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Assessment of Otolith Chemistry for Identifying Source Environment of Fishes in the Lower Illinois River, Illinois.

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Abstract

Knowledge of habitats used by fish throughout their life history is important for management and conservation of riverine fish populations and habitats. Naturally occurring chemical markers in otoliths have recently been used to determine natal origins and environmental history of fishes in a variety of marine and freshwater environments. However, to our knowledge no studies have examined the applicability of this technique in large floodplain rivers in the U.S.A. We evaluated otolith microchemistry and stable isotopic composition as tools for determining origins of fishes in the lower Illinois River, its tributaries, and floodplain lakes. Fishes were collected from eight sites during summer 2006 and two additional sites in spring 2007. Water samples were obtained from these 10 sites plus one additional tributary during summer and fall 2006 and spring 2007. Otolith and water samples were analyzed for $\delta^{18}O$ and a suite of trace elements; otoliths were also analyzed for $\delta^{13}C$. Tributaries, floodplain lakes, and the Illinois River possessed distinct isotopic and elemental signatures, principally driven by differences in $\delta^{18}O$ and $\delta^{13}C$ among floodplain lakes, the Illinois River, and tributary streams. Otoliths reflected differences in water chemistry among habitats. Relationships between water and otolith $\delta^{18}O$ and Sr:Ca were not significantly different among species, but some differences in relationships between water and otolith Ba:Ca among species were detected. Linear discriminant function analysis with a leave-one-out jackknife procedure on otolith $\delta^{18}O$ and $\delta^{13}C$ indicated that fish may be classified back to environment (Illinois River, tributary, or floodplain lake) of capture with 80-98% accuracy. Otolith microchemistry and stable isotope analyses provide a potentially effective means for determining recruitment sources and environmental history of fishes in the Illinois River. 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 emigrate from floodplain lakes or tributaries.

Introduction

Knowledge of habitats used by riverine fishes throughout their life history is important for management and conservation of lotic fish populations and the habitats upon which they depend (Schlosser, 1991; Fausch et al., 2002). Many fishes in large, regulated rivers are thought to depend on connectivity between the river channel and floodplain lakes to access off-channel habitats for spawning and larval nursery (Bayley and Li, 1992; Gozlan et al., 1998; Nunn et al., 2007). Tributary streams may also contribute to fish assemblages in large rivers, at least near their confluences (Brown and Coon, 1994; Robinson et al., 1998; Kiffney et al., 2006). Primary and secondary channels within the river itself represent another potentially important recruitment source and larval nursery habitat for some riverine fishes (Humphries et al., 1999; Scheimer et al., 2001; Dettmers et al., 2001; Keckeis and Scheimer, 2002). Despite evidence that any of these habitats (floodplain lakes, tributaries, and river channel) may contribute substantially to reproduction and recruitment in riverine fish populations, their relative importance as natal environments for fishes in large rivers has not been quantified. Uncertainty regarding the relative importance of source habitats for fishes in large, regulated rivers indicates a need for new techniques to determine environmental history of individual fishes in these ecosystems.

Like many other large navigable rivers, the Illinois River, which flows from the Chicago, IL area southwesterly to its confluence with the Mississippi River near St. Louis, MO, historically had much interaction with its floodplain (Starrett, 1971). However, levee construction and channelization during the past century separated the Illinois River from almost half of its floodplain (Starrett, 1971). Many lakes that historically had at least some connectivity to the Illinois River now exhibit little to no connectivity with the river; many of these floodplain lakes have subsequently experienced high sedimentation rates (Starrett, 1971). The Illinois River was one of the most productive commercial fisheries in the early twentieth century (Pegg and McClelland, 2004). However, due in part to anthropogenic habitat modifications, Illinois River fish populations were greatly reduced (Starrett, 1971; Pegg and McClelland, 2004). Some restoration of connectivity between the Illinois River and floodplain lakes has been conducted in select locations (Reuter et al., 2005; Schultz et al., 2007), reducing

floodplain lake sedimentation and providing fishes with access to these habitats for spawning and larval nursery (Csoboth and Garvey, 2008). However, the extent to which fishes produced in these restored floodplain lakes contribute to Illinois River fish populations is unknown. Restoration of river-floodplain lake connectivity may benefit both native and exotic fishes; connected floodplain lakes may be important source habitats for invasive Asian carps (bighead carp *Hypophthalmichthys nobilis* and silver carp *H. molitrix*) in the Illinois River (Pegg et al., 2002). However, Asian carps also spawn in the river's main channel (DeGrandchamp et al., 2007). Knowledge of the relative importance of the river channel and floodplain lakes to early life stages of Asian carps would be potentially valuable for developing strategies to control abundance of these exotic species that may be negatively affecting condition of native planktivorous fishes (Irons et al., 2007).

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 and Winkelman, 2008). Concentrations and stable isotopic compositions of some chemical elements in otoliths 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 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). Crook and Gillanders (2006) demonstrated the applicability of otolith microchemistry for identifying common carp recruitment sources in the Murray River, Australia. Stable isotopic composition of otoliths may also be useful for determining natal origin of fishes in freshwater environments and may distinguish fishes from locations that cannot be differentiated using otolith microchemistry (Dufour et al.,

2005; Whitledge et al., 2007). However, the applicability of otolith stable isotopic signatures as natural markers of fish environmental history in large river-floodplain ecosystems has not been assessed. Additionally, no studies have evaluated the applicability of otolith chemistry for identifying recruitment sources of fishes in the large, regulated rivers of the Midwestern United States.

The goal of this study was to determine whether otolith microchemistry and stable isotopic analyses may be useful tools for determining environmental history of fishes in the Illinois River, Illinois U.S.A. Specific objectives were to determine if water trace elemental and stable oxygen isotopic compositions differed among floodplain lakes, tributaries, and the Illinois River, to determine whether fish otolith microchemistry and isotopic compositions reflected those of environments (floodplain lakes, tributaries, and the Illinois River) in which they were captured, and to determine the accuracy with which individual fish could be reclassified to their collection locations based on otolith elemental and stable isotopic compositions. We also assessed whether relationships between water and otolith chemistry differed among fish species collected. Few studies have compared water-otolith chemistry relationships among species (Swearer et al., 2003; Hamer and Jenkins, 2007; Whitledge et al., 2007).

