5-1-2023

MULTI-SPECIES TROPHIC RESPONSE IN TRIBUTARIES OF THE OHIO RIVER ALONG A GRADIENT OF AN INVASIVE PLANKTIVORE

Justin Kowalski
Southern Illinois University Carbondale, jkwal96@gmail.com

MULTI-SPECIES TROPHIC RESPONSE IN TRIBUTARIES OF THE OHIO RIVER ALONG A GRADIENT OF AN INVASIVE PLANKTIVORE

by

Justin James Kowalski

B.S., University of Wisconsin – Stevens Point, 2018

A Thesis
Submitted in Partial Fulfilment of the Requirements of the
Master of Science Degree

School of Biological Sciences
in the Graduate School
Southern Illinois University Carbondale
May 2023

THESIS APPROVAL

MULTI-SPECIES TROPHIC RESPONSE IN TRIBUTARIES OF THE OHIO RIVER ALONG A GRADIENT OF AN INVASIVE PLANKTIVORE

by

Justin Kowalski

A Thesis Submitted in Partial

Fulfillment of the Requirements

for the Degree of

Master of Science

in the field of Zoology

Approved by:

Dr. James E. Garvey, Chair

Dr. Gregory W. Whitledge

Dr. James R. Lovvorn

Dr. Alison A. Coulter

Graduate School
Southern Illinois University Carbondale
March 22, 2023

AN ABSTRACT OF THE THESIS OF

Justin Kowalski, for the Master of Science degree in Zoology, presented on January 12, 2023 at Southern Illinois University Carbondale

TITLE: MULTI-SPECIES TROPHIC RESPONSE IN TRIBUTARIES OF THE OHIO RIVER ALONG A GRADIENT OF AN INVASIVE PLANKTIVORE

MAJOR PROFESSOR: Dr. James E. Garvey

1. Aquatic invasive species often have a gradient of abundance along connected systems as invasion occurs. Invading species effects on native species may not be apparent when the species first colonizes a new area but as abundance of the invader increases they may have detrimental effects to native ecosystems even when not fully established.

2. A gradient of Silver Carp (*Hypophthalmichthys molitrix*) abundance in the Ohio River exists as invasion has been slowed by the many navigation dams that exist on the river. I examined how the isotopic niche of four native species differed along the Silver Carp gradient in tributaries of the Ohio River using stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$). I also determined if the body condition of native species changed in tributaries of the Ohio River along the invasion gradient using relative weight.

3. Trophic dynamics of Gizzard Shad (*Dorosoma cepedianum*) and Largemouth Bass (*Micropterus salmoides*) differed along the Silver Carp gradient. Isotopic niche space was larger and relative weight was lower where Silver Carp were more abundant. Trophic dynamics of Bluegill (*Lepomis macrochirus*) and Smallmouth Buffalo (*Ictiobus bulbus*) did not differ along the Silver Carp gradient. Trophic chains were compressed where Silver Carp were abundant compared to where they were rare or absent.

4. Invasive Silver Carp may have community wide effects on native species. However, feeding pathways of native species may play an important role in determining how a native species will

be affected by invading Silver Carp as species that belong to the pelagic food web were most affected by Silver Carp invasion.

5. Future research should continue to focus on how native fish species from various trophic positions may be affected by Silver Carp invasion and how Silver Carp invasion plays a role in the complex interactions between biotic and abiotic factors in a complex, altered river system such as the Ohio River.

ACKNOWLEDGEMENTS

Funding for this project was provided by the United States Fish and Wildlife Service and the Center for Fisheries, Aquaculture, and Aquatic Sciences at Southern Illinois University - Carbondale. There are many people to thank for assisting with this project. Without assistance none of this would have been possible. I would first like to thank my committee members: my advisor James Garvey for his guidance, support, and for obtaining funding for the project along with this guidance leading to my professional growth, I would also like to thank Greg Whitledge, and Jim Lovvorn for their guidance, input, and contributions to my professional development. I would also like to thank Katie Zipfel from the West Virginia Department of Natural Resources, Mike Depew from the Pennsylvania Fish and Boat Commission, and Cassi Carpenter from Eastern Illinois University for their assistance in fish collection and valuable advice without which this project could not have been completed. I would also like to thank my fellow graduate students and technicians for allowing me to bounce ideas off of and assistance in processing samples in the lab. I would specifically like to thank Joe Rector, Patrick Padilla, Octavio Silva, and Michael Lucarelli for their help in the field collecting fish, and Azareah Carson, Zach Verzino, Meredith Moore, Clint Burton, and Christian Hutchison for their help processing samples in the lab. Finally, I would like to thank my family and friends for their support and patience throughout my education and completion of this project.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

The range expansion of invasive species often depends on a complex interaction between abiotic and biotic factors (Stohlgren et al. 2005; Cheng and Hovel 2010; Averett et al. 2016). Abiotic and biotic factors not only limit where species invade but also the rate at which invasion may occur (Cheng and Hovel 2010). There are two leading theories for how biotic variables influence species invasions. The first theory is that invasive species take advantage of an unoccupied niche. The unoccupied niche could either be unexploited or underutilized due to a decreased abundance in a native species (Seabloom et al. 2003; Sol et al. 2012; Pyron et al. 2017). In the case that the niche was vacated, the invading species could prevent native species from returning (Seabloom et al. 2003). The second theory is that invasive species outcompete or alter the environment and subsequently displace native species (Mills et al. 1994; Vitousek et al. 1996; Vander Zanden et al. 1999; Dangremond et al. 2010; DeBoer et al. 2018).

The range expansion of invasive species can often have lasting effects on established food webs, and invasive species often compete with native species (Mills et al. 1994; Vitousek et al. 1996; Vander Zanden et al. 1999; DeBoer et al. 2018; Bradley 2019; Cucherousset et al. 2020). Food webs are complex, providing a link between resources and consumers throughout an ecosystem (Paine 1980; Power 1990; Polis and Strong 1996; Vander Zanden et al. 1999). Within a system, consumers often rely on multiple prey resources and may utilize multiple habitats when feeding (Polis and Strong 1996; Vander Zanden et al. 1999). Changes in environmental conditions, both biotic and abiotic, can have lasting effects on established food webs, which can have direct or indirect effects on consumers (Vitousek et al. 1996; Harmon et al. 2009; Pyron et al. 2017; Bowes et al. 2020).

Competition for food resources with invasive species can have various effects on native species. Invasive species may displace native species from favorable feeding locations and alter behaviors causing a shift in their trophic niche (Vander Zanden et al. 1999; DeBoer et al. 2018; Wainright et al. 2021; Harris et al. 2022). The source of resources (Vander Zanden et al. 1999; Cucherousset et al. 2020) and selected prey (Vander Zanden et al. 1999; Freedman et al. 2012) of native species may shift. Trophic niche shifts may result in reduced condition or abundance of native species as sufficient resources to sustain the native population are no longer available (Vitousek et al. 1996; Solomon et al. 2016 Chick et al. 2020). The trophic position of the invading species plays a key role in the overall effect on native species. In their meta-analysis, Bradley et al. (2019) found that even though invaders of all trophic levels had negative impacts, invaders had higher effects on native species with lower or similar trophic positions.

Silver Carp (*Hypophthalmichthys molitrix*) is an invasive fish species that has established populations in the Mississippi River and many of its tributaries. Silver Carp were brought to the United States in the early 1970s for algae control in Arkansas aquaculture facilities, and escaped during flooding (Kolar et al. 2005). Since their escape, Silver Carp have been identified as an economic and ecological threat to aquatic ecosystems. The combined economic loss of Silver Carp and Bighead Carp to the Great Lakes recreational fishery alone could be as high as $139 million (Lauber et al. 2020). Silver Carp diets are composed of mostly zooplankton and phytoplankton, and Silver Carp can adapt their diet to match zooplankton and phytoplankton resources that are available in different systems (Williamson and Garvey 2005; Sampson et al. 2009; Hayer 2014; Tumolo and Flinn 2017). Past studies have suggested that Silver Carp can decrease zooplankton and phytoplankton abundance and alter the make-up of zooplankton composition in ecosystems following invasion (Sass et al. 2014; DeBoer et al.

2018). Decreases in abundance and changes in zooplankton and phytoplankton composition due to Silver Carp invasion could have detrimental ecological effects throughout the food web of aquatic ecosystems, especially on native planktivores and pelagic species.

One way that Silver Carp may affect native communities is through direct competition. Diets of Silver Carp overlap with native species that consume zooplankton and phytoplankton including Gizzard Shad and Bigmouth Buffalo (Sampson et al. 2009; Hayer 2014; Minder and Pyron 2018; Wang et al. 2018). The degree of overlap has been shown to vary depending on season, system, and productivity (Sampson et al. 2009; Minder and Pyron 2018; Wang et al. 2018; Coulter et al. 2019). Body condition of native planktivores declined following the establishment of Silver Carp (Irons et al. 2007; Pendleton et al., 2017). Changes to zooplankton and phytoplankton communities and native planktivores caused by Silver Carp may also affect native fish that do not consume plankton. Community wide changes in native fish assemblages have been observed following Silver Carp invasion (Solomon et al. 2016; Chick et al. 2020). Chick et al. (2020) concluded that the decline of sport fish in the Upper Mississippi River may be due to competition for zooplankton between larval and juvenile sportfish and Silver Carp. If Silver Carp are causing trophic changes in native species, it is important to understand how the trophic niche of native species from different feeding guilds are affected.

Previous studies have focused primarily on how Silver Carp affect the trophic status of only native planktivore species (Coulter et al. 2019; Wang et al. 2018; Harris et al. 2022; Lebeda et al. 2022). This study will investigate how Silver Carp affect native species at different trophic positions to examine how community dynamics of selected native species might be affected along an invasion gradient. This study will also examine if the effects of invasive Silver Carp might be distinguishable from the complex interactions that occur in large rivers such as the

Ohio River. The objectives of this study were to (1) quantify if there is a shift in trophic position of selected native species along a gradient of Silver Carp invasion, (2) determine how the isotopic niche space of selected native species change along a gradient of Silver Carp invasion, (3) determine if there is a shift in body condition of selected native species along a gradient of Silver Carp invasion, and (4) explore how observed results compare to expected trophic relationships expected as a result of large river concepts and how those relationships might differ when Silver Carp invasion occurs.

CHAPTER 2

METHODS

Study Area

The Ohio River is a large tributary to the Mississippi River that flows approximately 1578 km from the confluence of the Monongahela and Allegheny rivers in Pittsburgh, Pennsylvania to Cairo, Illinois where the river drains into the Mississippi River. The Ohio River is highly impounded with 19 navigation dams separating the main channel of the river into 20 pools. The Ohio River basin covers nearly 52,8357 km^2 and represents a variety of land uses including agricultural, forested, industrial, and urban.

Silver Carp invaded the Ohio River in the 1980s and have been captured as far upstream as the RC Byrd Pool near Gallipolis, Ohio. The invasion of Silver Carp in the Ohio River has been slowed by the many navigation dams resulting in three Silver Carp invasion zone categories defined by the United States Fish and Wildlife Service (ACRCC 2017). The “establishment category” has the highest abundance of Silver Carp where recruitment occurs. The “invasion category” is defined as the area where reproduction has been observed but no recruitment has been documented. The “presence category” is the farthest upstream Silver Carp have been observed and reproduction is likely not occurring. Finally, the present study included the section of the Ohio River upstream of the presence category where no Silver Carp have been documented, the “absence category”. The tributaries selected for the absence category are also upstream of where Bighead Carp have been found since Bighead Carp may also be affecting native fish populations. Three tributaries from each invasion zone category were selected for this study to represent an invasion gradient of Silver Carp in the Ohio River (Figure 1).

Sample Collection

Samples for this study were collected from each Ohio River tributary during July and August 2020 during base flow conditions. Collecting samples during base flow conditions reduced fluctuation in hydrology and standardized seasonal fluctuations in nutrient levels allowing for comparisons to be made among tributaries. If an adequate number of fish for analysis was not collected during this time period, fish were collected during September through October until a sufficient number of each species was captured. To test whether tissue stable isotope signatures changed between sampling events, at least five individuals of three species were collected during each time period at each site where fish were sampled during September and October. Each Ohio River tributary was sampled between the first dam or major tributary and 100 m from the confluence with the Ohio River. Five zebra mussel (*Dreissena polymorpha*) samples were collected within the section of river where fish sampling occurred to serve as $\delta^{15}N$ baselines to allow for comparisons to be made across tributaries. If zebra mussels were not collected within the section of river corresponding with fish collection, they were collected from the closest possible point to the fish collection area.

Native species selected for this study represent multiple trophic levels and are economically and ecologically important, including Largemouth Bass (*Micropterus salmodies*), Bluegill (*Lepomis macrochirus*), Gizzard Shad (*Dorosoma cepedianum*), and Smallmouth Buffalo (*Ictiobus bulbus*). At each site, up to 15 fish from each species were collected for analysis via boat electrofishing (Table 1). Total length, weight, and 2.5 cm x 2.5 cm muscle sample were collected from each individual fish. Each muscle plug was taken from the dorsal muscle, with skin and scales removed. Samples were frozen and transported to the laboratory. Samples were dried in an oven at 55°C for 48 hours, ground with a mortar and pestle, weighed with a microbalance, and analyzed by isotope mass spectrometry. Approximately 0.4 mg of dried fish tissue was used for each sample. Analysis of all tissue stable isotope samples was performed by the Southern Illinois University Mass Spectrometry Facility. Samples were analyzed for $\delta^{15}N$, $\delta^{13}C$, and C:N. If C:N ratio was >3.5 lipid content was normalized using the equation proposed by Post et al. (2007):

$$1)\quad \delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99 \text{ x C:N.}$$

Linear regressions were used to determine if normalization of stable isotope data to account for potential difference in $\delta^{15}N$ or $\delta^{13}C$ due to fish size were required (Appendix A).

