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RESEARCH ARTICLE

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

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Abstract Gizzard shad is a dominant planktivore/detritivore in the Wabash River, and populations crashed in the early 1990s. Previous work (1974–2008) identified a substantial shift in body-size structure and functional trait composition in the Wabash River fish assemblage between 1989 and 1996. Invasive Asian Carp appeared in the Ohio River basin including the Wabash River in the 1990s. Our goal was to test for temporal changes in assemblage composition and trophic structure relative to the invasion of Asian carp from the early 1990s. We hypothesized that establishment of Asian Carp was a contributor to the assemblage composition shift, and that their presence altered the trophic pathways and food sources of native fishes including Gizzard Shad (hostile takeover hypothesis). Alternatively, Asian Carp may have found success through capitalizing on an empty niche, likely left vacant by the decline in Gizzard Shad, or abundance changes in other trophic groups (opportunistic hypothesis). We utilized archival fish and mussel collections to test for trophic changes in the ecosystem using $\delta^{13}C$ and $\delta^{15}N$ stable isotope analyses. We examined stomach contents of the dominant planktivore/detritivore consumer, Gizzard Shad, from archival and recent collections to test for changes in the phytoplankton community. Stable isotopes indicated a community reliance on other, more deplete, carbon sources than indicated by the algae, and a slight increase between the $\delta^{13}C$ time periods. Although all functional feeding groups of fishes indicated some reduction in $\delta^{15}N$, the differences were only significant for omnivores, mussels, and planktivore/detritivores. Although Asian Carp may have contributed to the collapse of Gizzard Shad populations, other stressors were likely more important drivers of their decline. This is the first, albeit indirect, evidence of opportunistic "invasion" as opposed to the historically presumed hostile takeover model.

Keywords Fish assemblage structure · Stable isotope ratio · Invasive species

Introduction

Introduction of non-indigenous invasive species to riverine systems has altered ecosystems worldwide (Sala et al. 2000; Kolar and Lodge 2001; Olden and Poff 2005). Invasive species can induce cascade effects throughout riverine food webs, altering competitive interactions and trophic structure (Strayer et al. 2008; Delong 2010) and resource availability (Karatayev et al. 2014). Invasive species also have the capacity to trigger community assembly shifts (Anderson et al. 2009), alter nutrient cycling and processing rates (Sousa et al. 2009; Capps and Flecker 2013), and modify the assemblage composition in rivers (Olden and Poff 2005; Delong 2010). Aquatic invasive species with negative consequences in North American rivers include Zebra Mussels (*Driessena polymorpha*), Round Goby (*Neogobius melanostomus*), Rusty Crayfish (*Orconectes rusticus*), Asian Carp (Silver Carp, *Hypophthalmichthys nobilis*, and Bighead Carp, *H. molitrix*; Lodge et al. 1998;

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Kolar and Lodge 2001; Sakai et al. 2001). An understanding of the consequences of invasion may assist in preventing or limiting future negative effects.

Asian Carp (common name for Silver Carp and Bighead Carp) were introduced into the United States for aquaculture in the 1970s and escaped into the Mississippi River watershed during floods (Kolar et al. 2005). They subsequently spread into the Ohio River basin including the Wabash River, with rapid population growth (Coulter et al. 2013, 2015) that suggests the potential for impacts on native species. The plankton diet of Asian Carp overlaps with many native species (Sampson et al. 2009; Pongruktham et al. 2010). Although documentation of the effects of Asian Carp on native fishes is relatively limited (Sampson et al. 2009), the combination of high biomass and plankton consumption of these fish (Irons et al. 2007) suggests that their presence may lead to modifications to trophic pathways of native taxa. Irons et al. (2007) found decreased body condition in two likely competitor fishes (Gizzard Shad, *Dorosoma cepedianum*, and Bigmouth Buffalo, *Ictiobus cyprinellus*) in the Illinois River, following Asian Carp invasion. Stuck et al. (2015) suggested that Asian Carp have lower impacts in river ecosystems with little modification (e.g., dams, dredging). Yet, to date there is little focus on community effects or ecosystem alterations by Asian Carp invasion in the Mississippi River drainage (Delong 2010).

Gizzard Shad occur in high abundance in US reservoirs (Mundahl and Wissing 1987) and large rivers (Pyron et al. 2006), and contribute to nutrient dynamics through recycling and transport of nutrients (Schaus and Vanni 2000). Gizzard Shad selectively forage on zooplankton as larvae and expand their diet to include phytoplankton, zooplankton and detritus as adults (Drenner et al. 1982). In reservoirs Gizzard Shad consume plankton and switch to detritivory with decreased plankton and access to sediments, resulting in nutrient transfer from benthic to pelagic habitats (Schaus and Vanni 2000; Schaus et al. 2002). In the Ohio River, Gizzard Shad $\delta^{15}N$ signal was higher than expected and the $\delta^{13}C$ signal had high variation, indicating a wide variety of prey sources (Thorp et al. 1998). Zeug et al. (2009) also found high variation in $\delta^{13}C$ signal for Gizzard Shad in the Brazos River, Texas, and associated floodplain and oxbow habitats, potentially related to their seasonal prey switching (Schaus and Vanni 2000).

Potential causes of long-term river ecosystem change or assemblage changes include variation in nutrient or sediment inputs with land-use changes (Rabalais et al. 1996), industrial pollution, hydrologic alterations (Poff et al. 1997), invasive species, or natural population cycles of riverine organisms (Townsend 1989). Variation in river nutrients may result from changes in agricultural practices (Carpenter et al. 1998; Muenich et al. 2016). For example,

rapid transport of excess nutrients to rivers increases with rowcrop agriculture (Randall and Mulla 2001), tile drainage and channelized streams to rapidly move water from fields. Hydrologic alteration of river ecosystems occurs with dam operations, intensive land-use, water removal and myriad other impacts (Richter et al. 1996). Fish communities frequently respond to these stressors (Poff and Allen 1995; Walser and Bart 1999; Poff and Zimmerman 2010) and invasive species that are frequently more tolerant or even thrive under altered environmental conditions become an added synergistic threat (Früh et al. 2012).

