Abundance and distribution of early life stages of invasive carp in the Ohio River: Service Award Number F21AP02916

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), Ball State University (BSU), 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 at 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 2022 determined the extent of recruitment as below McAlpine L&D (Cannelton 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).

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.

2022 Project Objectives:

1) Determine the extent of bigheaded carp spawning activity in the Ohio River above Markland Dam.
2) Identify tributaries and areas of the Ohio River in which spawning of bigheaded carp occurs.
3) Determine the geographic 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) Estimate Hovey Lake recruitment potential and evaluate the feasibility of drain structure modifications to limit invasive carp recruitment from Hovey Lake.
6) Determine the propagule source of invasive carp in the Ohio River.

Project Highlights:

- Six sites were sampled above Markland Locks and Dam (RM 532) via ichthyoplankton tows from May to August, 2022. Suspicious eggs were sorted from samples collected at RM 305.2 (near Guyandotte River) and RM 356.4 (near Sciota River) and sent to Whitney Genetics Lab for verification of species. These eggs were verified as Freshwater Drum.
- Suspect *Hypophthalmichthys* larvae were collected in the Ohio River proper within J.T. Myers, Newburgh, and Cannelton Pools. Both Silver and Bighead Carp larvae were genetically confirmed in all three pools.
- Suspect *Hypophthalmichthys* larvae were collected in tributaries of Smithland Pool (Wabash and White rivers), J.T. Myers Pool (Green River), and Cannelton Pool (Deer Creek). Four genetically confirmed Silver Carp larvae were captured in the Green River and one was collected in Deer Creek. Nine suspicious larvae were caught in tributaries of R.C. Byrd Pool however genetic testing determined they were Quillback species and Bluntnose Minnow.
- Targeted surface trawling resulted in the first confirmed YOY invasive carp (Bighead and Silver Carp) being captured in Markland Pool of the Ohio River, two pools further upstream than previous detections.
- Targeted YOY Black Carp sampling occurred at 23 sites along the lower Ohio River. Nine YOY Black Carp were collected at one backwater site near Paducah, KY.
- Estimated total number of YOY invasive carp in Hovey Lake was 453,264 individuals, significantly lower than the 2021 estimate. Only 37 YOY invasive carp were captured passing through the Hovey Lake drain control structure, and net movement into the lake was insignificant.
- Water chemistry samples indicate many tributaries differ in Sr:Ca from the Ohio River. To date, 70 otolith microchemistry samples have been processed indicating a broad range of otolith core Sr:Ca. This indicates multiple recruitment sources of bigheaded carps in the Ohio River.

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 conducted ichthyoplankton tows at sampling sites within the R.C. Byrd (N = 1), Greenup (N = 1) Meldahl (N = 2), and Markland (N = 2) pools. Each sampling site was visited approximately five times from May 8 to August 6, 2022. 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.

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 = 3), McAlpine (N = 2) R.C. Byrd (N = 2) pools at least twice from June 2 to July 19, 2022, during ideal spawning conditions. 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 2 to July 19, 2022. 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:

Surface trawling effort was focused on tributaries and embayments within Smithland Pool (Mackey Bend Oxbow on Wabash River), J.T. Myers Pool (Hovey Lake drain), Cannelton Pool (Deer Creek, Millstone Creek, Clover Creek, Oil Creek, Yellowbank Creek, Poison Creek), and Markland Pool (Paint Lick Creek, Big Sugar Creek, Craigs Creek, Turtle Creek, Big Bone Creek, Laughery Creek, Hogan Creek, Arnold Creek, Grants Creek). From June 29 to September 7, 2022, a minimum of two trawls were conducted in each location. Additionally, Ball State University conducted surface trawl sampling throughout the summer of 2022 at Hovey Lake within the J.T. Myers Pool to document potential recruitment of invasive carp within the lake.

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 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 10 days. Areas were sampled with beach seine and backpack electrofishing as available. If YOY or juveniles were collected; length and weight were recorded, and the specimens were preserved for additional analysis as needed. Most sites (16 of 23) 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 attempted at four sites, but proved to be ineffective due to the difficulty of wading through deep, soft mud, which made sampling in general difficult at most sites.

Environmental variables:
A suite of habitat variables were collected at each surface trawl site including water temperature, water transparency, conductivity, pH, dissolved oxygen, maximum depth, average depth, tributary width, and presence/absence of woody debris and aquatic vegetation. Collection of environmental characteristics may determine preferred Ohio River tributaries for future invasive carp recruitment.

Hovey Lake recruitment:
YOY invasive carp were collected from Hovey Lake to estimate annual recruitment and YOY mortality. Sampling for YOY was conducted using boat electrofishing and surface trawling (3.7 m wide x 4 mm mesh, 76 cm x 38 cm mullet doors, 30 m towline). Collected YOY were frozen, and otoliths were removed after thawing. Otoliths were mounted on glass microscope slides using cyanoacrylate glue. Otoliths were polished using lapping film until the midplane was reached and circuli were visible. Daily growth data for cohorts of YOY was summarized by collection date to estimate hatch dates. Additionally, trap nets were set on both sides of the Hovey Lake drain control structure in an effort to determine YOY fish passage across the structure. HOBO (onset.comp.com) water level loggers were not functioning in 2022 and therefore no water level data within the Hovey Lake drain was captured.

Microchemistry:
Water samples were taken from the Ohio River and tributaries in August and September 2022. Tributaries that flow into J.T. Myers, Newburgh, Cannelton, McAlpine, and Markland pools were prioritized locations. Water samples were collected using a syringe filtration (0.45 µm pore size) technique and analyzed for Sr, Ba, and Ca concentrations. Additionally invasive carp otoliths were collected for microchemistry analysis. Invasive carp were sampled via electrofishing and a single otolith from 323 individuals was submitted to SIU for analysis; 168 from J.T. Myers Pool and 155 from Newburgh Pool to add to previously collected otoliths now totaling at least 250 individual fish from each J.T. Myers,

Newburgh, Cannelton, and McAlpine pools collectively in 2021 and 2022. Additional invasive carp otoliths from Markland Pool and above are being identified and will be sent for inclusion into the microchemistry analysis as well. Otoliths were embedded in epoxy, sectioned in the transverse plane, and sanded and polished to expose the otolith core. Sectioned otoliths were attached to glass microscope slides with double-sided tape in preparation for analysis by laser ablation-ICPMS.

Results:

Ichthyoplankton tows:
A combined total of 100 ichthyoplankton tows were conducted within the R.C. Byrd (N = 19), Greenup (N = 13), Meldahl (N = 33), and Markland (N = 35) pools (Table 1). A total of 70 suspicious eggs were sorted from tow samples in Greenup Pool, 20 of which were sent off to Whitney Genetics Lab for species confirmation. Five of the 20 samples returned genetic results, all of which were confirmed Freshwater Drum eggs. Additionally, 53 suspicious eggs and one suspicious larvae were identified from samples collected in Meldahl Pool; the suspicious larvae along with 10 eggs from Meldahl were sent off for genetic verification. Four of the eggs returned genetic results, all of which were confirmed Freshwater Drum; the suspicious larvae did not return genetic results. Several of the suspicious eggs from both Greenup and Meldahl pools varied in size, suggesting not all could be *Hypophthalmichthys* eggs, and many of the larger eggs lacked a large perivitelline membrane, which may have been removed by students during the sorting process. Removal of the perivitelline membrane may have effected the ability to get a genetic reading on some of the eggs.

A total of 55 ichthyoplankton tows were conducted within the mainstem Ohio River in the J.T. Myers (N=12), Newburgh (N = 12), Cannelton (N = 18), McAlpine (N = 12), and R.C. Byrd (N = 1) pools (Table 2). A total of 879 suspect *Hypophthalmichthys* larvae were collected in J.T. Myers Pool, four of which were submitted for genetic verification. Genetic results indicated one was Bighead Carp and three were Silver Carp larvae. Six suspect *Hypophthalmichthys* eggs and 881 suspect *Hypophthalmichthys* larvae were collected in Newburgh Pool; one egg and three larvae were submitted for genetic confirmation. The egg was confirmed a Silver Carp and the larvae were confirmed Silver and Bighead carps. In Cannelton Pool, 25 suspect *Hypophthalmichthys* larvae were morphometrically identified along with a couple suspicious larvea, 10 of which were submitted to Whitney Genetics Lab. Of these, five were confirmed Silver Carp, one was Bighead Carp, one was Grass Carp, two were Quillback species and one was Buffalo species. One suspicious larvae and three suspicious small YOY fish were pulled from McAlpine Pool samples but were genetically confirmed shiner species. One suspicious larvae from R.C. Byrd Pool was sent off for genetic confirmation which determined it was a shiner species.

An additional 75 ichthyoplankton tows were conducted in select tributaries of the Ohio River (Table 3; Figure 2). Multiple suspect *Hypophthalmichthys* eggs (N = 6), advanced eggs (N = 5), and larvae (N = 4) were captured in the Wabash River (above the White River confluence) in Smithland Pool, in addition to 10 suspect *Hypophthalmichthys* larvae captured in the White River. Six suspect *Hypophthalmichthys* larvae were captured in the Green River (J.T. Myers Pool), five of which were submitted for genetic confirmation; four of the five were confirmed Silver Carp, and one was a Freshwater Drum. Within Newburgh Pool, suspicious larvae were collected in Little Pigeon Creek (N = 8) and Anderson River (N =1); these specimens were determined to be shiner and sunfish species. Within Cannelton Pool, 12 suspect *Hypophthalmichthys* larvae were collected in Deer Creek, and one suspicious larvae was collected in both the Blue River and Silver Creek. Of these, one Silver Carp was confirmed in Deer Creek, along with several White Crappie, while the Blue River suspicious larvae was a buffalo. Nine suspicious larvae were caught in tributaries of R.C. Byrd Pool and therefore sent to Whitney Genetics Lab for species confirmation. The Raccoon Creek specimen was confirmed a Bluntnose Minnow, while the Kanawha River samples were all quillback species.