Methods

Fish and water samples were collected from 11 sites along the lower Illinois River (downstream from Peoria, IL; Fig. 1). These sites included two locations within the Illinois River channel (near Liverpool, Illinois, and Glades, Illinois), three tributaries of the lower Illinois River, and six backwater and floodplain lakes. Floodplain lakes included representatives with permanent and intermittent (during flooding) connections to the Illinois River and varied in the type of connection to the river (natural channel, ditch, or water control structure).

Two water samples were collected from each of the 11 sites during summer 2006 and again during both fall 2006 and spring 2007 to assess seasonal changes in stable isotopic and elemental compositions. One sample was collected during each season from each site for stable oxygen isotope analysis; a second water sample was collected seasonally from each site for analysis of a suite of twenty major, minor, and trace

elements (including Ca, Sr, Ba, Mg, and Mn). Water samples for stable oxygen isotope analysis were collected and stored in scintillation vials containing minimal air space and sealed with Parafilm to curtail evaporative loss and fractionation (Kendall and Caldwell, 1998). Water samples were analyzed for stable oxygen isotopic composition using a high-temperature conversion elemental analyzer (TC/EA) interfaced with a Thermo Finnigan Delta Plus XL® isotope ratio mass spectrometer. All stable isotope ratios were expressed in standard δ notation, defined as the parts per thousand deviation between the isotope ratio 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).

Fishes were collected from 10 sites during summer 2006 and spring 2007. 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*, and black crappie *Pomoxis nigromaculatus*) were collected where possible due to their recreational importance and widespread availability; temperate basses (yellow bass *Morone mississippiensis* and white bass *M. chrysops*), and freshwater drum (*Aplodinotus grunniens*) were also collected from several sites. Fishes were captured by alternating current (AC) and direct current (DC) electrofishing and trap netting at sites where a boat could be launched. At sites without boat access, fishes were captured by seining or 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. One otolith from each fish was analyzed for stable oxygen and carbon isotopic composition. Otoliths < 1 mg were pulverized whole with a mortar and pestle; 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 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 trace element (Sr:Ca, Ba:Ca, Mg:Ca, Mn:Ca) analysis. 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 and Hannigan, 2005; Ludsin et al., 2006); data are reported as element:Ca ratios (mmol/mol).

Least-squares linear regressions were used to characterize relationships between mean water and otolith signatures for each elemental or stable isotopic marker. ANCOVAs with Tukey's adjustment were applied to determine whether significant differences in otolith $\delta^{18}O$, Sr:Ca and Ba:Ca were present among species; mean water $\delta^{18}O$, Sr:Ca and Ba:Ca signatures from fish collection sites were used as covariates. Only species represented by ≥ 10 individuals that were collected from floodplain lake, tributary, and riverine collection sites were included in ANCOVAs. To increase sample sizes for comparisons of otolith chemistry signatures among species, otolith and water $\delta^{18}O$, Sr:Ca and Ba:Ca data from the nearby middle Mississippi River and upper Illinois River drainages were included in ANCOVAs (Whitledge, 2009; Zeigler, 2009).

Both univariate and multivariate approaches were used to assess differences in water and otolith trace element and stable isotopic signatures among individual sites and site types (floodplain lakes, tributaries, and the Illinois 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 in conjunction with inter-site differences in water chemistry were entered into a multivariate analysis of variance (MANOVA) and a discriminant analysis (CANDISC procedure in SAS®) to characterize the multivariate otolith chemistry signatures of the Illinois River, tributaries, and floodplain lakes; 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 the Illinois River channel, tributaries, and floodplain lakes. 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 (Illinois River, tributary, or floodplain

lake) based on their otolith chemical signatures. A p-value of ≤0.05 was considered significant for all statistical tests.

Results

Mean water $\delta^{18}O$ signatures were significantly different among site types (ANOVA, F=4.53, df=2, 29, p=0.022), with floodplain lake waters enriched in ^{18}O compared to the Illinois River (Fig. 2a). Tributary streams had an intermediate water $\delta^{18}O$ signature that was not significantly different from that of floodplain lakes or the Illinois River (Fig. 2a). Mean water Sr:Ca values did not differ among site types (ANOVA, F=1.69, df=2, 29, p=0.21), but differed at the individual site level (ANOVA, F=2.73 , df=10, 21, p=0.0251). One floodplain lake (Spring Lake, Tazewell County, Illinois) had significantly lower mean water Sr:Ca than all other sites, but no other sites differed among one another in water Sr:Ca. Mean water Ba:Ca values differed among some individual sites (ANOVA, F=3.09 df=10, 21, p=0.0142) and among site types (ANOVA, F=17.31 df=2, 29, p<0.0001). Tributaries had higher water Ba:Ca values than floodplain lakes and the Illinois River, but mean water Ba:Ca did not differ between the Illlinois River and its floodplain lakes (Fig.3). Mean water Mg:Ca and Mn:Ca both exhibited significant differences among sites (ANOVA, F=2.45 df=10, 21, p=0.0402 and ANOVA, F=5.09 df=10, 21, p=0.0008, respectively), and mean water Mn:Ca differed among site types (ANOVA, F=5.70 df=2, 29, p=0.0082). However, we did not consider these two markers further due to non-significant relationships between otolith and water Mg:Ca and Mn:Ca values (Mg:Ca: r^2=0.0455, p=0.3285; Mn:Ca: r^2=0.0334, p=0.4037)

Otolith $\delta^{18}O$ values were strongly correlated with water $\delta^{18}O$ values (r^2=0.7084, p<0.0001; Fig. 4). Mean otolith $\delta^{18}O$ values were significantly different among site types (ANOVA, F=188.56 df=2, 88, p<0.0001), with otoliths of fishes from floodplain lakes enriched in ^{18}O compared to otoliths from fishes collected in the Illinois River and tributaries (Fig. 2b). Mean otolith $\delta^{13}C$ values also displayed differences among site types (ANOVA, F=44.45 df=2, 90, p<0.0001), with otoliths from individuals collected in floodplain lakes enriched in ^{13}C compared to otoliths of fishes collected from the Illinois River and tributaries (Fig. 5).

