2021 MISSISQUOI WILD AND SCENIC FRESHWATER MUSSEL INVENTORY

January 13, 2022

Final Report Submitted to the Upper Missisquoi and Trout River Wild and Scenic Committee

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1. Introduction

In 2019, a collaboration between Arrowwood Environmental and the Missisquoi River Basin Association (MRBA) was developed to undertake an inventory of freshwater mussels on the lower reach of the Wild and Scenic section of the Missisquoi River in Berkshire and Enosburg, Vermont. The purpose of the inventory was to 1) document the presence of any rare mussel species within this reach and assess the health and abundance of these populations and 2) identify and assess the presence of freshwater mussel beds to use as potential long-term monitoring sites.

Funding was obtained through the Upper Missisquoi and Trout River Wild and Scenic (UMATR) Committee. This report includes a background section on freshwater mussels, methodology of the current survey and data analysis of the results.

2. Background

Freshwater mussels occupy a unique role in riverine ecosystems in North America in that they are a crucial keystone species in these habitats that are often overlooked. Their complex life histories and biology make them intimately tied to water quality and overall health of river systems. For this reason, they have been used as indicator species for assessing the overall health of riverine ecosystems (Aldridge et al. 2007). Unfortunately, recent and drastic declines in freshwater mussel populations have been documented across North America, with over 60% of the species considered threatened or endangered and 12% presumed extinct (Ricciardi, Neves, and Rasmussen 1998). This has caused conservationists to designate these taxa as the most imperiled fauna on the continent (Strayer 2004). These declines have been attributed to multiple factors such as decreasing water quality, dam construction, invasive species, aquatic habitat alterations and drastic sedimentation events (Lydeard 2004, Strayer 2004).

Unlike marine mussels, native freshwater mussels lack byssal threads used for attaching themselves to substrates. Instead, these animals extend their mantle (or “foot”) into the river or lake sediment to remain anchored. While this system allows them a limited amount of movement, excessive sheer forces associated with river currents can dislodge them. Freshwater mussels are primarily filter feeders, using their inhalant and exhalent siphons to filter phytoplankton, zooplankton, bacteria and particulate matter out of the water column. This feeding activity as well as bioturbation of the sediment by movement can have significant impacts on water nutrient dynamics and food web systems (Vaughn and Hakenkamp 2001).

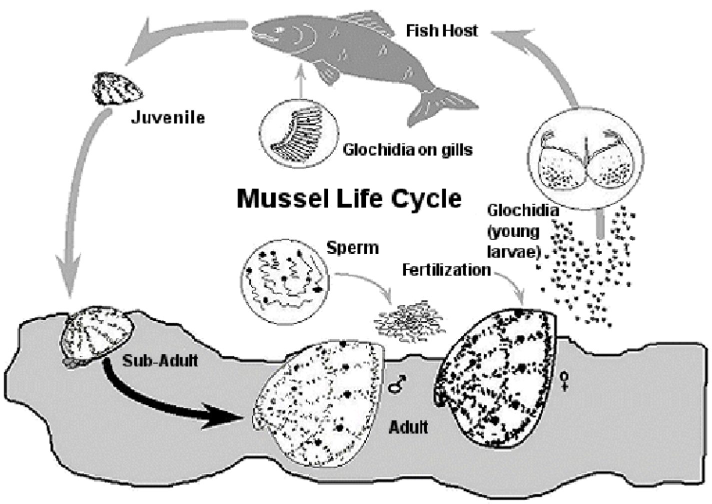


Image from U.S. Fish and Wildlife Service

One of the most fascinating aspects of the life history of these animals is the reproductive system. Reproduction is accomplished by male mussels releasing sperm into the water column which is inhaled by nearby females for internal fertilization of eggs. Unlike their marine cousins (and zebra mussels), native freshwater mussels do not have an independent, free-floating juvenile stage. The juvenile stage of freshwater mussels (called glochidia) are parasitic on host fish before they become independent. Some mussels are host generalist and can use any number of fish species for this stage while others are host specific, relying on one or two fish species for this stage of reproduction.

