbon (DIC) and metabolically-derived carbon (Kalish, 1991; Solomon et al., 2006). Observed differences in otolith $\delta^{13}C$ likely reflect differences in $\delta^{13}C$ of DIC between floodplain lake and riverine habitats. Although $\delta^{13}C$ of DIC was not measured, it is conceivable that floodplain lake DIC could potentially be enriched in ^{13}C compared to DIC in the Mississippi River due to higher rates of photosynthesis by aquatic primary producers in floodplain lakes (yielding higher photosynthesis:respiration ratios and enriched $\delta^{13}C$ values of DIC; Quay et al. 1986) or because the longer water residence time in floodplain lakes may enable equilibration of DIC with atmospheric CO_2 (Hoefs, 2004). Remineralization of particulate organic carbon in sediments is also a major source of DIC (Quay et al., 1986). Otolith $\delta^{13}C$ of fish collected in the Missouri River was enriched in ^{13}C compared to otolith $\delta^{13}C$ values of fish collected from the Mississippi River, likely reflecting differences in $\delta^{13}C$ of DIC of these two rivers (Fry & Allen, 2003). Higher $\delta^{13}C$ values of river DIC are associated with more mineral carbonate weathering and a relatively low contribution of isotopically light respired carbon (Hoefs, 2004). Differences in water and otolith Sr:Ca and Ba:Ca among locations sampled in this study are likely due primarily to variation in bedrock geology (Wells et al., 2003), such as occurs between tributaries on the Illinois and Missouri sides of the Mississippi River or between the Missouri and Upper Mississippi River basins.

Regardless of the mechanisms responsible for differences in water and otolith elemental and isotopic compositions among sampling locations, multi-parameter chemical "fingerprints" in otoliths established in this study will be useful for distinguishing among fishes from the Middle Mississippi River, its tributaries (including the Lower Missouri River), and floodplain lakes if observed differences in environmental signatures persist in future years. Several studies have reported interannual stability in

otolith isotopic and trace elemental signatures in some freshwater environments (Zimmerman & Reeves, 2002; Wells et al., 2003; Dufour et al., 2005; Munro et al., 2005; Ludsin et al., 2006; Whitledge et al., 2007; but see Schaffler & Winkelman, 2008). Mean water $\delta^{18}O$ values for the Middle Mississippi, Lower Missouri, Illinois, Big Muddy, and Meramec Rivers during this study were within the ranges of water $\delta^{18}O$ values reported for these rivers in prior studies (Frederickson & Criss, 1999; Coplen & Kendall, 2000; Winston & Criss, 2003), suggesting that $\delta^{18}O$ signatures of these rivers exhibit some stability across years. The Middle Mississippi River, its floodplain lakes and tributaries possessed distinct isotopic and elemental signatures that were reflected in fish otoliths despite some seasonal variation in water chemistry. Substantial inter-annual variation in environmental signatures may preclude the use of otolith chemistry for identifying natal origins of fishes during some years or may require that a "library" of environmental signatures be developed for multiple years and different classification models applied to fish from different year classes (Ludsin et al., 2006; Schaffler & Winkelman, 2008).

Otoliths from ten fish species (primarily centrarchids) were analyzed in this study, raising the possibility that inter-site differences in otolith chemistry may have been influenced by differences in relationships between water and otolith chemistry among species. However, differences in mean otolith $\delta^{18}O$, Sr:Ca, and Ba:Ca among collection sites incorporating data from individuals of all species reflected differences in water $\delta^{18}O$, Sr:Ca, and Ba:Ca among water bodies despite minor differences in species composition among collection sites. Additionally, Zeigler & Whitledge (2010) determined that there were no significant differences in relationships between water and otolith $\delta^{18}O$ and Sr:Ca signatures among the fish species collected in this study, consistent with the findings of some prior studies (Patterson et al., 1993; Whitledge et al., 2007). Differences in mean otolith $\delta^{13}C$ values between fishes from lake and riverine habitats were also consistent among species in the present study and in prior studies (Whitledge et al., 2007; Zeigler & Whitledge, 2010). Zeigler & Whitledge (2010) did find significant differences in relationships between water and otolith Ba:Ca among some of the fish species sampled in the present study; freshwater drum exhibited elevated otolith Ba:Ca values compared to several species of centrarchids. Species-specific incorporation of trace elements (Mg, Mn, Sr, and Ba) into otoliths has been noted in other studies (Swearer et al., 2003; Hamer & Jenkins, 2007). However, otolith Ba:Ca was the