Broadway et al. (2015) identified an assemblage shift from analyses of temporal variation in body-size structure and trophic composition in the Wabash River (Indiana, USA) fish assemblage from 1974 to 2008. Dramatic changes were detected in the dominant functional feeding groups between 1989 and 1993. Planktivorous/detrivorous Gizzard Shad (subsequently as planktivore), omnivorous Common Carp (*Cyprinus carpio*), and piscivorous Spotted Bass (*Micropterus punctulatus*) historically dominated the fish assemblage, and their decreased abundance (particularly Gizzard Shad) coincided with a dramatic increase in benthic invertivore fishes (Broadway et al. 2015). Following the community shift, energy transfer efficiency (and presumably nutrient transfer) increased (i.e., community size spectrum slopes flattened; Sprules and Munawar 1986), while food web capacity (ability to support secondary productivity) had no distinct temporal trends. This suggests fewer trophic linkages and energy pathways during the recent period (1993–2008). The food web changes observed occurred during a time when Asian Carp were first detected in the system. This begs the question, did Asian Carp contribute to the changes (i.e., as a driver of change), was their invasion and establishment simply opportunistic (i.e., they were able to take advantage of an empty niche left vacant by Gizzard Shad), did changes in abundances of native fishes impact Gizzard Shad abundance, or can none of these hypotheses be accepted?

Invasive species have frequently been decried as causes of change (e.g., Zebra Mussel; Strayer 2009). However, native communities are under constant pressure from multiple anthropogenic and natural stressors. It is reasonable to consider an alternative that many "invasive" species are simply opportunistically filling an energy void related to independent system changes (Gurevitch and Padilla 2004). Based on the traditional model of species invasion, we hypothesized that establishment of Asian Carp was a contributor to the assemblage composition shift, and that their presence altered the trophic pathways and food sources of native fishes including Gizzard Shad (hostile takeover hypothesis; Melbourne et al. 2007). One alternative is that Asian Carp found success through capitalizing on an empty niche, possibly left vacant by the decline in Gizzard

Shad (opportunistic hypothesis). Another hypothesis is that increased abundance of benthic invertivore fishes was partially stimulated by Asian Carp cycling of resources from the water column to the benthos. This is based on a potential shift to a greater consumption of Silver Carp fecal pellets (Yallaly et al. 2015) by benthic invertivores. If consumption of fecal pellets from Silver Carp is common in the Wabash River, this is a potential pathway that links pelagic to benthic production, similar to the observed shift in the Great Lakes with dreissenid mussel invasion (Higgins and Vander Zanden 2010). Specifically we assessed several complementary lines of evidence: (1) explicitly examine the timing of the Gizzard Shad decline, Asian Carp arrival and establishment, and the previously identified community shift (Broadway et al. 2015), (2) examine increased abundance of native benthic invertivore fishes that may have occurred with increased pelagic to benthic production from Silver Carp fecal pellets; with subsequent potential impacts on Gizzard Shad abundance through competition for benthic resources with benthic consumers (either aquatic macroinvertebrates or benthic invertivore fishes), (3) evaluate changes in algal composition in the diets of Gizzard Shad as the most direct competitor with Asian Carp, and (4) compare and contrast energy sources and food web position of key species before and after the community shift. As is frequently the case in ecology, especially when dealing with historic data sets, all desired data are rarely available, therein limiting the potential of obtaining a clear decision within hypothesis space. Thus we utilized a 'bulk of evidence and parsimony' approach based on a priori expectation (Table 1).

Methods

Site description

The Wabash River is a large Midwestern (USA) river (watershed of 85,000 km^2) threatened by agriculture, reservoir release management, manufacturing activities, and urban impacts during the past century (Gammon 1998). Details of the current (post-1998) Wabash River fish assemblage, physical habitats, and hydrologic variability are documented by Pyron et al. (2011; Pyron and Lauer 2004; Pyron and Neumann 2008).

Field and laboratory methods

We utilized the same data set from the Broadway et al. (2015) (Table 2); detailed collection methods may be found there. Fishes were collected during annual surveys during Jun–Oct from 1968 to 1998 using boat electrofishing with a Smith-Root Type IV GPP (Smith Root Inc., Vancouver, WA, USA) and in 2001–2008 with a Smith-Root 5.0 GPP with direct current voltage. Collections were distributed throughout the complete river distance, with effort concentrated from river km 300–530 (Fig. 1). Here we specifically extracted annual species abundances to compare and contrast the temporal abundance dynamics of Gizzard Shad, benthic invertivores, and Asian Carp (line of evidence #1 and #2, Table 1).

We performed stable isotope (δ^{13}C and δ^{15}N) analysis on preserved tissue samples to compare and contrast the energy sources and food web positions of key species (i.e., Gizzard Shad and Silver Carp) and the full fish assemblage before and after the community shift. Our stable isotope analyses used tissues from archived fishes from the Illinois Natural History Survey (INHS), Tulane University Biodiversity Research Institute, Ohio River Valley Sanitation Commission, and our own collections from the 2000s. We attempted to locate summer and fall collections from multiple locations of the Wabash River from 1958 to 2014. Our interest was in quantifying changes in trophic position (δ^{15}N) and basal energy sources (δ^{13}C) in fish tissues. We utilized both museum (INHS) and current collections of freshwater mussels, including Elephant Ear (*Elliptio crassidens*), Hickorynut (*Obovaria olivaria*), Maple Leaf (*Quadrula quadrula*), Monkeyface (*Quadrula metanevra*), Pimpleback (*Quadrula pustulosa pustulosa*) and Three Ridge (*Amblema plicata*). Herbivore fishes

