Characterizing Dietary Overlap Between Invasive Silver Carp *Hypophthalmichthys molitrix*, and Native Gizzard Shad *Dorosoma cepedianum*, in Kentucky and Barkley Reservoirs

M.S. Thesis Proposal

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Introduction

After the accidental escape and rapid expansion of Silver Carp (*Hypophthalmichthys molitrix)* into the Upper Mississippi River System (UMRS) in 1973, ecological consequences have been faced by managers while trying to control and study this invasive species. In the Mississippi River basin, altered flow and habitat may increase the probability of establishment of invasive species and many tributaries increase the likelihood of dispersal into connected drainages (Gido and Brown 1999). Rigorous approaches to detecting the effects of invasive species on native species require standardized data collection on the abundance of native biota before and after the establishment of the invasive species, in multiple invaded areas and multiple control areas where the invasive species is not established (Blossey 1999; Underwood 1992, 1994). Silver Carp is one of four species of Asian carps that have established reproducing populations in the UMRS (Chick and Pegg 2001; Raibley et al. 1995; Williamson and Garvey 2005).

Silver Carp is an omnivorous planktivore that consumes phytoplankton, zooplankton, and detritus (Xu and Xie 2004; Zhou et al. 2009, 2011). Most studies cite Silver Carp as primarily phytoplankton feeders, able to collect algae larger than 10 µm (Sieburth et al., 1978; Hampl et al., 1983; Smith, 1989; Vörös et al., 1997), while others suggest that Silver Carp is able to collect even nanoplankton (<10 µm) (Cremer and Smitherman, 1980; Xie, 1999; Görgényi et al., 2015). Their fused system of gill rakers allows them to retain particles as small as 3.2 µm in diameter and promotes retention and consumption of large quantities of zoo- and phytoplankton (Li and Dong 1996). Although Silver Carp is often described as phytoplanktivorous, depending on the composition of available food, the species also ingests a high proportion of zooplankton in its diet (Battonyai et al. 2015; Kolar et al. 2005). There is evidence that Silver Carp can alter zooplankton communities in the UMRS (Sass et al. 2014) and consume similar zooplankton species as native planktivorous fishes (Sampson et al. 2009), potentially leading to negative effects on native fishes through competition (Irons et al. 2007, 2011; Pendleton et al. 2017). Foraging competition between Silver Carp and native fishes is perpetuated by Silver Carp's diverse diet, fast growth rates, high fecundity rates, and rapid proliferation into new geographic sites (Xie and Chen 2001; DeGrandchamp et al. 2008; Cooke and Hill 2010).

The potential negative effects of Silver Carp are not limited to native planktivorous fishes because nearly all fish species in freshwater ecosystems begin their lives feeding on zooplankton (Chick and Van Den Avyle 1999). In lentic ecosystems forage fish assemblages can become negatively impacted by growing populations of Silver Carp. A food web model developed to project the effects of BHC on Lake Erie suggested that if they became established in the lake, they might eventually comprise up to 34% of the total fish biomass, and have negative effects on planktivorous fishes (e.g., emerald shiner *Notropis atherinoides*) and positive effects on some piscivores (e.g., smallmouth bass *Micropterus dolomieu*) (Zhang et al. 2016; L. N. Ivan et al. 2020). Within the Mississippi and Illinois River systems, they now comprise a large fraction of the total fish biomass, and have had significant negative effects on plankton and planktivorous fishes (Irons et al. 2007; Pendleton et al. 2017; Phelps et al. 2017).

The relatively rapid integration of Silver Carp into native food web assemblages is an important ecological dynamic to study. The addition of an exotic species, for example, could reduce the average size or lifespan of indigenous species, alter historical food web pathways, or decrease overall efficiency of resource use (Havens 1994). Research to date suggests bigheaded carps may be having negative effects on native fishes, particularly in the Illinois River (Irons et al. 2007, 2011; Solomon et al. 2016).

As Silver Carp expand their range through the upper Midwest, closer to the Laurentian Great Lakes and further into the southeastern reservoirs, the need for further research into the impact invasive Silver Carp have on lower trophic communities becomes increasingly valuable. Understanding the relationship between planktonic size, particle type and habitat selectivity of Silver Carp since they have established themselves in southeastern impoundments such as Kentucky and Barkley Reservoirs is important in the management of lower trophic communities, sportfish and forage fish populations. Observations from other systems such as the UMRS and Illinois River can be directly applied to continued research on these two reservoirs in order to document habitat selectivity and feeding strategies. With Gizzard Shad being a valuable prey source for sportfish such as Striped Bass *(Morone saxatilis)*, Largemouth Bass *(Micropterus salmoides)*, and Blue Catfish *(Ictalurus furcatus)*, examining the degree of dietary overlap they share with Silver Carp can give insight on how to better mange for sustainable populations of forage fish. Characterizing the variables such as seasonality, nutrient inputs and location on these reservoirs will also be important to understand the habitat selectivity of Silver Carp. Managers can then use results to make more informed decisions on how to predict movements of Silver Carp and continue removal efforts accordingly.