In order to attract host fish, gravid female mussels use one of a variety of techniques. Some mussel species modify part of their body (mantle) to resemble minnows or invertebrates. Others release glochidia in packages that resemble invertebrates, worms, eggs or larval fish. These lures elicit attacks by fish which, instead of a meal, get a mouth full of glochidia. It is thought that the type of lure used by a mussel can target a specific fish species by mimicking their preferred food item. For host generalists, glochidia are released as part of a large mucous web that can entangle fish indiscriminately.

In both circumstances, these small glochidia attach themselves to the gills of fish and feed on nutrients directly from the fish's bloodstream. When they have sufficiently matured, they release themselves from the fish's gills and anchor themselves to the substrate to begin their life as an adult mussel.

In Vermont, of the eighteen mussel species known to occur here, 15 of them are listed as uncommon, rare, threatened, or endangered. They occur in medium to large river systems and a variety of lakes, including large mussel populations in Lake Champlain. The Poultney and Missisquoi River basins contain some of the most diverse assemblages of freshwater mussels in the state. There are 9 mussel species known from the Missisquoi River Basin. Some species, such as the pink heelsplitter and the giant floater, are known only from the lower Missisquoi, below the fall-line. Six species are known to occur in the Missisquoi above the fall-line, though only a few of these are likely to occur above the Enosburg dam. See Table 1 below for a list of these species.

Table 1 List of native mussels known from the Missisquoi River

Species	**Common Name**	**S-rank**	**Distribution in Missisquoi**
Alasmidonta undulata	Triangle floater	S3-Uncommon	Throughout Missisquoi
Anodontoides ferrusacianus	Cylindrical papershell	S1S2-Endangered	Below fall-line and upstream to Sheldon
Elliptio complanata	Eastern elliptio	S5- Common	Throughout Missisquoi
Lampsilis ovata	Pocketbook	S2-Endangered	Below fall-line only
Lampsilis radiata	Eastern lampmussel	S5-Common	Below fall-line and upstream to Sheldon
Lasmigona compressa	Creek Heelsplitter	S2-Rare	Above fall-line upstream to Enosburg
Leptodea fragilis	Fragile papershell	S2-Endangered	Below fall-line only
Potamilus alatus	Pink heelsplitter	S2-Endangered	Below fall-line only
Strophitus undulatus	Creeper	S3-Uncommon	Throughout Missisquoi