Table 1 A priori expectations for hypotheses

| Line of evidence	If hostile takeover	If opportunistic
Concurrence of population dynamics and communities	Asian Carp initial increase followed by Gizzard Shad decrease and community shift	Gizzard Shad initial decrease prior to or concurrent with community shift and both prior to establishment of Asian Carp; benthic invertivore abundance increase followed by decrease in Gizzard Shad
Food web structure	Shift in δ^{13}C for Gizzard Shad and narrowing of community trophic space after establishment of Asian Carp	No changes in Gizzard Shad or community trophic space
Gizzard Shad diet	Shift in diet after establishment of Asian Carp toward less preferred algal species	No clear shift, or if shift not to less preferred

Table 2 Wabash River fishes by functional feeding group (FFG), abbreviations for Fig. 2, and percent abundance before and after assemblage shift in 1992

FFG	Common name	Genus species	Abbrev.	Pre	Post
Benthic invertivore	Black Redhorse	*Moxostoma duquesnei*	MODU	0.05	0.49
	Blue Sucker	*Cycleptus elongatus*	CYEL	1.08	2.33
	Freshwater Drum	*Aplodinotus grunniens*	APGR	2.31	27.37
	Golden Redhorse	*Moxostoma erythrurum*	MOER	1.38	1.16
	Northern Hogsucker	*Hypentelium nigricans*	HYNI	0.22	0.54
	Quillback Carpsucker	*Carpiodes cyprinus*	CACY	0.30	0.32
	River Carpsucker	*Carpiodes carpio*	CACA	3.30	13.97
	River Redhorse	*Moxostoma carinatum*	MOCA	0.04	0.35
	Shorthead Redhorse	*Moxostoma macrolepidotum*	MOMA	2.1	4.41
	Shovelnose Sturgeon	*Scaphirhynchus platorhynchus*	SCPL	0.53	0.71
	Silver Redhorse	*Moxostoma anisurum*	MOAN	1.16	3.03
	Smallmouth Buffalo	*Ictiobus bubalus*	ICBU	0.0	2.94
	Spotted Sucker	*Minytrema melanops*	MIME	0.004	0.0
	White Sucker	*Catostomus commersonii*	CACO	0.008	0.0
General invertivore	Bigmouth Buffalo	*Ictiobus cyprinellus*	ICCY	0.48	0.98
	Black Buffalo	*Ictiobus niger*	ICNI	0.03	1.17
	Bluegill	*Lepomis macrochirus*	LEMA	0.008	0.02
	Goldeye	*Hiodon alosoides*	HIAL	1.88	0.09
	Green Sunfish	*Lepomis cyanellus*	LECY	0.0	0.02
	Longear Sunfish	*Lepomis megalotis*	LEME	0.0	0.07
	Mooneye	*Hiodon tergisus*	HITE	0.72	0.12
	Redear Sunfish	*Lepomis microlophus*	LEMI	0.01	0.0
Herbivore-detritivore	Grass Carp	*Ctenopharyngodon idella*	CTID	0.0	0.28
	Highfin Carpsucker	*Carpiodes velifer*	CAVE	0.22	0.64
Omnivore	Common Carp	*Cyprinus carpio*	CYCA	14.13	10.83
	Channel Catfish	*Ictalurus punctatus*	ICPU	3.93	5.29
	Flathead Catfish	*Pylodictis olivaris*	PYOL	10.49	7.6
	Goldfish	*Carassius auratus*	CAAU	0.004	0.0
	Goldfish/Carp Hybrid	*Carassius/Cyprinus*	CACY	0.004	0.0
Parasite	Chestnut Lamprey	*Ichthyomyzon castaneus*	ICCA	0.0	0.03
	Silver Lamprey	*Ichthyomyzon unicuspis*	ICUN	0.05	0.01
Piscivore	American Eel	*Anguilla rostrata*	ANRO	0.17	0.0
	Black Crappie	*Pomoxis nigromaculatus*	PONI	0.04	0.14
	Blue Catfish	*Ictalurus furcatus*	ICFU	0.01	0.1
	Bowfin	*Amia calva*	AMCA	0.31	0.03
	Grass Pickerel	*Esox americanus vermiculatus*	ESAM	0.008	0.0
	Largemouth Bass	*Micropterus salmoides*	MISA	0.20	0.22
	Longnose Gar	*Lepisosteus osseus*	LEOS	5.40	1.78
	Sauger	*Sander canadensis*	SACA	0.52	0.83
	Shortnose Gar	*Lepisosteus platostomus*	LEPL	5.44	1.45
	Skipjack Herring	*Alosa chrysochloris*	ALCH	1.04	0.56
	Smallmouth Bass	*Micropterus dolomieu*	MIDO	0.63	1.18
	Spotted Bass	*Micropterus punctulatus*	MIPU	0.41	0.54
	Spotted Gar	*Lepisosteus oculatus*	LEOC	0.02	0.06
	Walleye	*Sander vitreus*	SAVI	0.05	0.13
	White Bass	*Morone chrysops*	MOCH	1.95	1.02
	White Crappie	*Pomoxis annularis*	POAN	0.27	0.2
	Yellow Bass	*Morone mississippiensis*	MOMI	0.01	0.0
Planktivore	Bighead Carp	*Hypophthalmichthys nobilis*	HYNO	0.0	0.32
	Gizzard Shad	*Dorosoma cepedianum*	DOCE	38.76	5.75
	American Paddlefish	*Polyodon spathula*	POSP	0.03	0.05

Table 2 (continued)

FFG	Common name	Genus species	Abbrev.	Pre	Post
	Silver Carp	*Hypophthalmichthys molitrix*	HYMO	0.0	0.87