Objectives

The main objectives of this project are to 1) Achieve a better understanding of contributors to habitat selectivity of Silver Carp, 2) further examine the composition of planktonic particles available for grazing in Kentucky and Barkley reservoirs, 3) identify selectivity for particle type and particle size by Silver Carp, and 4) compare the particle selectivity of Silver Carp to Gizzard Shad.

Study Sites

Kentucky Reservoir

Kentucky Reservoir was impounded in 1945 upon the completion of the six-year Kentucky Dam construction project by the Tennessee Valley Authority (TVA). Located approximately 35 km upstream from the confluence of the Tennessee and Ohio rivers, Kentucky reservoir provides a means of navigation through the lower Tennessee River, a source of hydroelectric power generation, flood control and outdoor public recreation activities such as boating, fishing and skiing. With 64,871 ha of surface water, 3,700 km of shoreline and spanning 296 km across Kentucky and Tennessee, Kentucky Reservoir is the largest impoundment in the eastern United States. Since 1994, Kentucky Reservoir has received either "fair" or "good" ecological health scores from TVA and is considered a eutrophic system. Specific sample sites for this project will occur at the Duck River, Big Sandy embayment and Sledd Creek embayment to include riverine, main reservoir and downstream lacustrine habitats.

Barkley Reservoir

Impounded in 1966 upon the completion of Barkley Dam by the U.S. Army Corp of Engineers, Barkley Reservoir is located 61 km upstream from the confluence of the Cumberland and Ohio rivers. Barkley Reservoir is also used for year-round navigation through the lower Cumberland river, hydroelectric power generation and public outdoor recreation activities. Though smaller than Kentucky Reservoir, Barkley contains 21,000 ha of surface water and 1,615 km of shoreline, a navigation channel at Cumberland River mile 32.8 that interconnects Barkley Reservoir to Kentucky Reservoir allows for boat traffic as well as passage for fish between the two reservoirs. Considered eutrophic as well, Barkley Reservoir sample sites for this project will occur at Saline Creek, Little River and Double Creek to also include riverine, main reservoir and downstream lacustrine habitats.

Methods

Sampling Protocol

Silver Carp Collection

In order to collect Silver Carp, the same methodology used by Padgett (2021) was applied in this study. Three gangs comprised of two monofilament gill nets (one Type-I and one Type-II) will be set at each site during the spring, summer and fall sampling seasons. Each Type-I gill net is comprised of two 45.7 m sections of 76.2 mm and 88.9 mm mesh and each Type-II gill net is also comprised of two 45.7 m sections with larger 101.6 mm and 108 mm mesh, respectively. Following a standard gill netting protocol, each gang of nets will be set perpendicular to shoreline where applicable to maximize catch efficiency of Silver Carp. Gillnets will be deployed in the evening, left to fish overnight and will be retrieved promptly the following morning when Silver Carp will be removed. To achieve a robust sample size to compare gill raker plankton composition, a total of four Silver Carp will be randomly selected from each net, per site, per season (i.e. spring, summer, fall).

Gizzard Shad Collection

To efficiently collect Gizzard Shad, the same custom electrified dozer trawl built by Midwest Lakes Electrofishing Systems (MLES) that was used by Padgett (2021) will be used. Equipped with a MLES Infinity series control box, receiving power from a Honda EU7000is generator, an electrical current will be distributed into the water via a 7-cable anode with the boat hull as the acting cathode. At least ten, five-minute trawls, travelling at 4.5 km/h (Hammen et al. 2019) along the relative shoreline where gillnets were deployed will occur to collect Gizzard

Shad. Amperes will be adjusted based on site water ambient conductivity and Gizzard Shad behavioral response using guidance from Miranda (2009). During each trawl, Gizzard Shad influenced by electrotaxis will be captured in the dozer trawl frame and will be contained in the cod end of the net. Captured Gizzard Shad will be removed from the cod end and immediately bagged and placed into an iced cooler until they are processed. Similar to the protocol implemented for the collection of Silver Carp, four Gizzard Shad will be selected at random when captured in dozer trawl transects to replicate the same sample size.

Plankton Collection

At each location where gillnet gangs will be set, the same 3m long by 5.08 cm in diameter polyvinyl chloride (PVC) integrated tube sampler as the one used by Padgett (2021) will be used to collect a representative plankton community sample from the upper 3 m of the water column. Following the same methodology as Padgett (2021) three subsamples will be taken at each gang, one approximately 25 m from the outside of each net and one at the midpoint between the two nets. Each subsample will be combined into a clean 5-gallon bucket, mixed thoroughly where a final 500 mL sample will be collected for processing in the laboratory. To collect the same representative plankton community sample at each dozer trawling transect where Gizzard Shad will be collected, three subsamples will be collected at the beginning, midpoint and endpoint of each five-minute trawl. Again, each subsample will be combined in a clean 5-gallon bucket, mixed thoroughly where a final 500 mL sample will be taken as done by Padgett (2021).

