**Title:** Early detection and evaluation of Invasive carp removal in the Ohio River

**Geographic Location:** Ohio River basin, extending from the J.T. Myers Pool (RM 845.9) to the R.C. Byrd pool (RM 279.2) along with the New Cumberland (RM 54.4), Montgomery Island (RM 31.7), Dashields (RM 13.3) and Emsworth (RM 6.2) pools of the Ohio River, in addition to the Wabash, Allegheny, and Monongahela rivers.

**Lead agencies:** Kentucky Department of Fish and Wildlife Resources (KDFWR) & West Virginia Division of Natural Resources (WVDNR)

**Participating Agencies:** Illinois Department of Natural Resources (ILDNR), Indiana Department of Natural Resources (INDNR), Pennsylvania Fish and Boat Commission (PFBC), Southern Illinois University (SIU), U.S. Fish and Wildlife Service (USFWS), West Virginia University (WVU)

#### **Statement of Need:**

Invasive species are responsible for undesirable economic and environmental impacts across the nation (Lovell and Stone 2005, Pimentel et al. 2005, Jelks et al. 2008). Negative impacts of Invasive carp in the United States are a major concern because of their tolerance and adaptability to a wide range of environmental conditions (Kolar et al. 2005, Zhang et al. 2016). Their ability to quickly colonize novel habitats with dense populations have caused significant impacts on tourism and recreation, and potentially threaten native ecosystems throughout the entire Mississippi River basin, including the Ohio River sub-basin. In response, it is necessary to gather information on invasive carp distributions, behavior, and population characteristics in the Ohio River basin (ORB). This information will be used to assess management actions related to their removal, suppression, and containment.

The tasks outlined in this document would add a sixth year of multi-agency and university surveillance and data collection focused on Invasive carp early detection and removal primarily above Cannelton Dam. Collaborative efforts have included fish community sampling, targeted Invasive carp sampling, and incorporation of unique data such as hydroacoustics. The primary goal of these projects is to provide an accurate population trend assessment of Invasive carp control and response efforts. In addition, fish community data may aid in determining impacts of carp on native fish assemblages. This project provides an ongoing, coordinated approach to assess Invasive carp management and suppression in the ORB.

### **Objectives:**

- 1. Evaluate management actions using changes in relative abundance, population characteristics, and distribution of invasive carps within intensive management zones.
- 2. Monitor long-term trends in native fish communities as indicators of change due to Invasive carp invasion.
- 3. Survey Invasive carp presence in upstream areas where they are rarely detected to inform response and containment efforts.
- 4. Determine spatial distributions (hotspots) and densities of Invasive carps in the lower Wabash River to inform and assess harvest.
- 5. Utilize hydroacoustics surveys to determine biomass densities and verify patterns of relative abundance for Invasive carp species within strategic management zones.

#### **Project Highlights:**

- With current sampling efforts being unable to capture Bighead, Grass and/or Black carps with any regularity, Silver Carp are still the primary focus of management efforts in the middle Ohio River. In the R.C. Byrd pool, captures of Silver Carp are increasing making both Silver and Bighead carp a priority in that pool. In other upstream pools located ahead of the Silver Carp invasion front, Bighead Carp continue to be the top priority.
- With the lower precision involved in tracking the long-term trends in abundance through Silver Carp catch rates, other methods for monitoring and evaluation, (i.e. hydroacoustics and occupancy modeling) are in development to estimate abundance and inform decision making.
- After the start of contract fishing in 2019, the next two years (2020-2021) of sampling resulted in relatively stable Silver Carp catch rates. An increase in CPUE in 2022 caused concern that there were some shifting characteristics of the overall population, but in 2023, those numbers were back to average indicating 2022 catches may have been an anomaly.
- Preliminary results of the Community Size Spectra (CSS) analysis suggests that invasive carp populations in the Cannelton to RC Byrd pools have not yet reach a threshold of abundance to negatively influence the size structure of the native fish community. This was corroborated by an analysis of zooplankton in the same pool. Baseline CSS are being developed for upstream pools.
- There was no substantial range expansion for either Bighead or Grass Carp during this project period, however, Black Carp have now been found in the upper Wabash River. Also, young Silver Carp <300mm were captured in Meldahl Pool during a fish tagging event in the spring of 2023.
- Hydroacoustics of the Wabash River showed that this technique is successfully tracking aggregations of bigheaded carp, as it does in the Illinois River. We have documented shifts in the distribution of densities and sizes of bigheaded carp showing that harvest indeed reduces local densities and causes carp to reaggregate.
- Successive years of positive eDNA results for invasive carp DNA in the Racine Pool and the Muskingum River suggest targeted sampling for carp may be warranted in those areas.

### **Methods:**

### *Clarification of this Document's Terminology*

With carp populations still expanding throughout the Mississippi River basin, they will undoubtedly move into new areas being managed by agencies that have no previous encounters with the species. And yet, at some point, each and every one of them will have to mitigate the impacts that these highly disruptive fish have on their resource. As a result, it has become increasingly important to clarify the terminology used in any related technical documents, which include these annual reports. Hence, the following is a list of defined terms that required further explanation in the project's previous reports.

- *Invasive Carp*: One of four fish species originating from the Asian continent (Silver Carp, Bighead Carp, feral/diploid Grass Carp, and Black Carp).
- *Bigheaded Carp*: One of two *Hypophthalmichthys* spp. (i.e. Silver (*H. molitrix*) & Bighead (*H. nobilis*) carp), or a hybrid of the two.
- *Community Size Spectra (CSS)*: An approach to describe the size structure of fish communities by quantifying the decrease in abundance among increasing body size classes.
- *Establishment Front:* Furthest upstream range of invasive carp where the population demonstrates both reproduction and successful recruitment.
- *Invasion Front*: Furthest upstream extent where invasive carp reproduction has been observed (eggs, embryos, or larvae), but lacks evidence of successful recruitment.
- *Presence Front*: Furthest upstream extent where adult invasive carp have been sampled, but there is no evidence of reproduction.
- *Targeted Sampling*: Use of standard sampling gear/techniques to target invasive carp while purposely excluding all other native species.

#### Objective 1:

### *Spring Standardized Targeted Sampling (Cannelton – R.C. Byrd)*

In the spring of each year, project partners conduct targeted sampling of invasive carp to obtain the data needed to estimate a relative abundance for the selected pools. The funding increases that were initially realized in 2021 continued to facilitate a large, targeted sampling effort in the current reporting period as well. During spring 2023 (11 April – 8 June), field crews from four agencies conducted targeted sampling for invasive carp in five pools of the Ohio River that stretched from Cannelton Pool (RM721) within the establishment front to R.C. Byrd pool (RM 237) within the presence front, excluding Meldahl Pool (Figure 1). Fixed sampling sites within each pool were pulled from a stratified-random design process completed in 2015. This produced an extremely high number of sites per pool and, although it would have been statistically ideal to sample all sites each year, funding and personnel are too limited to sample annually. Sites were chosen based on suitability of habitat and access, and approximately 24 fixed electrofishing sites were selected per pool. To ensure coverage within each pool, sites were divided between the mainstem river, island back-channels, tributaries/embayments, and dam tailwaters., with tributary sites being the most common. The mainstem river was the most abundant habitat type in each pool, but its size, depth and low-quality habitat created an area where it is very difficult to regularly sample invasive carp with the current gear-types. Tributaries are more vulnerable to the available gear used, therefore made up the majority of the sampling locations. This decision was also influenced by the abundance of telemetry data demonstrating that bigheaded carp spend a great deal of their time in these tributaries. In addition 8-12 gill net sites were incorporated into the targeted sampling within the last two pools

on the upstream end (Greenup  $\& RC$  Byrd) due to the lower abundances of bigheaded carps in these pools.

Electrofishing transects were conducted during the daytime and standardized at 900 seconds in a general downstream direction using a single dipper. Invasive carp were specifically targeted using increased driving speeds and allowable pursuit of fish upon sightings. During active sampling, most of the non-target species were ignored, but special attention was given to any small, shad-like species to avoid the possible misidentification of juvenile invasive carps. Relative abundance was inferred using CPUE data and compared to previous years to determine if there were changes in the mean and median fish caught per transect. Gill nets used in targeted sampling were typically 45m (150ft) in length, 3m (14ft hobbled to 10ft) in depth, and constructed of large mesh (12.5cm (5") bar mesh) with a foam core float line that keeps them suspended near the surface. The nets were set perpendicular from the shoreline and fished for two hours, during which noise and water disturbance is created with the intention of driving any bigheaded carps into the entanglement gear. Relative abundance was inferred using CPUE data and comparisons to previous years were only used to identify any changes in the number of fish caught per net as an indication of invasion advancement.

## *Assessing Invasive Carp Population Demographics*

Population demographics information was collected on a subset of fish, post-spawn, between August and October 2023. Field crews from four agencies (KDFWR, INDNR, WVDNR, and USFWS) sampled for invasive carp via boat electrofishing and gill netting. Data was used to determine sex ratios, length distributions, age distributions, and report body condition of fish collected in the Cannelton, McAlpine, and Markland pools. Length distributions were formed using 25 mm length bins. Ages were estimated using lapilliar otoliths (Cannelton:  $n = 251$ ; McAlpine:  $n = 244$ ; and Markland:  $n = 20$ ) encased in epoxy and thin-sectioned using agreed upon methods that were developed in 2021 during an invasive carp ORB workshop. Age distributions were summarized by percent total and visualized within a histogram.