3. Methods

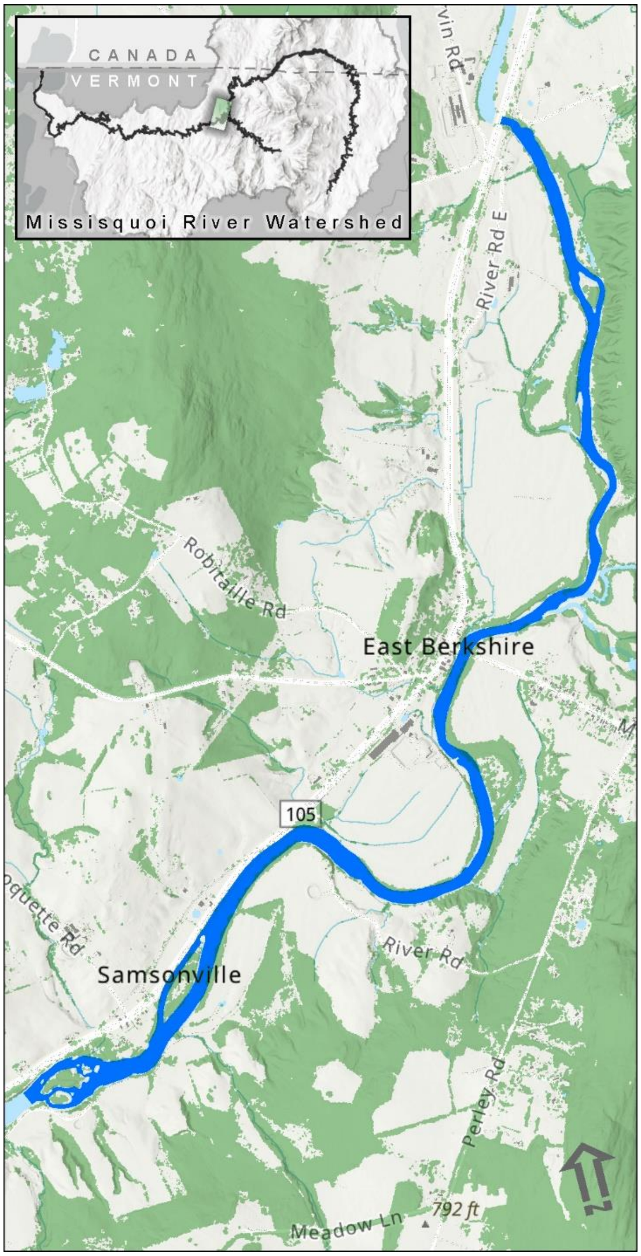


Figure 1 Study Area

The study area consisted of the lower reach of the Wild and Scenic section of the Missisquoi River from its downstream terminus in Enosburg upstream to Nutting Corners in Berkshire as shown in Figure 1. This stretch of river is approximately 4 miles in length and flows through a combination of agricultural lands and patches of forest.

Prior to field work, existing information on mussel distribution and abundance in the Missisquoi River Basin was obtained from the Vermont Natural Heritage Inventory. A digital map of the study area was uploaded into an iPhone X enabled with ArcGIS Collector and Survey123 field data collection applications. Field work was conducted on July 29, August 5 and 25, October 6 and 26, 2021. Precipitation events can create turbid water conditions, decreasing visibility and the ability to detect mussels. In order to avoid field work during sub-optimal conditions, flow conditions from the USGS 04293500 monitoring station in East Berkshire were consulted prior to each field visit to determine amount of flow in the river and recent flow trends. The summer of 2021 was characterized by mild drought and low-flow conditions ranging from 60-119 cubic feet per second during the July and August field visits. More moderate flows were present in October, with rates between 186-252 cubic feet per second during the field visits.

The river was accessed at road crossings. A kayak was used to navigate the river and visually scan appropriate habitat for the presence of freshwater mussel beds. In some cases, snorkeling was used to scan deeper areas or where substrate visibility from the kayak was limited. Mussels were found to be present at low levels (<0.25 mussels / m2) in sporadic locations throughout the study area. Since the focus of the study was to document mussel beds, these low-abundance occurrences were not mapped. A "mussel bed" was considered to be an aggregation of mussels existing at levels greater than 0.25 mussels/m2 and occupying an area >5m2. Once a mussel bed was encountered, the boundaries were mapped using GPS and mussels were surveyed using either a Timed Search or Quantitative Plot inventory. During the data analysis phase, the GPS points were converted into a polygon shapefile depicting the boundaries of each mussel bed. This methodology resulted in a comprehensive map of all mussel beds within the study area.

Timed Searches

The timed search method is well described in the literature (Strayer and Smith 2003) and consists of searching for mussels in appropriate habitat for a set period of time. The timed search data can be used to calculate a "Catch per Unit Effort" (CPUE) metric, typically expressed as number of mussels encountered per person hour. This is considered a qualitative metric because there is a lot of variability in survey efficiencies between surveyors and river conditions between sampling periods. However, CPUE technique is the best method for detecting rare, sparsely distributed individuals and it can be (cautiously) compared with CPUE data from previous inventories.

