Abundance and distribution of early life stages of invasive carp in the Ohio River: 2023 Technical Report

Geographic Location: Ohio River Basin

Participating Agencies: Indiana Department of Natural Resources (INDNR) Kentucky Department of Fish and Wildlife Resources (KDFWR), West Virginia University (WVU), United States Fish and Wildlife Service (USFWS), West Virginia Division of Natural Resources (WVDNR), Eastern Illinois University (EIU), Southern Illinois University (SIU).

Statement of Need:

The negative effects of Silver (*Hypophthalmichthys molitrix*) and Bighead Carp (*Hypophthalmichthys nobilis*), also known as invasive carp, have been widely documented throughout their introduced range. These effects are numerous and varied in nature, some with direct implications to native biota (Irons et al. 2007, Sampson et al. 2009). Additionally, Black Carp (*Mylopharyngodon* piceus) are becoming more prevalent in the Ohio River and pose a threat to native mollusks (Poulton et al. 2019). Research investigating what factors lead to invasive carp range expansion is critical for the control of these invasive fishes, and mitigation of the deleterious effects they can cause.

Extensive research efforts have been directed toward invasive carp reproduction in terms of timing, location, and environmental conditions. Invasive carp exhibit a boom-and-bust pattern of reproduction, with strong year classes usually linked with large, sustained flooding and critical temperature ranges (DeGrandchamp et al. 2007). Although some understanding of their reproductive requirements exist, evidence suggests spawning of these species is possible over wider environmental ranges (Coulter et al. 2013), and in more habitats (i.e., tributaries) than previously thought (Kocovsky et al. 2012). Juvenile invasive carp are extremely mobile and may also elicit clumped distributions among static environments, requiring a variety of different gear types to effectively sample various habitats throughout the Ohio River (Collins et al. 2017; Molinaro 2020). In addition, factors promoting successful reproduction and recruitment remain uncertain. Identifying these factors is critical in suppressing the spread of these invasive fishes into novel environments.

Previous confirmed invasive carp spawning events have occurred in downstream tributaries (i.e., Wabash River) and as far upstream as McAlpine Locks and Dam (L&D), and physical signs of spawning (i.e., spawning patches) have been observed as far upstream as Markland Pool for Silver Carp and Meldahl Pool for Bighead Carp. Reproduction of *Hypophthalmichthys* spp. was detected by the presence of genetically confirmed Bighead and Silver Carp eggs as far upstream as RM 463 (near Cincinnati, OH) in 2021. To support the Ohio River Fish Management Team (ORFMT) Basin Framework objectives (ORFMT 2014), this project was initiated in 2016 in an effort to improve capabilities to detect early stages of invasion and spawning populations of invasive carp (Strategy 2.8) and also monitor upstream range expansion and changes in distribution and abundance (Strategy 2.3). Results of sampling prior to 2023 determined the extent of recruitment as far upstream as Markland Pool, with the majority of young-of-year (YOY) and juvenile detections below Newburgh L&D in J.T. Myers Pool (Jansen and Stump 2017, Roth 2018, Jansen 2021). Specifically, Hovey Lake in J.T. Myers Pool is a known recruitment area and therefore has been a focus for research over the past couple years to evaluate the timing and conditions which allow YOY invasive carp to enter the lake.

In addition to the Basin Framework, this project directly supports the National Plan (Conover et al. 2007) by assisting in the forecast and detection of invasive carp range expansions (Strategy 3.2.4), determining life history characteristics (Strategy 3.3.1), and assembling information about the distribution, biology, life history, and population dynamics of Bighead and Silver Carp (Strategy 3.6.2). Additionally, the results of this project will help managers make informed decisions during future planning efforts regarding resource allocation for invasive carp deterrent and control strategies.

2023 Project Objectives:

1) Determine the upstream extent of invasive carp spawning activity in the Ohio River above Markland Dam.
2) Identify locations of the Ohio River in which spawning occurs.
3) Determine the extent and locations of invasive carp recruitment in the Ohio River.
4) Identify characteristics of potential invasive carp nursery areas when juvenile invasive carp are encountered.
5) Evaluate the feasibility of drain structure modifications to limit invasive carp recruitment from Hovey Lake.
6) Determine the propagule source of invasive carp in large tributaries of the Ohio River.

Project Highlights:

- Ohio River invasive carp reproduction appeared to be limited in 2023, as evident by lower than usual egg, larvae, and YOY captures throughout the field season.
- Eight sites were sampled above Markland Locks and Dam (RM 532) via ichthyoplankton tows from May to August, 2023. Suspicious eggs (n = 25) and larvae (n = 4) were sorted from samples and sent to Whitney Genetics Lab for verification of species. Most samples were genetically confirmed shiner species and Freshwater Drum; none were invasive carp.
- No *Hypophthalmichthys* eggs or larvae were collected in the Ohio River proper throughout 2023 sampling efforts.
- One *Hypophthalmichthys* larvae was collected in the Green River of J.T. Myers Pool. Invasive carp larvae were not captured in other sampled tributaries of the Ohio River.
- Targeted surface trawling effort expanded further upstream in 2023, but only one Silver Carp YOY was captured in J.T. Myers Pool.
- Targeted YOY Black Carp sampling occurred at 24 sites along the lower Ohio River. One YOY Black Carp was collected at one backwater site near Smithland, KY, and YOY invasive carp were collected at three sites along the lower Ohio River
- Ichthyoplankton sampling in the Wabash River and its tributaries visually identified 2,621 invasive carp larvae and 599 eggs. The Little Wabash River and adjacent mainstem Wabash River sites produced the greatest density of invasive carp larvae.
- YOY invasive carp appear to be entering Hovey Lake through the water control structure in Bayou Drain as soon as lake and river levels equalize, and corresponding flow through the structure becomes minimal.

Methods:

For analysis purposes and for the remainder of this report, the phrase "invasive carp" will be referring to Silver and Bighead carps (*Hypophthalmichthys* spp.) only. In addition, both "YOY" and "immature" are collectively referring to "juvenile" invasive carp; "YOY" will be defined as fish less than 150 mm, and "immature" will define fish between 150 to 400 mm (likely 1 to 2 years old) which have undeveloped gonads and are not capable of spawning. Adult invasive carp are defined as fish greater than 400 mm with mature, identifiable gonads. Additionally, the term "suspect *Hypophthalmichthys*" is referring to an egg, advanced egg, or larvae with morphometric characteristics aligning with bigheaded carps, while the terms “suspicious egg/larvae” refers to specimens that do not have 100% of the morphometric characteristics of bigheaded carps but still warrant genetic confirmation.

Ichthyoplankton tows:
To evaluate the extent of invasive carp spawning activity in the Ohio River above Markland L&D, West Virginia University and WVDNR conducted ichthyoplankton tows at sampling sites within the R.C. Byrd (N = 3), Greenup (N = 1) Meldahl (N = 2), and Markland (N = 2) pools. Each sampling site was visited approximately three times from May 22 to August 2, 2023. During each visit, four tows were conducted: three within the Ohio River proper, and one within the tributary or at the intake structure if the site was a previous EA Engineering larval sampling site. In addition, WVU conducted three ichthyoplankton tows at six stratified random main channel sites within both Markland and Meldahl pools, sampling each site three times throughout the same timeframe.

To further identify specific tributaries and areas of the Ohio River in which invasive carp spawning occurs, ichthyoplankton tows were conducted at tributaries within J.T. Myers (N=1), Newburgh (N = 2), Cannelton (N = 2), and McAlpine (N = 2) pools twice each from June 20 to July 6, 2023, during ideal spawning conditions (water temperatures between 64 to 80°F with moderate to high water 2-3 days after peak flow event). Additionally, tows were conducted on two occasions within the drain of Hovey Lake to determine presence and size of post-gas-bladder-inflation larvae. Lastly, the mainstem Ohio River was sampled in two to three locations within each the J.T. Myers, Newburgh, Cannelton, and McAlpine pools from June 20 to July 6, 2023. Three tows were conducted at each sampling site.

For all tows, a conical ichthyoplankton net (0.5 m, 500 µm mesh) was deployed from the bow of the boat. The boat was motored in reverse, pulling the ichthyoplankton net upstream for three minutes. The water volume sampled was recorded using a General Oceanics Flowmeter fitted to the ichthyoplankton net; depth (m) and water temperature (°C) were recorded using a boat-mounted depth sounder. All contents in the ichthyoplankton net were rinsed into a 500 µm sieve and preserved using 95% non-denatured ethanol (at an estimated ratio of nine parts ethanol to one-part sample volume) for physical identification in the lab. Suspect *Hypophthalmichthys* eggs and larvae were morphometrically identified (process outlined below) and a subsample were sent to Whitney Genetics Laboratory for genetic confirmation. For specific details on genetic identification results and methods employed by the Whitney Genetics Laboratory, refer to Appendix A.

Larval fish were initially sorted into non-invasive carp and potential invasive carp (suspicious) species using morphometric parameters provided by Auer (1982). Furthermore, early developmental characteristics outlined by Yi et al. (1998) and Chapman (2006) were utilized to physically identify suspect *Hypophthalmichthys* larvae, advanced eggs, and eggs from each sample (Figure 1). Invasive carp larvae were identified by the presence of an eye spot, and suspect *Hypophthalmichthys* were differentiated from Grass Carp (*Ctenopharyngodon idella*) and Black Carp (*Mylopharyngodon piceus*) using myomere counts. *Hypophthalmichthys* larvae have 38 to 39 myomeres, whereas Grass Carp larvae range from 43 to 45 myomeres and Black Carp have 40 and 41 myomeres. Suspect *Hypophthalmichthys* eggs were identified based on general size and presence of a large perivitelline membrane (5 to 6 mm in diameter). Suspect *Hypophthalmichthys* ‘advanced eggs’ were defined as the beginning of a yolk-sack larvae still contained within the perivitelline membrane. In most cases, suspicious eggs and larvae may not have every morphometric characteristic of invasive carp, however, due to their collection locations, several may have been vouchered and sent to Whitney Genetics Lab for genetic confirmation of species.

Surface trawl:
Targeted YOY invasive carp sampling using surface trawls took place in tributaries and embayments of the Ohio River from J.T. Myers Pool to Racine Pool. Due to YOY Silver and Bighead Carp being found in Markland Pool in 2022, crews put significantly more effort into sampling upstream tributaries in 2023.

Because several new crews began assisting with targeted surface trawling efforts, a collaborative training/demonstration event took place in Markland Pool on July 27th and 28th, 2023, to get familiar with trawling techniques and standardize gears as much as possible.

