Movements and Spawning of Bigheaded Carps in the Upper Wabash River, Indiana, USA: 2012 Update

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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 considerable 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 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 has been taken to prevent such an introduction through the installation of a physical barrier across Eagle Marsh. 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, are not fully understood.

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 both main channel and backwater habitats in the Illinois River where they preferred to remain near the river banks (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 novel 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 and tracked 163 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.

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}.

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.

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 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.

<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 RM406-165 (Figure 1). The VR2Ws were attached to custom platforms and anchors (Plates 1-2). 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.

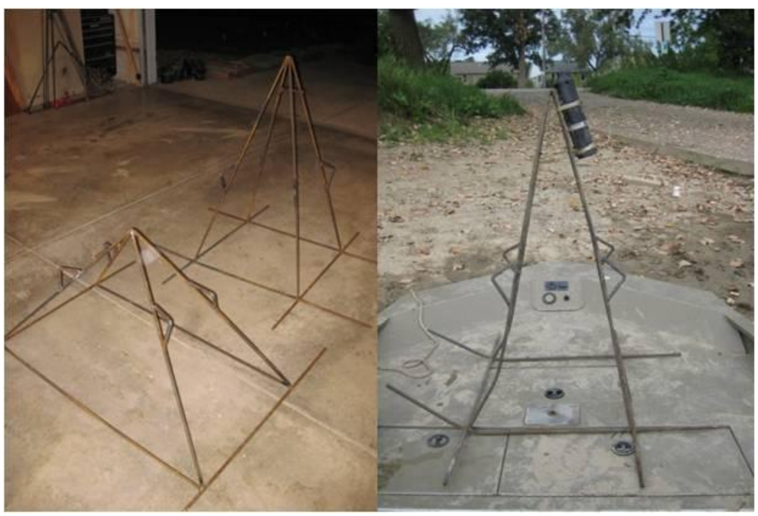

Plate 1. Larger deployment platforms for Vemco VR2W passive receivers (attached to the top of the stand in the picture to the right) deployed in deeper reaches of the Wabash River.

Plate 2. Smaller deployment system used for Vemco VR2W passive receivers in shallow reaches of the Wabash and Little Rivers.

