5-1-2013

IDENTIFYING ENVIRONMENT OF ORIGIN OF ILLINOIS RIVER ASIAN CARP VIA OTOLITH MICROCHEMISTRY AND STABLE ISOTOPE ANALYSES

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by

Jacob D. Norman

B.S., University of Missouri Columbia, 2008

A Thesis
Submitted in Partial Fulfillment of the Requirements for the
Master of Science Degree

Department of Zoology
in the Graduate School
Southern Illinois University Carbondale

May 2013

THESIS APPROVAL

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Approved by:

Dr. Gregory W. Whitledge,

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Southern Illinois University Carbondale
22 March 2013

AN ABSTRACT OF THE THESIS OF

JACOB D. NORMAN, for the Master of Science degree in ZOOLOGY, presented on 22 March 2013 at Southern Illinois University Carbondale

TITLE: IDENTIFYING ENVIRONMENT OF ORIGIN OF ILLINOIS RIVER ASIAN CARP VIA OTOLITH MICROCHEMISTRY AND STABLE ISOTOPE ANALYSIS

MAJOR PROFESSOR: Gregory W. Whitledge

Asian carp have rapidly expanded their range through much of the Mississippi River Drainage over the past 10 to 15 years. Silver and bighead carp are now the dominant fish species present along several reaches of the Illinois River. The upper Illinois River and shipping canals entering Lake Michigan are of great concern as pathways for Asian carp to enter the Great Lakes. Knowledge of reproductive habitats and dispersal pathways for these species may be valuable for ongoing and future efforts to control these exotic invasives. Previous studies have successfully identified spawning areas of native riverine species via otolith microchemistry, but this technique has not yet been applied to Asian carps. Both stable isotope and trace element ratios have been found to differ significantly among the large rivers of the Mississippi River drainage, enabling identification of natal environment for individual fish. The primary objective of this study was to identify differences in natal river origin and floodplain habitat use through the incorporation of trace elements (Sr:Ca) and stable isotopes ($\delta^{18}O$ and $\delta^{13}C$). Silver and bighead carp were collected via electrofishing and trammel netting along four reaches of the Illinois River from the Mississippi-Illinois River confluence at Grafton, IL to the upper segment of the Illinois River upstream of Starved Rock State Park. Sagittal otoliths were removed from both silver and bighead carp collected from each of the four reaches of the Illinois River for analysis of stable isotope ratios and trace element concentrations. Water samples were collected seasonally from the four reaches of the Illinois River and several of its associated floodplain lakes in addition to the Missouri, Upper Mississippi and Middle Mississippi Rivers to validate water signatures of the various river reaches. Results indicated the majority of adult Asian carp caught in the Illinois River originated from the Illinois. However, there was strong evidence indicating roughly twenty percent of captured adults were in fact immigrants from other sources; primarily the Middle Mississippi river and, to a lesser extent, the Missouri River. Stable isotope results indicated that Asian carps primarily used river channel rather than floodplain lake habitats during early life. The findings of this study suggest current Asian carp removal efforts should continue to be primarily directed within the Illinois River, however, the evidence of immigrant silver carp indicate expanding the control efforts into other rivers (Middle Mississippi River and Missouri River) will further support the control of Asian carp within the Illinois River.

ACKNOLWEDGEMENTS

I would like to extend my sincere thanks to my advisor Dr. Gregory Whitledge, for his continued guidance and support throughout my education at Southern Illinois University. I would also like to thank Dr. James Garvey and Dr. Matt Whiles for serving on my graduate committee and providing support in conducting and writing my thesis research. I would also like to thank the SIUC Fisheries and Illinois Aquaculture Center, SIUC Office of Research Development and the Illinois Department of Natural Resources for funding this research. Special thanks goes to David Dettman (Environmental Isotope Laboratory, Department of Geosciences, University of Arizona), Eric Freeburg and Robyn Hannigan (University of Massachusetts-Boston), Tim Howe and Norma Haubenstock (Alaska Stable Isotope Facility, University of Alaska-Fairbanks), Alan Shiller (Center for Trace Analysis, University of Southern Mississippi) and Mihai Lefticariu (Southern Illinois Mass Spectrometry Facility), for microchemistry and stable isotope analyses of otoliths and water samples. A special thanks goes to Dave Glover, Wes Bouska, Neil Rude, along with the rest of the faculty, staff, and students of the SIUC Fisheries and Illinois Aquaculture Center for all of their help and support. Finally I would like to thank my family and friends for their continued support and patience throughout my time spent on this research.

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INTRODUCTION

Non-native and invasive species present a growing problem in the U.S., accounting for changes in ecosystems, reduction in biodiversity and creating negative economic impacts to several enterprises including agriculture, forestry, fisheries and international trade (Lovell and Stone 2006). This issue has been widely acknowledged and documented for several decades (Simberloff 1996), accounting for an economic deficit of billions of dollars annually (Pimentel et al. 2000). According to Wilcove et al. (1998), the imperiled status of 400 of the 958 threatened or endangered species federally listed in the U.S. is attributed directly to competition or predation by invasive species.

Fisheries have felt an enormous negative influence from the introduction of non-native species. Over 100 fish species are federally listed as threatened or endangered in the U.S. (Tyus and Saunders 2000). During the 20th century, 30 fish species became extinct with invasives being at least partly to blame in 80% of those extinctions (Miller et al. 1989). The first documented introduced fish species described by DeKay (1842), was the goldfish brought over from Europe shortly after the seventeenth century. Today over 500 non-native fish taxa have been documented throughout the United States' inland waters, with 38% of these taxa established and reproducing (Nico and Fuller 1999). This has lead to a series of restrictions, policies and programs aimed to control the spread of non-natives and circumvent future introductions. The most notable regulations include Executive Order 11987, the Lacey Act, the Nonindigenous Aquatic Nuisance Prevention and Control Act, and the National Invasive Species Act (Nico and Fuller 1999).

Fish introductions can occur via several pathways, both accidental and intentional, including bait bucket releases, ornamental and or aquarium species being let go, intentional stocking to manage aquatic vegetation or to improve a current sport fishery, and by the emptying of ballast water (Li and Moyle 1993; Sampson et al. 2009). Once introduced, the success of introduced species is often attributed to their r-selected life history traits such as short generation times, rapid growth rates, outstanding dispersal and broad environmental tolerance (Lodge 1993). These ideal traits often allow introduced species to reach extremely high population densities in a short time frame (Lodge 1993; Williamson 1996) and out-compete the native aquatic fauna for food and habitat resources.

One particular group of invasive fish that is currently drawing lots of attention is bighead (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*). Collectively, these two species are often referred to as Asian carp. Their native waters comprise most of Eastern Asia, occurring in China, Vietnam, eastern Russia, Siberia and North Korea, where Asian carp account for over 60% of the total catch of fishes (Huang et al. 2001). Asian carp were first introduced into the U.S. in the early 1970s when they were brought into Arkansas to help control plankton blooms and associated water quality problems in aquaculture ponds (Henderson 1976). Flooding events during the early 1980s allowed some of these fish to escape from the aquaculture facilities and soon after they were observed in public waters of Arkansas (Freeze and Henderson 1982). Asian carp were identified in the Missouri River (MOR) by 1982 (Pflieger 1997), the Middle Mississippi River (MMSR) in 1983 (Burr et al. 1996), and the Illinois River (ILR) by the early 1990s (Irons et al. 2007). Currently bighead and silver carp have been identified in 23 and 16 states, respectively (Fuller 1999; Kolar et al. 2005).

Adult Asian carp are easy to identify, being described as deep-bodied and laterally compressed with a distinguishing ventral keel (Kolar et al. 2005). Silver carp can be distinguished from bighead carp by the ventral keel continuing past the pelvic fins to the throat. Juvenile Asian carp (15-150 cm total length) often resemble young shad but can be identified by the presence of a lateral line not visible on shad (Kolar et al. 2005). In their native waters Asian carp reach maturity by age three, with a life span of up to eight years (Kamilov 1985). However, Williamson and Garvey (2005) found Asian carp in the MMSR to reach maturity at age one with a maximum life span of only five years. This is possibly attributed to the high growth rates exhibited by young Asian carp in the MMSR plus the high density of juvenile fish found in the population. The age at which Asian carp reach maturity has been found to be strongly correlated with their first year growth rates (Kamilov 1987).

Due to their high reproductive success, Asian carp populations are rapidly expanding across the Mississippi River basin. In some cases such as the LaGrange reach of the ILR, Asian carp are now the dominant fish species (Irons et al. 2007). Densities of Asian carp sampled between 1990 and 2006 in the LaGrange reach increased linearly, while total biomass increased exponentially (Irons et al. 2007). This in turn has greatly affected species diversity and preferable fish catches by commercial fisherman (Sugunan 1997; Petr 2002). Both silver and bighead carp consume copious amounts of phytoplankton and zooplankton, a critical food component for many young of the year (YOY) native fish species and other filter feeders. Asian carp are excellent filter feeders capable of shifting the zooplankton community balance and disproportionately diminishing plankton (Burke et al. 1986; Xie and Yang 2000; Lu et al. 2002). The profuse consumption of plankton and zooplankton by Asian carp may potentially negatively affect the condition and health of other native species with similar diets. Besides affecting the native fish communities, Asian carp also present direct problems to the public that use the Illinois and connected rivers for recreation. Silver carp are excellent jumpers capable of leaping several feet out of the water. This poses a serious threat to boaters and their equipment as the turbulence caused by boat engines often brings silver carp sailing through the air striking windshields, boat equipment and boaters themselves. The rapid expansion of Asian carp in the Mississippi river drainage has driven researchers to better understand the reproductive and dispersal habits of these invasive species.

Asian carp reproduction in the Mississippi River watershed has been researched extensively. Bighead and silver carp require similar conditions for successful reproduction which in turn dictates establishment and dispersal potential. Asian carp eggs are bathypelagic, requiring ample flow to stay adrift in the water column and avoid settling to the bottom, becoming covered in sediments (Soin and Sukhanova 1972; Pflieger 1997; Schrank 2001). River lengths of over 100 km are typically required for suitable drift and successful spawning (Gorbach and Krykhtin 1980). In their native Asian waters, spawning peaks in late May with ideal water temperatures ranging from 22-26^{o} C (Jennings 1988). Similar reproductive habits appear to be present in U.S. waters. Studies have determined reproductive requirements and preferences in most of the Mississippi River drainage (Kolar et al. 2005) including the MOR (Schrank et al. 2001), ILR (Irons et al. 2007) and the Upper Mississippi River (UMSR) and MMSR (DeGrandchamp et al. 2007). The two most influential factors prompting spawning movements of Asian carp are suitable water temperatures and increase in flow.

While water temperatures and velocities both trigger spawning, the influence and contribution of both is unclear. For instance, Asian carp larvae were not observed in the MOR until both stable temperatures above 22^{o} C and a substantial increase in flow occurred simultaneously (Schrank 2001). DeGrandchamp et al. (2007) found similar results in the ILR during 2004-2005, however flow was found to be a more pertinent variable. Typical flood pulses occurred during 2004 and most female carp sampled resembled those of spawned-out fish, indicating that the spawning run had already occurred. In contrast, 2005 was a drought year with minimal spring flood pulses, resulting in a majority of the females sampled displaying signs of egg reabsorption. Studies conducted by Lohmeyer and Garvey (2008) on the MMSR and UMSR (pools 20, 22, 24 and 26) produced similar results. In 2005, larvae were only found in the MMSR and the downstream portion of pool 26. Ninety-nine point nine percent of larvae sampled in 2006 were found in the MMSR and pool 26. The MMSR is an open system, with any change in discharge being congruent throughout the upper and lower stretches while the UMSR is fragmented by locks and dams regulating flow among each pool. A strong flood pulse occurred in the spring of 2007, causing the locks and dams to remain fully open for 52 days and creating an open river environment with suitable discharge. During 2007, larval densities of 14.6 fish/m^3 were found in the upper pools sampled along the UMSR. These results clearly indicate that increased discharge during the typical spawning period is required for successful recruitment. While the exact driving forces of carp reproduction are still unsure it can be said that the knowledge of interactions between water temperature, discharge and recruitment is an intricate part in the understanding of Asian carp ecology (Schrank 2001).

Besides initiating spawning, annual floods also provide the required nursery habitats for juvenile Asian carp. Carp reproduction has been observed around a multitude of river habitats including behind gravel bars, sand bars, islands, dikes and at the mouths of tributaries with flows ranging from 0.3-3.0 m/s (Huet 1970; Jennings 1988; Kolar 2005). Asian carp have been observed spawning in the main channel of the ILR (Irons personal communication). Larvae hatch within 24 hours and begin to drift downstream (Etnier and Starnes 1993). Within 3-4 days larvae are capable of free swimming and exogenously feeding in the water column (Soin and Sukhanova 1972; Chapman 2006). By day 7 post-hatch the larvae begin to move in-shore and seek refuge in inundated floodplains and connected backwaters (Jennings 1988). While this is believed to be the normal pattern of early life habitat use by Asian carp, recently hatched larvae have been observed in unconnected stagnant backwaters near the ILR (DeGrandchamp 2007). Whether these fish were spawned in this unconnected backwater or drifted in when large flooding events temporarily inundated the floodplain is unknown. Better understanding of floodplain connectivity and Asian carp movements in and out of connected lakes during annual flood events will be essential for further insight into carp establishment.

