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THE ROLE OF SILVER CARP IN THE TROPHIC POSITION AND DIET OF RIVER OTTERS IN ILLINOIS

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THE ROLE OF SILVER CARP IN THE TROPHIC POSITION AND DIET OF RIVER
OTTERS IN ILLINOIS

By

Preston D. Feltrop

B.S., University of Missouri – Columbia 2011

A Thesis

Submitted in Partial Fulfillment of the Requirements for the
Masters of Science Degree

Department of Zoology
in the Graduate School
Southern Illinois University Carbondale
August 2015

THESIS APPROVAL

THE ROLE OF SILVER CARP IN THE TROPHIC POSITION AND DIET OF RIVER
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A Thesis Submitted in Partial
Fulfillment of the Requirements
for the Degree of
Masters of Science
in the field of Zoology

Approved by:

Dr. Clayton K. Nielsen, Chair

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AN ABSTRACT OF THE THESIS OF

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TITLE: THE ROLE OF SILVER CARP IN THE TROPHIC POSITION AND DIET OF RIVER OTTERS IN ILLINOIS

MAJOR PROFESSORS: Clayton K. Nielsen and Eric M. Schauber

Invasive prey species pose a threat to ecosystems and can alter food web and community dynamics. Populations of silver carp (*Hypophthalmichthys molitrix*), recognized with bighead carp (*H. nobilis*) as “Asian carp,” are growing rapidly in Illinois and may make up a large fraction of available prey for river otters (*Lontra canadensis*) in larger waterbodies. Asian carp occupy a considerably lower trophic level than most commonly recognized otter prey. My goals were 1) to assess the influence of consuming silver carp on the trophic position of Illinois otters using stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$), and 2) to assess the frequency of Asian carp in otter diets. I also compared the frequency of occurrence of prey groups (fish, crayfish, and amphibians) between land cover types and seasons. For my first goal, trappers collected tissue samples ($n = 30$) from harvested otters during November–April 2012–14, and I compared $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between otters from waterbodies with and without silver carp. I also measured $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of various prey types (silver carp, other fishes, crayfish, and amphibians) collected from otter harvest locations and used 2 common isotope mixing model programs, IsoSource and MixSIAR, to estimate relative contributions of prey types to otter isotopic signatures. Silver carp were primary prey for the Carlyle Lake (CL) otters ($n = 6$) based on mean MixSIAR ($25.7 \pm 18.7\%$) and IsoSource ($73 \pm 4.1\%$) contribution results, which constitute 6 of 8 otters harvested from an area containing silver carp. The other 2 otters from the Carlyle Lake Area (CLA) had similar MixSIAR contribution results but considerably lower IsoSource contribution results. MixSIAR provided a more evenly distributed contribution across

all sources, whereas IsoSource assigned high contribution estimates to select sources with signatures closest to the consumer signature. However, MixSIAR provides a useful tool to handle additional information and uncertainties, which are naively disregarded with IsoSource. I predicted otters at locations where silver carp were present would have a lower $\delta^{15}\text{N}$ value, but instead $\delta^{15}\text{N}$ values were higher for the CL otters than the otters at locations without silver carp present. However, the increased $\delta^{15}\text{N}$ signatures seem to be a result of elevated $\delta^{15}\text{N}$ of primary producers and potential otter prey in that system. I used sunfish [i.e., longear sunfish (*Lepomis megalotis*) and bluegill (*Lepomis macrochirus*)] as respective indicators of the isotopic baseline. Compared to local sunfish as a baseline, otters at the CLA did not show elevated or reduced $\delta^{15}\text{N}$ values compared to other sites. For the second goal, I estimated the frequency of occurrence of Asian carp otoliths and pharyngeal teeth in otter scat collected from 43 stream sites in central and southern Illinois during sign surveys in January–April 2013 and 2014. Consistent with previous studies, fish and crayfish were primary prey items for otters, followed by amphibians. Frequency of occurrence of crayfish increased from January–February to March–April, but frequency of occurrence of the other prey types remained similar between those periods. Land cover type did not seem to influence frequency of occurrence of prey types. Asian carp pharyngeal teeth and otoliths occurred in 2.6% of scat samples. However, I collected scat samples at only 6 of my 18 sites confirmed to have Asian carp present.

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CHAPTER I
THE ROLE OF SILVER CARP ON THE TROPHIC POSITION OF RIVER OTTERS IN
ILLINOIS

INTRODUCTION

The trophic position and ecological role of predators may be influenced by the presence and abundance of nonnative prey. The addition of species influences trophic interactions and may alter food web dynamics (Hobbs et al. 2009, Staniczenko et al. 2010, Pearse and Altermatt 2013, Strong and Leroux 2014). Trophic shifts as well as diet switching of generalist consumers have occurred in systems with species invasions (Vander Zanden et al. 1999, Roemer et al. 2002, Shaner and Macko 2011). Due to the complexity and variability of food webs, researchers have been challenged with determining the ecological impact of invasive species (Moyle 1986, Lodge 1993).

Non-native species can have positive and negative effects on food webs. In aquatic systems, Eurasian otters (*Lutra lutra*) have adapted to feeding on the non-native red-swamp crayfish (*Procambarus clarkii*), which are a primary prey resource during dry periods (Barrientos et al. 2013). Vander Zanden et al. (1999) reported lower prey-fish diversity, abundance, and trophic positions in lakes with smallmouth bass (*Micropterus dolomieu*) and rock bass (*Ambloplites rupestris*) invasions, causing predatory lake trout (*Salvelinus namaycush*) to shift from feeding on fish to consuming zooplankton. The energy pathway of benthic predators has changed to a pelagic trophic pathway after preying on invasive zebra mussels (*Dreissena polymorpha*), which obtain energy from the pelagic food web (Bulté and Blouin-Demers 2008, Locke et al. 2014). In Newfoundland, the introductions of 13 non-native terrestrial

mammal species have increased the available prey threefold and may have contributed to the increase of American marten (*Martes americana*) abundance (Strong and Leroux 2014). Black-capped chickadees (*Poecile atricapillus*) altered foraging behavior and hovering time to increase consumption of exotic *Urophora* larvae (Ortega et al. 2014). Barber et al. (2008) found gypsy moth (*Lymantria dispar*) outbreaks increase the abundance and shift the spatial distribution of cuckoos (*Coccyzus erythrophthalmus* and *C. americanus*), which could potentially alter the trophic impact of cuckoos throughout their distribution. Non-native species also can alter communities through various types of competition. The introduction of exotic pigs (*Sus scrofa*) replaced competition with predation as the primary ecological factor influencing the biotic communities in the California Channel Islands and nearly drove the island fox (*Urocyon littoralis*) to extinction due to apparent competition (Roemer et al. 2002). Although research about the impacts of invasive prey species on native predators is limited, there is evidence of measurable trophic impacts in ecosystems due to invasion (Barber et al. 2008).

Stable isotope analysis is a relatively new research tool that can be used to determine the role of species in ecosystems, most commonly applied to aquatic ecosystems. Stable nitrogen and carbon isotopes have been used in ecology studies to determine feeding relationships, trophic position, and energy flow through food webs (Vander Zanden and Rasmussen 1999, Hobson et al. 2000, Wengeler et al. 2010). Stable nitrogen isotopes ($\delta^{15}\text{N}$) can be used to indicate the trophic position of consumers (Michener and Lajtha 2007). Stable carbon isotopes ($\delta^{13}\text{C}$) can be used to trace food source and habitat associations (Freedman et al. 2012). Stable isotope values vary depending on the temporal and spatial variation of the isotopic composition of resources and are influenced by various environmental conditions, productivity levels, and amounts of nutrient loading (Post 2002, Michener and Lajtha 2007). Stable isotopes can be analyzed from a

variety of tissues or structures such as muscle, bone, hair, nails, and others. Different tissues or structures have varying isotopic turnover rates, with muscle samples typically representing isotopic signatures from a period of 2-8 weeks (Michener and Lajtha 2007, Freedman et al. 2012). Tissue-diet discrimination factors (DFs) are the difference between consumer tissue and prey $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values and are typically calculated from feeding studies or incorporated from the literature (Phillips 2012). Mixing models use stable isotope values to estimate diet contribution of prey sources to the consumer tissue, which reflects the assimilated diet. An assumption in mixing model calculations worth noting is that the consumer is in isotopic equilibrium with its prey sources (Michener and Lajtha 2007). If a diet shift between isotopically distinct prey has recently occurred, the consumer's tissue may not yet fully reflect the isotopic signature of its new diet due to tissue turnover and growth.

Silver carp (*Hypophthalmichthys molitrix*), often recognized as “Asian carp” along with bighead carp (*H. nobilis*), are invasive species of major concern that have increased in abundance and distribution throughout Midwestern U.S. river systems. Being filter feeders, they deplete available phytoplankton and zooplankton, hindering the growth and survival of native fish (Irons et al. 2007, Freedman et al. 2012). Silver and bighead carp can coexist spatially because silver carp larger than 26 mm primarily consume phytoplankton whereas bighead carp are mainly zooplanktivorous (Wang et al. 1989, DeGrandchamp et al. 2008). Silver carp reach sexual maturity quickly (age 2–3) and have high reproductive rates compared to native river-specialist species (Schrack and Guy 2002, Williamson and Garvey 2005). Larval silver carp rapidly grow (by age 1) too large to be consumed by most native predatory fish (Lodge 1993, DeGrandchamp et al. 2008). These attributes make silver carp prolific invaders with large impacts on invaded ecosystems.

North American river otters (*Lontra canadensis*; hereafter, otters) are apex aquatic predators that feed on a variety of aquatic organisms among different trophic levels. Otters maintain a high metabolic rate, which requires high food intake (up to 1–1.5 kg of fish per day; Serfass et al. 1990, Penland and Black 2009). Fish make up the greatest proportion of otter diets during winter (Stearns and Serfass 2005, Crait and Ben-David 2006, Crimmins et al. 2009, Barding and Lacki 2012). Otters prefer to prey upon slow-moving, top-water fish >10 cm in length (Serfass et al. 1990, Stearns and Serfass 2005, Crait and Ben-David 2006, Cote et al. 2008a) and juvenile and adult silver carp fulfill these conditions. Silver carp can dominate waterbodies, occurring in a much greater abundance than native fishes (Irons et al. 2007). These combined attributes could make silver carp particularly appealing and susceptible to otter predation. Silver carp and otters co-occur in larger waterbodies and their tributaries, whereas interaction in headwater streams and isolated lakes and ponds would be more rare (Lanszki et al. 2001, Freedman et al. 2012).

Asian carp occupy a lower trophic level than fish such as centrarchids and other cyprinids that otters typically consume in the greatest proportion (Freedman et al. 2012). As primary consumers, planktivorous filter-feeding fish such as Asian carp, gizzard shad (*Dorosoma cepedianum*), and bigmouth buffalo (*Ictiobus cyprinellus*) have lower $\delta^{15}\text{N}$ values than piscivorous fish (Freedman et al. 2012). Although Asian carp are exceptionally fast swimmers (77–128 cm/s for silver carp and 86–166 cm/s for bighead carp burst swim speed), they spend long durations in a stationary position at the surface of the water while feeding, which may increase their susceptibility to otter predation (Serfass et al. 1990, Kolar et al. 2007, Hoover et al. 2012). Silver carp can outcompete bighead carp and commonly occur in greater abundance (Williamson 2004). Silver carp can filter food particles less than half the size of bighead carp,

resulting in a primary diet of phytoplankton and a considerably lower $\delta^{15}\text{N}$ signature than bighead carp, which primarily consume zooplankton (Rogowski et al. 2009, Sampson et al. 2009, Zhou et al. 2009). Therefore, consuming greater proportions of silver carp than bighead carp may have a more significant impact on the trophic position of otters.

To my knowledge, there have been no studies on the impact of Asian carp on the diet of otters or any mammal species, and fisheries and wildlife biologists should acquire a greater understanding of their influence on native fauna given increasing distribution and abundance of Asian carp. Invasive prey species can alter food web and community dynamics and stable isotope analysis can be used to better understand the ecological impacts of Asian carp on otters (Vander Zanden et al. 1999, Roemer et al. 2002). The $\delta^{15}\text{N}$ value and trophic position of otters could be considerably reduced if they frequently consume silver carp because the other prey typically consumed by otters (other fishes, crayfishes, and amphibians) are primarily omnivores, insectivores, or piscivores. This could influence food web dynamics by potentially shifting the trophic link from typically consumed prey to prey in lower trophic levels. Furthermore, given the relative novelty of isotope research, comparisons between isotope analysis programs are important. Phillips et al. (2014) provided a descriptive comparison of IsoSource (Version 1.3.1, www.epa.gov/wed/pages/models/stable_isotopes/isosource/isosource.htm, accessed 04 Apr 2013) and MixSIAR (Version 2.1, <https://github.com/brianstock/MixSIAR/releases>, accessed 30 Sept 2014) but did not compare contribution results between programs. IsoSource and MixSIAR both employ mixing models to estimate ranges of source contributions for a consumer (Phillips and Gregg 2003). IsoSource has been widely used (Michener and Lajtha 2007, Bugalho et al. 2008, Shaner and Macko 2011, Newsome et al. 2012, Crowley et al. 2013) and MixSIAR is a more recent package that uses a Bayesian statistical framework to incorporate variation and

uncertainty with isotopic signatures, DFs, hierarchical random and fixed effects, individual random effects, covariates, and concentration dependence (Phillips et al. 2014).

