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An analysis of the preferences, morphometrics, and survey efficacy of the freshwater mussel community in the Greenup pool, Ohio River

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AN ANALYSIS OF THE PREFERENCES, MORPHOMETRICS, AND SURVEY EFFICACY OF THE FRESHWATER MUSSEL COMMUNITY IN THE GREENUP POOL, OHIO RIVER

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APPROVAL OF THESIS

We, the faculty supervising the work of Jacob Miller, affirm that the thesis, *An Analysis of the Preferences, Morphometrics, And Survey Efficacy of the Freshwater Mussel Community in The Greenup Pool, Ohio River,* meets the high academic standards for original scholarship and creative work established by the Department of Biological Sciences 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

The Greenup Pool is one of the most unique pools of the Ohio River in terms of habitat richness and anthropogenic impact both historical and current. The Greenup Pool spans 61.8 miles reaching from Greenup Dam in Greenup, KY to Robert C. Byrd Dam in Apple Grove, WV. We surveyed 17 sites to assess mussel communities within the Greenup Pool using the 2017 West Virginia Mussel Survey Protocol. At these sites, six 100-meter transects were surveyed for surface and subsurface mussels. Each transect was divided into 10-meter intervals. These surveys resulted in 4,041 live individuals from 21 species, including eight federally endangered *Plethobasus cyphyus*. This effort furthers our understanding of the Greenup Pool mussel community and identifies the outside bends and straightaways of the upper Greenup Pool as their preferred habitat. Habitat selection may be a result of lower historic impact in the Upper Pool and appears to be influenced by the coverage of fine sediment. Additionally, morphometric data indicates a severe lack of recent reproduction in the Greenup Pool, suggesting many species are only present as large, old individuals. The mussel community identified in this effort was compared to a prior Greenup Pool survey effort in 2017 using a similar site selection process and survey protocol. The analysis of the two efforts concluded the initial 2017 survey captured the mussel community based on the results of this effort. The combined data from both efforts is potentially the largest and most up to date mussel community resource for an individual navigational pool in the Ohio River.

OVERVIEW

The aquatic ecosystems of North America contain a vast array of biodiversity. The organisms inhabiting these ecosystems have developed specific and complex relationships over millennia of evolution (Dartnell, 2018). These organisms have prevailed through drastic changes in their environment by adapting and reproducing. However, since the arrival of Paleo-Indians in North America, aquatic ecosystems have been altered to better suit human needs (Haag, 2012). Aquatic systems have provided humans with drinking water, food, and transportation for thousands of years (Dartnell, 2018). In primitive times, humans were interwoven with these ecosystems and the alterations were often mild and localized (Haag, 2012; Cummings and Mayer, 1992). However, the impacts brought upon by the industrial revolution of the 19th century would subject these ecosystems to challenges of an all-new severity (Dartnell, 2018). While life since the 18th century has brought about great advancements in human history, aquatic ecosystems have experienced massive declines in species richness and abundance (Haag, 2012; Parmalee and Bogan, 1998). However, few taxonomic groups have experienced declines as severe as freshwater mussels (Unionida) (Haag, 2012).

North America is historically the most diverse place on the planet for freshwater mussels (Haag and Williams, 2013). As of 2017, 298 freshwater mussel species are recognized in North America (Williams et al, 2017). However, this group has experienced drastic population declines in the last century. Of the nearly 300 species described, it is estimated that 35 are now extinct (Williams et al, 2017; Center for Biological Diversity, 2020). Additionally, of the current extant species, it is estimated that over 70% are classified as threatened, endangered, or of special concern (Strayer et al, 2004). Freshwater mussel declines in North America are largely caused by

siltation, degradation of water quality, and disruptions in their reproductive cycle due to loss of host-fish (Watters et al, 2009).

Freshwater mussels occupy a wide range of aquatic habitats such as ponds, lakes, streams, and large rivers; however, lotic systems such as streams and rivers are greatly preferred (Watters et al, 2009). Large river systems contain the greatest species richness (FMCS, 2019). While most of the species richness comes from the many tributaries of large river systems, the mainstem populations are of special interest because they serve as a connection between fragmented mussel communities in smaller systems (Woolnough, 2006; Parmalee and Bogan, 1998). Unfortunately, compared to small stream communities, mussel faunas throughout North America's large rivers are poorly studied (Haag and Williams, 2013). Freshwater mussel communities in large rivers are particularly difficult to survey due to water depth, commercial traffic, strong currents, and low visibility (Kriege, 2017). In order to protect mussel communities in large rivers, we must understand their distribution, and the role of anthropogenic impacts and specific habitat variables in determining distribution. This information allows for a comparison to historical and future data to accurately detect population declines. To further support the ability to detect declines, efficient and replicable survey methods are of great importance.

IMPACTS TO FRESHWATER MUSSEL COMMUNITIES

As a result of the sensitive life history of the organisms, factors associated with historical and current anthropogenic activity are highly influential in freshwater mussel distribution (Watters et al, 2009). Freshwater mussels are sedentary organisms with daily movements that rarely exceed one meter (Schwalb et al, 2007). This limited mobility leaves them vulnerable to detrimental anthropogenic changes in their environment (Watters and Flaute, 2010). Additionally, because most freshwater mussels are slow growing, long-lived organisms, sudden

impacts can eliminate decades of mussel community development in a relatively short time period (Watters et al, 2009).

Siltation

The sedentary nature of freshwater mussels translates to a strong association with the benthic layer of the water bodies they inhabit (Haag, 2012). This association is important in understanding freshwater mussel populations in systems with heterogenous substrate compositions (Parmalee and Bogan, 1998). One of the greatest factors influencing substrate composition in large rivers is the presence of fine sediment, or siltation (Haag, 2012). Siltation is largely a result of the erosion caused by human activity along the banks of aquatic systems (USACE, 2019; Cordone and Kelly, 1961). Siltation causes impact for mussels by replacing the benthic layer with fine particles that are prone to shifting in changing water velocities (Haag, 2012). Siltation events can also bury existing mussels and restrict their ability to filter feed and reproduce (Watters et al, 2009). Heavy accumulation of fine sediment can result in an oxygen negative environment due to anaerobic bacteria respiration, further limiting freshwater mussels in areas of high siltation (Haag, 2012).

Degradation of Water Quality

Freshwater mussels primarily rely on filtering organic particles from the water column to obtain the necessary nutrients for their growth and survival, which leaves them prone to the intake of harmful pollutants in the water (Haag, 2012). Harmful pollutants include ammonia, heavy metals, and chemical anthropogenic compounds (i.e., pesticides or herbicides) (USACE, 2019). Harmful pollutants enter aquatic systems in several ways like surface runoff, industrial and wastewater discharges, agricultural runoff, point and non-point discharges from resource extraction activities and combined sewage overflows (CSO's) in urban and industrial riverine

systems. CSO's are a relic of historical development in cities along a rivers edge. These systems were designed to carry stormwater, domestic sewage, and occasional industrial sewage to disposal areas, which in the late 1800s, were typically local streams and rivers. While this was a major improvement in sanitation standards compared to previous methods, in the modern age this form of disposal is outdated. Under normal circumstances, sewage systems retain the storm/wastewater mix until it can be treated by modern treatment facilities. However, during high rainfall events, or even moderate rainfall events due to the continuous increase of impervious surfaces as a result of development, systems using CSO's become overloaded. In order to prevent the wastewater mix from backing up into the streets, the excess mixture is released into nearby rivers and streams. While this method is an improvement compared to historical practices, the exposure of untreated wastewater is still degrading water quality and impacting aquatic ecosystems in the modern age.

Loss of Host-Fish Availability

Freshwater mussels have a complex reproductive cycle that typically relies on an interaction with a fish host to reproduce. This interaction begins when an adult female mussel releases larval mussels known as glochidia (Haag, 2012). Once the glochidia have been released they must encounter an appropriate fish host. The glochidia attach to the host-fish, often on the gills or fins, where they encyst and remain until development is complete (Watters et al, 2009). After development is complete, the juvenile mussel detaches from the fish host and deposits into the substrate. If the substrate is suitable, a recently settled juvenile could remain in place for the duration of its lifespan. However, due to impacts (i.e., siltation) and natural variations of habitat in rivers, available habitat is often limited, resulting in the majority of juveniles not succeeding. While many mussel species are host-fish generalists, some species can only encyst and develop

while attached to specific species of fish (Woolnough, 2006). Due to this necessary interaction, mussel distribution is often limited by an aquatic system's fish community (Haag 2012). Like mussels, fish communities have experienced major declines due to anthropogenic activity (Haag 2012). The decline of fish communities has further lowered the frequency of interactions between mussels and host fish, halting them entirely for some species (Watters et al, 2009).

FRESHWATER MUSSELS OF THE OHIO RIVER

As with most aquatic systems, the Ohio River watershed has experienced significant impacts since the 1800s (Watters et al, 2009). Adequately assessing mussel communities for the entire river is challenging due to its large size, as the Ohio stretches nearly 1,000 miles and ranks second in total annual discharge in the United States (USGS, 1990). As a result, many sections of the Ohio River have not been surveyed for freshwater mussels. To understand the river better, ORSANCO established a "pool-level" bioassessment program as the primary means of bioassessment in the Ohio River. ORSANCO collects biological data by pool on several taxa and water parameters on a five-year rotation (ORSANCO, 2021). This group does not perform freshwater mussel surveys, however, using this "pool-wide" approach for mussels has been applied to the Greenup Pool in modern records.

THE GREENUP POOL

In 2017, Mitchell Kriege completed a statistically random mussel survey on a pool-wide scale on the Greenup Pool of the Ohio River (Kriege, 2017). The Greenup Pool is formed by the Greenup Lock and Dam in Greenup, KY at river mile (RM) 341.0 on the downstream end, and the Robert C. Byrd Lock and Dam in Apple Grove, WV at RM 279.2 on the upstream end (Fig. 1). The Greenup Pool contains diverse habitat and varying degrees of anthropogenic impacts. The upper section of the Greenup Pool has low levels of urbanization and industry (Kriege,

2017) but is influenced by agricultural development. Conversely, lower sections of the pool are strongly impacted by metropolitan areas, heavy industry, and the Greenup Dam (Kriege, 2017). One of the primary objectives of Kriege's study was to analyze the relationship between fish community data from ORSANCO's sampling efforts, and subsequent mussel community data at the same sites. ORSANCO's (2016) fish sampling sites were surveyed by Kriege for mussels under the 2016 West Virginia Mussel Survey Protocol (Clayton et al, 2016). The selection of these sites served two primary functions: (1) to examine the relationship between mussel and fish communities on a pool-wide scale; and (2) to contribute a statistically valid baseline data set for mussel communities throughout the Greenup Pool. Overall, the 2017 study by Kriege suggested a decline in multiple species compared to historic data. However, this alleged decline in species may be due to the lack of individuals collected in the upper pool (compared to historic data), and/or the randomness of the site selection itself (not targeting optimal habitat). To support the 2017 study by Kriege and strengthen our understanding of the mussel community of the Greenup Pool, I am conducting a second wave of sampling using different sites under the same survey protocol.

FOCUS OF STUDY

The goal of the following study is to further understand the mussel fauna of the Greenup Pool, this will be done in three chapters. Chapter I assesses the distribution of mussels in the Greenup Pool based on data collected for this study, including factors associated with distribution. Chapter II assesses the reproductive status of species found in the Greenup Pool. Chapter III compares the mussel community of the Greenup Pool using data collected for this study and previous data, primarily from the 2017 Kriege study. Additionally, Chapter III

analyzes the impact of survey protocol changes in large rivers and provides insight on what is required to characterize the mussel community of the Greenup Pool.

Figure 1. The Greenup Pool of the Ohio River
Aerial image of the Greenup Pool of the Ohio River, demonstrating its reach through many major metropolitan areas. Red lines represent the Lock and Dam systems that form the boundaries of the Greenup Pool.

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CHAPTER 1

INTRODUCTION

The lack of recent system-wide surveys in the Ohio River raises questions regarding the current state of the mussel community. Large river mussel communities are of great importance as they serve as a connection between the many tributaries and their associated mussel communities, which are otherwise fragmented (Stansbery, 1971). The lack of statistically sound data makes it difficult to assess the distribution of mussel communities. Their distribution is largely determined by anthropogenic impact, historical and present (Haag and Williams, 2013). In large rivers, impacts to mussel communities are often associated with major cities possessing a history of industrially focused ports (Haag, 2012). Large riparian cities introduce harmful pollutants that degrade the water quality of downstream ecosystems and destroy habitat through siltation associated with riparian destruction (USACE, 2019). The primary vehicle for the introduction of pollutants is combined sewage overflows (CSOs) that many "river cities" employ to prevent rainwater and sewage from overloading treatment plants and flooding city streets (USEPA, 2017). CSOs are considered a water pollution concern for nearly 800 cities in the United States (USEPA, 2017). Freshwater mussel abundance and richness is correlated with the concentration and distance from sewer outfalls (Gillis et al, 2017). The Ohio River drainage has the highest CSO density in the United States (USEPA, 2017). The Greenup Pool is no exception, containing 40+ CSOs, entering the river from Huntington, Ashland, and Ironton which occur in the middle and lower sections of the pool.

The development of major cities along large rivers has resulted in the destruction of riparian vegetation within the watershed. The removal of riparian vegetation, and land disturbance associated with development leads to erosion of exposed earth, introducing sediment

into the river (Haag, 2012; Parmalee and Bogan, 1998). Fine sediment is considered the most severe impact to freshwater ecosystems (Watters and Flaute, 2010). Sedimentation is largely dependent on water velocity (Ciborowski et al, 1977; Cordone and Kelly, 1961). Large, low gradient systems are more conducive to suspended particles settling over substrate. However, aquatic systems are not uniform, containing varying water velocities throughout. Water velocity is presumably influenced by stream morphology. In small streams, morphology is generally classified by riffle, run, pool, and glide morphology (WVDEP, 2004). Large, dammed rivers differ from small streams in that the structure of the river bottom is not as significant due to the depth, gradient, and navigational alterations. Additionally, a dammed river pool behaves differently with proximation to the upstream or downstream dam. In upstream sections, the river is typically shallower and moving faster. Water velocity can be expected to decrease closer to the downstream dam, creating habitat resembling a deep, silty lake. Therefore, large river morphology is better classified by the channel in the form of bends and straightaways (Buffington and Montgomery, 2013). Outside bends possess the highest velocity as water constricts and changes direction; alternatively, inside bends possess the lowest velocity as water disperses and collides with faster moving water. Straightaways are minorly influenced by redirections and typically possess mean stream velocities. Mean water velocity correlates positively with mean substrate particle size, as swifter water more easily displaces larger particles (Buffington and Montgomery, 2013). Dissolved oxygen, a limiting water parameter for aquatic organisms, is positively correlated with higher water velocity (Haag, 2012). Additionally, higher velocity translates to higher volume of water moving through an area, higher volume is correlated to more nutrients (food) available to aquatic organisms like freshwater mussels (Haag, 2012; Cummings and Mayer, 1992).

Fine sediments are composed of small inorganic and organic particles less than 0.063 millimeters (mm) wide (Bunte and Abt, 2001). There is some conflicting literature pertaining to the role of fines in the life cycle of freshwater mussels. The presence of fines may be crucial to the development of juvenile mussels, as fine sediment can provide micro habitats for pedal feeding mussels (Yeager, 1994). However, the vast majority of literature indicates that elevated fine sediment inputs may be harmful to freshwater mussel survival and reproduction (Haag, 2012). The small size of fine sediment particles increases the surface area available to microbial communities which consume large amounts of oxygen in the substrate (Haag, 2012). In areas of deep fine sediment accumulation, there is little to no oxygen circulation occurring just a few millimeters below the benthic boundary, indicated by methane gas emerging from benthic layers upon disturbance. The anerobic conditions caused by deep fine sediment accumulation influence benthic organism densities, including freshwater mussels (Haag, 2012). Additionally, substrate compositions dominated by fines are prone to shifting in swift water during high flow events. This could be problematic for sedentary organisms like freshwater mussels that may respond poorly to displacement, although the survivorship rate of displaced mussels is not fully understood (Haag, 2012). Fine sediment can also restrict filter feeding opportunities by blanketing mussels and isolating them from the water column (Watters et al. 2009). During reproductive events, fine sediment may limit the dispersion of sperm from male mussels and isolate female mussels from potential host fish (Watters et al, 2009). Further, the presence of fine sediment is correlated with suspended sediment, dependent on the velocity at the time of observation, suspended sediment can contain harmful particles which deteriorate water quality and limit freshwater mussel distribution (Bilotta and Brazier, 2008; Archamault et al, 2017). The Greenup Pool experiences heavy inputs of fine sediments and the overall negative impacts with

excess fine sediment are likely outweighing the benefits to pedally feeding juvenile mussels in the Greenup Pool.

The objective of this research was to determine the preferences of adult mussels relative to river morphology. Here, we present the methodology used to randomly sample the mussel community of the Greenup Pool and determine the preferences of that community based on three parameters, location within the pool (tied to anthropogenic impact), river morphology, and placement within the river channel (interval). Mussel community preferences will be gauged by abundance and species richness. Additionally, we present the substrate composition of the Greenup Pool based on those parameters. Mussel communities are hypothesized to be determined by substate composition which is determined by anthropogenic impact in the immediate and upstream area.

