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# Assessment of the Freshwater Mussel Community of the Upper Mahoning River Watershed and Factors Influencing Diversity and Abundance in Small Streams

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# ASSESSMENT OF THE FRESHWATER MUSSEL COMMUNITY OF THE UPPER MAHONING RIVER WATERSHED AND FACTORS INFLUENCING DIVERSITY AND ABUNDANCE IN SMALL STREAMS

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# ASSESSMENT OF THE FRESHWATER MUSSEL COMMUNITY OF THE UPPER MAHONING RIVER WATERSHED AND FACTORS INFLUENCING DIVERSITY AND ABUNDANCE IN SMALL STREAMS

MATTHEW T. BEGLEY

## ABSTRACT

Freshwater mussel communities have experienced drastic declines in diversity and abundance in many streams throughout North America. Among the reasons for these declines is the human-driven alteration of the landscape, as urban and agricultural use impart many known stressors to aquatic systems. Impairments include increased sedimentation, increased pollutants, increased flood frequency and intensity, and decreased diversity and abundance of many organisms, including fish, macroinvertebrates, and mussels. Attempts to explain the abundance and diversity of mussel communities using small-scale factors such as substrate type and flow velocity provided little to no predictive power. Instead, reach-scale variables, such as stream morphology and riparian vegetation, and catchment-scale variables, such as land use, performed better as predictors of mussel diversity and abundance. In this study, surveys of mussel communities were performed in Eagle Creek in 2013 and throughout the entire upper Mahoning River watershed in 2014. Stream morphology was assessed at the sites surveyed in 2014. No published surveys exist for the mussel community of the upper Mahoning River watershed, which is a headwater system in the upper reaches of the Ohio River watershed. The Eagle Creek watershed had the highest proportion of forested land in the upper Mahoning River watershed and supported the largest and most diverse mussel community, although evidence for recruitment was limited in this stream. Across the region, abundance and species richness were strongly correlated with drainage area. Abundance and species richness decreased with increased shear stress, electrical conductivity, and agricultural and urban land use. Conductivity was also correlated with agricultural land use, and no live mussels were found where conductivity exceeded 0.9mS. Overall, the upper Mahoning River watershed had a low diversity and abundance of freshwater mussels, likely due to the intensive anthropogenic land use. Even where conditions appeared better, historic land use may have obscured the relationship between in stream conditions and mussel abundance and diversity, as some populations may have experienced greater stressors in the past than today.

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# CHAPTER I

# INTRODUCTION

Freshwater mussels (Family Unionidae) inhabit streams and lakes throughout most of the world. The greatest diversity is reached in North America, with approximately 300 extant species (Lydeard et al. 2004, Williams et al. 1993). Unionids are distributed across the entire continent of North America, reaching their highest diversity in the Mississippi and Ohio River basins (Haag 2012). However, they are also one of the most imperiled groups, with the majority listed as threatened, endangered, or extinct (Lydeard et al. 2004). Recently, mussel populations have seen a decline in diversity and abundance due to a number of factors, including: pollution (Havlik and Marking 1987), dam construction (Watters 1996), overharvesting (Cummings and Graf 2010), and dreissenid invasions (Schloesser et al. 1996, Ricciardi et al. 1998). The vulnerable status of these animals makes their conservation more crucial. Unionids also provide an interesting model to test the impacts of human activity on stream ecosystems. Urban and agricultural land use (and a number of associated influences on streams) may have a powerful effect on mussel distribution and these factors can be compared among streams to identify conditions that are conducive to mussel presence. These conditions may be indicative of the quality of the ecosystem, to which mussels have the ability to contribute substantially.

Mussels are sedentary filter-feeders that burrow into the sediment of aquatic habitats, usually sand or mud but possibly also gravel and rocks (Cummings and Graf 2010) and can have significant impacts on the composition of nutrients in the water column and the benthos. They filter large quantities of water in a relatively short time, removing large amounts of algae from the water and excreting nutrients into the water and sediment (Vaughn et al. 2004). The presence of mussels has been shown in laboratory experiments to stimulate primary production (Vaughn et al. 2008). The act of burrowing can release nutrients by disturbing the sediments (Vaughn and Hakenkamp 2001). Mussel excretion includes feces and pseudofeces that are deposited into the sediment, where this nutrient source is available to benthic microbes, algae, and detritivores (Howard and Cuffey 2006). Mussel density has also been positively correlated with increased benthic macroinvertebrate densities in stream systems (Vaughn and Spooner 2006) and greater amounts of organic matter and algae in sediments (Spooner and Vaughn 2006), which have been tied to increased deposition of organic material in the sediment due to mussel presence (Howard and Cuffey 2006).

Not only do mussels have the potential to be a significant force in aquatic ecosystems, but the community composition (diversity) can also play an important role (Allen and Vaughn 2009, Spooner and Vaughn 2012). The composition of the mussel community in terms of number and proportions of species can have significant effects on stream functions such as primary production (Spooner and Vaughn 2012). Variation in temperature (daily and seasonal) and species interactions (community composition) can affect the behavior, metabolism, filtering rates, and excretion rates of mussels, leading to changes in the nutrient content of the water and the sediment (Spooner and Vaughn 2012). Clearly, the presence of native freshwater mussels is important to the aquatic communities they inhabit. Because the composition of these communities may be equally important in assessing the impacts of the decline of many mussel populations in North America, diversity and species richness, in addition to abundance, must be measured as a component of community health.

The distribution of freshwater mussels is highly dependent on their unique life history traits, which includes a parasitic stage. Males release spermatozeugmata ("sperm balls") into the water column, where they are filtered out by females (Graf and Cummings 2010). The females filter these packets from the water column through the same method as filter-feeding, where particles are filtered from the water by the gills and labial palps (Haag 2012). They use the sperm to fertilize eggs that are held in a modified portion of the gills called a marsupium (Haag 2012). These larvae develop in the mother's gills until they reach the glochidium stage, an obligate parasitic stage during their larval phase (Cummings and Graf 2010). The glochidium attaches to the gills or scales of a fish immediately after release from the mother (Haag 2012). These glochidia (which can range from <2000 to 10 million annually for a single individual (Haag 2013)) encyst on the gills of the host fish, where they feed off the fish and develop until they reach a necessary size (Haag 2012).

After they mature, juvenile mussels detach from the gills and enter the water column, where they fall to the stream or lake bed with the influence of possible water currents. Glochidia of *Actinonaias ligamentina* have been observed to travel almost

100m downstream, with most caught in a net placed 4m downstream from their release (Schwalb et al. 2010). These juvenile mussels settle to the bottom and must burrow into the substrate to avoid predation or dislodgement by moving water (Haag 2012). Some may bury themselves completely in the sediment during their first year (Balfour and Smock 1995). In the laboratory, 98.5% of juvenile (1-14 days old) *Villosa iris* were found to burrow completely beneath the sediment within 20 minutes (Yeager et al. 1994). Survival between the glochidial stage to juvenile stage is low; after settling to the bottom, survival is variable among species and increases with age thereafter (Haag 2012). The larval, juvenile, and adult stages are all susceptible to mortality caused by stressors associated with land use in the watershed.

The anthropogenic uses of land within a watershed play a large role in stream composition and function, which in turn may influence mussel populations. Agricultural and urban land use lead to predictable impairments on stream ecosystems, many of which have been identified as contributing to the loss or absence of mussels in these areas. Agricultural land can occupy a large portion of watersheds and can vary from as little as 5.7% (range=0-72%) in northern Minnesota, Wisconsin, and Michigan (Wang et al. 2003(a)), to 22.3% (range=6.5-38.4%) in Northern Georgia (Roy et al. 2003), and 42% (range=1-78%) in eastern Minnesota and Wisconsin (Wang et al. 2003(b)). In northwestern Ohio, agricultural land can occupy as much as 70-74% of a watershed (Krebs at al. 2010). Urban land may occupy a similar area of some watersheds, but often a much smaller area: from 0.5% (range=0-18%) in northern Minnesota, Wisconsin, and Michigan (Wang et al. 2003(a)), to 14% (range=1-86%) in eastern Minnesota and

Wisconsin (Wang et al. 2003(b)), and 15% (range=4.9-67%) in northern Georgia (Roy et al. 2003).

Agricultural land use can have significant impacts on the physical and chemical characteristics of streams. Application of fertilizers and removal of dense vegetation to destabilize soils may result in more material running off the land and into streams, increasing sedimentation and pollution (Allan et al. 1997; Jones et al. 2001). Erosion in the Sacramento River was estimated to be 56% greater between 1946 and 1997 (a time of increased agricultural development) than in the fifty years preceding this period, and approximately 150% greater for agricultural land compared to riparian forest from 1949-1997 (Micheli et al. 2004). Increased vegetation in riparian zones and stream catchments help to buffer the effects of agricultural land use and increased riparian vegetation is associated with decreased sedimentation and lower nutrient inputs compared to agricultural baselines (Boody et al. 2005). Meanwhile, agricultural land and decreased riparian cover have been positively correlated with increased nutrients in streams (Jones et al. 2001). Increased electrical conductivity was also positively correlated with increased agricultural land use in the Willamette Valley in Oregon, and ranged from <0.1mS/cm to ~0.7mS/cm (Pan et al. 2004). Benthic macroinvertebrate communities in Nevada streams were observed to be less diverse as electrical conductivity rose (Vander Laan et al. 2013).

Flood frequency and intensity can be escalated by agricultural land use. Removal of vegetation, and its ability to uptake excess water, and compaction of soils may be causes for the intensified flooding in these areas. Modeling of the effect of changing land cover on flooding in streams from the Raccoon River in Iowa has shown that frequency of floods, in particular severe floods, can be reduced if cropland is replaced by natural vegetation or more varieties of crops in rotation (Schilling et al. 2014). Increases in the amount of cropland and livestock in a watershed in England has been associated with increased number and duration of flood events at moderate levels of rainfall (<1 inch) (Archer et al. 2010).

The effects of agriculture on stream communities have been associated with decreased invertebrate (insect) richness and abundance in streams (Liess et al. 2012). Vondracek et al. (2005) found that catchment-scale and local-scale (riparian) land use was correlated with fish and macroinvertebrate assemblages and that wooded land and good riparian cover was associated with decreased turbidity in streams. Shade from woody and successional riparian vegetation helps to decrease maximum stream temperatures in the summer, providing an improved habitat for fish (Blann et al. 2002) and potentially a more robust source of hosts for mussels. The amount of nitrogen-15 in mussel tissue has been positively correlated with percent of agricultural land in the watershed, indicating direct uptake of human inputs by organisms in streams (Atkinson et al. 2014). Acute toxicity of mussels for ammonia concentrations (concentration at which 50% mortality occurred) were found to be 60-75% lower than U.S. Environmental Protection Agency standards for maximum concentrations of ammonia (Augspurger et al. 2003).

Urban land suffers from many of the same impacts on streams, as increased amounts of water enter streams from impervious surfaces (Dunne and Leopold 1978). This water often carries pollutants such as metals, nutrients, and salts to streams (Paul and Meyer 2001). Water column concentrations of metals such as chromium, copper, and lead had the highest levels in an urban stream compared to either a forested or agricultural stream in North Carolina (Lenat and Crawford 1994). Electrical conductivity and nutrient concentrations (carbon, phosphorous, ammonium) also were elevated in small streams near Melbourne, Australia, in response to increased impervious surface cover (Hatt et al. 2004). Elevated phosphorous levels were likewise correlated with impervious surface cover in Maryland (Kaushal et al. 2012). Macroinvertebrate communities changed from indicators of relatively good water quality to indicators of poorer water quality above 15% urban land cover in the watershed in Northern Georgia (Roy et al. 2003). Fish community index (Index of Biotic Integrity), trout abundance, and tolerant-fish abundance were found to have low levels with impervious surface cover above 11% (Wang et al. 2003(b)).

Because of the sedentary, benthic lifestyle of mussels, their habitats, and thus their distribution, are highly connected to the quality and stability of the benthic environment. Agriculture, which tends to decrease riparian vegetation and increase sediment loads to streams, has been correlated with declines in mussel diversity. Poole and Downing (2004) examined the change in the number of mussel species at sites between 1984-85 and 1998. They found that of 118 sites that had possessed the highest quality mussel habitat in Iowa in 1984-85, 47% had no live mussels in 1998 (compared to 6% in 1984-85) and even common species such as *Pyganodon grandis* and *Lampsilis siliquoidea* had experienced declines. Species richness increased or did not change in areas where <25% of the watershed was agricultural land (Poole and Downing 2004). For the highly-threatened genus *Epioblasma*, the loss of species may be linked as far back as 1000 years to the intensification of maize agriculture by Native Americans in the Southeast United States (Peacock et al. 2005).

Gangloff et al. (2009) found lower density and diversity of live mussels with increased urban land in two Alabama streams. Survival of experimentally caged mussels varied, as survival decreased from 100% at an upstream control site to 22% below a wastewater treatment discharge, where nutrient concentrations were also observed to be higher than the rest of the survey area (Gangloff et al. 2009). Brown et al. (2010) found *Potamilus inflatus* to be an order of magnitude less common at sites with residential development in the riparian zone compared to sites with riparian wetland forest near Baton Rouge, Louisiana. Sites where *P. inflatus* was not present were also found to have higher conductivity than sites where *P. inflatus* was present. Gillies et al. (2003) found increases in impervious surface cover in Atlanta, Georgia, between 1987 and 1997 to be linked to loss of mussel species in the watershed. Land use appears to have a significant impact on mussel communities, but what are the mechanisms responsible?

Early studies of mussel populations in streams aimed at identifying the type of substrates and local flow conditions that might be more closely associated with mussel presence (Strayer and Ralley 1993, Balfour and Smock 1995). However, these studies found few patterns among the microhabitat conditions and mussel abundance and diversity, and microhabitat factors were at best a weak predictor of mussel presence. Balfour and Smock (1995) surveyed a first-order stream in Virginia and found no significant associations between physical and chemical parameters (including sediment grain size, average water temperature, flow velocity, depth, and channel width) on *Elliptio complanata* abundance, although a slightly negative association with sediment in the 0.05-1.5mm class compared to larger sediment sizes was observed. However, substrate composition is not a useful indicator for species' preferences in most cases. Brim Box et al. (2002) surveyed Coastal Plain streams in Alabama, Florida, and Georgia, and found that only *Villosa lienosa* was significantly associated with substrate characteristics, though these characteristics (fine sediments, <0.063mm) may have been simply correlated with the stream bank habitats where this species is most often found. In New York, *Alasmidonta heterodon* was most likely to be found in the presence of fine sediments, and *A. varicosa* was most associated with medium sand (0.25-1.0mm), but the predictive power was minimal, and the authors stressed the weakness in association of mussel abundance with substrate size (Strayer and Ralley 1993).

Likewise, recent studies have failed to find clear relationships between mussel abundance and variables such as channel width, water depth and current velocity (Gangloff and Feminella 2007). Instead, the local-scale factors that influence mussel distribution are more likely related to the stability of sediments, intensity of flow, and intensity and frequency of flooding in a particular stream or section of a stream (Hardison and Layzer 2001, McRae et al. 2004, Gangloff and Feminella 2007, Daniel and Brown 2013). Mussels tend to colonize areas of flow refuge during floods (Strayer 1999). Mussel populations also appear to be correlated with regional characteristics (Vaughn 1997) such as watershed geology (Strayer 1983; Arbuckle and Downing 2002, McRae et al. 2004, Poole and Downing 2004) and land use (Poole and Downing 2004, Daniel and Brown 2013). Variation in mussel communities may be better explained by local habitat conditions relating to a larger group of factors, including complex hydraulic factors, and possible interactions of these conditions rather than one or a few simple measurements of stream morphology. However, stream size does play a role, as diversity has been observed to increase with increasing watershed area in streams in the Lake Erie watershed in Ohio (Krebs et al. 2010) and more broadly in the Ohio River watershed (Watters 1992).

Shear stress has been identified as a separate major factor limiting mussel presence. Shear stress is the pressure applied parallel to the stream bed in the direction of water flow due to the force of the water on the stream bed (Gordon et al. 2004). Mussels are more likely to be found where shear stress is low, allowing for juveniles to settle and develop to adults (Hardison and Layzer 2001). Shear stress, especially during periods of high flow, and sediment stability have been identified as key factors influencing mussel abundance in streams (Hardison and Layzer 2001, Howard and Cuffey 2003, Gangloff and Feminella 2007). Daraio et al. (2010) developed a model demonstrating that shear stress is negatively associated with settling of juveniles on the stream bed under high flow conditions ($>600m^3/s$) for large rivers. Thus, there will be a diminished mussel community in areas where excessive shear stress applied by peak flows removes juvenile mussels from the stream bed.

Many streams in North America have been experiencing increased temperatures due to a number of factors related to land use (Kaushal et al. 2010). In Tokyo, Japan, the average annual temperature of wastewater effluent rose from 17.2°C to 22.7°C from 1965 to 2004 (Kinouchi 2007). These temperatures may pose a threat to mussel survival at the current conditions in some areas and in many more streams if the trend continues. Transformation for *Anodonta suborbiculata* from glochidia to juvenile mussels on host fish was about 50% more likely at 10°C or 15°C than at 21°C, likely owing to suppression of fish immune systems at lower temperatures (Roberts and Barnhardt 1999). Pandolfo et al. (2010) found that the average lethal temperature for 50% (LT50) of juveniles was 33.1°C and 31.6°C for glochidia, although there is much variability among species. Juvenile burrowing behavior has also been seen to be reduced in *Lampsilis radiata* as temperature increases, preventing these individuals from reaching cooler temperatures in the sediment (Archambault et al. 2014). There may also be negative effects of high temperatures below these levels, such as changes in metabolism and availability of host fish.