My objective was to compare the trophic position of otters harvested from waterbodies with silver carp present to waterbodies with silver carp absent. I used $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ to estimate the impact of silver carp on the diet of otters. I predicted that otters harvested from areas where silver carp are present would occupy a lower trophic position. I also compared source contribution results of 2 common isotope mixing model programs.

STUDY AREA

I conducted research throughout southern Illinois at 13 sites where otters were harvested during the 2012–2013 and 2013–2014 trapping seasons (Figure I.1). One site (8%) occurred at a headwater (order 1–3) stream, 6 sites (46%) at 4–6 order streams, and 6 (46%) sites at larger rivers such as the Cache, Kaskaskia, and Saline rivers. One site (8%) occurred in an agriculturally dominated landscape (>70% agriculture land cover), 6 sites (46%) in forest, 1 site (8%) in urban, and 5 sites (38%) in a combination of the various land cover types. Silver carp occurred at 5 sites: the Carlyle Lake Area (CLA), the North Fork of the Saline River, Cache River near Belknap, the mouth of Bay Creek in Pope County, and Clear Creek Ditch (J. Stein, Illinois Department of Natural Resources, unpublished data). I focused on interior, more isolated waterbodies, which are farther from larger waterbodies such as the Mississippi and Ohio rivers, to reduce the amount of complexity and variability that occur in food webs with more inputs (Michener and Lajtha 2007). Otters can easily travel across the landscape but decrease movement in winter (Gallant et al. 2007, Janssens et al. 2008) when otters were trapped. Therefore, I presumed that an otter harvested from an isolated waterbody has fed mainly, or exclusively, from that waterbody during previous weeks and isotopic signatures of otter muscle

samples would reflect that diet. Mean temperature and precipitation for southern Illinois during summer are 24.7° C and 90.2 mm and 1.4° C and 115.7 mm during winter (J. Angel, Illinois State Water Survey, unpublished data).

METHODS

Consumer and Source Collection

I recruited Illinois trappers to collect tissue samples from otters they trapped from 5 November to 31 March during the 2012–13 and 2013–14 Illinois trapping seasons. I sent a collection kit with instructions and Whirl-Pak bags to trappers, who recorded date and time of capture, sex, weight, body length (nose to the tip of the tail), and capture location. Trappers collected a 40 mm-diameter plug of muscle from the right hindquarter near the vertebrae when they skinned each otter. Muscle tissue provides a long-term identifier (weeks) for the individual's isotopic signature that is less variable than other tissue samples (Wengeler et al. 2010, Freedman et al. 2012). Trappers stored the samples in individual Whirl-Pak bags in their freezer (approx. –20° C) (Jardine et al. 2003) until I collected the samples, at which time I asked the trappers to mark each otter capture location with Google Earth (Google Earth Version 6.1, www.google.com/earth/index.html, accessed 17 Oct 2011).

I collected otter prey items (i.e., fish, crayfish, and amphibians) during November to April 2012–2014 along 200-m transects, centered at otter harvest locations, to estimate the isotopic baseline of the system and prey contributions (Figure I.1). Transects were extended to 400 m at 4 sites to increase the number of prey samples collected. Sampling farther (3–5 km away, along the same waterbody) from the harvest location was necessary at the CLA, Square Pond, and North Fork of the Saline River because access to the location was impractical or impossible. I collected otter prey by seining, angling, hoop netting, backpack electrofishing (LR-

24, Smith-Root Co., Vancouver, Washington, USA), or boat electrofishing, depending on the size of the waterbody sampled. Waterbodies too deep to wade but lacking boat access were sampled using the backpack electrofisher operated from a canoe. Electrofishing is an effective method of sampling the top section of water, where otters are primarily foraging (Cote et al. 2008a). Fish were immediately euthanized with tricaine methanesulfonate (MS-222), crayfish and amphibians were immediately euthanized by decapitation, and all prey were then placed on ice until being stored at -20°C (Ben-David et al. 1997, Carabel et al. 2006, Mazumder et al. 2011). All prey capture and euthanization methods were approved by the Institutional Animal Care and Use Committee at Southern Illinois University (protocol #12-052).

I collected additional silver carp samples to compare their mean and variability of isotope values (i.e., $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) to different prey species and geographic regions in Illinois. I collected 35 adult silver carp from Horseshoe Lake in Alexander County during December 2013, the Kaskaskia River Lock and Dam in Randolph County during December 2013, and the Kaskaskia River directly below Carlyle Lake in Clinton County during April 2014. I did not capture any hybrid silver/bighead carp (*H. molitrix x nobilis*) and only captured 1 bighead carp during sampling.

Sample Preparation and Analysis

All samples were kept frozen until preparation for stable isotope analysis. I thawed the prey samples in warm water, identified each to species, measured total length, and rinsed with deionized water. I took muscle plugs just below the dorsal fin on fish ≥ 100 mm, whereas fish < 100 mm were processed whole. I dried otter and prey tissue samples in a drying oven at 60°C for 48–72 hrs, and then ground the dried samples to a fine powder using a mortar and pestle (Michener and Lajtha 2007, Wengeler et al. 2010). Because otter tissues varied among

individuals in the amount of lipid content, I applied the Folch et al. (1957) method of lipid extraction, which uses chloroform-methanol (2:1 by volume), to each otter tissue sample (Folch et al. 1957). I homogenized each sample and inserted 0.35–0.40 mg of the sample into a 3.5x5 mm tin capsule. I submitted tissue samples for stable carbon and nitrogen isotope analysis to the analytical chemistry laboratory at Southern Illinois University. The samples were combusted to gas and analyzed in the continuous flow isotope ratio mass spectrometer (IRMS). Isotope values were computed as $\delta = [[(R)_{\text{sample}}/(R)_{\text{standard}}] - 1] \times 1000$, where R is the ratio of the minor to major isotope (e.g., $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) (Jardine et al. 2003, Michener and Lajtha 2007). The values are expressed as per mil (‰) notation.

I used IsoSource and MixSIAR to quantify the contribution of prey species and groups to the isotopic value of each otter. For MixSIAR, I used uninformative priors and Markov Chain Monte Carlo (MCMC), running 3 chains of 100,000 iterations with a burn-in phase of 50,000 iterations and retaining every 50th posterior sample, resulting in 3,000 draws for the posterior distribution. I compared mean source contribution results (%) between IsoSource and MixSIAR using paired t-tests.

Mixing Model Constraints

I combined different prey species *a priori* that were ecologically similar and did not have statistically significant isotopic differences using Hotelling's T^2 test (Appendix A; Ben-David et al. 1997, Phillips and Gregg 2003, Phillips et al. 2005, Phillips 2012, Crowley et al. 2013). Combined sources are indicated by a (+) after one of the combined prey source names (e.g., white crappie⁺). Combining sources allowed me to reduce variation within the mixing space. The $\delta^{13}\text{C}$ values provide an additional isotopic signature to help characterize different sources.

Muscle DFs for otters are unknown because most otter isotope signatures have been analyzed using scat (spraint) and fur samples (Ben-David et al. 1997, Ben-David et al. 1998, Blundell et al. 2001, Wengeler et al. 2010, Crowley et al. 2013, Franco et al. 2013). Commonly accepted DFs are 3.4‰ (range 3–5‰) for $\delta^{15}\text{N}$ and 0.5‰ (range 0–1‰) for $\delta^{13}\text{C}$ (Post 2002). However, studies of river otters have typically used lower DF values for $\delta^{15}\text{N}$ and higher values for $\delta^{13}\text{C}$ (Kline et al. 1993, Cabana and Rasmussen 1994, Ben-David 1996, Ben-David et al. 1997, Ben-David et al. 1998, Wengeler et al. 2010, Franco et al. 2013). I applied the most commonly reported DFs used in stable isotope research on otters. I used DFs of 2‰ and 3‰ for $\delta^{15}\text{N}$ and 1‰ for $\delta^{13}\text{C}$ with IsoSource and MixSIAR. MixSIAR can analyze DFs with standard deviations so I also analyzed the data using a value of $2.5 \pm 0.5\text{‰}$ to account for uncertainty. I ran paired t-tests to compare mean source contribution results between the DF values within each program and also between programs using the same DF. I used the CL otters for comparison because the site contained a sufficient sample size whereas the other sites did not.

Silver Carp Contribution to Otter Diet

I analyzed 30 otter tissue samples collected from 13 sites during 2013–14. However, I was only able to estimate silver carp contribution to otter diet at the CLA sites because the other otter harvest locations either had no silver carp present, or I was unable to collect silver carp during sampling. I included the other otter harvest locations in my analysis as sites without silver carp present, although silver carp have been confirmed at 4 of those sites (J. Stein, Illinois Department of Natural Resources, unpublished data) during the summer when they are dispersed farther upstream (Coulter et al. 2012). Eight otter tissue samples were collected around the CLA, which includes Carlyle Lake (CL) ($n = 6$) and the Carlyle Lake Spillway (CLS) ($n = 2$), where silver carp are abundant (Figure I.1). I used the same prey species as sources for the CLA otters

but combined the sources differently between the CL and CLS otters. To reduce variance in the mixing space and increase the accuracy of the source contributions at CL, I combined highfin carpsucker (*Carpiodes velifer*) (n = 2) with gizzard shad (n = 5) and combined bluegill (*Lepomis macrochirus*) (n = 5) with white crappie (*Pomoxis annularis*) (n = 5). River carpsucker (*C. carpio*) (n = 1), largemouth bass (*Micropterus salmoides*) (n = 3), and silver carp (n = 13) were analyzed in the mixing models as independent sources. Gizzard shad and silver carp did not have significantly different isotopic signatures but I did not combine them because I was primarily interested in assessing the role of silver carp in the diet. I combined sources different from those I combined for the CL otters *a posteriori* for the CLS otters to reduce the number of potential source contribution solutions in IsoSource. The CLS otters were harvested from the same location approximately 3.4 km south of the CLS collection location. For these 2 otters, I combined bluegill with white crappie, but highfin carpsucker, gizzard shad, and silver carp were analyzed as independent sources. Largemouth bass and river carpsucker were omitted to simplify the mixing space and increase the precision of source contribution estimates.

I compared the mean $\delta^{15}\text{N}$ values of the CLA (CL and CLS) otters to the other 22 otters using a 2-tailed t-test. In an attempt to account for potential differences in food web baseline $\delta^{15}\text{N}$ among sites (Michener and Lajtha 2007), I repeated that t-test comparing CLA otters with other otters after using $\delta^{15}\text{N}$ values of local sunfish as a baseline to standardize otter $\delta^{15}\text{N}$ values (standardized otter $\delta^{15}\text{N} = \text{otter } \delta^{15}\text{N} - \text{mean sunfish } \delta^{15}\text{N}$). This assumes that these sunfishes occupy a consistent trophic position among sites. For mean $\delta^{15}\text{N}$ values of sunfish, I used longear sunfish (*Lepomis megalotis*) for 6 sites, bluegill for 2 sites, or mean values of both species for 5 sites. I compiled the primary prey sources ($\geq 50\%$ diet contribution) using IsoSource

for both DFs to provide a simplified list of prey species and types that primarily contributed to otter diet based on stable isotope analysis.

Silver Carp Isotope Comparison

To test whether $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures differed among silver carp sampled from the Kaskaskia River Lock & Dam, the CLS (also Kaskaskia River), and Horseshoe Lake between waterbodies and geographic locations in southern Illinois, I used a one-way analysis of variance (ANOVA) with Tukey's posthoc test for each element separately. I conducted all statistical tests with SPSS 19 (IBM Corp., Armonk, NY), with $\alpha = 0.05$.

RESULTS

The mean $\delta^{15}\text{N}$ value ($\pm\text{SE}$ throughout) of the CLA (CL and CLS) otters ($18.74 \pm 0.62\text{‰}$, $n = 8$) was higher ($t_{28} = 8.44$, $P \leq 0.001$) than other otters ($13.33 \pm 1.76\text{‰}$, $n = 22$; Figure I.2). However, $\delta^{15}\text{N}$ values of the other prey sources were also higher for the CLA than the other sites (Figure I.3), indicating a different $\delta^{15}\text{N}$ baseline. Relative to $\delta^{15}\text{N}$ of local sunfishes, the mean standardized $\delta^{15}\text{N}$ value for the CL and CLS otters ($1.59 \pm 0.38\text{‰}$, $n = 8$) was similar ($t_{28} = 0.19$, $P = 0.85$) to that of other otters ($1.70 \pm 1.72\text{‰}$, $n = 22$). The difference between the CLA otters and the other otters was less than the expected change required for a trophic level shift (95% confidence interval: -1.16 to 1.38‰). Changing DF value with IsoSource changed the primary prey source for 3 otters (Table I.1). IsoSource indicated that centrarchids were primary prey sources for 15 (DF = 2‰) or 11 (DF = 3‰) of 30 otters, potentially contributing to 38–100% of the diet. The 8 CLA otters had similar isotopic signatures to silver carp (Figure I.4), and IsoSource identified silver carp as the primary prey source for 6 CLA otters, potentially contributing to 30–95% of the diet (Table I.1).