The surface trawl measured 3.7 m wide, 0.6 m tall, and 5.5 m deep with 31.8 mm bar (number 12) netting. An additional layer of 4.8 mm mesh (35-pound delta) bag was attached externally to improve capture of small fishes. Additional foam floats were added to the top line of the trawl to provide extra buoyancy. Otter boards were 30.5 cm tall, 61.0 cm long, and each had a 12.7 cm diameter, 27.9 cm long "buoy style" PVC float attached to the top of the board allowing them to float. The trawl was deployed off the bow of the boat and attached with 24.4 m ropes. The boat was motored at 1.6 to 3.2 km per hour in reverse for five minutes before retrieving the net. In some locations it was not possible to complete five minutes of trawling, in which case sample time was documented. At the biologist's discretion, additional trawls were conducted at sites where either coverage was limited, or juvenile invasive carp were suspected. All invasive carp were identified to genus, measured to total length, and weighed.

Black Carp YOY Sampling:
KDFWR conducted targeted sampling for YOY Black Carp in the lower Ohio River from the confluence with the Mississippi River to above Smithland lock and dam. Sampling locations were chosen based on the hydrologic similarity to the location where YOY Black Carp were collected previously in Kentucky. Sampling effort did not exceed 30 days. Areas were sampled with beach seine and backpack electrofishing as accessible. If juveniles were collected; length and weight were recorded, and the specimens were preserved for additional analysis as needed. Most sites were sampled using a backpack electrofisher (Smith-Root LR-24) for variable durations depending on amount of habitat available to sample. Seining with 20' x 5' and 15' x 5' (1/8" mesh) seines was done at six sites, but proved to be difficult because of the deep, soft mud substrate.

Wabash River ichthyoplankton:
Eastern Illinois University (EIU) sampled ichthyoplankton in the Wabash River mainstem and four of its tributaries to monitor invasive carp (*Hypophthalmichthys* spp.) reproduction. EIU used a bow-mounted ichthyoplankton push net (Wildco), 0.5 meters in diameter, 2.5 meters in length, and 500 µm mesh. The tributaries sampled include the Vermilion, White, Embarras, Little Wabash rivers. At each tributary nine push net samples were collected: three within the tributary, three in the Wabash River above the tributary, and three in the Wabash River below the tributary. Each triplicate of samples consisted of a left bank, middle, and right bank sample to cover multiple areas across the channel. Each push net sample lasted five minutes and the volume of water filtered was estimated by a General Oceanics flow meter to achieve a target sample volume of 50 cubic meters. The contents of the net were emptied into a sample container and preserved with 95% non-denatured ethanol for identification in the lab. Invasive carp eggs and larvae were identified using meristic and morphometric features. A subsample of invasive carp eggs and larvae are in the process of genetic confirmation. A handheld YSI multiparameter meter was used to record temperature (°C). River discharge data (m3/s) from gauges nearest to sampling locations were obtained from United States Geological Survey.

Hovey Lake recruitment:
Hovey Lake is a known nursery area within the lower Ohio River, with YOY finding their way into the lake nearly every year. Excluding flood events, all water passing into and out of Hovey Lake must funnel through three culvert pipes at a control structure across Bayou Drain. Blocker boards can be installed within the culvert pipes during certain times of year to control the amount of water entering or leaving the lake. This control structure is operated by the Hovey Lake Fish and Wildlife property staff to manage the lake level primarily for waterfowl hunting opportunities. Multiple gears were used within Bayou Drain on both the river side and lake side of the control structure to evaluate the timing of YOY passage from the Ohio River into Hovey Lake.

Sampling for larval invasive carp was conducted using a conical ichthyoplankton net (0.5m, 500 µm mesh) deployed from the front of the boat. The boat was motored in reverse, pulling the ichthyoplankton net for three minutes (approximately 180 meters). This was repeated three times per side of the control structure. The water volume sampled was recorded using a General Oceanics Flowmeter fitted to the ichthyoplankton net; water temperature (^{o}C) was recorded using a boat-mounted depth sounder. All contents in the ichthyoplankton net were rinsed into a 500 µm sieve and preserved using 95% non-denatured ethanol (at an estimated ratio of nine parts ethanol to one-part sample volume) for physical identification in the lab. Suspect and suspicious *Hypophthalmichthys* larvae were sorted from all other native fish larvae and were counted and measured. A subset of some of the earliest suspect *Hypophthalmichthys* were sent to Whitney Genetics Lab for genetic confirmation of species.

A modified, rigid-frame surface trawl (herein called a beam trawl) was developed specifically for sampling in Bayou Drain with a small jon boat. Due to accessibility issues, a small jon boat was the only option for sampling the lake side of Bayou Drain. The standard 3.7 m wide surface trawl with otter boards that is used for other Ohio River YOY sampling was too large and produced too much drag to be able to effectively pull it with a small jon boat. The beam trawl consisted of a wooden frame measuring1.83 m wide and 0.61 m tall, with a 3.66 m long bag of 4.8 mm mesh (35-pound delta). Additional weight was added to the bottom of the frame to properly orient the trawl such that it floated upright in the water and just below the surface when towed. The trawl was deployed off the bow of the boat and attached with 20 m tow ropes. The boat was motored at 1.6 to 2.6 km per hour in reverse for the duration of the trawl before retrieving the net. A total of 14 minutes of trawling covering approximately 730 m was conducted on each side of the control structure each day. Individual tows lasted 3.5 to 5 minutes depending on obstacles in the water. After each tow, fish were sorted and YOY invasive carp were counted and measured.

Quadrafoil light traps were also deployed at Bayou Drain to passively sample for *Hypophthalmichthys* overnight. The light traps measured 30 cm diameter, 25 cm tall, with four entry slits of 5 mm (https://www.forestry-suppliers.com/p/78000/88740/watermark-quadrafoil-larval-fish-light-trap). A green Cyalume chemical lightstick (https://getcyalume.com/product/6-inch-green-snaplight-9-08076/) was placed in each trap and then traps were deployed to float below the water surface. Three to six traps were deployed on each side of the control structure – this number fluctuated depending on the amount of macroinvertebrate bycatch collected on previous sampling trips. Upon retrieval, all specimens concentrate in the bottom collection basin, water is drained through 250-micron mesh, and contents of the trap are rinsed into jars and preserved in 95% non-denatured ethanol.

In April, USGS installed a stream gage within Bayou Drain that monitors stream stage and velocity to compute streamflow in Bayou Drain. The gage is still being calibrated, but will be a valuable tool for assessing the exact flow conditions that allow invasive carp passage into Hovey Lake.

Microchemistry:
Water samples were taken from the Ohio River in J.T. Myers (N = 2), Newburgh (N = 3), Cannelton (N = 3), McAlpine (N = 3), and Markland pools (N = 3) during July and August, 2023. Additionally, three samples were collected from the Great Miami River (Markland Pool) throughout the same time period. Water samples were collected using a syringe filtration (0.45 µm pore size) technique and analyzed for Sr, Ba, and Ca concentrations.

Results:

Ichthyoplankton tows:
A combined total of 133 ichthyoplankton tows were conducted within the R.C. Byrd (N = 41), Greenup (N = 8), Meldahl (N = 42), and Markland (N = 42) pools (Table 1; Figure 2). Within those pools, 4 suspicious larvae were identified (one from Markland Pool, two from Meldahl, and 1 from R.C. Byrd) and sent to WGL for genetic confirmation. Two suspicious larvae were genetically confirmed shiner species, and two were Freshwater Drum. Likewise, a total of 25 suspicious eggs were sorted from tow samples and sent off to Whitney Genetics Lab for species confirmation. Twenty two of the 25 samples returned genetic results, none of which were invasive carp. Suspicious eggs were genetically confirmed as Freshwater Drum (n = 13), Shiner species (n = 4), Herring species (n = 4), and Silver Chub (n = 1). Most of the suspicious eggs from the upper pools varied in size and were typically much smaller than *Hypophthalmichthys* eggs; many lacked a large perivitelline membrane.

A total of 54 ichthyoplankton tows were conducted within the mainstem Ohio River in the J.T. Myers (N = 12), Newburgh (N = 12), Cannelton (N = 18), and McAlpine (N = 12) pools (Table 2). Zero *Hypophthalmichthys* eggs or larvae were collected in the Ohio River proper. Four suspicious larvae were collected in Newburgh Pool at RM 772.8 near Yankeetown, IN; These were genetically confirmed shiner species. In Cannelton Pool, ten suspicious larvae and two eggs were identified in samples collected from RM 662.9 near Leavenworth, IN; seven larvae and two eggs were submitted to Whitney Genetics Lab. Of these, the two eggs were Grass Carp, while the larvae were genetically confirmed as two Grass Carp, two Freshwater Drum, one herring species, and one chub species. No suspect eggs or larvae were collected from the mainstem McAlpine Pool samples.

An additional 48 ichthyoplankton tows were conducted in select tributaries of the lower Ohio River (Table 2; Figure 2). Three *Hypophthalmichthys* larvae were collected in tributaries of J.T. Myers Pool, one in Green River and two in Bayou Drain of Hovey Lake. Three additional Newburgh Pool tributary larvae were send for genetic confirmation and were determined to be Freshwater Drum and shiner species. No suspect invasive carp eggs or larvae were found in Newburgh or Cannelton Pool tributaries. Two suspicious larvae were pulled from Harrods Creek in McAlpine Pool, but were both genetically identified as shiner species.

Surface trawl:
Among project partners, surface trawling effort consisted of 162 tows totaling 13.2 hours of sampling. The majority of effort was expended in tributaries of Markland (4.7 hrs), Cannelton (3.2 hrs), and Meldahl (2.4 hrs) pools, followed by J.T. Myers (1.4 hrs), R.C. Byrd (1.0 hrs), Greenup (0.9 hrs), and Racine (0.3 hrs) pools (Figure 3). Only one YOY Silver Carp measuring 46 mm was captured in Hovey Lake's Bayou Drain in J.T. Myers Pool (not including data summarized in the *Hovey Lake recruitment* subsection). Despite extensive sampling efforts, zero YOY invasive carp were captured via surface trawls in Ohio River tributaries upstream of J.T. Myers Pool in 2023.

Black Carp YOY Sampling:
KDFWR sampled for YOY invasive carp at 24 sites along the lower Ohio River, 9 sites along the Mississippi River, 11 sites along the lower Tennessee River and 11 sites along the lower Cumberland River (Table 3; Figure 4). Five sites sampled had YOY invasive carp and one of those sites had one YOY invasive Black Carp. All specimens were fixed in formalin and identification was verified in the laboratory. The YOY Grass Carp and Black Carp were identified by removing pharyngeal teeth and comparing their morphology (Figure 5).

Wabash River ichthyoplankton:
EIU collected a total of 111 ichthyoplankton samples from May 25th to August 9th, 2023, across five sampling dates. Samples were taken from each tributary and corresponding main-stem Wabash River sites up to five times throughout the season, depending on access. An additional 15 samples were collected in the Embarras River near Charleston, IL, but results are not reported here as no visually identified invasive carp larvae or eggs were captured at these sites. The Vermilion River was inaccessible for the duration of the season and samples from the adjacent main stem Wabash River were taken on one occasion (June 6th). After the August 9th sampling event, water levels remained too low for the rest of the season to sample any sites.