Increased influx of sediments to streams due to agricultural and urban land use can have severe impacts on mussel populations. High concentrations of suspended solids may be causing reduced reproductive success. Osterling et al. (2010) found increased turbidity to be associated with fewer juveniles in the population and increased average age for *Margaritifera margaritifera.* Total suspended solids have been found to be associated with decreased proportions of gravid females but had no effect on fecundity or on sperm production in males for the mussel *Ligumia subrostrata* (Gascho-Landis et al. 2013). Increased sediment in the water column may result in decreased filtering effort by mussels, reducing the chance for females to filter sperm from the water column, or increased pseudofeces production, which may cause sperm to be rejected along with excess solid material (Gascho-Landis et al. 2013).

Suitable host fish are necessary for adequate mussel habitat, since the mussels cannot reproduce in the absence of host fish. The use of host fishes ranges from generalist (many species or families of hosts) to specialist (one or a few species of hosts). As invasive species move into new areas, the diversity of the fish community is diminished and host fish may be lost for some mussel species. Douda et al. (2013) found that glochidia of a host generalist mussel, *Anodonta anatina*, were unable to attach as effectively to invasive fish as to native fish. In a study of the Ontario rivers in the Lake Huron, Lake St. Clair, and Lake Erie basins, host fish presence was the most important factor, explaining 44% of the variation in mussel community composition (Schwalb et al. 2013), although host fish abundance was not related to mussel diversity among Ohio's Lake Erie tributaries (Krebs et al. 2010). Simply a reduction or loss of fish, possibly from the land use impairments mentioned above, could reduce or eliminate the ability of a mussel population to reproduce.

The abiotic and biotic factors mentioned above each influence mussel communities at various stages in the life cycle of a mussel. First, fish hosts must be available to disperse larvae. Then, sediments and flows must be stable enough to allow juveniles to settle and bury after leaving the host. Next, temperature and water quality must be suitable for juveniles and adults to persist and, finally, for proper timing and success of reproduction. Mussels are long-lived animals and impairment of juvenile recruitment may create an "extinction debt," where the long-term effects of habitat degradation on mussel populations may not be realized for many years. This was observed by Poole and Downing (2004), who found that the greatest loss in species richness over 15 years occurred in sites where the surrounding landscape had been changed most from the "historic" grassland and woodland to agricultural fields. Some species, especially those tolerant of disturbance and competition, may persist in these areas. However, their local extinction may occur as habitat continues to degrade and disappear (Tilman et al. 1994). Fragmented patches of suitable mussel habitat may be too far apart for successful dispersal and fertilization (Strayer 2008).

The size structure of mussel populations has often been observed to be skewed to an excess of larger individuals and relatively few small individuals (Haag 2012). If recruitment slows or stops, local extinctions will become more common. The freshwater mussel *Margaritifera margaritifera* has shown this pattern in Scottish (Hastie et al. 2000) and Swedish (Osterling et al. 2010; Osterling et al. 2014) streams, possibly due to insufficient recruitment of juveniles in some populations. Human impacts may cause lower recruitment, although sampling bias in some studies may overlook the small, hard-to-find juvenile mussels (Haag 2012). Some populations do show a more uniform distribution of sizes, indicating more consistent recruitment (Haag and Warren 2010). Balfour and Smock (1995) found an *Elliptio complanata* population in Virginia to consist of 90% individuals 4-6 years old, with a maximum age of 8, when they excavated sediment down to a clay layer. Lower than expected presence of small or young individuals may be an indication of discontinuous recruitment (Tevesz et al. 1985), and not a persistent lack of recruitment.

As mussels continue to suffer from anthropogenic impacts, procedures will need to be implemented to protect and augment current populations. Identification of diverse and abundant populations will indicate areas to be set aside from development or otherwise protected, sites that will provide the best habitat for harvesting (for aquaculture) and reintroducing populations, and sites where restoration of riparian vegetation will benefit mussels. Also, models of mussel presence can inform best practices for land use to help preserve current populations and guide stream restoration efforts where mussels are known to have been lost. Identifying areas of higher recruitment may be more important than simply identifying diverse or abundant communities. If the stream impairments often observed with agricultural and urban land use can be mitigated, increased recruitment can drive the return and persistence of mussels in many streams in North America.

All research was conducted in the upper Mahoning River watershed (8 digit HUC 05030103), which covers an area of 1472 km$^2$ in northeastern Ohio. This watershed lies in the northern reaches of the Ohio River watershed and borders the Lake Erie watershed, spanning five counties: Portage (containing the majority of the watershed), Trumbull, Stark, and Columbiana, and Mahoning. It is located in the Killbuck-Glaciated Pittsburgh Plateau physiographic region of northeastern Ohio (Ohio Division of Geological Survey 1988) and is composed of four major subwatersheds, from south to north: Mahoning River headwaters, Deer Creek, West Branch Mahoning River, and Eagle Creek (OEPA 2011). The proportion of land cover in each of the four major subwatersheds (Table 1) indicates that the primary land cover in the watershed is forested land (37%) but cropland (23%) and pastureland (17%) combined constitute a slightly greater proportion (OEPA 2011). Forested land cover increases from south to north as agricultural land decreases (OEPA 2011). Developed land reaches 20% in the Mahoning headwaters (southernmost subwatershed), but is only 7-11% of the other three subwatersheds.

Table 1: Four categories of dominant land cover in each of the four main subwatersheds of the upper Mahoning River. Cultivated crop and pasture/hay were combined as agriculture for analyses. Watersheds are oriented north to south moving from left to right across the table. From OEPA (2011) Upper Mahoning River TMDL Report.

| | Mahoning River Headwaters | Deer Creek | West Branch Mahoning River | Eagle Creek |
|---|---|---|---|---|
| Forest | 24% | 35% | 43% | 46% |
| Cultivated Crop | 30% | 26% | 16% | 20% |
| Pasture/Hay | 21% | 21% | 16% | 10% |
| Developed | 20% | 7% | 11% | 11% |

Stream impairments are widespread in the upper Mahoning River watershed and stem from a number of sources. Attainment status of sites mentioned below indicates a threshold level of quality based on habitat, fish, and macroinvertebrate community indices, with fish and macroinvertebrate standards varying by ecoregion and sampling method (Yoder and Rankin 1995). Full attainment indicates that all criteria meet the standards for the region, partial attainment with one or more indices below attainment but with a "fair" or above rating, and non-attainment with no indices above attainment or one group with a "poor" or "very poor" rating (Yoder and Rankin 1995). In the upper Mahoning watershed, 61% of sites in the 2011 Total Maximum Daily Loads report failed to meet full aquatic life standards (OEPA 2011). The report attributed most of the impairments (75%) to increased sedimentation, increased nutrient concentrations, and altered flow regimes. The report further identified the most likely sources of the first two impairments as agricultural (crop and livestock) and urban land use, and lowhead and large dams as the source of altered flow. Additionally, home septic systems have been identified as a major source of bacterial contamination in the watershed, although this has been primarily tied to non-attainment status for 95% of sites for recreational use (OEPA 2011).

The goals of this study are: 1) Catalog the diversity of mussels in the upper Mahoning River watershed, where a lack of published data exists; 2) Determine what, if any, hydrologic and land-use variables structure mussel communities in this area; and 3) Assess the viability of the Eagle Creek mussel community, which has the most intact community in the watershed. The study was performed in two complementary parts which were analyzed separately. First, a survey of the mussel community of Eagle Creek was conducted during the summer of 2013. Second, a survey of mussels and the environmental conditions at each survey site was performed throughout the upper Mahoning River watershed during the summer of 2014 to test the relationship between the environmental conditions and size and diversity of the mussel community. This research will fill a gap in data for the mussel communities of the upper Mahoning River watershed. This watershed is a headwaters for the upper reaches of the Ohio River and as such may have significant impacts on the quality of water that flows to the Ohio River. Small streams and headwaters, though often lower in abundance and diversity of mussels, may be important contributors to downstream communities in addition to water quality.

# CHAPTER II

# MATERIALS AND METHODS

### *2013 Eagle Creek Surveys*

During the summer of 2013, surveys were conducted at eight sites in Eagle Creek, a tributary to the Mahoning River. Additionally, surveys were conducted in the Mahoning River upstream and downstream of the confluence with Eagle Creek and one site approximately 3 miles upstream of this point. The Eagle Creek watershed (10-digit HUC 05030103-04) is the northernmost drainage of the upper Mahoning River watershed. In contrast to the entire upper Mahoning River watershed, most sites (nine of 16) in the Eagle Creek watershed met the full aquatic life use status (OEPA 2011). Furthermore, only one site on the main stem of Eagle Creek was classified as non-attainment for aquatic life (no partial attainment), and this site was outside of the study area.

Surveys of Eagle Creek were conducted upstream and downstream of five bridge crossings, one site in the Eagle Creek State Nature Preserve, one site at the Garrettsville waste water treatment plant, and one site at the mouth of Eagle Creek (Figure 1). Each bridge crossing was treated as a single site. The most upstream site was located at the waste water treatment plant, approximately 1 km downstream of a 3-4 m tall dam in Garrettsville. Dams were avoided in 2014 surveys, but the effect of this dam was examined in 2013 analyses. Surveys included only searches for freshwater mussels.

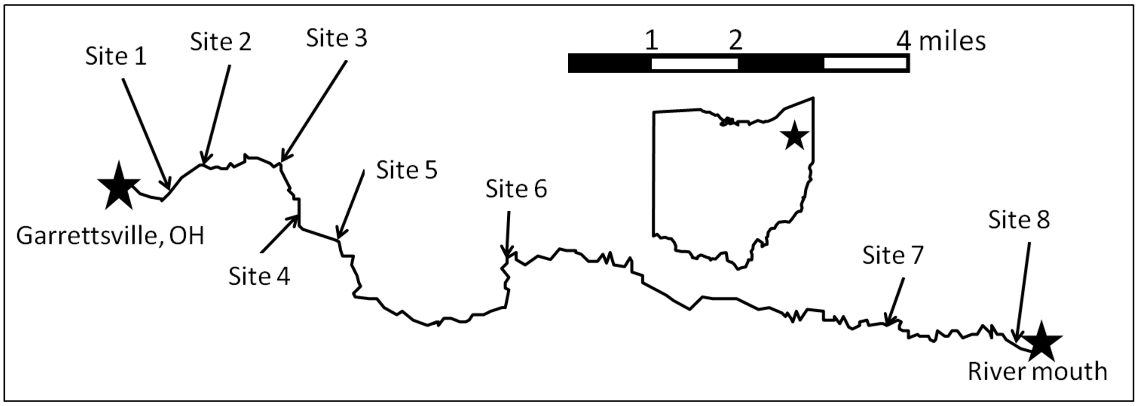


Figure 1: Survey sites in Eagle Creek (H'=Shannon diversity index; N=number of live mussels found; R=number of species of live mussels found).

Surveys were performed for approximately four person-hours at each upstream and downstream reach for all sites. GPS coordinates were recorded for the endpoints of each site. Surveys were started at the farthest downstream point to reduce the effect of suspended sediments on the effectiveness of visual searching. Wading was employed for seven of the eight sites, and mussels were found mainly through visual searches as water was shallow and clear in most areas. In deeper areas (~chest to shoulder height), mussel rakes were used to find mussels in the top layer of sediment. Mussel rakes were employed in the rest of the stream as well to find smaller and buried individuals. These rakes consisted of a bow rake with a basket attached of 1 cm mesh. Tactile searches were also used in more turbid water of moderate depth (~knee to waist deep). At the Eagle Creek river mouth and the surrounding section of the Mahoning River, surveys were conducted from kayak to facilitate access to sites. Consequently, surveys were restricted to the river banks and some other shallow areas because the depth in the center of the channel prohibited wading and tactile surveys and mussel rakes were not used. Likewise, the upstream site on the Mahoning River was restricted to visual and tactile surveys of the banks due to the excessive mid-channel depth.

Mussels were removed from the stream and identified to species by one observer (MTB) and measured for maximum shell length and age. Maximum length was measured to the nearest millimeter using calipers. Age was estimated by counting the dark lines on the outside of the shell when possible. Although less accurate than taking sections of shell from live mussels to count the internal annual growth rings (Veinott and Cornett 1996) our method did not require sacrificing any animals. At a minimum, the method of counting external growth lines should be sufficient to compare age differences among a single species at sites within the same stream. All live mussels were returned to the stream after completing each survey. Shells were collected and identified to species when possible, and are stored at Cleveland State University.

*2013 Analyses*

For the mussel community survey results, abundance (total # of live mussels), species richness (# of species), and Shannon diversity (H') was measured for each site in Eagle Creek. Shannon diversity is defined as:

$$-\sum p_i * \log(p_i)),$$

where $p_i$ is the proportion of live individuals to the overall abundance at a site, summed over all the species present at the site. These measurements were compared between sites to examine whether or not any patterns of distribution exist in lower Eagle Creek. The Mahoning River sites were not used in this analysis because this river is much larger than Eagle Creek and the surveys were less effective and thus not comparable due to the depth. The average length and age for *L. siliquoidea* at each site was compared with ANOVA in JMP7 (SAS Institute). Tukey's test of honest significant difference was used to determine which sites varied significantly from each other. Age and length data were recorded for all mussels, but only *L. siliquoidea* was present at all sites, and thus was the only mussel species used to examine size and age of live mussels.

### *2014 Upper Mahoning River Watershed Surveys*

Sites during 2014 sampling were located throughout the upper Mahoning River watershed. Twenty sites were chosen based on the sites used for the 2011 TMDL report from the Ohio EPA (Figure 2). These sites were located from the Ohio EPA Biological Monitoring web page (wwwapp.epa.ohio.gov/dsw/gis/ bio/index.php). Each was chosen in such a way to make use of the additional data from the TMDL report, which included drainage area and habitat quality and fish community metrics. Habitat quality was measured by the qualitative habitat evaluation index (QHEI). The fish community was assessed using the index of biotic integrity (IBI). These two indices were available for all but one site. The aquatic life use attainment categories were also provided for each site and include non (0), partial (1), and full (2) attainment statuses. All sites were located at least 10 m upstream of bridge crossings when present. Sites near dams (within 1.5-3 km) were not used to reduce the confounding effects of dam presence on mussel distribution. Indeed, two sites were removed after initial surveys: one site ~500m downstream of Dale

Walborn Reservoir (no live mussels found) and one site immediately below a lowhead dam at the Sebring public water intake (one live mussel found). Agricultural ditches were not included in the survey despite presence in the OEPA report, and one site was excluded due to being situated directly on a private cattle farm.

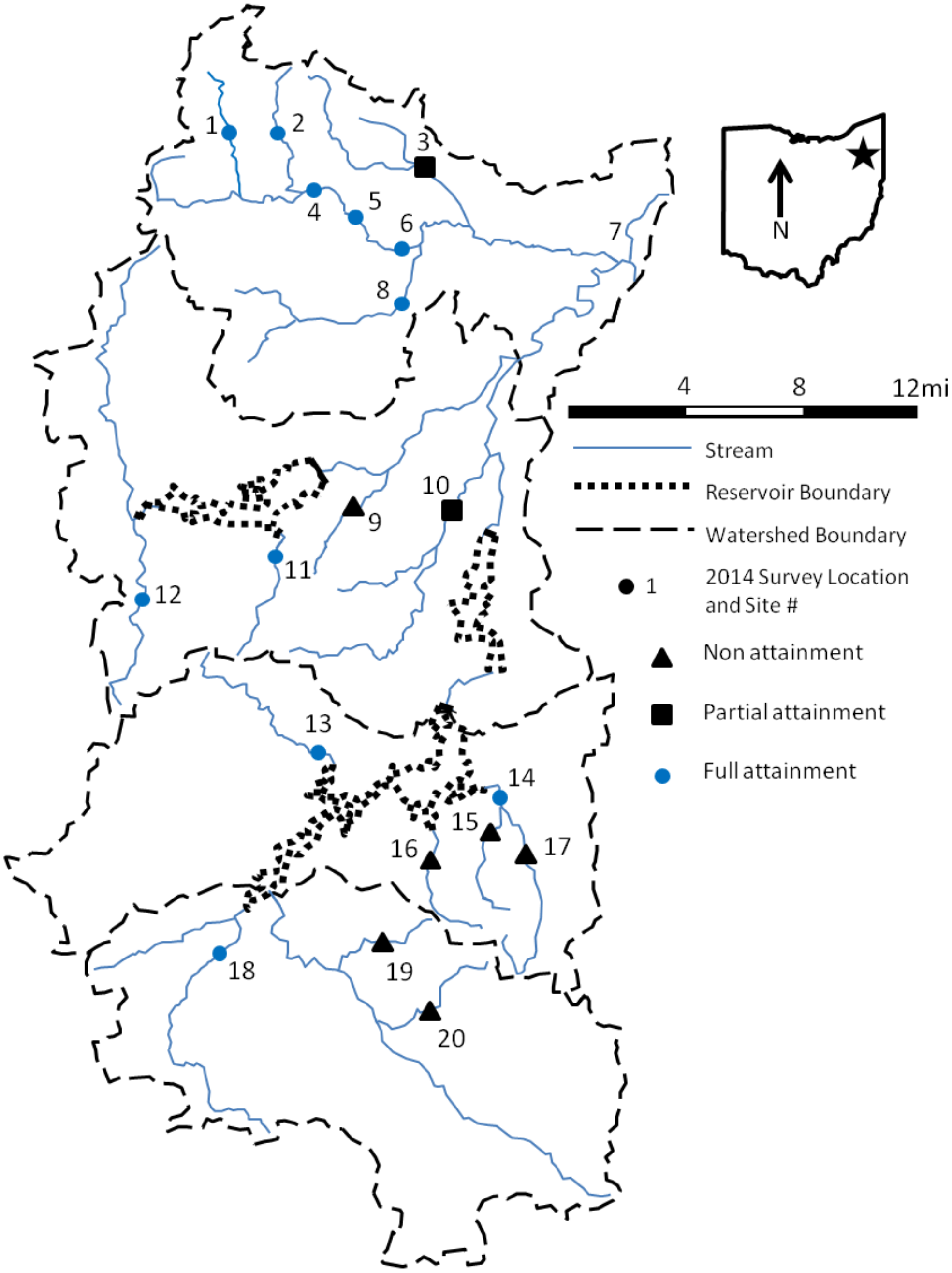


Figure 2: Map of the upper Mahoning River watershed with subwatershed and reservoir boundaries. Site #'s correspond to table 3. Only main streams in the four main subwatersheds and streams with sampling sites are shown. The northernmost portion is the Eagle Creek watershed, with sites 4, 5, and 6 in Eagle Creek. Sites 4 and 5 are contained within sites 2 and 4 from 2013 surveys of Eagle Creek, respectively.

