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Assessing the unionid assemblage of the Robert C. Byrd Pool, Ohio River

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ASSESSING THE UNIONID ASSEMBLAGE OF THE ROBERT C. BYRD POOL, OHIO RIVER

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Approved by
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Approval of Thesis

We, the faculty supervising the work of Ethan Hunter Bellamy, affirm that the thesis, *Assessing the Unionid Assemblage of the Robert C. Byrd Pool, Ohio River*, meets the high academic standards for original scholarship and creative work established by the Department of Biological Sciences Graduate Program and the College of Science. The work also conforms to the requirements and formatting guidelines of Marshall University. With our signatures, we approve the manuscript for publication.

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Dedication

This collection of work is dedicated to all the family and friends who couldn’t see this through with me. I hope I’ve made you proud.

Acknowledgments

First and foremost, I'd like to thank my thesis advisor Dr. Tom Jones. While deployed overseas prior to graduate school, Dr. Jones made sure to stay in touch and ensured me I had a place in his lab as soon as I returned home. Since then, Dr. Jones has become my mentor, dive instructor, and dear friend. He absolutely plays a vital role in who I am today, and I dare say without him, none of this would be possible.

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Abstract

Unionid mussels are the most imperiled taxa in the United States and are vastly understudied. The entire Robert C. Byrd (RCB) pool of the Ohio River is the study area. I selected sites from the 2019 and 2013 ORSANCO RCB pool assessments that utilize random site selection across the pool. I used SCUBA to survey and collect data on unionid diversity, reproduction, and habitat. I collected 1,083 individuals over 19 species in RCB pool. I compared results from RCB pool survey to two similar surveys conducted in Greenup Pool. Both richness and abundance in the Upper Section of Greenup Pool dwarfed all other sections in Greenup and RCB. Patterns of species redundancy are determined utilizing Nonmetric Multidimensional Scaling. RCB and Greenup Pool patterns are statistically similar. Data collected could serve as a baseline to site-specific surveys and have management implications for unionid assemblages in pools of large, navigable rivers.

Introduction

Unionid Overview

Unionid mussels are among the most imperiled taxon in the world. Of the 297 recognized species in North America over 70% are listed and less than a quarter (appx. 24%) are considered stable (Brown et al., 2010; Cummings & Graf, 2010; Williams et al., 1993a). North America has the highest unionid diversity of any continent yet management efforts have been deemed inadequate as populations continue to decline despite regulations such as the Clean Water Act of 1972 (Williams et al., 1993b). Unionids are understudied, especially with respect to their relationship with their environment (Box & Mossa, 1999; Cummings & Graf, 2010). This makes isolating habitat variables to manage for unionid assemblage conservation especially difficult. Industry requirements for protected species surveys in large navigable rivers drive many mussel surveys. Thus, most of our knowledge about mussel diversity in these areas is derived from pre-impact surveys to establish presence of threatened species, rather than from biomonitoring–oriented ecological surveys placed randomly to assess diversity and population-level parameters. This is especially concerning as large river species are amongst the most imperiled (Taylor, 1989)

Unionids can filter up to 10 gallons of water per day reducing excess sediment, bacteria, and algae suspended in the water column (Haag, 2012). Negative effects from loss of native unionid species are compounding. As water quality degrades, mussels die off, and thus a waterway's natural capability to restore themselves is diminished. This is significant as sediment is the leading pollutant in streams and rivers. Additionally, when abundant, mussels can reduce phytoplankton and increase water clarity by over 50%. This can lead to system level changes such as increasing fish and bird populations (Cummings & Graf, 2010). Freshwater mussels also help stabilize substrate, provide benthic refugia, and are an important food source for several fish and mammalian species (Brown et al., 2010; Clayton, 2023; Cummings & Graf, 2010).

In general, the unrivaled amount of loss of freshwater fauna has been attributed to stream channelization and damming, industrial development, urbanization, overexploitation, water pollution, and habitat destruction or degradation (Benke, A. C., 1990; Jones, 2022; Ricciardi & Rasmussen, 1999; Williams et al., 1993b) Unionids are among the most susceptible to these effects (Roe, 2004; Williams et al., 1993b). In addition to these issues, the button and pearl industry beginning in the mid-1800s killed thousands of tons of unionids (Clayton, 2023; Williams et al., 1993b). Cummings and Graf (2010) note that the historic and commercial harvest of unionids with the addition of habitat loss and degradation have left North American populations at levels unable to support sustainable future populations (Cummings & Graf, 2010).

The degradation of unionid abundance and habitat left the bivalves highly susceptible to invasion from foreign species. In the 1930s the invasive asian clam (*Corbicula fluminea*) was introduced. Later in 1988, zebra mussels (*Dreissena polymorpha*) were introduced into Lake St. Clair presumably through ballast water of cargo ships (Clayton, 2023; Haag, 2012; Roe, 2004; Williams et al., 1993a). These invasives, wherever found, have proven to decimate the already highly impacted unionid populations and in 1991, they would find their way to the Ohio River (Watters & Flaute, 2010; Williams et al., 1993a).

Unionids of the Ohio River

As the early European settlers made their way into the Ohio Valley in the latter 1700s, they were greeted by what was regarded one of the most beautiful rivers in North America. The shallow (avg. depth > 1ft), nearly 1,000 mile long pristine Ohio River sported a diverse habitat of numerous riffles and pools with occasional waterfalls (Taylor, 1980, 1989; Watters & Flaute, 2010). This diverse habitat was complemented by a diverse unionid assemblage to which Rafinesque reported at least 68 species in 1820; five times the total of unionid species in all of Europe (Taylor, 1980, 1989).

Unfortunately, almost as soon as the Europeans entered the valley so did degradation of its pristine nature. First forts, and then urban and trade centers began being built in places like Pittsburgh, Cincinnati, and Louisville. Pomeroy, a town located in this study's research study area, was also among the first settled. Along with urbanization came the need for lumber, agriculture, mining, and sewage (Taylor, 1989; Watters & Flaute, 2010). These industries were likely developed with complete disregard of the impact on the environment they cause, and thus habitat was degraded by siltation and contaminants. Today these areas are among the most void of unionid life (Watters & Flaute, 2010).

Degrading water quality and pollution due to urbanization is still an issue today for unionids, especially juveniles (Brown et al., 2010; Yeager et al., 1994). Increased urbanization means an increase in impervious surfaces and thus increased runoff of petroleum products and surfactants. Impervious surfaces and loss of riparian forest cover also affect flow regimes. Combined sewage overflows (CSOs) that pump untreated sewage during heavy rain events are also thought to be have a negative impact on unionids and are prevalent in urban centers (Gillis, et al., 2017; Kriege, 2018).

Williams et al. (1993) regards habitat destruction as the single most important threat to freshwater mussels and no single act may be more detrimental to unionid habitat than the damming of rivers. The first dam was constructed for navigational purposes on the Ohio River in 1885 and began the process of turning a heterogeneous lotic system into the deep, homogeneous lentic system we have today (Kriege, 2018; Watters & Flaute, 2010). From 1976 – 2018 high-rise dams replaced their smaller predecessors to maintain the minimum 9 ft. navigational channel. Today the Ohio River contains 19 dams to make 20 pools in total.

Dams convert free flowing lentic rivers into deeper lake-like systems and change the physical and biological environment (Williams et al., 1993a). Stable flow refuge and protection from scour, some of the most important factors for predicting unionid abundance, become scarce (Brown et al., 2010; Cummings & Graf, 2010). Instead of rifles and runs, there are only homogenous pools. This is especially problematic as lotic and lentic systems have distinctly different species that inhabit them. At best, disappearing species are replaced by more silt tolerant ones (Watters & Flaute, 2010). Taylor (1989) reports a 45% change in unionid composition within the last century and 17 species presumed extirpated.

The downstream section of these pools contains deposited sediment that can be several feet thick, and can become anoxic due to the chemical oxygen demand of the bacteria that occupy the silt (Kriege, 2018; Nogaro et al., 2008). This in turn can suspend unionid reproduction (Cummings & Graf, 2010). Loss of host species, particularly migrating fish, is also an issue for reproduction (Cummings & Graf, 2010; Williams et al., 1993a). Depth is greatest in these areas, reaching more than 50 ft in places. Most mussels occupy depths of three to fifteen feet and are only found deeper if the water is well oxygenated (Cummings & Graf, 2010). This is especially true for less tolerant species. Reproduction here is again an issue as unionid species rely on visually attracting host species with their lure which can prove to be difficult in 50 ft of low clarity water. One unionid species, *Elliptio crassidens*, is currently suffering from this specific issue as its only known host, the skipjack herring, is mainly a pelagic feeder. In the mid-1980s *E. crassidens*, along with *Quadrula quadrula*, was the most dominant species in the Greenup Pool of the Ohio River (A. C. Miller & Payne, 2000). In the last decade only two individuals less than 40mm in size have been located in the Ohio River (Patricia Morrison, personal communication 4/12/23). Today, due to the severed interaction between this species and its host species, *E. crassidens* reproduction is nearly nonexistent and the species is likely to soon be extirpated.

This study has two main project objectives: 1) determine the condition of unionid assemblage in the Robert C Byrd (RCB) Pool of the Ohio River by using a randomized, pool–scale approach, 2) identify any patterns in the unionid spatial distribution that may point to an underlying environmental gradient while assessing habitat variables for correlation with unionid abundance. Analysis questions for project objective one are composition based. They include: “What is the abundance and richness of the pool?”, “Does assemblage composition change with distance downstream?”, “Does assemblage composition change with distance from shore?”.

Analysis questions for project objective two are related to assemblage-scale spatial distribution patterns. They include: “Is there a pattern in the unionid *parallel distribution*?”, and “Is there a pattern in the unionid *perpendicular distribution*?”. The name of the distribution is in reference to its orientation to the riverbank. From this point forward, *parallel distribution* will represent the unionid distribution throughout the length of the pool, changing with river mile. *Perpendicular distribution* represents the unionid distribution changing toward the channel from the bank.

RCB pool results were compared to two projects in the Greenup Pool of the Ohio River that used the same surveys methods (Kriege, 2018; Miller, 2023). Comparison of summary statistics allows for further investigation into the condition of the unionid assemblage in each pool. Spatial distribution patterns within the pools are also compared to investigate whether unionid patterns are unique to each pool or the same. Similar pool patterns support the idea that unionids are responding to habitat variables and thus be more successfully managed for. Results from this study can serve as a baseline for further analysis and inform effective conservation practices for unionids inhabiting this decimated environment.

