Movements and Spawning of Bigheaded Carps in the Upper Wabash River, Indiana, USA: Final Project Report

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Introduction

Over 180 aquatic non-indigenous species (NIS) have been introduced into Great Lakes Basin waters to date, and new introductions are expected in the future. The so-called "bigheaded carps" (e.g., silver *Hypophthalmichthys molitrix* and bighead *H. nobilis*) are imminent threats to the Great Lakes given expected trajectories of nutrient flow disruption and food web alterations that will likely accompany their invasion of the Basin. While great effort has been expended to keep these species from entering the Great Lakes Basin via the Illinois River and its connection to the Chicago Sanitary and Ship Canal, an additional pathway for introduction has been identified at Eagle Marsh near Fort Wayne, Indiana. Eagle Marsh may provide a corridor for movement of these species between the Wabash and Maumee River basins during high water periods. The direct connection of the Maumee River with Lake Erie would therefore provide a means for introduction of bigheaded carp to the Great Lakes. Immediate action was taken to prevent such an introduction through the installation of a physical barrier across Eagle Marsh, and a permanent berm will be constructed to permanently separate the Wabash and Maumee River basins. However, the potential ranges and rates of movement by silver and bighead carps throughout the Wabash River, and especially into the Little River and Eagle Marsh, were not fully understood prior to berm construction, and reconnaissance to detect such movements was necessary.

Understanding the movements of invading species in novel environments is important for predicting potential impacts (DeGrandchamp et al. 2008), knowing where and when they utilize the environment for life history events like reproduction (Williamson and Garvey 2005), and for devising potential control strategies (DeGrandchamp et al. 2008). Bigheaded carp are known to make rapid, large scale movements that are usually associated with spawning (Abdusamadov 1987), and migrations may be triggered by several factors, including temperature (DeGrandchamp et al. 2008) and river stage/flow (Abdusamadov 1987; Peters et al. 2006; DeGrandchamp et al. 2008). For example, silver carp were found to move ≈10 km/day in the Illinois River and range over 250 miles (DeGrandchamp et al. 2008). The specific cues triggering bigheaded carp movements in the Wabash River watershed are as yet unknown, and such information is critical for devising control measures.

The extent and types of habitats used by bigheaded carp in the Wabash River are also unknown. For example, we currently have little knowledge of the use of smaller tributary rivers, like the Little River, by both silver and bighead carp during any stage of their life cycle. While silver carp were found to avoid main channel habitats in the Illinois River where they preferred to remain near river banks or in backwaters (DeGrandchamp et al. 2008), they currently occur in relatively high densities in Borrow Pit 1 (BP1), a backwater habitat, behind the Williamsburg Apartments in West Lafayette (River Mile 310, RM310), and in a main channel area at Logansport (RM351). From tracking conducted in 2011, we know that they occasionally occur in tributaries such as the Tippecanoe River. Determining habitat use by bigheaded carp in the Wabash River can help to devise strategies for control and prediction of invasion patterns in non-invaded river ecosystems.

Previous studies have successfully used telemetry to observe bigheaded carp movements in rivers (e.g., Calkins et al. 2012; DeGrandchamp et al. 2008). To date, we have tagged 300 individuals and successfully located 274 of these bigheaded carp in the Wabash River using ultrasonic tags and passive and manual tracking hardware to observe their movements. We have also monitored and recorded the habitat types that these tagged fish are utilizing. Ultimately, we expect these data to yield insight into the range of river and movement rates these fish may cover, as well as a characterization of their potential habitat. We have also conducted spring surveys to detect bigheaded carp spawning events at multiple sites in the upper Wabash River and one of its largest tributaries, the East Fork of the White River in an attempt to better understand the range of spawning activity and ecology in these fishes. Finally, we sampled to detect sites important for young bigheaded carp using mini-fyke nets at multiple locations in the middle Wabash River.

Methods

Tagging

Fish for acoustic tagging were collected using a 6 m electrofishing boat (Model SR16H; Smith-Root Inc., Vancouver, Washington) and a 6 m Polarcraft modified John boat outfitted with an electrofishing control box (Model VI-A; Smith-Root Inc., Vancouver, Washington). In both cases, the electrofishing equipment was powered by a generator, and adjustments were made to achieve a pulsator running at either 3-4 A of direct current at 30 pulses s^{-1} and 20-50% of range pulse width or 7-8 A of direct current at 120 pulses s^{-1}. Fish were also collected using gill nets set for < 30 min. Nets were borrowed from IDNR and had 10.16 cm bar mesh. Bighead carp from Oakdale Dam on the Tippecanoe River were collected using hook-and-line sampling. Hook-and-line collected fish were transported to the nearest Wabash River boat launch using an aerated fish hauler.

Candidate fish were anaesthetized using a custom-made mobile electroanesthesia unit (MEU). An AbP-3™ Pulsed-DC electrofishing box (ETS Electrofishing, LLC, Madison, Wisconsin) was used to generate an electrical field for the MEU (120 V, 30 Hz, 25% duty cycle, 7-15 s). The MEU induced loss of reflex almost instantaneously and recovery from anesthesia was relatively quick. Once loss of reflex was induced, each fish was weighed (g) using a HW-60KGL digital balance (±0.005 kg; A&D Co., Ltd., Tokyo, Japan) and measured for total length (cm). Each fish was also externally tagged using a Floy T-bar anchor tag (Model FD-68B; Floy Tag & Mfg. Inc., Seattle, Washington) inserted near the dorsal fin base. 2011 fish were classified as either silver, hybrid or bighead carp based on appearance, but 2012 and 2013 were classified using established DNA markers (Mia et al. 2005).

Vemco ultrasonic transmitters (Model V16-4L, 24 g, 16 mm diameter, 68 mm length) tasked for a nominal delay of 60 s were surgically implanted in the coelomic cavity of the carp. A 4-5 cm incision was made in the left side of the fish just dorsal and anterior to the anal fin in an area sterilized with Betadine (Walgreens Co., Deerfield, Illinois) where scales had been removed using a size 10 *or* 20 scalpel dipped in a 90% ethanol solution between surgeries. Transmitter weights were <2% of the fishes' weights in accordance with the recommended criteria from Vemco. After implantation, the incisions were closed using three absorbable monofilament sutures (PDS II, Ethicon Inc., Cornelia, Georgia). All fish were visually inspected to determine sex, if possible, although the gonads were often not visible during the surgeries. All fish handling was completed within a 2-minute time period. Fish were allowed to initially recover in the MEU. Once swimming ability had returned, fish were placed in an *in situ* pen until fully recovered, then released in the river. Recovery was defined as the return of normal orientation and swimming behavior post-surgery.

Tracking

Passive – Omnidirectional passive receivers (Vemco VR2W) were deployed on the river bottom in the Little River, Salamonie River, Tippecanoe River, and Wabash River between RM 406-165 (Figure 1). The VR2Ws were attached to custom platforms and anchors as detailed in previous reports. The size of each platform and anchor system was adjusted based on the water depth where it would be deployed. This combination of platforms and anchors was connected by 2-30 m steel cable for secure placement on the bottom of the river, and attached floats allowed for grappling of the cable to retrieve the VR2Ws for data downloads. Platforms were welded from rebar and anchors were cement-rebar structures deployed upstream of platforms that varied in weight from 26.3 kg to a single cinderblock. Passive receivers were occasionally tested to ensure their detection efficiency using a Vemco-supplied range testing tag, especially in shallow water.

Vemco VR2Ws were deployed in the river at smaller increments near tagging locations and at larger increments near the upper and lower boundaries of the study area as well as just upstream in the Tippecanoe and Little Rivers ultimately covering ~200 RM (Figure 1). Placement varied somewhat depending on access points. While this array covered considerably more area than the primary study site in the upper and middle Wabash River, this arrangement was judged sufficient to cover the full potential range of marked bigheaded carp based on maximum movements of silver (267 miles) and bighead (280 miles) carp observed in the Illinois River (DeGrandchamp et al. 2008). Data were downloaded approximately once a month during the summer and every three months the rest of the year. Eastern Illinois University (EIU) has deployed additional VR2W receivers downstream and has engaged in active tracking of their own tagged fish. EIU provided GPS locations for tagged bigheaded carp in cases where our tagged fish were located in the lower Wabash River.

