An Assessment of Silver and Bighead Carp (*Hypopthalmichthys* spp.) Movements and Spawning Activities in the Wabash River Watershed, Indiana – Phase I Annual Report

Submitted by: Alison Coulter, Graduate Research Assistant (astrandb@purdue.edu)
Reuben R. Goforth, Ph.D., Principal Investigator (rgoforth@purdue.edu)
Department of Forestry and Natural Resources, Purdue University
195 Marstellar Street, West Lafayette, IN 47907

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 "Asian carps" (e.g., silver *Hypophthalmichthys molitrix* and bighead *H. nobilis*) are large threats to the Great Lakes given expected trajectories of nutrient flow disruption and food web alterations that will likely accompany their introduction to 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 recently been identified at Eagle Marsh near Fort Wayne, Indiana. Eagle Marsh may provide a corridor for transfer of these species between the Wabash and Maumee River basins during flood periods, and the direct connection of the Maumee River with Lake Erie would therefore provide a means for introduction of Asian carp to the Great Lakes Basin. Immediate action to prevent such an introduction via transfer of adult Asian carp has been taken through the installation of a physical barrier across Eagle Marsh. However, the potential trajectories and rates of movement by silver and bighead carps throughout the Wabash River, and especially into the Little River and Eagle Marsh, have not been determined to date.

Understanding the movements of invading species in new systems 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). Asian 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 (Degrandchamp et al. 2008). The specific cues triggering Asian 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 Asian 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 both main channel and backwater habitats in the Illinois River (Degrandchamp et al. 2008), they currently occur in relatively high densities in the borrow pit behind the Williamsburg Apartments in West Lafayette (River Mile 310), and at the confluence of the Tippecanoe River with the Wabash River (River Mile 324). Determining habitat use by Asian carp in the Wabash River can help to devise strategies for control and prediction of invasion patterns.

Previous studies have successfully used telemetry to observe Asian carp movements in rivers (e.g., Degrandchamp et al. 2008). The tags used were ultrasonic transmitters (Vemco; Halifax, Nova Scotia) which emit a unique coded acoustic signal that can be detected using manual or passive receivers. Vemco VR2W stationary receivers can be placed in the river to continuously monitor movements and help to target the much more time intensive manual tracking activities. Once the general movements have been determined using the VR2Ws, a VR100 manual receiver can be used to pinpoint the locations of individual fish. The physical characteristics of these locations can then be determined using standard methods to better describe the occupied habitats.

We tagged and tracked 100 Asian carp in the Wabash River using Vemco V16-4L ultrasonic tags and Vemco VR2W passive and Vemco VR100 manual tracking hardware. We also conducted spring surveys to detect Asian carp spawning events at multiple sites in the upper Wabash River and one of its largest tributaries, the East Fork of the White River.

Methods

Tagging

Fish for acoustic tagging were collected at multiple locations in the Wabash River (Table 1) 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}. Total length and weight data from previous studies of Asian carp completed in fall of 2010 were used for comparison to tagged fish.

Candidate fish were anaesthetized using a custom-made mobile electroanesthesia unit (MEU, Plates 1-3). 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 (kg) 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.

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 (Plate 4). A 4-5 cm incision was made in the fish's left side 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 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 recover first in the MEU tank. Once swimming ability was recovered, fish were placed in a holding cage in the river until fully recovered.

Plate 1. Mobile electroanesthesia unit (MEU) tank with stainless steel electrodes and stand installed.

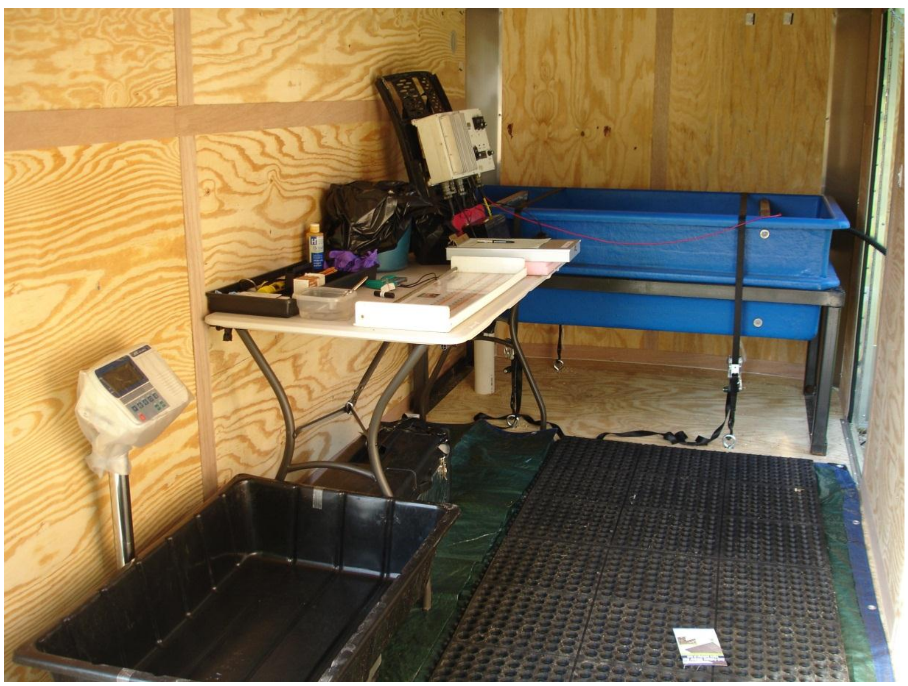

Plate 2. Completed mobile electroanesthesia unit (MEU) with tank, electrodes, AbP-3™ electrofisher, and associated fish processing and surgery equipment.


Plate 3. Mobile electroanesthesia unit (MEU) with flow through system of pumped river water in operation.


Plate 4. Surgical implantation of a Vemco 16-4L acoustic transmitter into a bighead carp. Magnified area depicts the surgical area post-suturing.

<u>Tracking</u>

Passive – Omnidirectional passive receivers (Vemco VR2W) were deployed on the river bottom in the Little River, the Tippecanoe River, and between Wabash River Miles 160-400. The VR2Ws were attached to custom platforms and anchors (Plates 5-6). 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 a 2-30 m underwater 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.

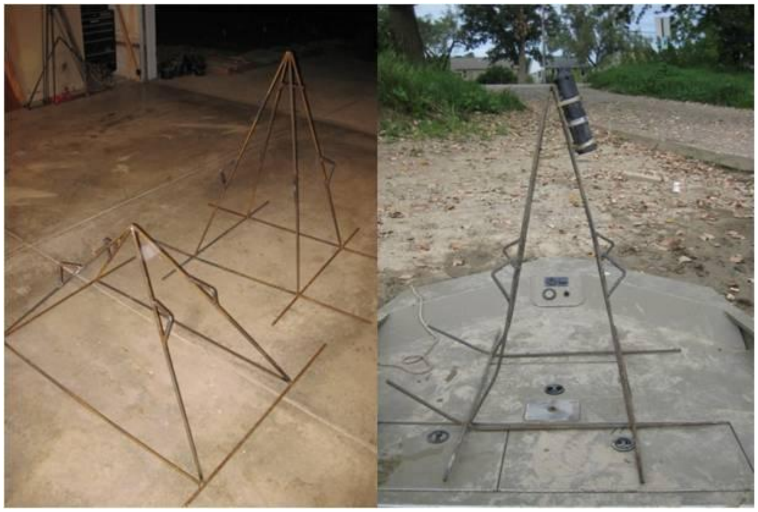

Plate 5. Larger deployment platforms for Vemco VR2W passive receivers (attached to the top of the stand in the picture to the right) deployed in deeper reaches of the Wabash River.