Both mean Sr:Ca values (Fig. 6) and mean Ba:Ca values (Fig. 7) in otoliths reflected mean water Sr:Ca and Ba:Ca (r^2=0.9247, p<0.0001 and r^2=0.6178, p<0.0001, respectively) signatures. No significant differences in mean otolith Sr:Ca were detected among site types (ANOVA, F=1.17, df=2, 89, p=0.32), although mean otolith Sr:Ca values differed at the individual site level (ANOVA, F=12.92, df=9,82, p<0.0001). Mean otolith Sr:Ca was significantly lower for one floodplain lake (Spring Lake) compared to all other sampling sites, consistent with differences in water Sr:Ca among sites. However, mean otolith Sr:Ca was not significantly different among fish from any other individual sites. Mean otolith Ba:Ca values did not differ among site types (ANOVA, F=1.83, df=2, 89, p=0.17), although mean otolith Ba:Ca dffered among some individual sampling sites (ANOVA, F=4.28, df=9, 82, p=0.0001).

Relationships between water and otolith $\delta^{18}O$ and Sr:Ca were not significantly different among species (p>0.05) for all pair-wise comparisons. However, relationships between water and otolith Ba:Ca differed among some species. Mean freshwater drum Ba:Ca was significantly different (p<0.05) from all other fish species except spotted bass (p=0.6616). Mean Ba:Ca for green sunfish was significantly different from that of bluegill (p=0.0019) and orangespotted sunfish (p=0.0357); mean Ba:Ca for largemouth bass was significantly different from that of bluegill (p=0.0011), orangespotted sunfish (p=0.0081), and yellow bass (p=0.0294).

Multivariate analysis of otolith data incorporating $\delta^{18}O$, $\delta^{13}C$, Sr:Ca, and Ba:Ca indicated that fishes from the Illinois River, its tributaries, and floodplain lakes possessed significantly different otolith chemistry signatures (Pillai's Trace Statistic: F=13.99 df=8,138, p<0.0001). A plot of the first two canonical variates from the CANDISC procedure in SAS illustrated the distinct otolith chemistry signatures among site types (Fig. 8). The first discriminant function (CAN1) from this model accounted for 99% of the total dispersion in the dataset. Otolith oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) isotopic compositions were correlated with CAN1 (correlation coefficients of 0.97 and 0.73, respectively; p<0.05), whereas otolith Sr:Ca was not associated with CAN1 (p>0.1), but was correlated with the second canonical variate (correlation coefficient of 0.89; p<0.05). Otolith Ba:Ca was not associated with CAN1 or CAN2 (p>0.05). Thus, $\delta^{18}O$ and $\delta^{13}C$ were the most important markers in defining otolith chemical fingerprints in multivariate

discriminant space. Linear discriminant function analysis of otolith data incorporating $\delta^{18}O$ and $\delta^{13}C$ with a leave-one-out jackknife procedure indicated that individual fish could be classified back to their environment of capture (Illinois River, tributary, or floodplain lake) with 80-98 % accuracy (Table 1). Neither the addition of otolith Sr:Ca nor otolith Ba:Ca data to the linear discriminant function analysis improved classification accuracy.

Discussion

Results indicated that fishes from the lower Illinois River, adjacent floodplain lakes, and tributary streams could be distinguished with a high degree of accuracy based on otolith $\delta^{18}O$ and $\delta^{13}C$. Fishes from a few individual sites also possessed distinct otolith Sr:Ca and Ba:Ca signatures. The naturally-occurring markers that best discriminated among fishes from the water bodies sampled in this study ($\delta^{18}O$ and $\delta^{13}C$, and to a lesser extent Sr:Ca and Ba:Ca) have frequently been among the most useful indicators of fish environmental history 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 (Illinois River, tributary, or floodplain lake) in this study were comparable to or greater than those of published studies using otolith 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 and Winkelman, 2008; Whitledge, 2009), marine (Campana et al., 1995), and estuarine (Thorrold et al., 1998; Gillanders and Kingsford, 2000) environments. Most misclassifications in this study occurred among fishes from the Illinois River and tributaries; only one individual collected in a floodplain lake was incorrectly classified as having come from the Illinois River, reflecting the distinct $\delta^{18}O$ and $\delta^{13}C$ signatures of floodplain lake and lotic environments in this study. Some misclassifications of fish to environment of capture may have been due to the presence of recent immigrants (Whitledge, 2009).

Otolith isotopic and elemental compositions were strongly correlated with corresponding water values and reflected differences in water $\delta^{18}O$, Sr:Ca, and Ba:Ca

among the Illinois River, its tributaries, and floodplain lakes and among individual sampling locations. Significant correlations between water and otolith signatures for these three naturally occurring chemical markers are consistent with results of prior studies (Patterson et al., 1993; Wells et al., 2003; Walther and Thorrold, 2008). Observed differences in water and otolith $\delta^{18}O$ between the Illinois River and its floodplain lakes 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 relative to the Illinois River. Whitledge et al. (2007) found similar differences in water δD (which undergoes similar fractionation processes) between floodplain ponds and the upper Colorado River due to higher evaporation rates of pond water. Otolith $\delta^{13}C$ also distinguished fishes from the Illinois River and its floodplain lakes, with otolith $\delta^{13}C$ values for individuals collected in floodplain lakes enriched in ^{13}C compared to fishes collected from riverine environments. 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; $\delta^{13}C$ of DIC in the Illinois River and tributaries is unknown, but is likely influenced by isotopically light respired carbon (Hoefs, 2004) from upstream municipal wastewater (e.g., from the Chicago metropolitan area) and other sources. Floodplain lake DIC might also be enriched in ^{13}C compared to that of the Illinois river due to higher rates of photosynthesis by aquatic primary producers or because the longer water residence time in floodplain lakes may enable equilibration of DIC with atmospheric CO_2 (Hoefs, 2004). Mechanisms responsible for the few observed differences in water and otolith Sr:Ca and Ba:Ca among locations sampled in this study are also unclear. Spring Lake's significantly lower Sr:Ca signature is likely due to its unique water source (groundwater from the Mahomet Aquifer) compared to other floodplain lakes and tributary streams sampled. Other observed differences in Ba:Ca signatures among sampling locations are likely due to local variation in bedrock geology (Wells et al., 2003).

