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FRESHWATER MUSSELS OF THE GREENUP NAVIGATIONAL POOL, OHIO RIVER, WITH A COMPARISON TO FISH HOST COMMUNITIES

A thesis submitted to
the Graduate College of
Marshall University
In partial fulfillment of
the requirements for the degree of
Master of Science
In
Biological Sciences
by
Mitchell David Kriege
Approved by
Dr. Thomas Jones, Committee Chairperson
Dr. Shane Welch
Dr. Ann Axel
Mr. Jeffrey Thomas

MARSHALL UNIVERSITY
MAY 2018

APPROVAL OF THESIS

We, the faculty supervising the work of Mitchell Kriege, affirm that the thesis, *Freshwater Mussels of the Greenup Navigational Pool, Ohio River, with a Comparison to Fish Host Communities*, meets the high academic standards for original scholarship and creative work established by the Biological Sciences Program and Marshall University. 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 Ohio River was historically a free-flowing system with diverse fish and freshwater mussel communities. Heavy industrialization, erosion from deforestation, and wide scale damming during the early-mid 20th century decimated riverine life. While mussel declines are well documented in the United States, in big river systems, freshwater mussel populations are poorly understudied. This thesis project mapped the mussel communities and site-specific sediments of the Greenup pool in the Ohio River for comparison to 2016 nighttime electrofishing data, provided by ORSANCO. Qualitative SCUBA surveys were performed at 18 randomly selected sites and two fixed sites between July and September. Each site consisted of six, 100 meter survey transects. Sediment was recorded in ten meter sections along each transect. I hypothesized that high fish-host richness and abundances will correlate with strong mussel communities. A secondary goal of my project was to identify areas which may warrent special protection due to the presence of federally endangered species. A total of 3,747 live mussels were collected from 23 species, including nine federally endangered Sheepnose (*Plethobasus cyphyus*). Using negative binomial regressions, fish host richness and abundances were not reliable predictors of freshwater mussel communities. The only exception was *Aplodinotus grunniens,* which acts as an inverse predictor of *Ellipsaria lineolata* populations. While there are few explanations to the broad spatial distribution of fish communities, freshwater mussel populations may be concentrated in the upper section of the pool due to heavy historical pollution and disturbances in the middle and lower Greenup pool.

CHAPTER I

FRESHWATER MUSSEL POPULATIONS OF THE GREENUP POOL, OHIO RIVER

INTRODUCTION

The Ohio is a large river system that harbors a diverse and unique freshwater mussel fauna (Watters and Meyers Flaute 2010; Haag 2012). However, small stream studies dominate freshwater mussel research. Effective sampling for mussels in a big river system is more complex due to water depth, commercial traffic, strong currents, and low visibility. To the author's knowledge, only one previous freshwater mussel survey in the Newburgh pool of the Ohio River has attempted to estimate the pool-wide mussel fauna using randomly generated data (ORSANCO 2017_1).

This study attempts to create a picture of the mussel communities throughout the Greenup pool of the Ohio River (Figure 1). This pool serves as an excellent model for a pool-wide survey due to high historical mussel and fish richness (Watters and Meyers Flaute 2010). In addition, riverine habitats and human influences vary greatly throughout the pool. Lower sections of the pool are strongly impacted by urban areas, industry, and the Greenup Dam. Conversely, urbanization and industry less heavily affect the upper section.

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

Brailing surveys for mussels were completed in the late 1980s and noted 16 species in the upper Ohio (Zeto 1987). However, these surveys are biased towards larger mussels or those filter-feeding on the substrate surface (Watters and Meyers Flaute 2010; Miller and Payne 2007). Burrowers or small species such as the genera *Epioblasma* and *Villosa* could have easily been overlooked. During the 1990s, Miller and Payne surveyed extensively in the Greenup pool for the United States Army Corps of Engineers (USACE) with surface supplied diving (2000). They used modern qualitative and quantitative survey methods to detect 30 species including three federally endangered mussels: *Lampsilis abrupta*, *Plethobasus cyphyus*, and *Obovaria* *retusa* (Miller and Payne 2000). However, their surveys were concentrated in the upper reaches of the pool, near Robert C. Byrd dam, and lack random sampling methods (Miller and Payne 2000).

Beginning in 2001, USACE surveys by Chad Lewis have discovered 28 species live within the vicinity of Robert C. Byrd dam (USACE 2017). However, these non-random surveys may not adequately represent the pool wide mussel fauna. My goal was to document the Greenup pool mussel fauna through randomized survey techniques utilizing SCUBA. I also attempted to identify locations which harbor federally threatened and endangered freshwater mussels. I hypothesized that the Greenup pool would support a total mussel richness of approximately 30 species with unionid communities heavily impacted below urban areas in the middle and lower pool.

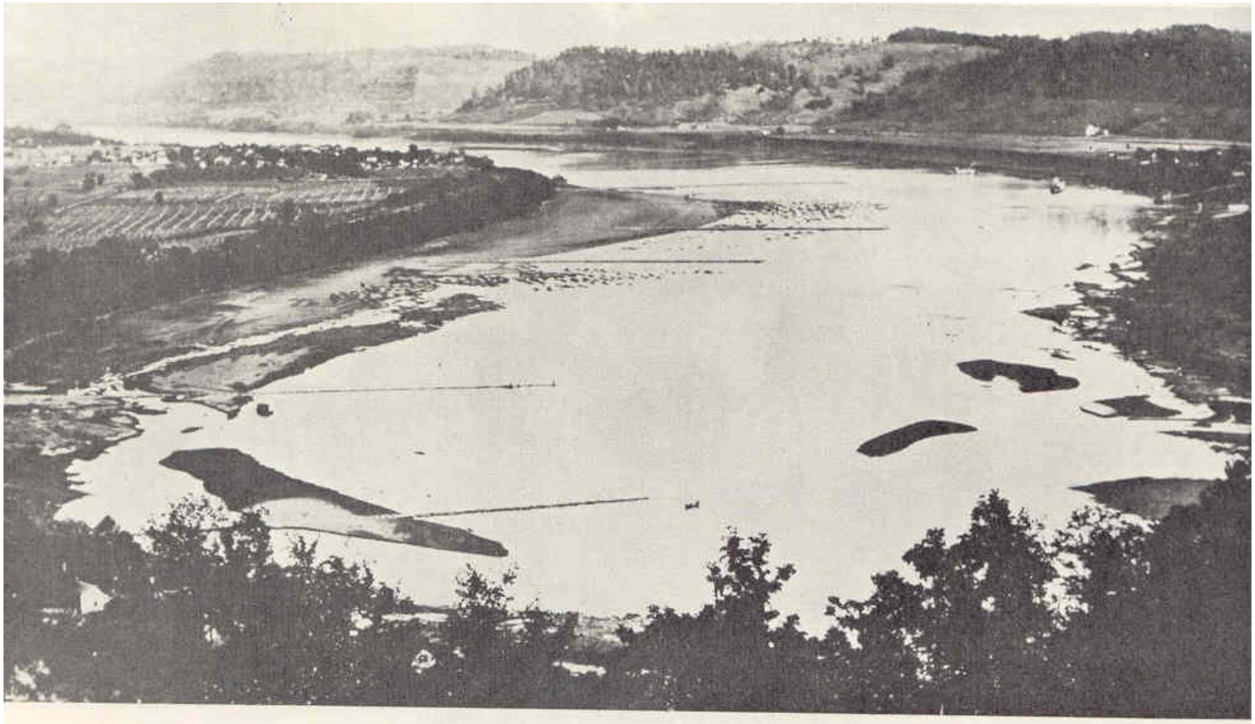
BACKGROUND

The 20th century in the United States saw massive ecological changes due to industrial growth and an exploding human population. General wildlife declines were seen broadly across the US but no group of species was impacted to the extent of freshwater mussels in the family Unionidae (Haag 2012; Watters and Meyers Flaute 2010; Watters, Hoggarth, and Stansbery 2009). Before encroachment by Europeans, roughly 300 species of unionids persisted in the eastern United States (Haag and Williams 2013). Unfortunately, approximately 10% of the 297 recognized species have gone extinct since the turn of the 20th century (Bogan 1993). The massive loss in mussel richness has been attributed to a combination of pollution, siltation, commercial harvest, loss of host fish, and alteration of riverine habitats (Haag 2012; Watters and Meyers Flaute 2010).

Ohio River History

The Ohio mainstem is a major US riverine system which is formed by the confluence of the Allegheny and Monongahela rivers in Pennsylvania. It flows southwest 1,579 km (981.1 miles) forming the borders of Illinois, Indiana, Kentucky, Ohio, and West Virginia. The Ohio River watershed also drains parts of New York, Alabama, Tennessee, Virginia, Georgia, Mississippi, Maryland and North Carolina. The Ohio empties into the Mississippi River at Cairo, Illinois (Taylor 1989; Watters and Meyers Flaute 2010). Over 25 million people, 10% of the US population, live within the Ohio River basin and the mainstem provides drinking water to three million people (ORSANCO 2018).

When European settlers arrived in the 1700's, the Ohio was a broad, free-flowing, shallow river meandering through pristine Eastern woodlands (Taylor 1989; Watters and Meyers Flaute 2010). Homesteaders quickly began to clear land along the fertile river banks for crops and livestock. By 1885, the completion of the first dam on the Ohio River for navigational purposes caused a major change in the mainstem morphology. Riffle and run habitats were converted to pools with a minimum depth of nine feet year round (Taylor 1989). By 1929, 51 wicket dams had been built within the Ohio mainstem, allowing for continuous commercial traffic on the river (Watters and Meyers Flaute 2010). Five of these dams were in the section covered by the modern-day Greenup pool (USACE 2014).



Ohio River as seen during a dry spell in the 1890s. The picture is an upriver view from the top of the hill (Seven Hills Farm) opposite Huntington, across from the former 26th Street ferrylanding. Five stone training dikes show prominently. The two nearer at hand were built about 3 feet high, so the river stage may be taken at about 2 feet. Overlooking the river in the distance, left of center, is Proctorville, Ohio

Figure 2. Historical (top) and modern (bottom) photo of the Greenup Pool above Proctorville, OH. Photo credit: United States Army Corp of Engineers (1979) (top) and Tom Jones (bottom).

Wicket dams were built on the Ohio River from the late 1800s until 1929 to raise water levels for commercial steamboat traffic (Figure 3). They consisted of large wooden "wickets" that resembled doors stacked across the river. Each wicket was held upward by a supportive iron rod and could be lowered during high flows (Steamboating the Rivers: Wicket dam n.d.). Wicket dams raised the river depth to a minimum of nine feet but were somewhat inefficient and did not completely eliminate riffle-run-pool habitats.

Figure 3. Ohio River wicket lock and dam number 18 at Reedsville, Ohio (USACE$_3$ n.d.).

The invention of modern tug boats and commercial barges encouraged the completion of more sophisticated dams. Beginning in the 1950s, high-lift, steel and concrete dams began to replace the outdated wooden, wicket dams. There are currently 20 dams spread throughout the Ohio mainstem to provide navigable waters for commercial traffic (USACE 2008).

Study Area

The Greenup pool forms a portion of the upper Ohio River mainstem (Figure 1). The pool is formed by Greenup Locks and Dam in Kentucky at river mile (RM) 341.0 and runs 61.8 miles up to RC Byrd Locks and Dam in West Virginia at RM 279.2 (Zeto 1987; $USACE_2$ n.d., USACE 2014). The pool is characterized by 39.2% straight flow and 60.8% river bends. Construction began on Greenup Locks and Dam in 1954 and was completed in 1962. The average depth is approximately 26 feet with a gradient drop of 0.4 ft/mile (ORSANCO 2011). Normal pool elevation is 515 feet. The upper section of the pool was formed by Gallipolis Locks and Dam in 1937 and renamed RC Byrd Locks and Dam in 1993 ($USACE_1$ n.d.)

Historical Mussel Fauna

The Greenup pool historically supported approximately 40 species of freshwater mussels (Watters and Meyers Flaute 2010). It has been estimated that the total mussel richness in the Ohio River has decreased by nearly 30% since the onset of human modifications (Taylor 1989). Records throughout the 1980s and 90s indicate a species richness of approximately 35 species throughout the upper Greenup pool (Dunn 1999_2; Miller and Payne 2000; Zeto 1987; Taylor 1980). Federally endangered species that historically occurred in the pool include: *Obovaria retusa, Lampsilis abrupta, Plethobasus cyphyus, Plethobasus cooperanus, Pleurobema plenum, Pleurobema clava, Epioblasma t. torulosa,* and *Cyprogenia stegaria* (Ecological Specialists, Inc. 2003_1; Taylor 1980). During Miller and Payne's work in the 1990s, *Q. quadrula* and *E. crassidens* were the dominant species (Miller and Payne 2000). Previous studies by Taylor in the 1980s also noted *E. crassidens* as the dominant species (Taylor 1980). However, these studies did not use modern survey methods and were biased towards large, thick-shelled species which tend to filter on the surface and strongly clamp onto brails. Modern data from the upper pool is concentrated around RC Byrd Locks and Dam. Beginning in 2001, transect surveys in the upper Greenup pool, within two miles of RC Byrd dam, have identified a richness of 28 species (USACE 2017).

Historical mussel data from the middle and lower Greenup pool is lacking. Heavy industrialization and strong anthropogenic influences may have limited researcher interest throughout these sections. Recent surveys for mooring and bridge projects have revealed an extremely limited mussel community (Table 1). Since 2003, the richness of the middle and lower pool has not exceeded 14 species. The middle and lower Greenup pool is dominated by *P. alatus*, a silt tolerant mussel (Hoggarth 2010; Fortenbery 2010, 2008; EnviroScience 2008_1, 2008_2, 2012; and Ecological Specialists Inc. 2003_1, 2003_2; Spaeth and Swecker 2015, 2016; Swecker 2009). The most successful middle and lower Greenup pool survey was completed by Swecker and Spaeth of ESI in 2016. They recovered 94 mussels representing 11 species from 800m^2 near Huntington, WV. Another mid-pool survey in 2010 by Dr. Michael Hoggarth for the Ironton, Ohio bridge replacement found 65 mussels representing eight species 500m^2 (Hoggarth 2010). No other surveys in the middle or lower pool have collected >50 live mussels (Table 1).

Table 1. Compiled live mussel data from the Greenup pool, Ohio River (Taylor 1980; Zeto 1987; Miller and Payne 2000; Dunn 1999_2; USACE 2017; Hoggarth 2010; Fortenbery 2010, 2008; EnviroScience 2008_1, 2008_2, 2012; and Ecological Specialists Inc. 2003_1, 2003_2; Spaeth and Swecker 2015, 2016; Swecker 2009).

Surveyor	**Year**	**Location**	**River Mile**	**Survey Method**	**Survey Area**	**Abundance**	**Diversity**
Taylor	1979	Upper	303, 302.3, 300, 299, 296, 283, 284,	Brailing/midden	Unknown	Unknown	17
Zeto	1985	Upper	289, 292	Brailing	Unknown	379	16
Miller and Payne	1992, ’93,’98	Upper	284, 287, 292, 290	Qualitative & quantitative diving	Unknown	8,163	30
Ecological Specialists, Inc	1990-1999	All sections	282-292, 294, 326-327, 340	Transects	Unknown	2,968	31
US Army Core of Engineers	2001-2017	Upper	279-80, 282	Transects	Unknown	7,235	28
Mainstream Commercial Divers	2010	Middle	318	Transects	780m²	5	2
Mainstream Commercial Divers	2008	Lower	336-337	Transects	1,000m²	2	1
Ecological Specialists, Inc.	2003	Middle	327	Transects	1200m²	0	0
M. Hoggarth	2010	Middle	237	Transects	2000m²	65	8
EnviroScience	2012	Middle	226, 227	Cells	Unknown	0	0
EnviroScience	2008	Lower	334	Transects	900m²	1	1
EnviroScience	2008	Middle	309	Transects	900m²	4	3
ESI (Swecker)	2009	Middle	312-13	Transects	3000m²	48	8
ESI (Spaeth & Swecker)	2015	Middle	325	Transects	550m²	39	9
ESI (Spaeth & Swecker)	2016	Middle	308	Transects	800m²	94	11

Impoundment Impacts

Modern dams along the Ohio replaced the natural riffle-run-pool habitat with a homologous, deep-water lotic system. These dams raised the average river depth to 23ft and inhibit natural flood pulses (Figure 2). The unnatural depth may limit mussel lure display efficiency and infestation success by separating benthic mollusks from pelagic fish hosts which spend their time in open water. Dams trap sediment by decreasing flow and destabilizing river banks with constricted flows and increased velocities below the outfall (Hagerty, Spoor, and Parola 1995). Additionally, the loss of current above dams causes fine particulates to accumulate on the river bottom, smothering aquatic life (Brim Box and Mossa 1999).

Riverine sedimentation reduces the available dissolved oxygen (DO) in the water by increasing total suspended solids (TSS) (Bilotta and Braizer 2008). Trapped sediments encourage microbial community growth and the decomposition of organic matter. Microbial cellular respiration in fine sediment constitutes an unnaturally high biological oxygen demand (BOD) and limits DO in benthic zones. Fine sediments also increase the benthic surface area, increasing the chemical oxygen demand (COD) (Nogaro, Mermillod-blondin, Montuelle, Boisson, and Gilbert 2008).

Increased sedimentation and reduced DO kills mussels and decreases the presence of unionid fish host species (Brim Box and Mossa 1999; Cordone and Kelly n.d.). Small benthic species such as darters (Percidae) that rely on shallow, riffle habitat may not venture into deep, oxygen-depleted pools, thus eliminating mussel assemblages by removing a pertinent stage in recruitment (Stauffer, Boltz, Kellogg, and Snik 1996). Reduced oxygen, changes in water depth/flow, and loss of host species have led to the severe disruptions of freshwater mussel populations in dammed systems (Haag 2012; Hornbach et al. 2014; Vaughn and Taylor 1999; Schwalb, Cottenie, Poos, and Ackerman 2011).

Commercial Traffic

Over 230 million tons of cargo is moved along the Ohio River every year (ORSANCO 2018). The Greenup pool was historically an important stop for barges carrying coal and iron. The Port of Huntington Tri-state is the largest inland United States port, ranked 4th nationally with 80 million tons of cargo, primarily coal, in 2000 (Huntington District Waterways Association n.d.). Huntington has been a traditional loading site for coal because of its connectivity to large metropolises along the Ohio River valley, extensive railroad system, and close proximity to southern Appalachian coal fields. Coal exports and riverside industries throughout the region fueled heavy barge traffic within the Greenup pool. In 2012, approximately 50,758 tons of cargo passed through the Greenup Lock and Dam (USACE_2 n.d.)

The middle and lower section of the Greenup pool is heavily impacted by barge traffic. Standard commercial barges are 12 feet (ft) tall and draft nine feet of water when fully loaded. A 15 barge chain and adjourned tug displays a volume of water equivalent to a 10 story building. Attached to each commercial tug is a 6-8 foot prop that requires 12 ft. of water. In shallow areas of the Ohio River, particularly in the optimal mussel habitat below dams, only three feet may separate the tug prop and benthic substrate. Therefore, commercial traffic may be heavily impacting fragile benthic habitat. Additionally, as the tugs pull into shore, they often drive their barges into the bank, crushing shoreline life. Meanwhile, propellers blast the river bottom, blowing benthic organisms downstream and compacting the substrate (Wolter and

Arlinghaus 2003). This heavy compaction and loss of heterogeneous substrates limits aquatic communities (Trautman 1986; Mueller Jr. and Pyron 2010).

Urban Runoff

Urban runoff has become a major issue for freshwater mussels in the 20th century. During rain events, point source and nonpoint source pollution enters streams from roadways, combined sewer overflows, and industry (Gromaire-Mertz, Garnaud, Gonzalez, and Chebbo 1999; Lee & Bang 2000). Cities create massive impervious landscapes that intensify flooding, scour stream substrates, contaminate runoff with salt and petroleum products, and raise water temperatures (Hasenmueller, Criss, Winston, and Shaughnessy 2017; Davidson and Gunn 2012). These extreme conditions limit urban aquatic life, including freshwater mussels (Gillis, Brim Box, Symanzik, and Rodemaker 2003). During his surveys of the upper Ohio in 1979, Taylor found no mussels for the first 90 miles below the metropolis of Pittsburgh which could likely be attributed to urban runoff and historical industrial point source pollution (Taylor 1980).

The middle portion of the Greenup pool is dominated by an urban landscape (Figure 1). Ashland, KY and Huntington, WV are the largest cities in the pool with populations of 21,378 and 48,113 people. Smaller localities including Proctorville OH, Ironton OH, Greenup KY, and Guyandotte WV also lie within the study area. Major cities such as Charleston WV and Pittsburgh PA lie upstream of the Greenup pool.

Combined Sewer Overflows

Combined sewer overflows (CSOs) are sanitary pipes built underground that carry storm water, raw sewage, and industrial waste water but constructed with overflow pipes that feed into local waterways. This system was designed to keep sewer treatment plants from becoming overloaded during intense storm events (EPA n.d.). Throughout these downpours, CSOs deliver a mixture of rain water, street runoff, and raw sewage into the river which can harm mussels and other aquatic life (Gillis et al. 2017).

A total of 42 CSOs enter the Greenup pool from the cities of Huntington/Kenova (24), Ashland (9), and Ironton (8) (Figure 4). An unspecified number also enter the pool from the small town of Cattletsburg, KY and cities along tributaries. Gillis et al. (2017) found that Canadian Unionidae populations dropped significantly below urban areas and documented a positive correlation between mussel richness and abundance with distance from sewer outfalls. Below outfalls, mussel populations were almost nonexistent and gradually rebounded moving downstream.

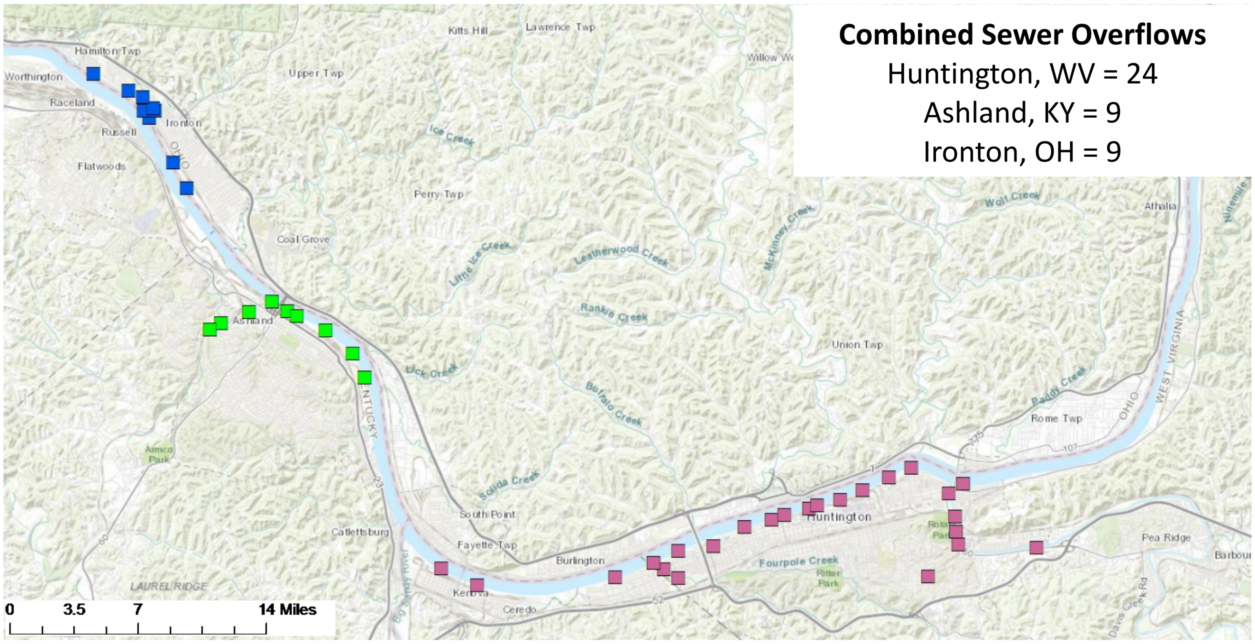


Figure 4. Combined Sewer Overflows (CSOs) in the Greenup Pool, Ohio River. The map includes 42 locations between the cities of Ironton, Ashland, and Huntington (Source: Personal collection).

Zebra Mussel Infestations

Zebra mussels (*Dreissena polymorpha*) are an invasive species native to southern Russian lakes (Mackie 1991; Ludyanskiy, McDonald, and MacNeill 1993). They are filter feeders which attach to hard underwater surfaces by abyssal threads (Mackie 1991; Ludyanskiy et al. 1993). Zebra mussels, unlike native unionids, do not rely on fish hosts. Instead, female zebra mussels release eggs which are fertilized in the water column and transform into free swimming veligers. Each female can produce over one million eggs a year, leading to explosive population growth (Ludyanskiy et al. 1993) (Figure 7). In the United States, their massive colonies clog water intake pipes and have been seen at densities of 750,000 animals per square meter (Ludyanskiy et al. 1993). The estimated cost from 2000 - 2010 to US and Canadian water users is >$5 billion in damages (Rosaen, Grover, Spencer, and Anderson 2012).

In addition to their economic harm, zebra mussels have wreaked havoc on native mussel populations since their arrival in the 1980s (Watters and Meyers Flaute 2010, Pilotto, Sousa, and Aldridge 2016; Gillis and Mackie 1994; Haag, Berg, Garton, and Farris 1993). The zebra mussel veligers attach to unionids and over time a large colony of *D. polymorpha* forms on the outer shell of the native mussel (Pilotto et al. 2016; Mackie 1991; Ludyanskiy et al. 1993; Matthews et al. 2014) (Figure 5-6).

Figure 5. *Fusconaia ebena* with a typical zebra mussel infestation, Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Figure 6. *Megalonaias nervosa* heavily infected by zebra mussels, Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

The weight of the zebra mussels interferes with the unionid's ability to move through the sediment and causes dislodgement during high water events (Haag 2012, Watters, Hoggarth, and Stansbery 2009). The abyssal threads also glue the mussel shells together, which inhibits feeding and reproduction (Haag 2012). Lastly, the zebra mussels steal food from native unionids as they congregate around the inflow syphon, encouraging stress and starvation (Mackie 1991; Ludyanskiy et al. 1993).

Zebra mussels were first noted in the lower mainstem Ohio River in 1991 and by 1994 they had reached Pittsburgh, PA (Watters and Meyers Flaute 2010). Their rapid spread through the Ohio mainstem may be due to zebra mussel attachment on boat hulls and upstream bound commercial barges (Matthews et al. 2014). Their numbers in the Ohio River peaked after the initial invasion but have since plateaued (Figure 7).

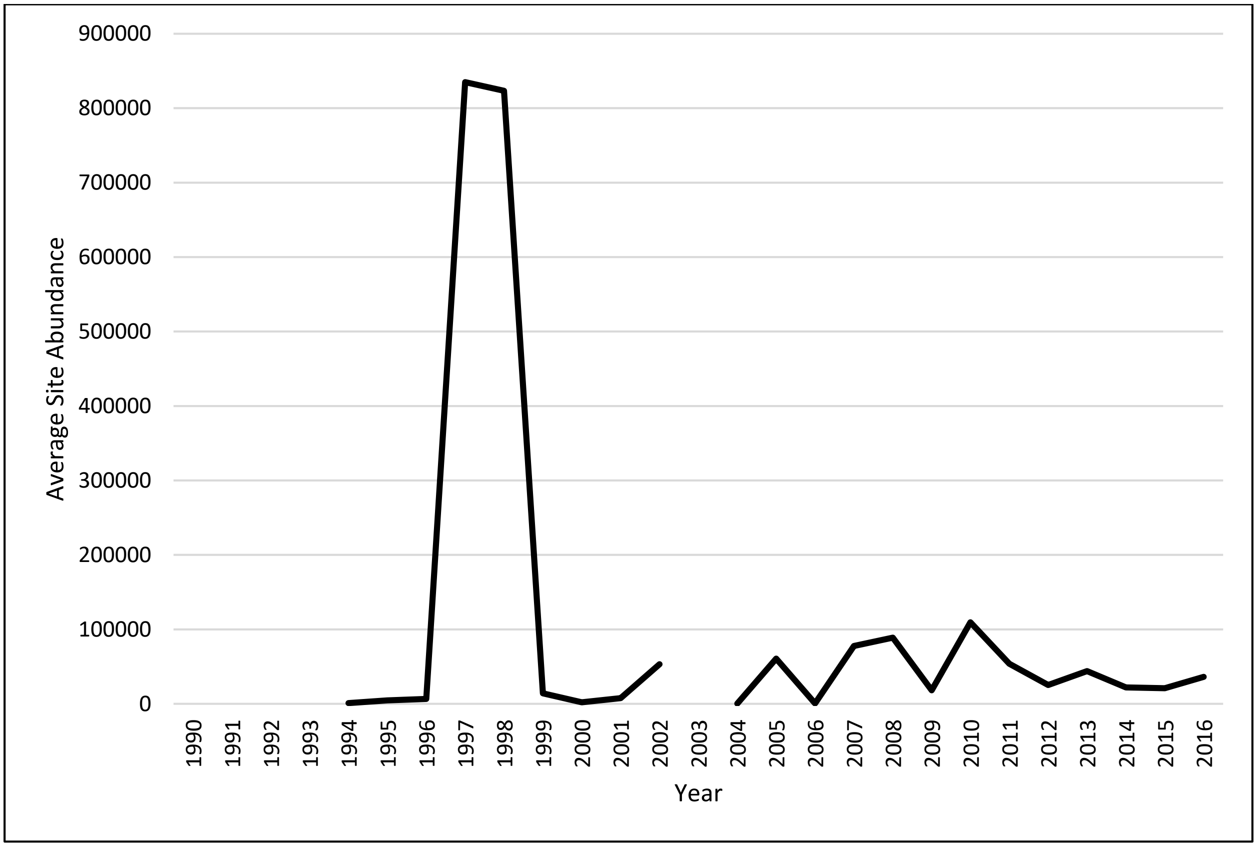


Figure 7. Zebra mussel infestation counts during ORSANCO surveys in the Ohio River (ORSANCO 2016_1).

METHODS

Mussel Survey

I performed freshwater mussel surveys at 20 locations in the Greenup pool of the Ohio River from July to September (Figure 8-12), 2017. All sites coincided with 2016 Ohio River Valley Sanitation Commission (ORSANCO) biological collection locations (ORSANCO 2016$_2$). Sites were split into the classifications “upper pool,” “middle pool,” or “lower pool” which were based on the density of prominent anthropogenic impacts and distance from dams. The upper pool contained 11 sites between RC Byrd Locks and Dam and the urban areas of Huntington, WV (Figure 8-9). The middle pool contained four sites situated within the cities of Huntington, WV and Ironton, OH (Figure 10-11). The lower pool was composed of five sites between the Greenup Locks and Dam and Ironton, OH (Figure 12).

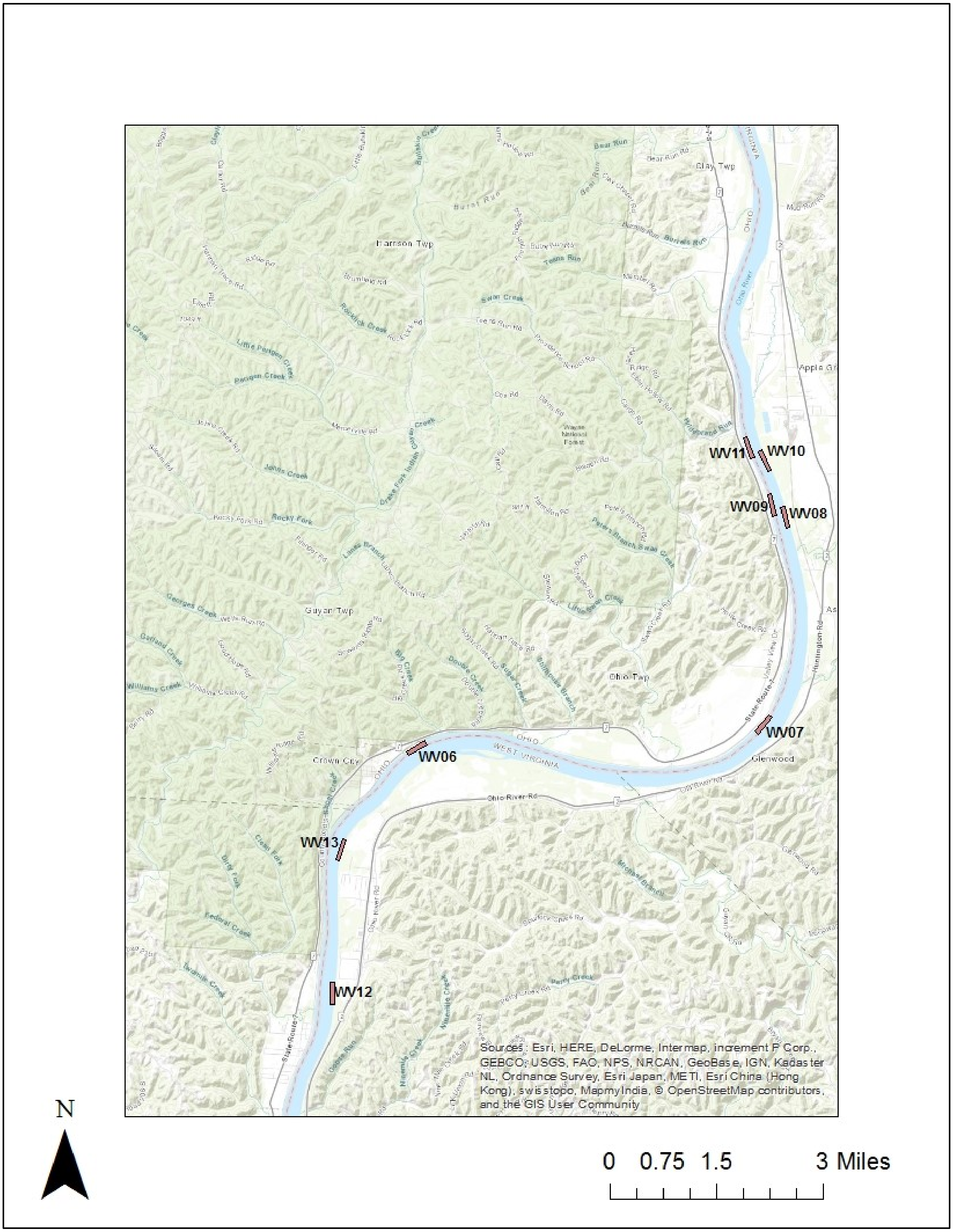


Figure 8. Eight upper Greenup pool sites, Ohio River (Source: Personal collection).