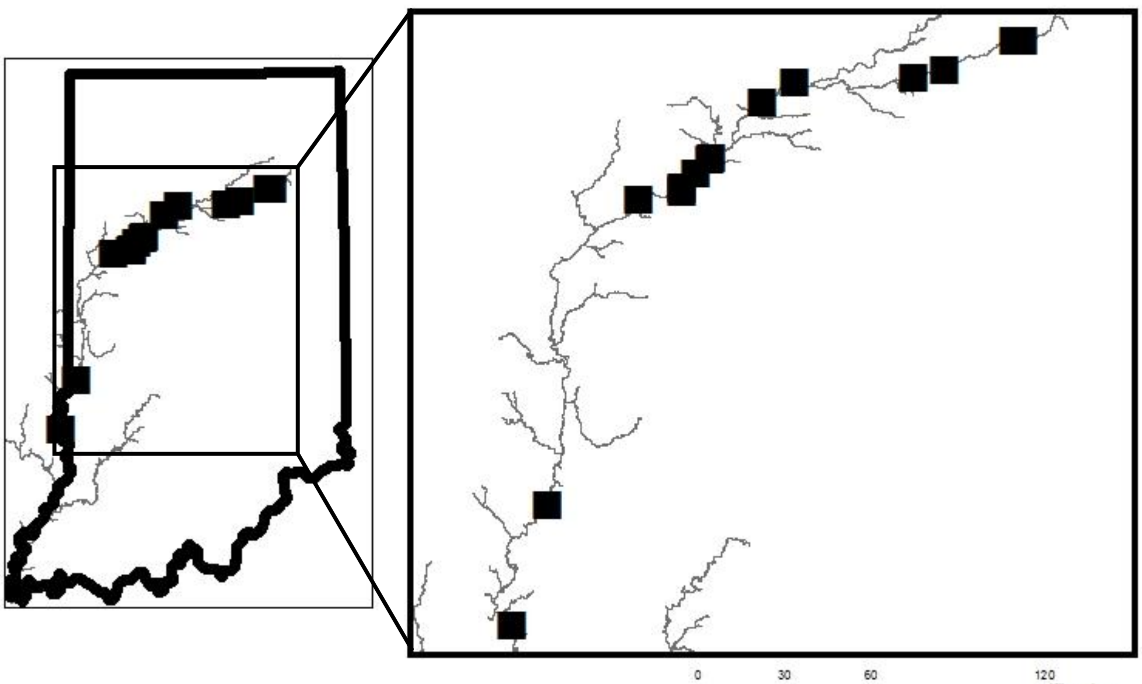

Figure 1. Placement of Vemco VR2W stationary receivers in the Wabash, Little and Tippecanoe Rivers. Each black square indicates the location of a VR2W.

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 will be deploying additional Vemco VR2Ws downstream of Terre Haute and they are willing to share information. As of 10/1/12 these had not yet been deployed (Sarah Huck, EIU, pers. comm.).

Active – Active tracking was accomplished by deploying hydrophones from boat or canoe depending on river conditions. Active tracking was primarily done between RM354 and RM271 (Covington, IN), and it was also conducted on one occasion from RM271 to RM239 (Montezuma, IN). Sections of river were tracked at least once every two weeks. Lengths of river sections tracked varied depending on method of travel. 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, 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).

Spawning Evaluations

We conducted spawning evaluations in both the upper Wabash River (RM 310-373) and the East Fork of the White River during summer 2012. Field crews conducted egg and larval sampling at selected sites on the rivers using paired bongo nets (500μm bucket mesh size) pulled in replicates of three. Bongo nets were towed from the bow of a 5 m John boat in a downstream direction for five minutes while the boat traveled in reverse. The volume of water sampled was quantified using a flowmeter (G. O. Environmental) attached inside the mouth of the bongo net. For tows done between 7/2 and 7/31, volume of water was based on an average volume of all tows done from 8/6 to 9/12 due to a mechanical malfunction in the flowmeter on those dates. At several sites, a stratified sampler (500μm mesh size; Plate 3) was used when the boat could not be launched to pull bongo nets. The stratified sampler was held vertically in the water for five minutes. This device was tested at RM 310 when eggs were known to be present and was found to capture eggs. The stratified sampler was used at RM 354 and 310. Two sampling events (May 2nd and May 17th) were conducted in the upper Wabash at RM373, 354, 340 and 320. Three sampling events (May 14th, June 5th and July 10th) were conducted on the East Fork of the White River with bongo pulls as described above at two locations.

Plate 3. Stationary stratified sampler used to assess spawning at RM354 and 310.

Ten eggs from each bongo net pull were identified under magnification using a Nikon SMZ1500 microscope (Nikon Instruments, Inc.), frozen, and preserved at -80ºC for later deoxyribonucleic acid (DNA) analysis (see below). Larvae were also preserved at -80ºC for later DNA verification. Bongo net sampling was conducted once water temperatures reached ≥15.6º C and were intensified with rising hydrographs, which have been found to be a trigger for spawning in bigheaded carp (Abdusamadov 1987; Peters et al. 2006; DeGrandchamp et al. 2008). Once eggs were detected, sampling continued on a weekly basis near RM 310 until eggs were not collected for three weeks. Depths (Model SM-5, Speedtech Instruments, Great Falls, Virginia) were taken at the start of each pull and water velocity (Marsh-McBirney Flo-mate Model 2000, Hach Company, Loveland, Colorado) 30 cm below the water surface was also taken prior to bongo pulls. Surface water temperature was also recorded just prior to sampling (HQ10 dissolved oxygen meter, Hach Company, Loveland, Colorado or YSI30 conductivity meter, YSI Inc., Yellowsprings, Ohio).

