ASSESSMENT OF FRESHWATER MUSSEL COMMUNITIES IN THE BASS CREEK WATERSHED, ROCK COUNTY, WISCONSIN

by

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DEDICATION

I would like to dedicate this professional project first to my husband, Matthew B. Becker, for always following me into adventurous unknowns. I appreciate your willingness to haul our kayaks back and forth, aid in Bass Creek exploration and mussel survey note-taking, and travel to Montana for courses and spring graduation. Second, to our daughters, Lily and Laura, who have been along every step through this Master of Science Program at Montana State University. You have also followed me into the adventurous unknowns and stayed with me on campus during field projects. You both have understood my love for learning and education is something that can never be taken away; believe in anything yoU.S.et your mind to. To you both, I also dedicate this project. Lastly, to my parents, Tim and Nancy, and sister, Sandy, you have motivated me to keep going, even when the going gets tough. To you, I also dedicate this final Master of Science Project.

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GLOSSARY

Channelization- straightening of stream channel to improve water flow.

Glochidia- parasitic larval stage, attaching hooks/suckers to host fish (Cummings & Graf, 2010).

Indian mounds- mounds created from ancient Native American mound-building societies to bury their dead, various in different shapes and groupings. Most are round or “conical,” and others are more linear in shape. Wisconsin has the highest concentration of mounds in the United States; all are protected as burial mounds. (Wisconsin Department of Natural Resources)

Middens- piles of dead shells (WI Mussel Monitoring Program, 2021).

Nacre- lustrous layer, also known as the mother of pearl layer; visible on the interior part of the shell. Continuously secreted and thickens shell with age. (Haag, 2012).

The prismatic layer- overlays the nacre, composed of sheets of calcium carbonate and a protein called conchiolin (Haag, 2012).

Periostracum- a thin protein layer covering the outer shell associated with a growing shell (Haag, 2012).

Relic- a mussel shell that is dead from apparent natural causes and does not contain meat or soft parts. Shells exhibit evidence of soiling, weathering, sediment, algal or mineral stains, vegetation, or other visual evidence on the interior. Shell has not been cooked or freshly cleaned (Illinois, 2020).

ABSTRACT

Within North America, freshwater mussels have endured large population declines and threatened to extirpation and extinction. Many factors contribute to the causes of imperilment, from overharvest to poor water quality and altered hydrological conditions. Mollusks are of ecological importance; they provide many aquatic functions and are indicators of stream health. Tools, like the Mussel Classification Assessment Tool (MCAT), have been created to guide mussel conservation in the Upper Midwest. Bass Creek in Rock County, Wisconsin, serves as my case study to: 1) access the viability of freshwater mussel community presence in Bass Creek, which currently has no mussels documented; 2) evaluate the abundance and community diversity of mussels in Bass Creek, where there is a lack of data; 3) apply MCAT to compare mussel assemblage health; and 4) to determine if other extrinsic parameters contribute to spatial differences in mussel communities. I performed mussel surveys in Bass Creek in September 2021. I also measured water quality parameters: water temperature, conductivity, pH, velocity, and dissolved oxygen. I found the three-ridged mussel (*Amblema plicata*) was the most dominant species, plain pocketbook was the most dominant live species and found farthest from Rock River confluence, and I found the highest mussel abundance closest to the confluence with the Rock River. I used MCAT protocols on Bass Creek; my replication indicated low recruitment, low species abundance, and low species evenness on a smaller spatial scale. My analysis from surveys and water quality point toward extrinsic factors, hydrological alterations, and habitat degradation as the primary driving factors of low mussel abundance. Although hydrological connectivity has been enhanced by removing Afton Dam, conservation implementation is still needed. My MCAT results illustrate mussel populations in Bass Creek are unstable but serve as a baseline for future studies.

INTRODUCTION

Ecological Importance of Mussels in Riparian Ecosystems

Freshwater mussels (Bivalvia: Unionidae) are a diverse group of fauna with more than 800 species that, except for Antarctica, have a worldwide range (Gomes-dos-Santos et al., 2019; Haag, 2012; Lopes-Lima et al., 2018; Sousa et al., 2021). Although freshwater mussels are distributed globally, mussel assemblages in the United States can be concentrated within community hotspots along interior river basins (Graf & Cummings, 2007; Haag, 2010; Haag, 2012; Lopes-Lima et al., 2018); especially within the Mississippi River Basin (Haag, 2012). Wisconsin has identified 50 species of native freshwater mussels (Gaumnitz, 2019).

Historically, freshwater mollusks have played a key role in maintaining the ecological integrity of aquatic systems (Gaumnitz, 2019). These taxa provide essential aquatic functions such as contributing to nutrient cycling, providing structural habitat and refugia for fish and aquatic insects to lay eggs, modifying substrate through burrowing and bioturbation, altering food webs, and providing water purification (Gomes-dos-Santos et al., 2019; Vaughn, 2018; Vaughn et al., 2008; and Woolnough et al., 2020). As mussels filter feed on particles within the water column, they serve as a sink of nitrogen and phosphorus within soft tissues and shells as they grow. A source of these nutrients from their reproductive parts or via excretion from protein breakdown under stress, long-term shell dissolution, or biodeposits (Gomes-dos-Santos et al., 2019; Spooner, 2013; Vaughn, 2018; Woolnough et al., 2020). Even after death, nutrients may be retained longer-term as shells are recalcitrant and take time to decompose (Atkinson & Vaughn, 2015). Likewise, mussels stimulate primary production. As these faunas burrow into stream sediments, nutrients are released into the water column, and their excretions support benthic macroinvertebrate populations (Spooner et al., 2013; Vaughn and Hakenkamp, 2001; Vaughn, 2018). Thus, mussels provide functions of ecological importance in riparian ecosystems, from increased primary production across trophic levels to improved water quality (Dunn, Zigler, and Newton, 2020); mussels are analogous to kidneys within freshwater systems (Gaumnitz, 2019).

Background and History of Mussels

Freshwater Mussel Life History and Traits

Life History: Native freshwater mussels are benthic macroinvertebrates found in various freshwater ecosystems (Ferreira-Rodriguez et al., 2019; Sethi et al., 2004; Sousa et al., 2021). Mussels have a unique life cycle in which recruitment and survival are based on a parasitic relationship between larva and fish hosts (Haag, 2012; Sousa et al., 2021). Mussel species consist of both males and females, with water temperatures as the primary cue for spawning (Haag, 2012). During spawning, males release sperm into the water, and through filter-feeding, females take in sperm, which fertilizes the eggs (Gaumnitz, 2019; Haag, 2012). The females hold the fertilized eggs until they become glochidia (Gaumnitz, 2019; Haag, 2012), which is the parasitic larval stage (Cummings & Graf, 2010) and are expelled by females when fish hosts are close (Gaumnitz, 2019). Mussels have adaptations to attract certain host species, e.g., stimulation of sensory hairs, and the glochidia attach to fish tissue, then undergo anatomical metamorphosis if the host is suitable (Haag, 2012). For example, *Lampsilis* cardium (plain pocketbook) mimics a minnow to draw fish near and has a package of glochidia that mimics worms to attract a potential host (Gaumnitz, 2019). During this parasitic relationship, glochidia receive nutrients from fish tissues. After metamorphism is complete, juveniles become detached, fall to the streambed (or other aquatic bottoms), and resume a free-living sedentary benthic lifestyle (Haag, 2012). Some species live to 100 years of age, such as the endangered spectaclecase mussel found in the St. Croix River in Wisconsin (Davis, 2021; Sousa et al., 2021).