Mussel surveys were performed at near-baseflow conditions between June and July to maximize search efficiency and maintain consistency among sites. Abiotic measurements were taken between June and September, also at baseflow conditions. Baseflow was established using the USGS Waterwatch monitoring system for Ohio (http://waterdata.usgs.gov/oh/nwis/rt) and checking water levels of several streams in the study area. All measurements, biotic and abiotic, were taken at least 5-7 days after a significant rain event (assessed if water levels raised more than a few inches). These precautions ensured comparable conditions across sampling.

Mussel searches were conducted in a similar manner as in the 2013 surveys, but were limited to 2-person hours at each site with at least two people searching per site. All surveys were conducted while wading in the stream. A 100m length was marked off for each site and the subsequent survey resulted in an exhaustive search of the defined reach at all sites, eliminating bias that could result from failing to search portions of a site. Mussels were collected and then identified to species, measured for maximum length, aged (when possible), and immediately returned to the stream. Total abundance, species richness (# of species), and Shannon diversity were recorded as in 2013 surveys. Shells were collected and recorded, and most shells were returned to the stream while a small number (~4-5 per site) were stored at Cleveland State University for voucher purposes.

Channel morphology was characterized by measuring stream width, average depth, bankfull width, bankfull height, and stream slope. Width was measured by stretching a measuring tape across the water surface of the wetted channel. The tape was then pressed against the stream bed along this same orientation to obtain the wetted perimeter, the distance of the cross section of the stream where the water touched the stream bed (Gordon et al. 2004). Average depth was calculated using a wading rod to measure depth at 1m intervals across the width of the stream and then taking the average of these measurements. Bankfull height was measured by identifying which bank had a shorter height between the water surface and the floodplain and then measuring this height. The bankfull level can be identified by several factors: change from sloped bank to flat land, from steep bank to gently sloped bank, change in vegetation from bare to vegetated or from grassy to shrubs and trees (Dunne and Leopold 1978). The bankfull width is the distance between the two banks at the bankfull height. Bankfull depth was calculated by adding the average depth to the bankfull height. Each of these measurements was taken at five transects 20m apart, starting at the most downstream portion of the mussel survey, and averaged to obtain a single value at each site. Slope was initially measured on site using a Suunto clinometer and stadia rods midstream between each transect, but the resolution of this device was not suitable to gain an accurate measure of slope. Instead, 7.5-minute topographical maps were used to measure the distance between successive elevation lines on either side of each site. The slope was then expressed as elevation change divided by distance (Gordon et al. 2004).

Estimated baseflow ($T_{base}$) and bankfull ($T_{bankfull}$) shear stress could both then be calculated using the measurements described above and the equation:

$$T=p*g*R*S$$

where p is the density of water, R is the hydraulic radius, and S is the slope of the stream (Gordon et al. 2004). Hydraulic radius can be estimated by using the average depth in wide (width:depth ~ 20:1) or rectangular channels (Gordon et al. 2004). Most sites met this criteria, and when hydraulic radius (average width*average depth / wetted perimeter) was plotted against average depth, linear regression resulted in an $R^2$ value of 0.99. Thus, average depth was used in place of hydraulic radius for the equation above. Using the average depth also allowed for more consistency between baseflow and bankfull calculations, as average depth was simply replaced with bankfull depth for calculation of $T_{bankfull}$.

A number of additional abiotic measurements were taken at each site at the same time as the measurements above, starting at the most downstream point of the reach to avoid disturbance to the stream bed that could influence measurements. Discharge was recorded once at each site using a digital flow meter (Hach FH950) and wading rod to measure the average velocity at 1m intervals across the width of the stream obtained at 0.6 of the maximum depth at each point (Gordon et al. 2004). The discharge was the sum of the average velocity times the average depth at each point. Conductivity and pH were measured in the middle of the stream for each transect using a hand held meter (Hanna Instruments 98130). Suspended solids were measured using a 120cm long turbidity tube, which is a narrow plastic tube with a Secchi disc painted on the inside of the closed end. The tube was filled completely with water, which was drained until the black and white markings of the disc could be distinguished. The water level at this point was recorded once at the midpoint of each transect and averaged for each site. Water that was clear enough to see through the entire tube was recorded as 120cm. The turbidity variable was removed from analysis due to the inability to resolve values greater than 120cm and because 82% of turbidity measurements exceeded this value.

Median particle size was recorded at each site by walking diagonally from bank to bank along the length of each site. At each step, one pebble was picked up at the tip of the observer's toes without looking down into the water to reduce bias in choosing a pebble. The pebble was then measured to the nearest millimeter, returned to the stream behind the observer, and another step was taken until 100 pebbles were measured along the 100m site. Clay, silt, and sand were not measured directly but recorded as 0.004mm, 0.03mm, and 0.5mm, respectively (Gordon et al. 2004).

Finally, land use was categorized from the Ohio EPA 2011 TMDL report for the upper Mahoning River watershed. This report provided percentage of forested, agricultural (crop + pasture), and developed (urbanized) land use in each of the four major subwatersheds mentioned above. These data were used in lieu of more accurate land use measurements surrounding the immediate area or catchment at each site. Although not site-specific, these data appear to be indicative of the general conditions throughout each watershed, and streams within the same major subwatershed have likely experienced similar impairments or buffers due to these conditions. Drainage area at each site was acquired from Ohio EPA's Division of Surface Water Biological Monitoring and Assessment site (http://wwwapp.epa.ohio.gov/dsw/gis/bio/index.php).

### *2014 Analyses*

Since survey scope and methods changed between 2013 and 2014, data for 2014 were analyzed separately. All analyses were performed in R (R Core Team 2013) unless otherwise noted. All variables were tested using the Shapiro-Wilks test for normality, and variables that were not normally distributed were transformed with $\log_{10}$, $\log_{10}+1$,or square-root prior to analyses, except for abundance and species richness, as these two response variables contained many zeros and should not be transformed (O'Hara and

Kotze 2010). All variables and transformations used are listed in Table 2. Shannon diversity was not used in analyses due to the low abundance at many sites which skewed calculations, and instead species richness served as an adequate measure of diversity. Pearson correlations between response variables (total abundance, richness, and diversity) and environmental variables were examined in SPSS (Ver. 19, IBM Corp.). Threshold values of variables were examined on the basis of presence or absence of live mussels.

Table 2: All variables for 2014 analyses. Summary statistics (minimum, maximum, mean) values are for variables pre-transformation. N=20 for all variables except QHEI and IBI (N=19).

| | Abbreviation | Explanation (units) | Transform (if used) | Minimum | Mean | Maximum |
|---|---|---|---|---|---|---|
| Mussel | | | | | | |
| | N | Total number of live mussels found | | 0 | 13.85 | 149 |
| | R | Species richness, number of species found | | 0 | 1.65 | 6 |
| | Diversity | Shannon diversity index | | 0 | 0.33 | 1.33 |
| Environmental | | | | | | |
| | Drainage | Drainage area at survey site ($km^2$) | $\log_{10}$ | 7.68 | 39.58 | 188.42 |
| | Pebble | Median grain size (mm) | $x^{1/2}$ | 0.030 | 13.55 | 46.50 |
| | Stress | Shear stress estimated at baseflow ($N/m^2$) | $x^{1/2}$ | 1.56 | 4.97 | 11.67 |
| | BankfullStress | Shear stress estimated at bankfull ($N/m^2$) | | 8.87 | 23.69 | 42.03 |
| | Forest | Percent forested land cover in watershed | | 0.24 | 0.39 | 0.46 |
| | Agriculture | Percent agriculture land cover in watershed | | 0.30 | 0.38 | 0.51 |
| | Developed | Percent developed land cover in watershed | | 0.070 | 0.11 | 0.20 |
| | pH | | | 7.47 | 8.03 | 8.50 |
| | Conductivity | Electrical conductivity (mS) | $\log_{10}+1$ | 0.45 | 0.78 | 1.59 |
| | Discharge | Discharge measured at each site ($m^3/s$) | | 0.0010 | 0.11 | 0.60 |
| | BFW | Bankfull channel width (m) | $\log_{10}$ | 5.57 | 10.29 | 25.48 |
| | Width | Baseflow channel width (m) | $\log_{10}$ | 2.42 | 5.90 | 14.62 |
| | BFD | Bankfull water depth (m) | | 0.73 | 1.18 | 1.60 |
| | Depth | Baseflow water depth (m) | $x^{1/2}$ | 0.10 | 0.26 | 0.70 |
| | Slope | Slope of stream reach | | 0.00064 | 0.0022 | 0.0053 |
| | QHEI | Qualitative habitat evaluation index | | 42.5 | 60.1 | 81.5 |
| | IBI | Index of biotic integrity | | 20.0 | 36.0 | 51.0 |
| | Attainment | Aquatic life use attainment status | | 0 | 1.2 | 2 |

First, the means of individual environmental variables were compared between sites where live mussels were present and sites where live mussels were not found using ANOVA, and then the means of all environmental variables were compared using MANOVA.  The associations among environmental variables and the associations between response variables and environmental variables were examined using principal components analysis (PCA).  The latter was done by labeling the points for each site with abundance and species richness, each on a separate biplot.  The advantage of using PCA is the ability to reduce a large number of environmental variables (p=14), which may be correlated, into a smaller number of principal components (usually ~2-4) which are linear combinations of the environmental variables that are not correlated (Johnson and Wichern 2007).  The number of principal components chosen should account for at least 70% of the total variation in the data to avoid much loss of information.  The first *n* components chosen can then be used in linear regression to predict abundance and species richness using stepwise regression and minimizing the Akaike Information Criterion.  This analysis was performed first without the QHEI and IBI variables and then adding these variables, due to the absence of these data from one site.

Next, canonical correlation analysis (CCA) was performed only for sites where live mussels were found.  CCA compares the correlation between linear combinations of two sets of variables and, like PCA, reduces the large number of environmental variables into a few canonical variables (Johnson and Wichern 2007).  The analysis thus gives a visualization of the association of each site and each species of mussel to the environmental variables.

Finally, a generalized linear model was produced using negative binomial and Poisson distributions for mussel abundance and species richness, respectively. Count data that contains a large number of zero's should be examined using a Poisson or negative binomial distribution rather than transforming the response variable to normality (O'Hara and Kotze 2010). This procedure has been used to model count data in other studies, ranging from insect abundance (Gardiner et al. 2014) to highway truck accidents (Miaou 1994). Poisson regression is appropriate when the mean and variance of the response are roughly equal (Ramsey and Schafer 2002). However, a negative binomial distribution is more appropriate when overdispersion (variance > mean) is present (Stamey and Beavers 2009). The best model was selected by minimizing the Akaike Information Criterion (AIC), which prevents overfitting by including a penalty when the number of variables is increased (Kutner et al. 2004). Another advantage of using the AIC is to compare the fit of non-nested models (Long 1997). A chi-squared test of the drop in deviance obtained by adding terms to the model was also used to determine whether additional variables could significantly improve the fit of the Poisson regression model (Ramsey and Shafer 2002). Separate models were produced using only variables that were not correlated significantly with drainage area, and which were identified in PCA and CCA as having stronger association (higher loadings) with abundance and richness than other variables. This procedure was done to remove the effect of stream size on mussel abundance and diversity so that the effect of other environmental variables could be explored.

# CHAPTER III

# RESULTS

## *2013 Eagle Creek Mussel Survey*

A total of 684 live mussels were found compared to only 103 shells in Eagle Creek in 2013 (Table 3). The live specimens were comprised of eight species, with four species accounting for 96% of live mussels. *Lampsilis siliquoidea* dominated the community at 72% of live mussels, followed by *Pyganodon grandis* (12%), *Lasmigona complanata* (7%), and *Strophitus undulatus* (6%). *L. siliquoidea* accounted for between 50-95% of live mussels at each site. The remaining four species (*Lasmigona compressa*, *Utterbackia imbecillis*, *Toxolasma parvum,* and *Elliptio dilatata*) made up the remaining 4% of live mussels. The river mouth was the only site in Eagle Creek where *U. imbecillis* was found. Additionally, three species were recorded only from old shells (no live specimens): *Lampsilis ovata, Amblema plicata,* and *Actinonaias ligamentina*.

Table 3: All live mussels and shells found during 2013 surveys of Eagle Creek, pooled for all sites (N=8 sites).

| **Species** | **Live** | **Shells** |
|---|---|---|
| *Lampsilis siliquoidea* | 493 | 59 |
| *Pyganodon grandis* | 80 | 13 |
| *Lasmigona complanata* | 48 | 12 |
| *Strophitus undulatus* | 39 | 9 |
| *Lasmigona compressa* | 9 | 4 |
| *Utterbackia Imbecillis* | 7 | 1 |
| *Toxolasma parvum* | 5 | 1 |
| *Elliptio dilatata* | 3 | 1 |
| *Lampsilis ovata* | 0 | 1 |
| *Amblema plicata* | 0 | 1 |
| *Actinonaias ligamentina* | 0 | 1 |
| **TOTAL** | **684** | **103** |

The results of two sites surveyed on the Mahoning River yielded only two species of live mussels (*L. siliquoidea* and *U. imbecillis*) and another species (*P. grandis*) represented only by shells.

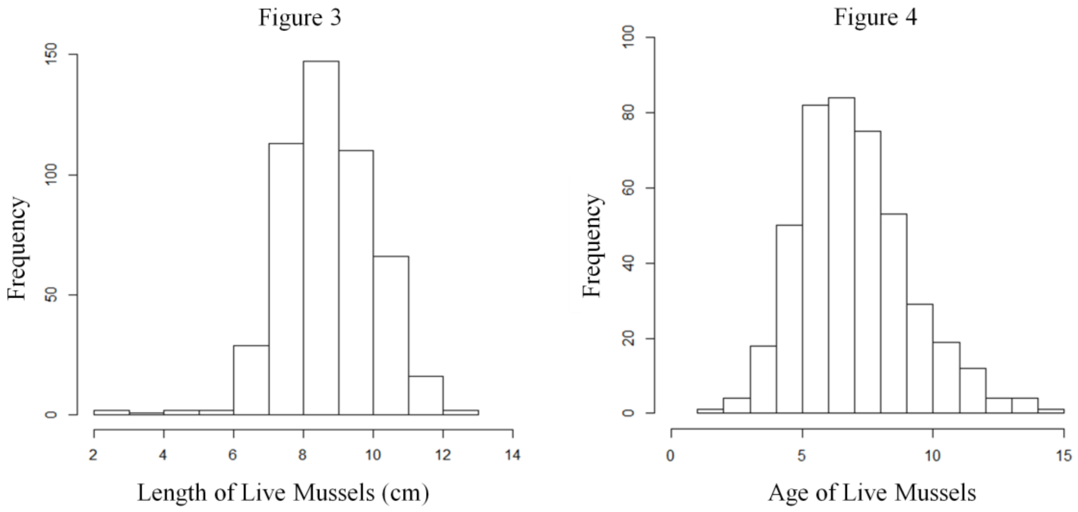
Abundance (N=# live mussels), species richness (R=# species) and Shannon diversity (H') for each site in Eagle Creek are shown in Table 4. Site numbers correspond to their position along Eagle Creek, with site 1 furthest upstream and site 8 at the river mouth. Live mussels were found at all sites, with the highest abundance at site 2 (N=171) and the lowest at site 4 (N=24). Species richness was similar at most sites, with four to six species found at each site except for site 1, where only 2 species were found (*L. siliquoidea* and *S. undulatus*). Shannon diversity fell into three categories from high to low. The highest diversity was found at sites 4 (H'=1.29), 5 (H'=1.34), and 8 (H'=1.29). Sites 5 and 8 also had the highest species richness (R=6). Sites 2, 3, 6, and 7 had intermediate diversity, with H' around 0.8-0.9. Site 1 had low diversity (H'=0.11), a result of the low species richness and the presence of only two individuals of *S.*

*undulatus*. As can be seen from Table 4, there does not appear to be any consistent pattern between distance from the river mouth and abundance, species richness, or diversity, with the exception of low richness and diversity at site 1.