Zebra mussels were used as a baseline to correct for natural variation in $\delta^{15}N$ among tributaries. A zebra mussel baseline was not available for Clover Creek so zebra mussel values from the Little Kentucky River were used. The Little Kentucky River and Clover Creek have a similar drainage in size, land use, and are close to each other so baseline levels of nitrogen and carbon were comparable. For each fish, corrected trophic position was calculated with the equation:

$$2)\quad TP = TP \text{ of baseline} + (\delta^{15}N_{fish} - \delta^{15}N_{baseline}) / 3.4.$$

Because primary productivity plays an important role in resource availability, I measured chlorophyll *a* concentration as a proxy for phytoplankton primary productivity at my study sites. To measure chlorophyll a, five 100 ml water samples were taken at each site with a Van Dorn water sampler within 1 m of the surface. Each sample was then filtered through a Whatman GF/F glass fiber filters (Whatman, Maidstone, United Kingdom). Chlorophyll a was extracted in acetone and then quantified with fluorometry with an Aquafluor Handheld Fluorometer (Turner Designs, San Jose, California, USA).

In addition to responding to invasive Silver Carp, trophic position and isotopic niche of native fishes may be affected by intraspecific competition for limited resources could potentially covary with Silver Carp abundance. For example, the total biomass of the Largemouth Bass population may be high in tributaries with high densities of Silver Carp. This high within-population biomass may lead to poor condition of individual Largemouth Bass not due to interspecific competition with Silver Carp, but due to high intraspecific competition. Thus, proportional biomass of each functional feeding groups of native fish guilds was compared for each pool of the Ohio River as a potential indicator of possible intraspecific competition. Fish data from the Ohio River Valley Water Sanitation Commission (ORSANCO) electrofishing sampling database were used to estimate total fish biomass in each Ohio River pool. Fish data from 2015 to 2022 were analyzed. Fish from the same functional feeding group should have similar isotopic signatures, so fish were grouped into functional feeding groups for analysis. Results for planktivores, piscivores, general invertivores, and benthic invertivores were included in this study since each of those functional feeding groups was represented by a study species in this study.

Data Analysis

To determine if native species trophic position was related to Silver Carp abundance, I used ANOVAs or Kruskal-Wallis tests to compare trophic positions among invasion categories depending on normality of the data. If differences existed a Tukey's test or Mann-Whitney U test was used to determine which invasion categories were different from one another. P-values were corrected using the Holm correction where applicable. Variation in mean carbon and nitrogen stable isotopes in relation to Silver Carp category was also assessed by constructing stable isotope biplots of carbon and nitrogen with points representing each species at each site.

To quantify if isotopic niches differed among species at varying Silver Carp abundances, I constructed population standard ellipses for each site. I then compared Bayesian estimates of ellipses area (SEA_c) for each species in each invasion category to determine if the area of ellipses differed between invasion categories (Jackson et al. 2011). Comparing SEA_c space allowed for identification of changes in niche space that may be occurring among Silver Carp categories. Standard ellipses were also be to analyze total isotopic overlap and isotopic overlap of Silver Carp onto selected native species. Examining isotopic niche overlap of Silver Carp and native species will help to identify if Silver Carp are consuming diet items with similar isotopic signatures to native species. Standard ellipses analyses were conducted in the SIBER package (Jackson et al. 2011)

Bootstrapped Layman metrics $\delta^{15}N$ range, $\delta^{13}C$ range, mean distance from individual points to centroid (CD), mean distance nearest individual neighbor (NND), and standard deviation of nearest neighbor (SDNND); Layman et al. 2007) were calculated for each species at each site. Layman metrics were used to describe factors that are contributing to possible differences in SEA_c space. For example, if $\delta^{15}N$ range and SEA_c space is larger in one Silver Carp category than another it would be concluded that increased SEA_c space for that species is driven by a reliance on food resources at a larger range of $\delta^{15}N$ between Silver Carp invasion categories. Differences in Layman metrics were compared for each species among invasion category and differences were determined by looking at overlap in 95% confidence intervals.

Body Condition

I used the length and weight data collected during sampling to generate relative weight (Wr) condition indices for each fish. Wr is a condition factor that uses species-specific standard length-weight regressions to compare predicted weight of an individual based on its length to the observed weight (Murphy et al. 1991). Each individual fish gets a value by dividing observed weight by the expected weight of that fish, values between 90 and 100 were considered average with 100 being the standard value, values below 90 were considered below average, and fish above 100 were considered above average. I then calculated a mean Wr for each species at each site using published standard equations (Table 2). Generalized linear models were used to test for a relationship between relative weight for all fish in each Silver Carp category and Silver Carp category for each native species, with random effect of tributary. For Gizzard Shad, a limited number of fish were captured that met the minimum length requirement for the standard weight equation. As a result, the establishment and invasion categories were combined into one group and the presence and absence categories were combined into another group for analysis.

Chlorophyll a

I constructed a linear regression of chlorophyll a against tributaries samples arranged from upstream to downstream to test for any trend in chlorophyll a concentration (µg/L) for the five chlorophyll a samples from the tributaries sampled in this study. Chlorophyll a values were plotted for each of the eleven study tributaries in order to evaluate if primary productivity changed along the longitudinal expanse of my study tributaries.

Fish Community Diversity

To determine if functional feeding group composition changes along the Ohio River each native fish species was assigned within a functional feeding group; functional feeding group was assigned based on documented diet preferences of species and was determined by using Pyron et al. (2017) and state and federal government online resources. Next, biomass was estimated by using standard equations of length-weight relationships (Murphy et al. 1991; Blackwell et al. 2000) to estimate weight (kg) for each species, multiplied by the number of each species at each length, divided by electrofishing time (hr). For species that did not have a standard equation, a standard equation of a closely related species was used. Biomass was then calculated for each functional feeding group by adding together biomass for each species making up a given functional feeding group. Proportion of biomass for each functional feeding group was calculated for each pool. Proportions of each functional feeding group for each pool were then plotted against river location (river miles) with correlation and non-linear regressions to determine if biomass of each functional feeling group changed from upstream to downstream. For all ANOVAs in this chapter normality and equality of variance were tested to determine if assumptions of the tests were met. All statistical analyses were performed in R (R version 4.0.5; R Core Team 2022). For all analyses alpha was set at 0.05.

CHAPTER 3

RESULTS

Trophic Position

Although targeted number of species were not caught for all species from all tributaries, adequate numbers of Gizzard Shad, Bluegill, and Smallmouth Buffalo were caught from each of the four Silver Carp categories to meet the minimum sample size required for analysis (Table 1). Largemouth Bass were captured in adequate number for analyses in three Silver Carp categories. Lengths of fish sampled varied greatly between tributaries (Table 1). Even though size differences did exist, examination of the data revealed that correcting for isotopic differences was not necessary for the data (Appendix A).

Zebra mussels were collected within the study area for all sites except the Beaver and Little Miami. Zebra mussels were collected near the confluence of the Ohio River. No downstream trend existed in carbon or nitrogen of zebra mussel baseline items. Baseline items in the establishment and absence categories had the highest $\delta^{15}N$ and lowest $\delta^{13}C$ values, and baseline items in the invasion and presence categories had the lowest $\delta^{15}N$ values and highest $\delta^{13}C$ values (Figure 2).

Trophic position of Gizzard Shad differed among Silver Carp categories ($F_{3,154} = 3.733$, $p < 0.01$). Relative to baseline items (i.e. zebra mussels) Gizzard Shad in the establishment tributaries had the lowest $\delta^{15}N$ of all Silver Carp categories, and Gizzard Shad in the invasion category tributaries had the highest $\delta^{15}N$ (Figure 2). Trophic position of Largemouth Bass also differed among Silver Carp categories ($\chi^2_2 = 32.126$, $p < 0.01$). Relative to baseline values Largemouth Bass $\delta^{15}N$ was inversely related to the abundance of Silver Carp with Largemouth Bass in the establishment category having the lowest $\delta^{15}N$ and Largemouth Bass in the presence category having the highest $\delta^{15}N$ (Figure 2). For Bluegill ($\chi^2_3 = 22.669$, $p < 0.01$) and Smallmouth Buffalo ($\chi^2_3 = 7.265$, $p = 0.0639$) there was no trend with trophic position and Silver Carp category. Relative to baseline values only $\delta^{15}N$ of Bluegill in the invasion category was higher than $\delta^{15}N$ of any other Silver Carp category (Figure 2).

Isotopic Niche Space as Ellipses

Ellipses area of Gizzard Shad differed among Silver Carp categories of Ohio River tributaries (Figure 3). The establishment category and invasion category tributaries of the Ohio River had larger ellipses areas than the presence and absence category tributaries, but did not differ from one another (Table 3). The absence category tributaries had a higher ellipses area than the presence category tributaries but this result was likely driven by high variation in isotopic signatures of Gizzard Shad among tributaries within the absence category (Table 3; Appendix B Figure B1). Ellipses area of Largemouth Bass differed among Silver Carp category (Figure 3). Largemouth Bass ellipses in the establishment and invasion categories had a larger area than the presence category ellipses, but did not differ from one another (Table 3). Bluegill and Smallmouth Buffalo ellipses area showed no trend relative to Silver Carp category (Table 3; Appendix B Figure B1)

Standard Ellipses Overlap of Silver Carp and native species

Total overlapping area of ellipses ($\delta^{15}N$ and $\delta^{13}C$) between Silver Carp and native species ellipses was low among all tributaries where Silver Carp were captured (Table 4**).** This is likely due to the ellipses area of native species being larger than the ellipses area of Silver Carp. In two of the three tributaries (Cumberland River and Wabash River) Silver Carp ellipses overlap within the native species ellipses was 67% or higher for all native species. Silver Carp overlap over Gizzard Shad was especially high with 86% overlap in the Wabash River and 100% in the

Cumberland River (Table 4). In Clover Creek overlap of Silver Carp over native species was lower (<51%) for all species (Table 4).

Isotopic Niche Space Described by Layman Metrics

Gizzard Shad $\delta^{13}C$ range confidence interval overlap indicated that both the establishment and invasion category $\delta^{13}C$ ranges were larger than the presence and absence category $\delta^{13}C$ ranges (Table 5; Appendix C Figure C1). There was no overlap between the establishment, invasion, and absence category and the presence category for distance to centroid, and establishment and absence category $\delta^{15}N$ range (Table 5; Appendix C Figure C1). Confidence intervals on $\delta^{15}N$ range for establishment and presence categories did not overlap for Largemouth Bass, indicating a difference in these categories (Table 5; Appendix C Figure C2). Bluegill (Appendix C Figure C3) and Smallmouth Buffalo (Appendix C Figure C4) showed no trends in Layman metrics relative to Silver Carp category (Table 5).

Body Condition

Gizzard Shad Wr decreased with increasing abundance of Silver Carp along categories ($F_{1,43} = 7.302$, $p < 0.01$). The establishment/invasion Ohio River tributaries were different than the presence/absence category ($z = -2.702$, $p < 0.01$). Mean Wr of Gizzard Shad in the absence/presence category was average (91.2), whereas Wr of Gizzard Shad in the establishment/invasion category was slightly below average (85.7). Mean Wr of Largemouth Bass differed among Silver Carp categories ($F_{2,44} = 8.887$, $p < 0.01$). The establishment category had a lower Wr than the invasion ($z = 3.849$, $p < 0.01$) and presence ($z = 3.597$, $p < 0.01$) categories. However, no difference between Wr of the invasion and presence categories ($z = -0.169$, $p = 0.984$) was observed. Largemouth Bass Wr in the establishment category (94) was average, and Wr in the invasion (112.8) and presence (112.1) categories were above average.

Bluegill and Smallmouth Buffalo showed no trend in Wr relative to Silver Carp category. Bluegill (93 – 96.2) and Smallmouth Buffalo (96.0 – 100.5) Wr was average for populations in all Silver Carp categories.

Production and Functional Feeding Group Longitudinal Gradients

Chlorophyll a concentrations increased downstream in terms of Silver Carp category ($F_{10,44} = 272.14$, $p < 0.01$; Appendix D Table D1). Chlorophyll a data were collected for 11 tributaries but not collected for the Beaver River. Benthic invertivore (including Smallmouth Buffalo) biomass decreased downstream and the relationship between river mile and biomass was strong, but there was no correlation trend for planktivores, piscivores, or general invertivores (Figure 4). When a nonlinear model was applied to planktivore biomass (including Gizzard Shad) was both low upstream and downstream but higher at mid river reaches ($F_{2,15} = 9.315$, $p < 0.01$).

CHAPTER 4

DISCUSSION

Food web dynamics of native fish species in tributaries differed longitudinally along the Ohio River. Specifically, downstream trends in larger isotopic niches and decreased Wr existed for two of my four study fish species. Additionally, food chain length was compressed in downstream tributaries. The Ohio River and its tributaries are a complex system and factors including the river continuum, river modifications, and the upstream invasion of invasive species including Silver Carp may affect the trophic dynamics of native species (Vannote et al. 1980; Ward and Stanford 1983; Thorp and Delong 1994; ACRCC 2017). Observed patterns in isotopic signatures and body condition of native fishes often changed in ways that suggest that complex relationships between physical and biotic processes may be contributing to the trophic dynamics of fish in the Ohio River tributaries.

For this study I sampled tributaries of the Ohio River because fish move between closely related tributaries and mainstem rivers, and fish communities in tributaries near the confluence are often similar to fish communities in the mainstem river. Movement between closely connected tributaries and mainstems may be seasonal or long term (Garvey et al. 2003; Fullerton et al. 2010; Koster et al. 2014; Pracheil et al. 2018). Fish communities in tributaries have been documented to closely resemble the fish community of the river that they flow into and have many of the same species (Pracheil et al. 2013; Laub et al. 2018; Dunn et al. 2018; Dunn and Paukert 2021). However, Thornbrugh and Gido (2010) found that fish assemblages were different upstream of the confluence of tributaries and mainstem rivers suggesting that some systems may have less connectivity between tributaries and mainstem rivers than others. Even though fish located in tributaries during the summer likely do not move between systems as much as other seasons (Koster et al. 2014; Prachiel et al. 2013), fish feeding in the Ohio River and moving between tributaries sampled and the Ohio mainstem may be a potential source or error in this study.