Length-weight relationships were derived from log10 transformed lengths and weights of captured fish. A single regression line was used to compare length-weight relationships to previous years. The equations developed for the ORB as well as other waterbodies are reported below (Tables 1 and 2) in the form of:

 $log10[Weight g] = a + b * log10[Length mm]$ 

Lastly, body condition was reported using relative weight equations developed by James Lamer (Lamer 2015). Condition was only reported using data from post spawn-fish, collected between the months of August and October. Differences in body condition were compared between Cannelton, McAlpine, and Markland pools in 2023 and for Cannelton and McAlpine in previous years.

# *Development of an Effective Monitoring Program*

With the invasive carps' tendency to behave much differently than native fish communities, KDFWR initiated a pilot study to determine whether occupancy modeling could become an effective substitute for current abundance measures that were initially developed for sportfish populations. During these efforts, all surveys included half-mile boat electrofishing transects that were conducted

in a downstream direction using a single dipper. All sites were visited on three occasions to account for imperfect detection. During each survey, a power goal was implemented with the intention of transferring a minimum of 3000 watts from water to fish (Gutreuter et al. 1995). At the conclusion of each transect, the presence/absence of carp was documented along with the data that was collected from captured fish. Invasive carp occupancy and detection were estimated via the use of a hierarchical model that is available in the 'unmarked' R package (Fiske and Chandler 2011).

Sampling efforts in 2023 took place in McAlpine Pool of the Ohio River, one pool upstream from the 2022 sampling conducted in Cannelton Pool. Following previous sample design, McAlpine Pool was divided into upper middle, and lower sections with 13 randomized sites in each. The proportion of tributary to mainstem river sites was based on the number of accessible tributaries within each river section.

# Objective 2:

## *Trends in Native Fish Communities*

Fish community monitoring was conducted in May and June 2023 at the tailwaters of Montgomery (New Cumberland Pool), Dashields (Montgomery Pool), and Emsworth (Dashields Pool) locks and dams on the Ohio River, the tailwater of Lock 8 (Pool 7) on the Allegheny River, and the tailwater of Braddock (Emsworth) lock and dam on the Monongahela River. Five consecutive 10-minute runs were conducted on each bank beginning either immediately downstream of the lock chamber or as close as possible to the dam wall for a total of 100 minutes of shock time. Electrofishing was conducted using an ETS MBS unit operated at 30% duty cycle, 60pps, and between 150-550 V pulsed DC. All fish species were targeted and enumerated in the field or retained for identification in the laboratory if field identification was not practical. Gamefish species were measured and weighed.

Fall fish community monitoring was conducted in the Greenup, R.C. Byrd, Racine and Emsworth pools of the Ohio River, Allegheny River, and Monongahela Rivers as well as the lower two miles of Chartiers Creek using gill nets and night electrofishing. For WV waters, electrofishing surveys were completed during the daytime in the Greenup and R.C. Byrd pools in October 2023. Only at mainstem sites were sampled to compliment WVU's community size-spectra analysis, but still using the same fixed sites identified in 2015. Electrofishing surveys in the Racine pool were conducted at the same fixed sites selected from a previous stratified-random design from 2022. Surveys consisted of 900 second timed transects beginning at the marked coordinates and continued downstream in the mainstem river and large tributaries. Surveys of small tributaries and embayments began at the marked coordinates and continued upstream to the completion of the timed transect, or until navigation was blocked, upon which the remainder of the timed transect was completed in the main channel just downstream of the mouth. All species were collected during these surveys. Schools of small fish (minnows and shad) were sub-sampled by dipping a portion of each school encountered. Small shad-like fish were examined closely to identify potential juvenile invasive carp. All fish were identified to species; non-minnow species were measured for total length (mm). Up to 20 fish of a single species per transect were measured for total weight (g). Gill net surveys were also conducted in fall 2023 at the same fixed sites as in previous years. Gill net sets consisted of two hour sets during the day using nets 45m (150ft) in length, 3m (14ft hobbled to 10ft) in depth, and constructed

of 10cm (5") with a foam core float line to keep them suspended at top water. Each net set was actively monitored, and effort was expended to run fish into the nets with boat noise. All by-catch was identified to species and recorded, and any non-target fish (excluding invasive carps) were released immediately after capture.

For community sampling in PA waters, 61 randomly selected sites in were sampled from September  $15<sup>th</sup>$  through October 30<sup>th</sup>. For each site, sampling consisted of a 2hr minimum gill net set using either 8cm, 10cm, or 13cm (3", 4", or 5", respectively) bar mesh as well as a 15-minute night electrofishing run (ETS MBS unit, 25% duty cycle, 60pps, 100-550volts). All individuals captured in gill nets were enumerated and gamefish were also measured. For electrofishing, a subset of ten individuals per species per 25mm size class greater than 125mm total length were measured and weighed for use in WVU's community size-spectra analysis. Individuals smaller than 125mm were identified, enumerated, and released if field identification was possible. Otherwise, individuals were retained for identification and enumeration in the laboratory. Laboratory identification is still ongoing.

Fish community monitoring was also conducted in the Greenup, R.C. Byrd, Racine and Montgomery pools of the Ohio River using seines in August and October 2023. Boat ramp seine hauls were conducted at select boat ramps located directly on or adjacent to the mainstem Ohio River in the Greenup, R.C. Byrd and Racine pools. One seine haul was conducted at each ramp with a 9m (30ft) seine with 0.5cm (3/16") mesh and a 1.8m (6ft bag) with smaller mesh (3mm or 1/8" mesh). Seine hauls were completed within the boundaries of the concrete structure boundary of each boat ramp. Beach seining was employed at six fixed locations in the Montgomery Pool using a 30m (100ft) seine with 1cm (3/8") mesh. One seine haul was conducted at each of the six locations. For all seine hauls, species readily identifiable in the field were enumerated and released; all other species were retained for identification and enumeration in the laboratory.

### *Using Community Size Spectra to Monitor the Impacts of Invasive Carp*

WVU staff continue to focus on applying community size-spectra to fish assemblage data (i.e. "community data") to assess food web level impacts of invasive carp and to establish benchmarks for restoration. Invasive carp management needs to work toward scientifically defensible targets but establishing those targets has been challenging. Community size spectra (CSS) describe the size structure of communities by quantifying the decrease in abundance among increasing body size classes and it accounts for all species captured in standard surveys. CSS have been used extensively as indicators of fishery sustainability (and over-fishing) and to set targets in marine systems and research in both marine and freshwater ecosystems has grown during the last decade due in large part to a large research investment by the European Union (Blanchard et al. 2014, Petchy and Belgrano 2010). The CSS essentially measures the ratio of large individuals to small individuals in the community and summarizes the immense complexity of food web dynamics into two simple parameters, the slope and centered y-intercept (termed elevation) of a line, which have direct biological meaning representing ecological efficiency and ecological capacity, respectively (Murry and Farrell 2014). The CSS slope and elevation are fairly stable in large river systems (Murry and Farrell 2014) but do react in predictable ways to environmental change including changes in species dominance (Broadway et al. 2015) and large-bodied low trophic position fish species (Murry et al. 2024). Large-bodied low trophic position fish, such as invasive carp will tend to reduce the slope of

the CSS (which is typically steeper under piscivore dominance). In 2023, WVU researchers completed the second year of their research efforts toward (1) understanding the dynamics of CSS relative the carp invasion, (2) evaluating the effectiveness of CSS as a community-level indicator of invasive carp impacts, (3) the use of CSS to establish community-level pool-specific restoration goals, and (4) evaluate the sensitivity of CSS to use as an early warning indicator.

WVU worked with state partners (KDFWR, WVDNR, PFBC, and USFWS) to collate existing community boat electrofishing data for CSS analyses. CSS was used to compare community size structure pre- and post-invasion in impacted pools to unimpacted (invaded vs non-invaded pools). This initial analysis established baseline conditions throughout all pools (e.g. mean CSS parameter values and degree of natural inter-annual and inter-pool variation). Data provided by state partners include fish lengths and weights derived from community electrofishing surveys conducted from 1994-present. Data for the Cannelton, McAlpine, Markland and Meldahl pools of the Ohio River was collected 2015-2020 by KDFWR. Data for the Greenup and R.C. pools were collected by WVDNR from 2016-2022. We received data for the La Grange pool of the Illinois River from the Illinois Natural History Survey from surveys conducted 1994 - 2021. We utilized earlier years' data in impacted pools as well as long-term averages in upstream unimpacted pools to serve as a reference (historic target condition). We assessed 'normal' or expected interannual variation in CSS from the upstream unimpacted (or lightly impacted) pools to provide a range in target values for conservation of native fishes.

In addition to the Ohio River fish data, WVU field crews collected monthly zooplankton samples. Zooplankton were identified to a broad taxonomic group (copepods, Cladocera, rotifer, veligers) counted and individuals were measured. Impacts of invasive carp were assessed by employing a BACI design and samples were compared to published surveys from 30 years earlier. Normalized Biomass Size Spectra (NBSS), an ataxic approach that expresses community structure as a function of body size instead of taxonomic identity was used to assess potential effects of invasive carp on zooplankton. Additionally, seasonality of the NBSS model and how it changed throughout the season was analyzed.