Plate 6. Smaller deployment system used for Vemco VR2W passive receivers in shallow reaches of the Wabash and Little Rivers.

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 (Figure 1). Placement varied somewhat depending on access points. While this array covered considerably more area than the primary focus area in the upper and middle Wabash and Little Rivers, this arrangement was judged sufficient to cover the full potential range of marked Asian 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 and retrieved from River Mile 165 VR2W on a 6-9 week rotation and from the other more upstream VR2Ws at 2-3 week intervals from 01-May through 31-Aug to detect movements of Asian carp during what is expected to be their most active time of the year. These upstream VR2Ws were then checked on a longer 4-6 week rotation through the remainder of the season.

Active – Active tracking was accomplished by deploying hydrophones from one of two tracking boats or a canoe depending on river conditions. Both an omnidirectional and directional hydrophone (Vemco VH110, and Vemco VH165, respectively) connected to one of two manual receivers (Vemco VR100) were used to locate and identify tagged Asian 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 fish was detected, the directional hydrophone was used to identify the fish's location within the river channel. Once a reading of 70 db or more was achieved on the manual receiver, the position of the tagged Asian carp was recorded using a handheld GPS (GPSMap 60, Garmin Ltd., Olathe, Kansas).

Habitat measurements were taken during manual tracking when tagged Asian 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 (Wentworth 1922): boulder, cobble, gravel, sand, fines, or hardpan.

Spawning Evaluations

We conducted spawning evaluations in both the upper Wabash River (River Miles 300-400) and the East Fork of the White River during the summer of 2011. Field crews conducted egg and larval sampling at selected sites on the rivers using paired bongo nets (333µm and 500µm bucket mesh size) pulled in replicates of three or more. Bongo nets were towed from the bow of one of two boats in a downstream direction for five minutes in an effort to detect the presence of Asian carp eggs and/or larvae. Eggs were identified under magnification using a Nikon SMZ1500 microscope (Nikon Instruments, Inc.) and/or sent out for deoxyribonucleic acid (DNA) analysis (see below). Larvae were preserved in a 10% formalin solution for later identification. Sampling activities were conducted once water temperatures reached $\geq 15.6^{\circ}$ C and were intensified with rising hydrographs, which have been found to be a trigger for spawning in Asian carp (Abdusamadov 1987; Peters et al. 2006; Degrandchamp et al. 2008). Sites were located longitudinally along the rivers to determine the spatial extent of spawning activities. Sampling continued on a weekly basis near River Mile 310 where Asian carp eggs were detected through late summer 2011 to determine spawning duration.

Eggs submitted for molecular verification were preserved at -80°C and sent to the USGS Upper Midwest Environmental Sciences Center, LaCrosse, Wisconsin (J. Amberg, USGS, pers. comm.). Genomic DNA was extracted from eggs and larvae using QIAamp DNA Mini Kit (Qiagen, Inc, Valencia, California) according to the manufacturer's instructions. Species identifications were analyzed in duplicate using both conventional polymerase chain reaction (PCR) and quantitative PCR (qPCR). Both types of PCR used 50 ng of genomic DNA and species-specific primers for silver carp and bighead carps (Jerde et al. 2011). Conventional PCR reactions (25 µL) were comprised of: 12.5 µL MangoMix (Bioline USA, Inc., Tauton, Massachusetts), 0.3 µM of each primer and molecular grade water. PCR conditions were as follows: 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 2 minutes for 45 amplification cycles and a final extension period of 10 minutes at 72°C on a Mastercycler® thermocycler (Eppendorf North America, Inc., Westbury, New York). Conventional PCR products were visualized on 1% agarose gel stained with GelRed™ (Phentix Research Products, Candler, North Carolina). Quantitative PCR reactions (20 µL) were comprised of: 10 µL SensiMix SYBR master mix (Bioline USA, Inc., Tauton, Massachusetts), 0.3 µM of each primer and molecular grade water with the same conditions as the conventional PCR on a Mastercycler® ep realplex 2 (Eppendorf North America, Inc., Westbury, New York). Melt curve analyses were used to determine assay specificity. All PCR products were compared with products from control DNA, which was extracted from fin-clips of silver carp and bighead carp.

Statistics

Total lengths and weights of fish tagged at each location were compared using a Kruskal-Wallace test. Fish tagged at Peru were not used in this analysis due to limited sample size ($n = 3$). The total length distribution of the tagged silver carp was segregated into five 7.9 cm size bins from 50-89.9 cm and these were compared to the distribution of all carp captured from the Wabash River (current and previous studies) using Chi-squared test of association. Total lengths and weights of fall 2010 fish were compared to tagged fish using Mann-Whitney U tests. Total lengths and weights of male and female fish were compared using two-sample, one-tailed t-tests. Total lengths of males, females, tagged and fall 2010 fish were regressed against weight. All tests were performed with $\alpha = 0.05$ using SAS 9.2 (2008 SAS Institute Inc., Cary, North Carolina).