I quantified increasing chlorophyll a concentrations in downstream tributaries of the Ohio River suggesting further connections between tributaries and longitudinal physical and biological processes in mainstem rivers. Dams also may affect connectivity between mainstem rivers and tributaries, and pooled water in the tributaries may result in tributaries having many of the same characteristics of nutrients and sediment transport as the dammed mainstem (Baxter 1977; Almeida et al. 2019). Almeida et al. (2019) suggested that low-head, run-of-the river dams on the Madeira River, South America, which are similar in structure to the low-head dams in the Ohio River, might have larger effects on tributaries than the mainstem in terms of shifts from lotic to lentic conditions.

In this study multiple tributaries were sampled from each Silver Carp category which integrated both mainstem influences and individual tributary upstream characteristics. Tributaries of large rivers often differ from one another including watershed size, tributary length, discharge, morphology, and nutrient availability (Bukaveckas et al. 2005; Dunn and Paukert 2021; Xiang et al. 2021). Including multiple tributaries from each Silver Carp category helped to account for differences that might exist between tributaries. Furthermore, combining tributaries that have their confluence with the Ohio River in the same Silver Carp category allowed for potential differences between tributaries to be accounted for while combining rivers that have similar abundances of Silver Carp.

Transport of nutrients and materials often occurs in predictable ways in rivers potentially explaining increased isotopic niche space increased in some species from upstream to downstream tributaries of the Ohio River. Specifically, the isotopic niche of Gizzard Shad increased in downstream tributaries. Layman metrics indicated this observed change was driven by differences in $\delta^{13}C$ which could indicate that Gizzard Shad were consuming resources from a wide variety of different sources. Fine particulate organic matter, sediment, and nutrients are transported downstream, resulting in large, high-order rivers often having deposits of sediment, nutrients, and autochthonous phytoplankton production (Vannote et al. 1980). Deposits of sediments and nutrients could lead to more diverse food resources for planktivores and detritivores downstream. In large rivers, such as the Ohio River and its large tributaries, coarse particulate organic matter from riparian areas in addition to autochthonous phytoplankton production could further increase the amount of food resources available to planktivores and detritivores (Thorp and Delong 1994; Sellers and Bukaveckas 2003).

Increased production and increased isotopic niche observed in this study in downstream tributaries could also be due to the effects of dams, and potentially other anthropogenic factors such as urban and agricultural pollution, that may reduce the effect of ecological processes occurring along the Ohio River continuum. The navigation dams on the Ohio River mainstem may affect the trophic dynamic of native species by altering natural flow regimes of rivers by blocking the transport of materials and increasing resource availability above the dam (Ward and Stanford 1983). However in contrast, the lack of a trend in carbon and nitrogen isotopic baseline values and biomass of some functional feeding groups demonstrates that dams may allow for upstream sites to have similar characteristics to downstream sites. The Ohio River and its tributaries might be an intermediate between the river continuum and dammed systems resulting in entirely lentic processes resulting in nutrient and sediment transport still occurring downstream but dammed sections of the river might not directly follow the River Continuum

Concept. Therefore, sections of river affected by dams might have nutrient and sediment loads that are not consistent with the River Continuum Concept (Ward and Stanford 1983). As a result characteristics associated with the most downstream portions of rivers might exist in mid or upper river sections including nutrient storage and higher productivity and phytoplankton production especially in areas upstream of dams (Wehr and Thorp 1997; Sellers and Bukaveckas 2003).

Some results of this study do not follow predictions associated with physical and biological processes expected as a result of the river continuum or dammed rivers and may be better explained by the presence of Silver Carp. The biomass of native planktivores was not highest downstream as would be expected if resources available to planktivore/detritivore species increased as predicted by physical and biological interactions in high order rivers (Miranda et al. 2019). If resources increased for Gizzard Shad as expected and equal or lower biomass of functional feeding groups exist, there should be more resources to go around resulting in higher body condition and subsequently Wr (Blackwell 2000). Interactions of physical and biological processes in rivers supporting downstream transport of materials also support higher biomass and abundance of piscivorous species, such as Largemouth Bass, that consume planktivores and detritivores (Vannote et al. 1980; Miranda et al. 2019). Increased food resources could also lead to increased Wr in piscivorous species as well. However, the results of this study indicate that the biomass of piscivores did not increase and that the Wr of Largemouth Bass decreased downstream.

The abundance of Silver Carp in the Ohio River and how Silver Carp possibly affect native species changes longitudinally in this river. Native planktivores and species that rely on planktivores as prey could be the most affected by Silver Carp as Silver Carp have been shown to decrease phytoplankton and zooplankton abundance and alter the make-up of zooplankton communities (Sass et al. 2014; DeBoer et al 2018). Silver Carp could benefit from diverse habitats associated with dams such as habitat heterogeneity and backwater and side channel habitats (DeGrandchamp et al. 2008; Calkins et al. 2012; Coulter et al. 2016). As a result, Silver Carp may be able to exist in higher abundances in highly dammed and altered systems such as the Ohio River system and its tributaries, and Silver Carp may be able to have larger effects on native species by altering zooplankton and phytoplankton composition (Sass et al. 2014; DeBoer et al 2018). Some of the results of this study do not follow the predictions associated with proposed longitudinal changes in rivers.

Even though isotopic niche space and overlap among species is expected to increase downstream as observed in this study, the invasion of Silver Carp may be contributing to the increased isotopic niches of Gizzard Shad and Largemouth Bass as changes in isotopic niche space have occurred following the invasion of Silver Carp in other rivers (Hayer 2014; Coulter et al. 2019; Wang et al. 2018; Harris et al. 2022; Lebeda et al. 2022). Most studies have found relatively high overlap between Silver Carp and Gizzard Shad isotopic niche space (Hayer 2014; Coulter et al. 2019; Wang et al. 2018; Harris et al. 2022; Lebeda et al. 2022) and diets (Sampson et al. 2009; Minder and Pyron 2018). Harris et al. (2022) compared overlap of bigheaded carp and native planktivores in areas of high and low abundance of the invader and found that there was less overlap between bigheaded carp and native species at high abundances of bigheaded carp suggesting that native species shifted their feeding habits due to competition with bigheaded carp.

I found altered trophic positions resulting in compressed trophic chains where Silver Carp were in highest abundance in the Ohio River, suggesting that this invader is having direct biotic effects on the food web. These results indicate that Silver Carp may be the cause of compressed trophic chains in these areas. There are conflicting findings as to how Silver Carp may affect the trophic position of native species. Freedman et al. (2012) found that overall species were more depleted in ^{15}N following Silver Carp invasion, species that were depleted included Gizzard Shad, Bluegill, Paddlefish, and Bigmouth Buffalo. Pyron et al. (2017) found that the trophic position of omnivores, planktivores (excluding Gizzard Shad), and mussels decreased in the Wabash River, USA following Silver Carp invasion, but trophic positions of other functional feeding groups including piscivores and invertivores were not different before or after Silver Carp invasion. These results along with the results from this study suggest that Silver Carp may be affecting the trophic ecology of native species, but other factors are likely contributing to some of the observed changes in native species isotopic signatures. Other studies examining the effects of invasive fish species trophic ecology of native species using stable isotopes found similar results to this study, especially when considering species at a similar trophic position. Vander Zanden et al. (1999) found that trophic position of Lake Trout decreased and source of carbon resources changed from littoral to more pelagic resources following an invasion of Smallmouth Bass in Canadian lakes. Additionally, Wainwright et al. (2021) found that native Bull Trout trophic position decreased following invasion of Lake Trout in lakes in the Rocky Mountains, USA.

Lower body condition of Gizzard Shad and Largemouth Bass in downstream tributaries could be another effect of competition of Silver Carp. Previous studies in the Illinois River suggest that decreased Wr of native planktivores (i.e. Gizzard Shad and Bigmouth Buffalo) may also be attributed to Silver Carp (Irons et al. 2007; Pendleton et al. 2017). Even though previous studies have not correlated the Wr of Largemouth Bass and Silver Carp the results found in this study makes sense. Gizzard Shad are known to be an important diet item to Largemouth Bass (Wolf and Phelps 2017; Anderson et al. 2021; Appendix E Table E2) Reduced condition of Gizzard Shad could lead to decreased Wr of Largemouth Bass if Gizzard Shad being consumed are no longer as nutritious as they previously were. Differences in Wr between Gizzard Shad and Largemouth Bass found in this study are likely biologically relevant. In their review of standard weight equations and use of Wr in fisheries, Murphy et al. (1991) found evidence from previous studies that differences in Wr can correlate to fat content in fish species. The same study also found that most studies use Wr values with a range of 10 (or +/- 5) to detect for differences in Wr. I saw a difference in Wr of 5 or more for both Gizzard Shad and Largemouth Bass among areas of Silver Carp abundance. Thus, the observed differences might reflect differences in fat content of those two species.

The trophic ecology of fish species, such as Bluegill and Smallmouth Buffalo, that rely less on pelagic planktonic resources may not be as affected by the invasion of Silver Carp because food sources consumed by these fish species might not be altered. Bradley et al. (2019) concluded that invading species at lower trophic levels had no consistent effects on native species communities as seen in this study. Pyron et al. (2017) also had results supporting complex relationships between Silver Carp and other variables in the Wabash River. One of these results was an overall decrease in $\delta^{15}N$ of planktivores, but not all planktivores including Gizzard Shad which was attributed to declines in Gizzard Shad populations before Silver Carp invasion (Pyron et al. 2017).

Future research should continue to examine how Silver Carp affect the trophic relationships of native species from various functional feeding group. Studies should focus on a variety of different functional feeding groups and different species within each functional feeding group as extensive research has found different responses of native planktivore species to Silver Carp (Sampson et al. 2009; Coulter et al. 2019; Minder and Pyron 2018; Harris et al. 2022). Exploring how Silver Carp effect functional feeding group population dynamics is important to consider. Understanding if/how Silver Carp play a role in the biomass of native species in the Ohio River, including the possibility of Silver Carp accounting for biomass that was previously comprised of native species biomass, may help to better understand the effects of their invasion. To better understand how Silver Carp may affect the biomass of native species, research should focus on complex interactions between biotic and physical variables that play a role in the ability of Silver Carp to invade farther upstream in the Ohio River system, but also affect the biotic resistance of native species to switch to alternate food sources. Additionally, the highly dammed nature of the Ohio River likely also plays a role in these complex interactions as not only is the upstream dispersal of Silver Carp disrupted but so is the transport of food resources downstream.

Table 1 - Summary of length range, δ^{15}N (‰), δ^{13}C (‰), trophic position (TP) values and standard error for native Gizzard Shad (G. Shad), Largemouth Bass (L. Bass), Bluegill, Largemouth Bass (L. Bass), and Silver Carp (S. Carp) from selected tributaries of the Ohio River, USA as a function of Silver Carp being established (Est, reproducing), adults present (non-reproducing, invasion; Inv), sighted (presence; Pres), or absent (Abs). δ^{15}N (‰), δ^{13}C (‰), and standard error for Zebra Mussels (Z. Mussels) was also reported.

Invasion Category	Tributary	Species	N	Length Range (mm)	δ^{15}N ± SE	δ^{13}C ± SE	TP ± SE
Est	Cumberland R.	G. Shad	15	74-215	12.35 ± 0.27	-26.26 ± 0.49	2.92 ± 0.08
		Bluegill	15	151-217	13.02 ± 0.23	-26.41 ± 0.28	3.11 ± 0.07
		S. Buffalo	15	343-689	13.05 ± 0.21	-27.53 ± 0.23	3.12 ± 0.06
		S. Carp	15	541-870	12.86 ± 0.13	-28.44 ± 0.25	3.07 ± 0.04
		Z. Mussels	5	-	9.23 ± 0.15	-29.87 ± 0.10	-
	Wabash R.	G. Shad	15	62-299	13.92 ± 0.35	-27.45 ± 0.39	2.49 ± 0.10
		S. Buffalo	15	196-568	13.98 ± 0.25	-27.35 ± 0.50	2.51 ± 0.07
		S. Carp	15	457-715	14.04 ± 0.18	-29.48 ± 0.26	2.53 ± 0.05
		Z. Mussels	4	-	12.25 ± 0.28	-29.11 ± 0.28	-
	Clover Cr.	G. Shad	15	53-374	10.40 ± 0.23	-30.25 ± 0.34	2.28 ± 0.07
		L. Bass	13	170-330	14.97 ± 0.22	-28.22 ± 0.10	3.62 ± 0.06
		Bluegill	15	100-154	12.24 ± 0.18	-29.14 ± 0.18	2.82 ± 0.05
		S. Buffalo	15	270-471	11.58 ± 0.29	-29.74 ± 0.21	2.63 ± 0.08
		S. Carp	15	605-909	12.96 ± 0.20	-28.97 ± 0.27	3.03 ± 0.06
Inv	Little KY R.	G. Shad	15	72-353	13.38 ± 0.22	-30.32 ± 0.63	3.16 ± 0.07
		L. Bass	14	186-416	16.69 ± 0.17	-27.86 ± 0.19	4.16 ± 0.05
		Bluegill	15	79-180	13.94 ± 0.24	-29.39 ± 0.54	3.32 ± 0.07
		S. Buffalo	13	380-621	12.45 ± 0.30	-29.71 ± 0.26	2.88 ± 0.09

