RUSTY CRAYFISH (*FAXONIUS RUSTICUS*) INTERACTIONS WITH BENTHIC MACROINVERTEBRATES IN THE MONOCACY RIVER

by

Kierstyn Higgins

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THESIS

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Accepted:

Eric Annis, Ph.D. Committee Member	Kristin Fisher, Ph.D. Director, Environmental Biology Program
Drew Ferrier, Ph.D. Committee Member	April M. Boulton, Ph.D. Dean of the Graduate School
Oney Smith, Ph.D. Committee Member	

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ABSTRACT

The rusty crayfish (*Faxonius rusticus*) is an invasive species with origins in the Midwestern Unites States, that has the capacity to alter benthic macroinvertebrate community structure through predation. Since 2007, *Faxonius rusticus* migrated downstream in the Monocacy River, establishing its presence as one of the predominant crayfish species in certain areas of the river. I analyzed changes in benthic macroinvertebrate communities in 2016 and 2021 at sites with and without a presence of *Faxonius rusticus* using biodiversity, density, and biomass as biological metrics. No significant changes in these metrics were detected in comparisons of with and without *Faxonius rusticus*. Some significant differences were detected between habitats. Additionally, macroinvertebrate taxonomic composition was not statistically significant across treatments with the exception of one genus. The lack of changes in macroinvertebrate communities is likely attributed to the significant decline in densities of *Faxonius rusticus* over the past five years. Because of this decline, I hypothesize that rates of predation by *Faxonius rusticus* are not high enough to be detected by field sampling in the Monocacy River.

DEDICATION

In loving memory of my father, Scott, for sharing 18 years of life full of wisdom with me.

In memory of Hans Wagner, for welcoming me to the department and helping me to develop a sense of belonging at Hood College.

Dedicated to my mother, Cheryl, Artie, and Steve for their unconditional support.

ACKNOWLEDGEMENTS AND SPONSORSHIP

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A special thank you to all the researchers in this field whose work I've utilized to help write this paper.

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INTRODUCTION

Invasive Crayfish in a Stream Ecosystem

Invasive species can significantly alter an ecosystem, causing permanent ecological and economic damage (Anderson et al. 2004). Invasive species are known to have negative consequences on indigenous flora and fauna through predation and competition, for example, the bullfrog has been shown to significantly reduce native frog species through predation (Adams 1999). In addition, the invasive aquatic plant, water chestnut, is capable of outcompeting native aquatic vegetation through the interception of sunlight (Groth et al. 1996). While the introduction of organisms into an ecosystem is a naturally occurring ecological process, humans have significantly accelerated the rate at which this process occurs through trade and travel. An important distinction is that not all non-native species have the characteristics of an invasive species (Davis et al. 2011). Additionally, there are many invasive species that have beneficial qualities. For example, the zebra mussel has been correlated with decreases in density of cyanobacteria in the Hudson River (Smith et al. 1998). This could be considered both an environmental and economic benefit to an ecosystem.

Since the mid-1900s Maryland's waterways have faced the introduction of non-native species of crayfish (Kilian et al. 2010). The primary sources of non-native crayfish introduction include the ornamental aquarium and pond organism trade, live-bait use, biological supply, and aquaculture (Hobbs 1989). Through the introduction of non-native crayfish into Maryland's freshwater bodies, native species have become increasingly threatened by predation, resource competition, and hybridization (Kilian et al. 2010).

The Potomac and Patapsco River basins are the main watersheds in northern central Maryland. Within these watersheds, there are a multitude of native crayfish species that could be particularly vulnerable to the introduction of invasive species. The state of Maryland is host to nine native crayfish species including *Cambarus acuminatus, C. bartonii, C. carinirostris, C. diogenes, C. dubius, Fallicambarus fodiens, Faxonius limosus, F. obscurus,* and *Procambarus acutus* (Kilian et al. 2010). Over the past 40 years, *F. limosus* has been displaced by an invasive crayfish species, *Faxonius virilis*, or the virile crayfish (Kilian et al. 2010). *Faxonius virilis* was first documented in Maryland in 1956 and was found in the Patapsco River (Schwartz and Meredith 1960). By the following year, *F. virilis* had displaced the two native crayfish, *C. bartonii* and *F. limosus* (Schwartz et al. 1963), to the Patapsco River basin. Until recently, *F. virilis* has become the predominant species throughout the major tributaries of the Potomac and Patapsco River.

In 2007, *F. virilis* was faced with a slightly smaller but even more competitive crayfish, *Faxonius rusticus*, also known as the rusty crayfish. The first record of *F. rusticus* crayfish in Maryland was found in the upper Monocacy River and Antietam Creek (Kilian et al. 2010). In 2010, *F. rusticus* was found for the first time in the Potomac River (Kilian and Ciccotto 2011). Over the past decade, *F. rusticus* has become increasingly more dominant in the Potomac River tributaries, particularly the Monocacy River, steadily displacing *F. virilis.* Since its initial introduction, *F. rusticus* has progressively moved further downstream from its original discovery location near Emmitsburg, MD, with the potential to dominate *F. virilis* in the lower Monocacy (Marinelli 2022, Selckman 2016). As of 2021, *F. rusticus* was advancing downstream at a rate of 2.66 km yr^{-1} (SD = 1.12) and covered 37.29 km over a span of 14 years (Marinelli 2022). With this progression downstream, native crayfish species will become increasingly more vulnerable to competition with *F. rusticus.*

Characteristics of Faxonius virilis

The virile crayfish, sometimes known as the Northern crayfish, is native to portions of the Great Lakes, Mississippi River, Missouri River, and Ohio River (Durland Donahou 2019). It was likely introduced to Maryland via improper bait disposal (Larson and Olden 2011).

Faxonius virilis prefers moderately flowing streams with ample coverage (Crocker and Barr 1968). It is a non-burrowing species that has a low tolerance for high flow rates (Maude and Williams 1983). Therefore, it is likely that this species will be found in pools or vegetation *F. virilis* can reach up to 13.1 cm carapace length (Durland Donahou 2019). It is an omnivorous species, known to feed on anything from plant detritus (Tran and Manning 2019), to fish eggs to juvenile aquatic reptiles and amphibians, to benthic macroinvertebrates (Recsetar and Bonar 2015). It is the dominant species of crayfish in the state of Maryland and is the main culprit for the displacement of *F. limosus* and *F. obscurus* (Kilian et al. 2010).

Characteristics of Faxonius rusticus

Faxonius rusticus or rusty crayfish is originally native to the lower Midwest (USA) including Missouri, Kentucky, Tennessee, Illinois, Indiana, Ohio, and Michigan (Charlebois and Lamberti 1996). It is thought that *F. rusticus* was introduced to Maryland by anglers using the species for bait (Kilian et al. 2010). *F. rusticus* can reach sizes of up to 5.8 cm carapace length (Charlebois and Lamberti 1996) with claws larger than *F. virilis*. *F. rusticus* consumes anything from small fish (Kreps et al. 2016) to fish eggs, aquatic macroinvertebrates (Morse et al. 2013), littoral periphyton and macrophytes (Charlebois and Lamberti 1996). *Faxonius rusticus* is a more aggressive forager and feeds at a higher rate than the virile crayfish (Morse et al. 2013). Like the virile crayfish, *F. rusticus* can be found in both lentic and lotic environments but *F. rusticus* is distinct in its ability to tolerate high flow rates of up to 40 cm/s (Perry et al. 2013). The velocity of a stream may cause morphological variations in the size and shape of *F. rusticus* (Perry et al. 2013). Since *F. rusticus* has been shown to displace *F. virilis* through competition (Hill and Lodge 1999), it is likely that the spread of *F. rusticus* could not only displace *F. virilis*, but also drastically decrease biomass and biodiversity of macroinvertebrate populations within the Monocacy River.

Importance of Benthic Macroinvertebrate Communities in Stream Ecosystems

Ecosystem functions are heavily influenced by biodiversity (Tilman 2001). Important ecosystem functions of a stream include habitat and sustenance for aquatic life, nutrient cycling, and water quality. Benthic macroinvertebrates account for most of the biodiversity in a stream and their diversity surpasses that of fish and macrophytes (Allan and Flecker 1993). Benthic macroinvertebrate assemblages can include crustaceans, bivalves, and worms, but are primarily comprised of insects. Many of these aquatic insects will begin their lives with an aquatic larval stage, only to metamorphose into terrestrial insects, including dragonflies, mayflies, and damselflies.

Benthic macroinvertebrates are essential to the structure of aquatic systems (dos Santos et al. 2016). These invertebrates are an integral part of the food chain, serving as prey for fish (Gilinksy 1984), amphibians (Salvidio et al. 1999) and even other macroinvertebrates (Morse et al. 2013). In addition to their importance as a food source, benthic macroinvertebrates also play an influential role in detrital processing (Webster and Banfield 1986). It is estimated that around 24% of leaf degradation in streams can be attributed to detritivores (Peterson and Cummins 1974). Nutrient cycling is extremely important in lotic ecosystems and is directly related to primary productivity within the stream (Essington and Carpenter 2000). Benthic macroinvertebrates can also be used as a measurement of water quality. Different species of macroinvertebrates have varying tolerances for levels of water quality. These water quality parameters usually include temperature, turbidity, dissolved oxygen, nutrient concentration, and any extraneous sources of pollution. The orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Tricoptera (caddisflies) are generally known as the EPT taxa, which are most sensitive to poor water quality. Benthic macroinvertebrate indices (BMI) are often used as indicators of stream health as they are an inexpensive and low-effort type of assessment. As benthic macroinvertebrates have low rates of mobilization, they provide a predictable, long-term assessment of the water quality of a stream (United States Environmental Protection Agency 2022).

Ecosystem Impacts

The impact of *Faxonius rusticus* as an invasive species is characterized by its behavior and diet (Morse et al. 2013). They are omnivorous, feeding on anything from algae to other macroinvertebrates. About 44-65% of their diet consists of detritus and in their native region of the Ohio River Valley, *F. rusticus* consumes the most animal matter during the summer and early fall months (Tran and Manning 2019). *Faxonius rusticus* is known to consume a broad range of macroinvertebrates from isopods to snails (Vollmer and Gall 2014). Smaller crayfish tend to have more selective diets, being more carnivorous while the larger crayfish lean towards a more omnivorous diet (Wilson et al. 2004). However, their diet composition can vary greatly since *F. rusticus* is considered a generalist species.

Crayfish are significant as both predators and prey within the food web (Kuhlmann and Hazelton 2007), but there are gaps in our knowledge of the impacts of *F. rusticus* on macroinvertebrate populations in streams and how it may differ from the current dominant species of crayfish. *Faxonius rusticus* is a much more aggressive predator than *F. virilis* (Morse et al. 2013), indicating that macroinvertebrate biodiversity and biomass could decrease because of its invasion and displacement of *F. virilis.* Less is known about how feeding behavior of *F. rusticus* differs between lentic and lotic systems. One meta-analysis comparing the impacts of *F. rusticus* on zoobenthic communities in a Wisconsin lake, found that while both non-*F. rusticus* and *F. rusticus* had negative impacts on total Gastropoda, *F. rusticus* also impacted Diptera and Ephemeroptera, while non-*F. rusticus* crayfish did not (Mccarthy et al. 2006). *Faxonius rusticus* significantly reduced Gastropoda, Ephemeroptera, Diptera, and Trichoptera (Mccarthy et al. 2006). In a northern-temperate lake, most individual macroinvertebrate taxonomic groups did decrease in abundance when *F. rusticus* increased (Wilson et al. 2004). While published works focusing on the impact of *F. rusticus* in streams are limited, in a Michigan stream, *F. rusticus* had a direct negative impact on benthic macroinvertebrate density and biodiversity (Charlebois and Lamberti 1996). This study site was revisited a decade later, only to find that where there was a presence of *F. rusticus*, invertebrate abundance had decreased (Bobeldyk and Lamberti 2006). From 2000 to 2004, The Maryland Department of Natural Resources conducted a biological stream survey of the upper Monocacy River, documenting the benthic taxa present in the watershed. Some of the benthic taxa that they found include species of Ephemeroptera, Trichoptera, and Diptera (Maryland DNR), suggesting that macroinvertebrate populations in the Monocacy might be vulnerable to predation by *F. rusticus*. Since benthic macroinvertebrates play a key role in functional diversity of a stream, it is a possible that reducing diversity adversely affect water quality and higher trophic levels.