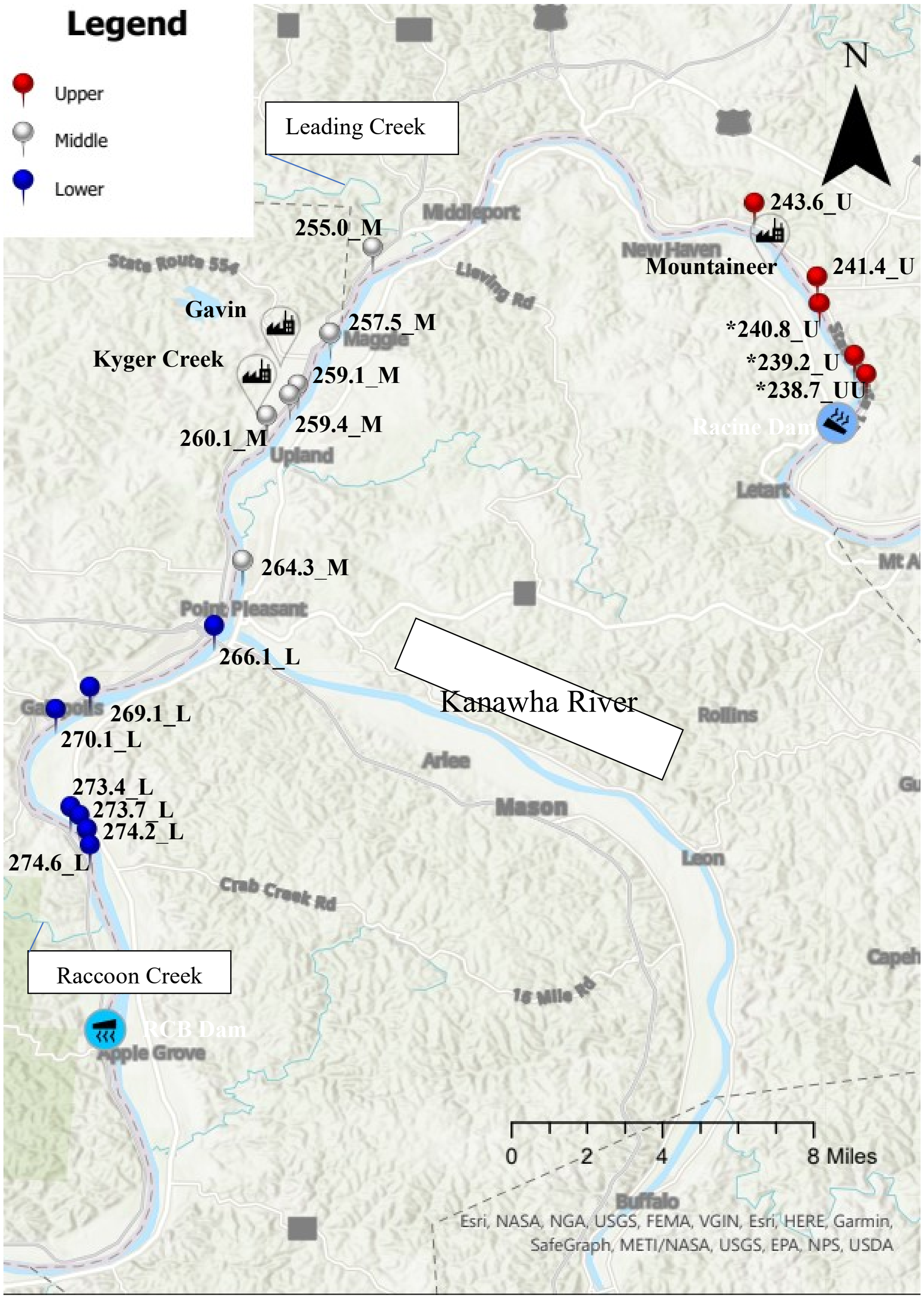
Methods

Study Area

The Robert C. Bryd (RCB) pool is 41.7 miles long, beginning at the Racine Locks and Dam (Ohio River Mile (ORM) 237.5) and ending at the RCB Locks and Dam (ORM 279.2) (Fig.1). The pool is bordered by Ohio and West Virginia with an average depth of 26 feet. The watershed is primarily forested (65.8%), but also struggles with heavy impact in the form of urbanization, industry, and agriculture (ORSANCO Biological Programs, 2013). Point Pleasant, WV and Gallipolis, OH are the main towns within the pool and lie near the middle section. Point Pleasant covers an area of 2.38 square miles with 4,031 people (*Point Pleasant (WV 25550) Profile,* 2023). Gallipolis covers an area of 3.62 square miles with 3,308 people (*Gallipolis, Ohio (OH 45631) Profile,* 2023). Three power plants and one chemical plant are also distributed across the pool. Pasture lands and row crops make up for 13.2% and 7.5% of land use respectively (ORSANCO Biological Programs, 2013). The Kanawha River also enters the Ohio River within this pool and has had a negative effect on fish communities downstream (ORSANCO Biological Programs, 2013). Other major tributaries to the pool include Leading Creek in the more northward reach and Raccoon Creek in the southward. These major tributaries were subject to approximately one billion gallons of untreated and partially treated acid mine drainage (AMD) in 1993 due to the emergency dewatering of Meigs Mine 31. The release of AMD had a significant impact on all aquatic life downstream (Fish and Wildlife Service, Interior, 2023).

Figure 1

Study Area – Robert C. Byrd Pool Sites



Note. Randomized, pool-wide 2019 and 2013 (*) ORSANCO assessment sampling sites in the Robert C. Byrd Pool of the Ohio River, West Virginia, USA

Data Collection

I collected data on the RCB pool during the 2021 and 2022 sampling season between August and October each year following 2022 WV Mussel Survey Protocol and WV collecting permits (West Virginia Division of Natural Resources Wildlife Resources Section, 2022). Utilizing this protocol allows for comparative analyses. Greenup Pool data was collected using the same study design and methodology by Jacob Miller and Mitchell Kriege (Miller, 2023; Kriege, 2018). Unionid data from these two surveys are aggregated and used for comparison to RCB in this study.

All sites came from the Ohio River Sanitation Commission (ORSANCO) assessments (ORSANCO Biological Programs, 2013, 2019). ORSANCO is responsible for biomonitoring of the Ohio River. Part of that assessment is sampling the biotic community. They randomly select sites to derive estimates at the pool-scale. This randomized, pool-scale study design is why ORSANCO assessment sites were used. Each pool is sampled on a five-year rotation and sites are named after the Ohio River Mile (ORM) for which they are located.

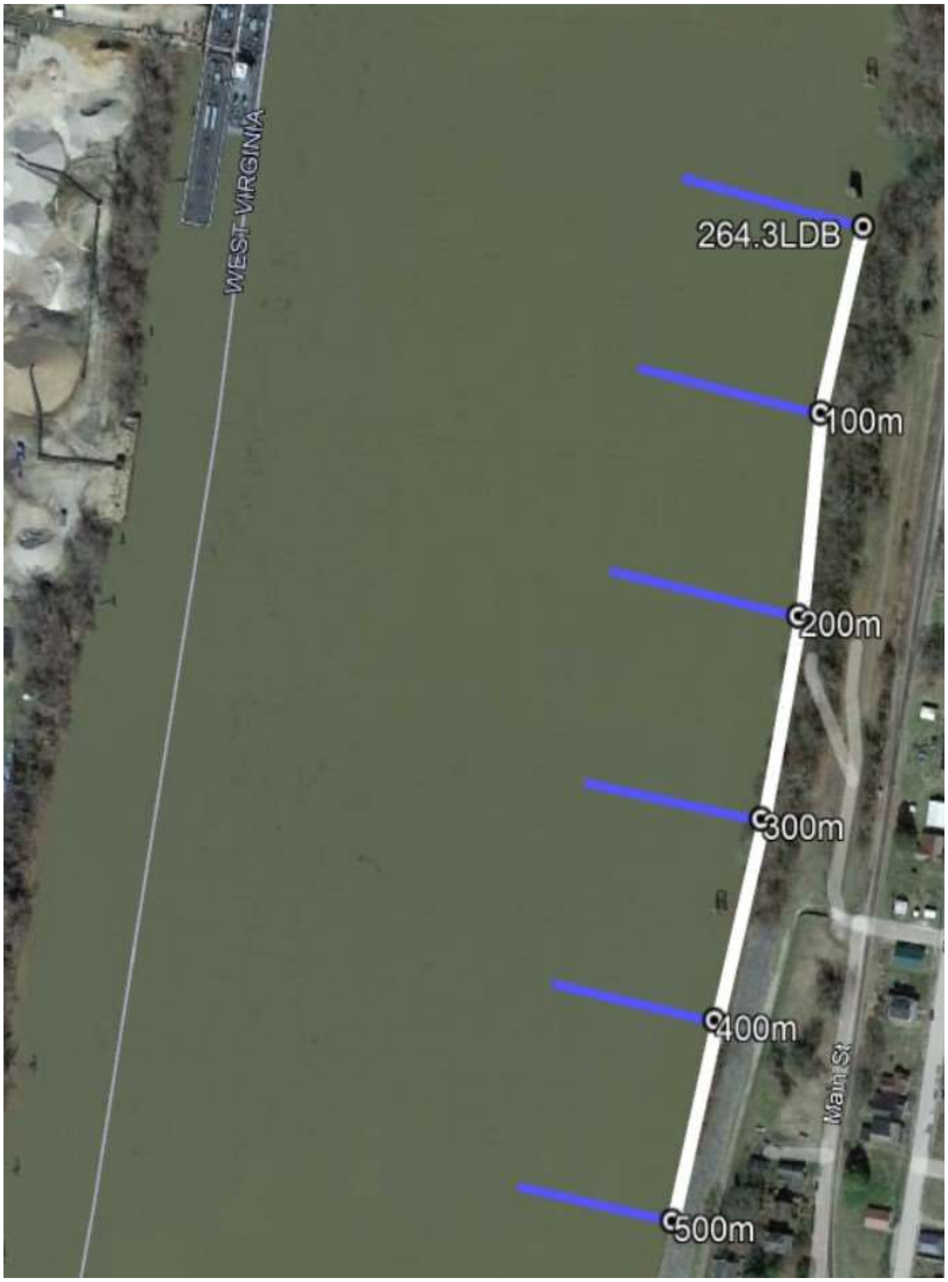
I selected eighteen total sites to use in this study (Fig. 1). Fifteen of those sites came from the 2019 ORSANCO assessment (ORSANCO Biological Programs, 2019). I selected three additional sites (238.7_U, 239.2_U, and 240.8_U) *a priori* from the 2013 ORSANCO assessment to have better representation of the pool's upper section (ORSANCO Biological Programs, 2013). I partitioned the pool into three separate sections: upper, middle, and lower (U, M, L). Sections were determined by considering impact density and distance from the upstream (Racine) dam and then evaluated for patterns in distribution (Kriege, Mitchell, 2018) (Fig. 1). I added an identifier to the site name to denote what section of the pool it belongs to (ex. *237.8_U). The asterisk identifies the site belonging to the 2013 ORSANCO assessment

(ORSANCO Biological Programs, 2013). The Upper Section contains five sites (*238.7_U, *239.2_U, *240.8_U, 241.4_U and 243.6_U). The Middle Section contains six sites (255_M, 257.5_M, 259.1_M, 259.4_M, 260.1_M, and 264.3_M). The Lower Section contains seven sites (266.1_L, 269.1_L, 270.1_L, 273.4_L, 273.4_L, 273.7_L, 274.2_L, and 274.6_L) (Fig. 1). Channel morphology is categorized as inside bend, outside bend, or straight away (I, O, S) and is also evaluated for species preference (Table 5).

Each site includes six transects spread out over a five-hundred-meter area with each transect spaced a hundred meters apart (Fig. 2). Each transect is one hundred meters perpendicular from the bank and segmented with ten 10 x 1-meter intervals (Fig. 2 and 3). The transect is marked by a lead core line lowered to the bottom with a tag denoting the interval number every ten meters. The lead line has an anchor on each end with a buoy line attached to the channel end. The surveyors utilize SCUBA to descend from the boat using the buoy line to reach the transect. Each interval is surveyed by the diver for a 5 minute minimum depending on substrate type (West Virginia Division of Natural Resources Wildlife Resources Section, 2022) (Fig. 3).

Figure 2

Transect Arrangement Within a Site



Note. Example of how six 100m transects (blue) within a single site are arrayed to constitute a 500m area.

Figure 3

Interval Arrangement Within a Transect

Note. Example of how ten 10 x 1m intervals constitutes a single 100m transect within a site.

I used Ocean Reef full face masks with Mercury wireless communication gear to audibly report to the boat the mussel bag number corresponding to the interval, substrate composition, and depth at the end of each interval. Live and dead unionids found within the interval are placed in a mesh bag and secured to the line at the end of each interval. All results were derived from numbers of live individuals. Deadshell provides evidence of species that may still occur in the pool. I visually estimated surface substrate composition using the modified Wentworth scale where bedrock is a large solid surface, boulder = >257mm, cobble = 65 – 256 mm, gravel = 2 – 64 mm, sand = < 2 mm, and fines are material that could be suspended in the water column (West Virginia Department of Environmental Protection, 2021). Once the transect is complete, the line is pulled and mussels from each interval are examined for species, sexual dimorphism, and length (mm). They are then placed back into the water in accordance with the 2022 WV Mussel Survey Protocol (West Virginia Division of Natural Resources Wildlife Resources Section, 2022).

Data Analysis

I utilized summary and interpretative statistics to reach project objectives. Both statistical approaches evaluated the assemblage as a function of river mile (*parallel distribution*) and distance from the bank (*perpendicular distribution*). First, I aggregated to the appropriate sample unit (pool, site, interval). I calculated summary statistics of the pool, site, and interval to get a general idea of the distribution and condition of the assemblage (Table 2-9). Dominate species, interval, substrate, average depth, and river morphology were also identified (Table 5 and 9). I calculated an estimate of the total number of individuals within 100 meters of both banks within the pool using the following series of equations (Table 2):

1. Pool Volume (gallons)

$$23{,}602{,}446.3\text{m}^2 \text{ (pool area)} \times 7.93\text{m (mean pool depth)} = 187{,}167{,}399\text{m}^3$$

$$187{,}167{,}399\text{m}^3 \times 264.172 \text{ gal/m}^3 \text{ (conversion factor)} = 4.94 \times 10^{10} \text{ gallons in RCB}$$

2. Pool Area of Interest (within 100m from each bank)

$$67{,}109.6\text{m (pool length)} \times 200\text{m (100m/bank)} = 13{,}421{,}920\text{m}^2$$

3. Percent AOI sampled within pool

50,000m^2 (single site area) x 18 sites = 900,000m^2

(900,000m^2 / 13,421,920m^2) x 100 = 6.71%

4. Estimation of total number of unionids within AOI of RCB

100% - 6.71% = 93.29% of AOI not sampled

93.29% x 1,083individuals (total collected) = 101,033 individuals (estimated remaining)

101,033ind. + 1,083 ind. = **102,116** estimated ind. in RCB Pool

5. Mean filtration rate of assemblage in RCB Pool

102,116 ind. x 8 gallons (mean unionid filtration rate) = **816,928** gallons filtered / day

6. Percent of pool filtered by assemblage each day

(816,928 gallons filtered / day / 4.94 x 10^10 gal.) x 100 = **0.004%**

Interpretative Statisics

I analyzed the distributions of the pool using free ordination and group testing (NMS and MRPP in PC-ORD v. 7.0, Peck, 2016). I plotted substrate composition and depth as a function of river mile to visualize their distribution across the pool (Figures 4 and 5). Substrate composition and depth was thought to potentially contribute to species redundancy.