Surface trawl:
The Mackey Bend oxbow of the lower Wabash River (situated in Smithland Pool of the Ohio River) was sampled on June 29, 2022. Six surface trawl tows totaling 30 minutes of effort produced 11 YOY Silver Carp, ranging in size from 33 to 48 mm total length. Additionally, two surface trawl tows totaling 10 minutes of effort were conducted on June 29th within the Hovey Lake drain, on the river side of the control structure. Thirty-eight YOY Silver Carp were collected in the drain, ranging from 21 to 35 mm total length.

Among six tributaries sampled in Cannelton Pool in July of 2022, 22 surface trawls were conducted totaling 105 minutes of sampling effort. Twenty-four YOY Bighead Carp and one YOY Silver Carp were collected in Cannelton Pool through this effort; 17 Bighead Carp in Clover Creek, six Bighead Carp in Millstone Creek, one Bighead Carp in Poison Creek, and one Silver Carp in Oil Creek. Cannelton Pool YOY Bighead Carp ranged from 37 to 65 mm total length, with a mean length of 48 mm. The single Silver Carp captured in Oil Creek measured 73 mm. Among the nine tributaries sampled in Markland Pool in August of 2022, 26 surface trawls were conducted totaling 128.5 minutes of sampling effort. Thirteen YOY invasive carp and one immature carp were captured; two YOY Bighead Carp in Paint Lick Creek, one immature Bighead Carp (190 mm) in Craigs Creek, one YOY Bighead Carp in Turtle Creek, and one Bighead YOY and nine YOY Silver Carp in Arnold Creek. The YOY Silver Carp ranged from 70 to 125 mm, and the YOY Bighead Carp ranged from 59 to 84 mm, total length. Figure 3 depicts locations where YOY invasive carp were captured in the Ohio River in 2022.

Black Carp YOY Sampling:
KDFWR sampled for YOY invasive carp at 23 sites along the lower Ohio River. A total of 67 fish species representing 17 families were collected or observed at the 20 sites sampled that had fish. Two sites sampled had age-0 invasive carp. This included one site that was a plunge pool of a tributary that was no longer connected to the Ohio River. The plunge pool contained YOY Grass, Silver, and Common Carp. A second site had Common, Grass, Silver, and Black age-0 invasive carps. The second site was a shallow backwater that was also no longer connected to the Ohio River. Nine YOY Black Carp were captured at this site, ranging in length from 56 to 71 mm total length (Figure 4). All specimens were fixed in formalin and brought back to the lab to be properly identified. The YOY Grass Carp and Black Carp were identified by pulling out pharyngeal teeth and comparing their morphology (Figure 5, Thomas, M. 2022)

Hovey Lake recruitment:
Sampling events were conducted during 10 weekly trips by Ball State University. A total of 334 YOY bigheaded carp were collected from Hovey Lake and the drain. Mean CPUE for Lake Hovey collections was 0.51 individuals / min electrofishing, and ranged from 0.02 to 3.0. Length-frequency histograms by collection date resulted in distinct growth of a single cohort (Figure 6). This relationship shows that YOY bigheaded carp became detectable in Hovey Lake proper in early June, 2022. The lengths of YOY captured each week showed a single recruitment cohort during 2022, which was not evident with age of YOY by week of capture (Figure 7). The regression of CPUE in Lake Hovey on date of collection did not result in a significant relationship and r-square was only 9.0 ($P < 0.4$, Figure 3).

The estimate of Z, the instantaneous daily mortality coefficient was 0.007. The regression of YOY total length on date of collection resulted in a significant relationship with an r-square of 72.8 ($P < 0.001$):
Total length (mm) = - 45,748 + 1.023 (date)

Density of YOY bigheaded carp in Hovey Lake was estimated as 0.08 individuals / m^2. Estimated total number of bigheaded carp YOY in 2022 within Hovey Lake was estimated at 453,264 (0.08 individuals / m^2 * 5,665,800 m^2 Hovey Lake area).

Trap net collections at the Hovey Lake drain control structure resulted in 8,865 fishes in 21 taxa with higher abundances collected in nets on the river side of the control structure. Catch rates were low until larval freshwater drum and larval gizzard shad were captured in early August. The species in highest abundances were gizzard shad, freshwater drum, bluntnose minnow and emerald shiner. Sportfish captured in trap nets at the control structure included channel catfish, walleye, bluegill, white crappie, green sunfish, and black bullhead. Numbers of YOY bigheaded carp captured in trap nets were low, ranging from 1 to 11 per trap night with a total of 37 for the summer.

Microchemistry:

Water samples were collected at five tributaries within Smithland Pool, three tributaries within J.T. Myers Pool, two tributaries within Newburgh Pool, eight tributaries within Cannelton Pool, four tributaries within McAlpine Pool, and six locations within Markland Pool. Additionally, water samples were collected from the mainstem Ohio River in each pool. In general, many tributaries differ in water Sr:Ca from the Ohio River (Figure 8). The few tributaries that overlap Sr:Ca with the Ohio River include Deer Creek, Sinking Creek, 14-Mile Creek, Indian-Kentuck Creek, and the Great Miami River. Some tributaries that enter Cannelton or Markland pools (Blue and Little Blue rivers, Hogan and Tanners creeks) overlap with water Sr:Ca of the Ohio River downstream of the Wabash, but not the section of the Ohio River that they flow into. In contrast, there is a lot of overlap in water Ba:Ca between the Ohio River and tributaries and among the tributaries themselves (Figure 9). The Anderson River stands out with high water Ba:Ca and the Salt, Kentucky, and Licking rivers have low Ba:Ca. In general Ba:Ca will be much less informative than Sr:Ca.

Over 400 otolith samples have been analyzed to date from fish collected during 2021. Analysis of remaining otolith samples from 2021 should be completed by the end of March, after which 2022 otolith samples will be processed. Data processing using ElementR software to convert raw elemental count data to molar element:calcium ratios is also in progress. To date, calculations have been completed for 70 otolith samples. A frequency distribution of otolith core Sr:Ca (reflecting early life history) for these 70 fish is shown in Figure 10. For these samples, the modal otolith core Sr:Ca is between 700 and 800 µmol/mol, and there is a broad range of otolith core Sr:Ca, from 201-3974 µmol/mol.

Discussion:

Results of the seventh 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.

Prior to 2021, the leading edge of genetically confirmed invasive carp reproduction was near the Salt River (RM 630) in Cannelton Pool. Data collected in 2021 confirmed Bighead and Silver carp spawning as far upstream as RM 463 near the Little Miami River, and therefore shifted the 'invasion front' further upstream. During 2022 sampling, the furthest upstream extent of genetically confirmed invasive carp spawning was back downstream in Cannelton Pool. There were some suspicious looking eggs collected in Meldahl and Greenup Pools that we were unable to get a genetic reading on, but other eggs in those same samples were determined to be Freshwater Drum. In 2021, several invasive carp eggs and larvae were collected from the Kentucky River which was the first time spawning was detected within a tributary of the middle Ohio River. In 2022, larvae were collected from the Green River suggesting spawning may be occurring there as well. It is possible that spawning within the Green River could contribute to larvae that find their way into the Hovey Lake drain. One confirmed Silver Carp larvae was collected in Deer Creek (lower Cannelton Pool; RM 718.7). It is somewhat unlikely that this specimen was spawned within the tributary due to both the relatively small watershed and recent field observations. Although our tributary sampling protocol prevents ichthyoplankton samples from being collected within a half mile from the tributary confluence with the Ohio River, recent field observations confirmed heavy Ohio River flows pushing into Deer Creek, presumably due to lock and dam operations nearby. The flows observed pushing into Deer Creek were not measured, but appeared to be significant enough that eggs and larvae from the Ohio River could easily be transported into the tributary over a half mile. These findings will be important to consider moving forward with management actions in the Ohio River basin.

Among the subsamples of suspected *Hypophthalmichthys* larvae and invasive carp-type eggs sent to Whitney Genetics Lab, most *suspect* larvae were confirmed to be Silver and Bighead Carp. There were also Grass Carp, Mosquitofish, Freshwater Drum, White Crappie, shiner sp., sunfish sp., buffalo sp., and quillback sp. genetically identified in our samples. As previously mentioned, we knew that not all eggs/larvae sent to Whitney Genetics Lab from 2022 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 and confidently identified by our 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.