Eggs submitted for molecular verification were preserved at -80 °C and genomic DNA was later extracted from eggs using QIAamp DNA Mini Kits (Qiagen, Inc, Valencia, California, USA) according to the manufacturer's instructions. Species identifications for May 8th, 11th and 16th 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). Primers and probes were designed in the D-loop region of the mitochondrial DNA of each species. All PCR reactions (25 μL) were comprised of: 10 μL TaqMan® Fast Universal Master Mix (Applied Biosystem, Foster City, California, USA), 0.3 μM of each primer and molecular grade water. PCR conditions were as follows: 94 °C for 10 s, 58 °C for 10 s, and 72 °C for 20 min for 40 amplification cycles and a final extension period of 10 minutes at 72°C on a Mastercycler® thermocycler (Eppendorf North America, Inc., Westbury, New York, USA). Amplicons were visualized on a 2% agarose gel stained with GelRedTM (Phentix 166 Research Products, Candler, North Carolina, USA). All PCR products were compared with products from control DNA extracted from fin-clips of silver and bighead carps. This analysis identifies the maternal species for each egg tested. Other eggs and larvae will be tested in a similar manner.

Statistics

Total lengths and weights of all fishes captured in 2010-2012 for tagging and all other reasons were regressed to generate length-weight relationships for both species.

Results

Silver carp captured throughout this study show a strong length weight relationship (R^2 = 0.8758; Figure 2). Relationship for bighead carp was not as strong but was admittedly based on a much smaller sample size (R^2 = 0.3822; Figure 3). New information on capturing bighead carp in the Wabash River should provide opportunities to capture more individuals of this specie and increase sample size for future analyses.

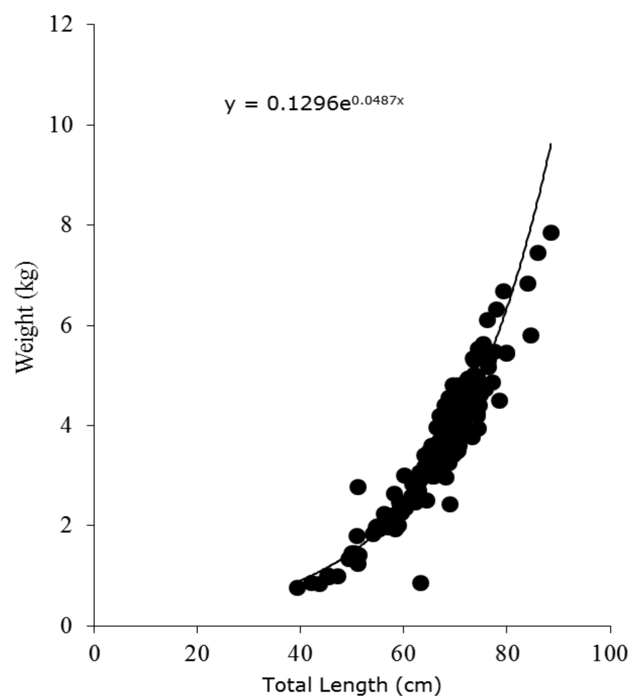

Figure 2. Length (cm) and weight (kg) relationship for silver carp (n = 214). Fish were surveyed over multiple seasons which could account for much of the variation seen around the regression.