MixSIAR and IsoSource Comparison

Different DFs provided varied contribution percentages within MixSIAR and IsoSource for the CL otters ($n = 6$) for the same 3 prey sources (white crappie⁺, river carpsucker, and largemouth bass; Appendix B). Comparing programs at the same DF value, MixSIAR estimated higher contribution percentages than IsoSource with the CL otters for 4 prey sources (white crappie⁺, gizzard shad⁺, river carpsucker, and largemouth bass) and lower contribution percentages for silver carp (Appendix B; $t_5 = 7.2$, $P \leq 0.001$ DF = 2‰, $t_5 = 16.86$, $P \leq 0.001$ DF = 3‰). MixSIAR estimated very similar contributions of silver carp across different DF values. However, silver carp contribution estimated by IsoSource was higher with a DF = 3‰ than a DF = 2‰ for 6 of 8 CLA otters (Appendix C).

The 2 programs yielded very different results when comparing contribution results of the CL otters with the CLS otters, which were harvested 3.4 km south of CL. MixSIAR estimated similar silver carp contribution for CL otters ($25.4 \pm 0.9\%$, DF = 2‰; $25.9 \pm 0.9\%$, DF = 3) and CLS otters ($24.0 \pm 18.0\%$, DF = 2‰; $28.1 \pm 20.0\%$, DF = 3‰). IsoSource, however, estimated much greater silver carp contribution for CL otters ($65 \pm 5.1\%$, DF = 2‰; $81 \pm 3.1\%$, DF = 3) than for CLS otters ($8.6 \pm 5.7\%$, DF = 2‰; $15.4 \pm 4.6\%$, DF = 3‰; Appendix C). Silver carp was the primary prey source in all but one combination of DF and program for the CL otters (Appendix C).

Silver Carp Isotope Comparison

Silver carp isotope signatures varied by waterbody for $\delta^{15}\text{N}$ values ($F_{2,32} = 137.86$, $P \leq 0.001$) and by site for $\delta^{13}\text{C}$ values ($F_{2,32} = 62.45$, $P \leq 0.001$). Mean $\delta^{15}\text{N}$ signatures for silver carp were similar ($P = 0.28$) between the Kaskaskia River Lock and Dam and the Kaskaskia River at CLS. However, mean $\delta^{15}\text{N}$ signatures for silver carp were lower ($P \leq 0.001$) at Horseshoe Lake

than the Kaskaskia River sites. Mean $\delta^{13}\text{C}$ signatures for silver carp were lower for Horseshoe Lake than the 2 Kaskaskia River sites (Figure I.5). Silver carp had lower $\delta^{15}\text{N}$ signatures than most of the prey sources from the same area, but a similar isotopic signature to gizzard shad (Figure I.4). The river carpsucker isotopic signature was considerably lower than any of the other prey sources from the CLA, but could be an anomaly due to limited sample size (Figure I.4).

DISCUSSION

I predicted that otters harvested from areas with silver carp present would occupy a lower trophic position, and therefore have lower $\delta^{15}\text{N}$ values, than otters harvested from areas with silver carp absent. Mean $\delta^{15}\text{N}$ values were higher for the CL and CLS otters than for other otters, opposite of the predicted pattern. However, the increased $\delta^{15}\text{N}$ signatures seem to be a result of elevated $\delta^{15}\text{N}$ of primary producers and potential otter prey in that system. Silver carp are more abundant at the CLA than the other sites (J. Stein, Illinois Department of Natural Resources, unpublished data) and were primary prey for the CL otters based on MixSIAR and IsoSource contribution results. I predicted that the dietary shift to prey at a lower trophic position than typical prey would lower the trophic position of these otters. Although mixing models calculated substantial contributions of silver carp to otter diets at CLA sites, the apparently high consumption rates of otters on Asian carp at these sites did not significantly lower otter $\delta^{15}\text{N}$ values compared to sites without Asian carp as predicted. I was unable to calculate actual otter trophic positions because that calculation requires $\delta^{15}\text{N}$ values of sources occupying the lowest trophic level (Cabana and Rasmussen 1996).

Otter $\delta^{15}\text{N}$ Signature and Primary Prey Comparison

All else being equal, increased $\delta^{15}\text{N}$ values indicate higher-level consumers. However, mean isotopic value should be interpreted with caution because all else is rarely equal due to

spatial and temporal variation in processes in the nitrogen cycle that influence primary producer and, consequently, consumer $\delta^{15}\text{N}$ values (MacLeod and Barton 1998, Finlay et al. 1999, Bode et al. 2003, Jardine et al. 2003). Mean $\delta^{15}\text{N}$ values were higher for the CL and CLS otters compared to the other otters. Freedman et al. (2012) reported lower $\delta^{15}\text{N}$ values for the fish community in areas of high Asian carp density after the invasion of Asian carp in the Illinois River. I would expect to find otters with a lower $\delta^{15}\text{N}$ value in areas with silver carp, but this does not seem to be the case just based on comparing mean $\delta^{15}\text{N}$ differences to otters from sites without silver carp present. The CLA otters could have a lower trophic position and still have a higher $\delta^{15}\text{N}$ value due to the higher $\delta^{15}\text{N}$ baseline of the CLA.

One way I accounted for some of the variance between systems was to use standardized $\delta^{15}\text{N}$ values. Primary consumers such as mussels and snails have been used in place of primary producers to indicate the system isotopic baseline because they provide a longer-term indicator with less seasonality for $\delta^{15}\text{N}$ signatures (Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999, Post 2002). However, I used sunfish (i.e., longear sunfish and bluegill) as respective indicators of the isotopic baseline because they are common prey sources for otters and were collected at each site in high enough abundance to provide reliable estimates of how mean $\delta^{15}\text{N}$ differed between sites. Compared to local sunfish as a baseline, otters at the CLA did not show elevated or reduced $\delta^{15}\text{N}$ values compared to other sites. I would expect to calculate standardized $\delta^{15}\text{N}$ differences with smaller or negative values if the otters are consuming prey from lower trophic levels but that was not evident in the results.

I used primary prey contributions to assess the difference in DFs within IsoSource as well as provide a more comprehensive view than just examining isotopic signatures for which prey sources Illinois otters are consuming. Although individual source contributions changed

depending on DF, I found little evidence of the DF having a notable influence on the primary prey sources identified by IsoSource. The primary prey source only changed for 3 otters between different DFs and could have changed for an additional 3 otters depending on interpretation of the contribution ranges. Silver carp seemed to be primary prey sources for all the CL otters (n = 6) with 1 otter possibly having white crappie⁺ as a primary source instead.

Silver Carp Contribution for CLA Otters

Silver carp had the highest contribution of any prey source for the CL otters for both MixSIAR and IsoSource even with different DFs. It is worth noting that gizzard shad contribution could have been underestimated in the mixing models as they have similar isotopic values as silver carp. Primary source contribution was split between 3 sources for the CLS otters when taking different programs and DFs into account. The difference in contribution results between the CL and CLS otters could be because the sources were combined differently or the sources did not accurately reflect the CLS otters' actual diet. Although the CLS otters were only harvested 3.4 km south of the prey collection site (CL), the otters were harvested near a rock quarry lake. I did not collect prey sources from the rock quarry lake near where the CLS otters were harvested and may not have sampled all prey sources in CL, so the proportion of their diet derived from the lake rather than the Kaskaskia River or CL is unknown. Shaner and Macko (2011) determined that approximately 25% contribution to the diet of generalists during resource pulses shifted their trophic position substantially. Therefore, the estimated contribution of silver carp to otter diets (about 25% according to MixSIAR, 44–92% according to IsoSource) could be sufficient to change otters' trophic position. These results are consistent with my prediction that otters would consume silver carp and, when available, silver carp could occur in enough proportion of the otter diet to lower their trophic position. Southern river otters (*Lontra*

provocax) occupied a lower trophic level based on scat and isotope analysis when their diet contained more crustaceans than salmonids (Franco et al. 2013). Diet shifts that lead to trophic shifts can cause complex trophic interactions and change food web dynamics (Vander Zanden et al. 1999, Shaner and Macko 2011). These food web changes will depend on the previous trophic structure and dynamics of the food web and could have significant implications to otters in areas of high silver carp densities (Vander Zanden and Rasmussen 1999).

Logically, I expected otters with high contribution of silver carp in their diet would occupy a lower trophic position. However, I found no evidence of that, and the possible explanations include: 1) otters from the CLA were at a lower trophic level, but that difference was obscured due to variability in the data, 2) silver carp are not actually at a lower trophic position than native prey at the CLA, or 3) silver carp are at a lower trophic level, but some or all otters outside the CLA also consumed a substantial proportion of silver carp or primary producers. The difference in land cover and increased percent of agriculture surrounding the CLA could contribute to the difference in $\delta^{15}\text{N}$ values compared to the other sites (Michener and Lajtha 2007). Additionally, the town of Carlyle located on the southern section of Carlyle Lake and a golf course 2.5 km away from the CLA sampling location could have both contributed to the variability of nutrients and inputs into the CLA (Cabana and Rasmussen 1996, Michener and Lajtha 2007). The higher $\delta^{15}\text{N}$ values of silver carp at the CLA compared to Horseshoe Lake are also consistent with the relatively higher $\delta^{15}\text{N}$ values of otters at the CLA so it seems that higher consumption of silver carp at the CLA may have influenced otter $\delta^{15}\text{N}$ values, just not in the predicted direction. Silver carp at the CLA could have consumed more zooplankton than previously acknowledged and occupied a higher trophic level. Also, the otters from areas without silver carp could have been consuming more primary consumers than previously thought.

Gizzard shad and common carp also occur in high abundances and could be appealing prey for otters.

MixSIAR and IsoSource Comparison

I compared the performance of MixSIAR and IsoSource, using the data collected from the CLA otters, because I was primarily interested in analyzing the trophic position of otters harvested from areas with Asian carp present. Compared with MixSIAR, IsoSource provided a lower variance around the mean contribution estimates, resulting in narrower potential contribution ranges (Benstead et al. 2006). My results are consistent with others who found that MixSIAR provides more evenly distributed contribution estimates across all sources, whereas IsoSource estimates high contribution values for sources with signatures closest to the consumer signature (Crowley et al. 2013, Phillips et al. 2014).

IsoSource has traditionally been the primary program used for stable isotope analysis and continues to be utilized (Michener and Lajtha 2007, Bugalho et al. 2008, Shaner and Macko 2011, Newsome et al. 2012, Crowley et al. 2013). It is a robust program capable of handling multiple sources and providing narrow contribution estimates (Phillips and Gregg 2003). However, IsoSource cannot incorporate the uncertainties that more recent programs such as MixSIAR can. MixSIAR incorporates a Bayesian approach to account for uncertainties among source and consumer isotopic values and uncertain DFs; DFs are the greatest sources of uncertainty in food web isotope studies (Stock and Semmens 2013, Phillips et al. 2014). MixSIAR is also beneficial if prior information about the system is already known or can be calculated to include in the mixing model (Phillips et al. 2014). Prior information of prey contribution parameters can be incorporated to develop more accurate posterior probability distributions of source contributions (Moore and Semmens 2008).

Discrimination Factor (DF) Comparison

The DF used for stable isotope analysis can greatly influence contribution results and the DF should be calculated from the same species and tissue being analyzed (Crowley et al. 2013, Phillips et al. 2014). At least 60% of previous studies have used discrimination factors different from the consumer species or tissue analyzed (Caut et al. 2009). Because the diet-tissue fractionation is unknown for otters (Crowley et al. 2013) and using an inaccurate DF can alter source contribution results (Newsome et al. 2012), I compared commonly used DFs for otters within each isotope analysis program. Contribution differences followed a similar directional relationship for different DFs within MixSIAR and IsoSource for pooled CL otters but contribution did not notably change for individual CLA otters. MixSIAR can account for greater variance and therefore seemed to be less sensitive to different DFs than IsoSource (Newsome et al. 2012, Stock and Semmens 2013).

Different DFs had little influence on the primary prey sources within IsoSource for all otters and did not change the primary prey source for the CL otters with either program. However, I am hesitant to report differences between primary prey sources for MixSIAR because the mean contribution estimates include high standard deviations and reporting only the mean values could misrepresent all feasible contribution solutions (Phillips and Gregg 2003). The contribution ranges were similar for different DFs across all sources for MixSIAR, not just the primary prey sources. However, contribution results had greater variance caused by different DFs within IsoSource for all sources compared to MixSIAR.