In 2021, the first confirmed YOY Silver Carp were captured in Cannelton Pool. The same pattern occurred in 2022, with YOY invasive carp being captured at several locations within Cannelton Pool again. Additionally, KDFWR received reports of YOY invasive carp in Markland Pool and captured two juveniles there in 2021. This prompted targeted YOY sampling to occur in Markland Pool in 2022, which resulted in multiple YOY and one juvenile bigheaded carps being captured. Invasive carp YOY were detected in three of nine sampled tributaries in Markland Pool, and a single 190 mm juvenile Bighead Carp was captured in Craigs Creek. It is unclear how long recruitment has been occurring within Markland Pool, but anecdotally, the low relative density of adult invasive carp suggest recruitment has been minimal in previous years. Regardless, these data confirm that the establishment front for invasive carp in the Ohio River has shifted further upstream. Interestingly, a relatively high percentage of the YOY invasive carp captured in 2022 were Bighead Carp, indicating Bighead Carp had favorable spawning conditions. Since this project's inception, the vast majority of YOY invasive carp captured have been Silver Carp, noticeably different than what was collected during 2022.

In addition to the targeted YOY sampling, three small (91mm, 104 mm, 107 mm) Silver Carp were captured in Oil Creek on April 19th during spring electrofishing efforts as part of the 'Early Detection and Evaluation' project. These fish were captured before any 2022 invasive carp spawning would have occurred, indicating overwinter YOY Silver Carp survival in Cannelton Pool. Because of presumed high mortality rates of YOY fish, we've previously questioned whether YOY invasive carp captured during summer sampling events truly indicates if recruitment is occurring in an area. After first documenting YOY Silver Carp in Oil Creek in 2021, then finding small age-1 Silver Carp there in spring of 2022, we are confident that presence of YOY in summer targeted sampling properly identifies areas of potential recruitment.

Efforts in 2022 revealed presence of age-0 Black Carp at a single location out of 23 sites sampled along the lower Ohio River in Livingtson, McCracken, and Ballard counties. The location is along the Ohio River shoreline at river mile 935 within the city limits of Paducah, which is 27 miles upstream of Gar Creek, where the single age-0 fish was collected in 2016. This occurrence is further evidence of Black Carp reproduction in the lower Ohio River drainage in western Kentucky. No large juvenile or adult Black Carp were captured or observed. 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.

Many juvenile invasive carp were again collected in 2022 in or near Hovey Lake in J.T. Myers Pool. Hovey Lake continues to demonstrate its importance as a nursery area for invasive carp recruitment in the Ohio River. To date, work at Hovey Lake has been able to estimate density of YOY invasive carp in the lake, and provide information on YOY growth rates, mortality, and approximate hatch dates. There was an attempt to quantify YOY fish passage through the Hovey Lake drain control structure, however very few invasive carp (N = 37) were captured, and their net movement into the lake was near zero. Additionally, limited environmental condition data was available, and therefore we are still unable to infer what specific conditions allow for YOY invasive carp to enter Hovey Lake. Because there were no high-flow events during late-May through June in 2021 or 2022, we are confident that YOY invasive carp are entering Hovey Lake through the only direct connection – through culvert pipes at a control structure located in the Hovey Lake drain (Bayou Drain). Unfortunately, we are still unable to determine exactly what conditions (flow direction and velocity through the control structure culvert pipes) are most conducive for passage of YOY invasive carp. A final report summarized work to date was completed by Ball State University.

Preliminary otolith microchemistry data indicates a broad range of otolith core Sr:Ca. These data suggest multiple recruitment sources of bigheaded carps in the study area, including tributaries with lower water Sr:Ca than the Ohio River and a few fish that likely originated in tributaries with high water Sr:Ca; the latter primarily enter Markland Pool or upriver. Data processing for fish caught in 2021 will be completed by early April, as well as using water chemistry data to estimate upper and lower limits of otolith Sr:Ca and Ba:Ca for different sections of the Ohio River and tributaries. This will allow us to infer early life environment for individual fish and to evaluate whether environments used during early life history differ among bigheaded carps collected from different areas. A comprehensive final report will be shared with the Ohio River basin partnership when remaining otolith samples are processed.

There has not been what we would consider a strong spawning event or year-class since this project was initiated in 2016. However, based on the presence of adult invasive carp as far upstream as R.C. Byrd Pool, the new findings of YOY invasive carp in Markland Pool in 2022, 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 ichthyoplankton tows 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. We do however recommend that a more uniform ichthyoplankton sampling design be considered, so that we not only have comparable data within the Ohio River, but among other sub-basins as well. Developing a sampling design that is robust, yet takes into consideration overall time commitment and the most efficient periods to be sampling should be a priority. A suggested approach would be to develop sampling designs that will best inform the FluEgg model (i.e. well documented larval development status at various locations), and back-calculate to locate potential mainstem spawning areas in the Ohio River. And as previously recommended, biologists should continue to use best management practices to preserve the DNA integrity of the samples.

Based on genetically confirmed results from the past three years, physical morphometrics can be successful in identifying *Hypophthalmichthys* advanced eggs and larvae from other native fish species. 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 more ‘suspicious’ eggs and larvae submitted to the genetics lab this year than in the past. 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 increasing invasive carp YOY sampling in the lower Ohio River with an emphasis on searching for YOY black carp. More of this work is planned for 2023. With regards to YOY Silver and Bighead carp sampling, we recommend more targeted sampling occur in Markland Pool and areas further upstream. Both KDFWR and WVU are now outfitted with surface trawls which will be used to greatly expand YOY sampling further upstream.

Additional work is still needed to quantify YOY invasive carp passage into Hovey Lake. We recommend INDNR conduct intensive sampling on both sides of the drain control structure in the spring of 2023 using a multi-gear approach. Light traps, ichthyoplankton tows, and surface trawling should be conducted at a minimum multiple times a week. Data collections should then be paired with a new USGS stream flow and velocity gage to determine precise flow conditions that allow for YOY invasive carp passage into the lake.

Other ongoing projects in the Ohio River basin are gathering data on presence of spawning patches on invasive carp; combining these data with information gathered through this project will help managers identify spatiotemporal patterns of invasive carp reproduction in the Ohio River. This information, along with recruitment patterns we have documented previously, can ultimately be used to identify sources of invasive carp population expansion throughout the basin, and help guide other ORFMT efforts such as deterrents and targeted removals.

Acknowledgements:

We would like to thank Zeb Woiak 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. An asterisk (*) denotes genetically confirmed *Hypophthalmichthys* samples, or the lack thereof, analyzed by Whitney Genetics Lab.

Sampling Information				Suspicious Samples to WGL (N)		Suspect *Hypophthalmichthys* (N)		
Pool	**Location**	**Transect Type**	**Tows (N)**	**Eggs**	**Larvae**	**Eggs**	**Advanced Eggs**	**Larvae**
Markland	RM 496.7 (near Hogan's Cr.)	Ohio River	10	0	0	0	0	0
Markland	Hogan's Creek	Tributary	5	0	0	0	0	0
Markland	RM 463.5 (near Little Miami R.)	Ohio River	14	0	0	0	0	0
Markland	Little Miami	Tributary	6	0	0	0	0	0
Medahl	RM 404.7 (near J.M. Stuart)	Ohio River	14	0	1	0	0	0
Medahl	J.M. Stuart Plant	At structure	6	0	0	0	0	0
Medahl	RM 356.4 (near Sciota R.)	Ohio River	13	5	0	0*	0	0
Greenup	RM 305.2 (near Guyandotte R.)	Ohio River	13	20	0	0*	0	0
R.C. Byrd	RM 276.1 (near Raccoon Cr.)	Ohio River	1	0	1	0	0	0*
R.C. Byrd	Raccoon Creek	Tributary	13	0	1	0	0	0*
R.C. Byrd	RM 265.1 (near Kanawha R.)	Ohio River	14	0	0	0	0	0
R.C. Byrd	Kanawha River	Tributary	19	0	8	0	0	0*

Table 2. Summary of ichthyoplankton tows collected in the Ohio River proper by the Kentucky Department of Fish and Wildlife Resources and Indiana Department of Natural Resources. An asterisk (*) denotes genetically confirmed *Hypophthalmichthys* samples, or the lack thereof, analyzed by Whitney Genetics Lab.

Sampling Information				Samples to WGL (N)		Suspect *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	4
J.T. Myers	RM 784.0 (near Green R.)	Ohio River	6	0	4	0	0	875*
Newburgh	RM 772.8 (near Little Pigeon Cr.)	Ohio River	6	1	3	1*	0	703*
Newburgh	RM 731.3 (near Anderson R.)	Ohio River	6	0	0	5	0	178
Cannelton	RM 718.7 (near Deer Cr.)	Ohio River	6	0	5	0	0	12*
Cannelton	RM 662.9 (near Blue R.)	Ohio River	6	0	5	0	0	13*
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	3	0	0	0*
McAlpine	RM 545.8 (near Kentucky R.)	Ohio River	6	0	1	0	0	0

Table 3. Summary of ichthyoplankton tows collected in Ohio River tributaries by the Kentucky Department of Fish and Wildlife Resources and Indiana Department of Natural Resources. An asterisk (*) denotes genetically confirmed *Hypophthalmichthys* samples, or the lack thereof, analyzed by Whitney Genetics Lab.