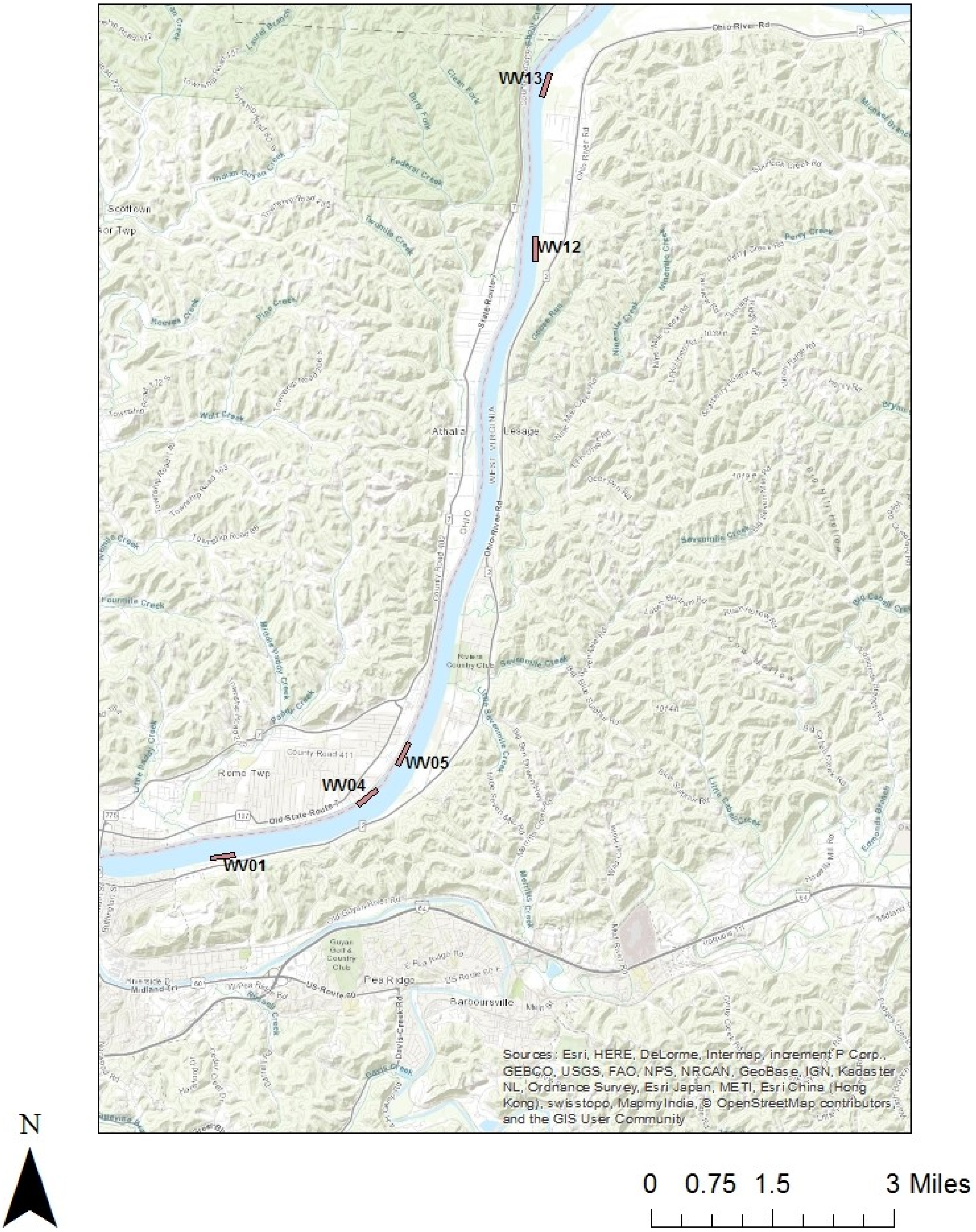


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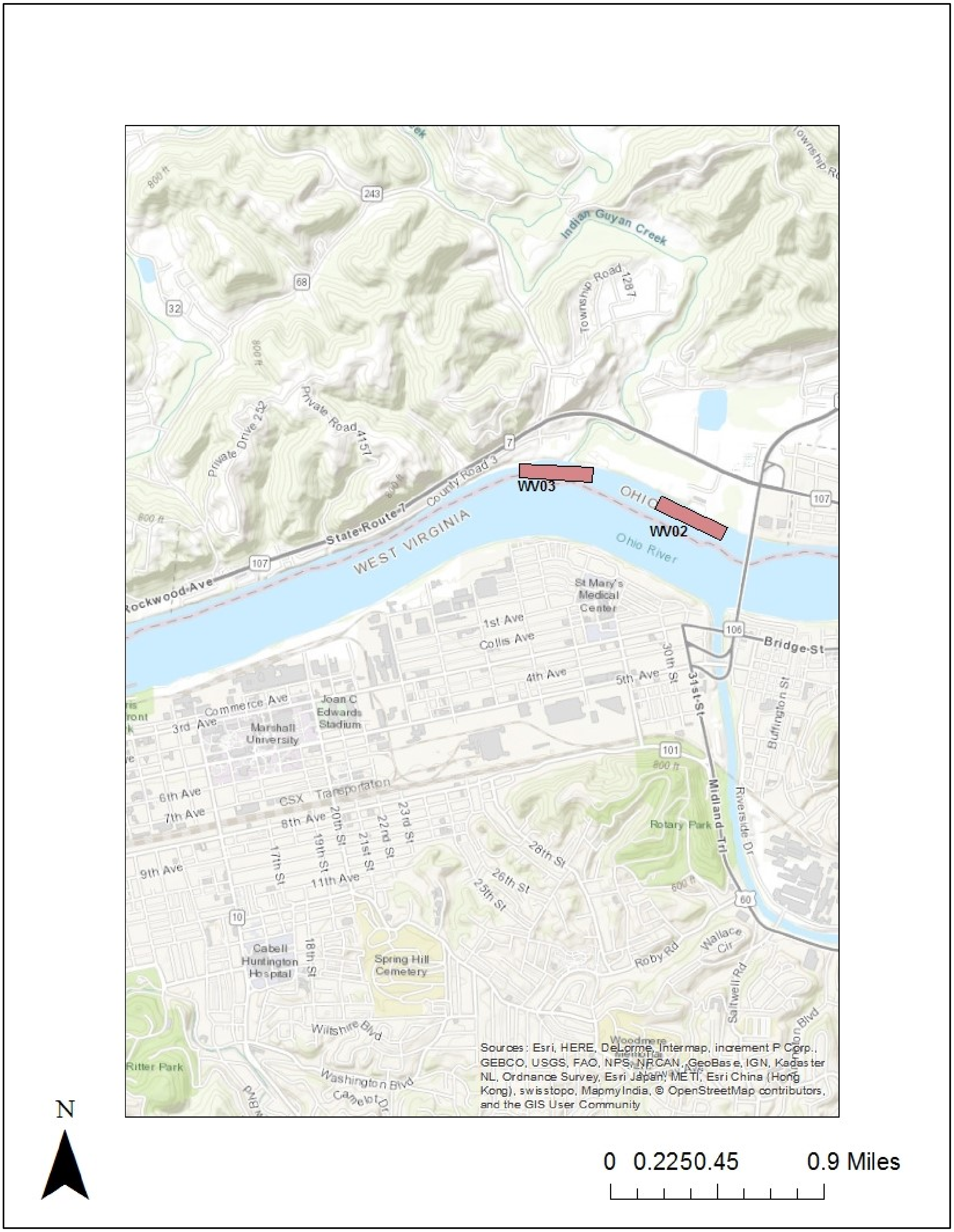
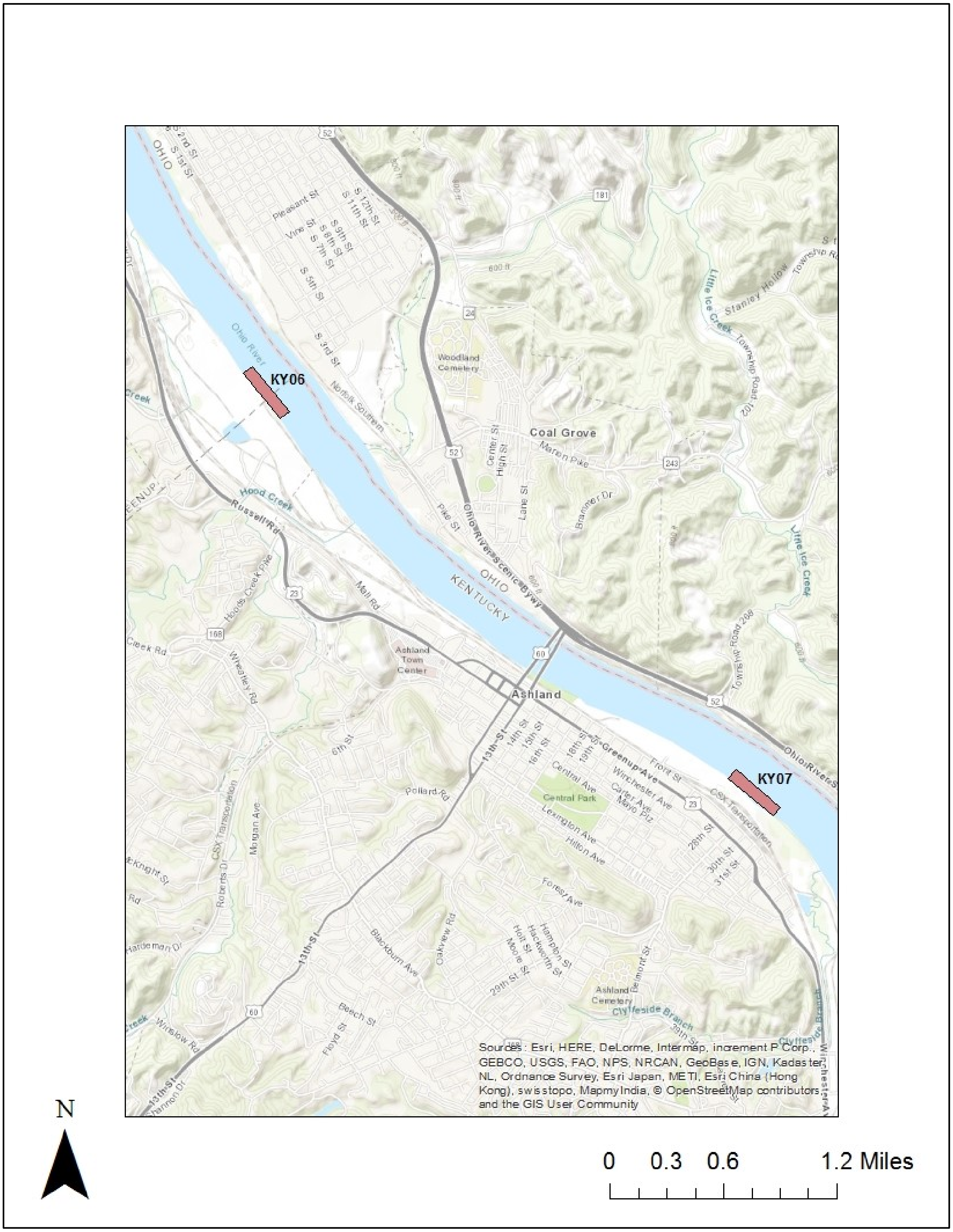
Figure 9. Five upper Greenup pool sites, Ohio River (Source: Personal collection).



Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

Figure 10. Two middle Greenup pool sites, Ohio River, near Huntington, WV (Source: Personal collection).



Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors and the GIS User Community

Figure 11. Two middle Greenup pool sites, Ohio River, near Ashland, KY (Source: Personal collection).

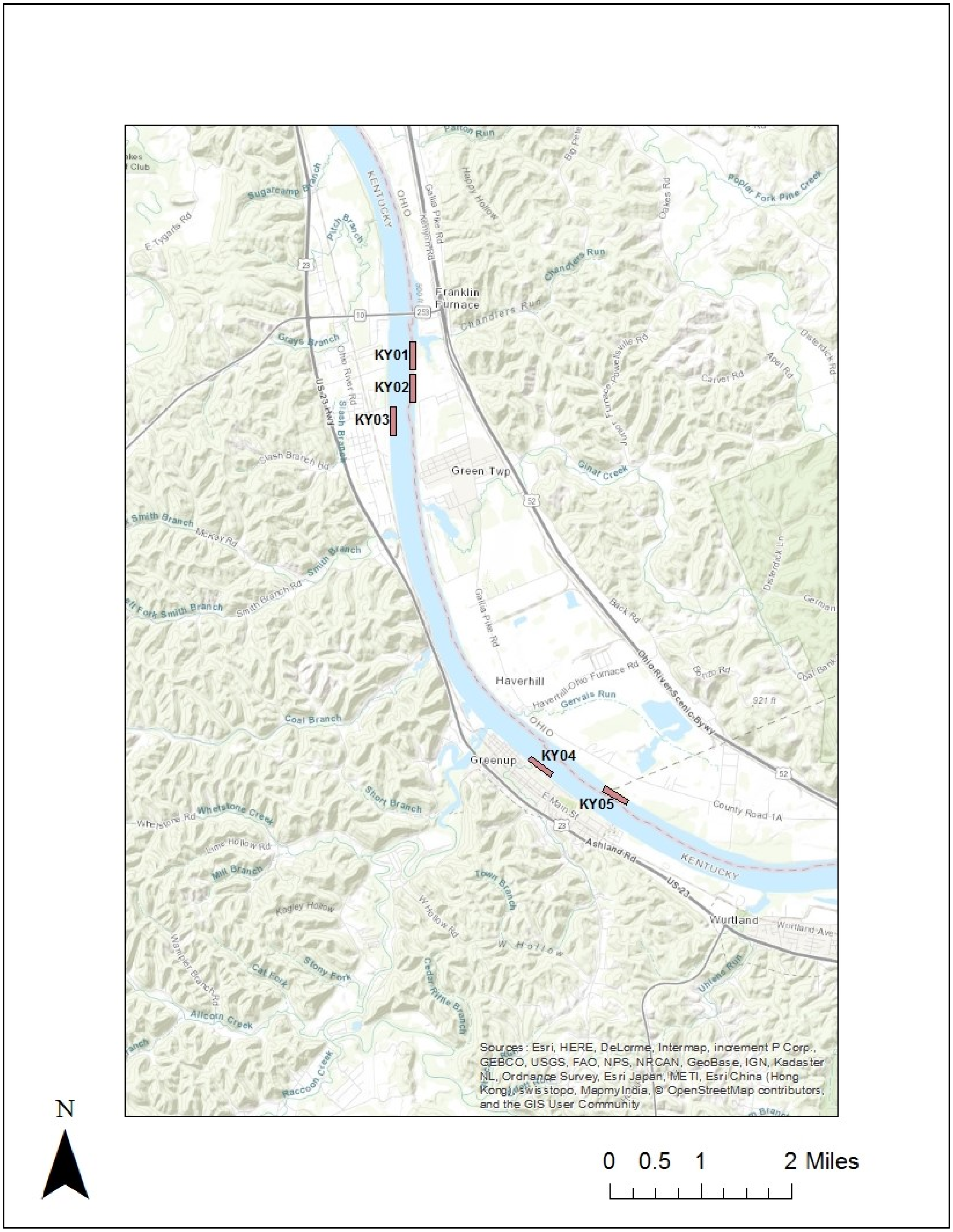


Figure 12. Five lower Greenup pool sites, Ohio River (Source: Personal collection).

The West Virginia mussel survey protocol was used as a reference model for experimental design (Clayton, Douglas, and Morrison 2016). Additionally, the study design mimicked ORSANCO mussel surveys in the Newburgh pool of the Ohio River in 2012 and 2017 but included twice as many transects (ORSANCO 2017$_1$). At each site, I used SCUBA to survey six systematic 100 meter transects. Each transect ran perpendicular from the river bank toward the channel and transects were spaced 100 meters apart to provide equidistant coverage of the 500X100 m area (Figure 13).

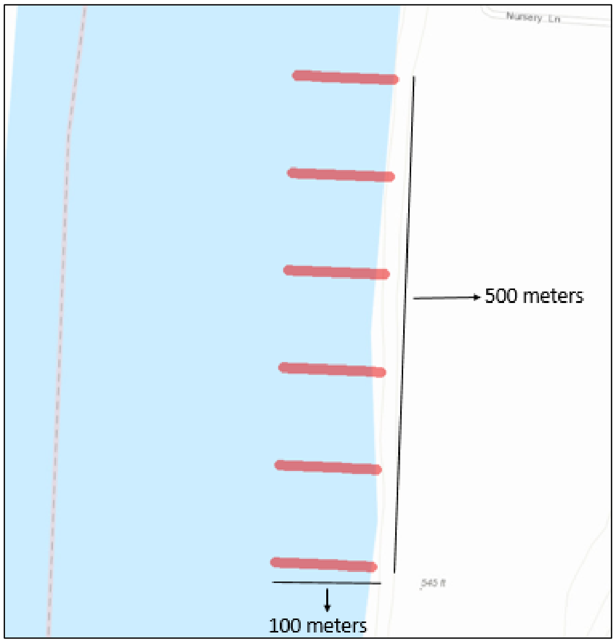


Figure 13. Typical site layout of six 100 meter transects running perpendicular from bank to river channel (Source: Personal collection).

Figure 14. Typical 100 meter transect layout. Each cell (intersect) is 1X10m² (Source: Google Earth Pro).

The transect classification number moved downstream at each site from “1” to “6.” Transects were split into ten 10 meter intervals to provide precise location data (Figure 14). The interval classification number “1” started at the shoreline. Transects were marked by running 100 meter lead lines from the bank toward the channel with an anchor at both ends, and 10 meter interval markers. Divers descended the buoy line to survey from the 10th interval to the bank. Transect standardized selection within the study did not provide randomized, defendable data. Therefore, site selection provided the random survey technique needed for statistical analysis.

Four different divers with freshwater mussel survey experience assisted during field efforts. They visually searched for siphons, flipped large debris, and used tactile sifting within the upper four inches of substrate. Mussel surveys were only performed when visibility exceeded 0.5m, a standard set by the WV Mussel Survey Protocol (Clayton et al. 2016). In heterogeneous substrates, a minimum of one min/m^2 of search time was required. Homogenous fine substrates did not have a time minimum requirement. Live mussels and deadshells (DS) were collected and stored in mesh bags for transport to the surface. Each bag corresponded to a specific transect and interval. Live mussels were identified, measured to the nearest millimeter, and returned to the site by broadcast scattering from a boat. During processing, mussels were kept out of the water for no longer than five minutes. The sex was listed for species exhibiting sexual dimorphism. If the individual was too young to identify sex, the shell was listed as a juvenile (J). Each live species was photographed and DS in good condition were retained as voucher specimens. Deadshell categories included: freshdead (shell shows no sign of wear and retains intact nacre and periostricum), weathered dead (shell shows signs of wear with tarnished nacre and periostricum), and subfossil (shell retains no nacre or periostricum).

Federally threatened and endangered individuals were measured for length, width, and height (mm). Voucher photographs and collection coordinates were sent to the appropriate state and federal agencies (USFWS, WVDNR, & KYDFWR) within 24 hours. All federally threatened and endangered mussels were returned by hand to optimal habitat within the site.

GIS

I used Arc Map 10.3.1 to map freshwater mussel populations within the Greenup pool. Mussel abundance and richness were calculated at the site, transect, and interval levels. CSO locations were provided by Ashland Sanitation District and Ohio EPA public data (Ohio EPA n.d). Barge impact sites indicated by parked barges, visible pins/stands, and shoreline barge repair structures were identified and marked in 2016 Google Earth images and converted to ArcGIS shapefiles

Pool Wide Mussel Abundance

I calculated the mussel abundances on a pool-wide basis within 100 meters of the shoreline. To determine the total pool area relative to survey design (within 100m of the bank), I calculated the average width of the river from 62 cross measurements (in meters), spaced one mile apart. I multiplied the total pool length (99,457.46m) by the average pool width to yield the total Greenup pool area (41,765,171.178m²). I then divided 200m (for both shorelines) by the average river width of 419.93m. I multiplied the resulting integer (0.4763m²) by the total Greenup pool area which returned the total area of our target population (19,891,293.054m²).

$$99{,}457.46m * 419.93m = 41{,}765{,}171.178m^2$$

$$\frac{200m}{419.93m} = 0.47623$$

$$0.4763 * 41{,}765{,}171.178m^2 = 19{,}892{,}751.03m^2$$

Each 500X100m² site contained 83.33 1X100m² transects. The total number of mussels from randomized sites (3,456 individuals) was multiplied by 83.33. Next, I calculated the total area of my sites by combining complete survey areas (500X100m²) from all 18 random sites (excluding WV11 and WV12). I then cross multiplied my total site area mussel abundance (287,988.48 individuals) per total site area (900,000m²) by total pool survey area mussel abundance (unknown) per total pool survey area (19,891,293.054m²) to estimate the complete mussel assemblage of the Greenup pool within 100 meters of both shorelines.

$$3{,}456\ individuals * 83.33 = 287{,}988.48\ individuals$$

$$(500m * 100m) * 18 = 900{,}000m^2$$

$$\frac{287{,}988.48\ individuals}{900{,}000m^2} = \frac{x}{19{,}892{,}751.03m^2}$$

RESULTS

My surveys in the Greenup pool of the Ohio River yielded 3,747 live mussels, found in 19 of the 20 sites (Table 2-5). Twenty-three species were collected live, including nine federally endangered *Plethobasus cyphyus* individuals. The common big river species, *Obliquaria reflexa* and *Quadrula pustulosa,* accounted for 55.31% of the relative abundance live mussel collections (Table 6). Thirteen of the 23 species were represented by relative abundances comprising <1% of the mussel collections (Table 6).

Table 2. Total mussel abundance collected in the Greenup pool, Ohio River.

Species	**Abundance**
Actinonaias ligamentina – Mucket	16
Amblema plicata – Threeridge	247
Ellipsaria lineolate – Butterfly	284
Elliptio crassidens - Elephantear	90
Fusconaia ebena – Ebony Shell	7
Fusconaia flava – Wabash pigtoe	4
Lampsilis cardium – Plain Pocketbook	51
Lampsilis siliquoidea – Fat Mucket	2
Lampsilis teres – Yellow Sandshell	1
Lasmigonia complanata – White Heelspliter	2
Leptodea fragilis – Fragile Papershell	3
Ligumia recta – Black Sandshell	398
Megalonaias nervosa – Washboard	21
Obliquaria reflexa – Threehorn Wartyback	1116
Plethobasus cyphyus – Sheepnose	9
Pleurobema cordatum – Ohio Pigtoe	47
Potamilus alatus – Pink Heelsplitter	212
Pyganodon grandis – Giant Floater	1
Quadrula metanevra – Monkeyface	244
Quadrula pustulosa – Pimpleback	958
Quadrula quadrula – Mapleleaf	27
Quadrula verrucosa – Pistolgrip	1
Truncilla truncata – Deertoe	6
Total Abundance	**3747**

Table 3. Lower Greenup pool mussel assemblages, Ohio River.

Site	*P alatus*	*A plicata*	*L recta*	*O reflexa*	Total
KY 03*	0	0	1	2	**3**
KY 01	2	0	0	6	**8**
KY 02	0	2	0	14	**16**
KY 05	0	0	1	0	**1**
KY 04	0	0	0	5	**5**

* denotes barge impact

Table 4. Middle Greenup pool mussel assemblages, Ohio River.

Site	*P alatus*	*L cardium*	*P grandis*	*A plicata*	*Q pustulosa*	*L recta*	*O reflexa*	Total
KY 06**	1	0	0	0	0	0	0	**1**
KY 07*	0	0	0	0	0	0	0	**0**
WV 02*	0	1	0	0	1	1	3	**6**
WV 03*	10	0	1	4	1	0	36	**52**

* denotes barge impact

** Denotes site with industrial/intake impacts

XX denotes revisit ORSANCO site

Table 5. Upper Greenup pool mussel assemblages, Ohio River.

Species	**WV01***	**WV04**	**WV05***	**WV06**	**WV07**	**WV08**	**WV09**	**WV10***	**WV11**	**WV12***	**WV13**
P alatus	11	10	16	35	5	35	36	5	33	13	1
L cardium	1	5	8	6	3	7	6	3	10	0	1
L complanata	0	0	0	1	0	0	1	0	0	0	0
P cordatum	12	7	2	18	0	1	1	0	4	2	0
E crassidens	16	0	12	4	1	0	11	0	43	3	0
P cyphyus	0	4	3	1	0	0	0	0	1	0	0
F ebena	2	3	0	1	0	0	0	0	1	0	0
F flava	0	0	1	2	0	0	0	0	0	1	0
L fragilis	0	0	0	1	0	1	0	0	0	1	0
P grandis	0	0	0	0	0	0	0	0	0	0	0
A ligamentina	0	2	2	3	0	1	3	0	4	1	0
E lineolata	8	21	16	90	30	16	21	7	54	21	0
Q metanevra	8	50	34	108	19	7	2	1	8	7	0
M nervosa	1	0	1	2	0	0	6	0	9	2	0
A plicata	19	22	10	56	11	22	23	2	52	24	0
Q pustulosa	34	107	72	353	59	37	60	6	134	62	1
Q quadrula	0	0	0	13	0	2	1	0	5	6	0
L recta	15	39	68	72	13	31	72	13	54	16	2
O reflexa	64	67	71	311	64	88	73	24	161	124	3
L siliquoidea	1	0	0	1	0	0	0	0	0	0	0
L teres	0	0	0	0	0	0	1	0	0	0	0
T truncata	0	1	0	3	1	0	0	0	0	1	0
T verrucosa	0	0	0	0	0	0	1	0	0	0	0
Total Live	**192**	**338**	**342**	**1081**	**206**	**248**	**318**	**61**	**573**	**284**	**8**

***** denotes barge impact

XX denotes fixed ORSANCO site

XX denotes revisit ORSANCO site

Table 6. Total Mussel Composition Collected from the Greenup Pool, Ohio River.

Species	**Percent (%) Relative Abundance of the total population**
Obliquaria reflexa – Threehorn wartyback	29.82
Quadrula pustulosa – Pimpleback	25.49
Ligumia recta – Black Sandshell	10.63
Ellipsaria lineolata – Butterfly	7.59
Amblema plicata – Threeridge	6.60
Quadrula metanevra – Monkeyface	6.52
Potamilus alatus – Pink Heelspliter	5.66
Elliptio crassidens – Elephantear	2.40
Lampsilis cardium – Plain Pocketbook	1.36
Pleurobema cordatum – Ohio Pigtoe	1.26
Quadrula quadrula – Mapleleaf	0.72
Megalonaias nervosa – Washboard	0.56
Actinonaias ligamentina – Mucket	0.43
Plethobasus cyphyus – Sheepnose	0.24
Fusconaia ebena – Ebonyshell	0.19
Truncilla truncata – Deertoe	0.16
Fusconaia flava – Wabash Pigtoe	0.11
Leptodea fragilis – Fragile Papershell	0.08
Lasmigonia complanata – White Heelsplitter	0.05
Lampsilis siliquoidea – Fat Mucket	0.05
Pyganodon grandis – Giant Floater	0.03
Lampsilis teres – Yellow Sandshell	0.03
Tritogonia verrucosa – Pistolgrip	0.03

Average mussel abundance by site was 187 animals with a standard deviation of ± 267, excluding negative values. Average richness by site was eight species. Site WV06 contained the maximum mussel abundance and richness with 1,081 individuals representing 20 species. KY07 exhibited the lowest mussel abundance and richness with zero live individuals encountered. The highest combined interval mean mussel richness throughout the pool was observed at intervals four through seven with a maximum of 14 species (Figure 15). Greenup pool mussel mean abundance was highest at interval five (Figure 16). Species richness and abundance was concentrated in the upper pool (Figure 17-18).

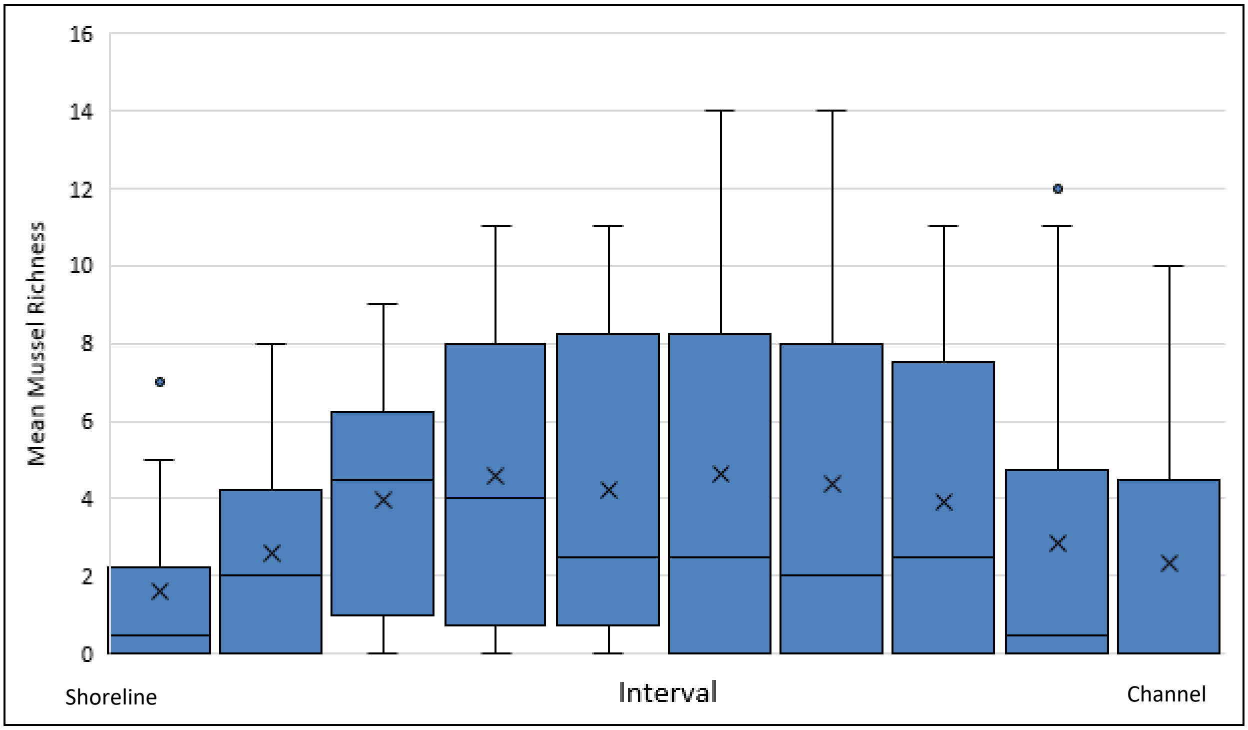


Figure 15. Mean mussel richness by interval in the Greenup pool, Ohio River. Each interval increases by 10m increase from the shoreline. (Source: Personal collection).

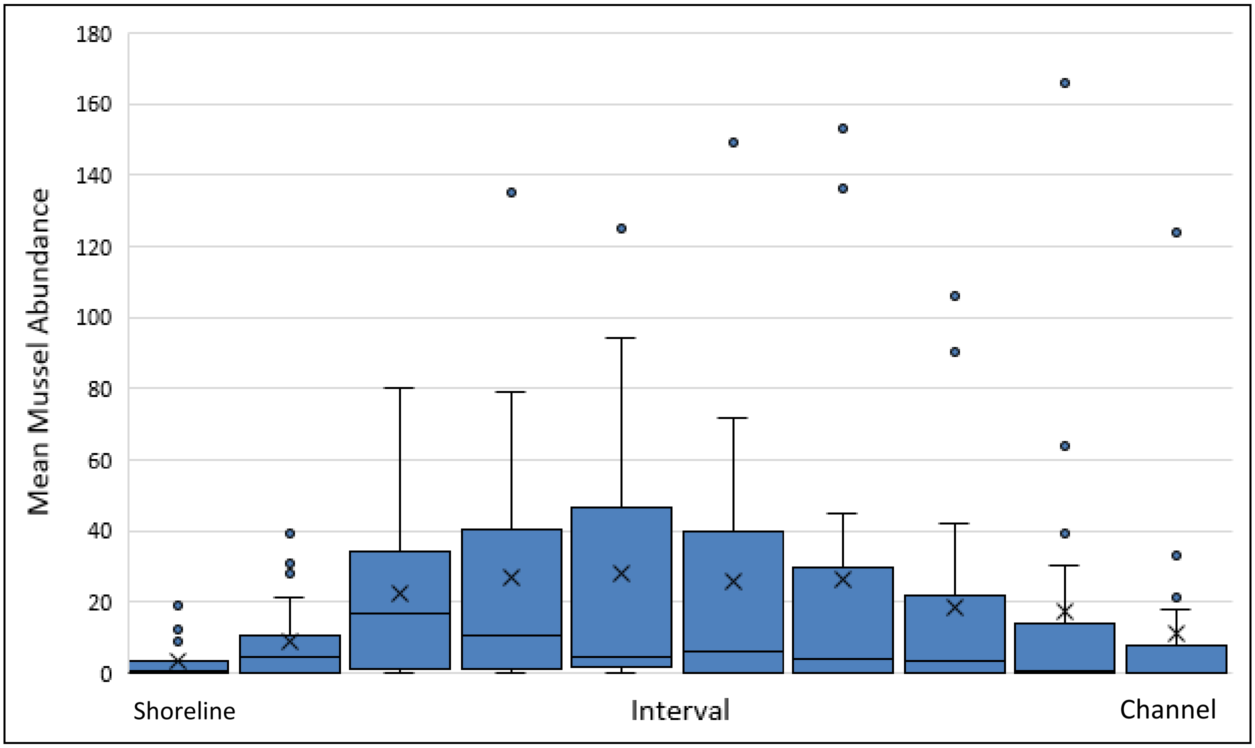


Figure 16. Mean mussel abundance by interval in the Greenup pool, Ohio River. Each interval increases by 10m increase from the shoreline (Source: Personal collection).

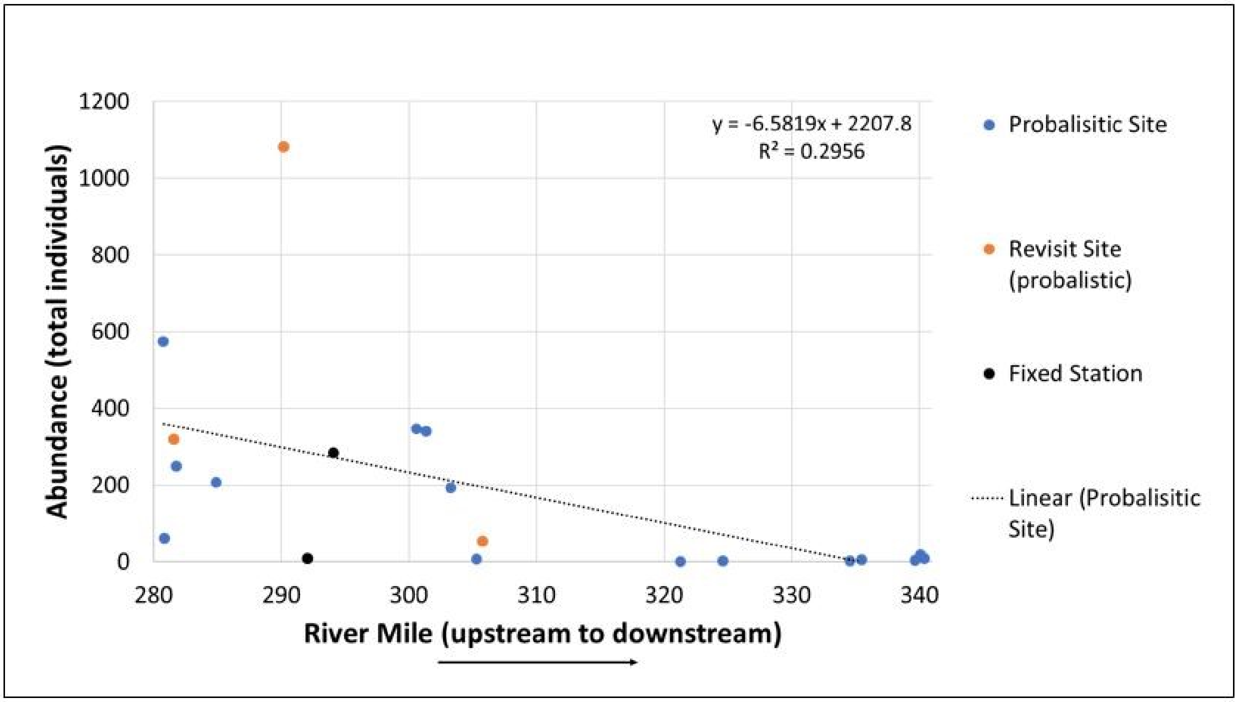


Figure 17. Mussel abundance counts by river mile in the Greenup pool, Ohio River (Source: Personal collection).

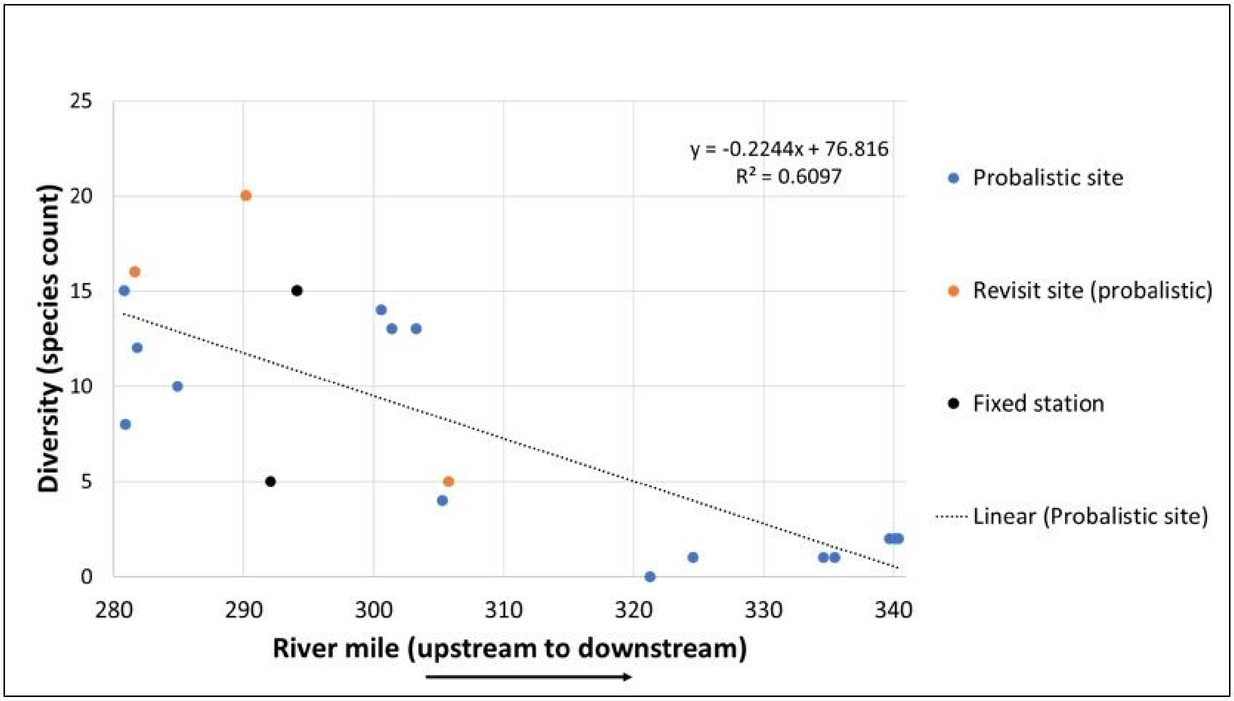


Figure 18. Mussel richness counts by river mile in the Greenup pool, Ohio River (Source: Personal collection).

Maximum mussel density was 5.6 mussels/m^2. Average mussel density was 0.32 individuals/m^2. Mussel density in 11 upper pool sites was 1.81 individuals/m^2 compared to a density of 0.0177 individuals/m^2 in nine middle and lower pool survey sites. Five additional species were added from the collection of 1,051 dead shell specimens (Table 7). *Obovaria retusa* was included but represented by a single subfossil specimen and has not been seen alive in the Greenup pool since 1992 (Miller and Payne 2000). It is considered extirpated from the state of West Virginia.

Table 7. Deadshell not found live during thesis surveys.

Species	**Abundance**	**Condition**
Truncilla donaciformis - Fawnsfoot	1	Weathered dead
Ptychobranchus fasciolaris - Kidneyshell	1	Weathered dead
Obovaria retusa - Ring Pink	1	Subfossil
Pleurobema sintoxia - Round Pigtoe	2	Weathered dead
Cyclonaias tuberculata - Purple Wartyback	1	Weathered dead

Using data from my 18 randomly selected sites (excludes WV11 & WV12), the total freshwater mussel population of the Greenup pool within 100 meters of both shorelines is an approximate estimate of 6.365 million animals. Each mussel averages eight gallons of water filtration per day (Haag 2012), yielding 50.92 million gallons of filtered water every 24 hours in the Greenup pool within 100m of both shorelines.

Middle and Lower Pool Results

The mussel communities in and below the urban areas at river mile (RM) 304.2 were limited (Figure 17-18). Nine sites downstream from Huntington, WV yielded 92 individuals from seven species. There were no sites in the industrial/urbanized middle or lower pool that contained more than five species or 52 individuals (Table 3-4). The average richness at middle/lower pool sites was 10.22 individuals and 1.8 species. Site WV03, across from Huntington, WV, exhibited the highest mussel abundance with 52 individuals representing five species (Table 4). WV03 contained 57% of the total live mussel collections from all nine middle and lower pool sites. Without WV03, the abundance and richness falls to 4.44 individuals and 1.5 species per site. The habitat in site WV03 mirrored that of upper pool sites and it was situated in close proximity to a tributary and receives minimal commercial traffic or CSO impacts compared to other urban study sites (Figure 10).

DISCUSSION

Changes in the Greenup Pool Mussel Assemblages

Watters and Meyers Flaute (2010) suggest that the Greenup pool historically supported approximately 40 species of freshwater mussels (2010). Intensive USACE surveys during the 1990s revealed 34 species present from 4,174 individuals (Dunn 1999_2; Miller and Payne 2000) (Table 1). By combining my thesis results with current USACE data from 2001-present, 10,982 individuals have been collected in the Greenup pool, representing only 28 species (USACE 2017). Although populations in the upper pool were extensive, the species richness was <30 which suggests that several species have been lost in the Greenup pool over the last 25 years or my surveys did not represent the actual mussel diversity present.

Declining species

There are five species in the Greenup pool which appear to be experiencing severe declines or have become extirpated. They include: *F. subrotunda*, *L. abrupta*, *O. subrotunda*, *P.*

sintoxia, and *T. donaciformis*. All five mussel species were regularly encountered during surveys performed by Heidi Dunn or USACE researchers in the 1990s (Dunn 1999_2; Miller and Payne 2000). They have been rarely collected or absent from the pool within the last 15 years (USACE 2017; Personal communication - Patty Morrison and Janet Clayton 2017).

Quadrula nodulata

Q. nodulata has not been found live in the Greenup pool since the 1990s (Dunn 1999_2; Miller and Payne 2000). However, it is a common member of many downstream pools including Markland (ESI 2014). In 2004, a live *Q. nodulata* individual was discovered in the Guyandotte River, a major tributary to the Greenup pool (USACE 2017). The Greenup pool may represent the northern extent of their range.

Obovaria retusa

Obovaria retusa is a federally endangered, big river mussel that has experienced severe declines throughout its range and may be extinct (Haag 2012). It has not been seen live in the Greenup pool since 1992 and was not encountered during thesis surveys (Miller and Payne 2000). I collected one subfossil deadshell specimen but this species has likely become extirpated from the Greenup pool of the Ohio River.