METHODS

Mussel Survey

We used site locations generated by ORSANCO for their 2011 sampling efforts. Site names identify the river mile (ex. 281.6), the side of the river/descending bank at which they occur (ex. RDB), and the morphological feature they occur in (ex. S=straightaway; site name=281.6RDBS). The site list provided by ORSANCO contains 20 sites. We used 16 of those sites, excluding fixed stations and dive hazards associated with commercial barge traffic. We used the West Virginia Mussel Survey Protocol as a reference for site preparation (Clayton et al, 2020). Survey sites consisted of six, 100-meter transects at each site. Transects were oriented perpendicular to the bank, extending from the shoreline into the river channel (Fig. 2). Transects were spaced 100 meters apart to sample a 500x100 meter area. The transects were numbered “1” to “6” beginning with “1” at the upstream point and ending with “6” at the downstream point.

Transect “1” was placed at the approximate coordinates from the ORSANCO collection. Transect lines were split into 10-meter intervals with interval “1” at the shoreline (Fig. 3). We created transects by laying weighted lead lines on the river bottom with an anchor on each end to secure them in place. A single buoy was placed on the channel end of the lead line to indicate the start point. Divers descended on the buoy line and surveyed along the lead line towards the bank. Lead lines were deployed by boat. Divers were deployed by boat or from the shore depending on conditions. Transect deployment was replicated at all sites excluding one, in which four transects were surveyed instead of six due to hazardous conditions.

We collected mussels within one meter of the transect line by wafting, visually searching for siphons, flipping large debris, and tactile sifting of the upper four inches of sediment. A minimum of one minute per square meter was spent searching during surveys. Live mussels and deadshell were collected and stored in mesh bags and attached to the lead line for transport to the surface. Each 10-meter interval had its own bag identified by its placement on the lead line, and a tag number which was relayed to the boat crew through full-facemask communication gear. On the surface, live mussels were identified, counted, sexed, and measured to the nearest millimeter. Deadshell was identified when applicable, but not included in individual counts. Species present in deadshell but absent from live collections were noted, but not included in species counts. The sex was listed for species exhibiting sexual dimorphism. Each live species was photographed and deadshell in good condition was retained as voucher specimens. Freshwater mussels were processed in a timely manner to ensure stress from handling was minimized. Mussels were submerged in fresh, flowing water while awaiting processing to avoid temperature fluctuations. Processed mussels were returned to the water and returned to the approximate capture location.

For federally threatened and endangered individuals we recorded length, width, and height in millimeters. Photographs, coordinates, and site river mile were reported to agency personnel within 24 hours. We returned threatened and endangered individuals to the water by hand placement in optimal habitat within the transect they were collected.

During transect searches, qualitative substrate composition was recorded for each interval. Composition was visually estimated by the diver and recorded in percentages (≥10%) in the form of fines, sand, gravel, cobble, boulder, bedrock, and other for a total of 100%. This information was relayed to the surface crew by the diver through full-facemask communication gear.

We applied linear regressions to mussel abundance and richness data treating river mile as the independent variable to analyze linear trends in the datum. We assigned survey sites to one of three classifications based on their location in the Greenup Pool and relation to points of major impact as upper, middle, or lower (U,M,L). Using SAS [9.4] (Copyright 2002-2012), Kruskal-Wallis tests were applied to mussel abundance and richness data treating pool location (U,M,L) as the independent variable to determine preferences in pool location. Survey sites were also classified based on river morphology to determine preferences in river position. Using aerial images, one of three classifications were assigned to each site, inside bend, outside bend, or straightaway (I,O,S). Using SAS [9.4], Kruskal-Wallis tests were applied to mussel abundance and richness data treating morphology (I,O,S) as the independent variable.

We applied a linear regression to the various substrate compositions treating river mile as the independent variable to analyze linear trends in the datum. Further, using SAS [9.4], Kruskal-Wallis tests were applied to the percent (%) fines treating both pool location and morphology as the independent variable.

RESULTS

The surveys at 16 sites yielded 4,041 live individuals from 21 species. Live mussels were collected at 14 of 16 sites (Table 1). The greatest abundance from a single site was 1,478 individuals (290.2RDBO). The lowest abundance by site where mussels were present was two individuals (sites 322.0LDBI and 329.2RDBO). The mean abundance from all sites was 252.56 individuals. The greatest richness for single site was 17 species (281.6RDBS). The lowest richness by site was two species, shared by four sites (Table 1). The dominant species was *Obliquaria reflexa*, with 1,462 individuals collected. Following *O. reflexa*, was *Cyclonaias pustulosa* with 1,091 individuals collected. Together, the two species totaled 2,530 individuals and represent 63% of the mussel fauna relative abundance (Table 2). An additional five species represented 30% of all individuals collected. The remaining 14 species represent less than 7% of all individuals collected. Within these fourteen species is the federally endangered *Plethobasus cyphyus*, which makes up 0.2% of the relative abundance (8 individuals).

The upper pool (6 sites) contains the highest abundance (3,501 live individuals) and richness (20 species) of the three navigational pool sections. Mussel density in the upper pool is 0.9725 mussels per m^2. The middle pool (5) sites yielded 516 live individuals from 16 species. Mussel density in the middle pool is 0.1842 mussels per m^2. The lower pool's (5) sites yielded 24 live mussels representing 6 species. Mussel density in the lower pool is 0.0080 mussels per m^2 (Table 3).

To capture the variation in habitat parameters along a 100-meter survey line, transects were broken up into ten, 10-meter intervals. This dissection allows for a structured and replicable look at trends in mussel communities and substrate compositions from a perpendicular perspective. On a pool-wide scale, the greatest mussel abundance and richness was centrally

distributed across the transects at intervals 4/5, which is 40 and 50 meters from shore (Fig. 4, Fig. 5). In most cases there was also a second peak in richness occurring further into the channel. The substrate composition at intervals 4 and 5 is predominantly fines, though this is not alarming as all intervals were dominated by fines (Fig. 6). However, the percent fines at intervals 4 and 5 are neither the highest nor lowest across the transects, as percent fines decrease linearly from interval 1 to 10 (Shore to channel). Mussel abundance and richness is the lowest at interval 1 where percent fines are greatest.

Regression analyses indicate a decline in freshwater mussel communities in relation to river mile. Mussel abundance is predicted from river mile by the formula: $y = -14.138x + 4613.2$, $R^2 = 0.3745$ (Fig. 7). Mussel richness is predicted from river mile by the formula: $y = -0.2836x + 95.395$, $R^2 = 0.6393$ (Fig. 8).

We used SAS [9.4] (Copyright 2002-2012) to analyze freshwater mussel abundance and richness data. Mussel abundance and richness were found to have a non-normal distribution and could not be normalized using standard transformations. Therefore, we used the non-parametric Kruskal-Wallis test. Mussel abundance and richness were independently analyzed against pool location (U,M,L) and river morphology (I,O,S).

There was a statistically significant difference in mussel abundance by pool location ($\chi^2 = 60.6801$, $df = 2$, $p < 0.0001$; Fig. 9). A pairwise two-sided multiple comparison post-hoc test was performed to determine significant difference in abundance between individual pool location classifications. Using the DSCF (Dwass, Steel, Critchlow-Flinger) method, all three classifications were significantly different from one another (U vs. M, U vs. L, and M vs. L, $p<0.0001$; Fig. 10). There was also a significant difference in mussel richness by pool location ($\chi^2 = 56.3502$, $df = 2$, $p < 0.0001$; Fig. 11). A pairwise two-sided multiple comparison post-hoc

test was performed to determine significant differences between individual pool location classifications. Using the DSCF method, all three classifications were significantly different from one another (U vs. M, $p = 0.0008$, U vs. L and M vs. L, $p < 0.0001$; Fig. 10). There was a significant difference in mussel abundance by morphology ($\chi^2 = 17.3338$, $df = 2$, $p = 0.0002$; Fig. 12). A pairwise two-sided multiple comparison post-hoc test was performed to determine significant difference in abundance between individual morphology classifications. Using the DSCF method, there was a significant difference in mussel abundance in straightaway vs. inside bend (S vs. I, $p < 0.0001$) and outside bend vs. inside bend (O vs. I, $p = 0.0231$), but there was not a significant difference in straightaway vs. outside bend (S vs. O, $p = 0.5696$) (Fig. 10). There was also a significant difference in mussel richness by morphology ($\chi^2 = 16.6705$, $df = 2$, $p = 0.0002$; Fig. 13). A pairwise two-sided multiple comparison post-hoc test was performed to determine significant difference in abundance between individual morphology classifications. Using the DSCF method, there was a significant difference in mussel abundance in straightaway vs. inside bend (S vs. I, $p < 0.0001$) and outside bend vs. inside bend (O vs. I, $p = 0.0238$), but there was not a significant difference in straightaway vs. outside bend (S vs. O, $p = 0.9602$) (Fig. 10). However, density increases from inside bends (0.0167 mussels per m^2) to straightaways (0.2864 mussels per m^2), but the highest average density was recorded in outside bends at 0.8757 mussels per m^2. Based on this method of calculation, mussel density for outside bends was over 300% (0.8757/0.2864*.100=0.3057) times greater than for straightaways.

Substrate composition for all (16) sites was 60.83% fines, 16.71% sand, 8.95% gravel, 5.82% cobble, 4.57% boulder, 0.52% bedrock, and 2.59% other (Table 4). Regression analysis indicates the dominant substrate (fines) could be predicted by river mile by the formula; $y = 1.1759x-301.83$, $R^2 = 0.5634$ (Fig. 14). In addition to being the dominant substrate, fines were

the only substrate type that increased linearly with river mile, therefore percent fines were the focus of additional analyses. Fines data was analyzed in Using SAS [9.4] (Copyright 2002-2012). Fines data was found to have a non-normal distribution and could not be normalized using standard transformations (Xsqaured, Xcubed, Xsqrt, Xinv, LogX), failing to meet the assumptions of a one-way ANOVA. Therefore, the non-parametric Kruskal-Wallis test was determined to be the most appropriate analysis. Percent (%) fines was analyzed against pool location (U,M,L) and morphology (I,O,S).

There was a statistically significant difference in percent fines by pool location (χ^2 = 52.4132, df = 2, $p < 0.0001$; Fig. 15). A pairwise two-sided multiple comparison post-hoc test was performed to determine significant difference in percent fines between individual pool location classifications. Using the DSCF (Dwass, Steel, Critchlow-Flinger) method, all three classifications were significantly different from one another (U vs. M and U vs. L $p < 0.0001$, M vs. L, $p = 0.0273$; Fig 10). There was not a statistically significant difference in percent fines by morphology ($\chi^2 = 3.2460$, df = 2, $p = 0.1973$; Fig. 16). A simple calculation of the mean % fine coverage results in inside bends; 77.81% fines, straightaways; 58.42% fines, and outside bends; 48.68% fines.

Post-hoc analysis supports the hypothesis that percent fine coverage influences mussel distribution across the three sections of the pool, as there was a significant difference in mussel abundance, richness, and percent fine coverage between each classification (Abundance: U vs. M, U vs. L, and M vs. L, $p<0.0001$, Richness: U vs. M, $p = 0.0008$, U vs. L and M vs. L, $p <0.0001$, Fines: U vs. M and U vs. L $p < 0.0001$, M vs. L, $p = 0.0273$; Fig 10).

DISCUSSION

Regression analyses suggest that freshwater mussel communities in the Greenup Pool decline moving downstream. Sites occurring further downstream generally had fewer individuals representing fewer species (Fig. 7; Fig. 8). This is supported by the pool location analysis, as mussel abundance and richness differ significantly between the three classifications. Further, both abundance and richness decrease in order from upper, middle, and lower pool. One factor contributing to this decline is demonstrated by the substrate composition regression analysis. As river mile increases, all substrate types decrease in percent coverage except for fines, which increases drastically (Fig. 14). This is supported by the pool location analysis, as an increase in percent fines translates to a decrease in mussel abundance and richness for the three classifications (U,M,L). Other factors likely influencing mussel distribution in the Greenup Pool include riverside cities like Huntington, Ironton, and Ashland and their associated combined sewage overflows (CSO's) (Fig. 17), riparian degradation, industrial activity, and commercial barge traffic.

Pool Location

This study identifies the upper section of the Greenup Pool as the most suitable habitat for freshwater mussels. The dominant impacts in the upper pool are limited to agricultural runoff and commercial traffic. The upper pool also lacks significant inputs from municipal CSOs and industrial discharge. Additionally, the banks of the upper pool are relatively forested in comparison to the middle and lower (Fig. 18). The presence of riparian vegetation and lack of major industrial activity limits the introduction of sediment. Further, the R.C. Byrd Lock and Dam increases water velocity in the upper pool, which restricts fine sediment deposition. Fines

occupied (36.92%) of the substrate samples in the upper pool, which is low in comparison to the percent fines of the middle (71.02%) and lower (79.35%) pool.

The middle pool is moderately impacted from industrial activity, commercial traffic, and point source pollution from the city of Huntington and CSO's. Additionally, the middle pool is impacted by its largest tributary, the Guyandotte River, an impaired river containing high levels of fecal cauliform, heavy metals, and industrial chemical compounds (WVDEP 2004). The substrate composition of the middle pool is dominated by fine sediment (71.02%). This can be attributed to the lack of riparian vegetation in the middle pool visible from aerial imagery (Fig. 19). The Guyandotte River also contributes large quantities of sediment to the middle pool (Fig. 20) (WVDEP 2004).

The lower pool was found to be the least suitable habitat in the Greenup Pool. The impacts to the middle pool continue through the lower pool and are only amplified in downstream areas as industrial activity, pollutants, and sediment accumulate (Fig. 21). The lower pool is further impacted by its largest tributary, the Big Sandy River, an impaired river that cuts through historic coal country and introduces heavy metals and sediment to the lower pool (ORSANCO 2021). The substrate composition of the lower pool is dominated by fine sediment (79.35%). In the downstream most sections of the lower pool, we recorded fine sediment accumulation that often exceeds 0.3 meters, leaving little opportunity for freshwater mussel colonization. The two downstream most sites, 331.8LDBS and 337.6RDBI, had nearly 100% deep fine sediment coverage and no live mussels. This accumulation is likely related to the Greenup Lock and Dam which acts as a barrier for fine sediment, trapping it in the deep, slow moving lower pool.

Morphology

Kruskal-Wallis analysis reveals a statistically significant difference in mussel abundance and richness when compared to river morphology. However, post-hoc analysis shows significant differences do not occur between each classification. For both abundance and richness, there was a significant difference between straightaway (S) and inside (I), and outside (O) and inside, but there was not a significant difference between outside (O) and straightaway (S) (Fig. 7). Additionally, the Kruskal-Wallis for fines shows there was not a significant difference in percent fines by morphology ($p = 0.1973$; Fig. 16). Within the bounds of the Kruskal-Wallis and DSCF post-hoc analysis, there is not sufficient evidence to suggest a relationship between mussel distribution, percent fine coverage, and morphology. However, mussel densities for the three classifications suggest otherwise.

Despite the results of the Kruskal-Wallis, there still appears to be a relationship between mussel density, percent fines, and morphology. However, it is important to consider why the statistical analysis contradicts what mussel density calculation suggest. First, the morphology classification does not take into account the pool location at which the sites occur. From prior analysis in this study, we know that pool location has a significant influence on mussel abundance and richness, and percent fines. For example, if a morphological classification (I, O, or S) occurs more frequently in the upper pool, mussel abundance and richness is more likely to be greater, percent fines is more likely to be lower, etc. Second, the Kruskal-Wallis functions on ranked sums, which may cause discrepancies in mussel abundance and richness by classification (Cody, 2013). In the case of outside bend (O) vs. straightaway (S), 2,417 mussels were collected in outside bends, and 1,581 were collected in straightaways. However, 8 of the 16 sites occurred in straightaways (5,520 m^2 of survey area), while 4 of the 16 sites occurred in outside bends

(2,760 m^2). As a result, mussel density calculations show a more appropriate comparison between the two morphology classifications (S density: 1,581 mussels/5,520 m^2 survey area = 0.2862 mussels per m^2; O density: 2,417 mussels/2,760 m^2 survey area = 0.8757 mussels per m^2). Lastly, mussel surveys conducted for this project were guided by transect lines oriented perpendicular to the bank, stretching 100 meters from the bank towards the channel. Transect placement resulted in a wide variety of habitat parameters encountered on a single transect line, including substrate compositions, water velocity, depth, and structure. Habitat parameters presumably influence mussel distribution in a perpendicular direction, likely dependent on the quality of habitat available, as well as species specific preferences. While it's challenging to fully understand the role of morphological classifications (I,O,S) in perpendicular habitat parameters, we can make some assumptions based on water velocity. Generally, water velocity is lowest at inside bends, greatest at outside bends, and moderate within straightaways. However, within these morphological classifications, water velocity is not uniform in a bank-perpendicular direction. Since water velocity determines substrate composition, especially percent fines, fine sediment coverage near the bank can differ greatly from fine coverage closer to the channel, creating perpendicular variation. This variation is amplified when comparing morphological classifications as the three types (I,O,S) behave quite differently. Essentially, a 100-meter transect in an outside bend is not going to perform the same as it would in an inside bend, or straightaway. Simply, the statistical analysis may not account for these discrepancies in perpendicular variation between the morphological classifications.