Shells: Mussel shell morphology is variable and can indicate age and growth, like tree rings. There are three primary layers: periostracum, prismatic layer, and nacre; the nacre is the mother-of-pearl inside the shell; it consists of calcium bicarbonate and conchiolin protein (Haag, 2012). Pearl formation starts when foreign objects, such as trematode parasites, get trapped between the mantle and shell (Haag, 2012). The outermost layer, the periostracum, protects the shell from dissolution. Mussel shell population differences are likely from differences in water chemistry, e.g., aquatic environments with low concentrations of calcium bicarbonate have thinner and smaller shells (Haag, 2012). Bivalves have adaptations to serve as an anchor to maintain position in sediments, such as a solid posterior ridge to decrease exposure to currents or tubercles (bumps) on the medial axis, disk sculptures, or heavy shells with the anterior center to stabilize in currents and sediments (Haag, 2012).

Impacts on Mussel Populations from Overharvest

Although these taxa play a critical role in aquatic ecosystems, they also were an important fishery in Midwest U.S. rivers. As early as the 1890s, mussels were harvested for button production, and shell factories spanned from Nebraska to Ohio (Temte, 1968). By 1899, there were more than 60 factories in the Midwest where 16 million pounds of shells were harvested (Temte, 1968). In 1908, a significant mussel decline was noticed, as mussel beds that covered streambeds and banks were decreased to a handful of mussels found every few miles

(Pritchard, 2001; Temte, 1968). The U.S. Bureau of Fisheries (now part of the U.S. Fish and Wildlife Service) created an initial mussel propagation program at the Fairport Biological Station in Iowa between 1908-1914; more successful propagations occurred in the early 1920s and into the 1930s (Pritchard, 2001; Temte, 1968). In 1916, a year after the Drinking Water Standards were adopted, the federal government started regulations and limitations on clamming (Temte, 1968). After World War II, the plastic button industry replaced shell buttons, and harvest decreased (Temte, 1968). By 1997, only 14 states allowed commercial harvest with permits required, and within Wisconsin, 2006 marked the close of all commercial clamming (Wisconsin Department of Natural Resources [WDNR], 2022). Current Wisconsin law states it is illegal to harvest live mussels from the waters of the State. Only dead shell collection is permissible unless species are threatened or endangered. One exception to this rule is the St. Croix National Scenic Riverway, where even dead shell collection is prohibited (WDNR, 2022).

Impacts on Mussel Populations from Water Pollution

Freshwater mussels are an indicator species for stream health because of their sensitivity to toxins, suspended and fine sediment, and changes in hydrological conditions (Atkins et al., 2014; Dunn, Zigler, and Newton, 2020). Mussel compositions have shifted since pre-European settlement, with freshwater systems now dominated by generalist species more tolerant of degraded water quality and greater fluxes in hydrological conditions (Haag, 2012). The domination of generalists leads to less dense and species-rich mussel assemblages in the Midwestern U.S. three-ridged mussel (*Amblema plicata*) is an example of a dominant riverine generalist species. It can be found in a wide range of habitats with low dissolved oxygen levels and lentic environments where pools of water are found behind gravel or sand bars or stream margins (Haag, 2012). Habitat degradation is correlated with decreases in North American freshwater mussel biodiversity; by the 1990s, 25 mussel species became extinct (Haag, 2012). There are 50 recorded mussel species in Wisconsin, but 11 are endangered, seven are threatened, seven more are considered a special concern, and two have been extirpated (Piette, 2005).

Documenting Populations as a Tool for Conservation

As several threats to freshwater mussel communities have been identified and studied, it is critical to assess current native freshwater mussel species and population distributions to guide conservation planning. Since 2009, the WDNR has cataloged native mussel presence statewide, with aid from citizen scientists, through the *Wisconsin Mussel Monitoring Program* (Gaumnitz, 2019). However, several rivers and streams within Wisconsin have not been surveyed for mussels (Piette, 2005). Tools have been created to assess environmental conditions to prioritize conservation actions, such as the Mussel Community Assessment Tool [MCAT] (Dunn et al., 2020). The MCAT provides a quantitative and consistent approach to evaluating mussel assemblage health by utilizing ten metrics and scoring criteria (Dunn et al., 2020). There are five primary topics: conservation status and environmental sensitivity, taxonomic composition, population processes, abundance, and diversity (Dunn et al., 2020). Within the five main topics, 10 metrics successfully assess the overall health of mussel assemblages: percentage of listed species, percentage of tolerant species, percentage of Lampsilini, percentage of fresh-dead, percentage ≤ 5 years old, percentage ≥ 15 years old, Q75 abundance, species evenness, tribe evenness, and expected number of species at a sample size of 100 mussels by rarefaction (ES_100) (Dunn et al., 2020).

Overharvest and water pollution from habitat alterations within the Midwestern U.S. have led to mussel assemblage declines and the need for conservation (Haag, 2012). Anthropogenic usages and changes have caused issues in water quality and habitat connectivity, which are problematic for sedentary and typically long-lived freshwater mussel inhabitants. Based on site history and land use, I hypothesize that mussel assemblages within Bass Creek will be characterized by low abundance, low species richness, and low mussel assemblage diversity, coinciding with low macroinvertebrate biotic indexes. My research will provide baseline data, fill in data gaps for the Rock River Basin, and contribute to the *Wisconsin Mussel Monitoring Program.* It will aid in a snapshot of additional factors that may contribute to the spatial distribution of mussel communities within this watershed. My data will be based on a short temporal scale, serve as a baseline for future monitoring, and continue to guide local conservation planning efforts.

CASE STUDY

Bass Creek will serve as my case study to assess how community assemblages of native freshwater mussels vary spatially on a localized scale within a watershed dominated by agriculture. I selected this watershed and creek because of its historical background, site history, and lack of mussel documentation. Several conservation efforts have been performed to increase water quality and hydrological connectivity. Adjacent to Bass Creek, agricultural land has been placed in permanent wetland conservation easements, and streambank stabilization projects were completed to decrease sediment loads (NRCS records).

Site Description

In its entirety, the Bass Creek watershed is within Rock County in south-central Wisconsin. It is a small watershed that is approximately 280 km^2 (109 mi^2) and nested within the Lower Rock Basin and the Mississippi River Basin (Figure 1). The land use is dominated by agriculture, and the physiography is considered the Southeast Glacial Plains, which consist primarily of glacial deposition from the Wisconsin Ice Age (Rock County Land Conservation Department [RCLCD], 2019).