## Objective 3:

### *Monitoring Ahead of the Invasion Front*

Targeted sampling for Invasive Carp was conducted in November 2023 in the Montgomery Pool and December 2023 in the New Cumberland Pool of the Ohio River. Sampling was conducted in the Montgomery Slough (RM 949.78 to 950.11) where positive eDNA hits for Bighead Carp were found historically. Gill nets used in sampling were 90m (300ft) in length,  $\sim$ 4m (12ft) in depth, and constructed of 8cm, 10cm, or 13cm (3", 4", or 5", respectively) bar mesh. Three gill nets were fished for approximately 24 hours each. Three gill nets of the same size and mesh were also fished in the New Cumberland Pool for approximately 24 hours each.

Incidental sampling for Asian Carp was conducted using boat electrofishing through targeted gamefish surveys on each of the Three Rivers. Nighttime boat electrofishing using a ETS MBS electrofishing unit operated at 60pps, 30% duty cycle, and 150-550V was conducted in March/April on Pool 4, Pool 5, Pool of the Allegheny River and the Maxwell and Elizabeth Pools of the Monongahela River. Sampling consisted of four non-overlapping 10-minute runs on each bank

beginning immediately downstream of the lock and dam for 80 minutes of total effort in each pool, with the exception of Pool 9 on the Allegheny River where sites were selected by targeting the best available habitat. Adult Sander species were targeted during these surveys and presence/absence of invasive carp species was recorded. Sampling in October occurred at four fixed sites in Pool 4 of the Allegheny River, four fixed sites in the Emsworth Pool, and five fixed sites in the Charleroi Pool of the Monongahela River for a total effort of 6.77hrs. Gear type and settings were the same as in the March Sander surveys. Black Bass were the primary target of the October surveys and presence/absence of invasive carp species was recorded. In November, nighttime boat electrofishing was conducted on the Monongahela River in the Grays Landing and Emsworth Pools, the Allegheny River in Pool 2 and Pool 7, and the Ohio River in the Montgomery and Dashields pools. Sampling was conducted via pulsed DC night boat electrofishing and gear type and settings were the same as in the spring Sander surveys. Sampling consisted of four non-overlapping 10-minute runs on each bank beginning immediately downstream of the lock and dam for 80 minutes of total effort in each pool. Adult Sander species were targeted during these surveys and presence/absence of invasive carp species was recorded.

To determine if Silver or Bighead Carps may be present in tributaries of the upper Ohio River, the USFWS Lower Great Lakes FWCO collected 90 water samples for eDNA analysis from each of seven tributaries in the R.C. Byrd, Racine Pool, Belleville, New Cumberland and Montgomery pools of the Ohio River in 2023 (Table 3). All eDNA sampling followed the USFWS (2023) Quality Assurance Project Plan. Following collection, eDNA samples were shipped to the USFWS Whitney Genetics Lab for processing and the results reported to state partners.

### Objective 4:

### *Spatial Distribution in the Wabash and White rivers*

Mobile hydroacoustic sampling was conducted by Southern Illinois University (SIU) during spring and fall months of 2023 in the Wabash River between Terre Haute, IN and the confluence with the Ohio River (Figure 2). The sites sampled were as follows: Crawleyville (5/11/23), Dogtown Ferry (4/27/23), Fay's Landing (5/10/23), Hustonville (4/18/23), New Harmony (5/12/23, 11/10/23), New Haven (4/26/23), St. Francisville (5/9/23), Terre Haute (4/17/23, 11/7/23), and Vincennes (4/19/23) (SIU FIGURE 1). Sampling during summer and most of fall was limited due to extremely low flow and discharge in the river (Figure 3). Hydroacoustic sampling consisted of two 200-kHz split-beam BioSonics DTX transducers that were horizontally oriented toward the center of the river while sampling. Each survey was approximately 5km long with multiple, non-overlapping, parallel passes covering the entire channel from bank to bank. The fish community was also sampled in the same reaches of the Wabash River using daytime electrofishing for determining size-specific species proportional abundances that are needed for analyzing hydroacoustic data. Electrofishing transects consisted of 900 seconds of pedal time following standardized long-term monitoring protocols (McClelland et al. 2012). Acoustic estimates from 2023 are being processed in Echoview and will be available by summer 2024. To date, SIU has been processing hydroacoustics data as per MacNamara et al. 2016, where fish acoustic targets are assigned as bigheaded carp or other species based on the relative proportion of fish within each size class from independent fisheries sampling. This is a simple approach that does not account for the variability within the fish assemblage estimates,

especially for large fishes. SIU will work with Carterville FCWO to explore the feasibility of processing acoustics data using a Bayesian hierarchical approach as described below.

A pilot project to assess the impact of targeted harvest removal of invasive carp in an oxbow of the Wabash River (near Grayville, IL) was conducted in March 2023. Block nets separated the oxbow from the Wabash main channel throughout the harvest event. Only the southwest portion of the Grayville oxbow was targeted for removal and assessment. Hydroacoustic sampling by SIU was conducted prior to harvest/sampling but after block nets were in place. INDNR also sampled the adult fish community via electrofishing in the oxbow prior to removal to inform hydroacoustic analyses. Gillnet harvest occurred for several days, followed by additional post-removal hydroacoustic sampling, and then removal of the block nets. Silver, common, and grass carp were harvested during the removal event and were therefore combined for hydroacoustic analyses and are referred to within this objective as 'invasive carp'.

### Objective 5:

## *Hydroacoustics Analysis*

The Carterville FWCO completed hydroacoustic sampling during October and November 2023 in the Cannelton and Newburgh pools of the Ohio River. Hydroacoustic data collection followed methods as described in the Large River Hydroacoustics Mobile Survey Standard Operating Procedure, Region 3 USFWS. Briefly, we deployed a BioSonics DTX echosounder multiplexing two, 200 kHz, side-looking split-beam transducers offset in angle to maximize water column coverage (Figure 4). Both transducers were deployed from the vessel's port side at a depth of 0.5 m on a bracket mounted to a mechanical rotator. The rotator ensured that the transducers tilted downward at appropriate angles such that the top edge of the shallow beam was parallel with the water surface. Hydroacoustic data collection was split among main channel, side channel, backwater, and tributary habitats. Within each pool, we collected hydroacoustic data in all side channels >0.8km in length, navigable tributaries (up to 3.2km from confluence), and backwaters because invasive carps often inhabit these areas. In the main channel, we selected 15% and 25% (based on analysis of data from full pool scans during 2021-2022) of available 0.8-km sites for data collection using a random sampling approach for Cannelton and Newburgh pools, respectively. This resulted in 109km and 90km of main channel transects for Cannelton and Newburgh pools, respectively. Transducer direction (shore vs thalweg) was randomly assigned to each main channel site. Both shore- and thalweg-facing transects were completed along each bank for all side channels with widths great enough to ensure sample area of thalweg-facing transects didn't overlap (i.e., thalweg facing hydroacoustic beams on opposite banks don't overlap in the middle of the side channel). In narrow side channels, two shore-facing transects were completed. Tributary data collection consisted of shore-facing transects with the boat centered within the channel and completed in both the upstream and downstream direction to ensure both banks were sampled. In backwaters, data were collected along the shoreline with the transducers facing towards shore. Calibration data were collected for both transducers prior to each survey to adjust hydroacoustic measurements.

Hydroacoustic data processing followed methods outlined in MacNamara et al. (2016) and the Large River Hydroacoustics Mobile Survey Standard Operating Procedure, Region 3 USFWS using

Echoview Version 13.0. Raw data and calibration files were imported into a mobile survey template for processing. Processing included a 1-m nearfield exclusion zone, bottom-line exclusions, and removal of bad data regions where wake disturbance or vegetation contributed to poor data quality. A single target detection algorithm (split beam method 2) facilitated the detection of individual fish targets using parameters suggested in Parker-Stetter et al. (2009). Using the equation developed by Love (1971), we estimated the target strength (TS) of 250mm fish during each survey and used that value as a TS threshold to remove fish less than 250mm from analyses. Groups of individual targets originating from the same fish were combined to make individual fish tracks to reducing the potential of overcounting. Fish targets and sample volume estimates were then exported from Echoview for further analysis.

To apportion hydroacoustic targets to fish species, the Carterville FWCO, INDNR, and KDFWR collected community data using an electrified dozer trawl and boat electrofishing. Community data collection followed the same hydroacoustics sampling design detailed above with two exceptions: 1) side channel and main channel sites were larger (1.6km) to ensure that sites were long enough to complete electrofishing transects and 2) due to logistical limitations, only 35 main channel community sites were sampled per pool (Figure 5). Deployments of both community sampling gears (dozer trawl and boat electrofishing) were planned for all sites, but deployment of the gear was at the discretion of the boat operator based on river conditions (e.g., water velocity and debris). Deployment of each gear was standardized to allow for comparisons among sites. The dozer trawl was deployed for 5-minutes at ~4.8km/h, following the Long-Term River Monitoring power goal tables to maximize catch. Boat electrofishing transects were 15 minutes in a general downstream direction with one dip netter. A power goal, intended to transfer a minimum of 3000 Watts from water to fish, was implemented (Gutreuter et al. 1995) at a 40% duty-cycle and 80 pulses per second (pulsed DC). All fish greater than 250mm were identified to species, weighed (g), and measured (total length; TL).