Objective

This study aimed to analyze the presence of *Faxonius rusticus* in the Monocacy River and its effects on macroinvertebrate populations. I hypothesized that there would be a significant decrease in macroinvertebrate density, biomass, and biodiversity in areas of the river where *F. rusticus* has an established presence. I tested this by comparing benthic macroinvertebrate diversity, density, and biomass between sites with and without *F. rusticus.* Based on literature that shows significant predation on macroinvertebrates from *F. rusticus* in lake systems, I expected to find statistically significant differences in abundance, biomass, and biodiversity between sites that have a presence of *F. rusticus* and those that do not.

METHODS

Sampling Sites

The upper Monocacy River watershed is a 635 km^2 watershed, located in the Potomac River basin, with a large portion lying in Frederick County, Maryland. The river spans 94 miles from Adams County, PA to its drainage point into the Potomac River. The surrounding land use consists mostly of agriculture, with the city of Frederick being the largest urban surrounding land use.

In 2016 (Figure 1) a group of students in the Annis Lab at Hood College, sampled eight sites along the Monocacy River, to quantify the presence of *Faxonius rusticus* and macroinvertebrate composition. In 2021 (Figure 2), I sampled 10 sites along the Monocacy River. The sites were chosen for both sampling years based on the invasion front and accessibility. Most sites are associated with a bridge crossing or adjacent roadway. The sites were intentionally chosen to provide an equal sample size of sites with and without a presence of *F. rusticus*. These sites are referred to as "*Faxonius rusticus* present" and "*Faxonius rusticus* absent". The 2016 sites are listed from North to South as follows: Route 140, Mumma Ford Rd., Route 77, Legore Bridge Rd., Links Bridge Rd., Devilbiss Bridge Rd., Biggs Ford Rd., and Monocacy Blvd. The 2021 sites are listed from North to South as follows: Route 140, Mumma Ford Rd., Route 77, Legore Bridge Rd., Devilbiss Bridge Rd., Monocacy Blvd, Pinecliff Park, Route 355, Michael's Mill Rd., and Park Mills Rd.

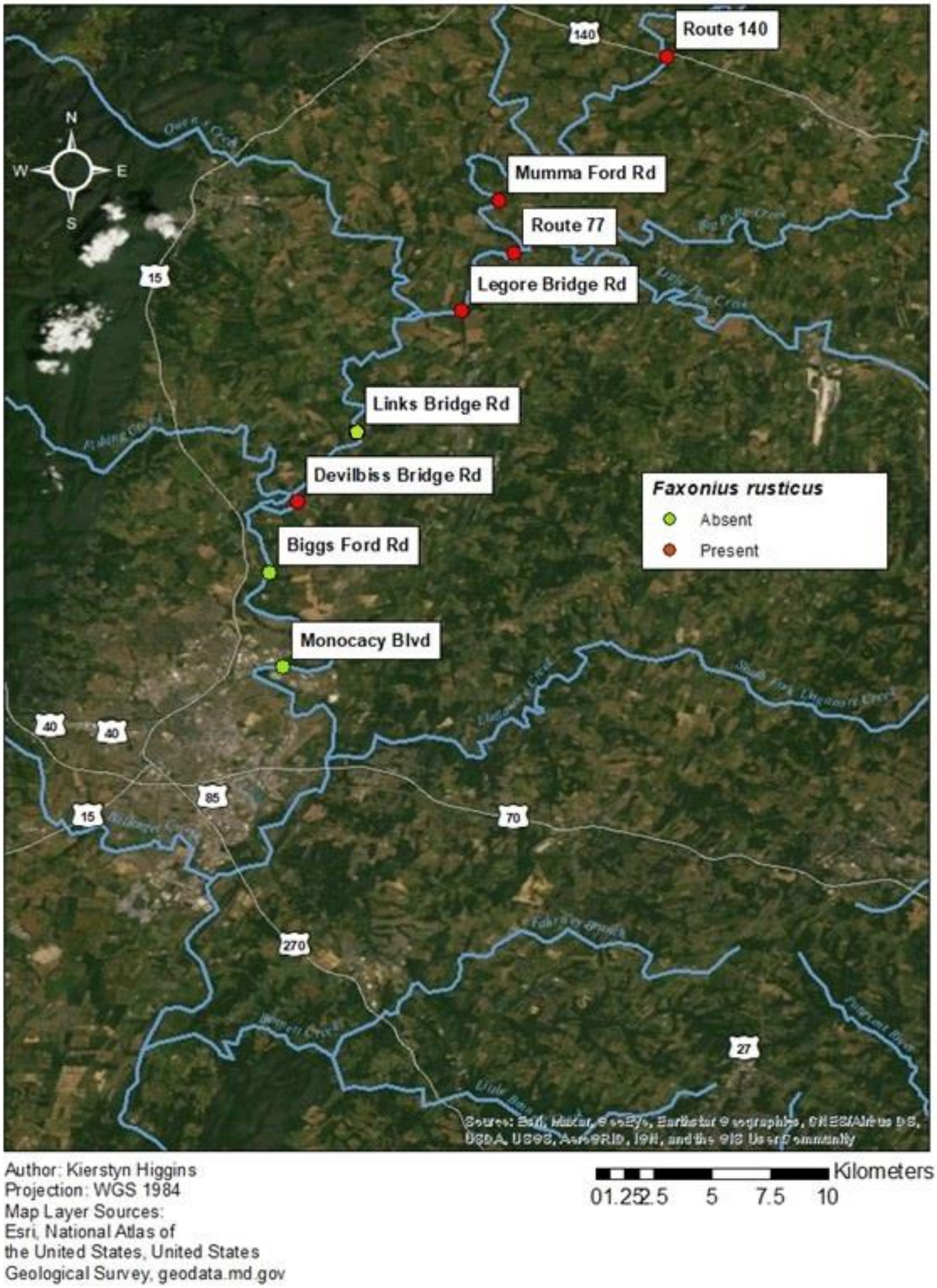


Figure 1. Monocacy River sites that were sampled in 2016 for crayfish and macroinvertebrates.

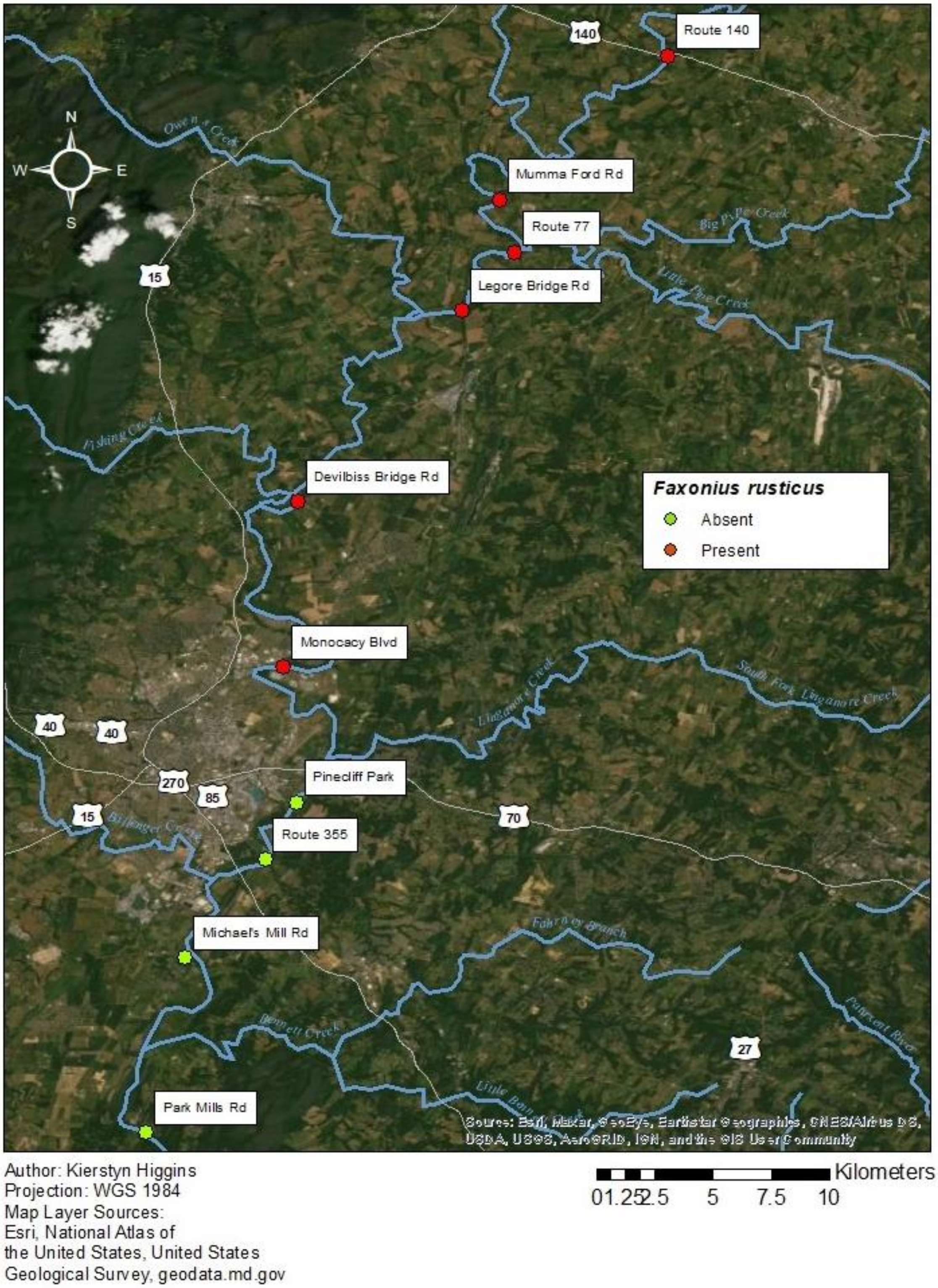


Figure 2. Monocacy River sites that were sampled in 2021 for crayfish and macroinvertebrates.

Temporal Factors

2016 sampling occurred throughout the month of June. In 2021, sampling occurred from mid-July to late August over a period of five weeks. There are a wide range of published macroinvertebrate studies in which sampling takes place between early May and late fall. It has been suggested that collection should not take place between mid-November and mid-April (Indiana Department of Environmental Management 2019). Sampling in the mid-summer allowed us to avoid hazardous water level conditions and maintain more consistent time intervals between sampling days.

Physical Parameters

Physical parameters were measured at each site including date, time, weather, habitat type, and flow rate. Habitat was characterized based on qualitative observations of flow and flow rate was measured using an OTT MF Pro flow meter (Table 1). A YSI probe was used to record temperature, and conductivity. A meter stick was used to record the depth of water at each site.

Crayfish Sampling

Crayfish sampling followed the quadrat method of DiStefano et al. (2003) and I stratified sampling by habitat based on qualitative observations of flow and substrate. I collected three samples at five different microhabitats within each site (vegetation, riffle, run, pool, and glide). For the comparisons in this study, only the habitats: riffle, run, and vegetation were used for analysis of impacts. In 2016, four samples were taken from the same three habitats. Results for pools and glides were recorded for an additional study. Samples were taken using a 1 m^2 quadrat sampler with four wooden legs 0.6 m tall, and 2.0 mm mesh walls weighted with chain. The sampler was placed in the substrate and the mesh walls contour to the substrate to prevent escape. The area within the sampler is then agitated by turning over rocks, disturbing by hand, and using a paddle to flush the contents into the cod end of the net. Any samples that were taken that did not have any crayfish were taken an additional time in an adjacent area. In these instances, the second sample was used. All crayfish within each quadrat were collected and stored on ice to be brought back to Hood College. Species, sex, and carapace length were recorded and the five largest *F. rusticus* and non- *F. rusticus* from each site were dissected. The cardiac stomachs were removed from the specimens and stored in 70% ethanol for stomach content analysis. A small number of crayfish were sacrificed for stomach analysis before proper identification took place and were originally identified as either *F. virilis* or *F. obscurus*, therefore, these crayfish were identified as "unknown non-*Faxonius rusticus*". However, for the purpose of this study this was not relevant as the comparisons were left at *F. rusticus*-present compared to *F. rusticus*-absent groups. No crayfish were released back into the river as per the Maryland Department of Natural Resources regulations and all samples were collected under permit #SCP202169 issued to Hood College by the Maryland Department of Natural Resources.