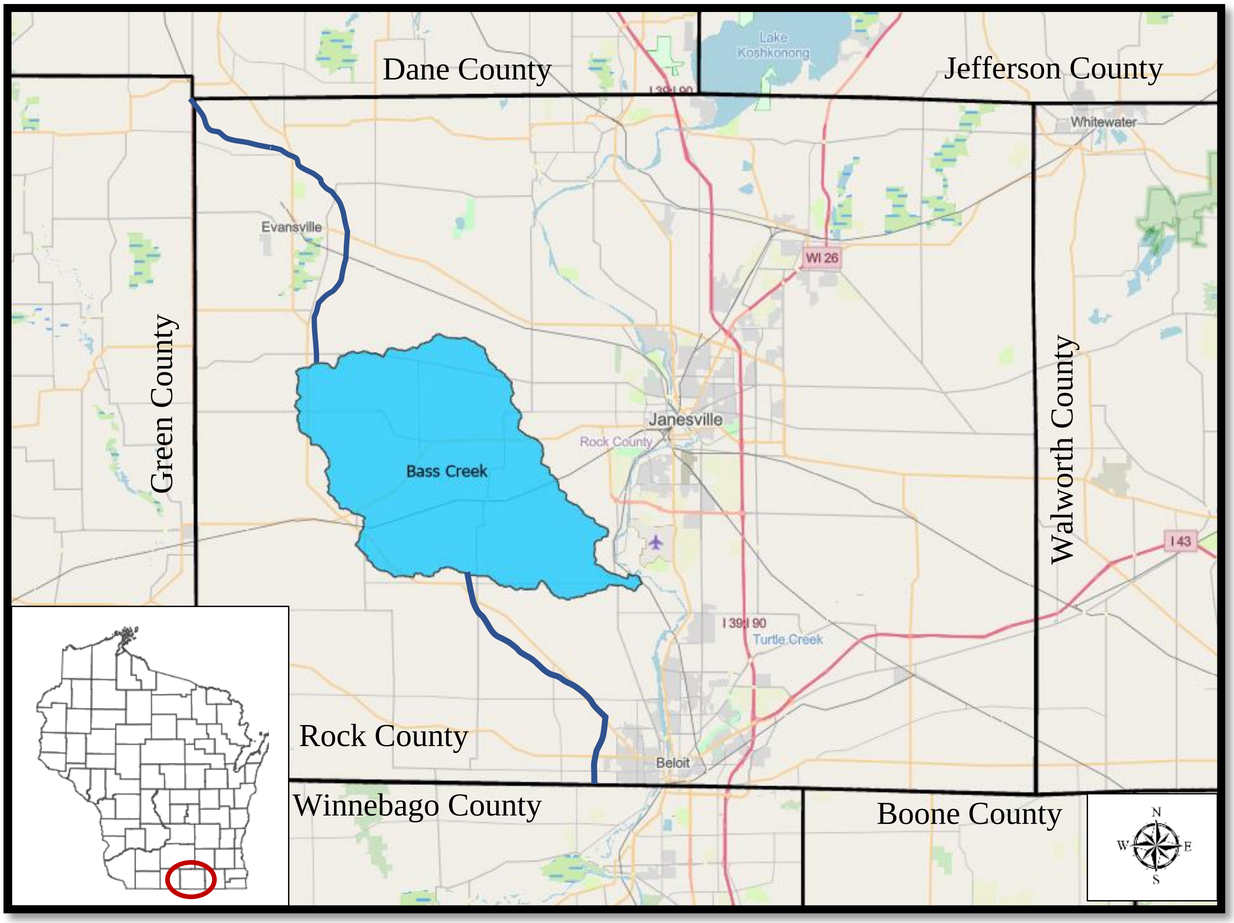

Figure 1. Location, Bass Creek Watershed- HUC 10 0709000212. Bass Creek is part of the Rock River watershed and drains to the East and South and on the west side of Bass Creek is the Sugar River watershed. The dark blue line indicates larger basin divides. The map was created in ArcPro and is not to scale.

Afton Dam and Archeology

A dam on Bass Creek, constructed before 1878, served as the power to a grist mill (RCLCD records). Over the dam’s 124-year life span, it was repaired several times. The priority for removal was for fish passage; part of this funding was provided by the Natural Resources Conservation Service (NRCS) through the Wildlife Habitat Incentives Program (WHIP). Afton Dam was approximately 1205 m (0.75 mi) upstream of the confluence with the Rock River.

The Wisconsin Historical Society was contacted to write a Cultural Resources report, to remove the dam. Two known Indian mound groups were found within the vicinity of the dam removal project on Bass Creek. One is known as the Afton Mill Group, located approximately 92 m (300 ft) east of the mill and about 15 m (50 ft) north of Bass Creek. The Afton Mill Group is a campsite, a group of four conical mounds and one tapering linear mound (Wisconsin Archeologist [WA], 1929). The Afton Mill Group indicates early anthropocentric use of Bass Creek, as mussels were an important food source for Native Americans with mussel shell pile discards, known as middens, found in Fulton, Township within Rock County (WA, 1929). Anthropocentric mussel exploitation may have caused the decline and recovery of native mussel species before the button industry (Appendix C).

Agriculture, Land Uses, and Water Quality

As it became settled, the southern part of Wisconsin had land converted from prairie and forests (Daniells, 1879). Poor farming practices have led to increases in soil movement and increased nutrients into waterways, cropping up to streambanks and contributing to poor stream quality for mussels (Poole & Downing, 2004; Haag, 2012). Similarly, Wisconsin drainage districts were formed and created channelized streams, decreasing sinuosity, increasing depths, and altering river habitat. The upper limits of Bass Creek consist of the Bass Creek Drainage District (DATCP, 2018; Rock County, 2022). Land use within the Bass Creek watershed is primarily agriculture. Bass Creek has exceeded limits for total phosphorus in 2014 and total suspended sediments in 2018 and is listed as impaired (RCLCD, 2020). Conservation efforts have been implemented to combat habitat degradation and poor water quality within the last two decades. Hundreds of acres have been placed into federal government programs, such as the

Environmental Qualities Incentives Program (EQIP) and Wetland Reserve Easements (WRE) (NRCS records). Although mitigations have been in place, more efforts may need to be implemented to continue water quality improvements, e.g., increased vegetation buffers and streambank stabilization projects, to decrease nutrient and sediment loads into Bass Creek. In general, mussel distributions are associated with the benthic habitats they inhabit, with agricultural land use connected to the quality of their homes. Current and past land should be considered primary environmental stressors on mussel distributions, species richness, and abundance.

Mussel Community Assessment Tool

Various conservation tools have been proposed to guide mussel conservation, such as the MCAT. My research hypothesis and data will aid in the following questions: Can MCAT be replicated on a smaller scale within the Mississippi River basin to direct conservation initiatives? Can fine-scale watershed patterns be extrapolated to large watershed scales to promote and guide conservation planning efforts? What other extrinsic factors, such as stream morphology and water quality, could influence mussel distributions?