To reduce bias in our hydroacoustic estimates, we used a Bayesian hierarchical model to account for uncertainty in TS measurements and a paucity of community data at hydroacoustic sites. For this analysis, we modified the methods described in DuFour et al. (2021). Briefly, we used a fitted quadratic regression model to calculate the probability of a fish being a Silver Carp given its length (Figure 6). Our most complex model describing the fish community included pool, habitat, TL, and  $TL<sup>2</sup>$  as fixed effects and community site nested within habitat and both community site and habitat nested within pool as random effects using a Bernoulli distribution. We compared the most complex model and four models containing a subset of variables from the full model using k-fold cross validation (CV). The most parsimonious model describing the community data had TL and  $TL<sup>2</sup>$  as fixed effects and Community Site as a random effect; therefore, the results of this model were used in subsequent calculations. We also modeled TS as a function of individual fish track to obtain a mean TS and credible intervals (CrI's) for each fish track. Mean TS and CrI's were converted to total length (TL) using the multi-species, side-aspect equation developed by Love (1971) (Figure 7). Importantly, TL based on TS is uncertain, as are the model parameters describing the fish community. To account for this uncertainty, we integrate across TL and the model parameters to estimate the probability that an individual is a Silver Carp based on its TS (for details see DuFour et al. 2021). To solve this integration, we used Monte Carlo simulations ( $n = 1000$ ) to estimate the number of Silver Carp at each site and converted this abundance to density by dividing by the

volume of water sampled by hydroacoustics (i.e., Wedge\_Volume\_Sampled). To examine the potential effects of habitat and pool on the Silver Carp density, we calculated the mean and 90% CrI's by habitat and pool. Non-overlapping CrI's were used to indicate significant differences between habitats and among pools.

Our models differ from those described in DuFour et al. (2021) in three ways. First, because Silver Carp make up a large proportion of fish between 500mm and 900mm in our community sampling, but Silver Carp < 500 or > 900 mm are rarely captured, we use a quadratic regression to describe the probability of a fish of a given length being a Silver Carp rather than a logistic regression as in DuFour et al. (2021). Second, following discussions of our analyses with M. DuFour, we determined that converting TS to backscattering cross section was not necessary and modelled TS directly. Third, we used k-fold CV rather than leave-one-out (LOO) CV for model selection because model diagnostics suggested that LOO CV likely resulted in biased model selection criteria and k-fold CV is a reliable alternative to this method (Vehtari et al. 2017).

### **Results:**

## *Spring Standardized Targeted Sampling (Cannelton – R.C. Byrd)*

During spring 2023, KDFWR and INDNR used 35.25 hours of targeted boat electrofishing to successfully collect a total of 394 fish across three different species of invasive carp, which included Silver (98.4%), Grass (1.2%) and Bighead (0.3%) carps (Table 5). As in previous years, most of the invasive carp were captured from the 48 electrofishing sites located within the Cannelton Pool  $(n =$ 326 invasive carp). The other 93 transects completed in two different pools of the middle to upper Ohio River contributed less than 18% of the overall catch ( $n = 68$  carp), which included 60 invasive carp from the McAlpine Pool and only 8 from Markland. Catch per unit effort slightly decreased in Cannelton Pool, with last years targeted monitoring results being the highest in years past with 8.70 invasive carp/transect. The 2023 average catch was 6.79 invasive carp/transect. The 2023 catch rates for Silver Carp in pools located directly upstream of Cannelton remained extremely low and included an average of 1.33 fish/transect for McAlpine and only 0.17 fish/transect in the Markland Pool. The average catch rates per pool for other invasive carp species also remained negligible. Upon completion of the 2023 targeted sampling efforts, KDFWR managed to capture a total of 5 Grass Carp and one Bighead Carp, which are similar results to those obtained in previous years.

Spring targeted boat electrofishing in the Greenup and R.C. Byrd pools by WVDNR zero invasive carps over 9.5hrs of effort. Spring gill netting in these pools yielded one Bighead Carp and one Silver Carp from 2700ft of net over 18 sets (Table 6). The most common bycatch species in these pools was Common Carp.

### *Assessing Invasive Carp Population Demographics*

By the end of the reporting period, a total of 17 Bighead Carp and 5 Grass Carp had been captured during the 2023 sampling efforts in the middle Ohio River. Most of the Bighead Carp were captured during fall gill net sampling efforts in Markland Pool to collect data for age & growth analysis and

the ongoing length/weight regression that is being constructed for Bighead Carp (Figure 8). The 5 Grass Carp captured in 2023 were via boat electrofishing in Cannelton Pool; total lengths ranged from 777 mm to 824 mm. With the small number of both Bighead and Grass Carp collected in 2023, there will be no additional demographics provided for either species.

The length frequency data collected during the 2023 reporting period was different than in previous years. Only about 1% of the fish caught in 2023 had total lengths of 600 mm or less (Figure 9). The majority of Silver Carp caught from the McAlpine Pool during this period had total lengths that ranged from 750 to 850 mm (29.5 – 33.5 in) and the length distribution did not fit an obvious bimodal pattern as it had in previous years. During 2023, the overall sample of Silver Carp obtained from McAlpine consisted of 48% male and 52% female fish, which was slightly more balanced than in Cannelton where males represented 41.3% of captures and females 58.7% (Figure 10). As in previous years, the sample of Silver Carp  $(n = 21)$  caught from the Markland Pool in 2023 exhibited a wide range of total lengths from 750mm to 1050mm, with around 24% of those captured being around 975mm (Figure 10).

Silver Carp sampled from the Cannelton Pool in 2023 were estimated to be between 2 and 12 years old (Figure 11). Age-4 and age-5 fish continued to be the most frequently sampled (63.0%) similar to both 2021 (65.0%) and 2022 (64.6%) samples. Silver Carp collected from the McAlpine Pool in 2023 exhibited a narrower age range of 4 to 11 years (Figure 12). However, the most frequently encountered age group of Silver Carp from McAlpine increased from 2022 to age-5 and age-6 fish, which made up more than 72% of the 2023 sample.

Body condition of Silver Carp collected in Fall 2023 was determined using relative weight  $(W_r)$ equations generated from over eight years of length-weight measurements (Figure 13). The average W<sub>r</sub> of Silver Carp collected from Cannelton in 2023 was nearly 108, which was an increase from the mean Wr of carp collected in past years (Figure 14). Similar comparisons of body condition for Silver Carp in the McAlpine Pool have determined that the mean  $W_r$  of fish caught in 2023 (108) was also up slightly from the average condition ( $W_r = 97$ ) of carp in 2022. A comparison of average body condition across three consecutive pools of the middle Ohio River continues to indicate that Silver Carp in Cannelton and McAlpine have a similar length-weight relationship, but in the Markland Pool, the same species appears to have substantially higher average relative weights (Figure 15).

### *Development of an Effective Monitoring Program*

Building on the previous project efforts in Cannelton Pool (2022), KDFWR moved sampling efforts upstream along the Ohio River in 2023. The occupancy project sampling design was implemented in McAlpine Pool, an area known to have a lower density of Silver Carp in comparison to the establishment front in Cannelton pool. Through July-August 2023, KDFWR conducted electrofishing transects at 39 sites that were visited on three different occasions for a total of 117 sampling events. Silver Carp were observed during at least one visit to 18 (46.2%) of these sites and during all three visits to 5 sites (12.8%). Data is being analyzed by WVU to provide further insight on the number of site visits needed for accuracy and the probability of Silver Carp occupancy and detection across the middle Ohio River.

#### *Native Fish Communities*

Fish Community sampling in the Greenup, R.C. Byrd and Racine pools of the Ohio River was conducted by WVDNR in October 2023 and consisted of 9.25hrs of effort. Electrofishing surveys yielded data from 48 fish species (Table 7). Gizzard Shad and Emerald Shiner constituted the bulk of collected fishes in both pools comprising approximately 38% and 15% of the total catch between all pools, respectively. Bluegill and Sauger were the most caught sportfish species. Smallmouth buffalo and Freshwater drum were the most caught non-sport fishes. Relative weights (where applicable) were within the mean for all species (Table 8). Seventeen gill net surveys (3,900ft of net) were conducted by WVDNR in the R.C. Byrd and Racine pools in fall 2023. Two Bighead Carp and one Silver Carp were removed from the R.C. Byrd pool during fall gill netting. Gill net bycatch included only two additional species of fish (Table 9).

Tailwater fish community monitoring by PFBC in Pool 7 of the Allegheny River, the Emsworth Pool of the Monongahela River, and the New Cumberland, Montgomery, and Dashields pools of the Ohio River was conducted in May/June 2023 and consisted of 1.67hrs of effort per pool using pulsed DC night electrofishing. Total number of species captured ranged from 30 to 37 at each of the five tailwaters sampled, with individual fish counts ranging from 985 to 1,311 fish captured at each of the tailwaters. Emerald Shiner, Mimic Shiner, Smallmouth Bass, and Walleye comprised approximately 55% of the total catch between all pools (Table 10). No Invasive Carp were captured during these surveys. Randomized pool wide fish community sampling took place in September and October 2023 on the Emsworth pool of the Ohio River and associated navigable tributaries. A total of 65 sites were sampled using night electrofishing and gill nets. Laboratory fish identification and data entry is still ongoing and will be reported on in next year's report. However, no adult invasive Carp species were captured or observed during the sampling events.