Perspective by Interval

Mussel abundance is concentrated on intervals 4 and 5 across pool location classifications (Fig. 22). Mussel abundance in the upper pool is centered around intervals 4 and 5 but is more

uniform compared to the intervals in the middle and lower pool. Additionally, mussel richness is equal at intervals 3, 4, and 10, again differing from the middle and lower pool. The upper pool appears to better support mussel communities in a broad, bank-perpendicular direction. Mussel abundance continues to occur primarily around intervals 4 and 5 across morphology classifications (Fig. 23). In outside bends, mussel abundance is greatest at interval 4-6, but remains high at intervals 7-10 in comparison to inside bends and straightaways. Additionally, mussel richness in outside bends is greatest at interval 10, which is not the case for inside bends and straightaways. This suggests outside bends better support mussel communities in broad, bank-perpendicular direction.

While there is great variation in bank-perpendicular mussel communities between pool location and morphology classifications, there are some noteworthy similarities. Prior analysis determined that the least suitable habitat for the pool location class was the lower pool, and for morphology inside bends act as the least suitable habitat. Interestingly, both low quality habitat classifications contain mussels at interval 10 with multiple intervals absent of mussels in the second half of the transects (Fig 22, Fig. 23). Interval 10 again stands out when considering what prior analysis determined as the most suitable habitat, pool location; upper pool, morphology; outside bend, as richness in the upper pool and outside bends is greatest at interval 10. Further, the percent fines for the upper pool and outside bends are lower and decrease rapidly from interval 1-10 (Fig. 24). Meanwhile, percent fines in the lower pool and inside bends are more uniform with little change from interval 1-10. In classifications with an increase in mussel abundance and richness at interval 10, it's reasonable to assume that the mussel community extends further into the channel than the standard 100-meter protocol, though future studies are needed to confirm this. However, this study does identify which classifications have greater

potential for mussel communities extending further than 100-meters based on those with high densities at interval 10. Further, the interval specific data indicates in part why the morphological classifications varied above as each feature has different mussel distributions and substrates.

Site 309.1RDBS

Located on the right-descending bank directly across from Huntington, WV, is site 309.1(RDBS) (Fig. 25). The mussel community sampled at 309.1 is one of the most thought-provoking topics of this study. This site contained 352 live individuals from 16 species, ranking 5th in abundance and 2nd in richness. Site 309.1 is the only site where *Pleurobema sintoxia* was collected, totaling seven live individuals. Additionally, 28 *Elliptio crassidens* (a species not federally listed, but showing decline in the Greenup Pool) were collected at 309.1, more than all other sites combined. However, the most intriguing aspect of site 309.1 begins with its central location in the middle pool, where historical anthropogenic activity has severely impacted aquatic organisms. No other site in the middle pool approached the abundance and richness found at 309.1. Site 309.1 is responsible for 68% of mussels collected in the middle pool and contains a representative of every species found at all other middle pool sites. Had it not been for site 309.1, the middle pool would have been more comparable to the lower pool in terms of mussel community. Further, the percent fines at site 309.1 was 78.85%, ranking 5th highest in all sites and 3rd in all sites where mussels were collected, and much higher than other sites with comparable abundance and richness (Table 1,3). The perplexing qualities of 309.1 can only be explained by a tributary directly upstream known as Symmes Creek (Fig. 23). The mussel community identified at site 309.1 suggests Symmes Creek is supplying the mussel communities downstream of its Ohio River confluence with beneficial elements (water quality, host fish, crucial nutrients) that may exceed what is available otherwise. However, the previous

suggestions do not consider the high percent fines observed. Water entering the Ohio River from Symmes Creek may possess adequate velocity to remove fine sediment from the benthic layer most of the year, as surveys were conducted in the driest months of the year, when the river is at its lowest.

Experimental Site 279.3

Site 297.3 occurs in the upmost reach of the Greenup Pool below the Robert C. Byrd Lock and Dam (Fig. 26). Site 279.3 was surveyed under the same protocol as all other sites in this study, however it was not part of the ORSANCO randomly generated site list, so it was not included in analysis or calculations. This site was chosen as an experimental site to assess potential mussel communities and substrate compositions influenced by the Greenup Lock and Dam. The upstream most transect was placed on the perimeter of the US Army Corps "restricted zone". Site 279.3 proved to be a unique environment unlike any other study sites. Extremely swift water and the lack of habitat (bedrock dominated substrates) indicated little rationale for mussels to occur within the site so only 4 transects were completed. Substrate compositions for site 279.3 were 24% sand, 4.5% cobble, 37.25% boulder, 34% bedrock, and 0.25% other. No live mussels were collected.

Table 1. Mussel Abundance and Richness by Site
Mussel species abundance, richness by site with GPS coordinates for each site within the Greenup Pool.

Site	Abundance	Richness	Latitude	Longitude
281.6RDBS	367	17	38.64855556	-82.1794444
283.0RDBS	339	14	38.62922222	-82.1730556
288.2RDBO	917	14	38.59502778	-82.2325
290.2RDBO	1478	16	38.59722222	-82.2683333
292.4LDBI	35	5	38.5725	-82.2897222
299.3LDBS	365	10	38.475	-82.3086111
307.3LDBS	72	10	38.42916667	-82.4263889
309.1RDBS	352	16	38.42543056	-82.4577778
311.6LDBS	6	2	38.40888889	-82.5002778
313.6RDBS	80	12	38.40425	-82.5361111
316.8RDBI	6	2	38.41603056	-82.5880556
321.1RDBO	20	5	38.47456111	-82.6133333
322.0LDBI	2	2	38.4781	-82.6280556
329.2RDBO	2	2	38.55468056	-82.7183333
331.8LDBS	0	0	38.56627222	-82.7638889
337.6RDBI	0	0	38.59861111	-82.8477778
Total	**4041**	**21**	-	-

Table 2. Mussel Species Composition in Greenup Pool
Total abundance of each mussel species collected and the percentage of the community those species occupied.

Species	Abundance	Percentage
O. reflexa	1462	36.18%
C. pustulosa	1091	27.00%
T. metanevra	286	7.08%
L. recta	276	6.83%
A. plicata	206	5.10%
P. alatus	155	3.84%
E. linelolata	301	7.45%
P. cordatum	73	1.81%
A. ligamentina	13	0.32%
F. flava	4	0.10%
Q. quadrula	33	0.82%
R. ebenus	10	0.25%
E. crassidens	52	1.29%
L. cardium	30	0.74%
L. siliquoidea	2	0.05%
L. teres	0	0.00%
L. complanata	3	0.07%
L. fragilis	1	0.02%
M. nervosa	27	0.67%
P. cyphyus	8	0.20%
P. grandis	0	0.00%
T. verrucosa	0	0.00%
T. truncata	1	0.02%
P. sintoxia	7	0.17%
Total	**4041**	**100.00%**

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Table 3. Mussel Densities by Classification

Mussel density by pool location (U=upper, M=middle, L=lower) and morphology (I=inside bend, O=outside bend, S=Straightaway). Classifications are assigned to a color based on their densities (Green=highest density, yellow=moderate, red=lowest).

Classification (U,M,L or I,O,S)	Density (# per m^2)
Upper Pool	0.9725
Middle Pool	0.1842
Lower Pool	0.0080
Inside Bend	0.1670
Outside Bend	0.8757
Straightaway	0.2862

Table 4. Substrate Composition by Site
Substrate compositions at each site, organized from smallest to largest (particle size).

Site	% Fines	% Sand	% Gravel	% Cobble	% Boulder	% Bedrock	% Other
281.6RDBS	58.50	3.33	5.67	10.00	13.67	3.00	5.83
283.0RDBS	21.67	11.00	20.33	19.33	24.67	0.00	3.00
288.2RDBO	28.67	48.75	20.17	1.75	0.00	0.00	0.67
290.2RDBO	26.83	23.83	28.00	17.00	3.83	0.00	0.50
292.4LDBI	58.50	3.33	5.67	10.00	13.67	3.00	5.83
299.3LDBS	27.33	43.00	19.17	4.33	1.33	0.00	4.83
307.3LDBS	37.18	41.28	13.97	3.08	3.21	0.00	1.28
309.1RDBS	78.85	0.26	8.33	5.77	3.59	0.26	2.95
311.6LDBS	68.08	23.08	1.92	2.44	1.15	0.00	3.33
313.6RDBS	75.77	7.56	9.49	5.26	1.03	0.00	0.90
316.8RDBI	95.25	2.50	2.00	0.25	0.00	0.00	0.00
321.1RDBO	42.31	31.79	6.79	12.31	5.64	0.00	1.15
322.0LDBI	61.67	27.69	1.67	1.67	1.41	2.05	3.85
329.2RDBO	96.92	0.00	0.00	0.00	0.00	0.00	3.08
331.8LDBS	100.00	0.00	0.00	0.00	0.00	0.00	0.00
337.6RDBI	95.83	0.00	0.00	0.00	0.00	0.00	4.17
Mean	**60.83**	**16.71**	**8.95**	**5.82**	**4.57**	**0.52**	**2.59**

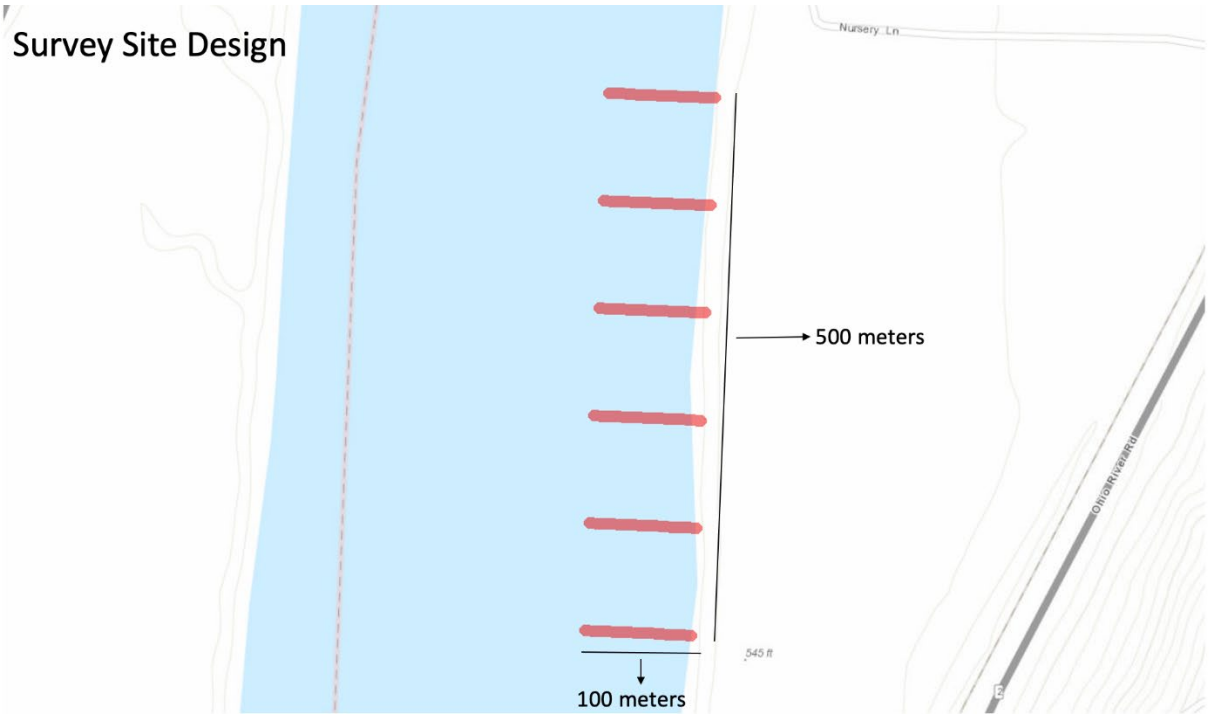

Figure 2. Survey Site Design at a Typical Straightaway
Site setup demonstrating spacing and orientation of transect lines (red) within the river (blue).

Figure 3. Diagram of Individual Transect with 10 Meter Intervals
Aerial image showing each 10-meter interval of a transect, survey area extends 1-meter away from the transect, creating 10 square meters of survey per interval.

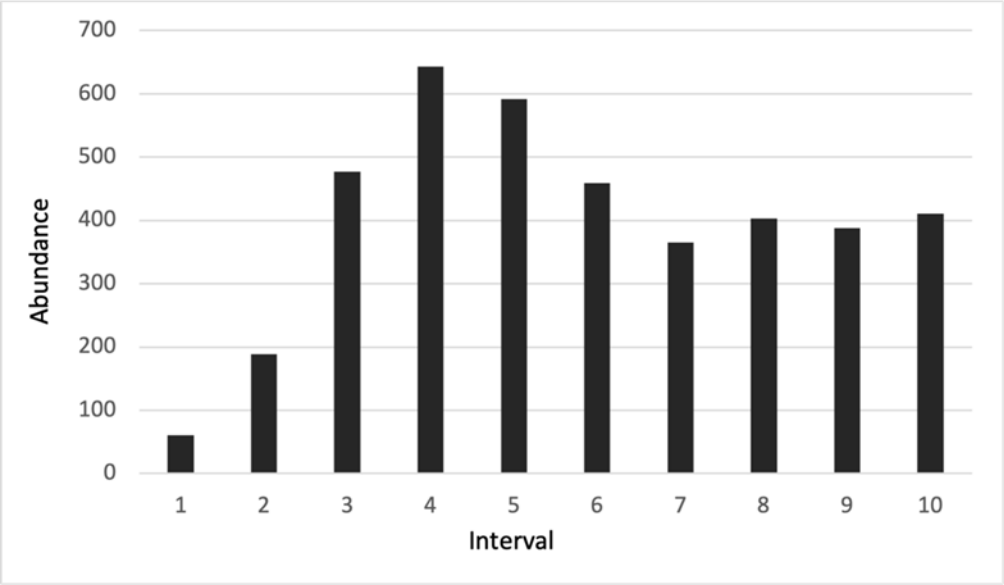

Figure 4. Mussel Abundance by Interval-All Sites
Bar chart showing mussel abundance at each interval from all sites. Peaks observed at intervals 4 and 5.

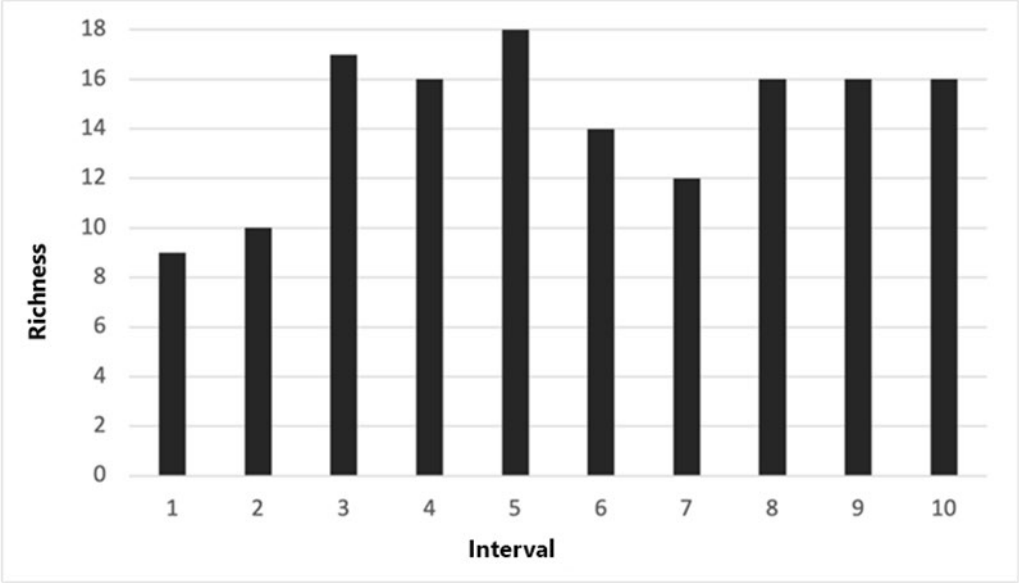

Figure 5. Mussel Richness by Interval-All Sites
Bar chart showing mussel richness at each interval from all sites. Peaks observed at intervals 3 and 5.

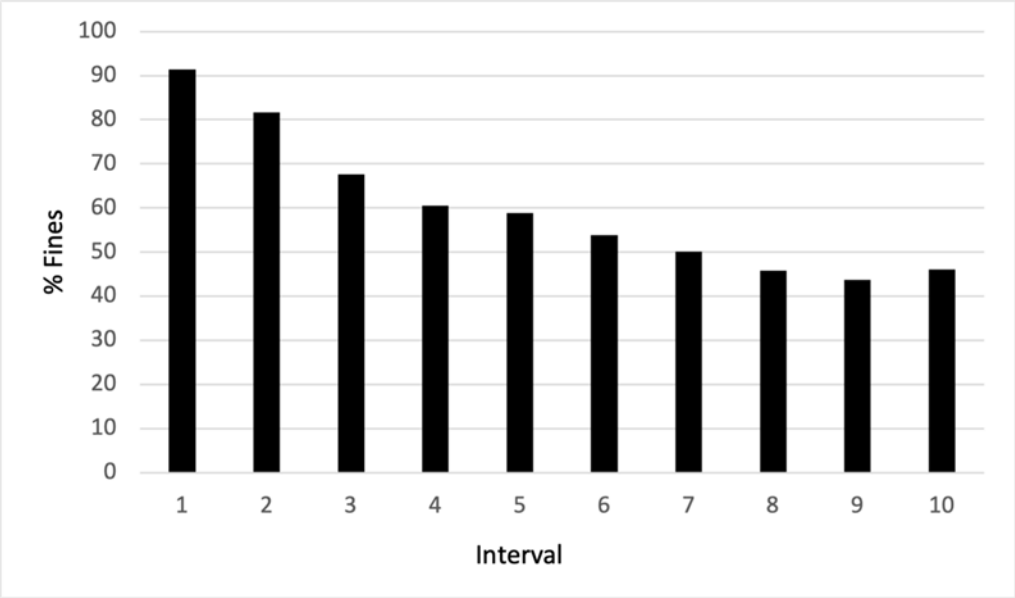

Figure 6. Percent Fines by Interval-All Sites
Bar chart showing the percentage of fine coverage at each interval from all sites. Highest percent fine coverage occurs at interval 1 (at the bank) and decreases linearly towards the channel.