Searching was conducted in shallow water (<2'deep) by using visual searches or, if conditions warranted, using a viewscope. Since all of the mussel beds encountered during the current inventory occurred in water less than 2' deep, no snorkeling or SCUBA work was required. In each case, every time a live freshwater mussel was encountered during the timed search, it was identified to species, recorded and placed back in the sediment. Dead valves were noted for rare mussel species but were ignored for common species. If an uncommon, rare, threatened or endangered mussel was encountered, the timer was stopped and the specimen was measured before returning to the substrate. Measurements were obtained by placing the mussel length-wise along a ruler and noting the length in millimeters. Once the pre-determined search time was completed, the number of mussels by species, along with length measurements was tallied.

Time intervals for searches varied depending on habitat and species abundance. If, at the end of this sampling period, there were many more productive habitat areas to sample at that location, the time was extended for varying periods depending on the amount of habitat present. At one location (Mussel Bed I) Lindsey Wight and Sara Lunn from MRBA were present to assist with field data collection. During this timed search, all mussels were measured (not just rare species) while the surveyor continued the timed search.

At each timed search location, data on river flow, substrate, depth and habitat type was also collected. Most timed searches were limited to the areas of the mussel beds. Some timed searches were conducted in areas of suitable habitat but where mussel beds were not obvious in order to determine if mussels were present at low density.

Quantitative Plots

The quantitative plot method was employed in the larger mussel beds when time allowed. Unlike the timed searches, this method allows for a more in-depth and quantitative sampling which can be repeated in subsequent years to determine population trends. This sampling method is based on the techniques outlined in Nalepa et al. 1996 and entails laying a 100' transect line over the mussel bed. Three random numbers between 1-100 were generated and a 1.0 m^2 quadrat plot was placed at each of these locations. Within the plot frame, all mussels were removed and excavation of the sediment down 4" deep was performed to detect any buried mussels that could not be seen from the surface. Each mussel was identified to species, measured lengthwise and placed back in the sediment. Additional data on sediment composition, water depth, flow, and habitat type was also collected for each plot.

Table 2 lists each of the mussel beds present in the study area along with the centerpoint coordinates and sampling technique employed. The map in Figure 2 shows the location of each of these mussel beds. A photo of each of these sites is presented in Appendix 1.

Table 2 All Mussel Beds Documented within the Study Area

Date	Site	Sample Method(s)	Latitude	Longitude	Size (ft2)
7/29/2021	Mussel Bed C	1 Meter Plot	44.94753913	-72.69830674	1423
7/29/2021	Mussel Bed D	1 Meter Plot and Timed Search	44.94530926	-72.69760137	18207
8/5/2021	Mussel Bed F	Timed Search	44.95169905	-72.69841815	5688
8/5/2021	Mussel Bed G	Timed Search	44.95019775	-72.69801374	8648
10/6/2021 and 10/26	Mussel Bed I	Timed Search	44.94235421	-72.69894403	13136
8/25/2021	Mussel Bed J	Timed Search	44.93820742	-72.70038514	4788
8/25/2021	Mussel Bed L	Timed Search	44.92489914	-72.71324612	3098
8/25/2021	Mussel Bed N	1 Meter Plot and Timed Search	44.92777206	-72.71993944	12962
10/6/2021	Mussel Bed P	1 Meter Plot and Timed Search	44.92103628	-72.73226994	12368

4. Results

The study area consists of a diverse array of riverine habitats suitable for freshwater mussels. A total of 9 mussel beds were documented within the study area as listed in Table 2. Locations and extent of these beds is shown in Figure 2 below. The location of mussels beds in a river is generally dependent on a complex interaction of fish host behavior, river geomorphology, flow and substrate (Newton, Woolnough, and Strayer 2008; Allen and Vaughn 2010). In the current study, most of the mussel beds occur in narrow, shallow areas along the banks of the river. They are also located in areas where river geomorphology creates slightly lower flows.