least informative of the four natural chemical markers used in this study, as few significant differences in otolith Ba:Ca were present among our collection sites. Thus, available evidence suggests that distinct multivariate otolith chemical fingerprints for the Middle Mississippi River, its tributaries, and floodplain lakes were driven primarily by differences in elemental and isotopic compositions among water bodies and were not an artifact of differences in fish species composition among our collection sites.

The accuracy with which fish were classified back to their environment of capture using linear discriminant function analysis demonstrates the potential applicability of multivariate otolith chemical signatures as indicators of recruitment sources and environmental history of fishes in the Middle Mississippi River, its tributaries, and floodplain lakes. Otolith microchemistry or stable isotopic compositions have been successfully applied to distinguish fish of floodplain lake and riverine origin in other river systems (Crook & Gillanders, 2006; Whitledge et al., 2007; Zeigler and Whitledge, 2010). Estimating the relative contributions of floodplain lake, tributary, and riverine habitats to fish populations in the Middle Mississippi River appears feasible via analysis of naturally occurring chemical signatures in otoliths. Potential specific applications of otolith chemistry in the Middle Mississippi River may include identification of the principle recruitment sources for fish species of recreational and commercial interest (e.g., ictalurids, centrarchids, *Ictiobus* spp.), species of conservation concern (e.g., shovelnose sturgeon *Scaphirhynchus platorynchus*, paddlefish *Polyodon spathula*, blue sucker *Cycleptus elongatus*, and chubs *Macrhybopsis* spp.), and invasive species (e.g., bighead carp *Hypophthalmichthys nobilis* and silver carp *H. molitrix*). However, characterization of relationships between water and otolith chemical signatures for species not sampled in this study will be required. The ability to reconstruct environmental history of individual fish using naturally occurring isotopic markers in otoliths may also facilitate efforts to quantify nutrient and energy subsidies to the Illinois River provided by fishes that immigrate to the river from floodplain lakes or tributaries (Polis et al., 1997).

Acknowledgments

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Table 1. Results of linear discriminant function analysis (LDFA) showing classification accuracy (determined by jackknife procedure) for individual fish to environment of collection based on otolith $\delta^{18}O$, $\delta^{13}C$, Sr:Ca, and Ba:Ca. Only fish with data for all four otolith chemistry parameters were entered into the LDFA.

Source Location	Assigned Location						% Correct
	Floodplain Lakes	Illinois Tributaries	Missouri Tributaries	Upper Mississippi River	Middle Mississippi River	Missouri River	
Floodplain Lakes	16	1	0	0	0	0	94
Illinois Tributaries	0	22	5	4	0	0	71
Missouri Tributaries	0	2	22	2	1	0	82
Upper Mississippi River	0	2	0	10	0	0	83
Middle Mississippi River	0	0	0	0	11	0	100
Missouri River	0	0	0	0	0	10	100

