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Fish Community Trends in the Greenup Pool, Ohio River

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FISH COMMUNITY TRENDS IN THE GREENUP POOL, OHIO RIVER

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APPROVAL OF THESIS

We, the faculty supervising the work of Nathaniel Kingsley Fleshman, affirm that the thesis, *Fish Community Trends in the Greenup Pool, Ohio River*, meets the high academic standards for original scholarship and creative work established by the Biological Sciences Program and the College of Science. This work also conforms to the editorial standards of our discipline and the Graduate College of Marshall University. With our signatures, we approve the manuscript for publication.

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ABSTRACT

Bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*H. molitrix*), collectively referred to as Asian carps, are invasive species that may negatively affect native species through plankton resource exploitation mechanisms. Bighead and silver carp escaped into the Mississippi River basin in the early 1970s and have been steadily migrating upstream. The Greenup Pool lies upstream of the current known invasion of Asian carp in the Ohio River. The Ohio River Valley Water Sanitation Commission (ORSANCO) has conducted probabilistic electrofishing community surveys in the Greenup Pool since 2006 on a five-year cycle. The objective of this study was to compare body condition of commonly collected fishes between ORSANCO survey years using relative weight and Fulton's condition factor. Channel catfish decreased in mean relative weight between 2011 and 2016, and sauger increased in mean relative weight between 2006-2011 and 2006-2016. The second objective of this study was to explore potential trends in fish community distribution in the Greenup Pool using nonmetric multidimensional scaling. The fish community appeared to show dissimilarity between the lower Greenup Pool and the middle and upper pools. This study will serve as a baseline for the Greenup Pool preceding the invasion of bigheaded carps for future studies and comparisons.

CHAPTER I

THE ASIAN CARP INVASION

INTRODUCTION

Bigheaded Carps

The term "Asian carp" predominantly refers to four species: the grass carp (*Ctenopharyngodon idella*), black carp (*Mylopharyngodon piceus*), bighead carp (*Hypophthalmichthys nobilis*), and silver carp (*Hypophthalmichthys molitrix*). The bighead and silver carp of the genus *Hypophthalmichthys*, the "bigheaded carps," are native to Asia but have been introduced into the United States and are becoming exceedingly problematic by exploiting resource availability, impacting native fish assemblages, and endangering human safety.

In their native range, bighead and silver carp extend from southeastern China to far eastern Russia. Both species occur in the Yellow River, Yangtze River, and Xi River, which are some of the world's longest rivers. While both species can occur in lacustrine systems, they require rivers or tributaries for spawning (Kolar et al. 2007; Stone et al. 2000). Bigheaded carps may exhibit broad reproductive variability. Bighead and silver carp reach sexual maturity in three to four years, which may be shortened or extended depending on environmental conditions (Kolar et al. 2007). Males typically reach sexual maturity one year before females. High water events in spring trigger spawning, but the duration of the spawn is variable. In their native range, bighead carp spawn from April until June, and silver carp from May until July or August (Kolar et al. 2007). Bigheaded carps may have a spawn that lasts until early fall or multiple spawn events in the United States (Garvey et al. 2006; Papoulias et al. 2006). Bigheaded carps spawn with respect to high water levels, velocities (≥0.7 m/s), turbidity, and a temperature gradient varying from 18-30°C (DeGrandchamp et al. 2007; Kolar et al. 2007). Both species exhibit tolerances to water temperature, dissolved oxygen, turbidity, and salinity (Kolar et al. 2007; Nico et al. 2020_2). Spawning locations are influenced by areas of mixed water, including tributary confluences, behind dykes, below sandbars, islands, and rock beds where eggs are released into these high-velocity currents. The eggs are semi-buoyant and require water column suspension to hatch, typically within 24 hours (Kolar et al. 2007). The eggs disperse into flooded backwaters, creeks, and floodplains, which serve as nursery habitat.

Bighead and silver carp have high fecundities, which increase with age and weight. The maximum weight of bighead and silver carp is 40 kilograms (kg) and 27 kg, respectively (Nico et al. 2020_1; Nico et al. 2020_2). Aging is difficult to determine for bighead and silver carp, but both species have long life expectancies (e.g., 10+ years) (Kolar et al. 2007). In the lower Missouri River, the mean bighead carp fecundity was found to be 226,213 eggs (Schrank and Guy 2002). Williamson and Garvey (2005) determined the fecundity of six silver carp from the Mississippi River, which had a range of 57,283 to 328,538 eggs. Another study of the middle Mississippi River found mean egg densities of 777,154 for bighead carp and 1,478,331 for silver carp (Garvey et al. 2006).

Bighead and silver carp are voracious, opportunistic planktivores. Bighead carp primarily feed on zooplankton, and silver carp preferentially feed on phytoplankton. When plankton densities and biomass are low, both species will opportunistically feed on either plankters or detritus (Kolar et al. 2007; Stone et al. 2000). Silver carp possess unique, highly developed sponge-like gill rakers compared to bighead carps' comb-like gill rakers. Silver carp can feed on particles as small as 3-4 micrometers, making them more efficient filterers than bighead carp (Kolar et al. 2007). Within one year, bighead carp have the potential to grow 2.7 kg and silver carp 5.4 kg (Kolar et al. 2007). High fecundity paired with rapid growth rates promotes protection from native predators. Alligator gar (*Atractosteus spatula*) has been suggested as a control mechanism for bigheaded carps, but this is unlikely to be effective due to: overall population decline, overexploitation, reduction in available spawning habitat, and approximately ten years required for individuals to reach sexual maturity (Buckmeier 2008; Perschbacher 2011). However, blue catfish (*Ictalurus furcatus*) have the potential to help reduce bigheaded carp populations. In Pool 26 of the Mississippi River, adult silver carp were found to be the major prey of blue catfish, comprising over 70% of their diet (Locher 2018). Blue catfish are native to the Ohio River and are regularly stocked for popularity as a sport fish, which could have implications for limiting the spread of bigheaded carps in the Ohio River.

Potential Impacts

Bighead and silver carp are significant invasive species primarily due to their feeding capabilities but also other life history strategies (e.g., high fecundity, tolerances) that allow them to infest unestablished waters and outcompete native species. Their aggressive feeding strategies influence the composition, abundance, and size structure of zooplankton and phytoplankton (Cooke et al. 2009; Kolar et al. 2007; Sass et al. 2014). This may reduce the amount of available plankters necessary for larval fish recruitment and native filterers, which may lead to increased competition, mortality, as well as to reduced biological fitness. Water quality may be reduced indirectly through reductions in plankton populations, resulting in increases in turbidity and chlorophyll-a (Nico et al. 2020$_2$). Schrank et al. (2003) examined the competitive interactions between age-0 bighead carp and paddlefish (*Polyodon spathula*), a native filter feeder, and found that bighead carp may negatively affect the growth of paddlefish when resources are limited. A later study of the Mississippi and Illinois rivers observed the diet overlap of paddlefish and both bigheaded carps were found to be minimal, with greater overlap of gizzard shad (*Dorosoma* *cepedianum*) and bigmouth buffalo (*Ictiobus cyprinellus*), which was likely attributed to prey size (Sampson et al. 2009). Irons et al. (2007) found a significant decline in gizzard shad and bigmouth buffalo body condition in the Illinois River following the Asian carp invasion from 2000 to 2006. Phelps et al. (2017) found that as silver carp relative abundance has increased from 1993 to 2012 in the Mississippi River basin, gizzard shad and bigmouth buffalo condition and relative abundance have decreased.

Silver carp pose a definitive human safety risk with their ability to launch into the air up to three meters (m) (Vetter and Mensinger 2016). This behavior is observed in response to outboard boat motors, but the biological significance is not understood. Vetter et al. (2017) modeled this behavior in response to a moving boat and determined that the direction in which the silver carp jumped was not random (e.g., jumping away from the boat). Areas of high abundance of silver carp may be difficult to navigate, boats and equipment may be damaged, and people may be significantly injured (Kolar et al. 2007).

History of the Ohio River and its Fishes

The Ohio River spans 1,579 kilometers (km) (981 miles) and six states (Illinois, Indiana, Kentucky, Ohio, Pennsylvania, and West Virginia), from the confluence of the Allegheny and Monongahela rivers in Pittsburgh, Pennsylvania, to where it joins the Mississippi River in Cairo, Illinois (ORSANCO 2020_1). Prior to European settlement in the 1700s, the Ohio River was an unrestricted, shallow river with coarse substrate, an abundance of adjacent hardwood forests, and wetlands (Pearson and Pearson 1989). Until the 1900s, woodlands were converted and wetlands were drained for agriculture and settlements, resulting in the development of large municipalities and an influx of fine sediments in the river (Pearson and Pearson 1989). With the first wicket dam constructed in 1885 by the U.S. Army Corps of Engineers (USACE), the morphology and water level of the river was drastically altered for navigation (Pearson and Pearson 1989). By 1929, the Ohio River consisted of 51 wicket dams which increased the depth of the navigation channel to nine feet (ft) (USACE, Great Lakes and Ohio River Division 2020). In the 1950s, the majority of these small, wooden wicket dams were phased out in favor of high-lift, steel and concrete dams, to accommodate the newer, larger towboats and barges (USACE, Great Lakes and Ohio River Division 2020). Nineteen high-lift locks and dams now occur along the length of the Ohio River (USACE, Great Lakes and Ohio River Division 2020). In present day, as one of the six major tributaries to the Mississippi River, the Ohio River carries the largest volume of water with an average depth of 7.3 m (24 ft) (ORSANCO 2020_1). The Ohio River basin now contains over 25 million people, approximately 10% of the U.S. population, and the mainstem serves as a source of drinking water for more than five million people (ORSANCO 2020_1).

The culmination of anthropogenic activity (e.g., agricultural, municipal, industrial) has drastically altered the historical native ichthyofauna of the Ohio River. Intrusion of fine sediments from historical logging and agricultural practices covered the coarse riverbed substrates, adversely impacting lithophilic fishes that required it for spawning (Pearson and Pearson 1989). Industrial effluents, mine drainages, and sewage discharges were largely unregulated prior to the 1960s, which effectively concentrated and reduced the populations of fishes sensitive to pollution (Pearson and Pearson 1989). At least three species, out of the total 159 species reported in the Ohio River, have been extirpated (Alabama shad, crystal darter, and lake sturgeon) (Pearson and Pearson 1989). The implementation of dams altered the natural flow and temperature regimes, morphology, aquatic habitat of the river, and reduced access to migratory fishes by creating physical barriers (Pearson and Pearson 1989; Lowman 2000). Water quality has improved since the enactment of the Clean Water Act (1972), and subsequently, fish community assemblages and populations have improved (Pearson and Pearson 1989; Emery et al. 1998; Thomas et al. 2004).

Bigheaded Carp Distribution in the Ohio River

The bigheaded carps were introduced into the United States in 1973 at a private fish farm in Arkansas for use as biological controls for plankton (Freeze and Henderson 1982). Several states, private aquaculture facilities, and wastewater treatment plants adopted these species to enhance water quality, likely facilitating their escape into the Mississippi River basin during high flood events. Both species were discovered in the Mississippi River basin in the early 1980s (Freeze and Henderson 1982; Jennings 1988). Bighead carp are more widely distributed than silver carp in the United States, with records in 27 and 23 states, respectively (Nico et al. 2020_1; Nico et al. 2020_2). Both bighead and silver carp are well established in the Mississippi, Missouri, Tennessee, Illinois, and Ohio River basins.

In the Ohio River, there are three classifications of bighead and silver carp establishment, which are defined within the Annual Performance Report for Asian Carp Research and Monitoring: establishment front, invasion front, and presence front (KDFWR 2019_3). The establishment front refers to areas with high densities of bigheaded carps and evidence of successful reproduction and recruitment. The invasion front refers to areas with evidence of reproduction but no evidence of successful recruitment. The presence front refers to areas with sparse numbers of individuals being observed but no evidence of reproduction or successful recruitment. The establishment front of bighead and silver carp resides within the Cannelton Pool, falling just below the McAlpine Dam at the Falls of the Ohio, Louisville, KY (Gillespie et al. 2016). The Kentucky Department of Fish and Wildlife Resources (KDFWR) suspect that bigheaded carps are beginning their expansion upstream in response to increasing densities within Cannelton Pool (KDFWR 2019$_1$). Reproduction has been observed upstream within the Meldahl Pool, but evidence of recruitment has not been detected (KDFWR 2019$_2$). The invasion front extends from McAlpine Dam to Greenup Dam. The Greenup Pool and R.C. Byrd Pool currently hold the presence front of silver carp in the Ohio River.

Little is known about bigheaded carp populations above Greenup Dam at Greenup, KY. Few reports of bighead and silver carp have occurred within the Greenup Pool. A single bighead carp was reported in 2014 at RM 280 in the Greenup Pool (Nonindigenous Aquatic Species Database 2020$_2$). A single adult silver carp was documented in the Greenup Pool at river mile (RM) 280 (USFWS 2016). In May 2022, two deceased bighead carp were found washed up on shore downstream of R.C. Byrd Dam near Apple Grove, WV in the Greenup Pool (Personal Communication – Jacob Miller, Thomas Jones 2022).

Slightly above the Greenup Pool at R.C. Byrd Dam near Apple Grove, WV, and upstream in R.C. Byrd Pool, bighead carp have been observed annually since 2015, and silver carp infrequently. In 2015, a single bighead carp was removed from the R.C. Byrd Dam old lock chamber (Personal Communication – Thad Tuggle). In 2016, a silver carp was reported from Raccoon Creek in the lower R.C. Byrd Pool by the Ohio Department of Natural Resources (ODNR) when it jumped into their boat (USFWS 2016; Nonindigenous Aquatic Species Database 2020$_1$). The confluence of Raccoon Creek is located less than five kilometers upstream of R.C. Byrd Dam. To the author's knowledge, the furthest upstream record of a silver carp was in the R.C. Byrd Pool below Winfield Dam on the Kanawha River near Winfield, WV in 2016 when it jumped onto a fishing access (Personal Communication – Katie Zipfel 2019). Four bighead carp were removed from R.C. Byrd Pool in 2016 between Raccoon Creek and R.C. Byrd Dam (USFWS 2016). In 2017, three bighead carp were removed near Raccoon Creek in R.C.

Byrd Pool (KDFWR 2018). In 2018, three bighead carp were removed from R.C. Byrd Pool between Raccoon Creek and R.C. Byrd Dam (KDFWR 2019$_1$). In 2019, eight bighead carp were removed from Raccoon Creek in R.C. Byrd Pool (Personal Communication – USFWS 2019). Eggs were found to be present in a female, but evidence of successful spawn or recruitment has not been found. This evidence of potential reproduction extends the invasion front of bighead carp to R.C. Byrd Pool. The furthest upstream record of a bighead carp in the Ohio River was from 2018 in New Cumberland Pool near Stratton, OH (Nonindigenous Aquatic Species Database 2020$_3$).

Current Monitoring and Mitigation Efforts

The Asian carp monitoring and mitigation effort is an ongoing collaborative process consisting of multiple federal, state, non-governmental, and local partners in the Mississippi River basin and its tributaries. The United States Fish and Wildlife Service (USFWS) leads most of these efforts by appropriating funds to various states, agencies, and partners. These efforts were augmented beginning in 2010 when bighead carp were listed under the Lacey Act, and even more so in 2014 with the enactment of the Water Resources Reform and Development Act (WRRDA) which authorized federal support to slow the spread of Asian carp in the Mississippi and Ohio River basins.

A significant amount of effort and funding has dealt with preventing Asian carp from reaching the Great Lakes. In the Illinois River, an area where population densities of Asian carp comprise as much as 63% of the biomass, there is a potential risk of them reaching Lake Michigan through the Chicago Area Waterway System (Garvey et al. 2015). The Great Lakes boasts a seven billion dollar sport fishing industry, and there is concern about Asian carp becoming established and negatively impacting the fishery (Alsip et al. 2019). The U.S. Army

Corps of Engineers operates a series of three electric barriers within the Chicago Sanitary and Ship Canal that are designed to prevent bigheaded carps from reaching Lake Michigan (USACE, Chicago District 2020). An additional electric barrier and deterrents are currently proceeding at Brandon Road Lock and Dam with a total estimated project cost of $830 million (USACE, Rock Island District 2020). To the author's knowledge, at the time of this writing, no bighead or silver carp have been observed or collected from Lake Michigan despite the presence of environmental DNA detections (Song et al. 2017). Three bighead carp have been collected from Lake Erie from 1995 to 2000 (Morrison et al. 2004).

In the Ohio River basin: Indiana, Kentucky, Ohio, West Virginia, and Pennsylvania continually implement the Ohio River Basin Asian Carp Control Strategy Framework aimed to prevent Asian carp expansion, monitor for early detection and response, and control populations (ORFMT 2014). Kentucky has taken the lead amongst the states in receiving funds from the USFWS and heads several projects in the Ohio River, including monitoring and response, abundance and distribution of early life stages, control and removal, containment and suppression, and tracking movement and distribution using telemetry (KDFWR 2020). A bighead carp tagged in 2013 moved from Meldahl Pool upstream over 200 river miles and passed through three dams, demonstrating their ability to travel great distances (Gillespie et al. 2016). Other projects consist of using experimental collection gears and methods, hydroacoustic barriers and bubble curtains, and incentivizing commercial harvest through contracts and fishing tournaments (KDFWR 2020).

The West Virginia Division of Natural Resources (WVDNR) has conducted sampling for Asian carp in the Greenup Pool as part of pool-wide community surveys since 2015, with R.C. Byrd Pool added in 2016 (Jackson et al. 2015; Stump et al. 2016). Biannual targeted sampling for Asian carp was introduced in 2017 to learn more about Asian carp abundance in each pool (USFWS 2017). Targeted sampling consists of gillnetting non-random sampling locations (e.g., tributaries, backwaters, etc.) using large mesh gill nets for two-hour net sets during the day and may combine electrofishing as part of the Long Term Resource Monitoring Program Procedures: Fish Monitoring (Gutreuter et al. 1995).

The Greenup Pool, Ohio River

The Greenup Pool flows 99.5 km (61.8 miles) from R.C. Byrd Locks and Dam at RM 279.2 (Apple Grove, WV) to Greenup Locks and Dam at RM 341.0 (Greenup, KY) (USACE, Huntington District 2020). Greenup Locks and Dam began operation in 1962, with R.C. Byrd Locks and Dam operating since 1937. This study uses a historical dataset from electrofishing surveys by the Ohio River Valley Water Sanitation Commission (ORSANCO) in the Greenup Pool, Ohio River. Species richness and abundance were compared between survey years using one-way ANOVA to determine changes in fish community assemblages. Fish body condition was used to assess changes over time of commonly collected fishes. Nonmetric multidimensional scaling (NMDS) was used to explore the relationship of fish community distribution with respect to pool morphology in the Greenup Pool. This study may be used to help determine future impacts to the fish community in the Greenup Pool following an invasion of bigheaded carps.

CHAPTER II

THE FISH COMMUNITY OF THE GREENUP POOL, OHIO RIVER

INTRODUCTION

The anthropogenic development of the Ohio River from a once unrestricted, shallow river to a navigable depth of nine feet covering its 1,579 kilometers (981 miles) has drastically altered the historical native ichthyofauna. The culmination of anthropogenic activity (e.g., agricultural, municipal, industrial) during the early 1900s resulted in increased fine sediments and pollution from largely unregulated industrial effluents, mine drainages, and sewage discharges (Pearson and Pearson 1989). Biomonitoring of fishes in the Ohio River began in the 1940s, which helped monitor and subsequently improve water quality (ORSANCO 2020_2; Thomas et al. 2004). The fish community serves as a necessary biological indicator of water quality in the Ohio River, which serves as a source of drinking water for over five million people (ORSANCO 2020_1). Species richness, species abundance, body condition, and community distribution provide valuable information about the relative condition of pool communities and potential water quality impairments. Invasive bigheaded carps pose a significant risk to native fishes due to their voracious feeding capabilities that may have deleterious trophic cascading impacts. This study may be used to help determine future impacts to the fish community in the Greenup Pool, Ohio River following an invasion of bigheaded carps.