I utilized two-dimensional Nonmetric Multidimensional Scaling (NMS) to analyze and compare the patterns in spatial distribution of the assemblages in the RCB and Greenup pool (Figures 6 - 13). This analysis is a nonparametric free ordination technique that reflects patterns of species redundancy in an ordination space unrestricted by any other variables i.e., it groups sites in an ordination space that are similar in species diversity and cooccurrence (species redundancy). Significant patterns of redundancy are evidence of potential underlying environmental gradients that may be affecting unionid species (Peck, 2016). Note that species redundancy here is being described as areas where composition and species cooccurrence is similar. It is not to be confused with redundancy relating to Redundancy Analysis (RDA).

I opted for a free ordination technique as opposed to guided because factors that drive unionid distribution are little understood (Box & Mossa, 1999). Data were highly skewed with a high degree of sparsity which warranted nonparametric testing.

To use this technique, several intermediate steps were needed. These steps include evaluating sparsity, choosing a relativization and distance measure, and determining the number of dimensions (Peck, 2016). The raw response (species) matrix of the RCB Pool Parallel Distribution ordination (Fig. 6) included the 19 live unionid species in the 18 sites.

I evaluated sparsity by determining the percentage of cells containing zero (count = 0), skewness, and variation (%CV) within the data. Percent zeros was 65.8% in the raw dataset. Over 50% generally means there is not enough useful information for the analysis to identify patterns in the dataset and no patterns will be detected (Peck, 2016). Species with less than three occurrences, meaning if they did not occur in at least three sites, were removed from the analysis. Eight species (*A. ligamentia, E. crassidens, L. complanata, L. ovata, L. siliquoidea, L. teres, M. nervosa, O. subtrotunda*) were removed for this reason and sparsity improved to 48.5%. This modification improved skewness from 2.6 to 2.0. Variation (%CV) improved from 195.3% to 139.8%. I used this method of data exploration in the remaining ordination solutions (Figures 7-13). Sparsity metrics for the final versions of each dataset are listed in Table 1.

A relativization by maximum was applied to all datasets to adjust the influence of highly abundant and dataset rare species in the analysis (Peck, 2016). This relativization downweights the influence of the highly abundant species (generalists) and upweights the influence of dataset rare species which allows the analysis to tell the story of the assemblage as opposed to only the generalists. This modification improved %CV to 31.5%.

The distance measure calculates similarities amongst all pairs of sample units. I used the Sorenson distance measure to analyze the heterogeneous response matrix as it is the most effective method for zero-rich datasets and focuses on the co-occurrences (redundancy) of the responses (species) (Peck, 2016).

A significant two-dimensional NMS solution (randomization test $p = 0.004$) with a final stress of 9.9 was chosen for interpretation after verifying consistency of interpretation among several NMS solutions. I then evaluated the two axes for significance (r squared > 40%). Significance is determined by the proportion of variance calculated as a proportion of variation in the reduced matrix relative to that in the original data matrix (Peck, 2016). Each species was then evaluated to determine whether it is correlated with the pattern of redundancy reflected by that axis.

I used this method of data analysis to generate the remaining ordination solutions with few exceptions. To produce the Robert C. Byrd Pool Perpendicular Distribution Ordination Solution (Fig. 8), I used the Chi squared distance measure to calculate ordination scores. This is due to data being near normally distributed and percentage of zeros are less than 20% (Table 1). Using the Chi squared distance measure, a significant two-dimensional NMS solution (randomization test $p = 0.02$) with a final stress of 6.2 was chosen for interpretation after verifying consistency of interpretation among several NMS solutions.

I deleted three sites (321.3_L, 331.8_L, and 337.6_L) with zero unionids found from matrices containing Greenup Pool data as matrix algebra does not allow for row totals equal to zero. For Greenup Pool ordination spaces (Fig. 9 and 10), a significant two-dimensional NMS solution (randomization test $p = 0.003$) with a final stress of 12.6 was chosen for interpretation after verifying consistency of interpretation among several NMS solutions. Combined pool ordination spaces (Fig. 11-13) best reflect a significant two-dimensional NMS solution (randomization test $p = 0.003$) with a final stress of 15.2 was chosen for interpretation after verifying consistency of interpretation among several NMS solutions.

Multi-Response Permutation Procedure (MRPP) is a multivariate nonparametric test of differences among groups. I used MRPP to test for differences in assemblage patterns of redundancy of pool location (U, M, L) and morphology (I, O, S). I also used MRPP to determine the similarity of the two pools unionid assemblage patterns. To do this, I optimized the data to correspond with NMS by modifying each dataset using a rank transformation (Peck, 2016).

Table 1

Sparsity Evaluation Metrics for All Datasets

Metrics	**RCB Parallel**	**RCB Perpendicular**	**Greenup Parallel**	**All Pools Parallel**
Skewness	2	0.7	2.4	3.1
% 0's	48.5	19.2	57.6	62.7
% CV	31.5	35.1	36	44.7

Note. Sparsity evaluation metrics from each dataset used to determine the viability for distance measures and ordination analysis.

Results

I recorded a total of 1083 live specimens comprised of 19 species in the RCB pool (Table 2). *Obliquaria reflexa* was the dominant species recorded across all 18 sites covering the pool and comprised nearly half (relative abundance = 43.5%) of the total live individuals collected. The Upper and Middle section produced similar counts in richness and abundance. Both were superior to the Lower section (Table 3). Interval 3 (30-40m) most often contained the highest abundance. Intervals 2-5 were the highest producing adjoined intervals. On average each site had 60.2 individuals and 8 species (Table 2). The maximum values recorded for abundance and richness are 268 individuals and 14 species. A total of 102,116 live unionid mussels were estimated to be in the first 100 meters of bank within the Robert C. Byrd Pool of the Ohio River. This assemblage has the capacity to filter 816,928 gallons of water a day, or 0.44 % of the entire pool each day (Table 1).

Robert C. Byrd Pool Summary

Table 2

Robert C. Byrd Pool Summary Estimates

Avg. Abundance (ind. / site)	60.2
Max. Abundance (ind. / site)	268
Total Abundance (all sites)	1083
Avg. Richness (sp. / site)	6.5
Max. Richness (sp. / site)	14
Total Richness (all sites)	19
Dominant Species	*O. reflexa*
Relative Abundance of Dominant Sp.	43.5
Avg. Density (ind/m^2)	0.15
Max. Density (ind/m^2)	0.44
Dominant Interval	3
Dominant Zone (group of 3 consecutive intervals)	2-4
Total Ind. within 100m from bank	102, 116
Pool Turnover (gal/day)	816, 928
Dominant Section	Upper - Middle

Note. Summary estimates of the RCB Pool investigated while describing its unionid assemblage. Only live specimens are included in this summarization. "Dominant" areas such as "Dominant Section" refer to where the highest counts in richness and abundance were recorded.

Obliquaria reflexa, *Ligumia recta*, and *Cyclonaias pustulosa*, (n = 471, 157, 139) are the three most dominant species and account for 70.8 % of the total assemblage collected. Only nine of the 19 species collected are not state or federal listed. One live *Obovaria subtrotunda* was found. It was not listed at the time of sampling. However, it, along with *Fusconaia subrotunda,* were federally listed under “Threatened” this year (Fish and Wildlife Service, Interior, 2023). Evidence (deadshell) of other federally listed species include *Fusconaia subrotunda, Plethobasus cyphyus,* and *Pleurobema clava*. Eight species occurred in less than four total sites and are considered dataset rare (Table 3).

Table 3

Robert C. Byrd Pool Species List

Species	Abundance	Composition	Occurrences	Status
Obliquaria reflexa - Threehorn Wartyback	471	43.5	17	Threatened (OH)
Ligumia recta - Black Sandshell	157	14.5	14	Threatened (OH)
Cyclonaias pustulosa - Pimpleback	139	12.8	10	
Amblema plicata - Threeridge	105	9.7	14	
Potamilus alatus - Pink Heelsplitter	69	6.4	12	
Theliderma metanevra - Monkeyface	38	3.5	7	Endangered (OH)
Lampsilis cardium - Plain Pocketbook	25	2.3	6	
Fusconaia flava - Wabash Pigtoe	21	1.9	5	
Ellipsaria lineolata - Butterfly	20	1.8	6	Endangered (OH)
Quadrula quadrula - Mapleleaf	12	1.1	7	
Megalonaias nervosa - Washboard	7	0.6	3	Endangered (OH)
Pleurobema cordatum - Ohio Pigtoe	5	0.5	4	Endangered (OH)
Lasmigonia complanata - White Heelsplitter	4	0.4	3	
Elliptio crassidens - Elephantear	3	0.3	2	Endangered (OH)
Actinonaias ligamentia - Mucket	2	0.2	2	
Lampsilis siliquoidea - Fatmucket	2	0.2	2	
Lampsilis ovata - Pocketbook	1	0.1	1	Endangered (OH)
Lampsilis teres - Yellow Sandshell	1	0.1	1	Endangered (OH)
**Obovaria subrotunda* - Round Hickorynut	1	0.1	1	Threatened ('23)
***Fusconaia subrotunda* - Longsolid	0	0.0	0	Threatened ('23)
***Plethobasus cyphyus* - Sheepnose	0	0.0	0	Endangered ('12)
***Pleurobema clava* - Clubshell	0	0.0	0	Endangered ('93)
****Leptodea fragilis* - Fragile Papershell	0	0.0	0	
****Pleurobema sintoxia* - Round Pigtoe	0	0.0	0	SPOC (OH)
****Reginaia ebenus* - Ebonyshell	0	0.0	0	Endangered (OH)
*****Cyprogenia stegaria* - Fanshell				Endangered ('90)
*****Lampsilis abrupta* - Pink Mucket				Endangered ('76)
*****Epioblasma triquetra* - Snuffbox Mussel				Endangered ('12)

Note. All species found within the Robert C. Byrd Pool, Ohio River during the 2021 and 2022 survey effort. An asterisk denote the species as one of the following: * live federally listed, ** deadshell only federally listed, ****species* = deadshell only, ****extirpated federally listed (no evidence found).

The Upper Section recorded the most individuals per site (mean abundance = 89.8 unionids) and had mean richness of 7.8 species per site. The Middle Section has the highest richness per site (mean richness = 8.5 species) and an average abundance of 86.2 individuals per site. The Lower Section only averaged 16.7 individuals per site and averaged only 3.9 species per site. Pool sections shared *O. reflexa* as the dominant species, but it is considerably more so in the Lower Section (*O. reflexa* relative abundance = 77.8%). Fines are the dominant substrate across all sections, but Large Woody Debris (LWD) became the second most dominant substrate in the Lower Section (Table 4).

Table 4

Robert C. Byrd Pool Section Summary Estimates

Section	Abundance	Mean Abundance	Relative Abundance (%)	Mean Richness	Dom. Species	Composition (%)	Dom. Int.	Dom. Zone	Dom. Morphology	Dom. Substrates	Mean Depth
Upper	449	89.8	41.5	7.8	O. ref	28.7	3	3-5	O/S	Fines - Gravel	16.7
Middle	517	86.2	47.7	8.5	O. ref	48.5	2	2-4	S	Fines - Gravel	21.2
Lower	117	16.7	10.8	3.9	O. ref	77.8	3	2-4	I	Fines - LWD	23.6

Note. Summary estimates of pool sections investigated while describing their unionid assemblage. Only live specimens are included in this summarization. “Dominant” is abbreviated as “Dom.”. “Interval” is abbreviated as “Int.”. Dom. Zone is comprised of three consecutive intervals with the highest counts.

Among the sites, 255_M has the most individuals collected and accounts for nearly a quarter (24.7%) of the sampled assemblage. It is second in richness only to site 238.7_U (richness = 14 species) and matched by 260.1_U (richness = 12 species). Only four of eighteen sites contained a species more dominant than *O. reflexa*. These include sites 238.7_U (*C. pustulosa*), 243.6_U (*L.recta)*, 259.4_M (*L.recta*), and 264.3 (*Amblema plicata*). *O. reflexa* is considerably more dominant in the Lower Section of the pool than the Upper or Middle sections (Table 5).

