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Status of Freshwater Mussels (Unionidae) in the French Creek Watershed, USA at the Onset of Invasion by Round Goby, *Neogobius melanostomus*

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Abstract: Freshwater mussels are an imperiled group of organisms that are vital to aquatic ecosystems. Services performed by freshwater mussels, coupled with their use for biomonitoring, make them an invaluable asset. *Neogobius melanostomus* (Round Goby), a recently introduced invasive species to the French Creek watershed, was once restricted to the watershed of Lake Erie in Pennsylvania. The Round Goby's propensity to consume *Dreissena polymorpha* (Zebra Mussel) and *Dreissena bugensis* (Quagga Mussel) in their native habitat raises concerns about this recent introduction into Pennsylvania's Allegheny River watershed. Since the discovery of their introduction within the watershed, we have followed the range expansion and dispersal rate of Round Gobies, which makes this study unique. The objectives of this study were to quantify baseline data on the contemporary diversity and abundance of unionid mussels in the upper French Creek watershed, and to explore potential habitat factors that influence or limit the size of the mussel populations. We gathered baseline data on freshwater mussel diversity and abundances across eight sites in the French Creek watershed and examined substrate particle size and host availability as potential limiting factors of the freshwater mussel distribution. Freshwater mussel surveys were conducted during the summer months (July–September) of 2017 using area-constrained surveys. Results showed a significant relationship between mussel diversity and substrate particle size ($p < 0.05$). From the data collected, we were able to calculate population estimates for the species found across the sample sites. Our results regarding the locations of native mussel populations and characteristics of their habitat provide the needed insight for establishing priority areas for the conservation of freshwater mussels, facilitating planning for protection, mitigation, and adaptation as the invasive Round Goby continues its spread.

Keywords: aquatic invasive species; Round Goby; freshwater mussels; French Creek watershed

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1. Introduction

The introduction and spread of aquatic invasive species are longstanding concerns in surface waters affecting biodiversity. Pimentel [1] estimated that some 50,000 plant and animal invasive species have been introduced to the United States. While some invasive species add value to the economy (e.g., from increased fish catches), the overwhelming majority cause major ecological changes to native plants and animals, as well as economic losses in agriculture, forestry, and food processing [1–5]. Economic losses associated with aquatic invasive species can be very large, estimated at $120 billion per year in the United States [1].

The Round Goby *Neogobius melanostomus* (Pallas) is a small benthic fish native to Eurasia, first introduced into the Great Lakes region of the United States by ballast waters of freighters [6]. The ability of the Round Goby to adapt to a wide variety of habitats and

environmental conditions, generating high abundance and widespread distribution, poses a threat to aquatic biota in freshwater streams and lakes. Over the past few decades, the prolific spread of this non-native fish has greatly disrupted aquatic ecosystems in the Great Lakes region, where it has changed the food web dynamics and displaced native species, with negative economic and ecological impacts [3,7].

Due to their high abundance and widespread distribution, Round Gobies can have wide-ranging effects on aquatic species through interspecific competition and predation [3,7]. They have been shown to directly compete with native fishes such as darters (Percidae), sculpins (Cottidae), and catfish (Ictaluridae) [3,7–11]. In addition to outcompeting native fish species for food resources, Round Gobies have been shown to prey on native fish embryos [12,13] and outcompete native sculpin species for spawning habitat [14]. Further, in the interaction of two invasive species, Round Gobies have been shown to prey on Zebra Mussels, where they can consume large quantities of juvenile Zebra Mussels per day [15]. The Round Goby's propensity to consume *Dreissena polymorpha* (Zebra Mussel) and *Dreissena bugensis* (Quagga Mussel) in their native habitat raises concerns about this recent introduction into the watersheds that host native mussel (Unionidae) populations.

While the Round Goby was previously restricted to the watershed of Lake Erie (Great Lakes Basin) in Pennsylvania, it has recently spread into the French Creek Watershed (Allegheny River/Upper Mississippi Basin). The introduction of the Round Goby to the French Creek Watershed first colonized a tributary to French Creek (LeBoeuf Creek) in August 2016 [16]. The invasion of an invasive fish has typically been documented and observed after their colonization of particular freshwater systems. For example, when the Round Goby was first observed in the tributaries of Lake Erie in the Great Lakes watershed, it had already become a dominant benthic fish. Our earlier discovery of the initial leading edge of the invasion by this species in this watershed, combined with the availability of background data on aquatic ecology in this region, affords an unprecedented opportunity to track the range expansion and dispersal rate of the Round Goby and its impacts on aquatic communities.