Federally Endangered *Plethobasus cyphyus* Trends

Plethobasus cyphyus is a federally endangered mussel that has not been common in the Greenup pool since modern surveys began in the 1980s. It has been consistently collected, but never composed >2% of the total mussel abundance (Zeto 1987; Miller and Payne 2000; Dunn 1999_2). Since 2001, USACE surveys have infrequently collected *Plethobasus cyphyus* during surveys below RC Byrd Lock and Dam. In 15 years of surveys, four live individuals were collected, comprising only 0.055% of the total USACE mussel fauna (2017).

P. cyphyus was the only federally threatened or endangered species collected during thesis dives. It accounted for 0.24% of my mussel abundances (Table 6). I collected nine individuals from 3,742 mussels; over twice as many *P. cyphyus* from half the total mussel abundance compared to the USACE surveys (2017). Our collections suggest that the area below RC Byrd Lock and Dam may not be optimal *P. cyphyus* habitat within the pool as previously assumed. All nine individuals collected were mature adults (Figure 19).

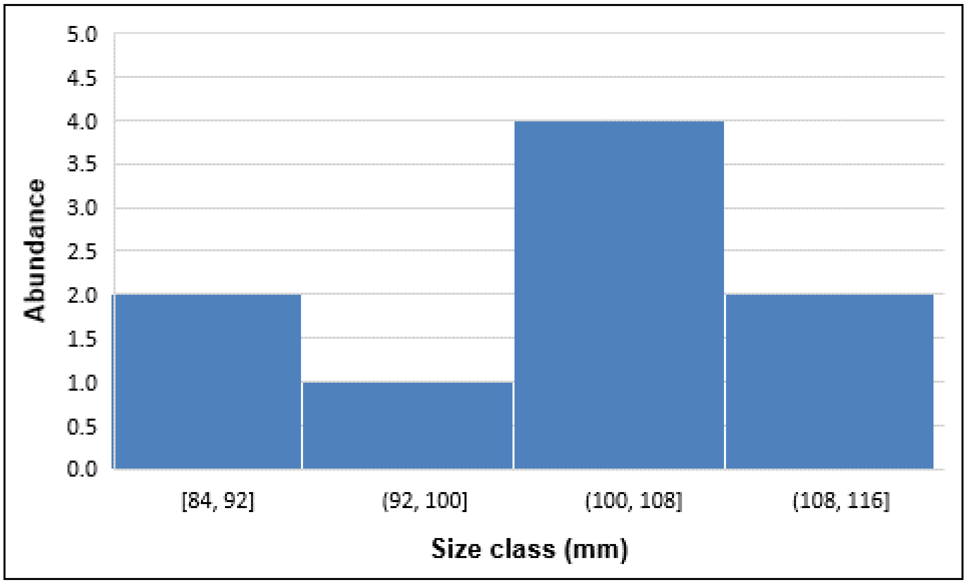


Figure 19. *Plethobasus cyphyus* size class counts, Greenup pool, Ohio River. Individuals over 30mm are considered adult mussels (Miller and Payne 2000) (Source: Personal collection).

The majority of my *P. cyphyus* encounters were made at semi-urban sites (7 specimens) with only one individual collected in the proximity of RC Byrd Lock and Dam. Surprisingly, four of these semi-urban specimens were found on an inside bend with substrates consisting of 10-70% fines over sand and gravel. Historically, *P. cyphyus* has been characterized as middle river species, rarely encountered in fines or inside bends (Oesch 1984; Parmalee and Bogan 1998). While transect data is not statistically defendable, my best site could contain over 330 *P. cyphyus* within the 500X100m area. Intensive surveys performed in the Markland pool by Environmental Solutions and Innovations (ESI) found *P. cyphyus* to show a strong affinity to sand habitat in outside intervals, 70-90 meters from shore (Spaeth, Anderson, and Swecker 2016). However, in the Greenup pool, few individuals were found in sediment dominated by sand and my *P. cyphyus* were concentrated 60 meters from shore (Figure 20).

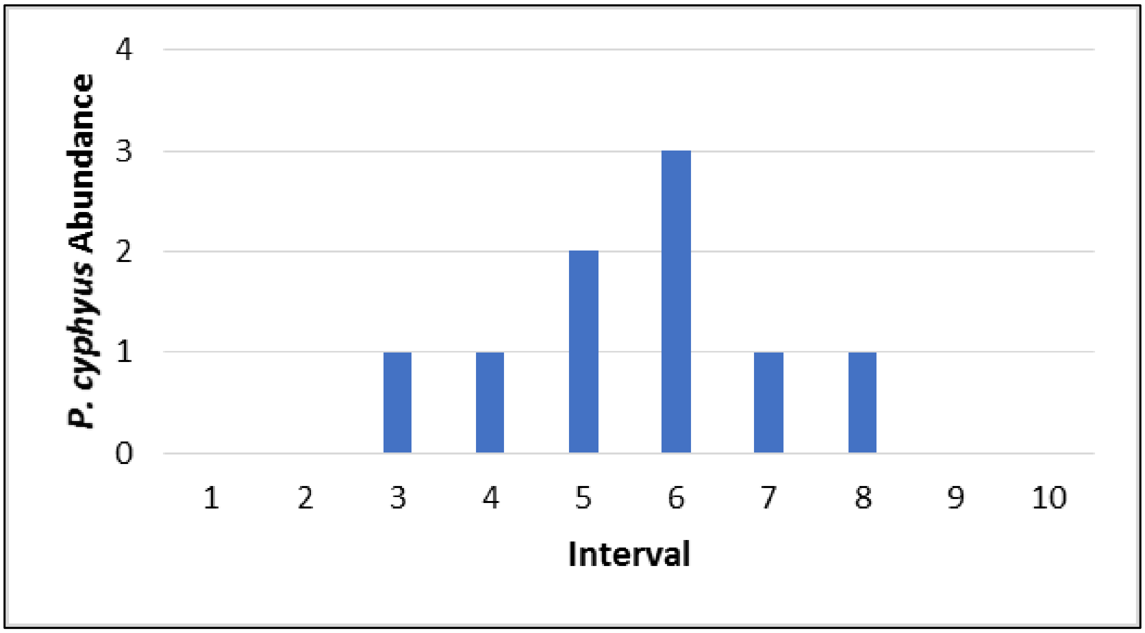


Figure 20. *Plethobasus cyphyus* abundance counts by interval. Each interval represents a 10m increase, with interval "1" beginning at the shoreline (Source: Personal collection).

My semi-urban sites, where 7 *P. cyphyus* were collected, are new locations for the species and warrant future surveys. Using my random sampling data, I predicted that the 100 meter area along the right and left descending banks for the entire Greenup pool contains approximately 16,547 *Plethobasus cyphyus*.

Commercial Traffic Impacts

The upper Allegheny River in Pennsylvania is a large, free flowing system which, unlike the Ohio River, experiences minimal commercial traffic impacts. It is a stronghold for three federally endangered species and exhibits a diverse mussel fauna that extends across the entire river width. Mussel beds are dictated by factors beyond their proximity to the river channel. In the Allegheny River, transects and survey cells do not show a decline in species abundance or richness moving channelward (Figure 21) (Swecker 2013). In the Greenup Pool and other sections of the Ohio, mussel populations begin to decline past 80 meters from shore; even if the depth remains constant (Figure 15-16) (ESI 2014). These trends show a stark contrast to the freshwater mussel distributions in the upper Allegheny River (Figure 21). Sites below Huntington, WV were heavily influenced by commercial traffic (Figure 22-23). Only 22 (7.5%), of the 291 visible barge impact sites from Google Earth occurred in the upper Greenup pool (Figure 22-24). Middle and lower sites exhibited low mussel abundance and richness in addition to a drop in mussel communities on outside intervals (Table 3-4). Propeller wash from commercial barge traffic may play a critical role in mussel distributions on intervals approaching the navigation channel and require further research.

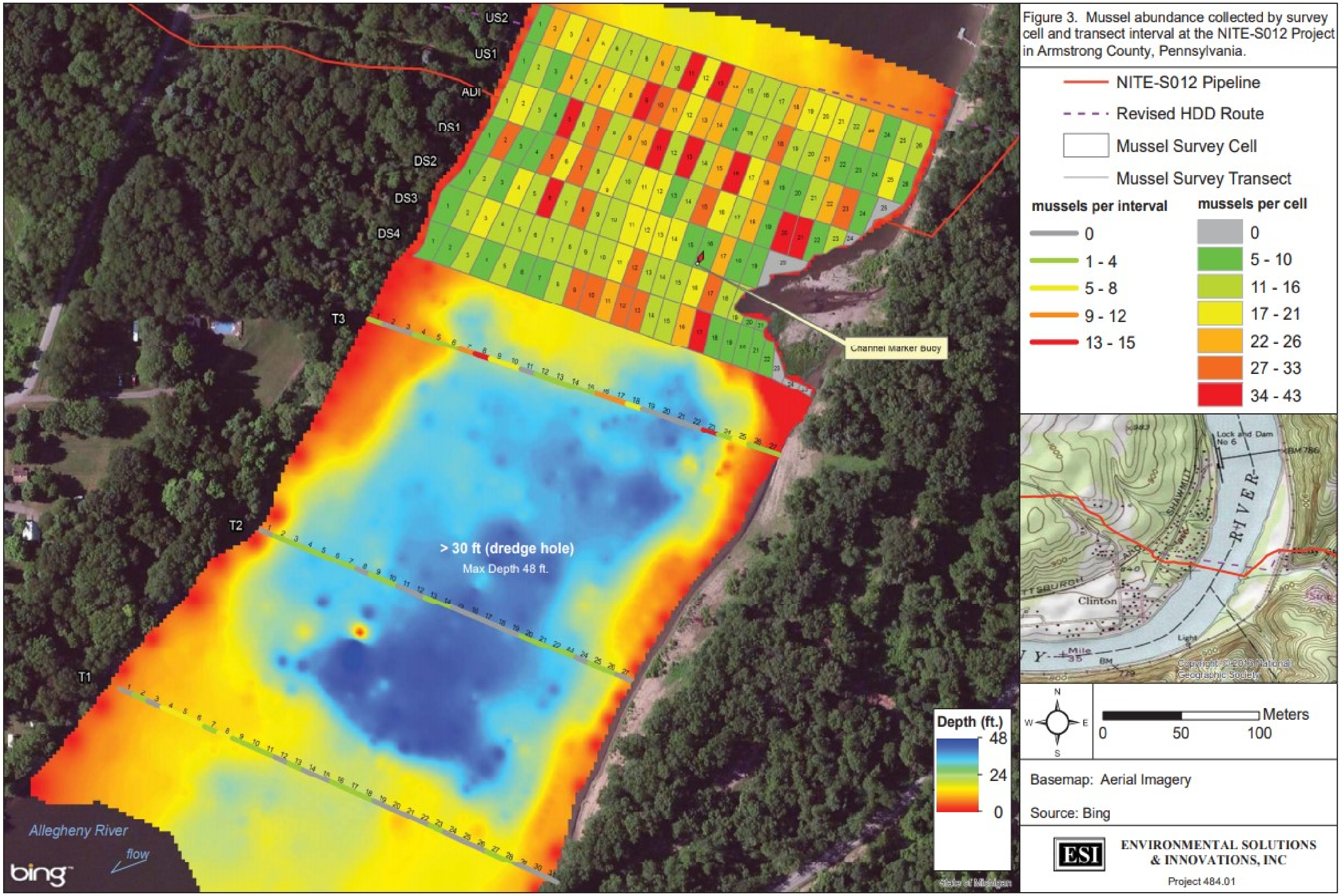


Figure 21. Mussel abundances collected by survey cell and transect interval on the Allegheny River (Swecker 2013).

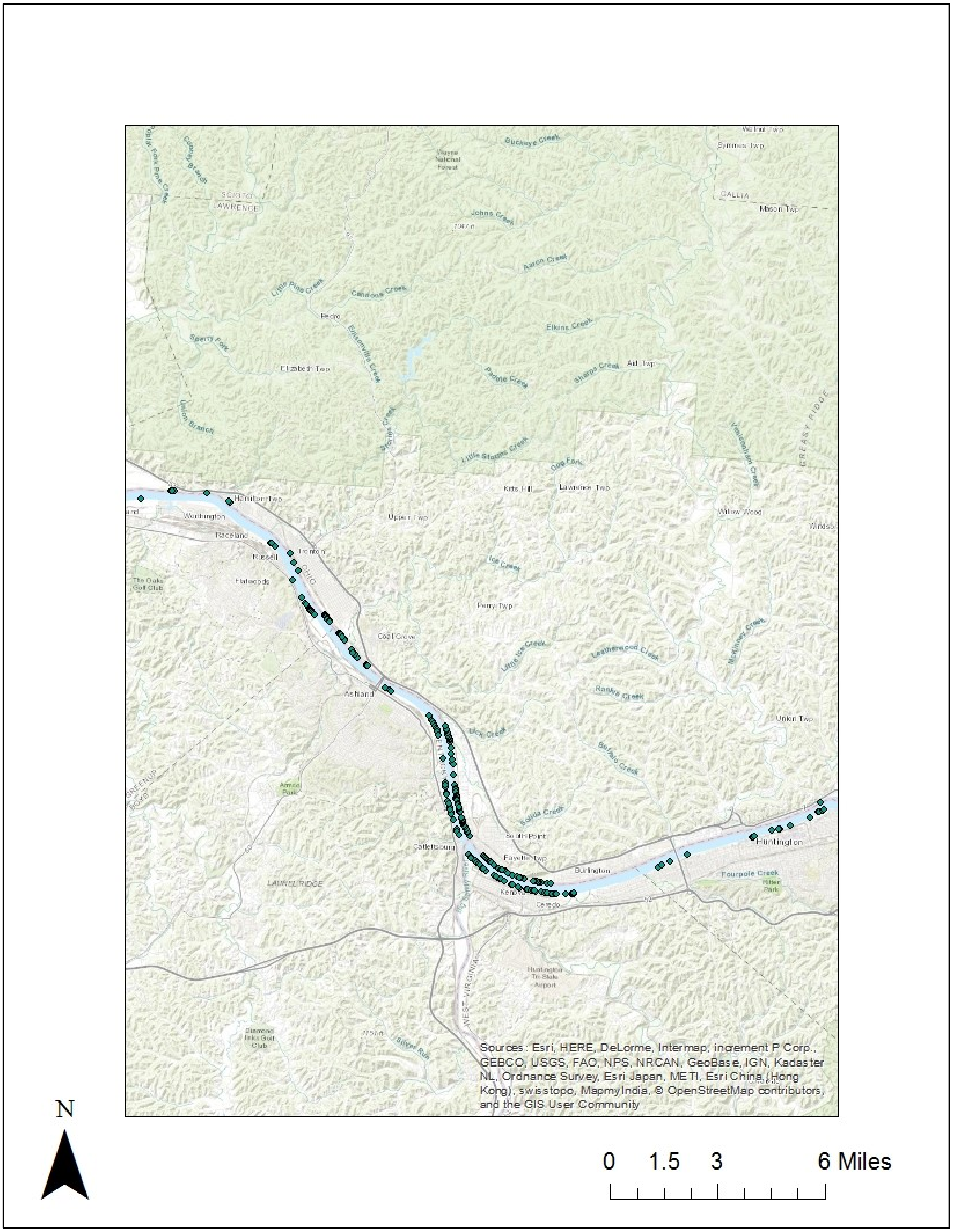
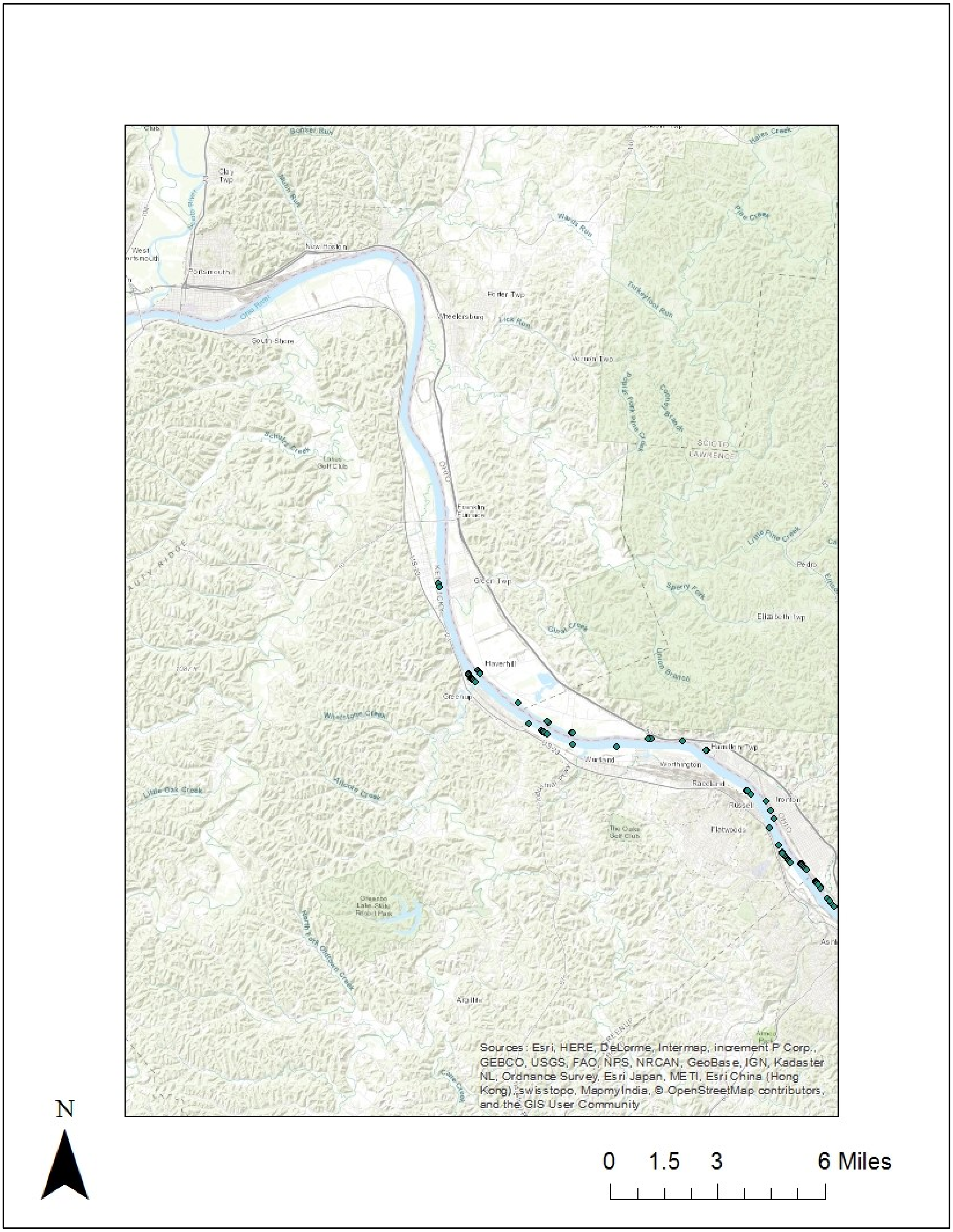
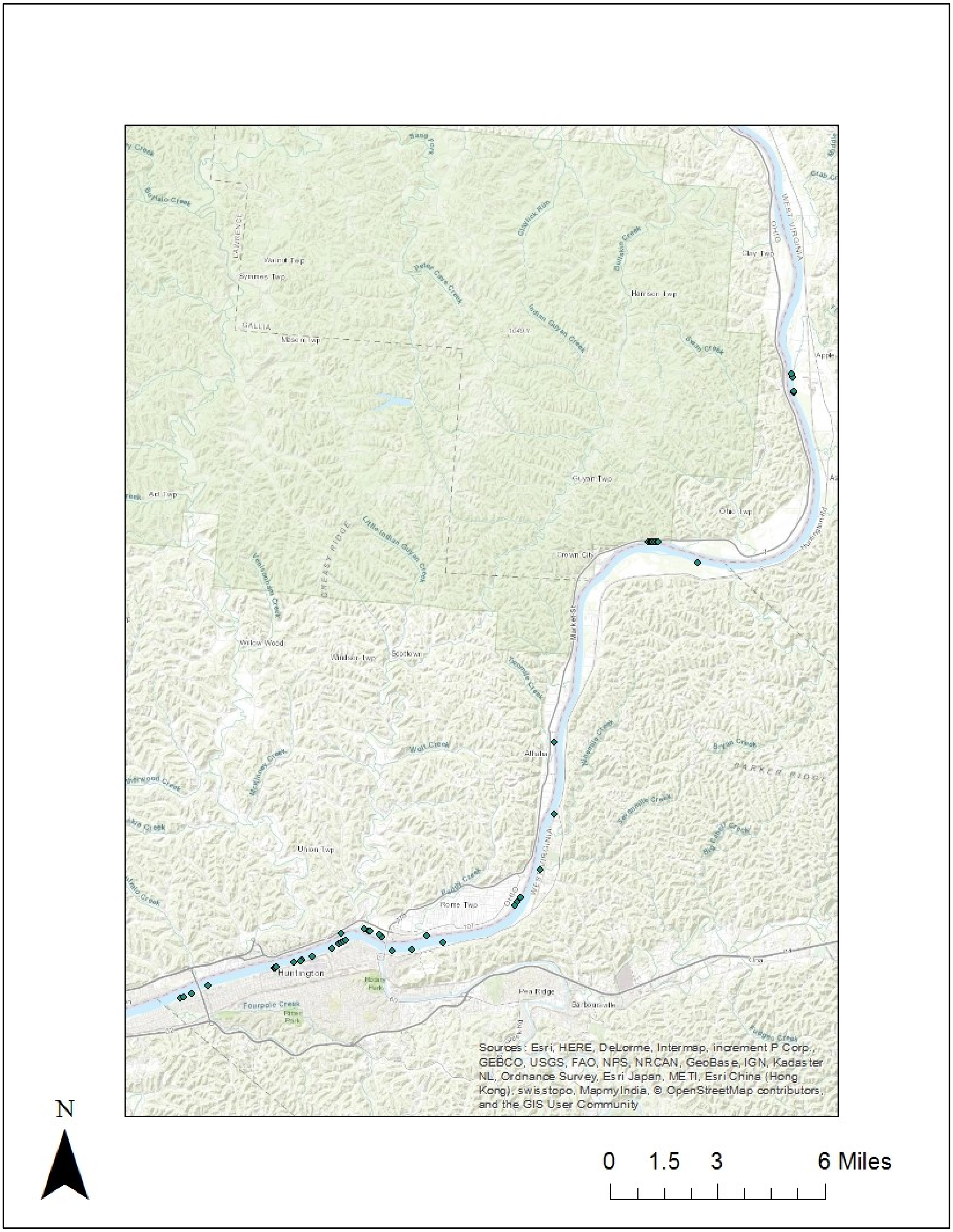


Figure 22. Barge impact sites on the middle pool bank (Source: Personal collection).



Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

Figure 23. Lower pool barge impact sites (Source: Personal collection).



Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

Figure 24. Upper pool barge impact sites (Source: Personal collection).

Zebra Mussel Observations

During their studies from 1993 - 1998, Miller and Payne noted that the zebra mussels had yet to heavily impact native mussels in the Greenup pool (Miller and Payne 2000). Unfortunately, my studies in 2017 suggest that zebra mussels are having a strong impact on native bivalves in the Greenup pool. Diver observations suggest over 95% of native bivalves encountered had zebra mussel infestations. Large species such as *Elliptio crassidens* and *Megalonaias nervosa* often harbored hundreds of individuals (Figure 6). Zebra mussel coverage appeared to be heaviest in the upper pool sites (WV11 & 09) below RC Byrd dam. At these sites, there was strong flow which kept a larger percentage of solid substrate silt free and inhabitable by zebra mussels.

Survey Comparisons

Intensive recent mussel surveys by USACE within the vicinity of RC Byrd Locks and Dam have turned up an additional five species live from my thesis collections (Table 7) (USACE 2017). Although these surveys are more localized, they do cover some of the best available habitat in the pool. The USACE collected 7,235 individuals from 2001 to 2017 but only found five additional species compared to my 2017 survey of 3,747 mussels (USACE 2017). Two of those five additional USACE species were collected as deadshell during thesis surveys (Table 7-8).

Table 8. Species found live during recent USACE surveys (2001-2017) which were not encountered alive during thesis surveys.

Species	**Abundance**
Elliptio diatata – Spike	1
Lampsilis ovata – Pocketbook	7
Pleurobema sintoxia – Round Pigtoe	2
Truncilla donaciformis – Fawnsfoot	1
Lasmigona costata – Flutedshell	2

Interestingly, the Flat Floater (*Anodontoides suborbiculata*) has only been found in the mainstem of the Greenup pool once (OSUM 1998). However, preliminary spot surveys in the backwaters of the Ohio River on Ice Creek, OH, indicate that it is very abundant in preferred habitats (Figure 25). My surveys poorly assessed species such as *A. suborbiculata*, which may only occur in creek mouths and backwaters of the Greenup pool. Due to an unknown survey area size by the USACE, my mussel recovery rate comparisons are unknown.

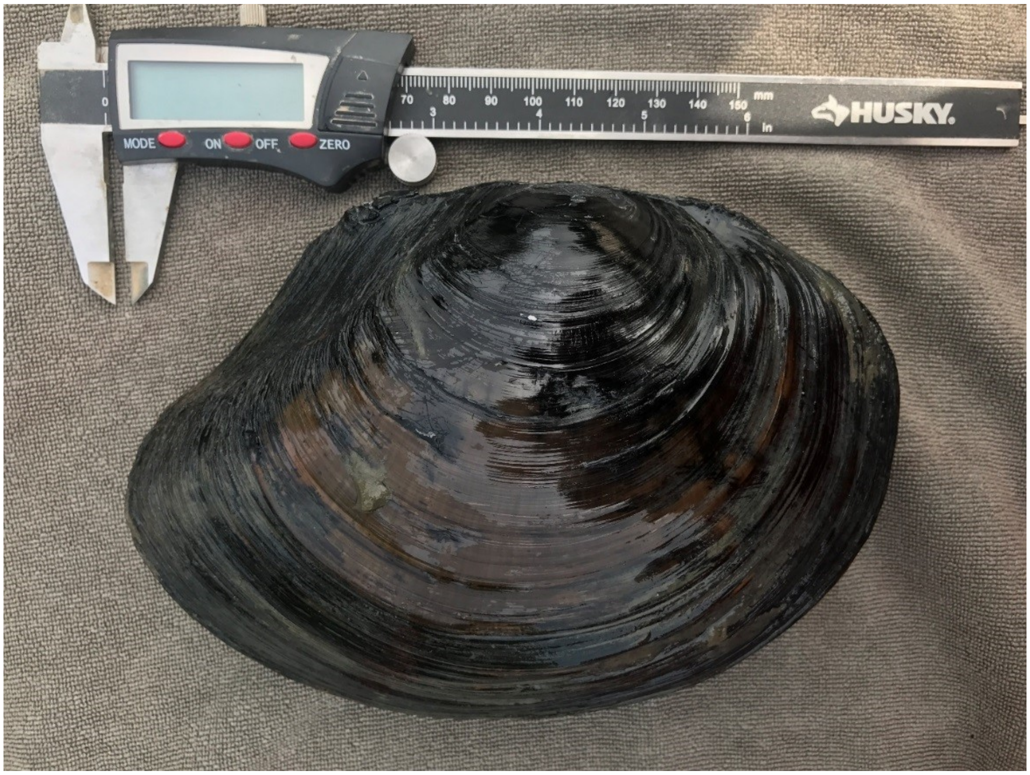


Figure 25. Flat Floater (*Anodonta suborbiculata*) from Ice Creek, OH. *A. suborbiculata* is a southern species that has recently expanded its range into the Greenup pool tributaries. However, it has only been encountered once in the mainstem in 1998 (OSUM 1998). Numerous individuals were observed in Ice Creek, OH backwaters. Deadshell was observed in Greenbottom Swamp, WV. Populations showed signs of strong recruitment with juveniles present (Photo credit: Tom Jones).

Thesis surveys performed in the poor-quality mussel habitat of the mid and lower pool recovered 92 individuals and seven species from nine sites (Table 3-4). The total survey area was 5200 m^2 equaling 0.0176 mussels/m^2. Although a survey comparison is not an optimal statistically sound method due to lack of random survey techniques from historical Greenup pool data, it does provide insight into mussel community distributions. Ten surveys in the middle and lower pool between 2003 and 2016 collected 258 individuals from 14 species at a density of 0.0231 mussels/m^2 (Table 1). Their total survey area was 11,130 m^2 (Hoggarth 2010; Fortenbery 2010, 2008; EnviroScience 2008_1, 2008_2 Ecological Specialists Inc. 2003_1, 2003_2;

Spaeth and Swecker 2015, 2016; Swecker 2009). The recovery density variation between thesis surveys and historical work is only 0.0055 mussels/m^2. However, the ten historical surveys collected seven more species than were found in my middle and lower pool sites but may be due to a large gap in the randomly generated sites between Huntington, WV and Ashland, KY.

Conclusions

Mussel populations declined sharply below urban/industrial areas downstream from Huntington, WV. In the middle and lower pool (37 miles long), 92 mussels representing seven species were collected from nine sites (Table 3-4). In the upper pool (24.8 miles long), 3,655 individuals representing 22 species were collected from 11 sites (Table 5). A combination of CSO discharge and physical destruction of substrate habitat by barge traffic may be a factor in the poor middle and lower pool mussel fauna (Figure 4) (Figure 22-24). While exceptions do exist (Figure 26), the upper Greenup pool supports a diverse mussel fauna. The upper pool is relatively free of barge impacts and concentrated urban CSO pollution (Figure 24). Future studies are needed to determine the impacts of CSOs and commercial traffic on freshwater mussel populations in the Greenup pool, Ohio River.

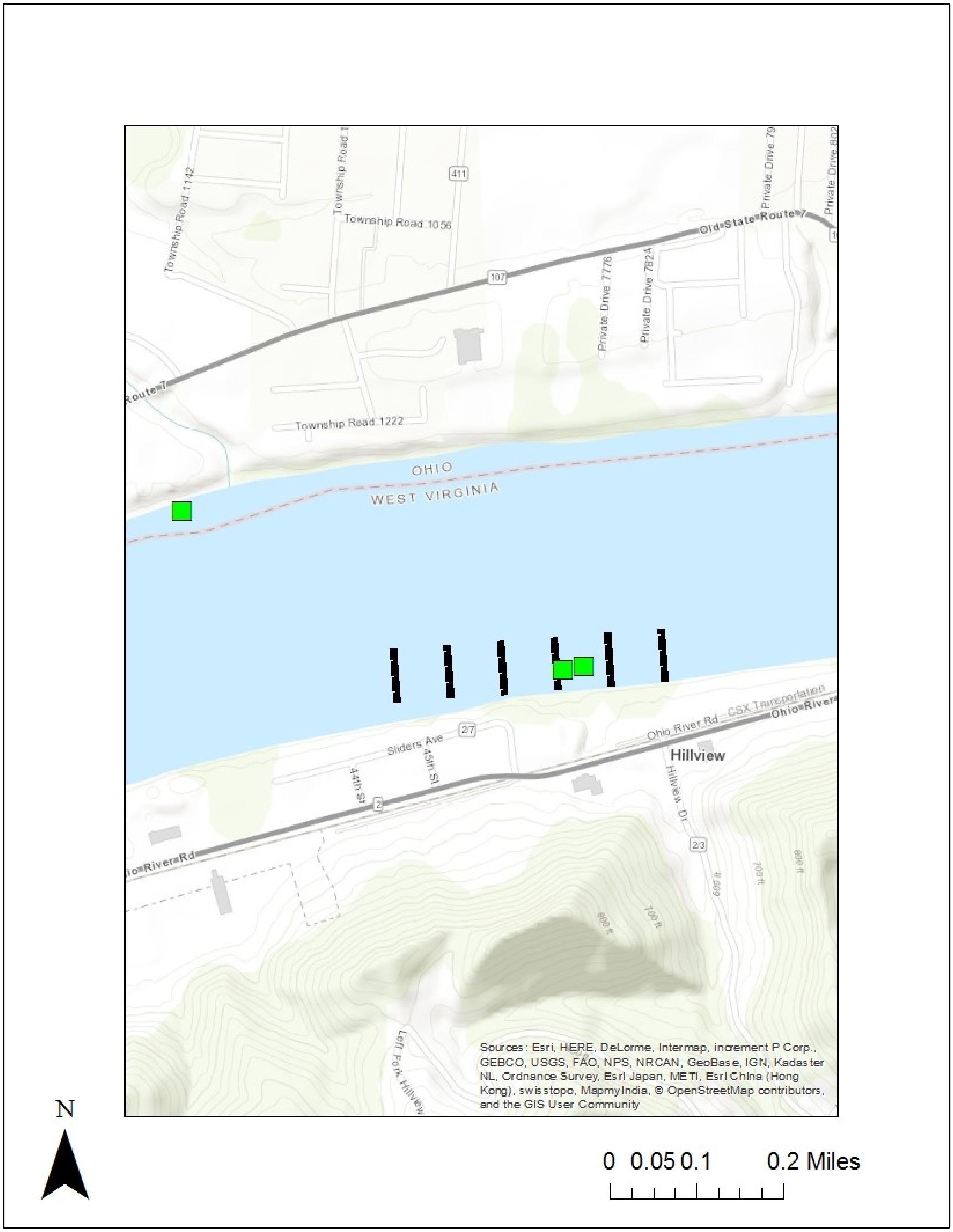


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Figure 26. WV01, an upper pool site with barge impacts but a strong mussel population. The green squares represent barge tie up pins (Source: Personal collection).

My survey design of six transects per 500X100m covered 1.2% percent of a total site area. Premier mussel beds could be missed between transects. Mussel density collection rates coincided with previous Greenup pool surveys but species richness was notably lower in my middle and lower pool thesis surveys (Hoggarth 2010; Fortenbery 2010, 2008; EnviroScience 2008_1, 2008_2 Ecological Specialists Inc. 2003, 2003; Spaeth and Swecker 2015, 2016; Swecker 2009). Large river mile gaps between randomized thesis sites yielded additional middle pool species for previous mussel surveyors (Spaeth and Swecker 2015, 2016; Swecker 2009). Further research is needed to determine the correct number of survey sites to define the pool wide freshwater mussel fauna.

CHAPTER II

SUBSTRATE COMPOSITION OF THE GREENUP POOL, OHIO RIVER

INTRODUCTION

Riverine substrate exerts a strong influence on freshwater mussel distributions (Brim Box and Mossa 1999). Fines are a common substrate in eastern US Rivers which can accumulate unnaturally in rivers due to anthropogenic erosion and reduced water velocity from damming (Watters et al. 2009, Haag 2012). Fines are composed of small organic and inorganic particles, less than 0.063 mm wide (Bunte and Abt 2001). Their small size increases the benthic surface area which promotes larger microbial communities and increases the sediment chemical oxygen demand (Nogaro et al. 2008). There is heavy debate as to whether fine sediments smother juvenile and adult mussels or provide micro habitats for feeding pedally (Brim Box and Mossa 1999, Haag 2012). My goal was to create a pool wide dataset of riverine sediments and determine their effect on freshwater mussel communities. I was particularly interested in the impact of fines on mussel richness and abundance.

BACKGROUND

Land use

Forest cover and urbanization dominate the Greenup pool (Figure 1). Row crops and livestock are primary sources of erosion and siltation (Grabowski and Gurnell 2016). These land practices are not as widespread along the Greenup pool mainstem as in Ohio River downstream sections. However, a narrow portion of the lower and upper pool flood plain are primarily agricultural (Figure 27-28).

Figure 27. Lower pool land use: Forested with moderate agricultural and urban coverage (Source: Google Earth Pro).

Figure 28. Upper pool land use: Heavily forested with light urbanization and moderate agricultural use (Source: Google Earth Pro).

Armored rip-rap banks line significant portions of the urban shorelines within the pool. Additionally, flood walls surround the cities of Guyandotte WV, Huntington WV, Ceredo WV, Cattletsburg KY, Ashland KY, and Ironton OH. These structures impede natural river pulses into the flood plain. Historically, iron mining and deforestation caused heavy erosion and greatly increased the sediment load of the Greenup pool. Over 97% of the forests in West Virginia have been clear cut within the last 200 years (Hiltz n.d.). Modern sources of erosion in the Greenup pool come from logging and improper farming practices, particularly within the Ohio Corn Belt (Blann, Anderson, Sands, and Vondracek 2009; Megahan and Kidd 1972). The Guyandotte and Big Sandy rivers are major tributaries to the pool. They drain the southern WV and eastern

Kentucky coal fields where strip mining and mountaintop removal activities produce significant sediment loads (Weaks 1982; WVDEP 2002).

Impoundment Impacts on Fine Sediment

Inhibited water flow from modern dams along the Ohio River encourages the accumulation of fine particulates on the substrate (Brim Box and Mossa 1999). Dams also weaken natural flood pulses that historically flushed sediments downstream (Schneider, Flörke, De Stefano, and Petersen-Perlman 2017). Suppressed water velocities and an increased sediment load from anthropogenic effects have combined to enhance fine sediment deposition in riverine systems (Hagerty et al. 1995).

Barge Traffic Substrate Impacts

Propeller scars from commercial barges occur where vessels move into shallow water (<4.5m) and strong currents from 1.8 - 2.4m tug propellers hit the river bottom. The fine sediment is blasted downstream and the remaining large debris becomes compacted (Wolter and Arlinghaus 2003). In the navigational channel, the substrate may be heavily impacted by barge traffic; even during normal water conditions. When water clarity is high, large plums of disturbed silt can be seen behind barges traveling upstream and disturbed fine sediment increases water turbidity up to five miles downstream (Figure 29-30). The water displaced by commercial traffic also pushes light sediments outward, toward the bank where naturally reduced velocities trap suspended particulates. (Figure 31-32). Additionally, the scouring of the river channel creates unnatural benthic drop-offs and exposes bedrock (Figure 33).