Biomonitoring in the Ohio River

The Ohio River Valley Water Sanitation Commission (ORSANCO) is an interstate governmental agency that was formed in 1948 to monitor water quality, conduct biological assessments, and set wastewater discharge standards in the Ohio River (ORSANCO 2020_2). Biomonitoring of fishes in the Ohio River and its tributaries was historically accomplished through rotenone lock chamber surveys beginning in 1957. In 1990, ORSANCO began implementing shoreline electrofishing to increase pool coverage due to the restrictions of lock chamber surveys. Since 2004, ORSANCO has employed a random sampling approach to monitor fish communities in all 20 pools of the Ohio River on a five-year rotational cycle (ORSANCO 2006). ORSANCO initiated electrofishing surveys in the Greenup Pool in 2006.

Study Purpose

The objectives of this study regarding ORSANCO's random electrofishing surveys in the Greenup Pool from 2006, 2011, and 2016 were: (1) to compare species richness and species abundance between survey years; (2) to compare body condition of 12 commonly collected fishes between survey years using relative weight and Fulton's condition factor; and (3) to attempt to assess the relationship of fish community distribution with respect to pool morphology (e.g., channel curvature, bank side, and pool section) using nonmetric multidimensional scaling. I hypothesized that the mean species richness and abundance between survey years has increased. I hypothesized that the average body condition of commonly collected fishes between survey years has increased.

Large rivers are notoriously difficult to sample due to their extreme variability and size. Electrofishing is primarily used for fish population and community assessments in large rivers and is standardized for use with Long Term Monitoring Programs (Gutreuter et al. 1995; ORSANCO 2020[3]). Species richness and abundance are frequently used in electrofishing surveys to assess fish community assemblages. Species richness and abundance were used instead of species diversity due to the inherent sampling bias of electrofishing (Bayley and Dowling 1993; Benejam et al. 2012). Benthic fishes, pelagic fishes, and fishes lacking swim bladders are frequently undersampled in electrofishing surveys, which does not represent the total diversity of fishes in the Ohio River.

I suspected that species richness and abundance may increase with time along survey dates due to observed trends in historical (1957-2001) water quality data (e.g., pH, dissolved oxygen, total/dissolved metals, etc.) (Thomas et al. 2004). Continued improvements to regulations such as ORSANCO Pollution Control Standards and USEPA Water Quality Standards support the continuation of improved water quality, which may subsequently help improve fish communities (ORSANCO 2019; USEPA 2022). The Greenup Pool exhibits different morphology (e.g., channel curvature) between the upper pool (upstream towards R.C. Byrd Dam) and the middle to lower pool (downstream from Huntington, WV to Greenup Dam). The upper pool is less impacted by urbanization, sedimentation, and pollutants compared to the middle and lower pools. A suite of environmental variables associated with disturbance increase as one moves downstream in the Greenup Pool: water depth increases, water velocity decreases, fine sediments increase, turbidity increases, and barge traffic increases. These factors likely influence fish community distribution in the pool. I suspected that species richness and abundance may decrease going from the upper pool to the middle and lower pool due to these conditions. Nonmetric multidimensional scaling was used to assess the relationship of fish community distribution with respect to river morphology. I suspected that there may be differences in fish community distribution between the upper pool, the middle pool, and the lower pool.

Relative weight and Fulton's condition factor were used to compare fish body condition changes between electrofishing survey years. Body condition serves as a surrogate of overall fish plumpness, or general health, which may be used as a proxy to assess improvement or decline of a species. Calculating the species richness, species abundance, condition of commonly collected fishes, and fish community distribution may help identify potential trends and determine changes in the fish community, which will provide important comparison pre-invasion data should bigheaded carps successfully invade the Greenup Pool.

METHODS

Study Area and Site Selection

The Greenup Pool (Figure 1) flows 99.5 km (61.8 miles) from R.C. Byrd Locks and Dam at RM 279.2 (Apple Grove, WV) to Greenup Locks and Dam at RM 341.0 (Greenup, KY) (USACE, Huntington District 2020). The pool has an average depth of 7.9 m (26 ft) with an average width of 338.63 m (1,111 ft) and a gradient drop of 0.12 m (0.4 ft) per mile (ORSANCO 2006). ORSANCO began probabilistic fish community surveys in the Greenup Pool in 2006, with subsequent surveys in 2011 and 2016. In 2006, 15 random sites were sampled (Figure 2). In 2011, 15 random sites were sampled, in addition to two revisit sites which were initially randomly determined (Figure 3). In 2016, 15 random sites and three revisit sites were sampled (Figure 4).

Figure 1. Satellite imagery of the Greenup Pool, Ohio River (Google Earth Pro).

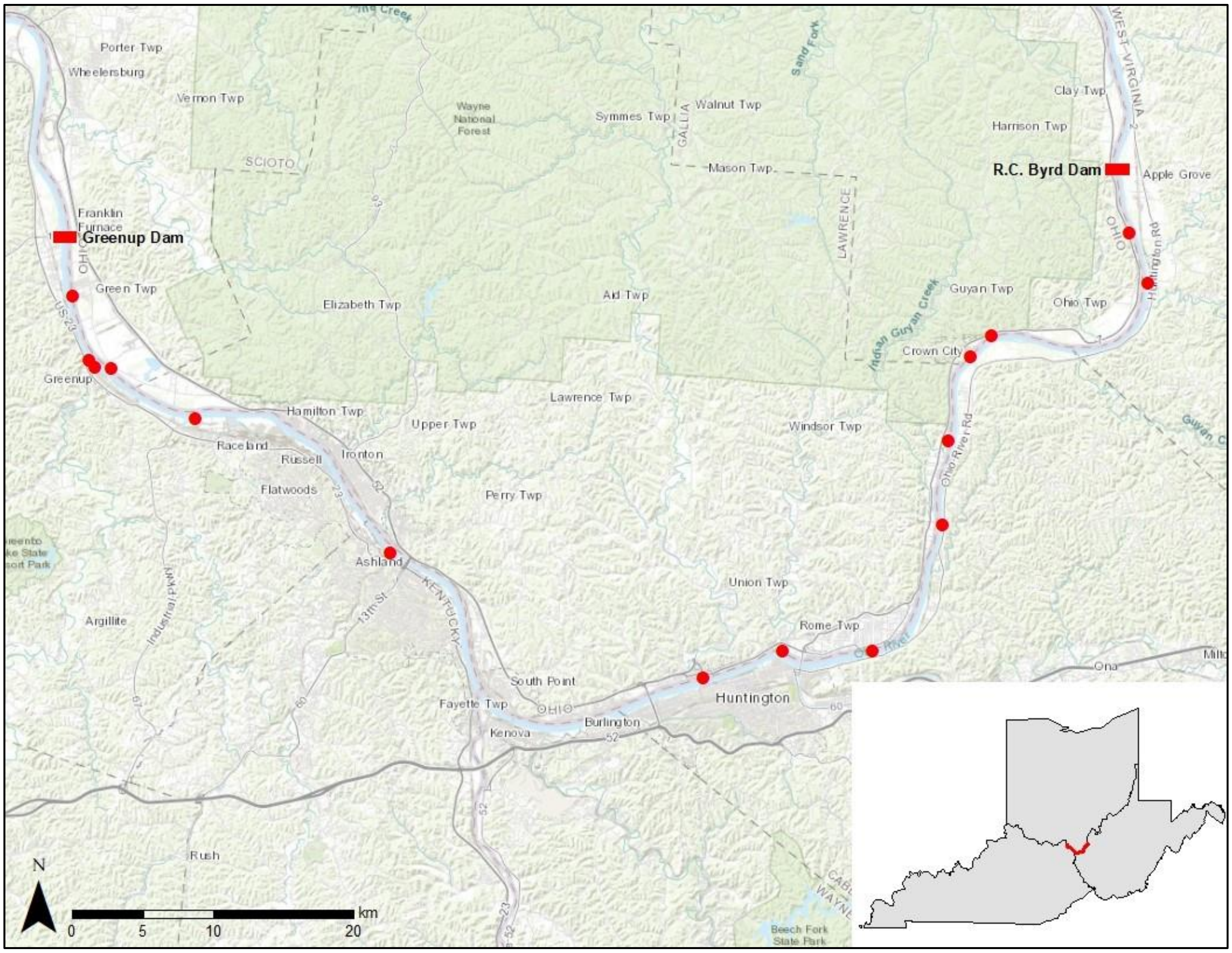

Figure 2. ORSANCO electrofishing sites within the Greenup Pool, Ohio River in 2006.

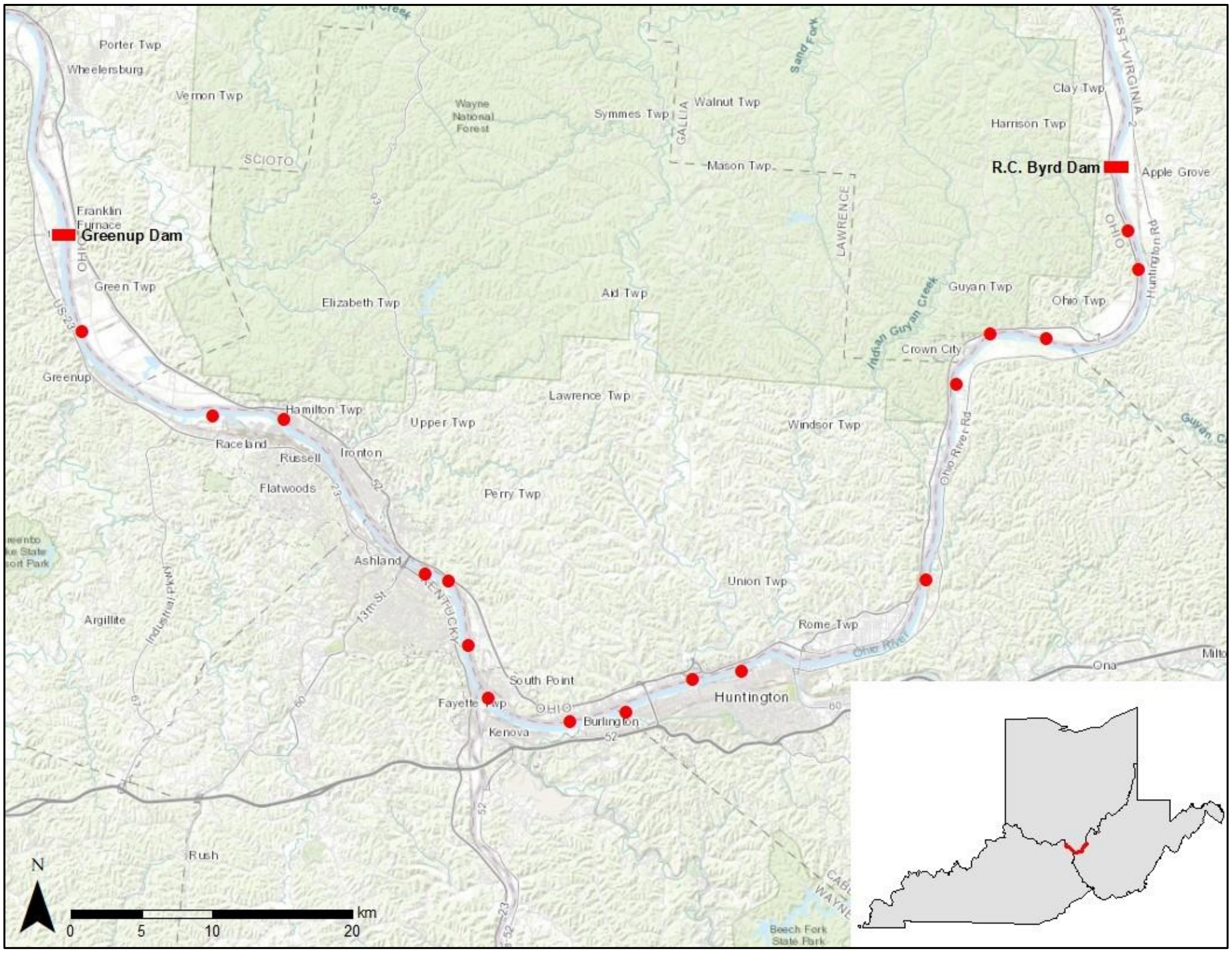

Figure 3. ORSANCO electrofishing sites within the Greenup Pool, Ohio River in 2011.

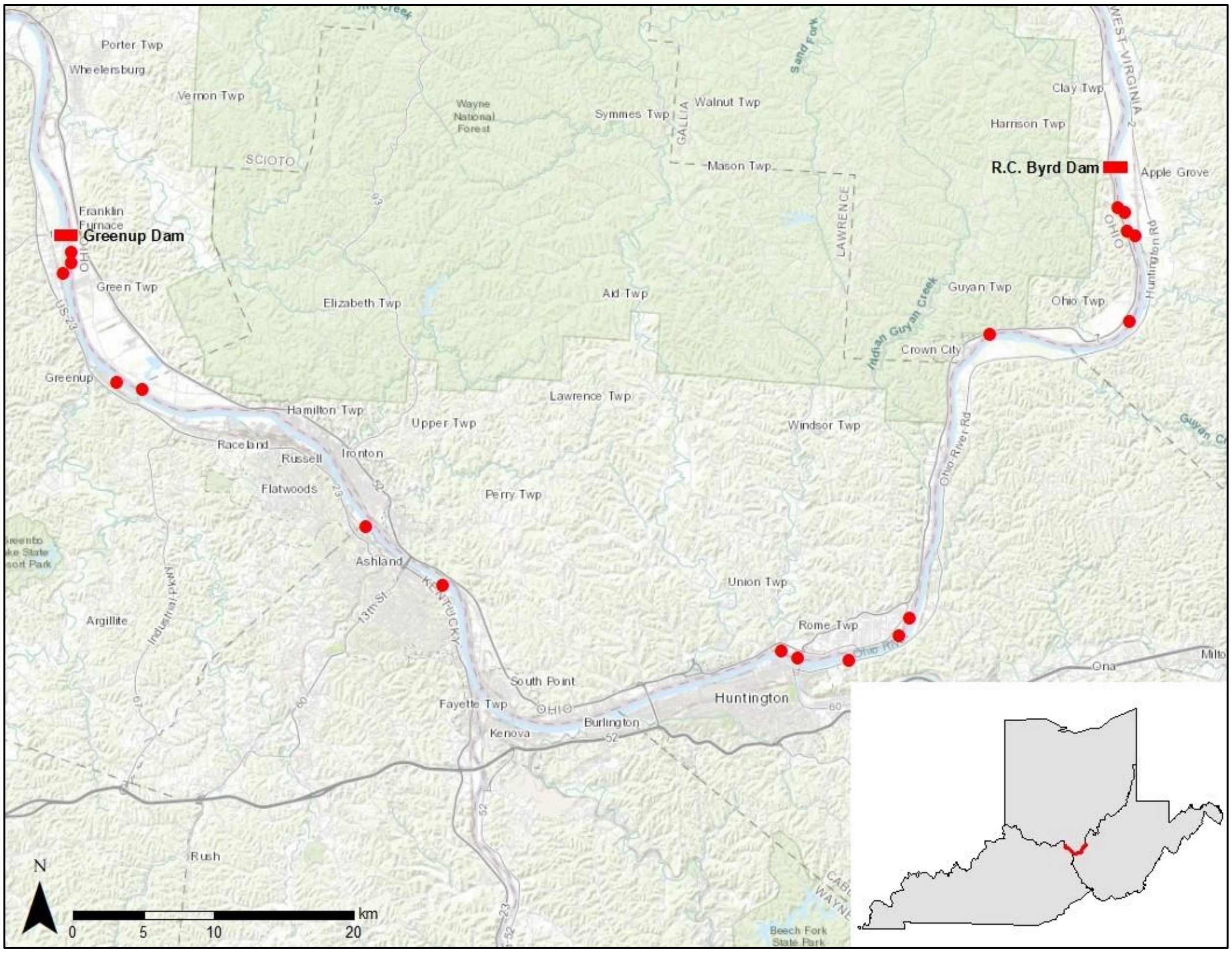

Figure 4. ORSANCO electrofishing sites within the Greenup Pool, Ohio River in 2016.

Fish Collection

Fish were collected by ORSANCO using nighttime pulsed direct current (DC) boat electrofishing between July 1 and October 31 for each survey year (ORSANCO 2011). This period of summer low flow represents water levels within one meter of "normal pool" which provides the most stable conditions for sampling. Deeper water may limit collection efficiency of benthic fishes by exceeding the electrical current range (six meters). Electrofishing was conducted at night along the shoreline to maximize individuals and species collected (ORSANCO 2011; Sanders 1992). A setting of 180-225 volts DC at 60-120 pulses per second with a duty cycle approaching 35% was used to achieve a target power of 6000-6500 watts

(ORSANCO 2020[3]). Each site measured 500 meters in length and followed parallel with the corresponding shoreline. Each site was electrofished starting from the upstream limit for a minimum of 30 minutes. The boat was maneuvered in a serpentine fashion within 30 meters from shore. All fish were stored in a live well until survey completion. Each fish was identified, inspected for DELTs (deformities, erosions, lesions, tumors), measured for total length (TL) to the nearest 3-centimeter (cm) size class, and weighed (kg) before being released. Questionable specimens were preserved in 10% formalin for later identification.

Species Richness and Abundance

Greenup Pool fish data from the 2006, 2011, and 2016 ORSANCO electrofishing surveys was used to assess species richness and abundance differences between survey years. Hybrid species were excluded from the analysis. Mean species richness and mean abundance between survey years was compared using one-way ANOVA and Tukey test. The null hypothesis for species richness stated that the mean species richness between survey years was equal (H_0: $\mu_{2006}=\mu_{2011}=\mu_{2016}$). The alternative hypothesis for species richness stated that the mean species richness between survey years was not equal (H_A: $\mu_{2006}\neq\mu_{2011}\neq\mu_{2016}$). The null hypothesis for species abundance stated that the mean species abundance between survey years was equal (H_0: $\mu_{2006}=\mu_{2011}=\mu_{2016}$). The alternative hypothesis for species abundance stated that the mean species abundance between survey years was not equal (H_A: $\mu_{2006}\neq\mu_{2011}\neq\mu_{2016}$). Species richness and abundance were plotted by site (river mile) with trendlines to examine linear correlation for each survey year.

Body Condition

Body condition was assessed for 12 commonly collected species using standard weight equations for ORSANCO Greenup Pool fish data from the 2006, 2011, and 2016 electrofishing surveys (ORSANCO 2016_1). In the case that standard weight equations were not available for a species, body condition was assessed using Fulton's condition factor. Only individual fish measurements were used for twelve commonly collected species. Weight (kg) measurements were converted to grams (g), and the 3-cm size class TL measurements were converted to centimeters by multiplying by 3. The TL (cm) measurements were then converted to millimeters (mm). Log_{10} standard weights were calculated for each individual using species-specific standard weight equations summarized in Blackwell et al. (2000), which were developed using large, national datasets. Individual fish collections that did not meet the minimum length requirements for each equation were excluded from the analysis. Log_{10} standard weights were converted to a standard weight (Ws) by scaling 10 to the power of each log_{10} standard weight value. The relative weight (Wr) was then calculated for each individual by the following equation: Wr = (Weight ÷ Standard Weight) * 100. Mean relative weights for each species were compared between sampling years using a Welch's ANOVA and Games-Howell test. The null hypothesis for Welch's ANOVA stated that the mean relative weights for each species were equal between survey years (H_0: $\mu_{2006}=\mu_{2011}=\mu_{2016}$). The alternative hypothesis stated that the mean relative weights for each species were not equal between survey years (H_A: $\mu_{2006}\neq\mu_{2011}\neq\mu_{2016}$).