My questions, posed above, will be addressed by four objectives: assessing the viability of freshwater mussel community presence in Bass Creek, which currently has no mussels documented; evaluating the abundance and diversity of mussel assemblages in Bass Creek, where there is a lack of data; applying MCAT on Bass Creek, to compare mussel assemblage health; and examining if other extrinsic parameters, such as stream morphology and water quality, contribute to spatial differences in mussel communities.

Methods and Materials

Biological Data Collection

Mussel Documentation: I performed website searches electronically using *iNaturalist* and the *Wisconsin Mussel Monitoring* pages to determine if mussels were present in Bass Creek. Through my investigations, in September 2021, no mussels have been documented for the Bass Creek watershed; however, my searches indicated mussel species had been recorded by other citizen scientists throughout Rock County, Wisconsin (Weinzinger, 2018). I also searched the *Wisconsin Natural Heritage Inventory Data* website for Rock County. Several mussel species were listed; however, this did not identify down to a sub-watershed scale for Bass Creek (WDNR, 2020).

Mussel Surveys: I conducted initial rapid qualitative surveys in September 2021, following survey methods for freshwater mussels in wadable streams within Wisconsin (Piette, 2005). My qualitative surveys allowed me to prioritize four survey locations on Bass Creek within a 13 km (8 mi) reach; the four sites were downstream of South Johnson Road to about 38 m (125 ft) south of old Afton Dam (Figure 2). I set up a transect tape at each of the four wadable sites, where I performed surveys approximately 30 m (98 ft) up and downstream of each transect, and I performed searches for two hours. When I found dead shells, they were included in counts. If the dead shells I found were intact and connected, I would count two individual shells. Following Piette’s methods, I inserted my hands 15 cm (6 in) into the sediment, fanned them to expose shells, and rubbed my hands against banks covered in water (Piette, 2005). I used a small bathyscope, after fanning, to observe specimens.

Figure 2. Survey Locations. I performed four transect surveys on Bass Creek, approximate locations indicated by yellow pins. I set up transects between South Johnson Road and upstream from the confluence with the Rock River. The image is not to scale. (Google Earth, 2022).

<u>Mussel Survey Reporting:</u> I took photographs of each freshwater mussel specimen and uploaded them to the *Wisconsin Mussel Monitoring* page on *iNaturalist* (Appendix B). I initially identified each shell with mussel identification guides (Graft & Cummings, 2022), and I confirmed through *iNaturalist*. My photograph identifications were reviewed by other citizen scientists and Wisconsin Department of Natural Resources mussel biologist Jesse Weinzinger for agreement of all mussel identifications, down to the species level (Appendix B). In Wisconsin, it is mandatory to provide summaries of findings for each survey project to the Wisconsin Department of Natural Resources. Data reporting forms are provided in the *Wisconsin Mussel Monitoring Program Training Manual* (2018) and include personnel, the reason for the survey, site description,

methods, datasheets, maps of sites, and a summary of results. However, more recently, *iNaturalist* has been used to submit observations (Weinzinger, 2018).

Extrinsic Factors

Habitat and Stream Morphology: I stretched a 50 m transect tape across Bass Creek and measured water depth with a meter stick at one-foot intervals along the transect tape and included the bank left and bank right heights. I took photographs at each transect to document vegetation for general habitat descriptions through Guidelines for Evaluating Fish Habitat in Wisconsin Streams by Simonson and colleagues 1993 (Piette, 2005; Appendix C). Additionally, I recorded riffles and runs, instream vegetation, main riparian land use, and primary substrate. I determined the primary substrate as the substance covering the largest area of each transect area, e.g., cobble, gravel, sand, or silt, as indicated on the Wisconsin Mussel Sampling Data Sheets, then uploaded them into the *Wisconsin Mussel Monitoring* page on *iNaturalist*.

Water Characteristics: I collected additional abiotic parameters, including air temperature, water temperature, conductivity, pH, velocity, and dissolved oxygen. The greatest depth was 56 cm (22 in), with clarity to the stream bottom; therefore, I did not measure turbidity at any survey sites. I used a *Hanna* HI98129 to measure water pH, electric conductivity, total dissolved solids, and *Sper Scientific* Dissolved Oxygen Pen to measure dissolved oxygen and water temperatures. I used a UNI-T TT363 handheld anemometer to gather wind speed and air temperature. I measured stream velocity using the float method. I dropped a tennis ball upstream 30 m (98 ft) and timed the movement across the transect tape (Water Action Volunteers, 2015). I conducted the float method four times at different areas along the transect and then calculated the mean velocity for each site. The parameters I collected represent grab samples during times of mussel surveys, serving as a baseline and a short-temporal scale of extrinsic factors that may play into mussel community preferences (or avoidances). I compared samples to WDNR grab samples taken in 2013, 2016, and 2017.

Mussel Classification Assessment Tool

From the mussel survey data I collected, I analyzed 10 metrics based on the MCAT, and I compared parameters for each transect (Dunn et al., 2020; Table 3). The percentage of mussel species listed endangered or threatened federally and by bordering States was selected by Dunn et al. (2020) for environmental sensitivity. I electronically searched bordering State websites to find native freshwater mussel species listed as threatened and endangered. By adding up the total number of threatened and endangered mussel species I found in Bass Creek, dividing by the total number of specimens, and multiplying by 100, I obtained a percentage (Table 3). A tolerant species, classified by Dunn et al. (2020), is *A. plicata* (three-ridged) and was selected to measure disturbed assemblages. I calculated the percentage of tolerant species by adding the number of *A. plicata* I documented in Bass Creek, dividing by the total number of specimens, and multiplying by 100 (Table 3). The percentage tribe of Lampsilini was used to compare the dominance (or lack of) of the number of individual mussel species. By adding the total number of Lampsilini tribe specimens I found in Bass Creek, dividing by the total number of individuals, and multiplying by 100, I obtained a percentage of tribe Lampsilini in Bass Creek samples (Table 3). Dunn et al. (2020) used the percentage of fresh-dead mussels to compare recent mortality for population processes; however, I found no fresh-dead shells during surveys (Table 3). As part of the population processes, Dunn et al. (2020) used the percentage of mussel species ≤ 5 years and ≥ 15 years to represent recruitment and old ages of assemblages. I calculated the percentage of species ≤ 5 years of age by adding specimens ≤ 5 years old, dividing by the total number of specimens, and multiplying by 100 (Dunn et al., 2020). I repeated the calculations for mussels ≥ 15 years of age by taking the sum of mussel shells ≥ 15 years old, dividing by the total number of specimens, and multiplying by 100 to obtain a percentage. (Table 3). I calculated the abundance at the 75th percentile using Excel (Q75, 3rd quartile) (Table 3). This metric provides more information about the spread of abundance than just the mean or median (Dunn et al., 2020). Lastly, I calculated three diversity metrics: Pielou's evenness at the species level and tribe level and rarefaction richness at 100 individuals. I used the following equations to calculate the MCAT diversity metrics:

Shannon-Weiner Index	$H' = -\sum_{i=1}^{s} P_i Ln P_i$	Equation (1)
Pielou's Index	$J = (-\sum_{i=1}^{s} P_i Ln P_i)/LnS$	Equation (2)
rarefaction richness	$E(S_{100}) = \sum_{i=1}^{S} \left[1 - \left(\frac{\frac{N-Ni}{n}}{\frac{N}{n}}\right)\right]$	Equation (3)

I first calculated Shannon's Index (not the initial MCAT) for species and tribe levels, *H'* (equation 1). *H'* is the index score with the sum of species, S, with the proportion of individuals belonging to the *i*th species, P_i (Shannon, 1949). I also calculated Pielou's Index (equation 2), where *H'* is divided by H'_{max}, the maximum possible of *H'*, indicated by *J* in Pielou's equation, if every species/tribe was equally represented throughout Bass Creek (Feldman, 1970; Table 3). I used rarefaction richness to compare richness to an equal number of individuals (Dunn et al., 2020). For rarefaction richness (equation 3), S is the number of species found within data collection at random; N_i is the total number of individual species; N is the total number of specimens in the sample, the sum of N_i; n is the sample size (in this case 100); $(\frac{N}{n})$ is the number of combinations that could be selected from a set of N (Krebs, 2017; Simberloff, 1972). This metric is typically used to scale down and not scale up, and does not seem necessary for the small spatial scale for Bass Creek.

Results

Mussel Species

Transect one is the farthest away from the confluence of the Rock River and approximately 400 m (1315 ft) downstream of South Johnson Road. I found only one mussel species on a gravel bar, A. ferussacanius (cylindrical papershell). Transect two is approximately 255 m (835 ft) downstream of transect one. At transect two, I found only one live mussel *L. cardium* (plain pocketbook). I found this mussel in the middle of the creek, in sandy sediment. Transect three is approximately 400 m (1315 ft) downstream of transect two. I found only a broken mussel shell of *L. complanate* (white heelsplitter). I noticed the substrate consisted of smaller rock/gravels. Transect four is approximately 225 m (740 ft) downstream of transect three and approximately 46 m (150 ft) downstream of the old Afton Dam. Transect four is also only about 1205 m (3950 ft) upstream of the confluence with the Rock River. I found transect four had the most species (10) and the most live species. Through collecting data, the three-ridge mussel was the dominant species, followed by plain pocketbooks (Table 1). I documented and noticed the oldest living mussels at transect four and greater than 20 years of age (Table 1). After I completed initial scouting and transect surveys, I found four tribes with 10 species of mussels in Bass Creek (Table 1). I uncovered and documented 43 relic shells and seven live species for 50 specimens. In transect two, *L. cardium* (plain pocketbook) was the species I found alive the most and the farthest upstream (Table 1). I also found F. flava (*wabash pigtoe*) and *A. plicata* (three-ridge) alive, each with one specimen. I found transect four had the most specimens, eight of the 10 cataloged. Of all sites I surveyed, transects one and two had the lowest species richness, with only one out of 10 species cataloged (Table 1).

Table 1. Mussel Species. I used the *Wisconsin Mussel Monitoring iNaturalist* page and Graft & Cummings (2022) for tribe, species, and genus identification. Relic shells do not have meat or soft tissue, with wear and visual presence of sediment, mineral stains, vegetation, etc. Locations with an asterisk indicate initial surveys, which were untimed and only included visual observation and photographs taken and submitted to *iNaturalist*. My results display similar mussel species assemblages in small streams in the Mississippi region (Haag, 2012).

Tribe	Scientific Name	Common Name	Location	Number of Species Live	Number of Relics/Shells
Amblemini	*Amblema plicata*	Three-ridge	Upstream of Transect*, Transect 4	1	13
Amblemini	*Anodontodies ferussacanius*	Cylindrical Papershell	Transect 1	-	2
Pleurobemini	*Eurynia dilatate*	Spike	Upstream Transect 3*, Transect 4	-	4
Pleurobemini	*Fusconaia flava*	Wabash Pigtoe	Upstream Transect 4*, Transect 4	1	3
Lampsilini	*Lampsilis cardium*	Plain pocketbook	Upstream of Transects 3 & 4*, Transects 2 & 4	5	3
Lampsilini	*Lampsilis siliquoidea*	Fat Mucket	Upstream Transect 4*, Transect 4	-	5
Anodontini	*Lasmigona complanate*	White Heelsplitter	Upstream Transect 3*, Transects 3 & 4	-	5
Lampsilini	*Ligumia recta*	Black Sandshell	Transect 4	-	2
Lampsilini	*Ortmanniana ligamentina*	Mucket	Upstream Transect 3*, Transect 4	-	2
Anodontini	*Pyganodon grandis*	Giant Floater Mussel	Upstream Transect 4*	-	3
Lampsilini	Unknown	Unknown	Upstream Transect 4*	-	1
Totals				7	43

Habitat and Stream Morphology

I noticed woody vegetation increased from transect one to two (Appendix C). I found transect two had a deeper stream bottom with thalweg on the left bank, and I observed the stream bed was mostly sand with mucky organic materials on the banks (Appendix A). The left bank was eroded with several tree roots exposed (Appendix C). Water velocity was quicker when I compared it to transect one. I calculated velocity at 30 m/s (Table 2).

Bass Creek has increased sinuosity from transect two to three, and woody vegetation shaded most of the Creek (Appendix C). From my creek expedition, this stretch had downed trees in the creek and required portaging. Canopy cover shaded the transect area I selected. The stream bed consisted of smaller pea-sized gravels and silts, which I documented (Appendix C). I calculated velocity at 30 m/s (Table 2).

By kayaking downstream, I noticed from transect three to four that Bass Creek becomes less sinuous, has less tree cover, and residential properties are closer to the stream banks (Appendix C). Slightly upstream of County Highway D, I noticed exposed bedrock on the right bank. As I set up transect four, I observed residential properties adjacent to bank right, and the area was slightly shaded from surrounding vegetation. The stream substrate I documented was primarily gravel, although there was a mix of larger and smaller substrates like sand and cobble present; streambanks were not exposed. I calculated the velocity at 21 m/s (Table 2).

<u>Water Quality</u>

I measured the dissolved oxygen (DO), ranging from 1mg/L to just above 12 mg/L, with transect one recording the lowest and transect three and two as the highest. The pH I gathered is alkaline and ranges from 7.7 to 8.2. I found transects three and four had the most elevated pH, and transect one had the lowest. The conductivity ranged from 657 to 675 µs/cm and was lowest at transects two and four and highest at transect one. Total dissolved solids (TDS) I measured ranged from 323 to 339 ppm, with the lowest found at transect two and the highest found at transect four. My velocity calculations ranged from 30 to 52 m/s (Table 2).

Transect four is closest to the confluence with the Rock River and has been more connected for the longest time, as it is downstream of the old Afton Dam. I viewed aerial imagery, and upstream of transect one is highly channelized. Transect one is farthest from the confluence and has had less time for mussel community establishment since Afton Dam was removed in 2002. Although distance and connectivity are part of the picture, water quality parameters also provide a snapshot into mussel inhabitance. Transect one had less favorable water quality, e.g., lowest dissolved oxygen level (1mg/L), slower velocity (41 m/s), second-highest total dissolved solids (335 ppm), and highest conductivity (675 µs/cm) (Table 2). I compared my water quality parameters to WDNR data from 2013, 2016, and 2017. WDNR collected data for dissolved oxygen levels that ranged from 4-17.5 mg/L and pH that ranged from 7.4-7.9 at County D in Afton. My results from 2021 are comparable to previous WDNR findings. (Table 2).