The ability of the non-native, invasive Round Goby to adapt to a variety of habitats and environmental conditions poses a threat to native aquatic life in tributary systems and inland lakes. The recent introduction of Round Goby to portions of the French Creek watershed is a large concern given their potential to consume native freshwater mussels [7,15–17]. Due to its unique and rich biodiversity and outstanding water quality, French Creek is nationally renowned as one of the most important streams in eastern North America and is identified as a globally significant watershed by the Nature Conservancy. In French Creek, the introduction and spread of the Round Goby threatens a diverse native freshwater mussel (Unionidae) fauna. French Creek is considered a stronghold for native freshwater mussels and fishes in the Northeast, as it is home to 80 species of fishes and 29 species of freshwater mussels. The first confirmed case of Round Gobies foraging on freshwater mussels within the upper French Creek watershed was reported in LeBoeuf Creek in 2019 [16], and thus the Round Goby is considered a potential threat to the native freshwater mussel populations in this watershed.

Freshwater mussels are an imperiled group of organisms that are vital to aquatic ecosystems, providing ecosystem services such as water filtration, and useful as a tool for biomonitoring. Although many researchers have studied environmental variables conducive to the colonization of unionid mussels, less is known about their specific habitat requirements. Factors limiting their colonization include hydraulic forces [18–22], substrate size [21–24], surface geology [19,22,25], and stream size [25]. Traditional habitat descriptions are mainly based on these abiotic characters and have proven unsatisfactory [26]. Quantitative tests of associations among mussel distributions and factors such as sediment grain size, current speed, water depth, and distance to shore have been shown to be ineffective at predicting the occurrence of mussels [26]. Studies have shown that even when freshwater mussels live in an area well defined by one of these factors at one site, they often occur in very different habitats at another site location. Past research has often provided

little insight into the primary factors that affect the distribution and locations of native freshwater mussels [26], and the locations of native mussels remain challenging to predict.

The diverse unionid mussel fauna found in the French Creek watershed are particularly vulnerable to predation by Round Gobies. The objectives of this study were to determine the distribution and abundance of unionid mussels in the French Creek watershed, and to determine the impact of substrate composition on the mussel population size. We gathered baseline data on freshwater mussel diversity and abundances across eight sites in the French Creek watershed and explored substrate particle size and host availability as potential limiting factors of the distribution of freshwater mussels.

2. Materials and Methods

2.1. Field Site Sampling

The study takes place in the French Creek Watershed, which is well known for its rich aquatic biodiversity. French Creek is a large, low-gradient, warm-water stream that flows through two states and five counties, as a fourth-order tributary to the upper Allegheny River (Figure 1). It originates in western New York, flows west, southwest into Pennsylvania and south, southwest to its confluence with the Allegheny River in Franklin, Pennsylvania. The creek is 187 km in total length with a drainage area of 3300 km^2.

Observational data on mussel populations and their habitats were collected in selected tributaries at eight locations throughout the watershed in July–September of 2017 and June of 2018 (see Figure 1). These monitoring sites were chosen based on accessibility and exploratory surveys. The site located in LeBoeuf Creek was at 41.909378, −79.986292 (Moore Road). In the French Creek, site locations were at 41.908119, −79.896937 (Route 97), 41.902636, −79.885662 (South Branch of French Creek), 41.902478, −79.972964 (Flatts Road), 41.893676, −79.989660 (French Creek Road), 41.860152, −79.993280 (Mystic Road), 41.771697, −80.1084469 (Venango), and 41.693969, −80.161017(Saegertown).

Previous studies from our research group have confirmed the presence of Round Gobies throughout the LeBoeuf Creek tributaries, with additional observations of their spread into the main channel of the French Creek [16,17]. At the time of this study, two of the sampling locations (LeBoeuf Creek at Moore Road and French Creek below the confluence with LeBoeuf Creek, denoted as sites 1 and 5 in Figure 1) have Round Gobies present, whereas they were absent from the other 6 sampling sites.