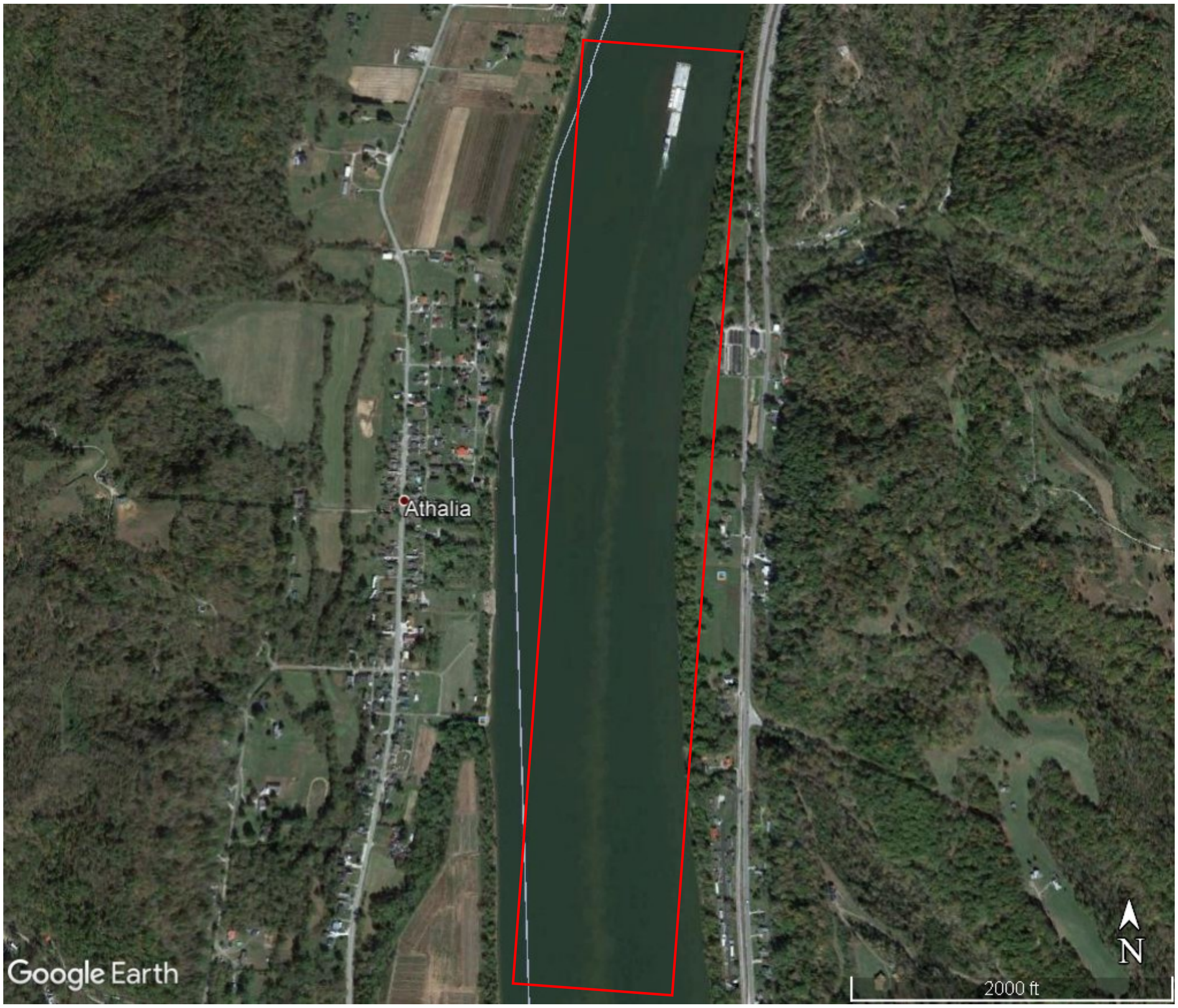


Figure 29. Barge Sediment Plume during clear water conditions (Source: Google Earth Pro).

Figure 30. Dilution of barge plume. Effects can be seen > five miles downstream (increased turbidity) (Source: Google Earth Pro).

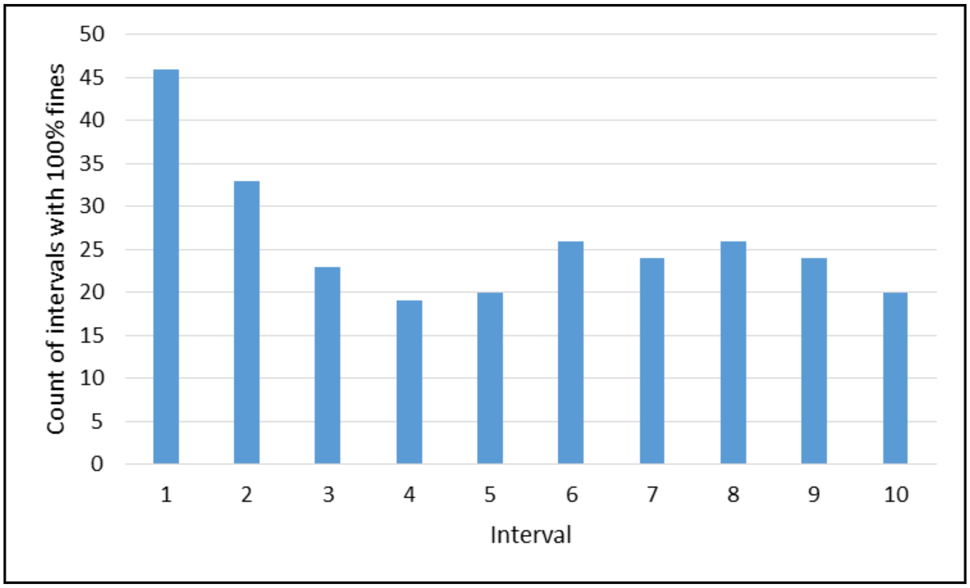


Figure 31. Counts of 100% fines coverage by interval where “1” represents the most shoreward sample location (Source: Personal collection).

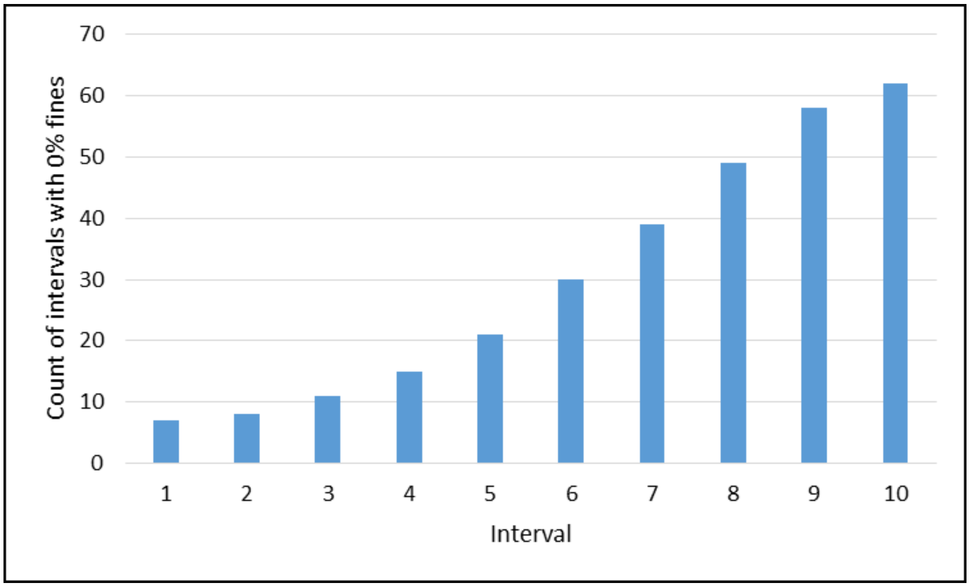


Figure 32. Counts of 0% fines coverage by interval where “1” represents the most shoreward sample location (Source: Personal collection).

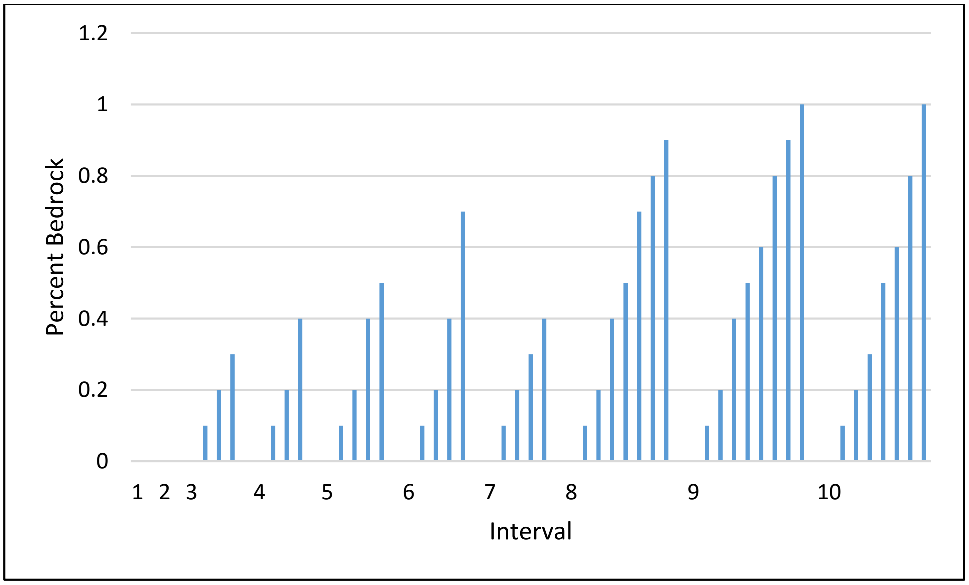


Figure 33. Counts of percent of bedrock substrate by interval. Each bar represents a percentage of an individual interval that contained bedrock. Groups represent a summation of all bedrock occurrences at each interval across all sites (Source: Personal collection).

METHODS

Substrate Collection/Classification

I performed substrate surveys at 20 sites in the Greenup pool of the Ohio River from July to September, 2017 (Figure 8-12). All sites coincided with 2016 ORSANCO biological collection locations and thesis mussel surveys. Sites were split into the classifications: “upper pool,” “middle pool,” or “lower pool” based on their proximity to prominent anthropogenic impacts and distance from dams. The upper pool contained 11 sites between RC Byrd Locks and Dam and the city of Huntington, WV (Figure 8-9). The middle pool contained four sites situated within the urban areas of Huntington, WV and Ironton, OH (Figure 10-11). The lower pool was composed of five sites between the Greenup Locks and Dam and Ironton, OH (Figure 12).

At each site, I used SCUBA to survey six 100 meter transects. Each transect ran perpendicular from the river bank toward the channel. All six site transects were spaced 100 meters apart to provide equidistant coverage of the 500X100 m area (Figure 13). The transect classification number moved downstream at each site from “1” to “6.” Transects were split into ten 10 meter intervals (Figure 14). The interval classification number “1” started at the shoreline. Transects were marked by running 100 meter lead lines from the bank toward the channel. Each line contained an anchor at both ends, a buoy to mark the starting interval, and 10 meter interval markers. Divers descended the buoy line to survey from the 10th interval to the bank. Sediment surveys were only performed when visibility exceeded 0.5m, a standard set by the WV Mussel Survey Protocol (Clayton et al. 2016).

Sediment percent coverage was observed by the divers as they moved through each interval during mussel surveys. The sediment composition was ranked as a percentage. For example, a typical interval may be categorized as 20% fines, 50% gravel, 10% cobble, and 20% sand. I classified sediments with a hybrid system that blended qualitative substrate properties and Forest Service particle size classifications (Table 9) (Bunte and Abt 2001). The sediment percent was written on slates for recopying on the surface or relayed by the diver to a boat crew via full face mask microphones. Transect standardized selection within the study did not provide randomized, defendable data. Therefore, site selection provided the random survey technique needed for statistical analysis of Greenup pool substrates.

Table 9. Greenup pool substrate classifications, Ohio River (Bunte and Abt 2001).

Substrate	Classifications
Boulder	Larger than 256 mm (Roughly larger than a human head)
Cobble	Between 64 and 256 mm (Roughly between the size of a human fist and head)
Gravel	Between 2 and 64 mm (Roughly to the size of a human fist)
Sand	Coarse hard grained particulate
Fines/Silt	Extremely fine loose sand, clay, and/or organic material
Bedrock	Imbedded rock surfaces covering >1m ^{2}
Woody Debris	Any benthic tree debris
Clay	Compacted clay particulates that can be penetrated
Hardpan	Compacted clay particulates that cannot be penetrated
Other	All other materials encountered including anthropogenic trash

GIS

I used Arc Map 10.3.1 to map site-specific sediment composition in the Greenup pool. Sediment was linked to each interval as a percentage. Barge impact sites indicated by parked barges, visible pins/stands, and shoreline barge repair structures were identified and marked in 2016 Google Earth images and converted to ArcGIS shapefiles.

Statistical Analysis

To understand the impact of fines on the Greenup pool mussel fauna, I used a negative binomial regression test to predict freshwater mussel abundance and richness by percent fines. I used a negative binomial regression to account for the large number of zeros in my data and lack of normality. For each negative binomial test, fines was the predictor (*x*) for mussel richness or abundance as the response (*y*) with 95% confidence intervals (CL) (Brim Box and

Mossa 1999). The null hypothesis stated that the regression coefficient is equal to zero (β=0). The alternative hypothesis stated that the regression coefficient is not equal to zero (β≠0). All statistical analyses were performed in SAS ® [9.4].

Table 10. Upper Greenup pool site classifications, Ohio River (RM 279 to 304).

Site	Morph type	Bank	Position	Fines	Sand	Gravel	Cobble	Bedrock
WV01*	Straight	LDB		64.4	0.2	13.6	16.7	0.3
WV08	Straight	LDB		26.7	17.3	6.3	26.7	0
WV10*	Straight	LDB		9	41.7	11.8	22	9.8
WV12*	Straight	LDB		48	22	8.3	6.3	8.2
WV09	Straight	RDB		27	9.8	11	16	19.5
WV11	Straight	RDB		13.8	7.2	17.8	8	9.5
WV04	Bend	RDB	Inside	26.7	19.2	42.3	7.2	0
WV05*	Bend	RDB	Inside	28.5	15.8	31.7	15.2	2
WV13	Bend	LDB	Inside	31.3	55.8	3.2	1.2	0
WV07	Bend	RDB	Inside	11.8	32.6	31.2	4.2	0
WV06	Bend	RDB	Outside	26.8	25.2	38.1	16.5	0

*Denotes site with barge impacts
XX Denotes fixed ORSANCO site
XX Denotes revisit ORSANCO site

Table 11. Middle Greenup pool site classifications, Ohio River (RM 304 to 333).

Site	Morph type	Bank	Position	Fines	Sand	Gravel	Cobble	Bedrock
Ky06**	Straight		LDB	26.2	25.2	38.2	16.5	0
Ky07*	Bend	Inside	RDB	44.8	42.8	1.6	0.5	0
Wv02*	Bend	Outside	RDB	28.6	47.8	19.1	1.2	0
Wv03*	Bend	Outside	LDB	63.4	23.8	2.5	0.5	0

*Denotes site with barge impacts

** Denotes site with industrial/intake impacts

XX Denotes revisit ORSANCO site

Table 12. Lower Greenup pool site classifications, Ohio River (RM 333 to 341).

Site	Morph type	Bank	Position	Fines	Mdepth	Gravel	Cobble	Bedrock
Ky01*	Straight		LDB	81.5	40cm	1.7	8.2	0.8
Ky02	Straight		RDB	92.7	>1m	0.3	0.5	0
Ky03	Straight		RDB	93.9	>1m	0.5	1.5	0
Ky04	Bend	Inside	LDB	94.8	20cm	0	0	0
Ky05	Bend	Inside	RDB	91.1	10cm	0.2	3.5	0.5

*Denotes site with barge impacts

Mdepth = Depth of fines (meters)

Freshwater Mussel Collections

My surveys in the Greenup pool yielded 3,747 live mussels, found in 19 of the 20 sites (Table 2-5). Twenty-three species were collected live, including nine federally endangered *Plethobasus cyphyus* individuals. Thirteen of the 23 species were represented by relative abundances comprising <1% of the total mussel collections (Table 6). Maximum mussel density was 5.6 mussels/m^2. Average mussel density was 0.32 individuals/m^2. Mussel density in 11 upper pool sites was 1.81 individuals/m^2 compared to a density of 0.0177 individuals/m^2 in nine middle and lower pool survey sites.

The mussel communities declined significantly in and below the urban areas of Huntington/Guyandotte WV at river mile (RM) 304.2 (Figure 17-18). There were no sites in the industrial/urbanized middle or lower pool that contained more than five species or 52 individuals (Table 3-4). The average richness at middle/lower pool sites (all below urban areas) was 10.22 individuals and 1.8 species. Site WV03 exhibited the highest mussel abundance below Huntington, WV with 52 individuals representing five species (Table 4). WV03 contained 57% of the total live mussel collections from all nine middle and lower pool sites. Without WV03, the abundance and richness falls to 4.44 individuals and 1.5 species per site.

Statistical Analysis

A negative binomial regression was used to assess the influence of percent fines by site as a predictor of freshwater mussel abundance in the Greenup pool. The model contained 18 observations (n=18), was slightly over dispersed (Value/DF=1.2723), and had a large negative estimate (-5.3007±1.2723). The 95% CL intervals did not contain zero [-7.7944 – -2.8070] and the p-value was less than 0.05 (P=<0.0001). There was a significant relationship between mussel abundance and percent fines

A negative binomial regression was used to assess the influence of percent fines by site as a predictor of freshwater mussel richness in the Greenup pool. The model contained 18 observations (n=18), was not over dispersed (Value/DF=0.6283), and had a negative estimate (-2.6046±0.6283). The 95% CL intervals did not contain zero [-3.8361 – -1.3731] and the p-value less than 0.05 (P=<0.0001). There was a significant relationship between mussel richness and percent fines

DISCUSSION

Mussel - Fines Analysis

Moving downriver from RC Byrd Locks and Dam, mussel populations steadily decreased past Huntington while the percent of fines increased at each site (Figure 34). Fines have historically been cited as a detrimental sediment to mussel colonization (Brim Box and Mossa 1999). In both analyses (mussel richness vs. percent fines and mussel abundance vs. percent fines), the negative binomial results yielded $\beta<0$ but had 95% CLs containing zero, and p-values >0.05. The negative binomial regression test indicates that fines act as a negative predictor of mussel abundance. For every one unit increase in fines abundance, the expected log counts of mussel abundance decreases by 5.3007 individuals.

Correspondingly, the negative binomial regression test indicates that fines act as a negative predictor of mussel richness. For every one unit increase in fines abundance, the expected log counts of mussel abundance decreases by 2.6046 species. However, these results should be viewed with caution as the model did not account for depth of fines, freshwater mussel population structure, or water velocity. Additionally, the lower pool exhibited drastic drops in freshwater mussel abundance/richness and a steady increase in fines deposition yet the sediment may be a single factor among many in determining freshwater mussel assemblages in the lower pool. Urban pollution (CSOs) and industrial activity may play a larger role in mussel survival. Silt tolerant species, such as *P. alatus*, *O. reflexa*, and *L. fragilis*, should have colonized the lower pool sites more heavily. Haag suggests that fines are not detrimental to mussel populations (2012). He believes that fines are, in fact, important for juvenile survival by allowing them to feed pedally (2012). Further research is needed to explore the lack of silt tolerant species in the lower pool and discount fines as a factor in freshwater mussel distributions.

Upper pool sites (RM 279 to 304)

RC Byrd dam creates a unique habitat the first 1-2 miles below its outfall in the upper portion of the Greenup pool. Swift currents from water moving through the dam inhibits the accumulation of fines. However, these strong currents do cause scouring in shallow sections (Emiroglu and Tuna 2011, Personal observation). Exposed rock and gravel dominate the sediment and a low percentage of fines yielded excellent water clarity (Figure 33) (Table 10).

The strong flow of upper pool sections also kept it moderately free of debris and logjams. This small section of the pool best mimics historical riverine conditions. The remaining upper pool is characterized by clean-swept substrate but the percent of fines steadily increases moving downstream toward Huntington, WV (Figure 34).

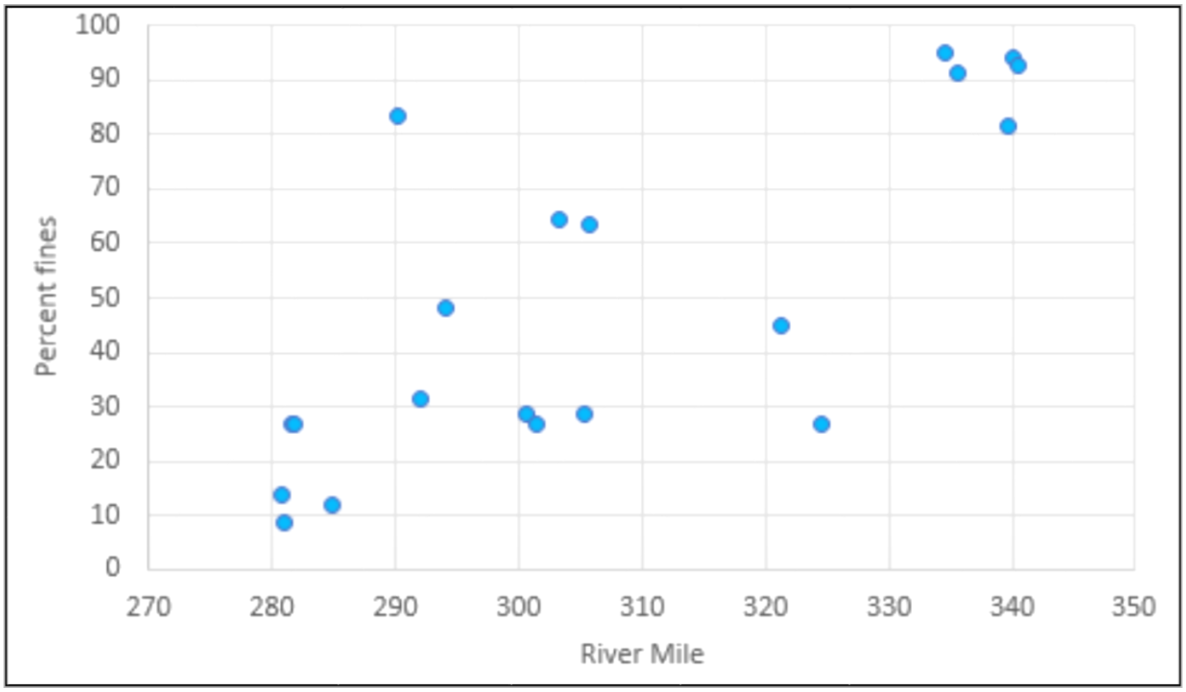


Figure 34. Percent of fines at each site by river mile moving downstream (Source: Personal collection).

Middle pool sites (RM 304 to 333)

Figure 35. Middle pool land use: Dominated by heavy urbanization and industrialization with moderate forest cover (Source: Google Earth Pro).

The middle portion of the Greenup pool is heavily impacted by urbanization and industry (Figure 35). The cities of Huntington, WV and Ashland, KY dominate the left descending bank (LDB). The Guyandotte and Big Sandy rivers also empty into this section of the pool. Both tributaries have been heavily impacted by coal mining (Weaks 1982; WVDEP 2002). This section of the pool begins to show moderate to heavy levels of sedimentation and numerous log jams were encountered (Table 11). Barge scours and industrial iron ore slag were also frequently observed at sites near Ashland, Ky.

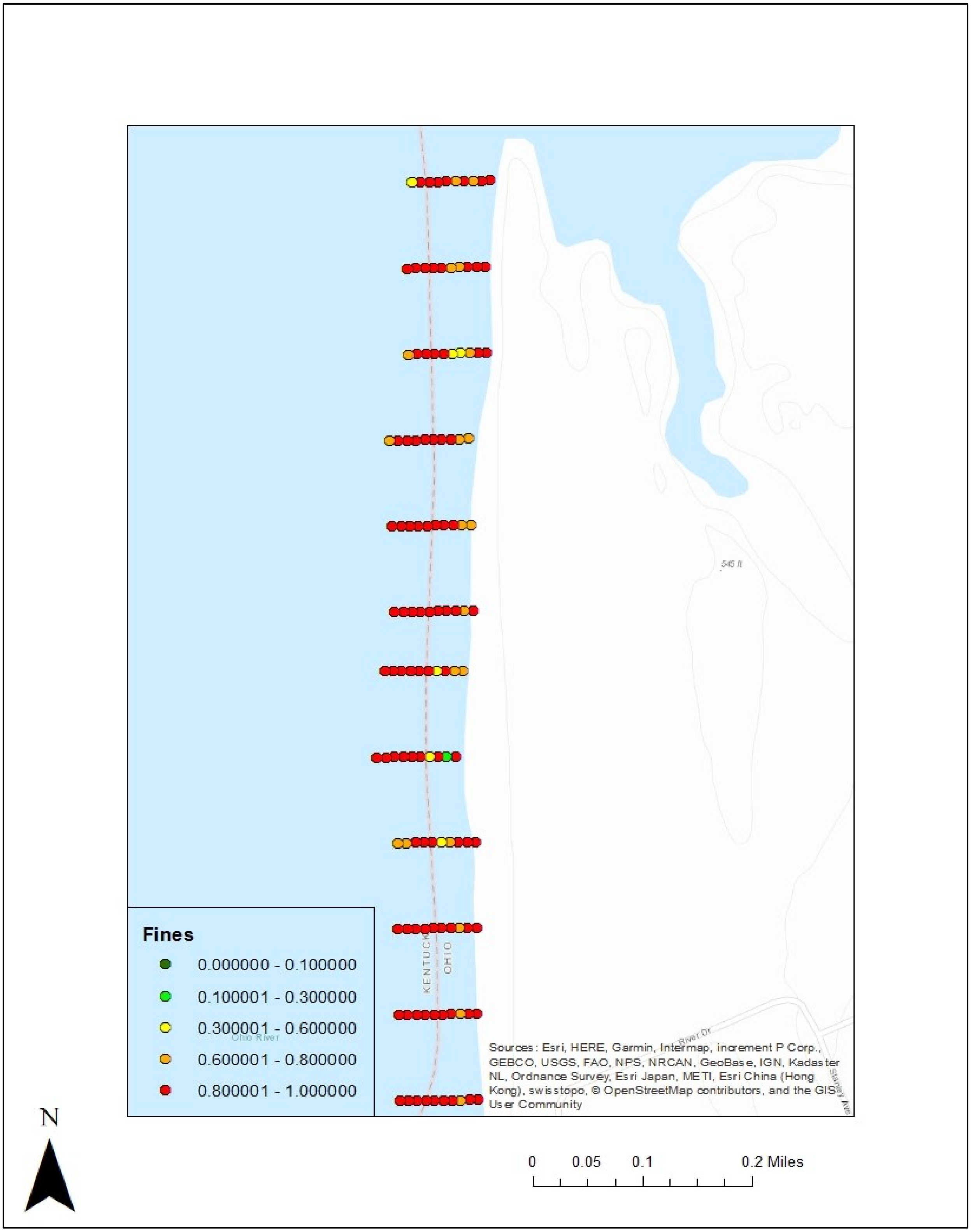
Lower pool sites (RM 333 to 341)

Heavy siltation, extreme depths, and minimal flow characterizes the lower section of the Greenup pool. Silt depths >1m were common with fines composing >80% of all substrates (Table 12). Large log jams dominated huge sections of my survey area. Depths up to 50 feet were encountered near Greenup Locks and Dam.

Impacts of Commercial Traffic on Sediment

Substrate observations during my project dives indicates that commercial traffic may impact fine sediments in the Greenup pool (Figure 29). Large propeller scars were observed at industrial loading sites in the lower pool (KY07 and KY06) and upper pool staging sites (WV10 and WV08) below RC Byrd Locks and Dam. Commercial impacts were also noted at WV12; a site where barges make 180° turns in the river (Personal observation). There were no visible impacts from above but the site contained heavy scour holes on the river bottom**.**

At all sites with commercial traffic influences, substrates appeared more compacted in outer transects, particularly at sites below RC Byrd dam (WV11, WV10, WV09, WV08). Exposed bedrock also becomes more common towards the center of the navigational channel which may be due to propeller wash (Figure 33). The counts of intervals with 100% fines coverage drops moving outward from the bank (Figure 31). Inversely, the counts of intervals with 0% fines coverage increases moving bankward (Figure 32). Propeller wash, water displacement, and surface waves from commercial traffic on the Ohio River may push fine sediments towards the bank. At three deep water sites (>45ft.) above Greenup Locks and Dam, fines exhibited homologous coverage throughout each transect (Figure 36) which suggests that no force exists in deep water to push the fine sediment shoreward. Reduced velocities from the dam may also play a role in fine sediment deposition at lower pool sites. Sediments could be suspended from commercial traffic and re-deposit in the same locations due to a lack of flow. Further research and measurements of commercial traffic propeller wash are needed to build upon these preliminary observations and explore Ohio River substrate patterns.



Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

Figure 36. Fine substrate deposition in lower pool intervals. Red circles indicate 80-100% fines coverage by interval while green circles indicate minimal fine deposition (Source: Personal collection).

Conclusions

Site level statistics supported my hypothesis that the percent of fines coverage impacts mussel populations. However, there are several confounding factors that my models could not take into account including depth of fines, freshwater mussel population structure, or water velocity. More research with randomized substrate data is needed to verify if freshwater mussels are impacted by the relationship of distance from shore and the percent of substrate coverage by fines. Additionally, the impact of commercial traffic on riverine substrates and freshwater mussel populations warrants future study.

CHAPTER III

FISH AND MUSSEL RELATIONSHIPS WITHIN THE GREENUP POOL, OHIO RIVER

INTRODUCTION

Larval freshwater mussels are an obligatory parasite to their fish or amphibian hosts (Haag 2012). Mussels can be host generalists or specialists (Watters et al. 2009). The parasitic host mechanism gives glochidia (larval freshwater mussels) a jumpstart of nutrients and disperses mussel populations (Woolnough 2006). If a host disappears from the ecosystem, the mussel population will be unable to recruit new cohorts. The fish-mussel relationship has been a focus of numerous studies throughout the 20th and 21st centuries (Barnhart, Haag, and Roston 2008; Hart 2014; Hauswald 2010; Watters et al. 2009; Irmscher and Vaughn 2015; Kelly and Watters 2010; Khym and Layzer 2000; O'Dee and Watters 2000; Sietman et al. 2017). Although most mussel species now have identified hosts, the spatial relationship between both parties is poorly understood.

Big rivers are notoriously difficult to sample for freshwater mussels and most surveys have been driven by commercial projects that lack statistical power due to non-random site selection (Hoggarth 2010; Fortenbery 2010, 2008; EnviroScience 2008, 2012; and Ecological Specialists Inc. 2003, 2003; Spaeth and Swecker 2015, 2016; Swecker 2009). Fish communities by comparison, have been studied and sampled on the Ohio mainstem using pool wide randomly sampled study designs since 2005 by ORSANCO. Currently, ORSANCO has collected over three million fishes from the Ohio River mainstem and its larger tributaries using night-time, shoreline electrofishing since 1991 and rotenone surveys of lock chambers from 1957-2005.

The goal of my study was to investigate the relationship between fish abundance/richness to freshwater mussel abundance/richness within the Greenup pool of the Ohio River. Although fish are highly mobile, I believe that fish and mussel abundance/richness will be positively related.

BACKGROUND

The upper Ohio River is a historically rich area for fish diversity (Sanders et al. 1999, Trautman 1986). However, damming, pollution and other anthropogenic influences throughout the 19th and 20th centuries have degraded the Ohio River ecosystem. The transformation of a shallow riverine system with riffle-run-pool habitat, to a homogenous, deep water system has altered the fish community in the Ohio River mainstem. The fish fauna is now dominated by generalists who do not rely on riffle habitats (Jacquemin and Pyron 2011). However, the Greenup pool still supports a diverse fish community with a richness >50 species (ORSANCO 2016_2, 2011).

Freshwater Mussel Reproduction

Freshwater mussels are obligate parasites on fish or mudpuppies for reproduction (Woolnough 2006). Host infestation occurs when the juvenile mussels, glochidia, encounter the appropriate host gills or fins (Haag 2012; Watters et al. 2009). The glochidia feed off of the hosts for several weeks and drop off at random into the substrate (Watters et al. 2009). This relationship may also be used for spatial distribution (Haag 2012). Some mussel species are generalists and can metamorphose on a variety of hosts while others are specialists on a single species or genus (Woolnough 2006). Common methods for mussels to facilitate contact between a host and their glochidia include: clamping fish hosts between their shells and pumping glochidia onto the gills, release of trot lines or webs of glochidia coated in mucus to ensnare fish, release of conglutinates in the form of matrices or insect mimics infested with glochidia (Haag 2012; Fuller 1974), and the use of a modified mantle as a lure to attract the fish hosts (Watters et al. 2009).

ORSANCO Involvement

In 1948, the Ohio River Valley Water Sanitation Commission (ORSANCO) was formed to monitor water quality, perform biological assessments, and set waste water discharge standards in the mainstem and tributaries of the Ohio River. ORSANCO is funded by a multi-state compact from states which share boundaries along the Ohio River or contain a section of the Ohio watershed (New York and Virginia). ORSANCO practices aquatic biomonitoring, which utilizes fish and aquatic invertebrates to assess riverine health (ORSANCO 2011). The presence or absence of sensitive species indicates environmental condition. ORSANCO surveys each pool of the Ohio River every five years and their sites served as locations for all mussel surveys for this thesis and provided a template for comparison between mussel and fish data.

METHODS

Fish collection

Fish community data for the Greenup pool was collected by ORSANCO via boat electrofishing. Electrofishing is a standard method of fish collection for researchers and government organizations in big rivers. An electrofishing unit is a device that is run from a generator which supplies a current to the water column below and around the boat. A setting of 180-225 volts DC at 60-120 pulses/ second with a duty cycle approaching 50% was used to achieve a target power of 6000W (ORSANCO 2017_2).

The pool was sampled between July 1st and October 31st when water was within one meter of the "normal flat pool." Sampling occurred at night when fish were most active, and many species moved into shallow water to feed on smaller fishes, invertebrates, and algae. Nighttime sampling maximizes the richness and abundance of species collected, giving an improved representation of the pool wide fish community (Sanders 1992).

ORSANCO surveyed 20 sites within the Greenup pool in 2016. Fifteen of the sites were randomly selected and three were revisit sites from 2011 sampling. However, these revisit sites were initially selected randomly. Two of the sites were preselected as reference locations and do not provide randomized data. During shocking, the boat began at the top of each 500-meter site and moved downstream for a minimum of 1,800 seconds. All available habitats were shocked within 100 feet from shore. However, deeper water did limit the collection efficiency as the electric current may not reach benthic species. Fish were placed into a live well until the entire site had been sampled. The specimens were then identified, measured, and inspected for abnormalities. Small fish, generally less than four cm, were retained and preserved in formalin for identification in the lab. Large fish and all game fish were released after data collection (ORSANCO 2011).

Mussel Survey

I performed freshwater mussel surveys at 20 locations (Figure 8-12) in the Greenup pool of the Ohio River from July to September, 2017. All sites coincided with 2016 Ohio River Valley Sanitation Commission (ORSANCO) biological and fish collection locations (ORSANCO 2016$_2$). Each transect ran perpendicular from the river bank toward the channel. All six site transects were spaced 100 meters apart to provide equidistant coverage of the 500X100 m area (Figure

13). The transect classification number moved downstream at each site from “1” to “6.” Transects were split into ten 10 meter intervals to provide precise location data. The interval classification number “1” started at the shoreline. Transects were marked by running 100 meter lead lines from the bank toward the channel with an anchor at both ends, and 10 meter interval markers. Divers descended the buoy line to survey from the 10th interval to the bank. Transect standardized selection within the study did not provide randomized, defendable data. Therefore, site selection provided the random survey technique needed for statistical analysis.

Divers visually searched for siphons, flipped large debris, and used tactile sifting within the upper four inches of substrate. Mussel surveys were only performed when visibility exceeded 0.5m, a standard set by the WV Mussel Survey Protocol (Clayton et al. 2016). In heterogeneous substrates, a minimum of one min/m^2 of search time was required. Homogenous fine substrates did not have a time minimum requirement. Live mussels and deadshells (DS) were collected and stored in mesh bags for transport to the surface. Each bag corresponded to a specific transect and interval. Live mussels were identified, measured to the nearest millimeter, and returned to the site by broadcast scattering from a boat. During processing, mussels were kept out of the water for no longer than five minutes. The sex was listed for species exhibiting sexual dimorphism. If the individual was too young to identify sex, the shell was listed as a juvenile (J). Each live species was photographed and DS in good condition were retained as voucher specimens. Deadshell categories included: freshdead (shell shows no sign of wear and retains intact nacre and periostricum), weathered dead (shell shows signs of wear with tarnished nacre and periostricum), and subfossil (shell retains no nacre or periostricum).

Federally threatened and endangered individuals were measured for length, width, and height (mm). Voucher photographs and collection coordinates were sent to the appropriate state and federal agencies (USFWS, WVDNR, & KYDFWR) within 24 hours. All federally threatened and endangered mussels were returned by hand to optimal habitat within the site.

GIS

I used Arc Map 10.3.1 to map freshwater mussel and fish populations within the Greenup pool. Mussel abundances and richness were entered at the site, transect and interval level. However, fish abundances and richness were only entered on a site basis. By joining fish and mussel data in Arc Map, I drew comparisons between fish hosts and obligate freshwater mussels.

Fish Host and Mussel Relationship Analysis

I used a negative binomial regression to test if mussel presence is predicted by fish host presence. I ran a negative binomial regression to account for zeros in my data, high means, and lack of normality. I assumed that the fish host acted as the predictor(x) and mussels as the response (y). Independence of samples was assumed. All statistical analyses were performed in SAS ® [9.4].

E. lineolata-A. grunniens and *L. recta-S. canadensis* were further analyzed using a negative binomial regression apart from the combined fish and mussel data. Each mussel species has a confirmed host and is a specialist or strongly prefers one fish host genus (Watters et al. 2009). Additionally, *E. lineolata, A. grunniens, S. canadensis*, and *L. recta* were abundantly collected (>100 individuals) in the Greenup pool. Both *E. lineolata* and *L. recta* are common big river species and may represent excellent models for future host-mussel studies. *Elliptio* *crassidens* and *Lampsilis teres* were also species of interest due to their rarity or lack of recent recruitment. However, both species did not meet the specifications for a statistical analysis.

RESULTS

Fish Richness and Abundance

During 2016 fish community surveys in the Greenup pool, ORSANCO collected 2,008 individuals from 46 species. Important mussel hosts collected include, *A. grunniens*, *S. canadensis*, and *L. osseus*. Darters and some pelagic species were poorly represented. The general fish abundance and richness showed almost no site trends throughout the pool with R^2 values <0.04 (Figure 37-38).

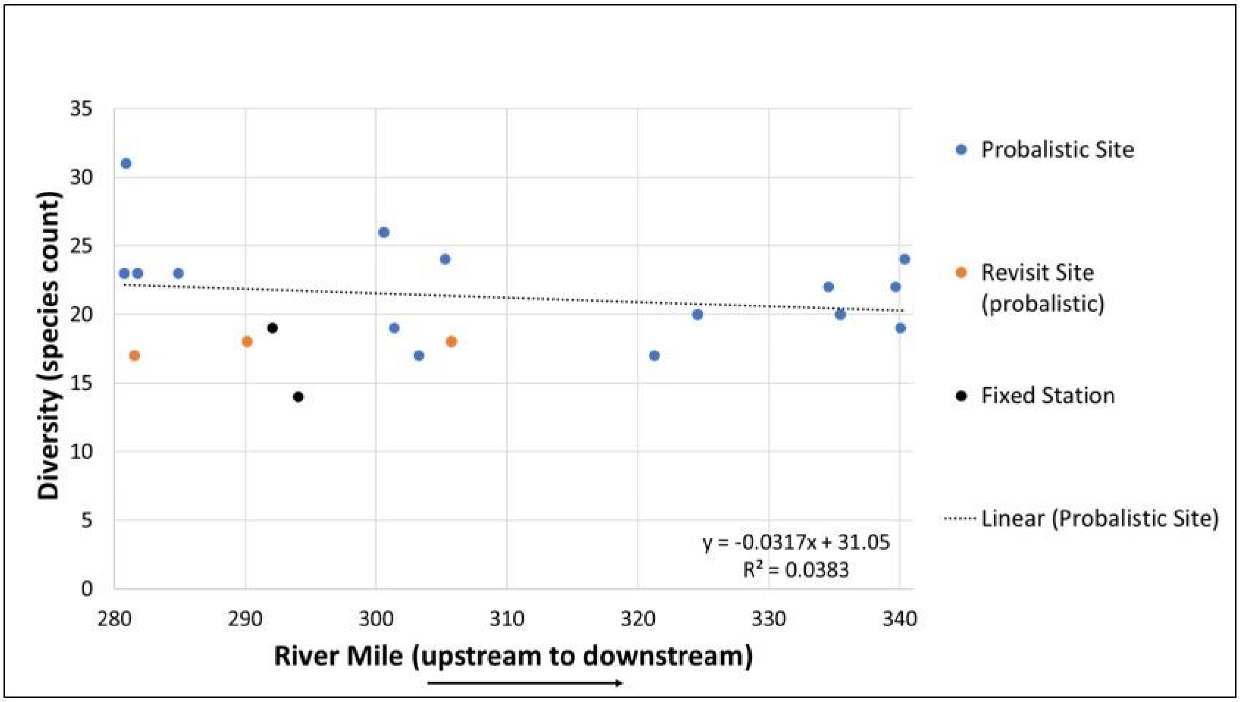


Figure 37. Fish richness counts by river mile in the Greenup pool, Ohio River (Source: Personal collection).