Silver Carp Isotopic Signatures

Silver carp $\delta^{15}\text{N}$ signatures were similar between the Kaskaskia River Lock and Dam and the Kaskaskia River at CLS but higher than Horseshoe Lake, which is 105 km south and in a

different watershed. Mean $\delta^{13}\text{C}$ signatures of silver carp also appeared to differ among sites. Typically, $\delta^{13}\text{C}$ values vary more than $\delta^{15}\text{N}$ values across habitats and geographic locations, although this was less evident in my results. The differences in carbon sources and primary production at the base of the ecosystem are likely why the $\delta^{13}\text{C}$ values were different between all sites (Freedman et al. 2012). However, $\delta^{15}\text{N}$ signatures differed between the Kaskaskia sites and Horseshoe Lake, indicating a higher nitrogen isotopic baseline for the Kaskaskia River sites. Therefore, silver carp isotopic signatures seem to vary depending on the isotopic baseline of the system and all sources should be collected from the system where the consumers were collected. Silver carp $\delta^{15}\text{N}$ signatures were considerably lower than most of the prey sources, including common prey of otters, but not the lowest I sampled. However, silver carp $\delta^{13}\text{C}$ signatures were more negative than any prey source from their respective system.

Ecological Implications

Apparent competition (Roemer et al. 2002) could play a role if otters frequently consume silver carp. Such abundant food could result in increased otter abundance, potentially producing an indirect negative effect on other prey of otters. Otters are highly susceptible to environmental contaminants, which were likely the reason for a decrease in the Illinois otter population during the 1900s (Woolf et al. 1997), and silver carp contain lower levels of pollutants, especially mercury, than higher-level consumers (Rogowski et al. 2009). Therefore, silver carp could provide a less contaminated food source in polluted waterbodies.

RECOMMENDATIONS

The combination of DF and program had varying levels of influence on the contribution results. Researchers must be cautious when selecting a DF, which should originate from the same species and tissue being studied (Crowley et al. 2013, Phillips et al. 2014). Without prior

information about the parameters of a system, IsoSource provides a clearer depiction of source contributions than MixSIAR. However, MixSIAR provides a useful tool to handle additional information and uncertainties, which are naively disregarded with IsoSource (Phillips et al. 2014). One of the developers of IsoSource even urges researchers to use Bayesian mixing models (Phillips et al. 2014).

Invasive prey species not only pose a threat to native ecosystems but can also alter community dynamics, which is why a better understanding of the impact of silver carp on otters should be a focus of future studies (Vander Zanden et al. 1999, Roemer et al. 2002). Additional research could provide insight into the potential implications of a trophic shift on the impact on the trophic structure of food webs containing otters and silver carp. Stable isotope analysis is an extremely valuable approach to trace elements through food webs and could be incorporated in future studies with additional approaches, such as fatty acid analysis, to provide a more comprehensive view of food webs (Benstead et al. 2006, Jaschinski et al. 2008, Leduc et al. 2009). For future research, I would focus on fewer study sites and increase the number of otter samples by personally trapping otters. I would conduct a thorough sampling effort on the fewer systems to provide a comprehensive catalog of sources. I would also concentrate sampling on baseline sources so I could calculate the trophic position of each otter.

Table I.1. Primary source contribution results ($\geq 50\%$) for different discrimination factors (DFs) from IsoSource for otter samples ($n = 30$) collected in southern Illinois, November–April 2012–14. I indicate combined species with a (+). I indicate sites where I recorded multiple prey sources when the maximum potential contribution was $\geq 50\%$ for each prey species with a (*).

| Site | Otter # | DF = 2% | | DF = 3% | |
|--------------------------------|---------|--------------------------------------|------------------------|--------------------------------|------------------------|
| | | Prey species | Contribution range (%) | Prey species | Contribution range (%) |
| Union Co. Refuge | 1 | Pirate perch ^{+a} | 63-75 | Pirate perch ^{+a} | 85-96 |
| | 2 | Pirate perch ^{+a} | 89-96 | Pirate perch ^{+a} | 96-100 |
| Cypress Cr. NWR | 3 | Devil crayfish | 49-54 | Devil crayfish | 78-81 |
| | 4 | Devil crayfish | 58-69 | Devil crayfish | 88-93 |
| McCorkle Cr. | 5 | Longear sunfish | 73-85 | Longear sunfish | 85-100 |
| | 6 | Central stoneroller/Longear sunfish* | 46-53/43-54 | Longear sunfish | 65-80 |
| Clear Cr. Ditch | 7 | Pirate perch | 100 | Pirate perch | 85-90 |
| Running Lake Ditch | 8 | Longear sunfish ^{+b} | 77-81 | Longear sunfish ^{+b} | 88-92 |
| Cache River - Mt. Pleasant Rd. | 9 | Blackspotted topminnow | 88-100 | Blackspotted topminnow | 88-100 |
| Camp Cr. | 10 | Flier sunfish | 89-90 | Flier sunfish | 100 |
| | 11 | Green sunfish | 72-85 | Pirate perch | 50-53 |
| North Fork Saline River | 12 | Longear sunfish ^{+c} | 71-75 | Longear sunfish ^{+c} | 54-59 |
| | 13 | Longear sunfish ^{+c} | 95-100 | Longear sunfish ^{+c} | 91-94 |
| | 14 | Longear sunfish ^{+c} | 95-100 | Longear sunfish ^{+c} | 92-97 |
| Square Pond | 15 | Pirate perch | 60-77 | Pirate perch | 39-57 |
| | 16 | Pirate perch | 63-74 | Pirate perch/Blackside darter* | 42-54/46-50 |
| | 17 | Pirate perch | 90-100 | Pirate perch | 70-81 |
| Bay Cr. Mouth | 18 | Gizzard shad | 69-78 | Gizzard shad | 48-60 |
| Cache River - Old Cypress Rd. | 19 | Flier sunfish | 75-79 | Flier sunfish | 93-100 |
| Cache River - Belknap | 20 | Flier sunfish | 54-60 | Flier sunfish | 80-84 |
| | 21 | Green sunfish | 93-100 | Green sunfish | 93-100 |
| | 22 | Green sunfish | 94-100 | Green sunfish | 94-100 |

Table I.1. Continued.

| Site | Otter # | DF = 2% | | DF = 3% | |
|--------------|---------|-------------------------------------------|------------------------|--------------|------------------------|
| | | Prey species | Contribution range (%) | Prey species | Contribution range (%) |
| Carlyle Lake | 23 | Silver carp | 38-76 | Silver carp | 61-72 |
| | 24 | Silver carp | 54-68 | Silver carp | 89-95 |
| | 25 | Silver carp | 54-72 | Silver carp | 78-92 |
| | 26 | Silver carp | 78-88 | Silver carp | 74-92 |
| | 27 | White crappie ^{+d} /Silver carp* | 38-56/30-52 | Silver carp | 76-87 |
| | 28 | Silver carp | 62-88 | Silver carp | 66-80 |
| Carlyle Lake | 29 | White crappie ^{+d} | 48-64 | Gizzard shad | 66-84 |
| Spillway | 30 | White crappie ^{+d} | 56-66 | Gizzard shad | 84-96 |

^{+a}Pirate perch combined with mosquitofish

^{+b}Longear sunfish combined with bluegill and green sunfish

^{+c}Longear sunfish combined with bluegill

^{+d}White crappie combined with bluegill

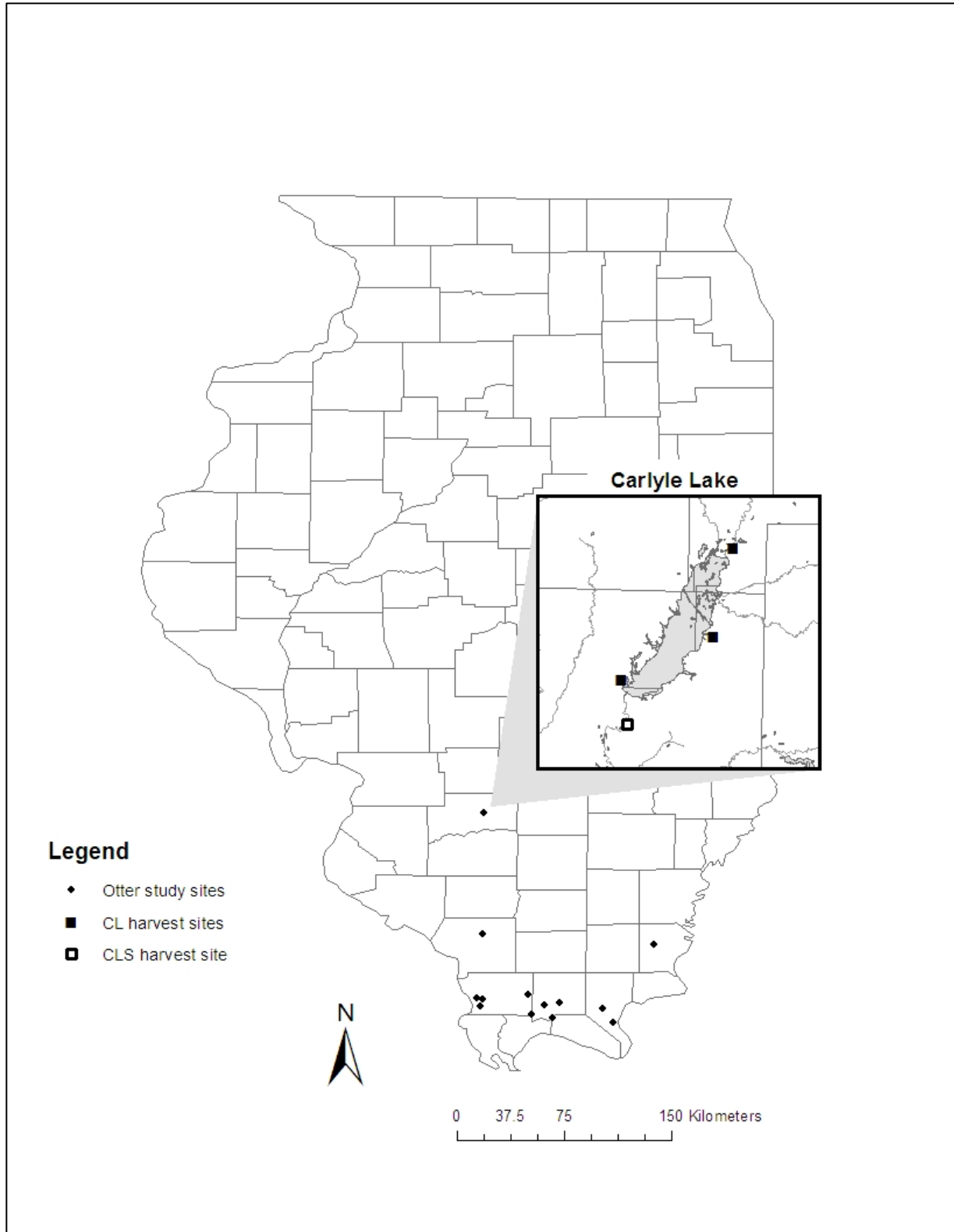


Figure I.1. Study sites (n = 13) of harvested otters (n = 30) in southern Illinois, November–April 2012–14. The enlarged section shows harvest locations (n = 4) for otters from Carlyle Lake (CL) (n = 6) and the Carlyle Lake Spillway (CLS) (n = 2).

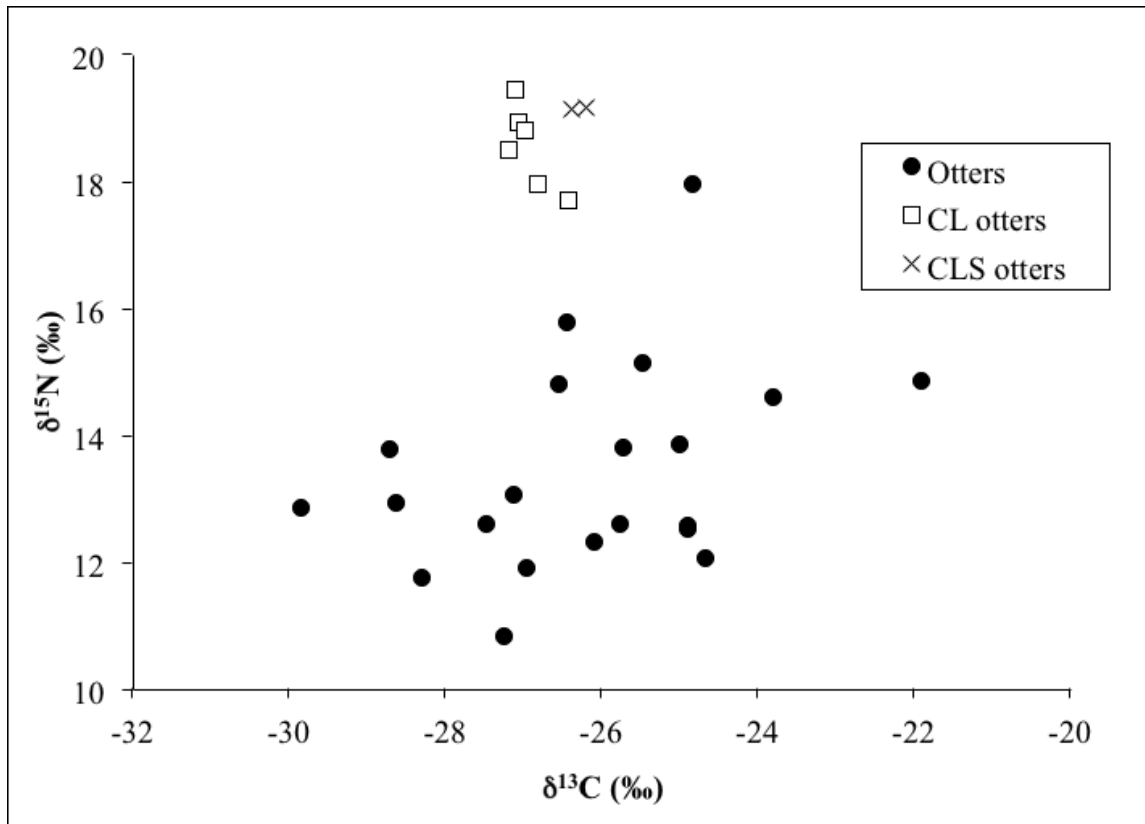


Figure I.2. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures for otter tissue samples collected at sites in southern Illinois, November–April 2012–14. Each symbol represents stable isotope values for otters from Carlyle Lake (CL) ($n = 6$) and the Carlyle Lake Spillway (CLS) ($n = 2$) compared to the otters from areas without silver carp ($n = 22$).