		S. Carp	6	655-765	13.21 ± 0.20	-29.40 ± 0.28	3.11 ± 0.06
		Z. Mussels	5	-	9.45 ± 0.08	-34.97 ± 0.14	-
	Licking R.	G. Shad	15	126-156	12.59 ± 0.20	-30.95 ± 0.15	3.60 ± 0.06
		Bluegill	15	60-180	11.65 ± 0.13	-28.43 ± 0.34	3.32 ± 0.04
		S. Buffalo	11	296-621	11.16 ± 0.25	-27.14 ± 0.30	3.18 ± 0.07
		Z. Mussels	5	-	7.16 ± 0.05	-31.26 ± 0.06	-
	Little Miami R.	G. Shad	5	206-577	12.04 ± 0.25	-29.09 ± 0.69	2.78 ± 0.07
		S. Buffalo	15	246-577	12.26 ± 0.22	-27.58 ± 0.29	2.86 ± 0.07
		Z. Mussels	5	-	9.32 ± 0.29	-30.64 ± 0.03	-
Pres	Big Sandy R.	G. Shad	15	85-151	11.00 ± 0.08	-28.46 ± 0.18	2.82 ± 0.02
		L. Bass	2	205-206	15.61 ± 0.11	-27.02 ± 0.15	4.18 ± 0.03
		Bluegill	7	95-135	11.79 ± 0.42	-26.85 ± 0.32	3.06 ± 0.12
		S. Buffalo	1	575	10.43	-24.51	2.66
		Z. Mussels	5	-	8.20 ± 0.05	-30.20 ± 0.11	-
	Guyandotte R.	G. Shad	15	87-109	11.69 ± 0.16	-28.31 ± 0.05	2.54 ± 0.05
		Bluegill	11	64-149	11.60 ± 0.29	-26.93 ± 0.33	2.52 ± 0.08
		S. Buffalo	15	298-560	10.93 ± 0.47	-25.84 ± 0.39	2.32 ± 0.14
		Z. Mussels	5	-	9.85 ± 0.08	-32.99 ± 0.08	-
	Kanawha R.	G. Shad	15	83-106	11.65 ± 0.12	-28.53 ± 0.07	3.31 ± 0.03
		L. Bass	14	215-296	15.37 ± 0.06	-28.13 ± 0.10	4.40 ± 0.02
		Bluegill	15	74-182	12.21 ± 0.20	-27.13 ± 0.43	3.48 ± 0.06
		S. Buffalo	12	264-457	11.80 ± 0.26	-28.82 ± 0.59	3.35 ± 0.08
		Z. Mussels	5	-	7.19 ± 0.09	-30.41 ± 0.08	-

Abs	Beaver R.	G. Shad	15	241-412	12.99 ± 0.18	-27.05 ± 0.25	2.75 ± 0.05
		Bluegill	15	77-190	13.73 ± 0.24	-25.81 ± 0.24	2.97 ± 0.07
		S. Buffalo	12	460-569	14.10 ± 0.10	-26.16 ± 0.39	3.08 ± 0.03
		Z. Mussels	5	-	10.44 ± 0.05	-29.61 ± 0.09	-
	Monongahela R.	G. Shad	15	55-74	13.00 ± 0.05	-29.75 ± 0.03	3.38 ± 0.01
		Bluegill	11	85-187	12.39 ± 0.29	-26.25 ± 0.47	3.20 ± 0.08
		S. Buffalo	14	490-767	11.32 ± 0.19	-25.66 ± 0.17	2.88 ± 0.06
		Z. Mussels	5	-	8.32 ± 0.04	-29.53 ± 0.11	-
	Allegheny R.	G. Shad	3	71-337	11.97 ± 0.07	-27.02 ± 1.55	2.89 ± 0.02
		Bluegill	15	123-176	12.07 ± 0.35	-26.28 ± 0.26	2.87 ± 0.10
		S. Buffalo	4	475-600	11.56 ± 0.34	-25.67 ± 0.32	2.72 ± 0.10
		Z. Mussels	5	-	9.12 ± 0.09	-30.58 ± 0.33	-

Table 2 – Intercept (a), slope (b), and minimum length used in standard equation ($\log_{10}(Ws) = a + b(\log_{10}\text{Total Length})$ used to calculate expected weight (Ws) based on length for Gizzard Shad (G. Shad), Largemouth Bass (L. Bass), Bluegill, and Smallmouth Buffalo (S. Buffalo). Standard weights were calculated based on lengths of fish caught in tributaries of the Ohio River, USA. Equations published in Murphy et al. 1991.

Species	Intercept (a; metric)	Slope (b)	Minimum Length (mm)
G. Shad	-5.376	3.170	180
L. Bass	-5.528	3.273	150
Bluegill	-5.374	3.316	80
S. Buffalo	-5.069	3.092	280

Table 3 - Probability that Bayesian ellipses area for Gizzard Shad (G. Shad), Largemouth Bass (L. Bass), Bluegill, and Smallmouth Buffalo (S. Buffalo) will be larger (>) or smaller (<) for one invasion category compared to another in the Ohio River, USA as a function of Silver Carp being established (reproducing; Est), adults present (non-reproducing; Inv), sighted (presence; Pres), or absent (Abs).

G. Shad		L. Bass		Bluegill		S. Buffalo	
Abs > Est	<0.01	Est > Inv	0.126	Abs > Est	0.296	Abs > Est	0
Abs > Inv	0.03	Est < Pres	0.034	Abs > Inv	0.468	Abs > Inv	<0.01
Abs < Pres	0	Inv < Pres	<0.01	Abs > Pres	0.012	Abs > Pres	0
Est > Inv	0.874			Est < Inv	0.338	Est < Inv	0.10
Est < Pres	0			Est > Pres	0.054	Est > Pres	<0.01
Inv < Pres	0			Inv > Pres	0.021	Inv > Pres	0

Table 4 - Standard ellipses overlap of Silver Carp (SVCP) and native species in the establishment (reproducing) category, including Gizzard Shad (GZSD), Largemouth Bass (LMBS), Bluegill (BLGL), and Smallmouth Buffalo (SMBUF) in the Cumberland River, Wabash River, and Clover Creek. Total overlap represents the portion of overlap between Silver Carp and the native species compared to the total area of both species' ellipses. Silver Carp overlap over a native species represents the portion of the native species ellipses also occupied by Silver Carp.

Cumberland River		Wabash River		Clover Creek	
Species Overlap	Percent Overlap	Species Overlap	Percent Overlap	Species Overlap	Percent Overlap
SVCP and GZSD Total Overlap	18%	SVCP and GZSD Total Overlap	22%	SVCP and GZSD Total Overlap	8%
SVCP Over GZSD	100%	SVCP Over GZSD	86%	SVCP Over GZSD	20%
SVCP and BLGL Total Overlap	21%	SVCP and SMBUF Total Overlap	24%	SVCP and BLGL Total Overlap	25%
SVCP Over BLGL	67%	SVCP Over SMBUF	73%	SVCP Over BLGL	36%
SVCP and SMBUF Total Overlap	31%			SVCP and SMBUF Total Overlap	27%
SVCP Carp Over SMBUF	82%			SVCP Over SMBUF	51%
				SVCP and LMBS Total Overlap	11%
				SVCP Over LMBS	15%

Table 5- Summary of Layman metrics for Gizzard Shad, Largemouth Bass, Bluegill, and Smallmouth Buffalo from tributaries among four categories of Silver Carp in the Ohio River, USA as a function of Silver Carp being established (reproducing; Est), adults present (non-reproducing; Inv), sighted (Presence; Pres), or absent (Abs). Metrics include δ^{15}N range, δ^{13}C range, size of Bayesian ellipses (SEA_C), distance to centroid (CD), nearest neighbor distance (NND), and standard deviation of NND (SDNND). Bootstrapped 95% confidence intervals reported below each value. Overlap in 95% confidence intervals denoted by letters, values with overlap have the same letters.

Species	Invasion Category	δ15N range	δ^{13}C range	SEA_C	CD	NND	SDNND
G. Shad	Est	5.10^{a}	7.56^{a}	9.49^{a}	2.43^{a}	0.203	0.359
		(4.22,5.46)	(6.67,7.73)		(2.10,2.75)	(0.12,0.30)	(0.24,0.47)
	Inv	4.31^{ab}	6.56^{a}	7.37^{a}	1.94^{a}	0.227	0.390
		(3.50,4.73)	(5.59,6.90)		(1.60,2.28)	(0.12,0.34)	(0.26,0.52)
	Pres	4.46^{ab}	2.23^{b}	1.66^{c}	1.07^{b}	0.088	0.206
		(3.44,4.95)	(0.79,2.90)		(0.87,1.27)	(0.04,0.14)	(0.08,0.36)
	Abs	3.47^{b}	4.11^{b}	4.56^{b}	1.80^{a}	0.126	0.229
		(3.05,3.66)	(3.55,4.23)		(1.53,1.98)	(0.05,0.19)	(0.12,0.35)
L. Bass	Est	2.25^{a}	1.07	0.95^{a}	0.715	0.156	0.237
		(1.67,2.54)	(0.53,1.22)		(0.51,0.91)	(0.04,0.27)	(0.10,0.35)
	Inv	1.87^{ab}	2.34	1.50^{a}	0.747	0.181	0.338
		(1.01,2.26)	(1.14,2.90)		(0.51,1.04)	(0.04,0.35)	(0.09,0.55)
	Pres	1.23^{b}	1.71	0.46^{b}	0.473	0.100	0.184
		(1.01,2.26)	(1.14,2.90)		(0.51,1.04)	(0.04,0.35)	(0.09,0.55)
Bluegill	Est	3.97^{ab}	5.72	4.71	1.64	0.219	0.427
		(2.48,4.77)	(3.87,6.52)		(1.34,1.97)	(0.09,0.35)	(0.19,0.66)
	Inv	3.15^{b}	6.20	4.23	1.60	0.199	0.321
		(1.85,3.85)	(4.92,6.69)		(1.26,1.96)	(0.10, 0.30)	(0.21,0.43)
	Pres	6.14^{a}	5.19	7.08	1.85	0.280	0.468
		(4.50,6.72)	(3.64,5.48)		(1.50,2.20)	(0.14,0.41)	(0.29,0.63)
	Abs	4.76^{ab}	5.76	4.09	1.30	0.204	0.425
		(3.78,5.18)	(3.03,6.95)		(1.02,1.64)	(0.10,0.32)	(0.20,0.66)
S. Buffalo	Est	5.67^{ab}	7.68^{ab}	6.54^{b}	1.82^{b}	0.222	0.414
		(3.77,6.61)	(5.13,9.04)		(1.54,2.15)	(0.12,0.33)	(0.23,0.64)
	Inv	4.15^{b}	5.36^{bc}	4.94^{b}	1.63^{b}	0.204	0.328

	(3.02,4.62)	(4.45,5.72)		(1.39,1.86)	(0.12,0.29)	(0.23,0.41)
Pres	7.14[a]	7.87[a]	13.81[a]	2.88[a]	0.341	0.528
	(6.10,7.57)	(5.93,8.67)		(2.42,3.36)	(0.17,0.51)	(0.36,0.74)
Abs	2.06[c]	3.97[c]	2.08[c]	1.04[c]	0.139	0.266
	(1.73,2.15)	(2.37,4.82)		(0.83,1.26)	(0.06,0.23)	(0.12,0.44)

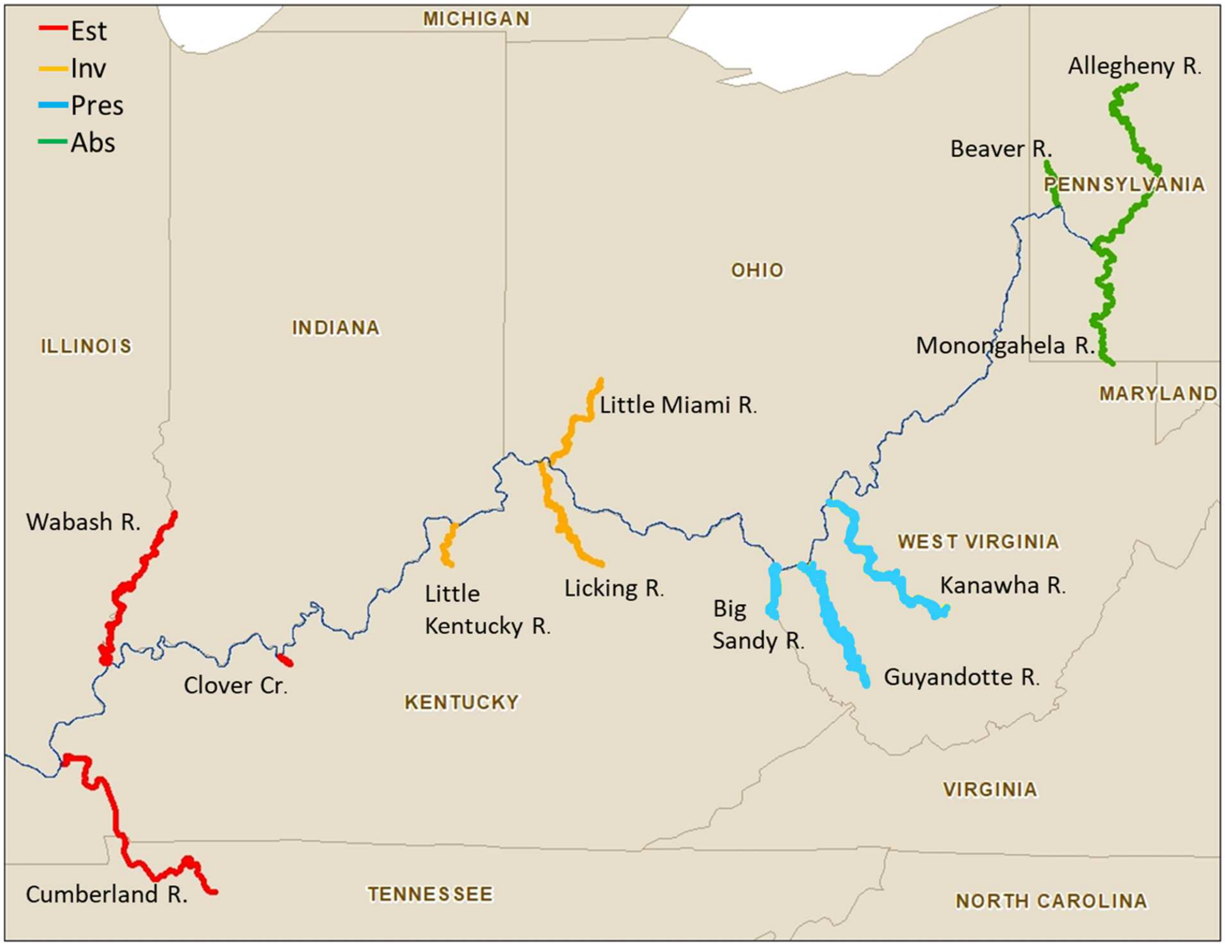

Figure 1 - Map of study tributaries of the Ohio River, USA. Tributaries included in this study are color coded to match the invasion category in which they belong. Including established (reproducing; Est), adults present (non-reproducing; Inv), sighted (presence; Pres), or absent (Abs).