Species that did not have standard weight equations were assessed using Fulton's condition factor. Fulton's condition factor (K) was calculated for select species using the equation: (K = Weight ÷ Total $Length^3$) * 100,000. Mean Fulton's condition factor for select species was compared between sampling years using a Welch's ANOVA. The null hypothesis for Welch's ANOVA stated that the mean Fulton's condition factor for each species was equal between survey years (H_0: $\mu_{2006}=\mu_{2011}=\mu_{2016}$). The alternative hypothesis stated that the mean Fulton's condition factor for each species was not equal between survey years (H_A:

$\mu_{2006} \neq \mu_{2011} \neq \mu_{2016}$). Welch's ANOVA was used to handle heteroscedasticity between survey years. All fish condition statistical analyses were performed in R version 3.6.1 (R Core Team 2019).

Nonmetric Multidimensional Scaling (NMDS)

Species count data from the 2006, 2011, and 2016 ORSANCO electrofishing surveys was used to examine species distribution with respect to pool morphology (channel curvature, bank side, and pool section). Channel curvature was categorized into three groups: inside bend, outside bend, and straightaway. Bank side was categorized into two groups: right descending bank (RDB) and left descending bank (LDB). Pool section was categorized into three groups: upper pool, middle pool, and lower pool (Figure 5). Data were log (x + 1) transformed to appropriately handle multiple zeros and non-normality. Hybrid species except for hybrid striped bass (*Morone chrysops x saxatilis*) were excluded from the dataset (Table 1). Nonmetric multidimensional scaling (NMDS) was used with Bray-Curtis distance measures and PC-ORD (7.07) to ordinate the dataset to assess the relationship between river morphology and fish community distribution (Mather 1976; McCune and Mefford 2016; Kruskal 1964). A random starting data configuration was used with 50 runs for the real data and 50 randomized runs to assess the stress and dimensionality of the data with a scree plot and Monte Carlo test. The stability of the final solution was assessed by plotting stress versus iteration number.

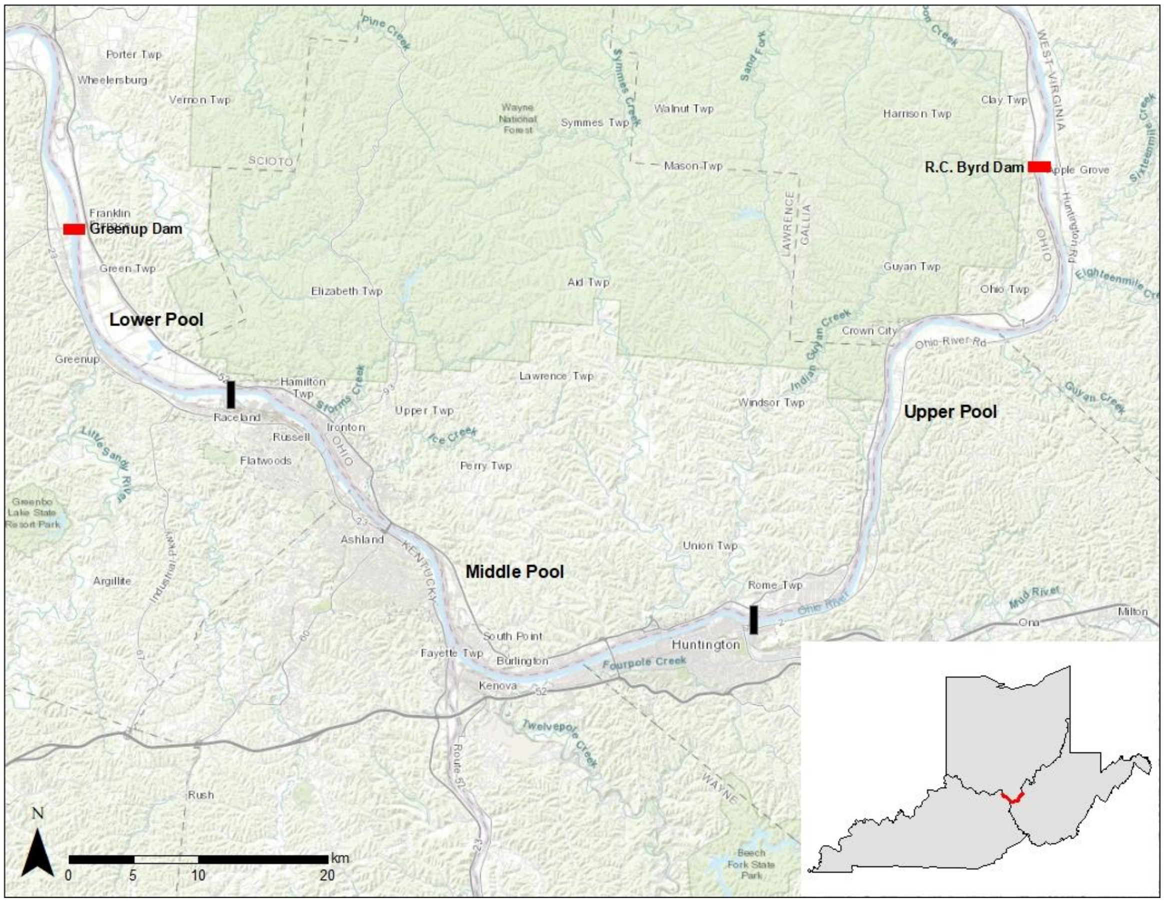

Figure 5. Upper, middle, and lower pool divisions of the Greenup Pool, Ohio River.

Table 1. Species codes used in the ORSANCO NMDS plots.

Species Code	Common Name
BiBuf	Bigmouth Buffalo
BkBuf	Black Buffalo
BkCra	Black Crappie
Blueg	Bluegill
BlMin	Bluntnose Minnow
Bowfi	Bowfin
BrBul	Brown Bullhead
BuMin	Bullhead Minnow
ChCat	Channel Catfish
ChDar	Channel Darter
ChShi	Channel Shiner
CoCar	Common Carp
CrChu	Creek Chub
EmShi	Emerald Shiner
FlCat	Flathead Catfish
FrDru	Freshwater Drum
GiSha	Gizzard Shad
GoRed	Golden Redhorse
GrSun	Green Sunfish
HiCar	Highfin Carpsucker
HSBas	Hybrid Striped Bass
LaBas	Largemouth Bass
Logpe	Logperch
LoSun	Longear Sunfish
LoGar	Longnose Gar
Moone	Mooneye
NHSuc	Northern Hogsucker
OrSun	Orangespotted Sunfish
Pumpk	Pumpkinseed
QuCar	Quillback Carpsucker
ReSun	Redear Sunfish
RiCar	River Carpsucker
RiDar	River Darter
RiRed	River Redhorse
RiShi	River Shiner
RoBas	Rock Bass
Sauge	Sauger
SiChu	Silver Chub
SiLam	Silver Lamprey
SiRed	Silver Redhorse
SmBas	Smallmouth Bass
SmBuf	Smallmouth Buffalo
SmRed	Smallmouth Redhorse
SfShi	Spotfin Shiner
StShi	Striped Shiner
SpBas	Spotted Bass
SpSuc	Spotted Sucker
Walle	Walleye
WhBas	White Bass
WhCra	White Crappie

RESULTS

Species Richness and Abundance

During the 2006 electrofishing community survey in the Greenup Pool, ORSANCO collected 1,349 individuals from 38 species (Table 2). Primary species collected (>5% of the total) were: bluegill (*Lepomis macrochirus* – 8.3%), emerald shiner (*Notropis atherinoides* – 6%), freshwater drum (*Aplodinotus grunniens* – 8.1%), gizzard shad (*Dorosoma cepedianum* – 20.2%), and sauger (*Sander canadensis* – 12.5%). Species richness showed no linear relationship with respect to site (R^2=0.0656) (Figure 6), with species abundance showing a relatively strong positive linear relationship with respect to site in the downstream direction (R^2=0.6495) (Figure 7).

Table 2. Species abundance from the 2006 electrofishing survey in the Greenup Pool, Ohio River.

Common Name	Species	Abundance	% Abundance
Black Buffalo	*Ictiobus niger*	1	0.07
Bluegill	*Lepomis macrochirus*	113	8.38
Bluntnose Minnow	*Pimephales notatus*	1	0.07
Brown Bullhead	*Ameiurus nebulosus*	1	0.07
Channel Catfish	*Ictalurus punctatus*	38	2.82
Channel Darter	*Percina copelandi*	20	1.48
Channel Shiner	*Notropis wickliffi*	67	4.97
Common Carp	*Cyprinus carpio*	8	0.59
Emerald Shiner	*Notropis atherinoides*	81	6.00
Flathead Catfish	*Pylodictis olivaris*	32	2.37
Freshwater Drum	*Aplodinotus grunniens*	110	8.15
Gizzard Shad	*Dorosoma cepedianum*	273	20.24
Golden Redhorse	*Moxostoma erythrurum*	35	2.59
Green Sunfish	*Lepomis cyanellus*	4	0.30
Highfin Carpsucker	*Carpiodes velifer*	4	0.30
Largemouth Bass	*Micropterus salmoides*	66	4.89
Logperch	*Percina caprodes*	10	0.74
Longear Sunfish	*Lepomis megalotis*	13	0.96
Longnose Gar	*Lepisosteus osseus*	20	1.48
Quillback	*Carpiodes cyprinus*	14	1.04
Redear Sunfish	*Lepomis microlophus*	1	0.07
River Carpsucker	*Carpiodes carpio*	52	3.85
River Darter	*Percina shumardi*	1	0.07
River Redhorse	*Moxostoma carinatum*	2	0.15
Sauger	*Sander canadensis*	169	12.53
Silver Chub	*Macrhybopsis storeriana*	37	2.74
Silver Lamprey	*Ichthyomyzon unicuspis*	1	0.07
Silver Redhorse	*Moxostoma anisurum*	6	0.44
Slenderhead Darter	*Percina phoxocephala*	1	0.07
Smallmouth Bass	*Micropterus dolomieu*	8	0.59
Smallmouth Buffalo	*Ictiobus bubalus*	50	3.71
Smallmouth Redhorse	*Moxostoma breviceps*	21	1.56
Spotfin Shiner	*Cyprinella spiloptera*	2	0.15
Spotted Bass	*Micropterus punctulatus*	42	3.11
Spotted Sucker	*Minytrema melanops*	5	0.37
Striped Bass	*Morone saxatilis*	1	0.07
White Bass	*Morone chrysops*	38	2.82
White Crappie	*Pomoxis annularis*	1	0.07
Total		**1349**	100

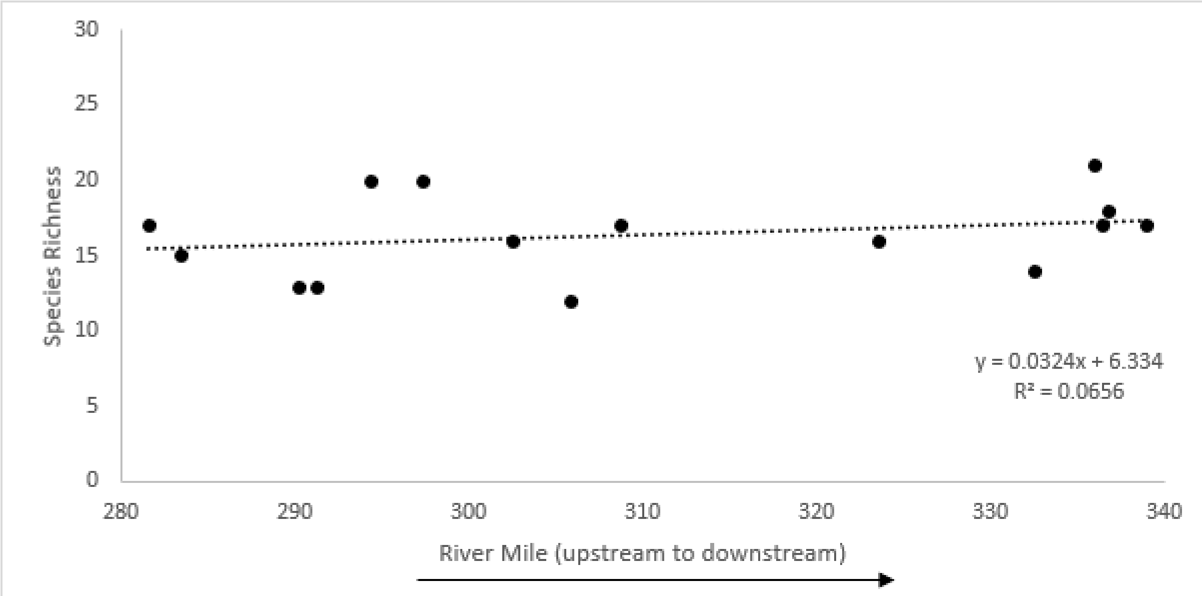

Figure 6. Species richness by river mile from the 2006 electrofishing survey in the Greenup Pool, Ohio River.

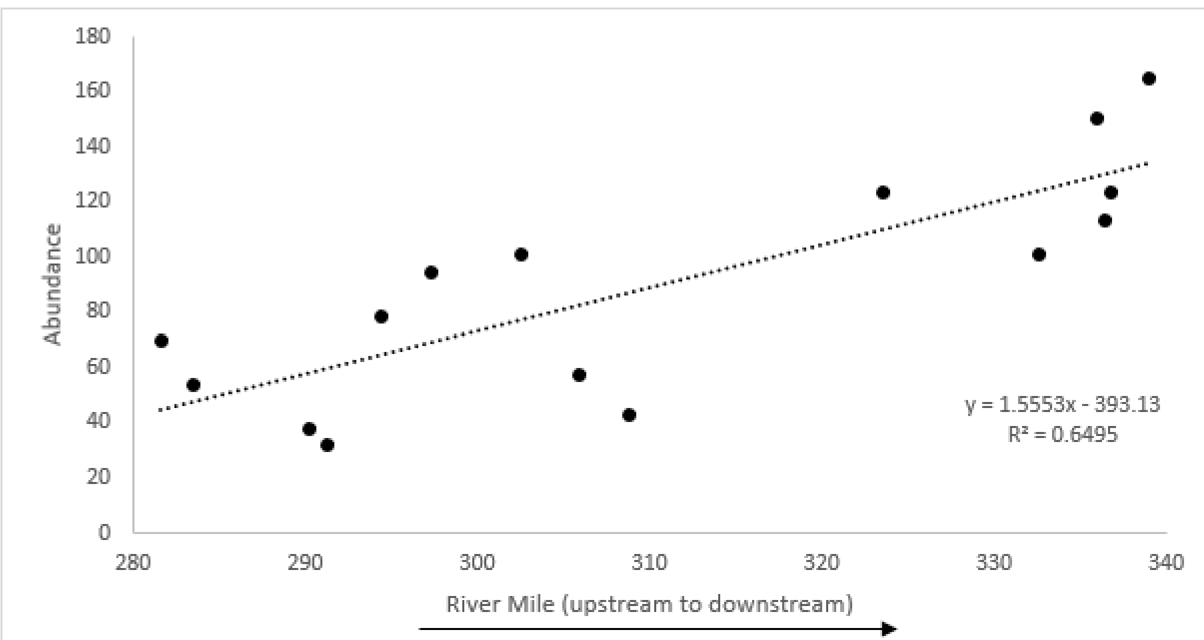

Figure 7. Species abundance by river mile from the 2006 electrofishing survey in the Greenup Pool, Ohio River.

During the 2011 electrofishing community survey in the Greenup Pool, ORSANCO collected 4,844 individuals from 41 species (Table 3). Primary species collected (>5% of the total) were: bluegill (7.0%), channel catfish (*Ictalurus punctatus* – 6.3%), channel shiner (*Notropis wickliffi* – 22.3%), emerald shiner (36.4%), and freshwater drum (6.9%). Species richness and abundance showed no apparent linear relationship with respect to site (R^2=0.0857, R^2=0.0109) (Figures 8-9).

Table 3. Species abundance from the 2011 electrofishing survey in the Greenup Pool, Ohio River.

Common Name	Species	Abundance	% Abundance
Black Buffalo	*Ictiobus niger*	1	0.02
Black Crappie	*Pomoxis nigromaculatus*	4	0.08
Bluegill	*Lepomis macrochirus*	340	7.02
Bluntnose Minnow	*Pimephales notatus*	4	0.08
Bowfin	*Amia calva*	1	0.02
Bullhead Minnow	*Pimephales vigilax*	25	0.52
Channel Catfish	*Ictalurus punctatus*	306	6.32
Channel Shiner	*Notropis wickliffi*	1085	22.40
Common Carp	*Cyprinus carpio*	13	0.27
Emerald Shiner	*Notropis atherinoides*	1767	36.48
Flathead Catfish	*Pylodictis olivaris*	40	0.83
Freshwater Drum	*Aplodinotus grunniens*	339	7.00
Gizzard Shad	*Dorosoma cepedianum*	180	3.72
Golden Redhorse	*Moxostoma erythrurum*	40	0.83
Green Sunfish	*Lepomis cyanellus*	3	0.06
Largemouth Bass	*Micropterus salmoides*	39	0.81
Logperch	*Percina caprodes*	2	0.04
Longear Sunfish	*Lepomis megalotis*	27	0.56
Longnose Gar	*Lepisosteus osseus*	51	1.05
Mooneye	*Hiodon tergisus*	4	0.08
Northern Hog Sucker	*Hypentelium nigricans*	2	0.04
Orangespotted Sunfish	*Lepomis humilis*	3	0.06
Pumpkinseed	*Lepomis gibbosus*	2	0.04
Quillback	*Carpiodes cyprinus*	12	0.25
Redear Sunfish	*Lepomis microlophus*	1	0.02
River Carpsucker	*Carpiodes carpio*	55	1.14
River Redhorse	*Moxostoma carinatum*	2	0.04
River Shiner	*Notropis blennius*	8	0.17
Rock Bass	*Ambloplites rupestris*	4	0.08
Sauger	*Sander canadensis*	96	1.98
Silver Chub	*Macrhybopsis storeriana*	12	0.25
Silver Redhorse	*Moxostoma anisurum*	24	0.50
Smallmouth Bass	*Micropterus dolomieu*	48	0.99
Smallmouth Buffalo	*Ictiobus bubalus*	26	0.54
Smallmouth Redhorse	*Moxostoma breviceps*	47	0.97
Spotfin Shiner	*Cyprinella spiloptera*	65	1.34
Spotted Bass	*Micropterus punctulatus*	129	2.66
Spotted Sucker	*Minytrema melanops*	1	0.02
Walleye	*Sander vitreus*	2	0.04
White Bass	*Morone chrysops*	26	0.54
White Crappie	*Pomoxis annularis*	8	0.17
Total		**4844**	100

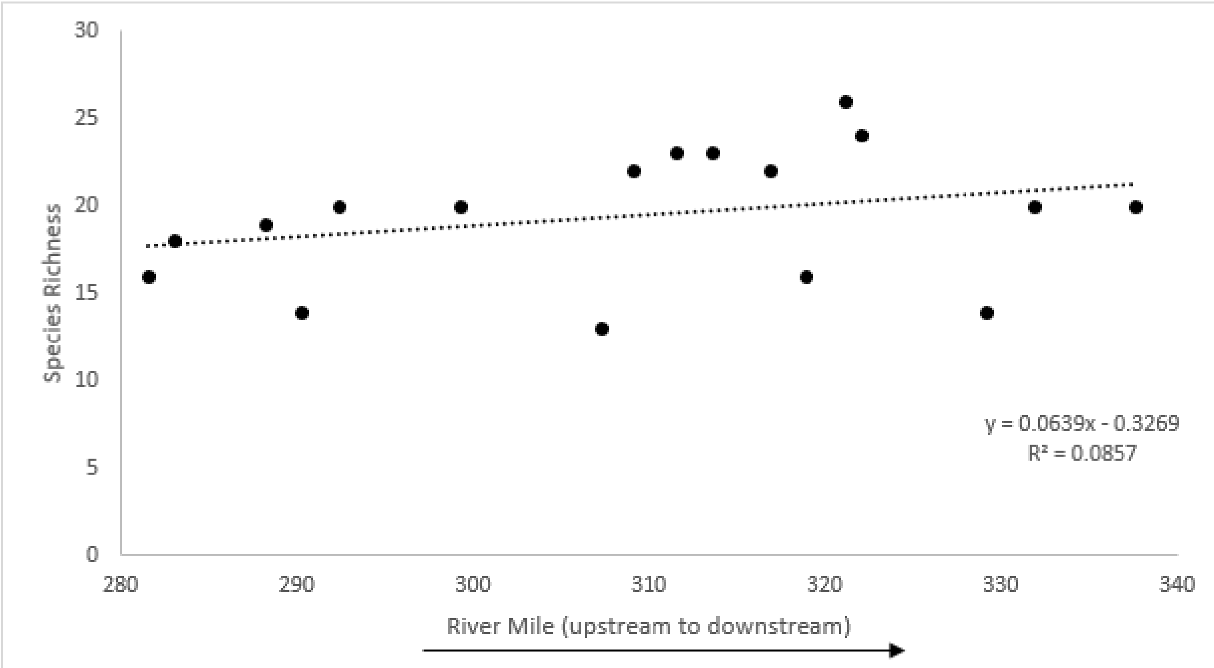

Figure 8. Species richness by river mile from the 2011 electrofishing survey in the Greenup Pool, Ohio River.