Regardless of the mechanisms responsible for differences in water and otolith elemental and isotopic compositions among our sampling locations, multi-parameter

chemical "fingerprints" in otoliths established in this study will be useful for distinguishing among fishes from the lower Illinois River, its tributaries, and floodplain lakes if observed differences in environmental signatures persist in future years. Other studies have reported interannual stability in otolith isotopic and trace elemental signatures in some freshwater environments (Zimmerman and 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 and Winkelman, 2008). Mean water $\delta^{18}O$ values for the Illinois River (-5.9‰ ± 1.0 ‰ SE) and tributaries (-4.3‰ ± 0.7 ‰ SE) during this study was within the ranges of $\delta^{18}O$ values reported by Coplen and Kendall (2000) for the Illinois River and other streams in Illinois and Missouri, respectively during November 1984-August 1987; these results suggest that the Illinois River and tributary $\delta^{18}O$ signatures are relatively stable across years. Summer 2006 water $\delta^{18}O$ values for the Illinois River were enriched in ^{18}O compared to $\delta^{18}O$ values reported by Coplen and Kendall (2000), perhaps due to the relatively warm, dry summer during 2006. However, floodplain lakes and the Illinois River possessed distinct $\delta^{18}O$ signatures that were reflected in fish otoliths despite some seasonal variation in water $\delta^{18}O$. Floodplain lakes differed among one another in their connectivity with the Illinois River, but all exhibited a distinct multivariate, isotopic signature that enabled highly accurate identification of individual fish captured in floodplain lakes based on otolith $\delta^{18}O$ and $\delta^{13}C$. Major floods would likely eliminate distinctions between the Illinois River and its floodplain lakes, although the effect of a short-duration flood (particularly one that occurs outside of the growing season) on otolith chemical signatures may be minimal. Substantial inter-annual variation in environmental signatures may preclude the use of otolith chemistry for distinguishing among fish of floodplain lake, tributary, and riverine origin during some years or may require a "library" of environmental signatures and separate classification models for assigning natal origin to fish from different year classes (Ludsin et al., 2006; Schaffler and Winkelman, 2008).

We found 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 $\delta^{18}O$, $\delta^{13}C$, and Sr:Ca among site types were consistent

among species. Most of the fish species collected in this study were centrarchids, which may account for the lack of significant differences in relationships between water and otolith $\delta^{18}O$ and Sr:Ca among species. In contrast, species-specific incorporation of trace elements (Mg, Mn, Sr, and Ba) into otoliths has been noted in other studies (Swearer et al., 2003; Hamer and Jenkins, 2007). Additional research should assess differences in relationships between otolith and environmental stable isotopic and microchemical signatures among fish species. While our findings suggest that models for determining environmental history of a particular fish species may sometimes be applicable to closely related species, water-otolith chemistry relationships should not be applied broadly across taxa without verification of their consistency among species.

The accuracy with which we were able to classify fish back to their environment of capture (Illinois River, tributary or floodplain lake) demonstrates the potential applicability of otolith oxygen and carbon isotopic compositions for determining recruitment sources and environmental history of fishes in the lower Illinois River drainage. Otolith microchemistry or stable isotopic composition have been successfully applied to distinguish fish of floodplain lake and riverine origin in other river systems (Crook and Gillanders, 2006; Whitledge et al., 2007). Estimating the relative contributions of floodplain lake, tributary, and riverine habitats to fish populations in the lower Illinois River appears feasible via analysis of naturally occurring chemical signatures in otoliths. Otolith stable isotopic signatures may be useful for identifying recruitment sources of both native fishes and exotic species such as bighead and silver carp; however, characterization of relationships between water and otolith chemical signatures for these species would 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).