Table 5

Robert C. Byrd Site Summary Estimates

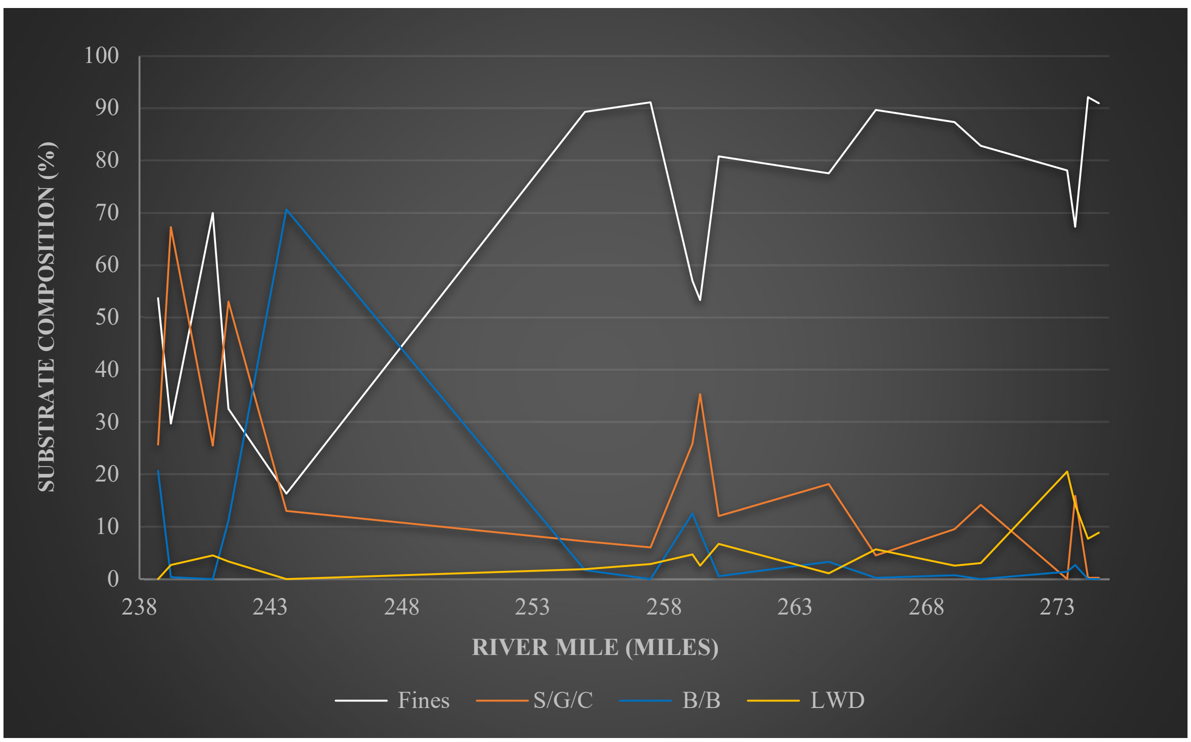
Sites	Abundance	Relative Abundance (%)	Richness	Dom. Sp.	Composition (%)	Dom. Interval	Morphology	Dom. Sub.	Composition (%)	Depth (avg.)
**238.7_U*	162	15	14	*C. pustulosa*	25.3	3	O	Fines	53.7	14.1
**239.2_U*	1	0.1	1	*O. reflexa*	100	4	I	Sand	34.7	17
**240.8_U*	83	7.7	9	*O. reflexa*	37.3	7	S	Fines	70	19.8
241.4_U	193	17.8	12	*O. reflexa*	30.1	3	S	Fines	32.5	18.8
243.6_U	10	0.9	3	*L. recta*	70	7	O	Bedrock	67.7	13.9
255_M	268	24.7	12	*O. reflexa*	56.3	2	S	Fines	89.3	23
257.5_M	71	6.6	11	*O. reflexa*	23.9	3	S	Fines	91.2	10.8
259.1_M	25	2.3	7	*O. reflexa*	52	3	S	Fines	57	24.3
259.4_M	18	1.7	4	*L. recta*	44.4	5	S	Fines	53.3	24.4
260.1_M	124	11.4	12	*O. reflexa*	49.2	2/3	S	Fines	80.8	21.4
264.3_M	11	1	5	*A. plicata*	27.3	3	S	Fines	77.6	23.3
266.1_L	13	1.2	3	*O. reflexa*	84.6	3	O	Fines	89.7	17.4
269.1_L	30	2.8	7	*O. reflexa*	73.3	3	S	Fines	87.3	21.1
270.1_L	3	0.3	2	*O. reflexa*	66.7	3	I	Fines	82.8	20.5
273.4_L	15	1.4	4	*O. reflexa*	80	4	I	Fines	78.1	26.5
273.7_L	16	1.5	3	*O. reflexa*	87.5	3	I	Fines	67.3	29.8
274.2_L	10	0.9	3	*O. reflexa*	50	4	I	Fines	92.2	25.8
274.6_L	30	2.8	5	*O. reflexa*	83.3	3	S	Fines	91	24.3

Note. Summary estimates of all sites investigated while describing their unionid assemblage. Only live specimens are included in this summarization. “Dominant” is abbreviated as “Dom.”. “Interval” is abbreviated as “Int.”. Dom. Zone is comprised of three consecutive intervals with the highest counts.

As river mile and distance from the upstream dam increases, the number of fines and depth also increase. Fines increased in an exponential fashion until around site 255_M (fines = 89.3%) and 257.5_M (fines = 91.2%). Nine sites downstream generally stayed between 80 and 90%. Exceptions occurred in sites at the ORM 259 with fines dropping to 57.0 and 53.3% respectively and a spike in the more heterogenous sand, gravel, cobble mix (S/G/C) substrate type. Other anomalies include the spike of bedrock (bedrock = 67.7%) at 243.6_U, and the sharp decline in depth at 257.5_M (depth = 10.8ft) (Figure 5 and 6).

Figure 4

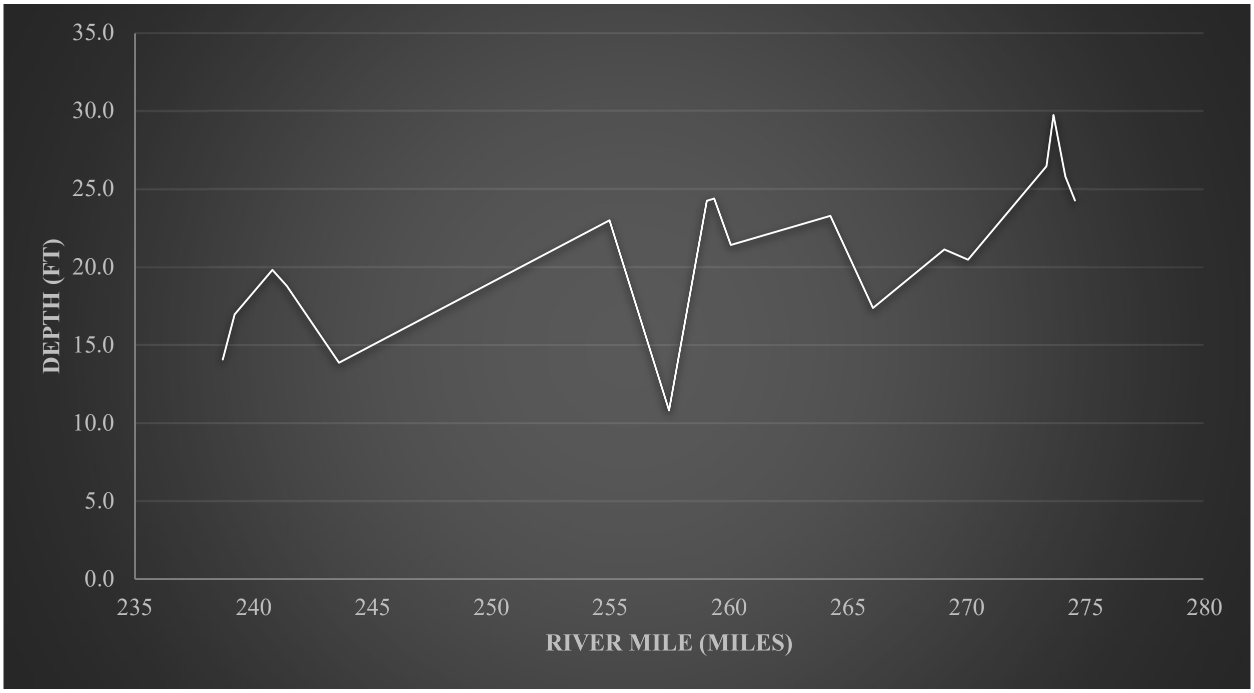
Benthic Habitat Distribution as a Function of Ohio River Mile



Note. This scatterplot reflects the distribution of the benthic habitat (substrate type and LWD) across all sites and thus within the pool. Early, fines become the dominant substrate by a large margin and LWD begins to increase toward the end of the pool.

Figure 5

Depth as a Function of Ohio River Mile



Note. Average depth sampled across all sites. Depth appears to generally increase with river mile that include several exceptions such as sites at ORM 259.

Greenup Pool Summary

Over 6000 live specimens comprised of 24 species were found over 34 randomized pool-wide distributed sites (Table 6) (Kriege, 2018; Miller, 2023). *Obliquaria reflexa* was the dominant species recorded and accounted for a third of the total abundance (relative abundance = 33.3%) of the live individuals collected (Table 6 and 7). The Upper Section dominated the dataset with 5304 live unionids found (Table 8). The average site had 178.7 individuals and 7.7 species. The maximum values recorded for abundance and richness are 1278.5 individuals and 18 species. A total of 566,437 live unionid mussels were found in the first 100 meters of bank within the Greenup Pool of the Ohio River. This assemblage has the capacity to filter 4,531,497 million gallons of water a day, or 5.18% of the entire pool every day. (Table 6).

Table 6

Greenup Pool Summary Estimates and Comparison

	RCB	Greenup
Avg. Abundance (ind. / site)	60.2	178.7
Max. Abundance (ind. / site)	268	1278.5
Total Abundance (all sites)	1083	6077
Number of Sites Sampled	18	34
Avg. Richness (sp. / site)	6.5	7.7
Max. Richness (sp. / site)	14	18
Total Richness (all sites)	19	24
Dominant Species	*O. reflexa*	*O. reflexa*
Relative Abundance of Dominant Sp.	43.5	33.3
Avg. Density (ind/m^2)	0.001	0.004
Max. Density (ind/m^2)	0.005	0.030
Total Ind. within 100m (ind.)	102,116	566,437
Pool Turnover (gal/day)	816,928	4,531,497
Best Section	Upper - Middle	Upper

Note. Summary estimates of the Greenup Pool investigated while describing its unionid assemblage. Only live specimens are included in this summarization.

Obliquaria reflexa, *Cyclonaias pustulosa*, and *Ligumia recta*, (n = 2025, 1592, 543) are the three most dominant species and together account for 68.4 % of the total assemblage collected. Half of the species collected are either state or federally listed. *Plethobasus cyphyus* was the only federally listed species found live (n = 14). Five species occurred in less than four total sites and are considered dataset rare (Table 7)

Table 7

Greenup Pool Species List

Species	Abundance	Relative Abundance	Occurrences	Status
Obliquaria reflexa **- Threehorn Wartyback**	2025	33.3	29	Threatened(OH)
Cyclonaias pustulosa **- Pimpleback**	1592	26.2	22	[redacted]
Ligumia recta **- Black Sandshell**	543	8.9	21	Threatened(OH)
Ellipsaria lineolata **- Butterfly**	454	7.5	17	Endangered(OH)
Theliderma metanevra **- Monkeyface**	402.5	6.6	16	Endangered(OH)
Amblema plicata **- Threeridge**	366.5	6.0	19	[redacted]
Potamilus alatus **- Pink Heelsplitter**	291.5	4.8	24	[redacted]
Elliptio crassidens **- Elephantear**	128.5	2.1	11	Endangered(OH)
Pleurobema cordatum **- Ohio Pigtoe**	93.5	1.5	13	Endangered(OH)
Lampsilis cardium **- Plain Pocketbook**	67.5	1.1	15	[redacted]
Quadrula quadrula **- Mapleleaf**	47	0.8	11	[redacted]
Megalonaias nervosa **- Washboard**	36.5	0.6	11	Endangered(OH)
Actinonaias ligamentia **- Mucket**	21	0.3	10	[redacted]
Reginaia ebenus **- Ebonyshell**	16	0.3	7	Endangered (OH)
Plethobasus cyphyus **- Sheepnose**	14	0.2	6	Endangered ('12)
Pleurobema sintoxia **– Round Pigtoe**	7	0.1	1	SPOC (OH)
Fusconaia flava **- Wabash Pigtoe**	6.5	0.1	5	[redacted]
Truncilla truncata **- Deertoe**	5.5	0.1	5	SPOC (OH)
Lampsilis siliquoidea **- Fatmucket**	3.5	0.1	4	[redacted]
Leptodea fragilis **- Fragile Papershell**	3	0.0	4	[redacted]
Lasmigonia complanata **- White Heelsplitter**	2.5	0.0	2	[redacted]
Pyganodon grandis **- Giant Floater**	1	0.0	1	[redacted]
Tritogonia verrucosa **- Pistolgrip**	1	0.0	1	[redacted]
Lampsilis teres **- Yellow Sandshell**	0.5	0.0	1	Endangered(OH)

Note. All species found within the Greenup Pool, Ohio River during the Miller and Kriege thesis collection (Kriege, Mitchell, 2018; J. Miller, 2023). An asterisk (*) denotes the species as a live federally listed species.

The Upper Section has the highest diversity and dominates all other sections in either pool (mean abundance = 357.1 individuals, mean richness = 11.8 species). Pool sections shared *O. reflexa* as the dominant species, but it is considerably more so in the Lower Section (*O. reflexa* relative abundance = 64.4%) (Table 8).