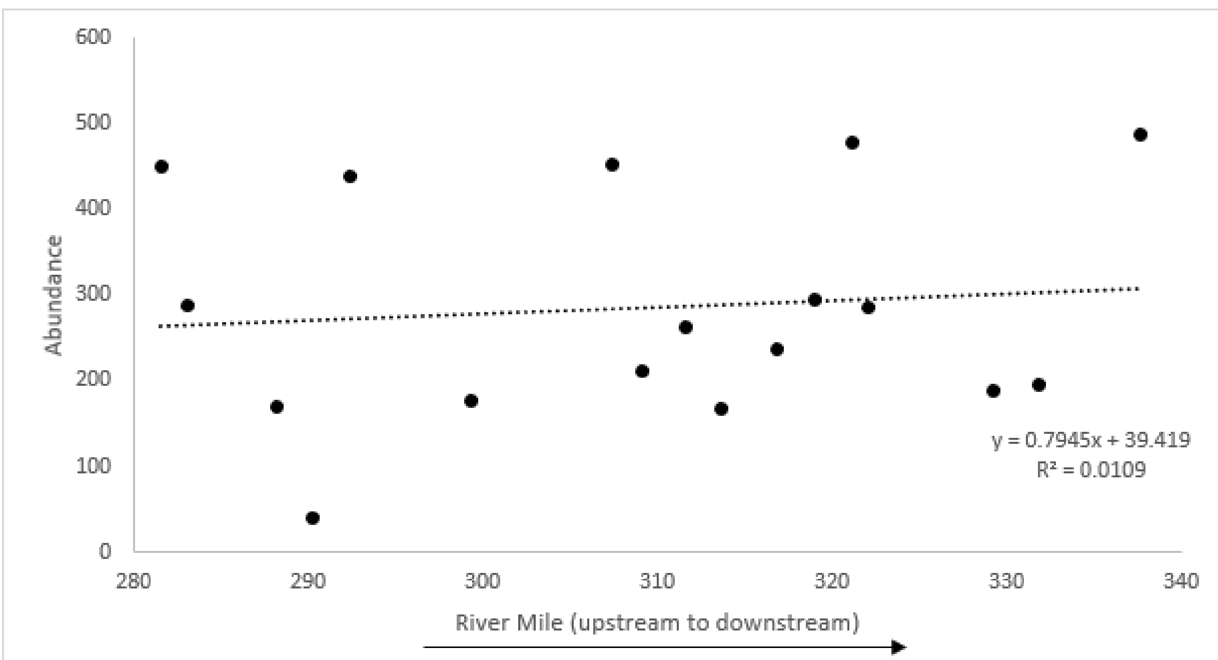

Figure 9. Species abundance by river mile from the 2011 electrofishing survey in the Greenup Pool, Ohio River.

During the 2016 electrofishing community survey in the Greenup Pool, ORSANCO collected 4,272 individuals from 43 species (Table 4). Primary species collected (>5% of the total) were: channel shiner (55.8%), emerald shiner (6.1%), gizzard shad (5.1%), and sauger (5.6%). Species richness and abundance showed no apparent linear relationship with respect to site (R^2=0.0481, R^2=0.1382) (Figures 10-11). Without the abundance outlier of 576 channel shiners at 301.4 RM, species abundance shows a moderately negative linear relationship with respect to site in the downstream direction (R^2=0.4138).

Table 4. Species abundance from the 2016 electrofishing survey in the Greenup Pool, Ohio River.

Common Name	Species	Abundance	% Abundance
Bigmouth Buffalo	*Ictiobus cyprinellus*	1	0.02
Black Buffalo	*Ictiobus niger*	3	0.07
Black Crappie	*Pomoxis nigromaculatus*	8	0.19
Bluegill	*Lepomis macrochirus*	207	4.85
Bluntnose Minnow	*Pimephales notatus*	2	0.05
Bullhead Minnow	*Pimephales vigilax*	19	0.44
Channel Catfish	*Ictalurus punctatus*	74	1.73
Channel Darter	*Percina copelandi*	1	0.02
Channel Shiner	*Notropis wickliffi*	2385	55.83
Common Carp	*Cyprinus carpio*	10	0.23
Creek Chub	*Semotilus atromaculatus*	1	0.02
Emerald Shiner	*Notropis atherinoides*	264	6.18
Flathead Catfish	*Pylodictis olivaris*	30	0.70
Freshwater Drum	*Aplodinotus grunniens*	124	2.90
Gizzard Shad	*Dorosoma cepedianum*	221	5.17
Golden Redhorse	*Moxostoma erythrurum*	149	3.49
Green Sunfish	*Lepomis cyanellus*	2	0.05
Highfin Carpsucker	*Carpiodes velifer*	10	0.23
Largemouth Bass	*Micropterus salmoides*	18	0.42
Logperch	*Percina caprodes*	16	0.37
Longear Sunfish	*Lepomis megalotis*	15	0.35
Longnose Gar	*Lepisosteus osseus*	57	1.33
Mooneye	*Hiodon tergisus*	2	0.05
Northern Hog Sucker	*Hypentelium nigricans*	1	0.02
Orangespotted Sunfish	*Lepomis humilis*	5	0.12
Quillback	*Carpiodes cyprinus*	4	0.09
Redear Sunfish	*Lepomis microlophus*	4	0.09
River Carpsucker	*Carpiodes carpio*	42	0.98
River Redhorse	*Moxostoma carinatum*	27	0.63
River Shiner	*Notropis blennius*	16	0.37
Sauger	*Sander canadensis*	240	5.62
Silver Chub	*Macrhybopsis storeriana*	11	0.26
Silver Lamprey	*Ichthyomyzon unicuspis*	1	0.02
Silver Redhorse	*Moxostoma anisurum*	44	1.03
Smallmouth Bass	*Micropterus dolomieu*	26	0.61
Smallmouth Buffalo	*Ictiobus bubalus*	20	0.47
Smallmouth Redhorse	*Moxostoma breviceps*	58	1.36
Spotfin Shiner	*Cyprinella spiloptera*	58	1.36
Spottail Shiner	*Notropis hudsonius*	3	0.07
Spotted Bass	*Micropterus punctulatus*	65	1.52
Spotted Sucker	*Minytrema melanops*	2	0.05
White Bass	*Morone chrysops*	18	0.42
White Crappie	*Pomoxis annularis*	8	0.19
Total		**4272**	100

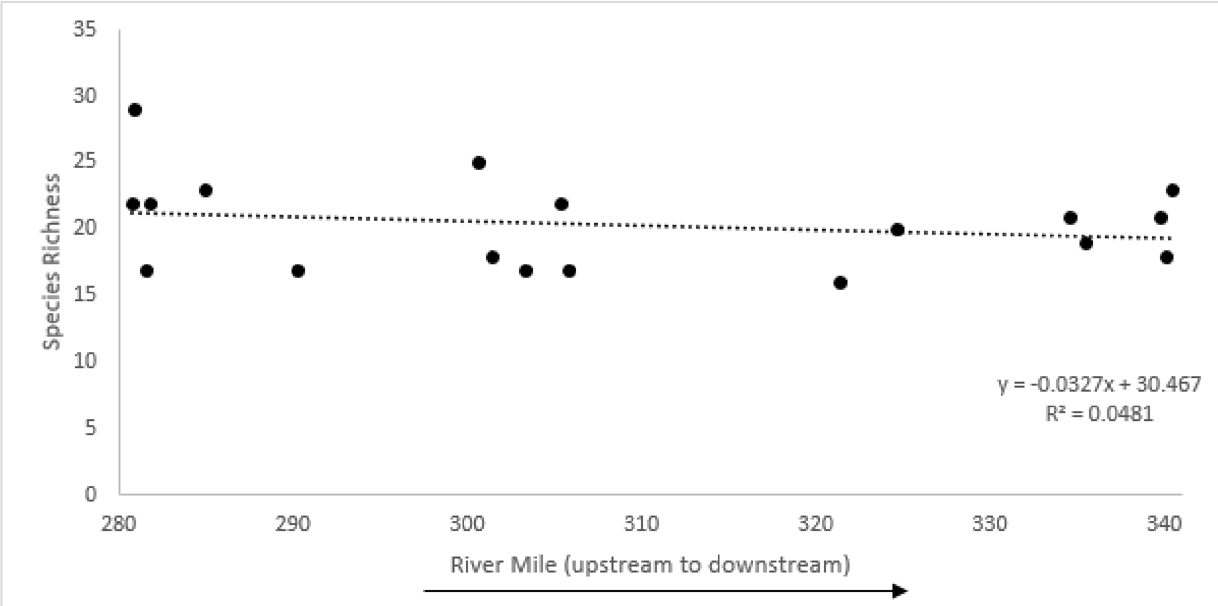

Figure 10. Species richness by river mile from the 2016 electrofishing survey in the Greenup Pool, Ohio River.

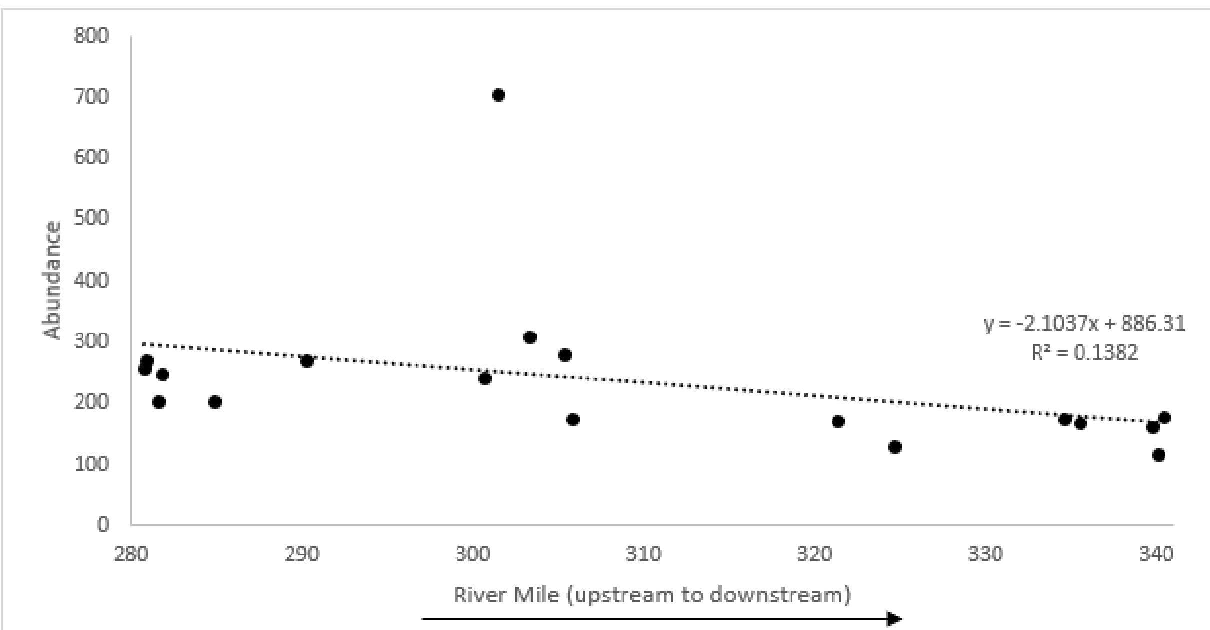

Figure 11. Species abundance by river mile from the 2016 electrofishing survey in the Greenup Pool, Ohio River.

One-way ANOVA detected a significant difference in mean species richness between survey years ($F(2, 47)=6.143$, $p=0.004$) (Figure 12). A Tukey post hoc test determined that there was a significant increase in mean species richness between 2006-2011 and 2006-2016 ($p=0.038$ and $p=0.003$, respectively). One-way ANOVA also detected a significant difference in mean species abundance between survey years ($F(2, 47)=13.03$, $p<0.001$) (Figure 13). A Tukey post hoc test determined that there was a significant increase in mean species abundance between 2006-2011 and 2006-2016 ($p<0.001$ and $p=0.001$, respectively). ORSANCO switched their boat electrofishing anode from a spider array to a sphere after 2006, but they concluded this did not impact performance (ORSANCO 2011). Total species abundances in 2011 and 2016 were amplified by channel shiners and emerald shiners, which are species that school and can occur unpredictably during electrofishing (Emery et al. 2003). Other species which increased in overall abundance include: bluegill, channel catfish, freshwater drum, golden redhorse (*Moxostoma erythrurum*), longnose gar (*Lepisosteus osseus*), river redhorse (*Moxostoma carinatum*), sauger, silver redhorse (*Moxostoma anisurum*), smallmouth redhorse (*Moxostoma breviceps*), and spotted bass (*Micropterus punctulatus*). Select species were poorly represented across all survey years (e.g., darters, pelagic species).

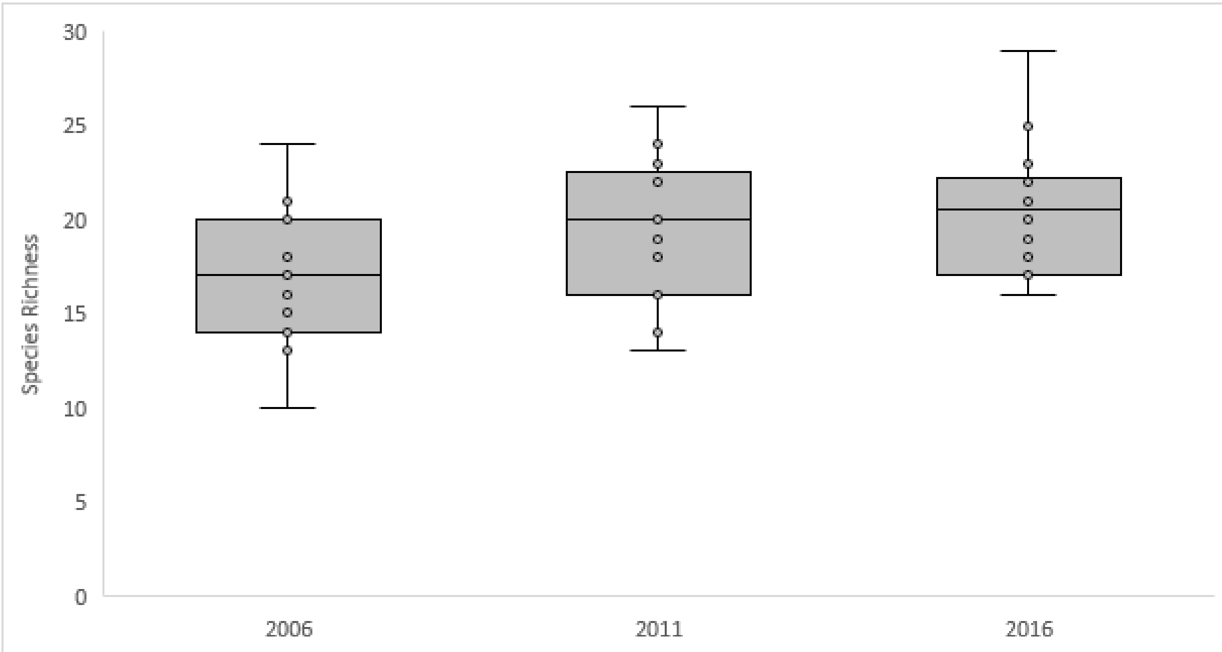

Figure 12. Species richness by electrofishing survey year in the Greenup Pool, Ohio River.

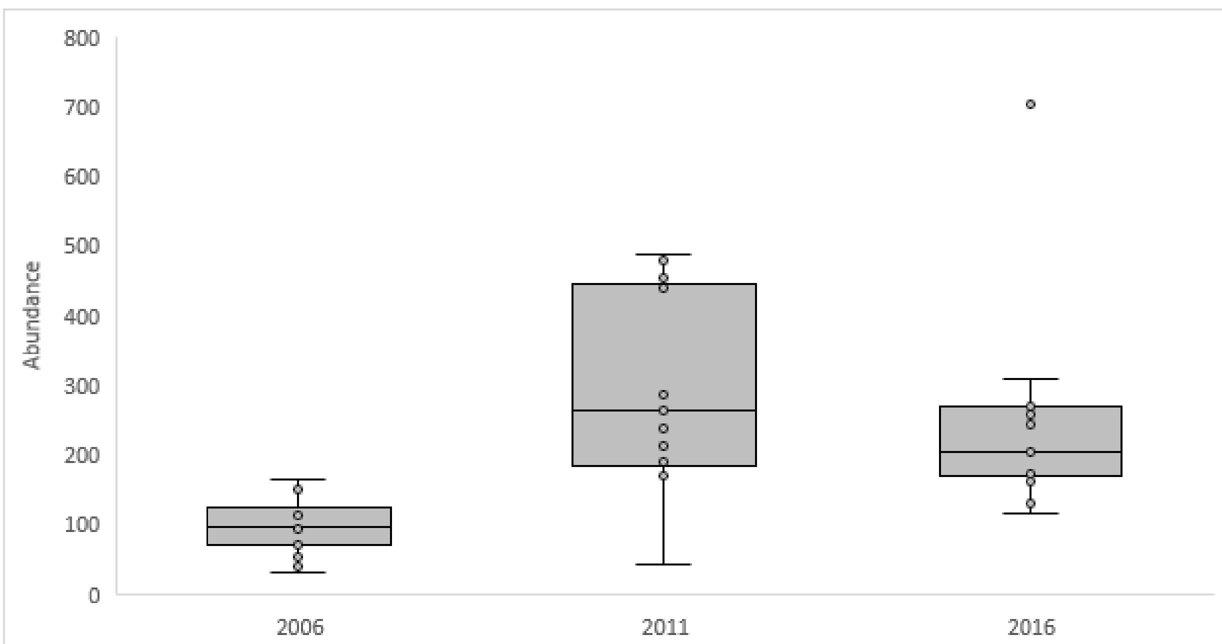

Figure 13. Species abundance by electrofishing survey year in the Greenup Pool, Ohio River.