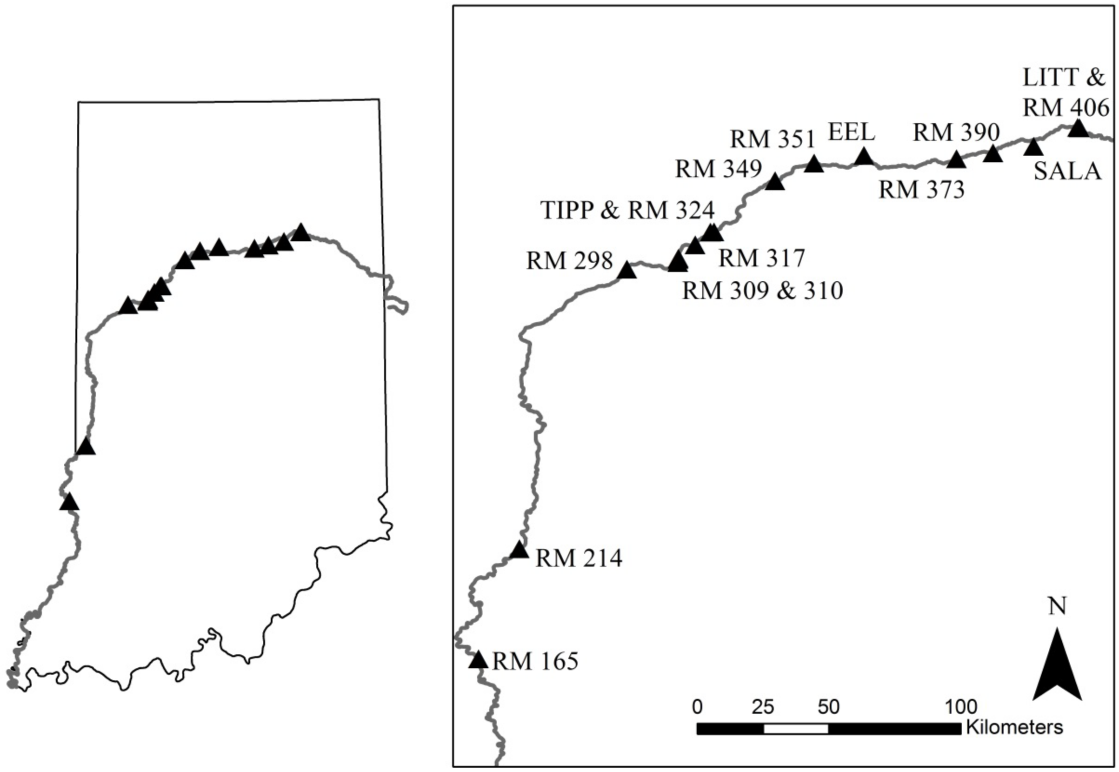

Figure 1. Placement of Vemco VR2W stationary receivers in the Wabash, Little (LITT), Eel (EEL), Salamonie (SALA) and Tippecanoe (TIPP) Rivers. Each triangle indicates the location of a VR2W and all are labeled with Wabash RM and/or the name of the tributary. Eel river stationary receiver was lost prior to downloading and so will not be referenced in the remainder of this report.

Active – Active tracking was accomplished by deploying hydrophones from a boat or canoe depending on river conditions. Active tracking was primarily done between RM354 (Logansport) and RM271 (Covington, IN). A 9 RM portion of the lower Tippecanoe River was also tracked. Sections of river were tracked at least once every two weeks. Lengths of river sections tracked varied depending on the vessel used. Up to 25 RM were tracked per day by boat and up to 16 RM were tracked per day by canoe. An omnidirectional hydrophone (Vemco VH110) connected to one of two manual receivers (Vemco VR100s) was used to locate and identify tagged bigheaded carp. First, the omnidirectional hydrophone was used to detect tagged carp in the vicinity of the tracking boat as it was piloted downriver at <5 mph. Once a reading of >75 db was achieved, the position of the tagged Asian carp was recorded using a handheld GPS (GPSMap 60c or GPS map 62s, Garmin Ltd., Olathe, Kansas).

Habitat measurements were taken when tagged bigheaded carp were detected. Depths (m) were measured using a hand-held depth finder (Model SM-5; Speedtech Instruments, Great Falls, Virginia). Similar to the methods used by Mueller and Pyron (2010), substrate type was determined using a 3 m or 6 m copper pipe to probe the bottom. Substrate type was categorized as one of six types: boulder, cobble, gravel, sand, fines, or hardpan (Wentworth 1922).

Monitoring of the spillways of the three main upstream dams for tagged fish was also conducted in October 2013 and monthly in summer 2014 (1-May, 2-June, 1-July and 6-August). These dams were the Roush, Mississinewa and Salamonie dams. Monitoring was done by dropping the hydrophone, attached to the VR100, into the dam outflow and allowing 10 min. for possible detections to register. This was done from shore and the hydrophone was placed as close to the stilling basin as possible given flow, access and length of the hydrophone cable.

Movements

When determining average movements, movement distance and rate were averaged at the individual level as movements coming from the same individual were likely not independent of each other. Movement distance and rate were compared between silver, hybrid and bighead carp using analysis of variance (ANOVA) with *post hoc* Tukey's tests. Distance and rate were also compared between years and/or months for each species depending on available data using either one or two factor ANOVAS. Movements were determined as the river distance between two consecutive detections for the same individual if these detections were > 50 m apart. Movements (sets of 2 detections) were considered independently from previous movements (i.e., a fish that traveled upstream then back to the same location was considered to have two movements not zero movement). Consecutive detections at the same GPS location or within 50 m of each other resulted in the fish being classified as stationary. Time spent as a stationary individual did not influence movement rates.

Statistical models were constructed using only visually and genetically identified silver and hybrid carp. Bighead carp were not included due to low sample size. Generalized linear mixed models were constructed to evaluate the impacts of ecological predictors and characteristics of the individual fish on movement distance and rate. Individual fishes were included as a random effect and full models also included: date, year, month, growing degree day (GDD, an indicator of temperatures in a given day), cumulative GDD (CGDD, an indicator of yearly temperature up to a particular date), water level (m), change in water level (over 24hrs), and sex. Month, year and sex were categorical variables, and the remaining variables were continuous. A base temperature of 10°C was used for GDD calculations. Water level was determined from a USGS river gage (Gage #03335500) as the value recorded on noon of the date, while change in water level was calculated as the difference in water level from noon the previous day to noon of the current day. An additional statistical model was created with a logistic response to examine movement probability (i.e., movements >50 m; 1 = move, 0 = no movement). This model used the same variables including individual fish as a random effect. All possible subsets of variables were run for each of the three models (distance, rate and probability) and models were evaluated with Akaike's Information Criterion with a small sample size correction (AICc). Models with ΔAIC < 2 were averaged using model averaging. The significant coefficients in these models were then examined for their effects on movement distance, rate and probability.

Habitat use was assessed based on data collected by the USGS using an acoustic doppler current profiler (ADCP; data used courtesy P. R. Jackson and E.M. Murphy, USGS). On 26 and 27-June-2012 and 17 and 18-June 2013, ADCP was towed in the thalweg for ≈10 mi stretch between Americus and West Lafayette and collected data on depth (m), velocity (cms^{-1}) and water temperature (°C) every 30 sec. Detections of bigheaded carp occurring within 2 weeks on either side of these sampling events and within the portion of river surveyed with ADCP were used to determine mean depth, temperature and water velocities utilized by tagged fish based on data from the nearest ADCP measurement. The mean depth, temperature and velocity utilized by the carp were compared to the mean of all of available habitat (i.e., mean of all the measurements) using one-sample t-tests. The mean of available habitat parameters were the means of all ADCP points measured.

Spawning

Drifting eggs were intermittently collected from multiple sites in the Wabash River in 2011 to detect presence/absence. To monitor the 2012-2014 spawning events for bigheaded carp, bongo nets were used to monitor egg production. Weekly bongo nets tows were conducted at RM310 once water temperatures were > 16 °C and continued until three consecutive sampling events detected no eggs. Three consecutive tows were completed during each sampling event. The bongo net was towed just below the surface of the water and the volume of water sampled was measured with a flowmeter (General Oceanics) in 2012, 2013, and 2014. Samples were taken back to the lab where eggs were counted. In 2012 and 2013, up to 30 eggs per week (10 eggs per pull) were frozen at - 80°C for later genetic analysis to determine if eggs were silver, bighead or hybrid carps. Eggs were also sampled periodically at five upstream sites in the Wabash River to assess the full extent of spawning in 2011 (1-June, 2-June, 14-June, 16-June), 2012 (2-May, 17-May), 2013 (29-May), and 2014 (1-June, 10-June, 25-June; Figure 2). The East Fork of the White River was also monitored for bigheaded carp spawning near Medora and Seymour ramps in 2012.

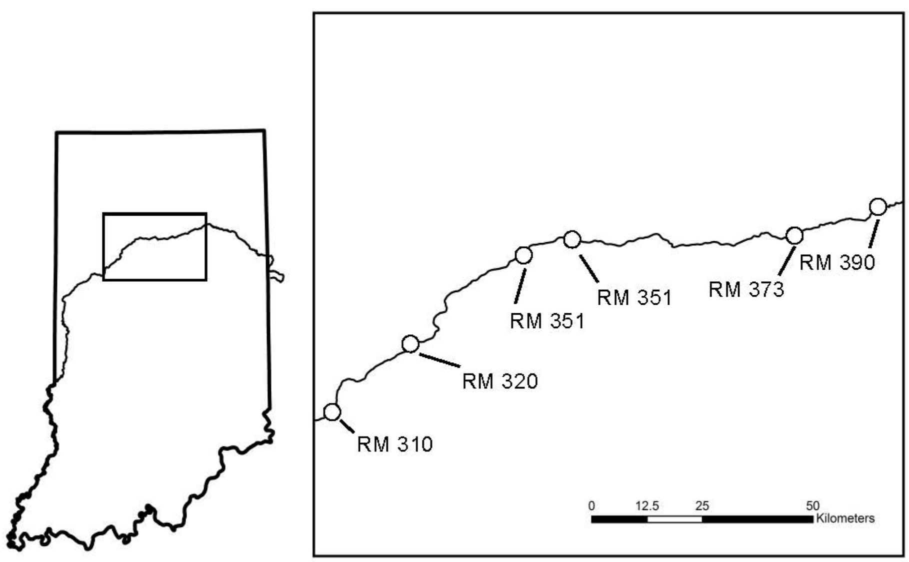

Figure 2. Locations of upstream sampling events in the Wabash River to determine upstream extent of spawning. Sampling was consistently done at RM 310 (West Lafayette). All other locations were sampled in 2011 and 2012 while only the two most upstream locations were sampled in 2013 and 2014.