In total, 2,621 larvae and 599 eggs were visually identified as invasive carp (Table 4). Additionally, 1,212 eggs had similar morphometry to invasive carp eggs but were smaller in diameter than average and were designated as 'potential eggs' (Table 4). A sub-sample of all eggs and larvae collected throughout the season are awaiting genetic confirmation.

Larvae were found in all the tributaries except for the Embarrass River tributary site. The Little Wabash River and adjacent mainstem Wabash River sites produced the greatest density of invasive carp larvae throughout the sampling season (Table 4). Peaks in larval density generally coincided with rapid rises in discharge in the tributary site and/or adjacent mainstem Wabash River sites (Figure 6). For example, on July 5th, peaks in larval abundance were observed in the mainstem Wabash River sampling location following a rapid river rise at New Harmony days prior (Figure 6). This contrasted with results from August 9th where only the Little Wabash River had a significant rise, resulting in relatively high density of larvae within the tributary, and the mainstem site below the tributary. Similar patterns were observed in the White River sites, just with relatively lower density levels to the Little Wabash sites (Figure 7). However, larvae were collected on June 7th from the Wabash River above the White River confluence that did not correspond to river rises in either the tributary, or mainstem Wabash River.

All sites except for the Embarras River tributary had visually identified *Hypophthalmichthys* eggs found in the samples. The eggs that were found in the Wabash River near the Embarras River tributary site are awaiting genetic confirmation as they were not clearly identifiable. The mainstem Wabash River near the Vermilion River had the highest density of eggs for a sampling event, followed by the White River and adjacent mainstem sites. The mainstem Wabash River sites near the Embarrass River also produced significant numbers of eggs, whereas the within-tributary sites produced very few. Final egg totals are subject to change as we complete genetic confirmation, thus results presented here represent a conservative estimate of invasive carp egg production for 2023.

Hovey Lake recruitment:
Bayou Drain of Hovey Lake was sampled on both sides of the water control structure from May 15th to June 23rd, 2023, via ichthyoplankton netting, surface trawling, and passive light traps. Ichthyoplankton netting effort consisted of 138 tows; 57 on the lake side of the control structure, 65 on the river side, and an additional 16 collections at the outflow of the control structure when water velocity was high enough to suspend the net. A total of 46 *Hypophthalmichthys* larvae were collected via ichthyoplankton nets. An additional 28 suspicious larvae were sent to WGL to genetically confirm species, however, none were determined to be invasive carp. The suspicious larvae were confirmed to be Orangespotted Sunfish, Common Carp, White Crappie, Freshwater Drum, Gizzard Shad, and shiner and buffalo species. Invasive carp larvae were first detected in ichthyoplankton nets on the river side of the control structure on May 25th, and on June 1st on the lake side of the structure. Ichthyoplankton nets captured invasive carp larvae from May 25th to June 8th, when densities ranged from 0.097 to 0.574 individuals/10 m^3, peaking on May 26th.

A total of 106 light traps were deployed over 11 sampling days. Traps were set 30 minutes before sundown and soaked overnight for approximately 14 hours. Light traps first collected *Hypophthalmichthys* larvae on May 19th on the river side of the control structure, yet didn't collect them on the lake side until June 13th. From May 19th to June 6th, the river side of the control structure had the highest catch rates, ranging from 1.67 to 15.83 invasive carp/trap night. From June 13th to June 23, the lake side of the control structure had higher light trap catches than the river side, which ranged from 0.33 to 3.25 invasive carp/trap night. Invasive carp ranging from 9 to 42 mm were captured via light traps (Figure 7).
Beam trawls efforts consisted of 141 tows, including 66 on the river side of the control structure and 72 tows on the lake side. Beam trawls first detected invasive carp on the river side of the control structure on May 31st, and later collected them on the lake side of the structure on June 5th. YOY Silver Carp catch rates were typically higher on the river side of the control structure, peaking at 9.7 fish/minute on June 8th. Silver carp captured via beam trawl ranged in size from 13 to 46 mm (Figure 8).

Collectively, gears captured more YOY invasive carp on the river side of the control structure (Figure 9). Fish showing up on the lake side of the control structure appeared to coincide with water levels equalizing, as highlighted by provisional data collected at the USGS stream gage station installed in Bayou Drain (Figure 10).

Microchemistry:
Mainstem Ohio River water samples were processed to further refine Sr:Ca signatures within the basin. Any sample with Sr:Ca value of less than 1334 µmol/mol can be definitively classified as tributary origin, while any samples with a value greater than 1438 µmol/mol can be classified as definitive Ohio River origin. Since 2021, 710 Silver Carp otoliths have been analyzed, the majority of which were collected in Cannelton and McAlpine pools. Tributary origin comprises 86.4% (n = 614) of Silver Carp otoliths sampled, while 11.0% (n = 78) originated in the Ohio River proper. To date, six Silver Carp have exhibited extremely high Sr:Ca core signatures, suggesting they may have originated much further upstream in the Ohio River Basin than the currently known recruitment front.

Discussion:

Results of the eighth year of the Abundance and Distribution of Invasive Carp Early Life Stages in the Ohio River project offer the most up to date information on the extent of invasive carp spawning and recruitment in the Ohio River. Collective efforts of ichthyoplankton tows, targeted surface trawls, and electrofishing directly addressed Basin Framework Strategy 2.8 by improving capabilities to detect early stages of invasion and spawning populations of invasive carp. This project continues to provide data to describe our current understanding of the distribution of invasive carp recruitment for the Water Resources Reform and Development Act (WRRDA) reporting. Moreover, knowledge acquired from this project directly informs planning efforts for future invasive carp deterrent, control, and other management strategies.

Excluding the Wabash River, invasive carp reproduction appeared to be very limited in 2023. The only significant river rise on the Ohio River throughout spring and summer of 2023 occurred in early May when water temperatures were below 65 F. There was no other high water event throughout the remainder of the summer, which likely limited spawning. During the early May event, water temperature was around 62 F during the crest, which is near the minimum temperature for Silver Carp spawning activity. Regardless, there appeared to be a small amount of spawning occur as suggested by post gas-bladder inflation larvae showing up in Bayou Drain of Hovey Lake on May 19th. A minor bump in river levels in early July likely produced some spawning, as we captured one *Hypophthalmichthys* larvae in the Green River. However, many gravid female Silver Carp were captured during various sampling efforts throughout the majority of the summer, again suggesting unfavorable Ohio River spawning conditions through much of 2023.

Among the subsamples of eggs and larvae sent to Whitney Genetics Lab, most were not confirmed to be Silver and Bighead Carp. There were a couple Grass Carp, shiners, and chubs that field crews suspected were *Hypophthalmichthys* but genetics confirmed otherwise. We knew that not all eggs/larvae sent to Whitney Genetics Lab from 2023 had every characteristic of *Hypophthalmichthys* species, however due to the proximity of where some were collected, we wanted verification to be safe. Invasive carp larvae can be readily identified by trained biologist, however due to staff turnover and the number of suspicious yet non-invasive carp larvae in our samples, we recommend a refresher training course to help further refine staff's ability to morphometrically identify larval invasive carp species confidently. Eggs remain inherently more difficult to discern and will likely need continued species confirmation through genetic methods.

More surface trawling effort was expended in 2023 than any other sampling year dating back to 2019 when trawling became the primary method for YOY collections. The increase in trawling efforts was driven by the 2022 collection of YOY Silver and Bighead carps in Markland Pool of the Ohio River, and subsequent interest from project partners to begin sampling for them further upstream. Despite the efforts, zero YOY invasive carps were captured upstream of J.T. Myers Pool. Surprising, even after finding YOY *Hypophthalmichthys* in Cannelton Pool in 2021 and 2022, none were collected there in 2023, further supporting the notion of limited spawning and recruitment in the Ohio River.

Efforts in 2023 revealed the presence of YOY Black Carp at a single location out of 55 sites sampled along the lower Ohio, Mississippi, Tennessee, and Cumberland River. The location was along the Ohio River shoreline at river mile 920, directly upstream of the Cumberland River confluence of the Ohio River. This site is 27 miles upstream of Gar Creek, where the single YOY fish was collected in 2018, and 15 river miles upstream of the site that YOY Black Carp were collected in 2022 (Figure 4). The 2022 and 2023 sites have similar habitat characteristics; both are close to the main river channel, shallow (< 1 m), muddy backwaters that may be a nursery area at higher water levels but can become isolated during low river stage. This occurrence is further evidence of consistent, albeit low, Black Carp reproduction in the lower Ohio River drainage in Western Kentucky. No juvenile or adult Black Carp were captured or observed during sampling. Although currently available collection data indicates Black Carp are now established and reproducing in the lower Ohio River drainage, it suggests their dispersal into the area has been more recent and they are less common than Grass and Silver carps.

Observed results for 2023 Wabash River ichthyoplankton sampling demonstrate there are multiple areas where invasive carp successfully reproduce throughout the Wabash River Basin, particularly in the Little Wabash River and the adjacent mainstem of the Wabash River. Although fate of larvae and recruitment dynamics are unclear for this species, these results suggest the Wabash River could be a significant source of YOY to the greater Ohio River Basin. Additionally, we documented spawning events in the White River, including significant numbers of invasive carp eggs. This location has not been sampled in the past by EIU and could represent an additional source of YOY within the basin. We found 2023 to have lower levels of invasive carp reproduction in the Wabash River Basin relative to past studies by EIU, likely due to low-water levels for the majority of the sampling season. Through this study and additional adult sampling, it appeared that gravid females retained their eggs later into the season than typically observed until there were significant rises in discharge. Two main peaks in larval and egg abundance were observed on July 5th, and August 9th and coincided with the relatively few discharge rises in the mainstem Wabash and its tributaries. Overall results of our 2023 sampling season show the Wabash River Basin is a potential source of invasive carp propagule pressure to the Ohio River Basin, even in years of less suitable hydrological conditions for their spawning. Continued monitoring over multiple years and varying hydrological conditions will be particularly important in future efforts, as well as a more detailed comparison to trends observed throughout the Ohio River Basin.