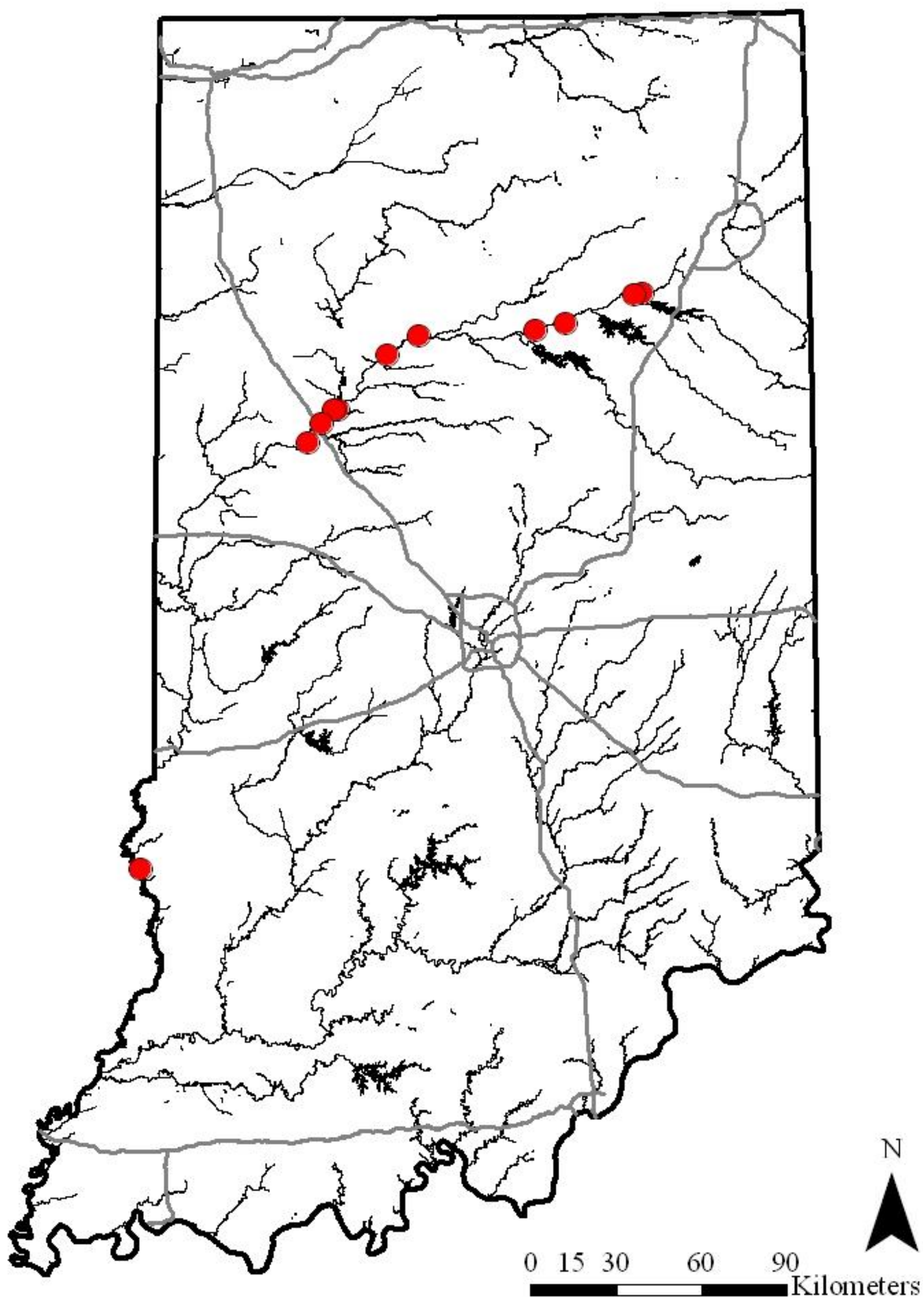

Figure 1. Locations of Vemco VR2W passive receivers. Gray lines represent interstate highways. River Mile locations of these are listed in Table 2.

Results

Tagged Fish Information

Surgeries to implant tags were conducted from 8-Apr to 2-Jun 2011. During this time 99 silver carp and one bighead carp were collected, tagged and then released at multiple locations along the Wabash River (Table 1). Mean weights of silver carp were not statistically different among the tagging locations ($H = -3.60$, $p = 0.166$), although mean total lengths were different ($H = 10.76$, $p = 0.005$). Post hoc analysis using Mann-Whitney U tests (Bonferonni corrected $\alpha = 0.017$) revealed that silver carp tagged at River Mile 310 were significantly smaller in total length than fish tagged at both River Miles 324 and 351 ($W = 322.0$, $p = 0.002$; $W = 511.2$, $p = 0.001$, respectively), although mean total lengths of silver carp tagged at River Miles 324 and 351 were not different from each other ($W = 761.0$, $p = 0.701$). The length-frequency distribution of tagged silver carp ($n = 99$) was not different from all observed silver carp ($n = 182$; $X^2 = 2.867$, $p = 0.580$; Figure 2). Total lengths of tagged fish ($n = 99$) and fall 2010 fish ($n = 83$) were also not different ($W = 2553.0$, $p = 0.742$). However, mean silver carp weights from fall 2010 ($n = 38$) were significantly lower than those of the tagged silver carp ($n = 99$; $W = 2158.5$, $p = 0.026$). Twenty-nine of the tagged silver carp had eggs visible during tagging and nine expressed milt. Mean female silver carp total length was not different from male mean total length ($t = -1.45$, $p = 0.167$), although females were significantly heavier than males ($t = -2.53$, $p = 0.022$). The smallest mature female silver carp (i.e., a fish with oocytes present) was 50.7 cm total length and the smallest male expressing milt was 58.3 cm. Length-weight regressions for silver carp from fall 2010 ($n = 38$; $R^2 = 0.749$, $p < 0.001$) and the tagged fish ($n = 99$; $R^2 = 0.835$, $p < 0.001$) were both significant (Figure 3). Regressions of male ($n = 9$; $R^2 = 0.934$, $p < 0.001$) and female ($n = 29$; $R^2 = 0.886$, $p < 0.001$) silver carp lengths and weights were also significant (Figure 4).

Table 1. Mean (± 1SE) total lengths and weights for Asian carp tagged at each location on the Wabash River.

		River Mile			
Species	Sample size/Measure	310	324	351	373
Silver Carp	n	24	20	52	3
H. molitrix	Mean Total Length (cm)	67.58±0.87	72.19±1.04	70.74±1.43	68.03±0.88
	Mean Weight (kg)	3.83±0.17	4.53±0.23	4.24±0.27	3.46±0.16
Bighead Carp	n	1	N/A	N/A	N/A
H. nobilis	Total Length (cm)	76.00			
	Weight (kg)	5.40			

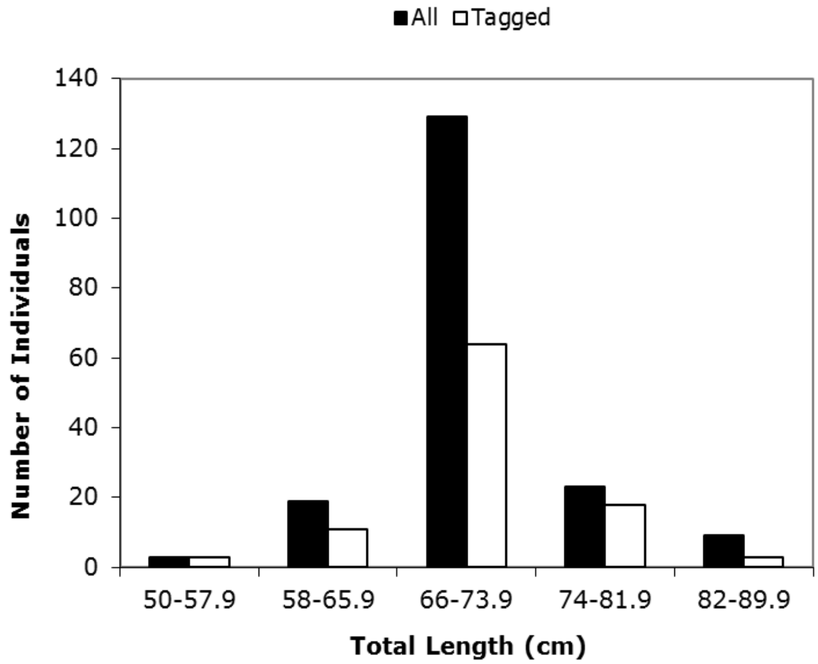

Figure 2. Silver carp length-frequency distribution of all fish observed to date (i.e., tagged and from fall 2010 (n = 182) and the tagged carp (n = 99). These distributions were not statistically different (X^2 = 2.867, p = 0.580).

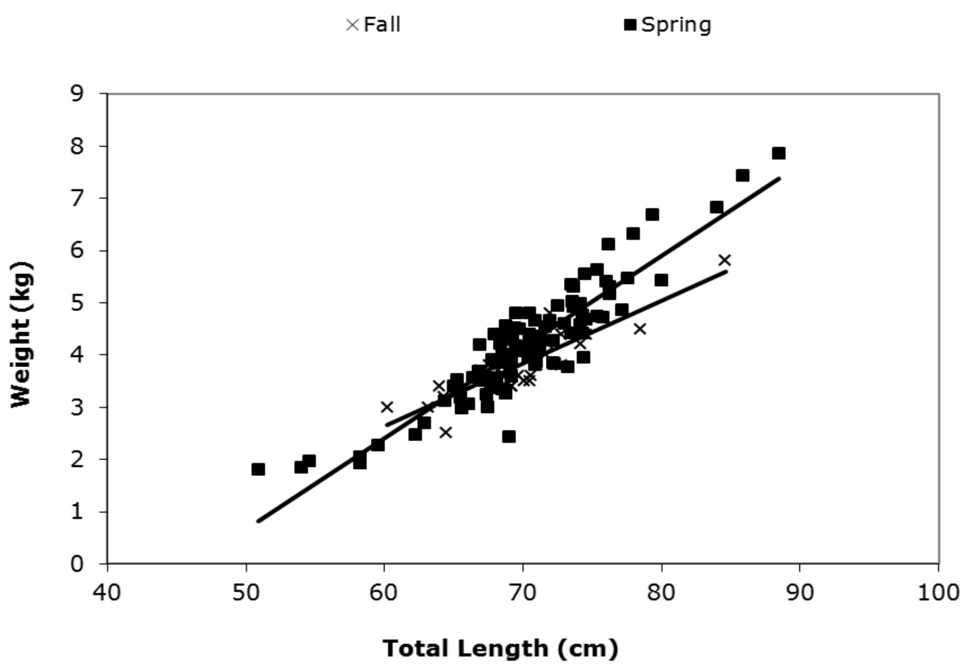

Figure 3. Length-weight relationships of the silver carp from fall 2010 (n = 138, R^2 = 0.749, p < 0.001) and those tagged with Vemco V16-4L transmitters (n = 99, R^2 = 0.835, p < 0.001).