2.2. Freshwater Mussel Surveys

Sample survey methods were developed using basic survey principles outlined by the U.S. Fish and Wildlife Service [27]. We established 10 m × 20 m quadrats using rebar and nylon rope. The number of quadrats used at each site was dependent on the width of the stream. For locations 20–39 m wide, we sampled one quadrat. For sites that were 40–79 m wide, we sampled two quadrats. We subdivide these larger quadrats into (50) 2 m × 2 m sub-quadrats (Figure 2). We sub-sampled five sub-quadrats in a diagonal fashion across the streambed, so that we surveyed a variety of habitats and flow regimes. We snorkeled each 2 m × 2 m square with no time-restriction. We disturbed surface debris, and excavated substrate until we reached clay and collected freshwater mussels whenever we found them. We placed mussels in mesh bags. After completely excavating a sub-quadrant, we transferred the mussels in marked bins for processing. We identified all mussels to species and marked them with color-coded tags before returning them to their respective sub-quadrats. Further details of our mussel surveys are available in Clark 2018 [28].

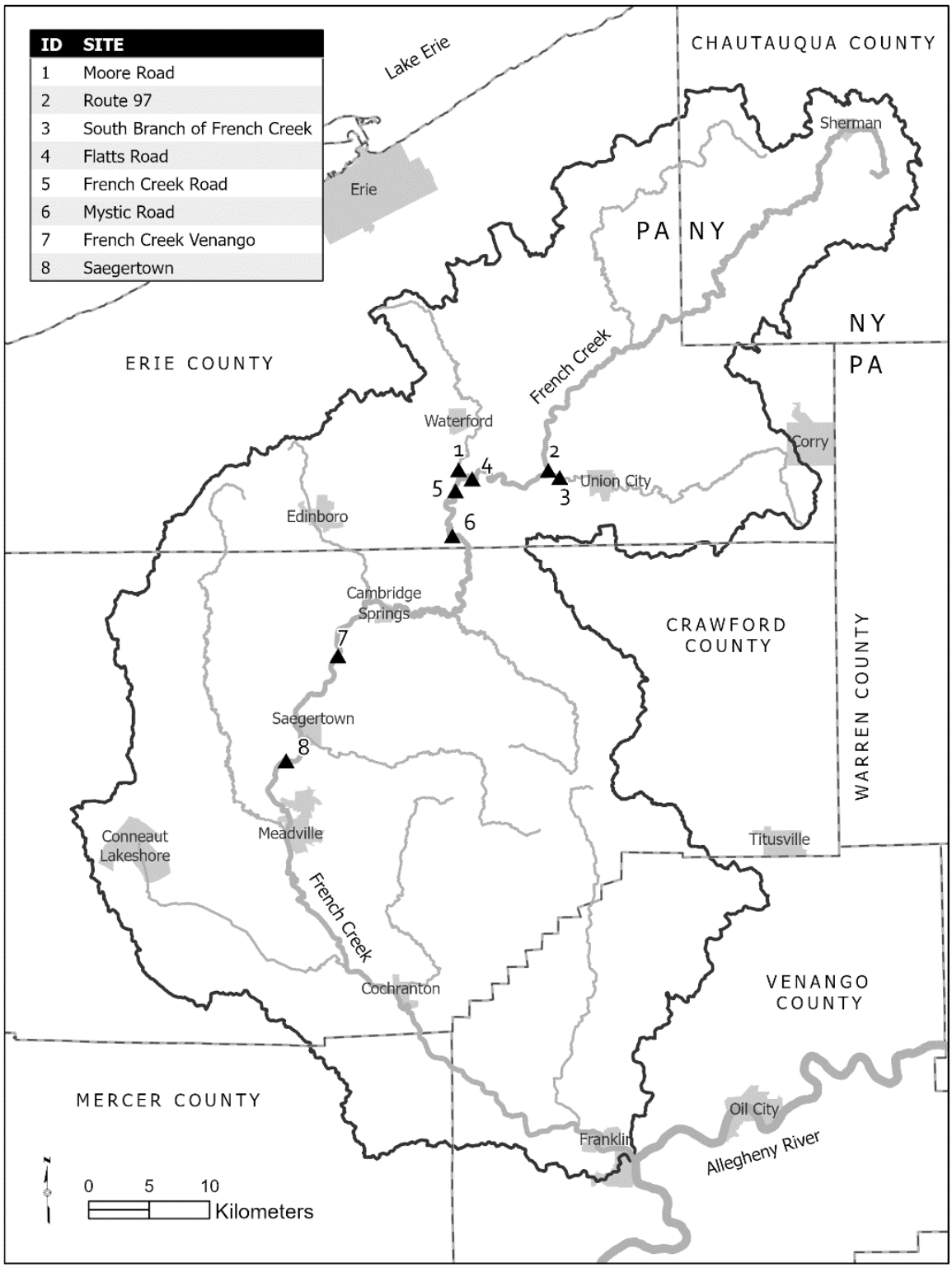

Figure 1. Map of the French Creek Watershed in New York and Pennsylvania, and the stream locations where native freshwater mussels were sampled.