Laboratory identification and data summary was completed for randomized pool wide fish community sampling from 2022 during summer 2023. In summary, 34 sites in the Montgomery Pool of the Ohio River (including lower Raccoon Creek and the lower Beaver River) and 21 sites in the Dashields Pool on the Ohio River were sampled using gill nets and night electrofishing. A total of 82 fish representing 10 species and 49 fish representing four species were captured using gill nets from the Montgomery and Dashields pools, respectively. Smallmouth Buffalo and Common Carp were the two most abundant species captured and comprised 81% of the gill net sample. Night electrofishing was performed for a total of 13.75hrs and captured 15,320 fish in the Montgomery Pool and 17,004 fish in the Dashields pool. Sixty-one different species were captured; however, the majority of the individuals sampled electrofishing were Emerald Shiners (81%; Table 11). No invasive Carp were captured during these fish community surveys. Data from these surveys has been compiled, QA/QC'd, and provided to WVU for use in their CSS analysis.

Thirteen boat ramp seine hauls were conducted by WVDNR in the Greenup, R.C. Byrd and Racine pools in Fall 2023. Seine hauls yielded 3,111 fish from 30 different species (Table 12). The number of fish collected varied greatly by site (9-646 individuals) and was likely due to river conditions. Channel Shiners were the most collected species in all pools, which is a contrast to previous years, where Emerald Shiners have dominated the catch. Young of year Gizzard shad were also more abundant in 2023 than in previous years. No invasive carps were collected. Mean diversity abundance over the sampling period will be used as a metric for the diversity of the small, more

littoral fishes of the mainstem Ohio River. Beach seining was conducted by PFBC on the Montgomery Island Pool in August 2023. No invasive carp species were collected. A total of 2,290 individuals of 20 different species were captured. Gizzard Shad and Emerald Shiner comprised 42% and 22% of the total catch, respectively (Table 13).

## *Using Community Size Spectra to Monitor the Impacts of Invasive Carp*

We used a community size spectra approach to evaluate fish community structure and compare size spectra across spatial and temporal gradients of silver carp abundances. Data provided by state partners allowed for the assessment of CSS across all pools. Results show the Illinois River CSS has changed predictably (i.e. lower slope) in response to the invasion of silver carp (Figure 16). While there has been an increase of silver carp in the Ohio River, densities have not reached the threshold required to impact CSS (Figure 17). While specific removal targets were not obtained from using CSS, CSS alongside adaptive management could be used for the management of silver carp. A baseline CSS has been developed for the Dashields pool, and one for the Montogomery pool is forthcoming. In addition, USFWS-Ohio River unit has collected monthly fish data across 4-6 pools of the upper Ohio River and that data will be used to develop baseline CSS in those pools and test scenarios to increase sampling efficiency in the future.

For more details, see Appendix A: The effect of silver carp on large river community size spectra. B.Novak. Master's Thesis, WVU 2023.

The zooplankton monitoring scheme was successfully integrated into the field sampling regime with ichthyoplankton tows in spring and summer 2023. Thirty tows were completed between May 22 and July 12 upstream of the Markland dam (Table 14). No differences in zooplankton community composition across the different carp invasion fronts were found. However, large-bodied copepods were more abundant and emerging earlier throughout the year while smaller-bodied rotifers were less abundant (Figure 18). No significant differences of NBSS models were found across carp invasion fronts, although there were trends in the data suggesting potential impacts could occur. Our findings suggest densities of invasive carp have not reached the threshold to produce negative effects on the zooplankton community. The NBSS models did change throughout the year and followed along with typical observed zooplankton phenology. A notable finding to report is that on average (across pools and months) *Dreissenid* veligers were over half of the organisms sampled.

For more details, see Appendix B: Effects of Invasive Species on Ohio River Zooplankton. S. Johnston. Master's Thesis, WVU 2023.

### *Monitoring Ahead of the Invasion Front*

Targeted gill net sampling for invasive carp by PFBC in the New Cumberland and Montgomery pools of the Ohio River did not collect any invasive carp species. A total of 45 individuals representing nine species were captured during targeted gill net sampling (Table 15). River Carpsucker and Hybrid Striped Bass were the two most common species captured and each comprised 27% of the total catch on the Ohio River, respectively. Additional scheduled sampling in mid-December was canceled due to high flows and excessive debris.

In addition, PFBC tracks incidental captures of Invasive carp through other various projects. Efforts in 2023 included targeted gamefish surveys for Sander spp in March/April and November at 11 tailwaters in the Allegheny, Monongahela, and Ohio Rivers and targeted surveys for black bass at 13 fixed sites in one pool of the Allegheny River and two pools of the Monongahela River in October 2023. No Invasive Carp species were captured or observed in any of the targeted gamefish surveys in March, April, October, or November 2023.

USFWS collected 950 eDNA water samples from seven tributaries within the Ohio River Basin (Table 2). Of those, six samples tested positive for invasive carp eDNA. Four samples were positive for the Bighead Carp marker, two in Mill Creek (Racine Pool), one in Tombleson Run (Racine Pool) and one in the Beverly Pool of the Muskingum River (Belleville Pool) whereas one sample in Tombleson Run and the Lowell Pool of the Muskingum River were positive for the non-specific invasive carp (either Bighead or Silver Carp) marker. This is the second consecutive year with a positive detection of invasive carp eDNA in the Beverly Pool of the Muskingum River. Continued sampling of these sites will guide the future need to use traditional sampling gear to monitor the invasive carp invasion front.

## *Spatial Distribution in the Wabash and White rivers*

In the Wabash River, a total of 5,385 fishes of 50 species were sampled in 2023 by SIU, Eastern Illinois University, and INDNR. Of these fishes, only 37 fish were bigheaded carp, suggesting that either invasive carp were rare in the river or that sampling with boat electrofishing was not efficient. In contrast, 210 and 133 bigheaded carp were sampled with similar effort in 2021 and 2022, respectively. Average sizes of bigheaded carp were similar among 2021 samples through 2023, although young-of-year bigheaded carp were only present in 2021 samples (Figure 19 SIU Figure 2).

Hydroacoustic data were successfully collected in the Wabash River in spring 2023. Summer sampling was not possible due to extremely low water levels (Figure 3). In fall 2023, low water levels again restricted access with the exception of the Terre Haute and New Harmony sites (Figure 3). All 2023 hydroacoustic data have been uploaded into Echoview with bottom lines assigned to echograms. Data are in the process of being analyzed for density estimates of bigheaded carp and should be available by summer 2024.

In the Grayville oxbow targeted harvest and sampling effort in March 2023, estimated invasive carp densities before harvest were highest near the center of the oxbow, slightly offshore (Figure 20 SIU Figure 4). Spatial distributions of the entire adult fish community were relatively evenly distributed throughout the oxbow prior to harvest for all fish sizes (Figure 21 SIU Figure 5). Gill nets removed 12,500 Silver Carp, weighing a total of 87,500 pounds (Figure 22 SIU Figure 6). Harvest reduced invasive carp densities (Figure 23 SIU Figure 7) and shifted their spatial distributions toward the eastern, nearshore area of the oxbow (Figure 21 SIU Figure 5). Following the harvest event, spatial distributions of the entire adult fish community shifted toward the southern portion of the oxbow closest to the main channel, especially for medium to large size classes (Figure 21 SIU Figure 5).

# *Hydroacoustics Analysis*

Community data comprised samples from 95 electrofishing and dozer trawl sites (Cannelton = 44, Newburgh = 51). Boat electrofishing collected more total fish  $>$  250 mm (n = 656) than dozer

trawling  $(n = 174)$ . A total of 158 Silver Carp were captured with more Silver Carp captured with boat electrofishing  $(n=112)$  than the dozer trawl  $(n=46)$ .

Model results suggest the greatest mean Silver Carp densities occurred in the Salt River and Little Pigeon Creek in Cannelton and Newburgh pools, respectively (for site-specific estimates, contact the Carterville FWCO). Silver Carp densities were less than 5 fish/1000m<sup>3</sup> at all sites. Longitudinal trends exist within Newburgh Pool but not Cannelton Pool with Silver Carp densities increasing in the downstream direction (Figure 24). Habitat differences existed both within and among pools. On average, tributaries had the greatest Silver Carp densities followed by backwater (Newburgh only), side channel (Newburgh only) and main channel sites (Table 17). Cannelton Pool had greater Silver Carp densities than Newburgh Pool in main channel habitats, whereas tributary density was greater in Newburgh Pool.