Adult Asian carp migrations have frequently been researched and documented. Individual adults have been observed traveling over 462 river km during the spawning season (DeGrandchamp 2008), similar to those of native river species such as paddlefish (*Polyodon spathula*) and pallid sturgeon (*Scaphirhynchus albus*). Daily movements have also been studied via mobile and stationary telemetry to better understand seasonal fish movements and habitat preferences. Water velocity and depth appear to be the two dominant factors influencing habitat selection (Kolar et al. 2005; DeGrandchamp 2008). Studies on both the MOR and lower ILR found Asian carp preferred water at least 3m deep and flows less than 0.3 m/s. Main channel and side channel borders were found to be the preferred habitats, with slightly under half of all tagged fish in the lower ILR occupying these areas (DeGrandchamp 2008) and close to 80 % in the MOR (Kolar et al. 2005). Aside from annual floods, adult Asian carp have been found to avoid the main channel and typically shallow backwaters as well. Several reasons are believed to account for these avoidances, including the high presence of boat and barge traffic in the main channel and poor oxygen levels coupled with higher temperatures in backwaters (DeGrandchamp 2008). The overall habitat selection of silver and bighead carp appears to be similar except for overwintering periods. Bighead carp move toward the larger tributaries while silver carp remain in the larger rivers in low flow pooled areas (Kolar et al. 2005).

Every year millions of new larvae are hatched out in the Mississippi River drainage. While adults have dispersed immensely throughout the drainage, the presence of larvae appears to be found almost exclusively along the unimpounded sections of the MSR, MOR and ILR (Lohmeyer 2007; DeGrandchamp 2007; Schrank 2001). This can likely be attributed to the long, continuous river stretches required by Asian carp eggs to hatch into larvae. This raises the question as to how each river is contributing to the overall Asian carp stock in the MSR drainage.

The ability of Asian carps to rapidly disperse and expand their ranges in the Mississippi River basin has led to concerns about these species invading the Great Lakes via the artificial Chicago Area Waterways that link the ILR drainage with Lake Michigan. The invasion into Lake Michigan could potentially alter the sensitive trophic structure present in the Great Lakes, posing threats to the economically important recreational and commercial fisheries in the Great Lakes. Large densities of Asian carp within Lake Michigan would also present the same dangers to recreational boaters already present within the ILR. The potential for movement of Asian carp from the ILR drainage and into Lake Michigan has been reduced via installation of 3 electrical barriers (2 operational, 1 currently being constructed) in the Chicago Sanitary and Ship Canal. However, the potential for Asian carp immigration into the Great Lakes is becoming more of a reality as two bighead carp have been found past the electric barriers in the past four years, including one fish in Lake Calumet, a direct unimpeded passage to Lake Michigan.

The present concern of Asian carp immigrating into Lake Michigan through the upper ILR has brought about additional control efforts to supplement the electric barriers. An enhanced commercial harvest has been implemented, intended to fish down the Asian carp population within the ILR, reducing overall carp abundance and impacts on the ILR system while also reducing the chances of fish circumventing the barriers. More direct control efforts have been conducted directly in the shipping canals to remove all Asian carp near the electric barriers; i.e. rotenone treatment removed the vast majority of fish from the shipping canal, followed by intense surveillance of the canal for new Asian carp immigrants post treatment. The ongoing commercial harvest is currently directed only within the ILR. The potential for Asian carp originating in other connected rivers to immigrate into the ILR raises questions towards the contribution of other environments supplying recruits to the ILR Asian carp stock. Also the relative importance of floodplain lakes serving as nursery areas to young ILR origin carp that ultimately recruit to the adult stock is also unknown. A means of tracking Asian carp movement, and determining life histories, will be required to fully understand the importance of all environments supporting Asian carp and help direct future control strategies.

Following animal movements can be achieved by two basic means: 1. Follow individuals directly via visible markers and transmitters, 2. the use of natural biological tags (Rubenstein and Hobson 2004). Traditional tagging or telemetry methods limit the number of fish in the study as combined capture and re-capture efforts are time consuming and are almost impossible to apply to early life stages of fish. The use of natural tags may be a more a feasible method as every individual already possesses a “mark” indicative of environments that an individual animal has previously occupied (Hobson 2008).

Both trace elements and stable isotopes have emerged as powerful means for determining migrations and natal origins, often capable of reconstructing environmental histories from adult fish (e.g., Wells et al. 2003; Brazner et al. 2004; Whitledge et al. 2007; Zeigler and Whitledge 2010). Identifying fish populations and stocks via otolith microchemistry has experienced rapid growth as an effective tracking tool over the past decade (Campana and Gagne 1995; Severin et al. 1995; Campana et al. 2000). The elemental fingerprints stored in fish otoliths have proven to be a powerful tool of fish stock identification (Kalish 1990; Campana and Gagne 1995; Campana et al. 1994). The use of otoliths for reconstructing fish environmental history relies on two main properties of these structures: 1) otolith growth is continuous throughout the lifespan of a fish and once otolith material is deposited, it is not reabsorbed or altered (Campana and Neilson 1985) and 2) Otoliths contain a record of fish age and growth. Elemental and stable isotopic signatures within otoliths can be related to locations of annuli to identify past environments occupied by an individual fish, including natal environments (Radtke et al. 1990; Kalish 1993; Gillanders 1996).

Thirty one elements have been identified in fish otoliths (Campana 1999). However, only a select few elements and stable isotopes have been found to produce dependably distinct signatures among rivers or river-floodplain habitats. Stable oxygen and carbon isotope ratios ($\delta^{18}O$ and $\delta^{13}C$) have both been used to identify source environment fish not only in the Mississippi and Illinois Rivers (Whitledge 2009; Zeigler and Whitledge 2010; Zeigler and Whitledge 2011), but are among the most commonly used isotopic signatures for reconstructing fish environmental history (e.g. Thorrold et al. 1997; Campana et al. 2000; Dufour et al. 2005; Durbec 2010; Schloesser et al. 2010). Zeigler and Whitledge (2010) characterized significantly different water $\delta^{18}O$ signatures between the floodplain lakes and main channel of the ILR, classifying native species back to the environment in which they were captured with 98% accuracy. Stable oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) isotopic ratios consistently exhibit heavier (more enriched = less negative) values in floodplain lakes compared to the main channel. The enriched values of water $\delta^{18}O$ found in floodplain lakes can be attributed to longer water residence times (Hoefs 2004) which results in greater evaporation of the lighter oxygen isotope (^{16}O), commonly referred to as evaporative fractionation. Observed differences in otolith $\delta^{13}C$ between fish that reside in rivers and floodplain lakes likely reflect differences in $\delta^{13}C$ of dissolved inorganic carbon (DIC) between floodplain lake and riverine habitats. $\delta^{13}C$ of DIC in the Illinois River 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). Sr:Ca analysis has also routinely been used to reclassify fish back to natal habitats (e.g. Bath et al. 2000; Wells et al. 2003; Zeigler and Whitledge 2010). Strontium concentrations in the MOR are higher than any of the other major rivers in the Mississippi River basin (Zeigler and Whitledge 2011), primarily due to the bedrock geology of the MOR basin (Wells et al. 2003). The MMSR (section of the Mississippi River between St. Louis and Cairo, IL), influenced by the MOR, can be expected to exhibit the next highest Sr:Ca values. Water Sr:Ca values in the ILR have consistently been lower than in the MOR and MMSR (Zeigler and Whitledge 2011).

The use of trace elements and stable isotopes may be an effective tool in determining both the primary recruitment sources (ILR, MOR, MSR) and the critical habitats for early life stages for Asian carp. The rehabilitated lakes along the lower ILR could potentially provide the ideal nursery habitat needed for young Asian carp. Stable isotope analysis could therefore be used to document the use of floodplain lakes by young carp. The purpose of this study was to develop an environment-origin (MOR, MS rivers and ILR including floodplain lakes) reclassification system for ILR Asian carp via otolith microchemistry and stable isotope analysis. The information obtained from this study will greatly enhance our knowledge of Asian carp origins and dispersal habits and will highlight environments and river reaches that are primarily responsible for contributing recruits to the Asian carp stock in the ILR.

Objectives

1. Determine if water Sr:Ca and $\delta^{18}O$ differed spatially (MOR, UMSR, MMSR, ILR and floodplain lakes) during this study (2010-2011) and were consistent with results of prior research. This objective tested the null hypotheses that there were no differences in water chemistry among these environments.

2. Determine if Sr:Ca and $\delta^{18}O$ values found in the ambient water were strongly correlated with those found in known origin Asian carp otoliths. This objective tested the null hypotheses that there were no significant relationships between water and carp otolith Sr:Ca and $\delta^{18}O$.

3. Determine if the water-otolith Sr:Ca and $\delta^{18}O$ relationships observed in native ILR species were comparable to known origin Asian carp. This objective tested the null hypotheses that water-otolith Sr:Ca and $\delta^{18}O$ relationships of known origin Asian carp and other ILR species were not different.

4. (A). Determine the relative contribution of natal environments (MOR, MMSR or ILR) to stocks of adult bighead carp and silver carp in the ILR, from samples of adult fish collected during 2010 and 2011, via analysis of otolith core Sr:Ca ratios.

(B). Determine the relative contributions of early life nursery habitats (river channel or floodplain lakes) to stocks of adult bighead carp and silver carp in the ILR, from samples of adult fish collected during 2010 and 2011, via otolith core $\delta^{18}O$ and $\delta^{13}C$ analysis.

5. Determine whether the principle environments (MOR, UMSR, MMSR, ILR or floodplain lakes) contributing to Asian carp stocks in the ILR, differed among river reaches (in the ILR), species, size (age) of fish and year classes of fish during 2010 and 2011. This tested the null hypotheses that the proportions of Asian carp that originated from each of these environments did not differ among river reaches, species, size classes, ages or year classes.

METHODS

Study Area

Samples were obtained from four large rivers and selected tributaries in the Mississippi River and Missouri River basins including the Missouri, Kansas, Mississippi and Illinois rivers between August, 2010 and October 2011. The current threat to Lake Michigan focused primary sampling efforts for Asian carp on the ILR. The ILR system (Figure 1.1; Irons et al. 2007) is characterized by a multitude of habitat types, with a much straighter, narrower river with minimum floodplain habitat along the upper reaches of the river closer to Lake Michigan. Restoration efforts along several reaches of the middle and lower ILR have reintroduced a portion of the complex floodplain and backwater lake habitat that used to characterize most of the river. This diverse habitat resulted in sampling efforts being divided into three sections of the ILR including: 1. the Alton reach, which extends from the confluence of the ILR and UMSR at Grafton, IL upstream to the LaGrange lock and dam, 2. the LaGrange-Peoria reach from the LaGrange lock and dam upstream to the Starved Rock lock and dam, 3. the Starved Rock reach extending from the Starved Rock lock and dam upstream to the origin of the ILR at the confluence of the Des Plaines and Kankakee rivers. The LaGrange and Peoria reaches were grouped into one continuous reach as both the water chemistry and floodplain connectivity are similar.

Field Sampling

Seasonal (June, August and October) water samples were collected during 2010 and 2011 from a suite of sites along the ILR, UMSR, MMSR and MOR to monitor both spatial and temporal variations in the water chemistries of potential natal environments for Asian carp present in the ILR. Sampling along the ILR included the main channel at Starved Rock, Havana and Kampsville, IL and floodplain lakes including Chautauqua, Anderson, Chain and Swan. Main channel samples of the other rivers were collected from Hamburg, IL on the UMSR, Chester, IL on the MMSR and St.Charles, MO on the MOR. Two water samples were collected from each site on each collection date to assess seasonal changes in stable isotopic and elemental compositions. One sample was used for stable oxygen isotope analysis; a second for Sr:Ca analysis. Samples for stable oxygen isotope analysis were collected in 20-ml scintillation vials containing minimal air space and sealed with Parafilm to curtail evaporative loss and fractionation (Kendall and Caldwell 1998). Samples were analyzed for stable oxygen and carbon 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 for water $\delta^{18}O$):

$$\delta^{18}O\ (‰) = [(Rsample / Rstandard) - 1] \times 1000;$$

where R represents $^{18}O/^{16}O$. Samples for analysis of water strontium:calcium ratio (mmol/mol) were also collected in 20-ml scintillation vials and stored on ice or refrigerated until analysis by high-resolution, inductively coupled plasma mass spectrometry (HR-ICPMS) at the Center for Trace Analysis, University of Southern Mississippi.

Adult Asian carp (335 – 1170 mm total length; n = 477) sampling in each ILR reach began in August 2010. Sampling efforts were equally distributed throughout each reach. Subsequent field sampling resumed the following spring 2011 to enhance sample size in selected reaches and obtain multi-year data. Due to Asian carp sampling difficulty and time constraints, unequal sample sizes occurred among all reaches and years. A minimum of 50 adults per species per reach was achieved. Samples were collected with a tandem effort of boat-mounted AC electrofishing and set experimental trammel nets. Trammel nets (35.56-cm-bar outer mesh, 7.62-cm-bar inner mesh) were deployed prior to electrofishing in attempts to drive fish into the nets. Previous studies have found electrofishing to be among the most effective sampling methods to collect silver carp (Williamson and Garvey 2005), while trammel netting appears to be the best means for sampling bighead carp. In order to process all fish in a timely manner and eliminate the time and effort of hauling large quantities of fish back to the lab with limited space, all fish were processed in the field and carcasses discarded back into the river.