Mean egg density was calculated using flowmeter readings (eggs/m^3). Multiple environmental variables were evaluated to determine their influence on spawning activity (presence and density of eggs). The flowmeter malfunctioned July 2012, and so average volumes from the surrounding dates with similar river discharge (as judged by USGS river gage) were averaged and that mean value was used to determine egg density. Environmental predictors of egg density were examined using linear models based on DOY, days since detection of spawning, water temperature, gage height, change in gage height over 24 hr, day length (hours to nearest 0.01) and CGDD. The initiation and cessation of spawning activity (spawning = 1, no spawning = 0) was modeled using Firth's penalized likelihood method for logistic regression (Firth 1993; Heinze and Schemper 2002; Heinze et al. 2013[logistf package]), based on the variables above. Only data collected from before and during the spawning season were used to predict the beginning of spawning, and only data from during and after the spawning season were used to predict the end of spawning activities. Backward stepwise selection will be used to determine the best model for predicting egg density, spawning and cessation of spawning based on AIC corrected for small sample size for egg density and profile likelihood ratio test for the logistic models (spawning initiation and cessation). Models were generated with data from 2012 and 2013, and then best models were tested using data from 2014 to evaluate their effectiveness. Correlation analysis was used to determine if amounts of hybrid carp eggs changed across the spawning season. We did this to determine whether hybrid bigheaded carp exhibit temporally segregated spawning compared to silver carp.

We conducted sampling for bigheaded carp young-of-year (YOY) in October and November 2014. Mini-fyke nets constructed following criteria outlined in Long Term Resource Monitoring Program for the Upper Mississippi River (available at http://www.umesc.usgs.gov/documents/reports/1995/95p00201.pdf) were set overnight at multiple locations near West Lafayette, Attica, Covington and Montezuma (Figure 3). The presence of bigheaded carp eggs close to hatching had previously been confirmed near West Lafayette, so sites downstream from that location were targeted for sampling. Mini-fyke nets were set in low flow areas such as backwaters, stream confluences and behind sandbars. Areas containing aquatic vegetation were also targeted within these locations. Nets were checked the following morning and the catch was identified to genus for *Notropis* spp. and *Pimephales* spp. and to species for all others. This was done to minimize processing time and thus minimize mortality of native fishes captured.

Genetics

Eggs and larvae collected in 2012 and 2013 as well as fin clips taken from tagged bigheaded carps (2012 and 2013) were tested to determine whether the samples came from silver, bighead or hybrid carps. DNA was extracted according to manufacturer's instructions using QIAamp mini DNA kits (Qiagen Inc., Valencia, California, USA). Extracted DNA was tested with polymerase chain reaction (PCR; CPX96, Bio-Rad laboratories, Inc., Hercules, California, USA) using 50 ng genomic DNA. Each sample was tested in duplicate with three established primers (Mia et al. 2005; Hmo1, Hmo3, and Hmo11). Negative controls using nuclease free water were included in each run. Reactions totaled 20 µl and consisted of 5 µM each of forward and reverse primers, 50 ng µL-1 template DNA, SensiMix (Bioline USA, Inc., Tauton, Massachusetts, USA) and nuclease-free water. Conditions for PCR were as follows: 94 °C for 2.5 min, 50 °C for 45 s, and 72 °C for 1 min for 45 amplification cycles with an extension period of 72 °C for 10 minutes. Results of PCR were visualized with gel electrophoresis on a 2% agarose gel run for 75 min at 3.8 Vcm-1. Results of electrophoresis were interpreted using established basepair sizes (Mia et al. 2005).

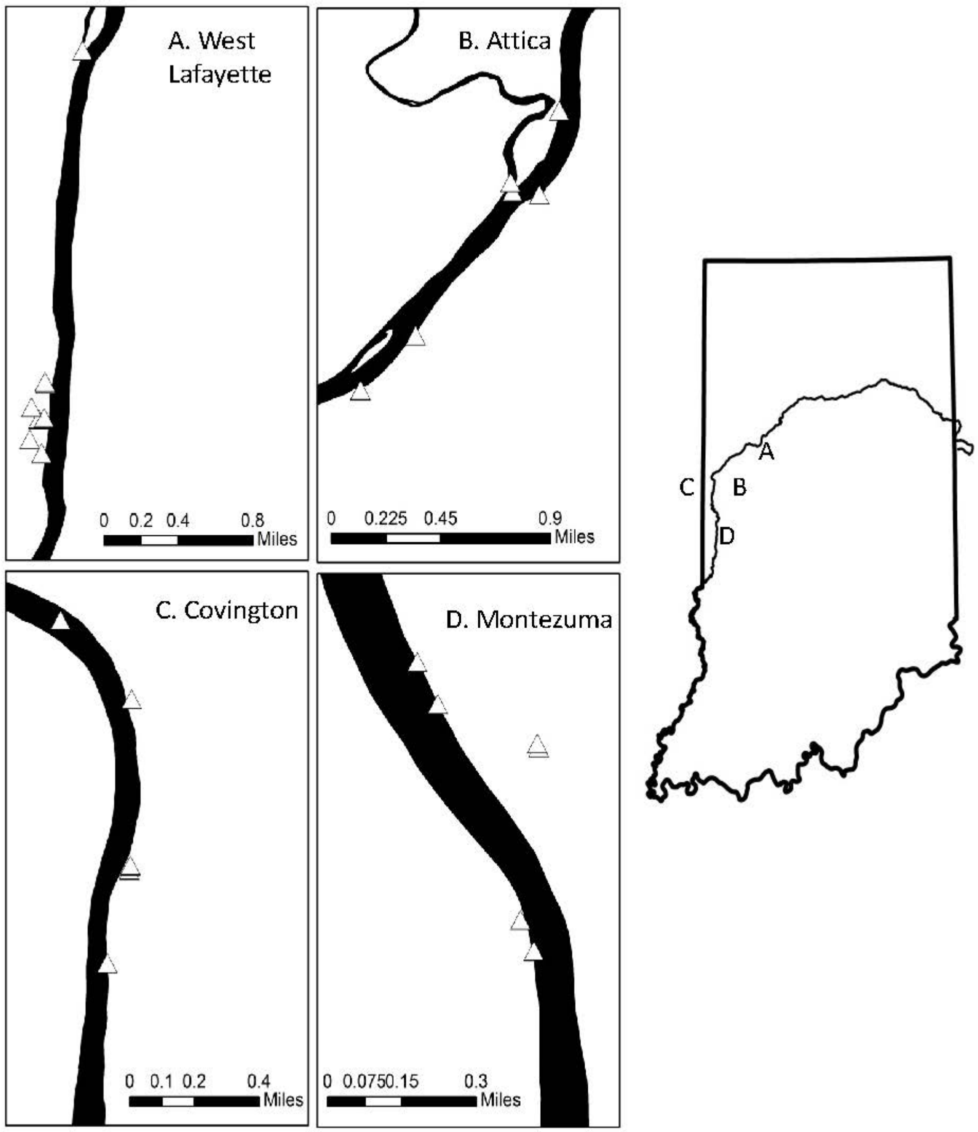

Figure 3. Locations of mini-fyke nets set overnight. Locations were targeted to backwater and other low- and non-flowing areas including flooded stream mouths, downstream of sandbars and downstream of bank outcroppings. Backwater maps were not available resulting in some points appearing out of the water.

Results

Movements

2014 update

Three additional silver carp were tagged in April 2014 bringing the total number of tags deployed to 300 (271 silver, 11 bighead, 18 hybrid; 100 of these silvers were from 2011 and not genetically tested). 229 different individuals were detected in 2014. No tagged fish were located in dam spillways on the Missisinewa, Salamonie, and Wabash Rivers. However, small (≈300 mm) silver carp were observed jumping in the Missisinewa spillway on several of our visits. One tagged silver carp was detected moving up the Tippecanoe River (24-Jul and 29-Jul 2014) which was the first instance of an individual involved in the telemetry study moving up a tributary more than 2 km (i.e., the location of the Tippecanoe River VR2W). No tagged individuals were detected on the Little River VR2W. Data from stationary receivers was last downloaded in August 2014 and so no data are available for 2014 after this date. Ice likely contributed to the loss of several upstream stationary receivers where anchors were located but cables attaching the stationary receivers to these anchors were frayed and broken (Forks of the Wabash, Wabash, Peru).

Genetic and visual results for tagged bigheaded carps were compared for 2012 and 2013 and indicated that there was a ≈9% chance of misidentifying a hybrid as a silver carp. There was a < 1% chance of identifying a silver carp as a hybrid, a hybrid as a bighead or a bighead as a hybrid.