Table 4: Mussels collected in Eagle Creek with sites numbered in order from upstream (Site 1) to downstream (Site 8). N=total number live mussels found. R=species richness, or number of live species found. Diversity=Shannon diversity index.

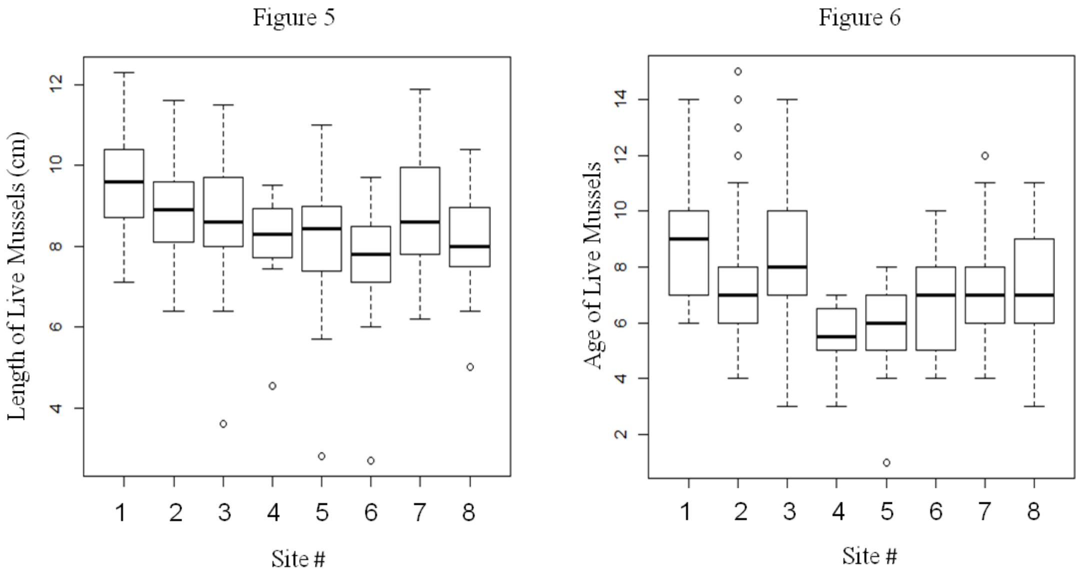
| | **Site 1** | **Site 2** | **Site 3** | **Site 4** | **Site 5** | **Site 6** | **Site 7** | **Site 8** | **Total** |
|---|---|---|---|---|---|---|---|---|---|
| *Lampsilis siliquoidea* | 89 | 129 | 47 | 12 | 18 | 55 | 103 | 40 | 493 |
| *Pyganodon grandis* | 0 | 19 | 9 | 2 | 6 | 8 | 21 | 15 | 80 |
| *Lasmigona complanata* | 0 | 2 | 1 | 3 | 4 | 12 | 22 | 4 | 48 |
| *Strophitus undulatus* | 2 | 18 | 11 | 0 | 2 | 1 | 0 | 5 | 39 |
| *Lasmigona compressa* | 0 | 1 | 0 | 6 | 1 | 0 | 1 | 0 | 9 |
| *Utterbackia Imbecillis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 7 |
| *Toxolasma parvum* | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 1 | 5 |
| *Elliptio dilatata* | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 3 |
| **N** | 91 | 171 | 68 | 24 | 33 | 77 | 148 | 72 | 684 |
| **R** | 2 | 5 | 4 | 5 | 6 | 5 | 5 | 6 | 8 |
| **Diversity** | 0.11 | 0.83 | 0.88 | 1.29 | 1.34 | 0.88 | 0.88 | 1.29 | 1.00 |

Average shell size (N=493) and age (N=436) was computed for live *L. siliquoidea* from all sites in Eagle Creek in 2013 (Figures 3 and 4). Fewer mussels were used in age estimates due to the inability to accurately count growth rings on older mussels and severely worn mussels. The average length for all sites was 8.75cm (median=8.64cm), with a minimum length of 2.7cm and a maximum of 12.3cm, with almost 99% of individuals 6.0cm and longer. The average age was 7.47 years (median=7 years), with a minimum age of 1 year and a maximum of 15 years. Only 5.3% of live mussels were age 4 or younger, and 1.1% were below age 4.



Figures 3 and 4: Lengths (Fig. 3) and ages (Fig. 4) of live *L. siliquoidea* found in Eagle Creek during 2013 surveys measured as the longest length of the shell from anterior to posterior and estimated age by counting external growth lines.

Results of one-way ANOVA comparing average length and average age of live *L. siliquoidea* between sites showed that there was a significant difference among sites ($p<0.0001$ for both length and age). A Tukey test of honest significant difference was used to compare sites based on age and length. There does not appear to be much variation in length among sites but the largest and oldest mussels on average were found at site 1 (Figures 5 and 6). Mussels at site 1 were significantly larger (mean=9.7cm; $p<0.0001$) than those from all other sites, while differences among all other sites were not significant. Site 1 also had the oldest mussels (mean=9.0 years), significantly older than all but site 3 (mean=8.6 years) which was significantly older than the rest of the sites except for site 8 (mean=7.4). Although not significantly different than other sites, site 4 had the youngest (mean=5.6 years) and the second smallest mussels (mean=8.1cm), behind site 6 (mean=7.7cm). Although the oldest and largest mussels were found furthest upstream, there did not appear to be any other pattern moving downstream on Eagle Creek.



Figures 5 and 6: Lengths (Fig. 5) and ages (Fig. 6) of live *L. siliquoidea* found at each site in Eagle Creek during 2013 surveys. Dark lines inside each box represent the mean length and age for that site. Lower and upper edges of boxes correspond to first and third quartiles, respectively. Whiskers extend to most extreme points that lie within 1.5 times the interquartile range (quartile 3-quartile 1) from the box.

*2014 Upper Mahoning River Watershed Survey*

Mussels were present at ten of the twenty sites surveyed throughout the upper Mahoning River watershed in 2014 and totaled 277 live individuals (Table 5). Eight species of mussels were found, with a maximum of six species at one site. The highest abundance was 149 live mussels with the next highest falling to 44, then just 35 live mussels. Abundance was very low at all remaining sites (N≤13). The most abundant species was *E. dilatata* (N=144), most of which (N=117) were found at one site, and this species was only found at two sites. The second most abundant was *L. siliquoidea* (N=75), followed by *P. grandis* (N=20). These two mussels remained the most widespread, and were found at seven sites, while *L. compressa* and *S. undulatus* were found at 4 sites each, and one species, *Amblema plicata*, was again found only as an old shell in Eagle Creek in 2014. One additional species, *Lasmigona costata*, was found live in Eagle Creek in 2014, although it had not been found in the larger 2013 surveys. Shells were found at three sites where no live mussels were found and only consisted of long-dead *L. siliquoidea* and *P. grandis* with one fresh dead *L. siliquoidea* shell at site 13 still containing tissue.

Table 5: Results for live mussels found during 2014 surveys. Only sites with live mussels are shown. N=total number live mussels found. R=species richness, or number of live species found. Diversity=Shannon diversity index.

| **Site** | **1** | **4** | **5** | **6** | **8** | **10** | **12** | **14** | **15** | **17** | **Total** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *L. complanata* | 0 | 0 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| *L. compressa* | 9 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 1 | 14 |
| *L. siliquoidea* | 0 | 39 | 2 | 15 | 0 | 8 | 5 | 5 | 0 | 1 | 75 |
| *A. ligamentina* | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *P. grandis* | 0 | 4 | 2 | 6 | 5 | 0 | 0 | 1 | 1 | 1 | 20 |
| *S. undulatus* | 1 | 0 | 2 | 6 | 5 | 0 | 0 | 0 | 0 | 0 | 14 |
| *E. dilatata* | 0 | 0 | 0 | 117 | 0 | 27 | 0 | 0 | 0 | 0 | 144 |
| *L. costata* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| **N** | 10 | 44 | 10 | 149 | 13 | 35 | 5 | 7 | 1 | 3 | 277 |
| **R** | 2 | 3 | 4 | 6 | 4 | 2 | 1 | 3 | 1 | 3 | 8 |
| **Diversity** | 0.33 | 0.41 | 1.33 | 0.81 | 1.22 | 0.54 | 0 | 0.8 | 0 | 1.1 | 1.33 |

Significant correlations among response and environmental variables from 2014 surveys were mainly associated with measures of stream size (Table 6). Drainage area and discharge were the variables most strongly correlated with abundance and species richness, both with a positive relationship, indicating larger and more diverse mussel communities in larger streams in the upper Mahoning River watershed. Drainage area and discharge were also significantly positively correlated with discharge, width, bankfull depth, and bankfull width, indicating that larger streams have larger drainage areas. The negative correlation between drainage area and slope indicated flattening of the terrain as one moves into larger streams. Drainage area was also positively correlated with aquatic life attainment status, indicating better aquatic communities in larger streams in the area. Abundance and species richness were significantly positively correlated with the EPA variable QHEI but not with the EPA variable IBI. QHEI and IBI were significantly correlated with each other and with drainage area. IBI was also correlated significantly and positively with percent forest land cover and negatively with percent agricultural land cover. Overall, larger streams in the area appear to have larger and more diverse populations of mussels, along with better habitat.

Neither conductivity nor land use variables were individually correlated with abundance or species richness, but land use did appear to have an effect on conductivity, and possibly levels of pollutants, in this system. Conductivity was positively correlated with percent agriculture and developed land but negatively correlated with percent forest. Percent forest was also inversely related to percent agriculture and percent developed land. However, agriculture and developed land were significantly correlated with each other.

Table 6: Pearson correlations (top number) and p-value (bottom number) for all variables used in 2014 analyses;  * indicates significance at the p<0.05 level; ** indicates significance at the p<0.005 level.

| | N | R | Drainage | Attainment | Pebble | Stress | BankfullStress | Forest | Agriculture | Developed |
|---|---|---|---|---|---|---|---|---|---|---|
| N | 1 | 0.700** | 0.688** | 0.290 | 0.077 | -0.270 | -0.374 | 0.311 | -0.323 | -0.053 |
| | | 0.001 | 0.001 | 0.214 | 0.747 | 0.249 | 0.104 | 0.183 | 0.165 | 0.824 |
| R | | 1 | 0.847** | 0.397 | -0.059 | -0.343 | -0.395 | 0.383 | -0.322 | -0.278 |
| | | | <0.001 | 0.083 | 0.804 | 0.139 | 0.085 | 0.096 | 0.167 | 0.235 |
| Drainage | | | 1 | 0.646** | -0.025 | -0.362 | -0.407 | 0.395 | -0.403 | -0.103 |
| | | | | 0.002 | 0.918 | 0.116 | 0.075 | 0.085 | 0.078 | 0.666 |
| Attainment | | | | 1 | 0.265 | 0.041 | 0.047 | 0.393 | -0.409 | -0.086 |
| | | | | | 0.259 | 0.865 | 0.845 | 0.087 | 0.073 | 0.718 |
| Pebble | | | | | 1 | 0.097 | 0.213 | 0.292 | -0.277 | -0.213 |
| | | | | | | 0.685 | 0.367 | 0.211 | 0.236 | 0.366 |
| Stress | | | | | | 1 | 0.664** | 0.266 | -0.294 | -0.047 |
| | | | | | | | 0.001 | 0.257 | 0.209 | 0.843 |
| BankfullStress | | | | | | | 1 | 0.058 | -0.006 | -0.222 |
| | | | | | | | | 0.809 | 0.979 | 0.347 |
| Forest | | | | | | | | 1 | -0.950** | -0.526* |
| | | | | | | | | | 0.000 | 0.017 |
| Agriculture | | | | | | | | | 1 | 0.243 |
| | | | | | | | | | | 0.301 |
| Developed | | | | | | | | | | |
| | | | | | | | | | | 1 |

Table 6 (continued): Pearson correlations (top number) and p-value (bottom number) for all variables used in 2014 analyses; * indicates significance at the p<0.05 level; ** indicates significance at the p<0.005 level.

| | pH | Conductivity | Discharge | BFD | BFW | Width | Depth | Slope | QHEI | IBI |
|---|---|---|---|---|---|---|---|---|---|---|
| N | 0.271 | -0.258 | 0.658** | 0.400 | 0.533* | 0.490* | 0.210 | -0.393 | 0.540* | 0.338 |
| | 0.247 | 0.273 | 0.002 | 0.080 | 0.015 | 0.028 | 0.375 | 0.087 | 0.017 | 0.158 |
| R | 0.450* | -0.321 | 0.657** | 0.386 | 0.592** | 0.674** | 0.160 | -0.434 | 0.487* | 0.321 |
| | 0.047 | 0.168 | 0.002 | 0.093 | 0.006 | 0.001 | 0.501 | 0.056 | 0.034 | 0.181 |
| Drainage | 0.523* | -0.335 | 0.760** | 0.531* | 0.767** | 0.764** | 0.221 | -0.518* | 0.612* | 0.614* |
| | 0.018 | 0.148 | <0.001 | 0.016 | <0.001 | <0.001 | 0.348 | 0.019 | 0.005 | 0.005 |
| Attainment | 0.355 | -0.259 | 0.413 | 0.178 | 0.493* | 0.462* | 0.053 | 0.005 | 0.737** | 0.884** |
| | 0.124 | 0.270 | 0.071 | 0.453 | 0.027 | 0.040 | 0.823 | 0.984 | <0.001 | <0.001 |
| Pebble | -0.304 | -0.040 | -0.271 | -0.528* | 0.155 | -0.021 | -0.544* | 0.359 | 0.200 | 0.269 |
| | 0.193 | 0.866 | 0.248 | 0.017 | 0.515 | 0.929 | 0.013 | 0.120 | 0.413 | 0.265 |
| Stress | 0.178 | -0.243 | -0.263 | 0.024 | -0.195 | -0.011 | 0.448* | 0.610** | 0.245 | 0.205 |
| | 0.453 | 0.303 | 0.263 | 0.922 | 0.410 | 0.962 | 0.049 | 0.004 | 0.312 | 0.399 |
| BankfullStress | -0.101 | 0.076 | -0.461* | -0.288 | -0.081 | -0.171 | -0.263 | 0.894** | 0.183 | 0.163 |
| | 0.672 | 0.749 | 0.041 | 0.218 | 0.734 | 0.472 | 0.262 | <0.001 | 0.452 | 0.505 |
| Forest | 0.300 | -0.703** | 0.391 | 0.189 | 0.353 | 0.406 | 0.322 | 0.043 | 0.374 | 0.456 |
| | 0.199 | 0.001 | 0.088 | 0.425 | 0.127 | 0.076 | 0.166 | 0.857 | 0.115 | 0.050 |
| Agriculture | -0.348 | 0.613** | -0.414 | -0.271 | -0.392 | -0.501* | -0.423 | 0.027 | -0.377 | -0.468* |
| | 0.133 | 0.004 | 0.069 | 0.247 | 0.087 | 0.025 | 0.063 | 0.909 | 0.111 | 0.043 |
| Developed | 0.130 | 0.482* | -0.011 | 0.133 | -0.048 | 0.091 | 0.154 | -0.230 | -0.100 | -0.126 |
| | 0.584 | 0.031 | 0.963 | 0.577 | 0.840 | 0.701 | 0.516 | 0.329 | 0.682 | 0.606 |

Table 6 (continued): Pearson correlations (top number) and p-value (bottom number) for all variables used in 2014 analyses; * indicates significance at the p<0.05 level; ** indicates significance at the p<0.005 level.

| | pH | Conductivity | Discharge | BFD | BFW | Width | Depth | Slope | QHEI | IBI |
|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1 | -0.442 | 0.541* | 0.428 | 0.388 | 0.542* | 0.431 | -0.191 | 0.339 | 0.416 |
| | | 0.051 | 0.014 | 0.060 | 0.091 | 0.014 | 0.058 | 0.421 | 0.155 | 0.077 |
| Conductivity | | 1 | -0.329 | -0.337 | -0.095 | -0.165 | -0.433 | 0.114 | -0.187 | -0.271 |
| | | | 0.157 | 0.146 | 0.689 | 0.488 | 0.057 | 0.633 | 0.444 | 0.261 |
| Discharge | | | 1 | 0.576** | 0.575** | 0.585** | 0.420 | -0.506* | 0.309 | 0.348 |
| | | | | 0.008 | 0.008 | 0.007 | 0.065 | 0.023 | 0.198 | 0.144 |
| BFD | | | | 1 | 0.504* | 0.613** | 0.735** | -0.624** | 0.110 | 0.198 |
| | | | | | 0.023 | 0.004 | <0.001 | 0.003 | 0.653 | 0.415 |
| BFW | | | | | 1 | 0.874** | 0.121 | -0.279 | 0.548* | 0.512* |
| | | | | | | 0.000 | 0.610 | 0.233 | 0.015 | 0.025 |
| Width | | | | | | 1 | 0.414 | -0.363 | 0.634* | 0.530* |
| | | | | | | | 0.069 | 0.116 | 0.004 | 0.020 |
| Depth | | | | | | | 1 | -0.404 | 0.098 | 0.121 |
| | | | | | | | | 0.077 | 0.691 | 0.622 |
| Slope | | | | | | | | 1 | 0.158 | 0.079 |
| | | | | | | | | | 0.517 | 0.747 |
| QHEI | | | | | | | | | 1 | 0.704 |
| | | | | | | | | | | <0.001 |
| IBI | | | | | | | | | | 1 |

Of the four major subwatersheds in the upper Mahoning River, the Mahoning headwaters had the highest proportion of agricultural and urban land use, and the subwatershed directly north, Deer Creek, had the second highest proportion of agricultural and urban land use. Only 4% of live mussels and just four species were found in these two subwatersheds. The next subwatershed, West Branch Mahoning River, had 14% of the live mussels found but only of two species, and its level of agricultural land use was intermediate between the Eagle Creek and Deer Creek watersheds. The northernmost subwatershed was that of Eagle Creek, which flowed through the highest proportion of forested land and possessed the least amount of agricultural and developed land. While 40% of sites were located in this subwatershed, it supported 82% of live mussels and eight species were found in 2014. Of the three sites without live mussels but where shells were found, two were located in the Deer Creek watershed and one in the Mahoning Headwaters.