Along with adult Asian carp samples, young of the year (YOY) silver and bighead carp ranging from 75 mm to 100 mm total length were obtained from known origin locations including the UMSR, MOR and the lower Kansas River. Known origin fish were those that did not have the potential of drifting down from upstream reaches containing different water chemistries (i.e. fish drifting into the MMSR from the ILR, UMSR or MOR will not be of known origin). The YOY Asian carp collected in the lower Kansas River were subsequently transported to research ponds at the U.S. Geological Survey's Upper Midwest Environmental Sciences Center in LaCrosse, WI. These transported fish provided an opportunity to test the effectiveness of otolith microchemistry for detecting movement of Asian carp among locations with different water Sr:Ca signatures. Several adult Asian carp were collected from disconnected lagoons in Chicago area parks and Harlow Island backwater along the MMSR (disconnected from the river channel during the 18 months prior to fish collection). Otolith edge samples were used from these fish for additional known origin signatures. Both YOY and adult otolith edge samples were paired with concurrent water samples from each site to characterize relationships between water and otolith chemistry for Asian carp.

Otolith Stable Isotope and Trace Element Analyses

Both lapilli otoliths were removed from each fish with non-metallic forceps, rinsed in distilled water, patted dry and placed in microcentrifuge tubes until preparation for analysis. One otolith from each fish was analyzed for carbon and oxygen stable isotope ratios. Otoliths from known-origin YOY fish were analyzed whole; an edge sample was used on known-origin adults removed from Chicago area lagoons. A core sample centered on the otolith nucleus was obtained from each adult fish from the ILR. All otolith material was obtained using a New Wave Research micromill. Otoliths were initially embedded in epoxy and sectioned into 1.3-mm thick sections (transverse cut) with a low-speed Isomet saw. Otolith sections were then affixed to glass microscope slides with a fine drop of superglue for the micro milling procedure. Sample thickness of each cross section was measured with a digital caliper before milling to ensure accurate drilling depth on the otolith surface. If thickness was inconsistent among samples (> .05 μm), adjustments were made in the micromilling program software to ensure consistent drilling depth. Slides were affixed to the micromill stage with double sided tape and drill X-Y offset was calibrated. Calibration was accomplished by manually drilling a small reference hole in the epoxy block next to the embedded otolith and following subsequent on-screen instructions. Drilling to obtain sub-samples of otolith material for stable isotope analysis consisted of three parallel lines roughly 1000 μm in length and 150 μm apart within the nucleus of the otolith. All drill settings were fixed as follows: drill depth = 150 μm, scan speed = 70 μm/sec, drill speed = 5%, cut width = 50 μm, plunge speed 244 μm/sec). After completion of the drilling process, slides were carefully removed from the stage and loose material was tapped onto weighing paper. A fine probe or pick was occasionally necessary to clean out loose material stuck in the drilled core. Three hundred micrograms of powdered otolith material were transferred from the weigh paper into a glass vial (Labco Exetainer) and sealed with a cap containing a rubber septum prior to analysis. Resolution of stable carbon and oxygen isotope analysis using this procedure corresponded to approximately the first year of a fish's life. All otolith isotopic analyses were conducted 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 $\delta^{13}C$, ‰).

The second lapilli otolith from each fish was prepared for analysis of Sr:Ca ratio. The otolith was embedded in Epo-fix epoxy, sectioned in the transverse plane using an ISOMET low-speed saw, and polished to reveal annuli. The 1.3-mm thin section was prepared for analysis under a class 100 laminar flow hood and handled only with nonmetallic acid-washed forceps. All polished samples 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 then stored in acid-washed polypropylene Petri dishes in a sealed container until analysis. The otolith sections were analyzed for strontium and calcium concentrations using a Perkin-Elmer ELAN 6000 inductively coupled plasma mass spectrometer (ICPMS) coupled with a CETAC Technologies LSX-500 laser ablation system. A laser ablated transect was run 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). Otolith microchemistry data were reported as Sr:Ca ratios (µmol/mol).

Data Analyses

Water data analyzed in this study included both 2010-2011 samples concurrent with this research and previous data (2006 – 2010) to test for long term stability within each sample site. A nested two way ANOVA was conducted to assess water Sr:Ca differences among rivers and over sampling years (2006-2011). A nested two way ANOVA was also conducted to examine water $\delta^{18}O$ differences between river channel and flood plain lakes and over sampling years (2006-2011). Both tests were followed by Tukey's HSD test for multiple comparisons. ANOVA results were used to identify all habitats that could be distinguished by their Sr:Ca or $\delta^{18}O$ signatures and, by extension, could also be distinguished and identified as natal environments for individual Asian carp based on otolith Sr:Ca or $\delta^{18}O$. A least-squares curve-linear regression was applied to known origin Asian carp otolith Sr:Ca values and corresponding water Sr:Ca values from their collection locations to characterize relationships between water and otolith Sr:Ca. A lest-squares linear regression was applied to known origin Asiacn carp otolith $\delta^{18}O$ values and corresponding water $\delta^{18}O$ values to characterize the relationship between water and otolith $\delta^{18}O$. Analysis of covariance (ANCOVA) was used to test whether relationships between otolith and water $\delta^{18}O$ differed between Asian carp and native ILR centrarchids (centrachid data from Zeigler and Whitledge 2010).

Identification of natal environment for adult Asian carp collected from the ILR (fish of unknown origin) using otolith Sr:Ca and $\delta^{18}O$ required characterization of the ranges of otolith Sr:Ca and $\delta^{18}O$ "signatures" representative of each potential natal environment. The UNIVARIATE procedure in SAS was used to determine fifth and ninety-fifth percentiles of water Sr:Ca values from each river (MOR, MMSR, and ILR) during 2006-2011. Proc GLIMMIX (identity link normal distribution) in SAS was then used to calculate 95% confidence limits around predicted otolith Sr:Ca values that corresponded to these 5th and 95th percentiles of water Sr:Ca for each river using the regression relationship between otolith and water Sr:Ca. The 95% confidence limits around predicted otolith Sr:Ca values were used as thresholds that defined the upper and lower limits of expected otolith Sr:Ca signatures for each river. The ranges of expected otolith Sr:Ca signatures for each river were used to identify natal river for adult Asian carp of unknown origin that were collected in the ILR. Natal river was assigned to individual, unknown-origin fish by matching otolith core Sr:Ca (first 100 µm of the laser transect, reflective of early life history) with otolith Sr:Ca signatures defined for each river. Proc GLIMMIX (identity link normal distribution) was also used to determine otolith δ^{18}O threshold values that distinguished fish that used floodplain lake and river channel habitats during early life. Individual, unknown-origin fish from the ILR were classified as having otolith core δ^{18}O values indicative of floodplain lake or river channel residency during early life using the limits of expected otolith δ^{18}O signatures defined for each of these two habitats.

Chi square tests were used to assess differences in relative frequencies of fish with otolith core Sr:Ca signatures indicative of the different natal environment categories (ILR, ILMS, MMSR, MSMO, or MOR) for all individuals combined and by species, river reach (Alton, LaGrange-Peoria, and Upper River) and year. Chi square tests were also used to assess differences in relative frequencies of individuals with floodplain lake and river channel otolith core δ^{18}O signatures for all individuals combined and by species, river reach (Alton, LaGrange-Peoria, and Upper River) and year. Changes in the distributions of otolith core Sr:Ca and δ^{18}O values with fish total length were evaluated using two dimensional Kolmogorov-Smirnov (2DKS) tests(Garvey et al. 1998). A *P*-value of ≤ 0.05 was considered significant for all statistical tests, and all statistical analyses other than 2DKS tests were performed using SAS 9.2 (SAS Institute, Inc. Cary, NC).

RESULTS

Water Sr:Ca and $\delta^{18}O$

Seasonal water samples (n = 77) taken from the ILR, UMSR, MMSR and MOR and ILR floodplain lakes analyzed for Sr:Ca revealed significant differences among environments ($F_{4,55} = 87.50$, $P < 0.0001$) (Figure 1.2). Further analysis via Tukey's HSD test revealed similar mean water Sr:Ca between the ILR and UMSR. The MOR displayed the highest mean water Sr:Ca at 3.25 mmol/mol, followed by the MMSR at 2.15 mmol/mol. Mean Sr:Ca values for the UMSR, ILR, and ILR floodplain lakes were not significantly different from one another, but were all significantly lower than mean Sr:Ca values for the MOR and MMSR. No significant differences in water Sr:Ca among years (2006-2011) were present within the four rivers ($F_{14,31} = 1.66$, $P = 0.12$). Analysis of water $\delta^{18}O$ samples (n = 77) taken from all river sites plus several connected floodplain lakes along the ILR revealed significant differences between river and floodplain lake habitats ($F_{1,66} = 28.06$, $P < 0.001$) (Figure 1.3). This analysis also revealed significant temporal variation of $\delta^{18}O$ within sites ($F_{9,66} = 2.60$, $P = 0.01$). Temporal variation was then further analyzed by habitat (river and floodplain lake), which revealed significant temporal variation of water $\delta^{18}O$ within floodplain lakes but not river sites (lake: $F_{4,24} = 5.60$, $P = 0.01$; river: $F_{4,42} = .87$, $P = 0.50$).

Relationships Between Water and Otolith Chemistry and Classification Models for Determining Natal Environment of Asian Carp

Asian carp otolith Sr:Ca values were very strongly correlated with water Sr:Ca signatures ($r^2 = 0.95$, $P < 0.001$)(Figure 1.4). Otolith Sr:Ca transect data of YOY Asian carp captured in the Kansas River (water Sr:Ca = 3.51 mmol/mol) and transported to research ponds in LaCrosse, WI (water Sr:Ca = 0.87 mmol/mol) further demonstrated that changes in water Sr:Ca experienced by Asian carp during their lifetime as a result of habitat shifts are reflected by changes in otolith Sr:Ca (Figure 1.5). Asian carp otolith $\delta^{18}O$ also exhibited a strong relationship with associated water $\delta^{18}O$ signatures ($r^2 = 0.94$, $P = 0.0013$) (Figure 1.6). ANCOVA analysis of regression relationships between water and otolith $\delta^{18}O$ for Asian carp and centrarchids revealed an insignificant interaction between species and otolith $\delta^{18}O$ ($F_{1,23} = 2.70$, $P = 0.11$) suggesting consistent relationships between water and otolith $\delta^{18}O$ between Asian carp and centrarchids from the ILR drainage.

Fifth and ninety-fifth percentiles of water Sr:Ca during 2006-2011 for rivers that are potential sources of Asian carp in the ILR were as follows: (ILR = 1.108 mmol/mol; 2.106 mmol/mol, MMSR = 1.655 mmol/mol; 2.989 mmol/mol, MOR = 2.622 mmol/mol; 3.992 mmol/mol). Predicted otolith Sr:Ca values used as thresholds (calculated using proc GLIMMIX in SAS) in the classification model for determining origin of adult Asian carp were as follows: ILR highest value = 1180 mmol/mol; MMSR lowest value = 760 mmol/mol; MMSR highest value = 2796 mmol/mol; MOR lowest value = 1954 mmol/mol. Thus, adult Asian carp collected from the Illinois River which had otolith core Sr:Ca values < 760 mmol/mol were designated as having originated from the ILR; fish with otolith core Sr:Ca values between 760 mmol/mol and 1180 mmol/mol were classified as having originated in either the ILR or MMSR; fish with otolith core Sr:Ca values between1180 mmol/mol and 1954 mmol/mol were determined to have originated in the MMSR; fish with otolith core Sr:Ca values between 1954 mmol/mol and 2796 mmol/mol were classified as immigrants to the ILR that originated in either the MMSR or the MOR; and Asian carp with otolith core Sr:Ca values > 2796 mmol/mol were classified as having originated in the MOR.

Threshold otolith $\delta^{18}O$ values (calculated using proc GLIMMIX in SAS) that distinguished residency in floodplain lake and river channel habitats were as follows: floodplain lake lightest otolith $\delta^{18}O$ value = -6.94 ‰; river heaviest otolith $\delta^{18}O$ value = -6.00‰. Thus, early life habitat of individual adult Asian carp collected from the ILR (fish of unknown origin) was determined from otolith core $\delta^{18}O$ as follows: fish with otolith core $\delta^{18}O$ > -6.00 ‰ originated from floodplain lakes; fish with otolith core $\delta^{18}O$ < -6.00 ‰ and > -6.94 ‰ were classified as being of uncertain origin (river or floodplain residency during early life could not be determined for these individuals); and fish with otolith core $\delta^{18}O$ < -6.94 ‰ originated from river channel habitat.

Natal Environments of Illinois River Asian Carp

The majority of adult Asian carp collected from the ILR originated in the ILR; an estimated six to 24 percent of fish sampled were immigrants that originated in the MMSR or MOR (Figure 1.7). The proportions of fish determined to have originated in each river (ILR, MMSR, or MOR) were unequal (χ^2 = 948.31, P < 0.001). Further analysis revealed a significant difference in the relative frequency of individuals from the different potential natal rivers between silver and bighead carp (χ^2 = 83.65, P < 0.001)(Figure 1.8). Of the 195 bighead carp sampled, 190 possessed an ILR otolith core Sr:Ca signature, with the other 5 fish displaying an otolith core Sr:Ca signature that represented either an ILR or MMSR origin. In contrast, ten percent of silver carp collected were immigrants to the ILR (primarily from the MMSR) and an additional 28% of adult silver carp exhibited an otolith core Sr:Ca signature that represented either an ILR or MMSR origin. Frequency distribution of natal environments for both silver carp and bighead carp did not differ among the three reaches of the ILR (silver carp χ^2 = 7.52, P = 0.48; bighead carp χ^2 = 3.82, P = 0.15 (Figures 1.9 and 1.10.) or sampling years (silver carp χ^2 =

1.86, P = 0.76; bighead carp χ^2 = .02, P = 0.89). The distribution of otolith core Sr:Ca values did not change significantly with total length of Asian carp (D = 0.016, P = 0.80)(Figure 1.11).