Macroinvertebrate Sampling

Macroinvertebrate samples were taken using a Surber sampler (Parker 2018), while practicing the same disturbance methods used for crayfish collection. Sampling areas were also selected using the same methods used for crayfish sampling. In 2021, five macroinvertebrate samples were taken from the three microhabitats at each site: riffle, run and vegetation. This is because glides are physically similar enough to runs, with only a slightly slower flow and less turbulence than runs (Bisson et al. 1982, Milan et al. 2009), therefore we presume that macroinvertebrate composition would be similar enough between the two, and that pools are generally too deep to effectively use a Surber sampler. In 2016, four samples from each habitat were taken. Macroinvertebrate samples were preserved in 70% ethanol and brought back to Hood College. Macroinvertebrate samples were sorted from any detritus and sediment and placed in 20 ml glass scintillation vials filled with 85% denatured ethanol. Any samples that contained specimens too large to fit into the vials (bivalves, gastropods, etc.) were stored in 50 ml glass specimen jars filled with 85% ethanol. Crayfish found within macroinvertebrate samples were identified but not all were stored due to their size. Macroinvertebrates were to the order level in 2016 and genus level in 2021. Any organisms that could not be identified to genus were classified at the nearest taxonomic level possible. All chironomids were identified to the family level, due to the complexity of proper genus identification of Chironomidae. Any distinctly different chironomids were identified as Chironomidae, following a sequential ID number.

From this data, I calculated biodiversity using the Shannon-Wiener Diversity Index (Lloyd et al. 1968) (Equation 1) for each sample.

$$\boxed{H = -\sum_{i=1}^{s} p_i ln p_i}$$

Equation 1. Shannon-Wiener Diversity Index (H-value).

These H-values were averaged for each site. I also calculated abundance as total number of individuals for each sample. These values were then averaged for each site as a measure of density (individuals/0.3 m^2). Densities were standardized to 1 m^2. An EPT Index was calculated for *F. rusticus*-present and *F. rusticus*-absent sites to investigate whether water quality is vastly different between the two treatments (Equation 2).

$$\boxed{\frac{Total\ EPT\ Taxa}{Total\ Taxa\ Found} \times 100\% = \%\ Abundance}$$

Equation 2. EPT Richness Index equation.

Once identified, samples were dried in a drying oven at 70 ℃ for 24 hours. The dry weight of each sample was recorded, and samples were then incinerated in a muffle furnace for 3 hours at 550 ℃. The ash weight was then recorded and subtracted from the dry weight to obtain an ash-free dry weight (AFDW), used as a measurement of biomass. Biomass was not measured for 2016 samples. Because not all crayfish from macroinvertebrate samples were preserved and that crayfish inherently weigh more than many of the other invertebrate taxa sampled, crayfish were not included in biomass measurements.

Stomach Content Analysis

The 10 largest *F. rusticus* and the 10 largest non-*F. rusticus* from each site sampled in 2021 were used for an exploratory stomach content analysis. If the site did not have *F. ructicus*, only the 10 non-*F. rusticus* were dissected. Due to the nature of crayfish dissection, occasional stomachs were too damaged to use for this analysis. In this case, the next largest crayfish from that category was used. Any macroinvertebrates found in the cardiac stomachs were identified to the lowest taxonomic group possible and documented accordingly.

Statistical Analysis

R was used for all non-parametric tests. Data was tested for normal distribution using the Shapiro-Wilk test. Since most of the groups were not normally distributed, non-parametric statistics were used for all tests. Mann-Whitney tests were used to compare the averages of sites with *F. rusticus* and without *F. rusticus* for H-value, density, and biomass metrics for macroinvertebrates (2021). Linear regressions, performed in Excel, were used for each of the three variables, once with the independent variable being density of *F. rusticus* (m^{-2}), and once with the independent variable being total crayfish density (m^{-2}). Due to the small sample size of *F. rusticus* absent sites, a series of pairwise Mann-Whitney tests was used to compare the averages for H-value, density, and biomass (2021) between habitats within both groups (*F. rusticus*-present and *F. rusticus*-absent).

For the 2016 data, Links Bridge, although surrounded by *F. rusticus*-present sites, it was treated as a *F. rusticus*-absent site since no *F. rusticus* were detected in sampling. Additionally, a series of Mann-Whitney tests were used to compare the average H-value, density, and biomass (2021) between the two groups (*F. rusticus*-present and *F. rusticus*-absent) for each habitat. Mann-Whitney tests were used to compare densities between the two groups for all taxa.

RESULTS

Crayfish Composition

Density of each crayfish species was calculated for both 2016 and 2021 sites, to quantify crayfish composition. Four species of crayfish were found including *Faxonius rusticus, Faxonius virilis, Faxonius obscurus, and Cambarus bartonii.* In 2016, *F. rusticus* was absent from Links Bridge Rd, Biggs Ford Rd, and Monocacy Blvd (Figure 3). *F. rusticus* shifted downstream 12.72 kilometers over the past 5 years between sampling efforts, from Devilbiss Bridge Rd. to Monocacy Blvd (Figure 3). *F. obscurus* was only present at sites where *F. rusticus* was absent.

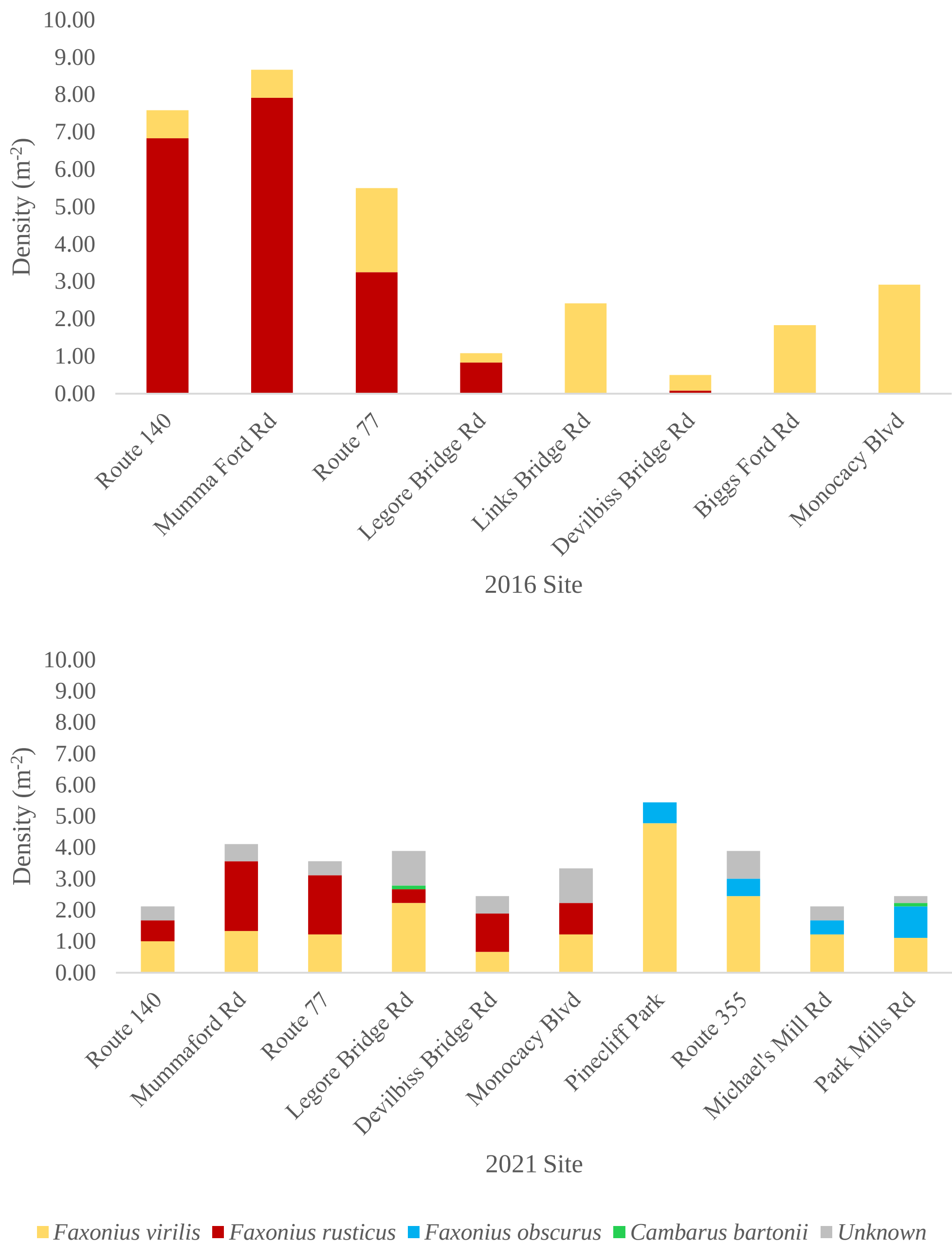


Figure 3. Density (m^{-2}) of crayfish species sampled in 2016 (top) and 2021 (bottom).

Biodiversity Comparisons of Macroinvertebrates

There was no significant difference in the average H-values ($p = 0.786$) determined for macroinvertebrates between *F. rusticus*-present and *F. rusticus*-absent sites in 2016 (Figures 4, 5). H-value was slightly higher in *F. rusticus*-absent sites (Figure 5), but the difference was ultimately not statistically significant. There was no significant difference in H-value at *F. rusticus*-present sites between vegetation and run habitats ($p = 0.095$), vegetation and riffle ($p = 0.056$), or riffle and run ($p = 0.548$) (Figure 6). H-value was not statistically different between any of the *F. rusticus*-absent habitat comparisons: vegetation and run ($p = 0.2$), vegetation and riffle ($p = 0.2$), and run and riffle ($p = 1.0$). Additionally, in comparing habitats at *F. rusticus*-present sites with *F. rusticus*-absent sites, no significant differences were found: vegetation ($p = 0.393$), riffle ($p = 0.786$), and run ($p = 0.786$) (Figure 6). There was no significant correlation between the density of *F. rusticus* and H-value ($p = 0.432$, $R^2 = 0.105$) (Figure 7). There was also no significant correlation between overall density of crayfish and H-value ($p = 0.513$, $R^2 = 0.075$) (Figure 8).

In 2021, The was no significant difference in the average H-values ($p = 0.609$) between *F. rusticus*-present and *F. rusticus*-absent sites in 2021(Figures 4, 5). There was no significant difference in H-value between any of the *F. rusticus*-present habitat comparisons (Figure 6): vegetation and run ($p = 0.394$), vegetation and riffle ($p = 0.093$), and riffle and run ($p = 0.065$). At *F. rusticus*-absent sites, H-value was significantly higher at runs than vegetation ($p = 0.029$) but not between vegetation and riffle ($p = 0.114$) or riffle and run ($p = 0.486$). When comparing *F. rusticus* present with *F. rusticus*-absent sites, runs at *F. rusticus*-absent sites had a significantly higher H-value than runs at *F. rusticus*-present sites (0.019), but there were no statistically significant differences between H-values of vegetation ($p = 0.762$) and riffle (p = 0.476). Additionally, there was no significant correlation between the density of *F. rusticus* and H-value (p = 0.554, R^2 = 0.045) (Figure 7). No significant correlation was detected between overall density of crayfish and H-value (p = 0.293, R^2 = 0.137) (Figure 8).

