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BIGHEADED CARP SPATIAL REPRODUCTIVE DYNAMICS AND POPULATION GENETICS IN LARGE
RIVER TRIBUTARIES

A Thesis
Submitted for the Requirements for the Degree of
Master of Science

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FACTORS INFLUENCING BIGHEADED CARP SPATIAL REPRODUCTIVE DYNAMICS IN ILLINOIS AND WABASH RIVER TRIBUTARIES

ABSTRACT

Fisheries managers are devoting considerable effort, time, and funding towards limiting the spread of invasive Bighead and Silver (bigheaded) Carp. A better understanding of factors that influence spatial patterns of bigheaded carp reproduction can help fisheries managers prevent their spread. To examine factors that influence spatial patterns of reproduction, we sampled ichthyoplankton using drift nets and larval push nets in three tributaries of the Illinois and Wabash Rivers March-September 2016-2018. We compared relative abundances of bigheaded carp eggs and larvae among tributaries using catch per unit effort (CPUE). Abiotic data were analyzed from individual study tributaries and pooled among tributaries with substantial versus minimal evidence of bigheaded carp reproduction. In total, 5,689 larval bigheaded carps were captured during three sampling seasons. Of the six study tributaries, we captured the most bigheaded carp larvae in the Sangamon River, followed by the Little Wabash, Embarras, Spoon, Mackinaw, and Vermilion Rivers. Based on previous literature, we investigated differences in watershed area, discharge, water temperature, turbidity, and free-flowing stretches of river in our study tributaries. In general, tributaries with greater relative abundance of bigheaded carp eggs and larvae had larger watersheds, greater discharge, lower secchi depths (higher turbidity), and longer free-flowing stretches than those with minimal evidence of reproduction. This study provides a thorough investigation of bigheaded carp reproduction in six large river tributaries and will help fisheries managers better monitor and mediate the spread of these invaders by helping prioritize potential monitoring locations.

INTRODUCTION

Predicting reproductive success in novel ecosystems is crucial for understanding the probability of an introduced species establishing itself (Coulter et al. 2013). Bighead Carp *Hypophthalmichthys nobilis* and Silver Carp *H. molitrix* (hereafter termed "bigheaded carps") are two highly invasive fish species causing deleterious effects on native food webs throughout much of the Mississippi River basin (DeBoer et al. 2018). Among other traits making these fishes successful invaders is their high reproductive capacity (Garvey et al. 2006).

Bigheaded carps rely on flowing water for reproduction (Kocovsky et al. 2012) and are thought to require 40-100km of free-flowing river for their eggs to incubate and hatch (Krykhtin and Gorbach 1981; Yi et al. 1988; Garcia et al. 2015). However, Murphy and Jackson (2013) suggest this length is influenced by water temperature, current velocity, and dispersal of eggs. Further, Murphy and Jackson (2013) propose river reaches as small as 25 km may be sufficient for bigheaded carp egg survival.

Water temperature and flow influence the reproductive events of most pelagic-spawning riverine fishes (Dudley and Platania 2007), including those of bigheaded carps (Kocovsky et al. 2012). However, invasive bigheaded carp have selected different habitats and demonstrated greater reproductive plasticity than in their native range (Deters et al. 2012; Coulter et al. 2013), which is thought to have facilitated their expansion. To better understand the reproductive plasticity of bigheaded carps, it is important to study their reproduction in a variety of systems.

The Illinois River (IL) is a highly impounded river thought to have the highest density of Silver Carp in the world (Sass et al. 2010) and has a direct connection to the uninvaded

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Laurentian Great Lakes through the Chicago Area Waterway System (CAWS). Currently, a series

of electric barriers serve as defense against invasive Bighead Carp, Black Carp

Mylopharyngodon piceus, Grass Carp *Ctenopharyngodon idella*, and Silver Carp (collectively

termed "invasive carps") from entering Lake Michigan and potentially impairing the $7 Billion

annual fishery of the Great Lakes (ASA 2008). In contrast to the heavily impounded Illinois River,

the Wabash River is the largest free-flowing river east of the Mississippi River (Skibsted 2012),

with a relatively lower Silver Carp density (Stuck et al. 2015). Both the Illinois (Zhu et al. 2018)

and Wabash (Coulter et al. 2016) Rivers have established spawning populations of bigheaded

carps.

Relating observed reproduction in already invaded waterbodies to potential use of

Great Lakes tributaries would help fisheries managers better predict bigheaded carp invasions

and reproductive activity, should they enter the latter ecosystem. Great Lakes tributaries are

not as long as the Illinois or Wabash Rivers (Murphy and Jackson 2013), therefore tributaries of

large rivers make more applicable sites for studying bigheaded carp reproductive activity.

Tributaries provide reproductive habitat for various fishes (Douglas and Douglas 2000; Webber

et al. 2013) and can host populations of large river species (Brown and Coon 1994). Although

there is no direct connection to the Great Lakes, the Wabash River tributaries in this study have

generally greater discharge than those of the Illinois River, providing a larger range of systems

to study. Additionally, several tributaries of the La Grange Reach of the Illinois River have

known populations of Silver Carp (Sass et al. 2010) and ample free-flowing distance for

potential bigheaded carp reproduction. Aside from Deters et al. (2012) finding minimal

evidence of bigheaded carp spawning in Missouri River tributaries, there is limited literature on

bigheaded carp reproduction in large river tributaries. Murphy and Jackson (2013) suggest suitability for bigheaded carp spawning is determined by a complex relationship between hydraulic and water-quality characteristics. The goal of our study was to better understand reproduction of bigheaded carps in Illinois and Wabash River tributaries and better predict potential use of Great Lakes tributaries for reproduction, should bigheaded carps invade.

During the 2016-2018 field seasons, we sampled bigheaded carp ichthyoplankton with the objectives of (1) determining to what extent individual tributaries of the Illinois and Wabash Rivers are used for bigheaded carp reproduction, and (2) elucidating the characteristics of the tributaries producing the most bigheaded carp eggs and larvae. We hypothesized that the most larval bigheaded carps would be sampled in Wabash River tributaries. We expected this because Wabash River tributaries have a more natural flow regime than Illinois River tributaries (Stuck et al. 2015) and bigheaded carp are known to use increases in water flow as a spawning cue (Kocovsky et al. 2012). Additionally, Wabash River tributaries generally have a higher discharge than the Illinois River tributaries of this study. Specifically, we expected tributaries with the greatest maximum discharge values to produce the most bigheaded carp eggs and larvae, as high discharge events have been associated with bigheaded carp spawning (Kolar et al. 2007). Further, rivers with longer free-flowing distance likely have more potential spawning sites and provide sufficient distance for eggs to incubate (Kocovsky et al. 2012; Murphy and Jackson 2013; Garcia et al. 2015). We also expected tributaries with warmer temperatures to be utilized more than cooler tributaries, as these could potentially be the first to have conditions suitable for spawning and bigheaded carp eggs incubate faster in warmer water (Kocovsky et al. 2012).

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METHODS

Study Sites

We selected three tributaries of the Illinois (Mackinaw, Sangamon, Spoon) and Wabash (Embarras, Little Wabash, Vermilion) Rivers as our study sites (Figure 1). All three Illinois River tributaries flow into the impounded La Grange Reach of the Illinois River, while all three Wabash River tributaries flow into the free-flowing lower section of the Wabash River. Four of our six study tributaries have notable impoundments (Table 2). We sampled tributaries monthly from March-September 2016-2018. Additionally, we sampled bi-weekly during June-August, as water temperatures are generally sufficient (> 17° C) for bigheaded carp spawning (Kolar et al. 2007; Coulter et al. 2013), and Coulter et al. (2016) previously sampled bigheaded carp eggs in the Wabash River during these months.

Sampling Techniques

We deployed ichthyoplankton drift nets at an upper, middle, and lower reach of each tributary (Table 1). At lower sites, we measured flow velocity (m/s) and sampled far enough upriver to ensure any ichthyoplankton sampled was derived from the tributary being sampled. Drift net openings were 0.25m height x 0.45m width with a 1.0m length 500μm mesh net to filter water and collect drifting larval fishes and eggs. Drift nets were positioned directly below the water surface. Flow was measured using a HACH flowmeter and used to determine the soak time of drift nets to standardize the amount of water filtered. Additionally, we sampled with bow-mounted ichthyoplankton push nets at the lower site of each tributary. We implemented push nets at middle sites in 2018 when water depth and boat access were adequate. Push net openings were 0.5m in diameter and had 3.0m of 500μm mesh net for sample collecting. Push

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nets were driven upriver for five minutes, also directly below the water surface. A General Oceanics flowmeter was mounted to the push net opening to gauge the volume of water filtered. We set drift nets and conducted ichthyoplankton pushes at a left, mid-channel, and right section of the river at each site. We recorded water depth (m), surface velocity (m/s), water temperature (°C), dissolved oxygen (mg/L), pH, conductivity (μs), specific conductivity (μs/L), and secchi depth (cm) at each site. When available, we used mean daily river discharge (meters^3/second) and water gauge height (m) from the furthest downstream United States Geological Survey (USGS) river gauge on each tributary for all days within our sampling season to compare discharge among tributaries.

Ichthyoplankton samples were immediately preserved in 95% EtOH after collection. Eggs and fishes were separated from debris and identified to family under a dissecting microscope using Auer (1982) and Chapman (2006) as references. Cyprinids were identified as *Hypophthalmichthys* spp. (bigheaded carps) if applicable (Chapman 2006). A subsample was tested genetically at the Georgia Southern University genetics lab to verify identification. Individual fish were distinguished as being a late-stage larva versus juvenile based on the presence or absence of a median fin-fold.