Extensive sampling within Bayou Drain from mid-May through June gave us the first look into the conditions that allow successful invasive carp passage from the Ohio River into Hovey Lake. As expected, YOY were first identified on the river side of the water control structure. One week later, as the water velocity flowing out of the lake subsided, YOY were detected on the lake side of the control structure. The multi-gear approach appeared to work well for capturing various sizes of YOY invasive carp, from post gas-bladder inflation larvae (~9 mm) and larger. Light traps captured invasive carp a few days earlier than ichthyoplankton nets, and captured the widest size range of YOY carp, suggesting they would be a good tool for early detection efforts in other potential recruitment areas throughout the basin. Although the installed water velocity gage is not yet calibrated, and therefore its data is preliminary, visual observations suggest that carp passage into the lake is limited when high velocity water is flowing out of the lake through the water control structure. Another year of data collections is needed to confirm, but keeping YOY carp from entering Hovey Lake may be as simple as closely monitoring drain flows and installing blocker boards immediately when the lake and river water levels nearly equalize. Interestingly, although we captured YOY on the lake side of the control structure, multiple surface trawling attempts in Hovey Lake proper did not capture any. If Hovey Lake water levels drop quickly and stay low, exposed mud flats and beaver dams may act as secondary barriers, keeping YOY trapped in the drain and out of the lake proper. The apparent limited invasive carp reproduction in 2023 may have drastically lowered the amount of YOY attempting to traverse Bayou Drain, therefore results from a "normal" spawning year may provide better insight for the quantity of carp entering the lake.

Preliminary results from otolith microchemistry data suggest a small percentage of Silver Carp spawned much further upstream than previously thought. Individual fish data will be investigated to better understand what may be happening. Unfortunately equipment issues with the laser ablation system caused additional delays and no new otoliths were processed. Gathering additional otolith samples from upstream river reaches may help better understand potential recruitment sources of the upper Ohio River.

There has not been what we would consider a strong spawning event or year-class since this project was initiated in 2016, and 2023 had exceptionally low spawning activity. However, based on the presence of adult invasive carp as far upstream as R.C. Byrd Pool, the 2022 findings of YOY invasive carp in Markland Pool, a highly successful spawning event could quickly shift the current known extent of recruitment to pools farther upstream. Therefore, the spatial and temporal variation in invasive carp recruitment in the Ohio River emphasizes the need for continued long-term monitoring with this project as well as others within the basin. Efforts in this project provide valuable insight into factors promoting the reproduction and recruitment of invasive carp, and ultimately range expansion. Results support several Basin Framework and National Plan strategies and will be used by biologists to mitigate the spread of these invasive fishes.

Recommendations:

We recommend continued work towards a uniform ichthyoplankton sampling design throughout the Ohio River. In addition to informing the partnership of the extent of spawning, these data will continue to help locate specific tributaries or locations important for invasive carp reproduction. Having comparable data within the Ohio River, and among other sub-basins, will allow managers to prioritize control efforts. In addition, we recommend beginning to use ichthyoplankton tows as a tool to measure more than just presence/absence – quantifying density of invasive carp eggs and larvae will help evaluate changes in spawning success over time.

Based on genetically confirmed results from the past four years, physical morphometrics can be successful in identifying *Hypophthalmichthys* advanced eggs and larvae from other native fish species. However, a few native fish and Grass Carp stumped us this year. The identification of eggs is more difficult and should still be verified via genetic analysis. The use of a measuring device on a microscope to determine if the perivitelline membrane is 5 to 6 mm will help in sorting between non-invasive carp and invasive carp-type eggs. There were many 'suspicious' eggs and larvae submitted to the genetics lab again this year that weren't in fact invasive carp, but rather, buffalos, shiners, Freshwater Drum, and other species. Because of this, along with staff turnover, we recommend field staff involved in the physical identification of *Hypophthalmichthys* larvae and eggs be trained or take a refresher course on larval fish identification. We recommend the continued use of morphometric methodologies being paired with genetic confirmation of a subsample of specimens to accurately discern between invasive carp and native fish eggs and larvae.

KDFWR recommends continuing to develop and geographically expand invasive carp YOY surveys in the lower Ohio River Basin with an emphasis on searching for YOY Black Carp. Continued effort is planned for 2024. With regards to YOY Silver and Bighead carp sampling, we recommend continuing targeted sampling in Markland Pool and areas further upstream. Both KDFWR, WVU, and USFWS are now outfitted with surface trawls which will be used to greatly expand YOY sampling further upstream in 2024.

INDNR recommends conducting another season of intensive sampling on both sides of the Hovey Lake water control structure in the spring of 2024 using the multi-gear approach. Seasonal variation may change the timing and quantity of YOY invasive carp attempting to enter the lake. As soon as the new USGS stream flow and velocity gage is calibrated, we recommend monitoring water conditions remotely in the future to help inform when to shut off water flow between the Ohio River and Hovey Lake.

Acknowledgements:

We would like to thank Zeb Woiak, Aaron Johnson, and staff at the USFWS Whitney Genetics Laboratory for their help processing egg and larval samples for this project.

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Table 1. Summary of ichthyoplankton tows collected by West Virginia University and West Virginia DNR. Sampling took place between May 22 and August 2, 2023. An asterisk (*) denotes genetically confirmed *Hypophthalmichthys* samples, or the lack thereof, analyzed by Whitney Genetics Lab.

Sampling Information				Samples to WGL (N)		*Hypophthalmichthys* (N)		
Pool	**Location**	**Transect Type**	**Tows (N)**	**Eggs**	**Larvae**	**Eggs**	**Advanced Eggs**	**Larvae**
Markland	RM 528	Ohio River	3	0	0	0	0	0
Markland	RM 514	Ohio River	3	0	0	0	0	0
Markland	RM 496.7 (near Hogan's Cr.)	Ohio River	9	0	0	0	0	0
Markland	Hogan's Creek	Tributary	3	0	0	0	0	0
Markland	RM 484	Ohio River	3	3	0	0*	0	0
Markland	RM 473	Ohio River	3	0	0	0	0	0
Markland	RM 463.5 (near Little Miami R.)	Ohio River	9	3	1	0*	0	0*
Markland	Little Miami	Tributary	3	0	0	0	0	0
Markland	RM 452	Ohio River	3	3	0	0*	0	0
Markland	RM 449	Ohio River	3	3	0	0*	0	0
Medahl	RM 429	Ohio River	3	0	0	0	0	0
Medahl	RM 405	Ohio River	3	2	1	0*	0	0*
Medahl	RM 404.7 (near J.M. Stuart)	Ohio River	9	0	0	0	0	0
Medahl	J.M. Stuart Plant	At structure	3	0	0	0	0	0
Medahl	RM 396	Ohio River	3	0	0	0	0	0
Medahl	RM 373	Ohio River	3	4	1	0*	0	0*
Medahl	RM 356.4 (near Sciota R.)	Ohio River	9	3	0	0*	0	0
Medahl	Sciota River	Tributary	3	0	0	0	0	0
Medahl	RM 355	Ohio River	3	2	0	0*	0	0
Medahl	RM 344	Ohio River	3	0	0	0	0	0
Greenup	RM 305.2 (near Guyandotte R.)	Ohio River	6	0	0	0	0	0
Greenup	Guyandotte River	Tributary	2	0	0	0	0	0
R.C. Byrd	Raccoon Creek	Tributary	12	0	1	0	0	0*
R.C. Byrd	RM 265.1 (near Kanawha R.)	Ohio River	9	2	0	0*	0	0
R.C. Byrd	Kanawha River	Tributary	20	0	0	0	0	0

Table 2. Summary of ichthyoplankton tows collected by the Kentucky Department of Fish and Wildlife Resources and Indiana Department of Natural Resources. Sampling took place between June 13 and July 6, 2023. An asterisk (*) denotes genetically confirmed *Hypophthalmichthys* samples, or the lack thereof, analyzed by Whitney Genetics Lab.

Sampling Information				Samples to WGL (N)		*Hypophthalmichthys* (N)		
Pool	**Location**	**Transect Type**	**Tows (N)**	**Eggs**	**Larvae**	**Eggs**	**Advanced Eggs**	**Larvae**
J.T. Myers	RM 840.6 (near Hovey Lake)	Ohio River	6	0	0	0	0	0
J.T. Myers	Hovey Lake Drain	Tributary	6	0	0	0	0	2
J.T. Myers	RM 784.0 (near Green R.)	Ohio River	6	0	0	0	0	0
J.T. Myers	Green River	Tributary	6	0	4	0	0	1*
Newburgh	RM 772.8 (near Little Pigeon Cr.)	Ohio River	6	0	3	0	0	0*
Newburgh	Little Pigeon Creek	Tributary	6	0	0	0	0	0
Newburgh	RM 731.3 (near Anderson R.)	Ohio River	6	0	0	0	0	0
Newburgh	Anderson River	Tributary	6	0	0	0	0	0
Cannelton	RM 718.7 (near Deer Cr.)	Ohio River	6	0	0	0	0	0
Cannelton	Deer Creek	Tributary	6	0	0	0	0	0
Cannelton	RM 662.9 (near Blue R.)	Ohio River	6	2	7	0*	0	0*
Cannelton	Blue River	Tributary	6	0	0	0	0	0
Cannelton	RM 608.5 (near New Albany)	Ohio River	6	0	0	0	0	0
McAlpine	RM 595.8 (near Harrods Cr.)	Ohio River	6	0	0	0	0	0
McAlpine	Harrods Creek	Tributary	6	0	2	0	0	0*
McAlpine	RM 545.8 (near Kentucky R.)	Ohio River	6	0	0	0	0	0
McAlpine	Kentucky River	Tributary	6	0	0	0	0	0

Table 3. Summary of YOY invasive carp captures in Western Kentucky during 2022 and 2023.

2022	**# of Sites Sampled**	**Sites with YOY Invasive Carp**	**Sites with YOY Black Carp**
Ohio River	21	2	1
Mississippi River			
Tennessee River	2		
Cumberland River			
Total	23	2	1
2023	**# of Sites Sampled**	**Sites with YOY Invasive Carp**	**Sites with YOY Black Carp**
Ohio River	24	3	1
Mississippi River	9	2	
Tennessee River	11		
Cumberland River	11		
Total	55	5	1

Table 4. Sampling location, transect location, number of push net samples, number of invasive carp larvae collected, number of invasive carp eggs collected, and number of potential invasive carp eggs collected in the Wabash River Basin in 2023.