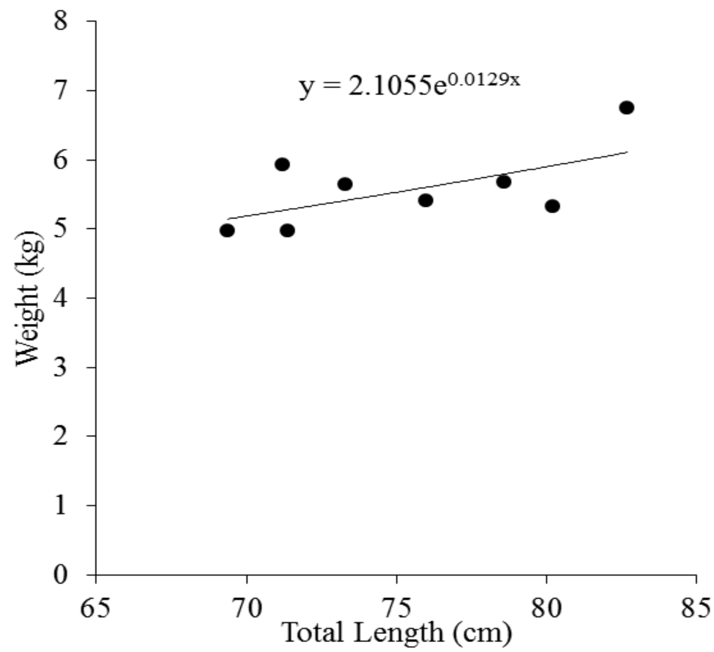

Figure 3. Length (cm) and weight (kg) relationship for bighead carp (n = 8). All measures are based on fall samples.

Initially, 200 tags were to be deployed by June 2012, but only 163 tagged bigheaded carps are currently at large in the Wabash River watershed. Unusually low water, levels and high water temperatures limited our ability to capture fish for tagging in 2012, thus limiting our ability to implant all of the 100 tags targeted for deployment in this second project year. The 37 tags not deployed in 2012 will be implanted in early 2013. Of these fish, one is a bighead carp and the rest are silver carp. We observed detections for 122 of the 163 at large fishes. There were 1,206,874 stationary receiver detections for 2011 and 2012. 2011 and 2012 were similar with RM351, RM309, and BP1 having the highest detection numbers (Table 1). As in 2011, we observed the use of a Wabash River tributary, the Tippecanoe River, by silver carp. The number of detections at RM309 was much higher in 2012 compared to 2011. Upstream detections and numbers of fish were lower for 2012 (RM390, 373 and 340.)

Table 1. Summary of the placement and detection history of the VR2W stationary receivers from 2011 and 2012. River miles were estimated from Hoggatt (1975). Goose Island VR2W was deployed in 8/2012 and has not been downloaded. Several VR2Ws have been lost in the Little River likely due to human tampering.

		2011		2012		
Location	River Mile	Detections	Individuals	Detections	Individuals	Last Download
Little River	2	0	0	-	-	9/30/2011
Huntington	406	5	2	3	1	8/16/2012
Wabash	390	8	4	3	1	10/23/2012
Peru	373	139	6	7	1	10/23/2012
Logansport	351	276607	32	243940	30	10/23/2012
French Post Park	340	268	18	77	18	10/23/2012
Americus	324	322	18	565	27	10/24/2012
Tippecanoe River	2	987	9	94	8	10/24/2012
I-65 Bridge	317	4095	29	36568	35	10/24/2012
Borrow 1	310	90855	24	283237	55	4/2/2012
Borrow 2	310	55765	11	58206	7	4/2/2012
26 Bridge	309	1375	15	145866	28	10/24/2012
Goose Island	298	-	-	-	-	-
Terre Haute	214	-	-	6	5	9/20/2012
Merom	165	42	2	37	7	9/20/2012

There were 347 active tracking detections in summer 2012 (Figure 4). Detections tended to be more clustered in the upper Wabash and more diffuse downstream. The pattern of detections through the summer was similar to 2011 for locations of clustering and pattern of movements. Timing of these movements did vary between years. When examined month by month, there are two areas where tagged carp could be found throughout the summer. These concentrations were located near RM 350 and RM 311. At both of these locations, mature males and females were present throughout the summer. Areas where fish concentrated were similar to 2011 with the exception of the Wabash-Tippecanoe confluence. In 2012, there were no bigheaded carp detected near the confluence after May. Many of the fish moved off downstream as the summer progressed while others stayed in deeper reaches of the river (Figure 5). The downstream movements of these fish tended to occur earlier in 2012 than 2011.