Statistical Techniques

We calculated catch per unit effort (CPUE) of bigheaded carp eggs and larvae as the total number sampled from each site divided by the amount of water filtered by that gear type. Specifically, we calculated CPUE as the number of eggs and larvae sampled per 100m^3 of water filtered. Amount of water filtered by drift nets was calculated using:

$$Water\ Filtered\ (m^3)$$

$$= Area\ of\ Drift\ Net\ Opening(m^2)\ \text{X}\ Water\ Velocity\left(\frac{m}{s}\right)\text{X}\ Duration\ of\ Sample\ (s)$$

Amount of water filtered by larval push nets was calculated using a General Oceanics 2030R

standard flowmeter and the following equations provided by General Oceanics (1980):

$$Water\ Filtered\ (m^3) = \left(\frac{3.14\ X\ Net\ Diameter^2}{4}\right)X\ Distance\ (m)$$

$$Distance\ (m) = \left(Flowmeter\ Counts\ X\frac{26,873}{999999}\right)$$

An analysis of variance (ANOVA) was performed to test for differences in mean secchi

depth, water temperature, and mean daily discharge among tributaries at lower sites pooled

for all three years and individual years. In the event where a variable was not recorded for a

given tributary, that variable was not used for analyses from other tributaries for that day.

Upon performing ANOVAs, we assessed statistical differences among tributaries using a Tukey's

post-hoc test. Secchi depth data was log_{10}-transformed and tested for homogeneity of

variances with a Fligner-Killeen test. Discharge and water temperature data were determined

non-normal after log_{10} transformation, so a Kruskal-Wallis one-way ANOVA was performed with

a Dunn's post-hoc test on the non-transformed data. Values were considered significant at α <

0.05 for ANOVAs and Tukey HSD post hoc tests.

To examine the relationship between environmental variables and ichthyoplankton

relative abundance, we used both simple and multiple regression analyses. Data were log_{10}-

transformed to ensure variables measured at different scales were of the same order of

magnitude and met the assumptions of normality and homogeneity of variance. Backward

model selection and Akaike information criterion (AIC) were used to determine the model of

best fit for the relationship between watershed area, coefficient of variation (CV) of mean daily discharge, free-flowing river length, and latitude for drift net egg CPUE, drift net larvae CPUE, larval push egg CPUE, and larval push larvae CPUE. We used the "step" function to remove the least informative variables and final models were based on AIC values. Predictor variables were removed until all remaining variables had AIC scores > the null model. All statistical analyses were performed using R version 3.6.0 (R Development Core Team 2019).

A total of 5,689 larval bigheaded carps were sampled from 2016-2018; 5,165 were

sampled by larval push nets and 524 from ichthyoplankton drift nets (Table 3). Across years,

1,040 larvae were collected in 2016, 30 in 2017, and 4,619 in 2018. Of these larvae, 4,427 were

captured in the Sangamon, 1,129 in the Little Wabash, 252 in the Embarras, 44 in the Spoon, 10

in the Mackinaw, and 10 in the Vermilion River (Table 3). Additionally, a total of 3,799

bigheaded carp eggs were sampled during this study. We sampled the most eggs in the

Embarras and Little Wabash Rivers with 1,746 and 1,386, respectively. Further, we captured

653 eggs in the Sangamon, 7 in the Mackinaw, 7 in the Vermilion, and 0 in the Spoon River

(Table 4). Since most (>98%) bigheaded carp larvae were captured at the furthest downstream

sampling site of a tributary, we focused on both ichthyoplankton catch and abiotic data taken

from those lower sites. Although egg CPUE was also highest at the downstream-most sites,

substantial bigheaded carp eggs were collected at other sites; therefore upstream, middle, and

downstream sites were all analyzed for egg data. At downstream sites, we sampled ~26,133 m^3

of water using larval push nets and ~55,045 m^3 of water using ichthyoplankton drift nets. When

all sites were combined for egg data, we sampled ~28,573 m^3 of water using larval push nets

and ~143,072 m^3 of water using ichthyoplankton drift nets. Larval push nets were deployed less

frequently due to the requirement of boat accessibility for using this gear, which was limited

during periods of low water.

Secchi depth (cm) was recorded a minimum of 27 times at the lower site of each study

tributary during the field seasons of 2016-2018. Secchi depth ranged from 20.1cm ± 1.9 (mean

± SE) in the Spoon River to 40.6cm ± 3.8 (mean ± SE) in the Vermilion River (Table 5). A Shapiro-

Wilks test revealed these data were not normal both before (P < 0.01) and after (P < 0.001) data was log-transformed. After $\log_{10}$ transformation, a Fligner-Killeen test showed the data met the assumption of homoscedasticity (P = 0.23). Overall, the model was significant (ANOVA: F = 8.15, df = 5, P < 0.001). A Tukey's post hoc test indicated significant (P < 0.01) differences in eight of the 15 comparisons. Notably, the mean secchi depth in both the Mackinaw and Vermilion Rivers were significantly greater than in the Embarras, Little Wabash, Sangamon, and Spoon Rivers (Figure 2). Mean secchi depth was not significantly different between the Embarras, Little Wabash, Sangamon, and Spoon Rivers (Figure 2); these being the tributaries with the most larval bigheaded carps captured from 2016-2018.

A total of 1,258 water temperature readings were recorded for our six tributaries. Water temperature during our study periods ranged from 21.88°C ± 0.92 (mean ± SE) in the Mackinaw River to 24.14°C ± 0.25 (mean ± SE) in the Embarras River (Table 5). When analyzed by individual years using a Kruskal-Wallis one-way ANOVA, no tributaries were significantly different in 2016 (P = 0.29), 2017 (P = 0.14), or 2018 (P = 0.22). When all three years of water temperature data were pooled, no tributaries had significantly different water temperatures (α = 0.003, Figure 3). All tributaries reached the 17°C and 22°C thresholds in all study years.

Daily discharge from March-September 2016-2018 ranged from 24.73 meters^3/second ± 1.38 (mean ± SE) in the Mackinaw River to 115.80 meters^3/second ± 6.60 (mean ± SE) in the Little Wabash River (Table 5). Our initial Kruskal-Wallis one-way ANOVA determined there were significant differences (χ^2 = 625.77, df = 5, P < 0.05) in mean daily discharge between tributaries. A Dunn's post-hoc test determined mean daily discharge was significantly different (P < 0.003) between all tributaries except the Spoon and Vermilion Rivers (P = 0.38, Figure 4).

Upon completing simple regressions, watershed area (Table 5) explained the most

variation (in order) in drift net larvae CPUE ($F_{1,4}$ = 21.89, P < 0.01, R^2 = 0.85), larval push larvae

CPUE ($F_{1,4}$ = 16.84, P = 0.01, R^2 = 0.81), drift net egg CPUE ($F_{1,4}$ = 3.53, P = 0.13, R^2 = 0.47), and

larval push egg CPUE ($F_{1,4}$ = 2.07, P = 0.22, R^2 = 0.34). Regarding multiple regressions,

watershed area was the best predictor, followed by CV of discharge for drift net egg and larvae

CPUE along with larval push larvae CPUE (Table 6). For larval push egg CPUE, CV of discharge

was followed by watershed area, length of free-flowing river, and latitude (Table 6).

Based on bigheaded carp egg catch and larval relative abundance (Tables 3 and 4), we

categorized tributaries into those with substantial, moderate, and minimal evidence of

reproduction. Substantial (Little Wabash and Sangamon Rivers) tributaries had > 1.0

larvae/100m^3 sampled in larval push nets (all years) and/or > 1,000 larval fish sampled with all

years combined. Moderate (Embarras and Spoon Rivers) tributaries had > 1.0 larvae/100m^3

sampled in larval push nets in an individual year and/or > 750 eggs sampled in multiple years.

Minimal (Mackinaw and Vermilion Rivers) tributaries had < 10 total eggs and ≤ 10 larvae

sampled in all three years combined.

DISCUSSION

Overall, this study provides a thorough investigation of bigheaded carp reproduction in large river tributaries. We found the highest number of bigheaded carp eggs and larvae in tributaries with the largest watershed areas, which subsequently had the highest discharge, greatest turbidity, and although not significant, tributaries with warmer water temperatures. These findings are not only helpful to fisheries managers within the Illinois and Wabash river basins, but also to better predict where bigheaded carps may spawn, should they invade the Laurentian Great Lakes.

Of our six study tributaries, we collected the most bigheaded carp larvae from (in order) the Sangamon, Little Wabash, Embarras, Spoon, Mackinaw, and Vermilion Rivers. We divided these tributaries into three groups; those with "substantial" (Little Wabash and Sangamon Rivers), "moderate" (Embarras and Spoon Rivers), and "minimal" (Mackinaw and Vermilion Rivers) evidence of bigheaded carp reproduction. Furthermore, larval push was our more efficient gear type, which was consistent with Roth et al. (in review, this issue). CPUE for the Mackinaw and Vermilion Rivers appeared to be biased high, as we were unable to use this gear in these tributaries during times of low flow when very few larvae of any species were captured. This lack of sampling likely resulted in fewer catches of zero for the Mackinaw and Vermilion Rivers in low flow conditions relative to the other four tributaries we sampled.