Table 2. Water Quality Parameters. At each transect, I calculated and measured water quality parameters during the time of mussel surveys.

Water Quality Parameters	Transect 1	Transect 2	Transect 3	Transect 4
Air Temperature	29°C	35°C	17°C	18°C
Water Temperature	18°C	23°C	17°C	16°C
Dissolved Oxygen (mg/L)	1.0	11.8	12.3	9.9
pH	7.7	8.1	8.2	8.2
Conductivity µs/cm	675	657	661	657
Total Dissolved Solids (ppm)	335	323	331	339
Velocity (m/sec)	41	30	52	35

Mussel Classification Assessment Tool (MCAT)

When calibrated to a small watershed scale, I calculated MCAT variables to determine tool efficacy to guide conservation planning (Table 3). In bordering states, three species are threatened: *Anodontodies ferussacanius* (cylindrical papershell), *Ligumia recata* (black sandshell), and *Eurynia dilatate* (spike). Based on similar species from Dunn et al. (2020), 28 % of species within Bass Creek are tolerant, and 36% of species surveyed were within the Lampsilini tribe (Table 3). Six mussels were between five and fifteen years of age, one was five or under, and 43 were at least 15 years. Recruitment for mussels is low, within approximately 13 km (about 8 mi) of Bass Creek, indicated by the lack of juvenile mussels found. Most mussel specimens found were relics with *L. cardium* (plain pocketbook) as the dominant live species.

A mix of parameters explains both stable and unstable mussel assemblages for Bass Creek, as defined by Dunn et al. (2020). Only half of the metrics calculated to fall within what Dunn et al. (2020) call the richest mussel assemblages, those with high recruitment and diverse ages and species, e.g., < 38% tolerant species; 35-40% *Lampsilini*; < 3% fresh-dead shells; 2-6% ≥ 15 years old; and > 16 species in a sample of 100 (Table 3). The other half of calculated MCAT parameters provide evidence of unstable mussel assemblages, those at risk of ecological decline, based on criteria from Dunn et al. (2020), e.g., < 4% of mussel species federal listed or listed by bordering states; < 49% of mussels ≤ 5 years of age (low recruitment); <13 mussels/m^2 in the 75th quartile; species and tribe evenness >0.8 (Table 3). The Bass Creek data indicates mussel assemblages have low recruitment rates, low species evenness, and do not have robust population dynamics, based on other studies by Dunn et al. (2020) and Haag and Warren (2010).

Table 3. Mussel Classification Assessment Tool (MCAT) Metrics. Modified for Bass Creek from Dunn et al. (2020).

MCAT Metrics	Bass Creek
Conservation Status and Environmental Sensitivity	
Percent listed federally and bordering[a] states	2
Percent tolerant[b]	28
Percent tribe Lampsilini	36
Population Processes	
Percent fresh-dead[c]	0
Percent mussels ≤ 5 years	2
Percent mussels ≥ 15 years	86
Abundance	
Abundance at the 75th percentile	2
Diversity	
Pielou's evenness (*J'*) at the species level[d]	0.0268
Pielou's evenness (*J'*) at the tribe level[d]	0.043
Expected number of species at a sample size of 100 mussels via rarefaction (ES_100)[e]	20

a Listed as a federal or state threatened or endangered species
b Tolerant species in Bass Creek
c Percent fresh-dead= (number of fresh-dead shells/ [fresh-dead shells+ live individuals] x100. Shells were considered fresh-dead if they had both valves attached, a flexible hinge line, and shiny nacre and if they were likely left by animals that died in the past few months
d Standard-diversity indices calculated using Shannon diversity indices and Pielou's evenness equation.
e Rarefaction richness was calculated using the rarefaction equation.

DISCUSSION

Based on MCAT metrics, which I calculated from the 2021 surveys, Bass Creek illustrates an aging population with low recruitment, low abundance, and low mussel assemblage richness. Low abundance could relate to extrinsic environmental stressors from anthropocentric land-use alterations associated with Bass Creek water quality impairments. Bass Creek watershed is agricultural land, although portions of Bass Creek consist of Wetland Reserve Easements (WRE).

Dams are a barrier, especially for host fish migrations and mussel distributions. The removal of Afton Dam in 2002 has increased the opportunity for fish passage and mussel assemblage dispersal, allowing Bass Creek to become more hydrologically connected to the Rock River. The mussel population within Bass Creek could be experiencing population dispersal growth farther upstream, which once had limited dispersal opportunities from the populations below old Afton Dam. Mussel dispersal is illustrated by the number of species I found primarily at transect four, downstream of the old Afton Dam, and the few species I documented farthest upstream.

I could not determine an exact age for each mussel; some shells were severely worn, and age was approximated. However, I estimated age by counting rings, then categorizing individuals into cohort groups, a time when they entered the mussel assemblage community in Bass Creek. In general, within Bass Creek, there is a lack of young mussels (under five years of age), where only one of the total 50 individuals represented this age group. This could indicate the low recruitment of mussels within Bass Creek. Yet, it could be explained by sampling bias, where small and hard-to-find juvenile mussels were overlooked (Haag 2012). If recruitment continues to be low or decreases, extirpation of species within Bass Creek could be common. Most of the species I found within Bass Creek are estimated to be at least 15 years of age (the oldest about 23 years old), with some between six and 13. Within the next decade, the six to 13 age cohorts' shells might be more abundant, and within a year or two, recruitments could replace these individuals.

Agricultural land uses might have a disproportionate effect on smaller tributaries than larger rivers and watersheds throughout the Rock River basin. Bass Creek serves as a baseline and example for small tributaries within the Rock River basin. Environmental stressors have led to low populations, but at least this indicates live mussels are present within smaller order streams. Small tributaries within the Rock River basin without impairments are likely to support higher abundance and mussel assemblage richness if the habitat is suitable. Additionally, stream conductivity suggests how agricultural land use can also be associated with pollutants from runoff. Conductivity was consistent among all surveyed sites and did not seem to have influenced low abundance or low mussel assemblage richness. Yet, current land uses could be concealed from past land uses, e.g., overharvest for buttons and food sources.

The Mussel Classification Assessment Tool has the strength to provide consistent parameters when assessing mussel assemblages (Dunn et al., 2020). My replication of MCAT ranked Bass Creek as having few environmentally sensitive species and aging populations with little recruitment. My MCAT scores also support the hypothesis that Bass Creek exhibits low abundance from past and current land uses. Low Pielou's evenness diversity MCAT metric scores also correspond with low macroinvertebrate scores (2.29) (WDNR records), indicating water quality impairments.