Sampling Information				Samples to WGL (N)		Suspect *Hypophthalmichthys* (N)		
Pool	**Location**	**Transect Type**	**Tows (N)**	**Eggs**	**Larvae**	**Eggs**	**Advanced Eggs**	**Larvae**
Smithland	Wabash River	Tributary	3	0	0	0	0	10
Smithland	White River	Tributary	3	0	0	6	5	4
J.T. Myers	Hovey Lake Drain	Tributary	4	0	10	0	0	1474*
J.T. Myers	Green River	Tributary	6	0	5	0	0	6*
Newburgh	Little Pigeon Creek	Tributary	6	0	5	0	0	0*
Newburgh	Anderson River	Tributary	6	0	1	0	0	0*
Cannelton	Deer Creek	Tributary	6	0	5	0	0	1*
Cannelton	Blue River	Tributary	6	0	1	0	0	0*
Cannelton	Silver Creek	Tributary	3	0	1	0	0	0
McAlpine	Harrods Creek	Tributary	6	0	4	0	0	0*
McAlpine	Kentucky River	Tributary	6	0	3	0	0	0*

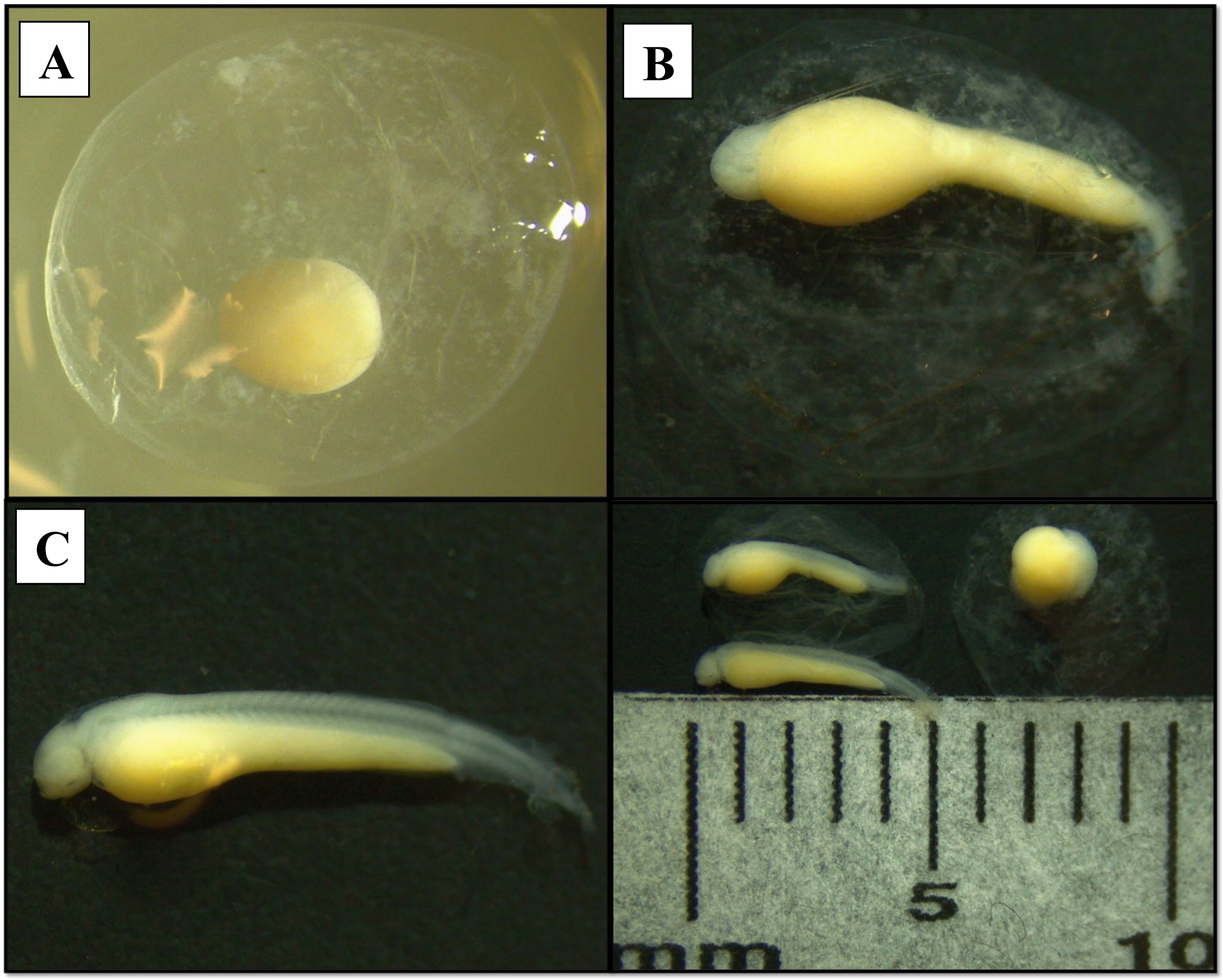

Figure 1. Developmental life stages of *Hypophthalmichthys* spp. with size comparisons. For the purposes of this report, pictures A, B, and C demonstrates specimens categorized as "eggs", "advanced eggs", and "larvae", respectively.

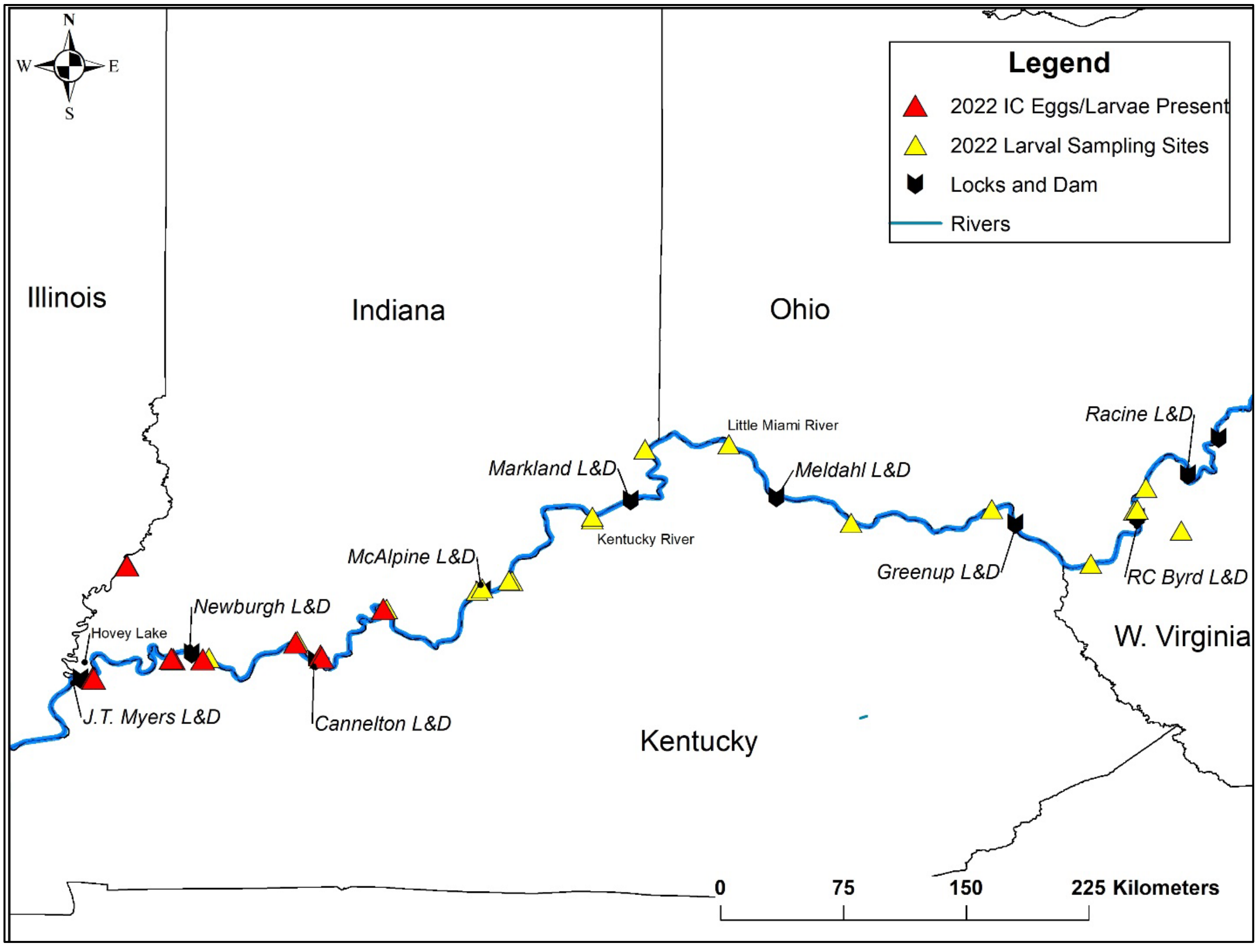

Figure 2. Map of 2022 study area of larval sampling sites. Black icons denote a locks and dam, yellow triangles indicate larval sampling sites, red triangles indicate locations where confirmed *Hypophthalmichthys* eggs, embryos, or larvae were collected.