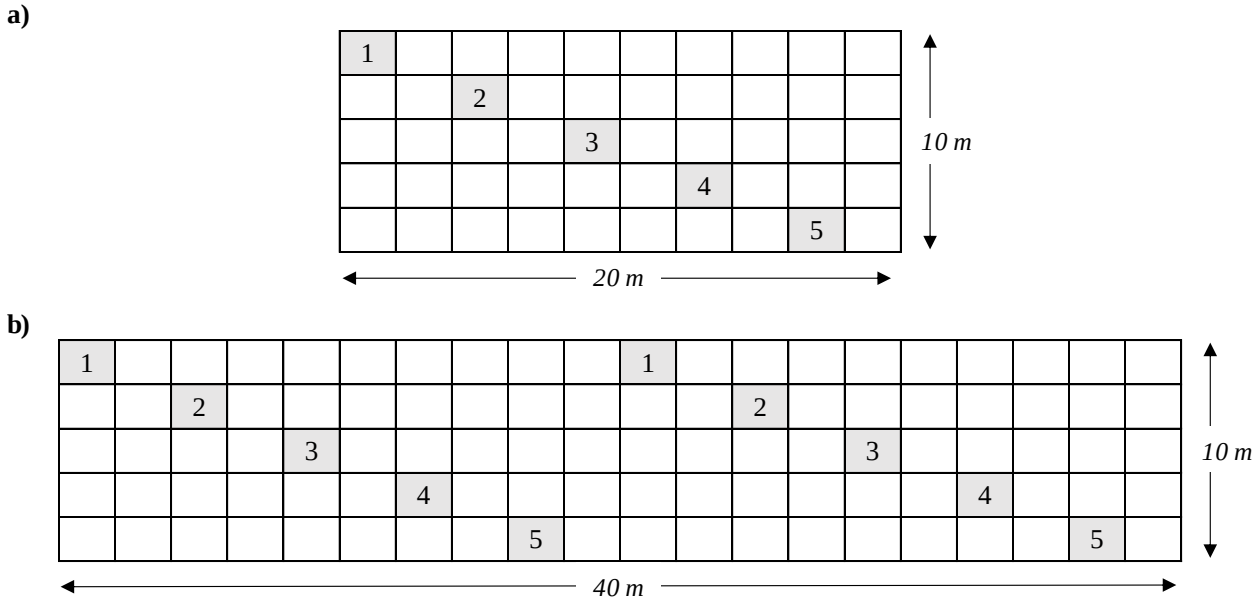

Figure 2. Stream sampling design, depicting 10 m by 20 m quadrats subdivided into 50 2 m by 2 m sub-quadrats. The sections sampled are highlighted in gray, for stream cross-sections that are (**a**) 20 m to 39 m wide and (**b**) 40 m to 79 m wide.

2.3. Fish Surveys

We compiled a listing of fish species that were likely or expected to be found at our sampling sites, based on historical records of observations and from our visual observations while sampling. We accessed historical aquatic survey collection records from the Penn State University Fish Museum. The museum contains over 12,000 collections made across Pennsylvania over the last 80 years, including 2100 collections within the French Creek watershed, providing a window into species distributions and their frequency of detections. Fish species were added to our list if they had been observed in any previous collections that had been conducted within 2 km of our sampling sites. Further, we added any additional fish species observed during our sampling efforts. Our research team is highly proficient in the identification of Pennsylvania fishes and could identify fishes by sight. The complete listing of fish species expected to be at each of our sampling sites is included as Supplementary Material accompanying this manuscript. For a complete list of fishes in the region and state, see Stauffer et al. 2016 [29].

2.4. Substrate Sampling

We quantified surface substrate index values using a 25 × 25 cm acrylic sheet marked with a grid of 25, 5 × 5 cm squares. We randomly selected three points in each sub-quadrate sampled during the study and placed flags at these positions. We positioned the center of the acrylic board over the flag's location and recorded the number of 5 × 5 cm squares covered by each rock [30,31]. Twenty-five categories of rock sizes were possible (R), and each category was a unique number of squares that a given rock occupied [30,31]. Given this sampling method, we quantified the surface substrate size in a 25 × 25 cm area around each flag [30,31]. The substrate index value (I) was determined by the sum of the number of rocks (n) we observed in each category multiplied by the category squared; $I = \sum nR2$ [30,31]. Therefore, the index values ranged from 25 to 625 and increased with substrate size [30,31].