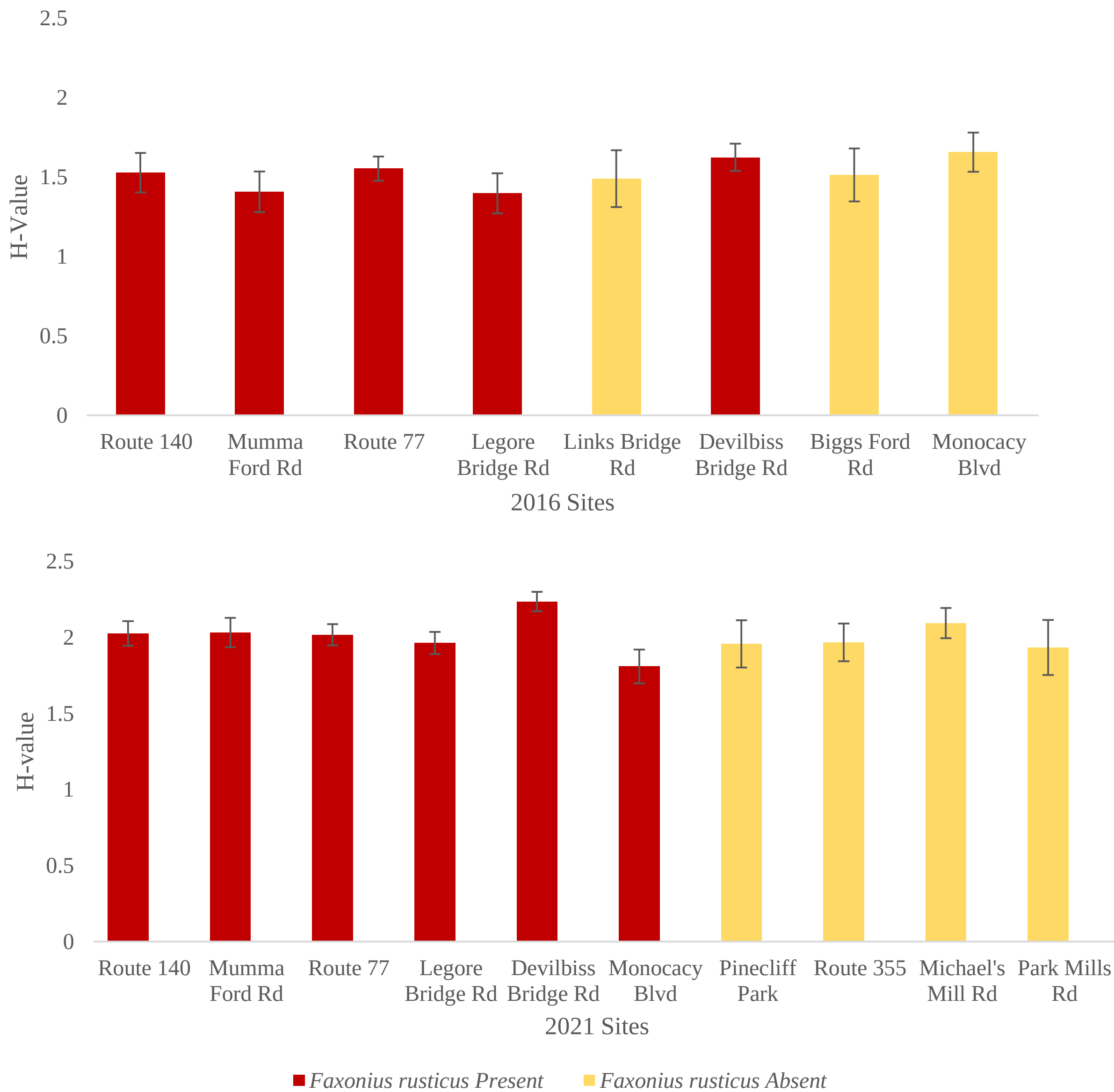


Figure 4. Average H-value at each site sampled in 2016 (top) and 2021 (bottom), characterized by presence or absence of *Faxonius rusticus*. Error bars denote ± 1 S.E.

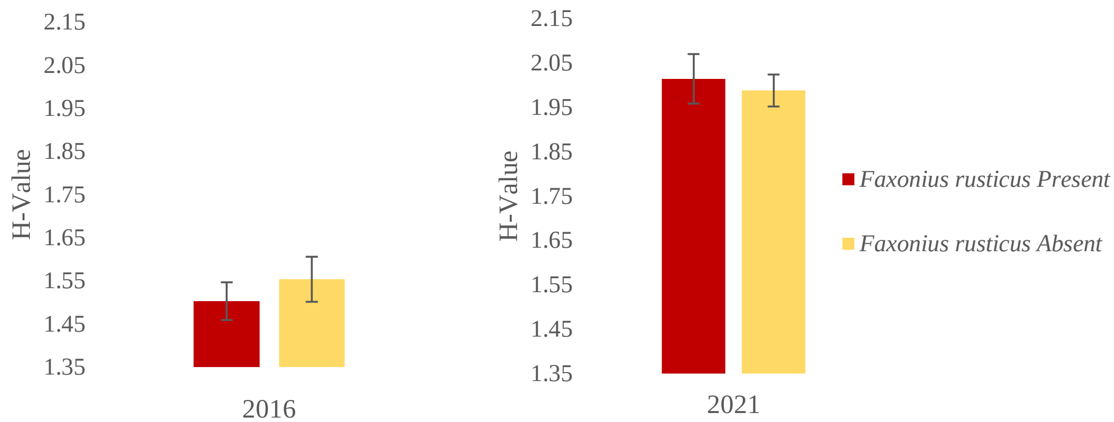


Figure 5. Average H-value at *Faxonius rusticus*-present and *Faxonius rusticus*-absent sites in 2016 (left) and 2021 (right). Error bars denote ± 1 S.E.

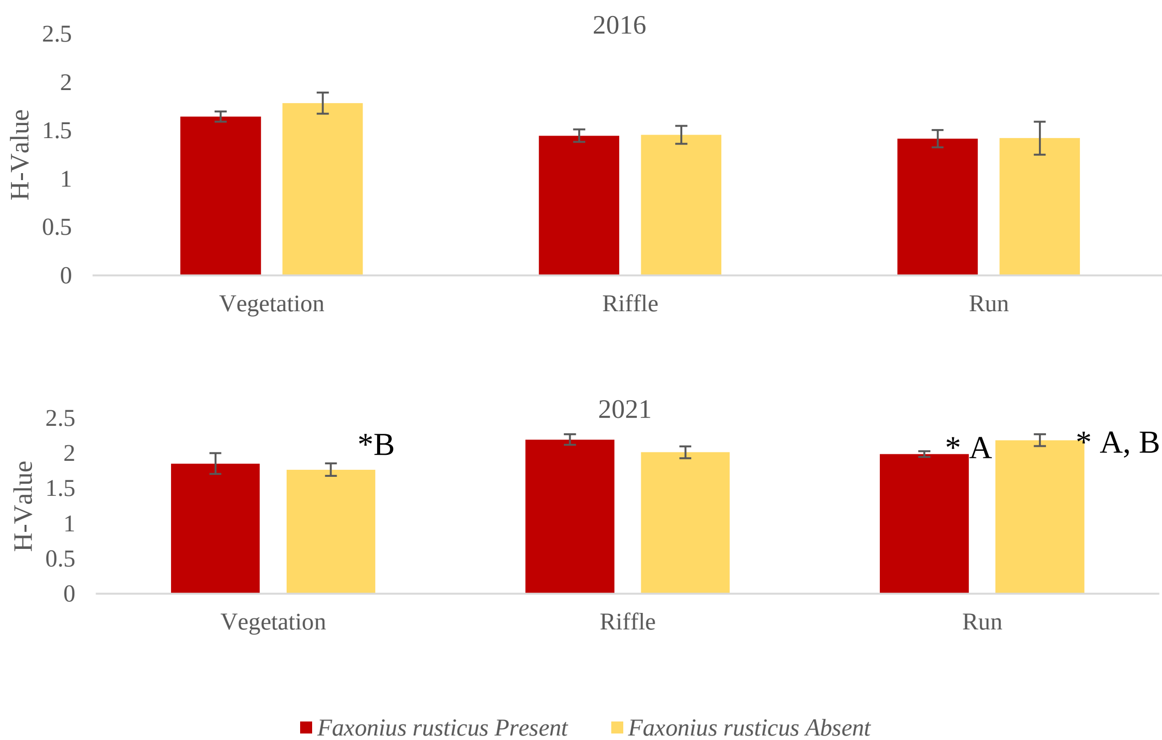


Figure 6. Average H-value of each microhabitat at *Faxonius rusticus*-present and *Faxonius rusticus*-absent sites in 2016 (top) and 2021 (bottom). Error bars denote ± 1 S.E. Asterisks denote statistically significant differences. Letters denote statistically different groups.

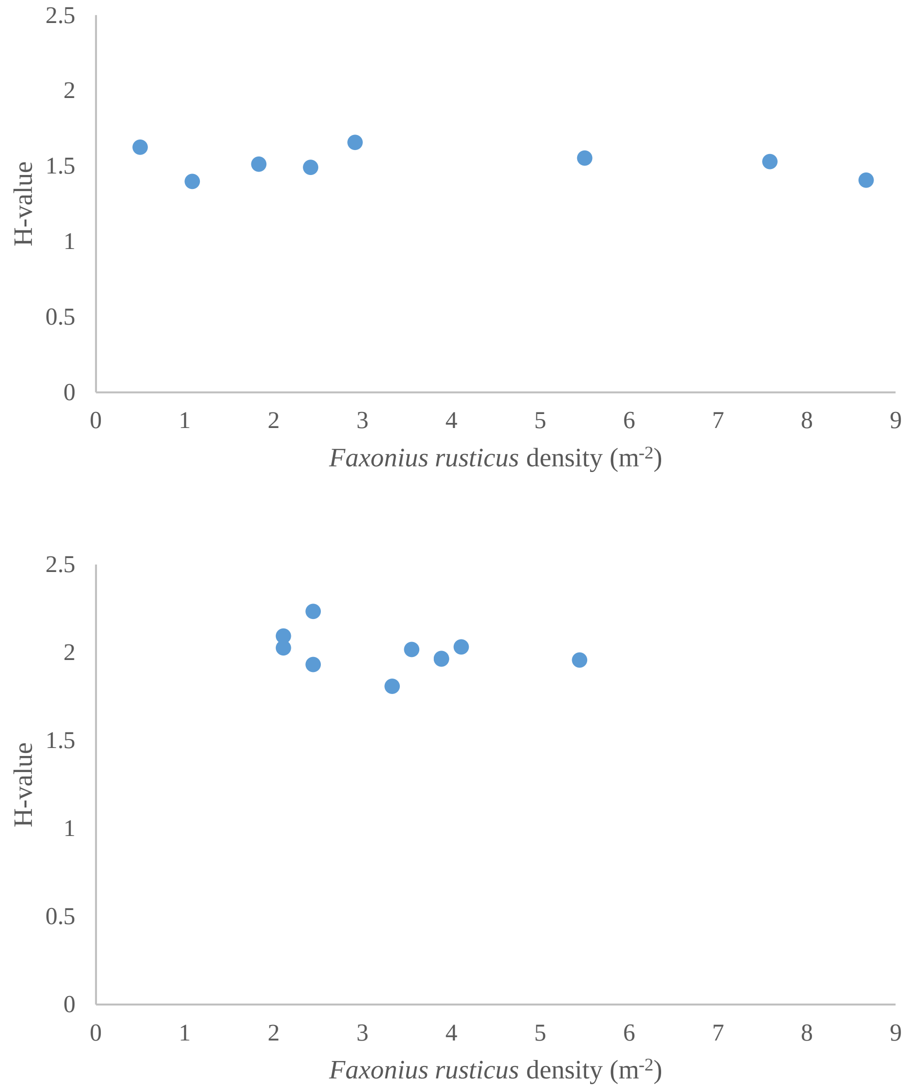


Figure 7. Plot of h-value as a function of density of *Faxonius rusticus* (m^{-2}) in 2016 ($p = 0.432$, $R^2 = 0.105$) (top) and 2021($p = 0.554$, $R^2 = 0.045$) (bottom).