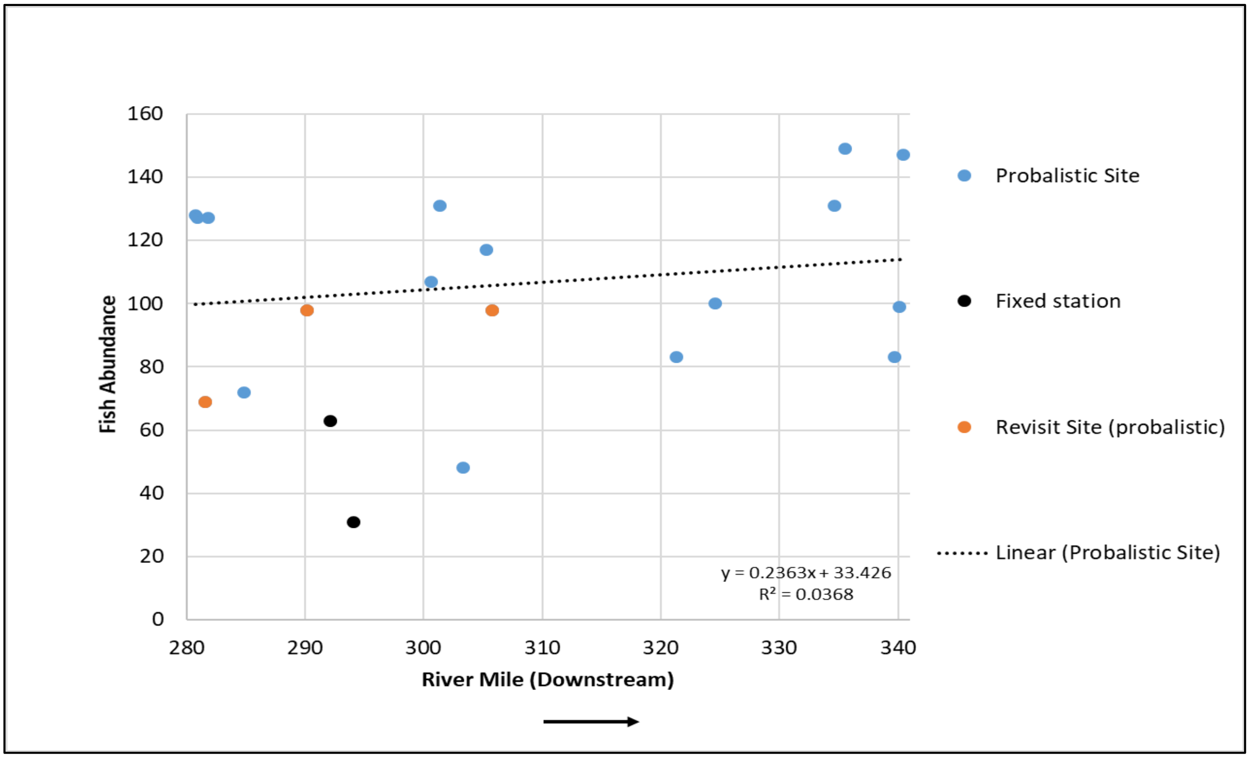


Figure 38. Fish abundance counts by river mile in the Greenup Pool, Ohio River (Source: Personal collection).

Mussel - Fish Abundance Analysis

A negative binomial regression was used to assess the influence of fish host abundance as a predictor of freshwater mussel abundance in the Greenup pool. The model contained 18 observations (n=18), was over dispersed (Value/DF=1.4288), and had a small negative estimate (-0.0061±0.0204). The 95% CL intervals contained zero [-0.0461 – 0.0338] and the p-value was >0.05 (P=0.7638). There was no significant relationship between fish host abundance and freshwater mussel abundance.

Mussel - Fish Richness Analysis

A negative binomial regression was used to assess the influence of fish host richness as a predictor of freshwater mussel richness in the Greenup pool. The model contained 18 observations (n=18), was over dispersed (Value/DF=1.2546), and had a small negative estimate (-0.0117±0.0582). The 95% CL intervals contained zero [-0.1258 – 0.1025] and the p-value was >0.05 (P= 0.8413). There was no significant relationship between fish host abundance and freshwater mussel abundance.

Elliptio crassidens

Elliptio crassidens was a historically dominant member of the Greenup pool mussel fauna (Miller and Payne 2000; Dunn 1999_2; Zeto 1987). It is long-lived, with a lifespan of up to 60 years (Haag 2012). However, a substantial reproductive event has not been observed in the last 35+ years. Taylor noted that only a large cohort was present in the upper Ohio in 1980 (Taylor 1980). Only two juvenile individuals have been observed in the upper Ohio mainstem in the last 25 years (Personal communication - Patty Morrison and Janet Clayton 2017).

I collected 87 live individuals and *Elliptio crassidens* populations were preferential to outer intervals (Figure 39). Data collected by the USACE throughout the 2000s, combined with my current results, reveal a cohort of individuals > 80 mm which dominate the shrinking population (Figure 40) (2017). Additionally, several of the smaller individuals I collected bias my population size structure due to injuries that physically cut the shells off on the posterior end (Figure 41). These shells are older than the measurements suggest. The host fish for *E. crassidens*, Skipjack Herring, was not collected in the Greenup pool during 2016 ORSANCO surveys (Watters et al. 2009; ORSANCO 2016_2) but is known from the pool. There were not enough host occurrences to run a statistical test.

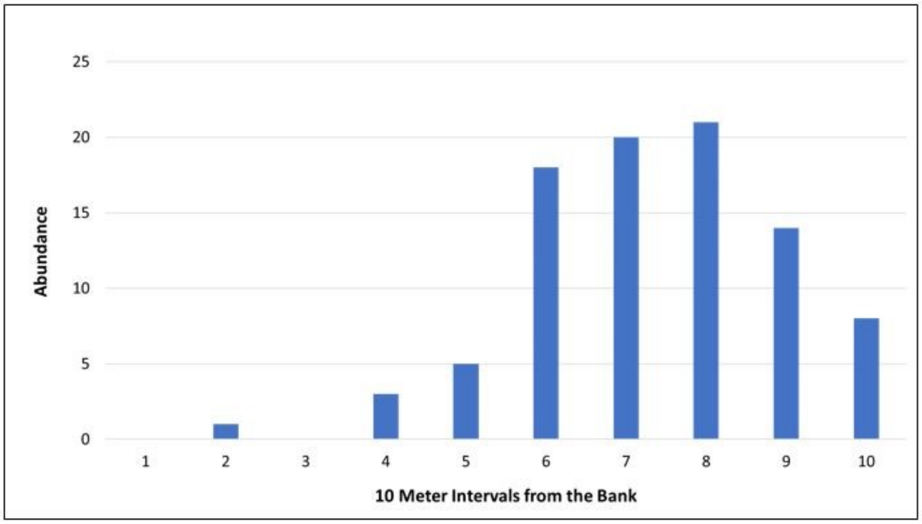


Figure 39. *Elliptio crassidens* abundance counts by interval, Greenup pool, Ohio River. Each interval represents a 10m increase, with interval #1 beginning at the shoreline (Source: Personal collection)

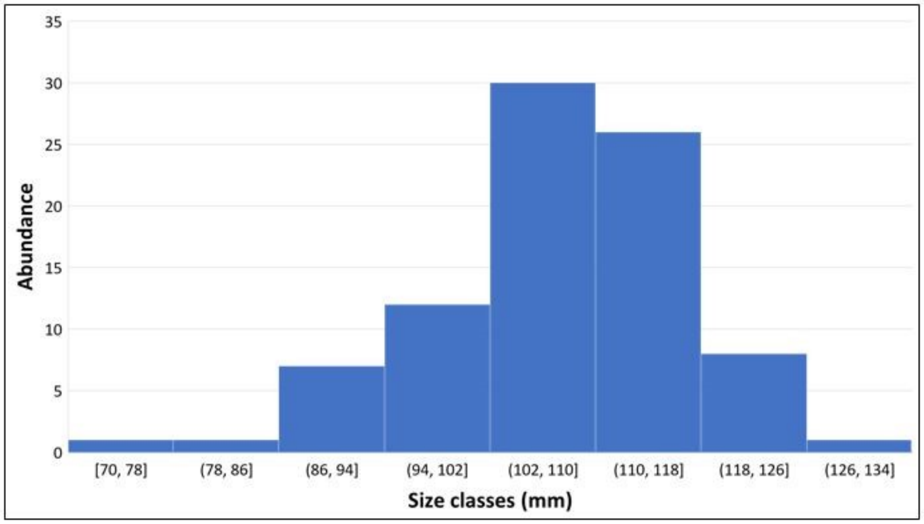


Figure 40. *Elliptio crassidens* size class count, Greenup pool, Ohio River (Source: Personal collection).

Figure 41. Injured *Elliptio crassidens* which skews size class data, Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Ligumia recta Analysis Results

Sander vitreus (Walleye) and *Sander canadensis* (Sauger) are the primary hosts for *Ligumia recta* (Haag 2012; Watters et al. 2009; Khym and Layzer 2000). *Ligumia recta* utilizes a lure to attract hosts and targets Walleye and Sauger due to their predator nature. ORSANCO collected zero Walleye and 211 Sauger during 2016 surveys in the Greenup pool. A total of 398 *L. recta* were collected during thesis surveys and the population shows signs of limited recruitment (Figure 59). However, large adults dominated the size classes (Figure 42). *Ligumia recta* show preference to outside intervals but could be found in all habitats (Figure 43).

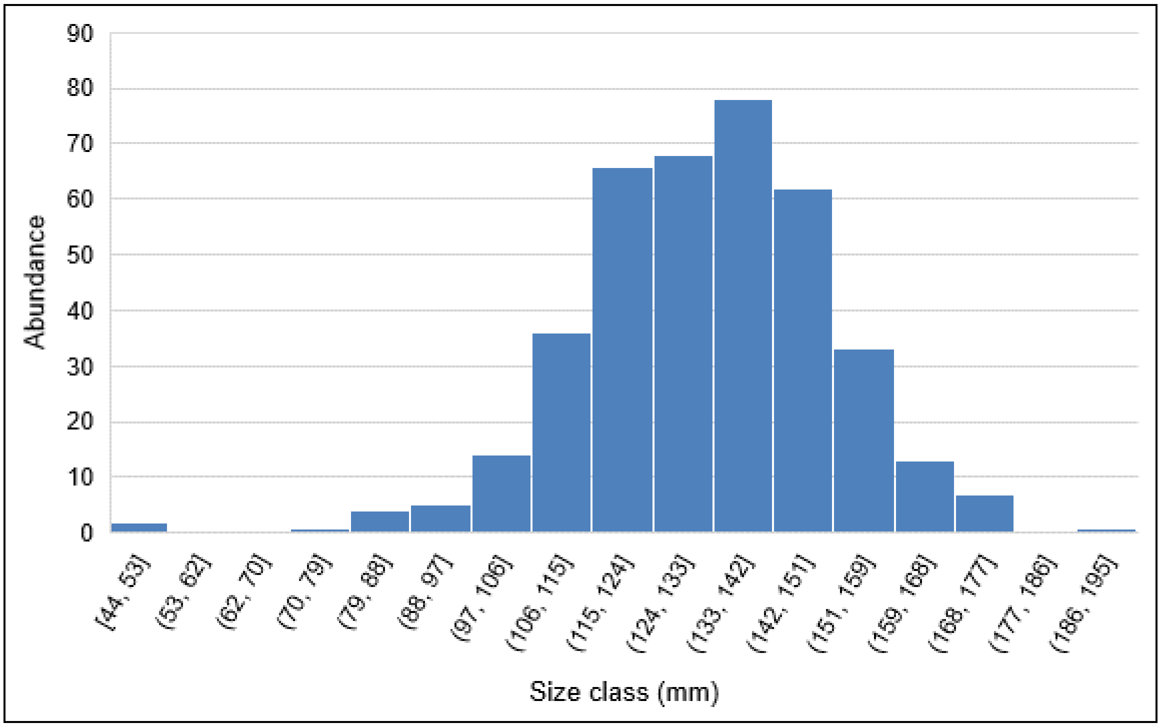


Figure 42. *Ligumia recta* size class count, Greenup pool, Ohio River (Source: Personal collection).

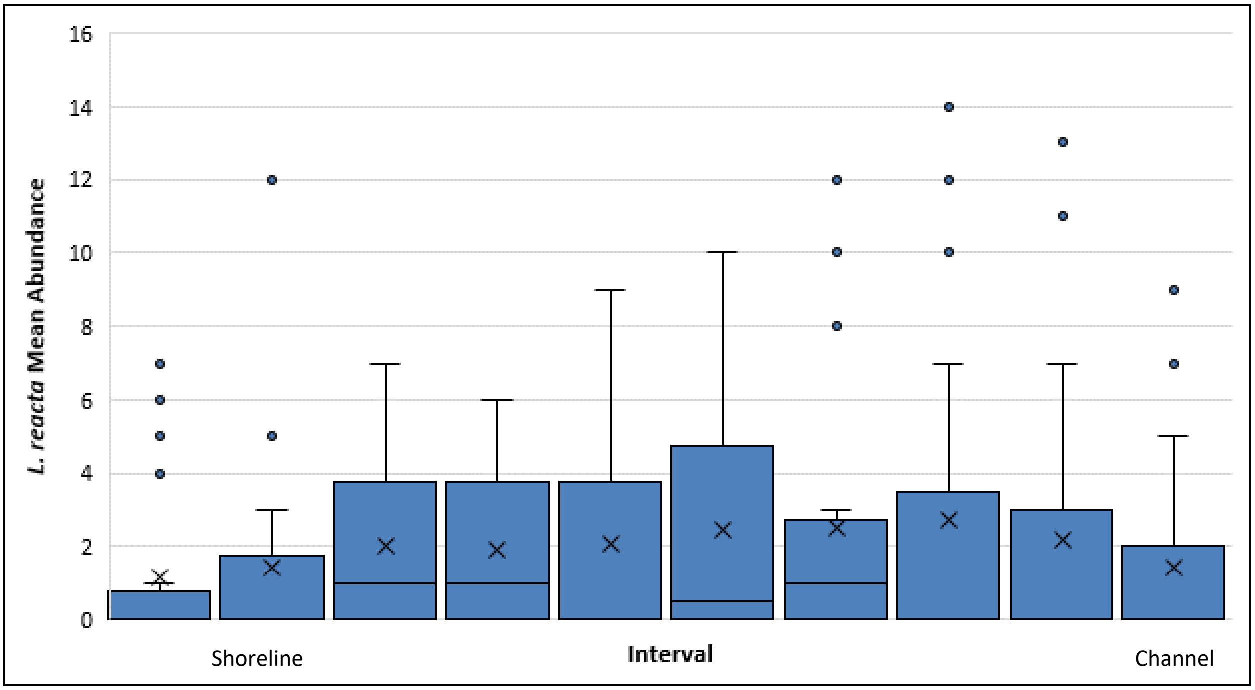


Figure 43. *Ligumia recta* mean abundance by interval, Greenup pool, Ohio River (Source: Personal collection).

A negative binomial regression test using *L. recta* as a predictor of *S. canadensis* with 18 observations (n=18) yielded a positive beta estimate of 0.0235. The 95% CL contained zero [-0.0708 - 0.01178] and the p-value was over 0.05 (p=0.6257±0.0481). The model was over-dispersed (Value/DF=1.2132) and failed to detect a relationship between *Ligumia recta* and *Sander canadensis*.

Ellipsaria lineolata Analysis Results

Ellipsaria lineolata showed signs of strong recruitment and a high affinity to outer transects (Figure 44-45).The Freshwater Drum is the only known host for *Ellipsaria lineolata* (Watters et al. 2009). ORSANCO collected 128 *Aplodinotus grunniens* during 2016 Greenup pool surveys. I encountered 284 live *E. lineolata* during thesis surveys and they were exclusively found within the upper portion of the Greenup pool. Inversely, *A. grunniens* were concentrated in the lower pool (Figure 46).

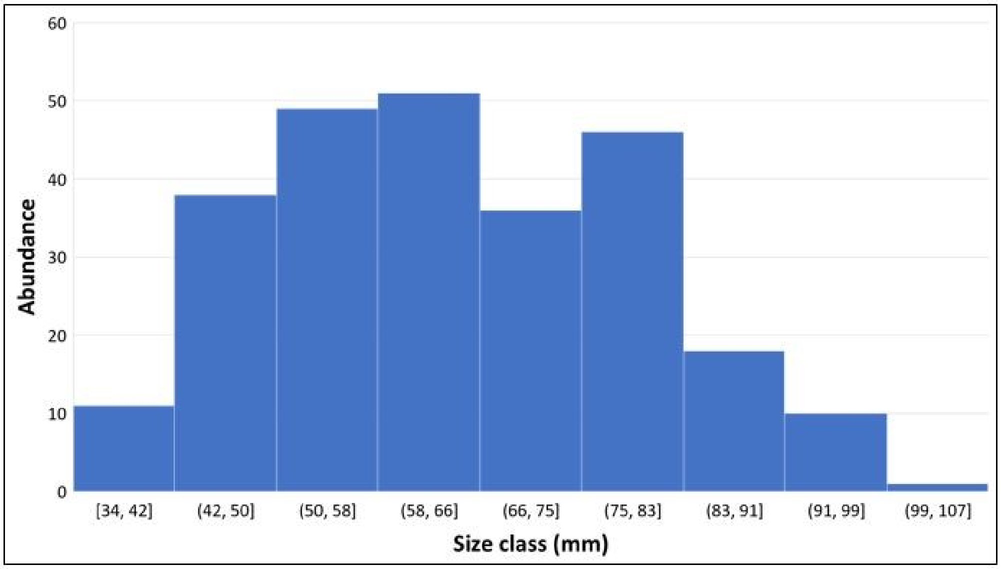


Figure 44. *Ellipsaria lineolata* size class count, Greenup pool, Ohio River (Source: Personal collection).

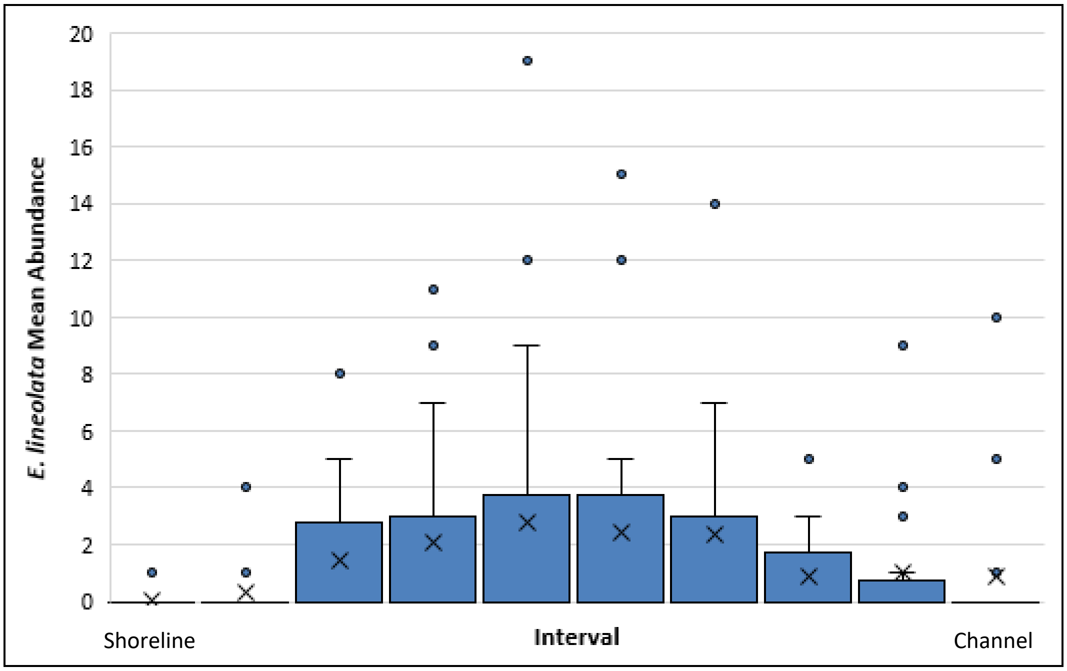


Figure 45. *Ellipsaria lineolata* mean abundance by interval, Greenup pool, Ohio River (Source: Personal collection).

A negative binomial regression test using *A. grunniens* as a predictor of *E. lineolata* with 18 observations (n=18) yielded a beta estimate of -0.3322. The 95% CL contained zero [-0.6097 to -0.0546] and the p-value was <0.05 (p=0.019). The model was not over-dispersed and revealed a negative relationship between *Ellipsaria lineolata* and *Aplodinotus grunniens*. The model did not account for seasonal fish movements, river clarity, mussel population structure, or water velocity.

Signs of Recruitment

L. recta and *L. cardium* were observed actively displaying by divers. Species that showed evidence of recruitment (individuals collected <30mm or significant gaps in population size structure) included *L. recta*, *L. cardium*, *P. alatus*, *P. cordatum*, *E. lineolata*, *Q. metanevra*, *A. plicata*, *Q. pustulosa*, and *O. reflexa* (Miller and Payne 2000). *Q. pustulosa* and *O. reflexa* exhibited the strongest signs of recruitment in the pool. Juveniles from *Ligumia recta* and *Lampsilis cardium* did not represent >1.9% of the total population collected.

DISCUSSION

Mussel – Fish Richness and Abundance Relationships

A negative binomial regression model between fish host and mussel communities had low power and failed to detect a correlation between the locations of fish and mussel abundance or richness. However, the model did not account for seasonality of fish collections, freshwater mussel population structure, or water velocity. Betas were less than zero ($\beta<0$) with 95% CLs containing zero and p-values over 0.05 which is puzzling since mussels theoretically colonize the best available habitat. Clean swept gravel, the preferred substrate for many mussel species, was common throughout the upper pool. This habitat supports diverse macroinvertebrate communities and should be prime hunting grounds for predatory host fish. Interestingly, many fish species such as the Freshwater Drum were concentrated in the lower pool which is dominated by fines and supports poor macroinvertebrate communities.

Minimal recruitment may act as a second explanation for the lack of mussel to fish relationships. Only 3-4 mussel species encountered exhibited strong signs of recruitment with multiple juveniles encountered (Miller and Payne 2000). All species showing signs of rigorous reproduction, except *Ellipsaria lineolata*, are host generalists (Watters et al. 2009).

Signs of recruitment

Q. pustulosa and *O. reflexa* showed vigorous signs of reproduction in the Greenup pool. However, their relatively even size class distributions are partially due to life history rather than recruitment success (Haag 2012). Small species such as *T. truncata* may be reproducing but their small size makes discovery during qualitative surveys unlikely (Hastie and Cosgrove 2002, Dunn 1999_1). Although visual freshwater mussel surveys are biased against small individuals, minimal juvenile encounters during thesis surveys may predict a future crash in the pool-wide mussel fauna, even among common species.

Ligumia recta Analysis

Sander canadensis, the host fish for *L. recta*, was commonly encountered throughout the Greenup pool and the Black Sandshell population showed signs of recruitment (Figure 59) (Figure 47). A negative binomial regression between *S. canadensis* and *L. recta* failed to detect a correlation between fish host and mussel populations. The model had low power with a 95% CL containing zero [-0.0708 - 0.01178] and a p-value >0.05 (p=0.6257) and did not account for seasonal fish movements, river clarity, mussel population structure, or water velocity. Likewise, count data by site showed no trends between *L. recta* and *S. canadensis* occurrences (Figure 47). However, *Ligumia recta* were observed displaying which suggests the timing of fish collection does overlap with *L. recta* breeding. Sporadic migration movements of Sauger may be a confounding issue that could not be accounted for by electrofishing (Kuhn, Hubert, Johnson, Oberlie, and Dufek, 2008; Pegg, Bettoli, and Layzer 1997)

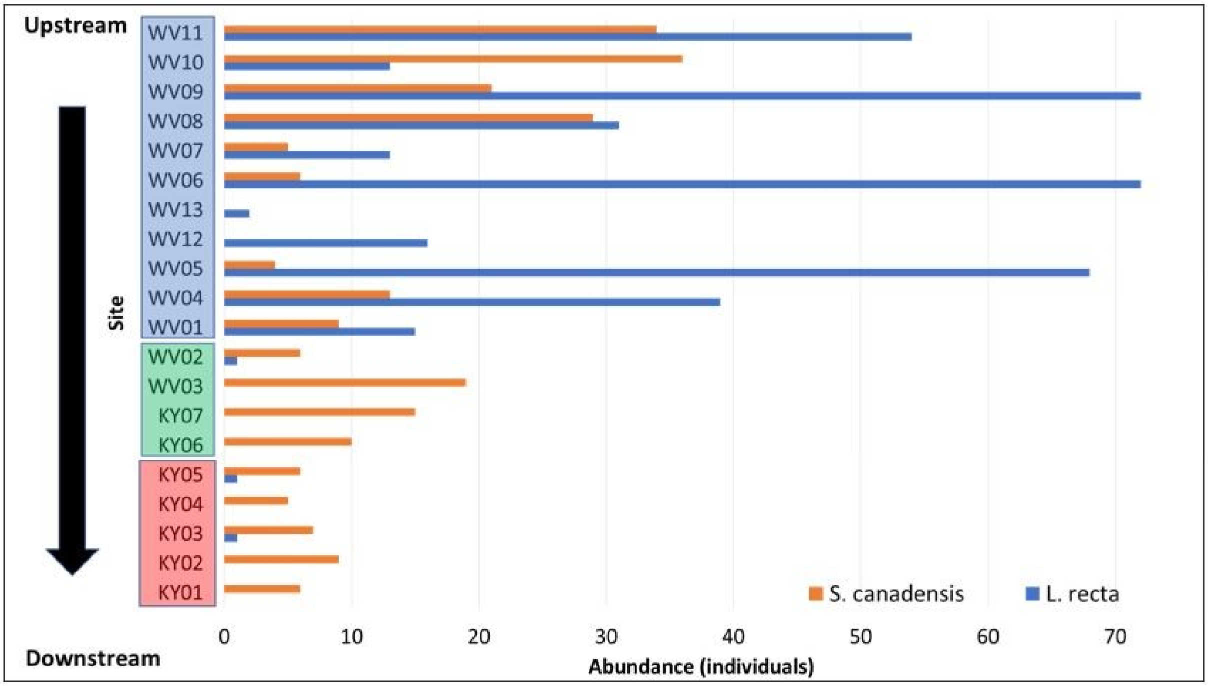


Figure 46. *Sander canadensis* and *Ligumia recta* abundance counts in the Greenup Pool, Ohio River. The blue boxes represent upper pool sites, green represents middle pool, and red represents lower pool sites (Source: Personal collection).

Ellipsaria lineolata Analysis

Ellipsaria lineolata was found exclusively in the upper Greenup pool sites. However, its host, *Aplodinotus grunniens*, was found predominantly in lower pool sites (Figure 46). A negative binomial regression test indicateed that *A. grunniens* act as a negative predictor of *E. lineolata*. For every one unit increase in *A. grunniens* abundance, the expected log counts of *E. lineolata* decreases by 0.3322 individuals. However, the model did not account for seasonal fish movements, river clarity, mussel population structure, or water velocity.

This inverse correlation is surprising, particularly since *A. grunniens* are molluscivorous. The explanation may lie in Freshwater Drum feeding habits. Fine silt substrate, which is easy to sift through, composes over 90% of the lower pool sediment. Instead of digging through rocks and gravel for mussels, the drum may find their meals easier to obtain in the lower pool.

Additionally, the reduced velocity in the lower pool may encourage drum to congregate above Greenup Locks and Dam to conserve energy.

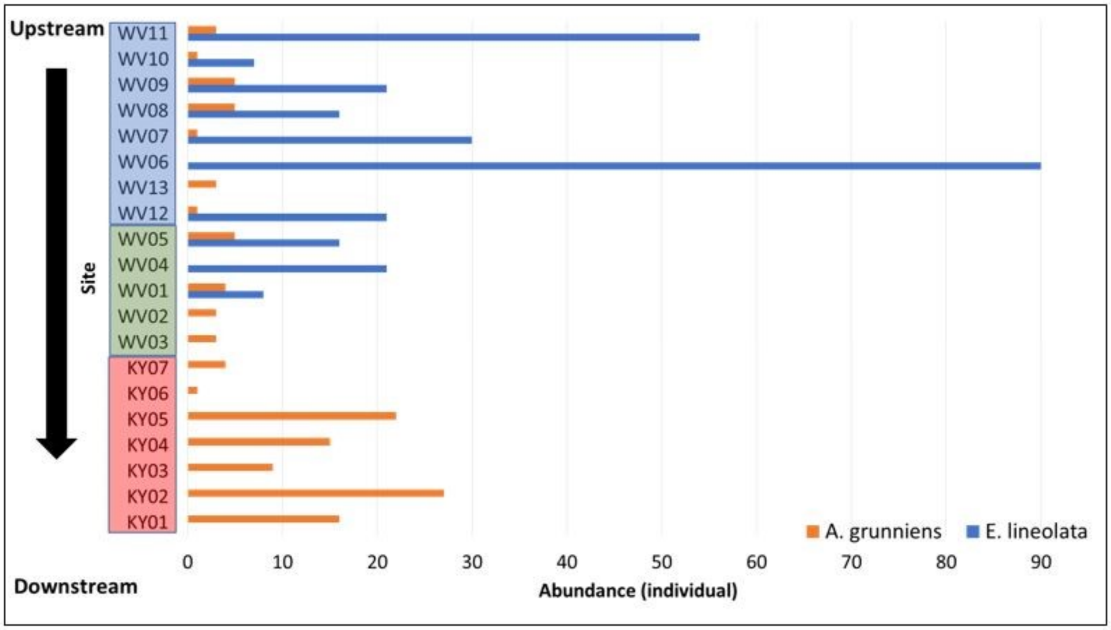


Figure 47. *Aplodinotus grunniens* and *Ellipsaria lineolata* abundance count in the Greenup Pool, Ohio River. The blue boxes represent upper pool sites, green represents middle pool, and red represents lower pool sites (Source: Personal collection).

***Lepisosteus osseus* – *Lampsilis teres* Interactions**

Lampsilis teres has been rare within the Greenup pool since at least the 1980s (Miller and Payne 2000; Taylor 1980). However, *L. teres* was historically a common member of the Upper Ohio mussel fauna (Haag 2012). Wendel Haag suggested that strong declines in *L. teres*' primary host fish, *Lepisosteus platostomus*, throughout the upper Ohio River may be the main factor in *L. teres* declines (2012). Walker, Kluender, Inebnit, and Adams noted that seasonal floods may play a key role in *L. platostomus* foraging (2013). Destruction of swamps and backwater habitat in the upper Ohio may be to blame for Shortnose Gar declines. *Lepisosteus platostomus* has not been collected in the Greenup pool since ORSANCO surveys began in 1957.

Due to the discovery of *L. teres* below RC Byrd Locks and Dam during thesis surveys and by USACE, a secondary host must be present (USACE 2017).

Lepisosteus osseus, along with other gar species, are recognized as viable hosts for *Lampsilis teres* (Watters et al. 2009; Keller and Reussler 1997). *L. osseus* is a common member of the fish fauna throughout the entire Ohio drainage and the only gar species collected in the Greenup pool by ORSANCO since fish surveys began in 1957. If the host viability for both species is comparable, the low densities of *L. teres* in the upper Ohio is perplexing. One solution may lie in the habits of both gar species. *Lepisosteus osseus* is a pelagic hunter that feeds almost exclusively on Cluepeidae (Richardson 2015). Inversely, *Lepisosteus platostomus* is a generalist, feeding on not only fish, but also amphibians and macroinvertebrates (Richardson 2015; Walker et al. 2013; Haag 2012). Due to its habitat niche, *L. platostomus* may simply be more likely to encounter displaying female *L. teres* as it hunts benthic habitats for macroinvertebrates such as crayfish (Haag 2012).

Collection locations of *L. teres* below RC Byrd Lock and Dam are in some of the shallowest sections of the pool and contain the highest number of *L. osseus* collected by ORSANCO (USACE 2017, Thesis Data) (Figure 48). All dead shell specimens and the single live individual were collected in rocky habitat on outside intervals > 30 m from shore. The location of shell collections is curious due to *L. teres*’s affinity to slow moving, silt ridden backwaters, which are common features throughout the middle and lower sections of the Greenup pool (Cummings & Mayer 1992; Watters et al. 2009). *L. teres* habitat within the Greenup pool would suggest that it may have adapted to a secondary host fish that does not share the same partiality for backwater habitat.

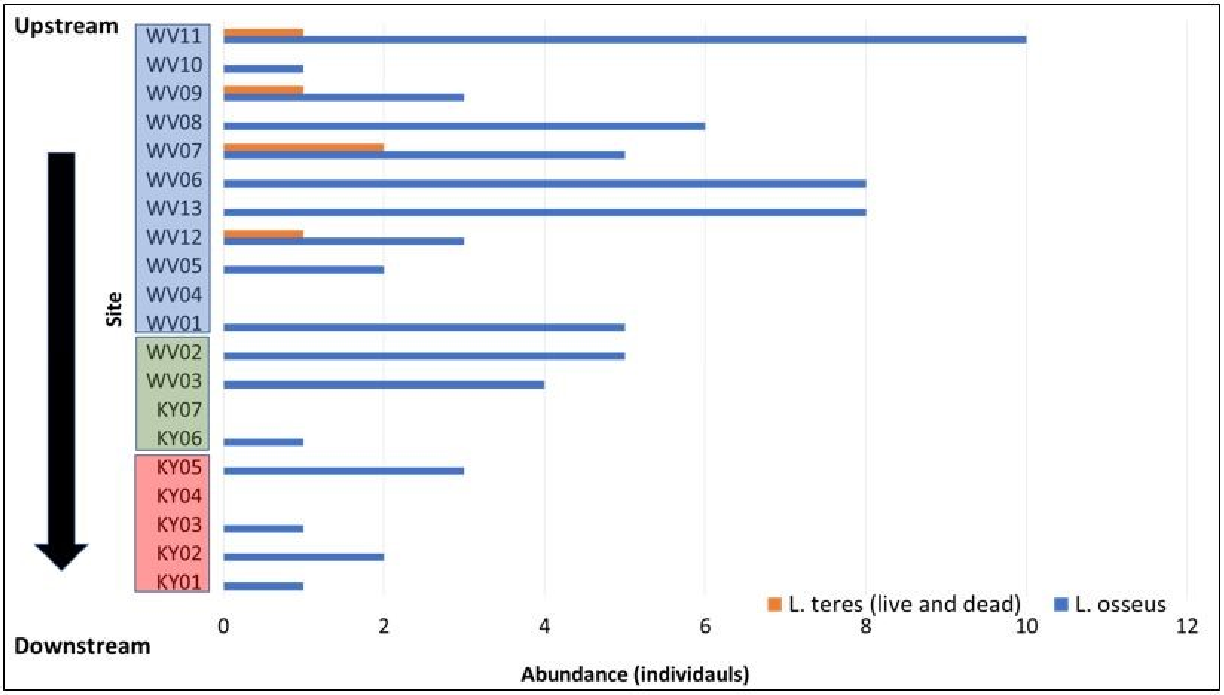


Figure 48. *Lepisosteus osseus* and *Lampsilis teres* abundances in the Greenup Pool, Ohio River. The blue boxes represent upper pool sites, green represents middle pool, and red represents lower pool sites (Source: Personal collection).

Elliptio crassidens Host Relationships

The explanation for *E. crassidens* recent lack of recruitment is perplexing. The most commonly cited reason for their reproductive failure is damming of the Ohio River mainstem (Miller and Payne 2000). While adult *E. crassidens* survive in the altered riverine system, juveniles may not.

The high lift dams also limit migrations for Skipjack Herring, the primary host for *E. crassidens* (Miller and Payne 2000, Kelner & Sietman 2000). The approximately 24 feet of water between the mussels and river surface may further hinder interactions between *E. crassidens* and the pelagic Skipjack Herring. The highest number of live or dead shell *E. crassidens* was observed within one mile below RC Byrd Lock and Dam, the shallowest section of the Greenup Pool (Figure 49). Their occurrences here may also be due to Skipjack Herring congregating below RC Byrd Lock and Dam as they attempt to move into the Belleville pool. No live or DS *E. crassidens* individuals were collected below Huntington, WV.

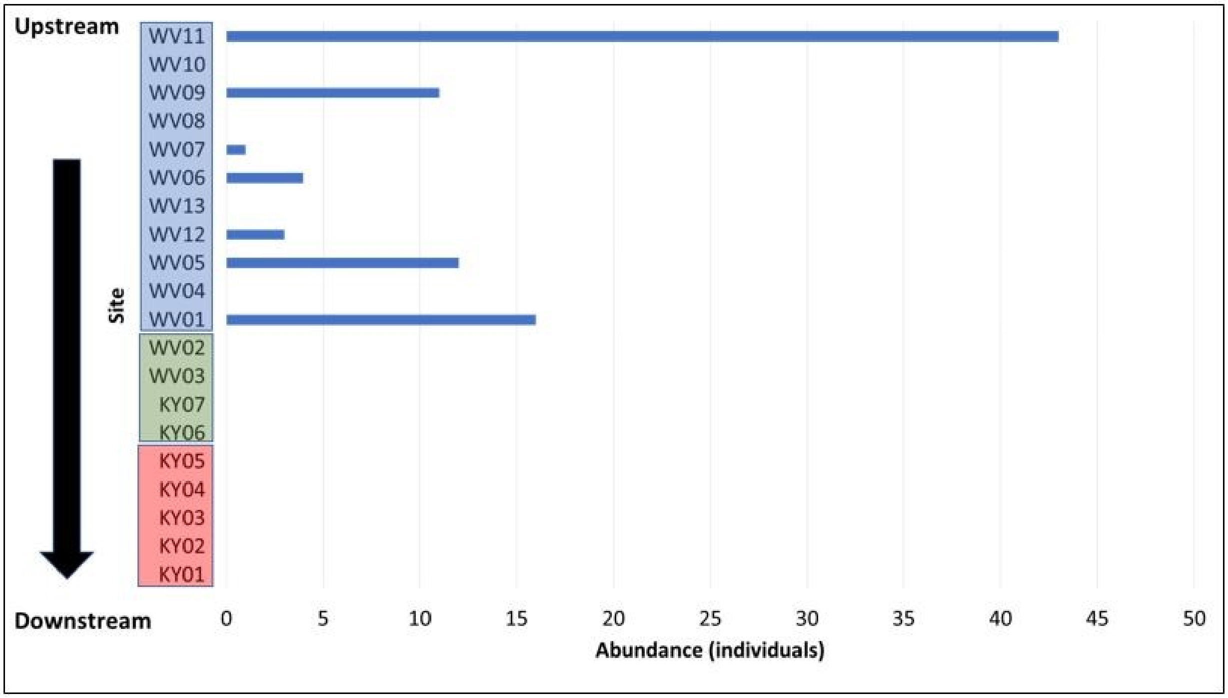


Figure 49. *Elliptio crassidens* abundances in the Greenup Pool, Ohio River. The blue boxes represent upper pool sites, green represents middle pool, and red represents lower pool sites (Source: Personal collection).