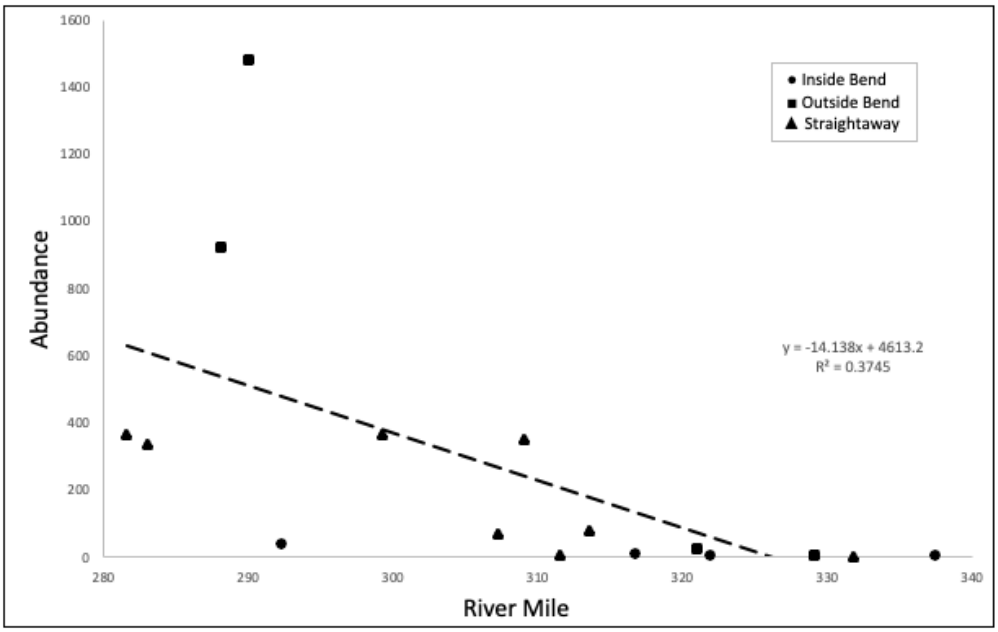

Figure 7. Mussel Abundance by River Mile
Scatter plot showing mussel abundance as river mile increases (moving downstream) and morphological classifications.

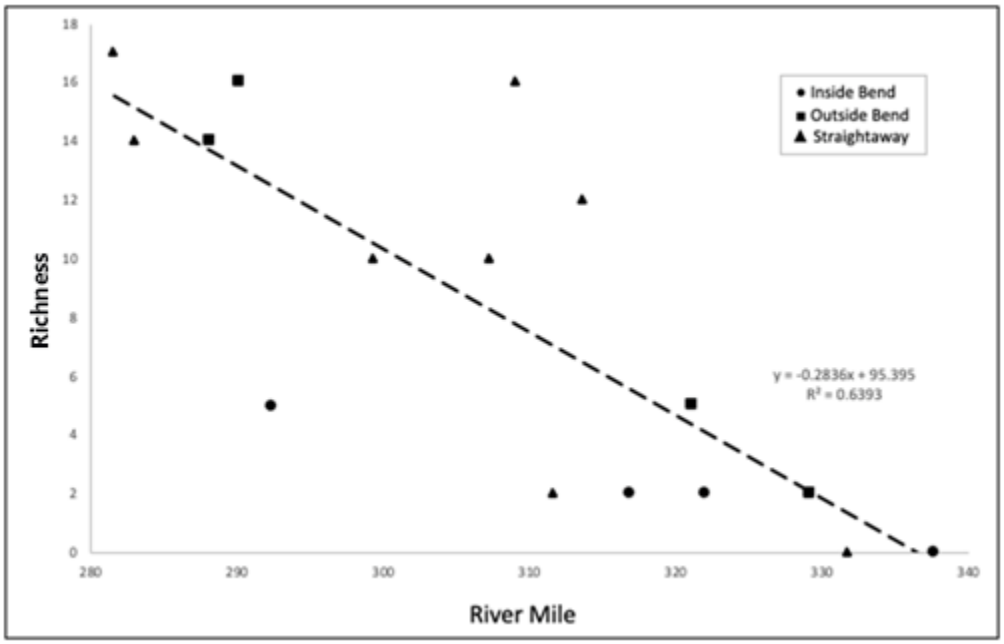

Figure 8. Mussel Richness by River Mile
Scatter plot showing mussel richness as river mile increases (moving downstream) and morphological classifications.

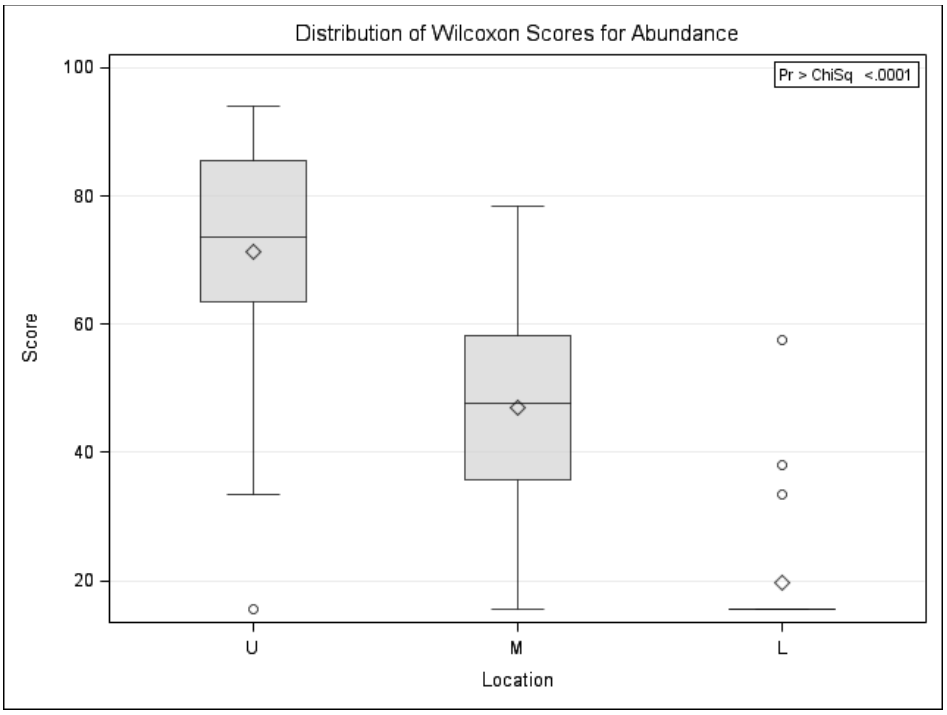

Figure 9. Ranked Mussel Abundance by Pool Location
Box and whisker plots showing the distribution of mussel abundance throughout the upper, middle, and lower pool.

Pairwise Two-Sided Multiple Comparison Analysis
Dwass, Steel, Critchlow-Fligner Method
Variable: Abundance

Location	Wilcoxon Z	DSCF Value	Pr > DSCF
U vs. M	4.6243	6.5397	<.0001
U vs. L	6.8248	9.6518	<.0001
M vs. L	5.3841	7.6142	<.0001

Pairwise Two-Sided Multiple Comparison Analysis
Dwass, Steel, Critchlow-Fligner Method
Variable: Richness

Location	Wilcoxon Z	DSCF Value	Pr > DSCF
U vs. M	3.6510	5.1632	<.0008
U vs. L	6.8442	9.6792	<.0001
M vs. L	5.3650	7.5872	<.0001

Pairwise Two-Sided Multiple Comparison Analysis
Dwass, Steel, Critchlow-Fligner Method
Variable: Abundance

Location	Wilcoxon Z	DSCF Value	Pr > DSCF
S vs. O	-1.0114	1.4304	0.5696
S vs. I	4.3901	6.2086	<.0001
O vs. I	2.6312	3.7211	0.0231

Pairwise Two-Sided Multiple Comparison Analysis
Dwass, Steel, Critchlow-Fligner Method
Variable: Richness

Location	Wilcoxon Z	DSCF Value	Pr > DSCF
S vs. O	-0.2713	0.3837	0.9602
S vs. I	4.3115	6.0974	<.0001
O vs. I	2.6214	3.7072	0.0238

Pairwise Two-Sided Multiple Comparison Analysis
Dwass, Steel, Critchlow-Fligner Method
Variable: Fines

Location	Wilcoxon Z	DSCF Value	Pr > DSCF
U vs. M	-5.7957	8.1964	<.0001
U vs. L	-6.1534	8.7022	<.0001
M vs. L	-2.5717	3.6370	0.0273

Figure 10. Post-hoc Analyses for All Kruskal-Wallis Test
Assemblage of tables demonstrating post-hoc analysis results for individual classification comparisons.

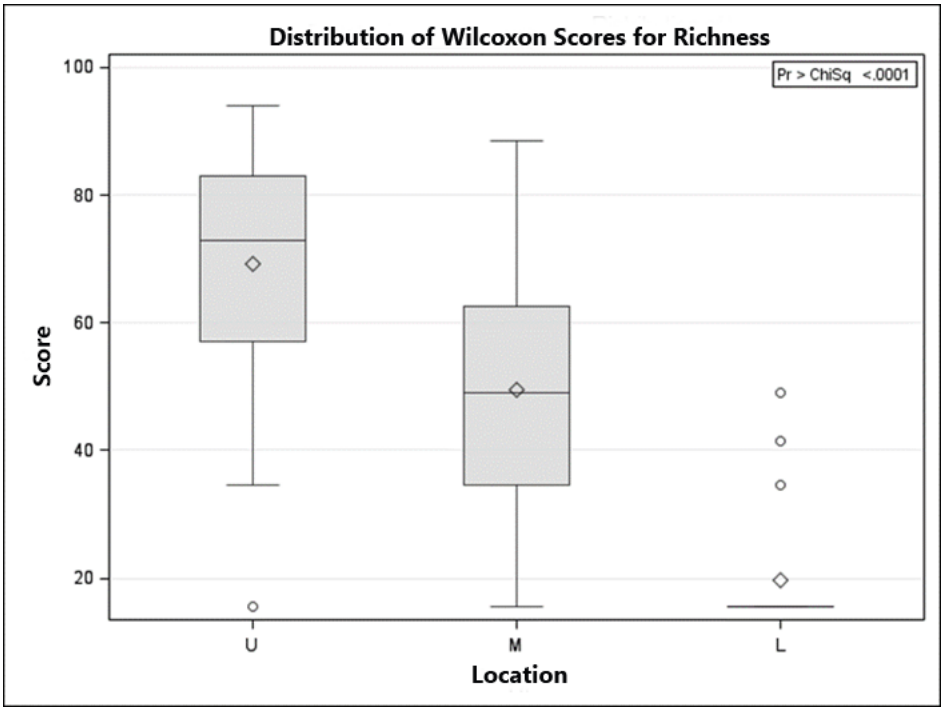

Figure 11. Ranked Mussel Richness by Pool Location Note
Box and whisker plots showing the distribution of mussel richness throughout the upper, middle, and lower pool.

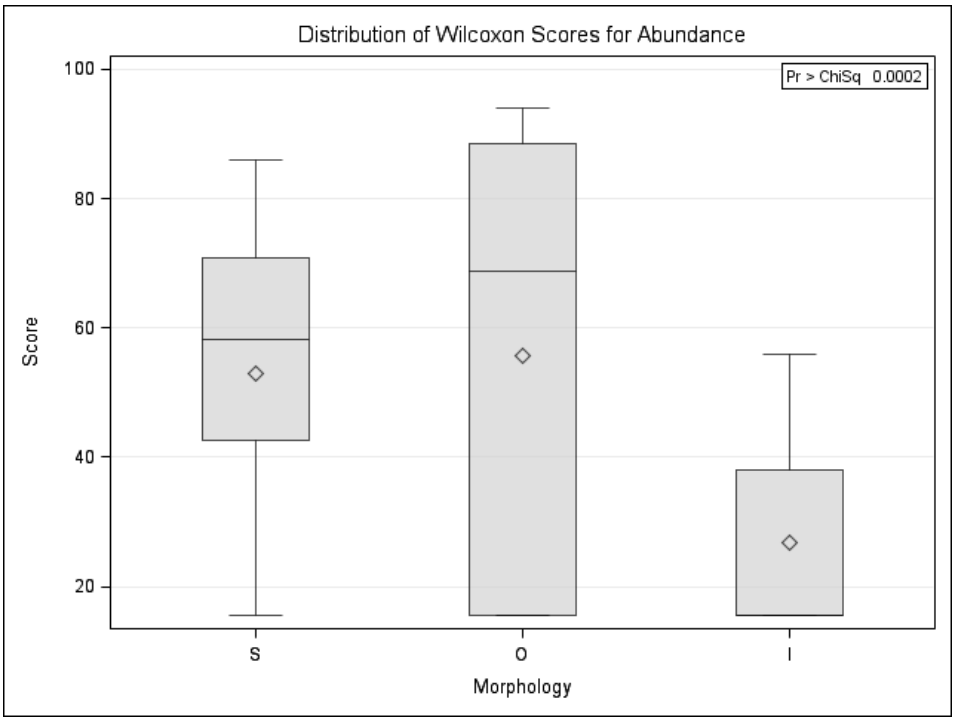

Figure 12. Ranked Mussel Abundance by Morphology
Box and whisker plots showing the distribution of mussel abundance throughout straightaways, outside bends, and inside bends.

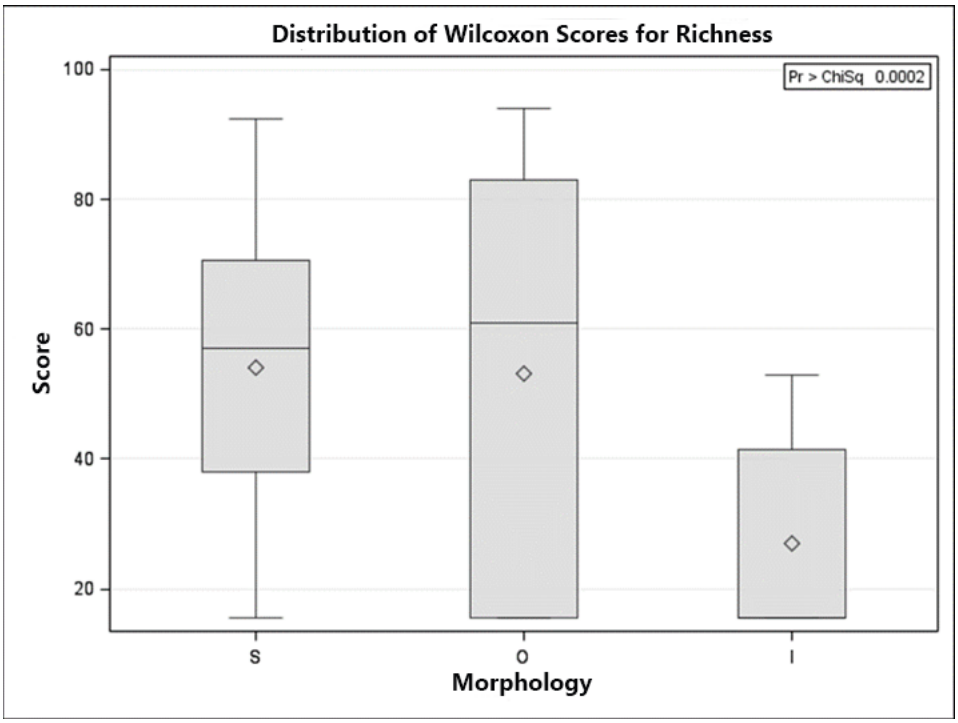

Figure 13. Ranked Mussel Richness by Morphology
Box and whisker plots showing the distribution of mussel richness throughout straightaways, outside bends, and inside bends.