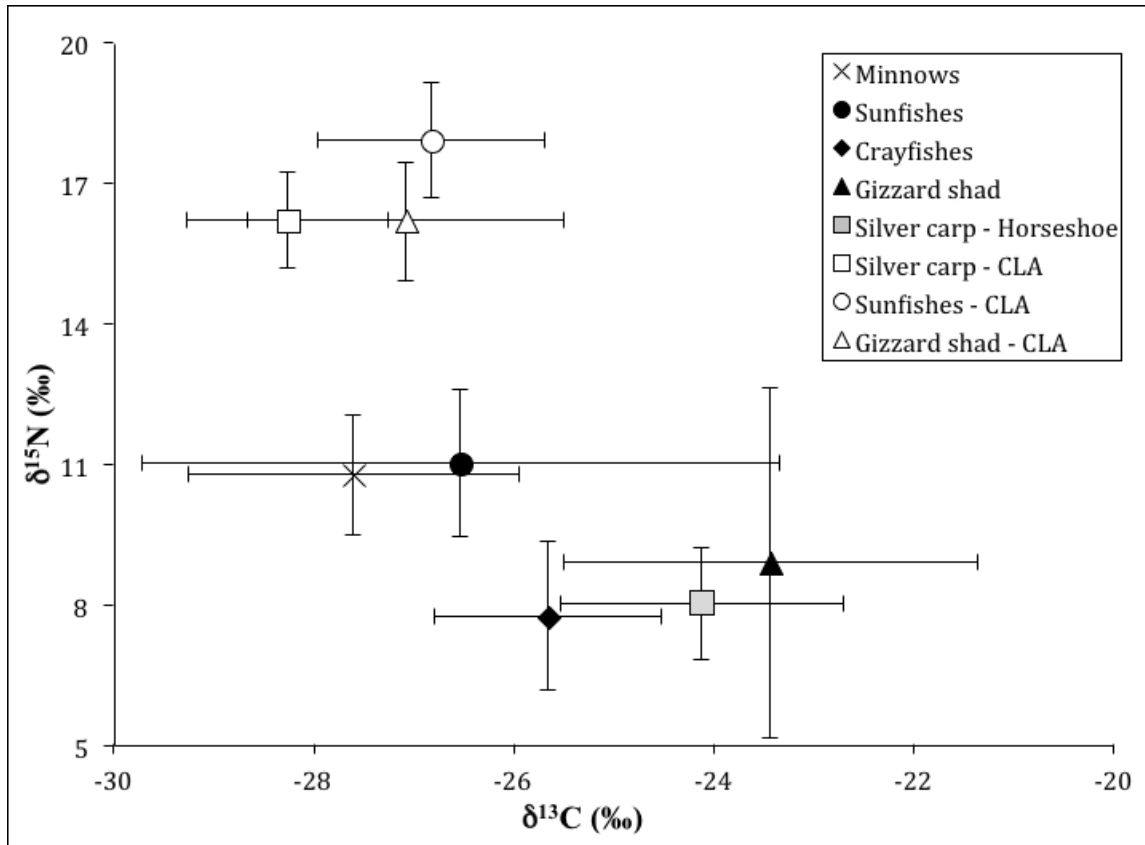


Figure I.3. Mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures for otter prey tissue samples collected at sites in southern Illinois, December–April 2013–14. Each symbol represents stable isotope values for silver carp ($n = 13$), sunfishes ($n = 13$), and gizzard shad ($n = 5$) at the Carlyle Lake Area (CLA) compared to minnows ($n = 45$), sunfishes ($n = 110$), crayfishes ($n = 5$), and gizzard shad ($n = 3$) from areas without silver carp ($n = 22$). Stable isotope values for silver carp ($n = 4$) from Horseshoe Lake are also included.

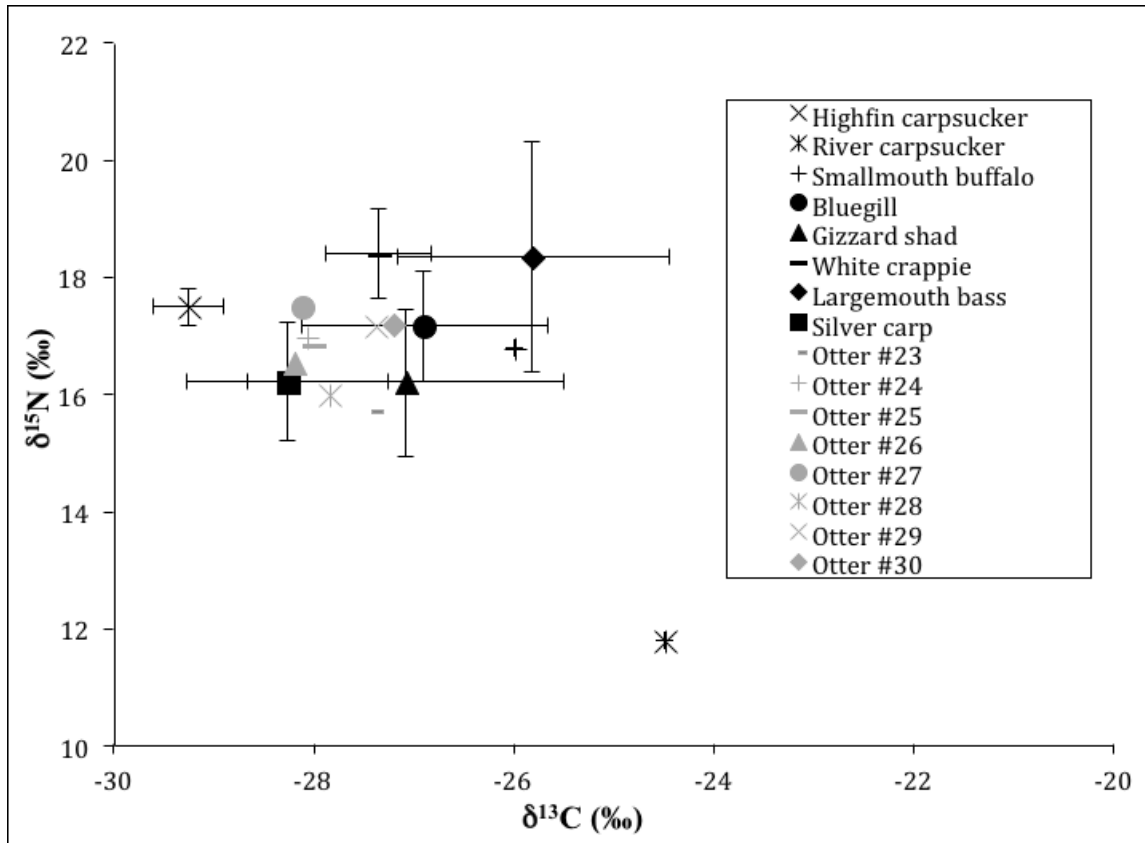


Figure I.4. Mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures for otter and prey tissue samples collected at the Carlyle Lake Area (CLA) in southern Illinois, December–April 2013–14. Each symbol represents stable isotope values for highfin carpsucker (n = 2), river carpsucker (n = 1), smallmouth buffalo (n = 1), bluegill (n = 5), gizzard shad (n = 5), white crappie (n = 5), largemouth bass (n = 3), silver carp (n = 13), and otters (n = 8). The otter isotope values were adjusted using a DF = 2‰ for $\delta^{15}\text{N}$ and DF = 1‰ for $\delta^{13}\text{C}$. A DF = 3‰ would result in each otter $\delta^{15}\text{N}$ value being reduced by 1‰.

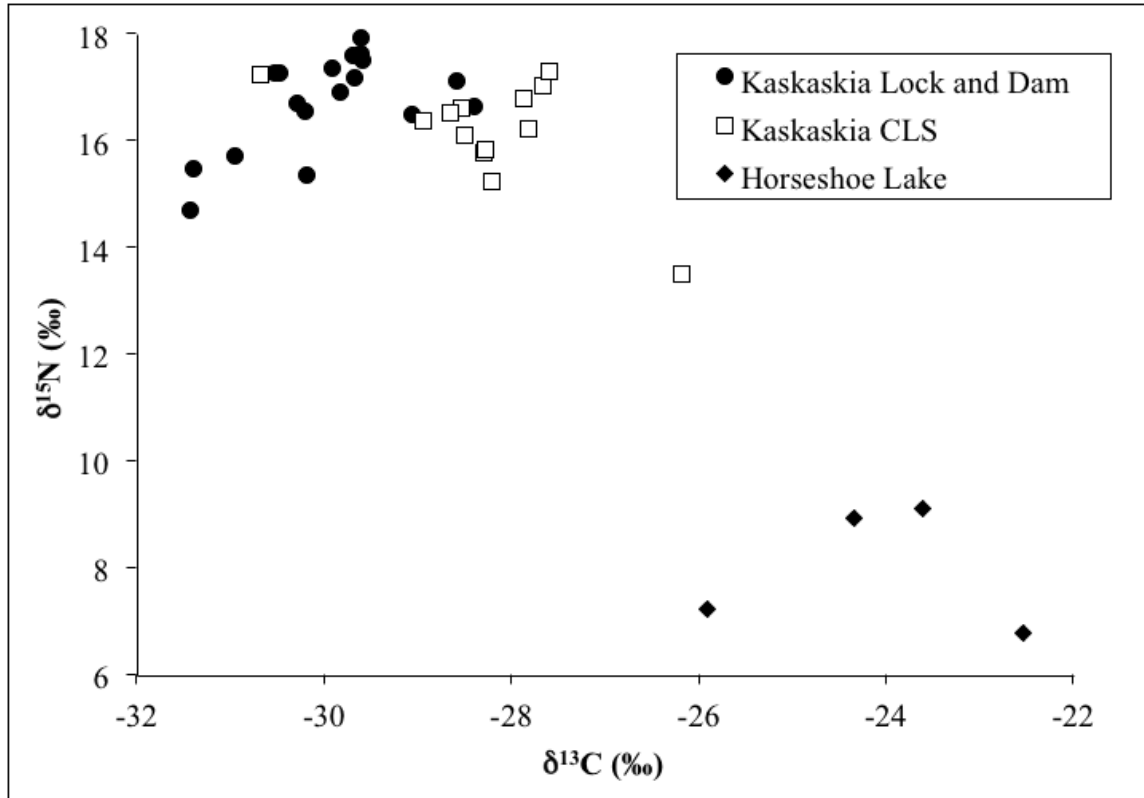


Figure I.5. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures for silver carp tissue samples collected at sites in southern Illinois, December–April 2013–14. Each symbol represents stable isotope values for silver carp from the Kaskaskia River Lock and Dam ($n = 18$), the Kaskaskia River at the Carlyle Lake Spillway (CLS; $n = 13$), and Horseshoe Lake ($n = 4$).