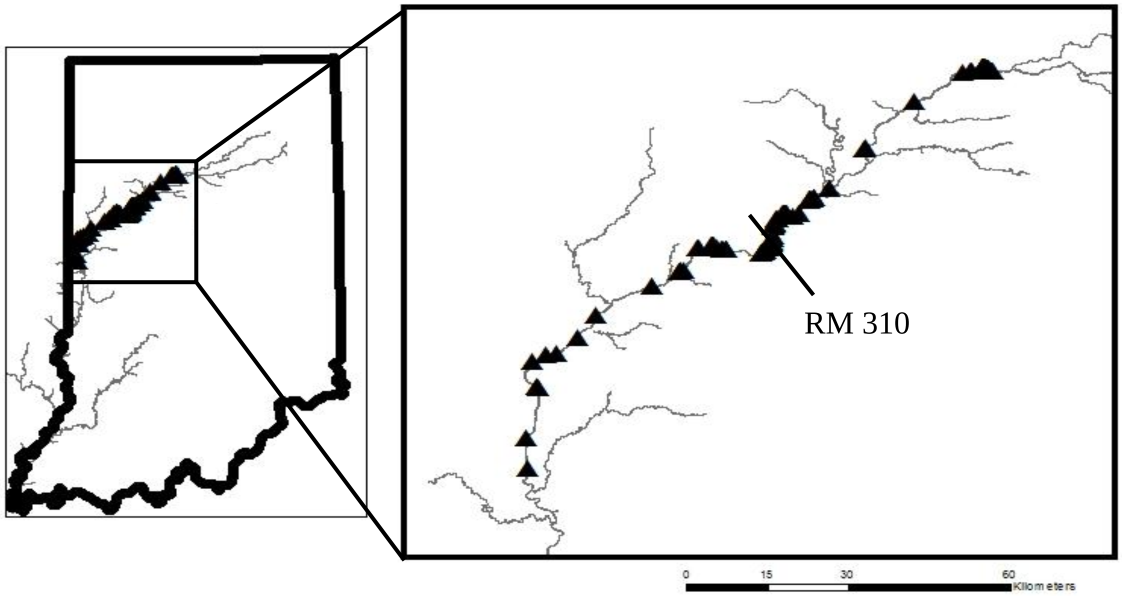

Figure 4. Active tracking detections for 2012 (n = 347). Each triangle represents a detection.

The largest upstream and downstream movements of fish occurred when river flows were higher. Many fish were stationary through summer 2011, were seen downstream during the winter and returned upstream in March-May. Of the movements quantified so far, the average movement rate was 0.5 km/day (n = 874) for 2011 and 2012. Movements quantified so far are all from summer and so average movement rate is likely to increase once spring and fall are calculated.

Tagged fish appeared to be extremely sedentary during winter, and tagged individuals began to enter and exit BP1 in spring 2012 (Figure 6). Combined with data from stationary receivers, mid to late March appeared to be a time fish moved upstream.