Mussel Species

Due to most *Lampsilis cardium* (plain pocketbook) found alive throughout the areas of Bass Creek I surveyed, this could indicate this species is tolerant and can thrive in conditions like those found in transects two and four (Table 2). It could also mean that *L. cardium* has been better at recruitment and species recovery since removing Afton Dam in 2002. The *L. cardium* specimen I found alive farthest upstream, in transect two, was estimated to be about eight years old. It could indicate how long it took the species to establish after the Afton Dam removal, e.g., a decade. While I found *Amblema plicata* (three-ridge) has the greatest abundance, most were relic shells, closest to the Rock River and only about 38 meters (125 feet) downstream from a once disconnected stream system. Although I found *Fusconaia flava* (wabash pigtoe) alive during an initial search, I did not collect water quality parameters during the initial investigations. I found this individual specimen near the County Highway D bridge with stream riffles. The streambed drops in elevation, and water flows quickly downstream towards its confluence with the Rock River.

CONCLUSION

Based on site history and current land uses within the Bass Creek watershed, mussel community populations have fluctuated between recovery and decline. However, I would argue mussel populations are experiencing a slow recovery since the removal of the Afton Dam and land placed into wetland easements two decades ago. The primary influence on the low abundance and species evenness could be from anthropocentric habitat alterations and associated Bass Creek impairments.

The Bass Creek watershed and the Rock River basin have similar physiographic features and land uses, e.g., agricultural uses, within Rock County, south-central Wisconsin, and sub-watersheds in Northern Illinois. Within the Rock River Basin, sub-watersheds such as Bass Creek have similar histories where mussel populations have existed, provided by evidence of middens and historical documentation on the amounts of shells and mussels harvested for buttons and pearls. Although my Bass Creek MCAT scores rank low, I propose that other degraded and impaired small order streams within the Rock River Basin would rank similarly. On the other hand, higher quality and more hydrologically connected small-order creeks and larger-order streams within the Rock River Basin would have more favorable MCAT metrics, supporting robust and healthy mussel assemblage health.

Through my replication of the MCAT, I agree it is a quantitative way to document mussel assemblage health and can act to guide conservation efforts. It uses a methodology to prioritize streams and watersheds of concern, where mussel populations are ecologically unstable, in continuous decline and threatened by extirpation. Therefore, I suggest it could be possible for MCAT to be used within several smaller sub-watersheds and in basins of similar land uses and physiographic origins, then use those scores to compare larger basins and regions, such as the Upper Mississippi Region studied by Dunn et al. (2020).

My MCAT metrics for the Bass Creek watershed demonstrate the potential recovery of mussel populations, and conservation practices should continue to improve aquatic and stream health. Although my numbers for mussel species did not necessarily support a healthy mussel community, it does provide a baseline for future conservation efforts and documentation. A prospective survey for the lower 13 km (8 mi) reach (South Johnson Road to the Rock River confluence) should be conducted in about five to ten years to assess changes in mussel assemblage population dynamics. I would also suggest mussel surveys be conducted upstream of South Johnson Road to the Village of Hannover, Wisconsin (warm stream habitat). Mussel surveys farther upstream would provide more information on mussel presence, dispersal, population dynamics, species richness, and species evenness. Future water quality surveys could also offer additional evidence of extrinsic factors to mitigate and reverse stressors, bolster mussel populations, and decrease the risk of extirpation.

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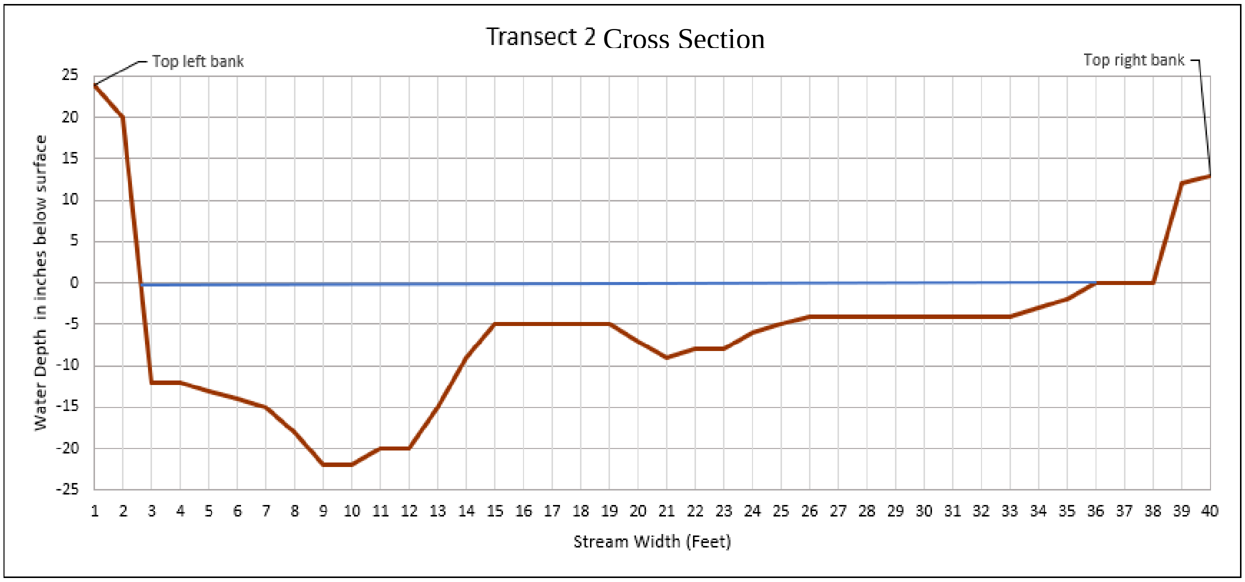
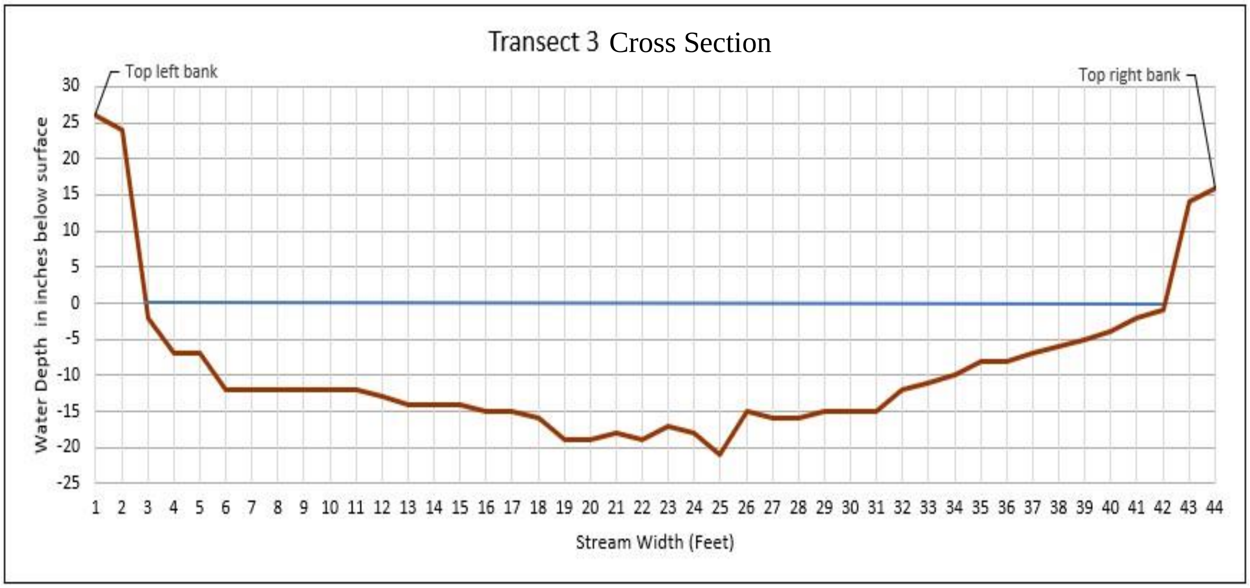
APPENDICES