Utilizing similar methods as Walleser et al. (2014) and Padgett (2021), the entire first gill arch from Silver Carp and Gizzard Shad will be removed using surgical scissors or a pair of fine bladed shears. Upon removal of the gill arch, a curved nozzle wash bottle will be used to thoroughly rinse the contents into a wide mouth 250 mL Nalgene bottle with purified drinking water. Silver Carp gill rakers will be rinsed until clean with 150 mL of purified drinking water and Gizzard Shad gill rakers will be rinsed until clean with 100 mL of purified drinking water. All plankton samples (i.e. plankton community samples and gill raker filtrates) will be kept in an iced cooler until being stored in a refrigerator at a stable 4˚C until processing occurs in the laboratory.

Plankton Sample Preservation

Plankton community samples collected from gillnet gang sites and corresponding dozer trawl transects will be preserved using 3% solution of 25% Glutaraldehyde. Raw gill raker filtrates collected from Silver Carp and Gizzard Shad will be preserved using a 6% solution of 25% Glutaraldehyde. Gill raker filtrates from Silver Carp and Gizzard Shad will be preserved using a higher concentration of 25% Glutaraldehyde due to the presence of blood and tissue that may enter the sample while rinsing occurs.

Data Analysis

Imaging Flow Cytometer

Water samples, along with gill raker filtrates from both Silver Carp and Gizzard Shad will be examined in the laboratory using a Flowcam 8000 imaging flow cytometer in a similar manner as done by Padgett (2021). Raw gill raker samples will be filtered using a series of mesh sieves before processing. A known volume from each individual sample will first be poured through a 1000 μm sieve, then filtering through a 300 μm sieve and finally filtering the sample through a 100 μm sieve. Filtrates from the 1000 μm and 300 μm sieves will be saved in 5 ml vials for further particle analysis under a standard light microscope in addition to the 4X objective lens of the FlowCam. The smaller filtrates from the 100 μm sieve (particles 4-100 μm) will be analyzed using the 10X objective lens of the FlowCam, where 1 mL replicates will be run and individual images will be taken of each particle. All plankton samples will be diluted using deionized water to achieve appropriate PPUI (particle per used image) and to prevent the flow cell from becoming clogged. Fluorescent triggers within each particle will be indicated and particles will be measured and grouped into three classifications, cyanobacteria, diatoms and other algae and detritus.

After the samples have been run through the FlowCam, a frequency histogram will be generated through VisualSpreadsheet Version 5, displaying values for particle type ratio, mean geodesic aspect ratio, mean area-based diameter (ABD), and maximum particle size. These metrics will be saved from the VisualSpreadsheet software, data will be saved into an excel spreadsheet for individual gill raker samples and water samples.

Statistical Analysis

Excel data summaries generated through VisualSpreadsheet Version 5 will be exported into R statistical computing software 4.2.1 where statistical analyses will be completed. Silver Carp and Gizzard Shad will be grouped into length bins to determine if there is a correlation between length and particle selectivity. Following the same statistical analyses as Sampson et al. (2009), nonparametric multidimensional scaling (NMDS) will be used to graphically illustrate potential overlap between the particle size and particle type. Additionally, aspects of seasonal availability of planktonic particles and particle selectivity will be analyzed using an analysis of similarity (ANOSIM) test in R package “vegan” (Sampson et al. 2009). A permutational multivariate analysis of variance (PERMANOVA) using the Bray-Curtis dissimilarity index will be used to justify seasonal dietary overlap between Silver Carp and Gizzard Shad based on gill raker filtrates and water composition samples from individual fish collection sites (Padgett 2021). Additionally, two-sample t-tests will used to compare differences in mean ABD, mean geodesic aspect ratio, and maximum particle size of gill raker filtrates between Silver Carp and Gizzard Shad, where p-values (≤ 0.05) will be accepted as statistically significant (Padgett 2021).

Implications

Findings from this study will provide state and federal agencies with current data on the status of lower trophic communities at seasonal and special levels on Kentucky and Barkley reservoirs. This information can then be compared to the habitat and planktonic particle type selectivity of Silver Carp to determine to how and why these non-native invaders are impacting indigenous species that rely on plankton communities for forage. Continuing this research on these unique systems will aid biologists in more sound management practices and eradication efforts allocated towards invasive carps, as well as the continued management of important forage fish populations such as Gizzard Shad.

Similar methods to this study could be used to investigate interactions between other indigenous planktivores, and Silver Carp. Future projects like this could also be applied to systems other than Kentucky and Barkley reservoirs that face the invasion of Silver Carp.

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