Table 8

Greenup Pool Section Summary Estimates and Comparison

Section	Abundance	Mean Abundance	Richness	Mean Richness	Dom. Species	Relative Abundance (%)	Dom. Morphology
Upper_RCB	449.0	89.8	15.0	7.8	*O. reflexa*	28.7	O/S
Upper_Gr.	5304.0	357.1	23.0	11.8	*O. reflexa*	33.0	S
Middle_RCB	517.0	86.2	15.0	8.5	*O. reflexa*	48.5	S
Middle_Gr.	714.0	102.0	16.0	8.4	*O. reflexa*	32.9	S
Lower_RCB	117.0	16.7	9.0	3.9	*O. reflexa*	77.8	I
Lower_Gr.	59.0	4.9	7.0	1.5	*O. reflexa*	64.4	S

Note. Summary estimates of pool sections investigated while describing and comparing their unionid assemblages. Only live specimens are included in this summarization. "Dominant" is abbreviated as "Dom.". "Interval" is abbreviated as "Int.". Dom. Zone is comprised of three consecutive intervals with the highest counts.

Among the sites, 290.2_U* has the most individuals collected and accounts for nearly a quarter (21.0%) of the sampled assemblage. It is second in richness only to site 281.6_U (richness = 19 species). Only six of thirty-four sites contained a species more dominant than *O. reflexa*. *O. reflexa* is considerably more dominant in the Lower Section of the pool than the Upper or Middle sections. No unionid mussels were found in three Lower Section sites (321.3_L, 331.8_L, and 337.6_L) (Table 9).

Table 9

Greenup Pool Site Summary Estimates

Sites	Abundance	Relative Abundance (%)	Richness	Dom. Sp.	Composition (%)	Morphology
280.8_U	573	9.43	15	*O. reflexa*	28.10	S
280.9_U	61	1.00	8	*O. reflexa*	39.34	S
281.6_U*	343.5	5.65	19	*O. reflexa*	30.70	S
281.8_U	248	4.08	12	*O. reflexa*	35.48	S
283.0_U	339	5.58	14	*O. reflexa*	32.15	I
284.9_U	206	3.39	10	*O. reflexa*	31.07	I
288.2_U	883	14.53	14	*C. pustulosa*	39.75	I
290.2_U*	1278.5	21.04	18	*O. reflexa*	33.44	O
292.1_U	8	0.13	5	*O. reflexa*	37.50	I
292.4_U	34	0.56	5	*O. reflexa*	35.29	I
294.1_U	284	4.67	15	*O. reflexa*	43.66	S
299.3_U	362	5.96	10	*O. reflexa*	62.15	S
300.6_U	346	5.69	14	*C. pustulosa*	29.48	I
301.4_U	338	5.56	13	*C. pustulosa*	31.66	I
303.3_U	52	0.86	5	*O. reflexa*	69.23	S
305.3_M	6	0.10	4	*O. reflexa*	50.00	O
305.8_M	192	3.16	13	*O. reflexa*	33.33	O
307.3_M	72	1.18	10	*O. reflexa*	34.72	S
309.1_M	352	5.79	16	*O. reflexa*	30.40	S
311.6_M	6	0.10	2	*O. reflexa*	66.67	S
313.6_M	80	1.32	12	*O. reflexa*	35.00	I
316.8_M	6	0.10	2	*O. reflexa*	66.67	I
321.1_L	20	0.33	5	*O. reflexa*	40.00	O
321.3_L	0	0.00	0	*N/A*	0.00	I
322.0_L	2	0.03	2	*O. reflexa*	50.00	I
324.6_L	1	0.02	1	*C. pustulosa*	100.00	S
329.2_L	2	0.03	2	*O. ref / C. pus*	50.00	O
331.8_L	0	0.00	0	*N/A*	0.00	S
334.6_L	1	0.02	1	*L. recta*	100.00	I
335.5_L	5	0.08	1	*O. reflexa*	100.00	O
337.6_L	0	0.00	0	*N/A*	0.00	I
339.7_L	4	0.07	2	*O. reflexa*	75.00	S
340.1_L	16	0.26	2	*O. reflexa*	87.50	S
340.4_L	8	0.13	2	*O. reflexa*	75.00	S

Note. Summary estimates of all sites investigated while describing their unionid assemblage. Only live specimens are included in this summarization. “Dominant” is abbreviated as “Dom.”. “Interval” is abbreviated as “Int.”. Dom. Zone is comprised of three consecutive intervals with the highest counts.

Interpretative Statistics

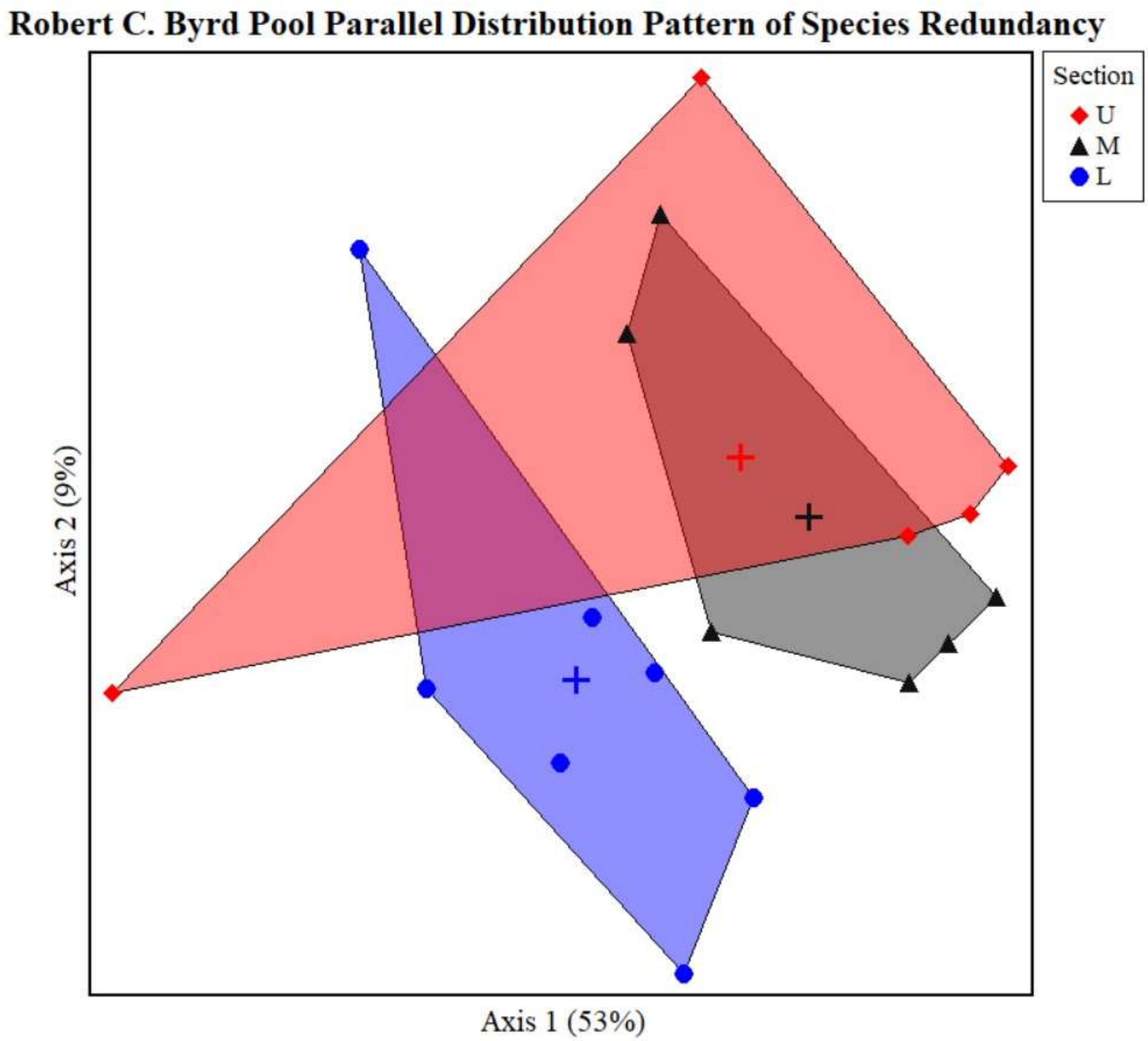
RCB Pool Parallel Distribution Analysis

The proportion of variance represented by NMS ordination axis 1 was 53%, while axis 2 represented an additional 12%. Six species were found to be trending with axis 1 including *Cyclonaias pustulosa* (r^2 = 70%), *Ellipsaria lineolata* (r^2 = 76%), *L. recta* (r^2 = 74%), *Potamilus alatus* (r^2 = 80%), *Pleurobema cordatum* (r^2 = 45%), and *Quadrula quadrula* (r^2 = 47%). No abiotic variables collected correlated with either axis.

MRPP results show Robert C. Byrd pool section patterns of redundancy are not all the same (MRPP, $p = 0.03$, $A = 0.13$). The Upper and Middle Section are not significantly different (MRPP, $p = 0.59$, $A = -0.03$). Both are significantly different from the Lower Section (MRPP, U-L: $p = 0.02$, $A = 0.15$; MRPP, M-L: $p = 0.02$, $A = 0.14$). Channel morphology types do not show a difference in pattern of unionid redundancy in the Robert C. Byrd Pool (MRPP, $p = 0.12$, $A = 0.07$). Straightaway and inside bend are the only types significantly different (MRPP, $p = 0.01$, $A = 0.12$).

Figure 6

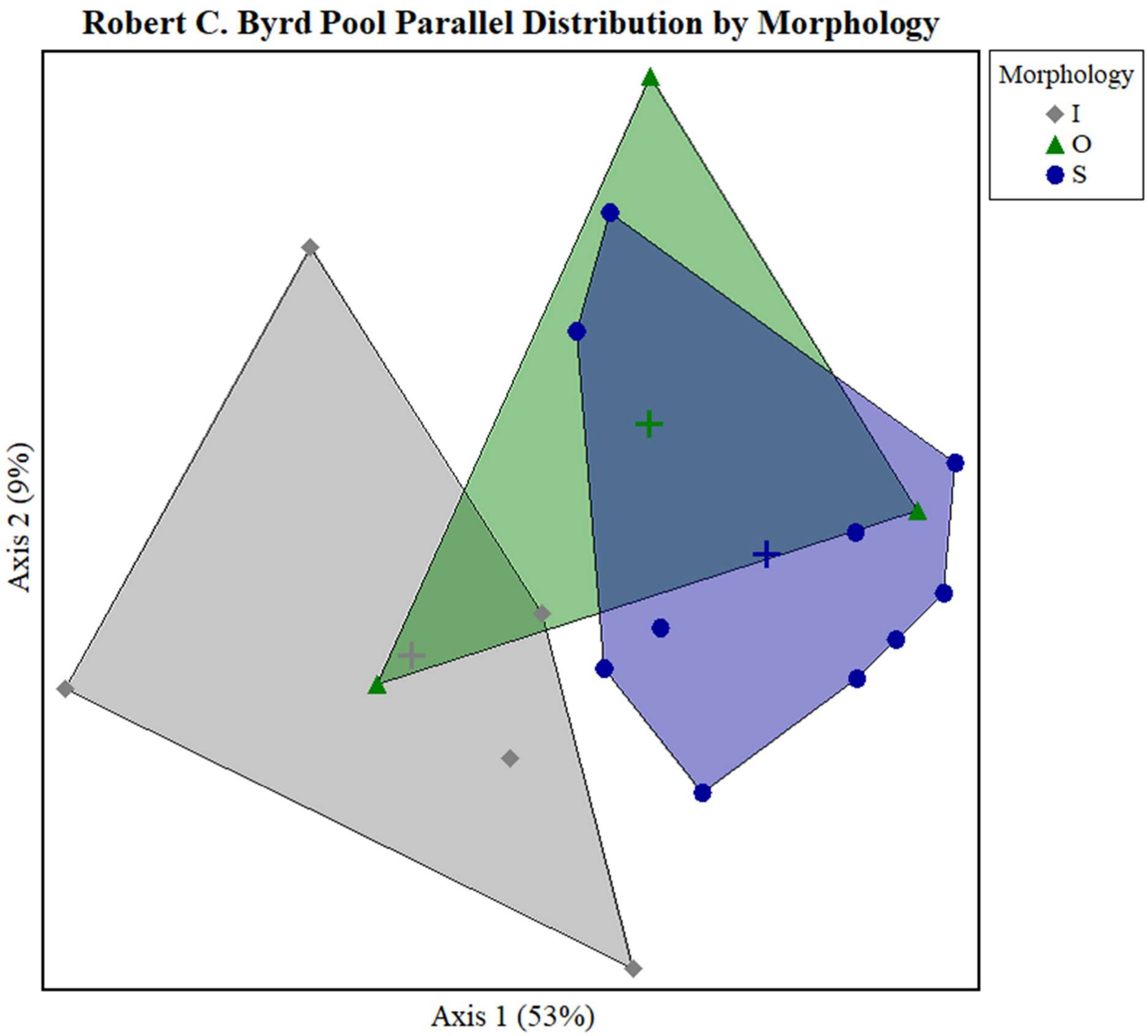
Robert C. Byrd Pool Parallel Distribution Ordination Space



Note. Robert C. Byrd Pool ordination solution with pool sections indicated by color reflecting a significant pattern of species redundancy among sites. Centroids are represented as "+". Centroids near another and polygons overlapping indicate similarity.