Sampling Location	Transect Location	Push Net (N)	Larvae (N)	Eggs (N)	Potential Eggs (N)
Vermilion	Wabash Upstream	3	1	115	385
Vermilion	Wabash Downstream	3	0	100	295
White	Wabash Upstream	15	7	90	0
White	Wabash Downstream	12	4	118	4
White	Tributary	15	5	65	89
Embarras	Wabash Upstream	12	69	70	55
Embarras	Wabash Downstream	12	7	30	47
Embarras	Tributary	12	0	0	47
Little Wabash	Wabash Upstream	9	503	6	0
Little Wabash	Wabash Downstream	9	1329	3	30
Little Wabash	Tributary	9	696	2	315
Totals		**111**	**2621**	**599**	**1212**

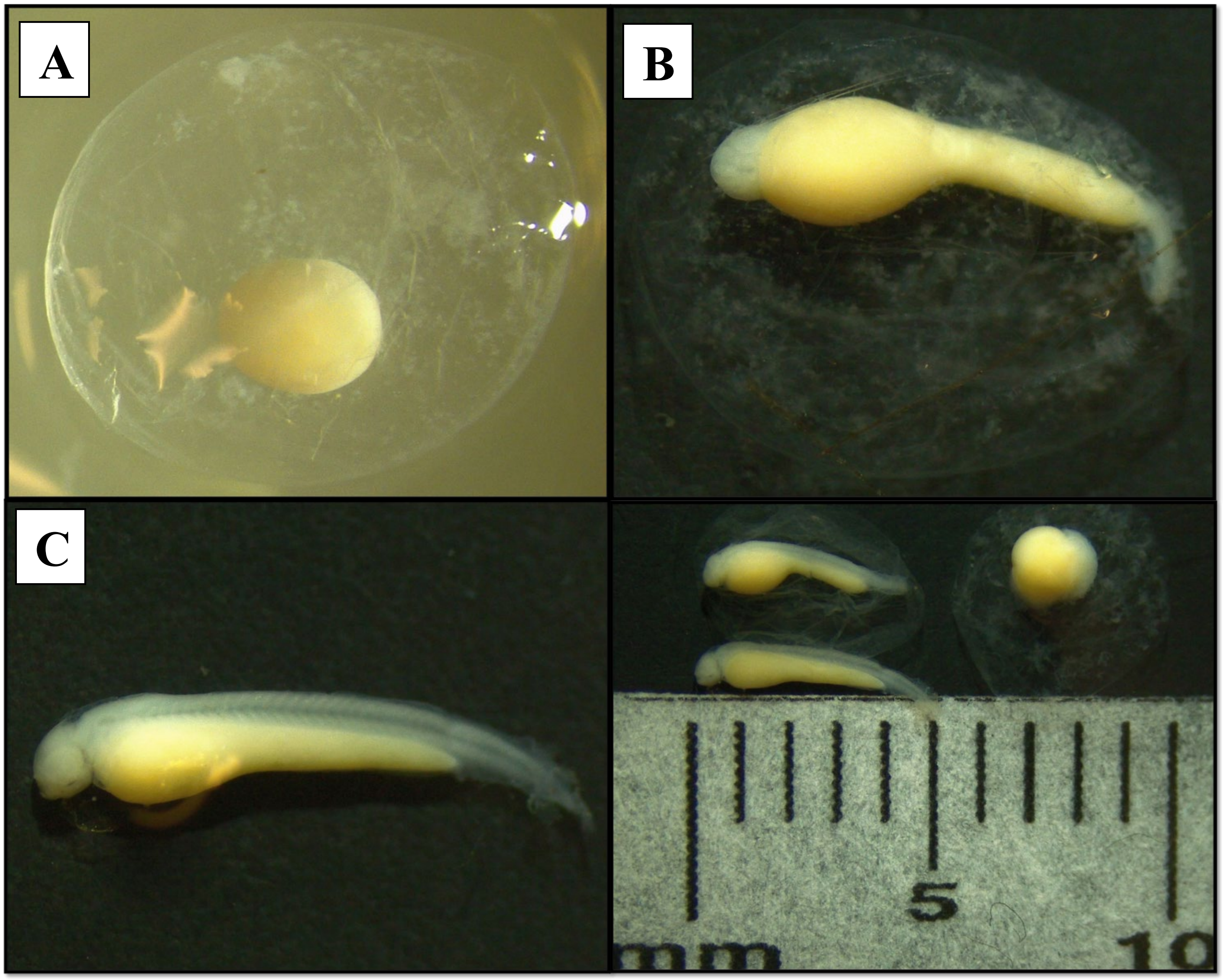

Figure 1. Developmental life stages of *Hypophthalmichthys* spp. with size comparisons. For the purposes of this report, pictures A, B, and C demonstrates specimens categorized as “eggs”, “advanced eggs”, and “larvae”, respectively.

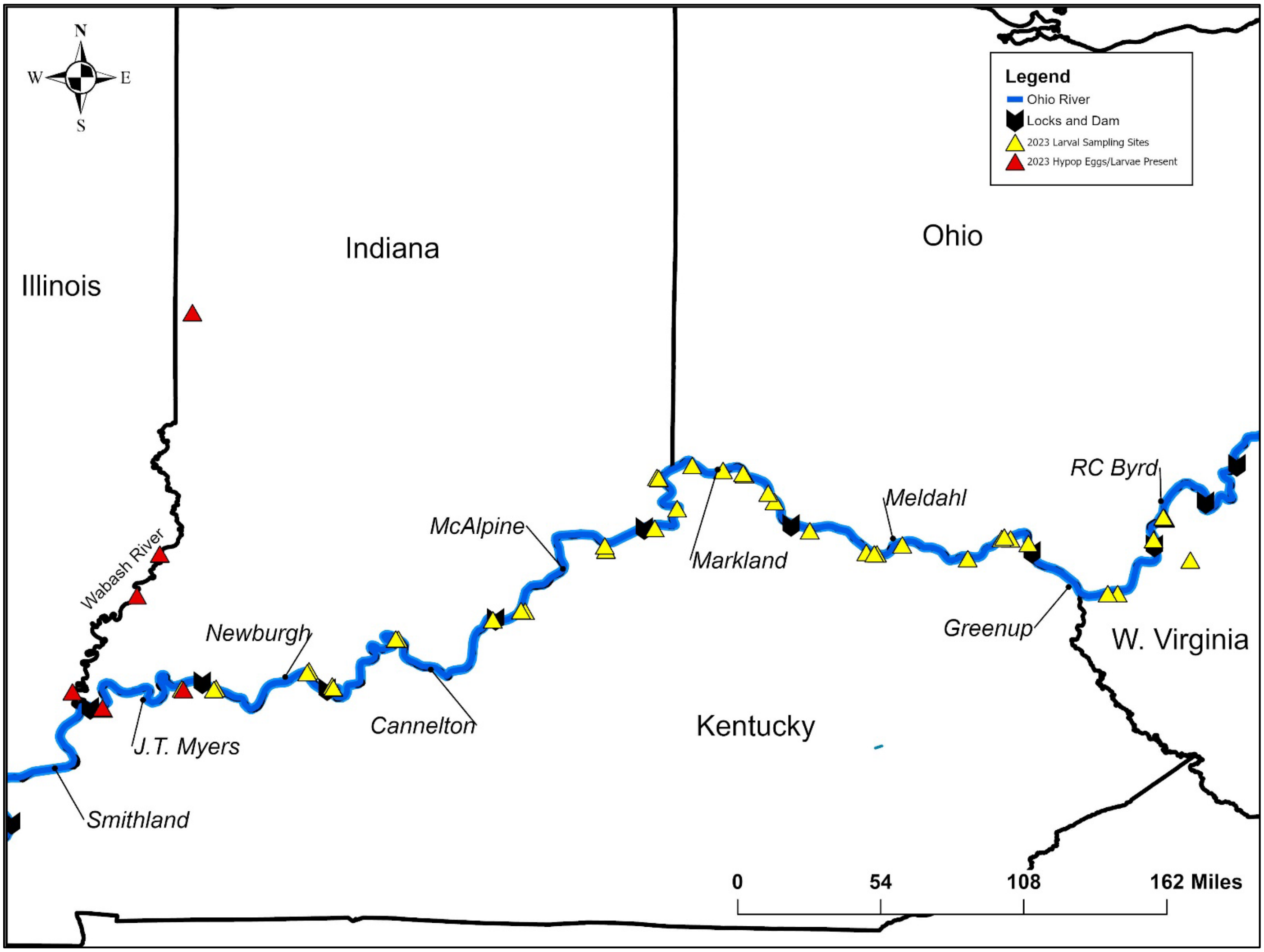

Figure 2. Map of 2023 study area of larval sampling sites. Black icons denote a locks and dam, yellow triangles indicate larval sampling sites, red triangles indicate locations where confirmed *Hypophthalmichthys* eggs, embryos, or larvae were collected.

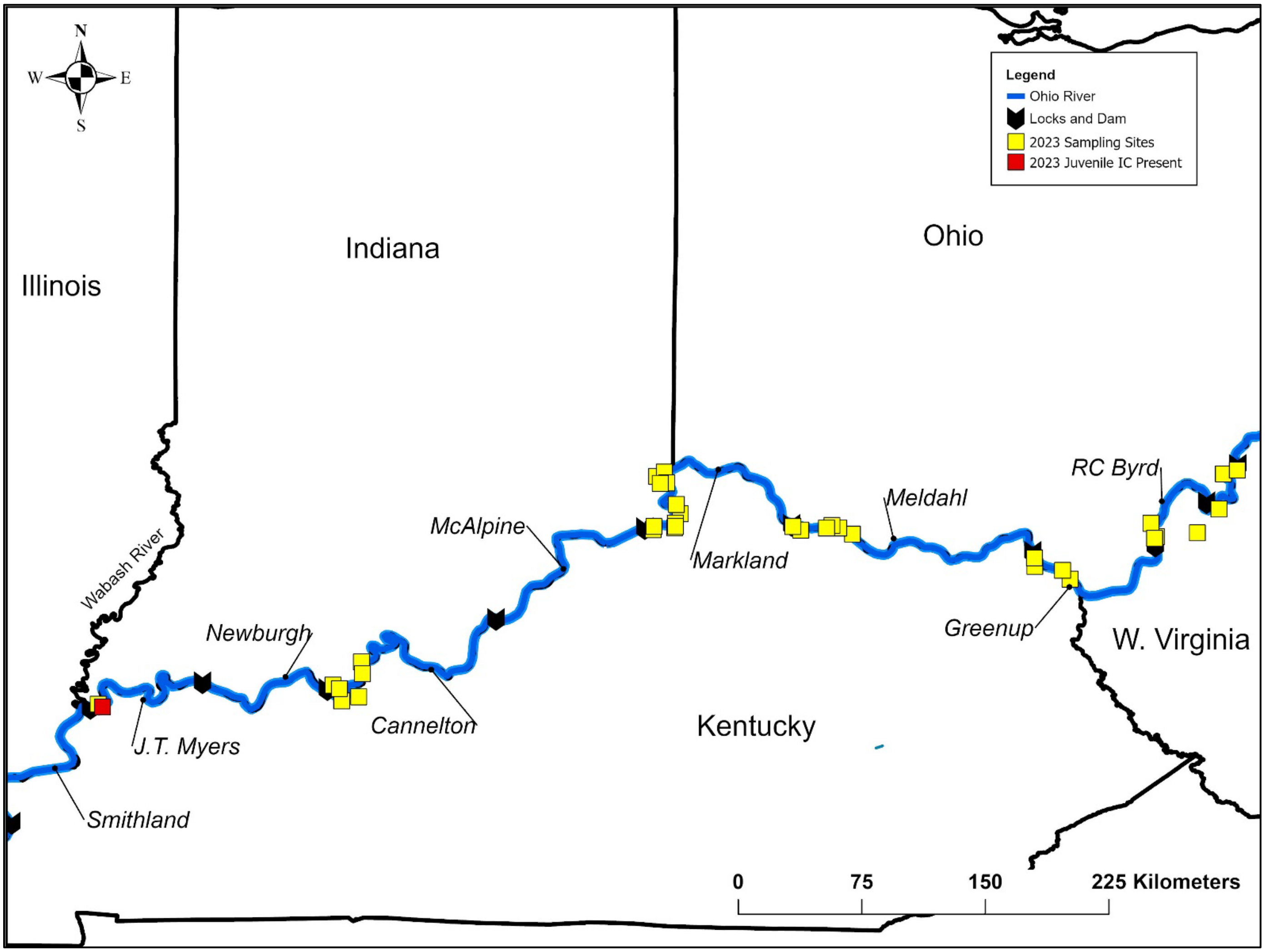

Figure 3. Map of 2023 study area of targeted juvenile sampling sites. Black icons denote a locks and dam, yellow squares indicate targeted sampling sites, red squares indicate locations where YOY or juvenile *Hypophthalmichthys* were collected.