The established otolith $\delta^{18}O$ cutoff values classified all ILR Asian carp collected into either floodplain lake, river channel or unknown origin. Thirty two fish (8.53%) used floodplain lake habitats during early life, 233 (62.13%) originated in river channel habitat and 110 (29.33%) fell into the unknown origin category due to overlapping otolith values predicted for river- and floodplain lake-resident fish (χ^2 = 164.30, P < 0.001) (Figure 1.12). The frequency distribution of individuals that used river channel and floodplain lake habitats during early life was significantly different between silver and bighead carp (χ^2 = 50.56, P < 0.001) (Figure 1.13). The frequency distribution of individuals that used river channel and floodplain lake habitats during early life also differed among the three reaches of the ILR for both species (bighead carp: χ^2 = 16.83, P < 0.01; silver carp: χ^2 = 37.43, P < 0.001). Two dimensional Kolmogorov-Smirnov analysis revealed a significant association between otolith core $\delta^{18}O$ and total length of Asian carp (D = .144, P < 0.001). More specifically, silver carp otolith core $\delta^{18}O$ values were significantly different with varying size classes (D = 0.1003, P = 0.002) (Figure 1.14). Silver carp greater than 501 mm total length displayed much greater variation (greater frequency of less negative values) in otolith core $\delta^{18}O$ among individuals (Figure 1.12). The distribution of bighead carp otolith core $\delta^{18}O$ values did not change with fish size (D = 0.04, P = 0.31).

DISCUSION

Water Sr:Ca and $\delta^{18}O$

The identification of spatially distinct water Sr:Ca values among the Illinois, middle Mississippi, and Missouri rivers was an essential component in the utility otolith microchemistry for identifying natal environment of Asian carp captured from the ILR. Statistically significant differences in water Sr:Ca among the rivers that represented potential sources of Asian carp to the ILR enabled development of a simple model to identify natal environment for individual fish. Spatial differences in Sr:Ca observed in this study were primarily driven by the bedrock geology of the MOR basin contributing to higher Sr:Ca values in the MOR (Wells et al. 2003). Previous studies have found similar spatial trends in water Sr:Ca among rivers in the middle portion of the Mississippi River basin (Smith 2010; Zeigler and Whitledge 2011;Phelps et al. 2012). Water Sr:Ca values measured in this study were within the range of water Sr:Ca for samples collected from the MOR, MMSR and UMSR durring 1996-2000 (Kelly et al. 2000) suggesting long term stability of water Sr:Ca signatures in the ILR, MMSR, and lower MOR. Similar stability of water Sr:Ca has been observed in other freshwater environments (Zimmerman and Reeves 2002; Wells et al. 2003; Munro et al. 2005; Whitledge et al. 2007) further supporting the strength of Sr:Ca as a chemical fingerprint(but see Schaffler and Winkelman 2008). Inter-annual consistency of differences in water Sr:Ca values among rivers enables use of a single classification model for identifying natal environment for Asian carp from otolith core Sr:Ca regardless of fish age.

Differences in water $\delta^{18}O$ values between river and floodplain lake habitats were useful as a secondary marker for determining early life habitat use by bighead and silver carps. Water $\delta^{18}O$ values generally followed expected patterns, with samples from floodplain lakes generally being more enriched (less negative $\delta^{18}O$ values) than river channel habitats. Longer water residence times within floodplain lakes result in a greater loss of the lighter isotope of oxygen (^{16}O) compared to river channels due to evaporative fractionation (Hoefs 2004). Previous studies have suggested inter-annual stability of water $\delta^{18}O$ in some freshwater systems (Wells et al. 2003; Dufour et al. 2005), including the ILR (Zeigler and Whitledge 2010; Smith 2010). Mean water values for the Illinois River during this study fell within the ranges of $\delta^{18}O$ values reported by Coplen and Kendall (2000) for the Illinois River during November 1984-August 1987 suggesting inter-annual stability of $\delta^{18}O$ within the ILR channel. Although mean ILR water $\delta^{18}O$ was consistent from year-to-year, some seasonal variation was observed. The variation associated with river water $\delta^{18}O$ can be attributed to seasonal fluctuations in discharge dependent upon precipitation and runoff. The 2006 $\delta^{18}O$ water data supports this, as severe drought conditions greatly reduced mean river discharge, allowing for greater fractionation to occur resulting in an enriched river $\delta^{18}O$ signature. Floodplain lakes exhibited more temporal variability in water $\delta^{18}O$ than river channel habitats, as connectivity to the river channel varied among years. During periods of isolation from the river, floodplain lakes diverge from the river's $\delta^{18}O$ signature due to greater evaporative fractionation of oxygen isotopes in the lakes. During years of intense flooding, floodplain lakes do not possess a distinct signature, or have only have a distinct signature during part of the growing season. The floodplain lake water data collected during 2010 and 2011 (both years of prolonged flooding), demonstrates this $\delta^{18}O$ depletion (more negative) effect on floodplain lakes. Future studies that use $\delta^{18}O$ or Sr:Ca to identify natal environment of fishes in the ILR, MMSR, or MOR and their floodplain habitats should continue to monitor water $\delta^{18}O$ or Sr:Ca to verify that differences among environments are still present.

Water-Otolith Relationships

The use of elemental strontium in inferring origin and habitat use of fishes has been documented across multiple environments and species. This is attributed to the simple passive process in which strontium replaces calcium normally deposited during otolith formation (Campana 1999; Gibson-Reinemer et al. 2009). The highly significant relationship between water and Asian carp otolith Sr:Ca indicates that otolith Sr:Ca can be used to reconstruct environmental history of Asian carp in areas where spatial differences in water Sr:Ca signatures are present, such as in the ILR, MMSR, and lower MOR. These findings are consistent with previous studies that have applied otolith Sr:Ca as a natural tracer of fish environmental history in both freshwater (Wells et al. 2003; Whitledge et al. 2007; Whitledge 2008; Gibson-Reinemer et al. 2009) and marine systems (Thorrold et al. 1998; Bath et al. 2000). The only peculiar finding was a non-linear relationship between water and Asian carp otolith Sr:Ca. Previous studies that assessed relationships between water and otolith Sr:Ca for other fish species have observed linear relationships (Wells et al., 2003; Walther and Thorrold, 2008; Zeigler and Whitledge 2010). Mechanisms responsible for the non-linear relationship between water and Asian carp otolith Sr:Ca are unknown.

Otolith Sr:Ca data for YOY Asian carp collected in the Kansas river and transported to research ponds in LaCrosse, WI demonstrated the ability to detect movement of Asian carp from one environment to another when the environments have distinct Sr:Ca values. The exact timing of fish movement among environments is difficult to determine precisely due poor readability of otolith annuli in bighead carp and silver carp. The otolith Sr:Ca data for fish moved from the

Kansas River to research ponds encompassed approximately 1-1.5 years, as all of the fish were less than 1.5 years old when they were harvested from the ponds. Variability in Sr:Ca in the beginning and middle portions of the laser-ablated transects across sectioned otoliths from these fish may reflect temporal variability in water Sr:Ca in the Kansas River or use of multiple environments during the first year of life for these fish prior to their initial capture and transport to research ponds. However, Sr:Ca at the edge of the otolith for each fish reflected the lower Sr:Ca of the research ponds. Future studies should investigate whether elemental and isotopic compositions of other aging structures such as post-cleithra or vertebrae reflect the chemical signatures of environments occupied by Asian carp and whether the more clearly visible annuli in these structures may enable more precise determination of the age or timing of movement among environments by Asian carp.

The highly significant linear relationship between water $\delta^{18}O$ and otolith $\delta^{18}O$ for Asian carp indicates that Asian carp otolith $\delta^{18}O$, like Sr:Ca, is an excellent natural marker for reconstructing environmental history of individual fish in areas where spatial differences in water $\delta^{18}O$ signatures are present. In the Illinois River, otolith $\delta^{18}O$ is useful for identifying use of floodplain lake vs. river channel habitats by young Asian carp. Several previous studies have observed strong linear relationships between water and otolith $\delta^{18}O$ and have used otolith $\delta^{18}O$ to identify source environment for other fish species (Patterson et al. 1993; Dufour et al. 2005; Zeigler and Whitledge 2010; Zeigler and Whitledge 2011). Results of ANCOVA suggested that the relationship between water and otolith $\delta^{18}O$ for Asian carp was consistent with the relationship between water and otolith $\delta^{18}O$ for native ILR centrarchids observed by Zeigler and Whitledge (2010). Previous studies have also found consistent relationships between water and otolith $\delta^{18}O$ among fish species (Patterson et al. 1993; Zeigler and Whitledge 2010).

Asian Carp Origins

Otolith core Sr:Ca data indicated that the majority of adult bighead and silver carp collected from all reaches of the ILR in 2010 and 2011 originated in the ILR, reflective of the established populations of both species in the ILR. Ten to thirty-eight percent of adult silver carp displayed otolith core Sr:Ca values indicative of fish that originated in the MMSR and, to a lesser extent, the MOR and subsequently immigrated to the ILR. The consistent proportion of immigrant silver carp among the three reaches of the ILR (Alton, Peoria-LaGrange, and Starved Rock) indicates that the relative importance of the MMSR and MOR rivers as sources of silver carp does not change with increasing distance from the mouth of the ILR and that once in the ILR, silver carp will move throughout its entirety. Silver carp telemetry data revealed similar movements throughout the ILR, especially during periods of increased flow (DeGrandchamp et al. 2008). In contrast, otolith core Sr:Ca data indicated that >97% of adult bighead carp collected from the ILR originated in the ILR itself, with only a few fish having potentially originated in the MMSR. This finding is somewhat surprising considering that bighead carp were detected and became established in the ILR prior to silver carp (Burr et al. 1996; Peters et al. 2006; Irons et al. 2007). Bighead carp are also known to move into and out of the ILR from the Mississippi River as adults based on telemetry studies (DeGrandchamp et al. 2008; Lohmeyer and Garvey 2009), but adult bighead carp present in the ILR appear to originate primarily in the ILR based on otolith core Sr:Ca. Consistency in the proportion of immigrant Asian carps within all reaches of the ILR between 2010 and 2011 suggests at least some temporal consistency in the relative importance of the ILR, MMSR, and MOR in providing recruits to the ILR Asian carp stocks. Results from the two dimensional Kolmogorov-Smirnov test revealed that the distribution of otolith core Sr:Ca values among individual silver carp (which is reflective of the relative frequency of individuals from different source environments) was independent of fish size (which is related to fish age) and provides further evidence for consistency in the proportion of immigrant silver carp contributing to the ILR stock across years.

The large proportion (62%) of Asian carp exhibiting otolith $\delta^{18}O$ core values reflective of use of river channel rather than floodplain lake habitat during early life is somewhat surprising considering that juvenile Asian carp have been described as having a high affinity for connected floodplain lakes, side channels, tributaries and backwaters (Abdusamadov 1987; Yi et al. 1988; Wang et al. 2003; Kolar et al. 2005). The 300 μg sample obtained from the core of each otolith for $\delta^{18}O$ analysis likely integrates environmental signatures over most of the first summer of life based on mean otolith mass of age-0 Asian carp sampled during fall (Average length: 199.93mm; Average otolith mass: 23.39 g; $R^2 = 0.95$). Thus the observed otolith core $\delta^{18}O$ values may be indicative of Asian carp predominantly, but not necessarily exclusively, using river channel habitats during their first year of life. It is very plausible that young Asian carp inhabiting the MMSR are using slack water environments within the main channel during early life due to the lack of true floodplain environments. The relatively low abundance of connected floodplain lake habitat along the ILR compared to the amount of river channel habitat and in comparison to the quantity of connected floodplain lake habitat historically present (Koel and Sparks 2002; Peg and McClelland 2004) may also account for the low percentage of Asian carp with otolith core $\delta^{18}O$ signatures indicative of floodplain lakes. While some Asian carp in the ILR are using floodplain lakes during their first year of life, otolith $\delta^{18}O$ data indicate that the river channel itself (including side channels, backwaters, and slack water, river-margin habitats) is capable of sustaining Asian carp recruitment.

The relatively low frequency of Asian carp with floodplain lake otolith core $\delta^{18}O$ signatures may also be due to periods of prolonged flooding during recent years that reduced or eliminated the distinction in water $\delta^{18}O$ signatures between the ILR and its floodplain lakes for part of the growing season. Hydrograph data from the ILR at Valley City (USGS, National Water Information System) revealed substantial flood pulses during the growing season each year from 2007 to 2010, whereas discharge did not exceed 20,000 cfs during June-November 2006 (Figure 1.15). Silver carp displayed greater inter-individual variation in otolith core $\delta^{18}O$ signatures with increasing size (age), with no fish < 501 mm total length having a floodplain lake otolith core $\delta^{18}O$ signature. The small range of otolith core $\delta^{18}O$ values among fish < 501 mm total length reflected use of river channel habitats by most of these individuals, many of which likely represent fish from 2007 or later year classes spawned during years in which substantial summer flood pulses occurred. Likewise, lower river discharge during summer 2005 and 2006 may explain the greater frequency (albeit still a relatively small percentage) of individual silver carp > 501 mm total length that displayed otolith core $\delta^{18}O$ values indicative of use of floodplain lake habitat as YOY. While Asian carp in this study were not aged, fish ages can be estimated based on previous research. Williamson and Garvey (2005) characterized age and growth rates of Asian carp in MMSR in 2003 and found that total length of most fish sampled was between 600 and 800 mm, with fish up to age 5 present. The mean total length of Asian carp in my study was 653.11 mm (± 171.58 mm SE). Assuming similar trends in age and growth in comparison to Asian carp in the MMSR, the majority of fish sampled from the ILR were likely between 3 and 5 years old.