#### **Discussion:**

In past years, Silver Carp populations displayed strong bimodal length frequency distributions in both the Cannelton and McAlpine pools. In 2023 that was no longer the case, with bimodal distributions weaking substantially indicating year-class strength for a single age-class. This may be evidence of a previously failed spawn or just an increase in mortality (or emigration) rates of the larger individuals. Additionally, the body condition of Silver Carp in the McAlpine and Cannelton pools showed higher mean relative weights than in previous years. Smaller length classes were not seen in the 2023 Markland Pool data as opposed to last year. This reflects the patterns seen historically in Markland which is dominated by larger fish that more than likely immigrated into that stretch of the river. The smaller range of length classes in Markland Pool, and the lack of bimodal length distributions in McAlpine and Cannelton pools have provided further evidence that we are in the midst of a long-term shift in the Silver Carp populations that have established themselves within the middle Ohio River. Additionally, during a fish tagging event in April of 2023, three silver carp each less than 300mm were captured in Meldahl Pool of the Ohio River. This indicates a need to target Meldahl Pool for age and growth data collection moving forward. More Silver Carp are being captured in the R.C. Byrd Pool and there are increasing positive eDNA hits in the Racine Pool indicating the need for continued monitoring possibly up to Racine Pool.

In response to the results of the 2021 Kentucky River occupancy pilot project and the 2022 sampling in Cannelton Pool, KDFWR continued to test if occupancy modeling could be applicable to the ongoing management of the invasive carp populations in the middle Ohio River. We continue to sample across a perceived abundance gradient so a fourth year of sampling will be conducted within the Markland Pool during summer 2024. During these efforts, the actual catches of invasive carp will continue to be tracked along with the presence/absence data. KDFWR expects that both the detection and occupancy probabilities will decline as the Silver Carp sampling efforts continue to shift further upstream. The objective of these combined efforts is to demonstrate how a less data intensive monitoring protocol can still be used to recommend specific sampling approaches.

#### *Community Size Spectra*

We observed trends in CSS slope (the Establishment front being flatter than both the invasion and presence front) on the Ohio River, although they were not statistically significant, suggesting that invasive carp densities may have not yet reached a level where they are impacting food web structure. Consistent with this suggestion are the results from the recent zooplankton survey of the Ohio River showing that large-bodied zooplankton did not decrease in abundance compared to before the Silver Carp invasion. This is in contrast to a study conducted on the Illinois River, where there is evidence of decreasing CSS in the presence of invasive carp. The on-going commercial and contract fishery harvest of Silver Carp in high density areas of the Ohio River could be slowing the spread of silver carp in the Ohio River through reduction of densities. These commercial and contract fishing efforts may be effective in keeping Silver Carp under the impact threshold.

### *White and Wabash Rivers*

The Wabash River is a major tributary of the lower Ohio River and possibly a source of bigheaded carp recruits throughout the Ohio River basin. Hydroacoustic sampling provides a quantitative benchmark by which the contribution of bigheaded carp from the Wabash River to the Ohio River sub-basin can be assessed through time. In 2021 and 2022, hydroacoustic sampling in the Wabash River revealed that densities of bigheaded carp were higher upstream. Thus, it is important to incorporate the spatial extent of distributions of carps in the river to be able to assess changes in density through time, especially as a function of harvest as a control. These data can be used to help direct harvest to control carp densities most efficiently. The frequency of hydroacoustics sampling in 2023 was reduced due to extremely low water levels during most of the summer and throughout the entire fall (Figure 3). Given these circumstances, we were still able to complete eleven sampling events and these data will be processed and available by summer 2024. SIU is currently working with Carterville FWCO to use a Bayesian hierarchical analysis to bring density estimates in the Wabash River into parity with the remainder of the Ohio River basin. This approach will incorporate a new measure of variability associated with the independent fish community sampling into density estimates.

The hydroacoustics used to assess the efficacy of harvest showed that this technique is successfully tracking aggregations of bigheaded carp in the Wabash River, as it does in the Illinois River (MacNamara et al. 2016). Shifts in the distribution of densities and sizes of bigheaded carp (almost exclusively silver carp) showed that harvest indeed reduces local densities and causes carp to reaggregate, likely as an attempt to avoid capture. This approach can be used to determine the relationship between acoustics density estimates and the true population size within a locality and is being explored further in 2024.

### *Hydroacoustics*

We found that both habitat and pool significantly affected mean Silver Carp density. These results support previous research evaluating density gradients across invasion fronts (MacNamara et al. 2016; Erickson et al. 2021) and Silver Carp habitat use (DeGrandchamp et al. 2008; Gillespie et al. 2017; Pretchel et al. 2018). Due to the paucity of backwater data  $(N = 1)$ , we lack confidence in any conclusions reached regarding the backwater data in Newburgh Pool. Our results suggest that Silver Carp densities are greater in tributaries than in either main channel or side channel habitats. This finding agrees with previous literature (Pretchel et al. 2018) that Silver Carp densities increase in tributary habitats. However, some literature suggest that tributary usage is less than mainstem usage in some Ohio River pools (Gillespie et al. 2017). These conflicting results suggest that fine-scale environmental characteristics may have a greater impact on Silver Carp habitat use than large-scale habitat features as suggested by Glubzinski et al. (2021).

The longitudinal trends in our data support previous literature (DeGrandchamp et al. 2008; MacNamara et al. 2016) and the findings from 2021-2022 hydroacoustic surveys describing invasion ecology within impounded rivers. Once populations become established upstream of a barrier, they expand their range upstream toward the next barrier. For Silver Carp, this expansion is often comprised of larger individuals (MacNamara et al. 2016; Lenaerts et al. 2021). The apparent longitudinal gradient in our Newburgh Pool density estimates may depict this upstream expansion but more information is needed to evaluate longitudinal changes in fish size within these pools.

Density estimates obtained from side-looking hydroacoustics have increased levels of uncertainty because bias is introduced from multiple sources. For example, target strength, which is converted to fish length, is a stochastic variable which depends on the physical (e.g., fish length and swim bladder presence) and behavioral (e.g., swimming direction and vertical movements) characteristics of the insonified fish (Foote 1980; Ona 1990; Boswell et al. 2009). For example, the orientation of insonified fish targets relative to the transducer greatly affects measured TS (Boswell et al. 2009; Johnson et al. 2019a). We use a side-aspect TS-TL equation to convert TS measurements to TL (Love 1971). This equation assumes fish are oriented perpendicular to the transducer at the time of sampling. Deviation from this assumption affects the total number of fish targets included in analyses and fish size estimated from TS (Boswell et al. 2009; Johnson et a. 2019a). Because we orient our transects parallel to the current, fish facing against or with the current will be oriented near-perpendicular to the transducer, validating our use of a side-aspect equation. In areas with reduced current (backwaters), target orientation relative to the transducer may deviate from perpendicular causing the use of Love's 1971 equation to bias density and size estimates. Some additional sources of bias in side-looking hydroacoustic estimates include near-surface effects on sound propagation (Balk et al 2017), subjectivity during processing (i.e., interpretation of echograms, exclusion lines, and editing of fish tracks), and apportioning of hydroacoustic targets to species using community data.

Although hydroacoustics accurately samples pelagic fish populations (Johnson et al. 2019b), the use of community data to apportion hydroacoustic targets to species can bias estimates. The tools we used to collect community data (dozer trawl and boat electrofishing) have size and species-related biases. For example, boat electrofishing is biased towards large individuals (Chick et al. 1999; Bayley and Austin 2002). Because community data are used to apportion hydroacoustic targets to species, these gear-specific biases are transferred to the hydroacoustic estimates. The combination of gears used here should reduce the effects of gear-specific biases, improving our assessment of the fish assemblage. Further, our Bayesian hierarchical models incorporate much of the uncertainty inherent to hydroacoustic estimates, reducing bias contributed by community sampling gears (DuFour et al. 2021).

Our use of Bayesian hierarchical modeling improves Silver Carp estimates by incorporating uncertainty from TS measurements, thresholding, and community sampling in the models. Previous methods ignored these sources of uncertainty, likely biasing density estimates. Additionally, this approach provides the capability of inferring the probability of a fish being a Silver Carp for lengths that have no community data. The ability to infer the probability of a fish target being a Silver Carp for areas lacking Silver Carp catch data improves our estimates by reducing the effect of sparse or missing community data. Further, this approach is applicable in multiple situations because it has the flexibility to incorporate different patterns within species composition data as well as variable data distributions within the hydroacoustics data, which are affected by site characteristics and sampling design.

### **Recommendations:**

- Targeted, standardized sampling should continue to add to our body of evidence indicating changes in relative abundances of invasive carps along the invasion front. In the meantime, occupancy modeling should continue to be explored to determine its use and efficacy in monitoring distributions and evaluating change in carp populations in the Ohio River, especially in areas of low abundance. Also, the absence of younger fish in Markland Pool this year as opposed to last, and the shift in body condition and length distributions in McAlpine and Cannelton pools, it is recommended that surveys for young-of-year recruitment continue in order to track any further changes in Silver Carp populations across the invasion front.
- With increasing catches of Silver Carp in the R.C. Byrd Pool, it is recommended that removal efforts increase in that pool to prevent any further invasion upstream.
- The eDNA project can be improved by increasing sample numbers and expanding the areas sampled. The Lower Great Lakes and the Carterville FWCOs recommend increasing eDNA sampling in West Virginia and adding two Kentucky reservoirs that are on the Salt and Licking rivers to the eDNA sampling.
- The new sampling design and analytical approach used during 2023 moved the hydroacoustic program closer to our goal of using side-looking hydroacoustics to evaluate Silver Carp densities within Ohio River pools. We recommend the continuation and further evaluation and development of the sampling design and analytical approaches to maximize the usefulness of the hydroacoustics program. Our results provide initial insights into Silver Carp densities throughout two Ohio River pools and the habitats within those pools. The approaches outlined within this report should be used in additional pools with established Silver Carp populations (e.g., Smithland Pool.), during future years to acquire a robust dataset that can be used to inform management decisions and evaluate the hydroacoustics program.