Overall

Detections collected over this 4 yr study totaled over 2 million from stationary receivers and just over 1,000 from active tracking (≈1200 hrs; Table 1). Backwaters tend to have high numbers of detections with the exception of Borrow 2 in 2013. Borrow 2 is often isolated from the channel depending on water level but 2013 water levels tended to be higher which may have allowed bigheaded carp to enter and exit rather than becoming trapped. 274 unique individuals of the 300 tags deployed had at least one detection over the study period. Receivers closer to the downstream and upstream boundaries of the study area tended to have fewer detections (i.e., from Wabash, IN upstream). Data were also more inconsistently available from downstream receivers due to burial in sediments and subsequent loss. Movement data were available for 147 genetically tested individuals (124 silver, 16 hybrid, 7 bighead carp); however, lack of sufficient data from >3 individuals precluded statistical testing for the hybrid and bighead carp groups except for between 2013 and 2014. Overall movement distances and rates were greater in bighead carp (distance: 37.57 ±72.39SD km; rate: 68.58 ±95.94SD km/week) than silver (distance: 12.06 ±12.24SD km; rate: 34.29 ±32.79SD km/week) and hybrid carp (distance: 9.85 ±10.61SD km; rate: 26.74 ±22.23SD km/week) (Figure 4). Hybrid carp did not have different movement distances ($F_{1,16}$ =3.96, $p = 0.065$) or rates ($F_{1,16}$ =0.58, $p = 0.457$) between 2013 and 2014. Bighead carp were also not different in movement distances ($F_{1,6}$ =1.48, $p = 0.278$) or rates ($F_{1,16}$ =3.57, $p = 0.118$) between 2013 and 2014. Genetically determined silver carp showed significant differences between years ($F_{2,668}$ =10.92, $p < 0.0001$; $F_{2,668}$ =6.75, $p < 0.0001$), months ($F_{8,668}$ =10.04, $p < 0.0001$; $F_{8,668}$ =23.80, $p < 0.0001$) and months between different years ($F_{9,668}$ =10.40, $p < 0.0001$; $F_{9,668}$ =5.60, $p < 0.0001$) in movement distance and rate, respectively.

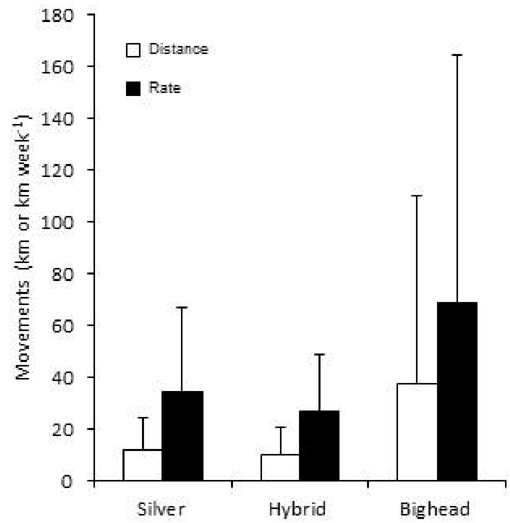

Figure 4. Mean (±1 standard deviation) movement distances (km) and rates (km week^{-1}) for silver, hybrid and bighead carp. Bighead carp had a significantly greater average distance moved (F_2=6.27, p = 0.002) and a significantly greater average movement rate (F_2=3.34, p = 0.038).

Silver and hybrid carps were not significantly different in movement rate and distance; thus, data from these two groups were combined for further analysis. Of all movements from silver and hybrid carps, individuals moved <50 m 93.6 % of the time (Figure 5). Individual fish were slightly more likely to move in the summer months (May-August) and were very likely to move in those months during 2012. Of the movements that occurred, there was a seasonal trend in directionality, with upstream movements generally occurring in spring and downstream movements occurring more in fall (Figure 6). The silver and hybrid carp combined data also indicated that significant differences existed among years ($F_{3,2273}$=7.95, p < 0.0001), months ($F_{7,2273}$=18.36, p < 0.0001), and months within years ($F_{21,2273}$=15.92, p < 0.0001; Figure 7) in movement distances. Only data from March – October were analyzed due to few to no movements > 50m in other months. *Post hoc* tests indicated that mean movement distance in 2011 was significantly smaller compared to 2013 and 2014 (Figure 8). Additionally, movement distance was significantly greater in March and April than July, and May had a significantly greater movement distance than June and July. April – August all had significantly smaller mean movement distances than September and October. The silver and hybrid carp combined data also indicated that significant differences existed among years ($F_{3,2273}$=45.43, p < 0.0001), months ($F_{7,2273}$=40.42, p < 0.0001), and months within years ($F_{21,2273}$=4.049, p < 0.0001; Figure 7) in movement rates. *Post hoc* testing indicated that 2013 had a significantly greater mean movement rate than the other three years (Figure 8). March had a significantly greater movement rate than April-August and October. April had a significantly greater average movement rate than July. May had a greater movement rate than July and August while June movement rate was significantly lower than July. All months tested had a significantly lower movement rates than September. June-August had significantly lower movement rates than October.

Table 1. Summary of active and stationary tracking from the four years. A zero indicates that there were no detections while a dashed line indicates that there was no receiver placed yet or that the stationary receiver was lost/damaged. Forks of the Wabash was previously called Huntington.

Receiver Name	River Mile	# Detections			
		2011	2012	2013	2014
Little River	2	0	-	0	0
Forks of the Wabash	406	5	3	10	-
Salamonie River	3.3	-	-	0	0
Wabash	390	8	3	12	-
Peru	373	139	7	43	-
Logansport	351	276607	243940	-	-
French Post Park	340	268	77	-	1222
Americus	324	322	565	103297	124076
Tippecanoe River	2	987	94	3924	-
I-65 Bridge	317	4095	36568	11557	10176
Borrow 1	310	90855	283237	245016	186422
Borrow 2	310	55765	58206	30	733580
IN 26 Bridge	309	1375	145866	-	-
Goose Island	298	-	-	1476	-
Terre Haute	214	-	6	-	2075
Merom	165	42	37	-	-
Active Tracking		299	347	154	286
Eastern Illinois				3	

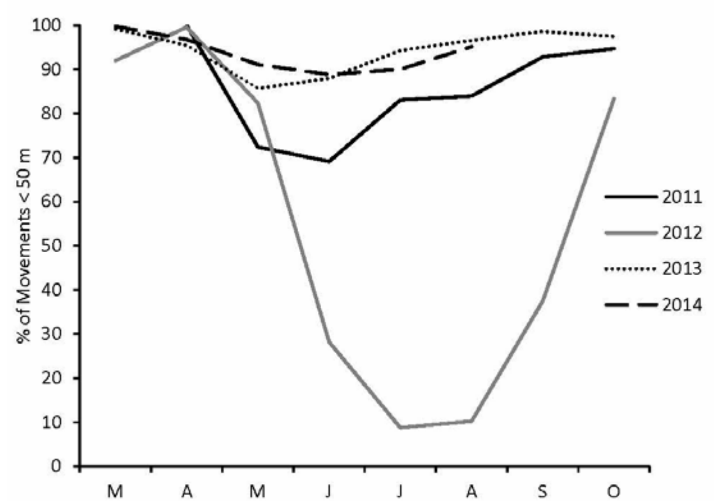

Figure 5. Percentages of silver and hybrid carp movements in a given month in each year that were <50 m (i.e., stationary).

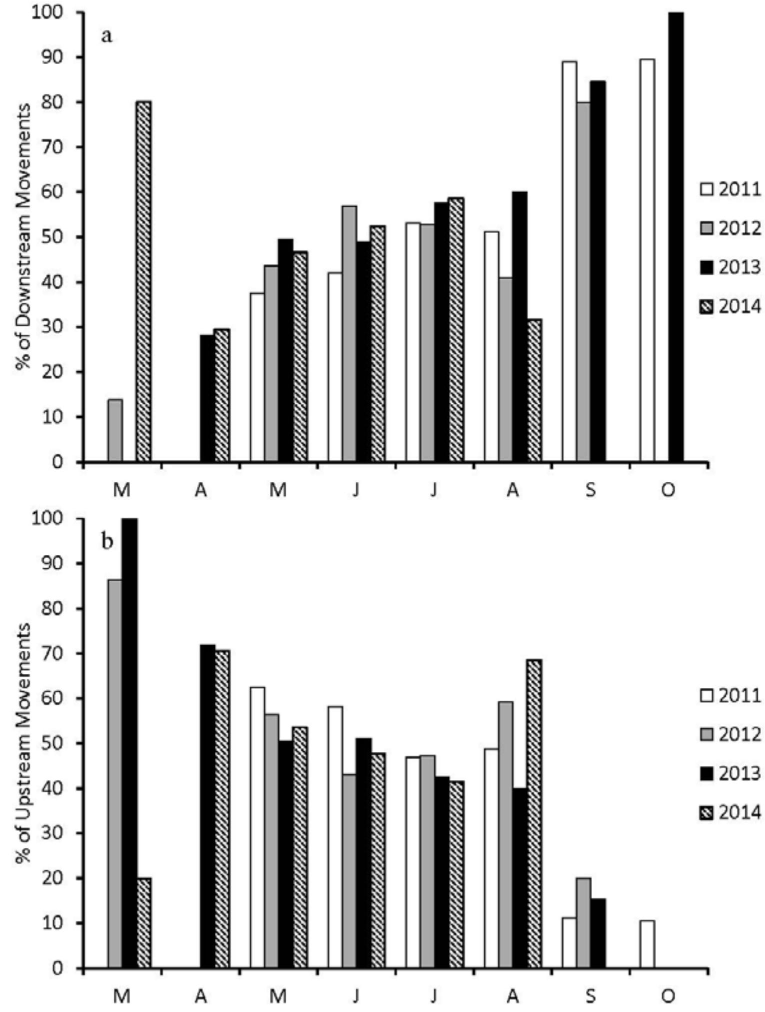

Figure 6. Directionality of the movements (> 50 m) of silver and hybrid carps presented as percentage traveling downstream or upstream by year.