CHAPTER II

ASIAN CARP IN THE DIET OF RIVER OTTERS IN ILLINOIS

INTRODUCTION

Otters are opportunistic aquatic predators that primarily consume fish, followed by crayfish and then amphibians (Lagler and Ostenson 1942, Greer 1955, Knudsen and Hale 1968, Swimley et al. 1998, Stearns and Serfass 2005, Crait and Ben-David 2006, Barding and Lacki 2012). Fish are consumed in the greatest proportion during winter (Stearns and Serfass 2005, Crait and Ben-David 2006, Crimmins et al. 2009, Barding and Lacki 2012), and fish families typically identified in the scat and gut contents of otters include centrarchids, cyprinids, and catostomids (Lagler and Ostenson 1942, Stearns and Serfass 2005, Crait and Ben-David 2006, Barding and Lacki 2012). Centrarchids have appeared in 11–36% of otter scats and cyprinids have appeared in 11–86% of otter scats (Lagler and Ostenson 1942, Stearns and Serfass 2005, Wengeler et al. 2010, Barding and Lacki 2012). Crayfish, where readily available, are typically consumed in greater proportion than fish during summer (Route and Peterson 1988, Roberts et al. 2008). However, crayfish are composed of a greater proportion of hard parts than other prey items, so the dietary importance of crayfish can be overestimated by scat analysis (Cottrell et al. 1996, Tollit et al. 1997, Marcus et al. 1998, van Dijk et al. 2007).

Asian carp abundance and distribution continue to increase throughout Illinois waterbodies and may influence available prey resources for otters. The effect of Asian carp on native fish and plankton communities are subjects of intense study (Williamson 2004, Kolar et al. 2007, Sampson et al. 2009, Chapman and Hoff 2011). Despite the many diet studies of otters, no published studies confirm that otters consume Asian carp outside of aquaculture ponds

(Lanszki et al. 2001, Lanszki and Molnár 2003, Kortan et al. 2007). Additionally, most diet studies in North America have either occurred before Asian carp arrived or occurred in areas without Asian carp (Ryder 1955, Knudsen and Hale 1968, Chabreck et al. 1982, Roberts et al. 2008, Crimmins et al. 2009, Barding and Lacki 2012). Determining the extent to which otters prey on Asian carp is crucial to further understanding the influence these invasive species have on otters and vice versa.

My objective was to estimate the presence of Asian carp in the diet of otters using scat analysis. I used frequency of occurrence to compare scat collected from waterbodies with Asian carp present to waterbodies with Asian carp absent and predicted otters would consume Asian carp when present. I also compared the difference in diet between seasons and land cover types and predicted there would be seasonal differences in the diet for crayfish and amphibians but no difference in the diet between land cover types.

STUDY AREA

I analyzed otter scat collected along waterbodies throughout central and southern Illinois (Figure II.1). Sign surveys for river otter were conducted at 120 bridge sites, selected from the Illinois Department of Natural Resources and Illinois Environmental Protection Agency stream database (A. M. Holtrop, Illinois Department of Natural Resources, personal communication). The sites captured a diverse array of freshwater habitats both with and without Asian carp present. Nineteen percent of sites occurred at 1–3 order headwater streams, 72% at 4–6 order streams, and 9% of sites at larger rivers such as the Saline, Little Wabash, Big Muddy, and Cache rivers. Thirty-nine percent of sites occurred in agriculturally dominated landscapes (>70% agriculture land cover) with 53% of those sites occurring in the northern half of the study area. The remaining sites were located in forest (27.5%), urban (2.5%), and other cover types (31%).

The sites in the southern portion of the study area occurred mainly in mixed hardwood forests (44%), grassland (21%), cropland (19%), wetland (8%), open water (6%), and urban (2%) (Luman et al. 1996).

METHODS

Otter scat was collected opportunistically along 400-m and 800-m stream transects, which began at road bridges, during January–April 2013 and 2014. A team of 2 technicians visited each site 4 times per season. The scat was stored in a Whirl-Pak bag, placed on ice as soon as possible in the field, and stored at -20° C (Mowry et al. 2011, Barding and Lacki 2012). I dried the scat samples at 60° C for 48 hours and then sifted the scat using a no. 18 (1.00 mm) long-handled sieve. (Mowry et al. 2011, Barding and Lacki 2012).

I recorded the presence of fish primarily by identifying scales in the sample. I primarily identified crayfish by their exoskeleton. Amphibians have more robust bones than fish and were discerned from small mammals by a lack of hair found in the scat sample. Prey types were identified using reference collections, taxonomic keys (Duellman and Trueb 1986, Daniels 1996), and photo references.

I examined scat for presence of Asian carp otoliths and pharyngeal teeth and calculated their percentage occurrence in the otters diet. Fish otoliths and pharyngeal teeth have commonly been used as identifying structures in prior otter diet studies (Greer 1955, Trites and Joy 2005, Cote et al. 2008*a*, Wengeler et al. 2010). Ruiz-Olmo et al. (1998) found European otters prefer to begin fish consumption by eating the heads but heads from larger fish (>30 cm) were less frequently consumed. I used physical references of Asian carp sagittal and lapilli otoliths and pharyngeal teeth in addition to photo references (D. C. Chapman, United States Geological Survey, unpublished data). It is not possible to visually distinguish silver carp from bighead carp

by examining their otoliths. However, silver carp have fine horizontal striations on the interior side of their pharyngeal teeth, whereas bighead carp teeth are smooth (Chu 1935, Yokote 1956, Spataru et al. 1983). Fish scales have been used in previous diet studies to differentiate species (Knudsen and Hale 1968, Crait and Ben-David 2006, Barding and Lacki 2012). However, differentiating Asian carp from other cyprinids, especially juveniles, using visual scale identification is particularly problematic and cannot be accomplished with confidence.

To categorize Asian carp presence at my survey sites, I compiled all fish sampling data from Illinois Department of Natural Resources for the stream sites where scat was collected and also referenced the online state stream database (<http://dnr.illinois.gov/IBICalculation/SelectSamplesForm.aspx>, accessed 02 Aug 2014). I used Geographic Information Systems to map Asian carp distribution because Asian carp were not present all survey sites. I determined the occurrence of Asian carp in otter scat collected from sites with Asian carp present (Figure II.1).

I used 2 x 2 contingency tables and Fisher's exact test to compare the frequency of occurrence of each prey type (fish, crayfish, and amphibians) in otter diet between late winter and early spring seasons (January–February, March–April). Given the average temperature from January–February was 3.1° C and from March–April was 10.3° C during the study period (www.wunderground.com, 2015), I predicted consumption of crayfish and amphibians would be higher during March–April than January–February. I used 2 x 3 contingency tables and Fisher's exact test to compare the frequency of occurrence of each prey type in otter diet between 3 land cover types: forest, agriculture, and mixed. I classified sites based on dominant land cover type (>50 % cover) within a 400m buffer around the survey location in a GIS. I defined mixed land cover as not having a dominant land cover type (<50 % cover). All statistical tests were considered significant at $P \leq 0.05$ and were conducted with SPSS 19 (IBM Corp., Armonk, NY).

RESULTS

I analyzed 155 otter scat samples from 43 sites: 56 (36.1%) samples from 2013 and 99 (63.9%) samples from 2014. Forty (25.8%) samples were collected as a solitary spraint and 115 (74.2%) samples were collected from 32 latrines. Asian carp were known to be present in 18 (15%) of the 120 surveyed sites. I collected otter scat from 6 of those sites but only 1 site had otter scat ($n = 2$) containing Asian carp remains. I found evidence of Asian carp in otter scat from 2 additional sites ($n = 1$ each) where there were no database records of Asian carp being present. Thus, Asian carp pharyngeal teeth or otoliths occurred in 4 (2.6%) scat samples.

Fish and crayfish were consumed in the greatest proportion, occurring in 140 (90.3%) and 87 (56.1%) scat samples, respectively. Amphibians occurred in 19 (12.3%) scat samples with 12 (63.2%) of those samples collected during January–February and 7 (36.8%) during March–April. I found hair (unknown species) in 4 (2.6%) scat samples, but the samples did not contain additional evidence of mammal consumption so the hair could potentially be from grooming. I found 220 otoliths in 48 (31.0%) scat samples and pharyngeal teeth in 6 (3.9%) scat samples from fish other than Asian carp. I found centrarchid otoliths in 26 (16.8%) scat samples.

Frequency of occurrence of prey items in the scat was similar between seasons for amphibians (95% confidence interval: -15.6 to 8.0% ; Table II.1). However, frequency of occurrence of crayfish increased from January–February to March–April and I found suggestive evidence that frequency of occurrence of fish decreased from January–February to March–April (Table II.1). Frequency of occurrence was similar between land cover types (forest; $n = 14$, agriculture; $n = 15$, mixed; $n = 14$) for each prey type (Table II.2).

DISCUSSION

I provide the first definitive evidence of North American river otters consuming Asian carp. The lack of Asian carp remains in scat collected from sites with Asian carp present could be the result of a limited number of samples or heads from larger fish (>30 cm) being less frequently consumed (Ruiz-Olmo et al. 1998). Also, otters consume prey in relation to abundance (Melquist et al. 2003, Kruuk 2006, Penland and Black 2009) so Asian carp abundance could have been low, potentially due to the downstream movement of Asian carp in the winter (Coulter et al. 2012), at the sites where I did not find evidence of Asian carp in the scat samples. Interestingly, I found evidence of Asian carp in scat samples from areas with no previous confirmation of Asian carp being present. Monitoring and sampling of Asian carp in Illinois are ongoing because they are prolific dispersers (Sampson et al. 2009, Freedman et al. 2012). Therefore, discovery of new sites containing Asian carp is not unexpected. Additionally, otters could have been foraging in nearby waterbodies containing Asian carp.

My findings are consistent with previous studies confirming fish and crayfish as primary prey items, followed by amphibians (Greer 1955, Knudsen and Hale 1968, Serfass et al. 1990, Stearns and Serfass 2005, Barding and Lacki 2012). The high proportion of fish present in the diet corresponds with previous studies indicating a high reliance on fish as prey during winter (Stearns and Serfass 2005, Crait and Ben-David 2006, Crimmins et al. 2009, Barding and Lacki 2012). Frequency of crayfish occurrence increased during March–April. I expected that crayfish consumption would increase in the summer potentially due to the increased crayfish availability and possibly a decreased ability of otters to capture fish due to their increased swimming speeds with warmer water temperatures (Erlinge 1968, Flint 1977, Wardle 1980). The frequency of occurrence for amphibians did not appear to differ seasonally. Although amphibians are typically

more available in warmer months, their proportion in the diet was similar between seasons and with reported frequencies (Ryder 1955, Knudsen and Hale 1968, Stearns and Serfass 2005, Roberts et al. 2008). Frequency of occurrence was similar between seasons for amphibians and fish, although a difference could be present for fish depending on interpretation of *P*-values. The time frames I set for the seasons were a fairly short range and could have potentially been too short to detect differences in diet. The difference in crayfish proportions could be attributed to greater seasonal fluctuations in abundance (Jędrzejewska et al. 2001).

I did not find evidence that land cover types influenced the frequency of occurrence of prey types at the sampled sites. Prey availability is the primary factor that influences the diet composition of otters and not different habitats (Kemenes and Nechay 1990). Jędrzejewska et al. (2001) found otter diets depended on habitat types. However, habitat types were defined by waterbody size and type and are likely not comparable to the habitat types I used in this study.

I found a considerable number of fish otoliths in the otter scat samples. Otoliths are characteristic for many species of fish and can be easily identified (Cote et al. 2008a, b; Crimmins et al. 2009). Although Asian carp otoliths can be exceptionally small (<2 mm) and difficult to discover in scat, otoliths can be a feasible option for identifying fish species in otter diets (Cote et al. 2008b). I found centrarchid otoliths in each sample containing Asian carp otoliths so otters appear to still be consuming their commonly identified prey in addition to Asian carp according to scat analysis. I only found fish pharyngeal teeth in 6 scat samples. However, pharyngeal teeth provide a valuable method for determining the difference between silver carp and bighead carp (Yokote 1956, Spartaru et al. 1983).

RECOMMENDATIONS

Otoliths and pharyngeal teeth enable efficient identification of fish species in otter diet; either in addition to fish scale identification or used solely when searching for a particular species of interest. I suggest future otter dietary studies involving Asian carp use pharyngeal teeth as a distinguishing structure to differentiate silver carp from bighead carp. I also recommend focusing the study on waterbodies with a high abundance of Asian carp as otters likely consume Asian carp less frequently with lower densities. Asian carp populations will continue to expand and increase in abundance, so future studies may also focus on the effect of the Asian carp invasion on otter diets.