Watershed area and CV of yearly discharge were the best predictors of bigheaded carp egg and larvae relative abundance in our study tributaries. We predicted high discharge variation and "flashiness" to be associated with greater egg and larval CPUE, as Silver Carp are thought to move up tributaries during times of increased flow and have been documented

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exhibiting this in tributaries of the Mississippi and Missouri Rivers (Deters et al. 2013; Vallazza, personal communication). However, CV of discharge was negatively correlated with all CPUEs aside from larval push eggs. We suggest this to be a factor of small, flashier tributaries being used less for reproduction than those with sustained higher discharges. Regarding the influence of latitude, warmer water temperatures at lower latitudes can decrease time needed for egg incubation (Kocovsky et al. 2013) and increase egg and larvae survival. However, we believe our study tributaries are close enough in proximity where this likely has a minimal effect. The statistical outcome of latitude predicting egg and larval relative abundance is potentially confounded by three of our four southern-most tributaries having the highest discharges and longest free-flowing stretches, which allow for a greater chance of successful reproduction (Camacho et al. This Issue).

We analyzed water temperature regimes of our six study tributaries because bigheaded carps require 17°C to spawn, but 22-26°C is preferred (Jennings 1988; Coulter et al. 2013). No tributaries had significantly different mean temperatures in either of the three individual years or when all years were pooled. All study tributaries reached both the 17°C and 22°C thresholds in all three years without a discernible pattern in the order of warming. Although the two tributaries with minimal evidence of reproduction (Mackinaw and Vermilion Rivers) had the two lowest mean water temperatures, this was likely not enough to have biological effects. We found no appreciable differences in this study, however, warmer Great Lakes tributaries flowing in from regions such as southern Wisconsin and southern Michigan are likely more suitable for bigheaded carp spawning than coolers tributaries in the far-northern parts of those states.

An increase in discharge (Zhang et al. 2000; Schrank et al. 2001; Wang et al. 2008) and adequate flow (Huet 1970) are primary cues for bigheaded carp reproduction. DeGrandchamp (2007) concluded that while high water may not be critical for reproductive success, it may augment egg and larvae survival. Further, Sullivan et al. (2018) states that sustained periods of high discharge can lead to strong Silver Carp year-classes. Further, the two tributaries with the highest larval bigheaded carp CPUE had the highest mean discharge values (Little Wabash and Sangamon Rivers), whereas the two tributaries with minimal evidence of reproduction (Mackinaw and Vermilion Rivers) had the lowest mean discharge values.

Although often covarying with watershed area and discharge, turbidity may also influence recruitment of bigheaded carps (Chang 1966; Verigin et al. 1978; Fermin 1990; Deters et al. 2013) and turbid water may reduce predation on bigheaded carp larvae (Reichert et al. 2010). However, Krykhtin and Gorbach (1981) found no relationship between turbidity and Grass Carp or Silver Carp reproduction. More recently, Deters et al. (2013) concluded that turbidity positively correlated with bigheaded carp egg production in the Missouri River; supporting our findings that our least turbid rivers had the least evidence of reproduction, although this is likely a product of discharge and watershed area. Most rivers where bigheaded carps have reproductive success are turbid, regardless of the mechanism leading to this success (Kocovsky et al. 2012). Although the relationship between turbidity and bigheaded carp reproduction may not be causal, this characteristic is likely indicative of a waterbody supporting bigheaded carp reproduction. Thus, Great Lakes tributaries with greater turbidity should be prioritized with regards to sampling for potential bigheaded carp reproduction if they enter the system.

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In their native range, bigheaded carp spawning sites are often highly turbulent from tributary confluences or other hard points such as islands (Huet 1970; Yi et al. 1988). While bigheaded carp eggs are semi-buoyant, adequate turbulence is required for eggs to maintain suspension in the water column (Soin and Sukhanova 1972; Pflieger 1997; Deters et al. 2013). In Summer 2017, we visually witnessed Silver Carp actively spawning where the Embarras River converges with the Wabash River. Eggs fertilized at this and similar locations, along with resulting larvae, would not have been sampled by our gear, because we sampled upstream of confluences to avoid capturing ichthyoplankton that may have originated from the mainstem river. However, our two tributaries where we caught the most larvae both have notable inputs; the Skillet Fork flows into the Little Wabash River in White County (IL) and Salt Creek flows into the Sangamon River in Menard County (IL), creating areas of turbulence and potential spawning sites. Moreover, the Little Wabash and Sangamon Rivers were the only seventh order rivers in this study. The confluence of two sixth order rivers, as described above, likely provides adequate turbulence for bigheaded carp eggs to stay in the water column during times of relatively higher flow.

Eggs of bigheaded carps were thought to require 100 km of free-flowing river to incubate and hatch (Krykhtin and Gorbach 1981; Yi et al. 1988), but recently, Murphy and Jackson (2013) suggest that this length depends on water temperature, current velocity, and dispersal of eggs. Further, Murphy and Jackson (2013) propose river reaches > 25 km may be sufficient for bigheaded carp egg survival. Five of our six study tributaries had > 100 km of free-flowing and the Vermilion River has 37 km of free-flowing river downstream from the Danville Dam (Danville, IL). Although we found minimal evidence of bigheaded carp reproduction in the

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Vermilion River, we did capture ten larvae and seven eggs in this tributary between 2016 and 2018. Further, the Danville and Ellsworth Dams were both removed in 2019, increasing the length of free-flowing river available and potentially making the Vermilion River more suitable for bigheaded carp reproduction. We recommend further sampling to evaluate how these dam removals and increased free-flowing river length affect future bigheaded carp reproduction.

Silver Carp exhibit highly plastic reproductive traits that allow them to spawn over a wider range of hydrological conditions, a more protracted time period, and in smaller rivers than reported from their native range (Coulter et al. 2013). Due to this plasticity and our biweekly sampling design, some spawning events may have been missed. Further, Cyr et al. (1992) states that most surveys of larval fish are based on few, large samples. Although many of our mean CPUE values were influenced by individual days with large catches, we had a large sample size during the three years of this study to consider. Additionally, the phenomenon of many sampling events with low catches in concurrence with few events of large catches reflects the sporadic nature of bigheaded carp reproduction (Zhang et al. 2000) and is supported by the high weekly variation in Coulter et al. (2016). However, we were likely to document evidence of substantial reproduction that took place in our study tributaries due to our extensive sampling efforts over three seasons and using both passive (drift nets) and active (larval push) gears in a variety of hydrological regimes.

Although adult abundance certainly influences recruitment (Garvey 2007) and production of eggs and larvae, we do not have relative abundance data for adults in our tributaries. CPUE of adults was not part of the scope of this project, but would be insightful in the future. Further, adults have been observed moving from mainstem rivers into tributaries

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with rising discharge during likely times of spawning (Vallazza, personal communication); therefore, resident tributary population values may not be indicative of the spawning stock using a particular tributary to reproduce, especially with the protracted spawning patterns (Coulter et al. 2013) of these species.

Although our study rivers flow into larger rivers (Illinois or Wabash), whereas tributaries of Lakes Erie and Michigan flow into a lentic system, this study provides a basis for determining higher priority Great Lakes tributaries to monitor for bigheaded carp reproduction, should Bighead or Silver Carp invade either of these systems. While watershed land use varies between the Great Lakes tributaries and our study system, which can affect runoff and variables such as turbidity (Knoll et al. 2003), we recommend prioritizing tributaries that have larger watersheds and have greater discharge, turbidity, and length of free-flowing stretches. For example, the St. Joseph River (Michigan) is one of the southern-most Lake Michigan tributaries, generally has a high discharge, and although not necessarily free-flowing, fish ladders intended for salmonids would likely be used by Silver Carp to move upriver, as they have been observed leaping as high as 276 cm (Stell 2018). Contrastingly, the Menominee River forms part of the Wisconsin-Upper Michigan border. The Menominee warms later and has an impassible dam, aside from a monitored fish-elevator, just 4 km upriver from Lake Michigan (Porter 2019). This dam would prohibit bigheaded carps from reaching viable stretches of free-flowing river for reproduction. It should be noted, however, that Great Lakes tributaries like those of the Illinois or Wabash Rivers with minimal evidence of bigheaded carp reproduction should still be monitored, especially considering the high reproductive plasticity observed in bigheaded carps (Coulter et al. 2013). Our study, along with Coulter et al. (2013) and Murphy and Jackson (2013), shows

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bigheaded carps are capable of spawning in shorter rivers than previously considered viable.

Further, with climate change expected to contribute to warmer water temperatures (Meisner

1990; Flebbe et al. 2006; Lyons et al. 2010), the length of river necessary for larval bigheaded

carp development will likely decrease (Kocovsky et al. 2012); therefore, increasing the

likelihood of these fishes successfully reproducing in shorter tributaries.

This study provides an examination of bigheaded carp reproduction in large river

tributaries and will help fisheries managers better assess the risk of bigheaded carp spawning in

various systems. We recommend further ichthyoplankton sampling of these six and potentially

other tributaries, as Silver Carp populations continue to grow in the Wabash River (Carpenter,

personal communication), and two dams were removed from the Vermilion River in 2019,

increasing free-flowing river stretches and allowing access to new habitats. The findings

presented in this paper and continued sampling should assist fisheries managers in better

prioritizing sites to monitor the potential further spread of bigheaded carps.