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### **Tables:**

Table 1. Length-Weight equations and the estimated weights of Silver Carp (450mm & 800mm) for eight different systems that contribute to the Mississippi River Basin. Published data for systems outside of the Ohio River Basin was obtained from Hayer et al. 2014.



Table 2. Length-Weight equations and the estimated weights of Bighead Carp (450mm & 800mm) at five locations within the Mississippi River Basin. Published data was used for river systems located outside of the Ohio River Basin.





Table 3. Location, number of samples and results of eDNA sample collection conducted in the Racine, Belleville, New Cumberland and Montgomery pools of the Ohio River in 2023.

Table 4. Number of silver carp radio-tagged at each location in the Wabash River system 2021-2023





Table 5. Electrofishing effort and the resulting total catch by the number of fish, number of species, and catch per unit effort (fish per transect) of three species of Invasive carp captured in five pools of the Ohio River from spring targeted sampling in 2023. 95% confidence intervals are in brackets.

Table 6. Total catch of targeted gill netting conducted in the Greenup and R.C. Byrd pools of the Ohio River in 2023.



electronshing surveys conducted in the Greenup, K.C. Dyrd and Kacine pools in 2023.									
	<b>Greenup Pool</b>			<b>Racine Pool</b>			R.C. Byrd Pool		
	2.5 hrs (10 Transects)		5.5 hrs (22 Transects)			1.25 hrs (5 Transects)			
		<b>Mean CPUE</b>				<b>Mean CPUE</b>			<b>Mean CPUE</b>
<b>Species</b>	N	Wr	no/hr (95% CL)	${\bf N}$	Wr	no/hr (95% CL)	N	Wr	no/hr (95% CL)
<b>Black Buffalo</b>	1		0.4(0.8)	1		0.2(0.4)	$\overline{a}$	$\frac{1}{2}$	
<b>Black Crappie</b>	1	$\overline{a}$	0.4(0.8)	$\mathbf{1}$	$\overline{\phantom{a}}$	0.2(0.4)	$\overline{\phantom{0}}$	$\qquad \qquad \blacksquare$	
<b>Bluegill Sunfish</b>	9	$\overline{\phantom{a}}$	3.6(2.1)	187	92	34.0 (29.7)	8	$\overline{\phantom{0}}$	6.4(8.8)
<b>Bluntnose Minnow</b>	$\overline{\phantom{a}}$	$\qquad \qquad \blacksquare$	$\blacksquare$	$\overline{\mathcal{L}}$	$\overline{\phantom{a}}$	0.7(0.8)	$\overline{\phantom{0}}$	$\qquad \qquad \blacksquare$	
Bowfin	$\overline{4}$	$\overline{\phantom{a}}$	1.6(1.7)	$\overline{\phantom{a}}$	$\overline{\phantom{a}}$	$\qquad \qquad -$	$\overline{\phantom{a}}$	$\overline{\phantom{a}}$	$\overline{\phantom{a}}$
<b>Bullhead Minnow</b>	1	$\qquad \qquad \blacksquare$	0.4(0.8)	10	$\overline{\phantom{a}}$	1.8(2.1)	$\overline{\phantom{0}}$	$\qquad \qquad \blacksquare$	
Channel Catfish	8	92.9	3.2(2.6)	7	87.8	1.3(1.2)	$\overline{\phantom{m}}$	$\overline{\phantom{a}}$	$\qquad \qquad \blacksquare$
Channel Shiner	69	$\overline{\phantom{a}}$	27.6(32.1)	35	$\overline{\phantom{a}}$	6.4(5.1)	19	$\overline{\phantom{0}}$	15.2(19.3)
Common Carp	8	114	3.2(2.6)	15	107.8	2.7(1.7)	$\overline{\phantom{a}}$	$\overline{\phantom{a}}$	
Eastern Banded Killifish		$\overline{\phantom{a}}$	$\overline{\phantom{0}}$	$\overline{c}$	$\frac{1}{2}$	0.4(0.5)	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$
Emerald Shiner 250		$\overline{\phantom{a}}$	100.0(50.0)	179	$\overline{\phantom{a}}$	32.5 (17.9)	79	$\overline{\phantom{a}}$	63.2(55.8)
Flathead Catfish	1	77.1	0.4(0.8)	$\mathbf{1}$	84.8	0.2(0.4)	$\overline{\phantom{m}}$	$\overline{\phantom{0}}$	
Freshwater Drum	44	104.4	17.6(11.4)	25	101.4	4.5(2.2)	6	92.9	4.8(4.6)
Gizzard Shad 520		94.7	208.0 (145.2)	606	88.9	110.2(81.1)	176	88.3	140.8 (98.6)
Golden Redhorse	52	$\overline{\phantom{a}}$	20.8(11.9)	7	$\overline{\phantom{a}}$	1.3(1.5)	$\mathbf{1}$	$\frac{1}{2}$	0.8(1.6)
Green Sunfish	1	$\overline{\phantom{a}}$	0.4(0.8)	6	$\overline{\phantom{a}}$	1.1(2.1)	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	$\frac{1}{2}$
Highfin Carpsucker	$7\phantom{.0}$	$\overline{\phantom{a}}$	2.8(2.6)	8	$\overline{\phantom{a}}$	1.5(1.5)	1	$\qquad \qquad -$	0.8(1.6)
Hybrid Striped Bass	10	100.3	4.0(3.1)	14	96.8	2.5(1.9)	3	92.5	2.4(3.1)
Hybrid Sunfish	$\blacksquare$	$\overline{\phantom{0}}$		$\mathbf{1}$		0.2(0.4)	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	
Johnny Darter		$\overline{a}$	$\overline{\phantom{0}}$	$\mathbf{1}$		0.2(0.4)	$\overline{a}$	$\overline{\phantom{m}}$	$\overline{a}$
Largemouth Bass	12	106.2	4.8(3.1)	25	98.1	4.5(2.2)	$\overline{a}$	$\overline{\phantom{m}}$	
Logperch	$\,8\,$		3.2(2.3)	$\blacksquare$	$\frac{1}{2}$	$\overline{\phantom{0}}$	$\overline{a}$	$\overline{\phantom{0}}$	
Longear Sunfish	$\overline{4}$	$\blacksquare$	1.6(1.3)	13	$\overline{\phantom{0}}$	2.4(3.7)	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	
Longnose Gar	-13	77	5.2(4.7)	9	82.6	1.6(1.3)	5	78	4.0(2.5)
Mirror Carp		$\frac{1}{2}$	$\overline{\phantom{0}}$	$\mathbf{1}$	$\frac{1}{2}$	0.2(0.4)	$\overline{a}$	$\frac{1}{2}$	$\overline{\phantom{a}}$
Muskellunge	$\overline{\phantom{a}}$	$\overline{\phantom{a}}$	$\overline{\phantom{0}}$	1	80.3	0.2(0.4)	$\overline{\phantom{0}}$	$\overline{\phantom{a}}$	$\overline{\phantom{a}}$
Northern Hog Sucker	7	$\overline{\phantom{a}}$	2.8(2.0)	5	$\qquad \qquad -$	0.9(0.9)	3	$\overline{\phantom{a}}$	2.4(4.7)
Orangespotted Sunfish	$\mathbf{1}$	$\overline{\phantom{a}}$	0.4(0.8)	2	$\overline{\phantom{a}}$	0.4(0.5)	$\overline{\phantom{0}}$	$\overline{\phantom{a}}$	$\overline{\phantom{0}}$
Quillback Carpsucker	5	$\overline{\phantom{a}}$	2.0(1.7)	29	$\overline{\phantom{a}}$	5.3(7.7)	$\overline{4}$	$\overline{\phantom{0}}$	3.2(6.3)
Redear Sunfish	$\overline{\phantom{a}}$	$\overline{\phantom{a}}$	$\qquad \qquad -$	11	95.2	2.0(1.4)	$\overline{\phantom{0}}$	$\overline{\phantom{a}}$	$\qquad \qquad -$
River Carpsucker	13	91.1	5.2(3.5)	10	96.4	1.8(1.2)	2	95.3	1.6(3.1)
River Redhorse 16		$\blacksquare$	6.4(4.2)	6	$\overline{\phantom{a}}$	1.1(1.8)	5	$\blacksquare$	4.0(4.9)
Spotfin Shiner	3	$\overline{\phantom{a}}$	1.2(1.7)	2	$\overline{\phantom{a}}$	0.4(0.5)	1	$\overline{\phantom{m}}$	0.8(1.6)
Sauger	60	80.5	24.0(15.5)	46	82.4	8.4(4.4)	9	75.6	7.2(4.6)
Silver Chub	$\mathbf{1}$	$\frac{1}{2}$	0.4(0.8)		$\overline{\phantom{a}}$	$\qquad \qquad -$	$\overline{\phantom{m}}$	$\qquad \qquad -$	$\overline{\phantom{a}}$

Table 7: Total number of fish captured per pool including abundance (Catch Per Unit Effort (CPUE)) and condition (Relative Weight (Wr; where applicable)) estimators during fall community electrofishing surveys conducted in the Greenup, R.C. Byrd and Racine pools in 2023.