There appeared to be no difference in environmental conditions on average between sites with mussels and sites without mussels. Combining all variables for MANOVA indicated that no significant differences existed between sites where mussels were or were not present (p=0.17), although power was low since *n* (# observations= 10) for each group was smaller than *p* (# environmental variables=14). Individual variables were compared using ANOVA (Table 7), and two variables showed significant differences between sites with mussels and sites without mussels. Width was significantly different (p=0.039), and larger on average for sites with mussel (7.16m) than for sites without mussels (4.64m). Drainage area was also significantly larger (p=0.0041) for sites with mussels (61.11km$^2$) than for sites without mussels (18.05 km$^2$). These results are not unexpected given that drainage area and width were strongly correlated with mussel abundance. Overall, though, there was no evidence for a difference between sites with mussels and sites without mussels.

Table 7: Means of environmental variables and results of ANOVA comparing sites with live mussels to sites without live mussels presents. Means given are for untransformed variables.

| | **Drainage** | **Pebble** | **Stress** | **Bankfull Stress** | **Forest** |
|---|---|---|---|---|---|
| **No mussels** | 18.05 | 13.95 | 5.51 | 24.3 | 0.37 |
| **Mussels** | 61.11 | 13.15 | 4.43 | 23.1 | 0.42 |
| **p-value** | 0.0041 | 0.94 | 0.65 | 0.79 | 0.13 |

| | **Agriculture** | **Developed** | **pH** | **Conductivity** | **Discharge** |
|---|---|---|---|---|---|
| **No mussels** | 0.401 | 0.129 | 7.93 | 0.89 | 0.04 |
| **Mussels** | 0.355 | 0.098 | 8.14 | 0.67 | 0.17 |
| **p-value** | 0.27 | 0.091 | 0.093 | 0.12 | 0.073 |

| | **BFD** | **BFW** | **Width** | **Depth** | **Slope** |
|---|---|---|---|---|---|
| **No mussels** | 1.13 | 8.63 | 4.64 | 0.27 | 0.00235 |
| **Mussels** | 1.23 | 11.94 | 7.16 | 0.26 | 0.002 |
| **p-value** | 0.32 | 0.089 | 0.030 | 1 | 0.52 |

The EPA variables QHEI and IBI were not included in MANOVA to maintain a balanced design to improve the reliability of the results. However, they were tested individually using a one-tailed t-test under the alternate hypothesis that each measure was greater for sites with live mussels present. The results indicated that QHEI was greater on average (p=0.04) for sites with mussels (QHEI=64.6) than sites without mussels (QHEI=56.1). There was no significant difference in IBI (p=0.19), although IBI was slightly higher for sites with mussels (IBI=38) than sites without mussels (IBI=34).

Although there were few correlations with environmental variables and few significant differences between sites with and without mussels, several threshold effects suggested that other environmental variables impose limits on the presence of mussels. No mussels were found at sites with conductivity greater than 0.9mS, which was also correlated with the land use variables. Only one live mussel was found at sites with drainage area less than 20.5km$^2$, width less than 4.5m and bankfull width less than 7.5m. Depth and bankfull depth did not appear to show a threshold. Only one live mussel was found at sites with QHEI score of 54 or less. Only four live mussels were found at sites in the non-attainment category for aquatic life use, but four out of the 11 sites with full aquatic life attainment status also had no live mussels. Of these sites, two had the highest observed bankfull shear stress (one of which had drainage of just 10.8km$^2$), one was located in the Mahoning Headwaters watershed (highest agricultural and urban land use), and the last had a drainage of only 18.4km$^2$.

The results of the principal components analysis (PCA, without the QHEI and IBI variables), indicated that the first four components explained 83% of the total variation in the environmental data (Table 8), and these four components were retained. The loadings for the first component (37% of total variation) appeared to be an average of most of the variables (loadings ~0.25-0.35), slightly weighted toward stream size. Drainage area, discharge, and width had the strongest association with component 1. Bankfull shear stress was not a strong factor in component 1, but was negatively associated with forested land cover. Forested land and agricultural land were negatively associated with each other, while conductivity was positively associated with agricultural land, mirroring the correlations observed above. Stream slope was negatively associated with the baseflow and bankfull width and depth measurements.

Table 8: Principal components and loadings for PCA without EPA variables. Proportion of variance is the contribution of each component to the overall variation in the environmental data. Loadings less than 0.1 are not shown.

| Variable | Comp 1 | Comp 2 | Comp 3 | Comp 4 |
|---|---|---|---|---|
| Drainage | 0.354 | | 0.251 | |
| Pebble | | -0.247 | 0.496 | |
| BankfullStress | -0.184 | -0.37 | -0.116 | -0.398 |
| Stress | | -0.375 | -0.463 | -0.18 |
| Forest | 0.241 | -0.41 | 0.111 | 0.192 |
| Agriculture | -0.264 | 0.356 | | |
| Developed | | 0.321 | -0.207 | -0.356 |
| pH | 0.278 | | -0.184 | -0.188 |
| Conductivity | -0.225 | 0.295 | | -0.416 |
| Discharge | 0.351 | | | |
| BFD | 0.329 | 0.127 | -0.238 | |
| BFW | 0.297 | | 0.281 | -0.414 |
| Width | 0.344 | | | -0.393 |
| Depth | 0.268 | | -0.465 | 0.11 |
| Slope | -0.254 | -0.382 | | -0.278 |
| **Proportion of Variance** | 0.37 | 0.21 | 0.14 | 0.10 |
| **Cumulative Proportion** | 0.37 | 0.58 | 0.73 | 0.83 |

Component 2 (21% of total variation) appeared to be a measure of the effect of land use on other environmental variables. Forested land was positively associated with bankfull and baseflow shear stress, which was the opposite of component 1. This result seemed unexpected, as forested land should buffer against shear stress by maintaining floodplain connectivity, but this relationship may just be a result of the positive association of slope with forested land in this component. Conductivity was again associated with agricultural and developed land, indicating a measure of the effect of land use on water quality in this component. Grain size was also positively associated with shear stress, indicating stream bed instability, as smaller particles would not be present in unstable sediments with increased shear stress.

Component 3 accounted for 14% of the total variation and was mainly a measure of pebble size, baseflow stress, and depth (loading>0.4). Baseflow shear stress was negatively associated with pebble size and positively associated with depth, which could be a measure of streambed stability, as smaller grain size in the presence of increased shear stress would indicate stable sediments. Component 4 (10% of total variation) was mainly a measure of baseflow and bankfull width, conductivity, developed land cover, and bankfull stress. All five variables were positively associated with each other in this component, which appeared to be a measure of stream size and the effect of developed land on conductivity.

Backward stepwise multiple regression with these four components showed that components 1 and 3 were most useful in predicting species richness ($R^2$=0.57) and abundance ($R^2$=0.40). Both components were significant ($p \leq 0.05$) for species richness, while only component 1 was significant for abundance. However, component 3 was not strongly significant ($p$=0.038) for the species richness model, and may not be significant due to relaxation of the assumption for normality in linear regression. However, component 1 was highly significant for both species richness ($p<0.001$) and abundance ($p$=0.004) and alone still explained 48% and 34% of variation, respectively. In both models, the parameter estimates were positive for component 1 and negative for component 3. This indicates that increased stream size (component 1) was associated with increased mussel abundance and species richness. Component 3 had positive loading for grain size and negative loading for baseflow shear stress, so a negative value for component 3 suggests that smaller grain size and increased bed stability are associated with increased abundance and richness.

The biplot for the first two principal components (Figure 7), which explained 58% of variation, shows sites plotted as points with the label for each site corresponding to the number of live mussels found at that site. The five sites without mussels in the upper-left portion of the biplot were associated with increased agricultural and developed land and increased conductivity. Two of these sites were in the full attainment category for aquatic life use. Towards the bottom, other sites with few or no mussels were associated with increased baseflow and bankfull shear stress. The highest abundance sites at the right side of the biplot were associated with increased baseflow and bankfull depth and width, as well as increased discharge, indicating larger streams. These sites were also associated with lower baseflow and bankfull shear stress, smaller grain size, and decreased agricultural and developed land. Species richness followed the same patterns described above (Figure 8).

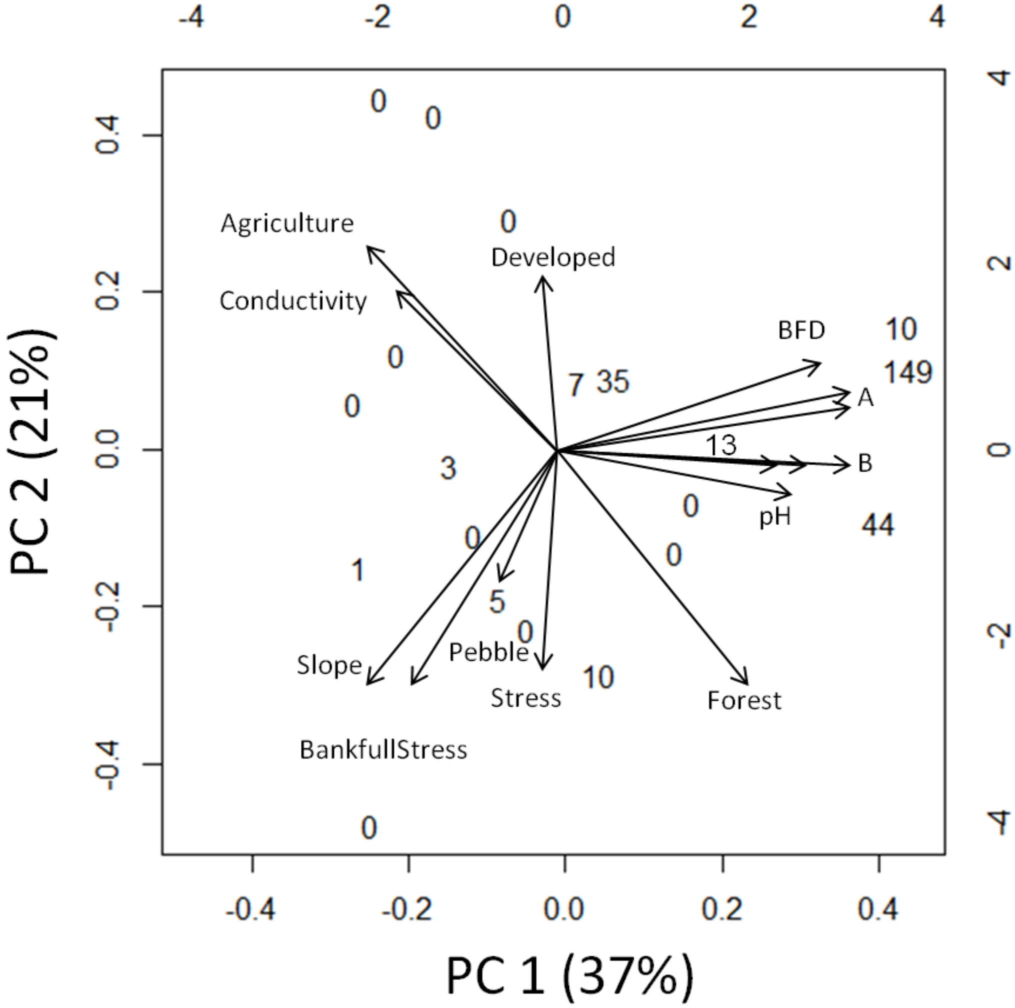


Figure 7: Biplot for the first two principal components, which account for 58% of the total variation in the environmental data. Points represent sites and are labeled with the abundance of live mussels at that site. Arrows indicate increasing influence of each variable, with length corresponding to loadings from Table 6 and direction corresponding to association with each component. The two arrows represented by the letter A indicate, from top to bottom, discharge and drainage area. The three arrows represented by the letter B represent, from left to right, depth, bankfull width (BFW), and width.

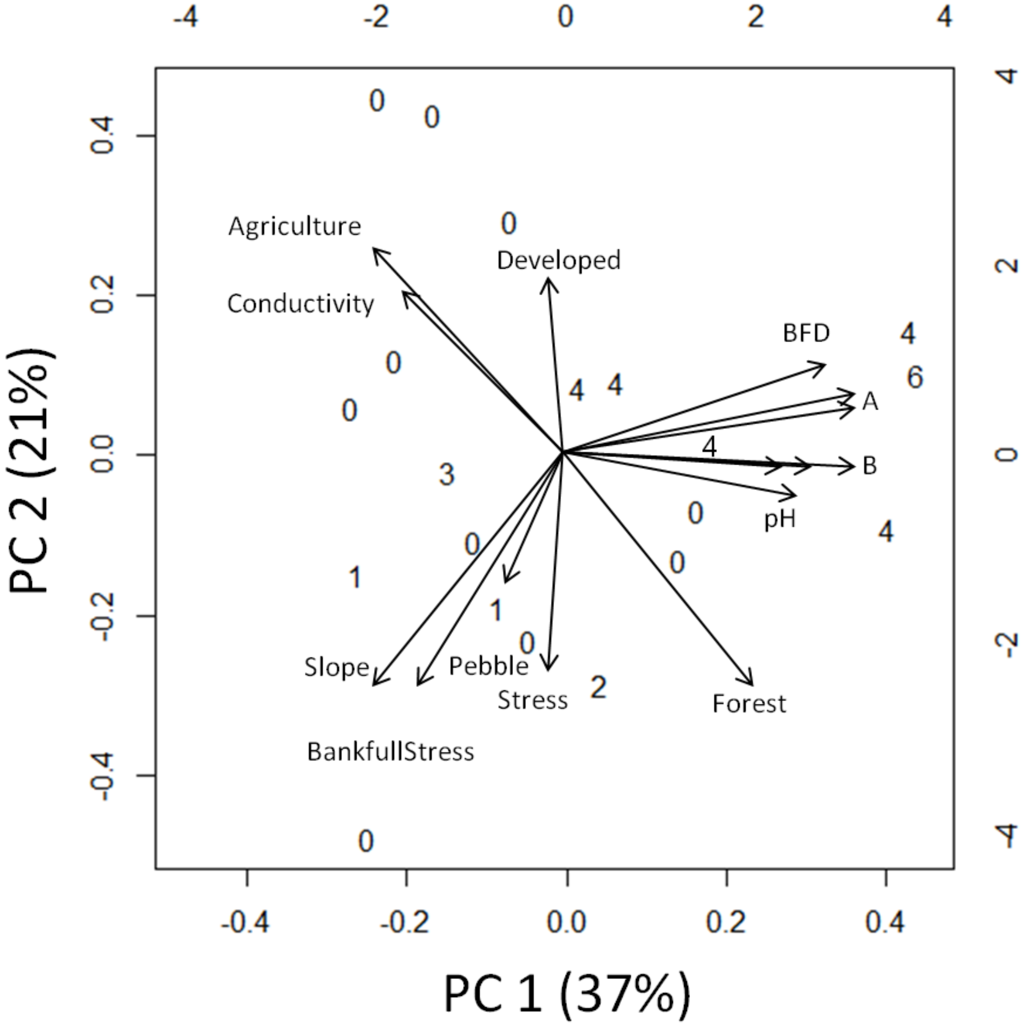


Figure 8: PCA biplot for the first two principal components with points labeled with species richness of live mussels at that site. The two arrows represented by the letter A indicate, from top to bottom, discharge and drainage area. The three arrows represented by the letter B represent, from left to right, depth, bankfull width (BFW), and width.

Component 3 was also plotted against component 1 (Figure 9) in the same manner as Figure 8.  Few or no live mussels grouped to the left side of the biplot and an intermediate to high abundance on the right side.  Therefore, abundance appeared to increase from the lower left to the upper right portion of the plot, going from increased shear stress, conductivity, and agricultural and developed land in the lower left to increased stream size in the upper right.  Again, the same pattern was observed for species richness.