TABLES

Table 1.1 - Sampling sites including river kilometers (km) from confluence, stream order, and nearest municipality of three tributaries of the Illinois River (Mackinaw, Sangamon, and Spoon Rivers) and three tributaries of the Wabash River (Embarras, Little Wabash, and Vermilion Rivers) sampled from March-September 2016-2018. All municipalities are within the state of Illinois unless otherwise noted.

River Basin	Tributary	Site	River km	Stream Order	Nearest Municipality
Illinois	Mackinaw	Upper	122	5	Congerville
		Middle	82	6	Mackinaw
		Lower	<1	6	Pekin
	Sangamon	Upper	208	5	Decatur
		Middle	137	5	Riverton
		Lower	<1	7	Beardstown
	Spoon	Upper	113	5	London Mills
		Middle	61	6	Seville
		Lower	<1	6	Havana
Wabash	Embarras	Upper	193	6	Charleston
		Middle	146	6	Greenup
		Lower	<1	6	St. Francisville
	Little Wabash	Upper (2016)	357	4	Neoga
		Upper (2017,2018)	127	6	Albion
		Middle	51	7	Carmi
		Lower	<1	7	New Haven
	Vermilion	Upper	37	5	Danville
		Middle	19	6	Georgetown
		Lower	<1	6	Cayuga, IN

Table 1.2 - Notable impoundments on study tributaries including distance to mouth (km) and height (feet) among three tributaries of the Illinois River (Mackinaw, Sangamon, and Spoon Rivers) and three tributaries of the Wabash River (Embarras, Little Wabash, and Vermilion Rivers) sampled from March-September 2016-2018.

River Basin	Tributary	Barrier	River km	Height (ft)
Illinois	Mackinaw	NA		
	Sangamon	Decatur Dam	210.3	33
	Spoon	NA		
Wabash	Embarras	Charleston Spillway	194.4	29
	Little Wabash	Lake Mattoon Dam	360.5	46
	Vermilion	Danville Dam	35.4	11

Table 1.3 - Mean yearly catch per unit effort (CPUE) in fish/100m^3, standard error (SE), and total catch (n) of larval bigheaded carp by drift nets (DN) and larval pushes (LP) in three Illinois River tributaries (Mackinaw, Sangamon, and Spoon Rivers) and three Wabash River tributaries (Embarras, Little Wabash, and Vermilion Rivers) from March-September 2016-2018 and for all years combined.

		DN			LP		
	Tributary	CPUE	SE	n	CPUE	SE	n
2016	Mackinaw	0	0	0	0	0	0
	Sangamon	0	0	0	0.06	0.06	1
	Spoon	0	0	0	0	0	0
	Embarras	0	0	0	0.70	0.70	20
	Little Wabash	1.12	0.44	99	40.22	23.17	916
	Vermilion	0.01	0.01	1	0.19	0.14	3
2017	Mackinaw	0.05	0.05	1	0.47	0.47	4
	Sangamon	0.03	0.03	1	0.09	0.09	1
	Spoon	0.08	0.05	3	0.08	0.08	1
	Embarras	0.03	0.03	1	0.20	0.14	2
	Little Wabash	0.08	0.05	5	1.00	0.69	8
	Vermilion	0	0	0	0.31	0.22	3
2018	Mackinaw	0.04	0.03	2	0.52	0.36	3
	Sangamon	4.43	2.11	276	123.05	79.94	4148
	Spoon	0.19	0.08	10	1.51	0.97	30
	Embarras	0.27	0.12	41	0.24	0.15	5
	Little Wabash	0.96	0.65	81	0.95	0.51	20
	Vermilion	0.07	0.04	3	0	0	0
2016-2018	Mackinaw	0.02	0.01	3	0.27	0.15	7
	Sangamon	1.53	0.73	277	61.56	40.2	4150
	Spoon	0.09	0.03	13	0.54	0.34	31
	Embarras	0.10	0.04	42	0.39	0.25	27
	Little Wabash	0.80	0.29	185	17.98	10.2	944
	Vermilion	0.03	0.01	4	0.16	0.08	6

Table 1.4 – Total catch of bigheaded carp eggs caught in drift nets and larval pushes fished on three tributaries of the Illinois River (Mackinaw, Sangamon, and Spoon Rivers) and three tributaries of the Wabash River (Embarras, Little Wabash, and Vermilion Rivers) from March-September 2016-2018.

Tributary	2016	2017	2018	Sum
Mackinaw	7	0	0	7
Sangamon	0	0	653	653
Spoon	0	0	0	0
Embarras	984	0	762	1746
Little Wabash	475	0	911	1386
Vermilion	7	0	0	7

Table 1.5 – Watershed area (meters^2), mean discharge (meters^3/second), secchi depth (cm), water temperature (°C) and standard error (SE) from the Mackinaw, Sangamon, Spoon, Embarras, Little Wabash, and Vermilion Rivers from March-September 2016-2018.

Tributary	Watershed	Discharge	SE	Secchi	SE	Temp	SE
Mackinaw	2,942	887	52	33.9	2.6	21.88	0.92
Sangamon	13,888	3,886	198	22.2	1.3	23.77	1.02
Spoon	4,817	1,132	62	23.1	1.9	23.34	1.06
Embarras	6,320	3,123	239	21.4	1.5	24.15	0.24
Little Wabash	8,386	4,090	233	20.1	1.5	23.99	0.22
Vermilion	3,367	1,307	79	40.6	3.8	23.06	0.26

Table 1.6 – Multiple regression model selection results including response variable, model predictor variables, Akaike weights (W_i), R^2, and P-values of the best model for predicting relative abundance (CPUE) of bigheaded carp (*Hypophthalmichthys* spp.) eggs and larvae caught in drift nets and larval push nets. Variables include log-transformed watershed area (LogArea), log-transformed coefficient of variation of mean daily discharge (LogCVQ), log-transformed unimpounded river length (LogLength), and log-transformed latitude of downstream-most sampling site of each tributary (LogLat). Environmental data was analyzed from March-September 2016-2018.