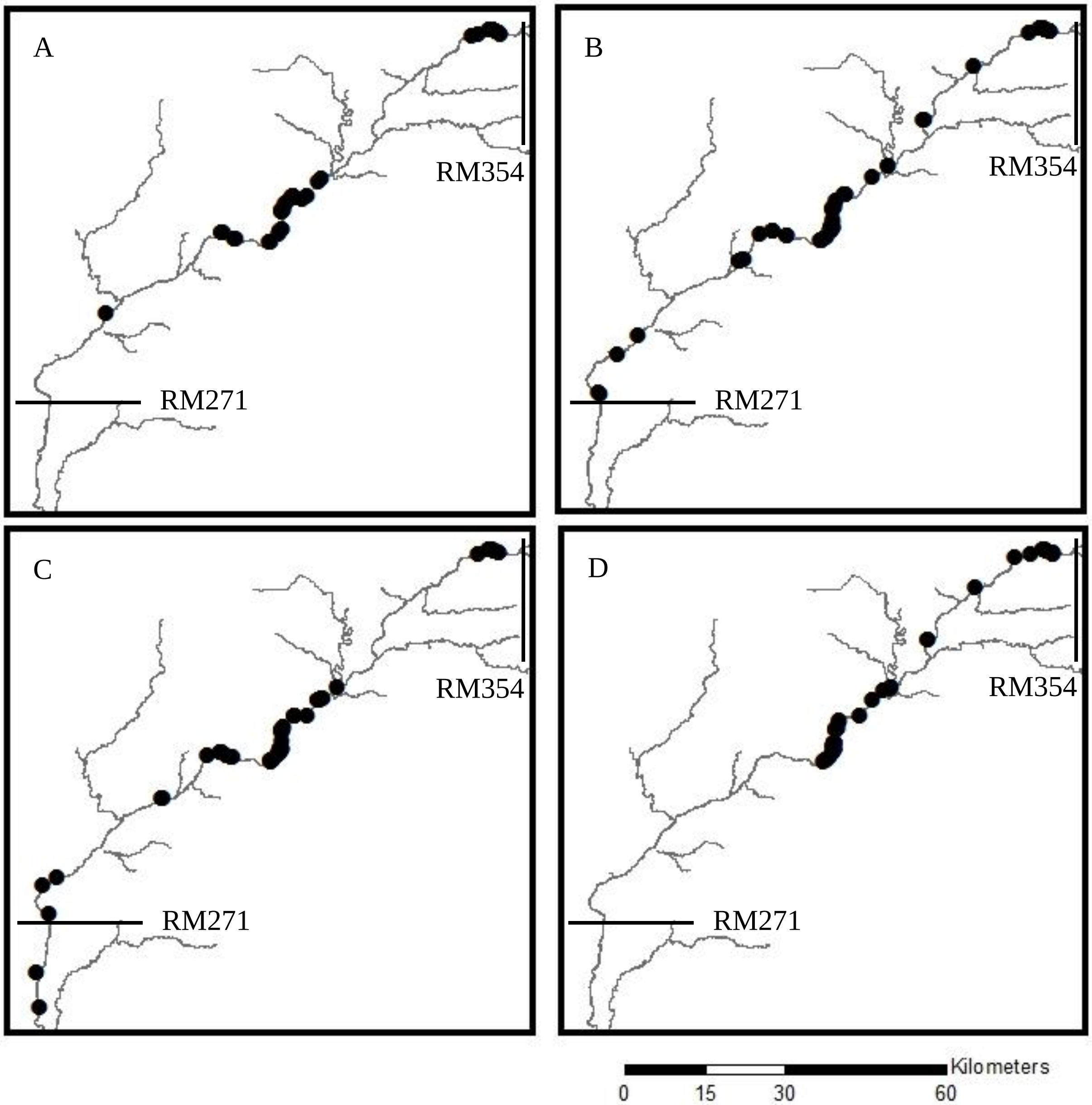

Figure 5. Active tracking detections over summer 2012. Detections broken down by month (A) May (n = 65), (B) June (n = 84), (C) July (n = 127), and (D) August (n = 71). As stated in methods, active tracking was done mostly between RM354 and 271.

Figure 6. Data from the VR2W stationary reciever located in BP1. Each row of dots represents data for one fish while each individual dot represents the detection of that individual on a certain date. A gap indicates that fishs' absence from BP1. 55 fish are represented in this figure.

Spawning of bigheaded carp for 2012 was first documented on 8-May-2012. This initial spawning even appeared to coincide with a small fall in hydrograph, but spawning activity did not appear to be otherwise linked with hydrograph. For the remainder of 2012, hydrograph was uncharacteristically steady or slightly declining. In 2011, egg density appeared linked with changes in temperature. For 2012, the highest temperatures caused reductions in spawning activity late in the season (Figure 7). Of eggs suspected to be bigheaded carp eggs, average diameter was 2834.586 μm (n = 354). The last eggs observed in bongo net tows were observed on 28-Aug-2012. Eggs from May 2012 samples submitted for DNA verification were verified as silver carp. Eggs collected in later samples will be tested in winter 2012-2013 as described above and densities represented in Figure 7 will be adjusted accordingly.