Figure 7

Robert C. Byrd Pool Parallel Distribution by Morphology Ordination Space



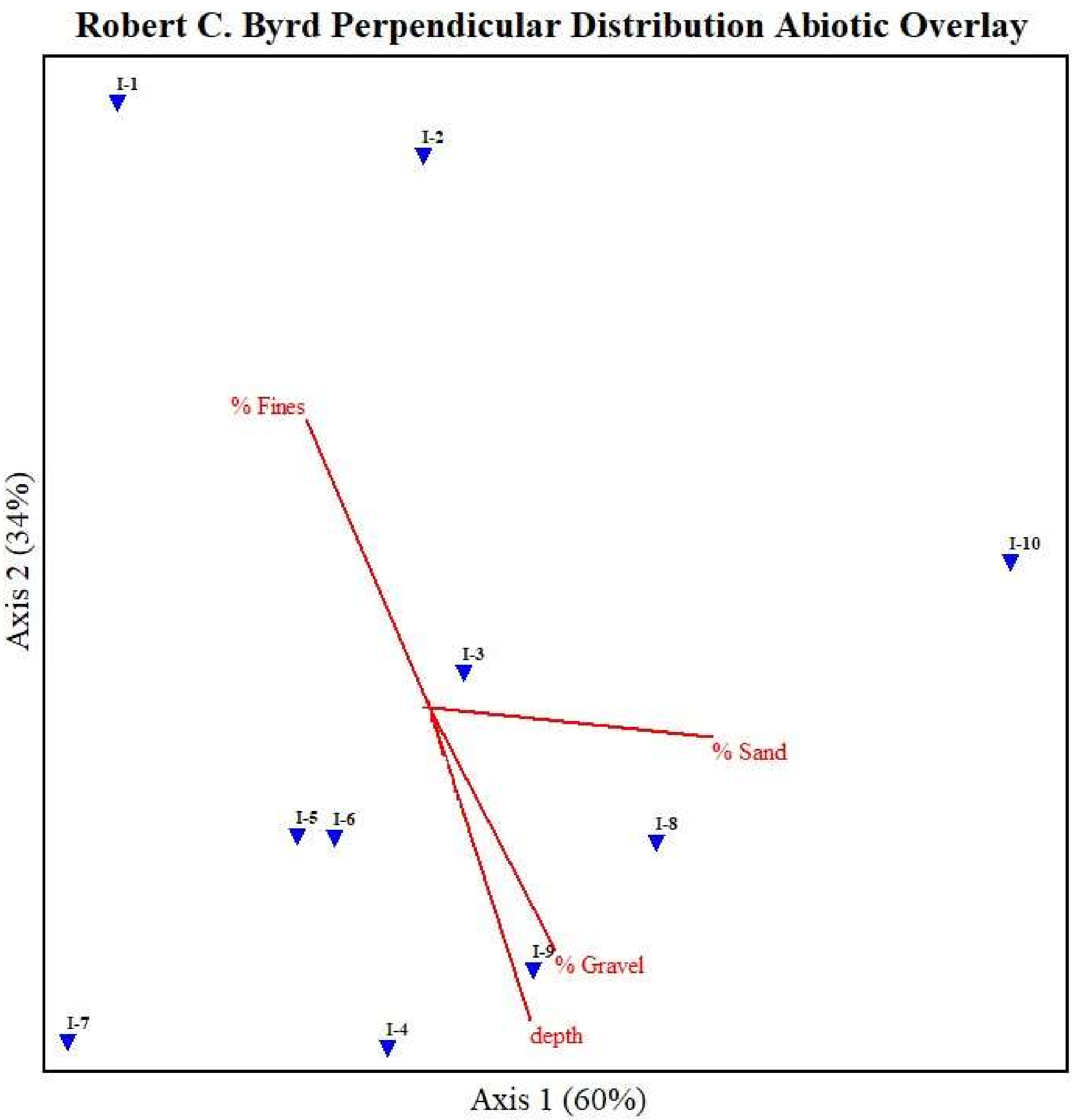
Note. Robert C. Byrd Pool ordination solution with morphology types indicated by color reflecting a significant pattern of species redundancy among sites. Centroids are represented as "+". Centroids near another and polygons overlapping indicate similarity.

RCB Pool Perpendicular Distribution Analysis

The proportion of variance represented by NMS ordination axis 1 was 60%, while axis 2 represented an additional 34%. Seven species were found to be trending with axis 1 including *Lampsilis cardium* (r^2 = 55%), *Ligumia recta* (r^2 = - 47%), *Megalonaias nervosa* (r^2 = 45%), *Potamilus alatus* (r^2 = - 44%), *Pleurobema cordatum* (r^2 = 48%), and *Quadrula quadrula* (r^2 = - 60%) . Fines are correlated (r^2 = - 51%) with Axis 1. Sand (r^2 = 78%), gravel (r^2 = 52%) , and depth (r^2 = 46%) are correlated with Axis 1 as well.

Figure 8

Robert C. Byrd Pool Perpendicular Distribution Ordination Space



Note. Robert C. Byrd Pool Perpendicular Distribution ordination solution reflecting a significant pattern of species redundancy among intervals.

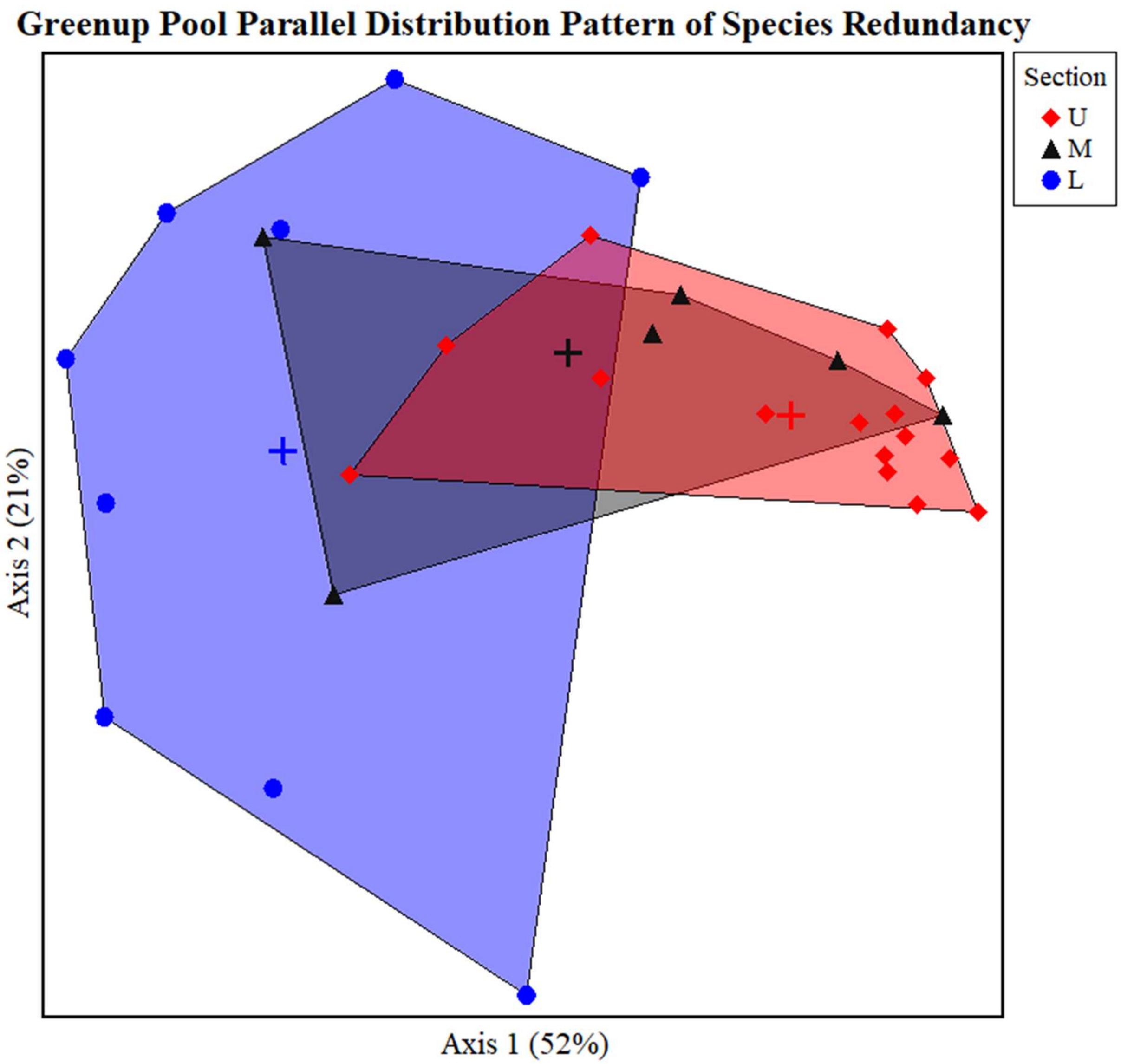
Greenup Pool Parallel Distribution Analysis

The proportion of variance represented by NMS ordination axis 1 was 52%, while axis 2 represented an additional 22%. Ten species were found to be trending with axis 1 including *Potamilus alatus* (r^2 = 75%), *Lampsilis cardium* (r^2 = 66%), *Reginaia ebenus* (r^2 = 43%), *Actinonaias ligamentia* (r^2 = 66%), *A. plicata* (r^2 = 76%), *Quadrula quadrula* (r^2 = 56%), *Ligumia recta* (r^2 = 75%), *Obliqueria reflexa* (r^2 = 69%), *Truncilla truncata* (r^2 = 44%), and the federally listed *Plethobasus cyphyus* (r^2 = 47%).

MRPP results show Greenup Pool section patterns of redundancy are not all the same (MRPP, $p = 0.00$, $A = 0.19$). The Upper Section pattern of redundancy is significantly different than the Middle and Lower Section (MRPP, U-M: $p = 0.0498$, $A = 0.06$; MRPP,U-L: $p = 0.00$, $A = 0.22$). Middle and Lower Section patterns are not statistically different (MRPP, $p = 0.07$, $A = 0.07$). Channel morphology types do not show a difference in pattern of unionid redundancy in the Greenup Pool (MRPP, $p = 0.75$, $A = -0.02$). No morphology type was statistically different than the other.

Figure 9

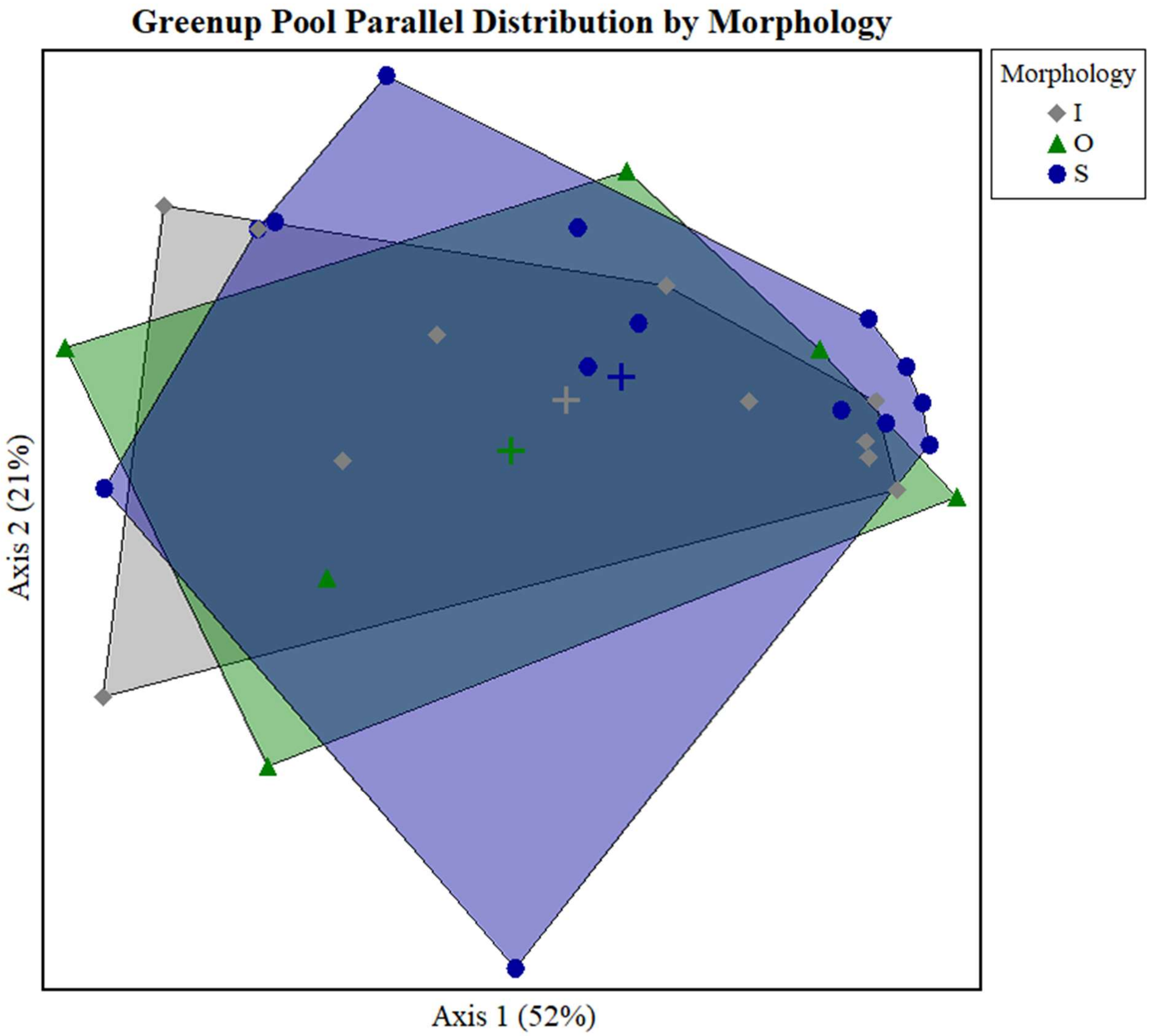
Greenup Pool Parallel Distribution Ordination Space



Note. Greenup Pool ordination solution with pool sections indicated by color reflecting a significant pattern of species redundancy among sites. Centroids are represented as "+". Centroids near another and polygons overlapping indicate similarity.