Chi-square tests indicated that bighead and silver carp differed significantly in early life habitat use, with a greater proportion of bighead carp exhibiting floodplain lake $\delta^{18}O$ values in their otolith cores compared to silver carp. This could be attributed to the very large proportion of bighead carp originating in the ILR, with a more extensive connected floodplain than the MMSR or MOR. However, the small total number of Asian carp displaying floodplain lake signatures may equally account for these results by making a relatively small difference in the percentage of "floodplain lake" fish statistically significant for comparisons between the two species. Results also indicated significantly higher percentages of bighead carp and silver carp that used floodplain lake habitat during early life among individuals captured in 2011 compared to fish collected in 2010. However, relative abundance of individuals with floodplain lake otolith core $\delta^{18}O$ signatures was low during both years, suggesting that these results may have little biological meaning. The frequency of fish with floodplain lake otolith core $\delta^{18}O$ signatures also differed significantly among ILR reaches for both species. As with other comparisons, however, the low overall occurrence of floodplain lake signatures in adult carp likely limits the meaningfulness of these findings. Interestingly, the highest frequency of occurrence of floodplain lake otolith core $\delta^{18}O$ signatures occurred in fish collected from the Starved Rock reach for both species. These data indicate upstream movement of Asian carp within the ILR, as the Starved Rock portion of the ILR lacks connected floodplain lake habitat.

Future Directions for Research

Both trace element and stable isotope analyses have been demonstrated to be useful for identifying natal environment of bighead carp and silver carp in the Illinois River. Future estimates of the contributions of different rivers to Asian carp stocks in the ILR would likely be more precise if otolith Sr:Ca data from YOY Asian carp (fish that would almost certainly be of

ILR origin) were used to define the limits of the otolith Sr:Ca signature for ILR-origin fish rather than using limits defined by water Sr:Ca data and the relationship between water and otolith Sr:Ca established in this study. The latter approach is subject to greater uncertainty due to variance associated with the relationship between water and otolith Sr:Ca entering into calculations of upper and lower limits of otolith Sr:Ca signatures indicative of fish from each river. Otolith chemistry data for Asian carp collected from other rivers (e.g., UMSR, MOR) would be useful for identifying recruitment sources of these invasive species in other portions of the Mississippi River basin. This information would assist in identification of important sources of Asian carp at a larger spatial scale and patterns of movement between rivers. Future stable isotope analysis within the ILR would also prove practical to further characterize the use of floodplain lakes during early life. This approach would prove the most effective following prolonged periods of low water, producing consistent differences in floodplain and river channel $\delta^{18}O$. Monitoring otolith core Sr:Ca annually would be potentially valuable for assessing the influence of enhanced commercial harvest on the proportion of immigrants to the ILR. If the ILR Asian carp stock is effectively fished down, the proportion of immigrants may increase thereby reducing the reproductive potential of the ILR stock. Identifying the degree (percent) of Asian carp immigration into the ILR from other sources will assist biologists attempting to model population dynamics of Asian carp within the ILR.

The findings of this study have demonstrated the strong utility of otolith trace element and stable isotope analyses in identifying environment of origin for Asian carp (and native species to a lesser extent) within large river systems. Expansion of chemical otolith analyses within freshwater systems may prove useful outside of large rivers. Migratory species within large reservoirs and natural lakes containing several tributaries could plausibly be traced back to tributary of origin assuming distinct chemical signatures can be identified. Discerning the proportions of wild to hatchery reared fish from a given body of water may also be attainable through trace element and stable isotope analyses. This information would aid fisheries managers in determining natural recruitment and survival of stocked fish. There is also potential to expand this rapidly developing science outside fishes or aquatic species. Identifying distinct chemical signatures within wetlands, or the organisms that inhabit them (invertebrates, macrophytes, waterfowl, fish, etc.) may allow biologists to trace migratory species across wetlands and environments. This data would aid natural resource managers in modeling energy flow pathways within wetlands and the associated impacts on population dynamics of several important wetland and migratory species.

MANAGEMENT IMPLICATIONS

To date, minimal knowledge of early life histories of Asian carp has prevented effective removal efforts of young carp. The ability to target habitats with aggregations of young carp and eradicate as many fish as possible before they reach sexual maturity could greatly impact Asian carp population densities. Population models for Asian carp in the ILR suggest that targeting multiple life stages (including juveniles) may be required to control Asian carp abundance. This study has highlighted the Asian carps' ability to reproduce without high levels of connection to associated floodplain habitats and suggests that any removal efforts targeting YOY Asian carp will need to include slack water habitat in the river channel. Islands, sandbars, dikes and other natural current breaks all possess ideal slack water environments that may harbor young Asian carp.

Ongoing removal efforts targeting adult Asian carp through enhanced commercial harvest have been directed only within specific reaches of the ILR (primarily downstream from Starved Rock, with some commercial harvest further upstream) and have not included other rivers (e.g., the middle Mississippi and Missouri Rivers). This study has demonstrated that immigrants from the MMSR and, to a lesser extent, the MOR contribute to stocks of adult Asian carp, especially silver carp, in all three reaches of the ILR. Expansion of the geographic scope of enhanced commercial harvest of Asian carp to include the MMSR and perhaps the MOR will likely be required to effectively control the abundance of adult silver carp in the ILR and thus limit their impacts on the ILR ecosystem and their probability of invading Lake Michigan.

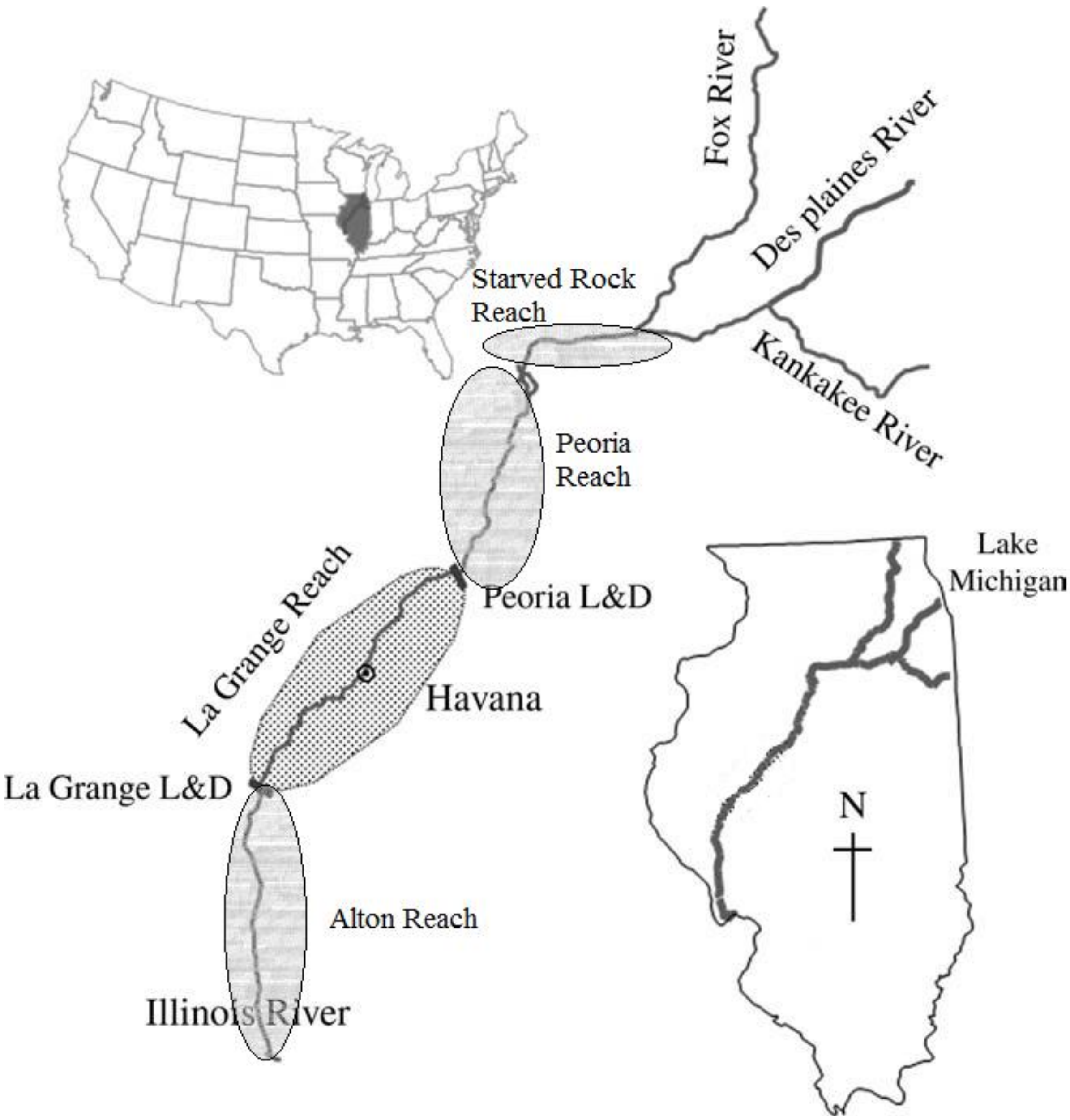

Figure 1.1. Detailed map of the Illinois River highlighting all reaches. The LaGrange and Peoria Reaches were treated as one unit due to similar habitat characteristics.

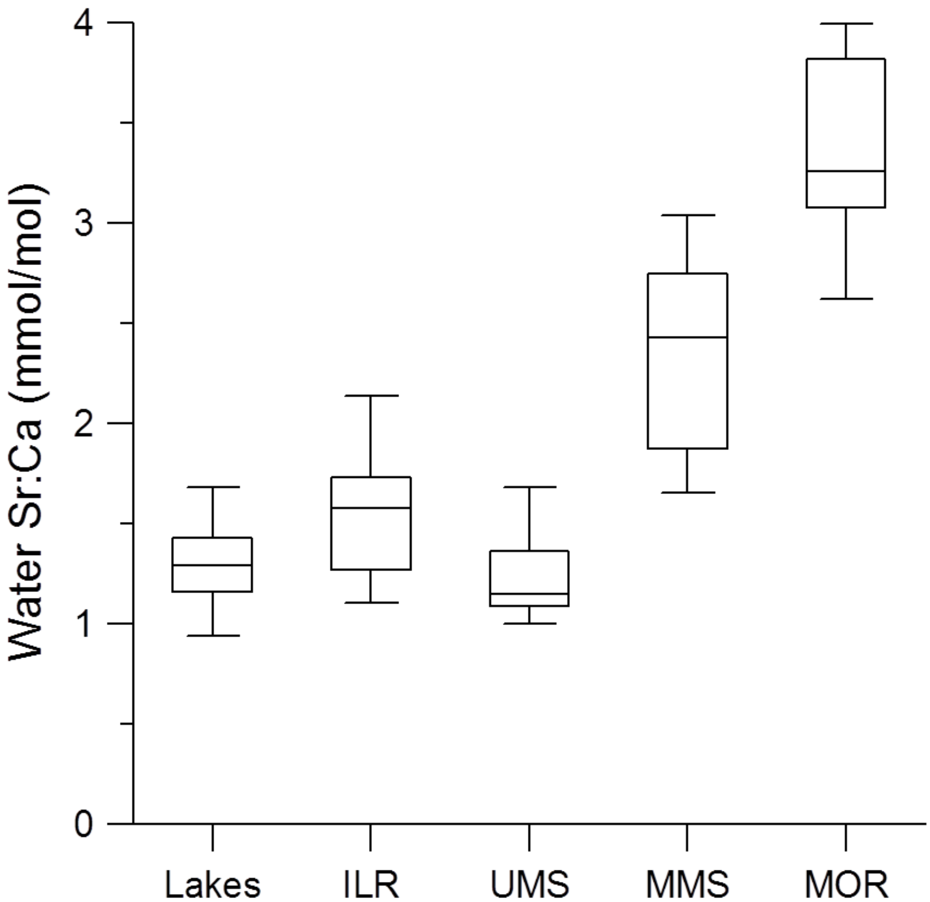

Figure 1.2. Water Sr:Ca data displaying the range, median, and inter-quartile range from all environments (Lakes = Flood plain lakes (n = 28), ILR = Illinois River (n = 23), UMS = Upper Mississippi River (n = 6), MMS = Middle Mississippi River (n = 9), MOR = Missouri River (n = 10)) sampled during June, August, and October 2006-2011.