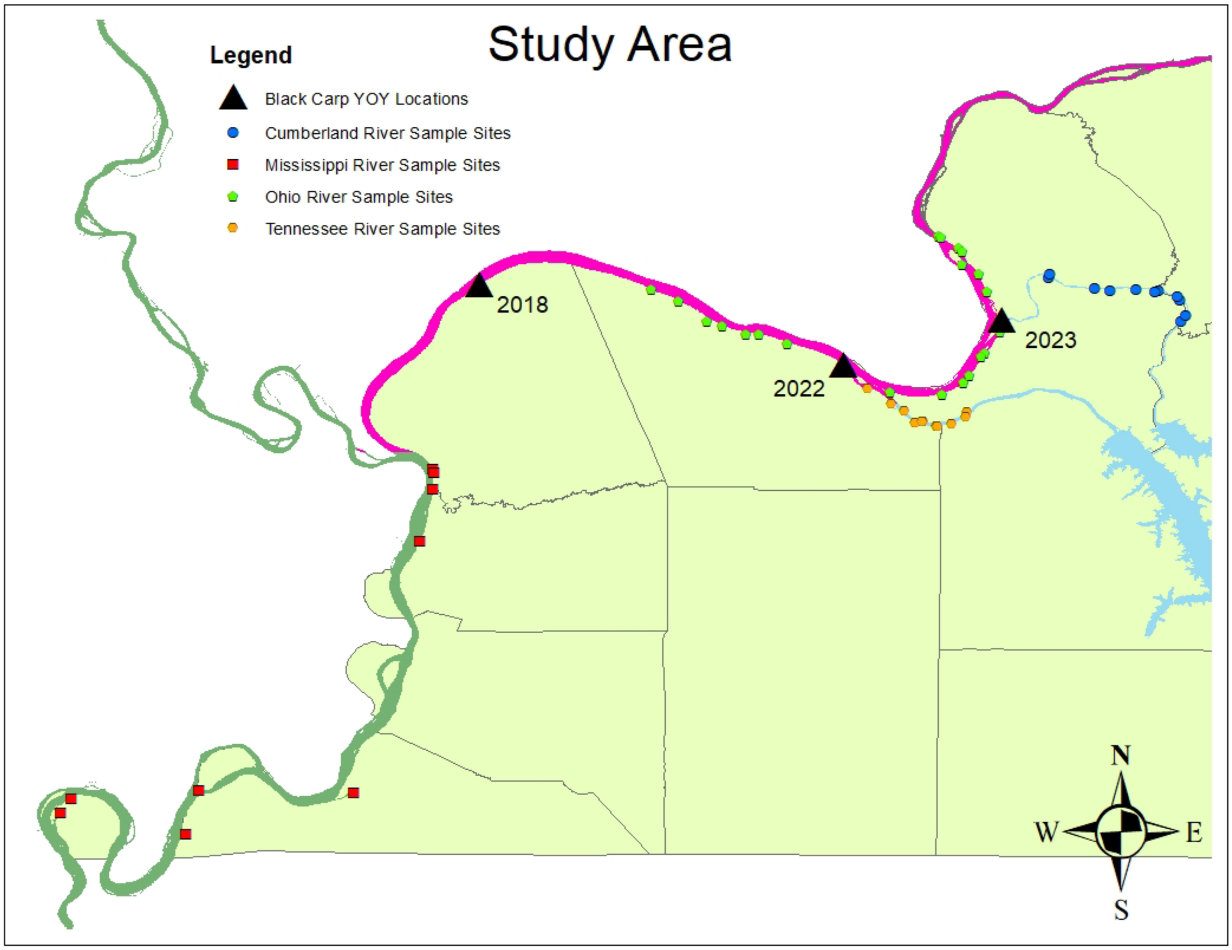

Figure 4. Site localities sampled in the lower Ohio River for YOY invasive carps during 2023, as well as locations of YOY Black Carp captures in 2018, 2022, and 2023.

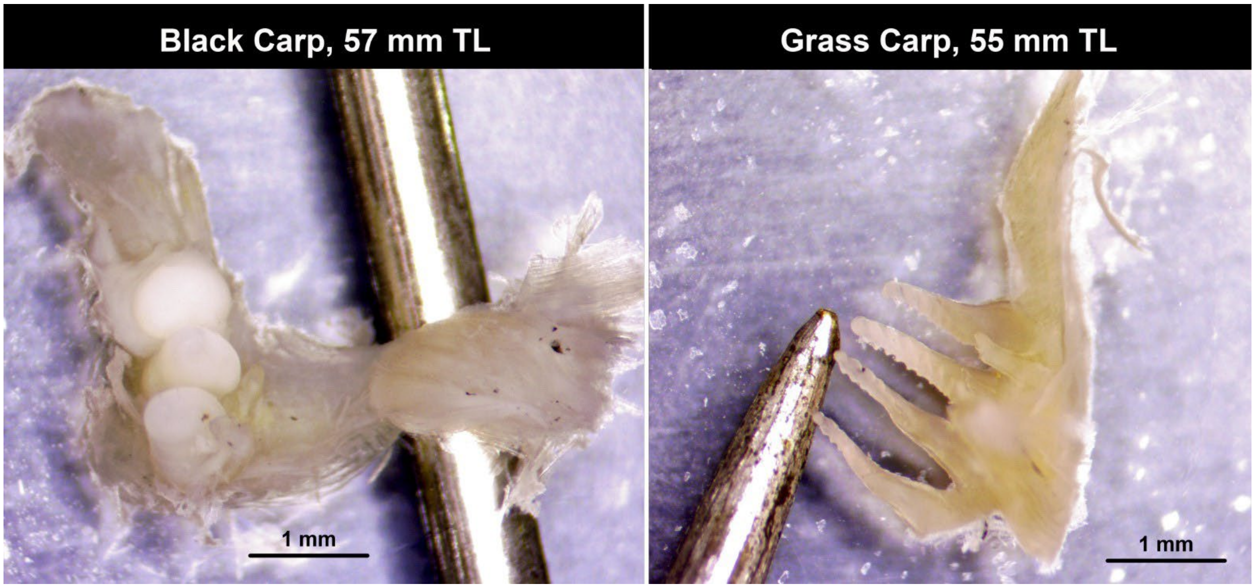

Figure 5. Comparison of pharyngeal tooth morphology between YOY Black and Grass carps of approximately the same size. Shown for each species is the dissected right pharyngeal arch. Black Carp has single row of 4 molar-like teeth (3 are visible). Grass Carp has two rows of slender, grooved teeth: 4 on inner row (visible) and 2 on outer row (obscured).

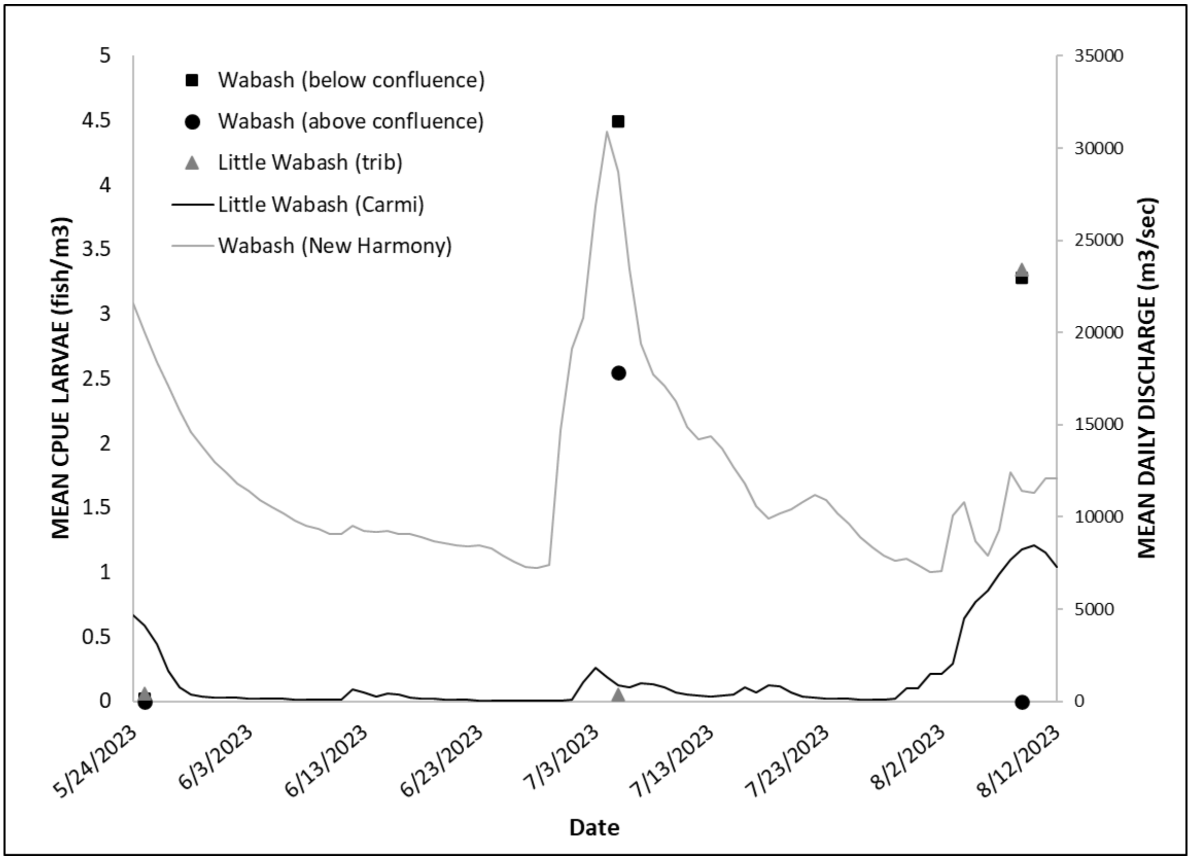

Figure 6. Catch per unit effort (fish/m^3 H2O filtered) of invasive carp larvae collected in the Little Wabash River (tributary site, grey triangles), and the Wabash River above (black circles) and below (black squares) the confluence of the tributary from May 25th to August 9th, 2023. Mean daily discharge (m^3/sec) is shown on secondary y-axis for the Little Wabash at Carmi (black line), and Wabash River at New Harmony (grey line).