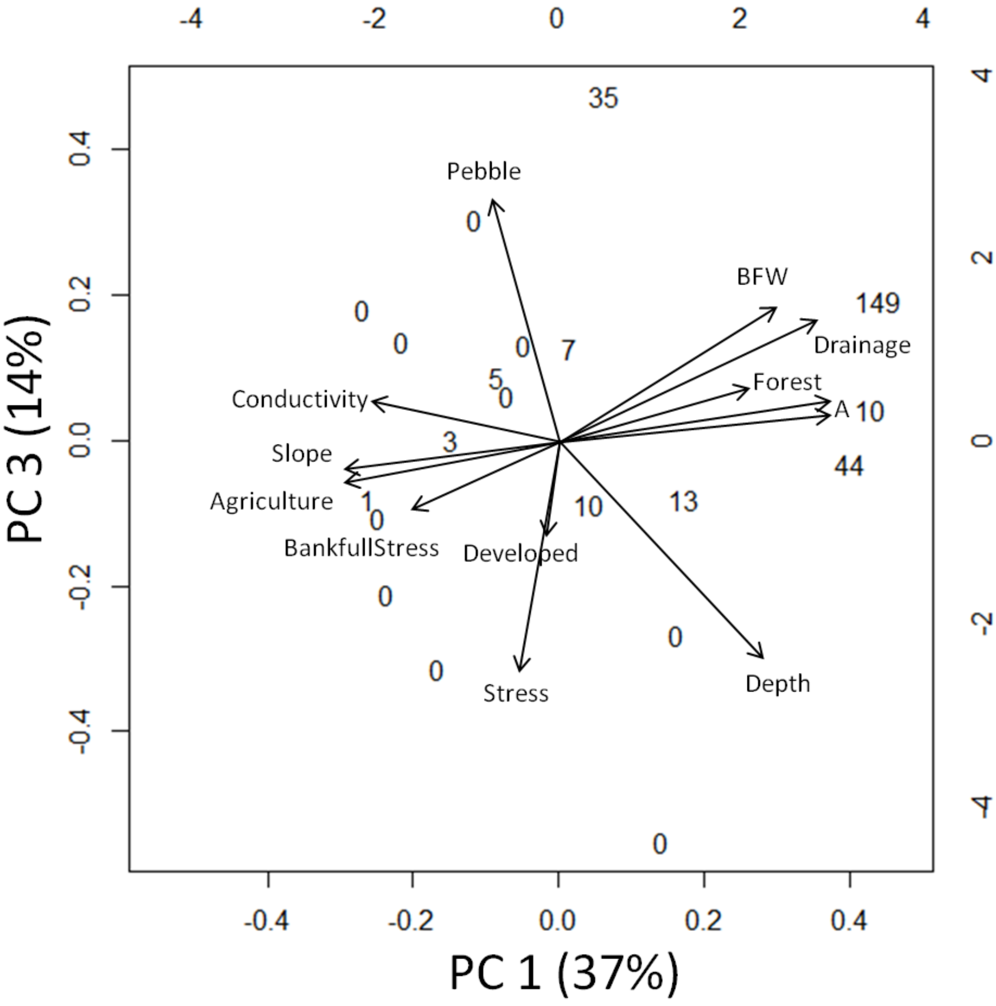


Figure 9: PCA biplot for components 1 and 3, which account for 51% of the total variation in the environmental data. Points are labeled with abundance of live mussels at that site. The two arrows at the right labeled with the letter A represent, from top to bottom, width and discharge.

When the QHEI and IBI variables were added back to the PCA analysis, with one site removed, the first four components still explained 79% of the total variation and the patterns were similar to those above. All four components had loadings similar in sign and magnitude to those in the PCA without QHEI and IBI (Table 9). The biplot for the first two components (Figure 10) suggested that most of the sites without mussels were associated with increased developed and agricultural land, increased conductivity, increased baseflow and bankfull shear stress, and increased grain size. Conductivity was once again closely positively associated with agricultural land use. Increased QHEI and IBI scores were negatively associated with agriculture and positively associated with forest land cover and appeared more associated with sites with live mussels present, although not very strongly.

Table 9: Principal components and loadings for PCA with EPA variables. Proportion of variance is the contribution of each component to the overall variation in the environmental data. Loadings less than 0.1 are not shown.

| **Variable** | **Comp.1** | **Comp.2** | **Comp.3** | **Comp.4** |
|---|---|---|---|---|
| Drainage | 0.341 | | 0.279 | |
| Pebble | | -0.329 | 0.369 | 0.166 |
| BankfullStress | | -0.407 | | -0.346 |
| Stress | | -0.305 | -0.442 | -0.303 |
| Forest | 0.267 | -0.29 | | 0.335 |
| Agriculture | -0.285 | 0.237 | 0.105 | -0.226 |
| Developed | | 0.27 | | -0.424 |
| pH | 0.275 | | -0.139 | -0.177 |
| Conductivity | -0.219 | 0.159 | 0.268 | -0.39 |
| Discharge | 0.305 | 0.173 | | |
| BFD | 0.271 | 0.245 | -0.223 | |
| BFW | 0.297 | | 0.316 | -0.171 |
| Width | 0.353 | | 0.156 | -0.217 |
| Depth | 0.229 | 0.133 | -0.471 | |
| Slope | -0.14 | -0.449 | | -0.243 |
| QHEI | 0.261 | -0.19 | 0.208 | -0.246 |
| IBI | 0.277 | -0.19 | 0.179 | -0.144 |
| **Proportion of Variance** | 0.34 | 0.21 | 0.14 | 0.10 |
| **Cumulative Proportion** | 0.34 | 0.55 | 0.69 | 0.79 |

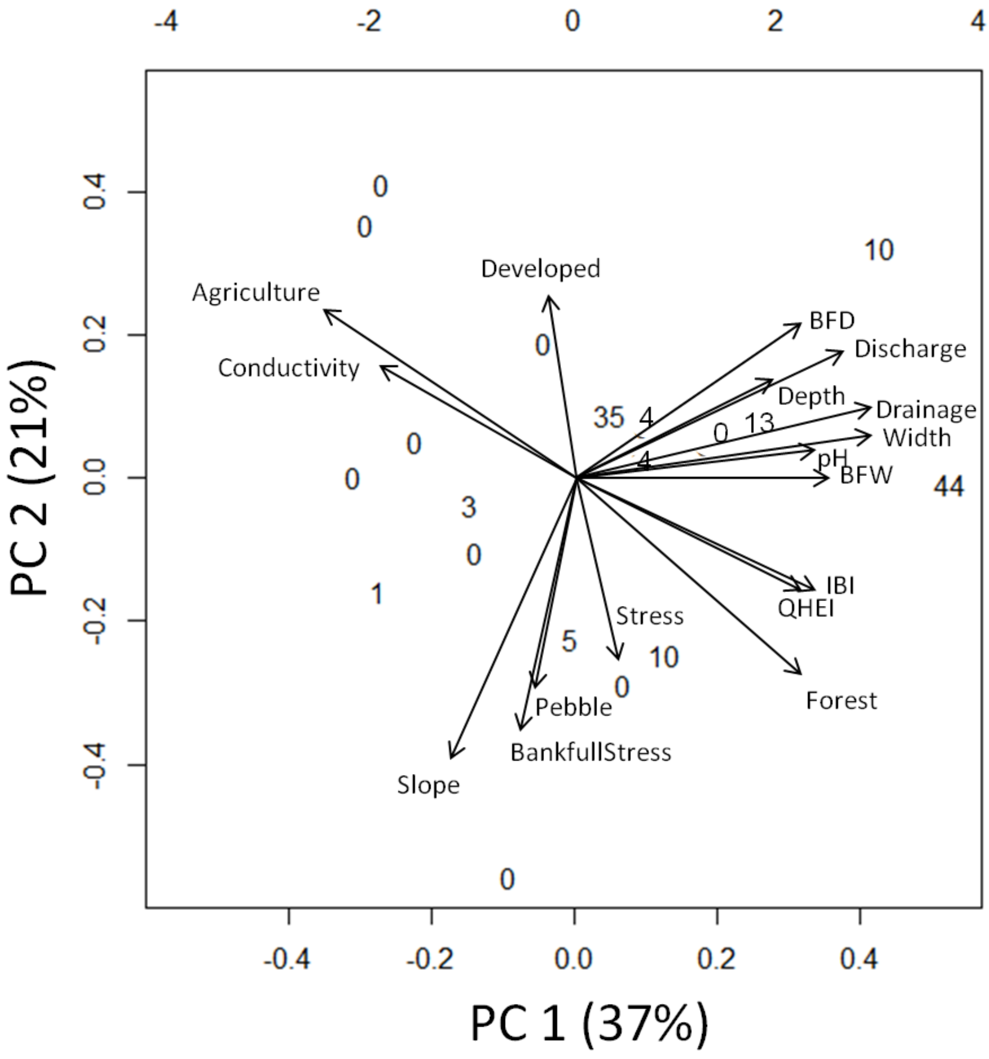


Figure 10: PCA biplot for the first two principal components based on loadings from Table 7, which account for 54% of the total variation in the environmental data. Points are labeled with abundance of live mussels at that site.

The results of the canonical correspondence analysis (CCA) (Figures 11-13) tested variation among sites where mussels were found and among species (points) as they related to environmental variables (arrows). The first three axes explained 88% of the variation among species and sites with respect to environmental variables. The first two axes explained 39% and 29% of the variation, respectively (Figure 11). *Lampsilis siliquoidea* and *P. grandis* were found above the zero line for axis 2, which is associated with increased baseflow and bankfull shear stress, agricultural land use, conductivity, bankfull depth, and pH. *Elliptio dilatata* was associated with increased discharge and grain size. *Lasmigona compressa*, and to a lesser degree *S. undulatus*, were associated with lower conductivity and intermediate shear stress. *L. compressa* in particular appeared to be associated with smaller streams, as it was located far to the left on axis 1, in the opposite direction of discharge. *Lasmigona complanata* was relatively in the center, but slightly associated with increased forest and discharge. Two species, *A. ligamentina* and *L. costata* were each represented by one individual, and may not provide an accurate interpretation for these species' association with environmental variables.

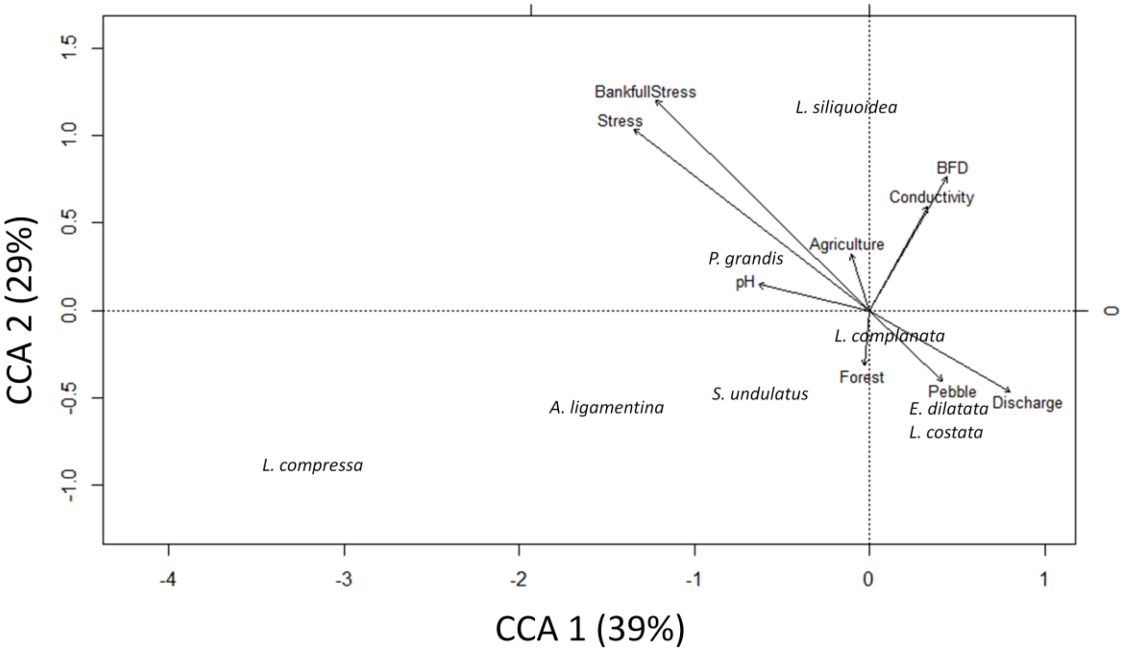


Figure 11: Plot of the first two canonical axes for CCA, which account for 68% of the variation in environmental data. Arrows indicate increasing influence of each variable, with length corresponding degree of influence and direction corresponding to association with each axis. Species are plotted according to their association with each environmental variable.

Since the CCA analyses was 3 dimensional, plots of axes 1 and 3 (Figure 12), which account for 59% of the variation, and axes 2 and 3 (Figure 13), which account for 49% of the variation, were examined and a few relationships mentioned above remained apparent. First, *L. siliquoidea* always appeared to be associated with increased shear stress and agriculture, although agriculture did not have a strong effect, decreased discharge, and with increased conductivity, more so than all other species. *Lasmigona compressa* appeared to have the greatest preference for decreased discharge after *L. siliquoidea*. *Elliptio dilatata* was most often associated with increased grain size, as was

*L. compressa* to a lesser degree. *Pyganodon grandis, L. complanata* and *S. undulatus* were associated with increased pH.

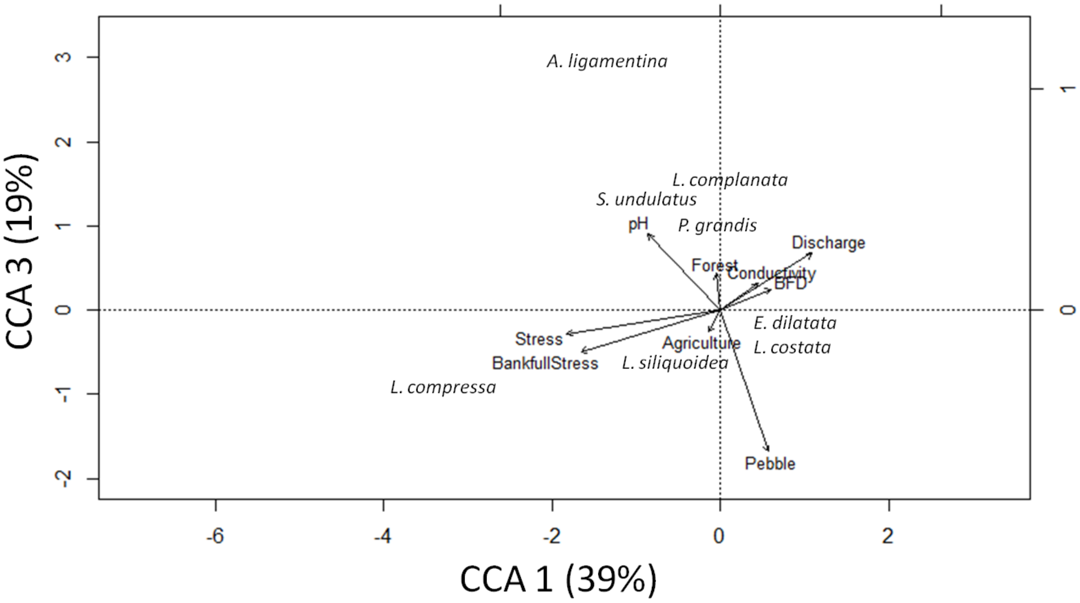


Figure 12: Plot of axes 1 and 3 for CCA, which account for 59% of the variation in environmental data.

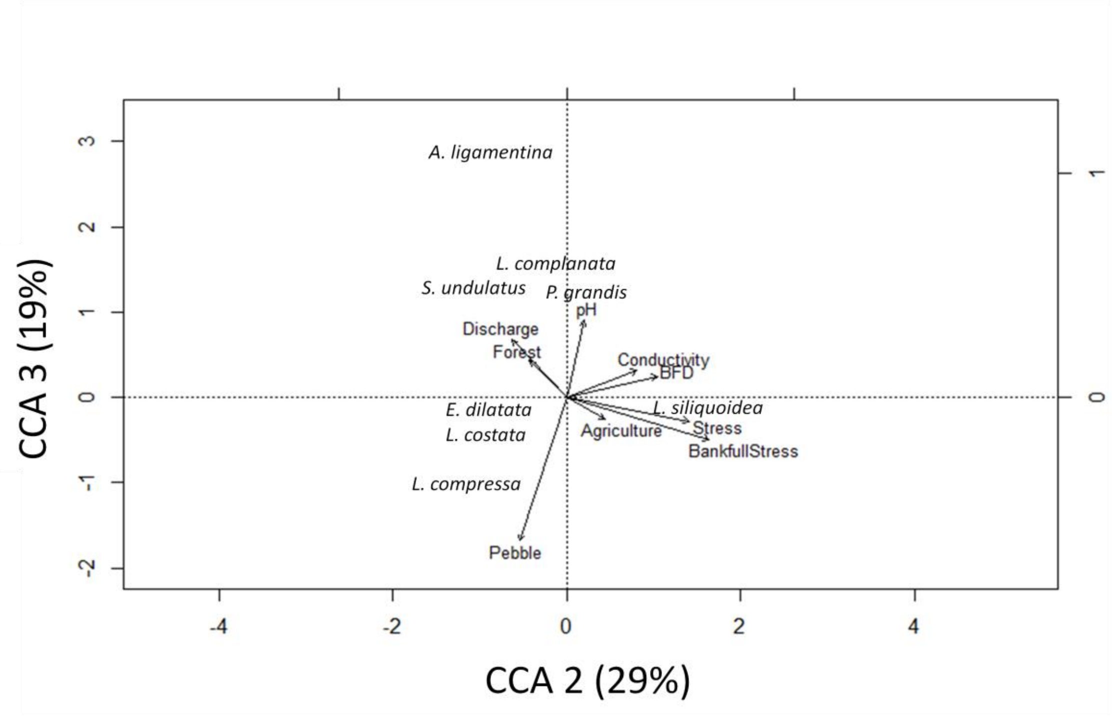


Figure 13: Plot of axes 2 and 3 for CCA, which account for 49% of the variation in environmental data.