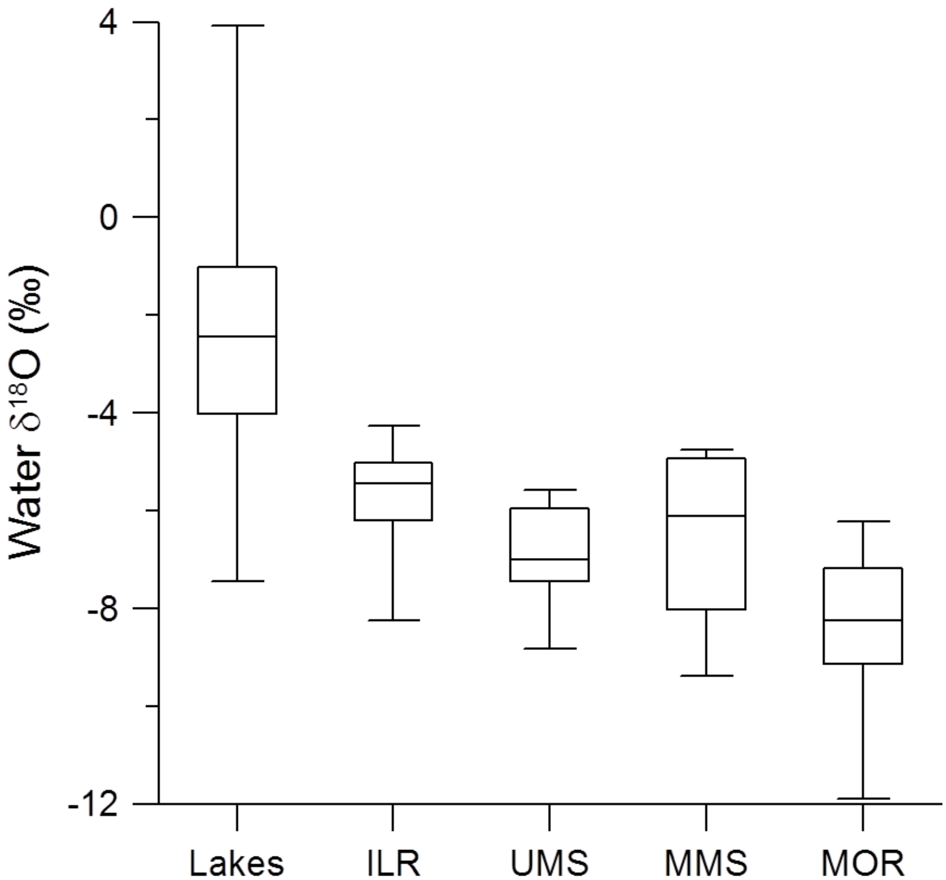

Figure 1.3. Water $\delta^{18}O$ data displaying the range, median, and inter-quartile range from all environments (Lakes = Flood plain lakes (n = 29), ILR = Illinois River (n = 22), UMS = Upper Mississippi River (n = 6), MMS = Middle Mississippi River (n = 10), MOR = Missouri River = (n = 10)) sampled during June, August, and October 2006-2011.

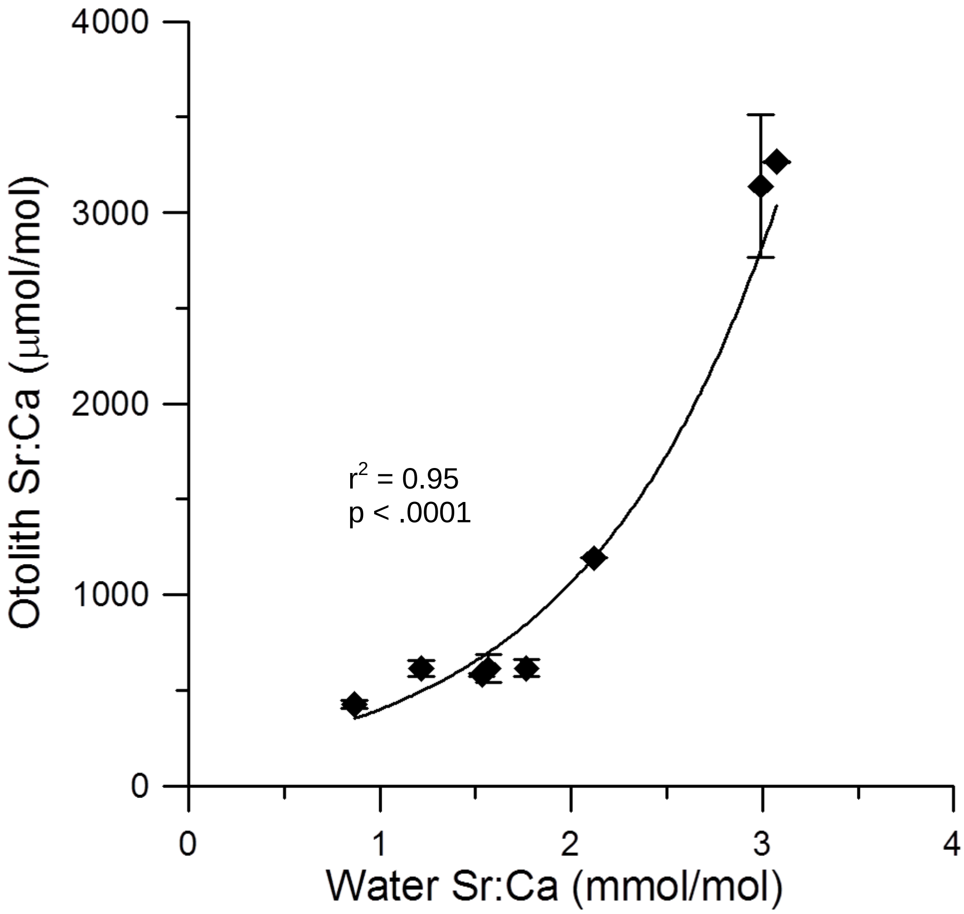

Figure 1.4. Regression analysis of known-origin Asian carp otolith Sr:Ca signatures and corresponding water Sr:Ca values from fish collection locations. Symbol represent means for fish from each location ± SE.

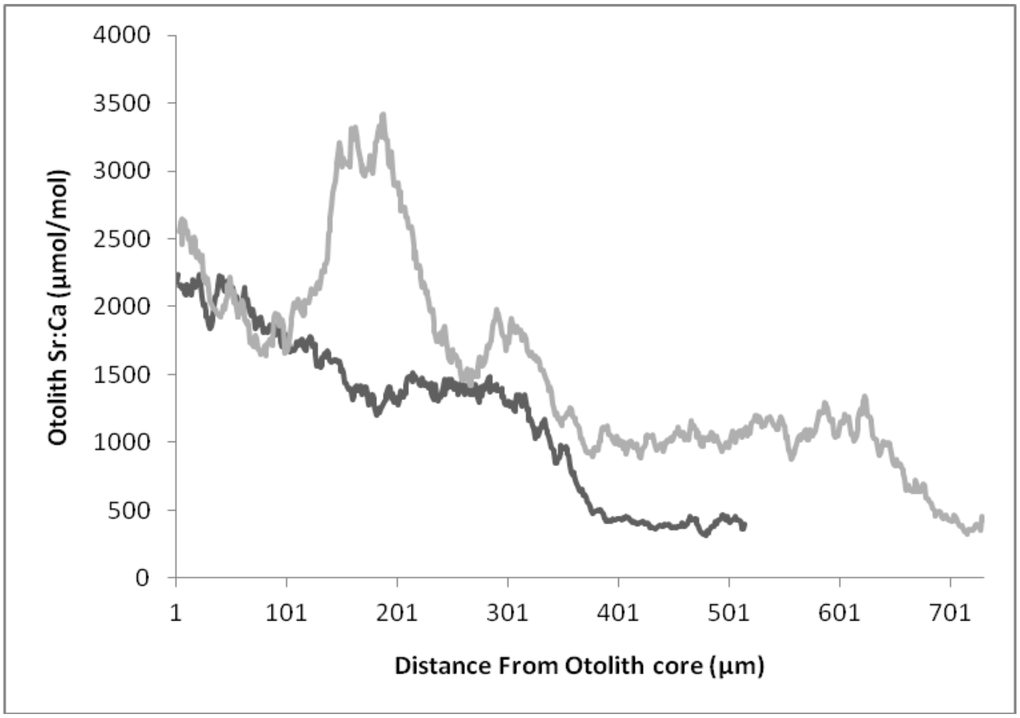

Figure 1.5. Otolith Sr:Ca transect data from two known-origin YOY Asian carp captured in the Kansas River (otolith core extends ~100µm from the center) and transported to USGS research ponds in LaCrosse, WI.

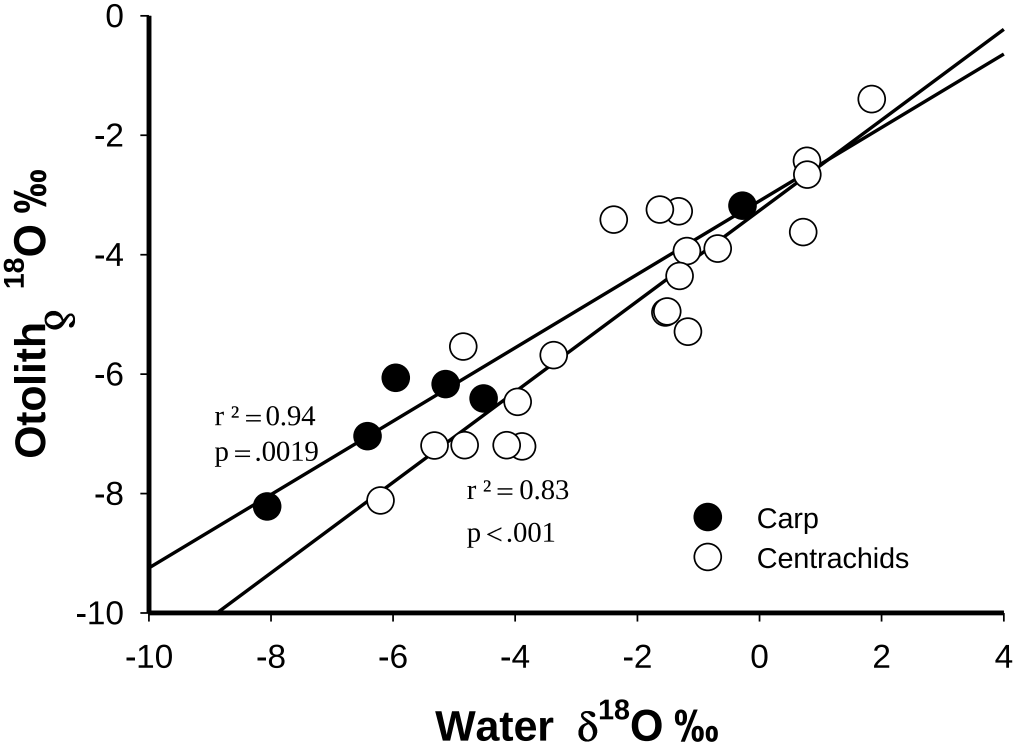

Figure 1.6. Regression Analysis of both known-origin centrarchid and known-origin Asian carp otolith δ^{18}O signatures plotted against water δ^{18}O values from fish collection locations. Symbol represent mean otolith δ^{18}O for fish from each collection location. Centrarchid data obtained from Zeigler and Whitledge (2010).

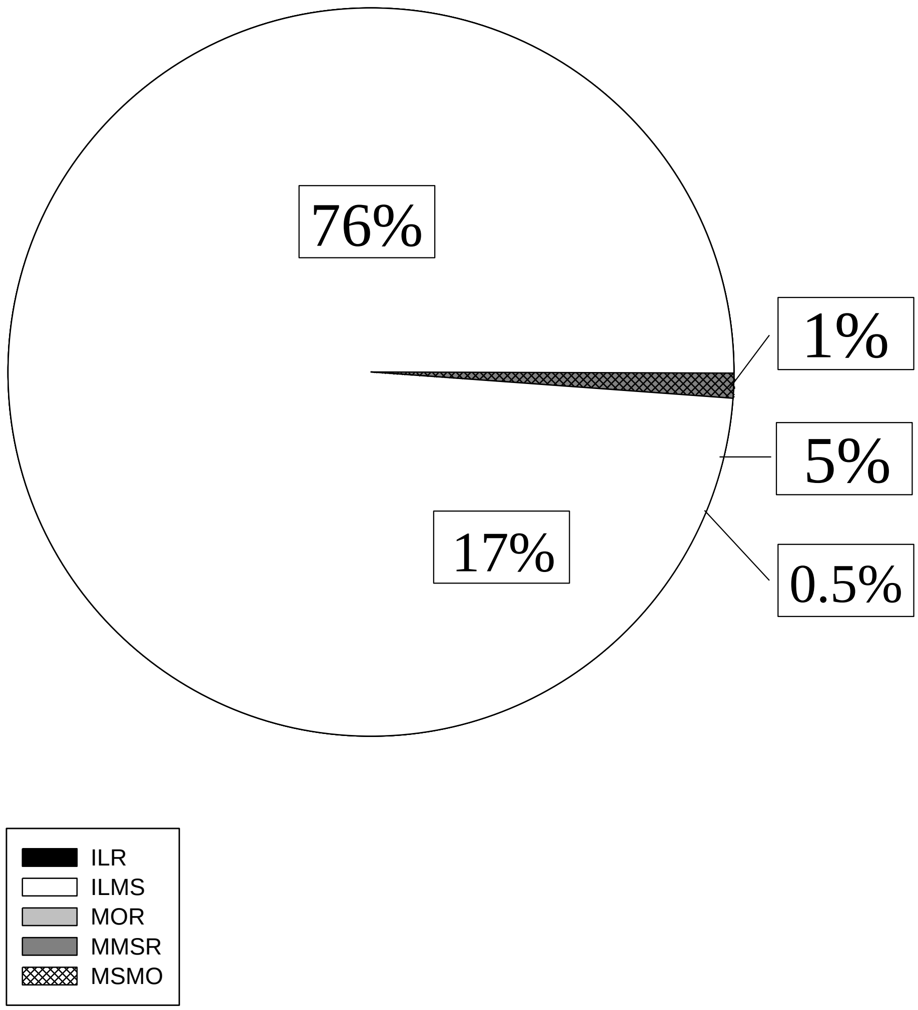

Figure 1.7. Pie chart showing natal origin of adult Asian carp (silver carp and bighead carp combined; n = 455) caught within the three reaches of the Illinois river (Alton, LaGrange/Peoria, and Starved Rock) during 2010 and 2011. (ILR = Illinois River origin; ILMS = Illinois River or middle Mississippi River origin; MOR = Missouri River origin; MMSR = middle Mississippi River origin; MSMO = middle Mississippi River or Missouri River origin).