Fig. 1 Map of Wabash River watershed and Indiana. *Bold line* indicates sampling area
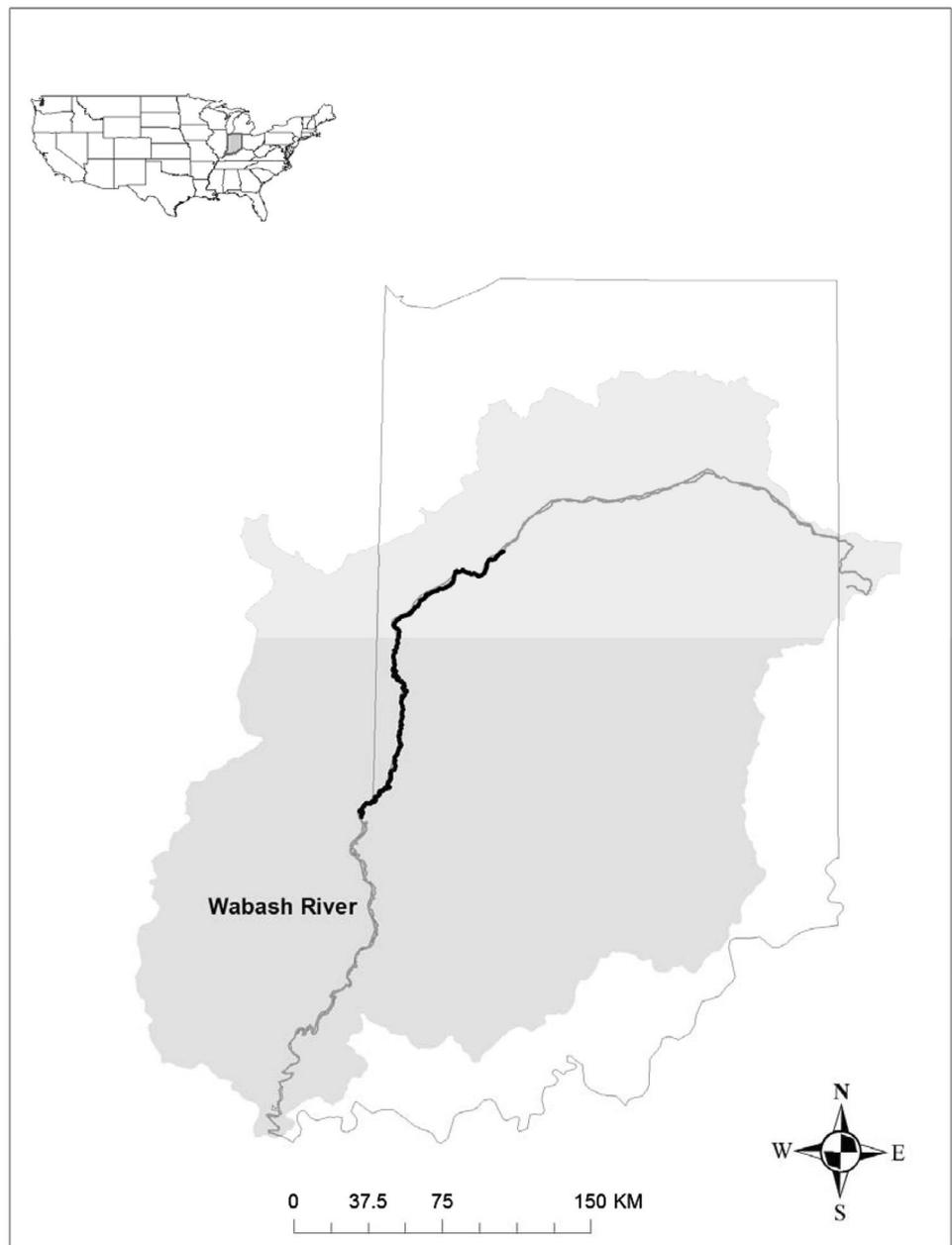
included Gizzard Shad, Emerald Shiner (*Notropis atherinoides*), Mississippi Silvery Minnow (*Hybognathus nuchalis*), and Spotfin Shiner (*Cyprinella spiloptera*). Piscivores were Spotted Bass, and benthic invertivores included Blue Sucker (*Cycleptus elongatus*), Freshwater Drum (*Aplodinotus grunniens*), and Redhorse Suckers (*Moxostoma* spp.).

All fishes were preserved in formalin and stored in 70% ethanol, while mussels were fixed and stored in 95% ethanol. For fishes, a portion of dorsal muscle tissue was removed from each specimen; for mussels, a portion of mantle tissue was removed from each specimen. Samples were dried at 60 °C for 48 h, ground and weighed into tin capsules. Stable isotope ratio analysis was performed at the University of Arkansas Stable Isotope Laboratory (UASIL, Fayetteville, AR, USA), using an elemental analyzer (Carlo Erba NC2500; Milan, Italy) coupled to a Finnigan Delta

Plus (Breman, Germany) isotope ratio mass spectrometer. Isotopic determinations of $\delta^{13}C$ and $\delta^{15}N$ were normalized to Vienna PeeDee Belemnite and atmospheric N_2 scales, respectively, using a combination of internal and international standards. Fixation and storage by formalin-ethanol or ethanol have little effect on $\delta^{15}N$ values, however they can alter $\delta^{13}C$ values, generally making samples more ^{13}C deplete (Lau et al. 2012). This can be problematic if the goal is to identify specific energetic sources in an ecosystem. However, because our goal was not identification of specific C sources or developing C source mixing models, but identifying temporal shifts in source utilization within taxa (i.e., not "did the C source change from x to y", but more generally, "was there a change in C source"?), and samples were treated the same within taxa, we elected not to adjust our $\delta^{13}C$ values. Additionally, species-specific correction factors have been determined for very few taxa, and researchers need to use care in identifying sources using $\delta^{13}C$ between taxa, especially with different preservation methods (Lau et al. 2012). To facilitate comparison of contemporary and museum specimens collected over 56 years, values for $\delta^{13}C$ were corrected for the Suess effect (reduction in baseline atmospheric $\delta^{13}C$ over the past 200 years) using Eq. 4 from Verburg (2007). We used 2014 as our baseline year and calculated a correction factor based on the collection year of a sample. This resulted in a $\delta^{13}C$ correction of up to $-1.60‰$ (for samples collected in 1958).