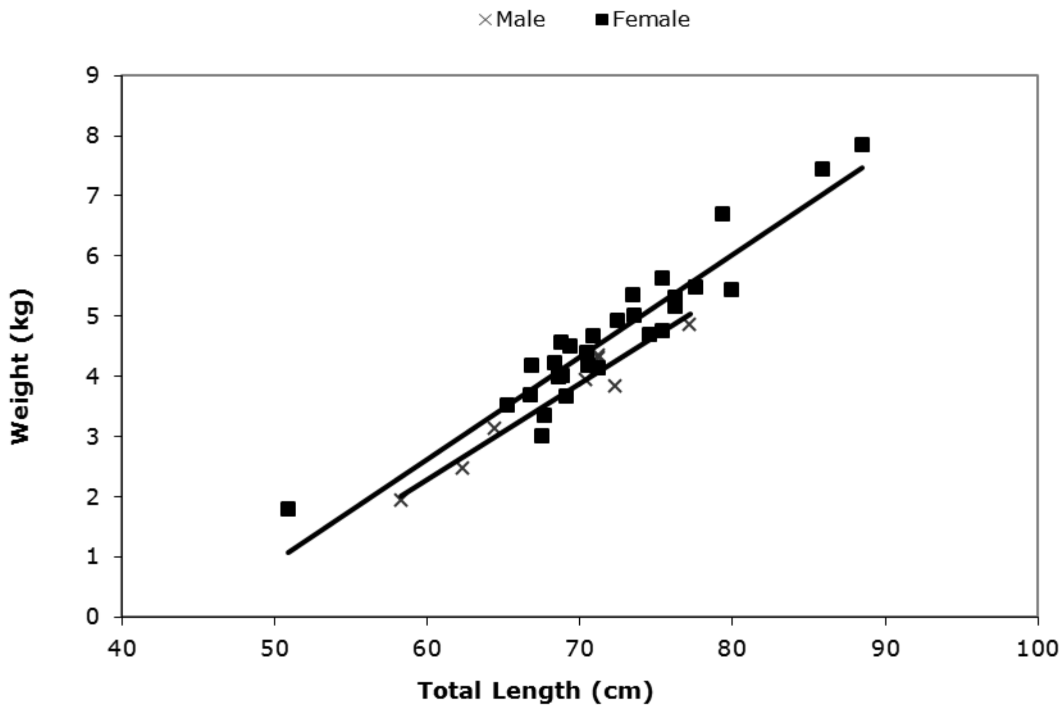

Figure 4. Length-weight relationships of male (n = 9, R^2 = 0.934, $p < 0.001$) and female (n = 29, R^2 = 0.886, $p < 0.001$) silver carp tagged in this study.

<u>Movements</u>

Of the 100 tagged fish, 69 were detected at least once post-surgery. This included the one tagged bighead carp which was detected approximately one month post-surgery in the large borrow pit at River Mile 310. Of all 69 fish detected, 24 were only detected once during this first phase of the project, including the single tagged bighead carp (Figure 5). The majority of fish with ≥25 detections were fish that remained near stationary receivers, especially the VR2W deployed at River Mile 351. There were 299 total active tracking detections and 62,849 passive detections.

Average depth was 2.74 m for all manual tracking detections which were spread between River Mile 351 and 371 (Figure 6). Greater numbers of upstream detections occurred earlier in the season and this shifted to greater numbers of downstream detections later in the season (Figure 7). The most upstream detection of a tagged fish (fish 31692) occurred on the Vemco VR2W passive receiver at River Mile 406, just downstream of the convergence of the Little and Wabash Rivers. The most downstream detection of a fish (fish 31649) was through manual tracking between River Miles 288 and 371. No study fish were detected on the most downstream (River Mile 165) or most upstream (Little River Mile 2) VR2Ws, although the number of detections at other receivers varied greatly (Table 2).

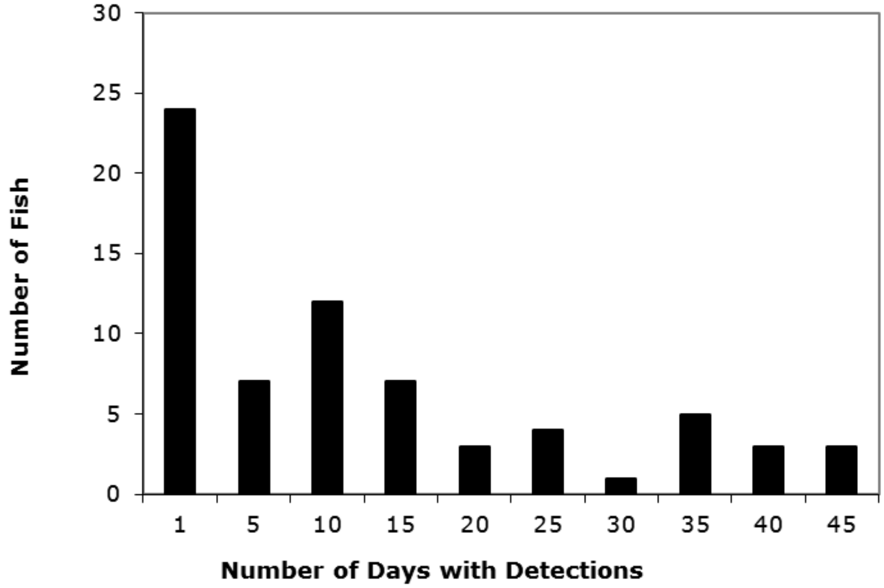

Figure 5. Frequency distribution of the number of days with detection information for each of the 69 fish detected.