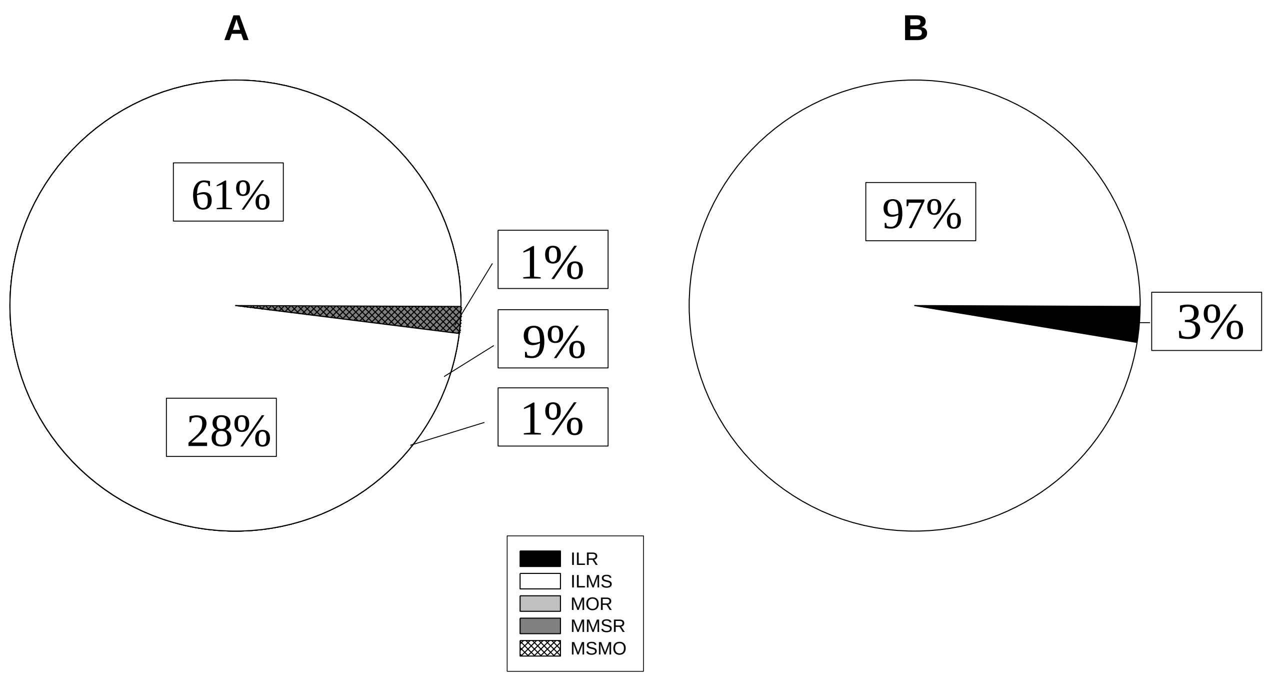

Figure 1.8. Pie chart showing the comparison of natal origins of: A) adult silver carp (n = 260) and B) bighead carp (n = 190) collected from the three reaches of the Illinois River (Alton, LaGrange/Peoria, Starved Rock) during 2010 and 2011. (ILR = Illinois River; ILMS = Illinois River or middle Mississippi River origin; MOR = Missouri River; MMSR = middle Mississippi River; MSMO = middle Mississippi River or Missouri River origin).

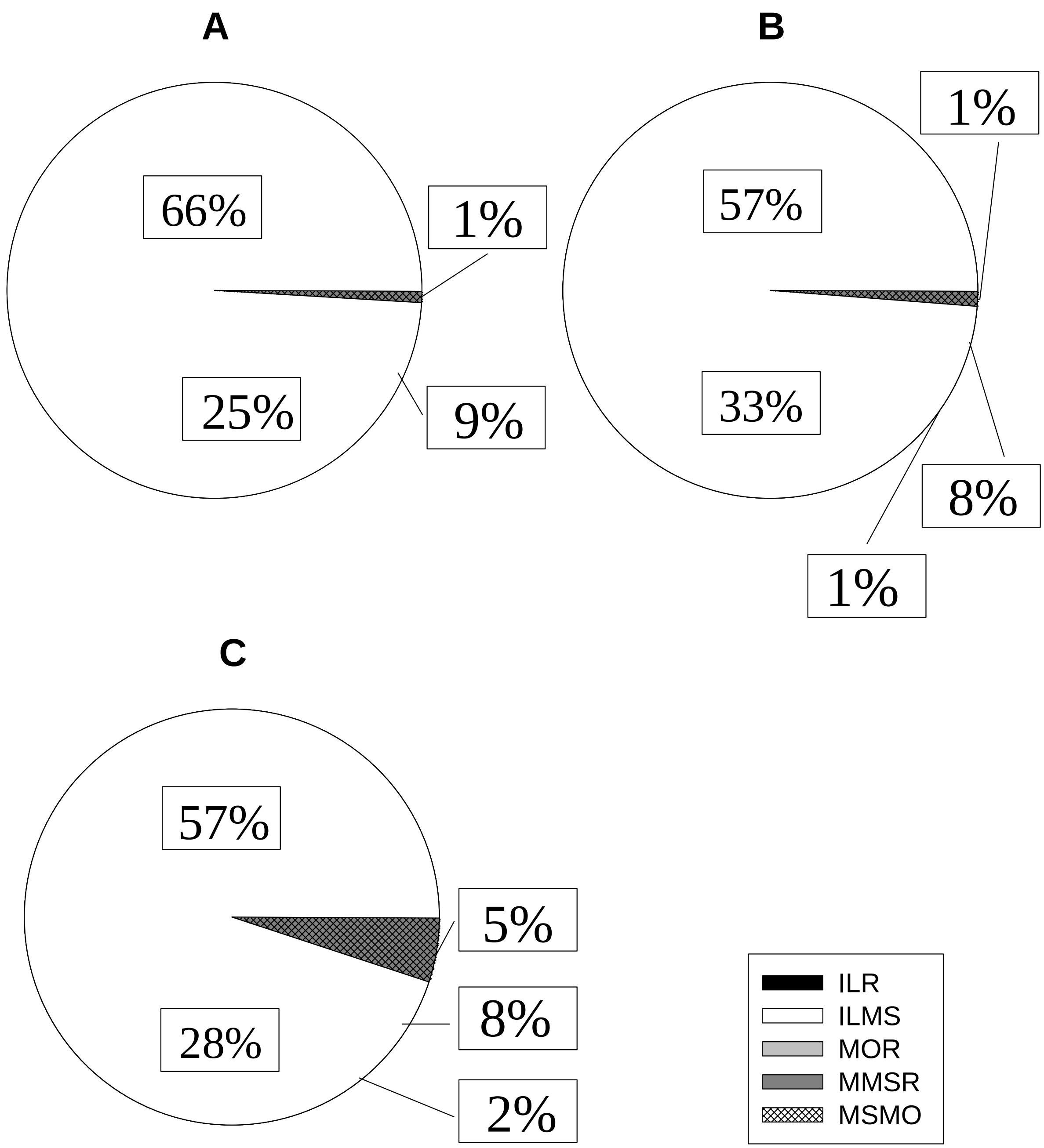

Figure 1.9. Pie charts showing the distribution of natal origins for adult silver carp captured in: A) Alton pool (n = 114), B) LaGrange/Peoria pool (n = 86), and C) Starved Rock pool (n = 60). (ILR = Illinois River; ILMS = Illinois River or middle Mississippi River origin; MOR = Missouri River; MMSR = middle Mississippi River; MSMO = middle Mississippi River or Missouri River origin).

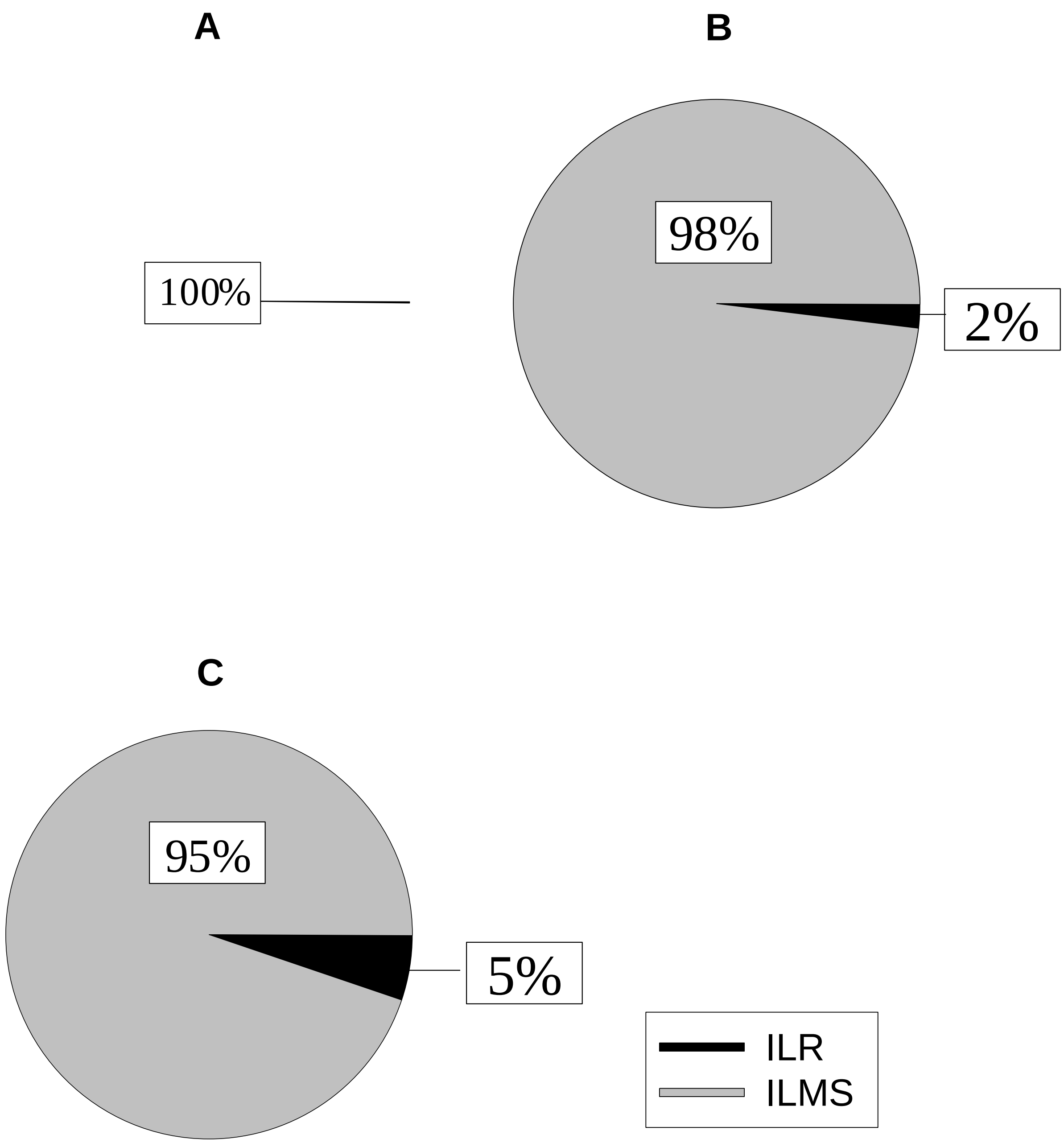

Figure 1.10. Pie charts showing the distribution of natal origins for adult bighead carp captured in A) Alton pool (n = 63), B) LaGrange/Peoria pool (n = 54), and C) Starved Rock pool (n = 78). (ILR = Illinois River; ILMS = Illinois River or middle Mississippi River origin; MOR = Missouri River; MMSR = middle Mississippi River; MSMO = middle Mississippi River or Missouri River origin)

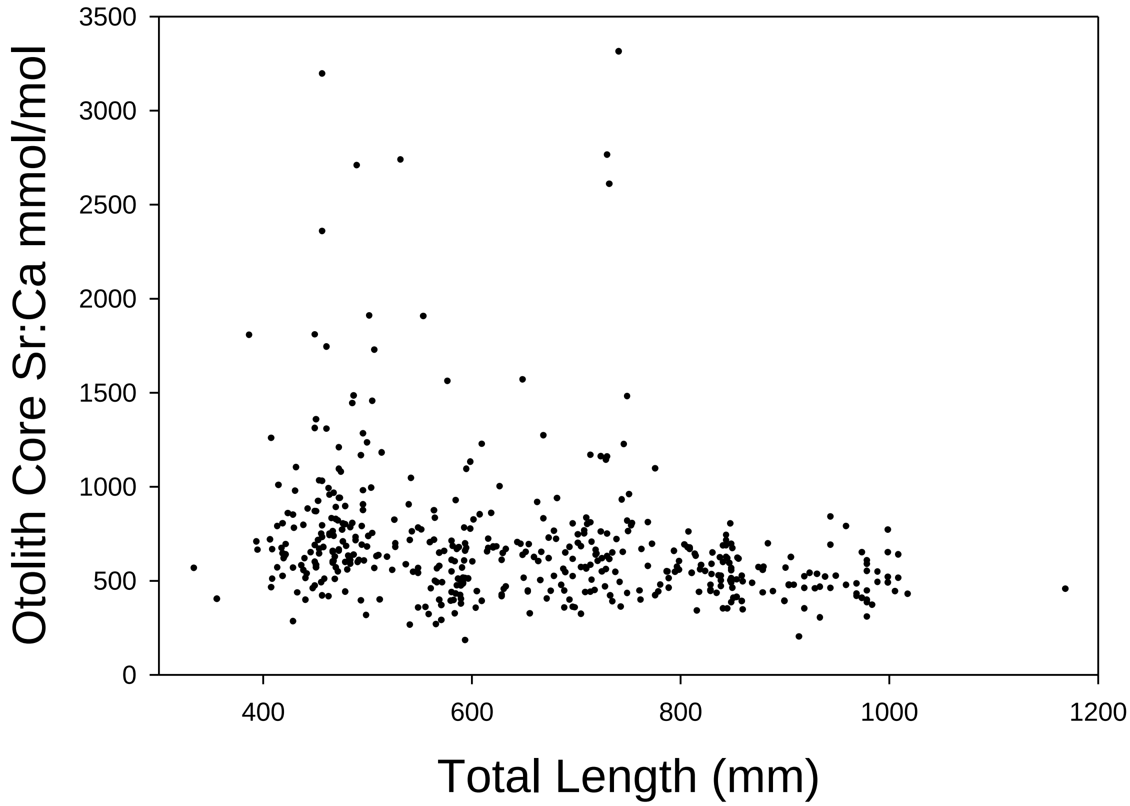

Figure 1.11. Adult Asian carp (n = 455) otolith core Sr:Ca signatures plotted against total length for individual fish collected from the Illinois River during 2010-2011.