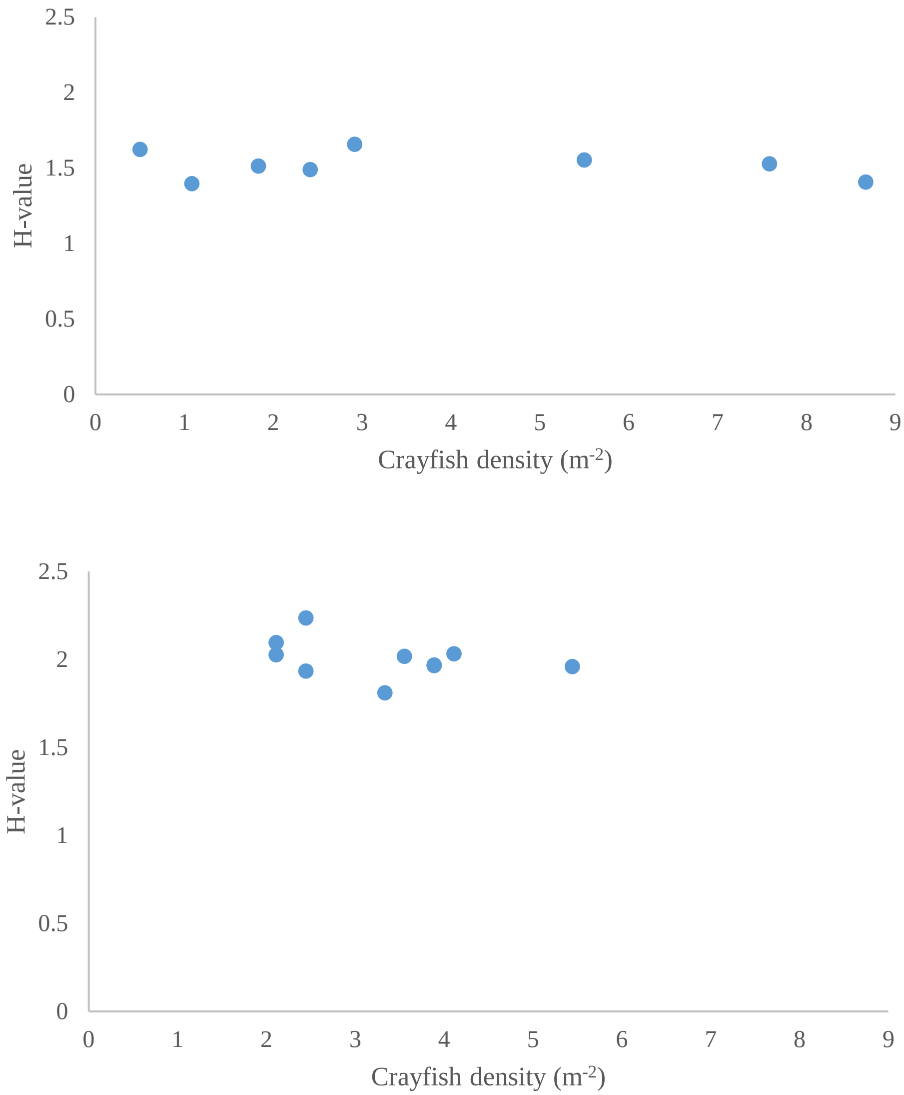


Figure 8. Plot of H-value as a function of total crayfish density (m^{-2}) in 2016 ($p = 0.513$, $R^2 = 0.075$) (top) and 2021($p = 0.293$, $R^2 = 0.137$) (bottom).

Density Comparisons of Macroinvertebrates

In 2016, there was no significant difference in density of macroinvertebrates between *F. rusticus*-present and *F. rusticus*-absent sites (p = 0.571) (Figures 9, 10). There was also no significant difference between densities in habitats at *F. rusticus*-present sites: vegetation and run (p = 0.222), vegetation and riffle (p = 0.310), and run and riffle (p = 0.222) (Figure 11). At *F. rusticus*-absent sites, there was no significant difference in densities between vegetation and run (p = 0.400), vegetation and riffle (p = 0.200) and run and riffle (p = 0.700) (Figure 11). When comparing habitats at *F. rusticus*-present sites with *F. rusticus*-absent sites, no significant differences were found: vegetation (p = 0.250), riffle (p = 0.571), and run (p = 0.250) (Figure 11). There was no significant correlation between the density of *F. rusticus* and density of macroinvertebrates (p = 0.537, R^2 = 0.066) (Figure 12). No significant correlation was detected between overall density of crayfish and density of macroinvertebrates (p = 0.570, R^2 = 0.057) (Figure 13).

In 2021, there was no significant difference in density of macroinvertebrates between *F. rusticus*-present and *F.rustcius*-absent sites (p = 0.476) (Figures 9, 10). The density of macroinvertebrates was significantly higher at riffles than at vegetation at *F. rusticus*-present sites (p = 0.004), however there was no significant difference between vegetation and run (p = 0.065) or riffle and run (p = 0.179) (Figure 11). At *F. rusticus*-absent sites, there was no significant difference in density detected between habitats: vegetation and run (p = 0.114), vegetation and riffle (p = 0.057), and riffle and run (p = 0.2) (Figure 11). When comparing *F. rusticus*-present with *F. rusticus*-absent sites, there were no statistically significant differences between densities of the three habitats: vegetation (p = 0.392), riffle (p = 0.914), and run (p = 0.476) (Figure 11). Additionally, there was no significant correlation between the density of

F. rusticus and density of macroinvertebrates ($p = 0.578$, $R^2 = 0.040$) (Figure 12). No significant correlation was detected between overall density of crayfish and density of macroinvertebrates ($p = 0.686$, $R^2 = 0.022$) (Figure 13).

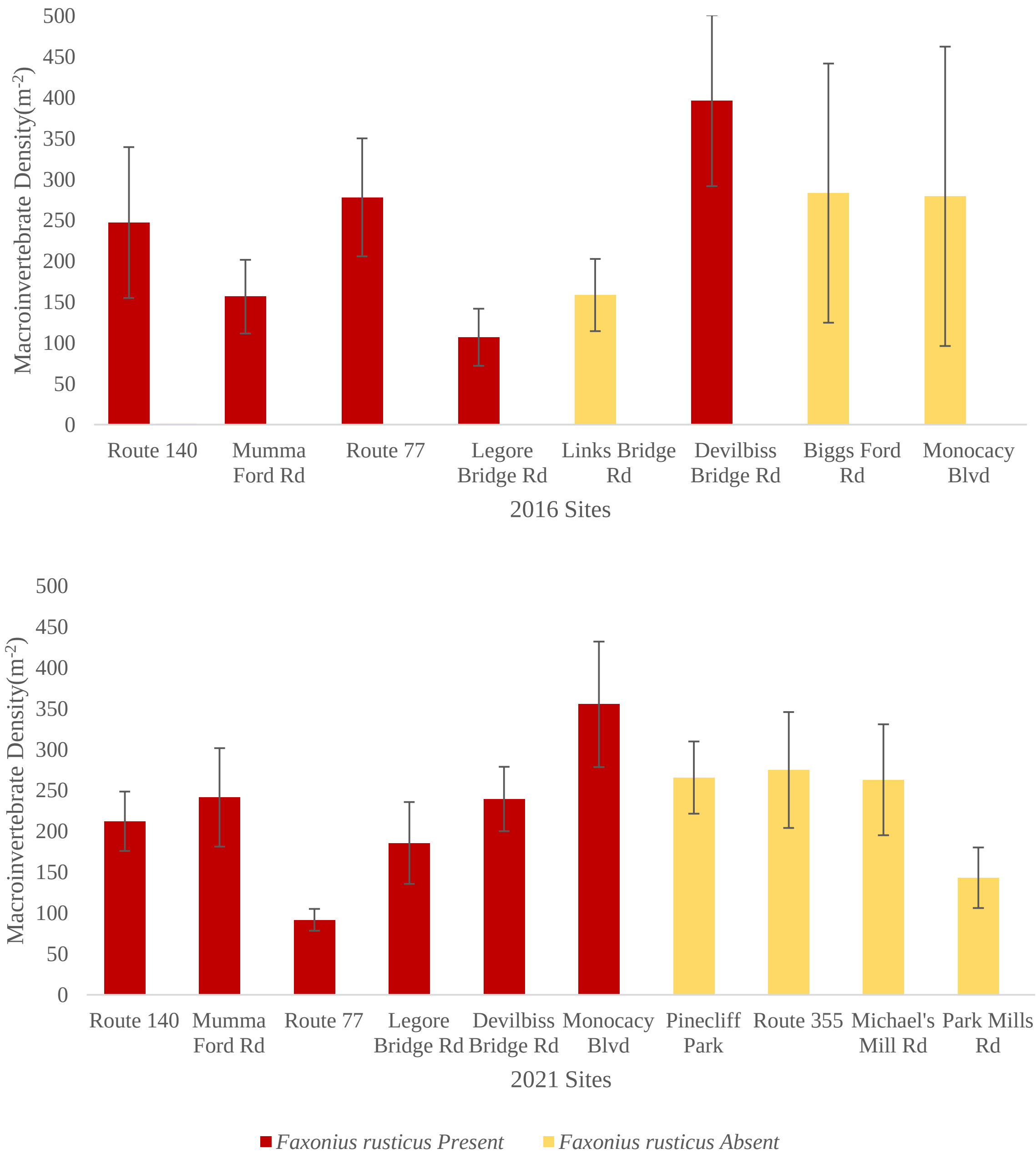


Figure 9. Average density of macroinvertebrates (m^{-2}) at each site sampled in 2016 (top) and 2021 (bottom), characterized by presence of *Faxonius rusticus.* Error bars denote ± 1 S.E.

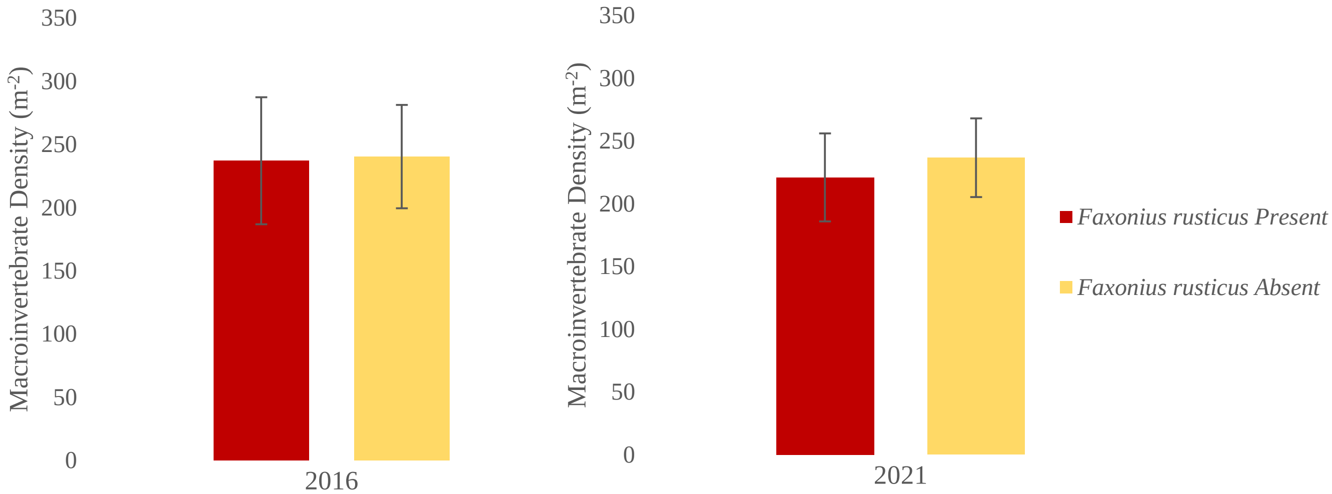


Figure 10. Average density of macroinvertebrates (m^{-2}) at *Faxonius rusticus*-present and *Faxonius rusticus*-absent sites in 2016 (left) and 2021 (right). Error bars denote ± 1 S.E.