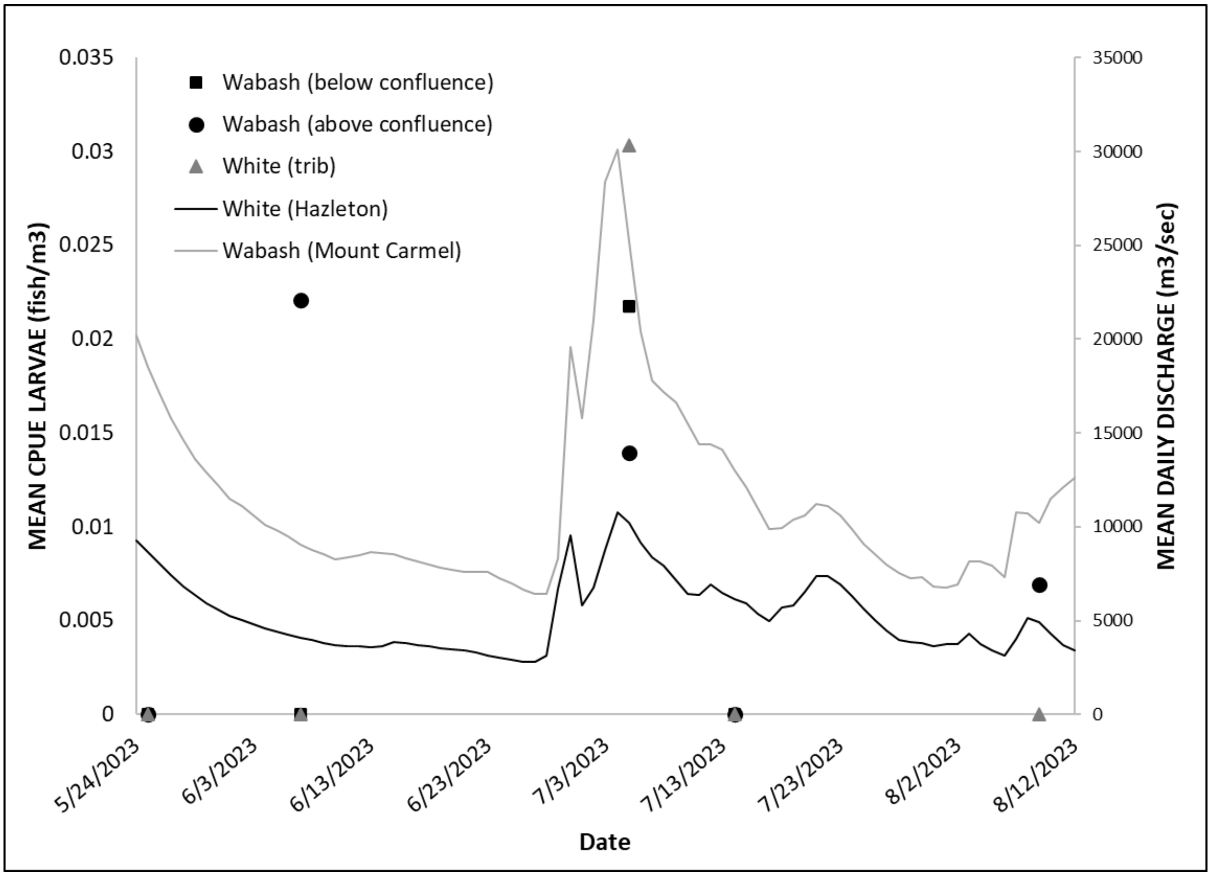

Figure 7. Catch per unit effort (fish/m^3 H_2O filtered) of invasive carp larvae collected in the White River (tributary site, grey triangles), and the Wabash River above (black circles) and below (black squares) the confluence of the tributary from May 25th to August 9th, 2023. Mean daily discharge (m^3/sec) is shown on secondary y-axis for the White River at Hazleton (black line), and Wabash River at Mount Carmel (grey line).

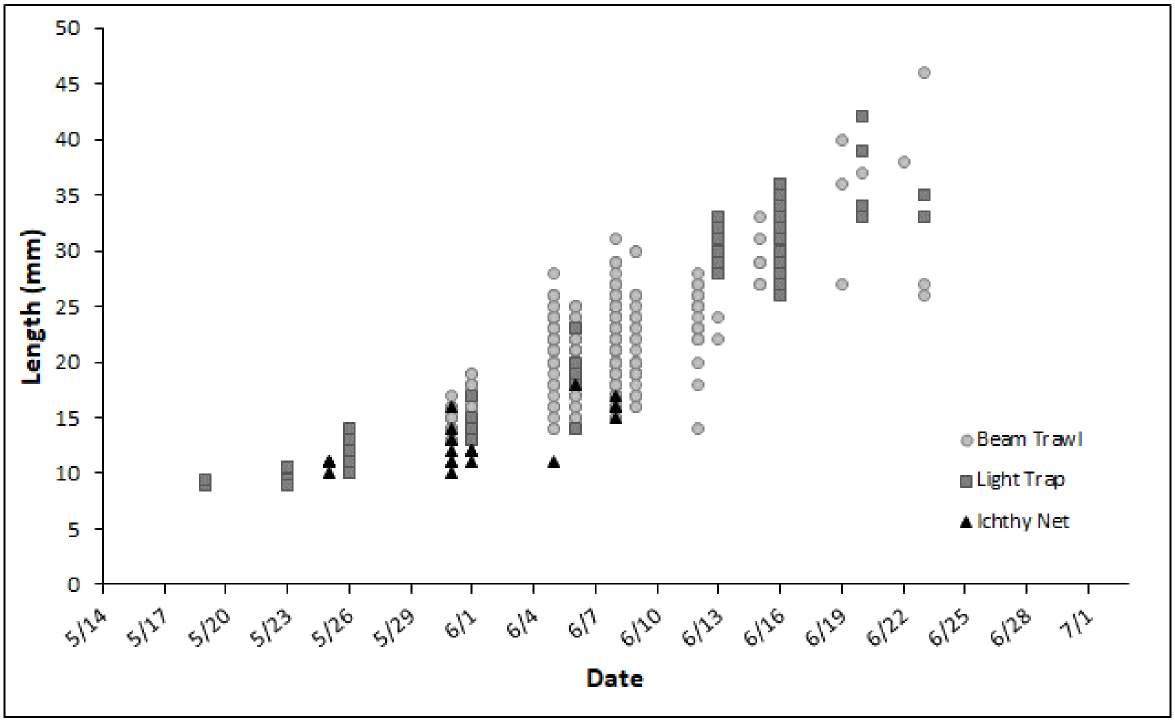

Figure 8. Length (mm) of YOY invasive carp captured within Bayou Drain of Hovey Lake during spring sampling efforts using beam trawls (circles), light traps (squares), and ichthyoplankton nets (triangles).

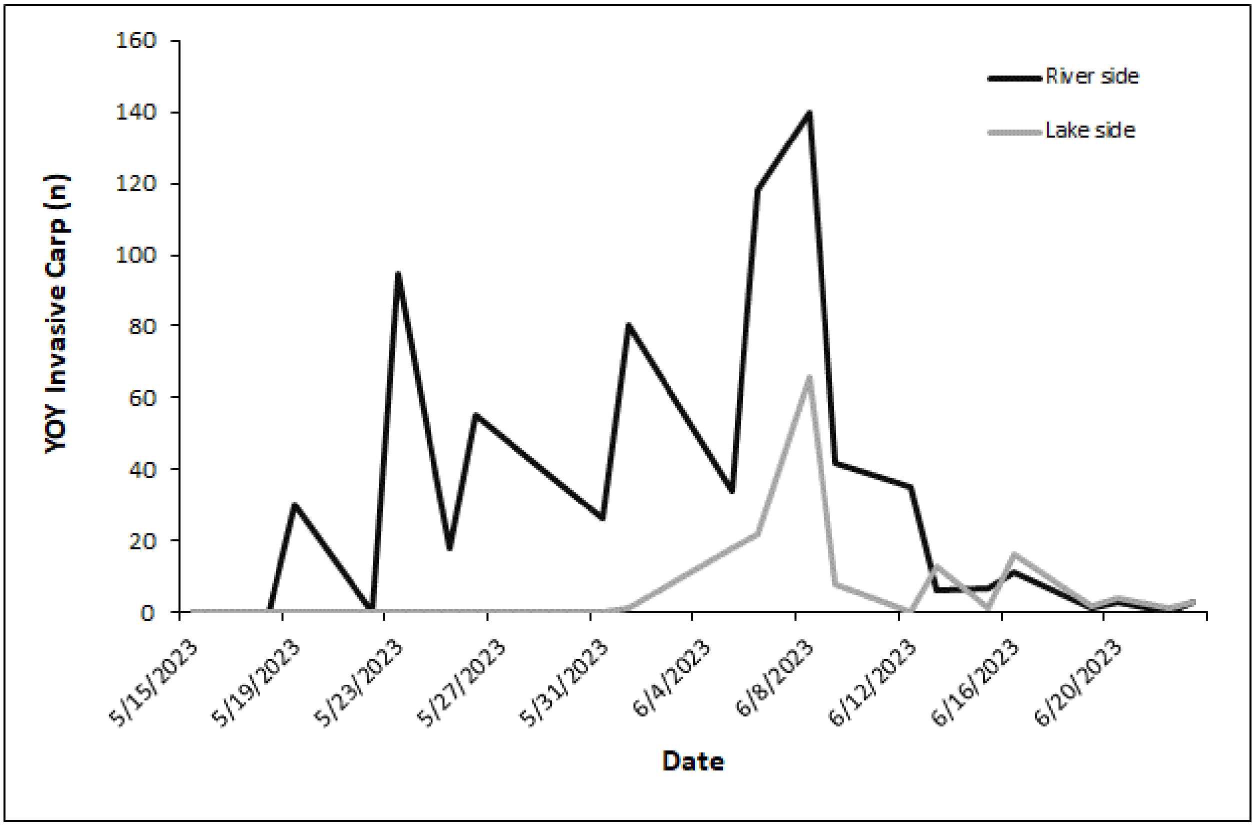

Figure 9. Number of YOY invasive carp captured within Bayou Drain of Hovey Lake during spring sampling efforts. Black line indicates the number captured on the river side of the control structure, while the grey line indicates the number captured on the lake side of the control structure.