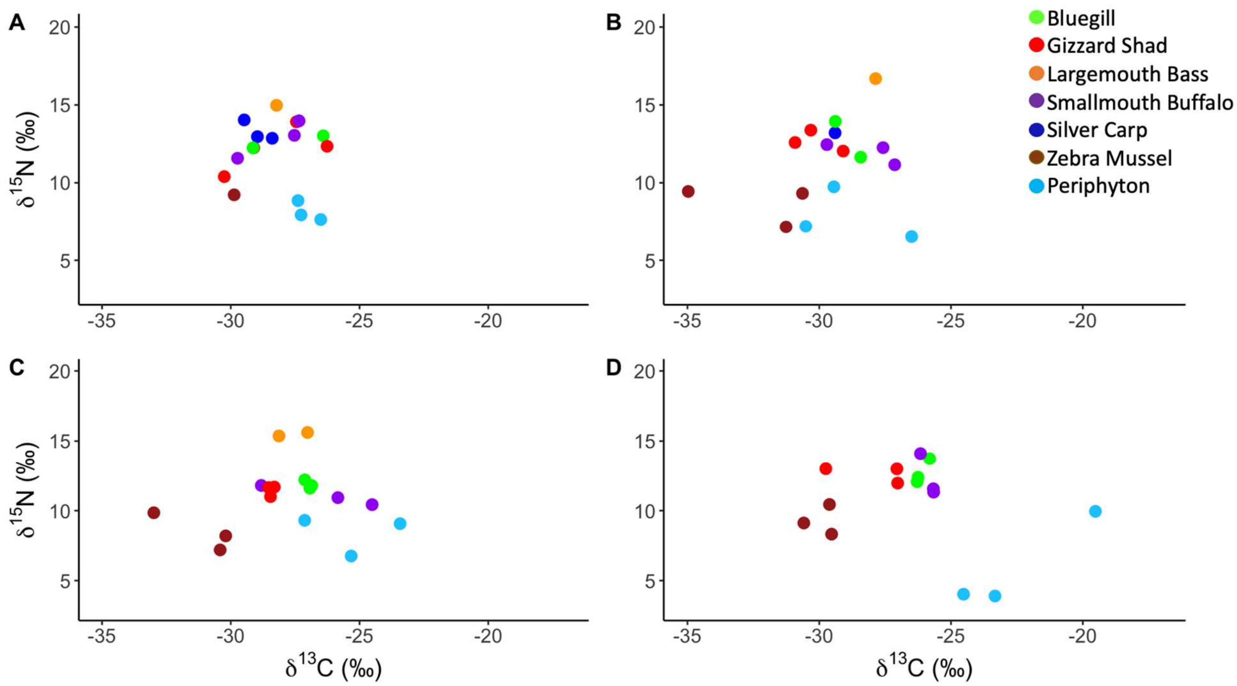

Figure 2 – Mean values of $\delta^{15}N$ and $\delta^{13}C$ for Gizzard Shad, Largemouth Bass, Bluegill, Smallmouth Buffalo, and Silver Carp for populations for each Silver Carp category in tributaries of the Ohio River, USA as a function of Silver Carp being (A) established (reproducing), (B) adults present (non-reproducing), (C) sighted (Presence), or (D) absent. Zebra mussel and periphyton $\delta^{15}N$ and $\delta^{13}C$ are also reported. Each point represents a mean value of each species from an individual tributary.

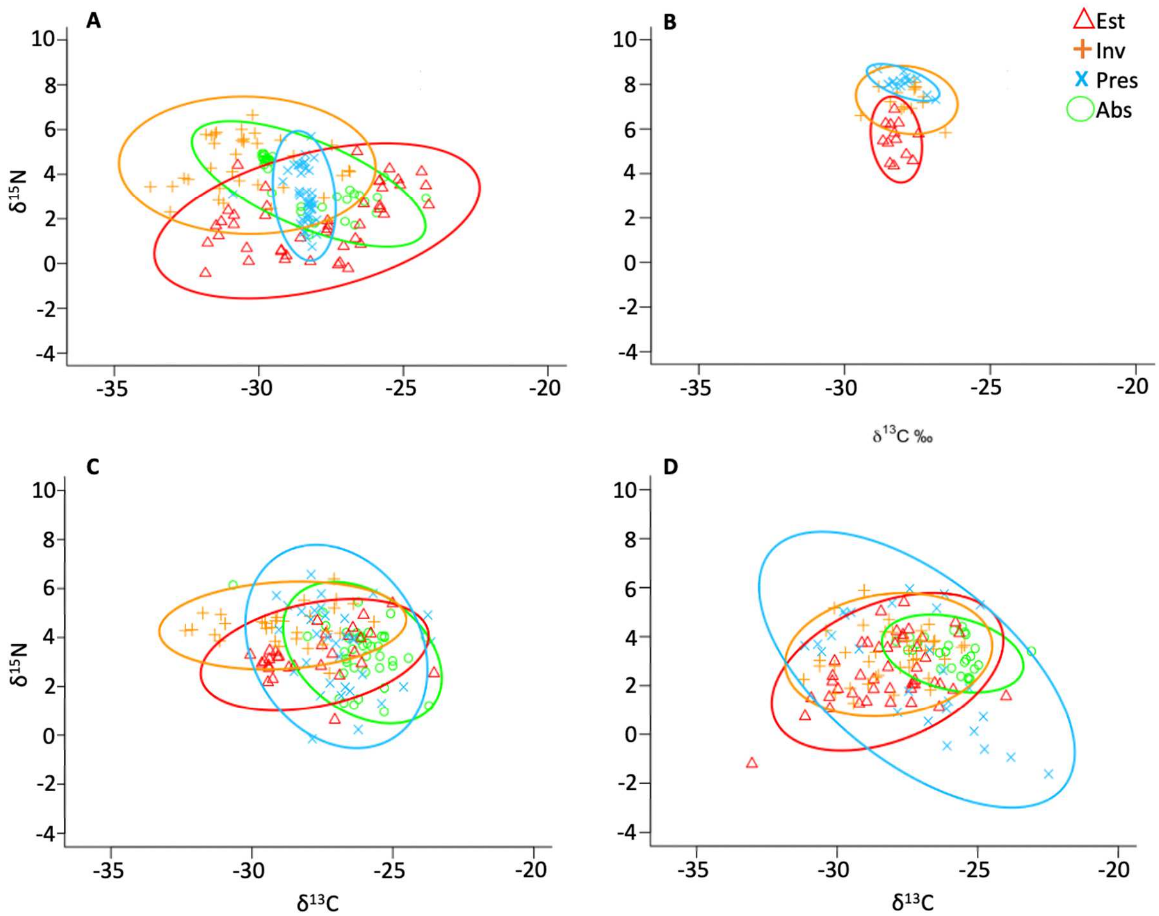

Figure 3 – Standard ellipses (A) Gizzard Shad, (B) Largemouth Bass, (C) Bluegill, and (D) Smallmouth Buffalo for populations for each Silver Carp category in tributaries of the Ohio River, USA. as a function of Silver Carp being established (reproducing; Est), adults present (non-reproducing; Inv), sighted (Presence; Pres), or absent (Abs). All $\delta^{15}N$ values are reported as corrected values with zebra mussel $\delta^{15}N$ as the baseline.

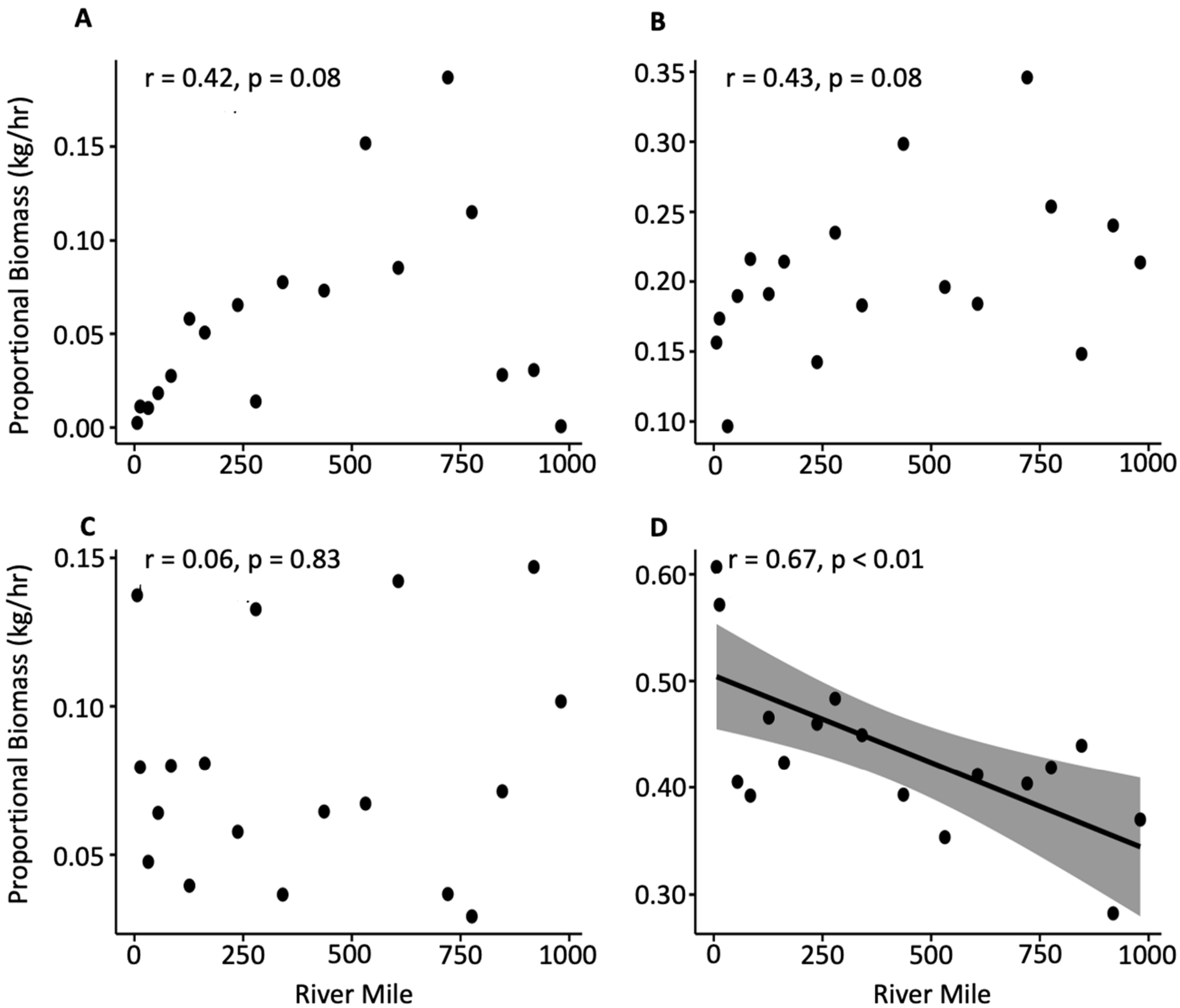

Figure 4 - Correlation of the proportion of community biomass (kg/hr) comprised by (A) planktivore, (B) piscivore, (C) general invertivore, (D) benthic invertivore in the Ohio River, USA. Proportions are for each pool of the Ohio River from upstream (left on the graph) to downstream (right on the graph). A p-value of <0.05 indicates significant correlation between proportion of community biomass and river mile.

REFERENCES

ACRCC. 2017. Asian Carp Annual Interim Reports for the Mississippi River Basin 2017 Ohio River Asian Carp Partnership Upper Mississippi River Asian Carp Partnership.

Almeida, R. M., S. K. Hamilton, E. J. Rosi, J. D. Arantes Jr., N. Barros, G. Boemer, A. Gripp, V. L. M. Huszar, P. C. Junger, M. Lima, F. Pacheco, D. Carvalho, A. J. Reisinger, L. H. S. Silva, and F. Roland. 2019. Limnological effects of a large Amazonian run-of-river dam on the main river and drowned tributary valleys. Scientific Reports 9(1):16846.

Anderson, C. A., R. L. Anderson, J. Wang, N. Gillespie, E. G. Lampo, N. N. McClelland, L. E. Solomon, R. Pendleton, and J. T. Lamer. 2021. Juvenile silver carp and bighead carp as forage for predatory fish in the LaGrange reach of the Illinois River. North American Journal of Fisheries Management https://doi.org/10.1002/nafm.10681.

Averett, J. P., B. McCune, C. G. Parks, B. J. Naylor, T. DelCurto, and R. Mata-González. 2016. Non-native plant invasion along elevation and canopy closure gradients in a middle Rocky Mountain ecosystem. PloS one 11(1): e1047826.

Baxter, R. M. 1977. Environmental effects of dams and impoundments. Annual Review of Ecology and Systematics 8(1):255-283.

Blackwell, B. G., M. L. Brown, and D. W. Willis. 2000. Relative weight (Wr) status and current use in fisheries assessment and management. Reviews in Fisheries Science 8(1): 1-44

Bowes, R. E., J. H. Thorp, and M. D. Delong. 2020. Reweaving river food webs through time. Freshwater Biology 65(3): 390–402.

Bradley, B. A., B. B. Laginhasb, R. Whitlockc, J. M. Allend, A. E. Batese, Genevieve Bernatchezf, Jeffrey M. Diezg, Regan Earlyh, Jonathan Lenoiri, Montserrat Vilàj, and C. J. B. Sortef. 2019. Disentangling the abundance–impact relationship for invasive species. PNAS 112(20): 9919-9924.