Body Condition

Twelve commonly collected species were examined for changes in body condition between the 2006, 2011, and 2016 ORSANCO electrofishing surveys: bluegill, channel catfish, flathead catfish (*Pylodictis olivaris*), freshwater drum, gizzard shad, golden redhorse, longnose gar, river carpsucker (*Carpiodes carpio*), sauger, smallmouth buffalo (*Ictiobus bubalus*), smallmouth redhorse, and spotted bass. All species except for golden redhorse and smallmouth redhorse were examined using relative weight. Relative weight is used to assess the body condition of a fish from its weight relative to a standard weight. Fish that are above or below a relative weight of 100 represent fish that are considered to be in better or poorer condition relative to the standard condition, respectively.

Channel catfish individual relative weights for 2006 (n=25), 2011 (n=59), and 2016 (n=34) were plotted across total length (mm) (Figure 14). Five individuals exceeded the standard weight. Statistically significant differences between mean relative weights were determined by Welch's ANOVA ($F(2, 50.636)=8.182$, $p=0.0008$). A Games-Howell post hoc test determined that there was a significant decrease in mean relative weights between 2011 and 2016 ($p=0.0004$).

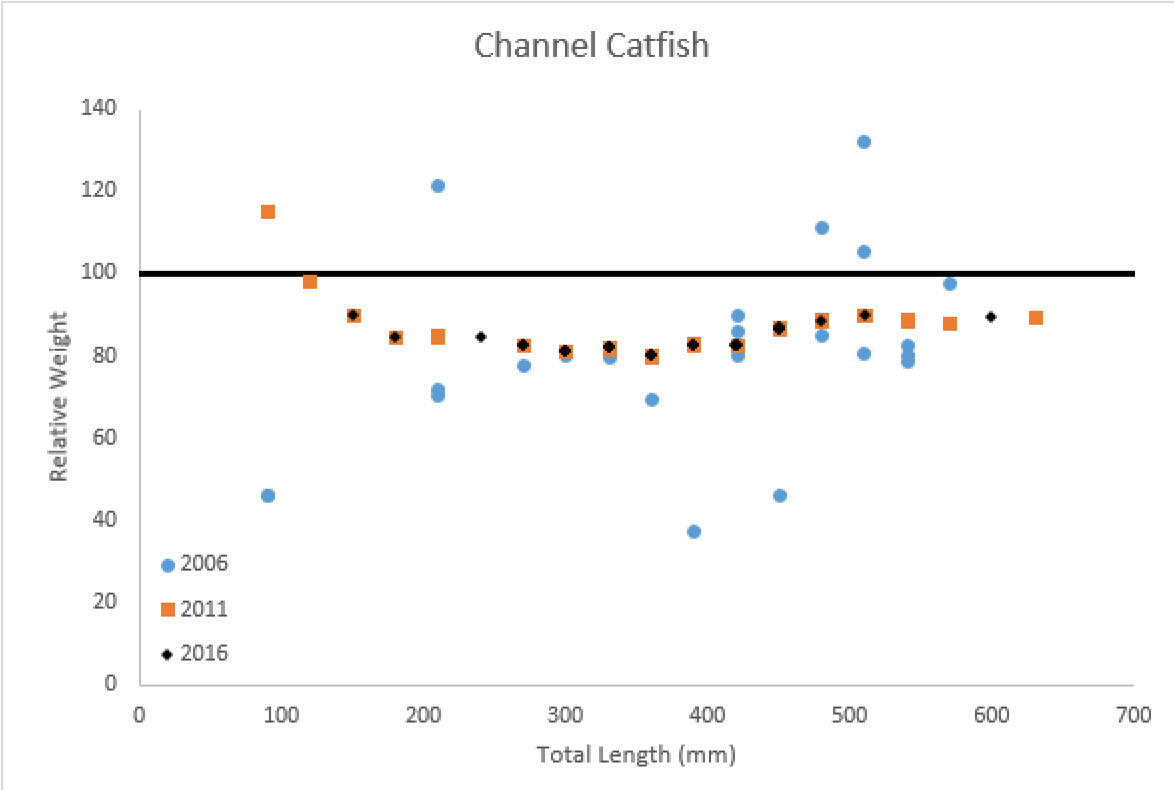

Figure 14. Channel catfish individual relative weights across total length (mm) for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

Sauger individual relative weights for 2006 (n=35), 2011 (n=27), and 2016 (n=41) were plotted across total length (mm) (Figure 15). No individuals exceeded the standard weight. Statistically significant differences between mean relative weights were determined by Welch's ANOVA ($F(2, 53.734)=6.2482$, $p=0.0036$). A Games-Howell post hoc test determined that there was a significant increase in mean relative weights between 2006 and 2011 ($p=0.005$), and 2006 and 2016 ($p=0.004$).

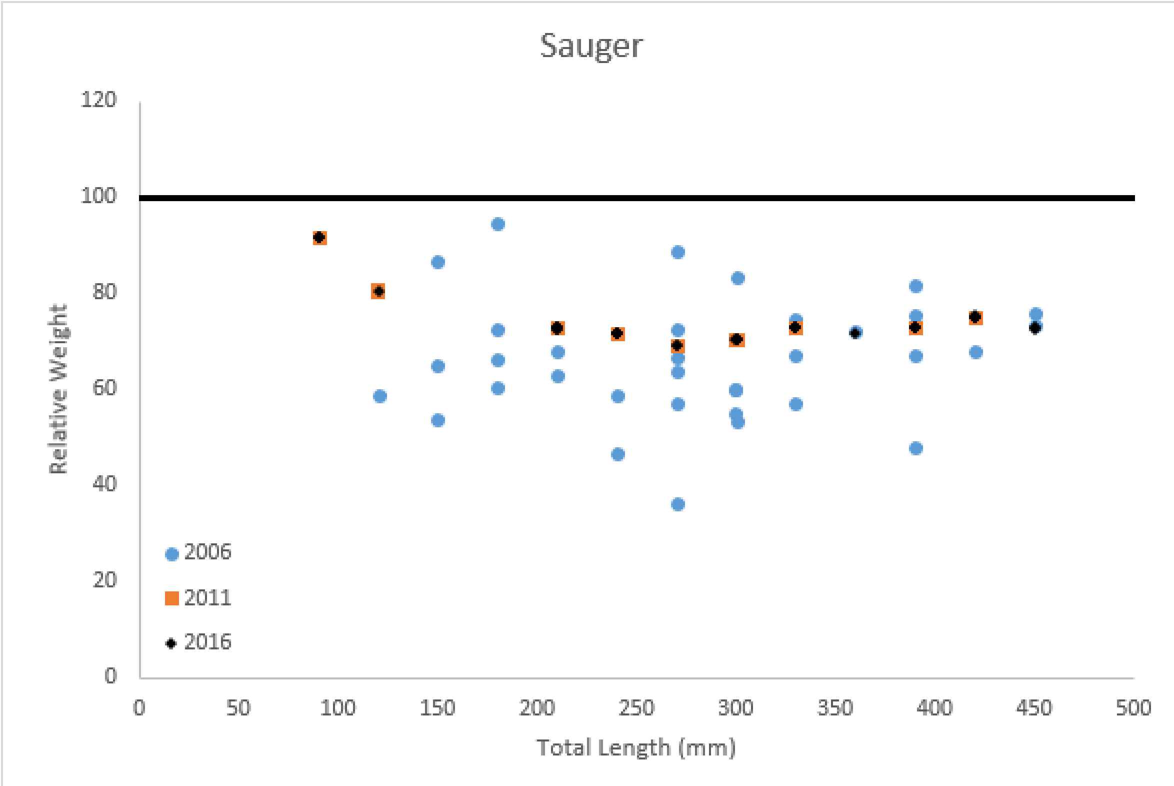

Figure 15. Sauger individual relative weights across total length (mm) for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

Freshwater drum individual relative weights for 2006 (n=20), 2011 (n=27), and 2016 (n=23) were plotted across total length (mm) (Figure 16). Two individuals exceeded the standard weight. Welch's ANOVA failed to detect a significant difference in mean relative weights between years ($F(2, 37.715)=0.2201$, $p=0.8034$).

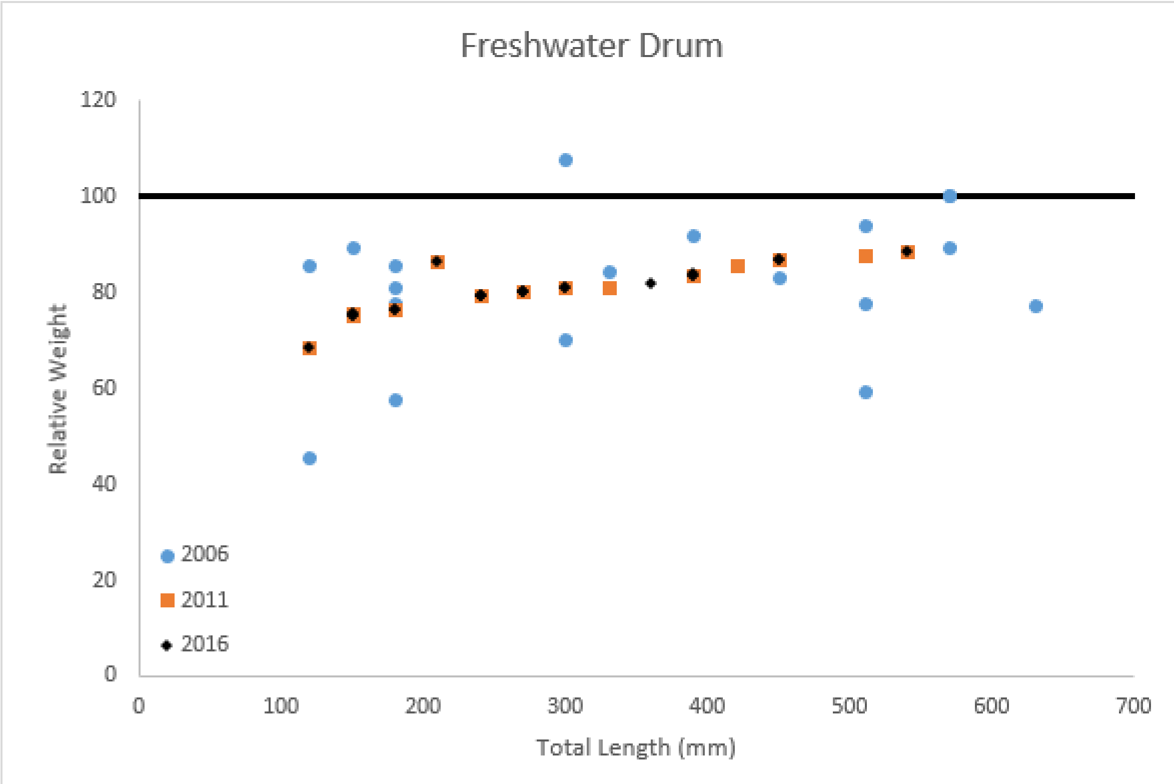

Figure 16. Freshwater drum individual relative weights across total length (mm) for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

Gizzard shad individual relative weights for 2006 (n=20), 2011 (n=27), and 2016 (n=27) were plotted across total length (mm) (Figure 17). A single individual exceeded the standard weight. Welch's ANOVA failed to detect a significant difference in mean relative weights between years ($F(2, 37.076)=2.1447$, $p=0.1314$).

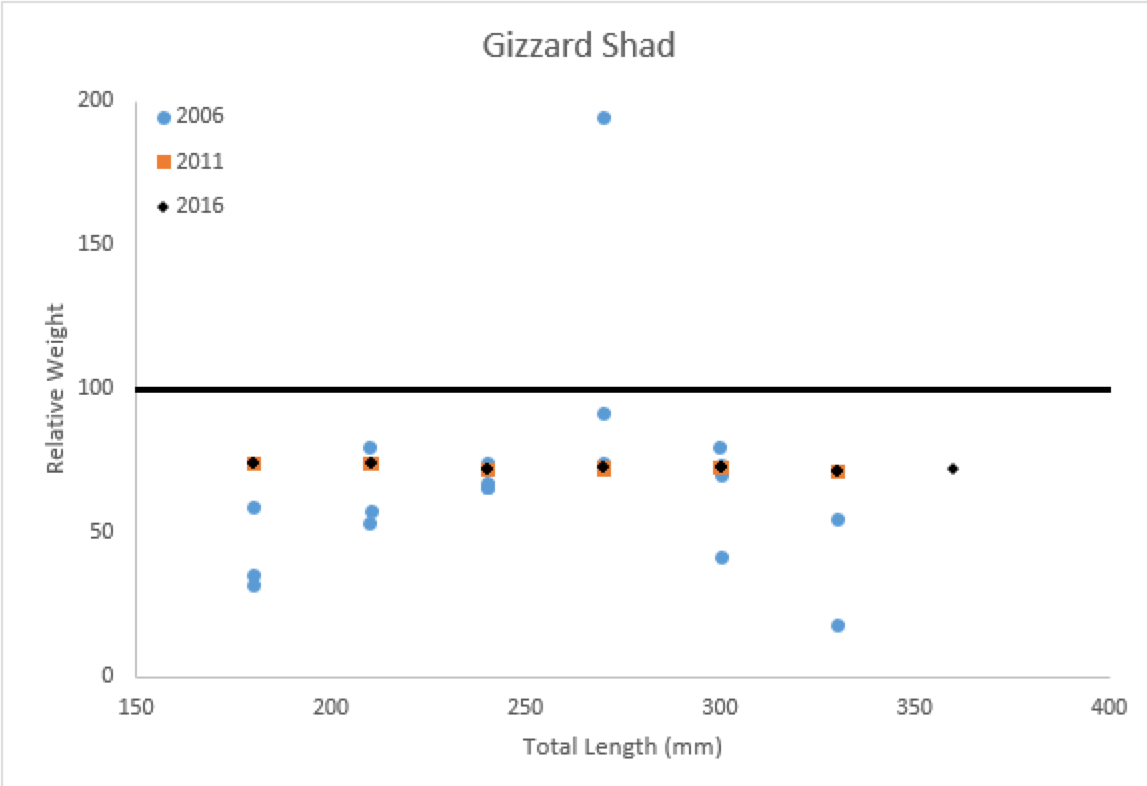

Figure 17. Gizzard shad individual relative weights across total length (mm) for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

Longnose gar individual relative weights for 2006 (n=23), 2011 (n=34), and 2016 (n=39) were plotted across total length (mm) (Figure 18). Four individuals exceeded the standard weight. Welch's ANOVA failed to detect a significant difference in mean relative weights between years ($F(2, 38.943)=1.1578$, $p=0.3248$).

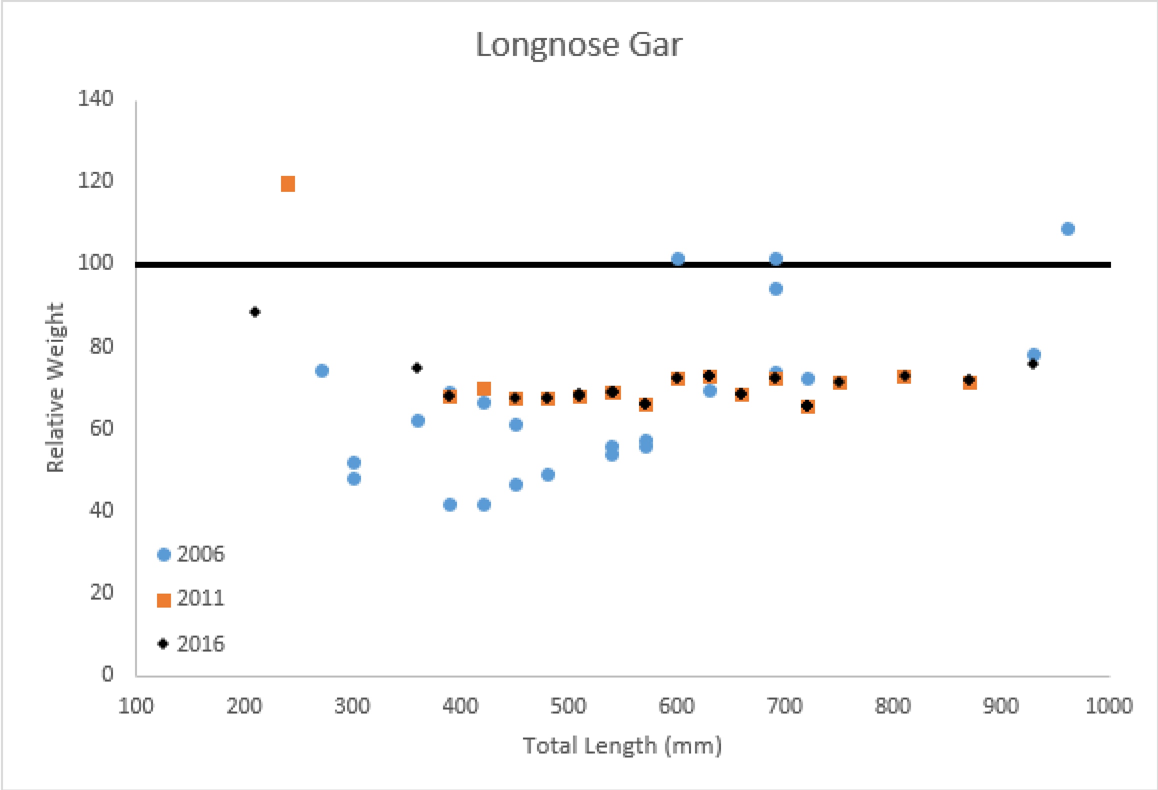

Figure 18. Longnose gar individual relative weights across total length (mm) for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

River carpsucker individual relative weights for 2006 (n=23), 2011 (n=20), and 2016 (n=15) were plotted across total length (mm) (Figure 19). Three individuals exceeded the standard weight. Welch's ANOVA failed to detect a significant difference in mean relative weights between years (F(2, 34.820)=2.0782, p=0.1404).