Response Variable	Predictive Model	W_i	R^2	P
Drift Net Eggs	LogArea - LogCVQ	0.55	0.73	0.14
Drift Net Larvae	LogArea - LogCVQ	0.54	0.93	0.02
Larval Push Eggs	LogArea + LogCVQ + LogLength - LogLat	0.65	0.99	0.15
Larval Push Larvae	LogAeea - LogCVQ	0.51	0.92	0.02
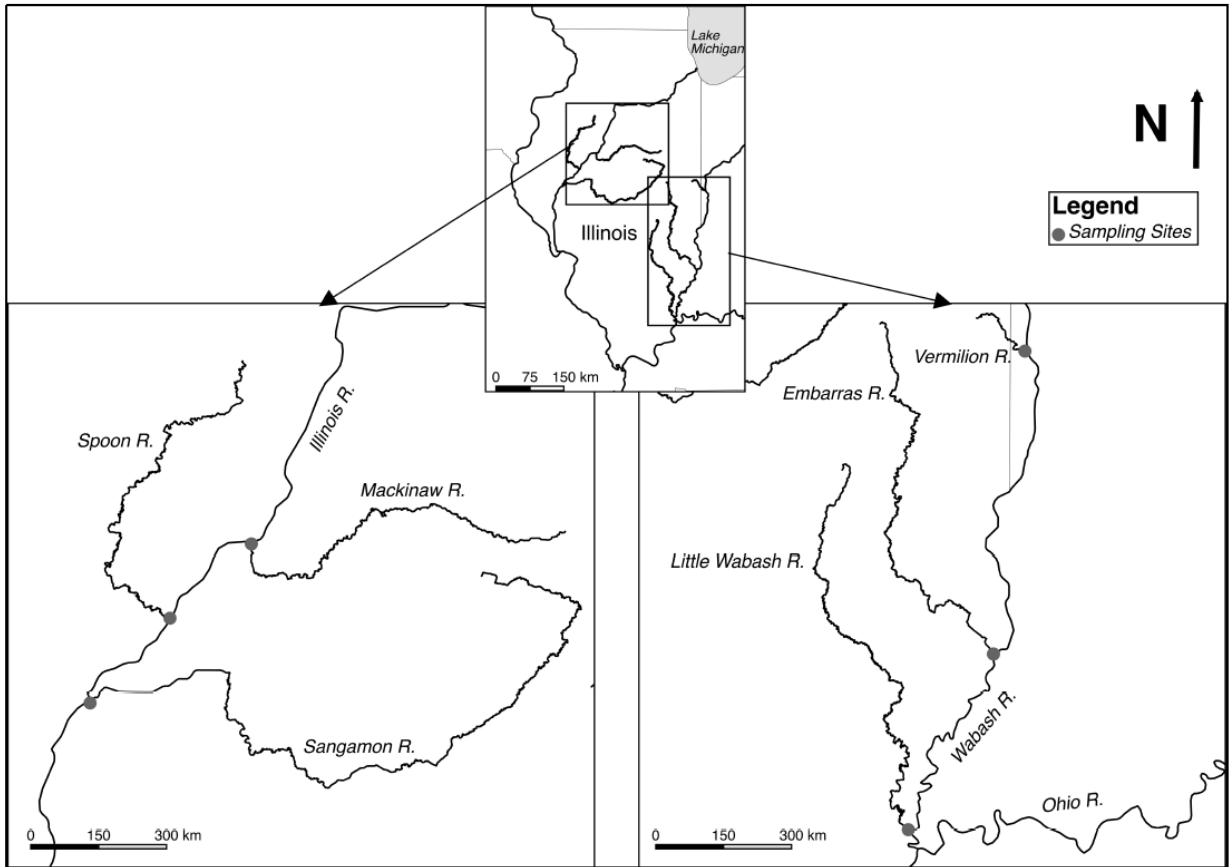
Figure 1.1 – Study area including the Mackinaw, Sangamon, Spoon, Embarras, Little Wabash, and Vermilion Rivers (Illinois and Indiana).
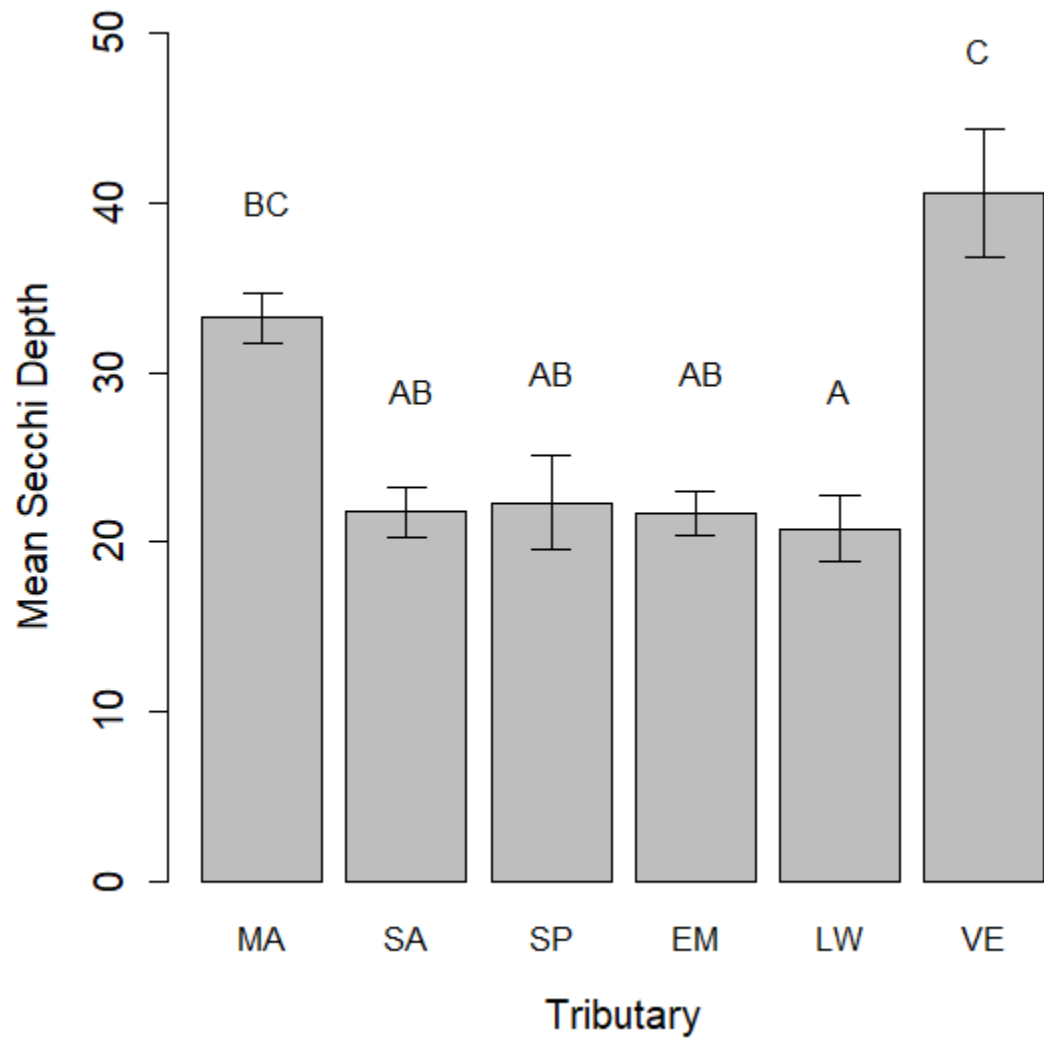
Figure 1.2 – Mean secchi depth (cm) from the Mackinaw (MA), Sangamon (SA), Spoon (SP), Embarras (EM), Little Wabash (LW), and Vermilion (VE) Rivers during 2016-2018 field seasons. Different letters denote significantly different mean secchi depth.
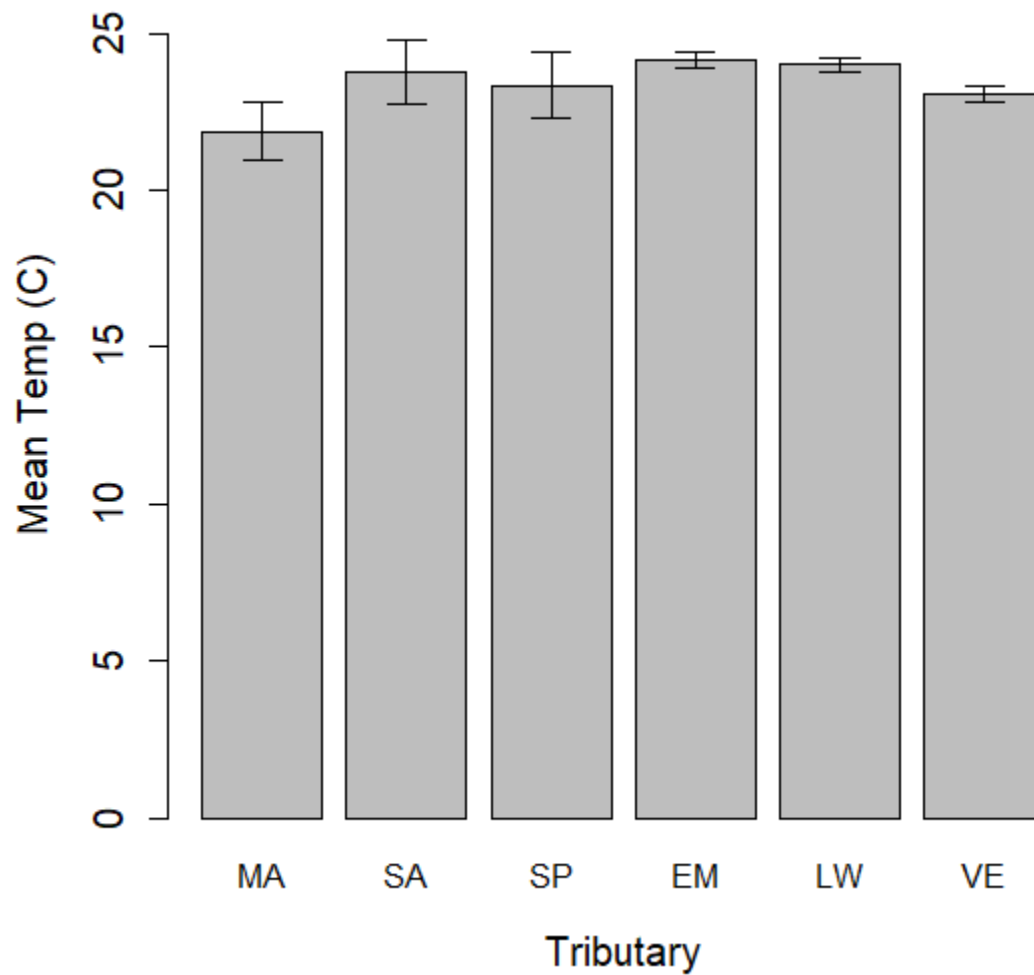
Figure 1.3 – Mean water temperature (°C) from the Mackinaw (MA), Sangamon (SA), Spoon (SP), Embarras (EM), Little Wabash (LW), and Vermilion (VE) Rivers during 2016-2018 field seasons.
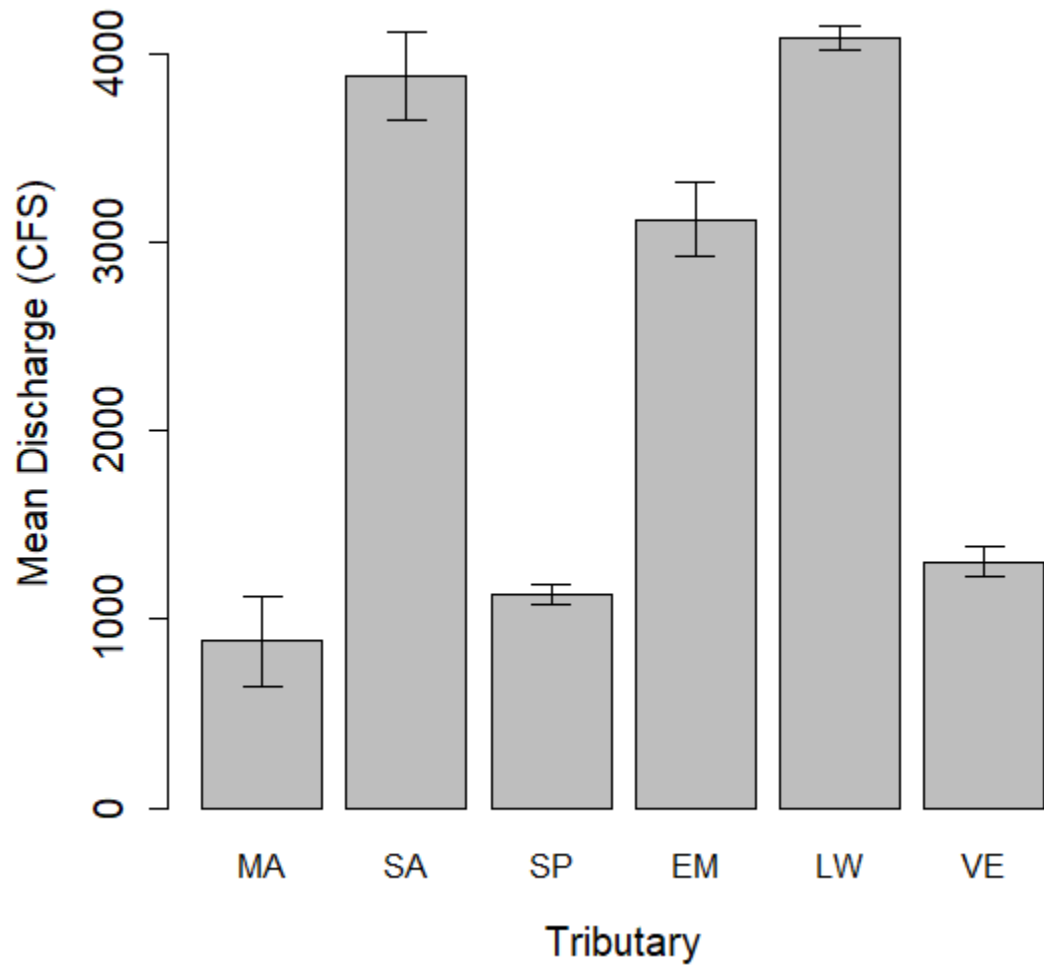
Figure 1.4 – Mean river discharge (m^3/second) from the Mackinaw (MA), Sangamon (SA), Spoon (SP), Embarras (EM), Little Wabash (LW), and Vermilion (VE) Rivers during 2016-2018 field seasons.

