Project Title: Historical assessment of Invasive Carp reproduction upstream of Lock and Dam 19 in the UMR

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Location of Activities: UMR, Iowa/Illinois/Missouri.

Statement of Need:
Invasive carp continue to increase in abundance and distribution above LD 19 in the Upper Mississippi River. This increase may be due to either dispersal of adults and/or through successful reproduction and recruitment in newly invaded areas. Adult invasive carp move upstream past LD 19, but movement rates are relatively low for the number of adults encountered in pools 19 and above. Conversely, larval invasive carp have been captured in pools 19-17 on an annual basis since 2014; yet captures of juvenile invasive carp in the fall are quite rare. The lack of juvenile captures could indicate that larvae are not successfully surviving until the juvenile phase or that juveniles are very difficult to locate and capture despite extensive sampling efforts that have occurred in the UMR. Age structure of adult invasive carp above LD 19 indicates recruitment is consistent. However, whether recruitment of these fish into the population above LD 19 is occurring due to adults migrating upstream through LD 19 or due to recruitment of offspring produced above LD 19 is unknown.

Otolith microchemistry can be a useful tool to identify locations where recruited fish are produced. Previous otolith microchemistry work on the Upper Mississippi River indicated <20% of adult invasive carp recruited above LD 19. However, this analysis was conducted with a limited number of fish, and it remains unknown whether this recruitment was the result of one large year class or due to low levels of consistent recruitment on an annual basis. Understanding where recruitment is occurring, and the frequency of recruitment (e.g., annually or periodically) is critical for implementing management actions such as commercial harvest and installation of barriers. Therefore, we propose to assess the historical recruitment of invasive carp above LD 19 using adult age structure to determine if they are recruiting above LD 19 and if so, the temporal variation in recruitment. This information will be critical to assess if the larvae being produced above LD 19 annually are not recruiting or if they are surviving but going undetected despite extensive sampling efforts.

Silver Carp will be collected by commercial anglers in pools 17-19, fish will be measured for total length, and otoliths will be removed for age estimation. Because an estimated 15% of the population was produced above LD 19, we will need to collect approximately 1,000 fish per pool for an adequate sample size. Fish will be randomly subsampled per 1 cm group and age-length keys will be used to develop age-frequency histograms for each pool. Once age-structure is determined, we will assess which age-classes are considered recruited to the gear and can be used for Maciena's catch curve analysis to assess historical recruitment of Silver Carp in pools 17-19 of the Upper Mississippi River. Most Silver Carp captured above LD 19 are 5-10 years old, with peak catches not occurring until age 6-7. Therefore, we anticipate having approximately five age-classes where recruitment can be assessed (6–10-year-old fish). Otolith microchemistry will be completed on approximately 60 fish per year class at Southern Illinois University to assess if their natal origin is above or below LD 19. The proportion of Silver Carp produced above versus below LD 19 per year class will be applied to the age-structure of fish captured from each pool to construct separate age-frequency histograms for fish originating from above versus below LD 19. Finally, catch curves will be constructed and residuals will be used to estimate year class strength for fish originating above and below LD 19 for both pools 17, 18, and 19. Finally, environmental conditions (e.g., mean June discharge) can be used to predict variation in recruitment based on environmental conditions.

Project Objectives:

1. Assess the historical occurrence and magnitude of Invasive Carp recruitment upstream of LD 19 in relation to environmental conditions.