Finally, to evaluate changes in algal diet composition of Gizzard Shad we removed preserved stomach contents of Gizzard Shad when possible. We recognize that Gizzard Shad are planktivores and detritivores (Schaus and Vanni 2000), but we were interested in algae as diet items based on their presence in all Gizzard Shad stomachs. We were able to sample Gizzard Shad from museum collections collected in 1964, 1997, 1999, and 2000. To better understand taxonomic changes in the algal community, we removed a subsample of stomach contents from 3 to 20 individuals from each time period and algal cells were enumerated. Algal cells were counted using a Palmer-Maloney nanoplankton counting chamber. At least 300 individual cells or colonies of algae were counted per Gizzard Shad stomach. Algae were identified to major groups (typically phylum) at 400× magnification using a light microscope. Stomach contents from 2 to 4 fish from each collection year were pooled (plus a pooled sample from Gizzard Shad collected in 2009), collected on a glass fiber filter, and analyzed for $\delta^{13}C$ and $\delta^{15}N$.

Data analysis

We used CPUE as a proxy for abundance over time to estimate the long-term trends in planktivore (primarily Gizzard Shad), benthic invertivore, piscivore, omnivore, and Asian Carp abundance.

To determine changes in the trophic structure of the fish assemblage over the past 40 years, we visually assessed $\delta^{13}C$ - $\delta^{15}N$ biplots and compared mean $\delta^{13}C$ and $\delta^{15}N$ isotope ratios for functional feeding groups (FFG) using mixed effects analysis of variance (ANOVA), followed by Tukey's HSD for post-hoc comparisons. The stable isotope analyses used three species of planktivore fishes, five omnivore fishes, six benthic invertivore fishes, two piscivore fishes, and six mussel species (Table 3). We were limited by availability of archival material and live mussels in the river (Fisher 2006) resulting in reduced temporal and spatial coverage, compared to our fish assemblage data set. The time period was divided into two categories as the majority of our available museum samples were from pre- and post-Asian Carp introduction (ca. 1992), with a large period in-between (~20 years). Due to variation in sample sizes of each species in the functional feeding groups, we used species as a random factor nested within each FFG and Type III sums-of-squares in the mixed-effects ANOVA. When there was no significant interaction between FFG and time-period, the ANOVA was re-run without the interaction term. If there was a significant interaction between FFG and time period, data were separated and individual Welch corrected t-tests were used to test for individual group differences.

We assessed changes in the algal assemblage in Gizzard Shad stomachs using two approaches. First, due to differing numbers of fishes available in each year, we pooled the algal information into two time periods 1964 (n = 20) and 1997–2000 (n = 27). We then used a log-likelihood ratio (G) test to determine differences in proportional abundance of the major algal groups between the different collection years. Our expected proportional abundance was based on the 1964 data. Second, we used linear regression to determine if there was a change in $\delta^{13}C$ or $\delta^{15}N$ with time in the stomach contents of Gizzard Shad, including an additional sample from 2009.

We set $\alpha = 0.05$ for all statistical analyses. Statistical analyses were performed in R (version 3.1; R Core Team 2014). For the ANOVA analyses we used the packages 'lme4' (Bates et al. 2014) and 'car' (Fox and Weisberg 2011); Tukey HSD tests were done using the package 'agricolae' (de Mendinburu 2014).

Results

Planktivore (Gizzard Shad) abundances historically showed high interannual variation, but declined steadily throughout most of the observed time period (Fig. 2). Asian Carp did not appear in surveys until 1999 when

Table 3 Fish and mussel collections used for isotope analyses

| Species	Collection years	N	Functional feeding group
Silver Carp	2013	5	Planktivore
Gizzard Shad	1958–2009	80	Planktivore/detritivore
Mississippi Silvery Minnow	1958	19	Planktivore
Spotted Bass	1964–1997	23	Piscivore
White Bass	2009	2	Piscivore
Blue Sucker	2007	2	Benthic invertivore
River Carpsucker	2004	4	Benthic invertivore
Freshwater Drum	1994–2013	21	Benthic invertivore
Black Redhorse	2007	15	Benthic invertivore
Shorthead Redhorse	1961–2007	28	Benthic invertivore
Silver Redhorse	1964	1	Benthic invertivore
Spotfin Shiner	1960–2009	63	Omnivore
Mimic Shiner	1958	5	Omnivore
Emerald Shiner	1958	5	Omnivore
Bluntnose Minnow	2004	10	Omnivore
Silver Chub	2004	11	Omnivore
Hickorynut mussel	2014	12	Planktivore
Three ridge mussel	1988–2014	11	Planktivore
Elephant ear mussel	1966–1988	6	Planktivore
Monkeyface mussel	2014	10	Planktivore
Maple leaf mussel	2014	5	Planktivore
Pimpleback mussel	2014	4	Planktivore