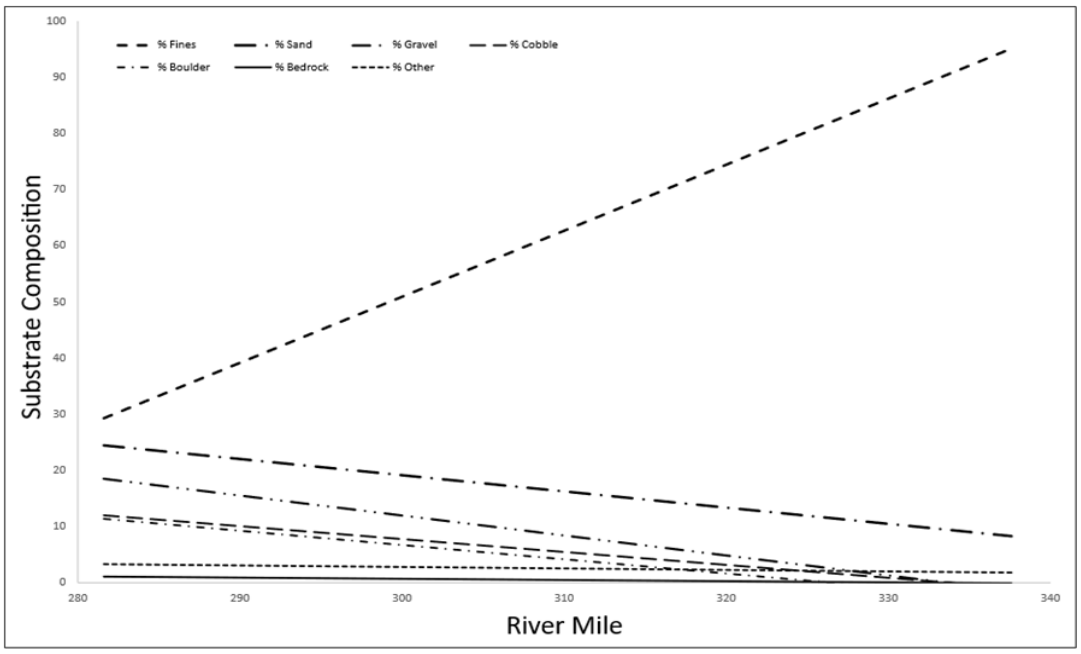

Figure 14. Substrate Composition by River Mile
Substrate composition by river mile, each substrate type is assigned a unique line texture.

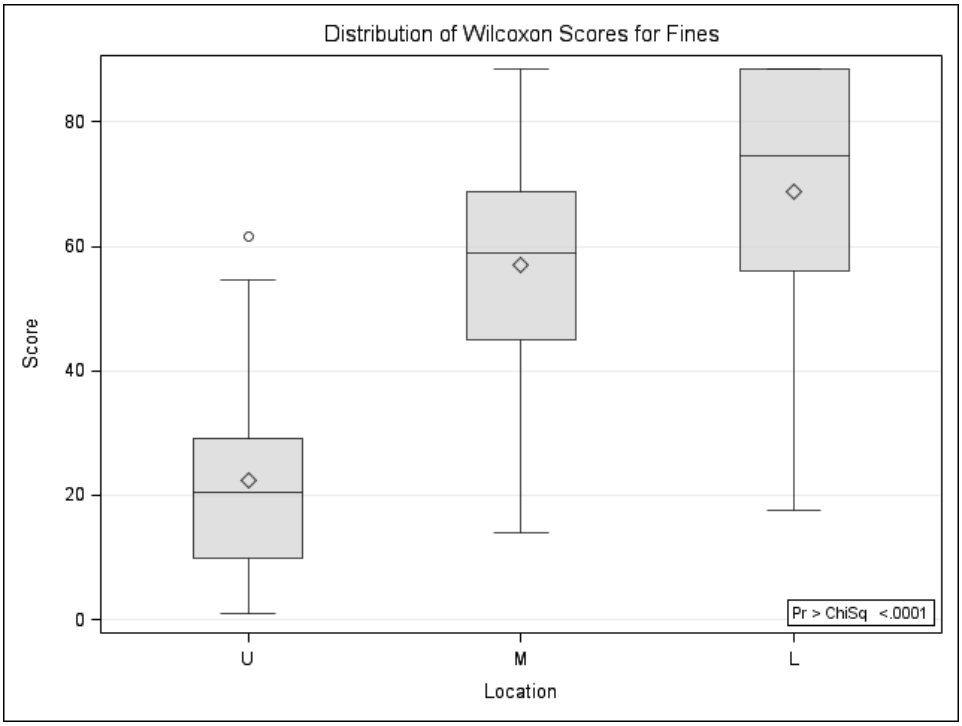

Figure 15. Ranked Percent (%) Fines by Pool Location
Box and whisker plots showing the distribution of percent fine coverage in the upper, middle, and lower pool.

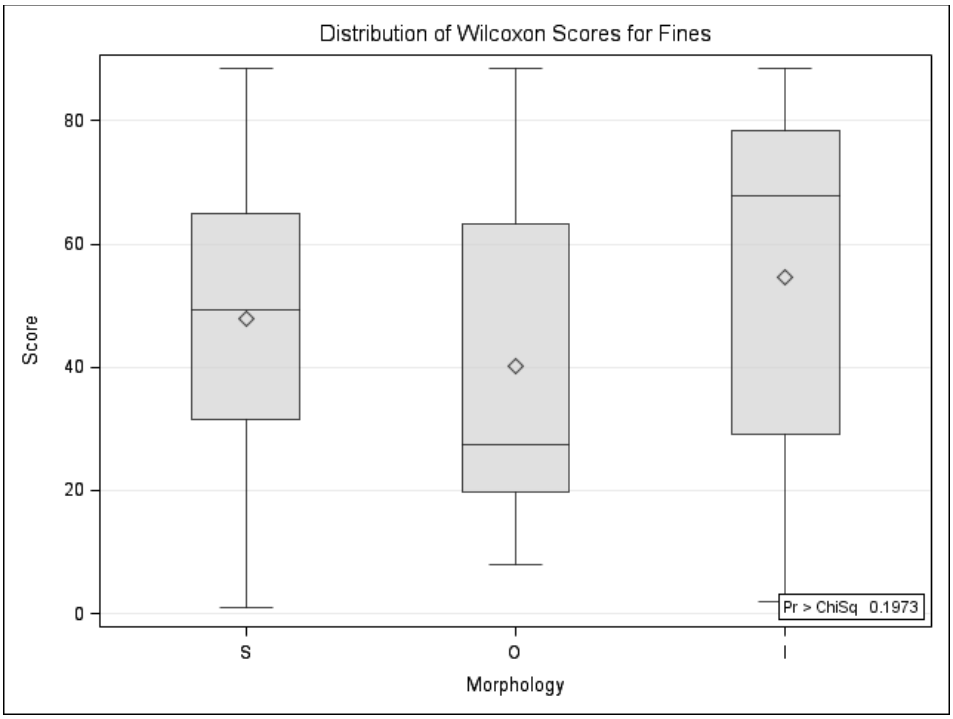

Figure 16. Ranked Percent (%) Fines by Morphology
Box and whisker plots showing the distribution of percent fine coverage in straightaways, outside bends, and inside bends.

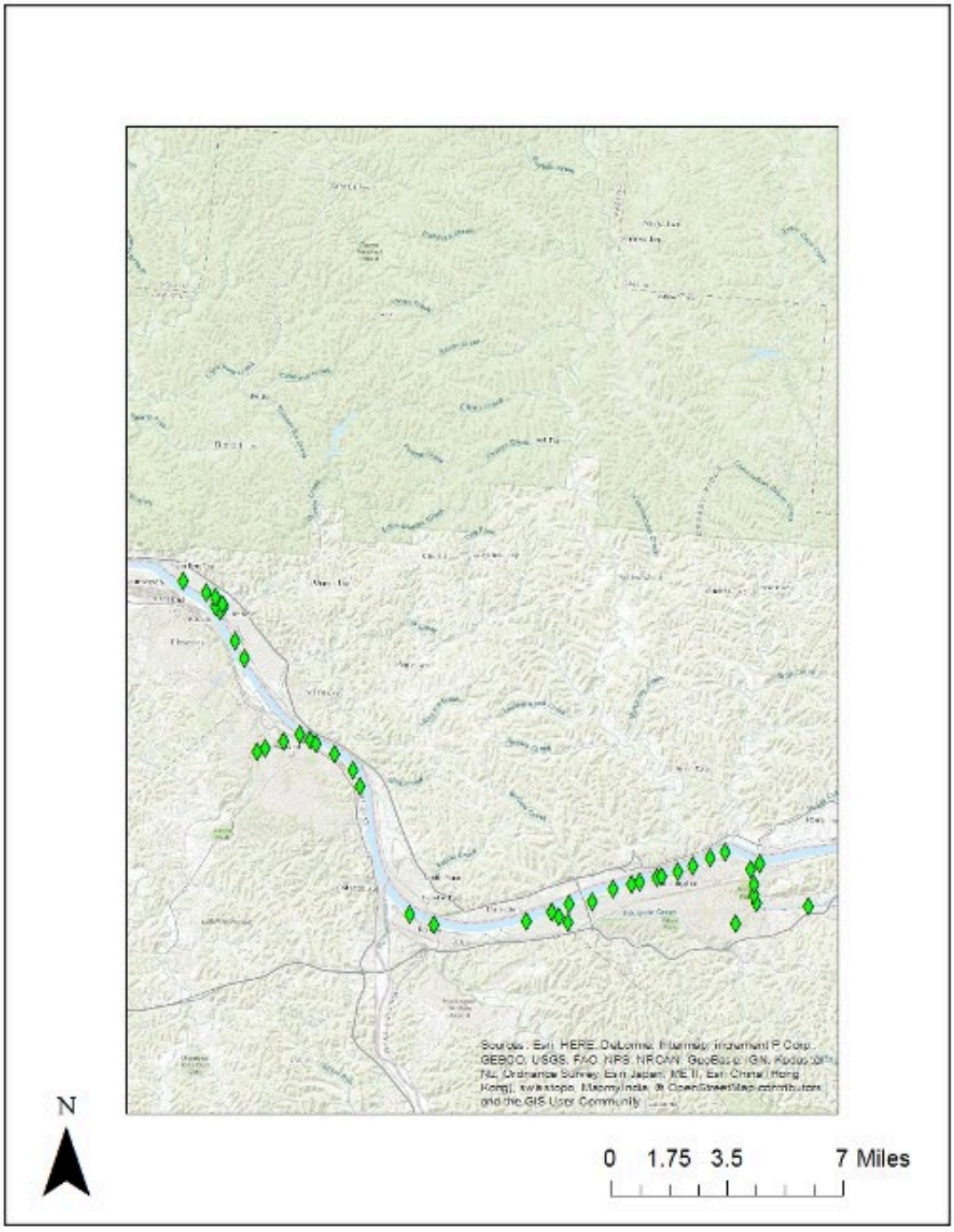

Figure 17. CSO Locations in the Greenup Pool (Kriege 2017)

Aerial view of the middle and lower Greenup Pool, showing the density of Combined Sewage Overflows (CSO's) on the river's edge and surrounding tributaries.

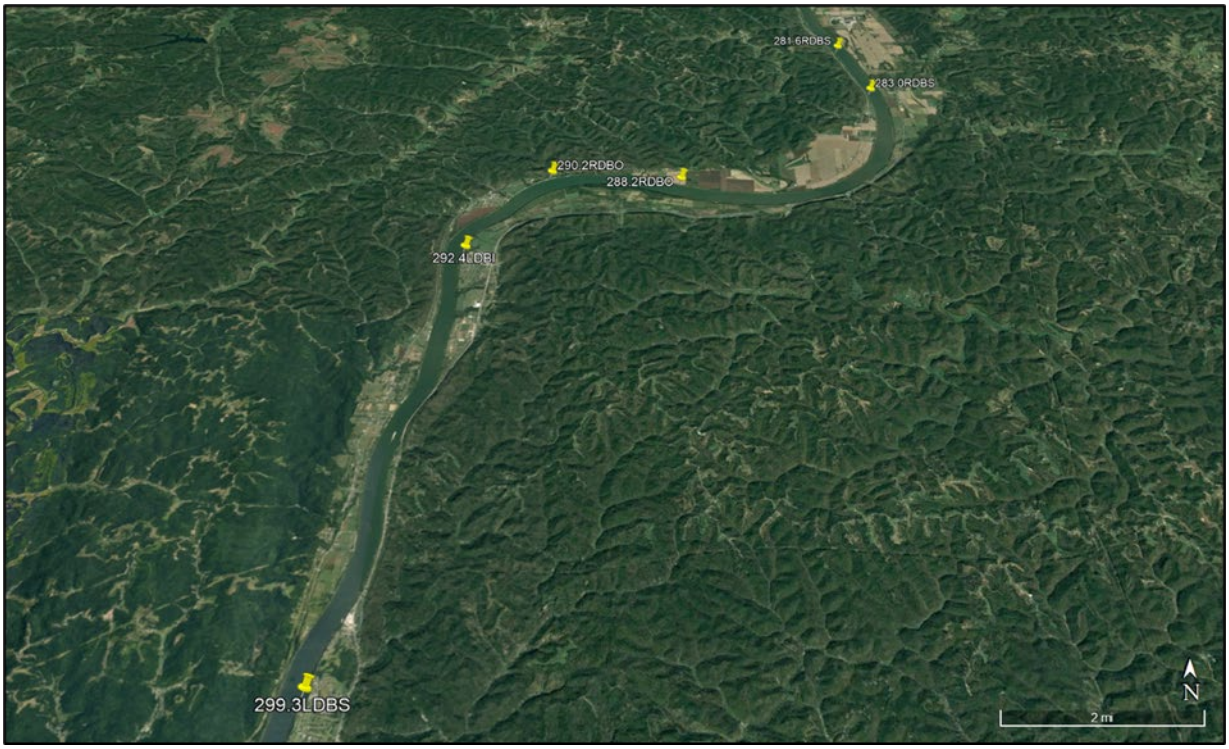

Figure 18. Upper Greenup Pool Sites
Aerial image of the upper Greenup Pool sites and surrounding land use.

Figure 19. Middle Greenup Pool Sites
Aerial image of the middle Greenup Pool sites and surrounding land use.

Figure 20. Introduction of Sediment at Confluence of Guyandotte River
Aerial image of the confluence of the Guyandotte River to the middle Greenup Pool, introduction of sediment is visible from aerial image.

Figure 21. Lower Greenup Pool Sites
Aerial image of the lower Greenup Pool sites and surrounding land use.

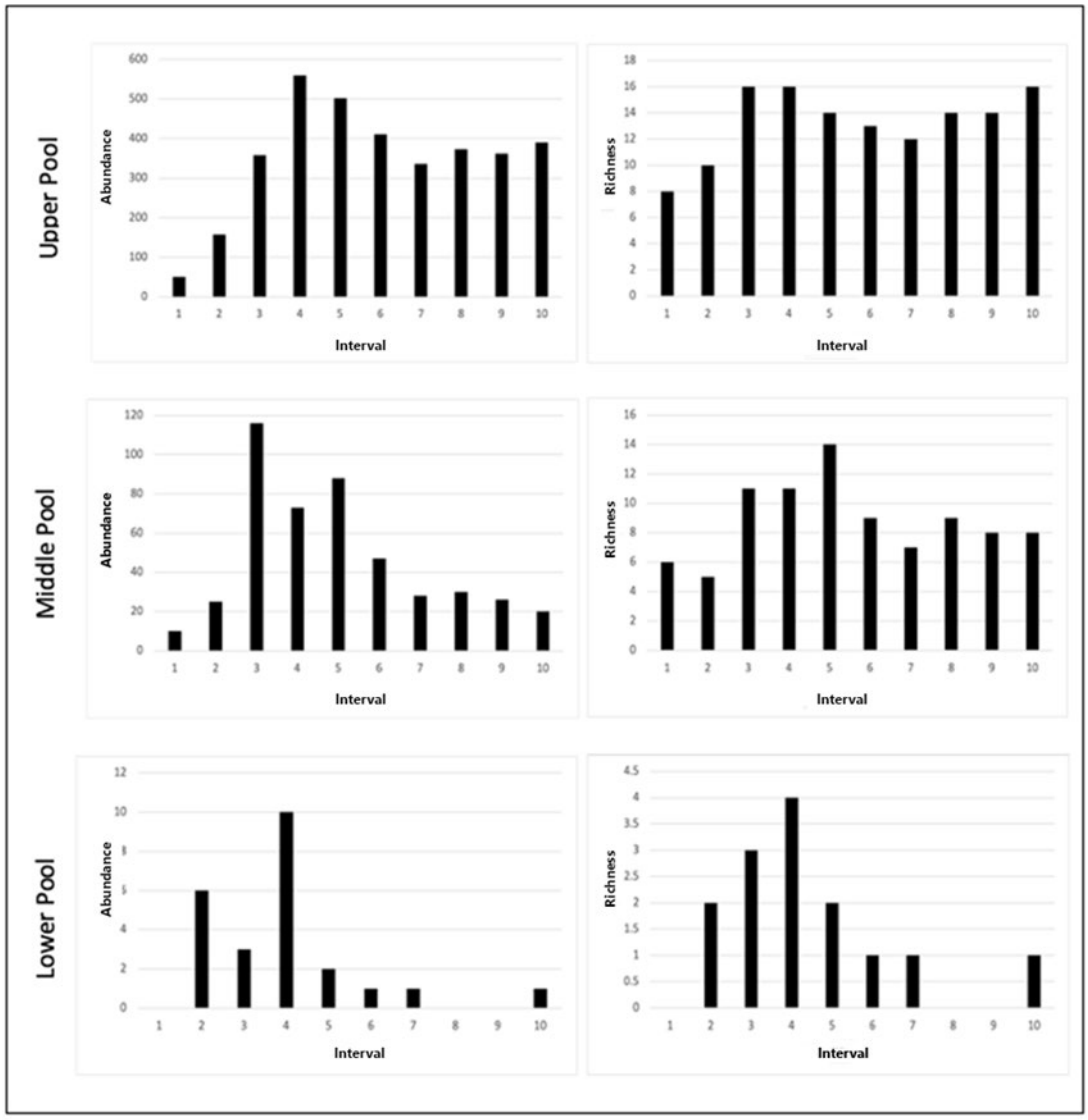

Figure 22. Mussel Abundance and Richness by Interval-Pool Location
Assemblage of bar charts showing the various occurrences of mussels at each interval in the upper, middle, and lower pool.