Project Highlights:

Invasive carp reproduction regularly occurs upstream of LD 19 and adult age structure indicates consistent recruitment; yet juvenile life stages are rarely found. We collected a total of 526 otoliths (505 from Silver Carp) from commercially harvested bigheaded carp (664-1,300 mm TL) upstream of Lock and Dam 19 (LD 19) during fall 2022 to assess historical recruitment patterns upstream of LD 19. Due to the apparent absence of juvenile life stages, we integrated otolith microchemistry (Sr:Ca and Ba:Ca; µmol/mol), oxygen stable isotope ($\delta^{18}O$, ‰), and year-class strength metrics to retrospectively assess Silver Carp recruitment upstream of LD 19. We measured otolith Sr:Ca, Ba:Ca, and $\delta^{18}O$ to infer early life environment (ELE) for 480 Silver Carp (sample failure <5%). We assessed the historical occurrence and magnitude of Silver Carp recruitment to identify recruitment sources and identified environmental correlates of year-class strength. Our results may provide managers evidence of the role environmental conditions play in the recruitment dynamics of invasive carp to better direct future control strategies such as juvenile recruitment monitoring, adult removal, and barrier management to slow invasive carp spread near the invasion front in the UMR.

Methods:

We collected otoliths from 505 Silver Carp were collected commercially harvested upstream of LD 19 (pools 16-19) between October and December 2022. Fish sampling activities making this study possible stem from a partnership between the Illinois Department of Natural Resources, Illinois Natural History Survey, and contracted commercial fishers aimed at reducing invasive carp densities through targeted removal; focusing efforts where these are most abundant near LD 19. Fishers captured carp using a variety of fishing nets: trammel nets, gill nets, hoop nets, and seines. Trammel and gill nets had 76 mm bar mesh size and gill nets were 183 m long and 3 m tall. Commercial fishers drove invasive carp into the nets using loud pounding or motorboat noises. We euthanized fish according to Protocol 16-09 approved by the Institutional Animal Care and Use Committee at Western Illinois University. For each euthanized fish, we recorded total length (mm), weight (kg), gonad weight (g), and sex. We removed the lapilli otoliths from the inner ear (following instructions previously detailed in Seibert and Phelps 2013; Sullivan et al. 2018, Anderson et al. 2021). We rinsed otoliths using lint-free cleaning wipes and distilled water. Once dried, we stored otoliths in individually labeled 0.6 ml microcentrifuge tubes. As part of the laboratory processing (January-October 2023), we embedded all the otoliths in epoxy EpoxiCure2 resin (Buehler, Illinois) following manufacturer guidelines and sectioned two 1 mm-thick transverse cuts through the core for each otolith-resin piece using a double Buehler Isomet low-speed saw (Buehler, Illinois; Whitledge et al. 2019). One of the otoliths in the pair was set aside for age estimation, and the other for both otolith microchemistry and oxygen isotope analyses.

We first estimated fish age using sections obtained from the second lapillar otolith embedded in epoxy. We glued each section to a labeled microscope slide and submerged the section in glycerol. We used a Leica S8 APO stereomicroscope and a Leica MC170 HD microscope camera (Leica, Wetzlar, Germany) to estimate age from annuli counts of each individual fish otolith section. We did not count the outer edge annulus given that the fish were caught later in the year (fall 2022; following guidelines by Mark W Fritts, USFWS, Personal Communication). All otolith sections are archived at the Illinois Natural History Survey's Illinois River Biological Station, Havana, IL. Three experienced readers independently estimated ages with no knowledge of fish length. When the age estimates differed, readers discussed and arrived at a consensus.