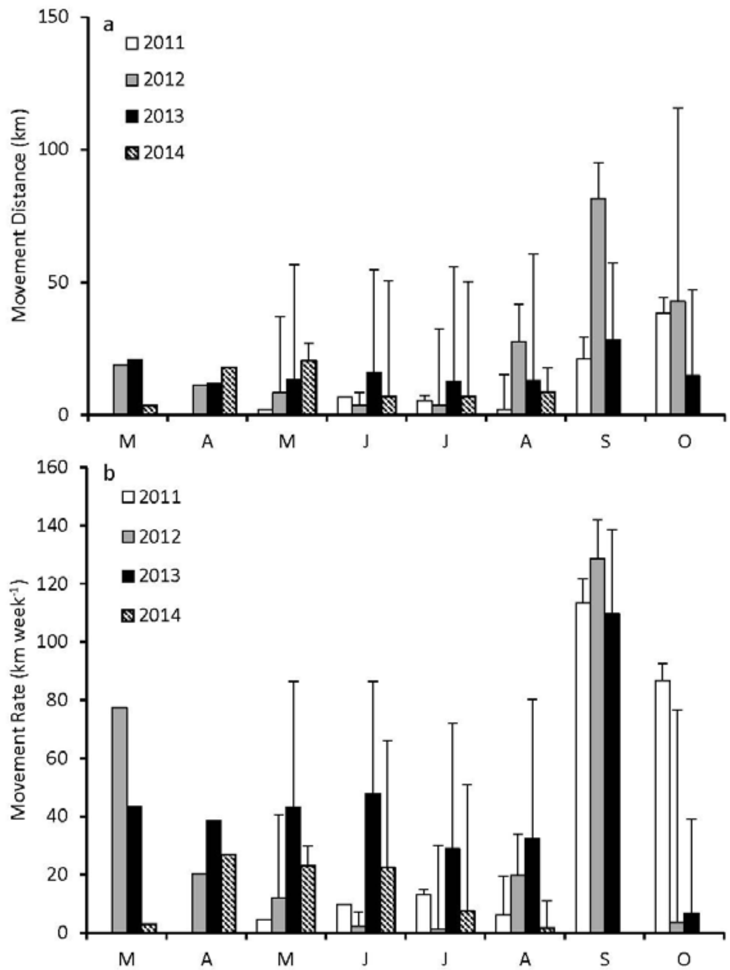

Figure 7. Average (±1 standard deviation) movement a) distances and b) rates for silver and hybrid carps by month.

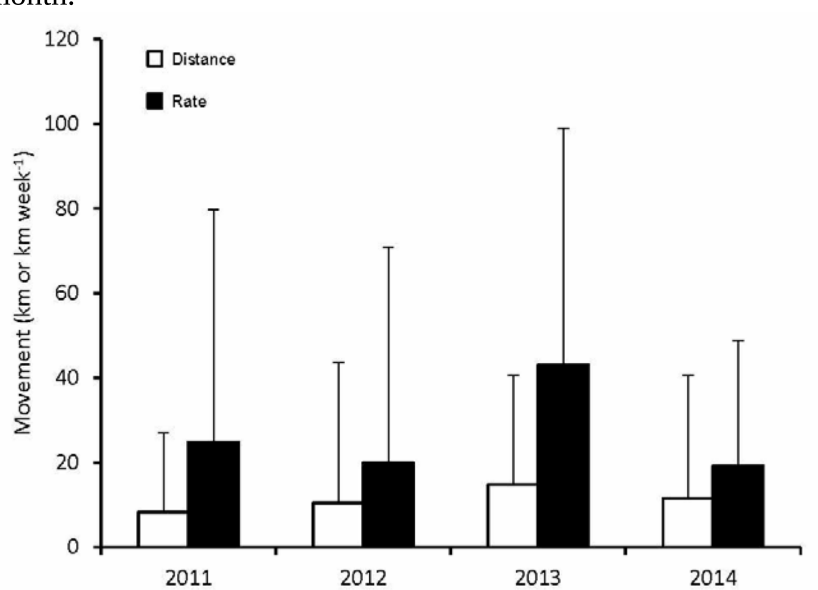

Figure 8. Average (±1 standard deviation) movement distance and rate by year for silver and hybrid carps.

Generalized linear mixed models for movement distance were run using only data from silver carp and detections collected from March-October, as hybrid and bighead carps generally had limited movements and very little movement was detected in the other months. Best models (< 2 ΔAIC) for movement distance all included gage, DOY, month and year (Table 2). Model averaging of these best models for movement distance resulted in a significant positive effect of gage, a negative effect of DOY, and positive effects for all months and years (Table 3). The months of September and October and the 2013 study year had the greatest positive effects on movement distance. However, fit of this averaged model was relatively poor (R^2=0.19). The best models for movement rate included CGDD, GDD, gage, DOY, month, and year (Table 4). Coefficients from model averaging indicated a significant negative impact of CGDD but a positive influence of GDD on movement rate. All study years had a positive effect on movement rate, with 2013 having the most positive effect. Additionally, gage and DOY also had significant positive effects on movement rate. March and September had significant positive effects on movement rate, while summer months had a negative impact (May-August). Fit of the movement rate model averaging was better than the movement distance model (R^2=0.27).

Table 2. Models of movement distance of silver carp. Model parameters, Akaike's Information Criterion corrected for small sample size (AICc), ΔAIC and AIC weight are all shown. Models listed were used in model averaging (Table 3). All models contained individual silver carp as a random effect.

Model	AICc	Δ AIC	Weight
Gage+DOY+Month+Year	19996.2	0	0.38
GDD+Gage+DOY+Month+Year	19996.6	0.41	0.31
CGDD+Gage+DOY+Month+Year	19997.8	1.60	0.17
CGDD+GDD+Gage+DOY+Month+Year	19998.0	1.82	0.15

The best models of movement probability all contained CGDD, gage24, DOY, month, year and sex (Table 5). Model averaging results indicated negative effects of CGDD and DOY and a positive effect of change in gage height over 24 hours. March and April had significant negative effects on movement probability, while all other months except May had significant positive effects (Table 3). All years had a significant negative effect on movement probability with 2012 being the least negative. Sex also had a significant negative influence on movement probability, with males being the least negative.

Table 3. Coefficients resulting from averaging the best models for silver carp movement distance, rate and probability. Averaged coefficients are listed with their corresponding p-value in italics. Coefficients are cumulative growing degree day (CGDD), growing degree day (GDD), current gage height (m, gage), change in gage height over 24 hours (m, gage24), day of year (DOY), months (March-October), years (2011-2014), and sex (male, female or unknown [unk]). All models contained individual silver carp as a random effect.

	Movement Distance		Movement Rate		Movement Probability	
CGDD	-0.0005	*0.726*	-0.013	*0.0008*	-0.0003	*0.0005*
GDD	0.085	*0.525*	0.911	*0.0003*	0.003	*0.603*
Gage	1.445	*≤0.0001*	1.319	*0.042*	0.0002	*0.980*
Gage24			0.596	*0.6001*	0.126	*≤0.0001*
DOY	-0.081	*0.005*	0.242	*≤0.0001*	-0.017	*≤0.0001*
March	15.92	*0.003*	39.71	*≤0.0001*	-2.750	*≤0.0001*
April	13.52	*0.009*	-14.94	*0.105*	-1.47	*≤0.0001*
May	15.58	*0.003*	-26.73	*0.005*	0.599	*0.054*
June	15.13	*0.01*	-30.57	*0.004*	1.49	*≤0.0001*
July	19.50	*0.002*	-43.35	*0.0002*	1.67	*≤0.0001*
August	25.76	*0.0003*	-45.27	*0.0005*	1.77	*0.0002*
September	40.40	*≤0.0001*	38.19	*0.013*	1.29	*0.019*
October	49.43	*≤0.0001*	-3.33	*0.848*	1.63	*0.009*
2011	13.19	*0.007*	41.41	*≤0.0001*	-2.750	*≤0.0001*
2012	17.62	*≤0.0001*	47.87	*≤0.0001*	-1.91	*≤0.0001*
2013	22.19	*≤0.0001*	68.18	*≤0.0001*	-3.61	*≤0.0001*
2014	15.92	*0.003*	39.71	*≤0.0001*	-3.71	*≤0.0001*
Female					-2.75	*≤0.0001*
Male					-1.28	*≤0.0001*
Unk					-2.20	*≤0.0001*

Table 4. Best models for silver carp movement rate. Model parameters, Akaike's Information Criterion corrected for small sample size (AICc), ΔAIC and AIC weight are all shown. Models listed were used in model averaging (Table 3). All models contained individual silver carp as a random effect.

Model	AICc	Δ AIC	Weight
CGDD+GDD+Gage+DOY+Month+Year	22498.1	0	0.605
CGDD+GDD+Gage+Gage24+DOY+Month+Year	22499	0.85	0.395

Table 5. Best models to predict the movement probability of silver carp. Model parameters, Akaike's Information Criterion corrected for small sample size (AICc), ΔAIC and AIC weight are all shown. Models listed were used in model averaging (Table 2). All models contained individual silver carp as a random effect.