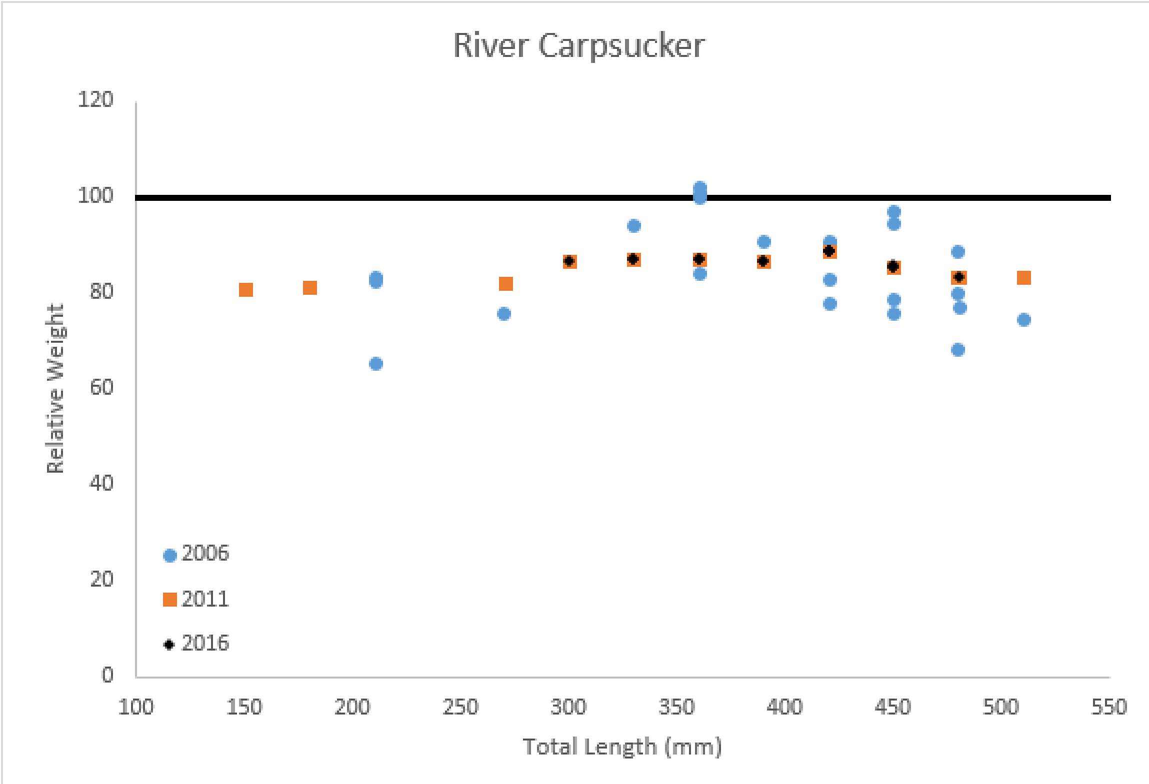

Figure 19. River carpsucker individual relative weights across total length (mm) for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

Smallmouth buffalo individual relative weights for 2006 (n=27), 2011 (n=20), and 2016 (n=16) were plotted across total length (mm) (Figure 20). No individuals exceeded the standard weight. Welch's ANOVA failed to detect a significant difference in mean relative weights between years (F(2, 34.366)=1.4733, p=0.2433).

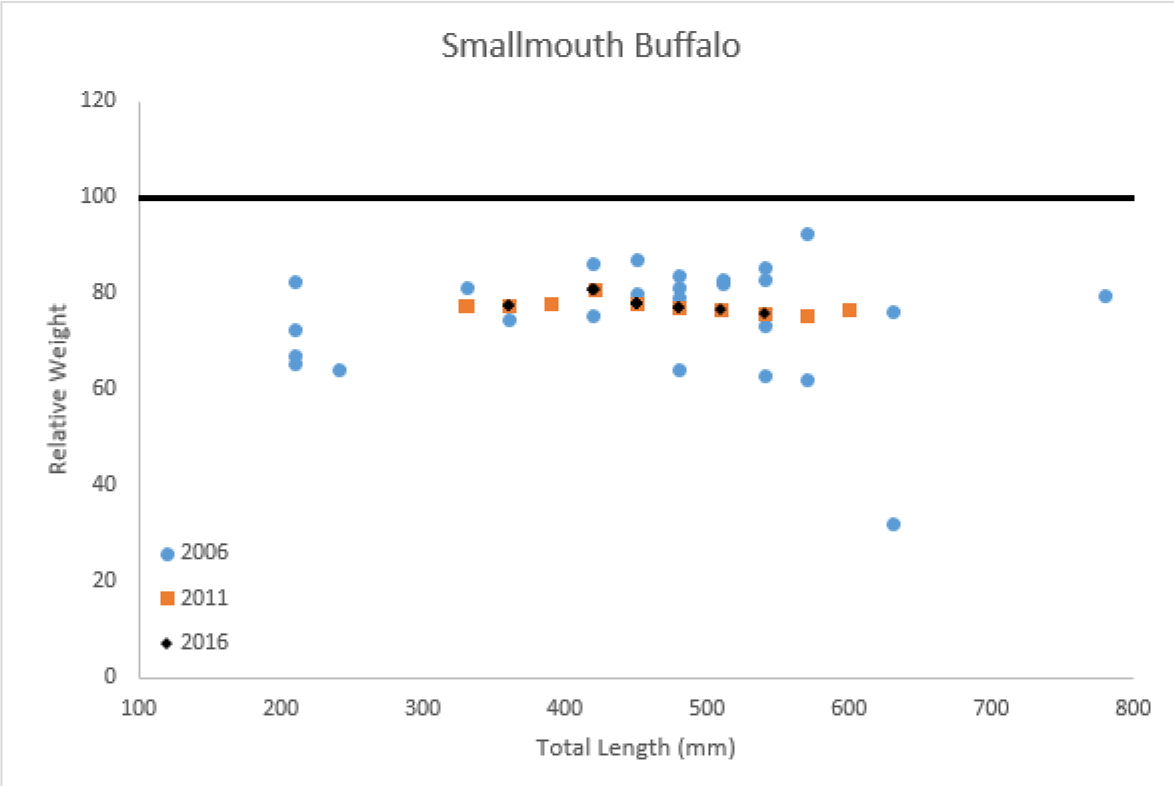

Figure 20. Smallmouth buffalo individual relative weights across total length (mm) for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

Bluegill individual relative weights for 2006 (n=11), 2011 (n=18), and 2016 (n=16) were plotted across total length (mm) (Figure 21). A single individual exceeded the standard weight. Welch's ANOVA failed to detect a significant difference in mean relative weights between years ($F(2, 20.601)=0.0515$, $p=0.9499$).

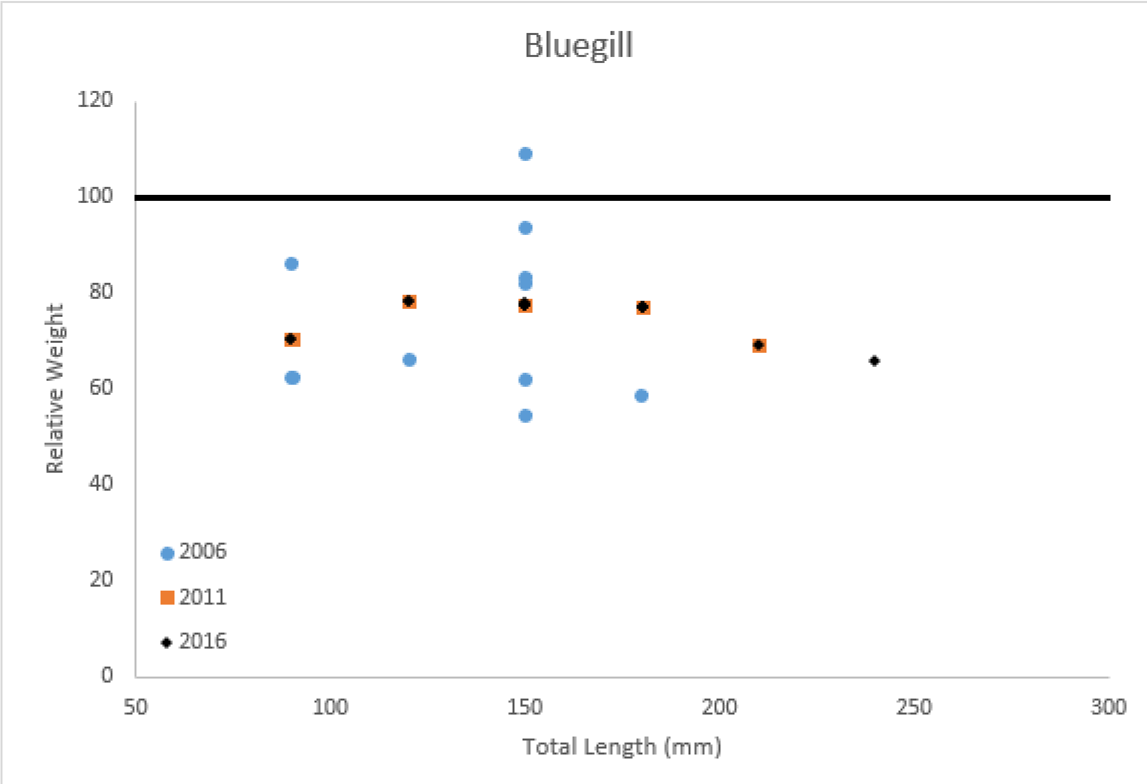

Figure 21. Bluegill individual relative weights across total length (mm) for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

Flathead catfish individual relative weights for 2006 (n=25), 2011 (n=36), and 2016 (n=24) were plotted across total length (mm) (Figure 22). Three individuals exceeded the standard weight. Welch's ANOVA failed to detect a significant difference in mean relative weights between years ($F(2, 45.415)=0.6153$, $p=0.5449$).

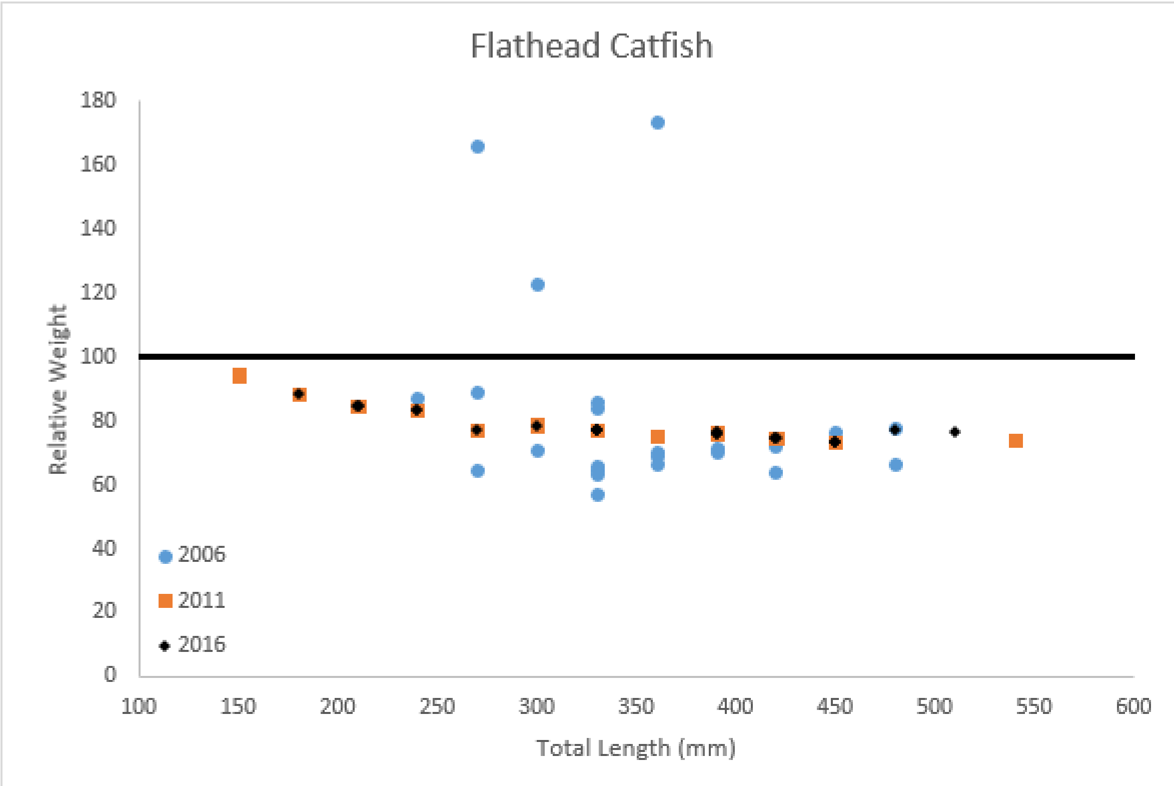

Figure 22. Flathead catfish individual relative weights across total length (mm) for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

Spotted bass individual relative weights for 2006 (n=12), 2011 (n=28), and 2016 (n=26) were plotted across total length (mm) (Figure 23). Three individuals exceeded the standard weight. Welch's ANOVA failed to detect a significant difference in mean relative weights between years ($F(2, 24.218)=0.1913$, $p=0.8271$).

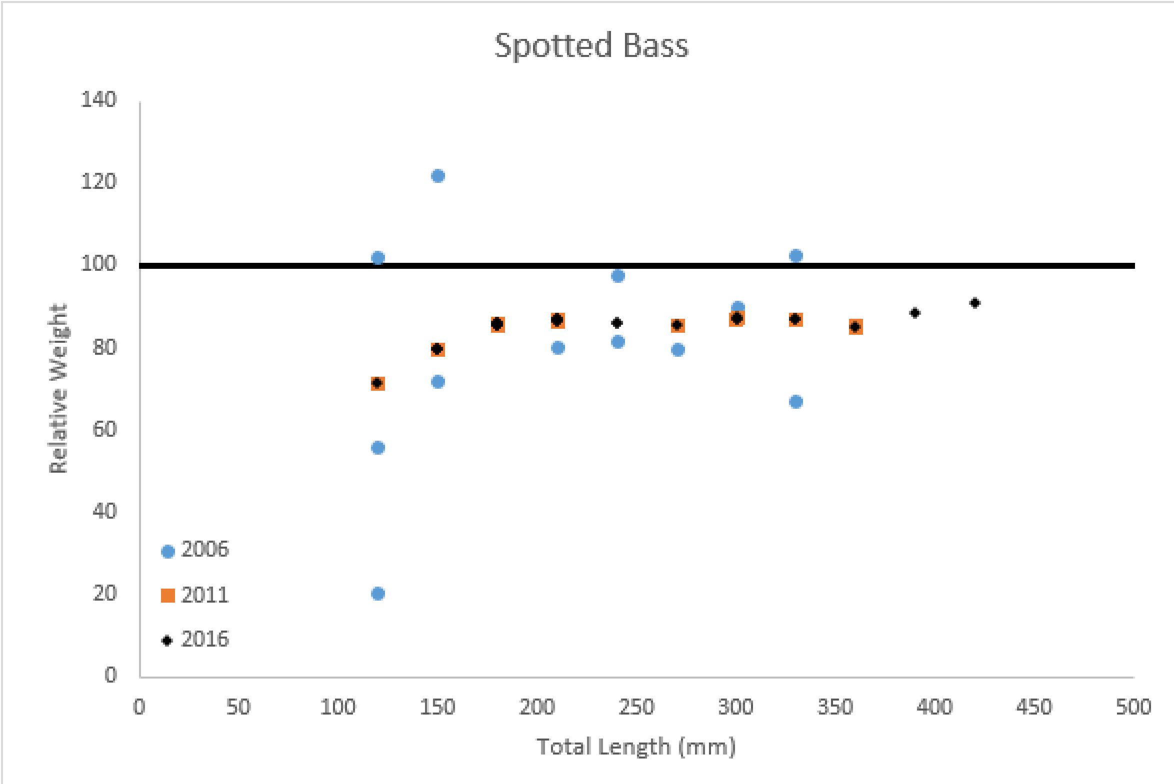

Figure 23. Spotted bass individual relative weights across total length (mm) for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

Golden redhorse and smallmouth redhorse were evaluated using Fulton's condition factor (K) since published standard weight equations were not available at the time of this study. Fulton's condition factor is the ratio between the observed weight and the expected weight of a fish based on its length. Fish that are above or below K=1.0 represent fish that are considered to be in a better or poorer condition relative to their length, respectively. Length-weight relationships of golden redhorse (Figure 24) and smallmouth redhorse (Figure 25) showed good fit (R^2=0.8846, R^2=0.9384). Golden redhorse individual K values for 2006 (n=20), 2011 (n=21), and 2016 (n=52) were plotted across total length (mm) (Figure 26). Smallmouth redhorse individual K values for 2006 (n=15), 2011 (n=20), and 2016 (n=29) were plotted across total length (mm) (Figure 27). Welch's ANOVA failed to detect a significant difference in mean

Fulton's condition factor between years for either golden redhorse ($F(2, 38.330)=0.0472$, $p=0.9539$) or smallmouth redhorse ($F(2, 29.215)=2.0218$, $p=0.1505$).

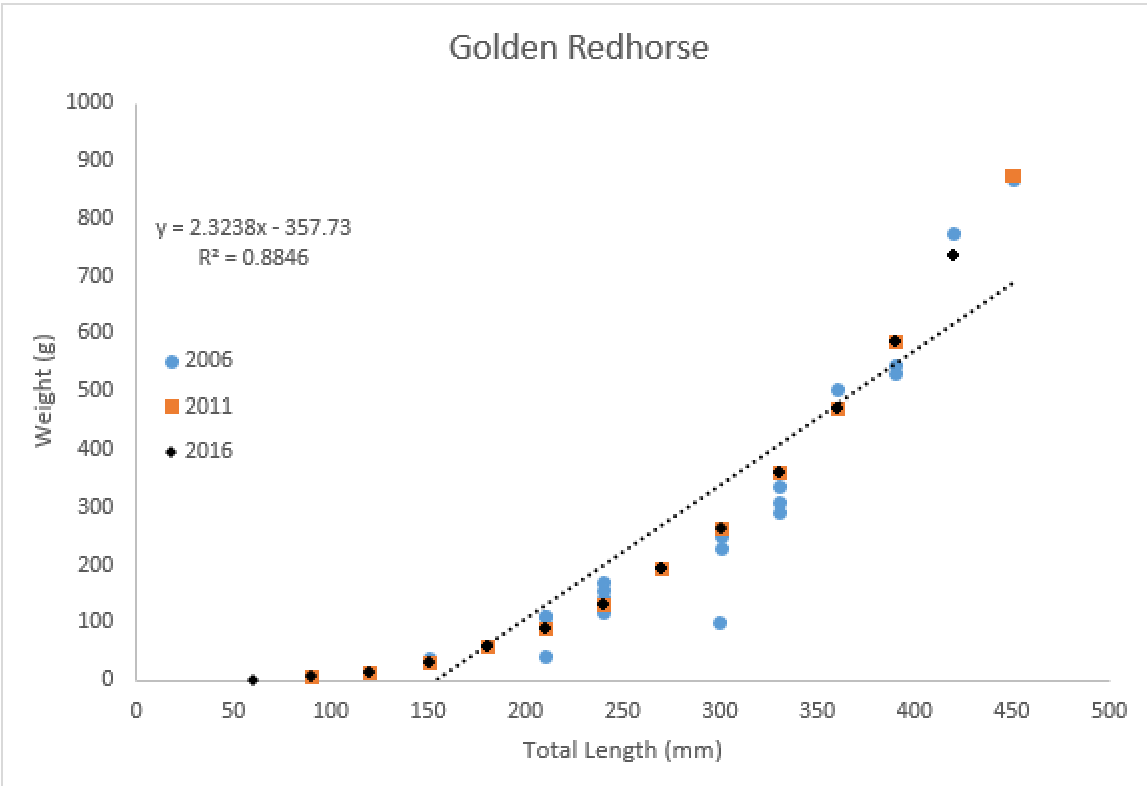

Figure 24. Golden redhorse length-weight relationship showing good fit for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