Poisson regression was used to model species richness with environmental variables despite the variance-to-mean ratio of 2.4, because the mean of species richness was less than five which means that this excess variance was likely not to be a problem (Ramsey and Schafer 2002). Many models were tested (Table 10), including a null model (intercept only) and models with drainage area and other variables correlated with drainage area showed the best fit. The chi-squared drop in deviance test showed that the model with drainage area only (model #2) was a significantly better fit than the null model (p<0.0001), but the rich model (model #4) was not significantly better than the drainage area-only model (p=0.052) and the AIC for model #2 was only slightly higher than that of model #4. Thus, drainage area alone appears to be the best explanatory variable for species richness. The parameter estimate of 2.45 indicated a significantly positive relationship between drainage area and species richness, however, the use of transformations and the Poisson distribution make interpretation of this estimate difficult.

Table 10: Poisson regression models for species richness. AIC is Akaike's Information Criterion. Residual deviance is an indication of the fit of the model and is used to calculate the chi-squared drop in deviance statistic.

| Model # | Terms in Model | AIC | Residual Deviance | Significant Variables ($p<0.05$) |
|---|---|---|---|---|
| 1 | Null | 84.9 | 53.3 | yes |
| 2 | Drainage | 56.4 | 22.8 | Drainage |
| 3 | Drainage+BFD+BFW+ Width+Slope | 56.75 | 15.16 | Drainage, Width |
| 4 | Drainage+BFD+BFW+ Width+Slope+ Attainment | 55.91 | 10.32 | Attainment(2), Drainage, Width, BFW |
| 5 | Stress | 82.8 | 49.2 | None |
| 6 | BankfullStress | 79.2 | 45.6 | BankfullStress |
| 7 | Conductivity | 81.4 | 47.8 | Conductivity |
| 8 | Stress+ BankfullStress | 81.1 | 45.5 | None |
| 9 | Stress+ BankfullStress + Pebble | 83.1 | 43.9 | None |
| 10 | Stress+ BankfullStress + Pebble+ Depth | 65.9 | 22.32 | None |
| 11 | Stress+ BankfullStress + Pebble+ Depth+ BankfullStress*Pebble + Stress*Depth | 56.75 | 15.16 | All |
| 12 | Stress+ BankfullStress + Pebble+ Depth+ BankfullStress*Pebble + Stress*Depth+ Forest | 64.9 | 15.3 | Stress, Pebble, Depth, BankfullStress*Pebble, Stress*Depth |

The same procedure was performed for abundance (Table 11) using negative binomial regression due to the large variance-to mean ratio of 83.5. The chi-squared drop in deviance test was not able to be computed for the negative binomial model, so models were assessed based on AIC alone. The model with drainage area alone had the lowest AIC, so drainage area appears to be the best explanatory variable for mussel abundance, as well. The parameter estimate of 5.05 for drainage area indicates a significant increase in abundance with increasing drainage area, but again interpretation is difficult.

Table 11: Negative binomial regression models for abundance of live mussels. AIC is Akaike's Information Criterion.

| Model # | Terms in Model | AIC | Significant Variables ($p<0.05$) |
|---|---|---|---|
| 1 | Null | 115.1 | yes |
| 2 | Drainage | 98.8 | Drainage |
| 3 | Drainage+BFD+BFW+ Width+Slope | 100.93 | Drainage, Width, BFW |
| 4 | Drainage+BFD+BFW+ Width+Slope+ Attainment | 102.33 | Drainage, Width, BFW, Slope |
| 5 | Stress | 115.3 | None |
| 6 | BankfullStress | 113.8 | None |
| 7 | Conductivity | 111.0 | Conductivity ($p<0.001$) |
| 8 | Stress+ BankfullStress + Pebble+ Depth | 117.5 | None |
| 9 | Stress+ BankfullStress + Pebble+ Depth+ BankfullStress*Pebble + Stress*Depth | 109.29 | All |

Poisson and negative binomial regression models were produced for species richness and abundance, respectively, after removing drainage area and variables correlated with drainage area (Tables 8 and 9). The remaining variables were grain size, shear stress, bankfull shear stress, forest land cover category, conductivity, and depth. Forested land cover was sufficient for land use due to the correlation with agriculture and developed land, and was converted to a categorical variable. For both species richness and abundance, the model with the lowest AIC included grain size, baseflow and bankfull shear stresses, average depth, and the interactions baseflow shear stress*depth and bankfull shear stress*grain size. These two interaction terms significantly improved the model for species richness compared to a model containing only these four first order terms ($p<0.001$) and these terms had negative parameter estimates for both models, indicating a negative influence on abundance and diversity. Baseflow and bankfull shear stress had positive parameter estimates, indicating increased richness and abundance with increased shear stress, which was unexpected. Although the AIC was slightly higher than for the model above, conductivity was highly significant in the negative binomial model for abundance, with a negative parameter estimate, indicating that conductivity may have a limiting effect on the abundance of mussels. Again, the interpretation of these estimates is difficult and fit of these two models may be poor, and are no better than the models with drainage area alone.

# CHAPTER IV

# DISCUSSION

Although mussel abundance was high in Eagle Creek in 2013 and 2014, abundance throughout the rest of the upper Mahoning River watershed was much lower. Diversity was also quite low in the upper Mahoning River watershed, with ten extant species and 13 total species, including species not represented by live specimens. Mussel communities in the area have likely experienced declines from historical levels—at least two species (*A. plicata* and *L. ovata)* may have been extirpated from the study area, as evidenced by the presence of large, heavily worn shells but no live mussels across two survey years. However, live individuals of these species could have been missed due to the small proportion of sites that were surveyed relative to the total amount of stream habitat in the watershed. Possible causes for reduced abundance and diversity relate to diverse environmental stressors that appear to be related to human changes to the landscape and associated stream impairments. The watershed has areas of high agricultural land cover and some urbanization, although other areas have large tracks of forested land still intact. Overall, stream size plays a larger role in explaining the mussel communities that persist, possibly through increased habitat diversity and by buffering against these stressors.

The Eagle Creek watershed has the highest proportion of forested land in the upper Mahoning River watershed, so more intensive surveys were conducted in 2013 for that subwatershed. The diversity of Eagle Creek was low, but the abundance of live mussels (N=684) compared to dead shells (N=103) seems a promising sign of relatively low adult mortality. Observation of a large ratio of live to dead mussels is contrary to studies in some streams in the nearby Lake Erie watershed of Ohio, where the number of shells found greatly outnumbers live mussels (Krebs et al. 2010(a), Krebs et al. 2010(b)). *Lampsilis siliquoidea, P. grandis,* and *L. complanata* are generally widespread in Ohio and at least *L. siliquoidea* and *P. grandis* are host-generalists (Watters et al. 2009). Thus, it is not unexpected that these species would be the most common, as they are also two of the most common species in large rivers in the nearby Lake Erie watershed of Ohio (Krebs et al. 2010(a)). Similarly widespread are *S. undulatus* and *T. parvum*, although *T. parvum* is not generally abundant where found (Watters et al. 2009).

The largest and oldest *L. siliquoidea* were found at the most upstream site of the survey, approximately 1km downstream of a dam in Garrettsville, OH. Diversity and species richness was also lowest at this site. Almost all of the stream bed between this site and the dam is composed of bedrock, which is not suitable mussel habitat, so this was the most extreme upstream habitat below the dam. Dams serve as barriers to migration of mussels and host fish, and the population at this site may be experiencing a lack of recruitment due to the absence of populations upstream and limited dispersal ability from downstream populations. The distributions of *Leptodea fragilis* and *Potamilus alatus* are generally confined to downstream portions from dams in rivers in Ohio, Indiana, and West Virginia (Watters 1999, Krebs et al. 2010(a)), as host fish rarely cross these barriers especially moving upstream. The dam in Eagle Creek appears to be a general barrier to unionids, as brief surveys above the dam discovered only one live individual and just a few shells of *S. undulatus* but many Sphaerid clams (Begley personal observation, JM Clark personal communication). Species richness and abundance may increase with downstream distance from dams (Vaughn and Taylor 1999), and while that was not strongly observed along the whole length of Eagle Creek in 2013, there was a significant impact on the mussel community from the dam in Garrettsville, OH.

There also appears to be a major under representation of young mussels in Eagle Creek, which comprised only about 5% of the *L. siliquoidea* population in 2013. The size structure of mussel populations has often been observed to be skewed to an excess of larger individuals and relatively few small individuals, although sampling bias in some studies may overlook the small, hard-to-find juvenile mussels (Haag 2012). Some small (<6.0cm) and young (<5 years) mussels were found in 2013 surveys, suggesting that sampling techniques were adequate to find small individuals when they were present. The freshwater mussel *Margaritifera margaritifera* has shown a similar pattern in Scottish (Hastie et al. 2000) and Swedish streams (Osterling et al. 2010; Osterling et al. 2014), possibly due to insufficient recruitment of juveniles in some populations. Hardison and Layzer (2001) suggested that *A. ligamentina* populations in Kentucky were highly skewed towards older individuals due to regulation of rivers by dams and the scouring effects of water releases from those dams to remove juveniles from the stream bed. Populations in other studies do show a more uniform distribution of sizes, indicating recruitment can vary (Haag and Warren 2010). Balfour and Smock (1995) found an *Elliptio complanata* population in Virginia to consist of 90% individuals 4-6 years old, with the maximum age of 8, with excavation of sediment down to a clay layer. Lower than expected presence of small or young individuals may also be an indication of discontinuous recruitment (Tevesz et al. 1985), and not a persistent lack of recruitment. However, if recruitment slows or stops, local extirpations will become more common, and this may be the case in other streams throughout the upper Mahoning River watershed, especially those impacted by human land use.

The relationship between live mussel numbers to those of shells in Eagle Creek in 2013 seems to contradict the possibility of an aging population. If the population is skewed towards older individuals, it would be likely that more shells would be found as older mussels die. However, this may be evidence of discontinuous recruitment rather than a total lack of recruitment. The vast majority of mussels in Eagle Creek in 2013 were estimated between five and ten years old. In five to ten years, shells of these animals may become more abundant in the stream and a year or two with relatively high recruitment may replace the recently dead mussels. While an exact age cannot be given for each mussel, the estimated age can be used to place each individual into a year in which they likely joined the population. For *L. siliquoidea*, most glochidia are released from June through August (Watters et al. 2009). By examining hydrographs for this time period between 2005 and 2012 (Figures 14 and 15), there appears to be a possible relationship between recruitment and hydrology. Between 2005 and 2009, water levels were generally below 40-50cfs$^{-1}$ with few peaks over 100cfs$^{-1}$, with the exception of 2006. These would be years that were better represented in 2013 surveys of Eagle Creek. In 2010 to 2012 hydrographs, there appear to be more sharp peaks above 100cfs$^{-1}$. Potentially, higher base flows and more numerous flooding events could be responsible for decreased recruitment of juveniles in these years. Future surveys between five and ten years from now would be valuable in assessing this assumption without exerting too much stress that comes with repeated handling of live mussels.

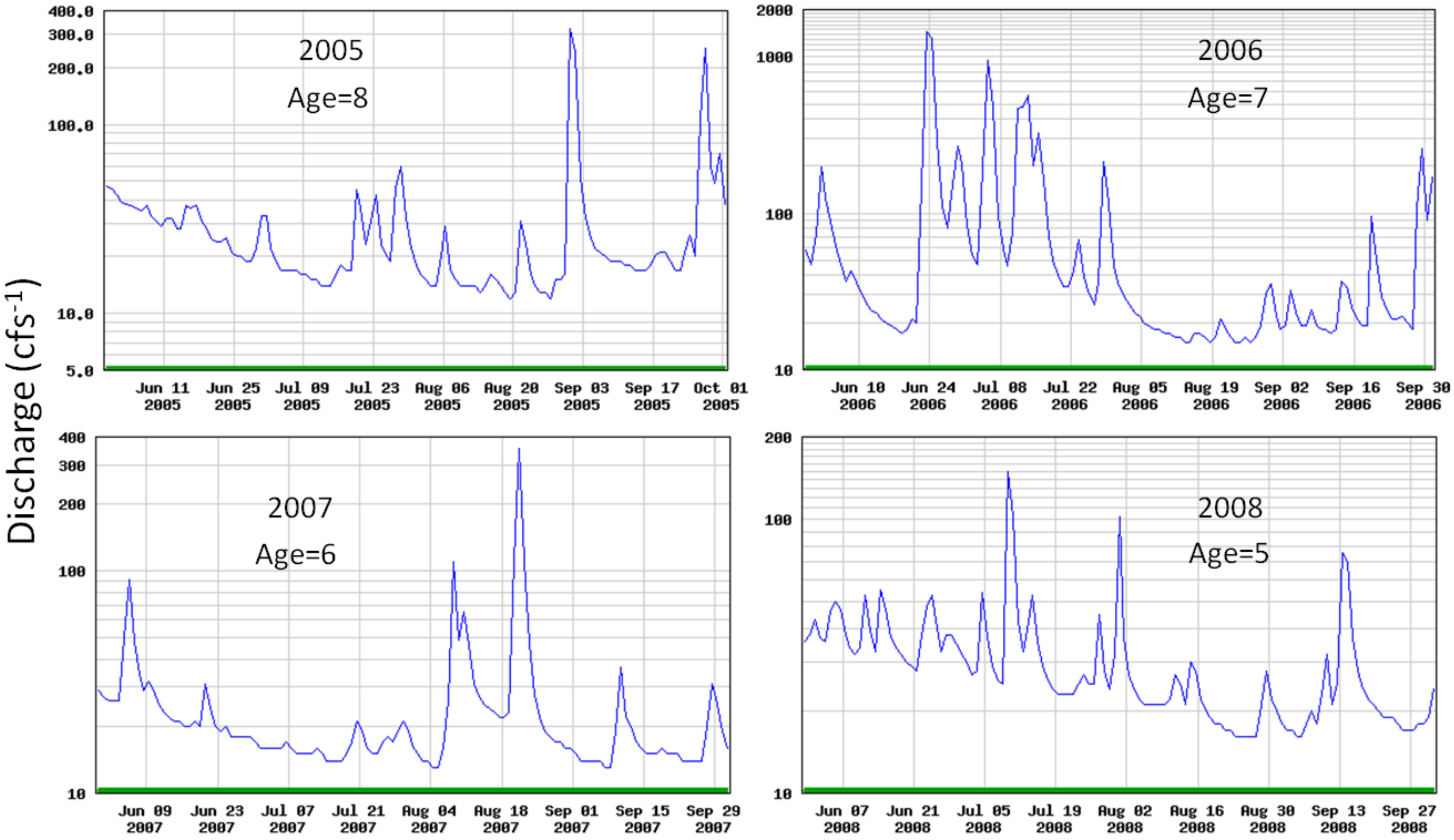


Figure 14: Hydrographs for Eagle Creek from June through September between years 2005 and 2008. Note logarithmic scale on the y-axis and different ranges for the y-axis on each plot.