Figure 10

Greenup Pool Parallel Distribution by Morphology Ordination Space



Note. Greenup Pool ordination solution with morphology types indicated by color reflecting a significant pattern of species redundancy among sites. Centroids are represented as "+". Centroids near another and polygons overlapping indicate similarity.

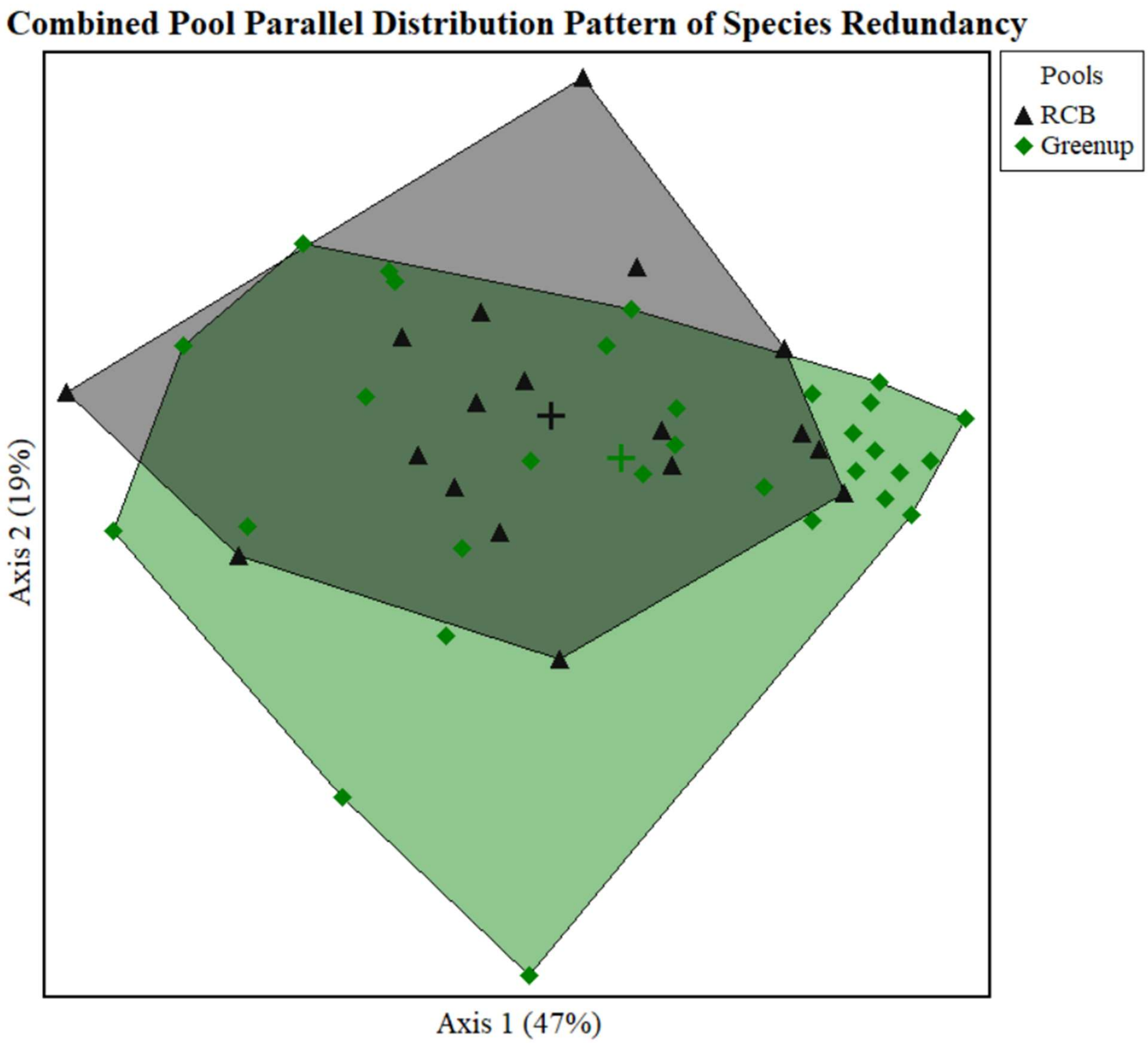
Combined Pool Parallel Distribution Analysis

The proportion of variance represented by NMS ordination axis 1 was 47%, while axis 2 represented an additional 18%. Eleven species were found to be trending with axis 1 including *Actinonaias ligamentia* (r^2 = 62%), *Amblema plicata* (r^2 = 75%), *Ellipsaria lineolata* (r^2 = 60), *Lampsilis cardium* (r^2 = 69%), *Ligumia recta* (r^2 = 76%), *Lampsilis siliquoidea* (r^2 = 41%), *Megalonaias nervosa* (r^2 = 51%), *Potamilus alatus* (r^2 = 74%), *Quadrula quadrula* (r^2 = 54%), *Truncilla truncata* (r^2 = 41%), and the federally listed *Plethobasus cyphyus* (r^2 = 45%).

MRPP results show the Robert C. Byrd and Greenup Pool patterns of species redundancy are not significantly different (MRPP, $p = 0.09$, $A = 0.01$). Pool section patterns of redundancy of the combined pool dataset are not all the same (MRPP, $p = 0.00$, $A = 0.18$). Each pool section is significantly different than the other (MRPP, U-M: $p = 0.03$, $A = 0.04$; U-L: $p = 0.00$, $A = 0.22$; M-L: $p = 0.00$, $A = 0.11$). Channel morphology types do not show a difference in pattern of unionid redundancy in the combined pool dataset (MRPP, $p = 0.55$, $A = -0.01$). No morphology type was statistically different than the other.

Figure 11

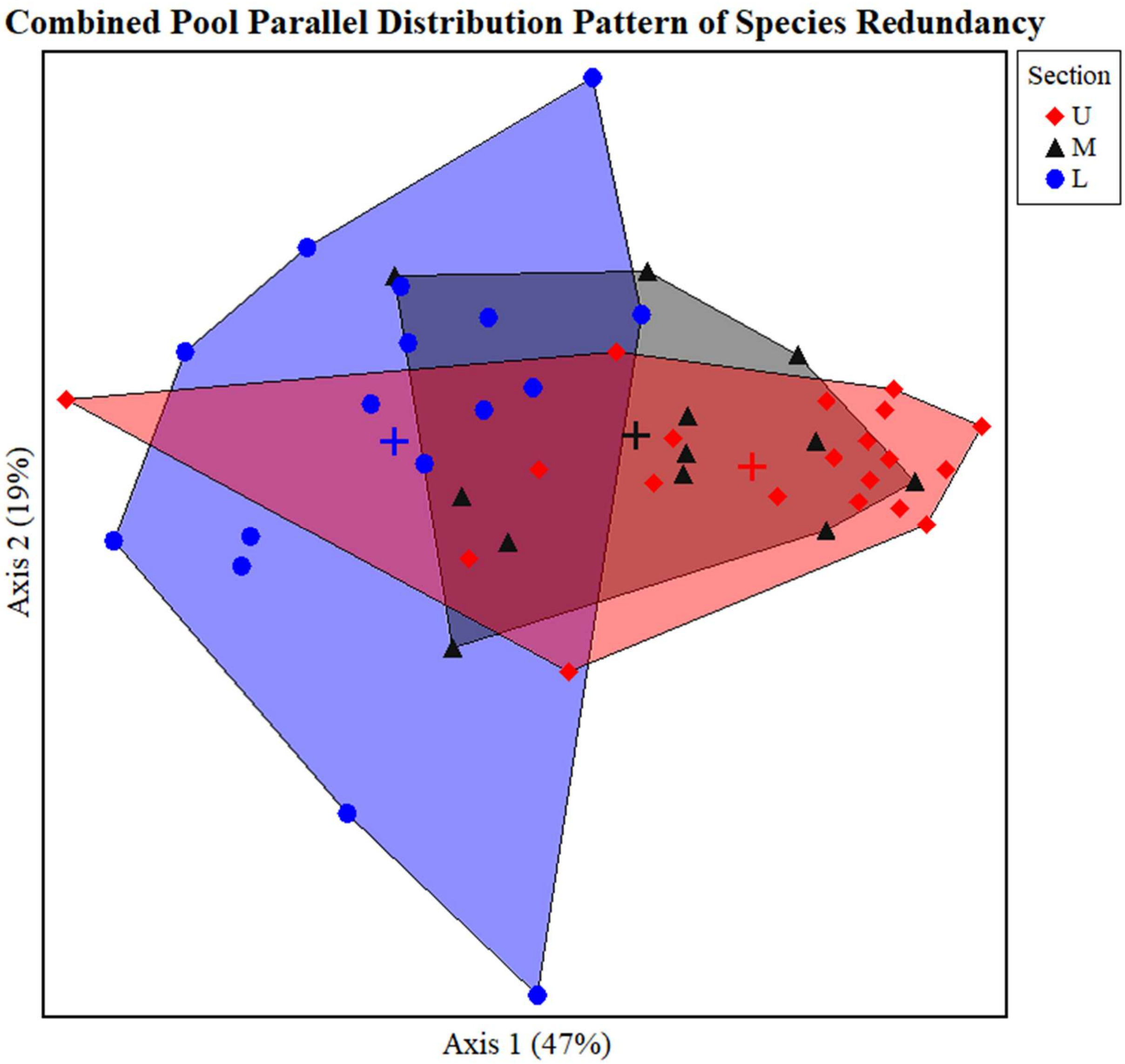
Overlaid RCB and Greenup Pool Parallel Distribution Ordination Space



Note. Combined ordination solution with RCB and Greenup pools indicated by color reflecting a significant pattern of species redundancy among sites. Centroids are represented as "+". Sections are denoted by the letter on the site name and can be qualitatively compared. Centroids near another and polygons overlapping indicate similarity.