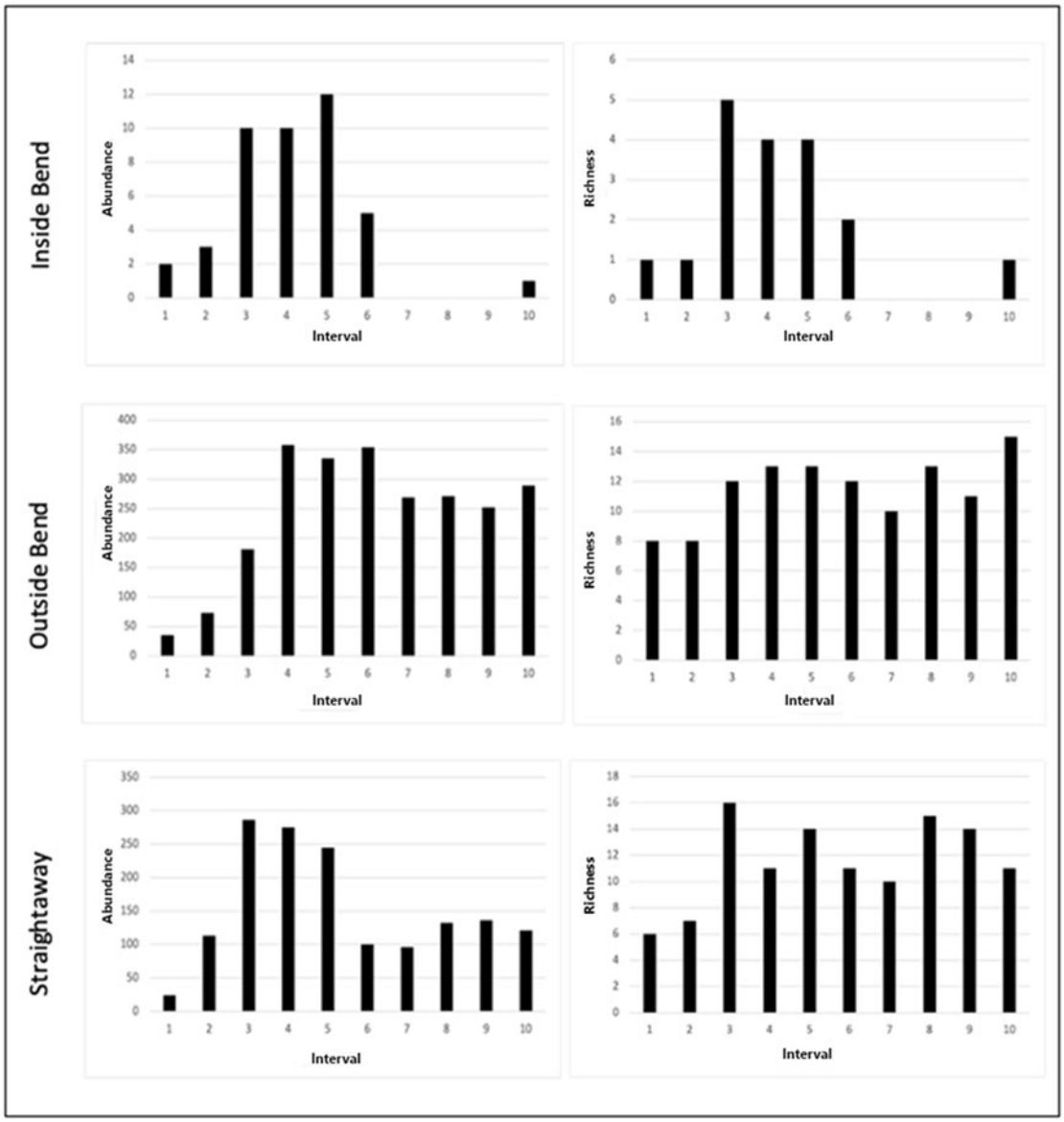

Figure 23. Mussel Abundance and Richness by Interval-Morphology
Assemblage of bar charts showing the various occurrences of mussels at each interval in straightaways, outside bends, and inside bends.

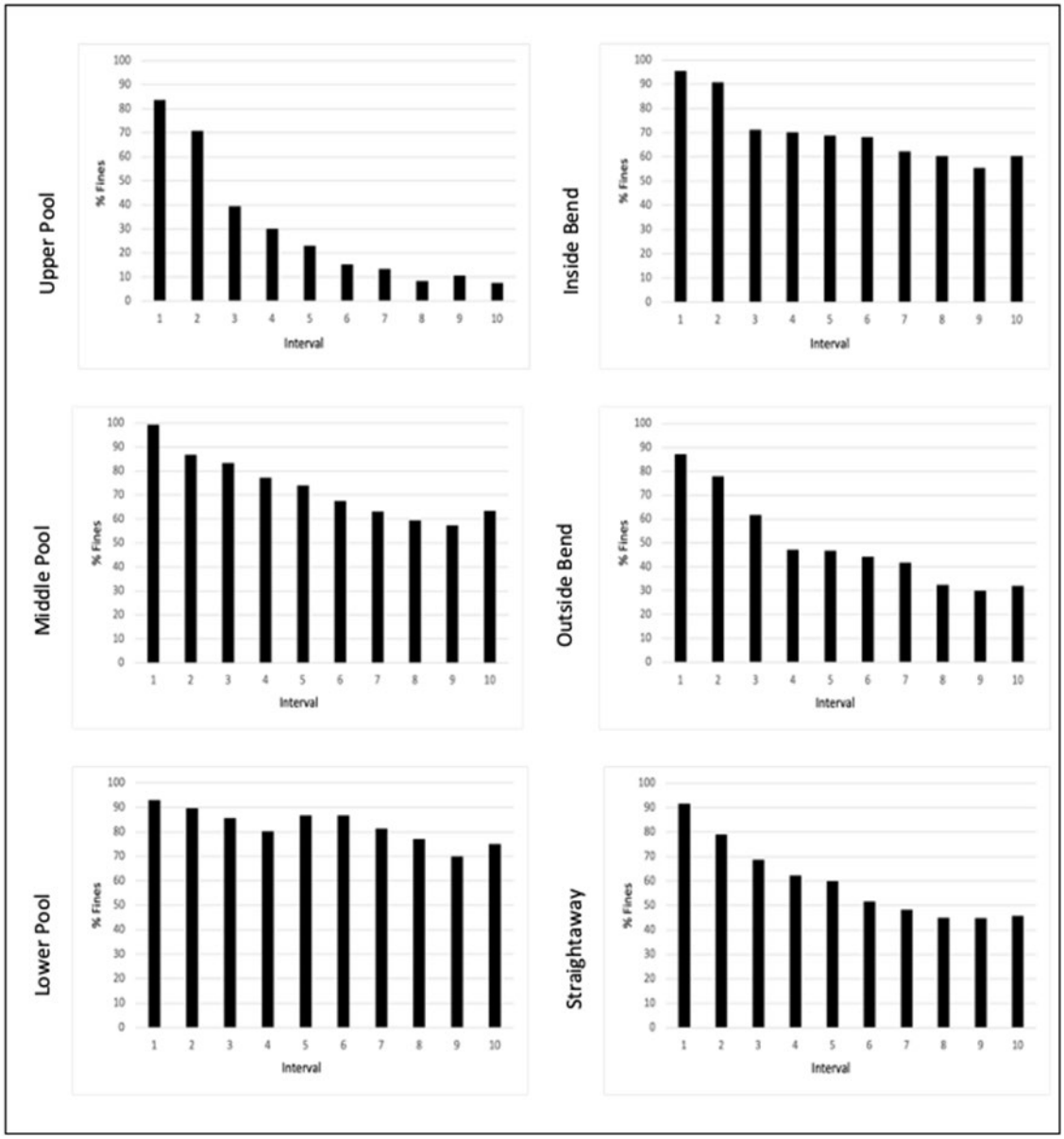

Figure 24. Percent Fines by Interval for Pool Location and Morphology
Assemblage of bar charts showing the various percent fine coverage at each interval for all classifications.

Figure 25. Site 309.1 and Symmes Creek
Aerial image showing the location of site 309.1 in close proximity to the confluence of Symmes Creek.

Figure 26. Experimental Site 279.3 and the Robert C. Byrd Lock and Dam
Aerial image of site 279.3 showing close proximity to Robert C. Byrd Lock and Dam. Swift water from the tailrace is visible from aerial image.

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CHAPTER 2

INTRODUCTION

Of the near 300 species of freshwater mussels in North America, almost all rely on a parasitic stage in which they attach to a fish host (Watters et al, 2009). The interaction between larval mussels (glochidia) and host-fish is presumably a major limiting factor in freshwater mussel distribution (Haag, 2012). Host-fish specificity for mussels is highly variable; prolific widespread species are often non-discriminatory and can parasitize many species of fish including non-natives (Watters et al, 2009; Barnhart and Haag, 2008). In contrast, uncommon or localized species often have high host-fish specificity and are commonly compatible with just one species of host-fish (Watters et al, 2009). Due to this obligatory relationship, it's reasonable to suggest that some mussel species are not recruiting simply because the required host species no longer occur within reach.

Like mussels, fish communities have experienced precipitous declines throughout the 19th and 20th centuries (Haag, 2012). Among the most severe impacts to fish communities, is the construction of the lock and dam systems (Haag, 2012; Woolnough, 2006). During the industrial boom of late 19th and early 20th centuries, lock and dam systems were constructed so engineers could control the water levels of major rivers (USACE, 2019). This was necessary for the transportation of commercial goods on the river at a new level of efficiency (USACE, 2019). This method was in fact so efficient that > 500 million tons of commercial goods are still transported by barge each year on navigable waters today (USACE, 2019). Unfortunately, the construction of these dams abruptly changed the ecology of free-flowing aquatic systems (Wolter and Arlinghaus, 2003). The construction of dams drastically increased water levels and replaced natural riffle-run-pool habitat with a continuous deep-water system, severely limiting the habitat

richness of the river (Watters and Flaute, 2010). This decline in habitat resulted in drastic changes in benthic substrate composition (Watters and Flaute, 2010). Changes in substrate composition left many fish species with substrate-specific reproductive requirements unable to reproduce (Haag, 2012; Cordone and Kelly, 1961). In addition, naturally occurring flood pulses were severely restricted, again, disrupting the reproductive requirements for many fish species (Haag, 2012). Perhaps the greatest impact to fish reproduction was the physical barrier the dams created for migratory fish species (Haag, 2012). Disruptions to fish reproductive cycles resulted in localized extirpations of numerous species throughout their historical ranges, limiting reproductive opportunities for many species of obligatory mussels (Watters and Flaute, 2010; Vaughn and Taylor, 1999). For mussel species who host-fish species still occur, the increased water level further separated mussels physically from host-fish, presumably lowering the frequency of the necessary reproductive interaction.

Determining the exact age of a freshwater mussel is difficult, though, some literature suggests they can be aged based on the number of "rings" found on the shell (Haag, 2012; Cummings and Mayer, 1992). However, the rings on a mussel shell can be influenced by factors other than age, so this method is better suited for an approximation of age than exact age (Haag, 2012). For large samples, counting rings on each mussel is tedious and time consuming, so mussels are commonly measured by length along the hinge line as a more efficient alternative. As a mussel grows older, the shell increases in length, with the greatest growth occurring in the first 2-5 years for most species (Haag, 2012). Therefore, "small" mussels, relative to adult sizes for some species, indicate recent recruitment.

Some species of freshwater mussel display sexual dimorphism, particularly members of the Lampsilinae family (Haag, 2012). Sexual dimorphism in mussels is identifiable due to the

inflation of the shell in females, known as the marsupium, where glochidia are stored prior to release (Haag, 2012). Since males do not store glochidia, the extra space in the shell is not needed, therefore males lack the inflated shell character (Haag, 2012). Fertilization success is presumably dependent on population density. Male mussels release sperm into the water column, female's siphon in the sperm to fertilize their eggs, therefore, males must occur upstream from females to complete fertilization (Haag, 2012).

Here, we present the size characteristics for the mussel species collected in this study, and identify which species show signs of recent reproduction. Additionally, we present the sex ratios of the sexually dimorphic species collected. We hypothesize mussel species occurring frequently to show sign of recent reproductive success.

METHODS

Upon collection (See chapter 1 methods) we identified, measured, and sexed (where applicable) each individual mussel. Measurements were recorded from the longest point parallel to the hinge line of each mussel. For *P. cyphyus,* a federally endangered species, we also collected shell width and depth measurements (required by permit listed species). Detecting young of year is difficult under the survey technique used for this study, therefore, we used mussel length data to determine which species show sign of reproduction in the Greenup Pool. We excluded species that were collected less than ten times to ensure our inferences were being made on enough individuals to represent the species. For all species included in this determination, individuals < 30 mm are considered juveniles (Haag, 2012; Miller and Payne, 2000). We calculated average lengths for all species to be used in future comparison. We calculated sex ratios and average length by sex for the four species collected that exhibit sexual dimorphism.

RESULTS

The freshwater mussel community of the Greenup pool appears to be deficient in recent reproduction events. Of the 21 species collected, only 5 species included individuals less than 30 mm, with few individuals at that size (Fig. 27, Table 5). Mussels < 30 mm are commonly accepted as juveniles (Haag, 2012; Miller and Payne, 2000). Surveys from the 1990s in the Greenup Pool indicate juvenile mussels made up as much as 40% of all mussels collected (Miller and Payne 2000). The two most abundant species, *O. reflexa* and *C. pustulosa*, have the smallest mean length, suggesting frequent reproduction, though neither species reach large sizes relative to other species. Other species collected with individuals < 30 mm include *E. lineolata*, *A. plicata*, and *P. alatus*. Less common mussels like *A. ligamentina, E. crassidens,* and *R. ebenus* have a more constricted size distribution, indicating a lack of recent reproduction. Sex was recorded for four species displaying sexual dimorphism: *L. cardium, E. lineolata, P. alatus,* and *L. recta*. Populations were male dominant for *L. cardium*, *E. lineolata*, and *L. recta* (Table 6). Additionally, males exhibit greater length (mm) than females for all four species recorded for sexual dimorphism.

DISCUSSION

Length and sex data are a common component to freshwater mussel surveys. Collecting length and sex data on a pool-wide scale allows for comparison in future studies. An increase in average size for a particular species in the Greenup Pool over time may indicate a decline in reproduction. Further, this data can be compared across the Ohio River mainstem providing insight to the reproductive status of mussels in other navigational pools. By comparing average size from a large population, inferences about the reproductive status can be made where

comparing juvenile mussel presence may not be entirely representative based on the increased difficulty of detecting juvenile mussels and the cyclic nature of freshwater mussel reproductive events. The cyclic nature of reproduction events includes factors associated with the time of year at which surveys are conducted, as reproductive timelines vary between species, even varying year to year (Haag, 2012). The cyclic nature of reproduction events is supported by Miller and Paynes surveys from the 1990s, as juvenile mussel collections varied, making up 0-40% of all mussels collected across survey years, 1992, 1993, and 1998 (Miller and Payne, 2000). It is likely no coincidence that the 5 species collected with individuals < 30 mm are within the top 7 species in terms of individuals collected (Fig. 27; Table 5). *A. plicata* and *P. alatus* were outranked only by *T. metanevra* whose smallest individual fell just outside the 30 mm threshold at 31.5 mm, and *L. recta* who showed great variation in length, suggesting a complex population dynamic, and is a relatively long bodied mussel, making it easier for young individuals of the species to fall outside of the 30 mm threshold (Fig. 27).

Understanding the trends of species size across their range can provide insight to the health of populations in regard to reproductive success, and the associated limiting factors. The Elephant-ear Mussel (*Elliptio crassidens*) was once a dominant species in the Greenup Pool according to historical records (Miller and Payne, 2000). However, *E. crassidens* made up just over 1% of mussels found in this study, with zero evidence of recent recruitment. This decline is a result of numerous impacts, but the decline in host-fish interaction is likely the primary factor. *E. crassidens'*s host-fish is the Skipjack Herring (*Alosa chrysochloris*), a migratory species who populations and range have been severely limited by the construction of dams (Haag, 2012; Lawrence, 2016). Species with adult dominated populations like *E. crassidens* are perhaps functionally extinct in the Greenup Pool, facing extirpation from their current range once the

aging adults perish, a trend that haunts mussel communities throughout North America (Woolnough, 2006).

Of the four sexually dimorphic species recorded, three species populations were male dominant. Male dominance within a population came as a surprise, because in most organismal reproductive settings, only one male is required to fertilize multiple females, tilting population dynamics towards female dominant. However, mussels are unique in their limited mobility, and non-discriminatory mate selection through broadcast sperm dispersal. Female mussels are reliant on the intake of sperm from males that occur upstream; therefore, fertilization success depends entirely on upstream male presence within a local population (Haag, 2012). Sex biased populations have been documented in mussels, but the ecological significance is poorly understood as sex ratios show no relation to taxonomic classification (Haag, 2012). Male dominant populations for three of four species may be a coincidence in this study, or perhaps these populations have adapted sex ratios to increase the likelihood of a male occurring upstream of a female. Alternatively, the male biased populations may be attributed to higher mortality rates for females, as they could be more vulnerable to predation when luring or releasing glochidia at the benthic transition (Haag, 2012). Additionally, sex ratio detection is further skewed by the various burrowing characteristics of the two sexes. Some species show strong seasonal variation in their benthic layer position, but collections may be biased as mussels higher in the benthic layer are more easily detected (Haag, 2012). In all four species where sex was recorded, males were longer. There is currently no evidence suggesting a benefit to males being larger (Haag, 2012). The size difference between males and females is likely a result of females expending energy for growing the marsupium and glochidia, energy that is directed towards shell growth in males.