Model	AICc	Δ AIC	Weight
CGDD+Gage24+DOY+Month+Year+Sex	12798.5	0	0.47
CGDD+GDD+Gage24+DOY+Month+Year+Sex	12799.1	0.54	0.36
CGDD+Gage+Gage24+DOY+Month+Year+Sex	12800.5	2.00	0.17

Comparisons of telemetry data with USGS ADCP data could only be done for silver carp as there were no detections for bighead and hybrid carps in the section of river monitored during the given time periods. In 2012, tagged silver carp were found in deeper water habitats ($\bar{X}_{Occupied}$ = 1.76 m ±0.49SD, $\bar{X}_{Available}$ = 1.03, n = 6, t = 5.41, p ≤ 0.0001) but not in 2013 ($\bar{X}_{Occupied}$ = 4.09 m ±0.71SD, $\bar{X}_{Available}$ = 3.96, n = 13, t = 0.67, p = 0.52). In both years, silver carp appeared to prefer lower water velocity habitats (2012: $\bar{X}_{Occupied}$ = 29.67 cms^{-1} ±10.04SD, $\bar{X}_{Available}$ = 36.20 cms^{-1}, n = 6, t = -9.24, p ≤ 0.0001; 2013: $\bar{X}_{Occupied}$ = 98.14 cms^{-1} ±8.85SD, $\bar{X}_{Available}$ = 108.82 cms^{-1}, n = 13, t = -4.35, p ≤ 0.001) and higher temperature habitats (2012: $\bar{X}_{Occupied}$ = 27.05°C ±0.26SD, $\bar{X}_{Available}$ = 25.67°C, n = 6, t = 9.64, p ≤ 0.0001; 2013: $\bar{X}_{Occupied}$ = 22.62°C ±0.12SD, $\bar{X}_{Available}$ = 22.40°C, n = 13, t = 6.32, p ≤ 0.0001).

Spawning

<u>2014 update</u>

Genetic processing of eggs and larvae from previous years was completed and most of the findings are discussed in the section below. Larvae collected from the East Fork of the While River in 2012 were genetically verified as not being bigheaded carp. Larvae collected from the Upper Wabash in 2013 at RM373 (Peru) were also genetically verified as not being bigheaded carp. A total of 938 eggs were tested for classification as silver, hybrid and bighead carp. As there is limited genetic material available from a single egg, 20% of eggs showed amplification with one or none of the three primers despite a rerun of the PCR when sufficient template material was available. Therefore, these eggs were not classified. Five of the eggs tested showed results from gel electrophoresis that were inconsistent with bigheaded carps.

Production of bigheaded carp eggs was first detected on 27-May-2014 and was last detected on 3-Sep-2014. Peak egg production occurred on 30-Jul-2014 at a density of 17.8 eggs/m^3. Upstream sampling for egg production yielded one bigheaded carp egg at RM390 (Wabash) in 2014.

No young-of-year bigheaded carps were collected during fall sampling efforts in 28 net nights. Average CPUE of all fishes captured was 9.3 ±13.4SD individuals/hr. Catch was dominated by bluegill (*Lepomis macrochirus*), *Notropis* spp. and *Pimephales* spp. at all sites. Catches also included: white crappie (*Pomoxis annularis*), black crappie (*P. nigromaculatus),* green sunfish (*L. cyanellus*), orange spotted sunfish (*L. humilis*), largemouth bass (*Micropterus salmoides*), pumpkinseed (*L. gibbosus*), warmouth (*L. gulosus*), freshwater drum (*Aplodinotus grunniens*), river carpsucker (*Carpiodes carpio*), channel catfish (*Ictalurus punctatus*), stonecat (*Noturus flavus*), bowfin (*Amia calva*), rainbow darter (*Etheostoma caeruleum*), mottled sculpin (*Cottus bairdii*) and creek chub (*Semotilus atromaculatus*).

Overall

Spawning of bigheaded carps occurred over a protracted season and across a variety of conditions (Table 6). In general, spawning began in late May and ended in September, with varying densities of eggs occurring across the season (Figure 9; Table 7). 2011 spawning data were presence/absence, and 2011 data were thus not included in most analyses. Genetic analyses of eggs from 2012 and 2013 spawning seasons showed that hybrids accounted for a higher percentage of collected eggs in 2013 (Figure 10). However, the percentages of hybrid eggs did not vary across the spawning season (2012: $r = -0.023$; 2013: $r = 0.028$), and there appears to be no separation in spawning seasons (Figure 11; Table 8). Silver carp eggs were 85% ±3.0SE of the bigheaded carp eggs collected, while hybrids and bighead carp usually represented about 11% ±3.7SE and 3.7% ±1.8SE of collected eggs, respectively.

Upstream spawning yielded eggs up to RM390 in 2011 and 2014 (1 egg on 1-June). No eggs were documented at upstream locations in 2012 and 2013. Additionally, no genetically confirmed eggs or larvae were located in the East Fork of the White River in 2011 and 2012. However, suspected eggs were collected, although the eggs were damaged in preservation and transit and could not be genetically tested to confirm their identity.

Model selection indicated that the presence of eggs at spawning initiation was predicted by CGDD (Table 9), and this model successfully predicted spawning initiation in 2014. No eggs were found in the river until the model predicted probability of spawning initiation was 85% (Table 10). However, the model to predict spawning cessation did not successfully predict the end of spawning in 2014.

Table 6. Summary of spawning conditions for bigheaded carp from 2012 - 2014 spawning seasons.

	Date			Water Temperature (°C)		Egg Density (eggs m^{3-1})		CGDD	
Year	Start	Max	End	Min	Max	Min	Max	Start	Peak
2012	7-May	1-Jul	27-Aug	18.5	28.6	0.07	526.67	293.5	910.5
2013	19-May	3-Jun	9-Sep	16.5	25.5	0.005	10976.63	228.1	365.3
2014	27-May	30-Jul	3-Sep	19	26	0.04	17.76	227.7	951.2

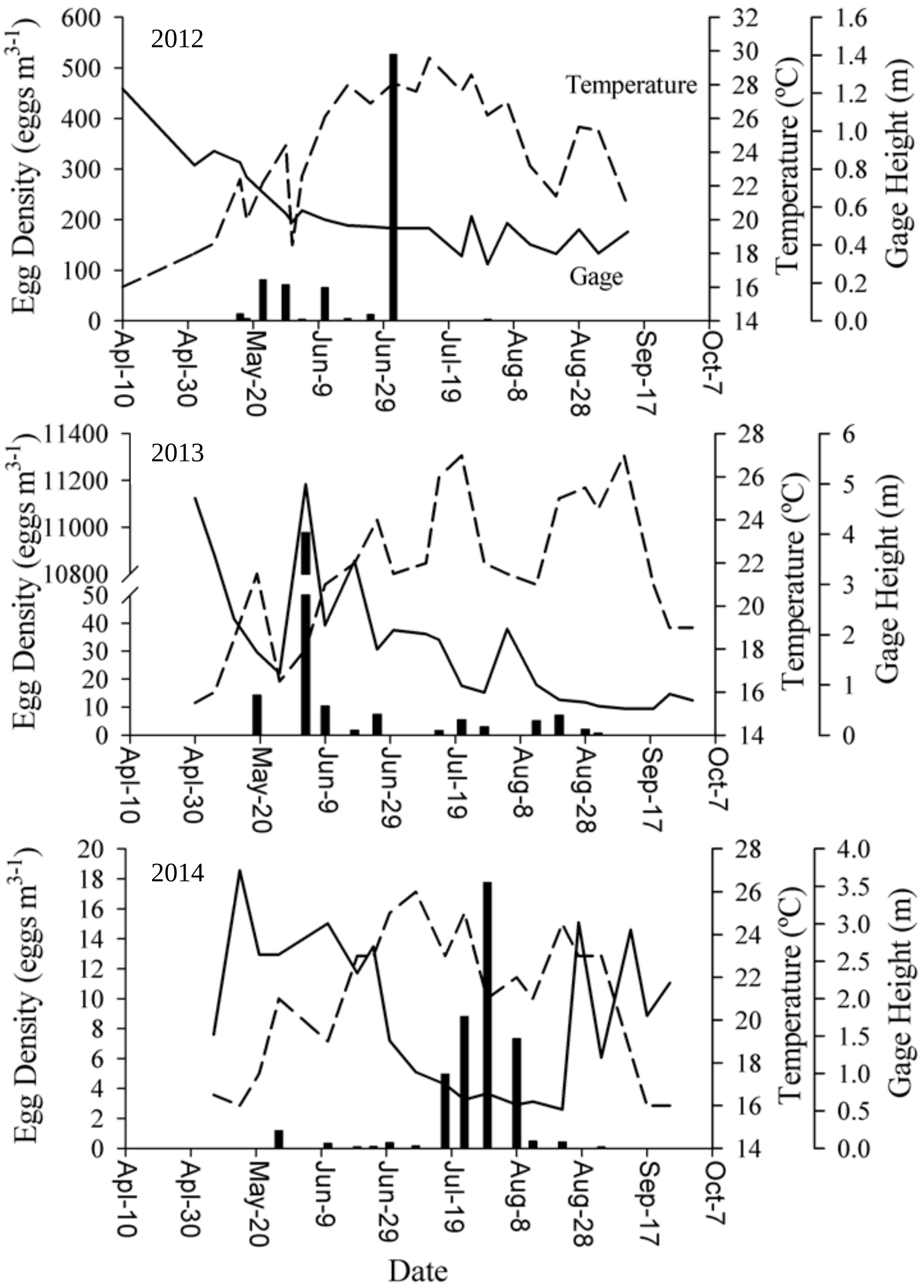

Figure 9. Summary of bigheaded carp egg densities (bars) collected at varying water temperatures (dashed line) and river gage heights (solid line) in 2012-2014. Egg densities are listed in Table 7.