Bukaveckas, P. A., D. L. Guelda, J. Jack, R. Koch, T. Sellers, and J. Shostell. 2005. Effects of point source loadings, sub-basin inputs and longitudinal variation in material retention on C, N and P delivery from the Ohio River basin. Ecosystems 8(7):825-840

Calkins, H. A., S. J. Tripp, and J. E. Garvey. 2012. Linking silver carp habitat selection to flow and phytoplankton in the Mississippi River. Biological Invasions 14(5):949-958.

Cheng, B. S. and K. A. Hovel. 2010. Biotic resistance to invasion along an estuarine gradient. Oceologia 164(4): 1049-1059.

Chick, J. H., D. K. Gibson-Reinemer, L. Soeken-Gittinger, and A. F. Casper. 2020. Invasive silver carp is empirically linked to declines of native sport fish in the Upper Mississippi River System. Biological Invasions 22(2):723–734.

Coulter, A. A., E. J. Bailey, D. Keller, and R. R. Goforth. 2016. Invasive Silver Carp movement patterns in the predominantly free-flowing Wabash River (Indiana, USA). Biological Invasions 18(2):471-485.

Coulter, A. A., H. K. Swanson, and R. R. Goforth. 2019. Seasonal variation in resource overlap of invasive and native fishes revealed by stable isotopes. Biological Invasions 21(2): 315–321.

Cucherousset, J., L. Závorka, S. Ponsard, R. Cereghino, and F. Santoul. 2020. Stable isotope niche convergence in coexisting native and non-native salmonids across age classes. Canadian Journal of Fisheries and Aquatic Sciences 77(8): 1359–1365.

Dangremond, E. M., E. A. Pardini, and T. M. Knight. 2010. Apparent competition with an invasive plant hastens the extinction of an endangered lupine. Ecology 91(8): 2261 – 2271.

DeBoer, J. A., A. M. Anderson, and A. F. Casper. 2018. Multi-trophic response to invasive silver carp (Hypophthalmichthys molitrix) in a large floodplain river. Freshwater Biology 63(6):5 97–611.

DeGrandchamp, K. L., J. E. Garvey and R. E. Colombo. 2008. Movement and habitat selection by invasive Asian carps in a large river. Transactions of the American Fisheries Society 137(1):45-56.

Dunn, C. G., B. L. Brooke, R. A. Hrabik, and C. P. Paukert. 2018. Intensive sampling reveals underreported use of great-river tributaries by large-river fishes in Missouri. Southeastern Naturalist. 17(3):512-520.

Dunn, C. G. and C. P. Paukert. 2021. Accounting for dispersal and local habitat when evaluating tributary use by riverine fishes. Ecosphere 12(8):e03711

Freedman, J. A., S. E. Butler, and D. H. Wahl. 2012. Impacts of invasive Asian carps on native food webs. Page Illinois-Indiana Sea Grant.

Fullerton, A. H., K. M. Burnett, E. A. Steel, R. L. Flitcroft , G. R. Press, B. E. Feist, C. E. Torgersen , D. J. Miller and B.L. Sanderson. 2010. Hydrological connectivity for riverine fish: measurement challenges and research opportunities. Freshwater Biology 55 (11): 2215–2237

Garvey, J. E., S. Welsh, and K. J. Hartman. 2003. Winter Habitat Used by Fishes in Smithland Pool and Belleville Pool, Ohio River. Final combined project report. Carbondale, IL.

Harmon, J. P., N. A. Moran, and A. R. Ives. 2009. Species Response to Environmental Change: Inpacts of Food Web Interactions and Evolution. Science 323(5919): 1347–1350.

Harris, B. S., J. A. Deboer, and J. T. Lamer. 2022. Trophic reorganization of native planktivorous fishes at different density extremes of bigheaded carps in the Illinois and Mississippi rivers, USA. Biological Invasions (24) 3013–3031.

Hayer, C.-A. 2014. Fish assemblage structure, trophic ecology and potential effects of invading Asian carps in three Missouri River tributaries. PhD Diss., Natural Resource Management Department, South Dakota State University.

Irons, K. S., G. G. Sass, M. A. McClelland, and J. D. Stafford. 2007. Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, U.S.A. Is this evidence for competition and reduced fitness? Journal of Fish Biology 71(Supplement D): 258–273.

Jackson, A. L., R. Inger, A. C. Parnell, and S. Bearhop. 2011. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology 80(3): 595–602.

Kolar, C., D. Chapman, W. Courtenay Jr., C. Housel, J. Williams, and D. Jennings. 2005. Asian Carps of the Genus Hypophthalmichthys (Pisces, Cyprinidae) -- A Biological Synopsis and Environmental Risk Assessment. Page Environmental Research.

Koster, W. M., D. R. Dawson, D. J. O'Mahony, P. D. Moloney, and D. A. Crook. 2014. Timing, frequency and environmental conditions associated with mainstem–tributary movement by a lowland river fish, Golden Perch (Macquaria ambigua). PLOSone 9(5):e96044.

Laub, B. G., G. P. Thiede, W. W. Macfarlane, and P Budy. 2018. Evaluating the conservation potential of tributaries for native fishes in the upper Colorado River basin. Fisheries 43(4):194-206

Lauber, T. B., R. C. Stedman, N. A. Connelly, R. C. Ready, L. G. Rudstam, and G. L. Poe. 2020. The effects of aquatic invasive species on recreational fishing participation and value in the Great Lakes: Possible future scenarios. Journal of Great Lakes Research 46(3): 656-665.

Layman, C. A., D. A. Arrington, C. G. Montana, and D. M. Post. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88(1):42-48.

Lebeda, D. D., A. M. Lebeda, and M B. Flinn. 2022. Isotopic niches of Silver Carp and two native planktivores in a large reservoir. Freshwater Science 41(4): 000-000

Mills, E. L., J. H. Leach, J. T. Carlton, and C. L. Secor. 1994. Exotic Species and the Integrity of the Great Lakes. BioScience 44(10): 666–676.

Minder, M., and M. Pyron. 2018. Dietary overlap and selectivity among silver carp and two native filter feeders in the Wabash River. Ecology of Freshwater Fish 27(1):506–512.

Miranda, R. V. Granzotti, and D. J. Dembkowski. 2019. Gradients in fish feeding guilds along a reservoir cascade. Aquatic Sciences 81(1):1-11.

Murphy, B. R., D. W. Willis, and T. A. Springer. 1991. The relative weight index in fisheries management: status and needs. Fisheries 16(2): 30-38.

Paine, R. T. 1980. Food Webs: Linkage, Interaction Strength and Community Infrastructure. Journal of Animal Ecology 49(3): 666–685.

Pendleton, R. M., C. Schwinghamer, L. E. Solomon, and A. F. Casper. 2017. Competition among river planktivores: are native planktivores still fewer and skinnier in response to the Silver Carp invasion? Environmental Biology of Fishes 100(10):1213–1222.

Polis, G. A., and D. R. Strong. 1996. Food Web Complexity and Community Dynamics. American Society of Naturalists 147(5):813–846.

Post, D.M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, and C G. Montana. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152(1):179–189

Power, M. E. 1990. Effects of Fish in River Food Webs. Science 250(4982): 811–814.

Pracheil B. M., P. B. McIntyre, and J. D. Lyons. 2013. Enhancing conservation of large-river biodiversity by accounting for tributaries. Research Communications 11(3):124-128.

Pracheil, B. M., J. Lyons, E. J. Hamann, P. H. Short, and P. B. McIntyre. 2018. Lifelong population connectivity between large rivers and their tributaries: A case study of shovelnose sturgeon from the Mississippi and Wisconsin rivers. Ecology of freshwater fish 28(1):20-32.

Pyron, M., J. C. Becker, K. J. Broadway, L. Etchison, M. Minder, D. DeColibus, M. Chezem, K. H. Wyatt, and B. A. Murry. 2017. Are long-term fish assemblage changes in a large US river related to the Asian Carp invasion? Test of the hostile take-over and opportunistic dispersal hypotheses. Aquatic Sciences 79(3): 631–642.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Sampson, S. J., J. H. Chick, and M. A. Pegg. 2009. Diet overlap among two Asian carp and three native fishes in backwater lakes on the Illinois and Mississippi rivers. Biological Invasions 11(3):483–496.

Sass, G. G., C. Hinz, A. C. Erickson, N. N. McClelland, M. A. McClelland, and J. M. Epifanio. 2014. Invasive bighead and silver carp effects on zooplankton communities in the Illinois River, Illinois, USA. Journal of Great Lakes Research 40(4): 911–921.

Seabloom, E. W., W. S. Harpole, O. J. Reichman, and D. Tilman. 2003. Invasion, competitive dominance, and resource use by exotic and native California grassland species. PNAS 100(23): 13384-13389.

Sellers, T and P. A. Bukaveckas. 2003. Phytoplankton production in a large, regulated river: A modeling and mass balance assessment. Limnology and Oceanography 48(4):1476-1487.

Sol, D., I. Bartomeus, and A. S. Griffin. 2012. The paradox of invasion in birds: competitive superiority or ecological opportunism? Oecologia 196(2): 553-564.

Solomon, L. E., R. M. Pendleton, J. H. Chick, and A. F. Casper. 2016. Long-term changes in fish community structure in relation to the establishment of Asian carps in a large floodplain river. Biological Invasions 18(10):2883–2895.

Thornburgh, D. J. and K. B. Gido. 2010. Influence of spatial positioning within stream networks on fish assemblage structure in the Kansas River basin, USA. Canadian Journal of Fisheries and Aquatic Science 67(1):143-156.

Ward, J. V., and J. A. Stanford. 1983. The serial discontinuity concept of lotic ecosystems. Pages 29–42 in T. D. Fontaine and S. M. Bartell, editors. Dynamics of lotic ecosystems. Ann Arbor Science, Ann Arbor, Michigan, USA.

Stohlgren, T. J., D. Barnett, C. Flather, J. Kartesz, and B. Peterjohn. 2005. Plant species invasions along the latitudinal gradient in the United States. Ecology 86(9): 2298-2309.

Thorp, J. H., and M. D. Delong. 1986. The riverine productivity model: an heuristic view of carbon sources and organic processing in large river ecosystems. Oikos 70(2):305-308.

Tumolo, B. B., and M. B. Flinn. 2017. Diet of Invasive Silver Carp (Hypophthalmichthys molitrix) in a Mainstem Reservoir Ecosystem. Journal of the Kentucky Academy of Science 78(1):3–11.

Vander Zanden, M. J., J. M. Casselman, and J. B. Rasmussen. 1999. Stable isotope evidence for the food web consequesces of species invasion in lakes. Nature 401(6725): 464–467

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37(1): 130-137.

Vitousek, P. M., C. M. D'Antonio, L. L. Loope, and R. Westbrooks. 1996. Biological Invasions as Global Environmental Change. American Scientist 84(5): 468–478.

Wainright, C. A., C. C. Muhlfeld, J. J. Elser, S. L. Bourret, and S. P. Devlina. 2021. Species invasion progressively disrupts the trophic structure of native food webs. PNAS 118(45): e2102179118.

Wang, J., D. Chapman, J. Xu, Y. Wang, and B. Gu. 2018. Isotope niche dimension and trophic overlap between bigheaded carps and native filter-feeding fish in the lower Missouri river, USA. PLoS ONE 13(5): 1–14.

Wehr, J. D. and J. H. Thorp. 1997. Effects of navigation dams, tributaries, and littoral zones on phytoplankton communities in the Ohio River. Canadian Journal of Fisheries and Aquatic Sciences 54(2):378-395.

Williamson, C. J., and J. E. Garvey. 2005. Growth, Fecundity, and Diets of Newly Established Silver Carp in the Middle Mississippi River. Transactions of the American Fisheries Society 134(6):1423–1430.

Xiang R., L. Wang, H. Li, Z. Tian, and B. Zheng. 2021. Water quality variation in tributaries of the Three Gorges Reservoir from 2000 to 2015. Water Research 195:116993.

Wolf, M. C., and Q. E. Phelps. 2017. Prey selectivity of common predators on Silver carp (Hypophthalmichthys molitrix): controlled laboratory experiments support field observations. Environmental Biology of Fishes 100(9): 1139–1143.

APPENDIX A

METHODS OF STABLE ISOTOPE SIZE NORMALIZATION DETERMINATION

To determine if size normalization was required for any fish species, a linear regression comparing length and $\delta^{15}N$ were performed for each species at each site. If significant results existed, the r^2 value was evaluated, if the r^2 value was around or below 0.4 the relationship was considered weak and was not corrected. If the r^2 value was higher than 0.4, I looked to see if all fish collected were within a size range representative of the same life stage as size normalization is used to correct for potential ontogenetic shifts. I also looked to see if removing the largest or smallest fish eliminated the significant result since if removing the larges or smallest fish changes the results the observed effect might not reflect the population as a whole. If either of these two patterns were observed I did not correct the $\delta^{15}N$ values. The Gizzard Shad population from Clover Creek was the only population that did not meet any of these criteria, as a result I compared diet data from individuals in the population and did not observe any differences so $\delta^{15}N$ values were not corrected. I did not use this method for all populations since it is not a standard method and diets may only provide a snap shot of what fish are consuming.