Conclusions

In all negative binomial regression tests besides *Ellipsaria lineolate* and *A. grunniens*, my data was unable to support the hypothesis that fish hosts have an influence on freshwater mussel communities. I suspect that this may be due to the highly variable movements of fish. Seasonal or weather related movements may exert significant influences on fish movements.

During ORSANCO surveys, only one channel darter was collected. As a group, darters (*Percidae* & *Etheostoma*) are notoriously under represented by electrofishing methods due to their lack of a swim bladder (Jacobs and Swink 1982, Personal communication – Jeff Thomas 2017). Channel and river darters, both important mussel host species, were frequently observed during thesis survey dives in the Greenup pool (Watters et al. 2009). These data holes represent significant challenges to defining the relationship between freshwater mussels and fish. In addition to electrofishing limitations, larger determinants for mussel habitat selection such as food availability or water velocity preferences require further study.

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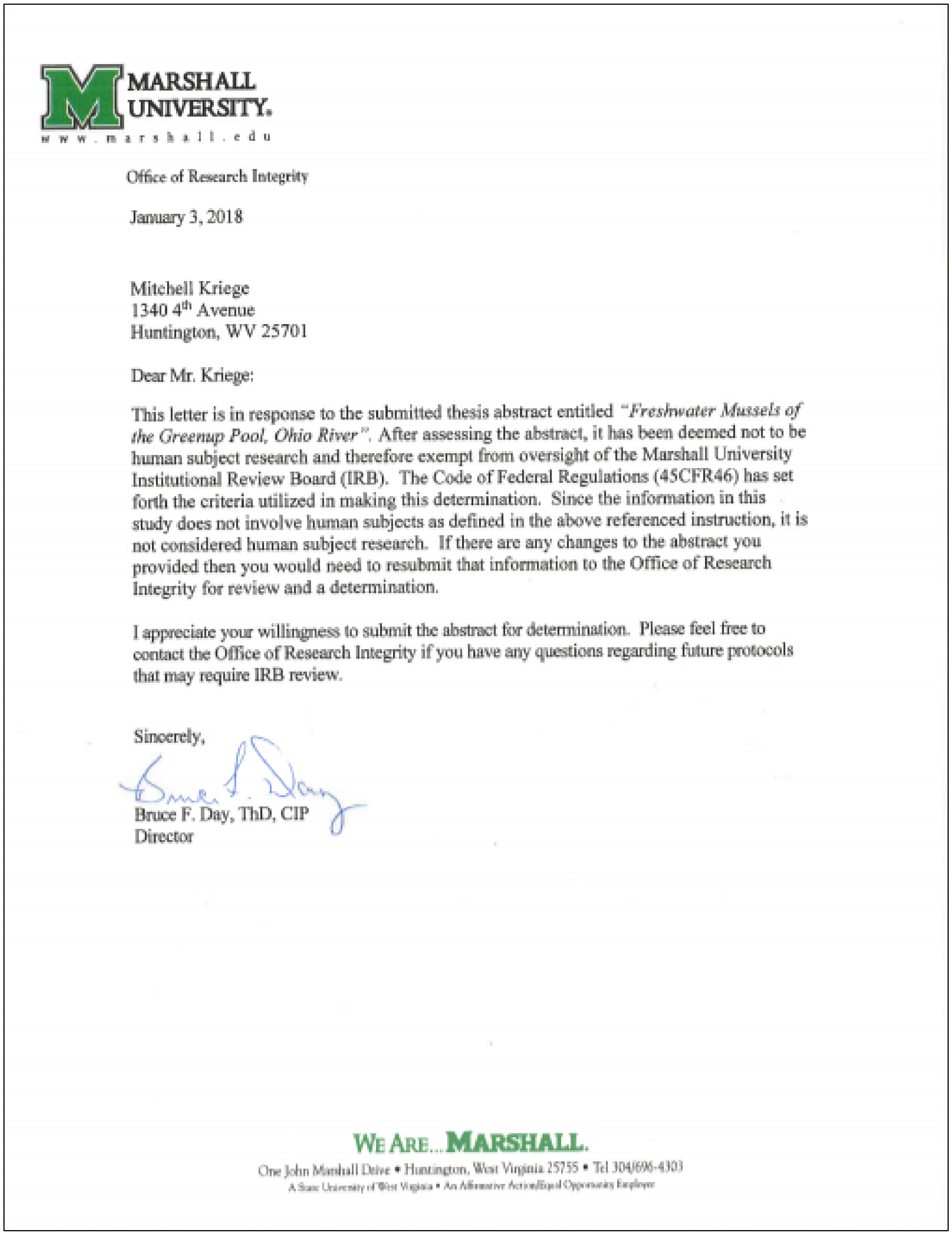
APPENDIX A: IACUC APPROVAL LETTER



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Office of Research Integrity

January 3, 2018

Mitchell Kriege
1340 4th Avenue
Huntington, WV 25701

Dear Mr. Kriege:

This letter is in response to the submitted thesis abstract entitled *"Freshwater Mussels of the Greenup Pool, Ohio River"*. After assessing the abstract, it has been deemed not to be human subject research and therefore exempt from oversight of the Marshall University Institutional Review Board (IRB). 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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APPENDIX B: SPECIES ACCOUNTS

Greenup Pool Freshwater Mussel Hosts

Table 13. Freshwater mussel laboratory and natural confirmed host species: *Q. metanevra, Q. pustulosa, Q. quadrula, T. truncata, T. verrucosa* (Watters et al. 2009; Freshwater Mussel Host Database 2017).

Species	***Q. metanevra***	***Q. pustulosa***	***Q. quadrula***	***T. truncata***	***T. verrucosa***
A. melas (Black Bullhead)	0	1	0	0	0
A. natalis (Yellow Bullhead)	0	0	0	0	1
A. nebulosus (Brown Bullhead)	0	1	0	0	1
A. grunniens (Freshwater Drum)	0	0	0	1	0
C. anomalum (Central Stoneroller)	1	0	0	0	0
C. spiloptera (Spotfin Shiner)	1	0	0	0	0
C. whipplei (Steelcolor Shiner)	1	0	0	0	0
I. punctatus (Channel Catfish)	0	1	1	0	1
M. storeriana (Silver Chub)	1	0	0	0	0
P. notatus (Bluntnose Minnow)	1	0	0	0	0
P. promelas (Fathead Minnow)	1	0	0	0	0
P. olivaris (Flathead Catfish)	0	1	0	0	1
R. atratulus (Blacknose Dace)	1	0	0	0	0
R. cataractae (Longnose Dace)	1	0	0	0	0
S. atromaculatus (Creek Chub)	1	0	0	0	0
Grand Total	**9**	**4**	**1**	**1**	**4**

Table 14. Freshwater mussel laboratory and natural confirmed host species: *F. flava, L. cardium, L. complanata, L. fragilis, L. recta, L. siliquoidea, L. teres* (Watters et al. 2009; Freshwater Mussel Host Database 2017; Khym and Layzer 2000; Kelly and Watters 2010; Haag 2012).

Species	***F. flava***	***L. cardium***	***L. complanata***	***L. fragilis***	***L. recta***	***L. siliquoidea***	***L. teres***
A. rupestris (Rock Bass)	0	0	0	0	1	1	0
A. grunniens (Freshwater Drum)	0	0	0	1	0	0	0
C. anomalum (Central Stoneroller)	0	0	0	0	1	0	0
C. spiloptera (Spotfin Shiner)	1	0	0	0	0	0	0
C. carpio (Common Carp)	0	0	1	0	0	0	0
E. jordani (Greenbreast Darter)	0	0	0	0	0	0	1
F. diaphanus (Banded Killifish)	0	0	1	0	1	0	0
L. osseus (Longnose Gar)	0	0	0	0	0	0	1
L. cyanellus (Green Sunfish)	0	1	1	0	1	1	0
L. gibbosus (Pumpkinseed)	0	1	0	0	1	0	0
L. humilis (Orangespotted Sunfish)	0	0	1	0	1	0	0
L. macrochirus (Bluegill)	0	1	0	0	1	1	1
L. megalotis (Longear Sunfish)	0	0	1	0	1	1	0
M. dolomieu (Smallmouth Bass)	0	1	0	0	0	1	0
M. salmoides (Largemouth Bass)	0	1	1	0	1	1	0
M. americana (White Perch)	0	0	0	0	1	0	0
M. chrysops (White Bass)	0	0	0	0	0	1	0
N. stramineus (Sand Shiner)	0	0	0	0	0	1	0
P. flavescens (Yellow Perch)	0	1	1	0	1	1	0
P. notatus (Bluntnose Minnow)	0	0	0	0	0	1	0
P. annularis (White Crappie)	0	1	1	0	1	1	0
P. nigromaculatus (Black Crappie)	0	2	1	0	1	1	0
S. canadensis (Sauger)	0	0	0	0	1	1	0
S. vitreus (Walleye)	0	2	0	0	1	1	0
S. atromaculatus (Creek Chub)	1	0	0	0	0	0	0
Grand Total	**2**	**11**	**9**	**1**	**15**	**14**	**3**

Table 15. Freshwater mussel laboratory and natural confirmed host species: *A. ligamentina, A. plicata, E. crassidens, E. lineolata, F. ebena* (Watters et al. 2009; Freshwater Mussel Host Database 2017).

Species	***A. ligamentina***	***A. plicata***	***E. crassidens***	***E. lineolata***	***F. ebena***
A. chrysochloris (Skipjack Herring)	0	0	1	0	1
A. rupestris (Rock Bass)	1	1	0	0	0
A. grunniens (Freshwater Drum)	0	0	0	1	0
C. anomalum (Central Stoneroller)	1	0	0	0	0
F. diaphanus (Banded Killifish)	1	0	0	0	0
H. alosoides (Goldeye)	0	0	0	0	1
L. cyanellus (Green Sunfish)	1	1	0	0	0
L. gibbosus (Pumpkinseed)	0	1	0	0	0
L. humilis (Orangespotted Sunfish)	1	0	0	0	0
L. macrochirus (Bluegill)	0	1	0	0	0
M. dolomieu (Smallmouth Bass)	1	0	0	0	0
M. salmoides (Largemouth Bass)	1	1	0	0	0
M. chrysops (White Bass)	1	0	0	0	0
N. buccatus (Silverjaw Minnow)	1	0	0	0	0
P. flavescens (Yellow Perch)	1	1	0	0	0
P. annularis (White Crappie)	1	1	0	0	0
P. nigromaculatus (Black Crappie)	1	1	0	0	0
P. olivaris (Flathead Catfish)	0	1	0	0	0
R. atratulus (Blacknose Dace)	1	0	0	0	0
S. canadensis (Sauger)	1	0	0	0	0
S. vitreus (Walleye)	1	0	0	0	0
S. atromaculatus (Creek Chub)	1	0	0	0	0
Grand Total	**16**	**9**	**1**	**1**	**2**

Table 16. Freshwater mussel laboratory and natural confirmed host species: *M. nervosa, O. reflexa, P. alatus, P. cordatum* (Watters et al. 2009; Freshwater Mussel Host Database 2017).

Species	***M. nervosa***	***O. reflexa***	***P. alatus***	***P. cordatum***
A. melas (Black Bullhead)	1	0	0	0
A. natalis (Yellow Bullhead)	1	0	0	0
A. nebulosus (Brown Bullhead)	1	0	0	0
A. grunniens (Freshwater Drum)	1	1	1	0
C. commersonii (White Sucker)	0	0	0	1
C. spiloptera (Spotfin Shiner)	0	0	0	1
D. cepedianum (Gizzard Shad)	0	1	0	0
I. punctatus (Channel Catfish)	1	0	0	0
L. osseus (Longnose Gar)	1	0	0	0
L. cyanellus (Green Sunfish)	1	0	0	0
L. macrochirus (Bluegill)	1	0	0	0
L. megalotis (Longear Sunfish)	1	0	0	0
M. salmoides (Largemouth Bass)	1	1	0	0
M. chrysops (White Bass)	1	0	0	0
N. buccatus (Silverjaw Minnow)	0	1	0	0
P. flavescens (Yellow Perch)	1	0	0	0
P. caprodes (Logperch)	1	0	0	0
P. phoxocephala (Slenderhead Darter)	1	0	0	0
P. notatus (Bluntnose Minnow)	0	0	0	1
P. annularis (White Crappie)	1	0	0	0
P. nigromaculatus (Black Crappie)	1	0	0	0
P. olivaris (Flathead Catfish)	1	0	0	0
R. atratulus (Blacknose Dace)	0	0	0	1
R. cataractae (Longnose Dace)	0	1	0	0
S. vitreus (Walleye)	0	1	0	0
S. atromaculatus (Creek Chub)	0	0	0	1
Grand Total	**18**	**6**	**1**	**5**

Table 17. Freshwater mussel laboratory and natural confirmed host species: *P. cyphyus P. grandis* (Watters et al. 2009; Freshwater Mussel Host Database 2017).

Species	***P. cyphyus***	***P. grandis***
A. rupestris (Rock Bass)	0	1
C. anomalum (Central Stoneroller)	1	1
C. auratus (Gold Fish)	0	1
C. spiloptera (Spotfin Shiner)	1	0
C. whipplei (Steelcolor Shiner)	1	0
C. carpio (Common Carp)	0	1
E. caeruleum (Rainbow Darter)	0	1
E. nigrum (Johnny Darter)	0	1
F. diaphanus (Banded Killifish)	1	1
G. affinis (Western Mosquitofish)	1	0
L. sicculus (Brook Silverside)	0	1
L. osseus (Longnose Gar)	0	1
L. cyanellus (Green Sunfish)	0	1
L. gibbosus (Pumpkinseed)	0	1
L. humilis (Orangespotted Sunfish)	0	1
L. macrochirus (Bluegill)	0	1
L. megalotis (Longear Sunfish)	0	1
M. storeriana (Silver Chub)	1	0
M. salmoides (Largemouth Bass)	0	1
M. chrysops (White Bass)	0	1
N. blennius (River Shiner)	1	0
N. hudsonius (Spottail Shiner)	1	0
P. flavescens (Yellow Perch)	0	1
P. mirabilis (Suckermouth Minnow)	1	0
P. notatus (Bluntnose Minnow)	1	1
P. promelas (Fathead Minnow)	1	0
P. vigilax (Bullhead Minnow)	1	0
P. annularis (White Crappie)	0	1
P. nigromaculatus (Black Crappie)	0	1
R. atratulus (Blacknose Dace)	1	1
R. cataractae (Longnose Dace)	1	0
S. atromaculatus (Creek Chub)	1	1
Grand Total	**15**	**22**

Freshwater Mussel Ecology and Results

Obliquaria reflexa – Threehorn wartyback

Figure 50. *Obliquaria reflexa,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

The Threehorn wartyback was the most common mussel found throughout the Greenup pool represented by 1,116 live individuals and 114 deadshell. Site density was 55.8 individuals/600m^2 with a transect density of 9.3 animals/100m^2. Strong populations of *O. reflexa* were found along the 3rd- 5th intervals as other mussel species started to taper off (Figure 51). It was one of the few mussel species found downstream of Huntington, WV (Figure 52). Dark color phases, as seen above in Figure 50, were commonly encountered. Oddities, such as an individual without its defining three horn feature were also recovered (Figure 53). *O. reflexa* are only known from large river systems in sand, gravel, and fines (Watters et al. 2009). *O. reflexa* is a host generalist (Table 16) (Freshwater Mussel Host Database 2017).

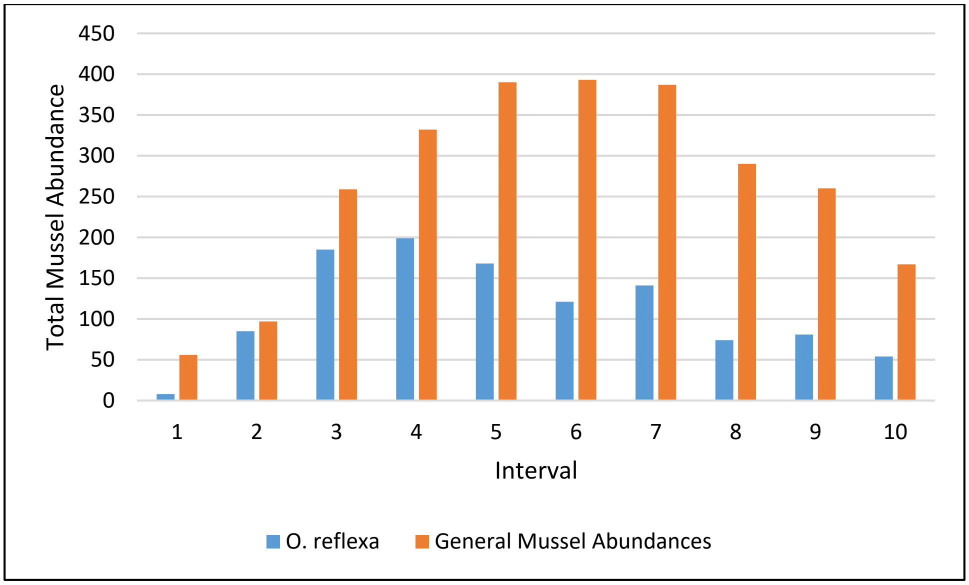

Figure 51. *Obliquaria reflexa* abundance counts versus general mussel abundance counts. *O. reflexa* is not included in the general mussel abundance. Each interval represents a 10m increase, with interval “1” beginning at the shoreline. Greenup pool, Ohio River (Source: Personal collection).

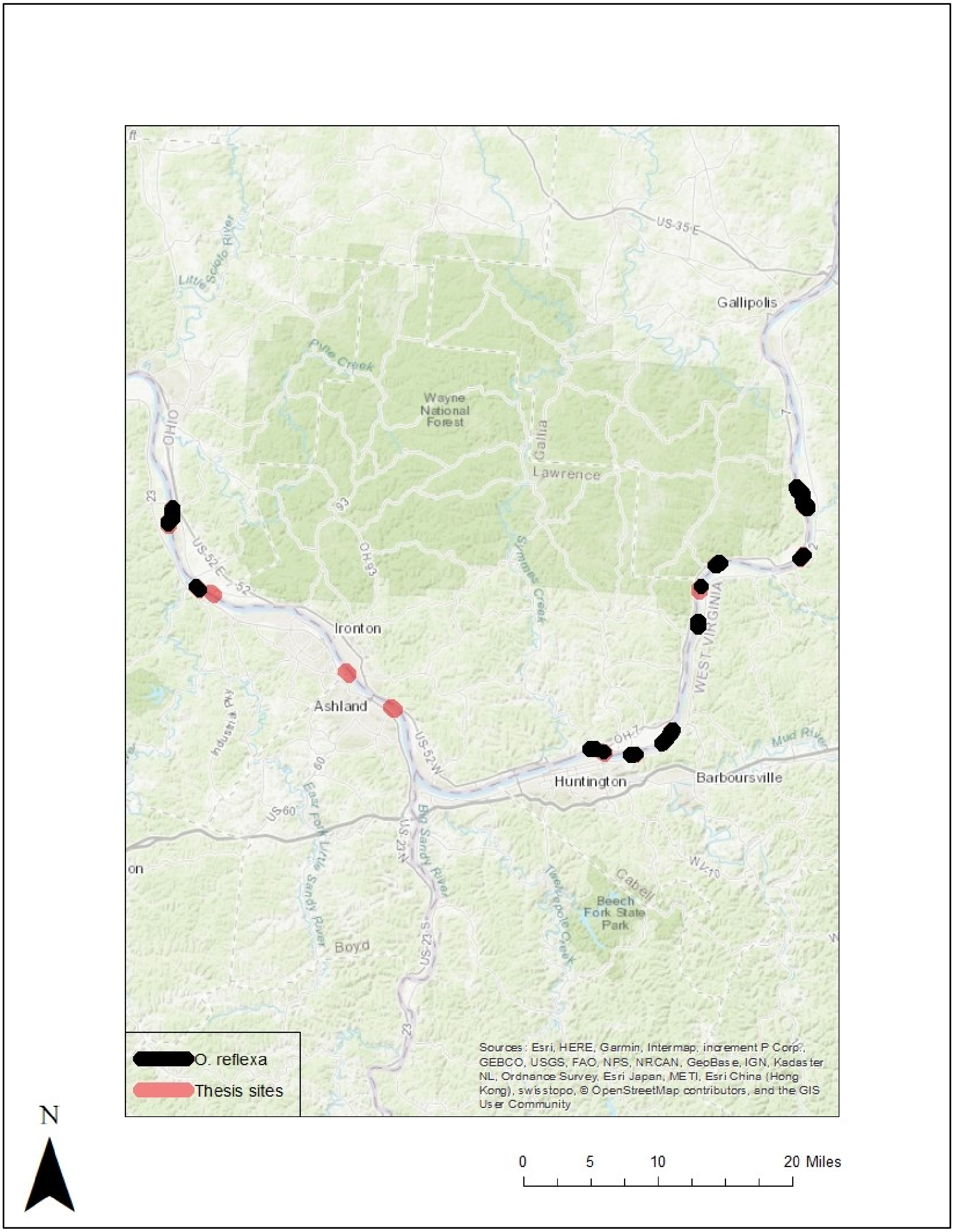

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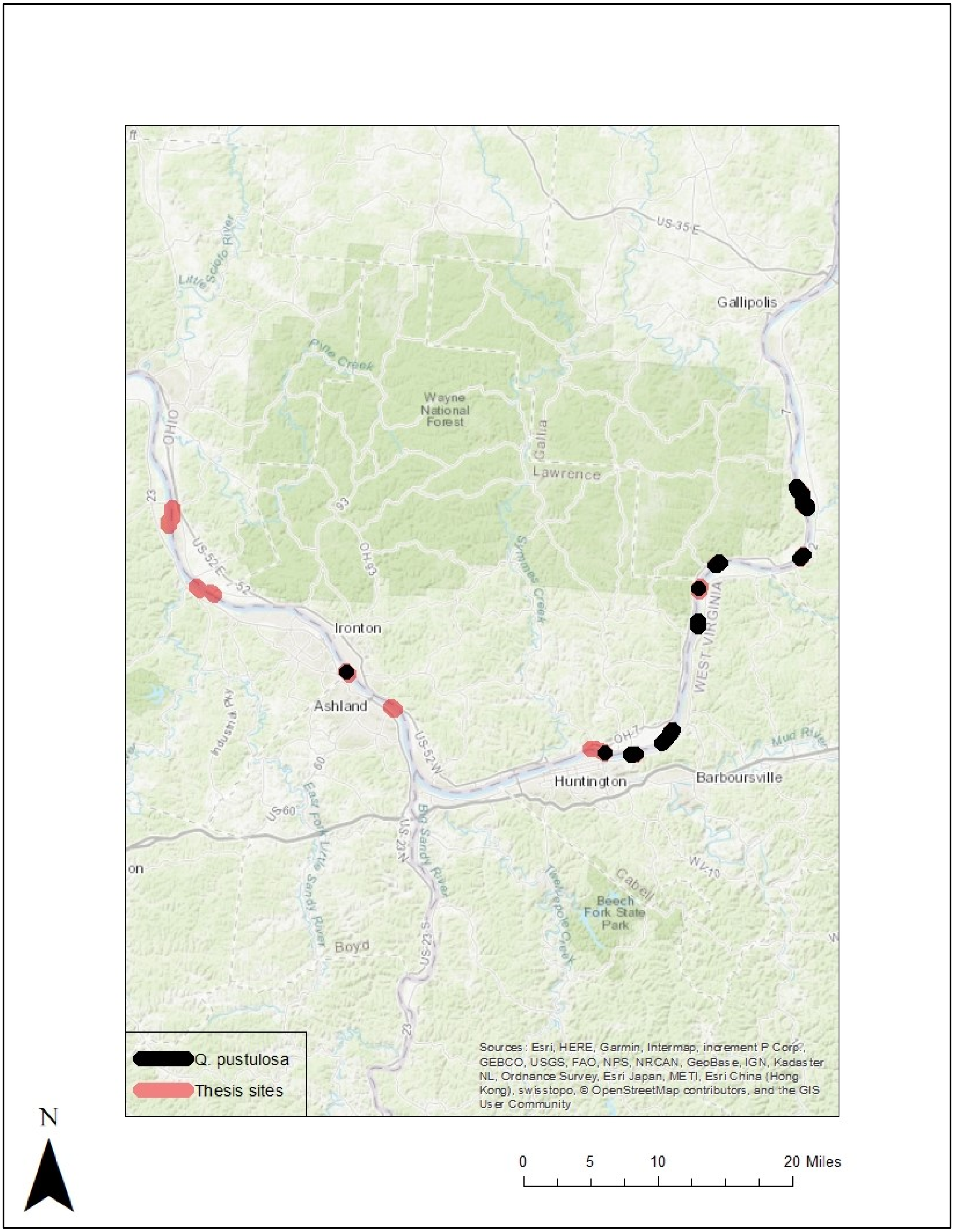
Figure 52. *Obliquaria reflexa* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Figure 53. *Obliquaria reflexa* without horns (middle individual). The middle individual represents a unique case in which a mussel has lost its defining characteristic making identification difficult (Photo credit: Mitchell Kriege).

Quadrula pustulosa – Pimpleback

Figure 54. *Quadrula pustulosa* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

The Pimpleback was the second most encountered mussel throughout the Greenup pool represented by 958 live individuals and 105 deadshell (Figure 55). Site density was 47.9 individuals/600m^2 with a transect density of 7.9 animals/100m^2. Multiple age classes were observed with evidence of strong recruitment (Figure 56). *Quadrula pustulosa* is a common member of most large to midsized river mussel assemblages and is a substrate generalist. In some sections of the Ohio, it may be the dominant species. *Quadrula pustulosa* is a catfish host specialist. (Watters et al. 2009). (Table 13) (Watters et al. 2009; Freshwater Mussel Host Database 2017).



Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

Figure 55. *Quadrula pustulosa* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Figure 56. Evidence of recruitment in *Quadrula pustulosa*, Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Ligumia recta – Black Sandshell

Figure 57. Female *Ligumia recta* (left) & male *L. recta* (right) Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Ligumia recta was a common species throughout the Greenup pool represented by 398 live individuals and 141 deadshell (DS) (Figure 58). Site density was 19.9 individuals/600m^2 with a transect density of 3.3 animals/100m^2. Multiple age classes were observed (Figure 59). The male to female sex ratio was 1.47:1. *Ligumia recta* is a big river specialist of sand and gravel whose primary fish host is *Sander canadensis* (Sauger) and S*ander vitreus* (Walleye) (Table 14) (Watters et al. 2009; Kelly and Watters 2010; Haag 2012; Khym and Layzer 2000; Freshwater Mussel Host Database 2017).

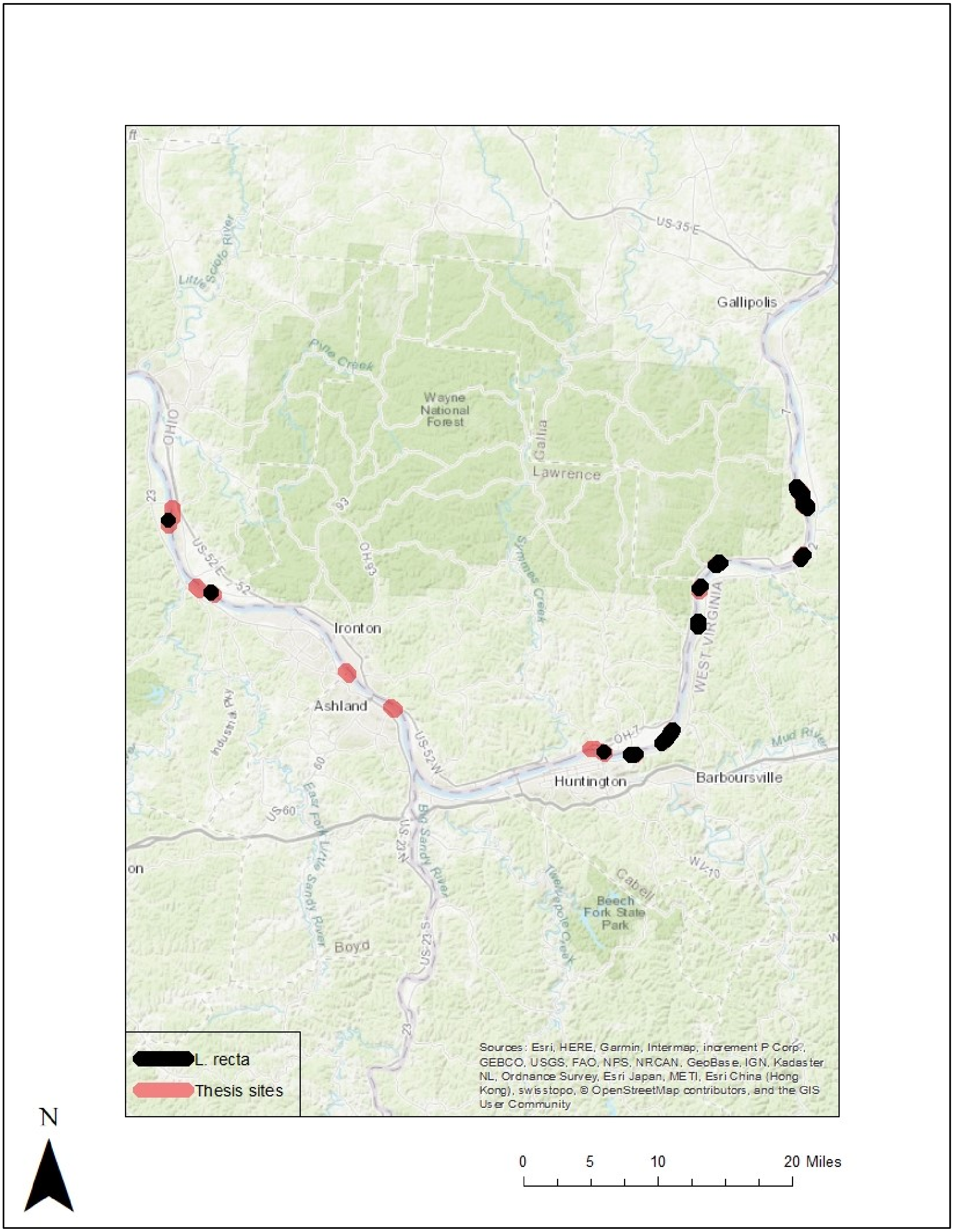


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Figure 58. *Ligumia recta* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Figure 59. Evidence of recruitment in *Ligumia recta*, Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

***Ellipsaria lineolata* – Butterfly**

Figure 60. Female *Ellipsaria lineolata* (left) & male *E. lineolata* (right), Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

The Butterfly was a common species encountered throughout the upper section of the Greenup pool represented by 284 live individuals and 22 deadshell (Figure 61). Site density was 14.2 individuals/600m^2 with a transect density of 2.4 animals/100m^2. The male to female ratio was 1:1.32 with multiple age classes observed (Figure 62). *E. lineolata* requires large river habitat with a sand/gravel substrate (Watters et al. 2009). The only known host for *E. lineolata* is *Aplodinotus grunniens* (Freshwater Drum) (Watters et al. 2009; Freshwater Mussel Host Database 2017) (Table 15). Female Butterfly mussels may sacrifice themselves during reproduction to molluscivorous host drum (Haag 2012).

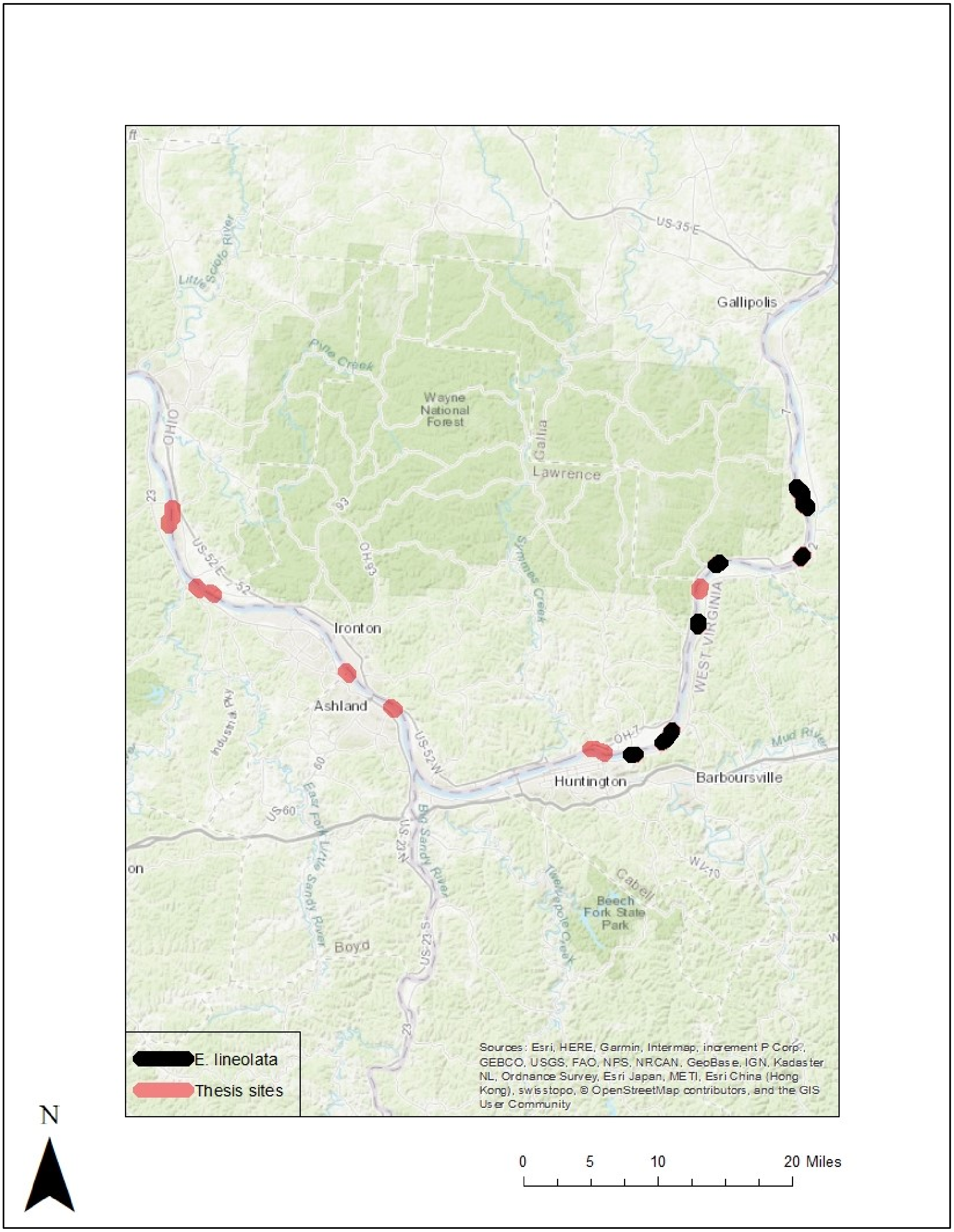

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Figure 61. *Ellipsaria lineolata* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Figure 62. Evidence of recruitment in *Ellipsaria lineolata*, Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Amblema plicata – Threeridge

Figure 63. *Amblema plicata,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Amblema plicata was encountered within all sections of the Greenup pool (Figure 64). I collected 247 live individuals and 219 deadshell. Site density was 12.4 individuals/600m^2 and with a transect density of 2.1 animals/100m^2. *A. plicata* is a heavy shelled mollusk which favors firm substrate in rivers, streams and lakes. The Threeridge is a host generalist (Table 15) (Watters et al. 2009; Freshwater Mussel Host Database 2017).