Three mussel species were documented within the study area: elliptio (*Elliptio complanata*), creeper (*Strophitus undulatus*) and the triangle floater (*Alasmidonta undulata*). The elliptio is the most common mussel in the state and is found in a wide variety of habitats in both river and lake systems. Both the creeper and the triangle floater are uncommon (S3-ranked) species found exclusively in riverine habitats. These patterns of abundance documented state-wide were also reflected in the frequencies documented in the current study. The data on relative abundance of each of these species is summarized in Table 3.

Table 3. Summary Data for Species Documented in the Study Area

Species	Common Name	S-rank	Total # found	Relative abundance
Alasmidonta undulata	Triangle floater	S3	5	0.3%
Elliptio complanata	Elliptio	S5	1373	94.7%
Strophitus undulatus	Creeper	S3	472	5.0%
	Total		1450	100.0%

Timed Searches

The timed searches yield data that is expressed in Catch-Per-Unit-Effort (CPUE) and is expressed in the number of mussels encountered per hour of searching for one person (i.e. per person hour). Table 4 shows the CPUE data for each site within the study area. CPUE for each site is broken out by species and a total for the site is presented in the final column. In some cases, multiple timed searches were conducted at a single site in different areas of the mussel bed (i.e. non-overlapping searches). These are shown as numbered searches in Mussel Beds I and L in the first column.

Table 4 CPUE data for mussel beds

CPUE (# of mussels encountered per person hour of search effort)				
Site*	**Triangle floater (*Alasmidonta undulata*)**	**Elliptio (*Elliptio complanata*)**	**Creeper (*Strophitus undulatus*)**	**Grand Total**
Mussel Bed-C	5	10.0	0.0	15.0
Mussel Bed-D	0	438.0	21.0	459.0
Mussel Bed-F	1.5	429.0	1.5	432.0
Mussel Bed-G	0	66.7	60.0	126.7
Mussel Bed-I #1	3	189.0	24.0	216.0
Mussel Bed-I #3	0	252.9	2.1	255.0
Mussel Bed-I #4	0	307.2	0.0	307.2
Mussel Bed-L #1	0	206.1	2.6	208.7
Mussel Bed-L #2	0	18.0	0.0	18.0
Mussel Bed-N	1	339.0	30.0	370.0
Mussel Bed-P	6	498.0	36.0	540.0
Grand Total	1.128	285.8	16.2	303.2