We inferred the ELE of each individual Silver Carp using a combination of otolith microchemistry (Sr:Ca and Ba:Ca; µmol/mol) and oxygen stable isotope analyses (δ18O, ‰) measured from aragonite material in the otolith primordium (Whitledge et al. 2019, Anderson et al. 2021). To obtain otolith Sr:Ca and Ba:Ca, and δ18O values, we first polished a resin-embedded otolith section from each individual Silver Carp for 90 s using 1,000 grit sandpaper and lapping film. We confirmed the presence of otolith primordium within each otolith section using a microscope. We fixed all individually labeled and ordered sections to microscope slides using double-sided tape and stored microscope slides in petri-dishes. We conducted all microchemical analyses (i.e., Sr:Ca and Ba:Ca only) using a Thermo XSERIES 2 inductively coupled plasma mass spectrometer (Thermo, Waltham, Massachusetts) coupled with an LSX-266 laser ablation system (CETAC Technologies, Omaha, Nebraska) at Southern Illinois University-Carbondale and following guidelines in Whitledge et al. (2019) and Anderson et al. (2021). We conducted a 30 s gas blank prior to laser ablation at the core of each otolith sample to subtract background counts of each element. We measured a standard (MACS-3; National Institute of Standards and Technology) to enable quantification and correction of possible instrumental drift for every 10-15 otolith core samples. To collect otolith δ18O values, we then drilled each laser-ablated section to also extract approximately 25-30 µg of carbonate material centered at the primordium (powder otolith aragonite) using a New Wave Research micro mill (New Wave Research, Fremont, California). Each subsample was placed on a 2 x 2 cm weighing paper and stored in a 0.6 ml disposable microcentrifuge tube to be analyzed at the Environmental Isotope Laboratory at the University of Arizona for oxygen stable isotope analyses (δ18O, ‰) using an automated carbonate preparation device (KIEL-III) coupled to a Finnigan MAT 252 gasratio mass spectrometer (Thermo Finnigan LLC, San Jose, California). We added dehydrated phosphoric acid to all our samples under vacuum at 70 °C. We used repeated measurements of NBS-19 and NBS-18 standard samples for δ18O and δ 13C, respectively, to determine isotope ratios.

For our elemental composition analyses involving microchemistry data, we first corrected for gas blank drift effects, and converted raw isotopic counts to elemental concentrations using the R shiny app: "ElementR" (Sirot et al. 2017) and converted the concentrations of strontium, barium, and magnesium to molar element:calcium ratios (Sr:Ca, Ba:Ca: μmol/mol) using custom functions in R. To visualize the variation in the elemental composition of our 2022 samples, we scaled values of Sr:Ca and Ba:Ca (μmol/mol), and δ18O (‰) constructing a principal component analysis (PCA) using the base R function prcomp().

With both microchemistry and oxygen isotopic data for individual samples, we inferred ELErelative to LD 19 by matching otolith measured Sr:Ca, Ba:Ca, and δ18O values with expected ranges of otolith Sr:Ca, Ba:Ca, and δ18O for reaches upstream or downstream of LD 19 (Whitledge et al 2019, Anderson et al. 2021). We calculated the range of water chemistry Sr:Ca, Ba:Ca, and δ18O values for each river upstream and downstream of LD19. We used linear regressions between environmental water samples and otolith elemental composition previously estimated for bigheaded carps (Norman and Whitledge 2015; Whitledge et al. 2019, Anderson et al. 2021) and corresponding 95% confidence limits used to develop a simplified early-life environment classification model relative to LD 19. We defined the categories of ELE "upstream of LD 19" and "downstream of LD 19" by reducing the following ELE categories predicted Sr:Ca, Ba:Ca, and δ18O value ranges for rivers or river segments either upstream or downstream of LD 19 established in Whitledge et al. (2019) and in Anderson et al. (2021). Since the microchemical/isotopic signature of rivers and/or river segments overlaps (e.g., Ba:Ca signature in rivers upstream of LD 19: Skunk, Iowa, and Cedar rivers, overlaps with the Ba:Ca signature of a river downstream of LD 19: Des Moines River), we excluded individuals with ELEs that match the rivers or river segments that cannot be confidently classified (i.e., "indeterminate relative to LD 19"). We calculated year-class composition as the proportion of adults from each year-class that we inferred recruited either upstream, downstream, or indeterminate relative to LD 19 for 480 Silver Carp individuals collected during 2022.