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ABSTRACT

Bighead and Silver Carp (bigheaded carp) are non-native fishes that have deleterious effects on the ecosystems they invade. Because of their negative effects on native species, fisheries managers devote substantial time and effort to limit the spread of these fishes. Better understanding of the patterns of bigheaded carp reproduction and dispersal can help manage these invaders. To determine spawning locations, we used ichthyoplankton drift nets and larval push nets in three Wabash River tributaries to capture bigheaded carp larvae in 2016 and 2017. Further, we used microsatellite loci to determine if genetic differences existed between larval bigheaded carp in our three study tributaries. In total, 1,246 larval bigheaded carp were collected from three tributaries, with the Little Wabash and Embarras River producing roughly 83% and 16% of larvae. Genetic analyses using various methods revealed high levels of genetic diversity in all three sites. Minimal evidence of inbreeding or outbreeding, as well as genetic difference between the upper Little Wabash and lower Embarras River samples help to improve our understanding of bigheaded carp reproduction and provide useful information for fisheries professionals to better mediate their spread.

INTRODUCTION

Invasive species generally have a smaller gene pool than that of natives and have substantially less time to disperse throughout their newly invaded range (Yang et al. 2015). However, invasive species are often under immense selective pressure (Chun et al. 2009) and often have high phenotypic diversity, increasing the potential for rapid evolution and genetic divergence (Lee 2002). Studies of genetic diversity and evolutionary changes can be useful for understanding the potential for colonization, establishment, geographic patterns of invasion, range expansion, lag times, and the potential for evolutionary responses to novel environments, including management practices (Sakai et al. 2001).

Genetic analyses are an increasingly popular tool used by fisheries researchers to gain insight regarding fish population structure (Gyllensten 1985) and determine if individual reproductive stocks exist within a population (Shaklee et al. 1990). Previously, a genetic stock has been defined as a local population that maintains recognizable genetic differentiation by separation of their spawning time or place (Bailey and Smith 1981). The delineation of genetic stocks has been used to better understand and manage the harvest of commercially or recreationally important fishes such as Lake Whitefish *Coregonus clupeaformis* (VanDeHey et al. 2009). Microsatellite loci can show genetic differences between populations separated by relatively short geographic distance (Koskinen et al. 2002) and can assist fisheries managers in examining population structure.

In the case of Bighead Carp *Hypophthalmichthys nobilis* and Silver Carp *Hypophthalmichthys molitrix*, hereafter termed "bigheaded carps", genetic tools afford the

ability to better understand reproductive patterns, determine if there is genetic isolation, and potentially determine if natal homing is exhibited (Freedman et al. 2005). In previous research, Stepien et al. (2019) found Silver Carp to exhibit high levels of genetic diversity and significant population differentiation across their North American range, including invasion fronts in the Illinois and Wabash Rivers. Although statistically significant genetic differentiation was found among populations in the Illinois, upper Mississippi, lower Mississippi, Missouri, and Wabash Rivers (Stepien et al. 2019), genetic structure of Silver Carp has yet to be examined on a finer scale within North America.

All previous genetic studies on Silver Carp have examined free-swimming juveniles or adults. This could potentially confound inferences of stock structure as Silver Carp exhibit high levels of dispersal (Coulter et al. 2016). Thus, individuals captured in one location may not represent individuals reproduced in that region. For example, a Silver Carp captured in the Illinois River was confirmed a full sibling of a lower Mississippi River individual (Stepien 2019). Additionally, the authors know of no Silver Carp genetics studies performed on populations within large river tributaries. For these reasons, we investigated population genetics of larval Silver Carp in tributaries of the Wabash River using larvae.

During the 2016-2017 field seasons, we sampled larval bigheaded carps with the objectives of (1) determining if genetic differences exist between study tributaries, and (2) investigating levels of genetic diversity within Wabash River tributaries. We hypothesized that Silver Carp would be genetically distinct between tributaries within systems, as they are known to spawn at relatively few, discrete sites in their native Yangtze River (Yi et al.

1988), indicating either a low rate of straying or minimal reproductive habitat. Additionally,

Stepien et al. (2019) found silver carp in the mainstem Illinois and Wabash Rivers to be

genetically different. Genetic differentiation between tributaries would be notable, as invasive

species generally have a smaller gene pool than that of natives and have had substantially less

time to separate throughout their newly invaded range. However, invasive species are often

under immense selective pressure (Chun et al. 2009) and often have numerous phenotypic

differences, increasing the potential for rapid evolution and genetic divergence (Lee 2002).

METHODS

Study Sites

We selected three tributaries of the Wabash River (Embarras, Little Wabash, and

Vermilion Rivers) as study sites (Figure 1). All three tributaries flow into the free-flowing lower

Wabash River. Tributaries were sampled monthly from March-September 2016-2017.

Additionally, we sampled bi-weekly during June-August in 2016-2017, as water temperatures

are generally suitable for bigheaded carp spawning during these months (Verigen et al. 1978;

Kolar et al. 2007).

Sampling Techniques

We deployed ichthyoplankton drift nets at an upper, middle, and lower reach of each

tributary (Figure 2.1). At lower sites, we intentionally sampled > 100m upstream of confluences

to avoid sampling ichthyoplankton that may have washed into a tributary from the mainstem

river. Drift net openings were 0.25m x 0.45m with a 1.0m 500μm mesh net to filter water and

collect drifting larval fishes and eggs. Flow was measured using a HACH flow-meter and used to

determine the soak time of drift nets using:

$$Soak\ Time\ (min) = 60 - \left(Flow\left(\frac{m}{s}\right) * 100\right)$$

Additionally, we sampled with bow-mounted ichthyoplankton push nets at the lower site of

each tributary. Push net openings were 0.5m in diameter and had 3.0m of 500μm mesh net for

sample collecting. Push nets were driven upriver for five minutes. A General Oceanics flow-

rocket was mounted to the push net opening to gauge the volume of water filtered. Drift nets

were set and ichthyoplankton pushes were conducted at a left, mid-channel, and right section

of the river at each site.

Samples were immediately preserved in 95% EtOH after collection. Larval fishes were identified to family under a dissecting microscope using Auer (1982) and Chapman (2006). Cyprinids were further identified to *Hypophthalmichthys* (bigheaded carp) if applicable. Fish were distinguished as being a late-stage larva versus juvenile based on the presence of a median fin-fold.

Laboratory Techniques

30 bigheaded carp were subsampled for genetic analyses. Individuals were subsampled to include fish from as many sampling events as possible. Bigheaded carp DNA was extracted using Qiagen DNeasy Animal Tissue Kit (Qiagen, Valencia, California) at Eastern Illinois University. For genetic analyses, all larvae were sent to the genetics laboratory at Georgia Southern University. Larval bigheaded carp were genotyped using a suite of 13 previously developed microsatellite loci (King et al. 2011). Microsatellites were amplified by multiplex polymerase chain reaction (PCR) using a common labeled M13F primer labeled with 5' 6-FAM or 5' HEX following Shimizu et al. (2002). The PCR was conducted at a final volume of 10 μL, containing 1X PCR buffer (50 mM KCl, 10 mM tris HCl pH 9.0), 2 mM $MgCl_2$, 200 μM of each dNTP, 0.1 unit Taq DNA polymerase, 0.9 μM of each PCR primer, and 1–20-ng template DNA. Thermal cycles were 94°C for 2 min, then 94°C for 30 s, 50–56°C for 30 s, and 72°C for 30 s, repeated 35 times, and finally 70°C for 5 min; this was done due to the different optimization thermocycler conditions for individual microsatellite loci. Products were then sent to the Georgia Genomics Facility (University of Georgia) for electrophoresis. Lane files were analyzed, and allele sizes were defined with Peak Scanner™ (Applied Biosystems, USA).