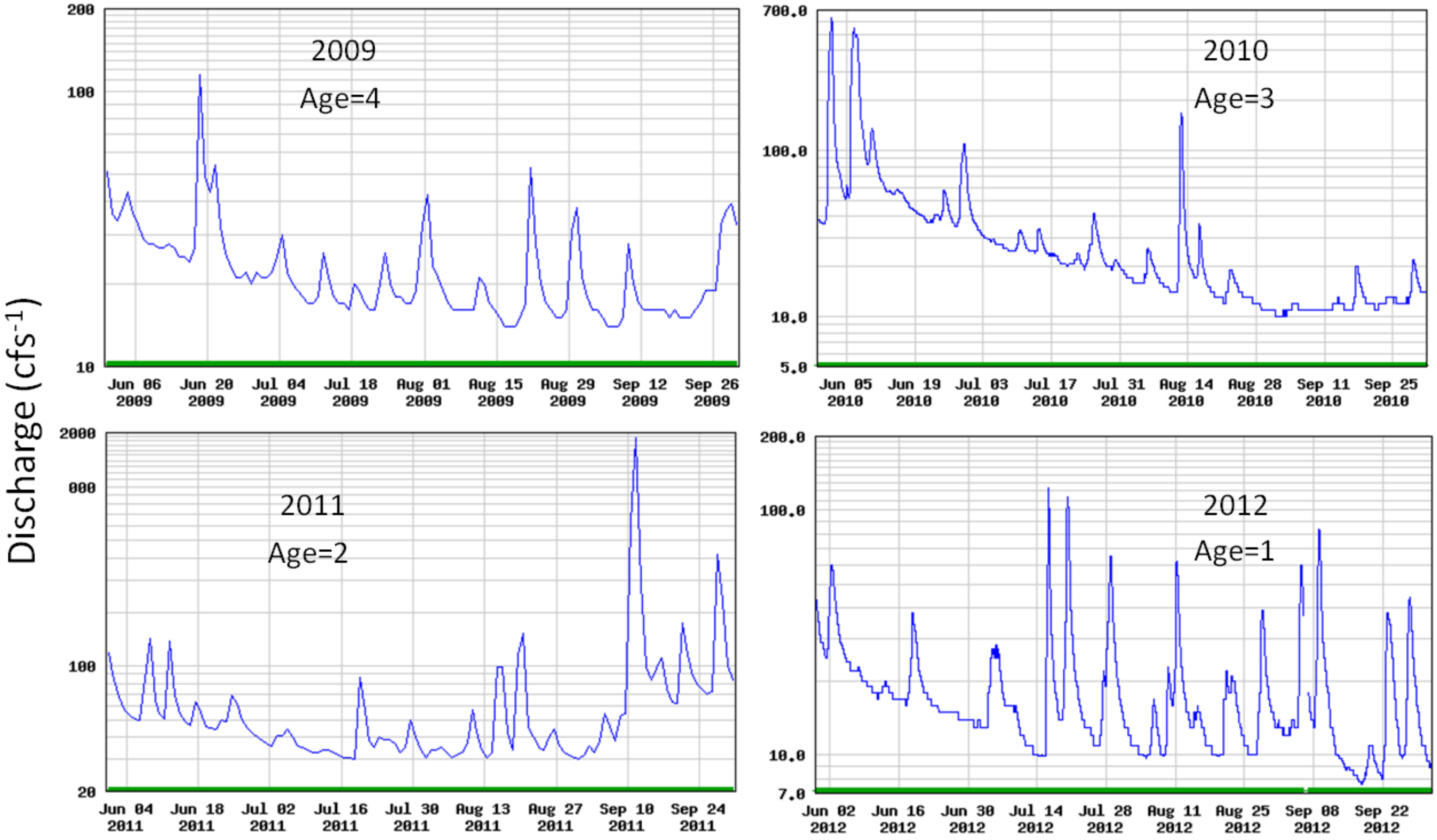


Figure 15: Hydrographs for Eagle Creek from June through September between years 2009 and 2012. Note logarithmic scale on the y-axis and different ranges for the y-axis on each plot.

The abundance and richness of mussels throughout the upper Mahoning River watershed in 2014 was extremely low compared to the abundance in Eagle Creek in 2013. However, the low abundance in 2014 surveys may have been a result of the small size of many of the streams surveyed. The strongest correlations were between variables indicative of stream size and mussel abundance and species richness, and were positively correlated. This result may come partially from the inability to survey several sites as effectively in larger streams, particularly in the Mahoning River which has two major reservoirs and depths in many locations that prohibit surveys by wading. Mussel abundance and species richness has been observed to increase in a downstream direction (i.e. from smaller to larger streams) in Alabama (Haag and Warren 1998) and Atlanta (Gagnon et al. 2006). Similarly, increasing watershed size is associated with increased species richness for historical conditions (extant and extirpated species) in tributaries of Lake Erie in Ohio (Krebs et al. 2010(a)). The strong relationship between drainage area and mussel abundance and species richness in this study may be a function of more diverse host fish assemblages in larger streams compared to headwaters (Haag and Warren 1998), and this was evidenced by the correlation between IBI score and drainage area. In Ohio River drainages, increased mussel species richness is associated with increased fish diversity in large rivers, with fish diversity predicted by drainage area, while richness in small streams and headwaters is directly related to drainage area (Watters 1992).

In addition to the effect of stream size on abundance and species richness, there does appear to be some relationship to land use. Agricultural and urban land use increase in a north to south direction in the watershed, and abundance followed an opposite trend, decreasing from north to south.  Species richness followed the same trend, with the exception of the West Branch Mahoning River watershed which only had two species present.  Declines in mussel abundance and diversity have been linked to agricultural (Poole and Downing 2004, Peacock et al. 2005) and urban land use (Gillies et al. 2003, Gangloff et al. 2009, Brown et al. 2010), and this pattern holds up in the current study.  Also, agricultural and developed land may have disproportionate effects on smaller streams compared to larger streams.  Although all streams in this study were relatively small, there was a relatively large range of discharge, width, and depth observed.  Since mussels were found in smaller streams in the Eagle Creek watershed, this suggests that for other smaller streams where no live mussels were found, stressors that accompany agricultural and urban land use may impact those streams more severely than larger streams.

Further support of the influence of land use on stream conditions is the association of conductivity with agricultural and urban land use, indicating possible pollutants from runoff associated with these types of land cover.  The threshold of mussel presence only below a conductivity level of 0.9mS suggests that conductivity, as a correlate to land use, is a limiting factor for the presence of mussels in some streams.  This is very close to the average of around 0.8mS found by McRae et al. (2004) in the River Raisin in Michigan for sites with no mussels present, although conductivity was not significantly different from sites with low or medium quality mussel communities as measured by abundance and species richness.  However, conductivity may be limiting on mussel abundance.

The effect of current land use on mussel communities, and aquatic systems in general, may be obscured by the unknown influence of land use in the past.  Maloney and

Weller (2011) found reduced nitrate levels in forested catchments that were agricultural in 1952, demonstrating a possibility of reduction of stream inputs from reforestation. However, they also found poor quality fish and macroinvertebrate communities in the same setting, indicating that agricultural land use can impair stream communities for many years. Past urban industrial land use also may contaminate soil with high concentrations of mercury, which peaked before 1940, and which in turn continue to contaminate aquatic systems today (Clark and Benoit 2009). In the upper Mahoning River watershed, many historic conditions cannot be easily observed today, but may contribute to the low abundance of mussels and low recruitment. Despite having the highest percentage of forested land in the study area, the Eagle Creek watershed community was still dominated by the pollution-tolerant species *L. siliquoidea* and *P. grandis* at 84% of live mussels.

While many environmental variables were cross-correlated, few hydrological variables were directly correlated with mussel abundance or species richness. Most streams were composed of clear water, measuring above the scale for turbidity, thus suspended solids were not a significant factor affecting mussel communities in this study. Baseflow and bankfull shear stress were weakly correlated with abundance and species richness. However, they were negatively correlated with mussels, indicating a possible negative influence of shear stress, as has been observed in other studies (Hardison and Layzer 2001, Howard and Cuffey 2003, Gangloff and Feminella 2007), although all of these studies have observed a stronger relationship.

The relationship between overall habitat quality (QHEI) and aquatic life attainment status with abundance and diversity indicate that a large group of environmental variables may be necessary to adequately predict abundance and species richness. QHEI provides a qualitative assessment of the habitat as a whole, incorporating a number of factors. Only one mussel was found at lower quality sites as indicated by low QHEI scores. Other qualitative metrics have been found to be similarly predictive of total mussel abundance (McRae et al. 2004), and site by site comparisons may be useful in small streams, as well (Lyons et al. 2007). Aquatic life use attainment status likewise relies on several factors, including the QHEI and IBI. Of the four sites with full attainment status but no mussels present, specific limiting factors were high bankfull shear stress, small drainage area, and high agricultural land use. The Ohio EPA's aquatic life designation system appears to be a useful indicator, especially for identifying sites where mussels are not likely to be found.

Thus, groups of variables and interactions among the variables appear to be good predictors of abundance and species richness, although the variation explained is somewhat low. The variables important here seem to point toward stream bed stability. Smaller grain size may indicate a stable stream bed in the presence of increased bankfull shear stress, which likely allows for the settling and establishment of juvenile mussels. Likewise, larger grain sizes would indicate that the smaller and lighter particles are more easily eroded by flood conditions. Increased stream bed stability had a positive association with abundance and species richness. Substrate stability at high flows has been recognized as an important factor influencing mussel distributions (Allen and Vaughn 2010). When variables correlated with stream size were removed, the interaction of increased particle size with increased bankfull shear stress indicated instability of the stream bed at high flows and a decline in mussel abundance and diversity in the upper

Mahoning River watershed, as expected (Allen and Vaughan 2010, Gangloff and Feminella 2007, Howard and Cuffey 2003). Reduced stability and increased disturbance of stream beds has also been linked to decreased fish biomass (Jellyman et al. 2013), which could have the effect of decreased mussel abundance due to loss of hosts.

Individual mussel species showed some variation in their tolerance to different environmental stressors, based on CCA results. Two widespread species in Ohio, *L. siliquoidea* and *P. grandis*, were also the most widespread in the upper Mahoning River watershed, perhaps due to their tolerance for increased disturbance (shear stress and conductivity) and agricultural land more so than other species found in the area. *Pyganodon grandis* can tolerate metal exposure and seems to display both a natural resistance (Cooper et al. 2013) and acclimation to resistance due to chronic exposure (Cooper et al. 2010). Glochidia and juveniles of *L. siliquoidea* showed acute and chronic toxicity to some pesticides, but not to others such as the widely-used compound atrazine (Bringolf et al. 2007). These two mussels may be more resilient to pollution, allowing them to persist in more urban and agricultural impacted watersheds, although possibly only at low levels. *Lasmigona compressa* and *S. undulatus* were associated with intermediate size streams (drainages between 23-92km$^2$) relative to other species and streams in the area and better water quality, while *E. dilatata* was restricted to relatively larger streams (drainages of 56km$^2$ and 188km$^2$) than other species with lower shear stresses. *Lasmigona complanata* appeared to show an intermediate tolerance for all factors relative to other species.

Only one species found in Eagle Creek, *L. compressa*, is listed as a species of concern by the state of Ohio (ODNR 2014). This is considered a species found mainly in areas of high water quality (Watters et al. 2009) and was found sporadically throughout the length of Eagle Creek. The presence of *U. imbecillis*, which was restricted to the river mouth, may be a result of migration from the Mahoning River, where it was also found. The almost lentic conditions of this area would facilitate presence of *U. imbecillis*, a mussel that may reproduce without the presence of a fish host (Watters et al. 2009). The conditions in Eagle Creek appear to be conducive to presence of at least common species and small numbers of the sensitive *L. compressa*, but evidence for recruitment is low.

### *Conclusions*

The Eagle Creek watershed appears to have the healthiest mussel communities in the upper Mahoning River watershed. Abundance and species richness are far higher than other portions of the watershed, and are likely associated with the lower proportion of agricultural and urban land, as well as an increase in forested land relative to the other three subwatersheds. Substrate may be more stable in these streams due to less pressure from agricultural and urban inputs. Water quality may be higher as well, with reduced pollutants in runoff from the adjacent land. However, recruitment may be low or sporadic in Eagle Creek. The rest of the watershed is physiographically similar to Eagle Creek, and there is no reason to suspect that mussels were not present throughout the upper Mahoning River watershed historically, especially with evidence of some shells in sites now dominated by agriculture and urban development. Populations may have already been long extirpated due to more intense land use, especially in these southern portions. The present concern is that Eagle Creek may experience a delayed extinction debt. Potentially, the more natural land cover found in the watershed provides a time delay from the effects of agricultural and urban land, which still make up 41% of the watershed and may have comprised a greater proportion in the past. Another possibility is that the Eagle Creek mussel community is recovering from past loss and recruitment may increase with time and may depend on hydrologic conditions during the juvenile stage. Local habitat characteristics are important in defining suitable habitat for mussels, and must be considered for reintroduction efforts. Efforts should consider stream size, as the smallest streams may not be able to harbor a robust mussel community, regardless of outside influences on the stream. Also, suitable morphological and hydraulic characteristics may not matter if the stressors from watershed land use, past and present, are not first mitigated or reversed.

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## APPENDIX

Table 12: Raw, untransformed data for 2014 surveys. Units can be found in Table 2.

| Site# | N | R | Drainage Area | Attainment Status | Pebble | Stress | Bankfull Stress | Forest | Agri-culture | Devel-oped | pH | Condu-ctivity | Discharge | BFD | BFW | Width | Depth | Slope | QHEI | IBI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 10 | 2 | 8.8 | 2 | 21 | 7.7 | 30.4 | 0.46 | 0.3 | 0.11 | 8.38 | 0.48 | 0.0284 | 1.12 | 8.26 | 6.13 | 0.28 | 0.0028 | 66 | 42 |
| 2 | 0 | 0 | 4.2 | 2 | 29 | 11.7 | 37.5 | 0.46 | 0.3 | 0.11 | 7.94 | 0.68 | 0.0225 | 0.73 | 5.57 | 3.77 | 0.23 | 0.0053 | 74 | 44 |
| 3 | 0 | 0 | 11.2 | 1 | 0.5 | 6.3 | 19.5 | 0.46 | 0.3 | 0.11 | 8.15 | 0.45 | 0.1286 | 1.37 | 7.47 | 5.09 | 0.45 | 0.0014 | 68.5 | 34 |
| 4 | 44 | 4 | 32 | 2 | 5.5 | 6.1 | 26.6 | 0.46 | 0.3 | 0.11 | 8.41 | 0.61 | 0.2925 | 1.60 | 25.48 | 14.62 | 0.37 | 0.0017 | 81.5 | 51 |
| 5 | 10 | 4 | 36 | 2 | 0.5 | 2.4 | 8.9 | 0.46 | 0.3 | 0.11 | 8.4 | 0.6 | 0.6 | 1.41 | 13.69 | 8.59 | 0.38 | 0.0006 | 61.5 | 40 |
| 6 | 149 | 6 | 73.6 | 2 | 11 | 2.6 | 10.8 | 0.46 | 0.3 | 0.11 | 8.29 | 0.56 | 0.537 | 1.47 | 17.4 | 8.64 | 0.35 | 0.0007 |  |  |
| 7 | 0 | 0 | 4.4 | 0 | 46.5 | 2.7 | 18.1 | 0.46 | 0.3 | 0.11 | 7.98 | 0.7 | 0.0102 | 0.88 | 8.18 | 4.02 | 0.13 | 0.0021 | 46.5 | 32 |
| 8 | 13 | 4 | 23.5 | 2 | 0.5 | 4.5 | 19.8 | 0.46 | 0.3 | 0.11 | 8.5 | 0.7 | 0.1398 | 1.22 | 8.74 | 5.96 | 0.28 | 0.0017 | 61 | 42 |
| 9 | 0 | 0 | 5.1 | 0 | 0.5 | 10.7 | 24.1 | 0.43 | 0.32 | 0.11 | 8.08 | 0.45 | 0.0284 | 1.58 | 7.32 | 5.41 | 0.7 | 0.0016 | 42.5 | 32 |
| 10 | 35 | 4 | 21.9 | 1 | 44 | 1.6 | 9.2 | 0.43 | 0.32 | 0.11 | 7.59 | 0.75 | 0.0202 | 1.08 | 12.78 | 7.74 | 0.18 | 0.0009 | 65 | 29 |
| 11 | 0 | 0 | 9.3 | 2 | 25.5 | 7.2 | 42.0 | 0.43 | 0.32 | 0.11 | 7.83 | 1.25 | 0.0436 | 1.16 | 20.52 | 8.5 | 0.2 | 0.0037 | 68 | 42 |
| 12 | 5 | 1 | 10.2 | 2 | 26 | 5.0 | 37.1 | 0.43 | 0.32 | 0.11 | 7.78 | 0.72 | 0.0518 | 1.25 | 7.84 | 4.8 | 0.17 | 0.003 | 61.5 | 44 |
| 13 | 0 | 0 | 7.2 | 2 | 10.5 | 2.8 | 18.3 | 0.35 | 0.47 | 0.07 | 7.47 | 0.74 | 0.0216 | 1.09 | 6.34 | 2.42 | 0.17 | 0.0017 | 54.5 | 38 |
| 14 | 7 | 4 | 19.1 | 2 | 13.5 | 3.8 | 18.8 | 0.35 | 0.47 | 0.07 | 8.11 | 0.79 | 0.0398 | 1.15 | 9.92 | 7.26 | 0.23 | 0.0017 | 74 | 47 |
| 15 | 1 | 1 | 3.7 | 0 | 9 | 6.2 | 34.3 | 0.35 | 0.47 | 0.07 | 8.06 | 0.68 | 0.001 | 0.95 | 6.24 | 3.01 | 0.17 | 0.0037 | 54.5 | 20 |
| 16 | 0 | 0 | 4.2 | 0 | 12 | 2.5 | 25.3 | 0.35 | 0.47 | 0.07 | 7.75 | 0.95 | 0.0024 | 0.98 | 6.84 | 3.17 | 0.1 | 0.0026 | 43.5 | 30 |
| 17 | 3 | 3 | 9.9 | 0 | 0.5 | 4.4 | 35.0 | 0.35 | 0.47 | 0.07 | 7.92 | 0.82 | 0.027 | 1.11 | 9.08 | 4.89 | 0.14 | 0.0032 | 56.5 | 26 |
| 18 | 0 | 0 | 17.4 | 2 | 14.5 | 3.0 | 23.9 | 0.24 | 0.51 | 0.2 | 8.37 | 0.9 | 0.0497 | 1.15 | 12.08 | 5.4 | 0.14 | 0.0021 | 60.5 | 42 |
| 19 | 0 | 0 | 3 | 0 | 0.03 | 4.0 | 18.7 | 0.24 | 0.51 | 0.2 | 7.85 | 1.15 | 0.0304 | 1.36 | 6.32 | 4.22 | 0.29 | 0.0014 | 49 | 20 |
| 20 | 0 | 0 | 4.5 | 0 | 0.5 | 4.3 | 15.6 | 0.24 | 0.51 | 0.2 | 7.83 | 1.59 | 0.0264 | 1.02 | 5.63 | 4.43 | 0.28 | 0.0016 | 54 | 28 |