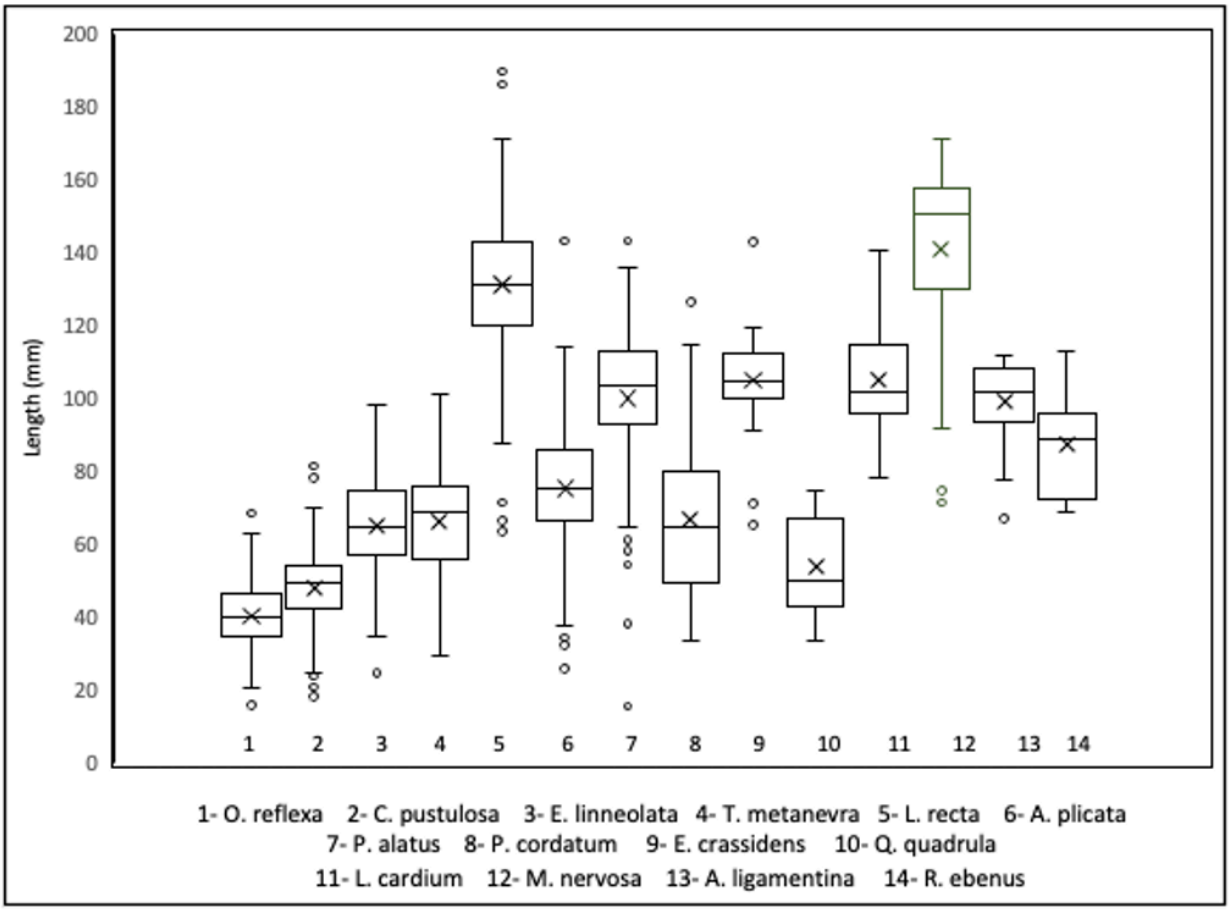

Figure 27. Size characteristics for species with >10 individuals collected
Box and whisker plots showing the distribution of shell length for various species.

Table 5. Number of Individuals and Average Length (mm)
Average lengths demonstrated in millimeters and number of individuals for each species collected.

Species	# of Individuals	Average Length (mm)
O. reflexa	1462	40.8195296
C. pustulosa	1091	48.1184109
E. lineolata	301	65.1622896
T. metanevra	286	66.1639706
L. recta	276	131.054887
A. plicata	206	99.0769231
P. alatus	155	100.004698
P. cordatum	73	66.9442623
E. crassidens	52	104.848148
Q. quadrula	33	53.8647059
L. cardium	30	104.783871
M. nervosa	27	140.839286
A. ligamentina	13	76.7906863
R. ebenus	10	87.4818182
P. cyphyus	8	91.0111111
P. sintoxia	7	92.6428571
F. flava	4	56.45
L. complanata	3	100.233333
L. siliquoidea	2	66.3
L. fragilis	1	70.2
T. truncata	1	26.7

Table 6. Sex Ratios and Average Lengths for Sex for Sexually Dimorphic Species
Sex ratios and average lengths for all (recorded) species that exhibit sexual dimorphism. Species exhibiting sexual dimorphism have an inflated posterior half when female.

Species	# of Males	# of Females	% Males	% Females	Male Avg. Length (mm)	Female Avg. Length (mm)
L. cardium	17	14	0.55	0.45	108.0235294	100.85
E. lineolata	159	135	0.54	0.46	68.96477987	60.84222222
P. alatus	49	91	0.35	0.65	106.7489796	99.67912088
L. recta	150	115	0.57	0.43	133.2773333	128.7165217

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CHAPTER 3

INTRODUCTION

The Greenup Pool historically supported 40+ species of freshwater mussel, eight of which are currently considered federally endangered (Watters and Flaute, 2010; Taylor, 1980). However, human activity has potentially reduced the mussel richness of the Greenup Pool by 30% in the last 2 centuries (Taylor, 1989). Unfortunately, there is reason to suspect the worst has yet to come, or at least be properly documented, as mussel communities throughout North America are still in rapid decline (Haag, 2012; Vaughn and Taylor, 1999; Cummings and Mayer, 1992). Surveys conducted by Miller and Payne in the 1990s collected 30 species from 8,163 individuals in the upper Greenup Pool (Miller and Payne, 2000). Other historical surveys using similar survey methods in the Greenup Pool have produced comparable results, with richness near 30 species (Table 7). However, the 2017 pool-wide surveys by Kriege yielded just 23 species from 3,747 individuals (Kriege, 2017). This comparison suggests a significant decline in mussel richness (5> species) in the last quarter century. However, it is worth noting that the surveys conducted by Miller and Payne were concentrated in the upper third of the Greenup Pool which has experienced significantly less historic impact. Conversely, Kriege's 2017 surveys occurred throughout the entire pool - including the middle and lower sections, where conditions are not as favorable for sensitive species. For most research prior to 1990, brailing was the primary method of unionid collection. Brailing targets larger mussels at the benthic boundary, discriminating against smaller, burrowing species (Taylor, 1980; Zeto, 1987). While techniques such as brailing can identify some mussel species within a given area, the entire mussel community is more thoroughly captured by qualitative SCUBA searches and other "diving"

oriented survey methods (Table 7). Qualitative SCUBA surveys are accepted as the most effective survey method for unit time (Haag and Williams 2013).

To understand the changes in a mussel community, specifically that of the Greenup Pool, replicable survey methods must exist and be conducted throughout some timeline. Many state agencies such as the West Virginia Division of Natural Resources (WVDNR) require freshwater mussel surveys for industry and development that involve aquatic systems (Clayton et al, 2020). The WVDNR provides a diving intensive survey protocol that must be followed by permitted individuals in order to move forward with the given project (E.g., bridge construction crossing a stream or river). This protocol was designed to detect threatened and endangered species and identify mussel communities within the work area and surrounding buffer zones (Clayton et al, 2020). The West Virginia Mussel Survey Protocol is responsible for majority of mussel surveys in the Greenup Pool and has laid a foundation for research-based projects such as Kriege's 2017 research which used the protocol exclusively for site preparation, survey area, survey time, etc. (Kriege, 2017). The sites selected by Kriege were based on randomly generated sites from the Ohio River Sanitation Commission (ORSANCO). The ORSANCO sites were not generated for mussel surveys; however, Kriege selected them as they are non-biased and generally distributed throughout the Greenup Pool (Kriege, 2017; ORSANCO, 2021). Kriege's work was the first pool-wide randomized mussel survey on the Greenup Pool, creating a baseline data set which allows further studies a point of comparison.

When compared to previous studies, the results of Kriege's 2017 surveys imply that the mussel community of the Greenup Pool is in decline as the total number of species encountered is lower than previous research suggests (Table 7). However, discrepancies in survey techniques, survey area, location of surveys in the pool, and the number of individuals collected

inhibits the ability to draw major conclusions about the status of the mussel community in the Greenup Pool in the last 20-30 years. To make stronger inferences about the status of the mussel community in the Greenup Pool, and the associated survey methods, Kriege's study was replicated using a different set of sites, under the West Virginia Mussel Survey Protocol.

Here, we leverage two massive efforts (2017 and 2020) to construct a thorough description of the mussel community of the Greenup Pool. Additionally, we compare the two efforts to detect specific differences in the mussel community observed. We hope to confirm the validity of capturing a full picture of the mussel community within the Greenup Pool by this pool-wide randomized sampling method. We hypothesize that this study will yield a similar mussel community to Kriege's 2017 research.

METHODS

We applied polynomial regressions to mussel abundance and species richness using the sum of each 10-meter interval, for 2017 and 2020. We found mussel abundance and richness to have a non-normal distribution that could not be normalized using standard transformations (Xsqaured, Xcubed, Xsqrt, Xinv, LogX), failing to meet the assumptions of an unpaired t-test. Therefore, we used the non-parametric Mann-Whitney U test to analyze the relationship between mussel abundance and richness between the 2017 and 2020 efforts. Further, species accumulation curves were applied to mussel community data from both efforts (2017 and 2020).

Species accumulation curves were generated for both 2017 and 2020 efforts using "R-Studio" using the "vegan" package and visualized using "ggplot2" (Fig. 28, Fig 29). Freshwater mussel abundance and richness data was analyzed using SAS [9.4] (Copyright 2002-2012). Mussel abundance and richness were independently analyzed between survey efforts (2017 and 2020).

RESULTS

Kriege's 2017 survey efforts yielded 3,747 mussels from 23 species. The 2020 efforts yielded similar results with 4,041 mussels from 21 species. The dominant species for both studies were the same *(O. reflexa* and *C. pustulosa*), making up over 50% of the live mussel collections in both 2017 and 2020 (Table 7). Three species observed in 2017 were not observed in 2020 (*P. grandis, L. teres, T. verrucosa*). One species was observed in 2020 that was not observed in 2017 (*P. sintoxia*) (Table 8). All other species collected in both studies occurred in similar densities (Table 8). *Plethobasus cyphyus* observations were similar in the two studies, with 9 individuals observed in 2017, and 8 individuals observed in 2020. All *P. cyphyus* were collected in the upper pool.

One site (290.2RDBO) was re-visited to assess survey efficacy and detect short-term (3 years) trends. The 2017 efforts at 290.2 produced 1,081 individuals from 20 species while the 2020 efforts produced 1,478 individuals from 16 species.

Analysis

Mann-Whitney U analysis indicates there is not a statically significant difference ($P>0.05$, two-tailed) in the distribution of mussel abundance between survey efforts (Mann-Whitney $U=290.00$, $n_1= 19$, $n_2= 17$, $P=0.9544$; Fig. 30). Analysis indicates there is not a statically significant difference ($P>0.05$, two-tailed) in the distribution of species richness between survey efforts (Mann-Whitney $U=287.50$, $n_1= 19$, $n_2= 17$, $P=0.9935$; Fig. 31).

DISCUSSION

The 2020 surveys yield similar results to Kriege's 2017 efforts. Analysis indicates there is not a significant difference between the survey efforts for abundance and richness. The combined data from 2017 and 2020 allows for more accurate comparisons between the 1990s and early 2000s as the number of individuals and survey area are much closer (Table 7). When considering the combined number of individuals (7,788) compared to the total species (24) there is evidence to suggest a decline in richness in the last 20-30 years. Given the abundance to richness ratio of the prior and present surveys in the Greenup Pool, the species not collected in the combined (2017 and 2020) efforts likely occur in very limited populations or have become extirpated from the pool. All species found in 2017 and 2020 efforts have been documented previously in the Greenup Pool.

The similarities between the 2017 and 2020 surveys suggest that additional surveys are not needed in the immediate future to capture the mussel community of the Greenup Pool. Based on the results of the analysis, it appears the 2017 effort captured the mussel community of the Greenup Pool. Further, the species accumulation curves generated for the two efforts are almost identical, respectively, and suggest low potential for increased richness in future sampling. The two surveys did not produce the same number of species, however, the species not encountered in both surveys occurred in very low densities and do not make up a significant component of the mussel community (Table 8). The species that were collected in both studies occurred in comparable percentages, despite being from entirely different sites. Additionally, the federally endangered *P. cyphyus* was collected 9 times in 2017, and 8 times in 2020. While densities for *P. cyhypus* were relatively low, it's encouraging that it was collected in a similar volume in both studies. *P. cyphyus* comprised 0.218% of the combined mussel community sampled (7,788

individuals). This is lower than a multi-decade synopsis of surveys by Ecological Specialists, Inc. (2000), who reported *P. cyphyus* comprising 0.4% of the mussel community in the Greenup Pool (Butler 2002). However, the sites surveyed for the synopsis were predominantly in the upper Greenup Pool (Ecological Specialists, Inc. 2000). When isolating the 2017 and 2020 collections to just the upper pool, *P. cyphyus* still comprises < 0.3% of the mussel community.

Krieges's sites were heavily weighted in the upper and lower Greenup Pool, by result of random site selection. This left questions about the middle Greenup Pool, where much of the anthropogenic impact enters the river from major cities and the confluence of impacted tributaries. Coincidentally, five of the randomly generated sites for this study occur in the middle pool (Fig. 32). These sites filled the gap in the transition between the upper and lower pool, which possessed strikingly different mussel communities in Kriege's study. This study suggests that the mussel community of the middle pool behaves more like that of the lower pool than the upper, with the greatest drop off in mussel abundance and richness occurring at or just above the city of Huntington and the confluence of the Guyandotte River.

Survey Methods

One of the major differences in the 2017 and 2020 surveys pertains to the survey protocol itself. For this study, surveyors spent a minimum of one minute of survey time per square meter of survey area. This method was not applied to the 2017 efforts. The additional time spent in the survey area allowed for surveyors to collect more mussels per interval. This is indicated by the difference in number of individuals observed per m^2 surveyed. The 2017 efforts produced 3, 747 individuals from 12,000 m^2 of survey area, whereas the 2020 efforts for this study produced 4,041 individuals from 9,400 m^2 of survey area. While more survey time did translate to more individuals collected, it does not appear that the additional time better indicates the mussel

community. Other than the time spent at each interval, the site set up and survey methods between the two studies were identical.

Site 290.2RDBO was the only 2017 site that was re-visited in 2020 to assess survey efficacy and determine short term trends in the mussel community. Site 290.2RDBO was documented as a strong mussel community in 2017, therefore it serves as a good model to assess the efficacy of a repeat survey under similar conditions and protocols. Site 290.2RDBO produced the greatest mussel abundance for any site in both studies, highest richness in 2017, and second highest richness in 2020. Total abundance in 2017 was 1,081 individuals from 20 species and 1,478 individuals from 16 species in 2020 (Table 9). The federally endangered *P. cyphyus* was collected in both efforts. The mussel community composition was similar between the 2017 and 2020 efforts with a minor discrepancy in total species, however, the species causing this discrepancy were collected in low densities (Table 9). The greater abundance from the 2020 surveys is presumably a result of the increased survey time per interval.

Adequately assessing freshwater mussel populations is paramount in conserving what remains of aquatic communities after two centuries of anthropogenic impact (Haag and Williams, 2013). The process of site selection using ORSANCO's random sites and the use of the West Virignia Mussel Survey Protocol, has resulted in a replicable survey method for future studies within the pool. The health of the mussel community in the Greenup Pool can now be accurately monitored in the future. This process can also be applied to other pools in the Ohio River as ORSANCO's sites extend well beyond the Greenup Pool. The ability to compare from pool to pool is now available, which will lead to a greater understanding of species distribution and decline in the Ohio River going forward. The data collected from the 2017 and 2020 efforts

is potentially the largest and most up to date mussel community resource for an individual navigational pool within the Ohio River system.

Table 7. Mussel Surveys in the Greenup Pool

Historical data of mussel surveys in the Greenup Pool, specifically large, high yield surveys were included. Hisotrical data suggests a decline in the mussel community of the Greenup Pool .