2.5. Diversity Index and Substrate Index Values

We ranked the number of host species and mussel species for each site using Brillouin diversity indices to compare these communities across the sample sites. We based diversity indices on 21 mussel species across the eight sample sites. We based substrate index values on 45 substrate samples (5 per quadrat) across the eight sample sites. During the duration of the study, we did not observe any freshwater mussel species at the South Branch of

the French Creek site; thus, we removed this site from the analysis. We analyzed the relationship between mussel diversity and host availability, and substrate and mussel diversity, using Pearson's correlation coefficient, and looked at the relationship between mussel Brillouin diversity and substrate index value using linear regression. While the datasets were limited in terms of the number of observations, we evaluated them for normality using Kolmogorov–Smirnov tests, which indicated that the data do not differ significantly from a normal distribution. We calculated population estimates for the five most abundant species of mussels at Moore Road, French Creek Road, Mystic Road, and Venango using the Jolly–Serber model in the R statistical framework [32–36].

3. Results

We collected a total of 4356 mussels distributed among 22 species (Table 1). The site at Venango yielded the highest number of mussels (n = 2813) and 15 species, whereas the site at Mystic Road yielded the greatest number of species (19) and n = 968 individuals. The following four species that are listed as endangered both by the USFWS and Pennsylvania were collected. A total of 29 *Epioblasma triquetra* (Rafinesque), Snuffbox, was collected at five sites. While these mussels were only found at five sample sites, at least one host species (i.e., *Cottus bairdii, Perca flavescens, Percina caprodes*) was present at all sample sites. *Epioblasma torulosa rangiana* (Lea), Northern Riffleshell, (45 individuals), were found only at Venango and Mystic Road. While this species was only found at two sites, at least one host species (i.e., *C. bairdii, Etheostoma caeruleum, Etheostoma camurum, Etheostoma nigrum, Etheostoma zonale*) was present at all sample sites. *Pleurobema clava* (Lamarck), Clubshell, (21 individuals) were found at four sites. One host species for this mussel was found at all sample sites (i.e., *Campostoma anamalum, Luxilus crysocephalus, P. caprodes, Percina maculate*). *Villosa fabalis* (Lea), Rayed bean (23 individuals), were found at four sites. At least one host species was present at all samples sites (i.e., *C. bairdii, E. blennioides, E. caeruleum, Etheostoma tippecanoe, P. caprodes*). *Pleurobema sintoxia* (Rafinesque), *Round Pigtoe,* (19 individuals), were found at four sites. At least one host species (i.e., *C. anomalum, Cyprinella spiloptera, Lepomis macrochrius, Pimephales notatus*) was present at all sample sites. *Simpsonaias ambigua* (Say), Salamander Mussel, an endangered species listed by Pennsylvania was not found, although its host (i.e., *Necturus maculosus*) is present in French Creek. *Quadrula cylindrica cylindrica* (Say), Rabbitfoot, which is listed as threatened by the USFWS and endangered by Pennsylvania, was found at three sites (n = 11). At least one host species (i.e., *E. caeruleum, L. crysocephalus*) was present at all sample sites. *Villosa iris* (Lea), Rainbow Mussel, is listed as threatened by both the USFWS and Pennsylvania and was collected at four sites (n = 23). At least one host species (i.e., L. *chrysocephalus, Lepomis cyanellus, Micropterus dolomieu, Micropterus salmoides, E. blennoides, E. caeruleum, Perca flavescens*) was located at all sample sites. Tables of the mussel species with corresponding fish host specimens found at each sampling site are included in the Supplementary Materials that accompany this manuscript.

Actinonaias ligamentina (Lamarck), Mucket, was the most abundant mussel (n = 1505) and was found at all sites, except for the South Branch of French Creek, where no mussels were collected. *Ptychobranchus fasciolaris* (Rafinesque), Kidneyshell, was also found at all sites with 1442 specimens collected. Both *Villosa iris* (Lea), Rainbow mussel, (n = 312) and *Elliptio dilatata* (Rafinesque), Spike mussel, (n = 701), were found at five of the six sites where mussels were found (Table 1). Population estimates for these species at each site are summarized in Tables 2–5.