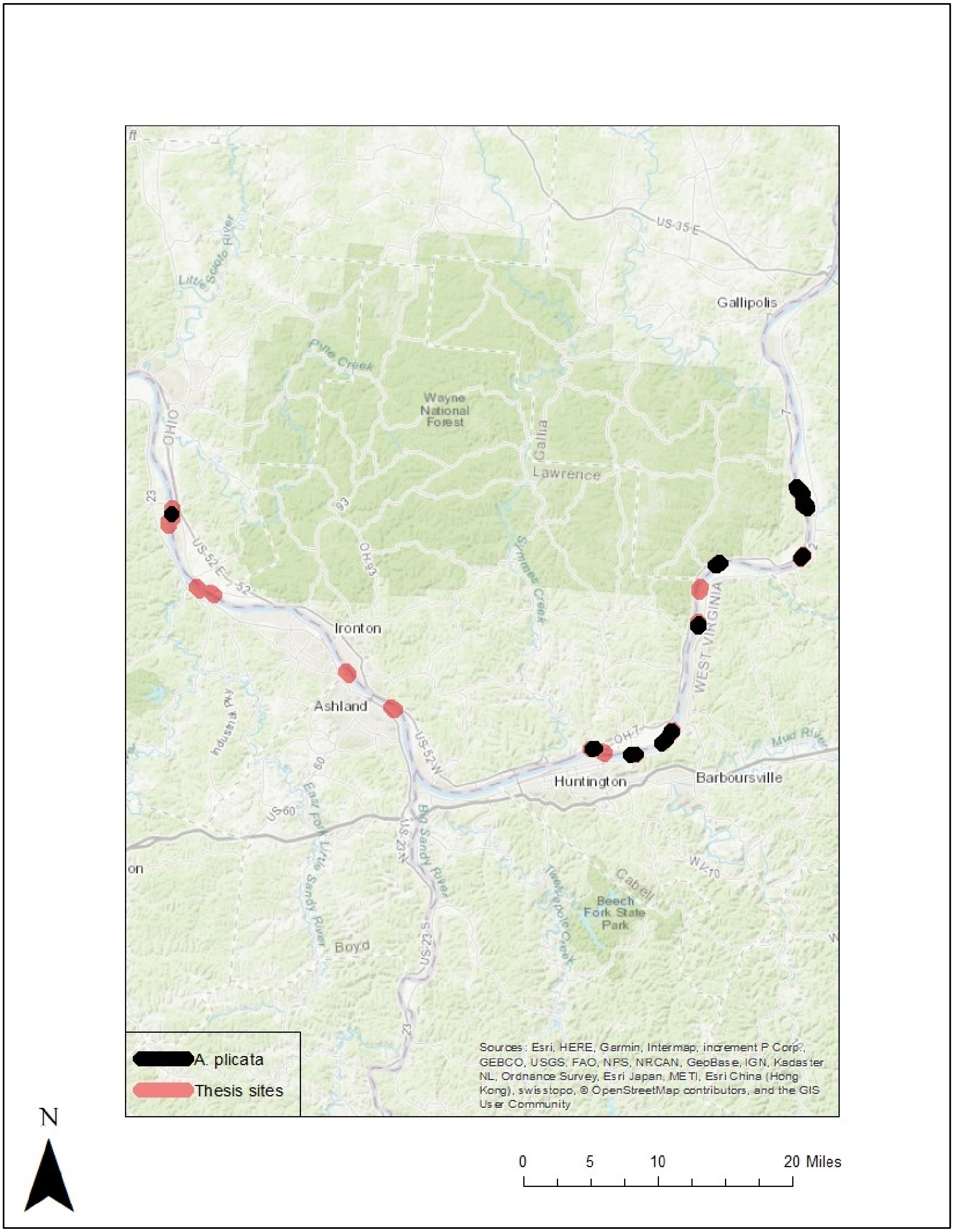

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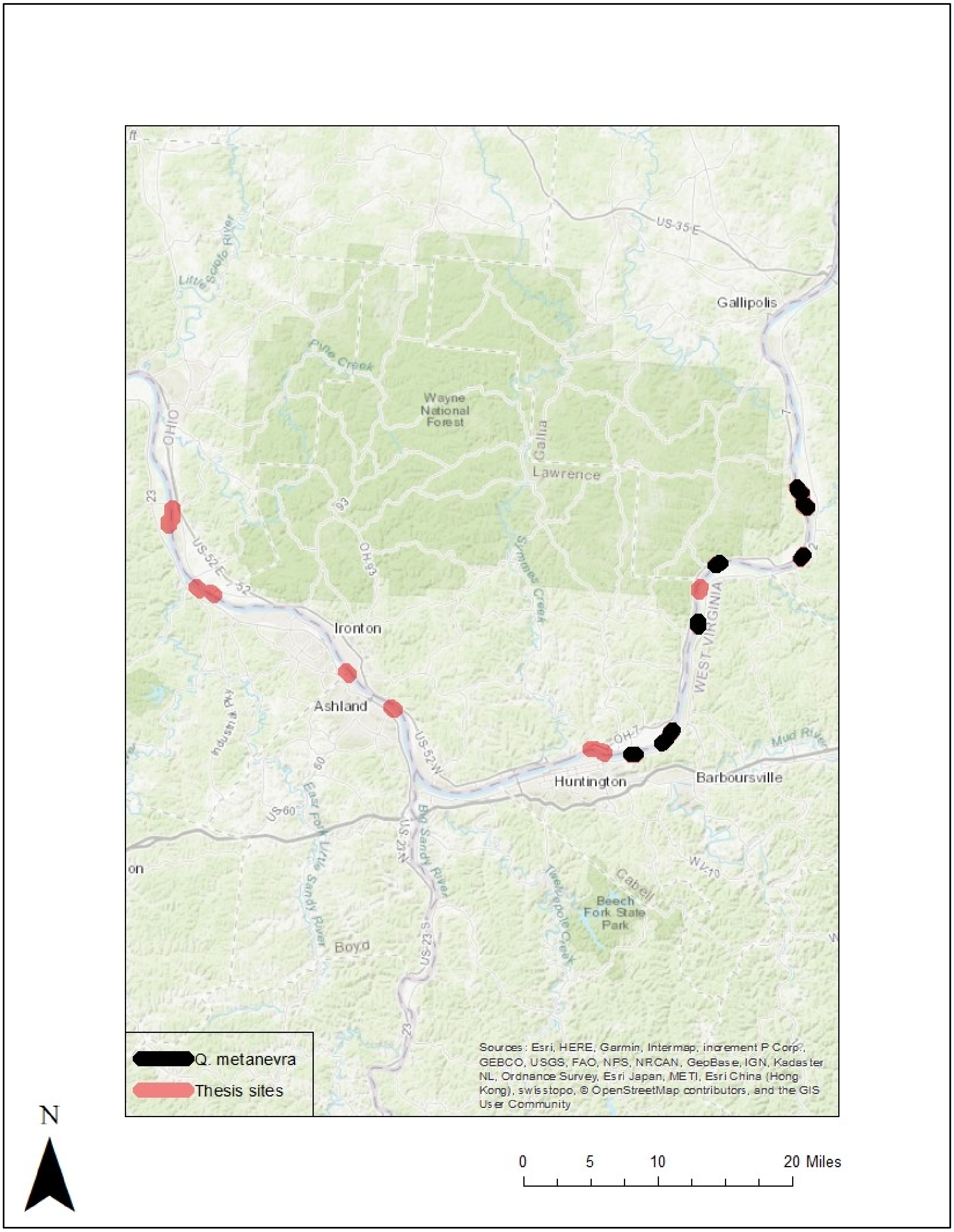
Figure 64. *Amblema plicata* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Quadrula metanevra – Monkeyface

Figure 65. *Quadrula metanevra,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Quadrula metanevra was frequently encountered in the upper Greenup pool (Figure 66). I collected 244 live individuals and 15 deadshell. Site density was 12.2 individuals/600m^2 with a transect density of 2.0 animals/100m^2. Multiple size classes were observed (Figure 67). *Q. metanevra* is a catfish host specialist in large rivers with sand and gravel substrate (Table 13) (Watters et al. 2009; Freshwater Mussel Host Database 2017).



Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

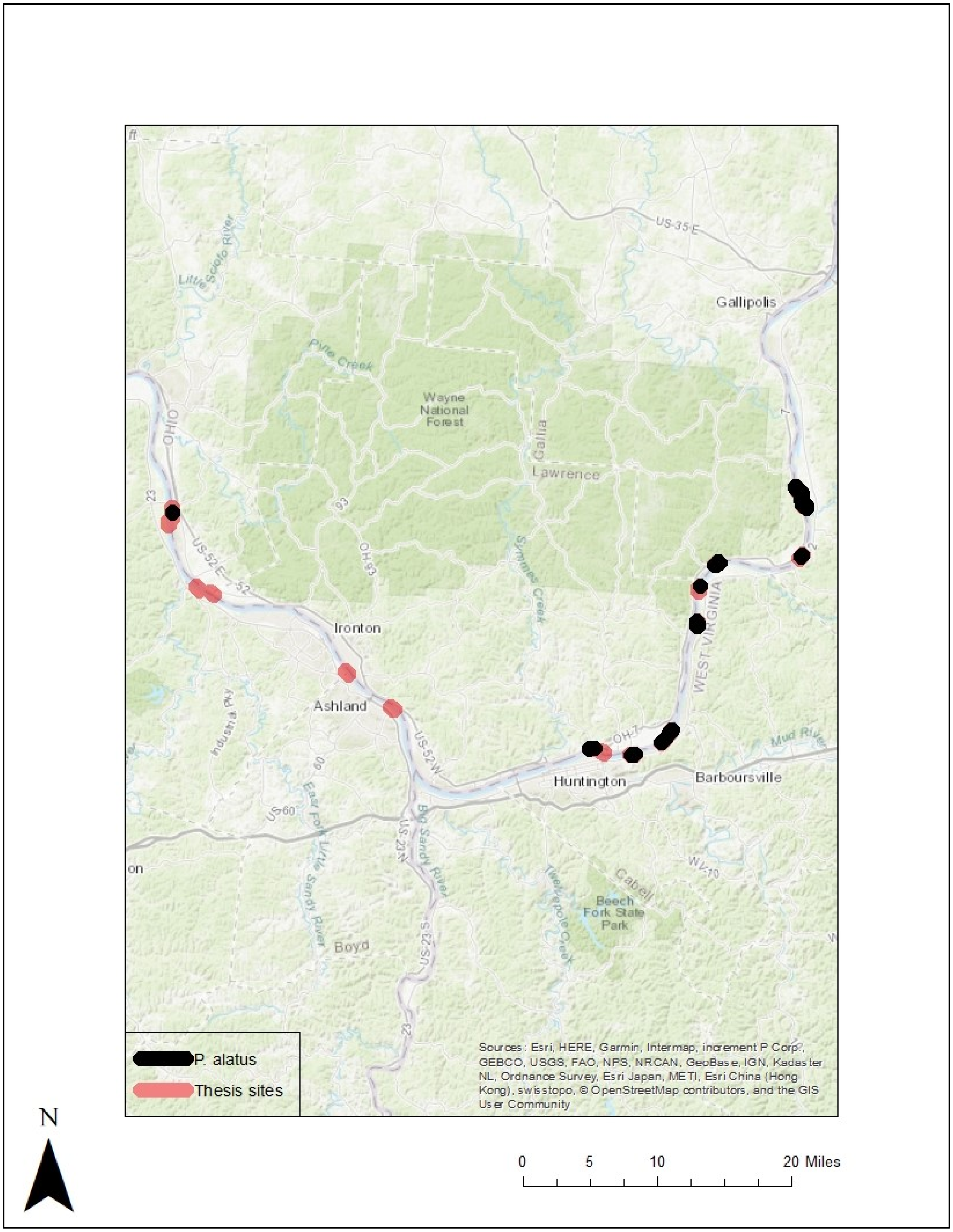
Figure 66. *Quadrula metanevra* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Figure 67. Evidence of recruitment in *Quadrula metanevra*, Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Potamilus alatus **– Pink Heelsplitter**

Figure 68. *Potamilus alatus,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

P. alatus was encountered throughout all three sections of the Greenup Pool (Figure 69). I collected 212 live individuals and 217 deadshell. Site density was 10.6 individuals/600m^2 with a transect density of 1.7 animals/100m^2. The Pink Heelsplitter is a common species throughout the Ohio River drainage. It is a thin shelled mussel which inhabits medium-large sized rivers in sand, mud or fines, usually out of the direct current (Watters et al. 2009; Hart 2014). *P. alatus* is host specialist on *Aplodinotus grunniens* (Freshwater Drum) (Table 16) (Watters et al. 2009; Freshwater Mussel Host Database 2017). Since *A. grunniens* are molluscivores, female *P. alatus* may sacrifice themselves during reproduction (Haag 2012).



Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

Figure 69. *Potamilus alatus* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Elliptio crassidens **– Elephantear**

Figure 70. *Elliptio crassidens,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Elliptio crassidens was encountered through the upper sections of the Greenup pool (Figure 71). I collected 90 live individuals and 101 deadshell. Site density was 4.5 individuals/600m^2 with a transect density of 0.75 animals/100m^2. *E. crassidens* is a heavy shelled mussel which inhabits sand and gravel substrates with current in large river systems. *E. crassidens* was historically a dominant member of the Greenup Pool mussel fauna (Taylor 1980). However, due to unsuccessful recent recruitment, the remaining population consists of aging adults > 80 mm with no juveniles observed. The host for *E. crassidens* in northern waters is *Alosa chrysochloris* (Skipjack Herring) (Table 15) (Freshwater Mussel Host Database 2017). Southern populations may also utilize *Alosa alabamae* (Alabama Shad) (Hart 2014).

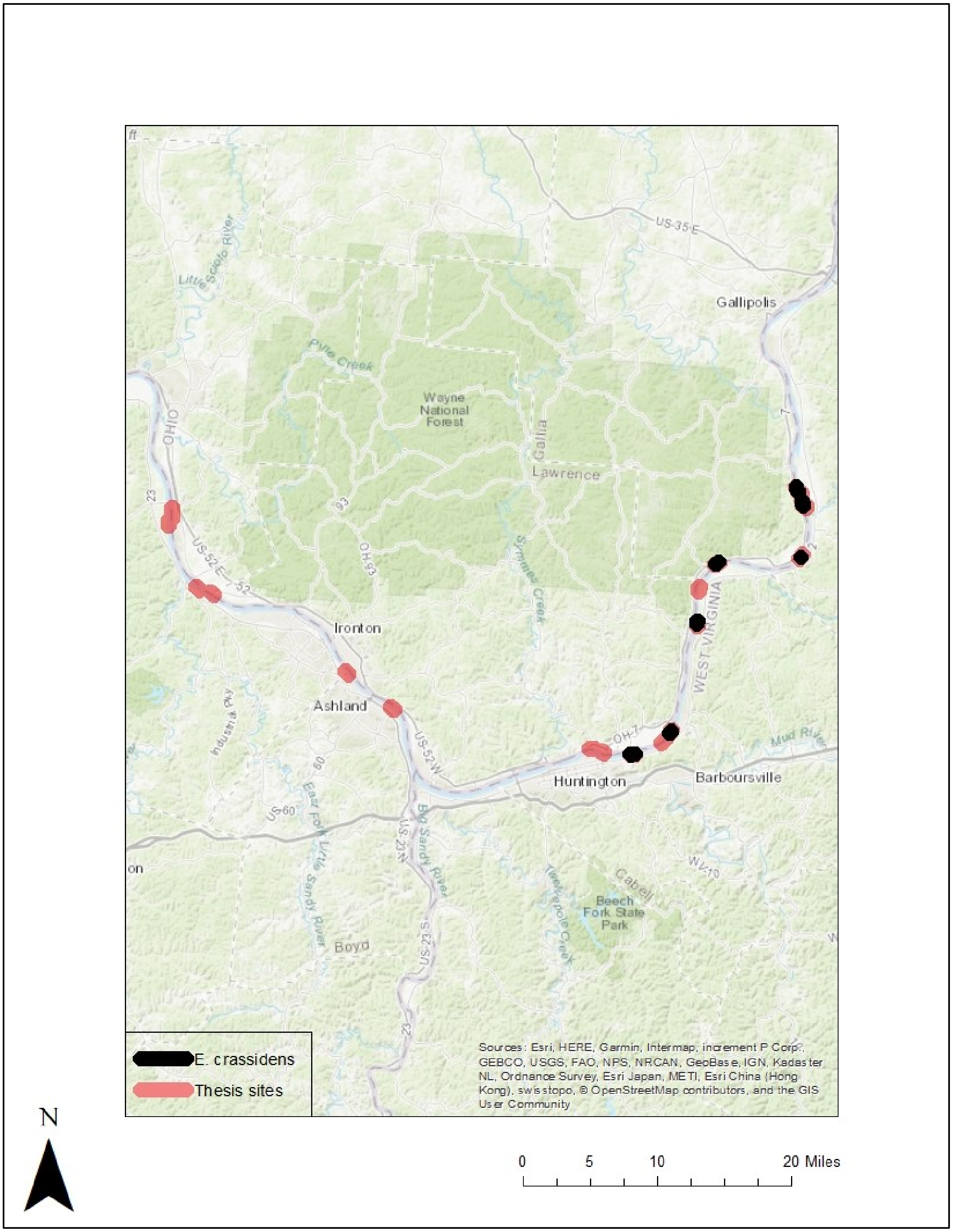


Figure 71. *Elliptio crassidens* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Lampsilis cardium – Plain Pocketbook

Figure 72. *Lampsilis cardium,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Lampsilis cardium was collected in the upper and middle sections of the Greenup pool (Figure 73). I collected 51 live individuals and 26 deadshell. Site density was 2.6 individuals/600m^2 with a transect density of 0.43 animals/100m^2. *L. cardium* is a host generalist but evolved to target predatory species due to its glochidial lure. During my surveys from August to September, 3-4 displaying female *L. cardium* were observed*.* The Plain Pocketbook is a host generalist but may specialize in predator species (Table 14) (Watters et al. 2009; Freshwater Mussel Host Database 2017).

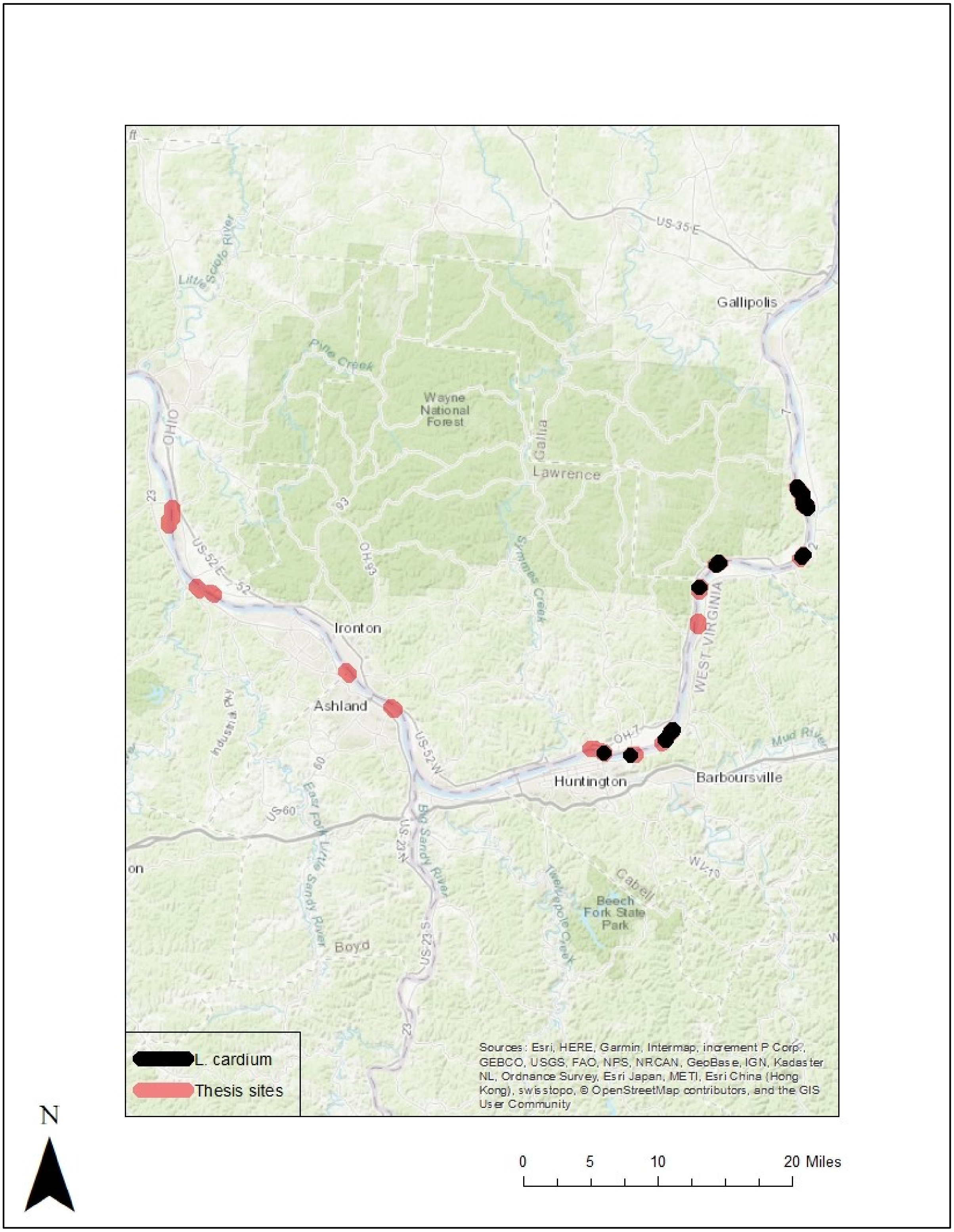


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Figure 73. *Lampsilis cardium* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Pleurobema cordatum – Ohio Pigtoe

Figure 74. *Pleurobema cordatum,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

P. cordatum was relatively common throughout the upper portion of the Greenup pool (Figure 74). I collected 47 live individuals and 12 deadshell. Site density was 2.4 individuals/600m^2 with a transect density of 0.4 animals/100m^2. My study area may lie on the northern edge of its range. A hundred miles south, in the Markland pool at Cincinnati, it is a dominant species in the mussel assemblage (ESI 2014). *P. cordatum* is a thick shelled mussel which inhabits sand or gravel substrate of large rivers in current. *P. cordatum* utilizes suckers and minnows as host species and did show signs of recruitment in the Greenup pool as three individuals < 30 mm were collected (Table 16) (Watters et al. 2009; Miller and Payne 2000; Freshwater Mussel Host Database 2017).

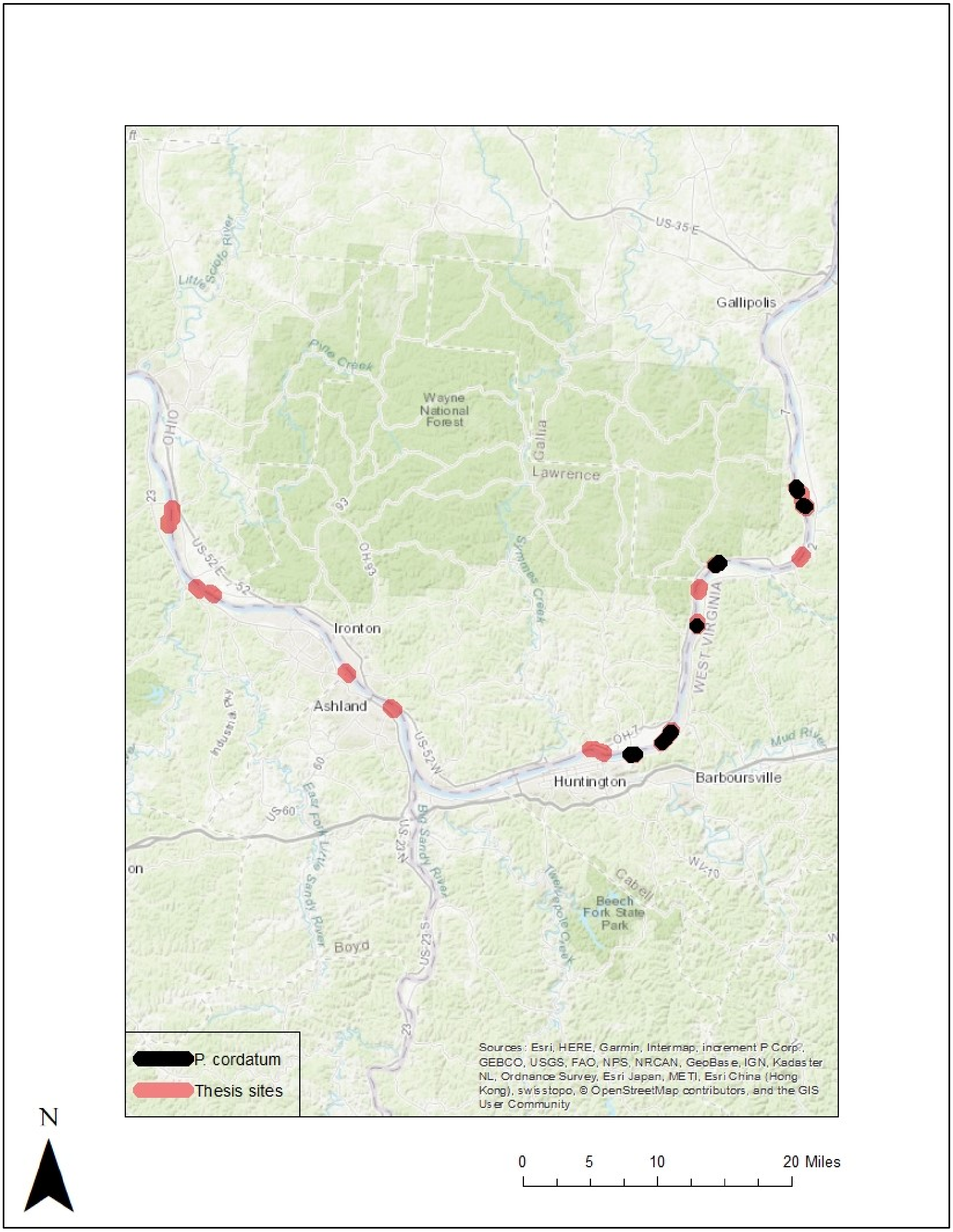

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Figure 75. *Pleurobema cordatum* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Quadrula quadrula – Mapleleaf

Figure 76. *Quadrula quadrula,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Quadrula quadrula was sporadically encountered throughout the upper Greenup pool (Figure 77). I collected 27 live individuals and 37 deadshell. Site density was 1.4 individuals/600m^2 with a transect density of 0.2 animals/100m^2. *Q. quadrula i*s a common member of many medium to large river systems in mud, sand, or gravel substrates. It was noted as a dominant species in the upper Ohio during surveys in the 1980s (Zeto 1987). However, *Q. quadrula* was found in relatively low numbers throughout my surveys, but it appears quite common in some adjacent Greenup pool backwaters (Figure 78). Many of the individuals I recovered were also older specimens, suggesting a decline in *Q. quadrula* populations. *Quadrula quadrula* is a catfish specialist; it’s only known viable host is *Ictalurus punctatus* (Channel Catfish) (Table 13) (Watters et al. 2009; Freshwater Mussel Host Database 2017).

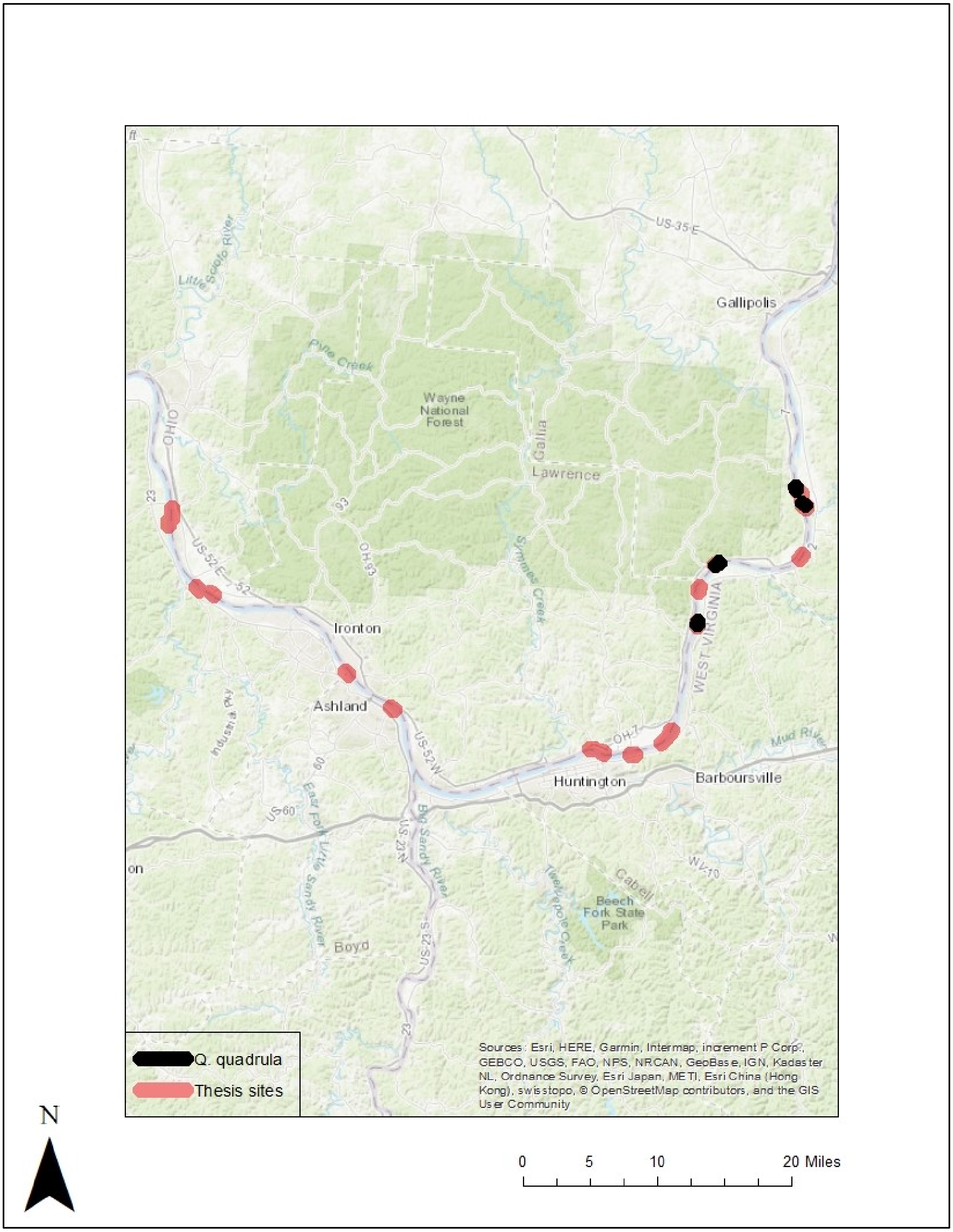

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Figure 77. *Quadrula quadrula* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Figure 78. ***Quadrula Quadrula*** collected during scouting surveys in Ohio River backwaters on Ice Creek, OH. Populations showed signs of rigorous recruitment (Photo credit: Tom Jones).

Megalonaias nervosa – Washboard

Figure 79. *Megalonaias nervosa,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

M. nervosa was found in moderate numbers at upper pool sites closest to RC Byrd Locks and Dam (WV12, WV11, WV09) (Figure 80). However, few individuals were found at other downstream sites. I collected 21 live individuals and nine deadshell. Site density was one individual/600m^2 with a transect density of 0.17 animals/100m^2. *M. nervosa* is a medium to large river species which inhabits areas with current and sand or gravel substrate (Watters et al. 2009). Populations show limited recruitment as no individuals were found below 96 mm. Additionally, sixty percent of the population was >150 mm. *Megalonaias nervosa* is a host generalist (Table 16) (Watters et al. 2009; Freshwater Mussel Host Database 2017).

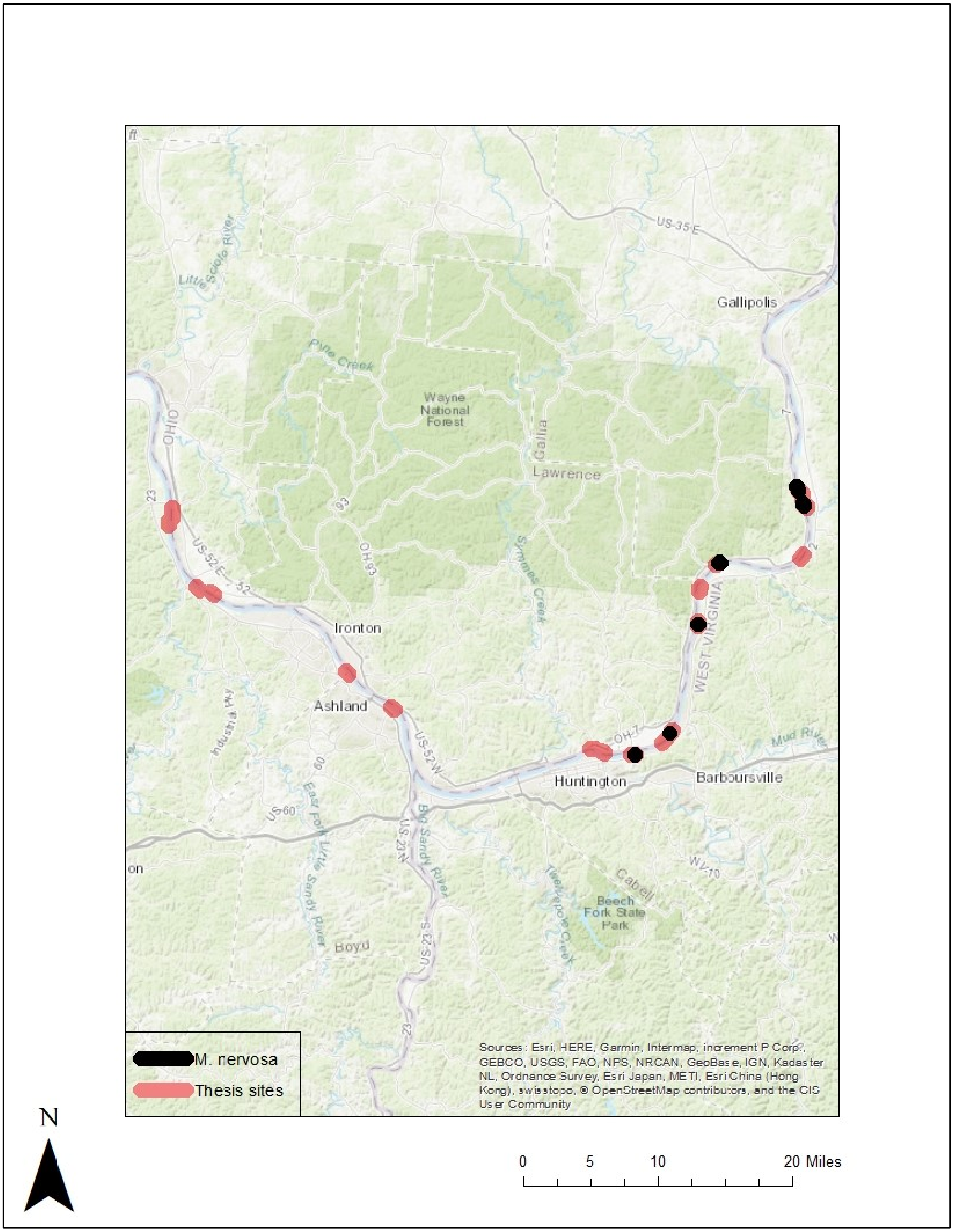

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Figure 80. *Megalonaias nervosa* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Actinonaias ligamentina – Mucket

Figure 81. *Actinonaias ligamentina,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Actinonaias ligamentina was found sporadically throughout the upper Greenup pool (Figure 81). I collected 16 live individuals and 11 deadshell. Site density was 0.8 individuals/600m^2 with a transect density of 0.13 animals/100m^2. *A. ligamentina* is a medium to large river species that prefers sand or gravel in areas of high current. The Mucket is a host generalist (Table 15) (Watters et al. 2009; Freshwater Mussel Host Database 2017).

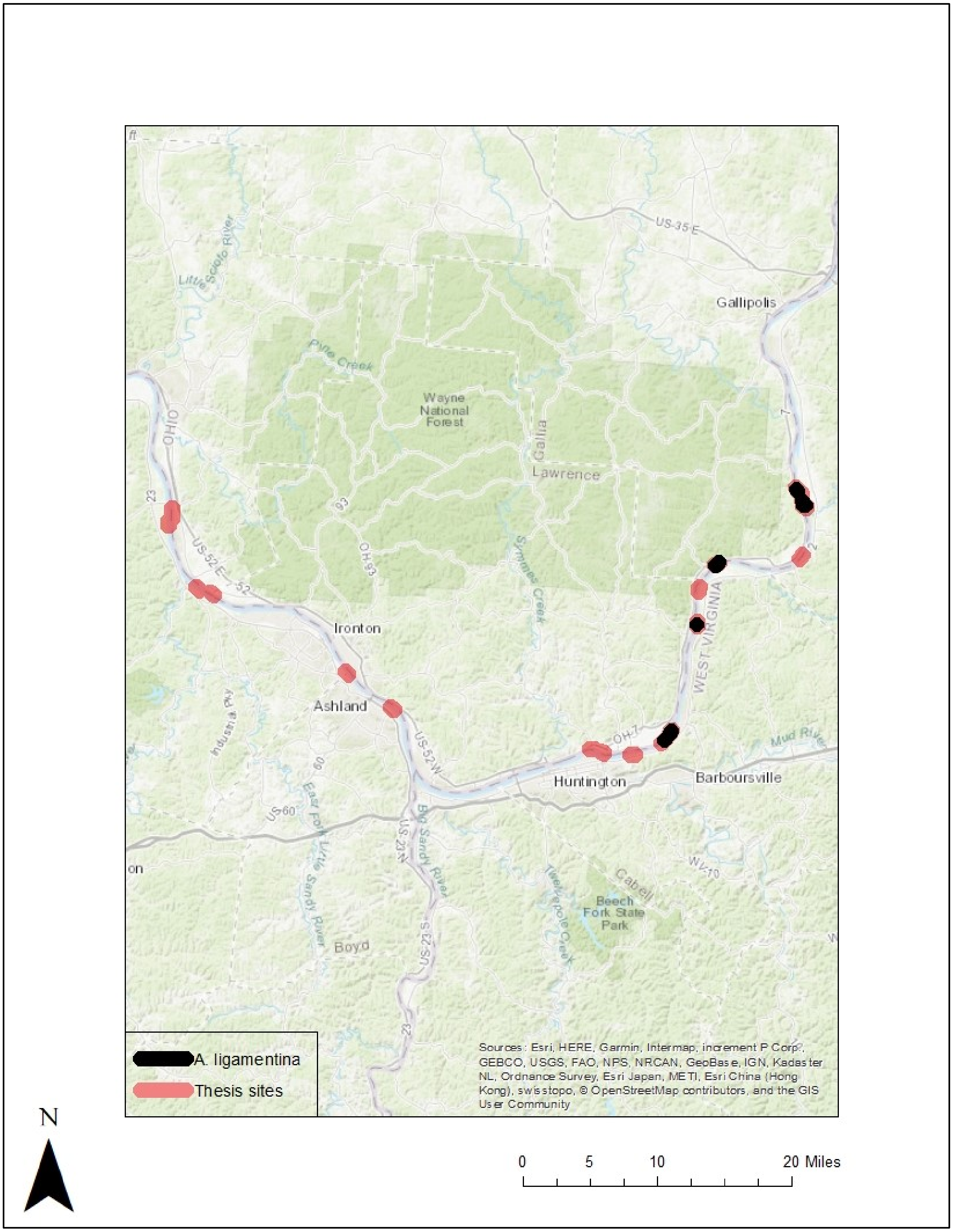

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Figure 82. *Actinonaias ligamentina* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

***Plethobasus cyphyus* – Sheepnose**

Figure 83. *Plethobasus cyphyus,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Plethobasus cyphyus was uncommonly encountered in the upper Greenup pool. Site maps were not included to protect the population locations of this federally endangered species. I collected nine live individuals and two deadshell. Site density was 0.45 individuals/600m^2 with a transect density of 0.08 animals/100m^2. The Sheepnose is a thick shelled, federally endangered mussel which prefers areas of strong current in large to medium sized rivers with sand and gravel substrate (Watters et al. 2009; Butler 2002). However, a strong population of individuals was found on an inside bend with a fines/gravel substrate. *P. cyphyus* occurs sporadically throughout the Ohio River mainstem and its larger tributaries. No juvenile individuals were encountered during my surveys. The Sheepnose is a host generalist, predominately utilizing minnows and shiners (Table 17) (Watters et al. 2009; Freshwater Mussel Host Database 2017).

Fusconaia ebena – Ebonyshell

Figure 84. *Fusconaia ebena,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Fusconaia ebena was uncommonly collected in the upper Greenup pool (Figure 85). I collected 7 live individuals and four deadshell. Site density was 0.35 individuals/600m^2 with a transect density of 0.06 animals/100m^2. The Ebonyshell is a thick shelled species that prefers the main current of large river systems with sand or gravel substrate (Watters et al. 2009). I recovered an unexpectedly low number of *F. ebena*. It is a dominant member of the mussel fauna in the Markland pool, approximately 100 miles downstream (ESI 2014). The Greenup pool may represent the northern edge of *F. ebena's* range. The primary host species for *F. ebena* in the Greenup pool is *Alosa chrysochloris* (Skipjack Herring) (Table 15) (Watters et al. 2009; Freshwater Mussel Host Database 2017).