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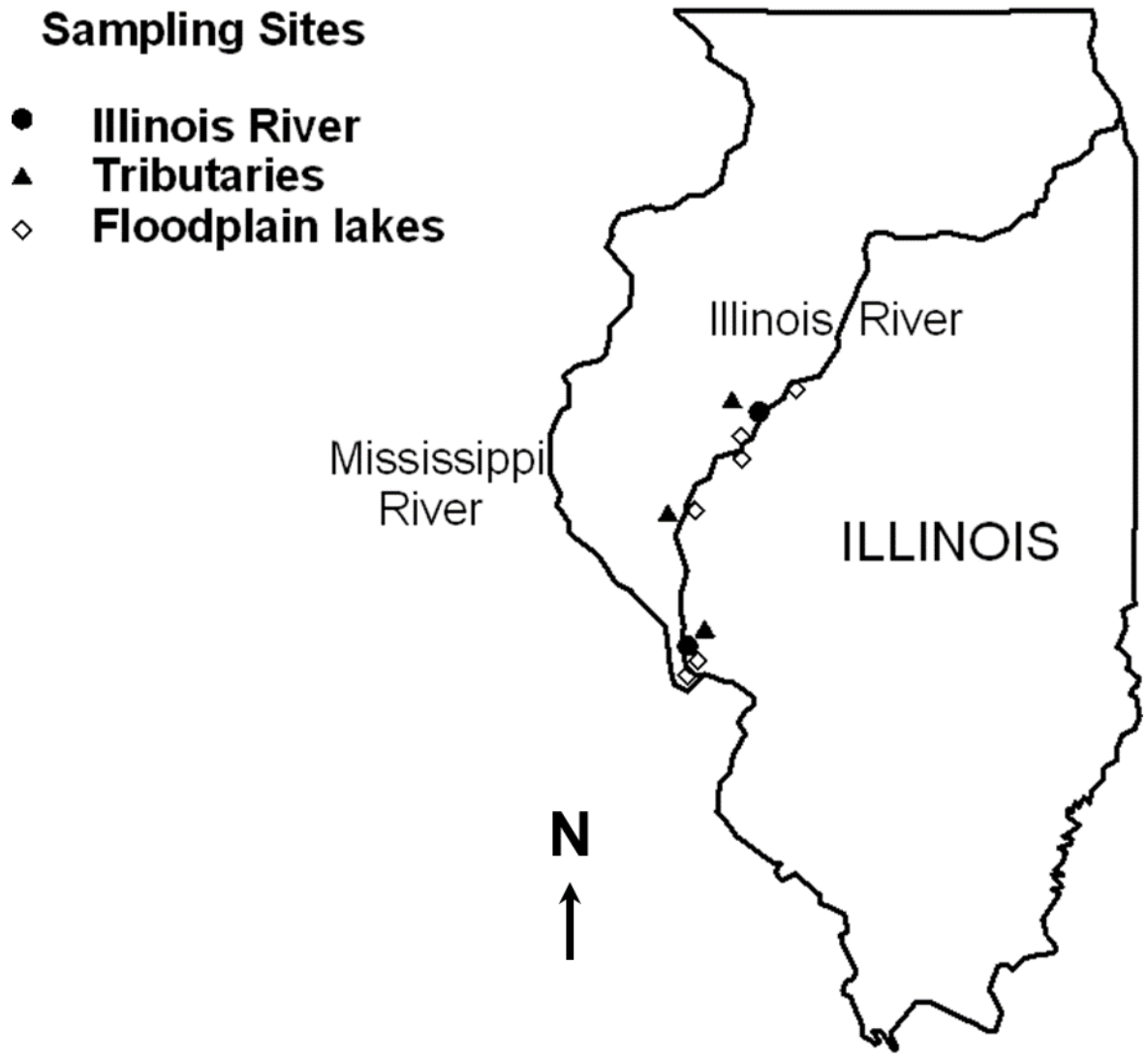
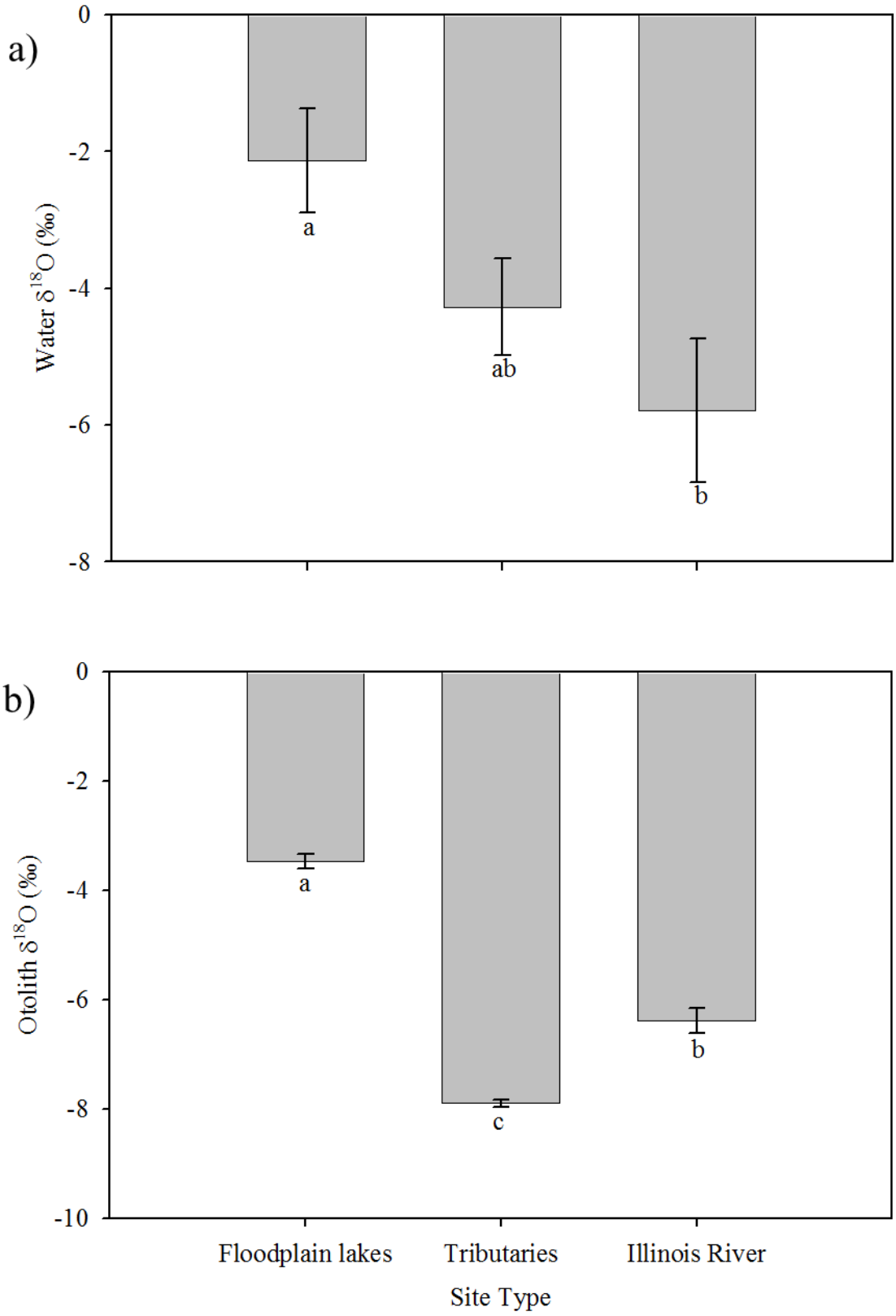
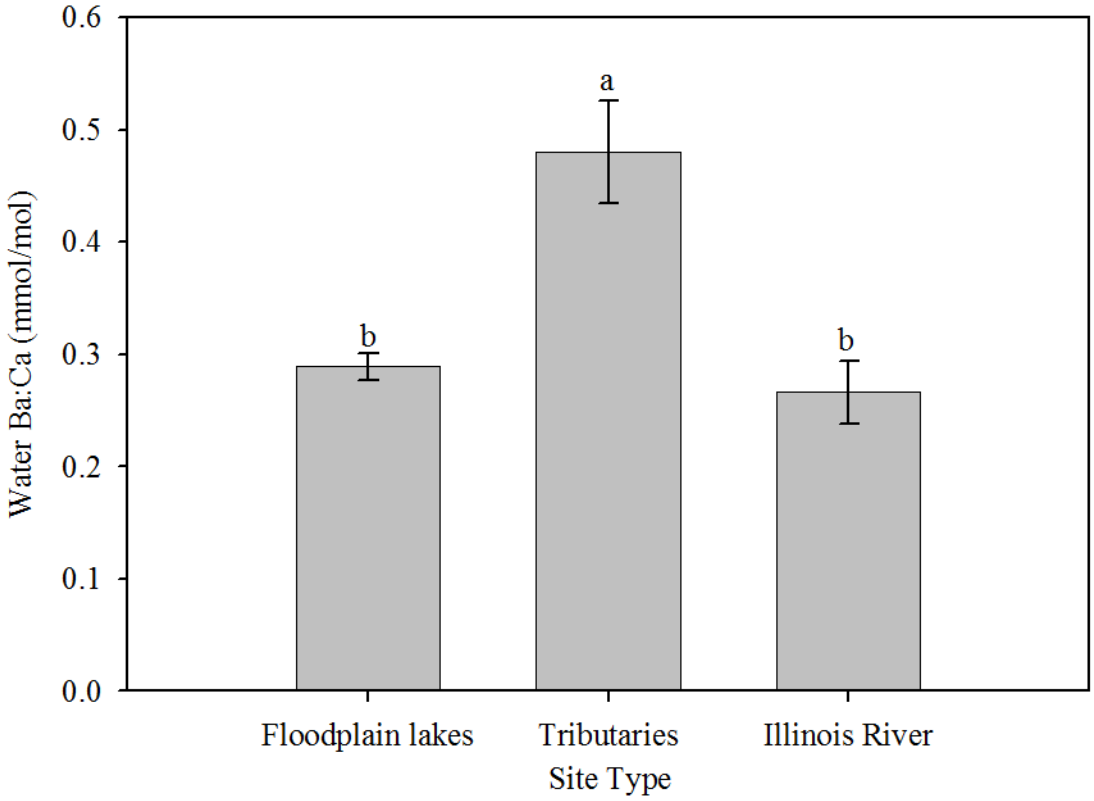
Table 1. Results of linear discriminant function analysis showing classification accuracy (determined by jackknife procedure) for individual fish to environment of collection based on otolith $\delta^{18}O$ and $\delta^{13}C$.