APPENDIX B

STANDARD ELLIPSES AREA OF NATIVE SPECIES

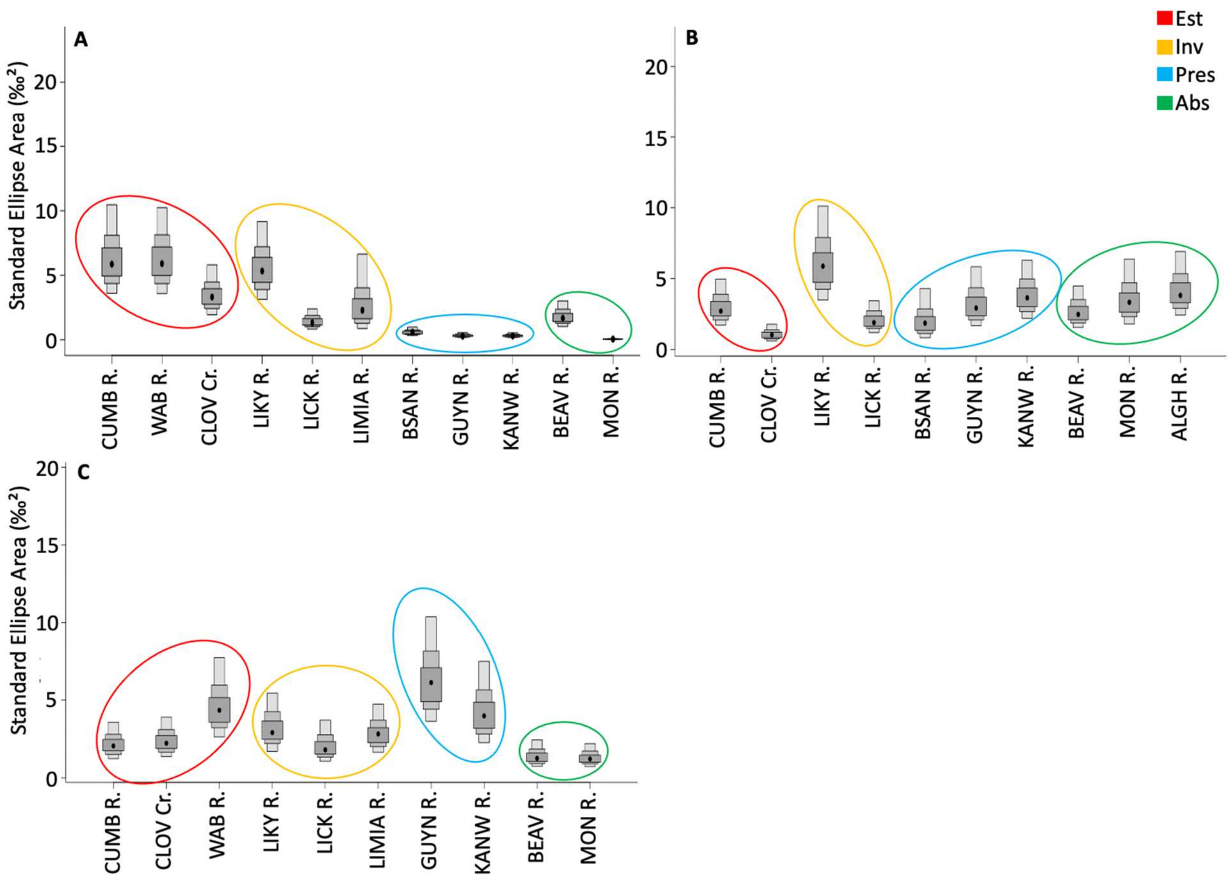

Figure B1. Ellipses area for each tributary for (A) Gizzard Shad, (B)Bluegill, and (C) Smallmouth Buffalo. Tributaries are grouped together by Silver Carp category including established (reproducing; Est), adults present (non-reproducing; Inv), sighted (Presence; Pres), or absent (Abs). Shaded boxes represent 50%, 75%, and 95% of ellipses sizes based on Bayesian estimates. Differences between individual tributaries in each invasion category could indicate that tributary effects are driving changes in ellipses area. Since Largemouth Bass were only captured in high enough numbers in one tributary from each invasion category individual tributaries were not compared for Largemouth Bass.

APPENDIX C

POPULATION LAYMAN METRICS CONFIDENCE INTERVAL OVERLAP

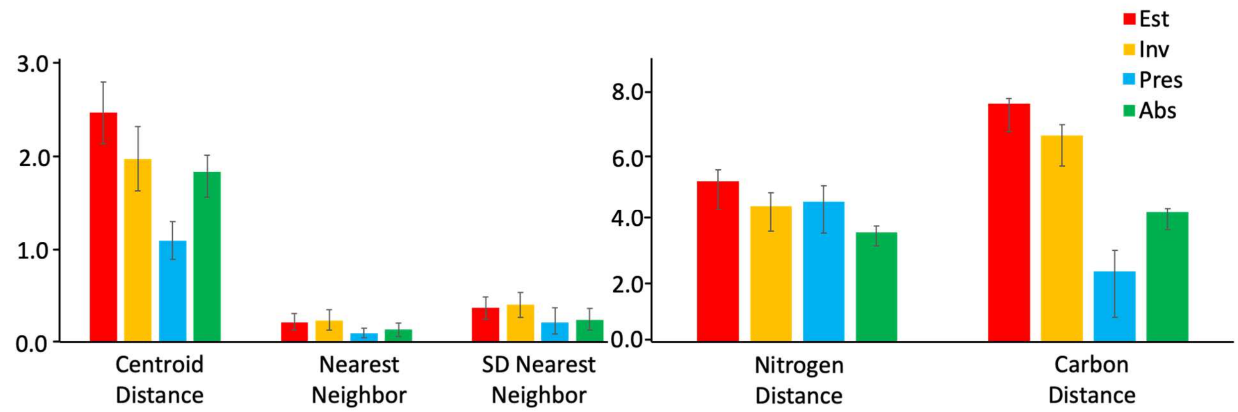

Figure C1**.** Layman metrics for Gizzard Shad in each invasion category including the established (reproducing; Est), adults present (non-reproducing; Inv), sighted (Presence' Pres), or absent (Abs) in the Ohio River basin. Metrics include $\delta^{15}N$ range, $\delta^{13}C$ range, size of Bayesian ellipses (SEA_C), distance to centroid (CD), nearest neighbor distance (NND), and standard deviation of NND (SDNND). 95% confidence intervals are also reported.

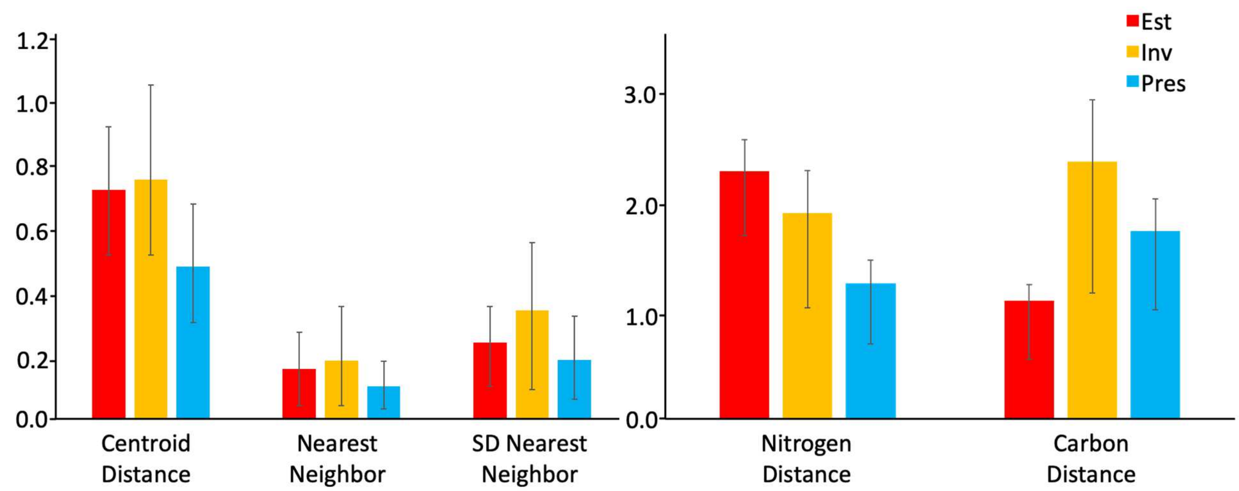

Figure C2. Layman metrics for Largemouth Bass in each invasion category including the established (reproducing; Est), adults present (non-reproducing; Inv), sighted (Presence' Pres), or absent (Abs) in the Ohio River basin. Metrics include $\delta^{15}N$ range, $\delta^{13}C$ range, size of Bayesian ellipses (SEA_C), distance to centroid (CD), nearest neighbor distance (NND), and standard deviation of NND (SDNND). 95% confidence intervals are also reported.

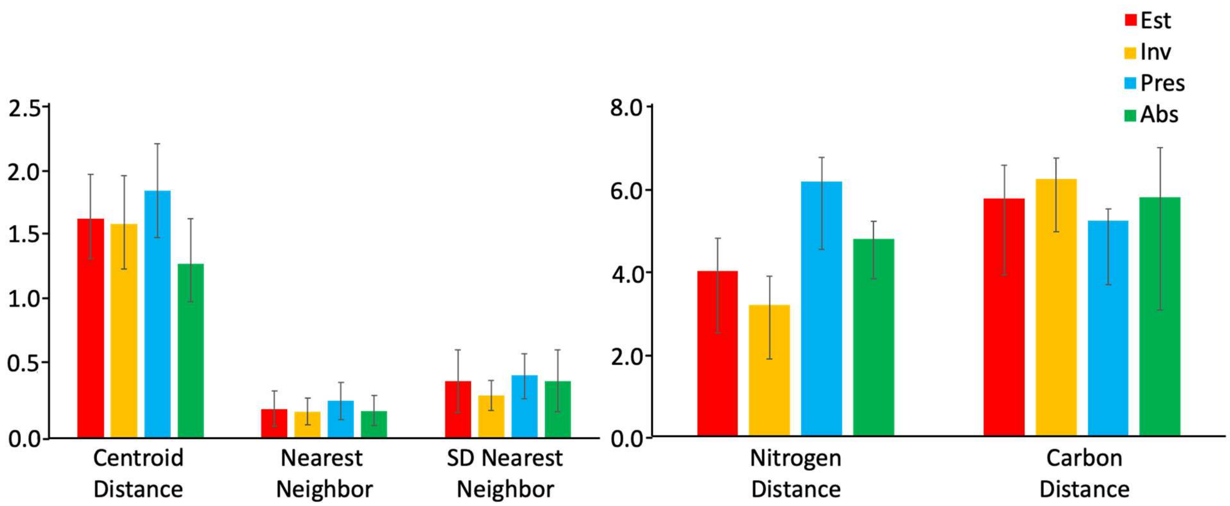

Figure C3**.** Layman metrics for Bluegill in each invasion category including the established (reproducing; Est), adults present (non-reproducing; Inv), sighted (Presence' Pres), or absent (Abs) in the Ohio River basin. Metrics include $\delta^{15}N$ range, $\delta^{13}C$ range, size of Bayesian ellipses (SEA_C), distance to centroid (CD), nearest neighbor distance (NND), and standard deviation of NND (SDNND). 95% confidence intervals are also reported.

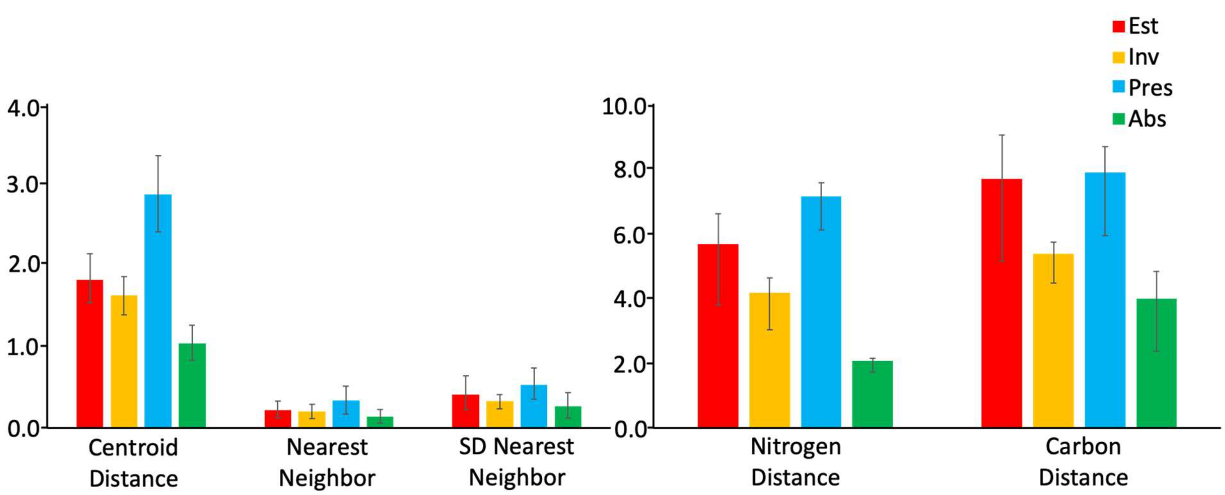

Figure C4. Layman metrics for Smallmouth Buffalo in each invasion category including the established (reproducing; Est), adults present (non-reproducing; Inv), sighted (Presence' Pres), or absent (Abs) in the Ohio River basin. Metrics include $\delta^{15}N$ range, $\delta^{13}C$ range, size of Bayesian ellipses (SEA_C), distance to centroid (CD), nearest neighbor distance (NND), and standard deviation of NND (SDNND). 95% confidence intervals are also reported.

APPENDIX D

CHLOROPHYLL A IN INVASION CATEGORIES

Table D1**.** Chlorophyll a concentrations (µg/L) for each tributary of the Ohio River sampled. Rivers from the establishment category are Clover Creek (CLOV), Wabash River (WAB), and Cumberland River (CUMB); invasion category are the Little Miami (LIMIA), Licking (LICK), and Little Kentucky River (LIKY); presence category are the Kanawha (KANW), Guyandotte (GUYN), and Big Sandy River (BSAN); and absence category include the Alleghany (ALGH) and Monongahela Rivers (MON). Standard error (SE) is reported for chlorophyll a values from each tributary.