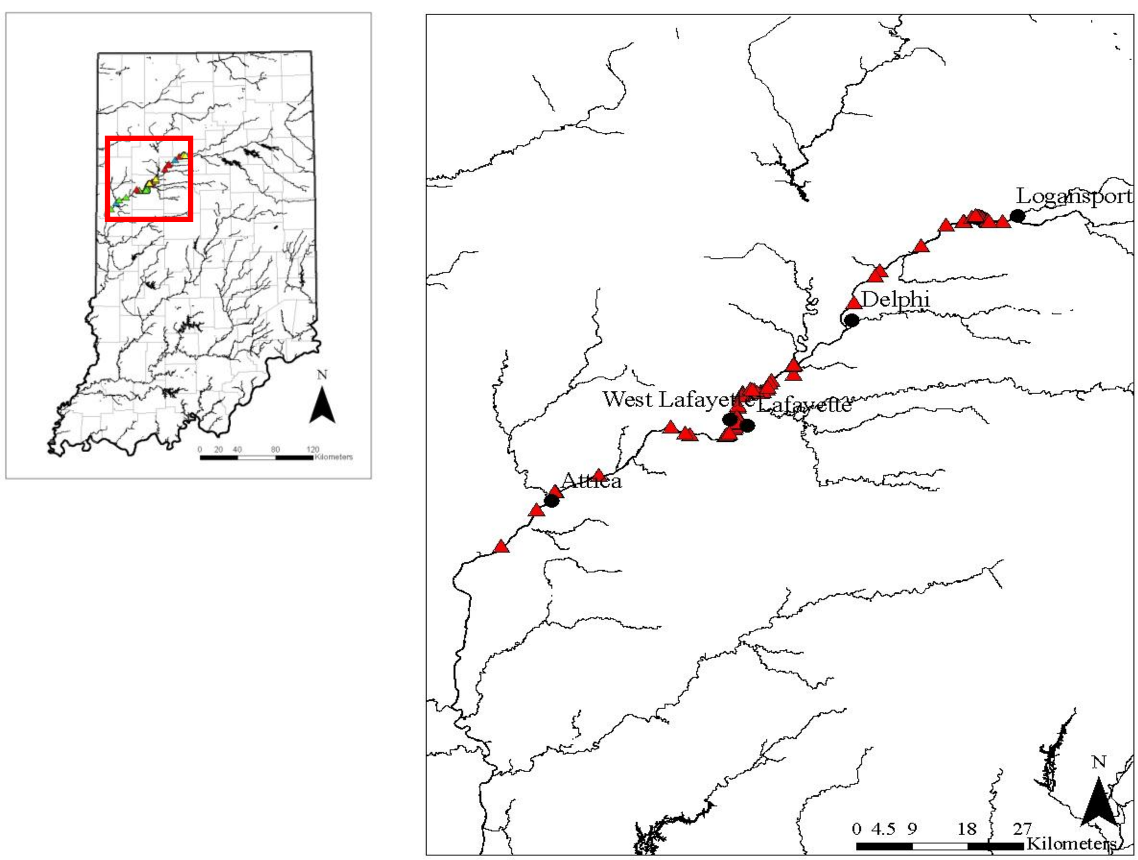

Figure 6. Summary of manual tracking detections for summer 2011 (n = 299).

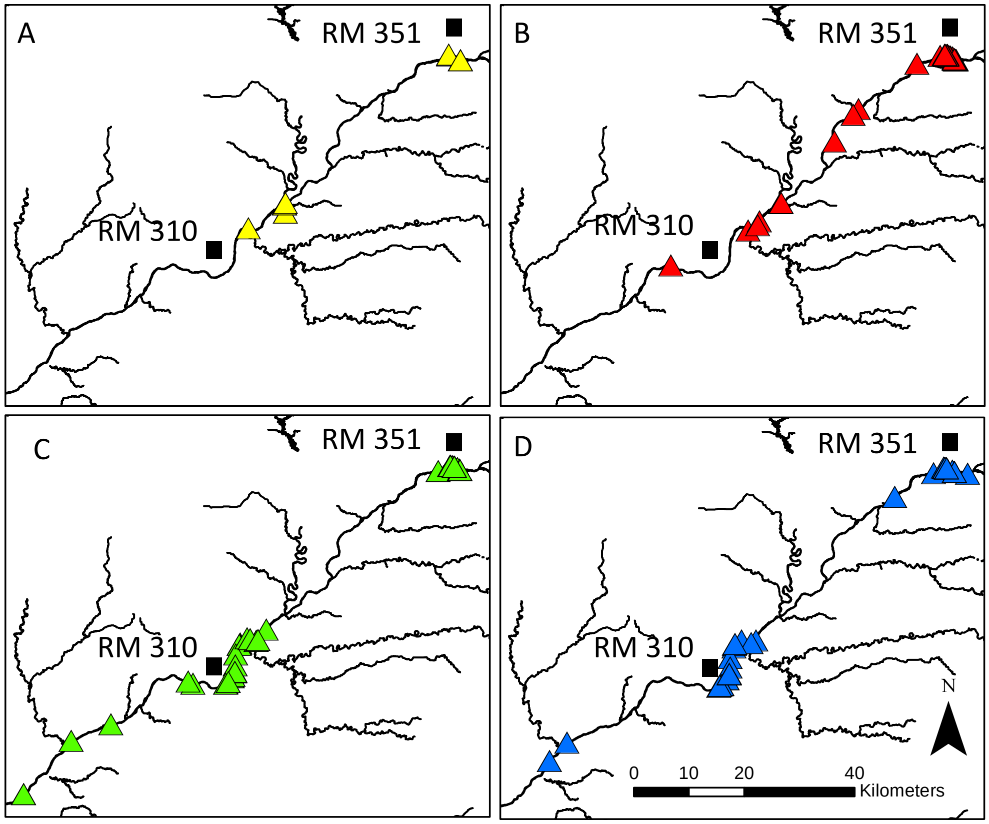

Figure 7. Manual tracking detections by month for A) May (n = 10), B) June (n = 64), C) July (n = 49) and D) August (n = 63). Approximate River Miles are listed next to the squares and each triangle represents a detection.

Table 2. Detection summaries for Vemco VR2W passive receivers. River Miles are for the Wabash River unless stated otherwise. River Miles were approximated based on Hoggatt 1975.

Receiver	River Mile	# Detections	# Fish	Last Download
Little River	2	0	0	9/30/2011
Huntington	406	1	1	9/30/2011
Wabash	390	5	2	8/8/2011
Peru	373	136	5	9/30/2011
Logansport	351	59554	25	8/4/2011
French Post Park	340	65	13	9/28/2011
Americus	324	307	13	9/28/2011
Tippecanoe River	2	513	7	9/28/2011
I65 Bridge	317	889	13	7/19/2011
26 Bridge	309	1375	13	7/30/2011
Merom	165	0	0	7/28/2011

Emergent patterns

Fish movements were often associated with rises in hydrographs in June and early July. As water levels dropped in July, tagged fish were observed to either concentrate in deeper pools in upstream areas or to move downstream (Figure 7). No tagged fish were detected downstream of the Lafayette/West Lafayette area until July 2011. The borrow pits near West Lafayette were likely staging areas for movements with fish exiting with rising hydrographs and then returning with falling hydrographs (Figure 8).

In general, several movement trends were apparent in the data. Nine individuals from borrow pits near West Lafayette moved upstream with rising hydrographs and then moved back downstream as the hydrograph fell (Figure 9). Four other individuals were also seen moving upstream with increasing hydrograph but were from other areas (Figure 10). Three individuals spent the entire summer in the borrow pits near West Lafayette. Five individuals stayed near West Lafayette, moving in and out of the borrow pits through the summer. Seventeen individuals spent June, July and August in two pools near Logansport and eight of these moved downstream with a rising hydrograph at the end of September (Figure 11).

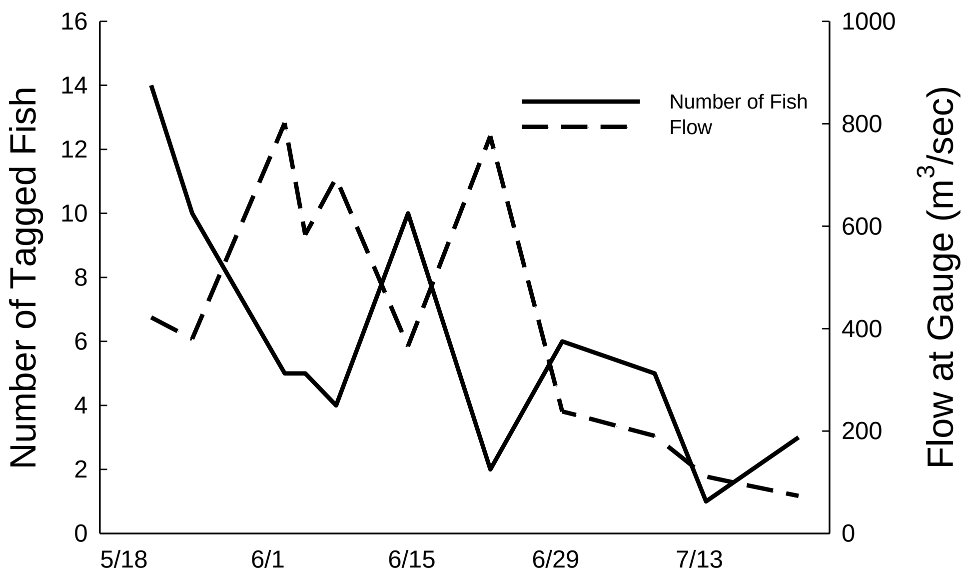

Figure 8. Numbers of tagged Asian carp in the larger borrow pit near River Mile 310 relative to the Wabash River hydrograph from Lafayette, IN, USGS gage. The solid line refers to the number of fish and the dashed line represents flow.