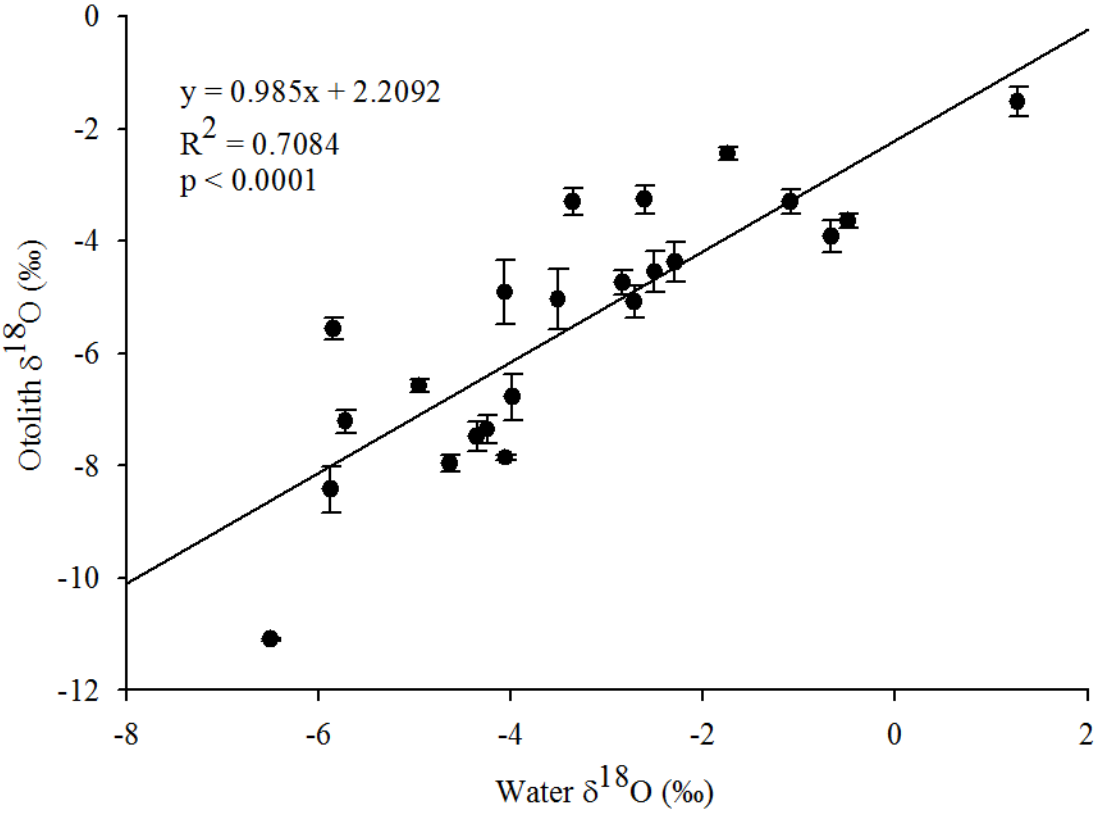
	Assigned Location			
Source Location	Floodplain lakes	Tributaries	Illinois River	% Correct
Floodplain lakes	53	0	1	98
Tributaries	0	16	1	94
Illinois River	0	4	16	80