Establishment			Invasion			Presence			Absence	
CLOV ±SE	WAB ±SE	CUMB ±SE	LIMIA ±SE	LICK ±SE	LIKY ±SE	KANW ±SE	GUYN ±SE	BSAN ±SE	ALGH ±SE	MON ±SE
5.33 ± 0.21	19.93 ± 0.38	5.99 ± 0.23	12.33 ± 0.60	7.99 ± 0.52	8.82 ± 0.21	2.42 ± 0.33	2.89 ± 0.06	5.19 ± 0.35	1.67 ± 0.15	2.17 ± 0.10

APPENDIX E

DIET DATA FROM NATIVE SPECIES IN OHIO RIVER TRIBUTARIES

Diets were analyzed to calculate a trophic position. For diet analysis stomachs were removed and preserved in 10% neutral buffered formalin. For planktivorous fish (i.e. Gizzard Shad, Silver Carp and Smallmouth Buffalo) foreguts were removed. Analyzing prey from foreguts will reduce the number of prey items that are unidentifiable due to digestive processes. For the remaining species, whole stomachs were removed and analyzed. In the lab, two different sets of methods were used to analyze diets. For planktivores, the identification process began by shaking samples vigorously to separate diet items from remaining stomach lining and mucus. Samples will then be homogenized in a known volume of Lugols iodine for Gizzard Shad and Silver Carp, and rose bengal for Smallmouth Buffalo. Two different stains were used to assist in identifying diet items based on what diet items were expected in each species diet. A 0.5 ml sample were removed and diet items identified to major taxonomic group (multi-celled chlorophyta, single celled chlorophyta, diatoms, cladocera, etc.). If necessary, multiple 0.5 ml samples were analyzed until at least 300 diet items are identified. For a subsample of Silver Carp and Gizzard Shad, two 1 ml sample slides were also observed to determine if any zooplankton were consumed. For the remaining species, all diet items were identified to the family level for insects and lowest taxonomic level possible for crayfish and fish. To quantify the proportion of detritus in Gizzard Shad diets, 0.5 ml sample were placed in a gridded microscope slide and analyzed at 40x. Two independent readers analyzed each sample by estimating the portion of the grid covered by detritus and portion of the grid covered by all diet items. The portion of the grid covered by detritus were divided by the portion of the grid covered by all diet items to determine proportion of detritus in each diet. For each individual consumer, conspecific diet items were enumerated. Numeric diet composition was calculated for each fish. Diet derived trophic position was calculated for each fish. First, prey species were assigned a trophic status. For example, primary producers were assigned a trophic position of 1, primary consumers a trophic position of 2, etc. Prey items that feed on multiple trophic levels were assigned an intermediate trophic position (e.g. 2.5). Trophic position of the consumer was calculated using the equation:

3) $TP = \Sigma(\text{Volume of prey species} \times \text{TP of prey type}) + 1$.

A mean diet-derived trophic position of each species was calculated for each site.

Detritus in Gizzard Shad Diets

There was a relationship between detritus in Gizzard Shad diets and Silver Carp abundance. Gizzard Shad in the presence category had lower detritus in their diet than Gizzard Shad in the establishment ($z = -5.090$, $p = <0.001$), invasion ($z = -2.657$, $p = 0.039$), and absence ($z = -3.128$, $p = 0.009$) categories. Gizzard Shad in the invasion category had moderately less detritus in their diets than the establishment category ($z = -2.500$, $p = 0.059$). Amount of detritus in Gizzard Shad diets in the absence category did not differ from the establishment ($z = 1.117$, $p = 0.677$) or invasion ($z = -0.948$, $p = 0.777$) category. However, only one site from the absence category accounted for most of the data for that invasion category.

Table E1**.** Diet data for Gizzard Shad in Ohio River Silver Carp categories captured in tributaries of the Ohio River, USA as a function of Silver Carp being established (reproducing; Est), adults present (non-reproducing; Inv), sighted (Presence; Pres), or absent (Abs). Plankton proportion is presented in proportion of each plankton group to all plankton identified in the diets of Gizzard Shad. Detrital proportion is presented as total estimated detritus in Gizzard Shad diets. Standard error is also reported (± SE)

Invasion Category	Chlorophyta	Cyanobacteria	Diatom	Euglena	Proportion Detritus
Est	0.242 ± <0.01	0.731 ± <0.01	0.012 ± <0.01	0.008 ± <0.01	0.786 ± 0.02
Inv	0.279 ± 0.04	0.709 ± 0.04	0.004 ± <0.01	0.006 ± <0.01	0.759 ± 0.04
Pres	0.231 ± 0.03	0.756 ± 0.01	0.003 ± <0.01	0.003 ± <0.01	0.645 ± 0.03
Abs	0.321 ± 0.07	0.657 ± 0.04	0.013 ± 0.01	0.007 ± <0.01	0.696 ± 0.07

Table E2**.** Diet data for Largemouth Bass in the Ohio River Silver Carp categories captured in tributaries of the Ohio River, USA as a function of Silver Carp being established (reproducing; Est), adults present (non-reproducing; Inv), sighted (Presence; Pres), or absent (Abs). Number of diet items consumed is representedas total number consumed by all Largemouth Bass in each Silver Carp category.

Diet Item	Silver Carp Category		
	Establishment	Invasion	Presence
Chironomid sp.	1		
Decopoda sp.		1	1
Dorosoma sp.		33	12
Lepomis sp.	1		
Micropterus sp.	1		
Trichoptera so.		1	
Unidentifiable Diet Item	3	1	1
Unidentifiable Fish sp.	6	12	5

Table E3**.** Diet data for Bluegill in the Ohio River Silver Carp categories captured in tributaries of the Ohio River, USA as a function of Silver Carp being established (reproducing; Est), adults present (non-reproducing; Inv), sighted (Presence; Pres), or absent (Abs). Number of diet items consumed is represented as total number consumed by all Bluegill in each Silver Carp category.

Diet Item	Silver Carp Category			
	Establishment	Invasion	Presence	Absence
Adult Chitonomid sp.	1			
Adult Coleoptera sp.	1			
Adult Flying Insect sp.	8	3	8	4
Adult Terrestrial Insect sp.	3			
Amphipoda sp.			36	7
Asellidae sp.			2	
Bivalva sp.	3	9	15	
Chaoboriade sp.		3		
Chironomid Larvae sp.	682	764	1043	394
Coleoptera Adult sp.		7	2	
Coleoptera Larvae sp.	3	1	141	
Decapoda sp.	10		3	2
Dipterian Pupae	15	19	20	236
Ephemeroptera sp.	17	1	33	1
Formicidae sp.		3		2
Gammeridae sp.				35
gastropoda sp.	3		1	1
Hemiptera sp.			1	
Hydrachidiae sp.	1		1	
Hymenoptera sp.				1
Odonata sp.	2		2	11
Orthropoda sp.			2	
Poduridae sp.				1
Pyralidae Larvae sp.				6
Trichoptera sp.	21	9	62	13

Table E4. Diet data for Smallmouth Buffalo in the Ohio River Silver Carp categories captured in tributaries of the Ohio River, as a function of Silver Carp being established (reproducing; Est), adults present (non-reproducing; Inv), sighted (Presence; Pres), or absent (Abs). Number of diet items consumed is represented as total number consumed by all Smallmouth Buffalo in each Silver Carp category.

Diet Item	Silver Carp Category			
	Establishment	Invasion	Presence	Absence
Amphipoda sp.				51
Araneae sp.				5
Bivalve sp.			3	8
Ceratopogonidae sp.	4	235	79	
Chironomid sp.	2585	6624	374	6873
Cladocera sp.	781	6064	1726	15149
Copepoda sp.	437	6956	2141	6934
Dipterian Pupae sp.	44	78	1	273
Gastropoda				1
Hermiptera	3			
Hirudinea	12	147	58	8
Hydracrina	2	4		28
Odonata				16
Ostracoda	16	581	389	34
Rotifera	227	75	103	123
Trichoptera	70	48	4	204

Trophic Comparisons Between Stable Isotopes and Diet Analysis

I compared diet derived trophic position against isotope derived trophic position for each species using paired t-tests. For the three species below diet derived trophic position was significantly different than isotope derived trophic position (Table E5; E6; E7). I did not compare the two trophic positions for Gizzard Shad since all the diet derived TPs would be 2.0. The few zooplankton I found in the diets may explain the differences but I have no way of combining that data with the phytoplankton data since they were not standardized in the same way. I also do not have zooplankton data for all the Gizzard Shad and most of the Gizzard Shad I subsampled did not have any zooplankton in their diets. I have a few theories for why this might have occurred. The first is that because of the diet items I found in the diets I could not do volumetric diet proportions to represent larger items contributing more to the isotope taken up by the consumer, but instead did numeric proportions. Also, my data has a small sample size that only represents a small period in time where as stable isotope signatures represent a longer time period. Finally, if Silver Carp are having an effect on the prey species of the species (i.e. Gizzard Shad consumed by Largemouth Bass) I would expect isotope derived trophic position to vary whereas prey trophic position would be the same for all tributaries.

Table E5. Diet and isotope derived trophic position (TP) of Largemouth Bass in tributaries of the Ohio River, USA. TP was compared using paired T-tests. Standard deviation is also reported (± SD).

Tributary	Diet Derived TP	Isotope Derived TP	t Statistic	df	p-value
Clover Creek	3.76 ± 0.44	3.60 ± 0.27	0.98	6	0.36
Little Kentucky	3.56 ± 0.16	4.15 ± 0.21	-8.21	8	<0.01
Big Sandy	3.75 ± 0.35	4.18 ± 0.04	-1.54	1	0.37
Kanawha	3.50 ± 0	4.40 ± 0.06	-49.19	11	<0.01

Table E6**.** Diet and isotope derived trophic position (TP) of Bluegill in tributaries of the Ohio River, USA. TP was compared using paired T-tests. Standard deviation is also reported (± SD).

Tributary	Diet Derived TP	Isotope Derived TP	t statistic	df	P-value
Cumberland R.	3.53 ± 0.08	3.121 ± 0.29	4.72	9	<0.01
Clover Cr.	3.50 ± <0.01	2.79 ± 0.23	9.58	9	0.01
Little Kentucky R.	3.50 ± 0.01	3.22 ± 0.41	1.95	8	0.087
Licking R.	3.50 ± <0.01	3.30 ± 0.18	3.44	9	<0.01
Big Sandy R.	3.50 ± 0	3.05 ± 0.33	3.61	6	0.0112
Guyandotte R.	3.50 ± <0.01	2.55 ± 0.27	10.01	7	<0.01
Beaver R.	3.53 ± 0.07	2.89 ± 0.30	6.51	9	<0.01
Monongahela R.	3.50 ± 0	3.00 ± 0.11	9.82	4	<0.01
Allegheny R.	3.51 ± 0.07	2.89 ± 0.40	4.59	9	<0.01
Kanawha R.	3.48 ± 0.05	3.51 ± 0.23	-0.35	9	0.73

Table E7**.** Diet and isotope derived trophic position (TP) of Smallmouth Buffalo in tributaries of the Ohio River, USA. TP was compared using paired T-tests. Standard deviation is also reported (± SD).

Tributary	Diet Derived TP	Isotope Derived TP	t Statistic	df	p-value
Wabash R.	3.56 ± 0.18	2.41 ± 0.33	9.75	7	<0.01
Clover Cr.	3.50 ± 0	2.79 ± 0.42	3.77	4	0.20
Little KY R.	3.44 ± 0.56	2.92 ± 0.30	2.42	8	0.042
Licking R.	3.41 ± 0.52	3.20 ± 0.24	1.06	9	0.32
Little Miami R.	3.56 ± 0.15	3.01 ± 0.90	2.01	9	0.075
Guyandotte R.	3.39 ± 0.55	2.52 ± 0.54	3.05	8	0.016
Kanawha R.	3.76 ± 0.25	3.37 ± 0.28	2.49	7	0.038
Beaver R.	3.54 ± 0.12	3.08 ± 0.16	10.00	9	<0.01
Monongahela R.	3.50 ± 0.01	2.93 ± 0.23	7.77	9	<0.01
Alleghany R.	3.50 ± 0	2.72 ± 0.20	7.77	3	<0.01

Levin's Niche Breadth Index

I used the numeric diet data collected to determine niche breadth using Levin's niche breadth index.

3) $B = Y2 / \Sigma Nj2$

This index will complement the niche breadth calculated with stable isotope analysis because it considers each diet species/group individually. Niche breadth was calculated for each individual and a mean niche breadth was calculated for each site (Table E8). I used ANOVAs for each fish species to compare each species mean niche breadth among sites. I looked at the proportion of detritus found in Gizzard Shad diets and use a generalized linear model with binomial family to determine if Gizzard Shad are consuming more detritus where Silver Carp are abundant. Levin's niche breadth did not differ among invasion categories for Gizzard Shad ($F_{3,8} = 0.0782$, $p = 0.97$), Bluegill ($F_{3,6} = 1.136$, $p = 0.407$), and Smallmouth Buffalo ($F_{3,8} = 0.0457$, $p = 0.986$) Since I only had Largemouth Bass from one tributary from each invasion category I could not run an ANOVA, but no pattern existed among the three tributaries

Table E8**.** Levin's niche breadth for Gizzard Shad (G. Shad), Largemouth Bass (L. Bass), Bluegill, Smallmouth Buffalo (S. Buffalo) in tributaries of the Ohio River, USA.

Tributary	G. Shad	L. Bass	Bluegill	S. Buffalo
Cumberland R.	3.19	-	3.19	3.13
Wabash R.	2.93	-	-	2.13
Clover Cr.	3.11	1.25	1.07	2.40
Little KY R.	3.07	1.12	1.12	3.35
Licking R.	3.67	-	1.18	2.72
Little Miami R.	1.92	-	-	2.02
Big Sandy R.	2.74	1.80	1.36	2.00
Guyandotte R.	3.13	1	1.97	3.21
Kanawha R.	3.27	-	1.39	2.90
Beaver R.	3.46	-	2.07	2.13
Monongahela R.	2.70	-	1.79	3.24
Allegheny R.	2.77	-	4.19	2.38

VITA

Graduate School
Southern Illinois University Carbondale

Justin J. Kowalski

jkwal96@gmail.com

University of Wisconsin - Stevens Point
Bachelor of Science, Fisheries and Water Resources, May 2018

Thesis Paper Title:
Multi-Species Trophic Response in Tributaries of the Ohio River Along a Gradient of an Invasive Planktivore

Major Professor: James E. Garvey