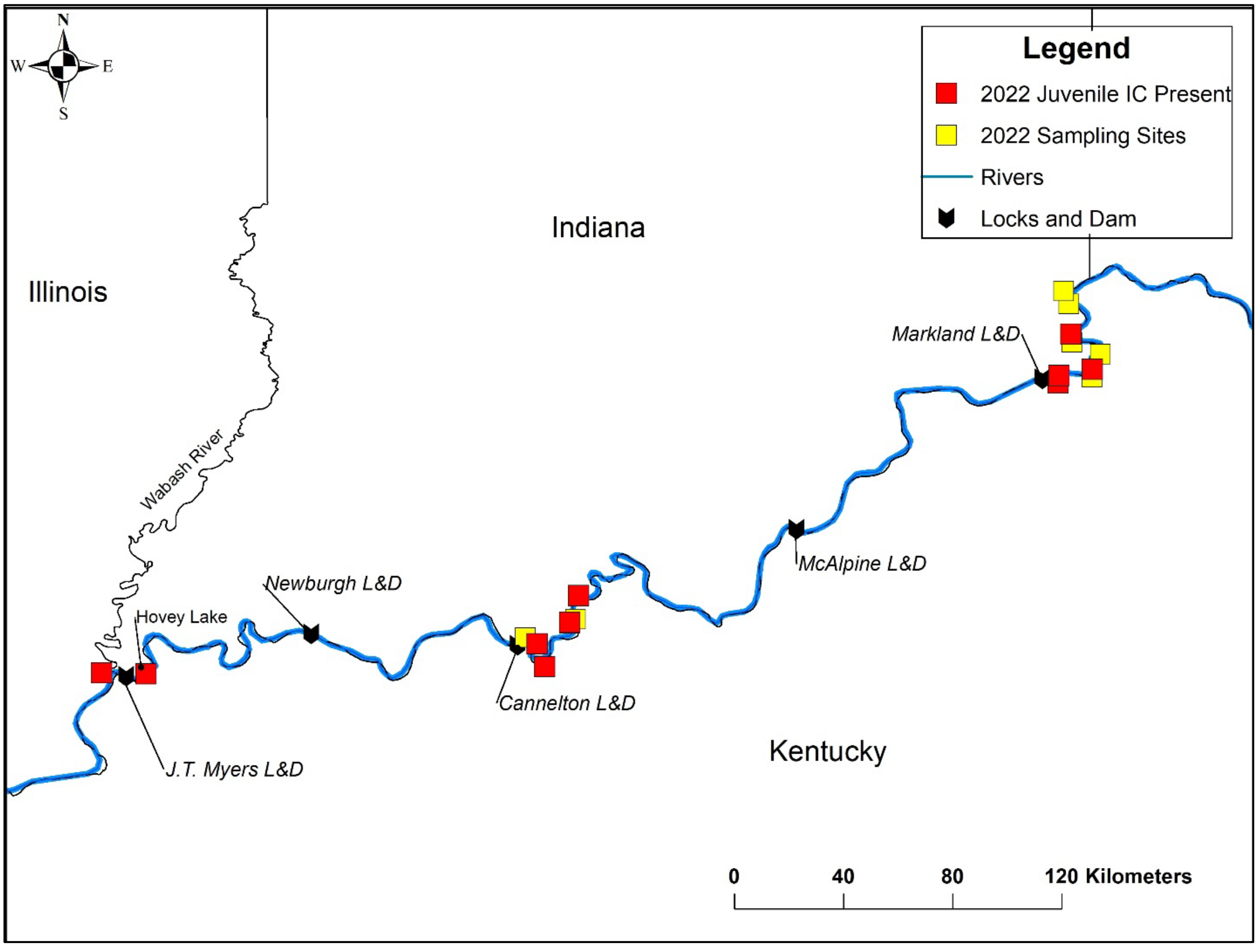

Figure 3. Map of 2022 study area of targeted juvenile sampling sites. Black icons denote a locks and dam, yellow squares indicate targeted sampling sites, red squares indicate locations where YOY or juvenile *Hypophthalmichthys* were collected.

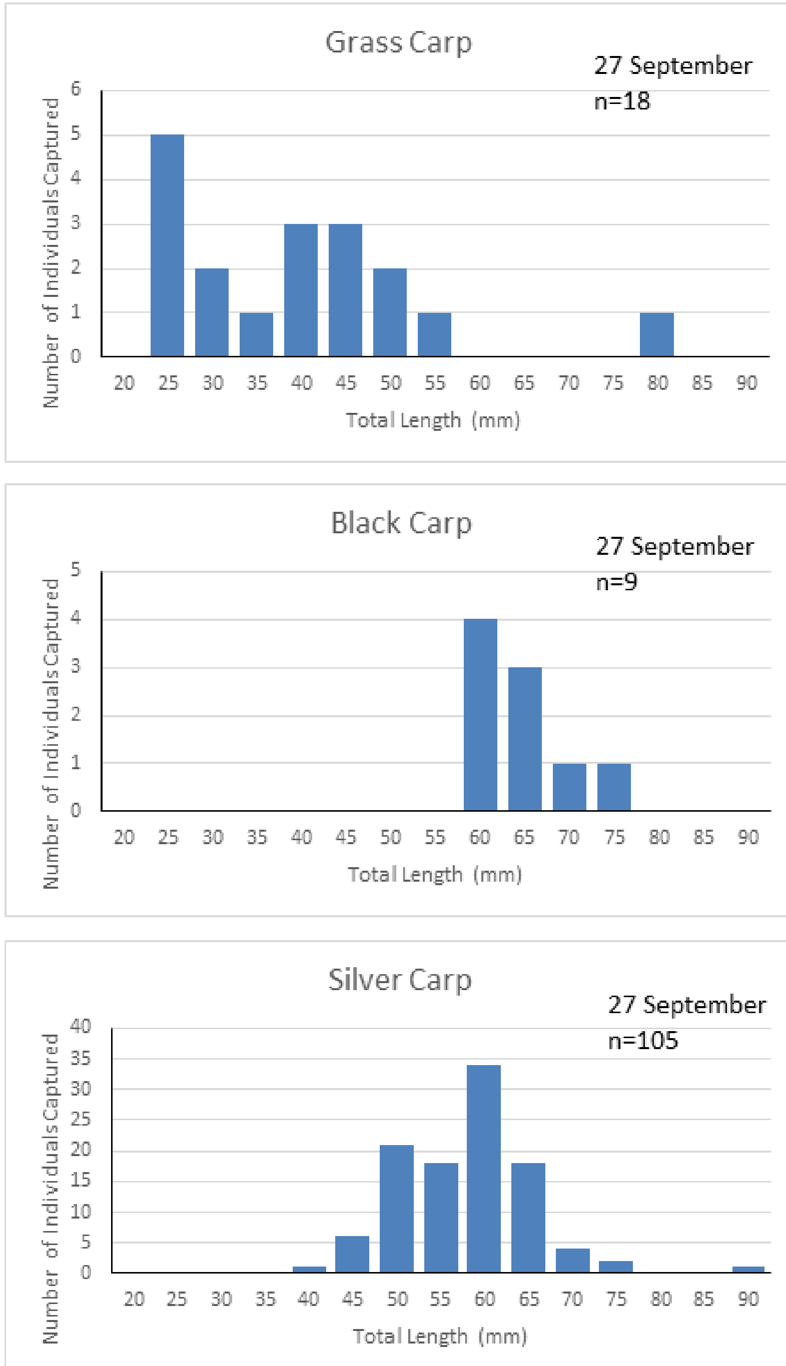

Figure 4. Length frequency distributions of Grass, Black and Silver carp collected in the shallow backwater along the lower Ohio River during targeted YOY Black Carp sampling.

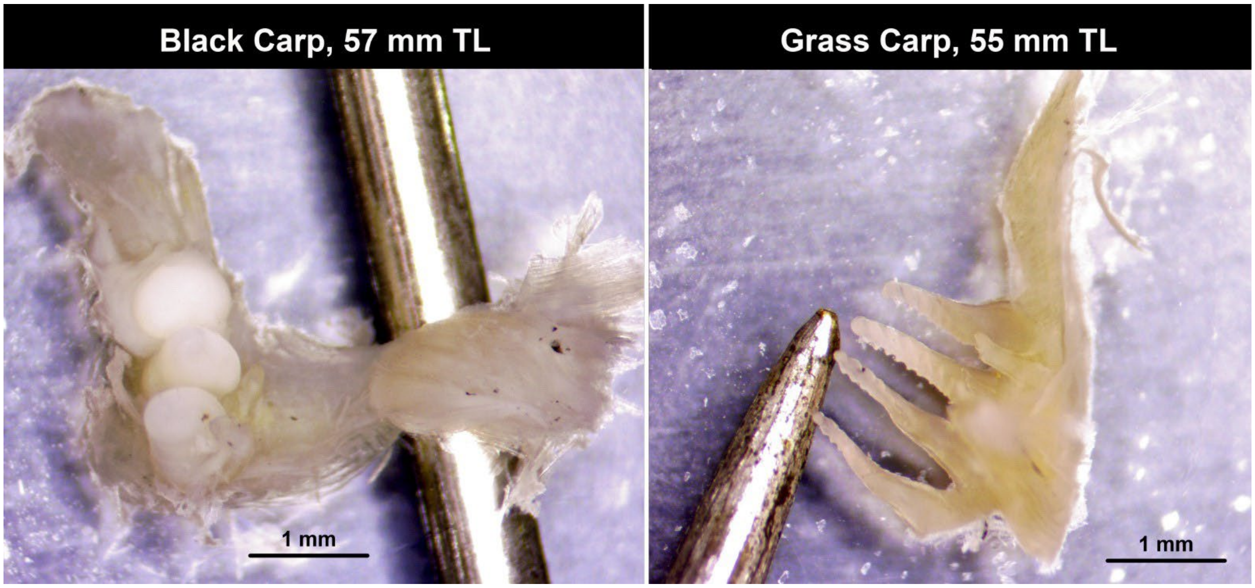

Figure 5. Comparison of pharyngeal tooth morphology between small juvenile Black and Grass carps of approximately the same size. Shown for each species is the dissected right pharyngeal arch. Black Carp has single row of 4 molar-like teeth (3 are visible). Grass Carp has two rows of slender, grooved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