Fig. 2 Catch per unit effort ($\ln + 1$ transformed) for fish trophic groups and Silver Carp by year
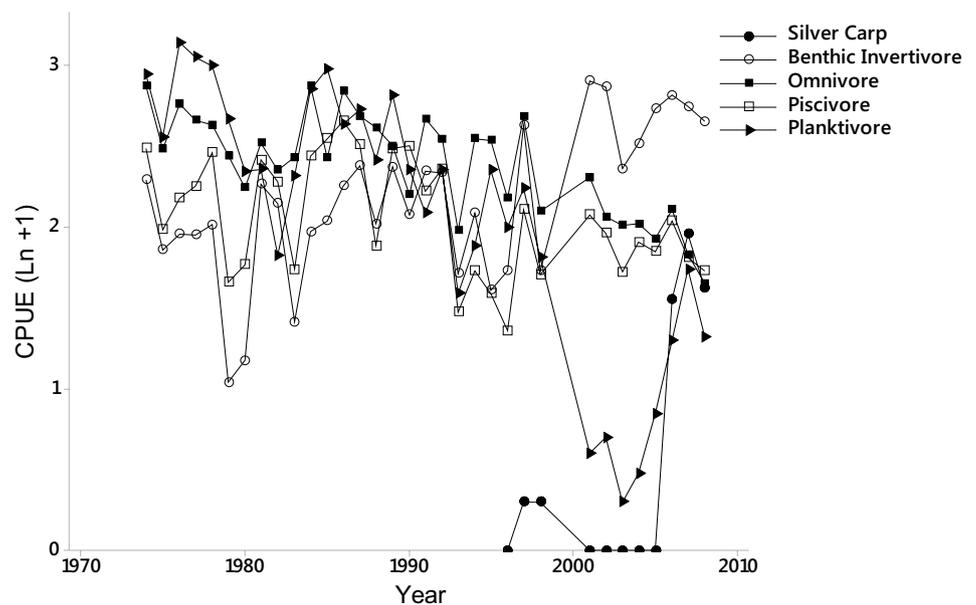
Gizzard Shad were already below their 1970s levels. The decline in planktivores appears to have been independent of the arrival of Asian Carp (Fig. 2). Omnivore and piscivore abundances declined steadily through the period. Benthic invertivores had cyclical variation through the period and increased during the last decades, simultaneous to planktivore declines.

Stable isotope biplots indicated a community reliance on other, more deplete, carbon sources than indicated by the algae (Fig. 3), and a slight increase in $\delta^{13}C$ between the time periods. Changes in $\delta^{15}N$ were less consistent, and interestingly, while $\delta^{15}N$ in algae increased between the time periods, many of the FFGs indicated a decrease of $\delta^{15}N$ in the post-1992 time period. Mixed effect ANOVA

Fig. 3 Mean (±SD) isotope ratios for δ¹³C and δ¹⁵N from fish, mussel, and algae samples prior to and after 1992
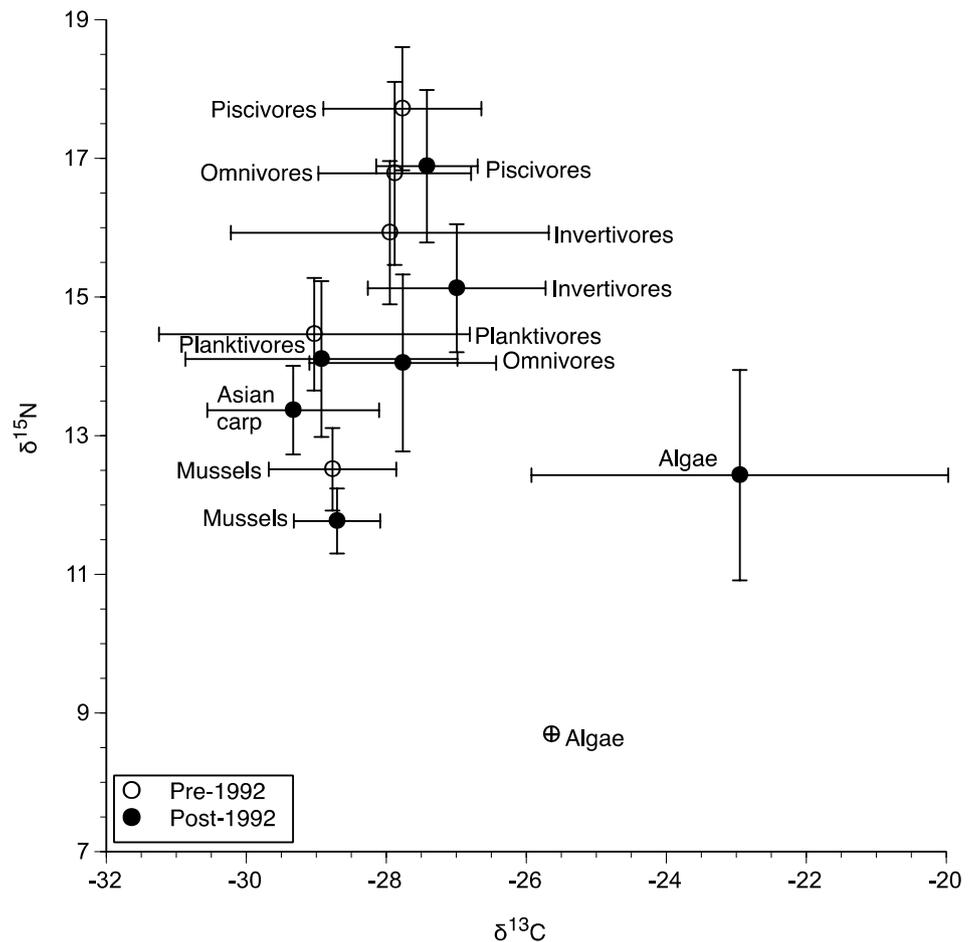
indicated no significant differences in $\delta^{13}C$ ratio between FFG ($F_{4,333} = 1.74$, $p = 0.189$) but there was a significant difference between the two time periods ($F_{1,335.96} = 12.84$, $p < 0.001$) and no significant interaction ($F_{4,275.33} = 0.8726$, $p = 0.481$). The mean $\delta^{13}C$ in fishes increased 0.53‰ between the two time periods, from −28.45 to −27.92‰. There were also significant differences in $\delta^{15}N$ ratio for both FFG and time period, however there was a significant interaction ($F_{4,238.74} = 30.76$, $p < 0.001$). Individual Welch corrected t tests were used to compare FFGs across the two time periods. Although all FFG indicated some reduction in $\delta^{15}N$, the differences were only significant for omnivores, mussels, and planktivores (Table 4). Removal

of the contemporary (2014) collection of five Silver Carp from the planktivore comparison, resulted in a marginally but non-significant difference ($t_{72.76} = 1.77$, $p = 0.08$), indicating that the decrease in planktivore $\delta^{15}N$ was potentially related to the presence of Silver Carp.