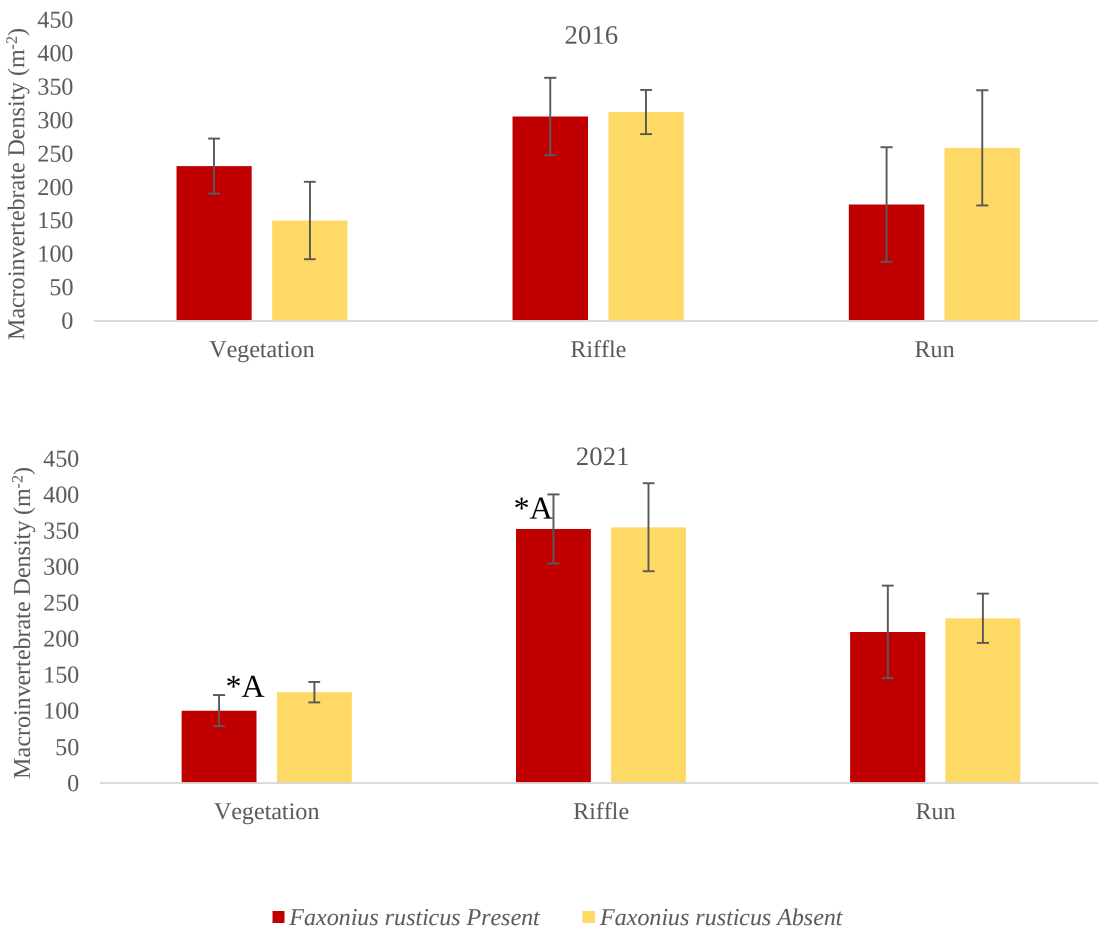
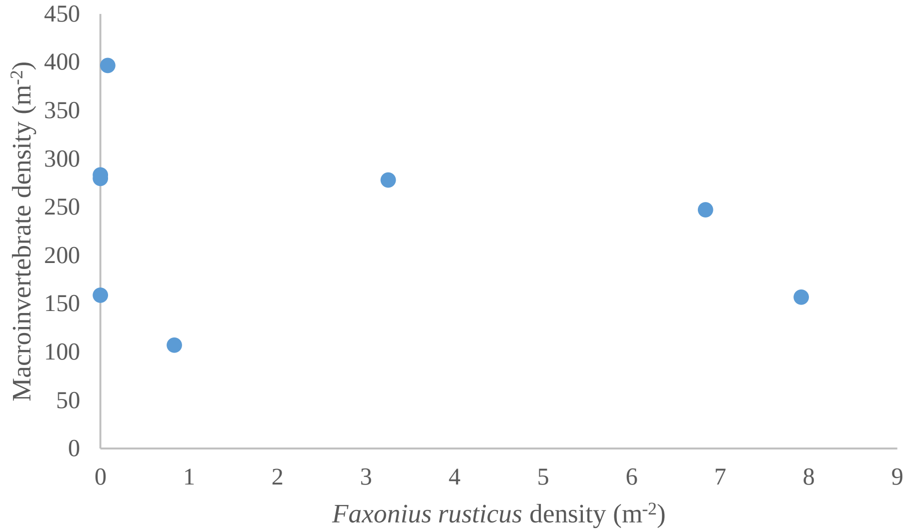


Figure 11. Average macroinvertebrate density (m^{-2}) of each microhabitat at *Faxonius rusticus*-present and *Faxonius rusticus*-absent sites in 2016 (top) and 2021 (bottom). Error bars denote ± 1 S.E. Asterisks denote statistically significant differences. Letters denote statistically different groups.



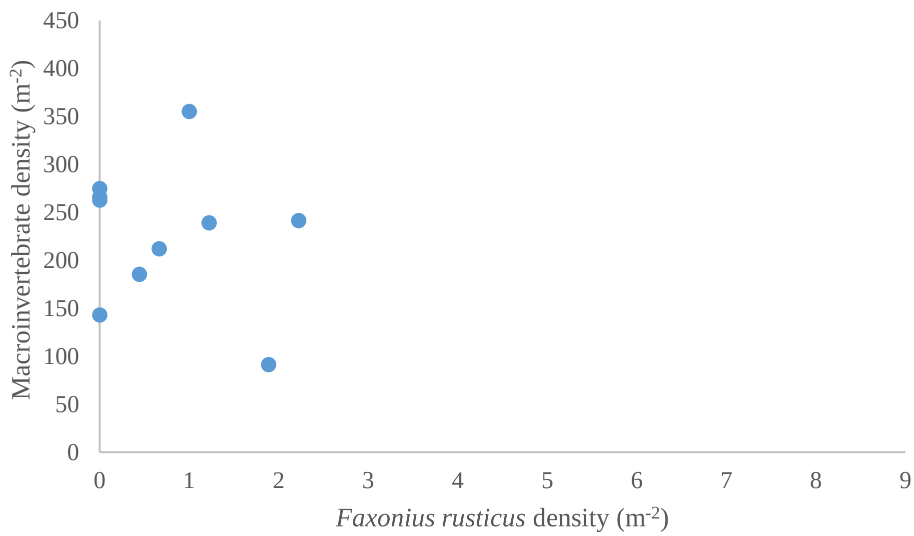
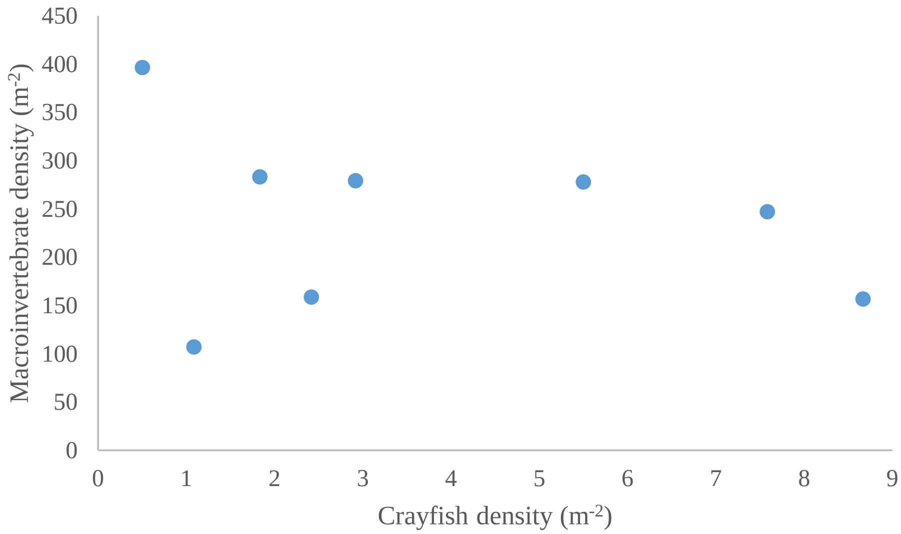


Figure 12. Plot of macroinvertebrate density (m^{-2}) against density of *Faxonius rusticus* (m^{-2}) in 2016 ($p = 0.537$, $R^2 = 0.066$) (top) and 2021 ($p = 0.570$, $R^2 = 0.05$) (bottom).



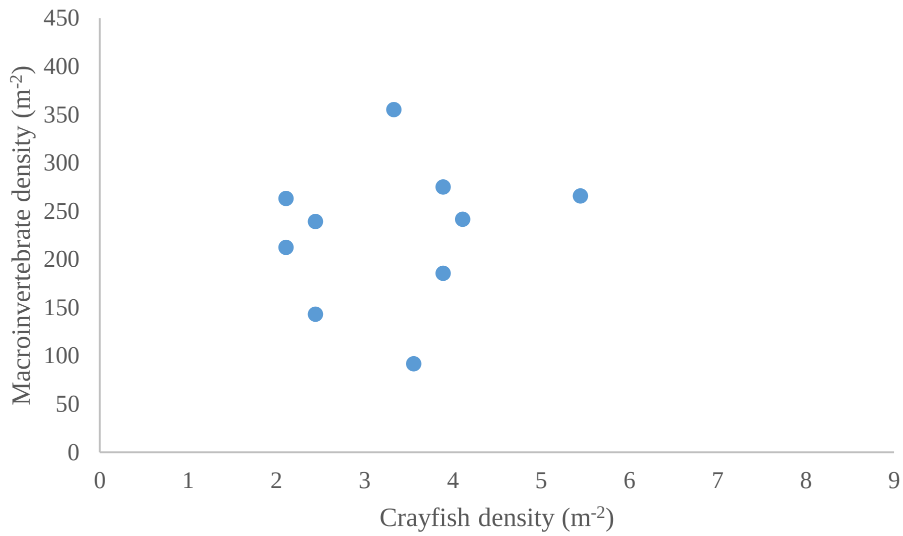


Figure 13. Plot of macroinvertebrate density (m^{-2}) against total crayfish density (m^{-2}) in 2016 ($p = 0.570$, $R^2 = 0.057$) (top) and 2021($p = 0.686$, $R^2 = 0.022$) (bottom).

Biomass Comparisons of Macroinvertebrates

In 2021, there was no significant difference in biomass (AFDW) at *F. rusticus*-present and *F. rusticus*-absent sites (p = 0.914) (Figures 14, 15). There was no significant difference in biomass between any of the *F. rusticus*-present habitat comparisons (Figure 16): vegetation and run (p = 0.818), vegetation and riffle (p = 0.589), and riffle and run (p = 0.699). At non-*F. rusticus* sites, there was no significant difference in biomass between vegetation and run (p = 0.686), vegetation and riffle (p = 0.343) or riffle and run (p = 1.0). When comparing *F. rusticus*-present with *F. rusticus*-absent sites, there were no statistically significant differences between biomass of the three habitats: vegetation (p = 1.0), riffle (p = .914), and run (p = .914) (Figure 16). There was no significant correlation between the density of *F. rusticus* and biomass (p = 0.889, R^2 = 0.003) (Figure 17). No significant correlation between total crayfish density and biomass was detected (p = 0.163, R^2 = 0.228) (Figure 18).

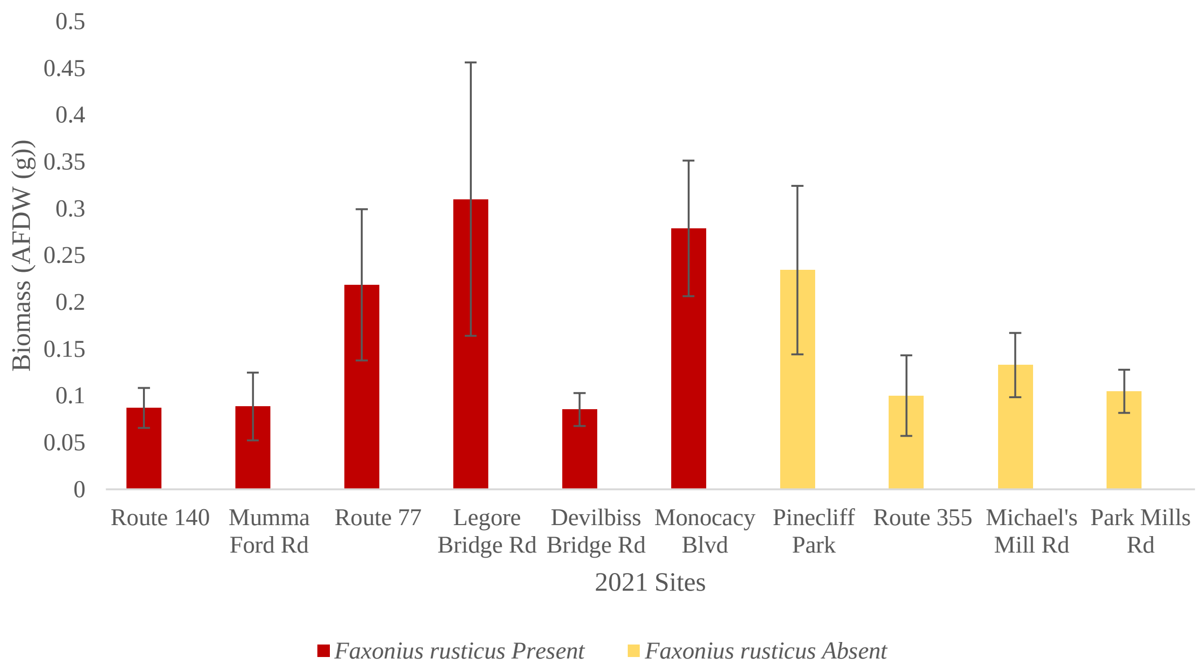


Figure 14. Average AFDW (g) at each site sampled in 2021, characterized by presence of *Faxonius rusticus*. Error bars denote ± 1 S.E.