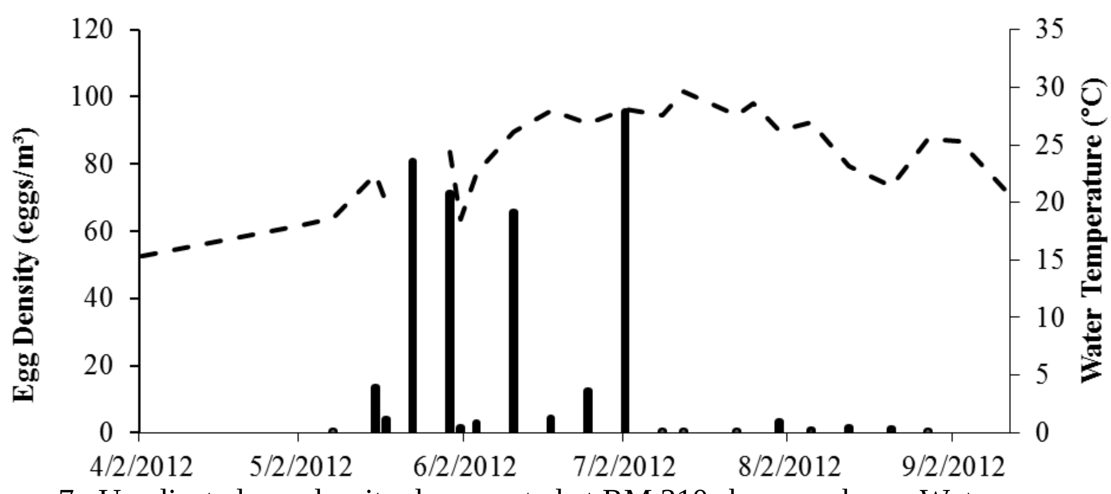

Figure 7. Unadjusted egg density documented at RM 310 shown as bars. Water temperature measured just prior to sampling activities is represented by the dashed line.

Bigheaded carp eggs were not found as far upstream in 2012 as in 2011. Eggs were only detected at RM 310 with the stratified sampler and bongo net, and at RM 322 by bongo net. No eggs were collected at RM373, 354 or 340. No eggs were obseved in samples collected from the East Fork of the White River. One larvae collected in the 10-Jul-2012 sample from the East Fork of the White River has yet to be tested for DNA confirmation (to be done in winter 2012-2013).

Discussion

We have many detections for multiple tagged fish to date, including fish tagged in 2011 that were not detected again after tagging in 2011 but were detected in 2012. This confirms that the number of tagged fish distributed in the Wabash River is high, and our methods for surgically implanting transmitters to achieve interannual tracking appears to be successful. We expect the number of fish detected from previous tagging events to continue to increase with each successive year. In general, tagged fish range over large areas of the Wabash River. Not surprisingly, silver carp moved downstream in the fall and tended to be stationary throughout the winter, presumably remaining in deeper, more thermally stable pools during that time. Overwintering occurred in BP1, a deep, low flow habitat that may provide an area of low energy use. Some tagged fish returned to similar locations both in summer 2011 and summer 2012 (e.g., RM351). Such fish remained largely immobile during both summers, presumably because they occupied the only deeper water refugia within the shallow upper Wabash. Tributary use declined in 2012. Silver carp, however, were still detected in the Tippecanoe River while tributary use had only previously been associated with bighead carp (Kolar et al. 2007). Once all the movements are quantified (i.e., based on cumulative data from 2011, 2012, and 2013), we expect to see statistically higher movement rates in the fall and spring compared to summer and winter. Such movements are generally consistent with those observed in both the native ranges of these fishes and in other novel ecosystems.