When assessing the algal community in Gizzard Shad stomachs, chrysophyta were excluded because they occurred in a single sample, at low abundance. There were significant differences between algal composition in the stomach contents of 1964 and 1997–2000 Gizzard Shad ($G_5 = 3872.1$, $p < 0.001$). The algae consumed by Gizzard Shad shifted from predominantly chlorophytes (~70%), to a mix of chlorophytes, diatoms, and euglenoids (~30% each),

Table 4 Results of Welch two sample t tests for functional feeding groups (FFG), pre- and post-1992 on $\delta^{15}N$ for fishes of the Wabash River

FFG	Pre-1992 δ¹⁵N	Post-1992 δ¹⁵N	Δδ¹⁵N	t value	d.f.	p
Piscivores	17.72	16.89	0.83	1.93	13.96	0.074
Omnivores	16.78	14.05	2.73	10.11	87.91	**<0.001**
Benthic invertivores	15.93	15.13	0.8	1.51	3.28	0.219
Planktivores	14.46	14.03	0.43	2.27	84.62	**0.026**
Mussels	12.51	11.77	0.74	4.06	17.99	**<0.001**

Significant differences are highlighted in bold

with a small decrease in the proportion of cyanobacteria (Fig. 4). Stomach contents indicated no consistent change in $\delta^{13}C$ ($p = 0.59$), which ranged from -26.79 to $-20.69‰$. However, there was a significant relationship between $\delta^{15}N$ and year ($R^2 = 0.84$, $p = 0.03$, Fig. 5), with $\delta^{15}N$ increasing in more recent samples, but the single early sample (1960's) showed strong leveraging.

Discussion

Broadway et al. (2015) used body size abundance patterns from the same database we used, and found that planktivores were replaced by benthic invertivores between 1989 and 1996. Gizzard Shad populations in the Wabash River declined steadily since the early 1970s, potentially as a result of increased nutrient loads and anthropogenic disturbances, and collapsed to near non-existent abundances by the early 2000's. Asian Carp were first detected in the Wabash River around 1995 (Kolar et al. 2005). Although Asian Carp may have contributed to the collapse of Gizzard Shad populations, other stressors were likely more important drivers of their decline (line of evidence #1, Table 1). The trophic status of Gizzard Shad remained constant ($\delta^{13}C$ and $\delta^{15}N$) over time, and similar to that of Asian Carp (line of evidence #2, Table 1). Concurrently, the diet of Gizzard Shad shifted toward a greater mixed consumption of diatoms, euglenoids, and chlorophyta, as opposed to a historically heavier reliance upon chlorophyta (line of evidence #3, Table 1). It is unclear however, if this shift is due to varied foraging locations, naturally declining chlorophyta abundances, or Asian Carp mediated competition. Based on assessment of the 'bulk of evidence and
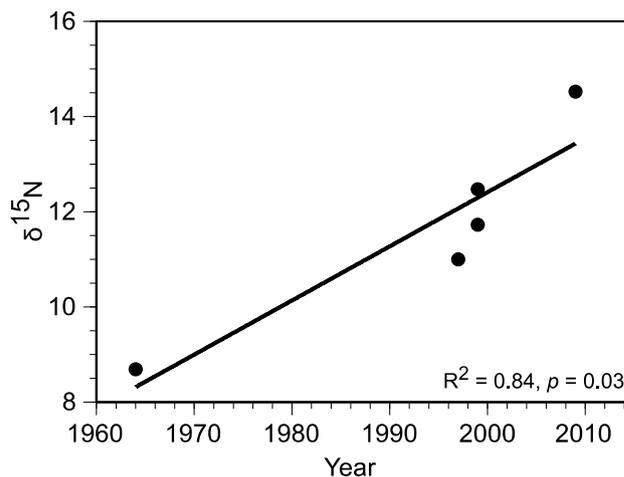

Fig. 5 $\delta^{15}N$ over time from gizzard shad stomachs. Contents from 2 to 5 individual fish at each date were combined to achieve an adequate sample mass

parsimony' table, our conclusions best support the opportunistic hypothesis, as opposed to a more traditional hostile takeover. The fact that Asian Carp have not yet become hyper-abundant as in other systems (Irons et al. 2007) suggests that whatever environmental changes that lead to the Gizzard Shad decline are likely continuing to restrict both Gizzard Shad recovery and Asian Carp expansion.

Changes in nutrient loading from wastewater treatment and agriculture, hydrological alterations or from multiple contributions may be contributing factors (Pyron et al. 2006). Nutrient inputs frequently increase with agricultural land-use (Fennessy and Cronk 1997) and increased human populations (Meyer and Turner 1994). The population