Figure 12

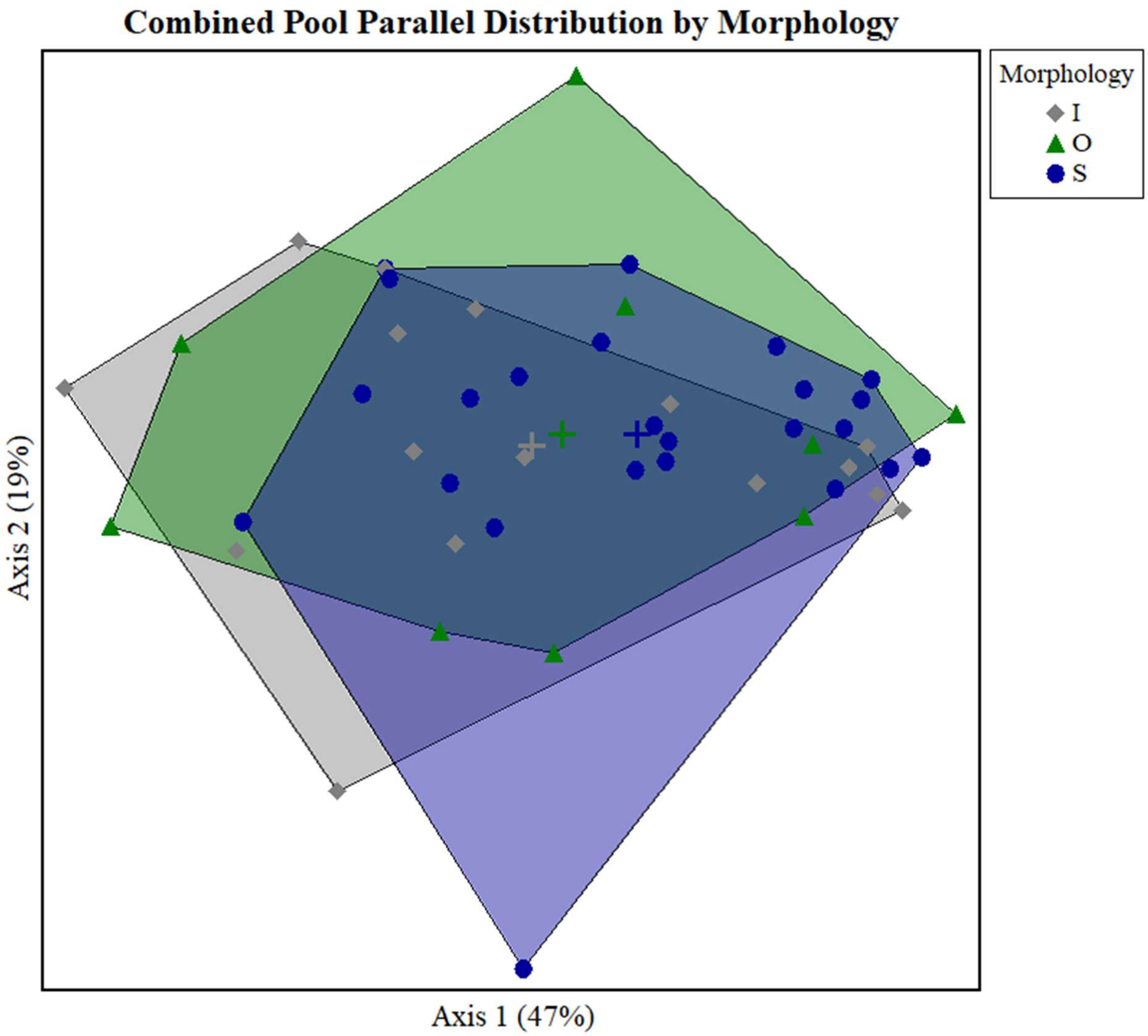
Combined Pool Parallel Distribution Ordination Space by Section



Note. Robert C. Byrd and Greenup Pool combined ordination solution with pool sections indicated by color reflecting a significant pattern of species redundancy among sites. Centroids are represented as “+”. Centroids near another and polygons overlapping indicate similarity.

Figure 13

Combined Pool Parallel Distribution Ordination Space by Morphology



Note. Robert C. Byrd and Greenup Pool combined ordination solution with morphology types indicated by color reflecting a significant pattern of species redundancy among sites. Centroids are represented as "+". Centroids near another and polygons overlapping indicate similarity.

Discussion

Pool Summary and Condition

This study provides baseline representation of the unionid assemblage inhabiting the Robert C. Byrd Pool. It also evaluates its condition through comparative analysis of the Greenup Pool and analyzes its spatial distribution for patterns of species redundancy. Large navigable rivers are notoriously difficult to sample for unionids due to depth, flow regimes, and recreational/commercial traffic. The requirement of SCUBA gear/training, small boat expertise, and multiple personnel make for additional logistical challenges with this type of sampling. For these reasons, most of what we know about unionids comes from smaller streams despite large river species being among some of the most impacted. The information we do have about large river unionids comes from site-specific consulting reports as opposed to a randomized, representative biomonitoring effort. Historic biomonitoring efforts either neglect unionids all together or are often done using brail surveys which are known to be biased toward certain species (Dolson, et al., 2023). The intensive biomonitoring-oriented sampling design of this study optimizes our understanding of the unionids in this pool of the Ohio River and others alike. Furthermore, it provides significant insight for assemblage – scale best management practices to be implemented as well as follow on studies interested in spatial and temporal relationships.

Robert C. Byrd Pool appears to be in a more degraded condition compared to the Greenup Pool (Table 6). This was an unexpected result as I hypothesized the RCB Pool to be in better condition due to less urbanization and subsequent impact. The RCB Pool receives a high level of potential impact for its size. In less than 50 miles of river there are numerous industries including three power plants, constant barge traffic, and the influence of the Kanawha River. The Kanawha influence seems to play some role in the low counts of the Lower Section as all sites below the

Kanawha belong to the Lower Section and have much lower values (Table 4). This is not surprising given the impact the lower Kanawha receives from urbanization and industry. For example, the lower 10 miles of the Kanawha River is dominated by continuous mooring pins on both banks which catch and accumulate fine sediments. While diving site 266.1_L below the mouth of the Kanawha, the substrate was thick with over a foot of fines and detritus material (Fig. 1).

The top and fourth sites highest in abundance demonstrate how difficult it can be to pinpoint relevant factors influencing unionids in big rivers. Site 255_M accounted for nearly a quarter (24.7%) of the RCB sampled abundance and is closest in proximity to the tributary devastated by the 1993 dewatering of Meigs Mine 31 (Table 5, Fig. 1) (Allman, Karyn, 2006). Despite an estimated 1 billion gallons of untreated and partially treated AMD entering the tributary, unionids of the mainstem seemed relatively unaffected during the investigative survey (Heidi Dunn, personal communication 4/12/23). Also surprising, fines was by far the dominant substrate at this site and depth was moderately high (Table 4, Fig. 4 and 5).

Site 260.1_M is just downstream of Kyger Creek Power Plant and was hypothesized to yield very few mussels. This site is the fourth most abundant and accounts for more than 10% of the sampled RCB assemblage. Site 260.1_M is directly below the warm water discharge of Kyger Creek Power Plant (Fig. 1). Sampling at this site occurred later in the season and water temperatures were noticeably higher than the surrounding area. Fish are known to use the sustained warmer temperatures of industrial discharges throughout the colder months of the year. This could account for the larger number of mussels found as host species are congregated in this small area for longer periods of time.

Fines were also clearly the dominant substrate at site 260.1_M and accounted for over 80% of its composition (Table 4). Both noteworthy sites scoring such a high level of fines adds to the debate as to how much substrate matters for unionid assemblages. I recommend future surveys of this kind with a goal to find significant substrate patterns related to unionids should focus on adjusting substrate data collection methodology. This study recorded substrate composition by whatever was on the benthic surface. Observation suggests there could be a difference in unionid response to the depth of fine sediments. A small layer of fines is seen throughout much of the pool whereas often feet of fine sediments are characteristic of the Lower Section of pools. To capture this difference, weighting fines by another variable such as embeddedness may be useful.

Regarding difference in pool section, I expected the upper reaches of pools to generally be the most diverse with numbers significantly dropping downstream due to upper reaches being a more natural, lotic environment. Because of the high-rise dams, water velocity slows, suspended sediments fall, and depth increases (Fig. 4 and 5) which I thought would account for the scarcity of unionids. When looking at Lower Section counts of both pools compared to others, this seems to obviously be the case (Table 8). However, Upper and Middle Section counts in the RCB Pool are very similar (Fig. 4). I believe this to be, at least in part, because of the lack of unionid refugia in the Upper Section of RCB.

Upon further evaluation of the comparison between the condition of the RCB and Greenup pools, an argument could be made that Greenup isn't as well off as it seems. The Upper Section in this pool accounts for over 80% of the 6,077 unionids found (Table 8). The federally listed Sheepnose (*Plethobasus cyphyus)* accounted for 14 of those individuals. The Upper Section of the Greenup Pool held 23 of the 24 species found in the while only 19 species were found in the entire RCB Pool. This section of the pool is mostly rural, and urbanization does not occur until the lower two thirds of the pool. At this point, the variation between the Greenup and RCB greatly reduces. In the case of the Lower Section, the RCB Pool values are higher (Table 8).

From this perspective, it seems as though only the more rural Upper Section of the Greenup Pool is superior to the RCB Pool. This reach is by far the most diverse area in over 100 miles of Ohio River. It is home to several federally listed species including *Plethobasus cyphyus, Pleurobema clava, Obovaria subrotunda, and Cyprogenia stegaria.* Despite these facts, barges can still often be found illegally banking on its shores. Industry is currently reshaping the landscape surrounding this reach as well.

Pool Pattern and Spatial Distribution

I found all ordination solutions to have a singular significant ($p > 0.05$, %CV > 40) axis to interpret (Fig. 6 – 13). The statistically significant (randomization test $p = 0.004$) two – dimensional ordination solution of the RCB Pool Parallel Distribution shows that the assemblage is not randomly distributed across the pool (Fig. 6). The trending species indicate that they may be responding to some underlying environmental gradient. This supports the idea that there is some combination of factors that account for unionid presence despite controversy over abiotic variables such as substrate composition. Two species, *P. cordatum* and *Q. quadrula,* demonstrate a weak but consistent association to this pattern where found ($r^2 < 50\%$). *O. reflexa* was not found to be trending despite being most abundant. Because of its generalist nature and abundance, it does not provide any useful information in determining why mussels are where they are.

I found the RCB Parallel Distribution pattern of species redundancy to be statistically similar to the Greenup Pool Parallel Distribution (Fig. 11). There is a unionid pool pattern and Figure 11 reflects the face of it. This is highly relevant as it indicates that not only are unionids not randomly distributed in pools, but they are responding to an unidentified environmental gradient in the same manner. This means if we could determine what environmental factors are significantly attributing to this gradient, we can more effectively manage and protect unionid assemblages at the pool scale. Federally listed *P. cyphyus* was among the species to respond with this pattern in both the Greenup (r^2 = 47%) and Combined Pool Parallel Distribution Analysis (r^2 = 45%).

Unionid distribution patterns in sections among pools do not mirror each other (Fig. 6, 7, 9-13). Sections in both the RCB and Greenup Pool which had the highest diversity and thus attributed the most information, were similar. In the RCB Pool, that is the Upper and Middle Section. In the Greenup Pool, that is the solely the Upper Section. However, if the two outlier sites were removed from the Middle Section in Figure 9, I believe the pattern in the Middle Section would be statistically similar to the Upper Section as it is in the RCB Pool. Sparsity within Lower Section of each pool left little information to determine a pattern of redundancy. It's clear from Figure 6, 9, and 12 that the pattern of Lower Section is distinct from the others, regardless of any outliers.

Relationships among morphology types in pools were also not uniform. Inside Bend spatial patterns, if any at all, were different than the Outside Bend and Straightaway in the RCB Pool. This is different from the Greenup and Combined Pool datasets where all morphology types were found to be the same.

I believe methodology will need to be revised to determine whether morphology type has a difference in unionid presence and spatial distribution. There is a high degree of variation among pool sections. In the classification of morphology for this study, the pool was treated as if all sections were the same. For example, there are only three sites in the RCB Pool classified as

Outside Bend. Two are in the Upper Section and one in Lower. Because of their difference in abundance, more replication of morphology types needs to occur in the same area so the difference in types will be relative to their location.

Study design could also be improved for pool section analysis. This is seen as the lower A– value suggests a less than ideal degree of heterogeneity within sections. Now that we have baseline data for these pools, a cluster analysis could be used to determine where differences in pools sections actually occur instead of choosing them a priori.

Most intervals are alike except for intervals 1, 2, and 10 (Fig. 8). Fines were most associated with intervals 1 and 2. Observation tells me interval 10 is also homogenous habitat. Thus, it makes sense they would be apart from the group. The first two intervals are typically deep with silt. Interval 10 typically consists of substrate like boulder and bedrock that is not fit for unionids to anchor themselves to the bottom. This interval is also more susceptible to barge traffic.

Conclusion

Both Robert C. Byrd and Greenup Pool share a statistically similar pattern of species redundancy. This means unionid assemblages are not randomly distributed across pools in the Ohio River. Unknown environmental gradients are driving their spatial distribution. Although this analysis led to more questions than answers about these factors, knowing they are there is a step in the right direction. Future funding and emphasis should be placed on examining these distributions and what is driving them. If these factors are identified, they could influence best management practices and greatly benefit unionid conservation efforts.

Management should be specified for pools of the Ohio River. Summary statistics indicate pools also seem to act as their own independent environments for unionids despite sharing similar patterns. Unionid protection and conservation should be focused on the upper reaches of pools. The Upper Section of the Greenup Pool distinguishes itself as the only unionid stronghold in over 100 miles of river while the lower sections in each pool are nearly void. The lower reaches of pools should then be used for necessary industry and development.

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Appendix A: IRB Approval Letter

MARSHALL UNIVERSITY.
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Office of Research Integrity

May 11, 2021

Hunter Bellamy
2110 Shore Street
Point Pleasant, WV 25550

Dear Hunter:

This letter is in response to the submitted thesis abstract entitled *"Mussels of the Robert C. Byrd Pool."* 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). The Code of Federal Regulations (45CFR46) has set forth the criteria utilized in making this determination. Since the information in this study does not involve human subjects as defined in the above referenced instruction, it is not considered human subject research. If there are any changes to the abstract you provided then you would need to resubmit that information to the Office of Research Integrity for review and a determination.

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

Sincerely,

Bruce F. Day

Bruce F. Day, ThD, CIP
Director

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