Table II.1. Frequencies of occurrence (%) of prey items for otter scat samples (n = 155) collected in southern Illinois during 2013–14. I used 2 x 2 contingency tables and Fisher's exact test ($df = 1$) to compare prey occurrence between seasons ($\alpha = 0.05$).

| Prey items | Season | | | | <i>P</i> -value |
|------------|------------------------------------|------|------------------------------|------|-----------------|
| | January–February (n = 108 scat) | | March–April (n = 47 scat) | | |
| | n with prey | % | n with prey | % | |
| Fish | 101 | 93.5 | 39 | 83.0 | 0.07 |
| Crayfish | 54 | 50.0 | 33 | 70.2 | 0.02 |
| Amphibian | 12 | 11.1 | 7 | 14.9 | 0.56 |

Table II.2. Frequencies of occurrence (%) of prey items by land cover types for otter scat samples ($n = 155$) in southern Illinois during 2013–14. I used 2 x 3 contingency tables and Fisher's exact test ($df = 2$) to compare prey occurrence between land cover types ($\alpha = 0.05$).

| Prey items | Land cover | | | | | | <i>P</i> -value |
|------------|----------------------------|------|---------------------------------|------|---------------------------|------|-----------------|
| | Forest ($n = 47$ scat) | | Agriculture ($n = 51$ scat) | | Mixed ($n = 57$ scat) | | |
| | n with prey | % | n with prey | % | n with prey | % | |
| Fish | 44 | 93.6 | 40 | 78.4 | 56 | 98.2 | 0.11 |
| Crayfish | 30 | 63.8 | 31 | 60.8 | 26 | 51.0 | 0.70 |
| Amphibian | 9 | 19.1 | 4 | 7.8 | 6 | 10.5 | 0.27 |

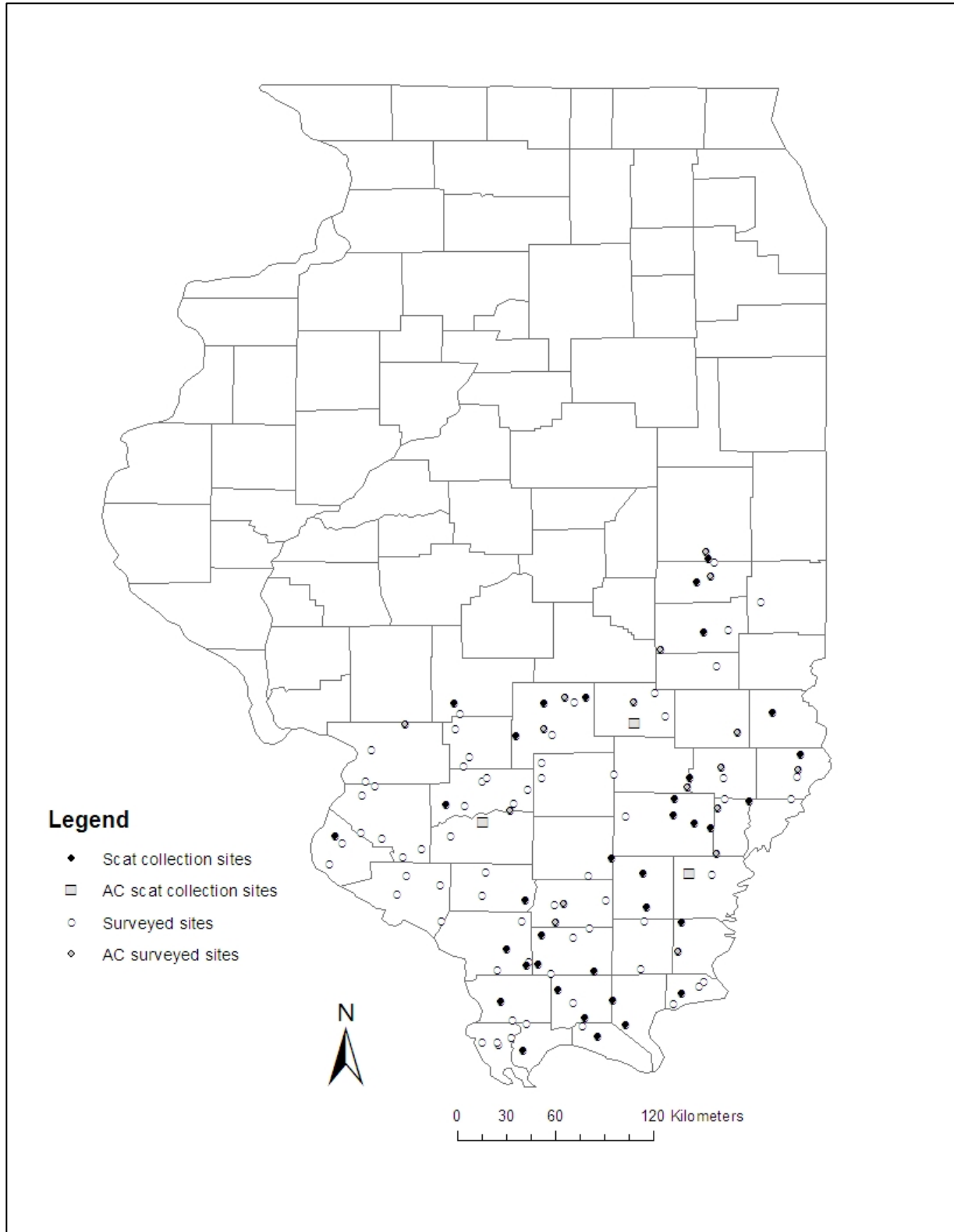


Figure II.1. Otter scat collection sites (n = 43), sites with Asian carp (AC) evidence in the scat (n = 3), total sites surveyed (n = 120), and surveyed sites with Asian carp (AC) present (n = 18) in southern Illinois, January–April 2013–14.

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APPENDICES

APPENDIX A. Prey sources with $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and source combinations for otter study sites ($n = 13$) in southern Illinois, November–April 2012–14. I indicate combined species with a (+).

| Site | Source name | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) | Combined species | |
|--------------------|------------------------------------|---------------------------|---------------------------|--------------------------|-----------------|
| Union Co. Refuge | Blackspotted topminnow | 9.04 | -26.12 | | |
| | Bluegill ⁺ | 10.49 | -27.72 | Bluegill | |
| | Pirate perch ⁺ | | | | Common carp |
| | | | 10.53 | -26.81 | Pirate perch |
| | | | | | Mosquitofish |
| | River carpsucker | 14.26 | -27.19 | | |
| Cypress Cr. NWR | Devil crayfish | 9.12 | -26.03 | | |
| | Longear sunfish | 11.63 | -27.37 | | |
| | Pirate perch | 11.45 | -28.70 | | |
| | White crappie | 12.05 | -29.93 | | |
| McCorkle Cr. | Central stoneroller | 12.05 | -23.68 | | |
| | Longear sunfish | 10.50 | -26.20 | | |
| | Virile crayfish | 6.25 | -24.44 | | |
| | Devil crayfish | 7.49 | -26.55 | | |
| Clear Cr. Ditch | Blackstripe topminnow | 9.36 | -25.28 | | |
| | Blanchard's cricket frog | 6.74 | -27.52 | | |
| | Green sunfish | 10.21 | -34.71 | | |
| | Longear sunfish ⁺ | | 10.18 | -28.84 | Longear sunfish |
| | | | | | Bluegill |
| | Pirate perch | 11.52 | -28.46 | | |
| Running Lake Ditch | Blackstripe topminnow ⁺ | 11.82 | -26.77 | Blackstripe topminnow | |
| | | | | Pirate perch | |
| | Frog/crayfish ⁺ | 9.95 | -25.99 | Blanchard's cricket frog | |
| | | | | White river crayfish | |
| | Longear sunfish ⁺ | | 11.03 | -30.05 | Longear sunfish |
| | | | | Bluegill | |
| | | | | Green sunfish | |
| | Ribbon shiner | 14.10 | -27.78 | | |

APPENDIX A. Continued.

| Site | Source name | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) | Combined species |
|-----------------------------------|------------------------------------|---------------------------|---------------------------|---------------------------------------|
| Running Lake Ditch Cont. | Warmouth ⁺ | 11.71 | -27.73 | Warmouth Orangespotted sunfish |
| | Blackspotted topminnow | 10.54 | -27.27 | |
| Cache River - Mt. Pleasant Rd. | Flier sunfish | 9.28 | -30.06 | |
| | Green sunfish | 14.67 | -27.51 | |
| | Longear sunfish | 11.48 | -27.51 | |
| Camp Cr. | Black bullhead ⁺ | 10.86 | -26.46 | Black bullhead Yellow bullhead |
| | Bluntnose minnow | 11.84 | -28.04 | |
| | Green sunfish | 10.93 | -26.11 | |
| | Longear sunfish ⁺ | 11.90 | -25.13 | Longear sunfish Bluegill |
| | Pirate perch | 10.23 | -28.08 | |
| North Fork Saline River | Blackstripe topminnow ⁺ | 11.74 | -25.87 | Blackstripe topminnow Pirate perch |
| | Largemouth bass | 12.75 | -27.05 | |
| | Longear sunfish ⁺ | 13.96 | -24.65 | Longear sunfish Bluegill |
| | Warmouth | 9.57 | -26.24 | |
| Square Pond | Blackside darter | 9.11 | -32.11 | |
| | Blackspotted topminnow | 10.66 | -27.67 | |
| | Longear sunfish ⁺ | 11.40 | -27.80 | Longear sunfish Warmouth |
| | Pirate perch | 10.12 | -28.06 | |
| Bay Cr. Mouth | Bluntnose minnow | 11.36 | -30.37 | |
| | Gizzard shad | 13.10 | -25.60 | |
| | Largemouth bass | 10.74 | -26.86 | |
| | Longear sunfish ⁺ | 12.85 | -28.86 | Longear sunfish Bluegill |

APPENDIX A. Continued.

| Site | Source name | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) | Combined species |
|----------------------------------|-------------------------------------|---------------------------|---------------------------|------------------------------------------------------------------|
| Bay Cr. Mouth Cont. | Warmouth ⁺ | 10.84 | -29.72 | Green sunfish |
| | | | | Warmouth |
| | | | | Orangespotted sunfish |
| | | | | Redear sunfish |
| Cache River - Old Cypress Rd. | Blackspotted topminnow | 11.27 | -27.84 | |
| | Brook silverside | 11.05 | -28.03 | |
| | Flier sunfish | 10.66 | -30.87 | |
| | Longear sunfish ⁺ | 11.89 | -28.18 | Longear sunfish Warmouth |
| Cache River - Belknap | Blackspotted topminnow ⁺ | 9.35 | -31.98 | Blackspotted topminnow Banded pygmy sunfish Emerald shiner |
| | Flier sunfish | 7.10 | -30.48 | |
| | Green sunfish | 10.26 | -29.72 | |
| | Longear sunfish | 10.67 | -30.91 | |
| | Spotted bass | 12.46 | -32.91 | |
| Carlyle Lake | Gizzard shad ⁺ | 16.56 | -27.69 | Gizzard shad Highfin carpsucker |
| | Largemouth bass | 18.33 | -25.80 | |
| | River carpsucker | 11.79 | -24.48 | |
| | Silver carp | 16.22 | -28.26 | |
| | White crappie ⁺ | 17.77 | -27.12 | White crappie Bluegill |
| Carlyle Lake Spillway | Gizzard shad | 16.19 | -27.07 | |
| | Highfin carpsucker | 17.49 | -29.25 | |
| | Largemouth bass | 18.33 | -25.80 | |
| | Silver carp | 16.22 | -28.26 | |
| | White crappie ⁺ | 17.77 | -27.12 | White crappie Bluegill |

APPENDIX B. Paired t-test results of differences for mean (\pm SE) source contribution results (%) between MixSIAR and IsoSource and different discrimination factors (DFs) within each program for Carlyle Lake (CL) otters (n = 6) in southern Illinois, April 2014. Results were significantly different with a *P*-value ≤ 0.05 . I indicated combined species with a (⁺).

| | | Source contributions | | | | |
|-----------------------------|-----------|-----------------------------|-----------------------------|----------------------------|-----------------------------|-----------------------------|
| Comparison | Group | White crappie ^{+a} | Gizzard shad ^{+b} | River carpsucker | Largemouth bass | Silver carp |
| MixSIAR | DF = 2‰ | 22.7 \pm 1.4 | 23.0 \pm 0.4 | 11.5 \pm 2.3 | 17.5 \pm 1.2 | 25.4 \pm 0.9 |
| | DF = 3‰ | 20.0 \pm 1.7 | 23.1 \pm 0.8 | 14.9 \pm 3.1 | 16.0 \pm 0.5 | 25.9 \pm 0.9 |
| | | $t_5 = 6.78, P \leq 0.001$ | $t_5 = 0.51, P = 0.63$ | $t_5 = 8.44, P \leq 0.001$ | $t_5 = 3.64, P = 0.02$ | $t_5 = 0.87, P = 0.42$ |
| IsoSource | DF = 2‰ | 21.2 \pm 17.5 | 8.1 \pm 4.4 | 4.0 \pm 6.7 | 1.8 \pm 0.9 | 65.0 \pm 14.3 |
| | DF = 3‰ | 2.9 \pm 4.8 | 3.8 \pm 1.3 | 12.0 \pm 10.4 | 0.4 \pm 0.3 | 81.0 \pm 8.8 |
| | | $t_5 = 3.12, P = 0.03$ | $t_5 = 2.20, P = 0.08$ | $t_5 = 4.17, P = 0.01$ | $t_5 = 3.46, P = 0.02$ | $t_5 = 2.28, P = 0.07$ |
| Between programs DF = 2‰ | MixSIAR | 22.7 \pm 1.4 | 23.0 \pm 0.4 | 11.5 \pm 2.3 | 17.5 \pm 1.2 | 25.4 \pm 0.9 |
| | IsoSource | 21.2 \pm 17.5 | 8.1 \pm 4.4 | 4.0 \pm 6.7 | 1.8 \pm 0.9 | 65.0 \pm 14.3 |
| | | $t_5 = 0.23, P = 0.83$ | $t_5 = 7.72, P \leq 0.001$ | $t_5 = 4.04, P = 0.01$ | $t_5 = 21.83, P \leq 0.001$ | $t_5 = 7.20, P \leq 0.001$ |
| Between programs DF = 3‰ | MixSIAR | 20.0 \pm 1.7 | 23.1 \pm 0.8 | 14.9 \pm 3.1 | 16.0 \pm 0.5 | 25.9 \pm 0.9 |
| | IsoSource | 2.9 \pm 4.8 | 3.8 \pm 1.3 | 12.0 \pm 10.4 | 0.4 \pm 0.3 | 81.0 \pm 8.8 |
| | | $t_5 = 11.66, P \leq 0.001$ | $t_5 = 30.94, P \leq 0.001$ | $t_5 = 0.97, P = 0.38$ | $t_5 = 86.79, P \leq 0.001$ | $t_5 = 16.86, P \leq 0.001$ |