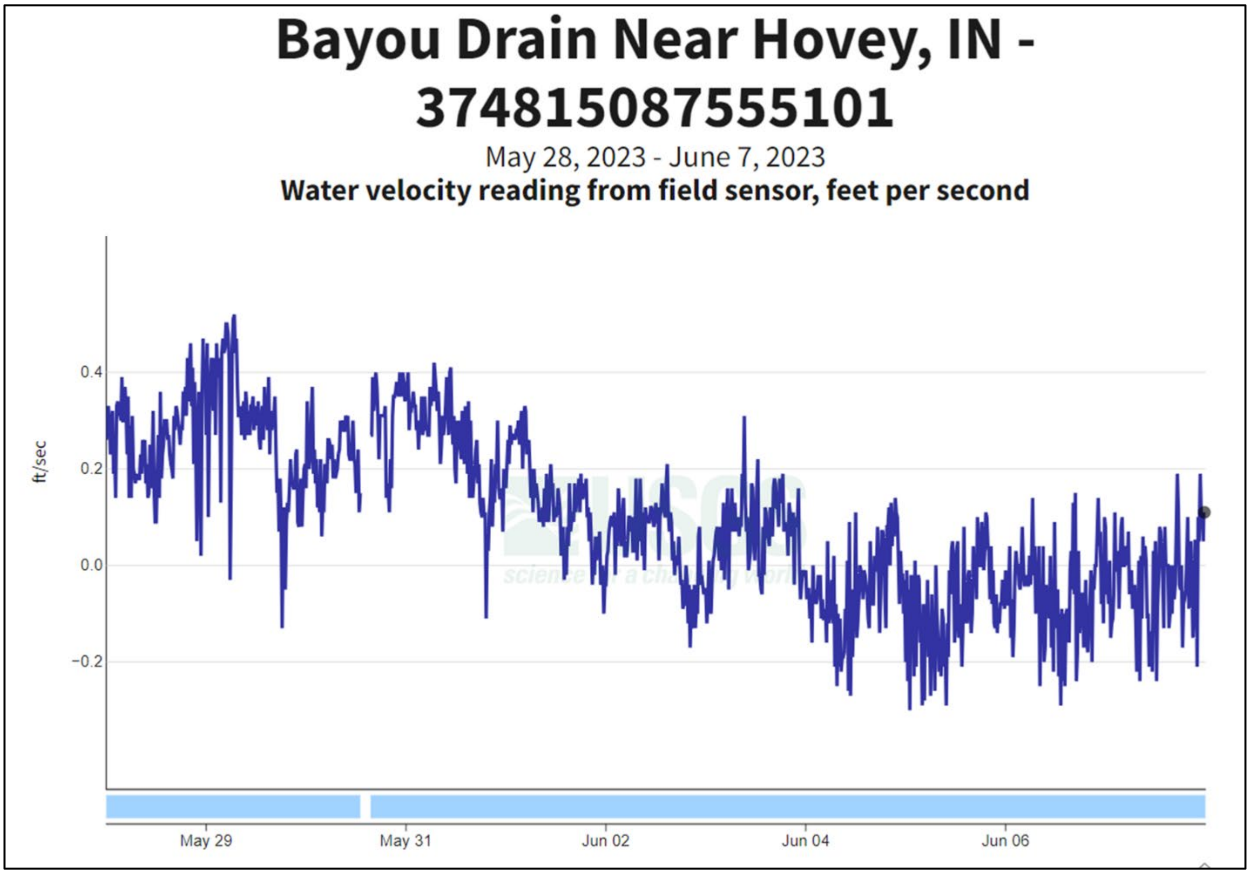

Figure 10. Water velocity readings from the monitoring gage installed within Bayou Drain of Hovey Lake near the water control structure during the week when lake and river levels began to equalize. Positive values indicate water flowing out of Hovey Lake into the Ohio River, while negative values indicate reverse flow into the lake. Note that data are still provisional and subject to change as calibrations are made. Debris near the control structure appears to be impacting the quality of data being collected.

WGL Report: March 27, 2024

Genetic Identification Via Sanger Sequencing

Indiana Department of Natural Resources
By: Zeb Woiak & Aaron Johnson

Samples (n=88) were received by the Whitney Genetics Lab from the Indiana Department of Natural Resources. Samples were kept in a -20°C freezer until they were processed by WGL lab staff.

Methods

We used our laboratory's standardized methods for Sanger sequencing. Samples were extracted using a modified version of the IBI Scientific gMAX extraction kit with a lyse and spin column (Qiagen) and a final elution volume of 200µL. For DNA extraction procedures and all further analyses, clean laboratory practices and appropriate anti-contamination precautions were used.

Samples were sequenced at the cytochrome c oxidase I gene (COI), which is commonly referred to as the 'barcode gene' and has been widely sequenced specifically for the purpose of species-level identification. This gene was amplified with a cocktail of four primers (VF2_+1, FishF2_+1, R1d_+1, FISHR2_+1) that are universal to most fish species (Ivanova et al. 2007; Ward et al. 2005). Amplification was accomplished with the Platinum™ Green Hot Start PCR mix (Invitrogen™ Life Technologies, Carlsbad, CA) in 25 µl reactions, using primers references above modified with M13 tags to streamline sequencing work. PCR products were cleaned with ExoSAP-IT® PCR Product Cleanup (Affymetrix, Santa Clara, CA). All PCR products were cycle sequenced in 1/16th BigDye Terminator v1.1 (Life Technologies, Carlsbad, CA) 20 µL reactions using both the forward and reverse primers.

Clean-up of the sequences before analysis was done with BigDye Xterminator kits (Life Technologies) to remove unincorporated bases. Sequence data were collected on an Applied Biosystems 3500XL Genetic Analyzer (Life Technologies). Sequences for each sample were trimmed, manually edited, and de novo assembled using the default Geneious assembler software. If a sample had one forward or reverse sequence that was low quality or failed, the other sequence was carried forward for all further analyses. Sequences were then compared to sequence data using the Basic Local Alignment Search Tool (BLAST) for all sequences in NCBI GenBank.

Results

Of the 88 samples 83 amplified (Table 1) and 33 could only be identified to genus. We sequenced both forward and reverse directions for 75 of the samples and used the assembled sequences for the final analysis. Six samples were successfully amplified but only matched ~94% to reference sequences in GenBank. These samples are marked with an asterisk because we do not have confidence in any species assignments that are less than 98% identical. Control samples were as expected. Sample failure may be due to low quality DNA or a failure in the sample processing. Final species assignments are in the attached table (Table 1). In the attached spreadsheet there are two tabs (1) Final Species Assignment and (2) Top 5 Blast Results. As mentioned above, some samples have final species assignments that are only assigned to a genus (e.g., *Notropis* spp.), but there is only a single species listed in their top 5 blast results. This is a result of a lack of genetic diversity between closely related species at the locus we analyzed.

FASTA sequences can be sent in a file which may be opened using Microsoft Notepad, if needed. Please let me know if you have any questions or concerns.

References

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Ward RD, R Hanner, PDN Hebert. 2009. The campaign to DNA barcode all fishes, FISH-BOL. Journal of Fish Biology 74:329–356.

Table 1. Species assignment based on sequence data from cytochrome c oxidase I (COI) mitochondrial locus. Results were derived using the Basic Local Alignment Search Tool and NCBI GenBank. Table values for samples that failed to sequence were populated with "*No Data*".
* Denotes samples with less than a 98% match to any reference sequence from NCBI GenBank.

IDNR Sample #	Final Species Assignment
1	*Notropis* spp.
2	*Notropis* spp.
3	*Notropis* spp.
4	*Aplodinotus grunniens*
5	*Aplodinotus grunniens*
6	*Notropis* spp.
7	*Notropis* spp.
8	*Hypophthalmichthys molitrix*
9	*Ctenopharyngodon idella*
10	*Ctenopharyngodon idella*
11	*No Data*
12	*No Data*
13	*Notropis* spp.
14	*Notropis* spp.
15	Pomoxis annularis
16	*Cyprinus carpio*
17	*Cyprinus carpio*
18	*Lepomis humilis*
19	*Lepomis humilis*
20	*Lepomis humilis*
21	*Lepomis humilis*
22	*Aplodinotus grunniens*
23	*Cyprinus carpio*
24	*Lepomis humilis*
25	*Lepomis humilis*
26	*Cyprinus carpio*
27	*Lepomis humilis*
28	*Lepomis humilis*
29	*Ictiobus* spp.
30	*Ictiobus* spp.
31	*Ictiobus* spp.
32	*Ictiobus* spp.
33	*Ictiobus* spp.
34	*Ictiobus* spp.
35	Hypophthalmichthys molitrix
36	Hypophthalmichthys molitrix
37	Hypophthalmichthys molitrix
38	Hypophthalmichthys molitrix
39	Hypophthalmichthys molitrix
40	Hypophthalmichthys molitrix

41	Hypophthalmichthys molitrix
42	Hypophthalmichthys molitrix
43	*Notropis* spp.
44	*Notropis* spp.
45	*Notropis* spp.
46	*Notropis* spp.
47	*Hypophthalmichthys molitrix*
48	*Ictiobus* spp.
49	*Notropis* spp.
50	Ictiobus spp.
51	*Notropis* spp.
52	*Ictiobus* spp.
53	*Lepomis humilis*
54	*Dorosoma cepedianum*
55	*Lepomis humilis*
56*	*Alosa* spp.
57	*Ctenopharyngodon idella*
58*	*Macrhybopsis* spp.
59	*Ctenopharyngodon idella*
60	*Aplodinotus grunniens*
61	*Notropis* spp.
62	*Aplodinotus grunniens*
63	*Aplodinotus grunniens*
64	Macrhybopsis storeriana
65	*Aplodinotus grunniens*
66	*Aplodinotus grunniens*
67*	*Alosa* spp.
68*	*Alosa* spp.
69	*No Data*
70	*Notropis spp.*
71	*Notropis spp.*
72*	*Alosa* spp.
73	*Notropis* spp.
74	*Aplodinotus grunniens*
75	*No Data*
76	*No Data*
77	*Aplodinotus grunniens*
78	*Aplodinotus grunniens*
79	*Aplodinotus grunniens*
80	*Aplodinotus grunniens*
81	*Aplodinotus grunniens*
82	*Aplodinotus grunniens*
83	*Aplodinotus grunniens*
84	*Notropis* spp.
85	*Aplodinotus grunniens*
86*	*Alosa* spp.
87	*Aplodinotus grunniens*
88	*Aplodinotus grunniens*