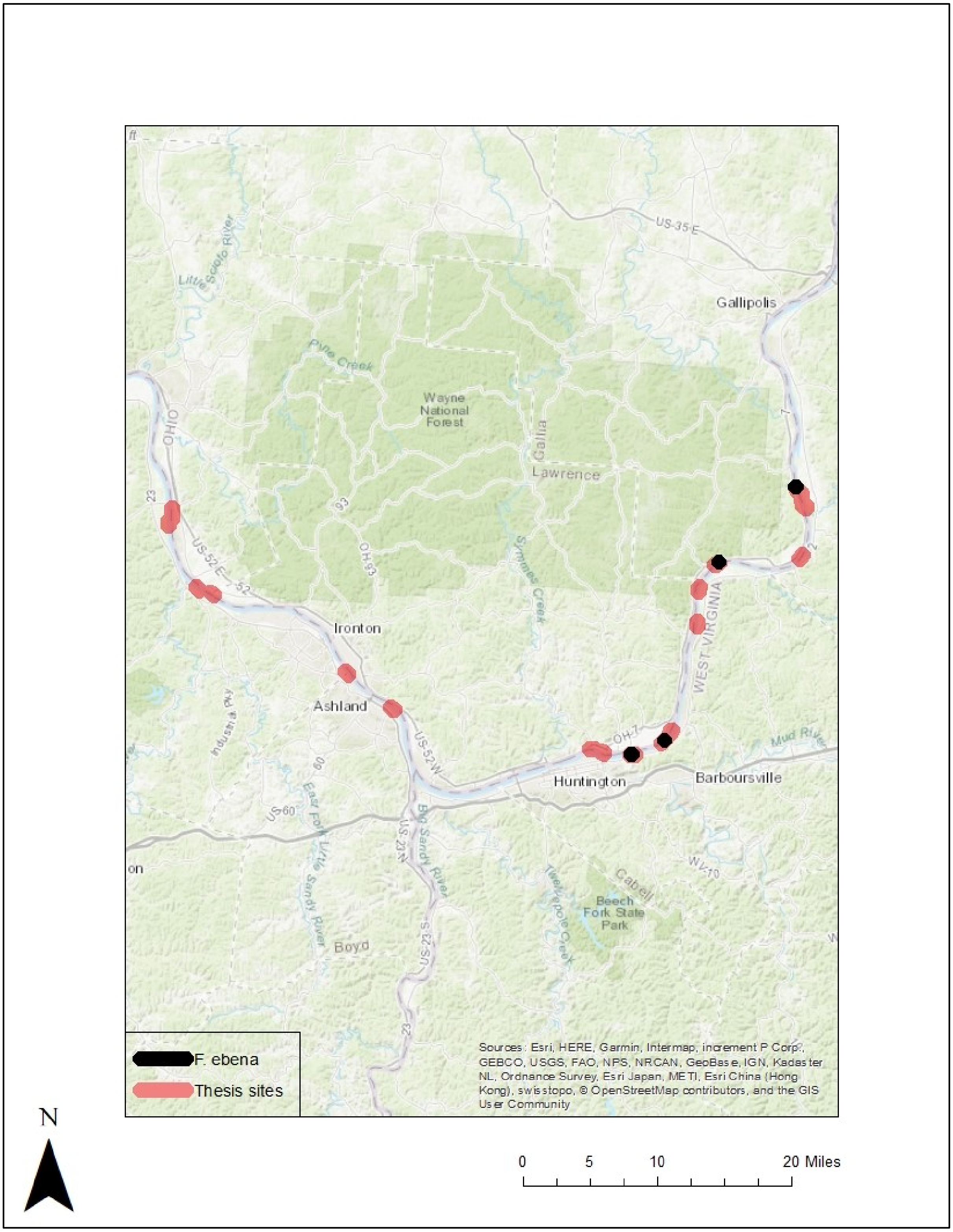

Figure 85. *Fusconaia ebena* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Truncilla truncata – Deertoe

Figure 86. *Truncilla truncata,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Truncilla truncata was uncommonly encountered in the upper Greenup pool (Figure 87). I collected six live individuals and five deadshell. Site density was 0.3 individuals/600m^2 with a transect density of 0.05 animals/100m^2 but its small size may skew abundance estimates. *T. truncata* is a small mussel species that inhabits medium to larger rivers in sand or gravel (Watters et al. 2009). The host fish for *T. truncata* is *Aplodinotus grunniens* (Freshwater Drum) (Table 13) (Watters et al. 2009; Freshwater Mussel Host Database 2017). Like *E. lineolata* and *P. alatus*, the Deertoe may also be a species in which females sacrifice themselves for reproduction (Haag 2012).

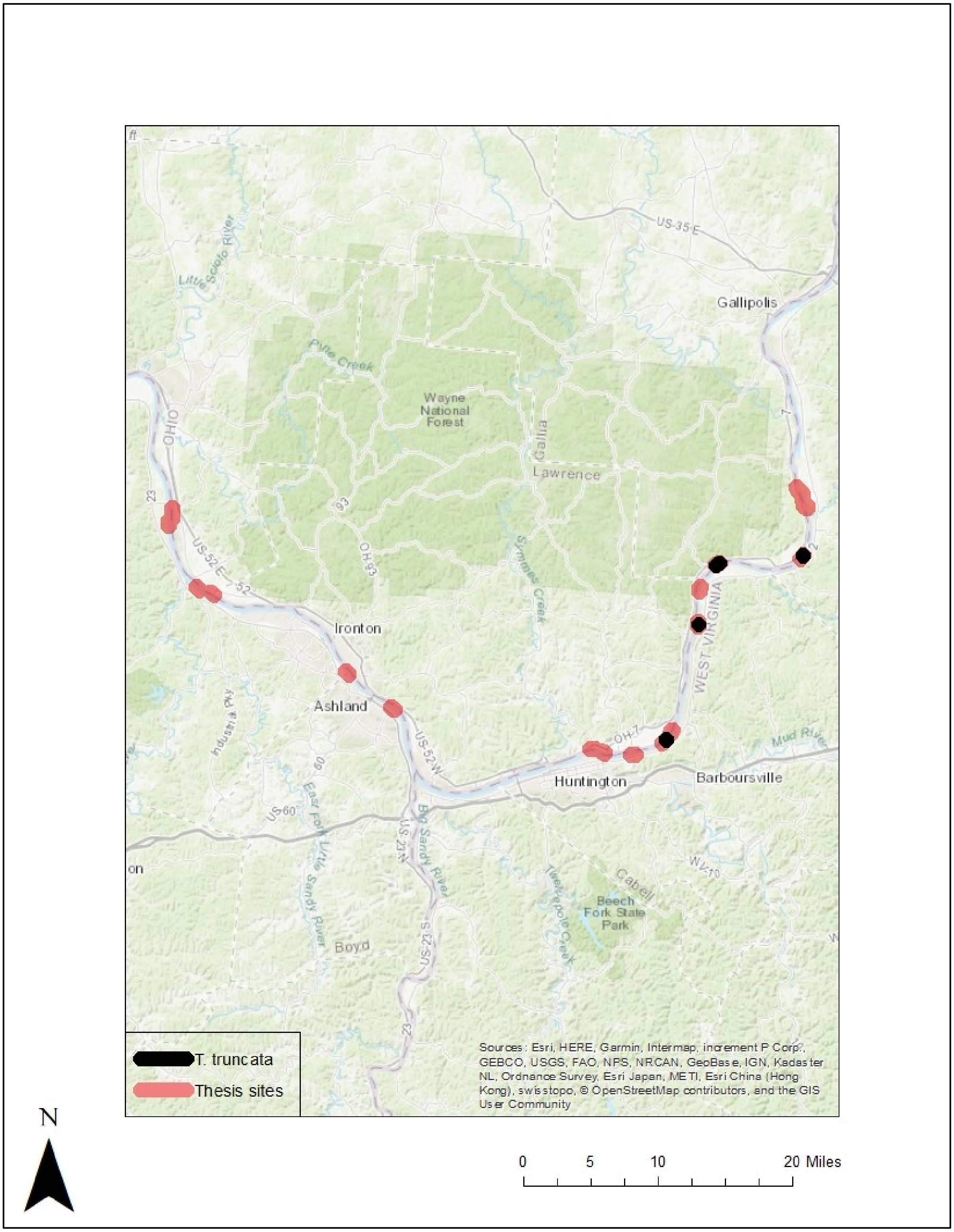

Figure 87. *Truncilla truncata* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

***Fusconaia flava* – Wabash Pigtoe**

Figure 88. *Fusconaia flava***,** Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Fusconaia flava was rarely encountered in the upper Greenup pool (Figure 89). I collected four live individuals and three deadshell. Site density was 0.2 individuals/600m^2 with a transect density of 0.03 animals/100m^2. *F. flava* is a hard shelled, habitat generalist species (Watters et al. 2009). It is a common mussel in many tributaries of the Ohio River but was rarely encountered live in the Greenup pool during thesis surveys. The Wabash Pigtoe has been transformed on *C. spiloptera* (Spotfin Shiner) and *S. atromaculatus* (Creek Chub) (Table 14) (Watters et al. 2009; Freshwater Mussel Host Database 2017).

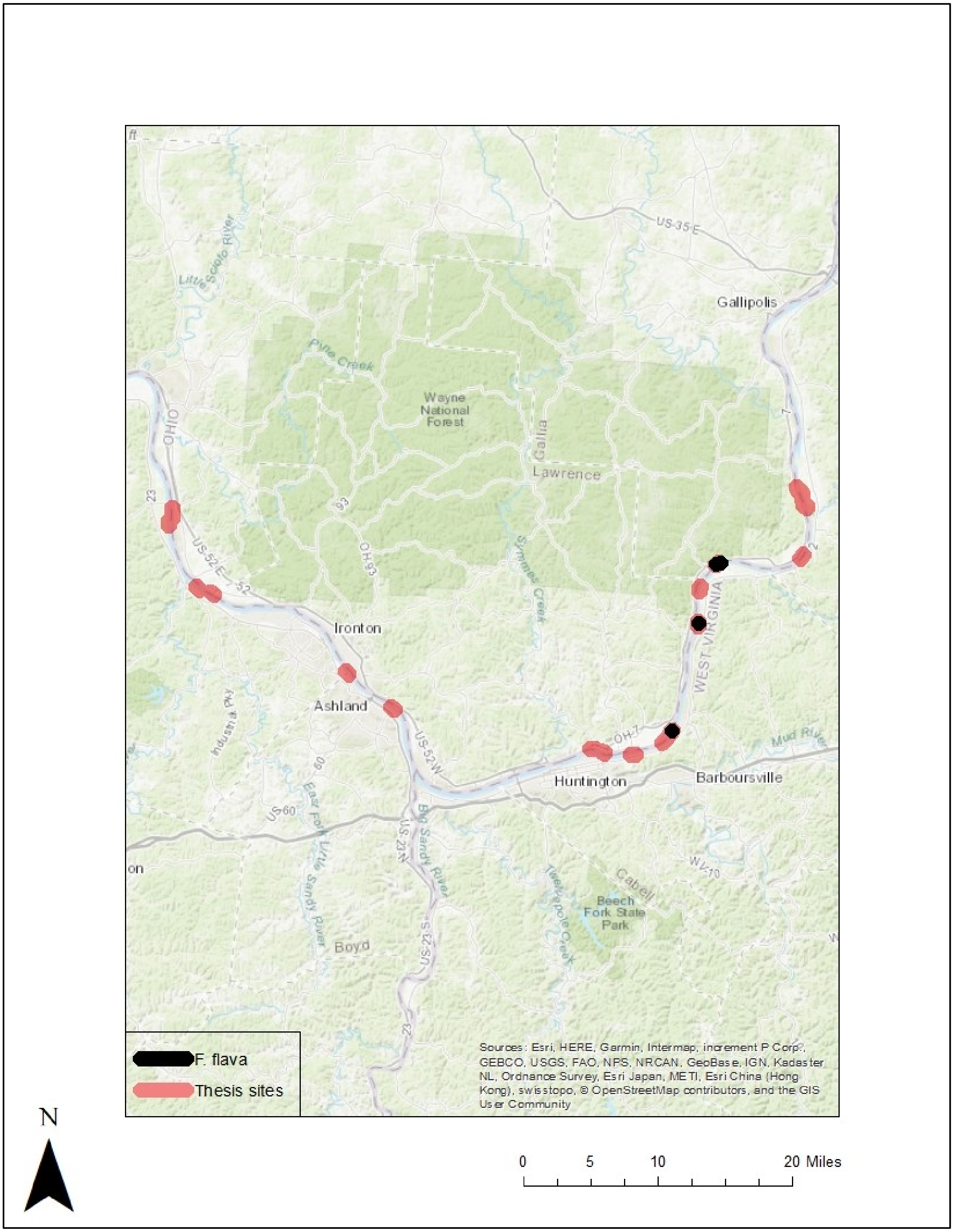

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Figure 89. *Fusconaia flava* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Leptodea fragilis – Fragile Papershell

Figure 90. *Leptodea fragilis*, Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Leptodea fragilis was rarely encountered in the upper Greenup pool (Figure 91). I collected three live individuals and six deadshell. Site density was 0.15 individuals/600m^2 with a transect density of 0.03 animals/100m^2. *L. fragilis* prefers mud or silt substrate in sluggish water of lakes and all stream sizes. It may be uncommon in the Greenup pool mainstem due to heavy current. However, its absence in the lower section of the pool that experiences minimal current is perplexing. The host fish for *L. fragilis* is suspected to be *Aplodinotus grunniens* (Freshwater Drum), although a successful laboratory infestation has never been observed (Table 14) (Watters et al. 2009; Freshwater Mussel Host Database 2017). Like *E. lineolata*, *P. alatus*, and *T. truncata*, the Fragile Papershell may also be a species in which females sacrifice themselves for reproduction (Haag 2012; Freshwater Mussel Host Database 2017).

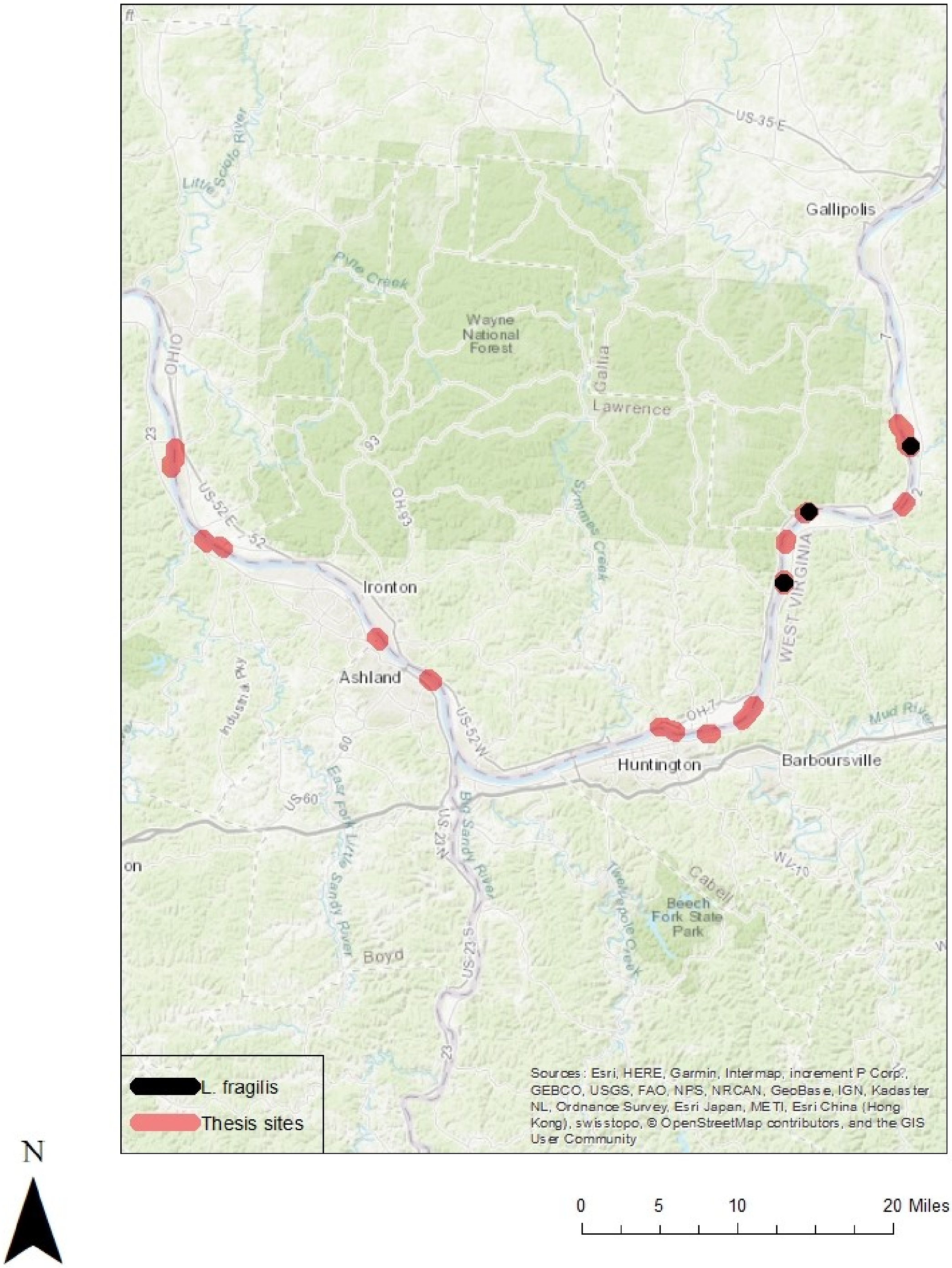

Figure 91. *Leptodea fragilis* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Lasmigonia complanata **– White Heelsplitter**

Figure 92. *Lasmigonia complanata,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Lasmigonia complanata was rarely encountered in the upper Greenup pool (Figure 93). I collected two live individuals and six deadshell. Site density was 0.1 individuals/600m^2 with a transect density of 0.02 animals/100m^2. *Lasmigonia complanata* prefers mud or silt substrate in sluggish water of lakes and all stream sizes. Like *L. fragilis*, the White Heelsplitter may be uncommon in the Greenup pool due to heavy current in the main-stem. Its absence in the lower section of the pool that experiences minimal current is surprising. The White Heelsplitter is a host generalist (Table 14) (Watters et al. 2009; Freshwater Mussel Host Database 2017).

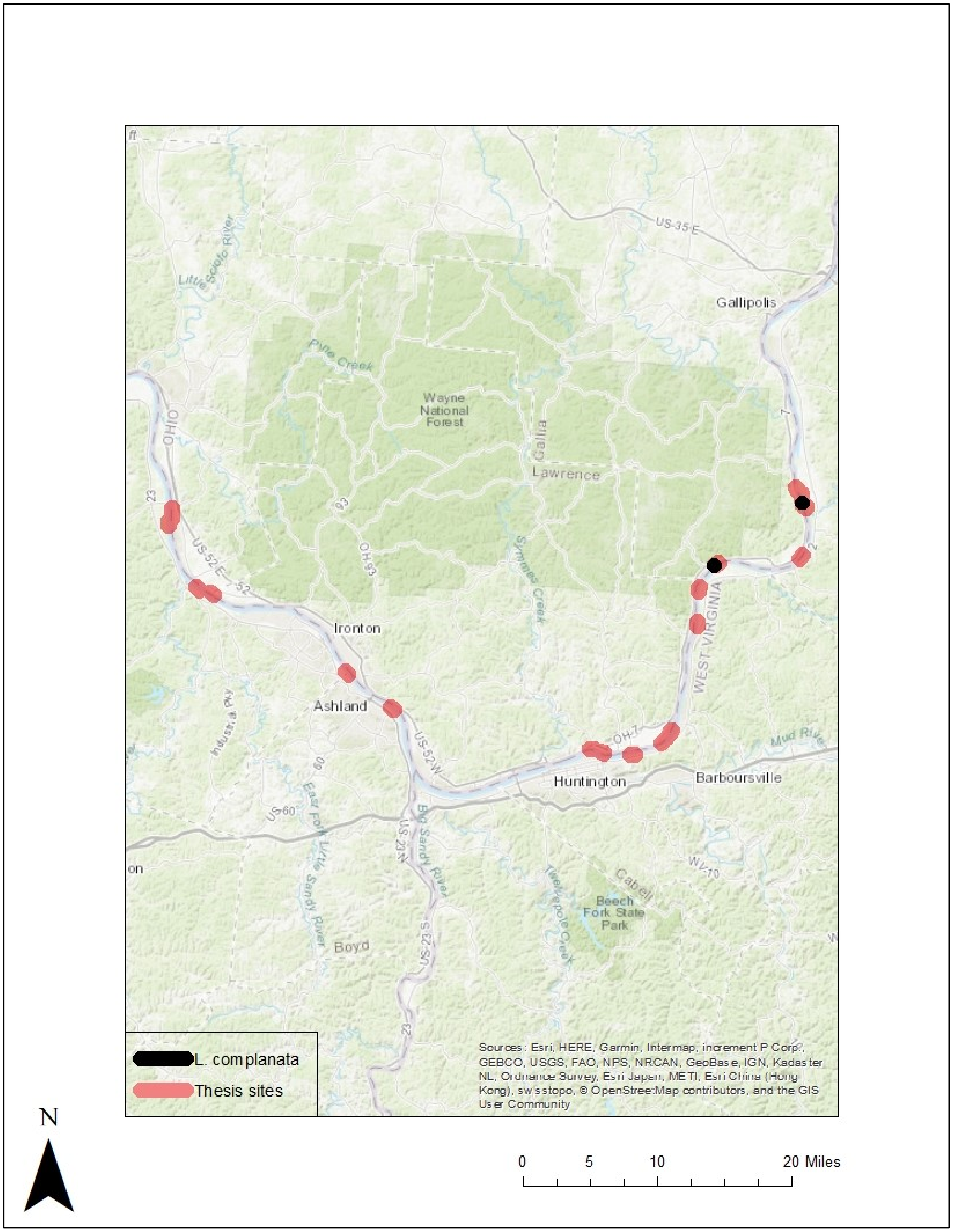

Figure 93. *Lasmigonia complanata* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Lampsilis siliquoidea – Fat Mucket

Figure 94. Female *Lampsilis siliquoidea*, Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Lampsilis siliquoidea was rarely encountered in the upper Greenup pool (Figure 95). I collected two live individuals and four deadshell. Site density was 0.1 individuals/600m^2 with a transect density of 0.02 animals/100m^2. *L. siliquoidea* is a common mussel that prefers slow current in fines or mud. It is found in lakes and streams of all sizes throughout the Ohio, Missouri, and Mississippi drainages (Watters et al. 2009; Cummings and Mayer 1992). Its rarity in the Greenup pool mainstem may be due to heavy current. *Lampsilis siliquoidea* is a host generalist (Table 14) (Watters et al. 2009; Freshwater Mussel Host Database 2017).

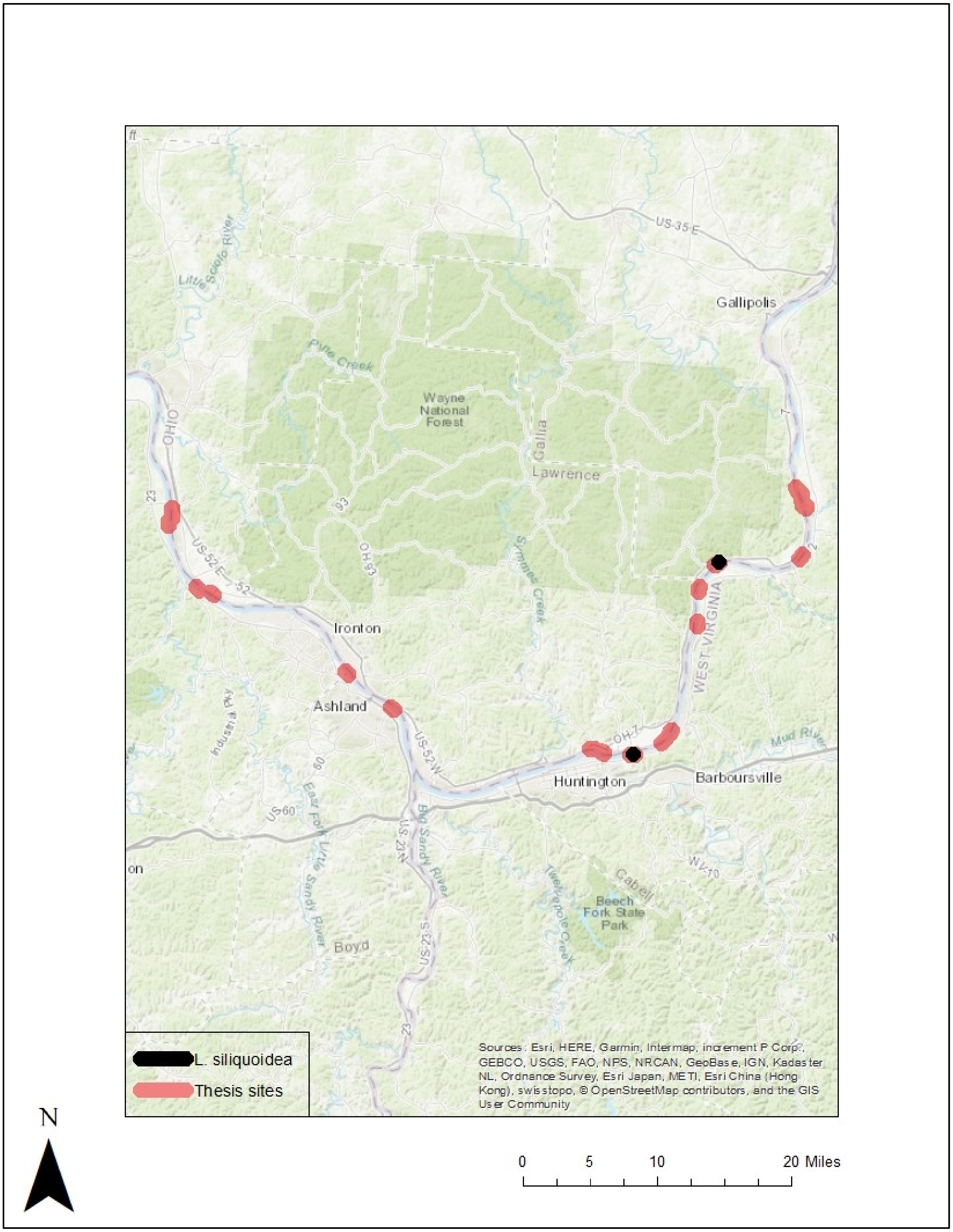

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Figure 95. *Lampsilis siliquoidea* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

***Pyganodon grandis* – Giant Floater**

Figure 96. *Pyganodon grandis*, Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Pyganodon grandis was only represented by one live individual in the middle Greenup pool (Figure 97). No deadshell was collected. Site density was 0.05 individuals/600m^2 with a transect density of 0.008 animals/100m^2. *Pyganodon grandis* is a common mussel found in lakes and streams of all sizes throughout the Ohio, Missouri, and Mississippi drainages. *P. grandis* prefers slow current habitat in fines or mud (Watters et al. 2009). Its rarity in the Greenup pool mainstem may be due to heavy current. *Pyganodon grandis* is a host generalist (Table 17) (Watters et al. 2009; Freshwater Mussel Host Database 2017).

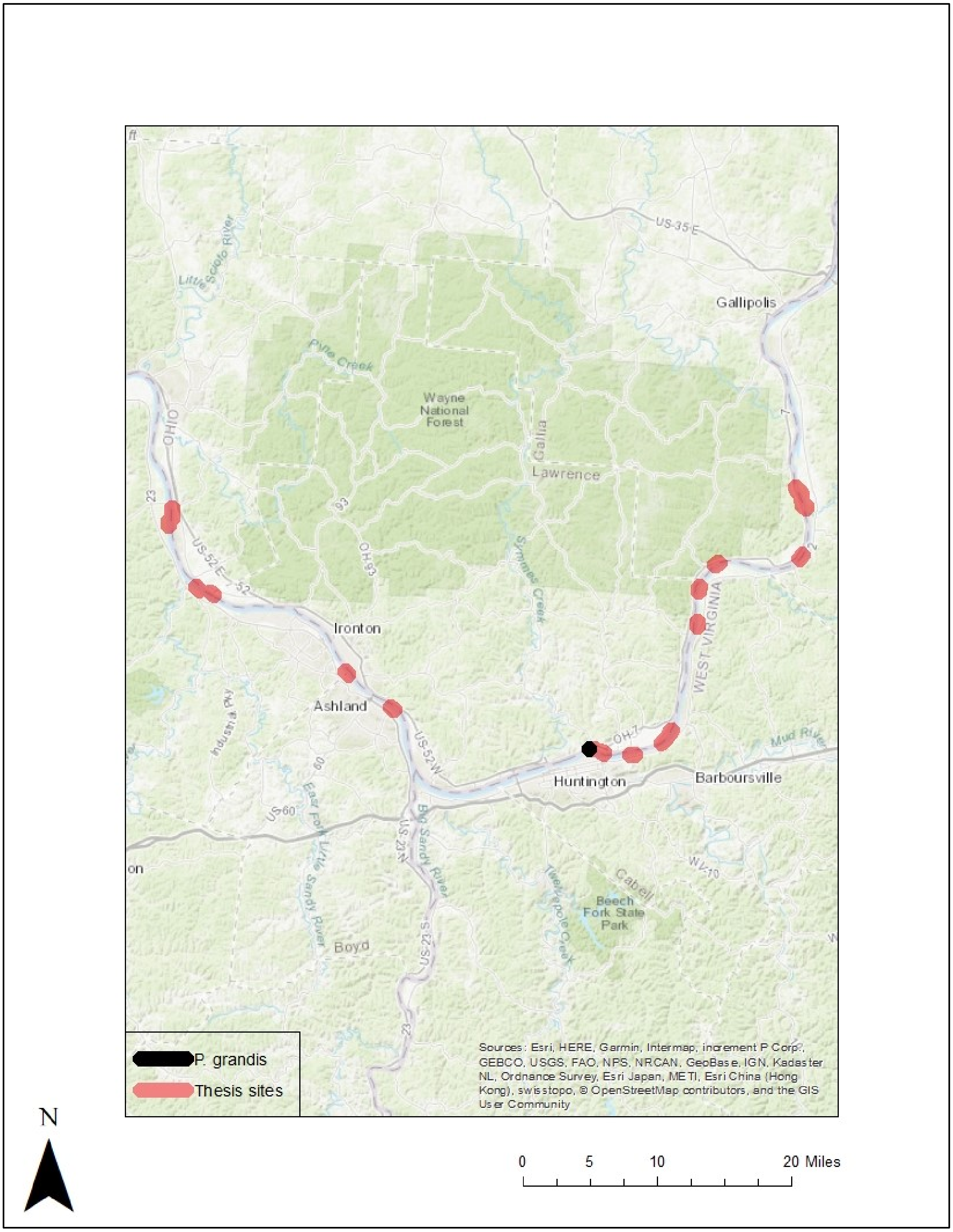

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Figure 97. *Pyganodon grandis* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

Lampsilis teres – Yellow Sandshell

Figure 98. *Lampsilis teres,* Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Lampsilis teres was only represented by one live individual in the upper Greenup pool near RC Byrd Lock and Dam (Figure 99). All deadshell specimens were also collected within the lock and dam vicinity. Site density was 0.05 individuals/600m^2 with a transect density of 0.008 animals/100m^2. *L. teres* is a southern species that prefers slack water in sand, mud or silt (Watters et al. 2009). It inhabits backwaters and the bank edges of large rivers (Personal observation). The Greenup Pool may lie on the northern edge of its range. Gar are often cited as the primary hosts, specifically *Lepisosteus platostomus* (Shortnose Gar) (Table 14) (Watters et al. 2009; Freshwater Mussel Host Database 2017; Haag 2012). In the Greenup pool, *L. osseus* (Longnose Gar) is the only present host species.

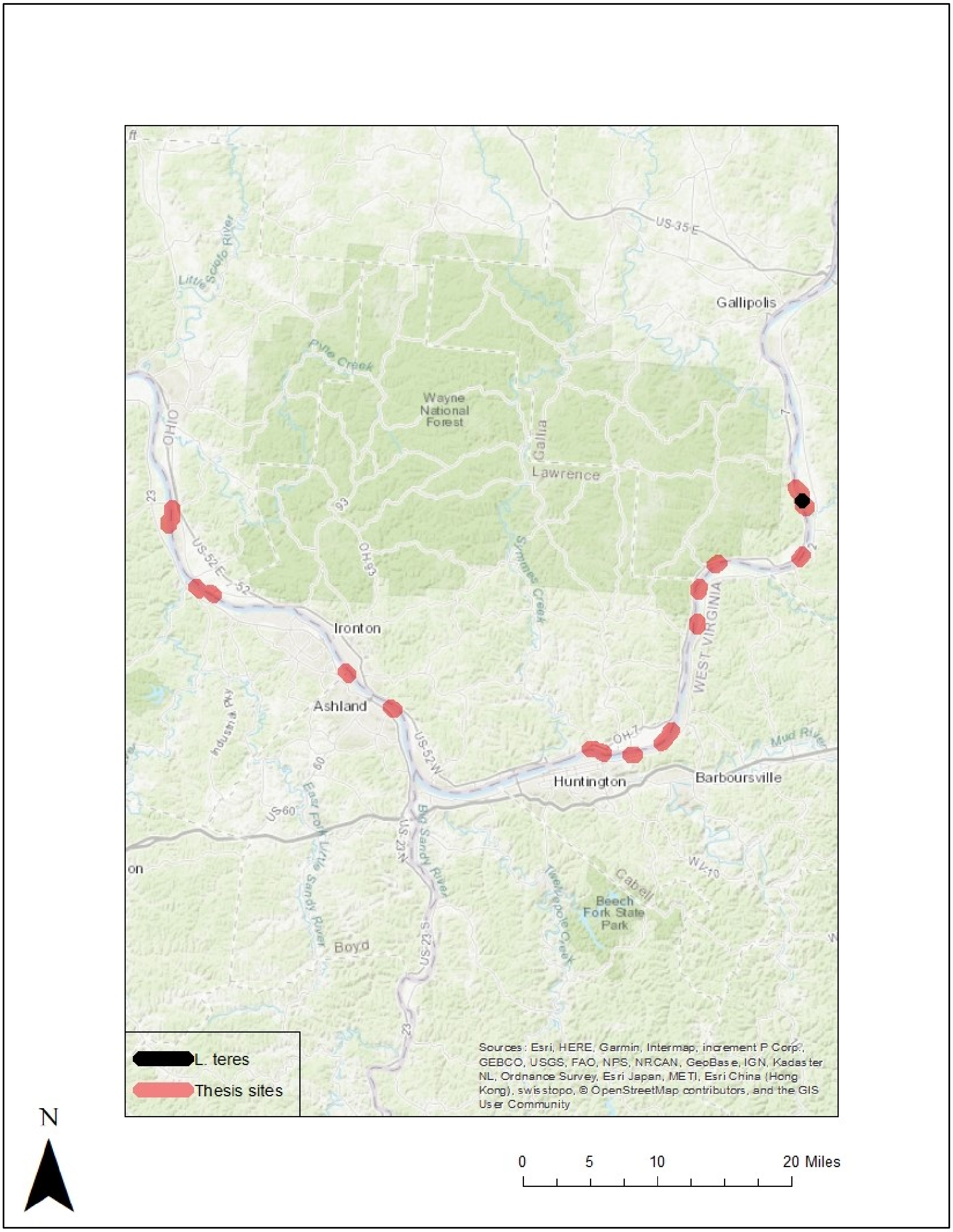

Figure 99. *Lampsilis teres* live collection sites in the Greenup pool, Ohio River (Source: Personal collection).

***Tritogonia verrucosa* – Pistolgrip**

Figure 100. *Tritogonia verrucosa*, Greenup pool, Ohio River (Photo credit: Mitchell Kriege).

Tritogonia verrucosa was only represented by one live individual in the upper Greenup pool (Figure 101). No deadshell was collected. Site density was 0.05 individuals/600m^2 with a transect density of 0.008 animals/100m^2. *T. verrucosa* is a hard shelled mussel species that prefers sand and gravel substrate (Watters et al. 2009). Its rarity in the Greenup pool mainstem may be due to a habitat preference of medium sized streams. The Pistolgrip is a catfish specialist (Table 13) (Watters et al. 2009; Freshwater Mussel Host Database 2017).

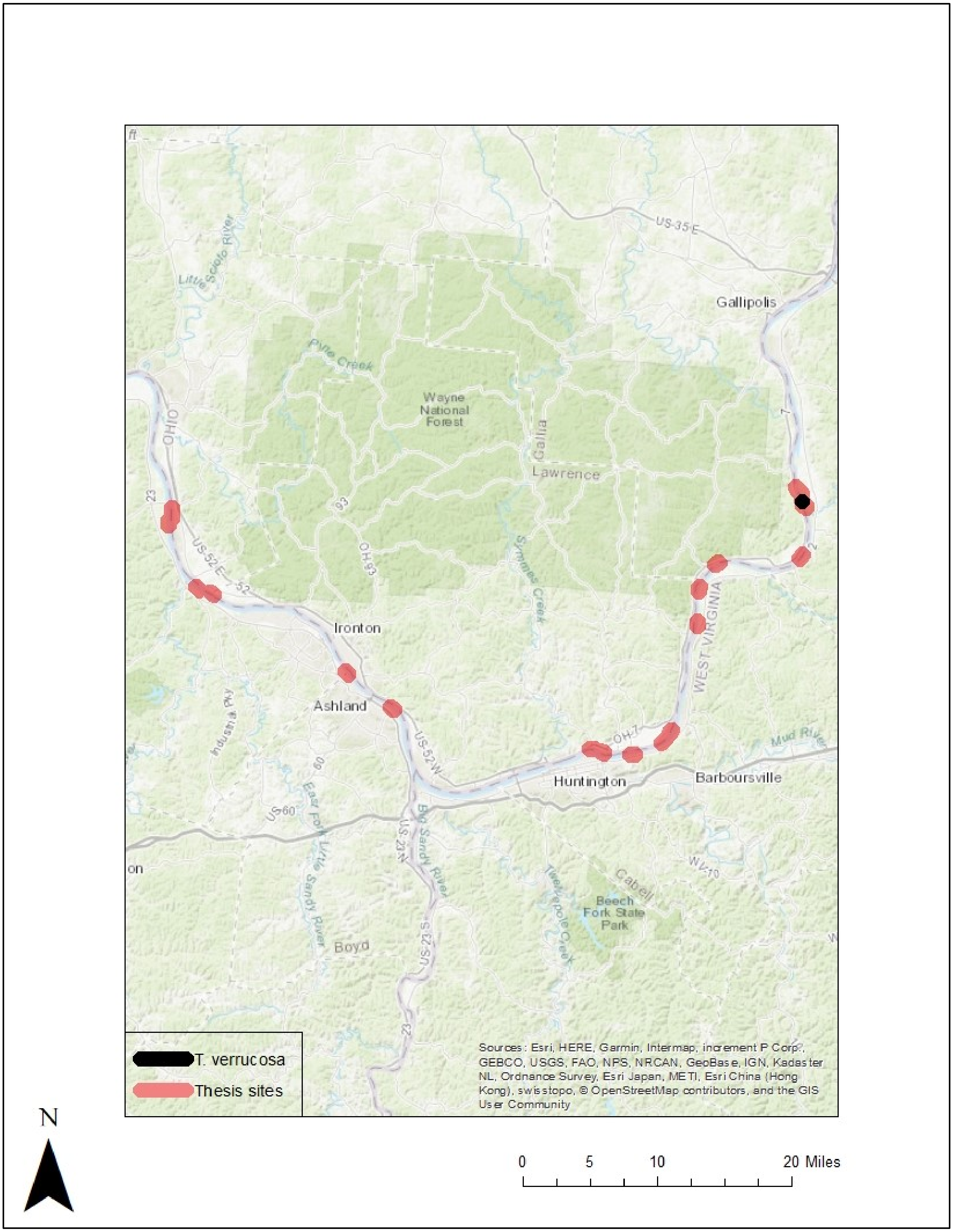

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Figure 101. *Tritogonia verrucosa* live collection sites in the Greenup pool, Ohio River.