Table 1. Occurrence of freshwater mussel species captured across the French Creek sampling sites. We report the total number of individuals caught over the duration of the study. Further reported are the summary statistics for each site, including the number of mussels and species observed, and their Brillouin diversity index values. Key: – none found, [1] federally endangered species, [2] federally threatened species, [3] state-endangered species, [4] state-threatened species.

Species	Route 97	Flatts Road	Moore Road	French Creek Road	Mystic Road	Venango	Saegertown	Total Number Across Sites
Actinonaias ligamentina (Lamarck)	4	9	40	72	440	928	12	1505
Alasmidonta marginata (Say)	5	2	34	–	3	10	–	54
Amblema plicata (Say)	–	–	9	–	9	–	–	18
Anodontoides ferussacianus (Lea)	–	–	–	1	–	–	–	1
Cyclonaias tuberculata (Rafinesque)	–	–	–	–	–	–	–	0
Elliptio dilatata (Rafinesque)	4	–	17	7	91	563	19	701
Epioblasma torulosa rangiana (Lea) [1,3]	–	–	–	–	11	34	–	45
Epioblasma triquetra (Rafinesque) [1,3]	8	–	2	5	13	1	–	29
Fusconaia subrotunda (Lea)	–	–	–	–	1	–	–	1
Lampsilis cardium (Rafinesque)	1	–	–	14	28	6	–	49
Lampsilis fasciola (Rafinesque)	4	1	–	6	13	1	2	27
Lampsilis siliquoidea (Barnes)	–	–	6	–	1	11	–	18
Lasmigona complanata (Barnes)	–	–	–	–	–	–	–	0
Lasmigona compressa (Lea)	–	–	–	1	–	–	–	1
Lasmigona costata (Rafinesque)	–	–	6	3	13	20	–	42
Ligumia nasuta (Say)	–	–	–	–	–	–	–	0
Ligumia recta (Lamarck)	3	–	–	5	12	6	3	29
Pleurobema clava (Lamarck) [1,3]	–	1	–	1	17	2	–	21
Pleurobema sintoxia (Rafinesque)	–	1	–	5	7	6	–	19
Ptychobranchus fasciolaris (Rafinesque)	35	6	69	46	119	1124	43	1442
Pyganodon grandis (Say)	–	–	2	2	–	–	–	4
Quadrula cylindrica cylindrica (Say) [2,3]	–	–	–	6	4	1	–	11
Simpsonaias ambigua (Say) [3]	–	–	–	–	–	–	–	0
Strophitus undulatus (Say)	–	–	–	1	3	–	–	4
Taxolasma parvus (Barnes)	–	–	–	–	–	–	–	0
Utterbackia imbecillis (Say)	–	–	–	–	–	–	–	0
Villosa fabalis (Lea) [2,4]	–	–	1	5	16	–	1	23
Villosa iris (Lea)	–	1	6	34	167	100	4	312
Total number of mussels	64	21	192	214	968	2813	84	–
Total number of species	8	7	11	17	19	15	7	–
Brillouin Diversity Value	1.23	1.23	1.66	1.93	1.78	1.36	1.23	–

Table 2. Population estimates for the four most common species of mussels at the *French Creek Road* sampling site. Species denoted by an asterisk are populations whose population estimate was less than the sample size. For these species, the true number of individuals observed is reported.

French Creek Road Mussel Species	July 2017	August 2017		September 2017		Total	
	Total Captures	No. Re- Captured	Total Captures	No. Re- Captured	Total Captures	Population Estimate	Standard Error
Kidneyshell-*Ptychobranchus fasciolaris* (Rafinesque) *	16	10	16	11	14	46	–
Spike mussel, Mucket-*Actinonaias ligamentina* (Lamarck)	16	12	25	24	30	26	1.2
Spike Mussel-*Elliptio dilatata* (Rafinesque) *	2	1	2	2	3	7	–
Rainbow mussel-*Villosa iris* (Lea)	12	9	13	7	9	15	2.4

Table 3. Population estimates for the four most common species of mussels at the *LeBeouf Creek Moore Road* sampling site. Species denoted by an asterisk are populations whose population estimate was less than the sample size. For these species, the true number of individuals observed is reported.