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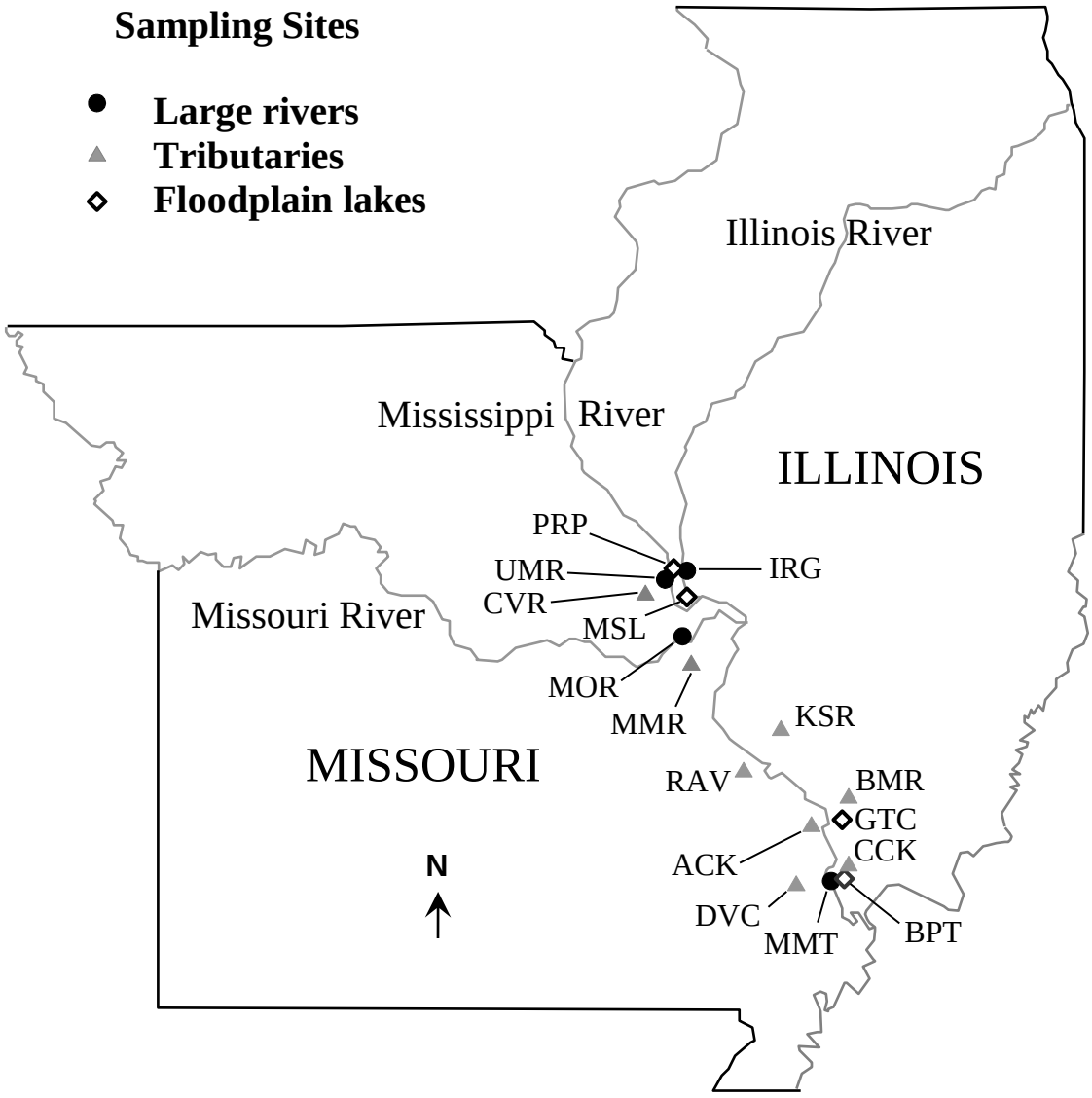
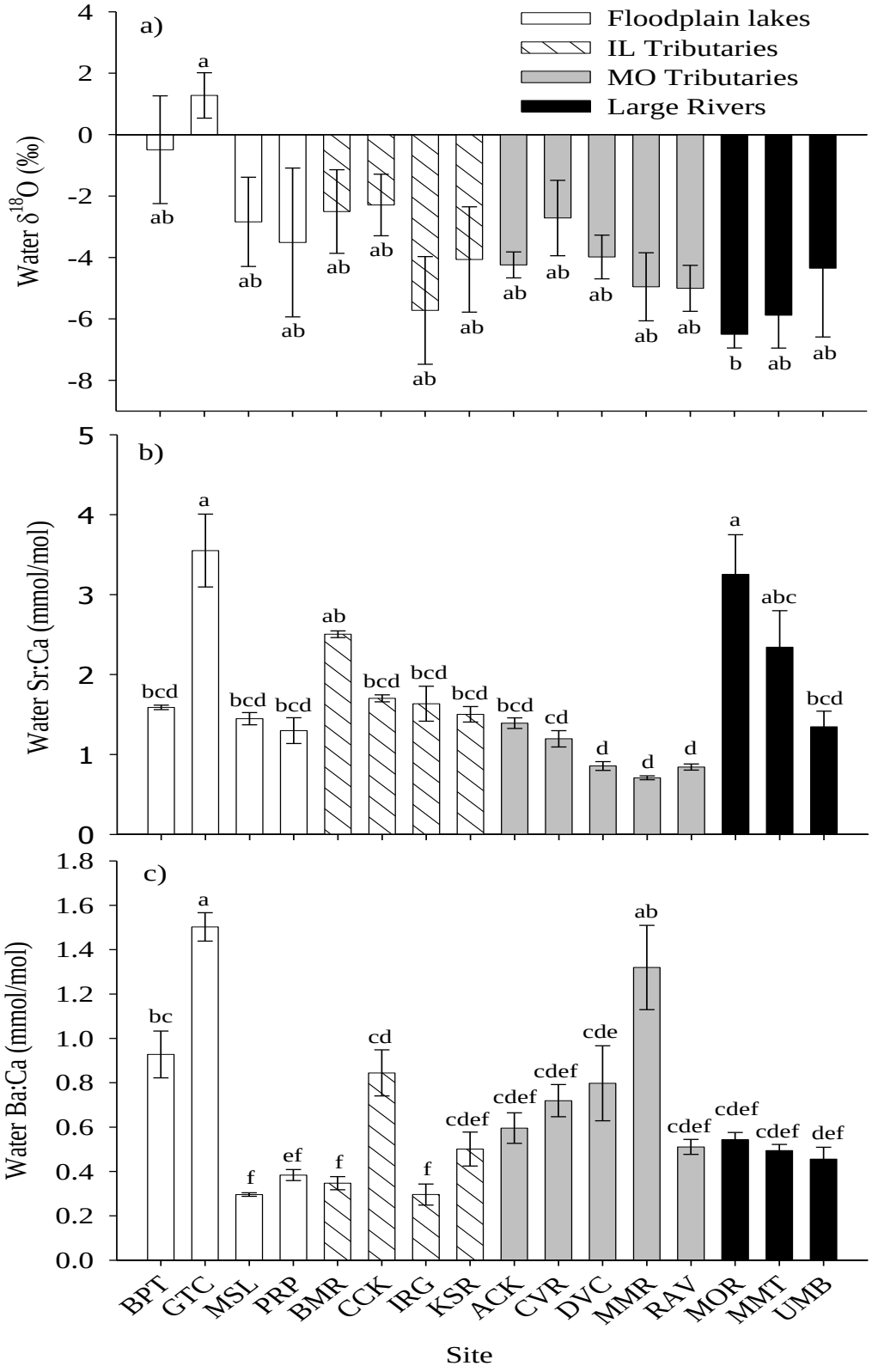