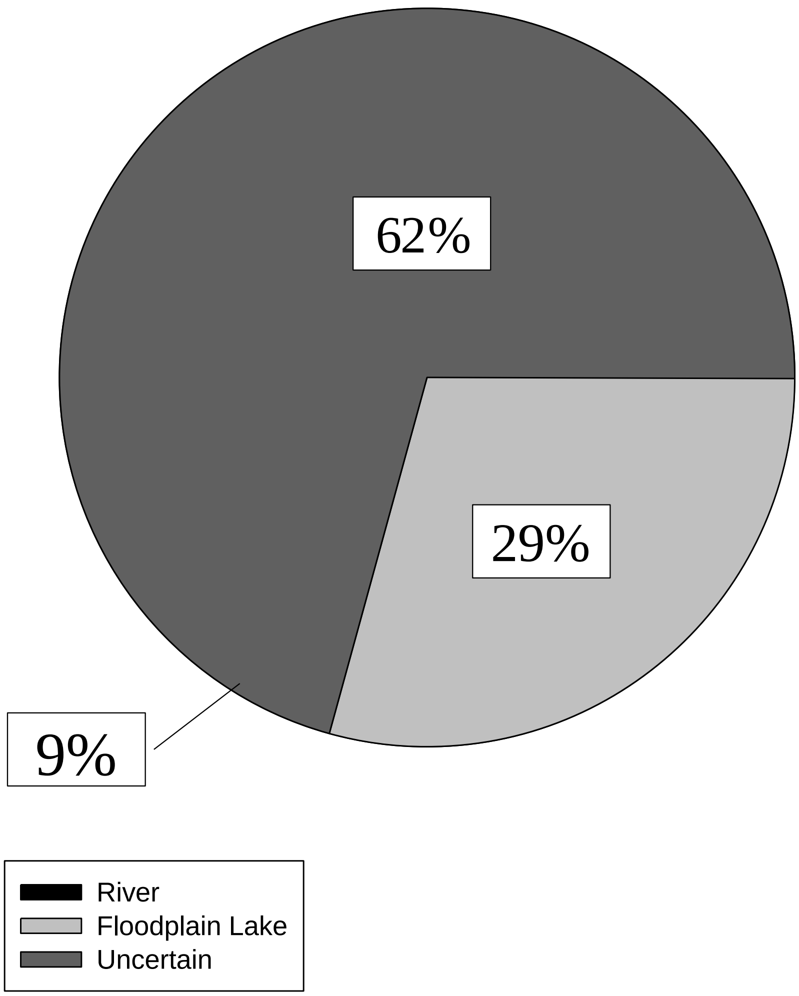

Figure 1.12. Pie chart showing distribution of habitats used during early life by adult Asian carp (bighead carp and silver carp combined; n = 375) caught within the three reaches of the Illinois River (Alton, LaGrange/Peoria, Starved Rock) during 2010-2011. Early life habitat (river or floodplain lake) could not be determined for individuals classified as “uncertain”origin.

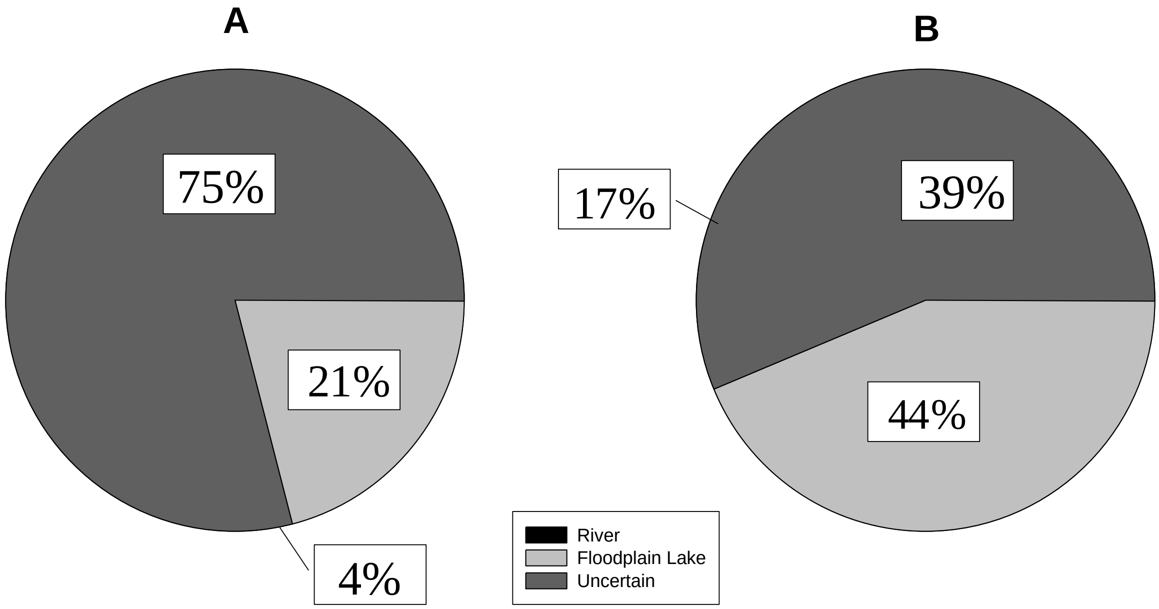

Figure 1.13. Pie chart comparing early life habitat use between A) silver carp (n = 238) and B) bighead carp (n = 137) caught within the three reaches of the Illinois River (Alton, LaGrange/Peoria, Starved Rock) during 2010-2011. Early life habitat (river or floodplain lake) could not be determined for individuals classified as “uncertain”origin.

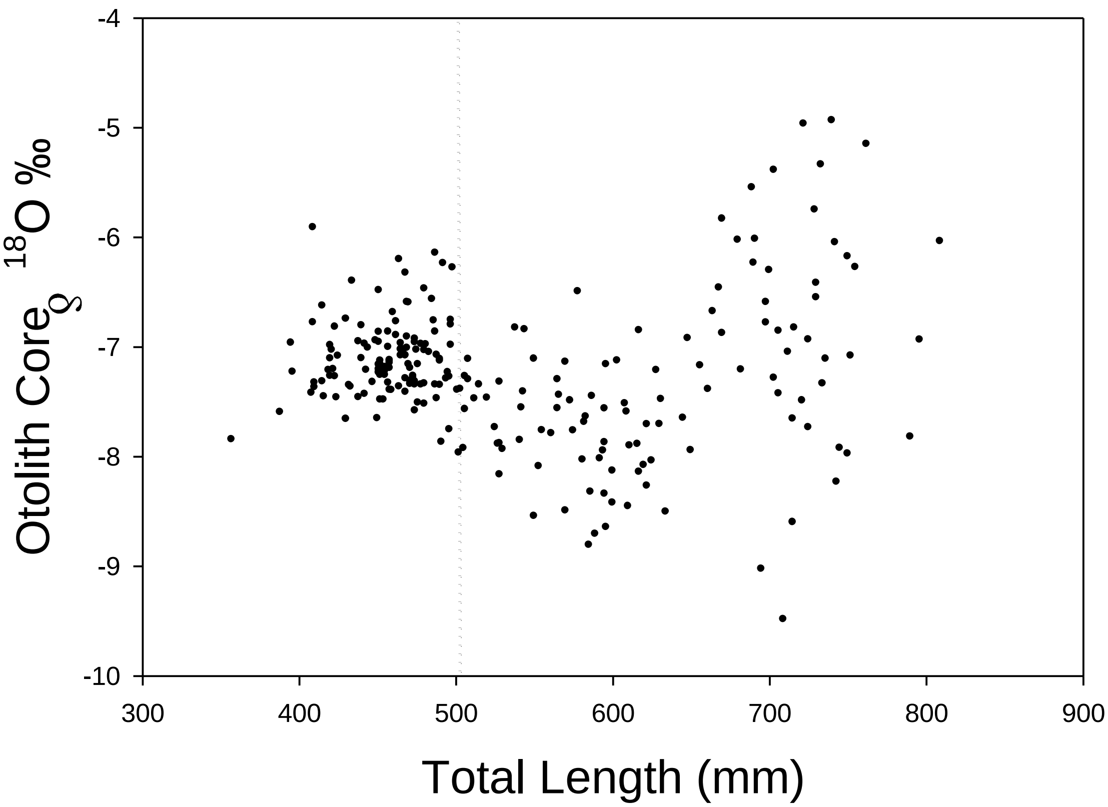

Figure 1.14. Adult silver carp (n = 238) otolith core δ^{18}O signatures plotted against total length for individual fish collected from the Illinois River during 2010-2011. Vertical dotted line represents total length at which a significant shift in the distribution of otolith core δ^{18}O values was detected using a two-dimensional Kolmogorov-Smirnov test.

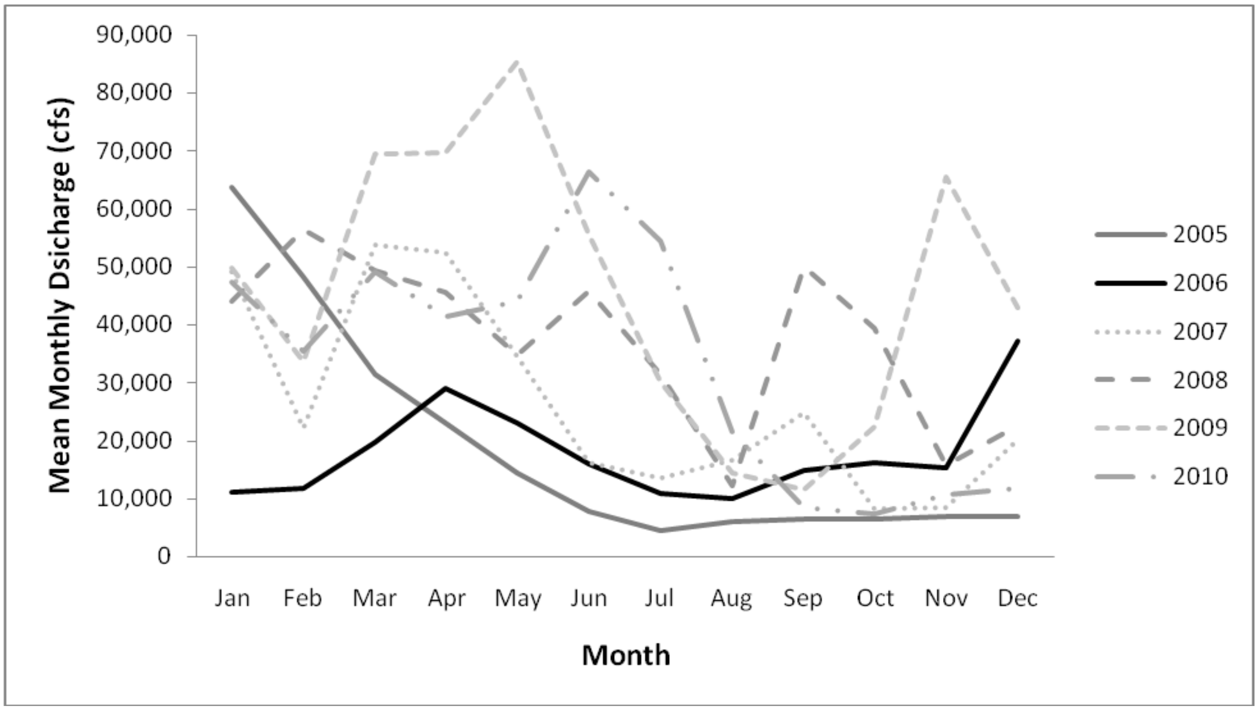

Figure 1.15. Mean monthly discharge (cfs) for the Illinois River obtained from the USGS gauge at Valley City, IL from 2005 through 2010. Both solid lines (2005 and 2006) display the prolonged drought conditions present during that two-year span (USGS.gov).

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