Some differences in timing of movements between 2011 and 2012 were likely due to differences in water temperature and flow regimes between the two years of study. Fish moved downstream in June and July 2012 compared with July and August in 2011, and many fish that resided at RM 351 in 2011 did not return that far upstream for summer 2012. Wabash River levels were considerably lower than most years due to drought conditions experienced in summer 2012, and this likely contributed to the decreased frequency of upstream movements observed. We are hopeful that hydrologic conditions will be more “normal” in 2013, thus allowing us to gather additional data under river conditions that are more conducive to carp movements. Such conditions would provide the opportunity to more definitively determine seasonal areas of concentration as well as upstream extent of movements.

It is notable that there was a substantial increase in stationary detections at RM309 from 2011 to 2012. The receiver at this location is in a deep pool just downstream from BP1 and BP2, both of which were isolated from the Wabash River for much of summer 2012. Large aggregations of bigheaded carp were visible just upstream of RM309. While this is not uncommon for BP2, BP1 usually remains connected to the river for most of the year and, in 2011, tagged fish were found moving in and out of BP1 throughout the summer. The increased number of detections on the stationary receiver deployed at RM309 may reflect alternate habitat use during the unusually low water levels of summer 2012 resulting in the isolation of BP1. Data downloaded from the VR2W deployed in BP1 would help to more definitively evaluate this hypothesis, although loss of the buoy on this receiver has made recovery impossible to date. We will continue to try to recover this receiver in 2013. Declining number of fish and detections upstream (e. g. RM373) were also likely caused by low water restricting the movements of tagged fish.

Tagged fish were detected by stationary receivers at both RM373 (Peru, IN) and RM390 (Wabash, IN) on 30-Mar-2012. This indicates that silver carp are using habitats in upstream areas of the Wabash River approaching the confluence with the Little River, although no detections have been observed on the stationary receiver on the Little River to date. There was also one fish detected at RM406 (Huntington, IN) on 13-May-2012, but this fish was moving downstream and was seen at RM 390 and likely moved upstream earlier in the year without being detected. We have lost two VR2Ws at the Little River site to date, and we suspect that these may have resulted from vandalism/theft. We are working on alternative strategies for this deployment to avoid these issues in the future and expect to have another VR2W in place by 20-Nov-2012. Regardless, there does appear to be consistency in timing of upstream movements in the spring that could make it possible to predict when fish will move upstream and perhaps where they are spawning. If such consistency is discovered in 2013, this information may be useful for future control efforts and also to predict the potential upstream extent of invasion in novel ecosystems.

Spawning in the Wabash did not appear as widespread as in 2011, likely due to low water levels. This restricted both fish movement and our efforts in egg collection. The furthest upstream bigheaded carp spawning was documented was RM322. Spawning began approximately one month earlier in 2012 compared to 2011, likely due to warmer water temperatures. Both 2011 and 2012 spawning ended in late August or early September. Based on egg measurements, bigheaded carp eggs may be smaller than egg diameters reported in other systems. Mature eggs were reported as being 3 to 4 mm in diameter while Wabash eggs are <3mm. Once egg photos are identified to stage, a more detailed comparison of egg diameters can be done. Bigheaded carp eggs were not documented in the East Fork of the White River however one larval fish was captured during a sampling event. It is difficult to assess spawning for 2012 due to the extreme low water levels, although, as in 2011, egg density through the spawning period does not appear closely related to either hydrology or temperature. It does appear that both temperature and hydrology are involved in starting the spawning season for these bigheaded carps. Once all data are collected (2011, 2012 and 2013), these potential cues and others (e. g. growing degree day) will be assessed to see if the initialization of spawning can be predicted.

In the future, the remaining ~600 eggs collected and photographed during summer 2012 will be DNA tested to verify that they are from bigheaded carps and to determine whether they are hybrid individuals. Larvae, including the one collected in the White River, and fin clips from tagged fish will also be DNA tested for verification and to determine hybrid status. Additional stationary receivers that could not be deployed in 2012 due to low water levels will be deployed in three tributaries to further monitor use of tributaries by silver carp. The remaining tags from this year and the new tags for 2013 will be deployed with a focus on including more bighead carp in the study. Therefore we expect to continue to detect more previously tagged fish next year.

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