LeBeouf Creek Moore Road Mussel Species	July 2017	August 2017		September 2017		June 2018	Total	
	Total Captures	No. Re- Captured	Total Captures	No. Re- Captured	Total Captures	No. Re- Captured	Population Estimate	Standard Error
Kidneyshell-*Ptychobranchus fasciolaris* (Rafinesque)	8	7	21	15	21	18	66.7	23
Spike mussel, Mucket-*Actinonaias ligamentina* (Lamarck)	6	4	8	7	8	15	18	2.5
Spike Mussel-*Elliptio dilatata* (Rafinesque) *	3	1	5	5	5	2	17	–
Rainbow mussel-*Villosa iris* (Lea) *	2	0	3	1	1	0	6	–

Table 4. Population estimates for the four most common species of mussels at the French Creek Mystic Road sampling site.

French Creek Mystic Road Mussel Species	July 2017	August 2017		September 2017		Total	
	Total Captures	No. Re- Captured	Total Captures	No. Re- Captured	Total Captures	Population Estimate	Standard Error
Kidneyshell-*Ptychobranchus fasciolaris* (Rafinesque)	44	25	40	31	35	48	3.6
Spike mussel, Mucket-*Actinonaias ligamentina* (Lamarck)	148	113	151	119	140	167	3.8
Spike Mussel-*Elliptio dilatata* (Rafinesque)	36	20	27	22	28	37	3.8
Rainbow mussel-*Villosa iris* (Lea)	102	34	49	10	16	56	7.1

Table 5. Population estimates for the four most common species of mussels at the French Creek Venango sampling site. Species denoted by an asterisk are populations whose population estimate was less than the sample size.

French Creek Venango Mussel Species	July 2017	August 2017		September 2017		June 2018	Total	
	Total Captures	No. Re- Captured	Total Captures	No. Re- captured	Total Captures	No. Re- Captured	Population Estimate	Standard Error
Kidneyshell-*Ptychobranchus fasciolaris* (Rafinesque)	219	190	270	246	300	172	529.3	30.4
Spike mussel, Mucket-*Actinonaias ligamentina* (Lamarck)	169	175	225	209	251	162	443.2	25.4
Spike Mussel-*Elliptio dilatata* (Rafinesque) *	120	82	144	105	137	78	240.1	20.8
Rainbow mussel-*Villosa iris* (Lea) *	51	6	9	11	15	25	28	6.4

The relationship between mussel Brillouin diversity index values diversity and substrate index values is shown in Figure 3. Results from the linear regression suggest a significant relationship between the mussel diversity index value and the substrate index value ($p < 0.05$). While the number of data points is sparse, this analysis shows that mussel diversity is negatively correlated with increasing substrate index value or larger surface substrate deposits (contained rocks measuring >175 sq cm). Sampling sites that had surface substrates with values larger than 50 typically had lower mussel diversities than those with values less than 50. The only "outlier" site that did not follow this pattern was LeBoeuf Creek at Moore Road. This site had a mussel diversity index value of 1.66 while scoring more than 65 for a substrate index value.

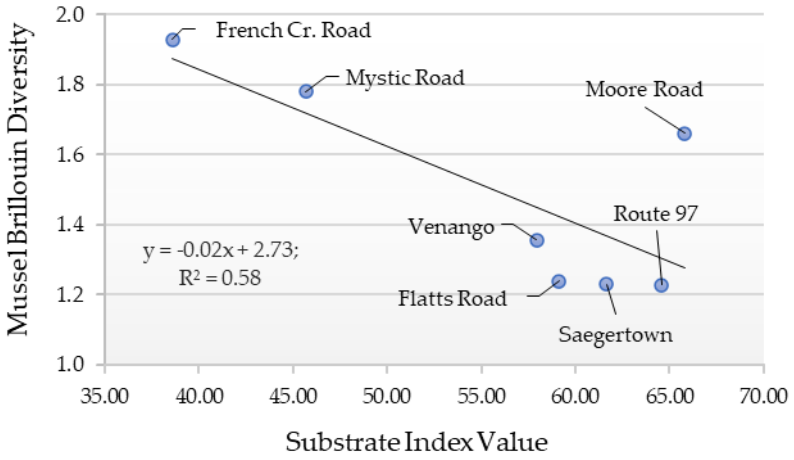

Figure 3. Relationship between Unionidae mussel Brillouin diversity values and substrate index values across the sampling sites in the French Creek watershed.

4. Discussion

While the unionid diversity in North America is the greatest in the world (with approximately 300 recognized species, [37]) this group of organisms is being subjected to a multitude of threats, including invasive species [38]. Freshwater mussels have been negatively impacted by human alteration to most lotic systems in North America and remain among the most imperiled group of fauna in freshwater systems; however, many species of mussels in French Creek are thriving [39].