Data Analysis

All microsatellite loci were tested for Hardy-Weinberg equilibrium (HWE) conformation using GENEPOP (Rousset 2008). Based on significant deviations from HWE, loci were individually tested for null alleles. Locus genotypes were then corrected for expected null alleles using FreeNA software (Chapius and Estoup 2007). GENEPOP (Rousett 2008) was used to calculate linkage disequilibrium. Allelic richness (A_r) was estimated for this corrected dataset with HP-RARE version 1.1 (Kalinowski 2005). Inbreeding coefficients (F_{IS}), observed heterozygosity (H_o), and expected heterozygosity (H_e) were estimated with GenAlEx version 6.501 (Peakal and Smouse 2006).

Overall, pairwise genetic differentiation between sites and groupings based off STRUCTURE analysis were measured by calculating the Φ_{ST} estimator of F_{ST} (Peakal and Smouse 2006) with GenAlEx 6.501; 9,999 permutations were used to approximate statistical significance. To compare pairwise genetic differentiation {Φ_{ST}/ (1- Φ_{ST})} and pairwise geographic distance (river miles), a Mantel's test was used to test for isolation by distance.

Bayesian clustering program STRUCTURE version 2.3.4 (Pritchard et al 2000; Falush et al. 2003) and GENELAND version 4.0.6 (Guillot et al. 2005) were implemented to determine the number of distinct genetic groups (k) in our samples. In STRUCTURE, independent runs were conducted for k = 1-8 using 10,000 burn-in steps and 100,000 post burn-in steps. The highest probability of k was identified by the maximum estimated natural log of the probability of observing the data, given the number of groups and the delta k method of Evanno et al. (2005). STRUCTURE HARVESTER was used to determine delta k for each cluster (Earl and Von Holdt 2012). For GENELAND, the k value was selected using the spatial, uncorrelated allele

frequencies model. After k was estimated, another run with the k value was performed to determine genetic group membership probabilities. Individuals were assigned to genetic groups if their q-value was ≥ 0.7. If an individual did not have q-values ≥ 0.70, it was placed into a mixture group.

RESULTS

A total of 1,246 larval bigheaded carp were sampled in Wabash River tributaries in 2016 and 2017 (Table 2.1). Of those captured, 1,033 bigheaded carp were from the Little Wabash, 206 from the Embarras, and 7 from the Vermilion River. Due to the lack of sample size, we removed fish from the Vermilion River from our analyses. Adequate (Nosova et al. 2019) numbers (n = 30 from each site) of bigheaded carp larvae were captured to analyze the lower Embarras River (EM), upper Little Wabash River (ULW), and lower Little Wabash River (LLW).

Upon analyses, locus 3 in a lower Little Wabash sample, and locus 6 and 7 in Embarras samples did not conform to Hardy Weinberg Equilibrium (HWE); all other loci conformed. Aside from those three loci, we found no null alleles or linkage disequilibrium present. Allelic richness (A_r) ranged from 7.12 to 7.69 (Table 2.2). Observed heterozygosity (H_o) ranged from 0.66 to 0.68 (Table 2.2). Expected heterozygosity (H_e) ranged from 0.72 to 0.74 (Table 2.2). Inbreeding coefficients (F_{IS}) ranged from 0.03 to 0.08.

Pairwise comparisons showed a significant genetic difference between EM and ULW samples (Φ_{ST} = 0.021, P = 0.002), (Table 2.3). LLW was not significantly different from EM (Φ_{ST} = 0.004, P = 0.147) or ULW (Φ_{ST} = 0.004, P = 0.163), (Table 2.3) samples. A Mantel's test did not detect a significant isolation-by-distance pattern between pairwise compared sites (r = 0.74, P = 0.47) (Figure 2.2).

Two groups (k = 2) were selected as the appropriate number of unique genetic population groups using GENELAND and STRUCTURE. All three sample sites had fish assigned to population groups 1, 2, and the mixture group (Figure 2.3; Table 2.4). Our EM sample (n = 30)

had 21 fish assigned to population group 1, 6 fish assigned to population group 2, and 3 mixed

fish (Figure 2.3; Table 2.4). Regarding our Little Wabash samples, LLW (n = 29) had 18 fish

assigned to population 1, 5 fish assigned to population 2, and 6 mixed fish (Figure 2.3; Table

2.4), while ULW (n = 29) had 14 fish assigned to population group 1, 6 fish assigned to

population group 2, and 9 mixed fish (Figure 2.3; Table 2.4).

DISCUSSION

Invasive species often have lower genetic diversity and have had less time to diverge throughout their invaded range (Yang et al. 2015) that natives. However, immense selective pressure can often increase the potential for rapid evolution and genetic divergence (Chun et al. 2009; Lee 2002). In the case of Silver Carp, our study further illustrates the ability of these fishes to invade new ranges while maintaining high genetic diversity.

Greater genetic diversity was found among our Silver Carp samples than those from the Illinois (IL), upper Mississippi (UM), lower Mississippi (LM), Missouri (MO), and Wabash (WA) Rivers (Stepien et al. 2019). Using similar microsatellite techniques, A_r ranged from 5.7 to 6.3 and H_o ranged from 0.57 to 0.60 in IL, UM, LM, MO, and WA samples (Stepien et al. 2019), while our Embarras and Little Wabash River samples ranged from 7.12 to 7.69 in A_r and 0.66 to 0.68 in H_o. Relative to Silver Carp found in Eastern European and Asian aquaculture, our samples had greater H_o than those documented in Bangladesh (Gheyas et al. 2006), Belarus (Nosova et al. 2019), and Pakistan (Nazish et al. 2018). Embarras and Little Wabash River samples had greater A_r than Silver Carp in Pakistan (Nazish et al. 2018), similar A_r values as Bangladesh samples (Gheyas et al. 2006), but lower than Belarus samples (Nosova et al. 2019). Regarding wild populations in their native range in China, our samples had greater H_o than those observed in two studies (Liao et al. 2006; Feng et al. 2014), but lower than two others (Wang et al. 2008; Guo et al. 2013). Embarras and Little Wabash River samples had lower A_r than one Chinese study (Guo et al. 2013), comparable to Liao et al. (2006), and greater than those in two other studies (Wang et al. 2008; Feng et al. 2014) on wild Silver Carp in their native range. This is notable, as genetic diversity at the edge of an invasion front is often lacking

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(Piaggio et al. 2017). Additionally, this indicates a lack of genetic bottleneck and high population viability, should the population continue to expand. This further exacerbates the need for vigilance in monitoring for these fish in new waters and potential mechanical removal using methods such as commercial harvest.

Embarras River Silver Carp were genetically different from those in the upper Little Wabash River while those in the lower Little Wabash River were not significantly different from those in the upper Little Wabash or the Embarras River. Our Mantel test showed no significant isolation-by-distance pattern, which may be influenced by a low number of sampling sites (n = 3), potentially leading to a type II error. It should be noted that our two genetically different sites were the furthest apart (Figure 2.2). Isolation by distance is commonly seen in various adult riverine fishes (Primmer et al. 2006; Sotola et al. 2017).

This is the only study to our knowledge that uses larvae to examine Silver Carp population genetics. Further, this is also the only study to our knowledge that investigates Silver Carp genetics on this fine of scale or in large river tributaries. With adult Silver Carp traveling approximately 10.6km/day (DeGrandchamp et al. 2006) and evidence of high dispersal, even among full siblings (Stepien et al. 2019), it was beneficial to investigate population structure using larvae. Silver Carp larvae generally can only disperse downriver (Kolar et al. 2007) and are theoretically more likely to be near the site they were spawned at, relative to adults. Future genetic studies of Silver Carp should compare adults to the larvae from our study and include samples of larval Silver Carp from other large river tributaries, such as those of the Illinois River.

Table 2.1 – Total catch of larval bigheaded carp by river reach in three Wabash River tributaries from March-September 2016-2017.

Tributary	Lower	Middle	Upper	Total
Embarras	205	1	0	206
Little Wabash	968	63	2	1033
Vermilion	7	0	0	7
Total	1180	64	2	1246

Table 2.2 – Sample size (n), observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{IS}), allelic richness (A_r), private allelic richness (PA_r), and median pairwise R_{ST} (MPR), of larval bigheaded carp sampled from the Embarras, lower Little Wabash, and upper Little Wabash Rivers from March-September 2016-2017.

	n	H_o	H_e	F_{IS}	A_r	PA_r	MPR
Embarras	30	0.68	0.72	0.030	7.12	1.33	0.001
Lower LW	29	0.66	0.74	0.080	7.69	1.66	-0.027
Upper LW	29	0.66	0.72	0.056	7.14	1.28	0.018

Table 2.3 – Pairwise genetic comparisons between lower Little Wabash (LLW), upper Little Wabash (ULW), and Embarras (EM) River Silver Carp from March-September 2016-2017; Φ_{ST} values are below diagonal blanks and significance values are above. * indicates significant pairwise differences after using Bonferroni corrections for multiple comparisons.

	LLW	ULW	EM
LLW		0.163	0.147
ULW	0.004		*0.002
EM	0.004	0.021	

Table 2.4 – Number of individuals assigned to genetic group 1, 2, or a mixture (3) from the Embarras (EM), lower Little Wabash (LLW), and upper Little Wabash (ULW) Rivers from March-September 2016-2017. Groups are based off Structure and Geneland analyses.