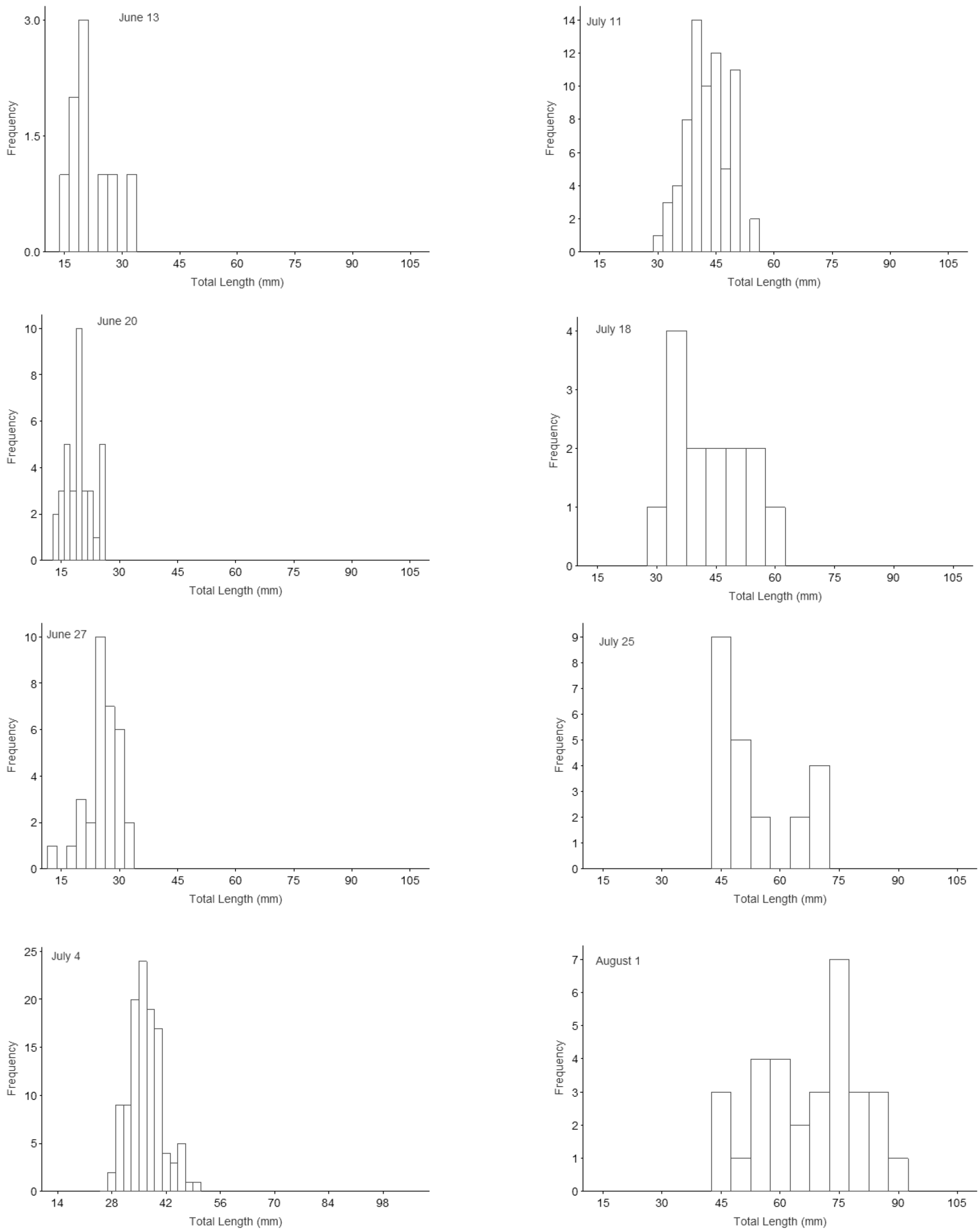

Figure 6. Length-frequency histograms for total length of YOY invasive carp by collection date from Hovey Lake in 2022.

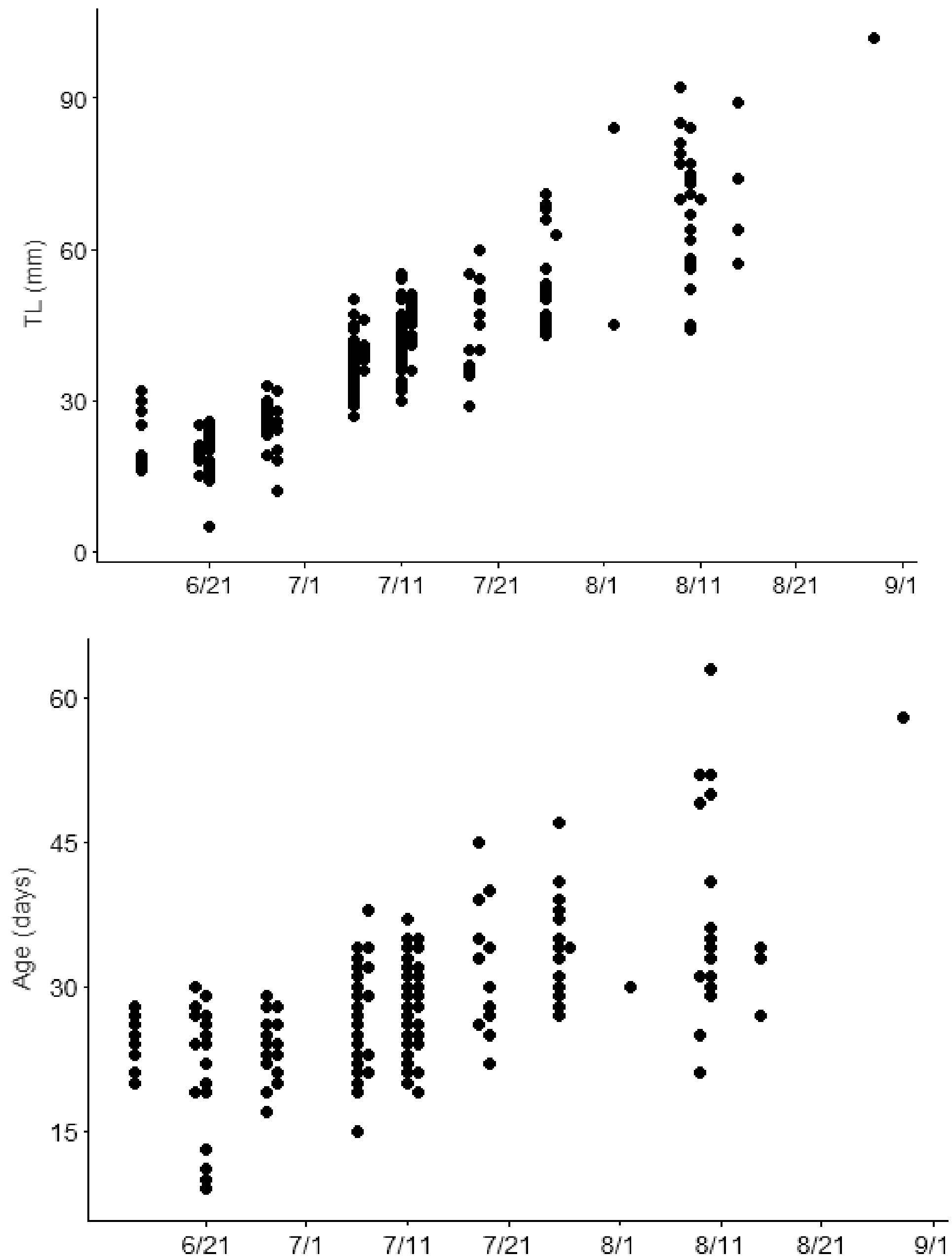

Figure 7. Total length of YOYinvasive carp by capture date (top) and ages of YOY by capture date (bottom) at Hovey Lake, 2022.