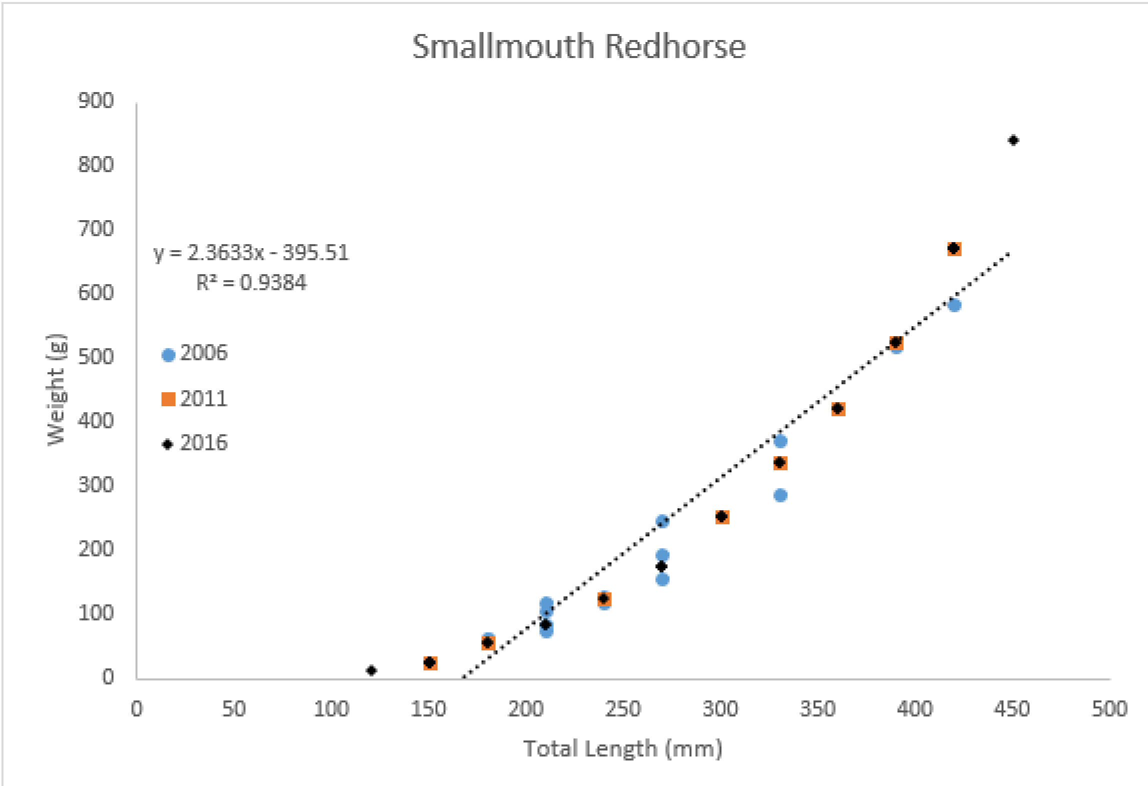

Figure 25. Smallmouth redhorse length-weight relationship showing good fit for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

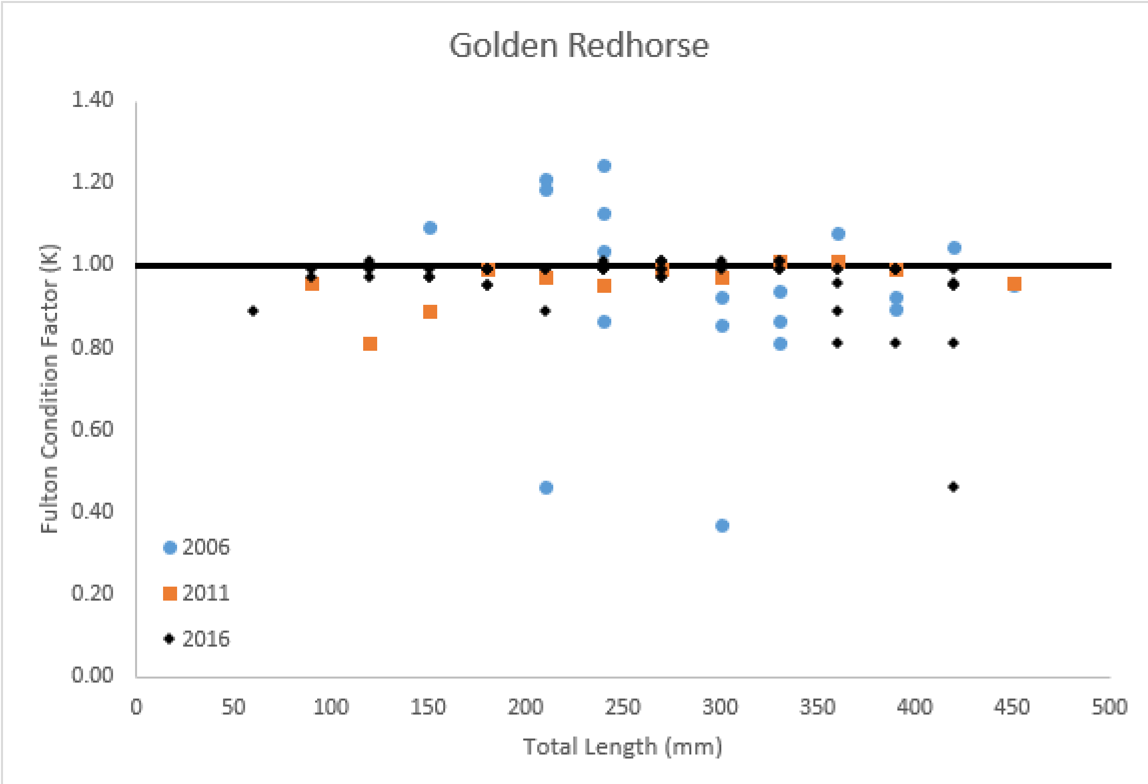

Figure 26. Golden redhorse individual Fulton's condition factor (K) values across total length (mm) for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

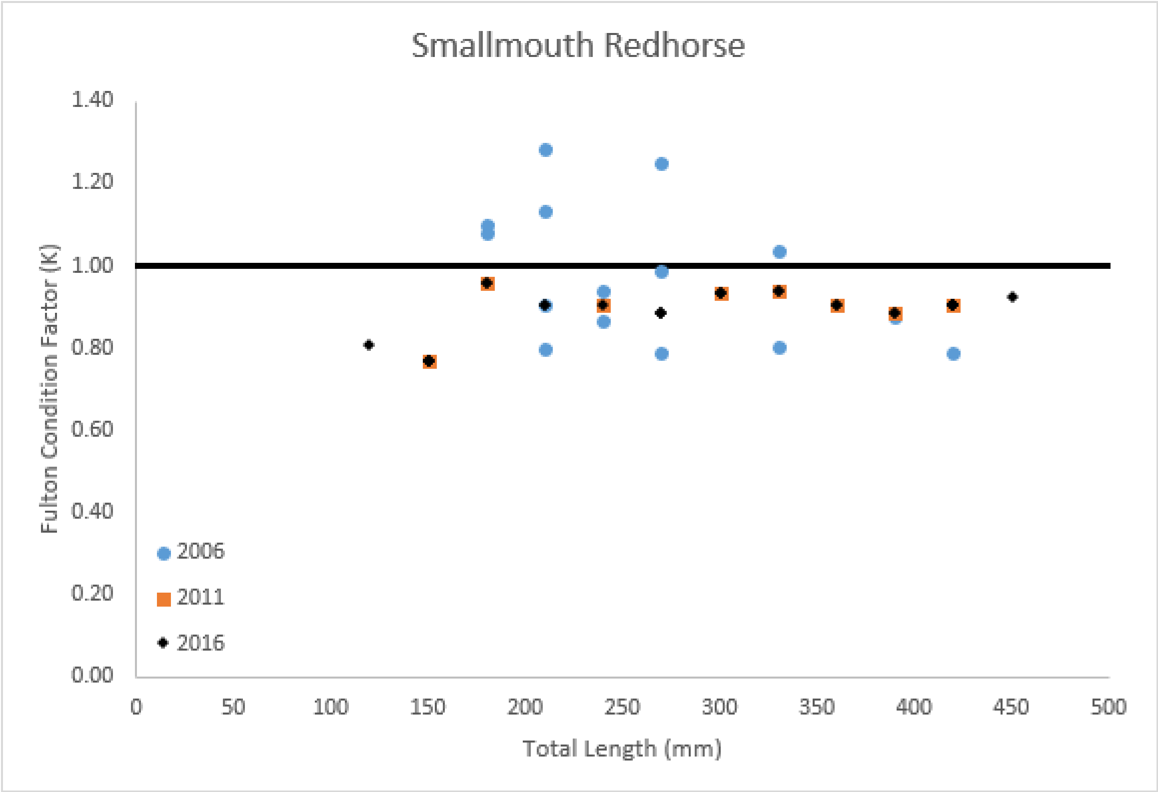

Figure 27. Smallmouth redhorse individual Fulton's condition factor (K) values across total length (mm) for 2006, 2011, and 2016 ORSANCO electrofishing surveys.

NMDS

The fish community appeared to show differences between pool sections in the Greenup Pool (Figure 28). The majority of species (86%) exhibited greater dissimilarity between the lower pool and the upper and middle pools. Species that were more associated with the lower and middle pool were: centrarchids (bluegill, green sunfish (*Lepomis cyanellus*), longear sunfish (*Lepomis megalotis*), redear sunfish (*Lepomis microlophus*), largemouth bass (*Micropterus salmoides*), silver chub (*Macrhybopsis storeriana*), and spotted sucker (*Minytrema melanops*). The fish community did not appear to show differences with respect to channel curvature or bank side (Figures 29-30).

The scree plot (Figure 31) of the stress (i.e., an inverse measure of fit), with respect to increasing dimensionality, shows that two dimensions are appropriate. Stress values for the real data runs should remain below 20 in order to be acceptable measures of fit. The minimum stress of the real data compared to the minimum stress of the randomized data (Table 5) shows that the best dimensional solutions provide a significant decrease, more so than expected by chance (Monte Carlo test, p=0.0196). This helps confirm that a two-dimensional solution is appropriate. The stability of the final ordination (Figure 32) plateaus around 40 iterations, showing that a stable solution was found and that it is acceptable, with a final stress of 18.61. The final solution was ordinated in a two-dimensional view with overlaid convex hulls of each pool morphological category (e.g., pool section, bank side, channel curvature). Points that are closer together are least dissimilar, and points that are further apart are most dissimilar. 81% of the variation was represented in the distance matrix (R^2=0.817) (Figures 28-30).

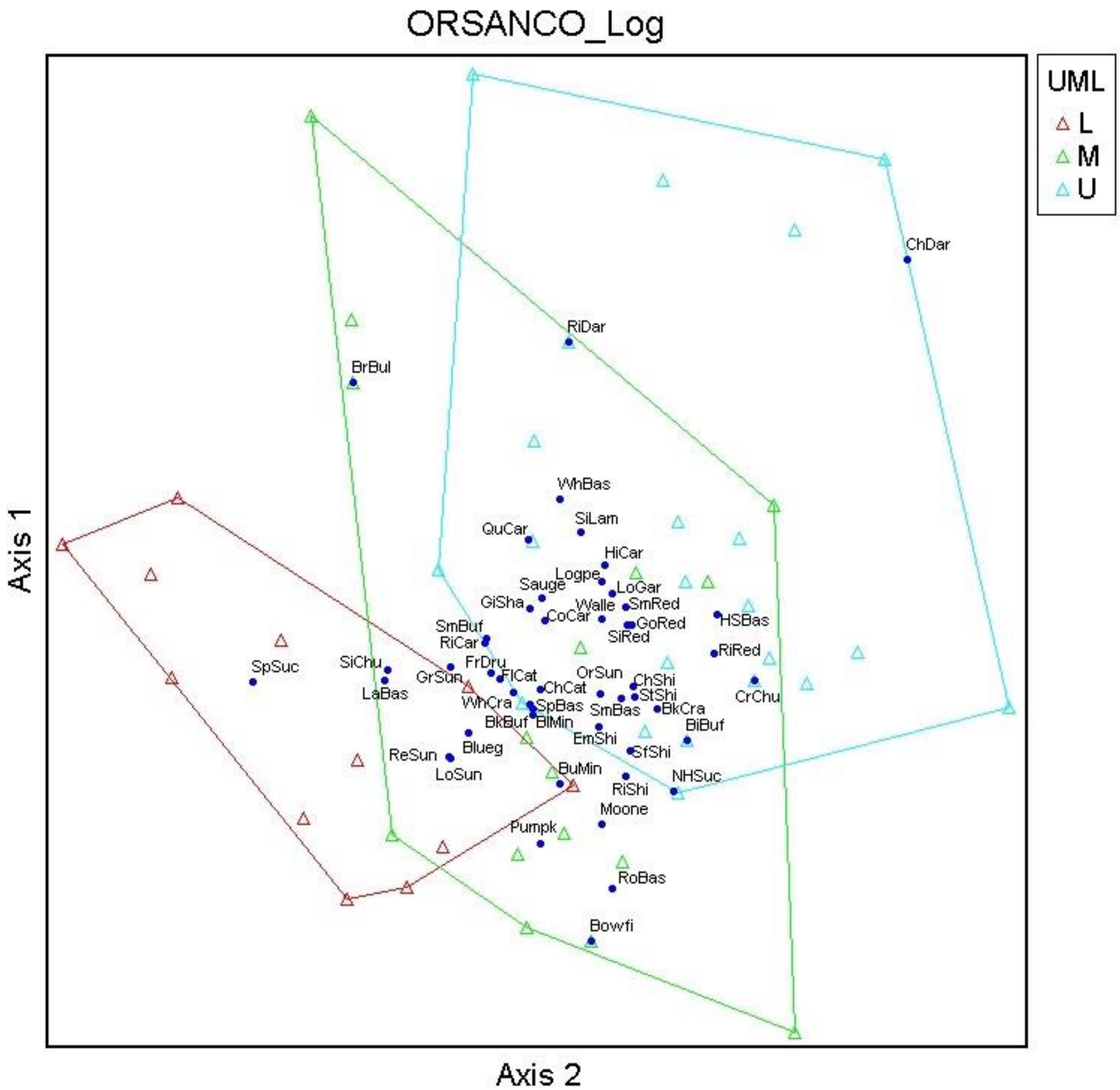

Figure 28. Species dissimilarity between sites with respect to upper (U), middle (M), and lower (L) pool in the Greenup Pool, Ohio River for the 2006-2016 ORSANCO dataset.

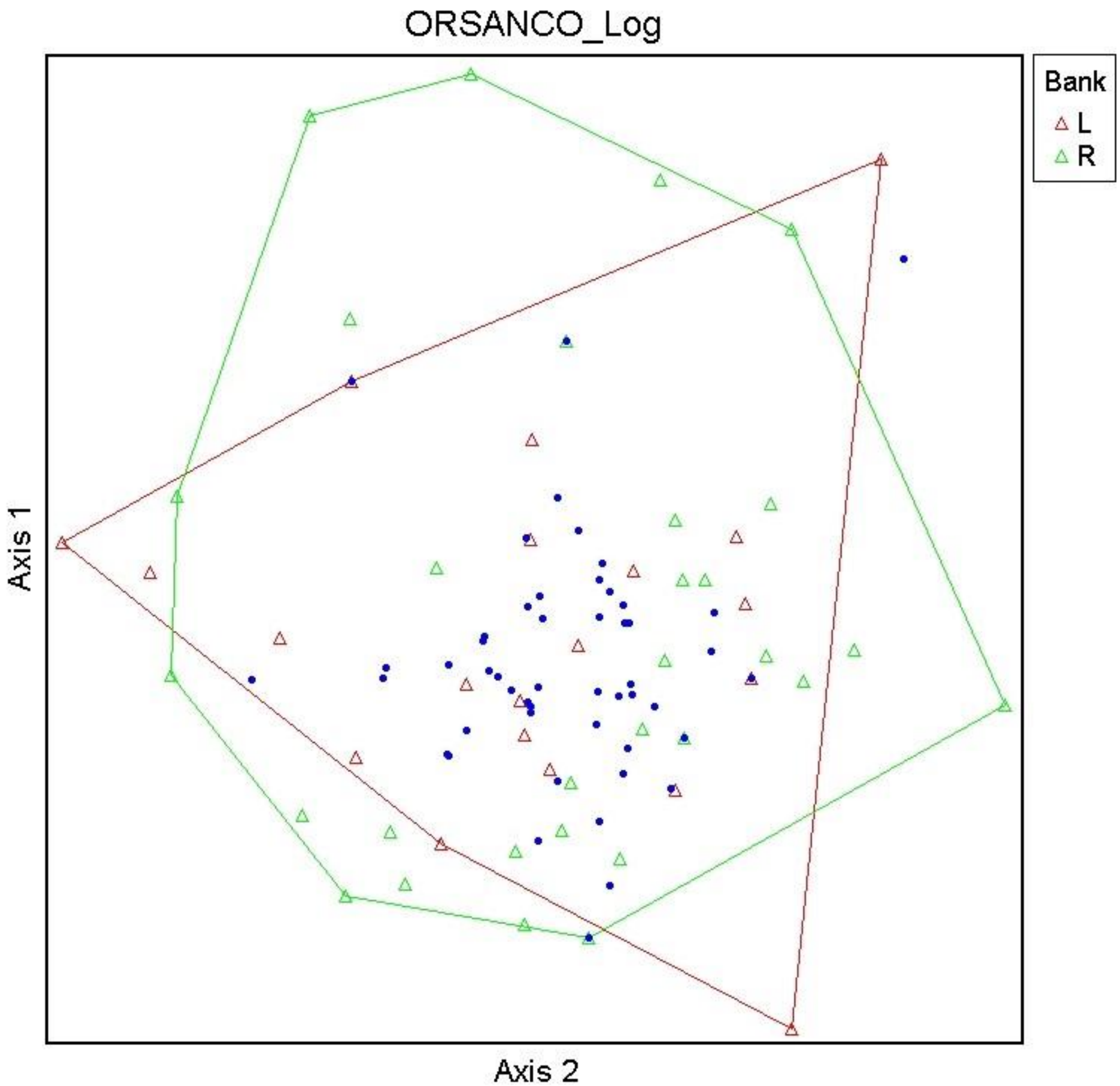

Figure 29. Species dissimilarity between sites with respect to left descending bank (L) and right descending bank (R) in the Greenup Pool, Ohio River for the 2006-2016 ORSANCO dataset.