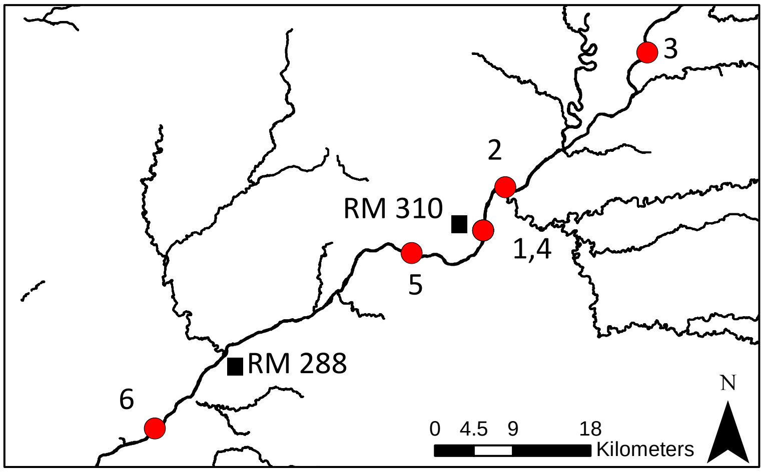

Figure 9. Telemetry data for silver carp 31623 tagged at River Mile 310, which exhibited movements similar to those of 8 other tagged fish. The fish used the borrow pit as a staging area and made upstream runs associated with rising hydrographs. Approximate River Miles are listed next to the squares while each circle represents a detection. Detection dates for sites were: 1) 5-May, 1-Jun, 3-Jun, 13-Jun, and 28-Jun; 2) 23-May; 3) 23-Jun; 4) 30-Jun and 5-Jul; 5) 7-Jul and 6) 29-Jul. Point 4 is obscured by point 1. Point 1 is the larger borrow pit.

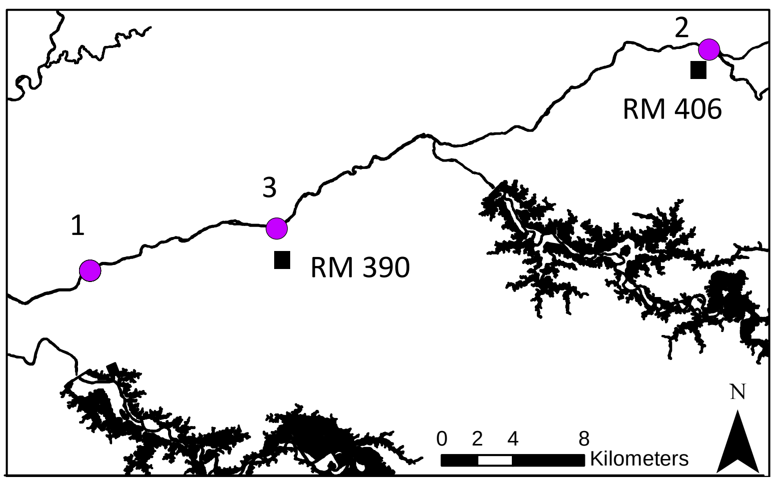

Figure 10. Telemetry data for silver carp 31692, tagged at River Mile 351, which demonstrated upstream movements associated with hydrograph changes. This fish was only detected on stationary receivers and ventured the farthest upstream. Approximate River Miles are listed next to the squares while each circle represents a detection. The dates of the detections at each site are: 1) 9-Jun through 13-Jun and 6-Jul, 2) 16-Jun, and 3) 5-Jul.

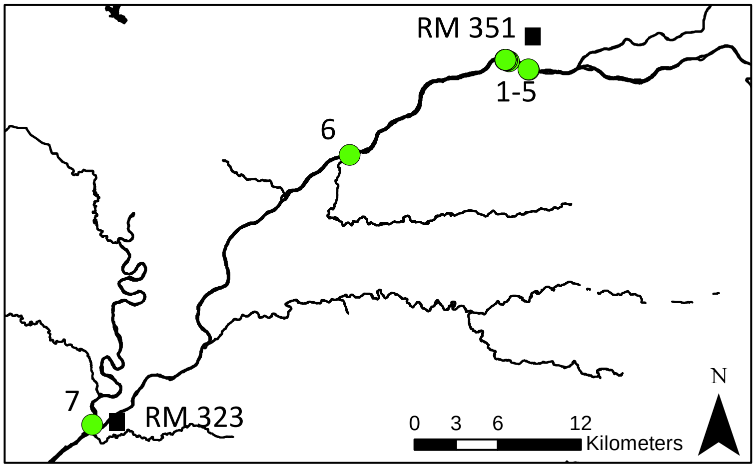

Figure 11. Telemetry data for silver carp 31636, tagged at River Mile 324. This fish moved upstream post-tagging to River Mile 351 where it remained until a rise in hydrograph in September when it moved downstream. Approximate River Miles are listed next to the squares while each circle represents a detection. The detection dates at each site were: 1) 8-Jun to 13-Jun, 16-Jun to 21-Jun, 26-Jun to 7-Jul, 10-Jul, 15-Jul, 20-Jul, and 27-Jul to 30-Jul; 2) 14-Jun; 3) 24-Jun; 4) 19-Jul; 5) 15-Aug; 6) 26-Sep; and 7) 27-Sep. Point 1 is the VR2W stationary receiver at River Mile 351.

Areas of Concentration

There were several areas where the tagged fish became concentrated during the study. The largest area of concentration occurred in July and August with declining hydrograph at River Mile 351 (Figure 12). There were two pools with 2-3 m water depths in close proximity at this location. It is likely that the fish were trapped in these pools as a result of extremely shallow water depths both up- and down-stream of River Mile 351. This assertion is further supported by the immediate downstream movement of nine tagged fish in September when the water levels rose substantially.

Two manmade borrow pits near River Mile 310 also served as areas of concentration during the study. Each of the two borrow pits contained at least one tagged fish throughout the summer, and there was generally >1 tagged fish in the larger borrow pit throughout the study period. This use of the borrow pit habitats was likely because of the lower flow and deeper water habitats characteristic of both. Two fish (68.9 cm TL, 4.005 kg, female; 71.0 cm TL, 4.345 kg, unknown sex) remained in the larger borrow pit throughout the summer. One fish (67.4 cm TL, 3.245 kg, unknown sex) that was detected in the smaller borrow pit throughout the summer was trapped due to loss of connectivity of that borrow pit with the mainstem of the river under low flow conditions. The borrow pits were usually higher in temperature than the main channel of the river which may have increased food availability. The area immediately outside of the borrow pits also had higher numbers of tagged fish detected, and these fish appeared to regularly move in and out of the borrow pits.