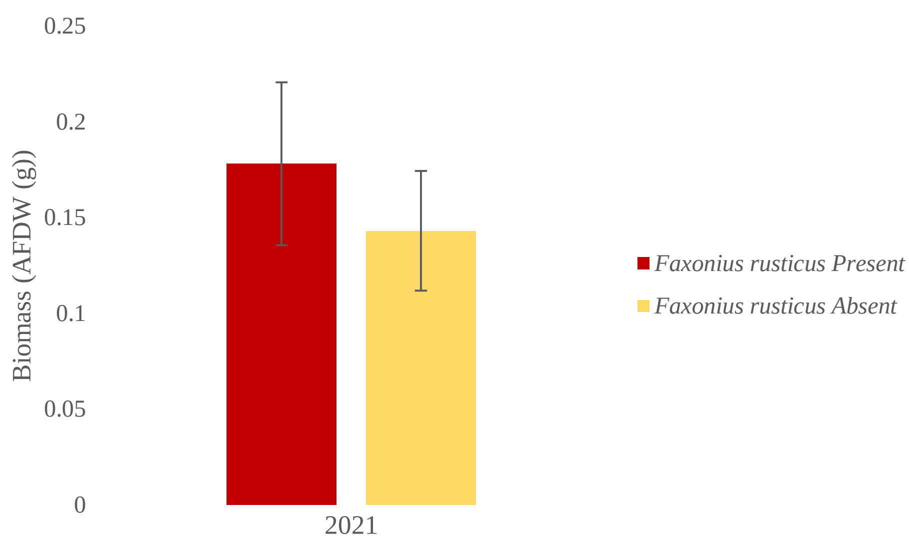


Figure 15. Average AFDW (g) of *Faxonius rusticus* present and *Faxonius rusticus* absent sites in 2021. Error bars denote ± 1 S.E.

Figure 16. Average AFDW (g) at each microhabitat at *F. rusticus*-present and *F. rusticus*-absent sites in 2021. Error bars denote ± 1 S.E.

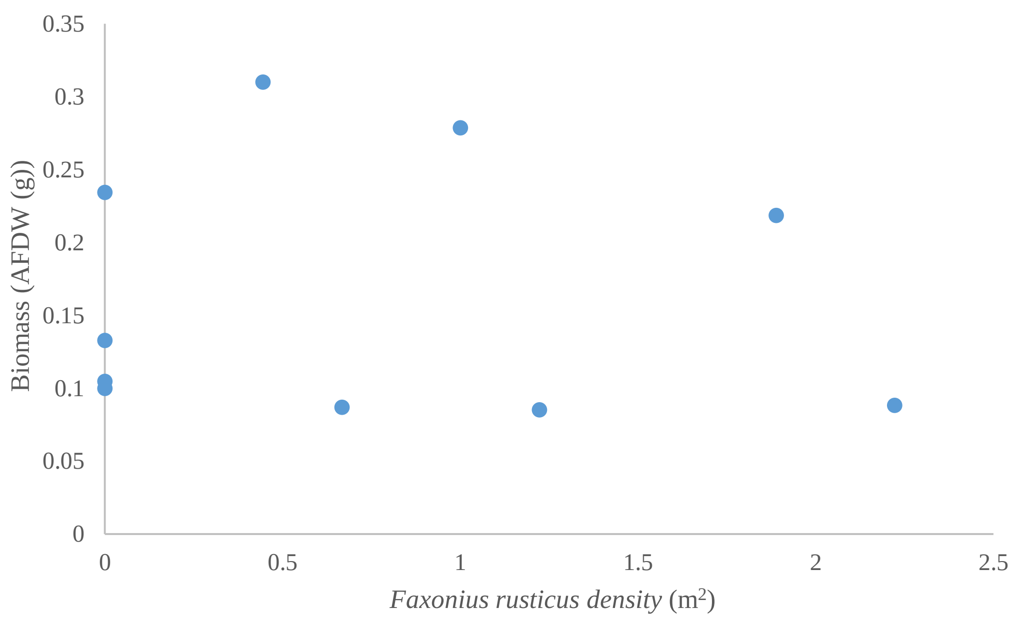


Figure 17. Plot of the average density (m^{-2}) of *Faxonius rusticus* at each site against the average AFDW (g) at each site in 2021($p = 0.889$, $R^2 = 0.003$).

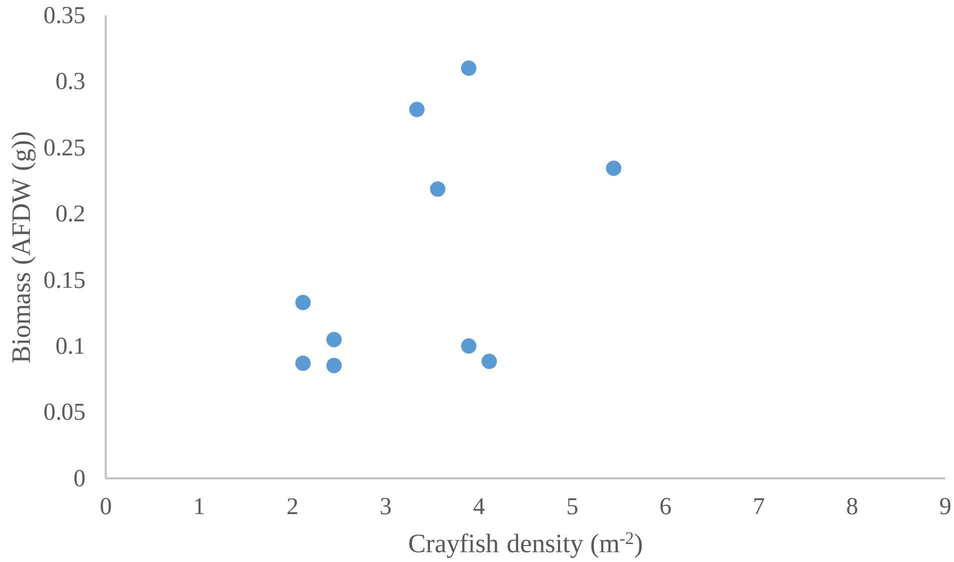


Figure 18. Plot of the average crayfish density (m^{-2}) at each site against the average AFDW (g) at each site in 2021($p = 0.163$, $R^2 = 0.228$).

Taxonomic Comparisons

In total 109 taxonomic groups were identified. Table 1 shows the average densities for each taxon at *F. rusticus*-present and *F. rusticus*-absent sites, and the accompanying p-value. Only one group was statistically different ($p = 0.031$) between *F. rusticus*-present and absent sites, which was the genus *Ameletus spp.*, also known as the comb-mouthed minnow mayfly. The density of *Ameletus spp.* Was statistically higher at *F. rusticus*-absent sites than at *F. rusticus*-present sites (Table 1). The EPT Richness Index was calculated for *F. rusticus*-present and *F. rusticus*-absent sites. *Faxonius rusticus*- present sites had 25.7% EPT taxa and *F. rusticus*-absent sites had 27.5% EPT taxa.

Table 1. Average density (m^{-2}) of all taxonomic groups identified in 2021 at *Faxonius rusticus*-present and *Faxonius rusticus*-absent sites and their accompanying p-values.

Genus	Density *Faxonius rusticus* Present Sites (m^2)	Density *Faxonius rusticus* Absent Sites (m^2)	p-value	Genus	Density *Faxonius rusticus* Present Sites (m^2)	Density *Faxonius rusticus* Absent Sites (m^2)	p-value	Genus	Density *Faxonius rusticus* Present Sites (m^2)	Density *Faxonius rusticus* Absent Sites (m^2)	p-value
Acentrella	0.30	0.44	0.717	*Glossosoma*	0.44	0.11	0.256	*Simulium*	0.19	0.33	0.542
Acroneuria	0.07	0.22	0.542	*Helichus*	0.04	0.00	0.540	*Stenacron*	1.33	2.50	0.392
Agarodes	0.04	0.00	1.000	*Helicopsyche*	0.07	0.00	0.540	*Stenelmis*	34.00	34.94	0.914
Agnetina	1.22	0.72	0.058	*Hemerodromia*	0.04	0.00	0.540	*Stylogomphus*	0.15	0.00	0.287
*Ameletus**	0.07	0.78	0.031	*Heptagenia*	1.70	1.50	0.914	*Stylurus*	0.04	0.00	0.540
Amnicola	0.11	0.00	0.540	*Hetaerina*	0.41	0.11	0.694	*Trepobates*	0.19	0.00	0.147
Ancyronyx	0.11	0.00	0.287	*Heterocloeon*	0.56	1.00	0.585	*Trichocorixa*	0.26	0.00	0.540
Anthopotamus	4.19	4.33	0.593	*Hexagenia*	0.04	0.00	0.540	Trichoptera	0.56	0.39	0.577
Antocha	0.00	0.17	0.307	*Hexatoma*	0.04	0.06	0.878	*Tricorythodes*	2.89	2.39	0.610
Apatania	0.00	0.06	0.307	*Hirudinea*	0.17	0.00	0.540	*Tubifex*	0.04	0.11	0.761
Argia	1.07	0.33	0.508	*Hydrochara*	0.00	0.06	0.307	*Viviparis*	0.04	0.00	0.540
Attenella	0.00	0.22	0.094	*Hydropsyche*	4.70	4.22	0.831				
Aulodrilus	0.15	1.89	0.299	*Hydroptila*	0.00	0.06	0.307				
Baetis	4.85	4.56	0.762	*Ischnura*	0.30	0.11	0.464				
Beloneuria	0.00	0.06	0.307	*Isonychia*	1.85	2.33	1.000				
Belostoma	0.07	0.00	0.540	*Laevapex*	0.07	0.17	0.429				
Boyeria	0.00	0.06	0.307	*Lanthus*	0.04	0.00	0.540				
Brachycentrus	0.78	0.17	0.570	*Leptoxis*	33.52	25.83	0.914				
Branchiura	0.04	0.00	0.540	*Leucrocuta*	4.41	3.44	0.762				
Caecidotea	0.30	0.00	0.540	*Limnophora*	0.00	0.06	0.307				
Caenis	3.37	1.22	0.521	*Lumbricina*	0.07	0.11	0.878				
Callicoryxa	0.00	0.06	0.307	*Maccaffertium*	3.37	9.89	0.109				
Calopteryx	0.19	0.19	0.908	*Macronychus*	1.85	0.56	0.238				
Campeloma	1.74	0.11	0.075	*Mesovelia*	0.04	0.00	0.540				
Cheumatopsyche	39.26	50.06	0.914	*Metrobates*	0.63	0.33	0.912				
Chimarra	5.37	3.06	0.915	*Microcylloepus*	14.85	14.44	0.476				
Chironomidae	8.15	2.78	0.109	*Myzobdella*	0.15	0.00	0.284				
Chironomidae 1	2.48	0.00	0.504	*Neogerris*	0.04	0.00	0.540				
Copelatus	0.04	0.06	0.878	*Neoperla*	0.07	0.22	0.542				
Corbicula	5.44	2.17	0.669	*Ophiogomphus*	0.00	0.06	0.307				
Corydalus	2.59	0.78	1.000	*Optioservus*	9.11	11.72	0.476				
Crangonyx	0.04	1.22	0.238	*Paragnetina*	0.19	0.17	1.000				
Curculionidae	0.00	0.06	0.307	*Parapoynx*	0.04	0.00	0.540				
Dibolocelus	0.00	0.06	0.307	*Peltodytes*	0.15	0.06	1.000				
Diplectrona	0.44	0.06	0.694	*Physella*	0.33	0.00	0.067				
Dolophilodes	0.00	0.06	0.307	*Pisidium*	0.22	0.94	0.065				
Dromogomphus	0.04	0.00	0.540	*Placobdella*	0.00	0.06	0.307				
Dubiraphia	0.19	0.06	0.397	*Planaria*	0.93	1.17	0.059				
Dysticidae	0.67	2.00	1.000	*Planorbella*	0.00	0.06	0.307				
Ectopria	0.26	0.00	0.147	*Planorbis*	0.04	0.00	0.540				
Elimia	0.00	0.72	0.307	*Prosimulium*	0.63	0.00	0.287				
Ephemerella	0.59	1.72	0.278	*Psephenus*	6.85	7.44	1.000				
Epicordulia	0.00	0.11	0.307	*Ranatra*	0.11	0.00	0.287				
Eurylophella	0.04	0.00	0.540	*Rhagovelia*	0.04	0.11	0.761				
Faxonius	0.37	0.17	0.438	*Rheumatobates*	0.07	0.06	1.000				
Ferrissia	0.41	0.39	1.000	*Rhithrogena*	0.04	0.11	0.350				
Fossaria	0.07	0.00	0.284	*Serratella*	3.30	6.89	0.284				
Gammarus	4.89	22.28	0.053	*Serromyia*	0.00	0.06	0.307				
Gerris	0.00	0.11	0.307	*Sialis*	0.37	0.17	0.504				

Stomach Content

Macroinvertebrates from the stomachs of the 10 largest *F. rusticus* and non-*F. rusticus* crayfish at each site were identified and organized into the table below (Table 2).