We compared proportions in the three inferred ELE categories within in our sample and between studies (Whitledge et al. 2019; Anderson et al. 2021) using *X2* tests. We then built separate catch-curves and calculate RVI for the distinct ELE categories. For the catch curve analysis, we first constructed age-frequency histograms from age estimates for all Silver Carp separately by inferred ELE to assess both the proportion of individuals produced upstream versus downstream of LD 19 in each year-class, annual recruitment variability, and the magnitude of annual recruitment upstream versus downstream of LD 19. To estimate recruitment variability, we calculated the recruitment variability index (RVI; Guy and Willis 1995) that varies from -1 to 1, where values closer to 1 represent consistent recruitment. We then used weighted catch-curve residual analysis on age-frequency distributions to quantify the magnitude of Silver Carp recruitment each year while reducing the relative impact of older ages with fewer fish (Maceina 1997; Maceina and Bettoli 1998). Studentized residuals ranged between −2 and 2 and we interpreted years with negative values of studentized residuals as years producing weaker year-classes and years with positive values of studentized residuals as years producing stronger year-classes (Maceina 1997). Last, we explored the degree of synchrony among subsets of individuals with inferred early life environment upstream, downstream, and indeterminate relative to LD 19. We conducted a Spearman's rank correlation test between studentized residuals resulting from separate catch curve analyses including individuals that we inferred ELE upstream versus ELE downstream of LD 19.

Obtaining information on ELE using microchemistry represented a necessary first step to assess both the year-class composition and magnitude of Silver Carp recruitment upstream of LD 19 (through catch curve analysis) in relation to environmental conditions (Maceina 1997; Sullivan et al. 2018). Following guidelines in Sullivan et al. (2018), we gathered environmental data to calculate variables hypothesized to influence year-class strength from the National Oceanic and Atmospheric Administration (NOAA; https://www. ncdc.noaa.gov/) weather stations and the U.S. Geological Survey (USGS; https://www.usgs.gov/) gage station located in Keokuk, Iowa (LD 19) in the Mississippi River from January 1, 2000 to December 31, 2022. From the daily discharge data (m3/s), we calculated baseflow as the ratio between the 7-day minimum daily discharge and the mean discharge per year, cumulative seasonal river discharge (e.g., the reproductive season from May to June, and the non-reproductive season from July to October), and the cumulative seasonal coefficient of variation of the mean daily discharge (Sullivan et al. 2018). From the daily temperature (°C) data, we also calculated annual degree days base (base 0°C; to facilitate comparison with previous literature on environmental predictors of Silver Carp invasion; Brook et al. 2023), mean temperature of the coldest quarter of a year, and mean temperature of the warmest quarter of a year (bioclimatic variables typically coded as BIO10 and BIO11, respectively). To explore variation in year-class strength represented by catch-curve residuals as a function of the environmental predictors we used Pearson's product-moment correlation coefficient (Pearson's r). We explored whether these environmental variables were correlated to Silver Carp year-class strength separately for fish with distinct inferred ELE categories (i.e., upstream or downstream to LD 19). To test the correlation between environmental variables and year-class strength we used the R function corr() but specifying Pearson's product-moment correlation in the arguments. We executed all analyses in R version 4.2.2, (R Core Team 2024) and R Studio (R Studio 2024) using the "tidyverse" environment (Wickham et al. 2019).

Project Activities:

We assessed the historical occurrence and magnitude of Silver Carp recruitment to identify recruitment sources and identified environmental correlates of year-class strength. All analyses have been completed. This includes analyses of otolith Sr:Ca, Ba:Ca, and $\delta^{18}O$ to infer ELE for 480 Silver Carp (sample failure <5%). We also calculated metrics representing the variation in temperature and discharge from 2009 to 2016, corresponding to year-classes represented in our sample to use as environmental covariates in residual regression models of year-class strength. Statistical analyses have been completed. This work has been presented at the Iowa Chapter of the American Fisheries Society, the Midwest Fish and Wildlife Conference, and the American Fisheries Society Annual Meeting. A manuscript with final project results and discussion will be ready to be submitted for agency and journal peer review in the coming few months. Our results provide managers evidence of the role environmental conditions play in the recruitment dynamics of invasive carp to potentially direct future control strategies such as juvenile recruitment monitoring, adult removal, and barrier management to slow invasive carp spread near the invasion front in the UMR.

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