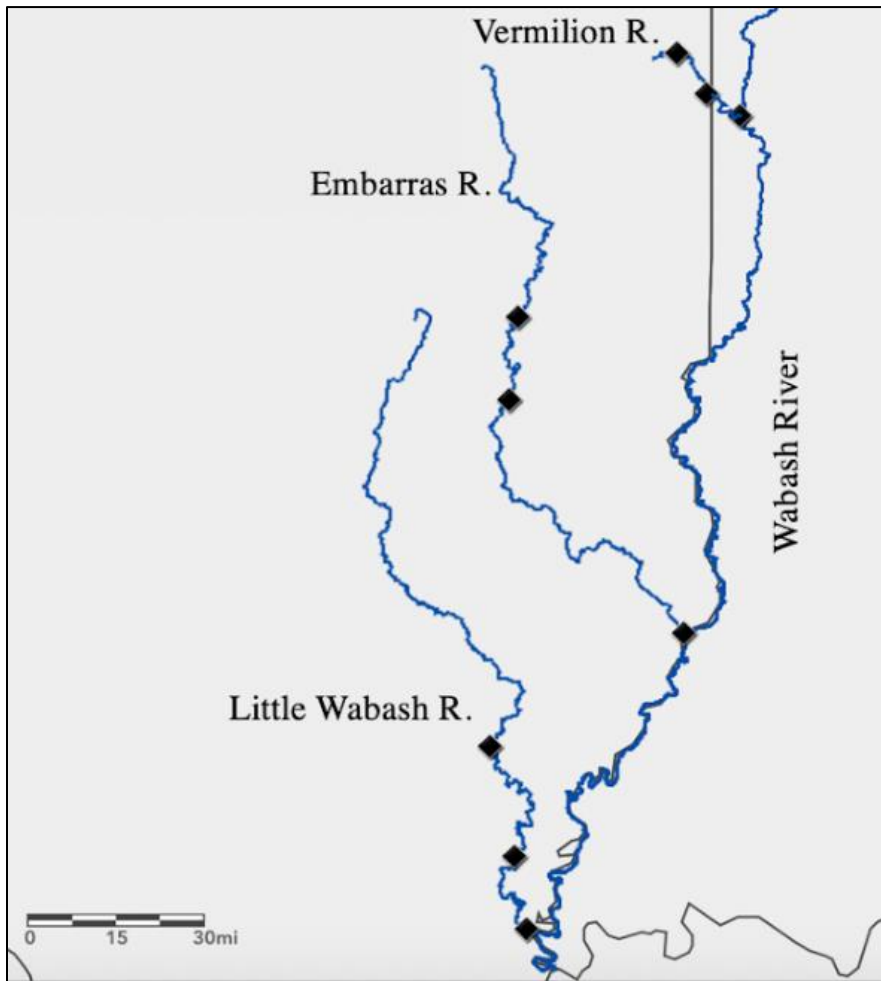| Site	Genetic Groups		
	1	2	3
EM	21	3	6
LLW	18	5	6
ULW	14	6	9

Figure 2.1 – Study area including upper, middle, and lower sampling sites on the Embarras, Little Wabash, and Vermilion Rivers (Illinois and Indiana).
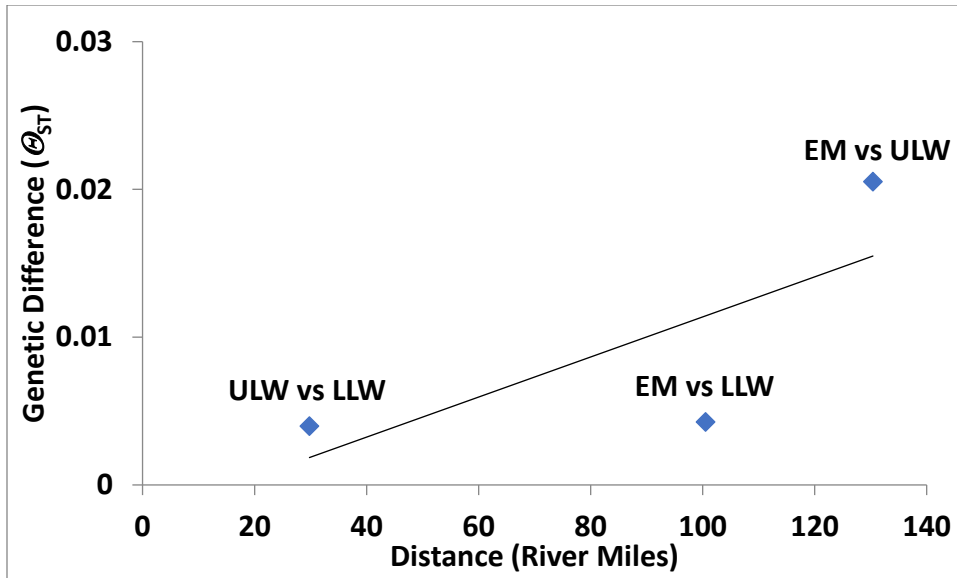
Figure 2.2 – Scatter plot with genetic difference (Φ_{ST}) among larval Silver Carp versus distance (river miles) for sites on the Embarras (EM), upper Little Wabash (ULW), and lower Little Wabash (LLW) Rivers, 2016. Mantel's test was not significant (correlation coefficient $r = 0.74$, $P = 0.47$).

Figure 2.3 – STRUCTURE plot for *k* = 2, illustrating groupings and genetic structure among larval Silver Carp samples taken from the Embarras, upper Little Wabash, and lower Little Wabash Rivers, 2016.

LITERATURE CITED

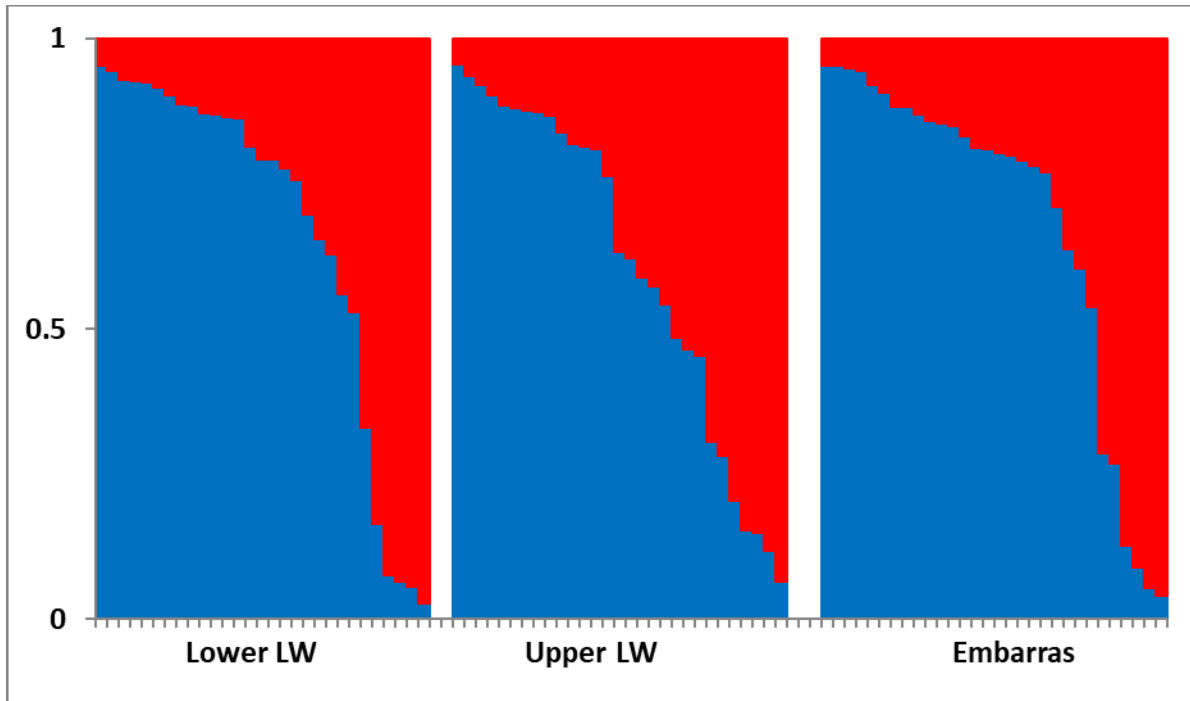Auer, N. A. 1982. Identification of larval fishes of the Great Lakes basin with emphasis on the Lake Michigan drainage. Great Lakes Fishery Commission, Ann Arbor, MI 48105 82(3):744.

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CONCLUSION

In summary, we found bigheaded carps are more likely to utilize tributaries with larger watersheds, higher discharges, and greater turbidity. Further, tributaries in larger watersheds are more likely to have prolonged periods of higher discharge and turbidity, making watershed area a good predictor of bigheaded carp utilization for reproduction. Using genetic analyses, we analyzed Silver Carp in the Embarras and Little Wabash Rivers and found high genetic diversity and minimal evidence of inbreeding or any genetic bottleneck. Based on these findings, fisheries managers should prioritize bigheaded carp monitoring in Great Lakes tributaries within larger watersheds. If found in new waters, we recommend agencies respond with rapid control/removal efforts, due to the high genetic diversity found at leading edges of the species range and thus a propensity to quickly expand, both in abundance and range.