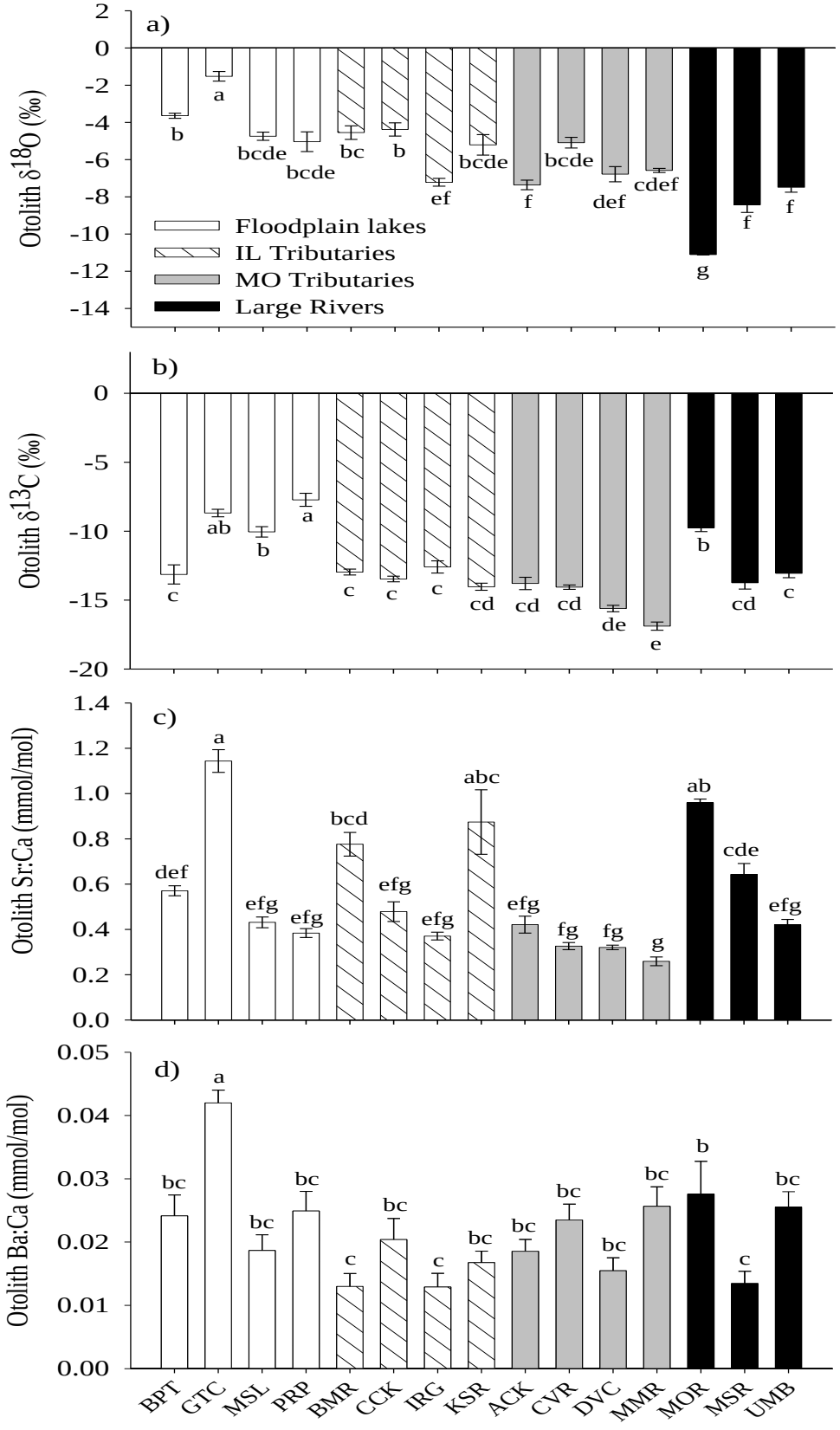
Fig. 4. Multivariate otolith chemical fingerprints for fish from the Mississippi River, its tributaries, and floodplain lakes based on the first two canonical variates obtained through linear discriminant function analysis including $\delta^{18}O$, $\delta^{13}C$, Sr:Ca, and Ba:Ca.