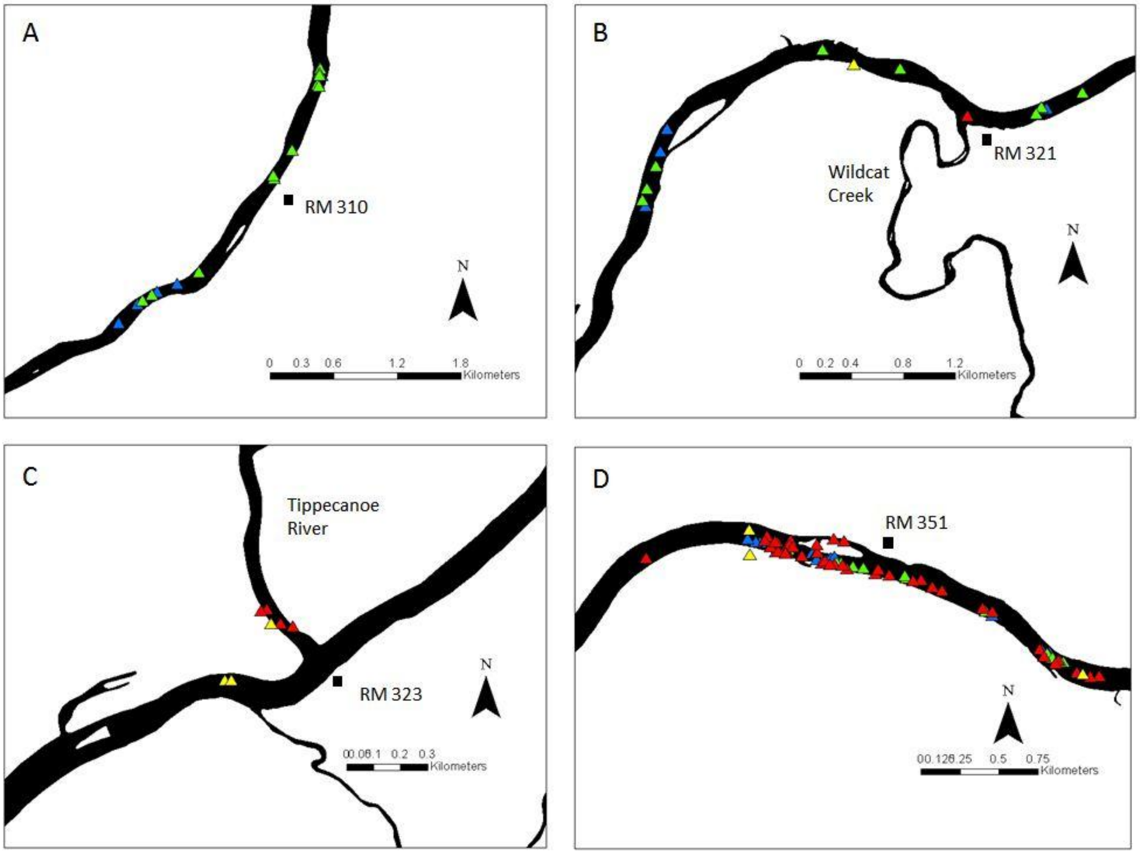

Figure 12. Areas of concentration for Asian carp in the middle Wabash River including A) West Lafayette/Lafayette, B) near the 65 overpass between West Lafayette and Americus, C) confluence of the Tippecanoe and Wabash Rivers and D) near Logansport. Gray lines are state and interstate highways. Each triangle represents a detection. Squares represent approximated River Miles. Colors correspond to month as in Figure 7.

<u>Spawning</u>

Paired bongo nets were deployed in the Wabash River from Wabash, IN, to West Lafayette, IN, and in the East Fork of the White River once water temperatures rose above 24°C. One location in the East Fork of the White River near Medora, IN, had one suspected Asian carp egg that was not found when the sample was processed and so could not be verified (Figure 13). One tow in Wabash, IN, yielded two Asian carp eggs that were at the gastrula stage. No eggs were found at Peru, although numerous eggs were recovered from bongo net pulls at Logansport, French Post Park, Americus and West Lafayette/Lafayette. Weekly tows near River Mile 310 continued to yield Asian carp eggs well into July despite the lack of substantial hydrograph changes. The latest date with molecularly confirmed Asian carp eggs was 21-Jul, although there was no substantial hydrograph change associated with this date.

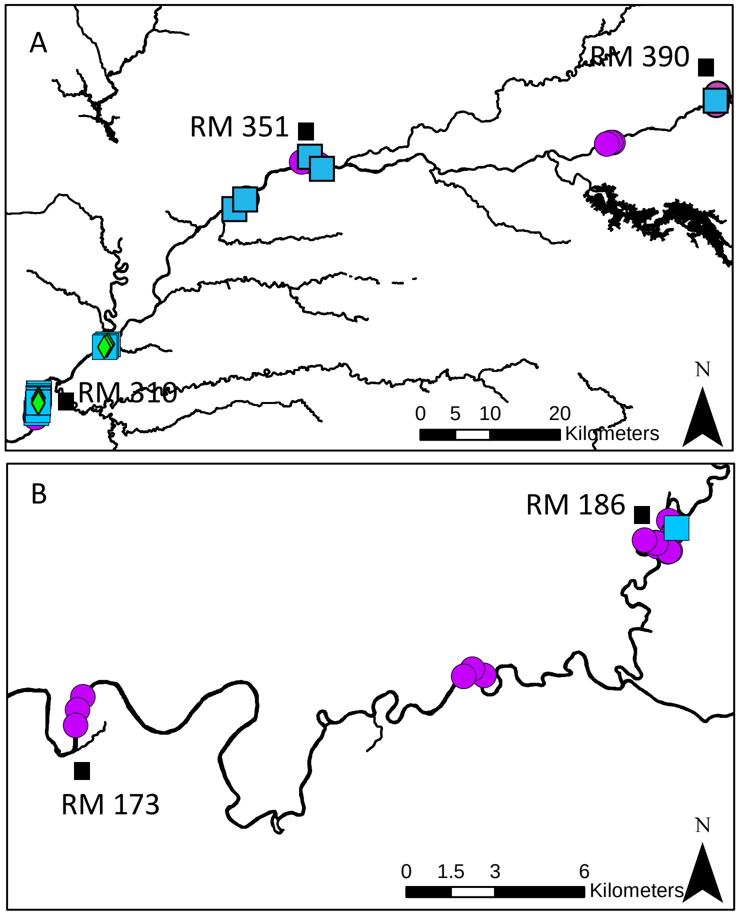

Figure 13. Summary of bongo net tows performed in summer 2011. Green diamonds indicate sample sites that yielded eggs confirmed as Asian carp through molecular analysis. Blue squares represent sites with Asian carp eggs confirmed under magnification, and purple dots represent sites where no eggs were found. Squares indicate approximated River Miles. A) Wabash River and B) East Fork of the White River.

Discussion

This first phase of our multi-year study of Asian carp movements in the Wabash River yielded multiple noteworthy results from which we can build our continued research. We now have some basic demographic information on silver carp in the middle and upper Wabash River that was heretofore unknown. Not surprisingly, mean total lengths were consistent across sex and time, although fish weights were not. As would be expected, mean weights of female silver carp were greater than males due to the gravidity of female fish at the time of tagging (i.e., during late spring). Female fish tagged into late May and early June consistently had large numbers of mature ova present, and although males expressing milt were also collected, gravid females were heavier than sexually mature males. Spawning condition likely also contributed to the difference in mean weights of fish from fall 2010 to spring 2011.