Table 7. Summary of bigheaded carp egg densities observed from 2012-2014 near West Lafayette, IN.

2012			2013			2014		
Date	Egg Density (eggs m^3-1)	Density SE	Date	Egg Density (eggs m^3-1)	Density SE	Date	Egg Density (eggs m^3-1)	Density SE
1-Apr-12	0.00	-	30-Apr-13	0.00	-	7-May-14	0.00	-
1-May-12	0.00	-	6-May-13	0.00	-	15-May-14	0.00	-
7-May-12	0.19	0.15	12-May-13	0.00	-	21-May-14	0.00	-
15-May-12	13.64	4.93	19-May-13	14.21	0.47	27-May-14	1.17	0.49
17-May-12	4.03	1.49	26-May-13	<0.01	<0.01	11-Jun-14	0.33	0.00
22-May-12	80.87	32.27	3-Jun-13	10976.63	0.00	20-Jun-14	0.10	0.05
29-May-12	71.42	31.86	9-Jun-13	10.38	2.96	25-Jun-14	0.10	0.04
31-May-12	1.64	44.42	18-Jun-13	1.72	0.20	30-Jun-14	0.37	0.09
3-Jun-12	2.80	1.36	25-Jun-13	7.39	2.41	8-Jul-14	0.16	0.03
10-Jun-12	65.71	17.42	30-Jun-13	0.07	0.03	17-Jul-14	4.96	0.88
17-Jun-12	4.26	2.75	10-Jul-13	0.22	0.06	23-Jul-14	8.80	1.05
24-Jun-12	12.32	3.88	14-Jul-13	1.63	0.17	30-Jul-14	17.76	7.62
1-Jul-12	526.67	461.50	21-Jul-13	5.50	0.74	8-Aug-14	7.33	1.30
8-Jul-12	0.30	0.08	28-Jul-13	2.99	0.22	13-Aug-14	0.46	0.08
12-Jul-12	0.07	0.04	4-Aug-13	0.05	0.03	22-Aug-14	0.41	0.12
22-Jul-12	0.23	0.02	13-Aug-13	5.08	0.40	27-Aug-14	0.00	-
25-Jul-12	0.00	-	20-Aug-13	7.06	1.11	3-Sep-14	0.09	0.03
30-Jul-12	3.27	0.09	28-Aug-13	2.02	0.32	12-Sep-14	0.00	-
5-Aug-12	0.71	0.10	1-Sep-13	0.70	0.04	17-Sep-14	0.00	-
12-Aug-12	1.57	0.18	9-Sep-13	0.12	0.04	24-Sep-14	0.00	-
20-Aug-12	1.21	0.26	18-Sep-13	0.00				
27-Aug-12	0.32	0.13	23-Sep-13	0.00				
2-Sep-12	0.00	-	30-Sep-13	0.00				
11-Sep-12	0.00	-						

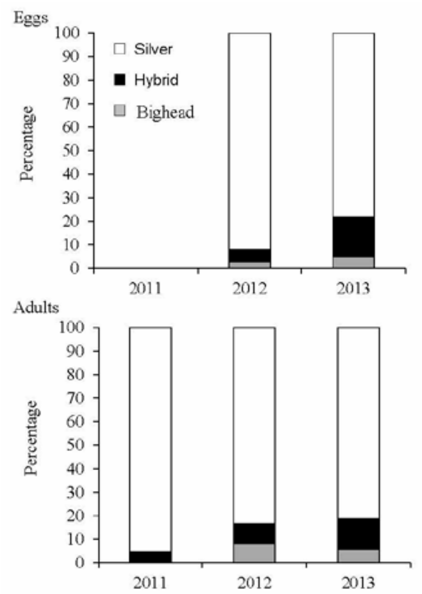

Figure 10. Percentages of bigheaded carp eggs and adults classified as silver, bighead and hybrid carps. No eggs were analyzed from the 2014 spawning season.

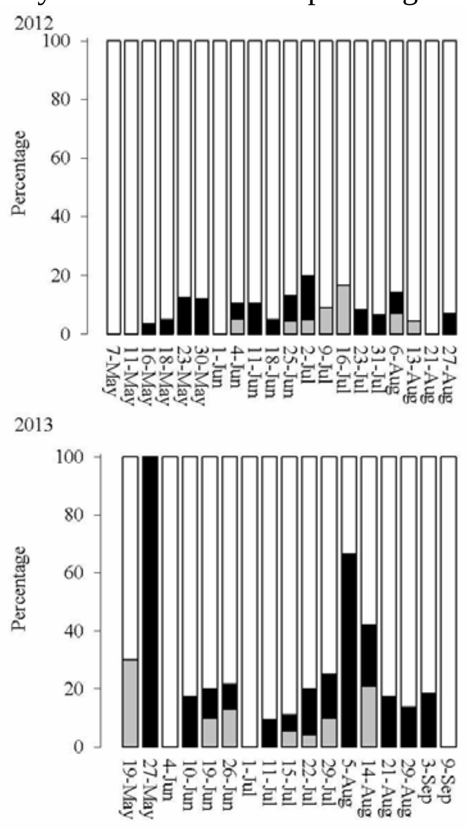

Figure 11. Occurrences of bigheaded carp eggs by type for both 2012 and 2013 spawning seasons. Silver carp are white, bighead carp are gray and hybrid carp are black.

Table 8. Summary of data presented in Figure 11. Percentages of eggs that were genetically determined to be bighead, hybrid and silver carp on specific dates as well as the total number of eggs tested on those dates.

Date	Bighead	Hybrid	Silver	Total
7-May-12	0.00	0.00	100.00	5
11-May-12	0.00	0.00	100.00	5
16-May-12	0.00	3.57	96.43	28
18-May-12	0.00	5.00	95.00	60
23-May-12	0.00	12.50	87.50	24
30-May-12	0.00	12.00	88.00	25
1-Jun-12	0.00	0.00	100.00	23
4-Jun-12	5.26	5.26	89.47	19
11-Jun-12	0.00	10.53	89.47	19
18-Jun-12	0.00	5.00	95.00	20
25-Jun-12	4.35	8.70	86.96	23
2-Jul-12	5.00	15.00	80.00	20
9-Jul-12	9.09	0.00	90.91	11
16-Jul-12	16.67	0.00	83.33	6
23-Jul-12	0.00	8.33	91.67	12
31-Jul-12	0.00	6.67	93.33	15
6-Aug-12	7.14	7.14	85.71	14
13-Aug-12	4.35	0.00	95.65	23
21-Aug-12	0.00	0.00	100.00	17
27-Aug-12	0.00	7.14	92.86	14
19-May-13	30.00	0.00	70.00	20
27-May-13	0.00	100.00	0.00	1
4-Jun-13	0.00	0.00	100.00	29

Table 8 Continued.

10-Jun-13	0.00	17.24	82.76	29
19-Jun-13	10.00	10.00	80.00	20
26-Jun-13	13.04	8.70	78.26	23
1-Jul-13	0.00	0.00	100.00	7
11-Jul-13	0.00	9.52	90.48	21
15-Jul-13	5.56	5.56	88.89	18
22-Jul-13	4.00	16.00	80.00	25
29-Jul-13	10.00	15.00	75.00	20
5-Aug-13	0.00	66.67	33.33	3
14-Aug-13	21.05	21.05	57.89	19
21-Aug-13	0.00	17.24	82.76	29
29-Aug-13	0.00	13.79	86.21	29
3-Sep-13	0.00	18.52	81.48	27
9-Sep-13	0.00	0.00	100.00	8

Table 9. Model selection results and coefficients for models predicting bigheaded carp egg density, spawning initiation and cessation. Coefficients of the best model are as listed, and the AIC_C value is listed for the egg density model while the likelihood values are listed for the initiation and cessation spawning models. P-values for model coefficients are listed in italics below each coefficient.

Model	Intercept	Gage	Date	CGDD	Model p	df	AIC_c /Likelihood Ratio
Egg Density	62.578	-6.74	-0.247		*0.003*	31	300.78
		0.152	*0.012*				
Spawning Initiation	-5.500			0.032	< *0.0001*	1	22.65
				< *0.0001*			
Spawning Cessation	8.754		-0.050	0.0054	*0.025*	2	26.92
			0.0003	*0.0004*			

Table 10. Model predictions for bigheaded carp spawning in the Wabash River for 2014. Egg presence and density are the actual values from the field. Density model spawning initiation and spawning cessation list the results of the best models detailed in Table 9.