Sampling Sites
Large rivers
Tributaries
Floodplain lakes
Illinois River
Mississippi River
ILLINOIS
Missouri River
MISSOURI
PRP
UMR
IRG
CVR
MSL
MOR
MMR
KSR
RAV
BMR
GTC
CCK
ACK
DVC
MMT
BPT
N


a)
b)
c)
Floodplain lakes
IL Tributaries
MO Tributaries
Large Rivers
Water δ18O (‰)
Water Sr:Ca (mmol/mol)
Water Ba:Ca (mmol/mol)
BPT
GTC
MSL
PRP
BMR
CCK
IRG
KSR
ACK
CVR
DVC
MMR
RAV
MOR
MMT
UMB
Site


a)
Otolith δ18O (‰)
2
0
-2
-4
-6
-8
-10
-12
-14
b
a
bcde
bcde
bc
b
ef
bcde
f
bcde
def
cdef
g
f
f
Floodplain lakes
IL Tributaries
MO Tributaries
Large Rivers
b)
Otolith δ13C (‰)
0
-5
-10
-15
-20
c
ab
b
a
c
c
c
cd
cd
cd
de
e
b
cd
c
c)
Otolith Sr:Ca (mmol/mol)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
def
a
efg
efg
bcd
efg
efg
abc
efg
fg
fg
g
ab
cde
efg
d)
Otolith Ba:Ca (mmol/mol)
0.05
0.04
0.03
0.02
0.01
0.00
bc
a
bc
bc
c
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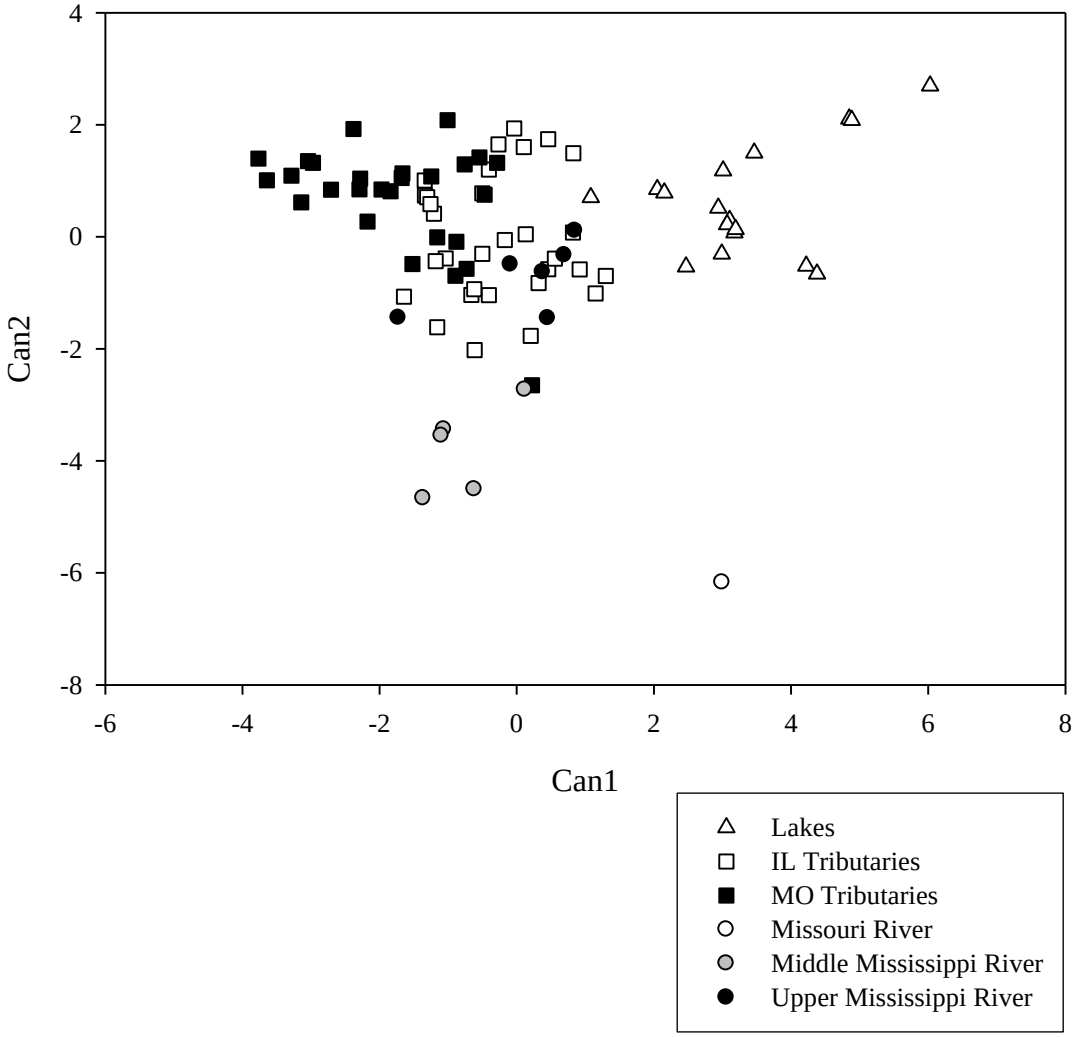
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Can2
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Lakes
IL Tributaries
MO Tributaries
Missouri River
Middle Mississippi River
Upper Mississippi River