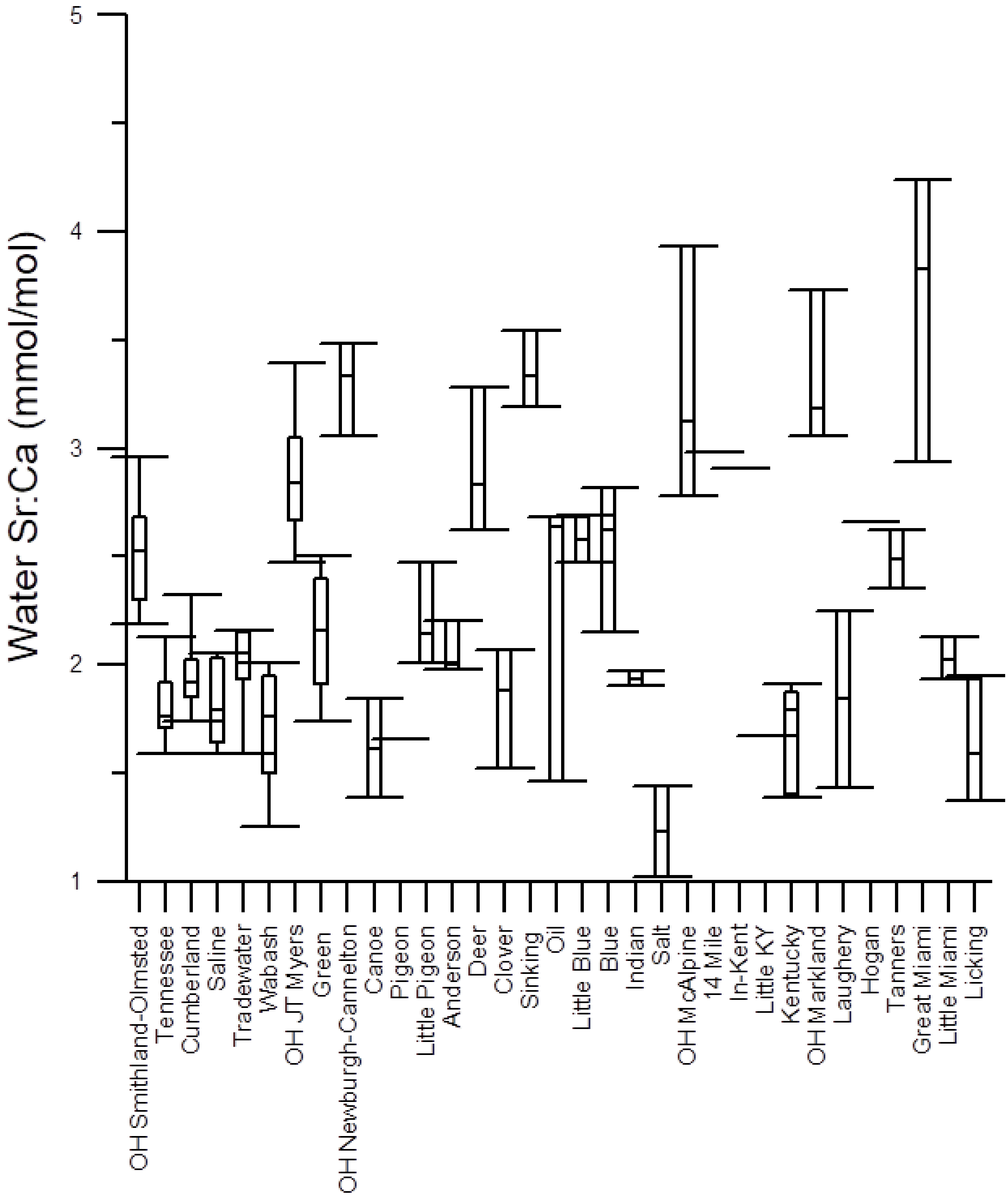

Figure 8. Boxplot of water Sr:Ca data from the Ohio River and tributaries that enter the river from Markland Pool and downstream, including data from 2022 water sample collections for this project and data from prior collections (when present).

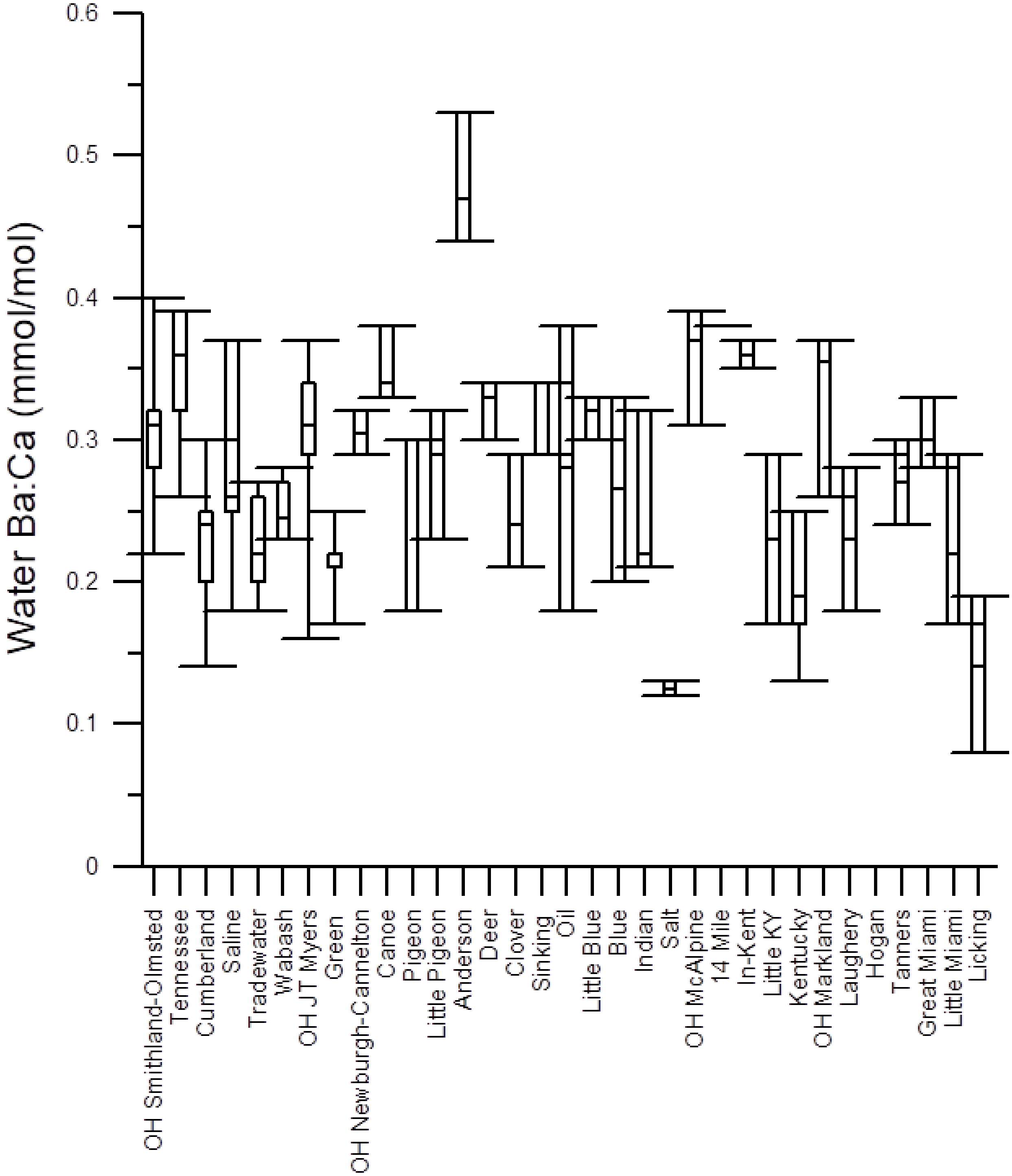

Figure 9. Boxplot of water Ba:Ca data from the Ohio River and tributaries that enter the river from Markland Pool and downstream, including data from 2022 water sample collections for this project and data from prior collections (when present).

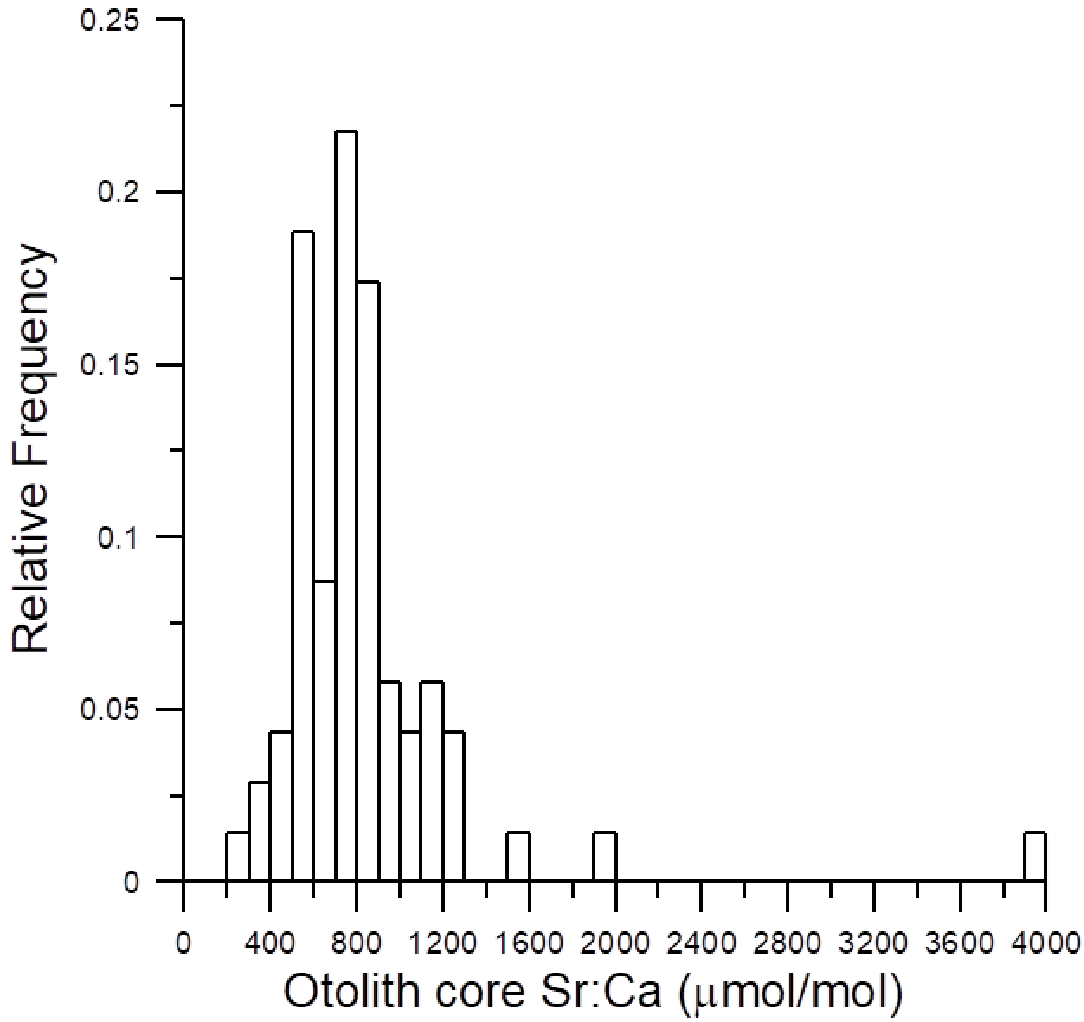

Figure 10. Frequency distribution of otolith core Sr:Ca from 70 invasive carp otoliths collected in the Ohio River during 2021 sampling.