DOY	Egg Presence	Egg Density ±SE (eggs m^3-1)	Density Model (eggs m^{3-1})	Initiate Spawning	Cease Spawning
127	0	0	-	0.06	-
135	0	0	-	0.29	-
141	0	0	-	0.50	-
147	1	1.17 ±0.49	8.38	0.85	1.0000
162	1	0.33	1.82	1.00	1.0000
171	1	0.10 ±0.05	4.05	1.00	1.0000
176	1	0.10 ±0.04	0.40	1.00	1.0000
181	1	0.37 ±0.09	7.63	1.00	1.0000
189	1	0.16 ±0.03	8.47	1.00	1.0000
198	1	4.69 ±0.88	7.33	1.00	1.0000
204	1	8.80 ±1.05	7.20	1	1.0000
211	1	17.76 ±7.62	4.90	1	1.0000
220	1	7.33 ±1.30	3.66	1	1.0000
225	1	0.46 ±0.08	2.12	1	1.0000
234	1	0.41 ±0.21	0.59	1	1.0000
239	0	0	-17.51	1	1.0000
246	1	0.09 ±0.03	-7.10	1	1.0000
255	0	0	-	-	1.0000
260	0	0	-	-	0.9999
267	0	0	-	-	0.9929

Discussion

Bigheaded carps appeared to exhibit clear seasonal trends in movement directionality, distance, and rate, with rapid upstream movements prior to spawning in the late spring and downstream movements in the fall. Silver carp were also found to occupy deeper, lower velocity, higher temperature habitats as well as pools around bridges and backwater areas where available. The spawning requirements of bigheaded carps appear different from their native range, as eggs were found in sections of the Wabash River with a small watershed size. Spawning activity was not solely influenced by changes in hydrograph, as suggested in the literature; instead the initiation of spawning was best predicted by a model composed of a single variable, CGDD. Finally, there were no detections of tagged bigheaded carps in the Little River during this study.

Movements of bigheaded carps can be extremely rapid and cover large distances. Movement rates for silver carp are similar to those previously documented in the Illinois River (DeGrandchamp et al. 2008). However, movement distances and rate appear influenced by a variety of environmental and individual characteristics with a general trend of increased movements in the spring and fall compared to the summer (May-August). Additionally, there is variation in this trend across years. It also appears individuals are more likely to move that during the summer, with drought years (i.e., 2012) increasing movement probability. In general, the averaged model for movement rate was better than the averaged model for movement distance. This may have been a result of the inclusion of stationary receiver data limiting the movement distances that were detected. However, this effect should have been reduced by the inclusion of the active tracking data as well as data from stationary receivers. Several additional environmental predictors may have contributed to variation observed during this study including phytoplankton density (Calkins et al. 2012). Despite potential additional sources of variation, trends in the movements of bigheaded carp exist and could serve as management targets for these species (e.g., removal efforts, spawning disruption efforts, etc.).

Movements of bigheaded carps exhibit habitat preferences that may also be exploited by managers. Silver carp were found to prefer deeper, higher temperature, lower velocity habitats compared to other available habitats. While they did not appear to select for deeper water in 2013, the water levels during the time period examined were high, and so fish may not have had to be as selective to allow them to remain in preferred deeper habitats. This supports previous findings from the Illinois and Missouri Rivers where silver carp preferred backwater habitats and bighead carp occupied areas behind wing dams (DeGrandchamp et al. 2008; Kolar et al. 2007). As large bodied, open-water swimming fishes, bigheaded carps may require deeper habitats, and as the Wabash River approaches baseflow in summer months, they appear to concentrate in deeper pools and backwaters. These concentrations may facilitate removal and control efforts. As discussed in previous reports, bigheaded carp also utilize areas around bridges which also likely have low velocities. These areas around bridges are also deeper and may serve to concentrate bigheaded carps. Overall, the trends observed in bigheaded carp movements and habitat use can be utilized to facilitate the removal of these fishes. For example, upstream movements prior and during spawning represent a potential time when bigheaded carps may invade upstream habitats and may also be a time when fish can be targeted using removal gears (i.e., weirs, hoop nets) in the main channel. Non-spawning months, when fishes concentrate in deeper, low velocity habitats, represent times during which harvest or piscicide treatments could be potentially used as control efforts in backwaters and pools. However, the potential impacts of such targeted removals on native fish species needs to be evaluated before such strategies can be implemented.

Bigheaded carps may regularly utilize tributary confluences as habitats based on observations during this study and in other studies (Kolar et al. 2007). Larger tributaries and dam spillways may therefore also represent potential habitats for targeted removal. It is likely that bigheaded carps would concentrate in any deeper water habitats available which, in the tributaries sampled, may be dam spillways. Tagged bigheaded carp were found to move up the Tippecanoe River, the largest tributary included in the study area. Additionally, smaller silver carp were observed jumping at the Mississinewa and Oakdale dam spillways. It is possible that usage of tributaries is regulated by the size of individual bigheaded carps. No tagged bigheaded carp were detected in the Missisinewa, Salamonie or Little Rivers, although tagged fish were detected in the Wabash River near its confluence with each of these rivers. It appears that smaller tributaries are less likely to be utilized by bigheaded carps, especially larger individuals, as they are already known to select deeper habitat. It appears that tributaries may be utilized by bigheaded carp, especially larger tributaries or potentially during high flow events which may expand available habitat.

Initiation of spawning in bigheaded carps appears to be linked to CGDD. While CGDD is included in risk assessments to determine if spawning of bigheaded carp is possible, spawning of these species was also thought to require significant changes in hydrograph. The findings of this work indicate that changes in hydrograph may not be necessary but may trigger more concentrated spawning as observed in 2013. High egg densities in 2013 demonstrate that changes in hydrograph once temperatures are appropriate for spawning can produce massive spawning events. Regardless of hydrograph, as observed in 2012, bigheaded carp still spawn and spawning is consistently spread across several months. The cessation of spawning activities was difficult to model and may occur after a set period of time rather than in response to any particular environmental cue. While no bigheaded carp YOY were collected in 2014, this does not necessarily mean that no recruitment from embryos to YOY occurred. Based on observations from other systems YOY bigheaded carp are patchily distributed through river systems (D. Chapman, USGS and S. Butler, UIL *personal communications*). Despite no detected recruitment in 2014, there appears to have been recent recruitment as the proportion of adult bigheaded carp < 400 mm collected during sampling for this study has increased from 2010-2013 (Coulter and Goforth *in prep*).

Hybrids were increasingly represented in both adult and egg samples collected, but physically appeared similar to silver carp. Reproduction of silver, bighead and hybrid carps occurred across the summer months, and there was no discernable difference in spawning season for these groups. This indicates that hybrid carps are likely to continue to increase in this system, and it has been suggested that hybrids may require difference management strategies due to ecological and biological differences compared to the parental types (Lamer et al. 2010). However, because silver carp are at a higher abundance in the Wabash River than bighead carp, hybrids are more likely to backcross with silver carp and may become increasingly similar to silver carp both ecologically and biologically. This is supported by the movements of silver and hybrid carps which were not significantly different from each other. Therefore, it is likely that any management actions for silver carp will also help to manage hybrid carps. Hybrid carp themselves may have implications for the invasion success of bigheaded carps. Invasive species, especially when the population is founded by only a small number of individuals, may be prone to genetic bottlenecks, inbreeding and founder effects. Hybridization is known to help overcome these effects. Therefore, it is likely that hybridization of bighead and silver carp could help to increase genetic diversity and overcome genetic bottlenecks. Plasticity and variation may also contribute to the success of bigheaded carps. Spawning across a variety of environmental conditions may contribute to the reproductive success of bigheaded carps in invaded ecosystems. For example, bigheaded carp reproduction detected in this study represents spawning of these fishes in a smaller watershed area than previously had been documented (Coulter et al. 2013). Increased genetic variation in a population that results from hybridization and backcrossing of silver and bigheaded carps may increase the likelihood of successful invasions by these species. Increased genetic heterozygosity can contribute to ability of individuals and a population to persist across a wide variety of environmental conditions. Therefore, continued increased numbers of hybrid bigheaded carps may help maintain genetic diversity in the population and increase their resilience to environmental change.

Summary
Movement and spawning behaviors of bigheaded carp observed in the upper middle Wabash River over this 4 year study illustrate that while there are some consistencies in these behaviors across invaded ecosystems (i.e., habitat preferences), there are also some substantial deviations in these traits between native and invaded ranges (i.e., watershed size required for spawning, environmental spawning triggers, duration of spawning season, etc.). In particular, egg production over a protracted spawning season and hybridization between silver and bighead carp in newly invaded ecosystems may be key factors that facilitate their successful establishment. On the other hand, mass movement events in the spring and fall and occupancy of habitats such as backwaters and pools during low flow periods may be useful for targeting these invasive fishes for removal as a control strategy. None of our tagged fishes were detected in the Little River or other tributaries (except the largest tributary in this study, the Tippecanoe River), suggesting that introduction of adult Asian carp across Eagle Marsh is unlikely even in the absence of the measures that have been taken to separate the Wabash and Maumee River basins at this location. Further, it appears unlikely that juvenile Asian carp will be present at locations sufficiently upstream in the Wabash River to provide introduction pressure at Eagle Marsh. Although there was evidence of spawning in upstream areas, it appears to be at very low levels, and even developing eggs at these locations would be carried far downriver before hatching. Still, it is clear that the biology and ecology of these species are somewhat unpredictable in newly invaded ecosystems, and while the threats may be extremely small at this time, there is potential that this might change in the future. Complete separation of the Wabash and Maumee watersheds via a constructed berm at Eagle Marsh is therefore warranted to avoid future introduction opportunities.

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