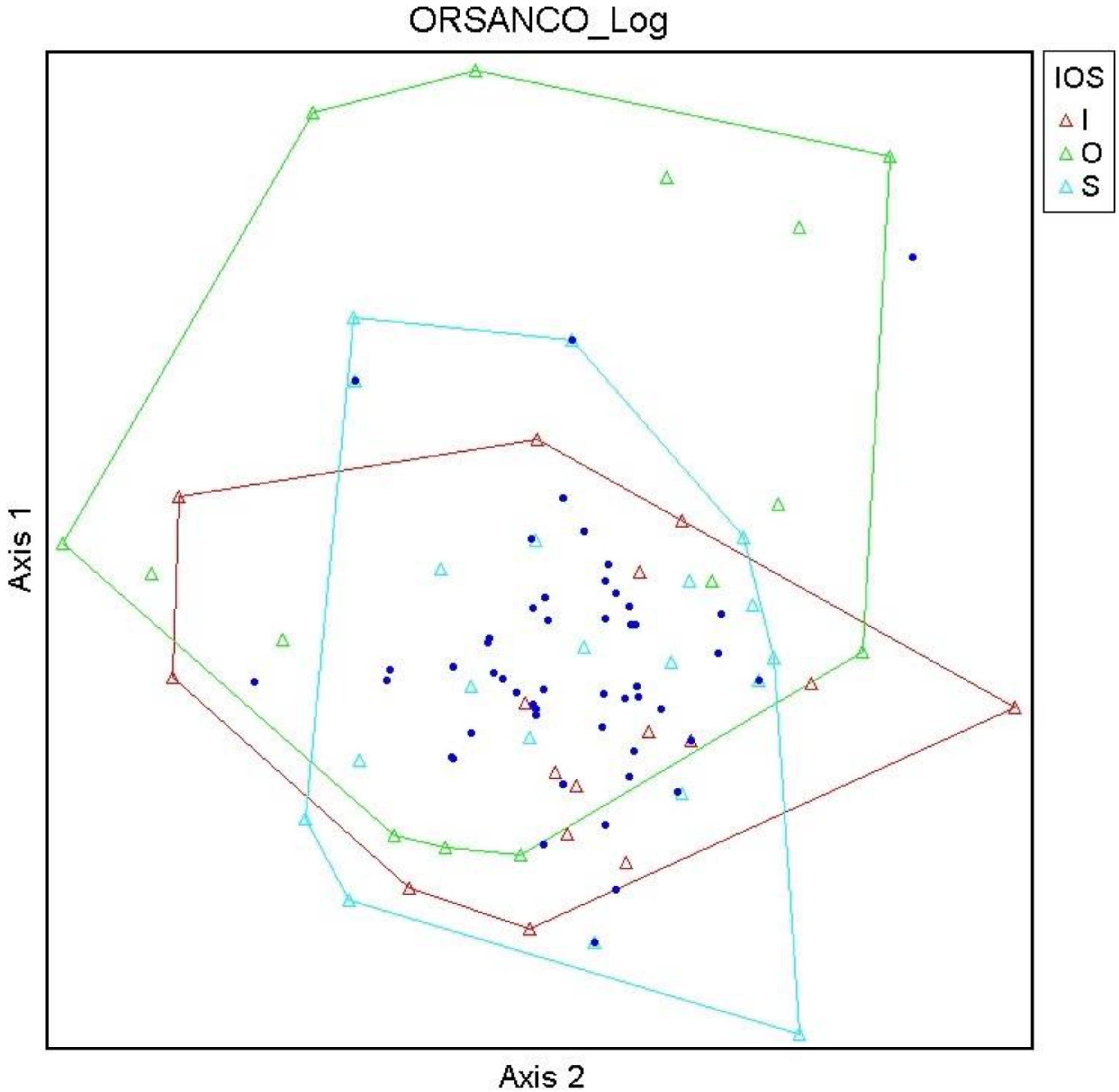

Figure 30. Species dissimilarity between sites with respect to inside bend (I), outside bend (O), and straightaway (S) channel curvature in the Greenup Pool, Ohio River for the 2006-2016 ORSANCO dataset.

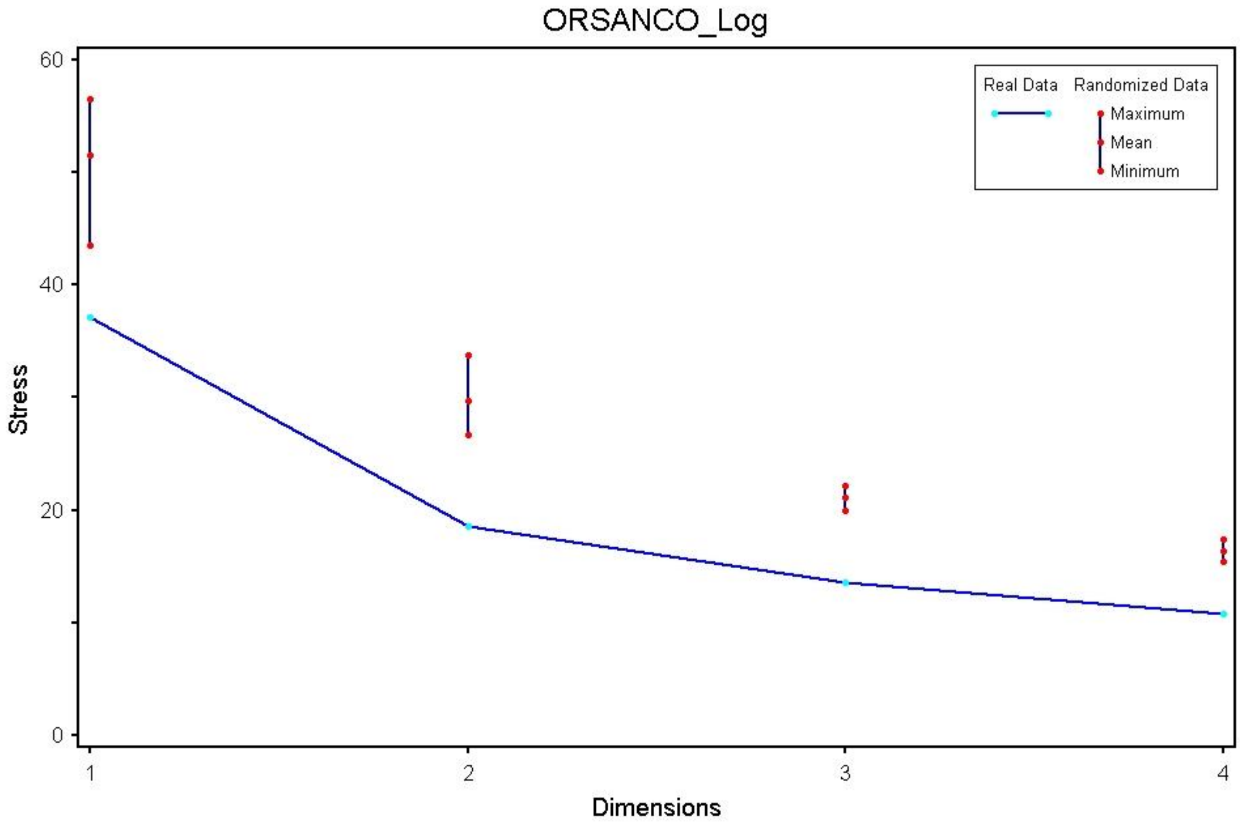

Figure 31. Scree plot showing stress decreasing with increasing number of dimensions for the 2006-2016 ORSANCO dataset.

Table 5. Stress in relation to dimensionality (axes) for the 2006-2016 ORSANCO dataset. Stress of the real data is compared to a randomized version of the data to determine if the dimensional solutions are significantly decreasing stress greater than expected by chance (p=0.0196).

Stress in real data 50 run(s)				Stress in randomized data Monte Carlo test, 50 runs			
Axes	Minimum	Mean	Maximum	Minimum	Mean	Maximum	p
1	37.086	47.271	56.574	43.547	51.527	56.563	0.0196
2	18.611	19.847	40.234	26.746	29.695	33.802	0.0196
3	13.617	13.892	14.738	19.977	21.095	22.121	0.0196
4	10.757	10.959	11.574	15.384	16.417	17.466	0.0196

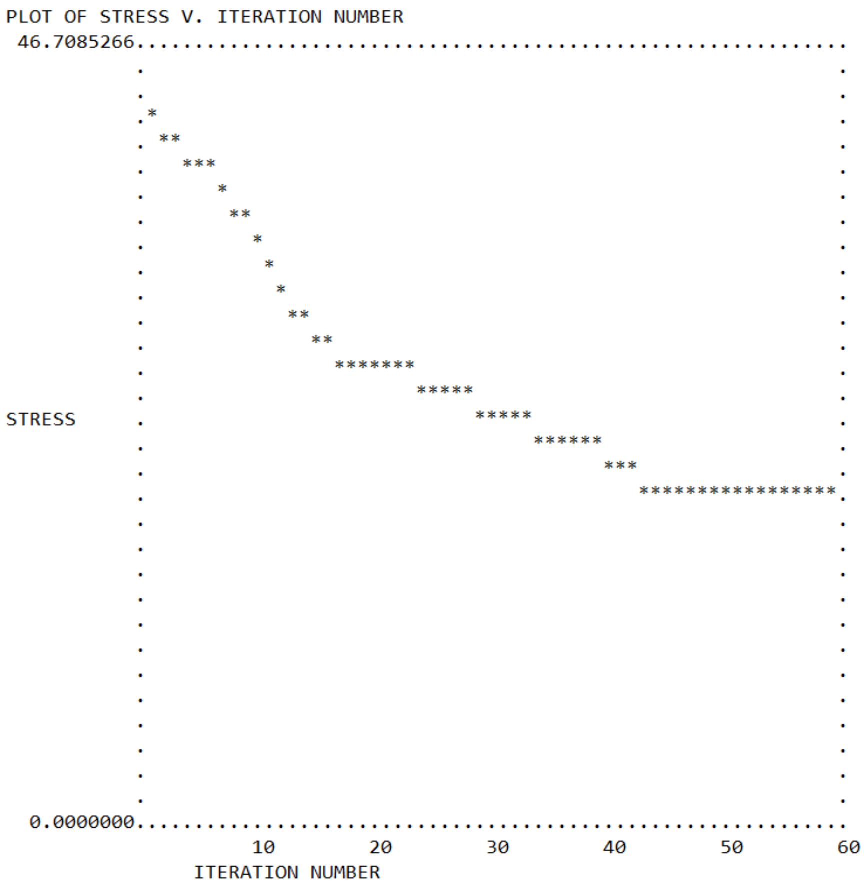

Figure 32. Stress decreasing with increasing number of iterations for the 2006-2016 ORSANCO dataset. The solution stabilizes around 40 iterations.

DISCUSSION

Species Richness and Abundance

The increases in species richness and abundance over the periods 2006-2011 and 2006-2016 suggest that the relative condition of the fish community has improved in the Greenup Pool. Increases in multiple species of redhorses (*Moxostoma*) support this conclusion, as redhorses are invertivores that are sensitive to water quality degradation (Emery et al. 2003; ORSANCO 2016$_2$). Potential increases in aquatic vegetation may have provided additional forage and habitat for macroinvertebrates and these fishes. Large increases in channel shiners and emerald shiners indicate good recruitment years, which provide forage for predatory fishes that also saw increases in abundance relative to the 2006 electrofishing survey (channel catfish, longnose gar, sauger, spotted bass).

Species abundance exhibited a strong positive linear relationship with respect to site in the downstream direction in 2006, which may have been due in part to the significantly lower numbers of species and abundances collected in 2006 compared to 2011 and 2016. For the 2006 survey, several species (e.g., bluegill, channel catfish, freshwater drum, gizzard shad, largemouth bass, spotted bass) were collected in larger proportions in the downstream sections of the Greenup Pool. Centrarchid species prefer more lentic conditions found more commonly in the middle and lower Greenup Pool, with channel catfish and freshwater drum being tolerant of these conditions (e.g., increased water depth, lower water velocity, increased turbidity, etc.). Larger proportions of these predatory species (e.g., channel catfish, largemouth bass, spotted bass) and more tolerant species may indicate increased pressure on forage fishes (e.g., shiners, young-of-year) which were undersampled in 2006. This suggests that 2006 may have overall been a poor year for the majority of the fish community in the Greenup Pool. Species richness did not exhibit a linear relationship with respect to site in 2006, which was unsurprising given the overall low numbers of species. For 2011, species richness and abundance did not exhibit a linear relationship with respect to site, which were spread relatively evenly throughout the Greenup Pool. The spread of channel and emerald shiners throughout the pool in 2011 indicate improvements in the relative condition of the fish community compared to the 2006 survey. Shiners provide a large source of food by converting energy in lower trophic levels, which when spread throughout the pool, have a greater potential to support fishes in more areas (e.g., upper, middle, and lower pools). With the exclusion of a channel shiner outlier, species abundance exhibited a moderately negative linear relationship with respect to site in the downstream direction in 2016. This was the expected relationship as the morphology, habitat, and conditions in the Greenup Pool are drastically different between the upper pool and the middle and lower pools. However, habitat and environmental conditions were not accounted for in this analysis and should be a point of focus in future studies. Upstream in the Greenup Pool, habitat and conditions would be expected to be more favorable for the majority of species as there is less impact from urbanization, sedimentation, combined sewer overflows (CSOs), and barge traffic (Kriege 2018). However, this was not observed across all survey years and appeared to differ between each survey year. Fish movement is highly variable and environmental conditions during sampling efforts during summer low flow (e.g., water temperature, dissolved oxygen, turbidity, etc.) as well as other factors (e.g., food resource availability, good/poor recruitment, etc.) may have a greater influence on fish community distribution between years than river mile in the Greenup Pool. Future studies are needed that account for these variables to help determine the relationship of species richness and abundance in the Greenup Pool.

Body Condition

Channel catfish abundance increased greatly between 2006 and 2011, likely leading to increased competition and subsequently decreased relative weight. Sauger abundance decreased between 2006 and 2011, likely leading to reduced competition and increased relative weight. Potential forage fishes (e.g., channel shiner, emerald shiner) increased in 2011 and 2016, possibly increasing sauger relative weight. Increases in forage potential would likely increase body condition of other predatory species (e.g., flathead catfish, longnose gar, spotted bass); however, this was not detected statistically. Sauger condition was the lowest mean of all the evaluated species for the initial 2006 electrofishing survey, which was similar to other evaluated species (e.g., gizzard shad, longnose gar). It is possible that 2006 was a poor year in the Greenup Pool (e.g., poor environmental conditions, limited food resources, etc.), leading to below-average conditions for these species during the summer. Channel catfish appeared to be within the same range of relative weights as other species that scored a higher condition (e.g., 80+), which suggests that channel catfish are still in poor condition relative to the other species.

For all 12 species comparisons between ORSANCO electrofishing survey years for relative weight and Fulton's condition factor, the majority of individuals consistently fell below Wr=100 or K=1.0 (94%). This suggests that despite increases in species richness and abundance, indicating overall improvements in the fish community between survey years, these species are in poor condition. Even though the 3-cm size class measurements are likely contributing to an overestimate of the actual total length of an individual and may result in a poorer body condition, a similar pattern was found in another dataset (Fleshman 2019). Relative to the standard weight of each species, body condition is poor for all 12 species in the Greenup Pool. This is alarming as the fish community in the Greenup Pool has been classified as in "healthy condition"

(ORSANCO 2016$_2$). A potential explanation is that fish were collected during summer low flow (July-October) for each sample year, resulting in a poorer body condition across all survey years. Summer is stressful on the majority of fishes due to increased water temperatures, lower dissolved oxygen, and increased metabolism. Additionally, many species reproduce in the spring and summer (e.g., all 12 species evaluated), which may reduce condition post-spawn. Future investigations of fish condition changes in the Greenup Pool should incorporate various sampling seasons to evaluate the holistic condition of each species.

One caveat to this analysis is that these data for the 12 species represent a small subset of the total fish measurements collected (<25%), which only include fish that were weighed individually rather than batch-weighed. Additionally, the implementation of 3-cm size class measurements after the initial 2006 electrofishing survey may reduce the detection of potential changes in fish body condition between the 2011 and 2016 surveys. While individually collecting length and weight measurements dramatically increases processing time, accurate measurement data is important to assessing fish condition.

NMDS

A large majority of fish species were more closely associated with the upper and middle pools compared to the lower pool. This likely reflects the higher level of disturbance experienced by the lower pool. Compared to the upper pool, the lower pool is greatly impacted by urbanization, sedimentation, CSOs, and barge traffic (Kriege 2018). Moving downstream in the Greenup Pool, water conditions typically associated with lower water quality increase such as: increased water depth, decreased water velocity, lower dissolved oxygen, increased turbidity, and increased fine sediments (Hassel et al. 1988).

One limitation of this analysis is that this ordination fails to factor in environmental variables, which may produce different solutions. Including site-specific water quality parameters (water temperature, dissolved oxygen, turbidity) and habitat data may reveal potentially different trends. An alternative potential explanation for the differences in fish community distribution by pool section is the seasonality of sampling. Fish are collected via electrofishing during summer low flow (July-October) for each sample year. Lower dissolved oxygen levels during summer low flow may lead fishes to migrate upstream where water velocity and dissolved oxygen may increase.

Centrarchids (bluegill, green sunfish, longear sunfish, redear sunfish, largemouth bass) prefer more lentic conditions and habitat, which is typical of the lower pool. As water depth increases and water velocities decrease, large woody debris and snags tend to accumulate, which provides additional habitat structure that centrarchids prefer (Crook and Robertson 1999). Decreased water velocities promote growth for algae and zooplankton, which various sunfish consume, in addition to spotted suckers and silver chub. Dissolved oxygen may also be lower due to: decreased water velocities, biological oxygen demand, and reduced primary productivity, which may help drive the major fish community upstream. Increased barge traffic in this section of the pool notably increases turbidity, resulting in limited light penetration and reduced primary productivity that may exclude the major fish community. Increased fine sediments in the lower pool drastically reduce available habitat for macroinvertebrates that may serve as a primary food source for species such as the redhorses, buffalo fish, and carpsuckers, which were all more associated with the upper and middle pool (Wood and Armitage 1997). Additionally, increased fines reduce available coarse substrate required for lithophilic spawning fishes (redhorses, shiners) and for other species that may benefit from coarse substrates (buffalo fish, carpsuckers)

(Mapes et al. 2015). Species associated with the upper and middle pool represent various feeding groups: catastomids (*Moxostoma*, *Ictiobus*, *Carpiodes*), cyprinids (*Notropis*), clupeids (gizzard shad), which are primarily invertivores and planktivores, but also piscivores like longnose gar, temperate basses (*Morone*), and percids (*Sander*). Less fine sediments and better water quality in the upper middle and upper pool likely provide better conditions for macroinvertebrates and fishes, which may have trophic cascading effects on the major fish community. This suggests that the fish community in the Greenup Pool may prefer the upper and middle pools compared to the lower pool. Similar trends were seen in the freshwater mussel community of the Greenup Pool, with very few mussels being found in the lower pool (Kriege 2018). Future analyses should include site-specific water quality parameters (water temperature, dissolved oxygen, turbidity) and habitat data to assess the holistic fish community distribution in the Greenup Pool and elicit different potential solutions.

Conclusions

The fish community of the Greenup Pool seems to have improved with increasing species richness and abundance since the initial electrofishing survey by ORSANCO in 2006. However, the consistent poor body condition of 12 evaluated species indicates that the fish community in the Greenup Pool is not faring well overall. This poses a great concern, particularly as bigheaded carps invade the Greenup Pool, which will likely lead to reductions in native fish condition, species richness, and abundances. Incorporating various sampling seasons and increasing sample sizes to include other species may provide a more holistic condition of the fish community in the Greenup Pool pre-bigheaded carp invasion. Above the Big Sandy River and the city of Huntington, the middle and upper pools provide better habitat and water quality that may be driving the fish community upstream. However, fish movement is highly variable, and it is possible that the community may be responding to stressful conditions during summer low flow when they are sampled. Future studies are needed that account for seasonality, environmental conditions, and habitat to further evaluate fish community distribution in the Greenup Pool. As bigheaded carp abundance increases in the Greenup Pool, fish community distribution will likely be affected through their deleterious trophic cascading impacts.

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Appendix A: IACUC Approval Letter

MARSHALL UNIVERSITY.
www.marshall.edu

Office of Research Integrity

August 9, 2022

Nathan Fleshman
3305 Maywood Drive
Ona, WV 25545

Dear Nathan:

This letter is in response to the submitted thesis abstract entitled *"Fish Community Trends in the Greenup Pool, Ohio River."* After assessing the abstract, it has been deemed not to be human subject research and therefore exempt from oversight of the Marshall University Institutional Review Board (IRB). Since this study only involves the use of fish survey data from the Ohio River Valley Water Sanitation Commission (ORSANCO) it is exempt from the Institutional Animal Care and Use Committee (IACUC). The Code of Federal Regulations (45CFR46) has set forth the criteria utilized in making this determination. Since the information in this study does not involve human subjects as defined in the above referenced instruction, it is not considered human subject research. If there are any changes to the abstract, you provided then you would need to resubmit that information to the Office of Research Integrity for review and a determination.

I appreciate your willingness to submit the abstract for determination. Please feel free to contact the Office of Research Integrity if you have any questions regarding future protocols that may require IRB review.

Sincerely,

Bruce F. Day, ThD, CIP
Director

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