Fish tagged at the three tagging sites that could be statistically compared showed a difference in mean total length with fish captured near River Mile 310 smaller than those at the two upstream locations. Unlike the other tagging sites where fish were captured from the river mainstem, fish at River Mile 310 were largely collected from within manmade borrow pits where they occurred in high densities. It is unclear as to why there would be a difference in the size of fish among the tagging locations, although there may be some level of stunting that is occurring for fish that primarily reside in backwaters, such as the borrow pits vs. the mainstem of the river. It is also possible that differences in total length among the tagging locations were temporally driven given that that fish at River Mile 310 were tagged at least 2 weeks prior to fish collected at the upstream tagging locations. Our results to date suggest that silver carp retreat to lower areas of the river as river water levels fall, and these areas may be used for overwintering as well. Differential movement rates of smaller vs. larger individuals upstream for spawning could explain the prevalence of smaller individuals earlier in the season at River Mile 310. Regardless, we expect to more explicitly address this during Phase II of the project.

Movements and behaviors exhibited by tagged individuals indicated several patterns that may have management implications, especially because they have not been previously reported. First of all, no tagged fish were detected in the Little River during this first phase of the study, and although we had planned to deploy VR2Ws in other smaller tributaries this summer, malfunctions and losses of several of our existing units made this difficult (although this will be rectified with the purchase of additional VR2Ws in Phase II). Although it is a considerably larger stream compared to the Little River, we did have 513 detections of seven tagged silver carp in the Tippecanoe River. While this does not necessarily suggest that Asian carp will use the Little River, these observations are nonetheless significant because although bighead carp have previously been reported to use river confluences, similar associations have not been reported for silver carp (Kolar et al. 2007). In the future, additional Vemco VR2W stationary receivers will be deployed just upstream in the Mississinewa, Salamonie and Eel Rivers. These rivers and streams encompass a wide range of tributary sizes and should provide us with the opportunity to further explore the use of tributaries as habitat by Asian carp.

Silver carp in the Wabash River exhibited upstream movements associated with rising spring and summer hydrographs consistent with those reported in past studies (e.g., Abdusamadov 1987; Peters et al. 2006; Degrandchamp et al. 2008). These movements were associated with spawning activities, and tagged fish generally remained in upstream areas in May and June and then moved downstream in later months as water levels dropped. However, some individuals remained upstream throughout the summer in two deeper pools surrounded by extremely shallow water near River Mile 351. These pools likely served as refugia for the silver carp during low water periods, and many of these individuals rapidly moved downstream once the water level rose in the late September. The confluence of the Tippecanoe and Wabash Rivers was another point at which silver carp tended to concentrate, among others, and all of these areas, especially the pools near River Mile 351, may be good candidates for control measures via targeted harvest during periods of low water. This may however present logistical issues as low water levels make work on this portion of the Wabash River difficult.

The manmade borrow pits near River Mile 310 also supported some of the highest concentrations of Asian carp in the study area. These borrow pits had slightly higher temperatures and considerably lower current velocities compared to the main channel, thus providing habitat that required the fish to expend less energy. A small, ephemeral natural backwater (River Mile 314) upstream from both of the borrow pits also had high numbers of silver carp present and low water current velocity, although no tagged fish were observed in that backwater during this phase of the study. Tagged fish were detected in a sheltered backwater behind an island near River Mile 351 until low water levels made it uninhabitable. In the spring and early summer, fish abandoned the borrow pits with rising hydrographs and appeared to use these artificial backwaters as staging areas for further upstream movements when spawning conditions were optimal. Once the hydrograph began receding, the fish tended to move back to these artificial backwaters. Even though these borrow pits were partially or fully isolated from the river depending on flow, some tagged individuals remained in them for the entire summer regardless of connectivity, indicating that they are a potentially suitable habitat. These fish included one known female (eggs visible during tagging) that was not detected outside of the borrow pit for spawning. This extensive use of backwater-like habitat is not surprising since the nearly still waters of the borrow pits and natural backwater were similar to the lentic habitats these species inhabit in their native distribution (D. Chapman, USGS, pers. comm.). Both natural and manmade backwaters may therefore be good targets for control measures via harvest, especially during low summer flows or receding hydrographs during the spawning season.

The occurrences of Asian carp eggs near River Mile 390, and suspected presence of eggs in the East Fork of the White River near Medora, IN, are especially notable results from Phase I. The watershed areas of both sites are roughly equal, and the occurrence of Asian carp eggs in rivers this small constitutes reproduction in the smallest waters known to date (D. Chapman, pers. comm.) although recruitment has not been documented. It is also very notable that we confirmed reproduction of silver carp in the Wabash River at Lafayette as late as 21-Jul, and, more importantly, this spawning event occurred without an accompanying rise in the hydrograph. While silver carp are known to spawn beyond the late spring, they have always been reported as spawning only in association with an increase in the hydrograph. The evidence of successful reproduction so far upriver and atypical spawning events will likely have substantial consequences for modeling efforts seeking to predict invasion trajectories of Asian carp in the Great Lakes since both factors may substantially affect invasion success of these species (D. Chapman, USGS, pers. comm.).

Acknowledgements

This project would not have been possible without the extensive input and help of our State of Indiana collaborators, Goforth Lab graduate students, personnel, and undergraduate technicians. Doug Keller with the Indiana Department of Natural Resources (INDNR) was instrumental in getting this project established and planned. Tom Stefanavage, Nate Nye, and Dan Wessel, also with the INDNR, helped to collect fish for tagging and also assisted with tracking data collection. Goforth Lab graduate students Jay Beugly, Conor Keitzer, Sam Nutile, and Caleb Rennaker helped to capture fish for tagging and also provided active and logistical support in tracking fish. Goforth Lab Coordinator Beth Bailey provided extensive logistical support and field data collection for this project. Undergraduate students Megan Gunn, Kristen Ruhl, Colleen Taylor, Tess Thoren, and Preston Sipe worked countless hours to help collect fish for tagging as well as collecting tracking data. Finally, this project would not have been possible without the generous financial support provided by the Indiana Department of Natural Resources, the US Fish and Wildlife Service, and the USEPA's Great Lakes Restoration Initiative.

Literature Cited

Abdusamadov, A. S. 1987. Biology of white amur, C*tenopharyngodon idella*, silver carp, *Hypophthalmichthys molitrix*, and bighead, *Aristichthys nobilis*, acclimatized in the Terek region of the Caspian Basin. Journal of Ichthyology 26: 41-49.

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

Hoggatt, R. E. 1975. *Drainage Areas of Indiana Streams*. U.S. Department of the Interior Geological Survey: Water Resources Division.

Jerde, C. L., A.R. Mahon, W.L. Chadderton W. L. and D.M. Lodge. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conservation Letters 4: 150–157.

Kolar, C. S., D. C. Chapman, W. R. Courtenay, Jr., C. M. Housel, J. D. Williams and D. P. Jennings. 2007. Bigheaded carps: A biological synopsis and environmental risk assessment. American Fisheries Society Special Publication 33, Bethesda, MD.

Mueller, R. Jr. and M. Pyron. 2010. Fish assemblages and substrates in the middle Wabash River, USA. Copeia 2010(1): 47-53.

Peters, L. M., M. A. Pegg and U. G. Reinhardt. 2006. Movements of the adult radio-tagged bighead carp in the Illinois River. Transactions of the American Fisheries Society 135: 1205-1212.

Wentworth, C.K. 1922. A scale of grade and class terms for clastic sediments. Journal of Geology 30: 377-392.

Williamson, C. J. and J. E Garvey. 2005. Growth, fecundity, and diets of newly established silver carp in the middle Mississippi River. Transactions of the American Fisheries Society 134: 1423-1430.