Table 8. Average relative weight (W<sub>r</sub>) calculated by species and pool from community surveys conducted in the Greenup, R.C. Byrd and Racine pools of the Ohio River 2023 by WVDNR (Bister et al. (2000) and Nuemann et. al. (2012)). Outliers were removed from analysis. Comparisons with the pool average from 2017-2023 data are also included.



# Table 9. Gillnetting effort (feet of net), catch and species counts from fall gill net surveys conducted in the Greenup and R.C. Byrd pools in 2023.



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Table 10. Total number of fish captured per pool and percent of total captured at five pools combined in the Allegheny, Monongahela, and Ohio Rivers during spring tailwater night electrofishing surveys in 2023.



(A=Allegheny, M=Monongahela, O=Ohio)



Table 11. Total number of fish captured per pool by gear type in the Montgomery and Dashields pools of the Ohio River in September and October 2022. GN = Gill Net, NTEF = Night Electrofishing.







Table 12. Fish captured by species and percent total abundance from boat ramp seine hauls conducted in the Greenup, R.C. Byrd and Racine pools of the Ohio River in Fall 2023.

2023 Ohio River Boat Ramp Seines									
	Racine (3 Sites)			R.C. Byrd (5 Sites)			Greenup (5 Sites)		
		$\frac{0}{0}$			$\frac{6}{10}$			$\frac{0}{0}$	
<b>Species</b>	N	Catch	Pool Ave	N	Catch	Pool Ave	N	Catch	Pool Ave
<b>Black Redhorse</b>		$\overline{\phantom{0}}$			$0.09\%$	$0.02\%$		$\overline{\phantom{0}}$	
Bluegill	19	8.88%	5.84%	4	$0.34\%$	1.55%		0.06%	0.98%



Table 13. Species and number captured with percent of total catch during annual beach seine surveys in the Montgomery Island Pool from 2023.





	# of zooplankton samples							
<b>Site</b>	Year	May	June	July	Aug	<b>Sept</b>	<b>Oct</b>	
Kyger Creek	2021				$\overline{\phantom{a}}$	$\overline{\phantom{0}}$		
Guyandotte River	2021				-	-	-	
Scioto River	2021				$\overline{\phantom{0}}$	-		

Table 14. Temporal distribution of zooplankton samples collected in the Ohio River 2021-2023.



Table 15. Total number of fish captured per pool from gill net sampling at Montgomery Slough (Montgomery Pool) and near Phyllis and Georgetown Islands (New Cumberland Pool) in the Ohio River in November/December 2023.

<b>Species</b>	<b>Montgomery Pool</b>	<b>New Cumberland Pool</b>	Total
Common Carp			
<b>Freshwater Drum</b>			
River Carpsucker			
Sauger			
Silver Redhorse			



Table 16. Number of unique fish detected at each receiver location in the Ohio River 2021-2023. For locations with multiple receivers (e.g. lock and dam structures) detections from all receivers were pooled, and the number of unique fish are reported.



Table 17. Number of sites (N) and mean and upper and lower 90% credible intervals (CrI) for Silver Carp density  $(SVC/1000m^3)$  within main channel (MC), side channel (SC), and tributary (TRIB), habitats within Cannelton and Newburgh pools.

			<b>Mean SVCP</b>	Average Lower 90%	<b>Average Upper</b>
Pool	<b>Habitat</b>	N	<b>Density</b>	CrI	90% CrI
Cannelton	MC	131	0.096	0.094	0.097
Cannelton	Trib	18	1.530	1.520	1.540
Newburgh	<b>BW</b>		1.68	1.67	1.69
Newburgh	MC	102	0.050	0.049	0.051
Newburgh	<b>SC</b>	34	0.113	0.110	0.115
Newburgh	Trib	5	1.860	1.840	1.880

**Figures:**



Figure 1. A section of the middle Ohio River consisting of six pools (Cannelton - Racine) that are colored according to the Silver Carp population's invasion status in 2023. A pool's status is reevaluated each year following the analysis of sampling data that's collected for several ongoing research projects in the Ohio River Basin.



Figure 2. Map of hydroacoustic sample sites sampled in the River in 2023.



Figure 3. Gauge height at USGS Gauge 03378500 graph at New Harmony, IN on the Wabash River indicating low water in sumer and fall 2023 reducing the ability to reach hydroacoustice sample sites.



Figure 4. Depiction of hydroacoustic beams with transducers offset to maximize water column coverage for two split-beam echosounders. Figure modified from McNamara et al. 2016.



Figure 5. Map of dozer trawl (red) and boat electrofishing (blue) sites in Cannelton and Newburgh pools during October 2022. Community data were used to apportion hydroacoustic targets to species. Black lines across the river indicate dam locations.



Figure 6. Estimated probability of a fish being a Silver Carp given its total length for the downstream portion of Deer Creek. The dark line is the median probability, and the gray-shaded areas represent the 90% (light) and 75% (dark) credible intervals, respectively.



Deer Creek DN | IN DS Deer Creek DN-1

Figure 7. Estimated mean TS (dB) and TL (cm) for all fish tracks at Deer Creek for the downstream, shore-facing transect. Black dots represent the estimated median TS and TL. Dark and light gray lines represent 75% and 90% credible intervals, respectively.



Figure 8. The log-transformed length-weight relationship of Bighead Carp collected from the middle Ohio River. The darker circles illustrate the length and weights of the fish sampled through 2022, while the Bighead Carp collected in 2023 and resulting regression line are provided in red. The dark line represents a regression equation (see Table 2) generated from all of the length-weight data collected between 2015 and 2023.



Figure 9. The length frequency distribution (25mm bins) for Silver Carp collected from the Cannelton, McAlpine and Markland pools in 2023.



Figure 10. Length frequency (25 mm bins) distributions for male and female Silver Carp collected from the Cannelton and McAlpine pools during 2023.



Figure 11. Age distribution of Silver Carp that were collected from the Cannelton Pool in fall 2023.







Figure 13. The log-transformed length-weight relationship of the Silver Carp collected from the middle Ohio River. Darker circles illustrate lengths & weights of Silver Carp sampled in 2015 – 2022. The length-weight data from 2023 and the resulting regression line is provided in red. The dark line represents the regression equation (see Table 1) generated from the entire Silver Carp length-weight dataset from 2015 through 2023.



Silver Carp Relative Weights (W<sub>r</sub>) | 2015 - 2023

Figure 14. Relative weight (W<sub>r</sub>) comparisons for Silver Carp captured from the Cannelton and McAlpine pools in August – October of 2015 through 2023. The standard weights needed for the  $W_r$  calculations were generated using the  $50<sup>th</sup>$  percentile regression methods outlined in Lamer et al, 2015.



Figure 15. Relative weight  $(W_r)$  comparisons for Silver Carp collected in August – October 2023 from the Cannelton, McAlpine and Markland pools of the middle Ohio River. The standard weights needed for the  $W_r$  calculations were generated using the 50<sup>th</sup> percentile regression methods outlined in Lamer et al, 2015.



Figure 16. CSS slope for the Illinois River. CSS slope flattened (higher ecological efficiency) over the data collection period. The first large silver carp spawn in the Illinois River was documented in 2000. The red line indicates when the silver carp from the spawning event started recruiting to electrofishing starting in 2004 when silver carp CPUE jumped from 2.29fish/hr to 12.29 fish/hr.



Figure 17. Regression of CSS graphed by pool for the Ohio River. The mean slopes from downstream to upstream are: Cannelton: -1.31, McAlpine: -1.64, Markland: -2.53, Meldahl: -1.86, Greenup: -1.84, RC Byrd: -176 No significant differences were determined.



Figure 18. Average NBSS model for zooplankton collected monthly (May-October) from Markland Pool to R.C. Byrd Pool in the Ohio River, with breakdown of size class density for each aspect of the zooplankton community for each month.



Figure 19. Boxplots of total lengths (mm) of silver carp sampled using electrofishing in the Wabash River during 2021 through 2023. Small fish, likely recruits were only sampled in 2021.



Figure 20. Spatial distributions of invasive carp before and after harvest in the Grayville oxbow of the Wabash River in March 2023. Note, the lower density scale in the post-harvest panel.



Figure 21. Spatial distributions and estimated sizes of the entire adult fish community sampled during hydroacoustic sampling pre- and post-harvest of invasive carp from the Grayville oxbow of the Wabash River in March 2023.



Figure 22. Bigheaded carp removed during the Grayville oxbow harvest removal in March 2023. Photo Credit: Justin Widloe.



Figure 23. Mean (standard error) estimated invasive carp densities from hydroacoustic sampling conducted pre- and post-harvest of invasive carp from the Grayville oxbow of the Wabash River in March 2023.



Figure 24. Hydroacoustically derived Silver Carp (SVC) density with river mile for main channel sites in Newburgh and Cannelton pools during October and November 2023. River miles decrease from downstream to upstream within the Ohio River (left to right on x-axis).