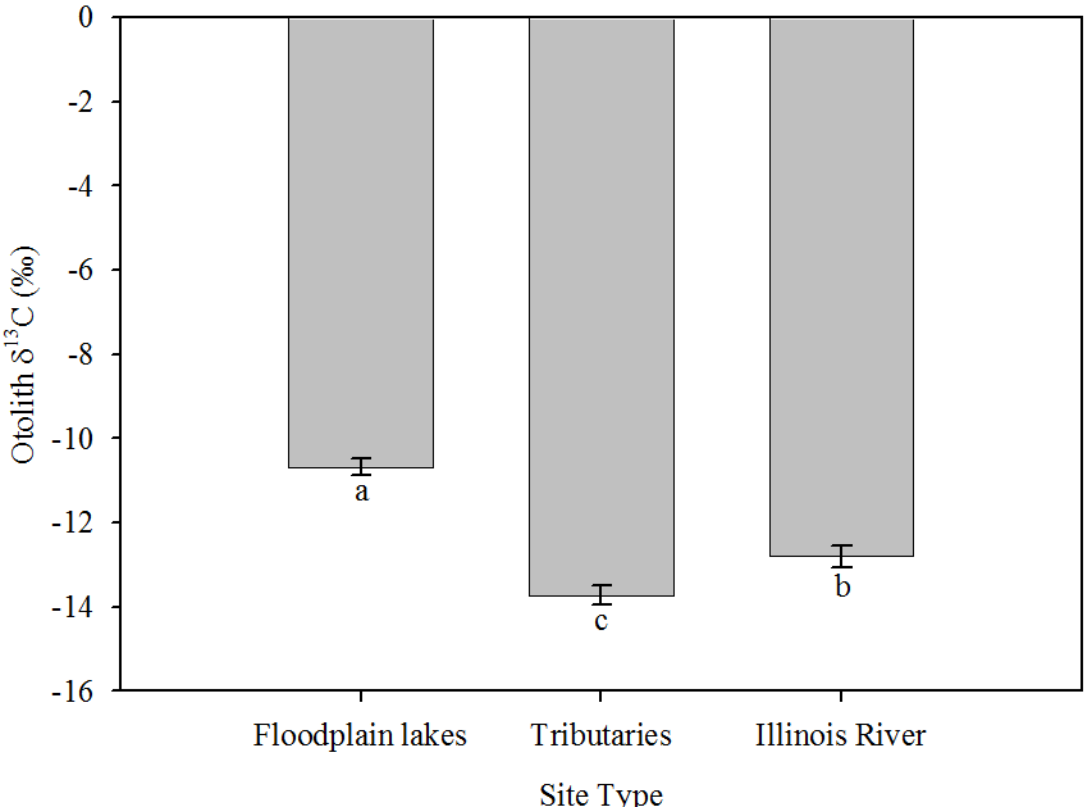
List of Figures

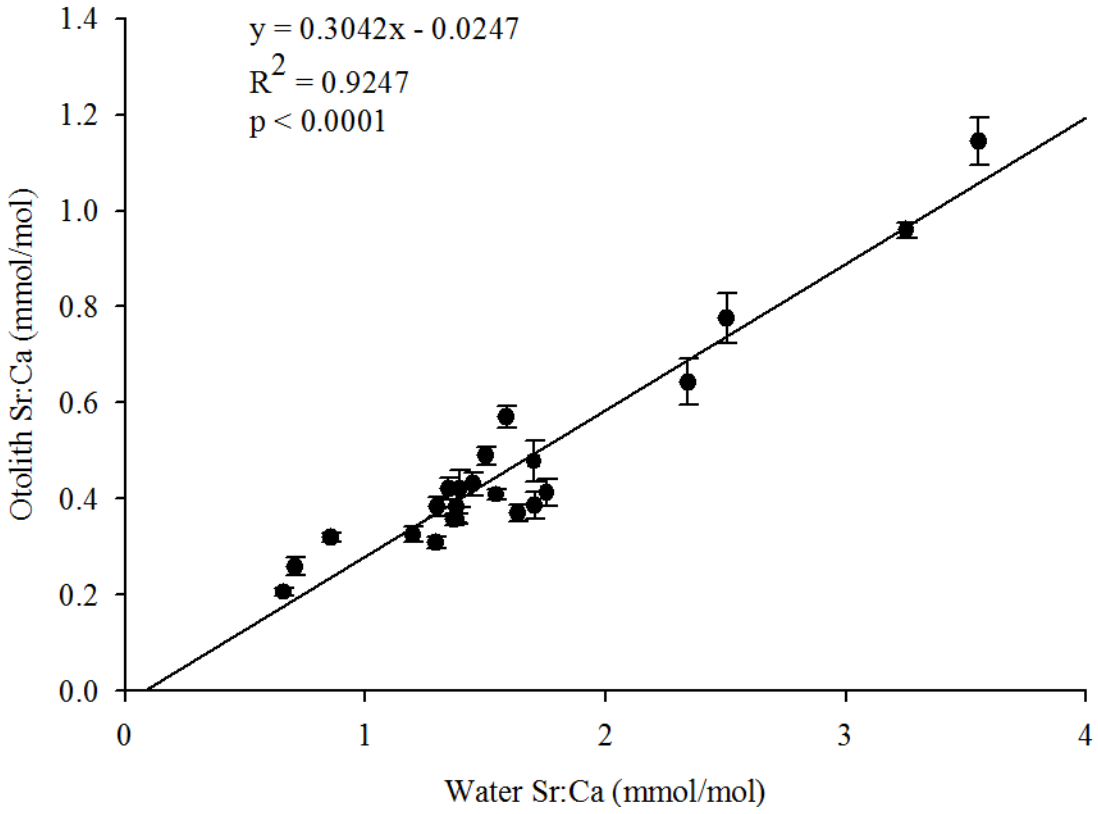

Sampling Sites
Illinois River
Tributaries
Floodplain lakes
Illinois River
Mississippi River
ILLINOIS
N