Fig. 4 Proportional abundance of major algal groups in 1964 and 1997–2000. Data are mean abundances from stomach contents of 20 (1964) and 27 (1997–2000) Gizzard Shad from the Wabash River
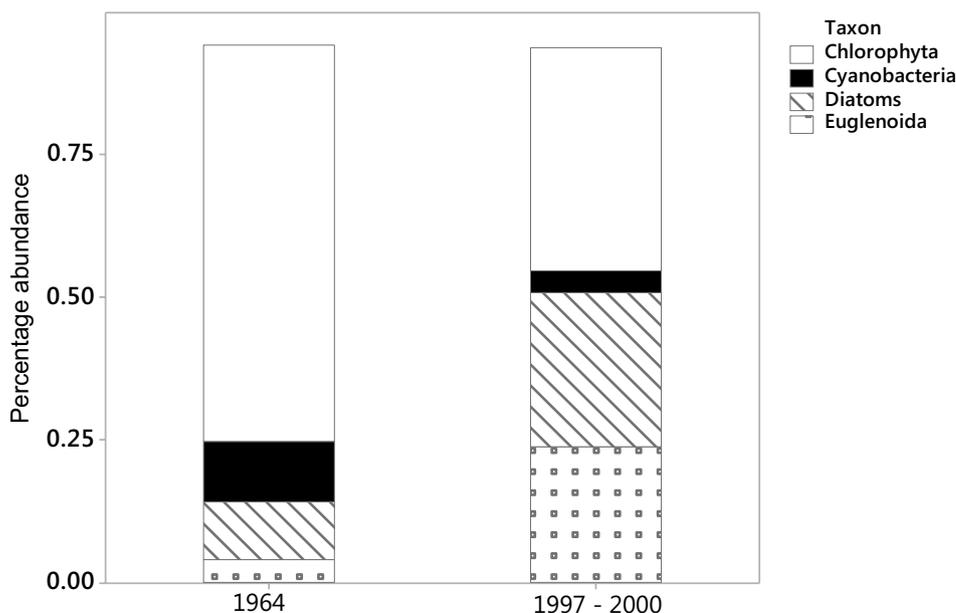
of Indiana increased 25% during the past four decades (http://www.stats.indiana.edu/population/PopTotals/historic_counts_states.asp, accessed 7/1/2014). Wastewater increased with higher human population in the US (Carey and Migliaccio 2009). For nutrients such as nitrogen, the leading source of nitrogen inputs to river systems is large-scale agriculture (Howarth et al. 2012). Indiana swine and poultry production intensity and land application of wastes increased since the 1970s, increasing and changing nutrient delivery to surface waters (Sims et al. 1998; Muenich et al. 2016). Additional influences in the watershed include hydrologic alterations with drainage practices of tile drainage and stream channelization, reservoir operations, and urbanization (Pyron and Neumann 2008). Flood and drought cycles in the watershed are likely altered with these impacts and precipitation changes in the Midwestern US (Pyron and Neumann 2008). Flood or drought events likely strongly influence fish assemblage structure. Pyron et al. (2011) identified four large Wabash River flood events in the past 30 years (1985, 1991, 2003, 2005) with the potential for effects on the fish assemblages of inner bend habitats. Because the community shift and the Gizzard Shad decline happened over a period of several years it is unlikely that these flood events were a primary driver.

The differences in $\delta^{13}C$ among the time periods indicated enrichment for the algae (from Gizzard Shad stomachs). We suggest that increased allochthonous sources (e.g., wastewater and agricultural pollution) may contribute to the nutrient pool (Chandra et al. 2005). However, our isotope ratio results indicate that mussels do not now or historically consume the algal component we sampled. Silver Carp appear to use similar carbon from algae/plankton as mussels and Gizzard Shad, based on the isotope ratio data (Fig. 3). In addition, all of the fish taxa use similar carbon sources (Fig. 3). The assemblage shift from one dominated by planktivorous Gizzard Shad to one dominated by benthic invertivores such as Freshwater Drum (Broadway et al. 2015), indicates a stronger reliance on benthic pathways in recent years.

The $\delta^{15}N$ signal for FFG did not respond consistently with time periods compared with changes in $\delta^{13}C$ for the same FFG. Differences among FFG are expected, as there is an approximate 3.4‰ enrichment of $\delta^{15}N$ per trophic level (Vander Zanden and Rasmussen 2001). The trophic level of omnivores decreased recently (2.7‰), suggesting a shift in diet. The decrease in trophic level for omnivores suggests trophic restructuring. A potential explanation is a shift to a greater consumption of Silver Carp fecal pellets (Yallaly et al. 2015). If consumption of Silver Carp fecal pellets is common in the Wabash River, this is a potential pathway that links pelagic to benthic production, similar to the observed shift in the Great Lakes with dreissenid mussel invasion (Higgins and Vander Zanden 2010). The

shifts in mussel $\delta^{15}N$ (0.74‰) and planktivores (0.43‰) were small which would be expected as they rely on basal resources.

Our stable isotope analyses of fish muscle tissue showed that planktivore Gizzard Shad did not shift $\delta^{15}N$ in recent collections compared to historical collections. The dietary signatures of Gizzard Shad are most similar to Silver Carp (Fig. 3). However, the algal stomach contents suggest a change in available food sources. Gizzard Shad and other planktivores can be size selective for food items (Drenner et al. 1982), and the decrease in larger, and presumably more nutritious chlorophytes recently suggests Gizzard Shad may be feeding in locations with less chlorophytes or chlorophytes may have been grazed down by Asian Carp. It is interesting that the $\delta^{15}N$ signature of Gizzard Shad stomach contents (algae in stomachs) increased without a concurrent increase in muscle $\delta^{15}N$, indicating that snapshots of stomach contents may not be indicative of the energy assimilated over time. The fish assemblage changed from high abundance of Gizzard Shad prior to introduction of Asian Carp, possibly with changes in nutrient delivery to the river. We suggest that competition with Asian Carp may be contributing to the lack of recovery of Gizzard Shad, but not related to the original decline.

Asian Carp may have successfully invaded the Wabash River through capitalizing on a practically void niche, left vacant by the decline of Gizzard Shad, thus reducing any competitive exclusion forces that may have been exerted by abundant Gizzard Shad populations. However, abundances of Asian Carp remain low in the Wabash River, which could be attributed to unfavorable ecosystem conditions that may be more intrinsically linked with benthic production and are likely limiting both the recovery of Gizzard Shad populations, and the hyper-abundances of Asian Carp abundances as observed in the Illinois River (Stuck et al. 2015). Should these conditions change, Asian Carp populations in the Wabash River may rapidly increase as occurred in other regions. Although we state these conclusions tentatively, to date little attention has been given toward the community effects or ecosystem alterations imposed by Asian Carp invasions. Future research should focus on comparing and contrasting habitats, nutrient dynamics, and fish assemblages across a range of systems where Asian Carp occur. This is the first, albeit indirect, evidence of opportunistic "invasion" as opposed to the historically presumed hostile takeover model.

and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

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