^{+a}White crappie combined with bluegill

^{+b}Gizzard shad combined with highfin carpsucker

APPENDIX C. Mixing model source contribution results (%) for MixSIAR and IsoSource (Bold) for each discrimination factor (DF) at Carlyle Lake (CL) and Carlyle Lake Spillway (CLS) in southern Illinois, April 2014. Mean (\pm SD) contribution results are provided with 95% credible intervals for MixSIAR and minimum and maximum ranges for IsoSource. I indicated combined species with a (+).

| Otter # | DF (‰) | Source contributions | | | | |
|---------|---------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|-----------------------------------------|-------------------------------------------|
| | | White crappie ^{+a} | Gizzard shad ^{+b} | River carpsucker | Largemouth bass | Silver carp |
| 23 | 2.5 \pm 0.5 | 19.4 \pm 15.6(1.5-51.0) | 22.3 \pm 17.1(1.6-55.5) | 16.9 \pm 12.7(1.1-41.1) | 15.8 \pm 13.6(0.9-43.5) | 25.6 \pm 18.4(1.9-60.2) |
| | 2 | 20.8 \pm 16.0(1.5-52.5) | 22.4 \pm 16.4(1.7-53.0) | 15.2 \pm 11.6(1.1-36.2) | 16.3 \pm 13.8(1.1-43.9) | 25.3 \pm 18.2(2.2-59.7) |
| | 3 | 18.6 \pm 15.4(1.4-50.2) | 21.9 \pm 17.0(1.5-54.6) | 19.2 \pm 14.4(1.3-46.5) | 15.9 \pm 13.7(1.0-43.9) | 24.4 \pm 17.7(1.8-58.4) |
| | 2 | 5.1\pm3.5(0.0-14.0) | 16.1\pm10.7(0.0-44.0) | 16.0\pm0.0(16.0-16.0) | 3.6\pm2.8(0.0-10.0) | 59.2\pm8.7(38.0-76.0) |
| | 3 | 0.6\pm0.8(0.0-3.0) | 2.9\pm2.7(0.0-11.0) | 28.5\pm0.6(28.0-30.0) | 0.2\pm0.4(0.0-1.0) | 67.7\pm2.3(61.0-72.0) |
| 24 | 2.5 \pm 0.5 | 22.8 \pm 17.0(1.7-55.5) | 22.9 \pm 17.0(1.6-55.1) | 11.5 \pm 10.0(0.7-30.7) | 17.2 \pm 14.6(1.2-46.5) | 25.8 \pm 18.4(2.0-60.7) |
| | 2 | 23.9 \pm 18.4(1.5-59.6) | 23.3 \pm 17.5(1.6-58.0) | 9.9 \pm 9.8(0.5-30.1) | 18.0 \pm 14.8(1.2-48.2) | 25.0 \pm 18.5(1.8-60.6) |
| | 3 | 20.5 \pm 16.3(1.3-53.7) | 23.8 \pm 17.7(1.8-56.8) | 12.6 \pm 10.8(0.7-33.7) | 16.3 \pm 14.0(0.9-44.4) | 26.8 \pm 19.0(1.9-62.6) |
| | 2 | 32.1\pm2.7(28.0-38.0) | 5.3\pm4.7(0.0-16.0) | 0.0 | 1.0\pm1.3(0.0-4.0) | 61.6\pm3.5(54.0-68.0) |
| | 3 | 0.4\pm0.6(0.0-2.0) | 1.8\pm1.9(0.0-6.0) | 5.5\pm0.5(5.0-6.0) | 0.1\pm0.2(0.0-1.0) | 92.3\pm1.6(89.0-95.0) |
| 25 | 2.5 \pm 0.5 | 21.5 \pm 16.9(1.4-54.8) | 24.0 \pm 17.7(1.7-57.2) | 11.7 \pm 10.2(0.8-32.1) | 16.6 \pm 14.1(1.0-45.3) | 26.2 \pm 19.0(2.1-62.4) |
| | 2 | 23.9 \pm 17.6(1.7-56.8) | 22.7 \pm 17.1(1.7-54.8) | 10.2 \pm 9.6(0.4-28.5) | 18.2 \pm 14.9(1.3-48.1) | 25.0 \pm 18.4(1.8-60.6) |
| | 3 | 20.6 \pm 16.5(1.3-53.9) | 23.5 \pm 17.5(1.9-57.3) | 13.6 \pm 11.2(0.7-35.4) | 15.5 \pm 13.7(1.0-43.3) | 26.8 \pm 19.3(2.4-63.1) |
| | 2 | 28.2\pm3.4(22.0-36.0) | 6.1\pm5.4(0.0-20.0) | 0.0 | 1.6\pm1.8(0.0-6.0) | 64.1\pm4.2(54.0-72.0) |
| | 3 | 1.2\pm1.5(0.0-4.0) | 4.4\pm4.0(0.0-14.0) | 8.2\pm0.5(8.0-10.0) | 0.5\pm0.9(0.0-2.0) | 85.8\pm3.6(78.0-92.0) |
| 26 | 2.5 \pm 0.5 | 20.3 \pm 16.3(1.4-52.5) | 23.5 \pm 17.8(1.6-58.5) | 13.1 \pm 11.5(0.7-35.5) | 16.5 \pm 14.1(1.1-44.7) | 26.6 \pm 19.4(1.9-62.8) |
| | 2 | 22.6 \pm 17.0(1.8-54.9) | 23.3 \pm 17.5(1.6-56.3) | 11.0 \pm 10.0(0.6-30.9) | 16.8 \pm 14.3(1.1-44.4) | 26.4 \pm 18.8(2.0-61.7) |
| | 3 | 19.5 \pm 15.8(1.3-51.2) | 23.7 \pm 17.6(1.7-57.3) | 15.1 \pm 12.2(1.0-38.7) | 15.6 \pm 13.8(0.8-43) | 26.1 \pm 19.1(1.9-61.7) |
| | 2 | 10.8\pm2.6(6.0-16.0) | 3.9\pm3.7(0.0-12.0) | 0.0 | 1.1\pm1.5(0.0-4.0) | 84.2\pm2.9(78.0-88.0) |
| | 3 | 1.5\pm2.0(0.0-8.0) | 5\pm4.7(0.0-16.0) | 9.5\pm1.1(8.0-12.0) | 0.3\pm0.8(0.0-2.0) | 83.7\pm4.4(74.0-92.0) |

APPENDIX C. Continued.

| Otter # | DF (‰) | Source contributions | | | | |
|----------|-------------------------|------------------------------|-----------------------------|-----------------------------|-----------------------------|------------------------------|
| | | White crappie ^{+a} | Gizzard shad ^{+b} | River carpsucker | Largemouth bass | Silver carp |
| 27 | 2.5±0.5 | 22.9±17.1(1.8-55.3) | 23.4±17.5(1.8-56.2) | 10.2±10.0(0.5-30.3) | 18.1±14.9(1.3-47.3) | 25.5±18.6(1.9-61.1) |
| | 2 | 23.7±18.1(1.5-58.1) | 23.4±17.8(1.7-58.9) | 9.3±9.4(0.5-27.7) | 19.4±15.7(1.3-50.3) | 24.3±18.5(1.4-58.8) |
| | 3 | 22.8±17.2(1.7-55.4) | 23.5±17.3(1.8-56.8) | 11.2±9.7(0.7-30.5) | 16.9±14.4(1.1-46.5) | 25.6±18.8(1.9-61.4) |
| | 2 | 47.2±4.0(38.0-56.0) | 7.3±6.5(0.0-26.0) | 0.0 | 1.9±2.1(0.0-8.0) | 43.6±5.2(30.0-52.0) |
| | 3 | 12.7±2.0(8.0-17.0) | 3.6±3.3(0.0-13.0) | 0.0 | 0.9±1.1(0.0-4.0) | 82.8±2.6(76.0-87.0) |
| 28 | 2.5±0.5 | 20.2±16.1(1.4-51.9) | 22.9±17.4(1.7-57.6) | 14.9±12.2(0.9-38.7) | 16.1±14.0(1-44.2) | 25.9±18.9(1.7-61.0) |
| | 2 | 21.0±16.2(1.6-52.2) | 22.9±17.2(1.6-55.7) | 13.2±10.9(0.8-34.2) | 16.4±14.0(1.1-44.5) | 26.5±19.0(2.0-61.6) |
| | 3 | 18.2±15.2(1-49.1) | 22.3±17.2(1.3-56.4) | 17.8±13.7(1.3-43.9) | 16.0±13.8(1-44.3) | 25.6±18.6(1.9-60.7) |
| | 2 | 3.5±2.9(0.0-10.0) | 9.9±7.7(0.0-30.0) | 8.0±0.0(8.0-8.0) | 1.6±1.7(0.0-6.0) | 77.0±6.1(62.0-88.0) |
| | 3 | 0.8±1.2(0.0-4.0) | 4.9±4.3(0.0-14.0) | 20.1±0.4(20.0-22.0) | 0.4±0.8(0.0-2.0) | 73.8±3.9(66.0-80.0) |
| Totals | | 21.3±16.6 12.0±2.3 | 23.1±17.3 5.9±5.0 | 13.1±11.1 8.0±0.3 | 16.8±14.2 1.1±1.3 | 25.7±18.7 73.0±4.1 |
| 29 | | White crappie ^{+a} | Gizzard shad | Highfin carpsucker | Silver carp | |
| | 2.5±0.5 | 26.8±19.5(1.9-63.8) | 24.9±17.8(2.2-58.3) | 21.2±16.2(1.7-30.6) | 27.1±18.9(2.4-62.6) | |
| | 2 | 29.3±19.5(2.5-65.3) | 23.8±17.6(2.0-56.9) | 22.5±17.4(1.4-56.7) | 24.4±18.1(1.7-59.0) | |
| | 3 | 25.6±19.3(1.8-63.3) | 27.7±18.8(2.8-63.1) | 18.8±15.0(1.2-48.9) | 27.9±20.0(2.0-65.9) | |
| | 2 | 56.6±3.9(48.0-64.0) | 24.9±7.0(10.0-38.0) | 6.4±4.3(0.0-14.0) | 12.2±7.6(0.0-26.0) | |
| 3 | 0.5±0.9(0.0-2.0) | 75.0±4.7(66.0-84.0) | 1.5±1.7(0.0-4.0) | 23.0±5.3(12.0-32.0) | | |
| 30 | 2.5±0.5 | 27.9±19.6(2.4-64.5) | 26.0±18.4(2.3-61.6) | 20.1±15.9(1.4-51.0) | 26.1±18.7(2.3-61.3) | |
| | 2 | 30.7±20.2(2.7-67.2) | 24.0±17.5(2.2-57.5) | 21.8±17.4(1.5-57.3) | 23.5±17.8(1.8-58.8) | |
| | 3 | 25.4±18.8(2.0-62.2) | 28.3±19.3(2.6-64.3) | 18.1±14.7(1.4-48.3) | 28.2±20.0(2.0-66.3) | |

APPENDIX C. Continued.

| Otter # | DF (%) | Source contributions | | | |
|----------|--------|-----------------------------|----------------------------|-------------------------|--------------------------|
| | | White crappie ^{+a} | Gizzard shad | Highfin carpsucker | Silver carp |
| 30 Cont. | 2 | 61.9±2.6(56.0-66.0) | 30.8±4.0(22.0-38.0) | 2.2±2.0(0.0-6.0) | 5.0±3.7(0.0-12.0) |
| | 3 | 0.7±1.0(0.0-2.0) | 90.0±3.1(84.0-96.0) | 1.6±1.6(0.0-4.0) | 7.7±3.9(0.0-14.0) |
| | Totals | 27.6±19.5 | 25.8±18.2 | 20.4±16.1 | 26.2±18.9 |
| | | 29.9±2.1 | 55.2±4.7 | 2.9±2.4 | 12.0±5.1 |

^{+a}White crappie combined with bluegill

^{+b}Gizzard shad combined with highfin carpsucker

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