Appendix A: Whitney Genetics Lab Report – 2022 Sampling.

WGL Report: April 10, 2023

Genetic Identification of Larval Fish

Indiana Department of Natural Resources
By: Zeb Woiak

Craig Jansen sent 98 samples to the Whitney Genetics Lab and were received by Zeb Woiak. Samples were kept in a -20^{c} freezer until they could be processed by WGL lab staff.

Methods

We used our laboratory's standardized methods that are common in many core sequencing facilities. Samples were extracted using a modified version of the IBI Scientific gMAX extraction kit with Qiagen Lyse and Spin columns 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 1 gene (UCOI), which is commonly referred to as 'the barcode of life' (Ward et al. 2009) and has been sequenced for over 138,653 animal species specifically for the purpose of species-level identification. This gene was amplified with a cocktail of 4 primers 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 (from references above) modified with M13 tags to streamline sequencing work. PCR products were cleaned up for sequencing with ExoSAP-IT® PCR Product Cleanup (Affymetrix, Santa Clara, CA) and then cycle sequenced in 1/16th BigDye Terminator v3.1 (Life Technologies, Carlsbad, CA) 20-µL reactions.

Clean-up of the sequences before analysis was done with BigDye Xterminator kits (Life Technologies) to remove un-incorporated bases. Sequence data was collected on an Applied Biosystems 3500XL Genetic Analyzer (Life Technologies). Sequences for each sample were edited by eye, trimmed, and aligned using Geneious DNA analysis software and compared to sequence data contained in GenBank using the Basic Local Alignment Search Tool (BLAST) for all sequences in NCBI GenBank. FASTAsequence files can be sent to you in a file which may be opened using Microsoft Notepad, if needed.

Results

66 of 98 samples successfully amplified at one or more genes (Table 1). 24 of the 66 samples could only be identified down to genus. For 23 of those samples, this is a result of a lack of genetic diversity between the closely related species at the locus we analyzed. However, for sample #41 it is likely because of mis-identified reference sequences in the NCBI GenBank database. Control samples were as expected. Sample failure may be due to low quality DNA or a failure in the sample processing. Please let me know if you have any questions or concerns.

Appendix A: Whitney Genetics Lab Report – 2022 Sampling.

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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 identification results based on sequence data from cytochrome oxidase 1 (UCOI) mitochondrial locus. Percent sequence match is between the observed sequence and the reference sequence of the stated length (total base pairs) from the BLAST search. Table values for samples that failed to sequence were populated with "-".

	UCOI				
Sample ID	Accession Number	Species	Common Name	% Match	Length
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	MG570455	Notropis spp.	Shiner species	99.7	602
5	MG570456	Notropis spp.	Shiner species	99.5	588
6	NC_033925	Notropis spp.	Shiner species	99.7	603
7	NC_033925	Notropis spp.	Shiner species	99.6	568
8	-	-	-	-	-
9	MG570456	Notropis spp.	Shiner species	99.5	589
10	NC_033925	Notropis spp.	Shiner species	100	601
11	-	-	-	-	-
12	-	-	-	-	-
13	-	-	-	-	-
14	-	-	-	-	-
15	-	-	-	-	-
16	-	-	-	-	-
17	-	-	-	-	-
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	-	-	-	-	-
22	-	-	-	-	-
23	-	-	-	-	-
24	-	-	-	-	-
25	-	-	-	-	-
26	-	-	-	-	-
27	-	-	-	-	-
28	-	-	-	-	-

29	-	-	-	-	-
30	-	-	-	-	-
31	-	-	-	-	-
32	-	-	-	-	-
33	-	-	-	-	-
34	-	-	-	-	-
35	-	-	-	-	-
36	-	-	-	-	-
37	ON259410	Hypophthalmichthys molitrix	Silver carp	100	607
38	ON259410	Hypophthalmichthys molitrix	Silver carp	99.8	605
39	OL825609	Gambusia affinis	Mosquitofish	100	597
40	JN027541	Notropis spp.	Shiner species	99.7	592
41	MF621712/OL457399	Lepomis machrochirus/ Lepomis cyanellus	Sunfish species	99.8/99.5	599
42	MT577130	Notropis spp.	Shiner species	99.8	585
43	JN027428	Notropis spp.	Shiner species	99.8	576
44	NC_033925	Notropis spp.	Shiner species	99.8	585
45	JN027428	Notropis spp.	Shiner species	100	572
46	NC_026528	Ictiobus spp.	Buffalo species	100	589
47	ON259410	Hypophthalmichthys molitrix	Silver carp	100	603
48	MG599474	Aplodinotus grunniens	Freshwater drum	99.7	594
49	OP001136	Hypophthalmichthys molitrix	Silver carp	100	565
50	OP001136	Hypophthalmichthys molitrix	Silver carp	100	568
51	OP001134	Hypophthalmichthys molitrix	Silver carp	100	595
52	OP001134	Hypophthalmichthys molitrix	Silver carp	99.8	580
53	OP456561	Ctenopharyngodon idella	Grass Carp	100	565
54	KX145578	Carpiodes spp	Quillback species	99.7	587
55	JN026920	Ictiobus spp.	Buffalo species	100	577
56	KX145578	Carpiodes spp	Quillback species	99.6	558
57	KF558281	Pomoxis annularis	White crappie	100	581
58	ON259410	Hypophthalmichthys molitrix	Silver carp	99.8	594
59	KF558281	Pomoxis annularis	White crappie	100	579
60	KF558281	Pomoxis annularis	White crappie	100	581
61	KF558281	Pomoxis annularis	White crappie	100	581
62	ON259410	Hypophthalmichthys molitrix	Silver carp	100	602
63	OL494289	Hypophthalmichthys nobilis	Bighead carp	99.8	602
64	OP001134	Hypophthalmichthys molitrix	Silver carp	100	586

65	OP001134	Hypophthalmichthys molitrix	Silver carp	99.8	582
66	ON259410	Hypophthalmichthys molitrix	Silver carp	100	610
67	MG599474	Aplodinotus grunniens	Freshwater drum	100	591
68	MG599474	Aplodinotus grunniens	Freshwater drum	100	588
69	MG599474	Aplodinotus grunniens	Freshwater drum	100	588
70	MG599474	Aplodinotus grunniens	Freshwater drum	99.3	594
71	MG599474	Aplodinotus grunniens	Freshwater drum	99.8	591
72	-	-	-	-	-
73	MG599474	Aplodinotus grunniens	Freshwater drum	99.8	560
74	MG599474	Aplodinotus grunniens	Freshwater drum	100	573
75	MG599474	Aplodinotus grunniens	Freshwater drum	100	584
76	MG599474	Aplodinotus grunniens	Freshwater drum	100	567
77	OP001134	Hypophthalmichthys molitrix	Silver carp	100	583
78	MK843772	Hypophthalmichthys nobilis	Bighead carp	100	585
79	OP001134	Hypophthalmichthys molitrix	Silver carp	100	574
80	OP050548	Hypophthalmichthys molitrix	Silver carp	99.7	577
81	OP001134	Hypophthalmichthys molitrix	Silver carp	99.8	581
82	OP001136	Hypophthalmichthys molitrix	Silver carp	100	566
83	OL494289	Hypophthalmichthys nobilis	Bighead carp	99.8	594
84	OP001136	Hypophthalmichthys molitrix	Silver carp	100	574
85	ON259410	Hypophthalmichthys molitrix	Silver carp	99.8	589
86	ON259410	Hypophthalmichthys molitrix	Silver carp	100	590
87	ON259410	Hypophthalmichthys molitrix	Silver carp	100	591
88	ON259410	Hypophthalmichthys molitrix	Silver carp	100	596
1 New	JN024863	Carpiodes spp	Quillback species	99.8	525
2 New	MG570458	Pimephales notatus	Bluntnose minnow	98.5	527
3 New	JN027540	Notropis spp.	Shiner species	99.8	494
4 New	KX145578	Carpiodes spp	Quillback species	100	526
5 New	-	-	-	-	-
6 New	JN024863	Carpiodes spp	Quillback species	100	526
7 New	JN024863	Carpiodes spp	Quillback species	99.8	530
8 New	JN024876	Carpiodes spp	Quillback species	99.6	527
9 New	KX145578	Carpiodes spp	Quillback species	100	517
10 New	JN024863	Carpiodes spp	Quillback species	99.8	531