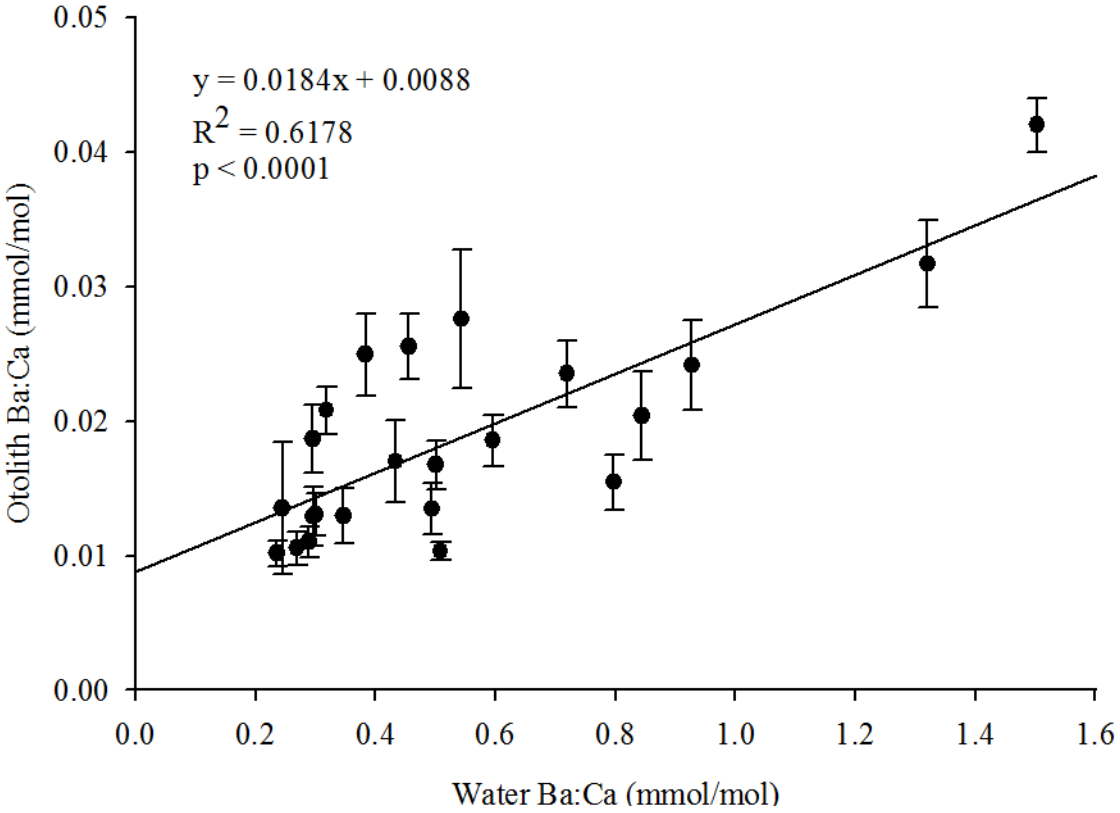

a)
0
-2
-4
-6
-8
Water δ18O (‰)
a
ab
b
b)
0
-2
-4
-6
-8
-10
Otolith δ18O (‰)
a
c
b
Floodplain lakes
Tributaries
Illinois River
Site Type

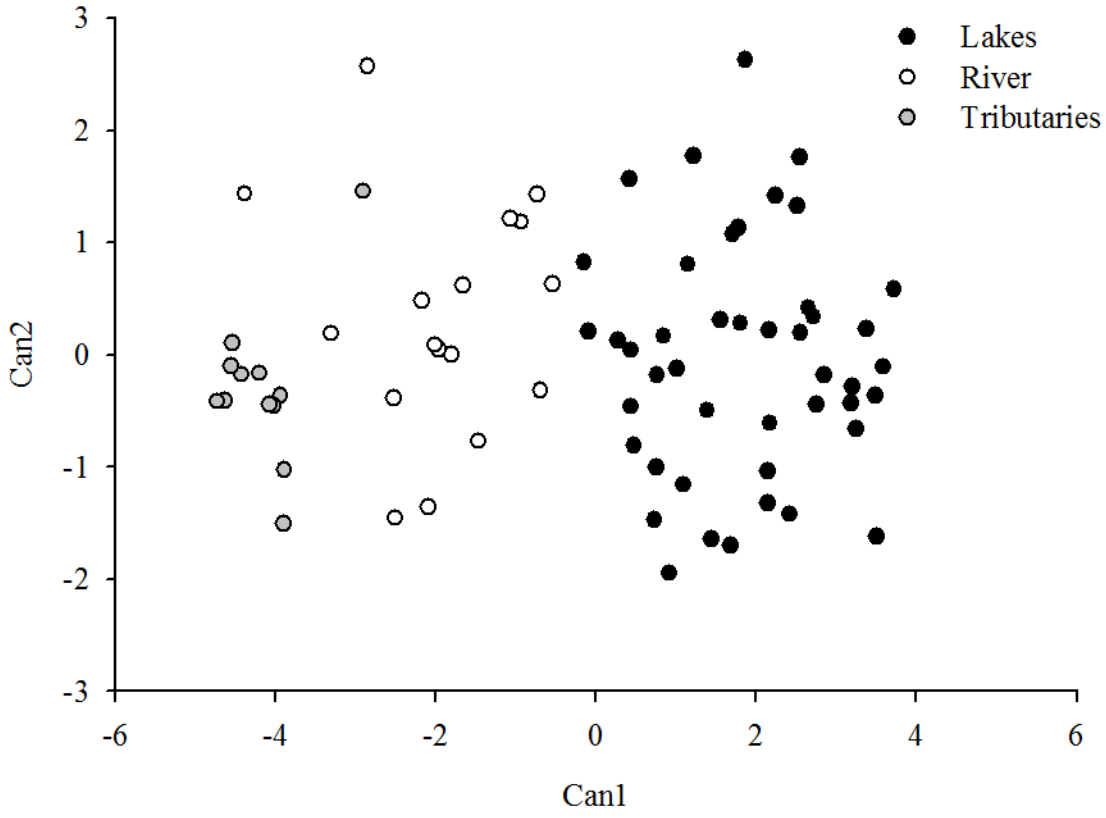

0.6
0.5
0.4
0.3
0.2
0.1
0.0
Water Ba:Ca (mmol/mol)
a
b
b
Floodplain lakes
Tributaries
Illinois River
Site Type


y = 0.985x + 2.2092
R2 = 0.7084
p < 0.0001
Otolith δ18O (‰)
Water δ18O (‰)
0
-2
-4
-6
-8
-10
-12
-8
-6
-4
-2
0
2


0
-2
-4
-6
-8
-10
-12
-14
-16
Otolith δ13C (‰)
a
c
b
Floodplain lakes
Tributaries
Illinois River
Site Type


y = 0.3042x - 0.0247
R2 = 0.9247
p < 0.0001
Otolith Sr:Ca (mmol/mol)
Water Sr:Ca (mmol/mol)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
1
2
3
4


y = 0.0184x + 0.0088
R2 = 0.6178
p < 0.0001
Otolith Ba:Ca (mmol/mol)
Water Ba:Ca (mmol/mol)
0.00
0.01
0.02
0.03
0.04
0.05
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6


Lakes
River
Tributaries
Can2
Can1
3
2
1
0
-1
-2
-3
-6
-4
-2
0
2
4
6