APPENDIX A

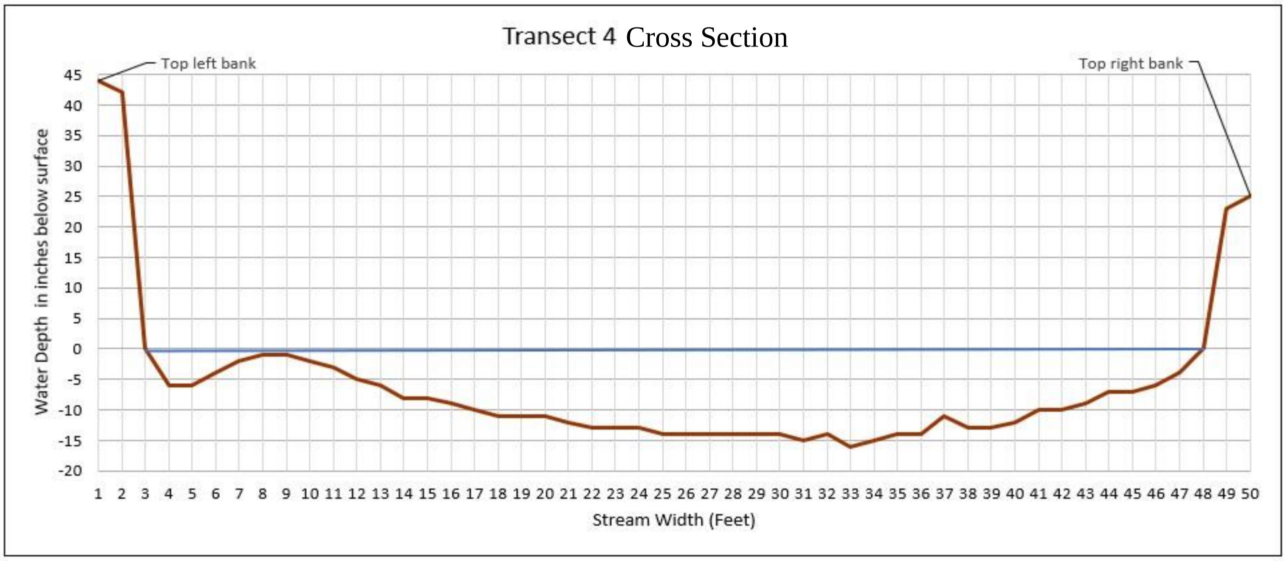
BASS CREEK CROSS-SECTIONS


Transect 1 Cross Section
Top left bank
Top right bank
Water Depth in inches below surface
40
35
30
25
20
15
10
5
0
-5
-10
-15
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
Stream Width (Feet)


Transect 2 Cross Section
Top left bank
Top right bank
Water Depth in inches below surface
25
20
15
10
5
0
-5
-10
-15
-20
-25
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
Stream Width (Feet)



Transect 3 Cross Section



Transect 4 Cross Section

APPENDIX B

MUSSEL SPECIMEN PHOTOGRAPHS

Amblema plicata (Three-ridge), transect four, alive.

Anodontodies ferussacanius (Cylindrical Papershell), transect one.

Eurynia dilatate (spike), transect four.

Fusconaia flava (wabash pigtoe), alive.

Lampsilis cardium (plain pocketbook), Transect two, alive.

Lampsilis siliquoidea (mucket), transect four.

Lasmigona complanate (white heelsplitter), transect four.

Ligumia recta (black sandshell), transect four.

Ortmanniana ligamentina (mucket), transect four.

Pyganodon grandis (giant floater mussel), upstream of transect four.

APPENDIX C

Transect Habitat Photographs

Transect 1, looking upstream.

Transect 1, looking downstream.

Transect 2, looking downstream at bank left.

Transect 3, looking upstream.

Transect 3, looking downstream.

Transect 4, looking upstream. Riprap upstream from Afton Dam removal.

Transect 4, looking downstream.

APPENDIX D

Buttons & Pearls

Mussel Exploitation and Potential Recovery

Buttons: John Boepple is considered the father of the mussel shell button industry, where he moved from Germany to the United States and specifically to Muscatine, Iowa, in 1891 (Pritchard, 2001; Temte, 1968). During the late 1890s and early 1900s, several mussel shell factories spanned from Nebraska to Ohio (Temte, 1968). By 1899, over 60 factories in the Midwest and Wisconsin, 16 million pounds of shells were harvested; the annual harvest was in the tens of millions in Wisconsin (Temte, 1968). Within a three-year time, between 1901-1903, prices of mussel shells almost doubled from $12 to $20/ton, primarily due to dwindling supplies (Temte, 1968). Waste was also a particular issue, as buttons were punched from the shells, and the rest of the shell was discarded; shells became used and used in the road “paving” (Temte, 1968). By the 1940s, the invention of plastic allowed buttons to be made more readily and at a cheaper price (Temte, 1968). During these 20 years, mussel populations had some time to recoup (Temte, 1968). Most companies quit producing buttons after WWII, and within Wisconsin, the clamming boom ended in Prairie du Chien in 1966 (Temte, 1968). The Bureau of Fisheries hired Boepple, the father of the mussel shell button industry, to study mussel natural history (Pritchard, 2001) and, in 1911, ironically cut his foot on the same species (White Heel Splitter) he discovered that initiated the rise of mussel shell exploitations for the button industry (Gaumnitz, 2019; Haag, 2012; Temte, 1968).

Pearls: From 1901-to 1903, Pearls were another mussel-exploited commodity. Assorted colors of freshwater pearls were found in distinct species, e.g., three ridge mussel species had blue, green, and lavender pearls (Temte, 1968). In 1902, upwards of $1,000 was paid for an excellent pearl in Beloit, Wisconsin (Temte, 1902). Rock County, Wisconsin, the button and pearl business boasted the Janesville Pearl and Button Company, significant to the area from 1899 until 1903 (Temte, 1968). Natural pearl hunting was eventually phased out due to cultural pearls in the 1960s (Temte, 1968). In 1969, clamming practices for the Rock River basin ended (Illinois Natural History Survey [INHS], 2012).