Surveyor	Year	Pool Location	Survey Method	Abundance	Richness
Taylor	1979	Upper	Brailing/midden	-	17
Zeto	1985	Upper	Brailing	379	16
Miller and Payne	1992, 1993, 1998	Upper	Qualitative & quantitative diving	8,163	30
Ecological Specialists, Inc	1990-1999	Upper, Middle, Lower	Transects	2,968	31
USACE	2001-2017	Upper	Transects	7,235	28
Kriege	2017	Upper, Middle, Lower	Transects	3,747	23
Miller	2020	Upper, Middle, Lower	Transects	4,041	21

Table 8. Observed Mussel Community in 2017 and 2020
Comparison of the mussel community of the Greenup Pool between 2017 and 2020. Survey methods and random site selection are comparable.

Species	Kriege (2017) Abundance	Miller (2020) Abundance	Kriege (2017) % of Community	Miller (2020) % of Community
O. reflexa	1116	1462	29.82	36.18
C. pustulosa	958	1091	25.49	27.00
T. metanevra	244	286	6.52	7.08
L. recta	398	276	10.63	6.83
A. plicata	247	206	6.60	5.10
P. alatus	212	155	5.66	3.84
E. lineolata	284	301	7.59	7.45
P. cordatum	47	73	1.26	1.81
A. ligamentina	16	13	0.43	0.32
F. flava	4	4	0.11	0.10
Q. quadrula	27	33	0.72	0.82
R. ebenus	7	10	0.19	0.25
E. crassidens	90	52	2.40	1.29
L. cardium	51	30	1.36	0.74
L. silliquoidea	2	2	0.05	0.05
L. complanata	2	3	0.05	0.07
L. fragilis	3	1	0.08	0.02
M. nervosa	21	27	0.56	0.67
P. cyphyus	9	8	0.24	0.20
T. truncata	6	1	0.16	0.02
P. sintoxia	0	7	0.00	0.17
T. verrucosa	1	0	0.03	0.00
L. teres	1	0	0.03	0.00
P. grandis	1	0	0.03	0.00
Total	**3747**	**4041**	-	-

Table 9. Observed Mussel Community at Re-visit Site (290.2RDBO) in 2017 and 2020
Species specific comparison of the mussel community of the Greenup Pool between 2017 and 2020.

Species	Kriege (2017)	Miller (2020)
O. reflexa	311	544
C. pustulosa	353	421
T. metanevra	108	137
L. recta	72	81
A. plicata	56	65
P. alatus	35	43
E. lineolata	90	114
P. cordatum	18	31
A. ligamentina	3	4
F. flava	2	0
Q. quadrula	13	10
R. ebenus	1	1
E. crassidens	4	5
L. cardium	6	11
L. silliquoidea	1	0
L. complanata	1	2
L. fragilis	1	0
M. nervosa	2	4
P. cyphyus	1	5
T. truncata	3	0
P. sintoxia	0	0
T. verrucosa	0	0
L. teres	0	0
P. grandis	0	0
Abundance	**1081**	**1478**
Richness	**20**	**16**

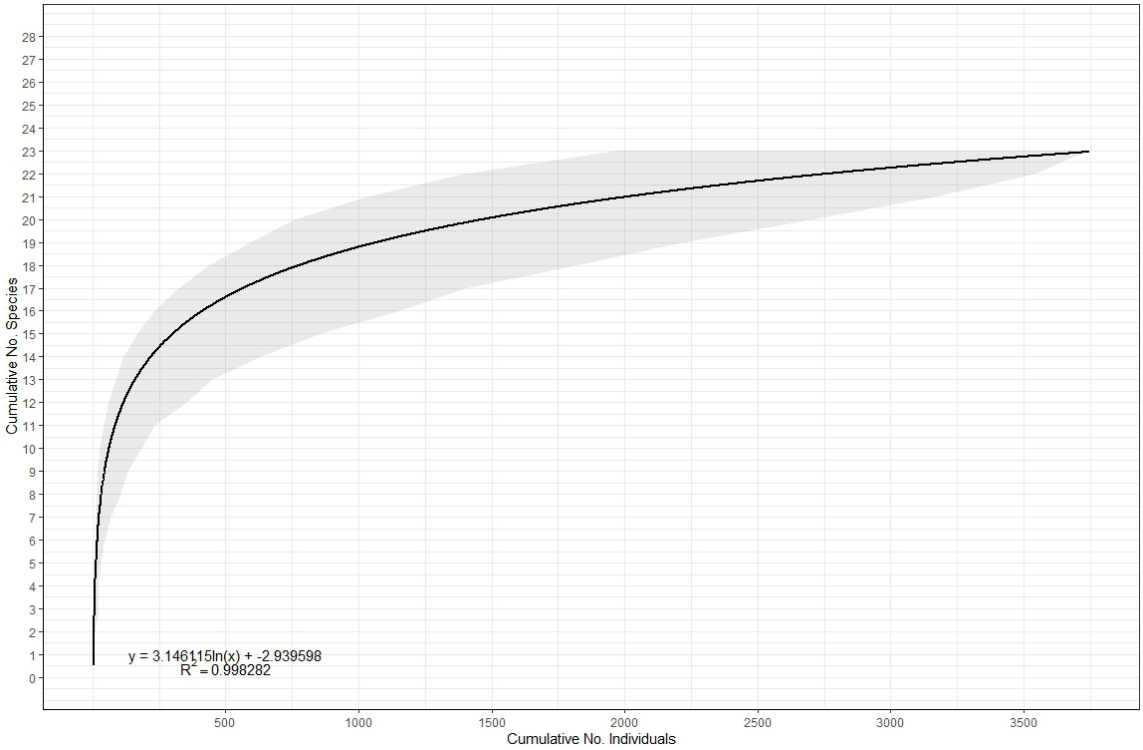

Figure 28. Species Accumulation Curve for Kriege Data (2017)
Species accumulation curve showing the proportion of number of individuals compared to the number of species for 2017 data set.

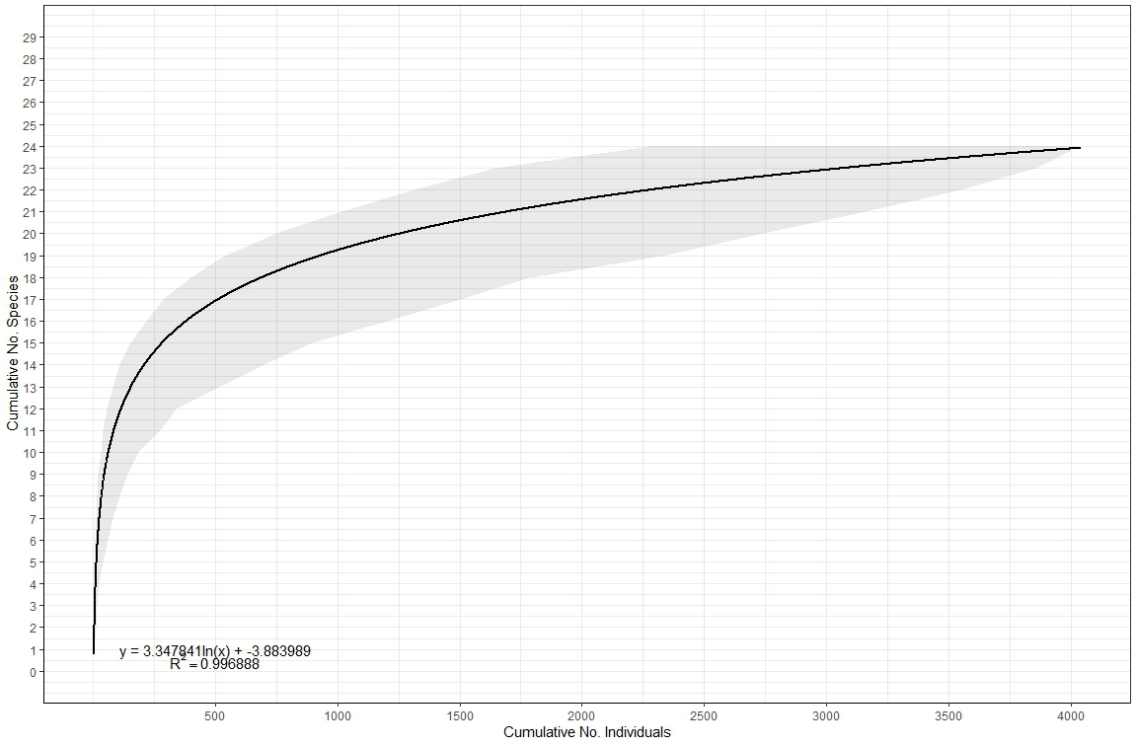

Figure 29. Species Accumulation Curve for Miller Data (2020)
Species accumulation curve showing the proportion of number of individuals compared to the number of species for 2020 data set.

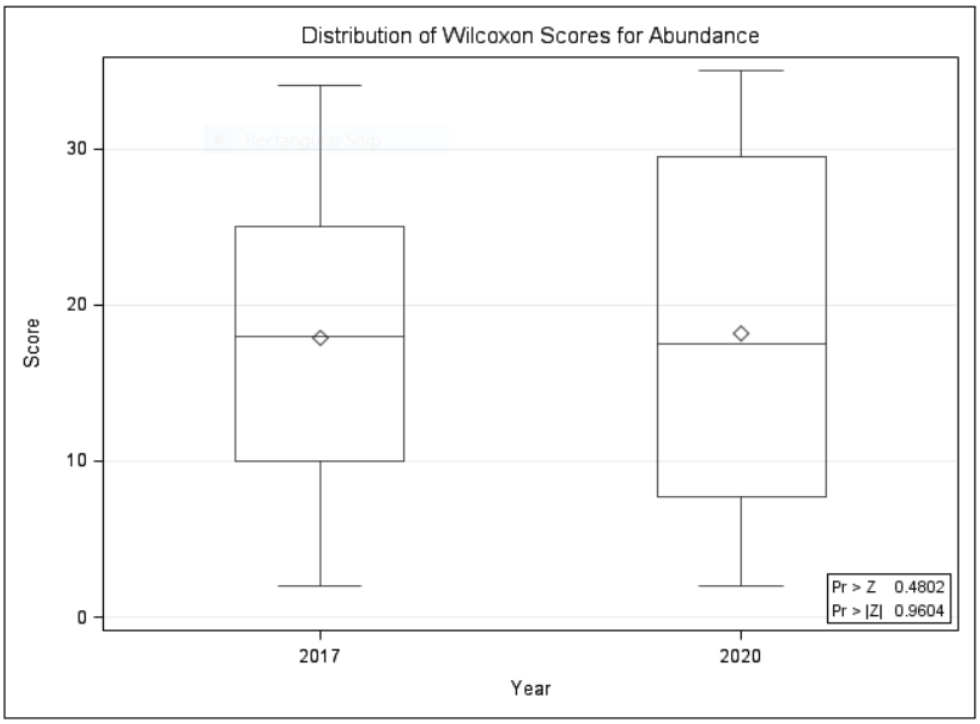

Figure 30. Ranked Mussel Abundance by Survey Effort (Year)
Box and whisker plots showing ranked mussel abundance between survey years (2017 and 2020).

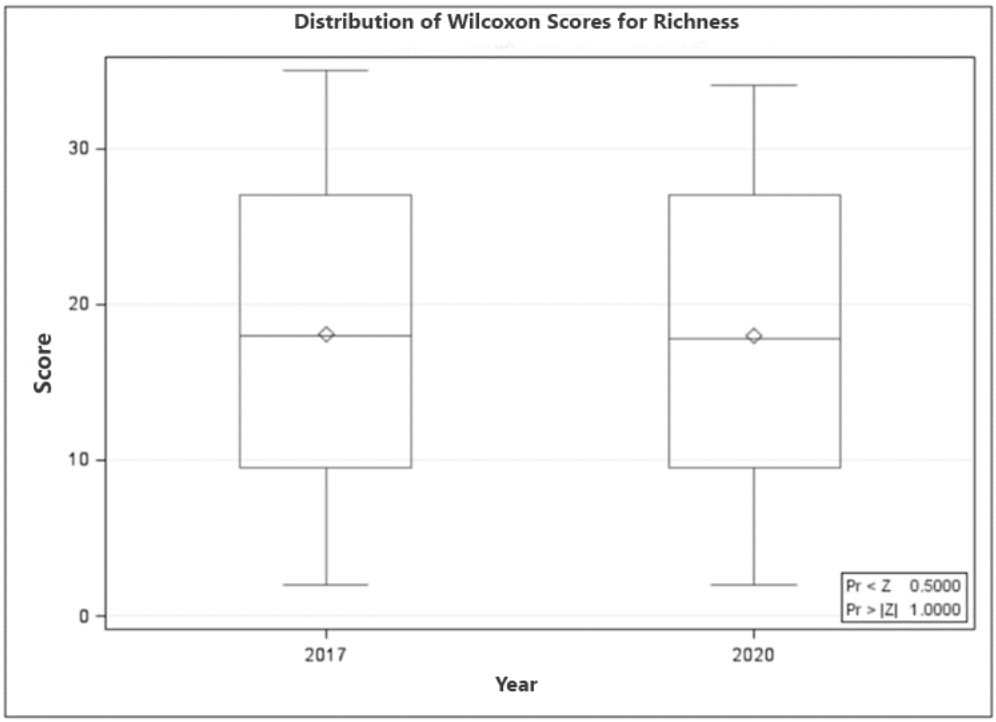

Figure 31. Ranked Species Richness by Survey Effort (Year)
Box and whisker plots showing ranked mussel richness between survey years (2017 and 2020).

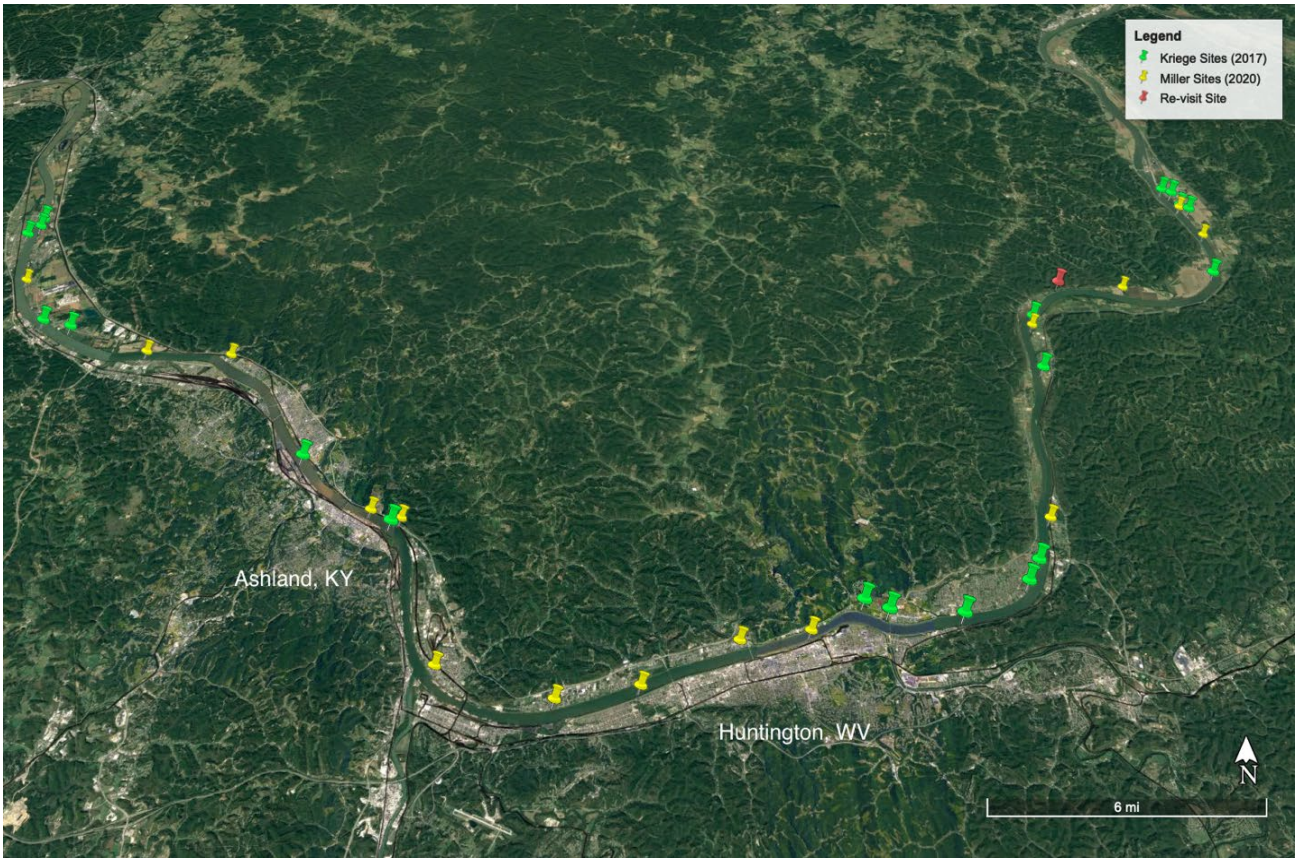

Figure 32. Site Distribution for 2017 and 2020 Efforts
Aerial image showing distribution of both 2017 and 2020 site locations throughout the Greenup Pool.

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APPENDIX: IRB APPOVAL LETTER

MARSHALL UNIVERSITY
www.marshall.edu

Office of Research Integrity

October 19, 2022

Jacob Miller
61 Mill Creek Crossing
Hurricane, WV 25526

Dear Jacob:

This letter is in response to the submitted thesis abstract entitled *"Unionid Bivalves in the Greenup Pool of the 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). This thesis does not require IACUC approval since it involves invertebrate research. The Code of Federal Regulations (45CFR46) has set forth the criteria utilized in making t/his 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