The introduction of invasive Round Gobies to the French Creek watershed poses a major threat to both native fishes and unionid mussels. Mussels are particularly vulnerable as the Round Goby threatens survival both directly through the consumption of juveniles [16] and indirectly through the displacement of their fish host species [3,7,40–43]. With over 80 species of fish and 29 species of freshwater mussels, French Creek is nationally renowned for its biodiversity and is the most species-rich stream in Pennsylvania. In this study, we observed the presence of 22 species of unionid mussels and their respective host species across the locations that we sampled (see Table 1). The fact that four mussel species that we observed are listed under the Endangered Species Act, with two species considered to be critically imperiled (having lost most of their historic global range), highlights the need for conserving and managing native freshwater mussel populations in French Creek.

From the observational data collected during our stream surveys, we calculated population estimates for the native mussel species found across most of the sampling sites. Population estimates and survival rates could not be estimated for species that had fewer than ten observations over the duration of sampling, and actual values for numbers of individuals and extant marks were reported for these species. While the dynamics of occupancy are complex, we do not predict that host availability is a limiting factor for mussels in French Creek based on the diversity of ichthyofauna present.

Considering habitat, our results showed a significant relationship between the diversity of mussels and substrate particle size ($p < 0.05$). Our findings showed that freshwater mussel diversity was greatest in substrates that contained high proportions of smaller surface sediments. These results align with previous descriptive habitat studies that also looked at substrate as a tool to identify suitable mussel habitat [23,44]. Similarly, our findings indicate that freshwater mussels in our system were found more frequently in well sorted sediments with lower surface substrate index values. It is unclear, however, whether the association is reflected as a preference, as these depositions of finer materials occur in areas with low hydraulic flow regimes, are potentially more stable, and possibly contain higher amounts of organic matter. Certainly, a substrate of well-sorted sediments supported a higher diversity of mussels and provided suitable spawning habitat for most of the associated hosts, as suggested by this as well as other studies [23,26,28].

We cannot explain the absence of mussels at the South Branch of the French Creek site. This site did not differ in substrate composition, water quality, or presence of potential host species. Perhaps the substrate is more mobile and shifts overtime, which was not a parameter we measured. Additionally, we cannot determine why relative abundance, species, and population sizes differ among stations. More information is needed about the colonization and competition among species of mussel. Perhaps colonization at a particular site is dependent upon which mussels colonize first as shown for macroinvertebrates on artificial substrates as shown by Stauffer et al. 1976 [45]. It remains a scientific challenge to understand the specific environmental conditions and habitat characteristics that limit unionid populations. Quantitative tests of associations between mussel distributions and factors such as sediment grain size, water velocity, water depth, and distance to shore have been shown to be ineffective at predicting the occurrence of mussels [26]. Moreover, studies have shown that even if mussels live in an area well defined by one of these factors at one site, they often occur in very different habitats at other sites [26]. More research needs to be directed at pinpointing the complex interaction of factors that support and limit unionid populations.

This study is unique since our prior research allowed us to identify the precise location and time of the introduction of the invasion by the Round Goby within the French Creek Watershed, whereas most studies are conducted once an invader is established and its presence subsequently realized. The contemporary data on native mussel populations presented in this study, combined with the prior work conducted on mussels in this watershed before the Round Goby was introduced [39,46], provide important baseline information on the distribution and abundance of unionid mussels in French Creek. Such information is needed to inform strategies for the conservation and protection of aquatic life. Stakeholders have undertaken important public education and outreach activities toward reducing the spread of the Round Goby and other aquatic invasive species in this region. There is strong potential for the invasive Round Goby to broaden its geographic distribution within freshwater stream networks in the USA, given the French Creek watershed's location in the upper headwaters of the vast Mississippi River Basin.

Supplementary Materials: The following are available online at http://www.hydroshare.org/resource/e72f2afe5eb54dfda6fe4465279a2fb3, Data and supporting information for this manuscript are provided in the HydroShare digital data repository of the Consortium of Universities for the Advancement of Hydrologic Sciences, Inc., [47]. In addition to the original data from the figures and tables presented in the manuscript here, the supplementary materials also include: Table S1. Mussel species with corresponding fish host specimens found at sampling sites. Table S2: Occurrence of fish species at sampling sites. Table S3: Coordinates of mussel sampling sites.

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