Table 2. Macroinvertebrates found in the stomachs of both *Faxonius rusticus* and non-*Faxonius rusticus* stomachs.

***F. rusticus* stomach**			**Non- *F. rusticus* stomach**		
Genus (or lowest taxonomic level)	**Common name**	**Frequency**	**Genus (or lowest taxonomic level)**	**Common name**	**Frequency**
Chironomidae	Non-biting midge	2	*Hydropsyche*	Net-spinning caddisfly	1
Stenacron	Flat-headed mayfly	1	*Actinoptera*	Unknown fish material	1
Hydropsyche	Net-spinning caddisfly	2	*Argia*	Dancer	1
Diptera	Unknown true fly	1	*Parapoynx*	Aquatic caterpillar	1
Odonata	Unknown dragonfly/damselfly	1	*Leucracuta*	Flat-headed mayfly	2
			Heptageniidae	Unknown flat-headed mayfly	1
			Coleoptera	Unknown beetle	1

Microhabitat Physical Parameters

The average for each of the physical parameters (flow (m/s), temperature (°C), depth (cm), and conductivity (μS/cm)) ± one standard error for each microhabitat is provided in the table below to better visualize the physical differences in these habitats (Table 3).

Table 3. Average physical parameters ± 1 standard deviation for each microhabitat.

	Vegetation	**Riffle**	**Run**
Flow (m/s)	0.061 ± 0.058	0.414 ± 0.194	0.278 ± 0.144
Temperature (°C)	26.527 ± 2.348	26.094 ± 2.108	26.091 ±1.781
Depth (cm)	16.790 ± 8.343	16.661 ± 6.425	24.205 ± 9.148
Conductivity (μS/cm)	414.671 ± 64.971	418.788 ± 61.153	429.331 ± 59.867

DISCUSSION

The community structure benthic macroinvertebrates in the Monocacy River does not appear to be threatened by predation from *Faxonius rusticus*. I hypothesized that there would be a significant difference in biodiversity, density, and biomass between sites with a presence of *F. rusticus* and sites without in the Monocacy River. Our results suggest that overall, there is no significant relationship between the presence of *F. rusticus* and the biotic indices of benthic macroinvertebrates. I saw no significant difference between the overall average H-value, density (m^2), and AFDW (g) at *F. rusticus*-present and *F. rusticus*-absent sites in 2016 and 2021. Additionally, I considered three different microhabitats (vegetation, riffle, and run) that may impact these variables. I did see a slight significant difference in the h-value of *F. rusticus*-present and *F. rusticus*-absent sites, when looking at only run habitats. However, all other significant differences were within either *F.rusticus*-present or *F. rusticus*-absent groups, between habitats. While I treated Links Bridge Rd as a *F. rusticus*-absent site in 2016, due to there being no *F. rusticus* detected in samples, this follows the assumption that as *F. rusticus* migrated through the site downstream, it did not alter the macroinvertebrate communities. However, metrics for this site were relatively consistent with the rest of the 2016 sites, so I perceive this as having a minimal impact on my results.

Impact of Faxonius rusticus Presence on Benthic Macroinvertebrates

Various studies performed on limnetic ecosystems have documented that *F. rusticus* has a negative impact on benthic macroinvertebrate abundance through predation (Kreps et al. 2016; Mccarthy et al. 2006; Wilson et al. 2004). *Faxonius rusticus* has been shown to decrease abundance of snails in a lake from > 10,000 to < 5 individuals m^{-2} (Wilson et al. 2004).

Additionally, some studies have shown this same relationship in stream ecosystems (Charlebois and Lamberti 1996; Bobeldyk and Lamberti 2006). In a Michigan river, sites sampled lacking *F. rusticus* had significantly higher densities of invertebrates compared to those with a presence of *F. rusticus* (Bobeldyk and Lamberti 2006). The results of our study in a lotic ecosystem are inconsistent with the findings in these experiments as we found that the presence of *F. rusticus* had no effect on benthic macroinvertebrate metrics. Most of the studies conducted in streams that show significant changes in macroinvertebrate assemblages from the *F. rusticus* used enclosures, which involves colonizing enclosures in the stream bed with crayfish to measure its predation (Charlebois and Lamberti 1996; Bobeldyk and Lamberti 2006; Kuhlmann 2016). Our study made use of a less controlled environment; therefore, it may suggest that these trophic dynamics behave differently in a completely "wild" system. Two experiments testing the effects of *F. rusticus* on invertebrates; one that consisted of strictly field sampling, like our methods and one enclosure experiment, showed that macroinvertebrate density and diversity did not change with *F. rusticus* relative abundance at field sampled sites (Kuhlmann et al. 2009). Additionally, they found that crayfish density did impact macroinvertebrate density in the enclosure experiments (Kuhlmann et al. 2009). This raises the question of whether enclosure experiments capture all the stochasticity that is the reality of lotic systems and whether this stochasticity can impact the way *F. rusticus* not only interacts with other species of crayfish but also lower trophic levels.

Impact of Crayfish Density of Benthic Macroinvertebrates

The density of *Faxonius rusticus* did not have a significant effect on biodiversity, abundance, or biomass of benthic macroinvertebrates in the Monocacy River. We saw no significant correlation between density of *F. rusticus* and any of these metrics. Additionally, we saw no significant correlation between overall crayfish density and the biotic indices. This is consistent with the hypothesis that crayfish species do not affect changes in macroinvertebrate communities in a NY stream (Kuhlmann 2016). Additionally, macroinvertebrate density tends to be most altered by high densities of crayfish (high = 10.6 crayfish/m^{-2}, low = 4.0 crayfish/m^2, range: 0-19.3 crayfish/m^2) (Kuhlmann 2016). In a Wisconsin lake, increasing densities of *F. rusticus* were correlated with a decrease in macroinvertebrate densities from 2100 to 176 m^{-2} (Nilsson et al. 2012). In 2016, we observed average crayfish densities ranging from 0.5-8.6 crayfish/m^2 (Figure 3). In 2021, we saw overall densities ranging from 2.1-5.4 crayfish/m^2 (Figure 3). Both overall crayfish densities and *F. rusticus* densities decreased overtime. These results are inconsistent with findings showing that crayfish density will generally increase post-invasion (Hansen et al. 2013b; McCarthy at al. 2006) and those invasive crayfish densities will often surpass the densities of native crayfish (Hansen et al. 2013c). Five years post-invasion, densities of *F. rusticus* dropped to 40-60% of the total population (Marinelli 2022). It is possible that the density of *F. rusticus* was not high enough to have a significant impact on macroinvertebrate diversity. Possible mechanisms for the decline in population could include habitat alteration, disease, and parasitism (Marinelli 2022). While the reason for this decline in the density of *F. rusticus* in the Monocacy River is outside of the scope of this study, it may be a possible explanation for results consistent with other studies were not observed. It also may be that density of *F. rusticus* across sites does not differ enough to detect any significant relationship between *F. rusticus* density and macroinvertebrate diversity and density. While *F. rusticus* densities were higher in 2016, macroinvertebrate taxa were only identified to order, so biodiversity is likely higher than calculated. However, overall densities would not change because of this.

Macroinvertebrate Taxonomic Composition

Benthic macroinvertebrate taxonomic composition in the Monocacy River does not vary based on the presence of *Faxonius rusticus.* I found that only one genus significantly differed in densities between *F. rusticus*-present and *F. rusticus*-absent sites, indicating that taxonomic composition was very similar between the two groups. In terms of taxon richness, *F. rusticus*-present sites had 90 different taxa while *F. rusticus*-absent sites had 81 different taxa. This is consistent with the finding in Kuhlmann (2016), that suggest that high densities of *F. rusticus* do not affect taxon richness. An interesting observation is that the amphipods belonging to the genera *Gammarus* and *Crangonyx* were only found in sites with no *F. rusticus* and the transitional invasion front of Monocacy Blvd. While this difference in density was striking, it was not statistically significant. Amphipods are frequently found in vegetation habitat, which is consistent with where most of the *F. virilis* were found in 2021 sampling (Marinelli 2022). Density of amphipods tends to decrease where abundance of *F. virilis* increases, specifically female *F. virilis* (Hanson et al. 1990). Relatively low densities of *F. virilis* has the capacity to reduce abundance and size-distribution of macroinvertebrates through predation (Hanson et al. 1990). It is possible that the previous invasion of *F. virilis* in the Monocacy River had some sort of preliminary impact on the macroinvertebrate communities within the river. However, we lack data on macroinvertebrates in the Monocacy River prior to 1956 to make this comparison.

Impact of Habitat on Benthic Macroinvertebrate Composition

Habitat seems to have a greater influence on macroinvertebrate communities than the presence of *Faxonius rusticus*. There is an important connection between physical-chemical variables such as temperature, conductivity, and flow and macroinvertebrate taxa. Benthic macroinvertebrate biodiversity tends to increase with lower temperatures, higher velocity, and lower conductivity (Nguyen et al. 2018). While temperature and conductivity did not substantially change across our three microhabitats, flow was a distinguishing factor for defining our habitats. For this reason, it makes sense that we would see lower biodiversity, density, and biomass within the vegetation samples where stream velocity slow. Many taxa of macroinvertebrates are highly sensitive to changes in these physical metrics. For example, vegetation, we tend to see more tolerant taxa here such as bivalves and gastropods, and less of the "EPT" (Ephemeroptera, Plecoptera, and Trichoptera) taxa who have a lower tolerance for these harsh conditions such as high temperatures and low oxygen concentrations (Gaufin and Tarzwell 1952). There was no substantial difference in the EPT Richness Index score for *F. rusticus*-present and *F. rusticus*-absent sites, suggesting that water quality is roughly the same at both sites. This implies that water quality is likely not a concern as a cause for any differences in species diversity or richness at *F. rusticus*-present and *F. rusticus*-absent sites in the Monocacy River. For comparison the median % EPT score in fifth order non-tidal Virginia streams was approximately 47%, but ranged from 0-70% (Virginia Department of Environmental Quality 2003). In North Carolina, free-flowing piedmont streams and rivers with a % EPT taxa richness between 24-31% throughout the months of July-September are considered to have "good" water quality (Lenat 1988), indicating that the Monocacy River may fall somewhere between "average" and "good" water quality depending on interpretation of the EPT score.

CONCLUSION

Invasive species have the capacity to do irreparable damage to an ecosystem. It is important to understand the mechanisms through which invasive species alter an aquatic system to better manage these highly valuable ecosystems. This study has shown that both the presence and density of *Faxonius rusticus* has a very limited impact on the macroinvertebrate community structure in the Monocacy River, which are results that have not been previously reported in the literature. Furthermore, I observed a declining trend in the density of *F. rusticus* over the course of five years, suggesting that there may be a shift in the dynamics of the invasion. Ultimately, the reason for this shift is yet to be determined but could give insight as to why we observed no significant change in macroinvertebrate composition. Future studies should further investigate the relationship between *F. rusticus* density and benthic macroinvertebrates, as well as potential causes of declines in *F. rusticus* numbers post-invasion. The results of this study imply that not all invasive species are damaging to an ecosystem and that biological origin may not be the best indicator of whether a species will become invasive. The results of this study may be used for implementing invasive species management strategies in the Potomac River basin. Better understanding the feeding behaviors of *F. rusticus* can help management strategists determine how threatening the species is in this ecosystem. While *F. rusticus* is still a concern in the Monocacy River due to its historically invasive capabilities, this study may encourage strategists to concentrate management efforts towards more destructive species.

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