**Sites where timed searches yielded no mussels are not shown*

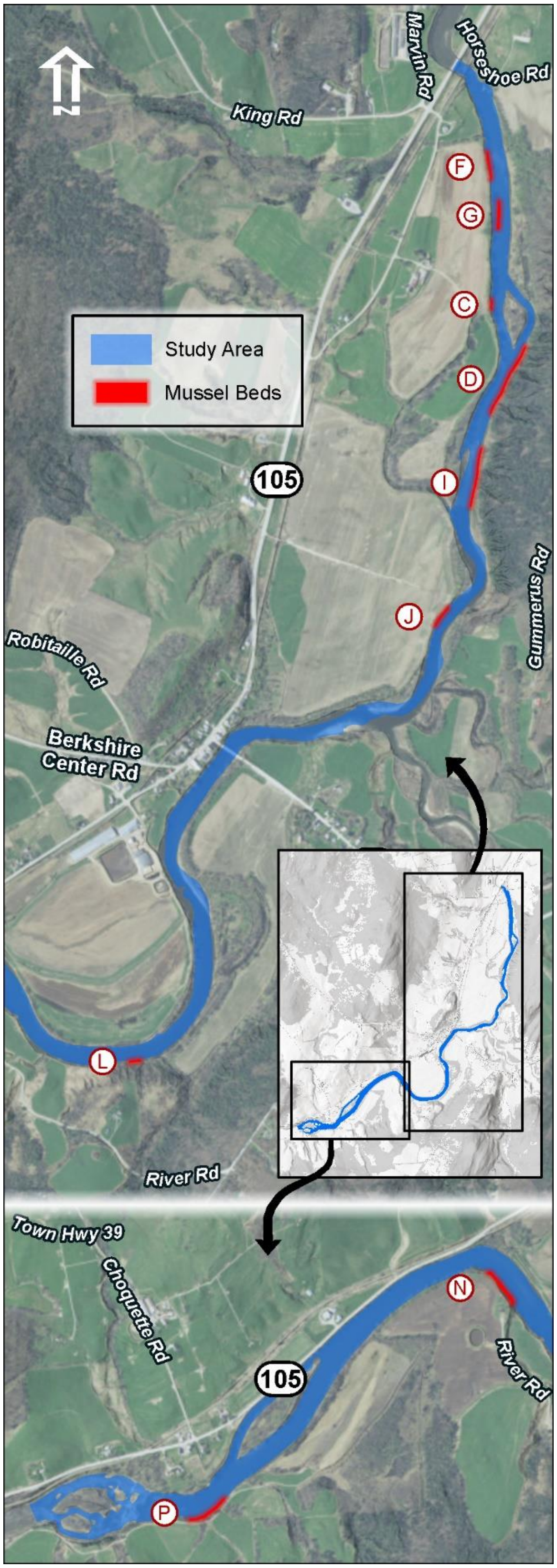


Figure 2 Map of Mussel Beds

The data show that the beds with the most abundant mussels are Bed P, D and F. In these beds, mussels were very abundant and, in some places within the mapped beds, extremely dense. Bed C had the lowest density of mussels and presumably represents an area where habitat is less favorable. In the case of survey #2 in Bed L, this area was sampled on the margins of the more productive Bed L where habitat suitability is lower.

The species composition of nearly all the mussel beds is overwhelmingly dominated by elliptio. This result is not surprising given that this species is known to be the most common mussel in the region and has a wide ecological amplitude. Mussel Bed G, however, exhibits a striking exception to this trend where nearly one-half of the mussels encountered were of the creeper. This bed is also the only one that is not located along the banks of the river, rather it is situated in a shallow riffle along a gravel bar. The creeper species tends to prefer cobble and gravel-dominated substrates in shallow runs and riffles. Similar mussel beds dominated by this species have been anecdotally noted elsewhere in the Missisquoi downstream of the Enosburg dam.

Quantitative Plots

Four of the documented mussel beds were sampled using the Quantitative Plot method. All species documented in the quantitative plots were elliptio. The results of this sampling are presented in Table 5 below.

Table 5 Number of Mussels per Square Meter

Site	Average # mussels / m2	Std Error
Mussel Bed-C	2.0	0.6
Mussel Bed-D	3.0	1.0
Mussel Bed-N	4.3	1.9
Mussel Bed-P	26.0	3.2

The data show that Bed P has the highest density of mussels of those that were sampled using the Quantitative Plots method. This is similar to the CPUE data which shows that this mussel bed has the highest density of mussels in the study area. Quantitative data for Beds D and N does not align very well with the CPUE data presented in Table 4. This could be the result of a high degree a variability in mussel abundance throughout the mussel beds. If some of the three 1m2 plot locations randomly land on an area with fewer mussels, this will be reflected in the data.

Population Demographics

By obtaining length measurements of mussels, information on the age structure of the populations can be obtained. Length-frequency histograms are often used to illustrate the demographics of mussel populations, with the smaller mussels representing younger individuals and larger animals representing older individuals. Though growth curves of mussels are not always linear and actual age estimates are under debate (Anthony et al. 2001), these histograms are useful for showing general trends. Data for the length-frequency histograms presented below are taken from mussels measured during both the Timed Searches and the Quantitative Plots. These histograms show the frequency of elliptio and creeper mussels in different length categories. Too few triangle floater mussels were present to result in useful demographic data; this species is therefore not shown.

Figure 3 Length-Frequency Histogram for Elliptio complanta (n=264)

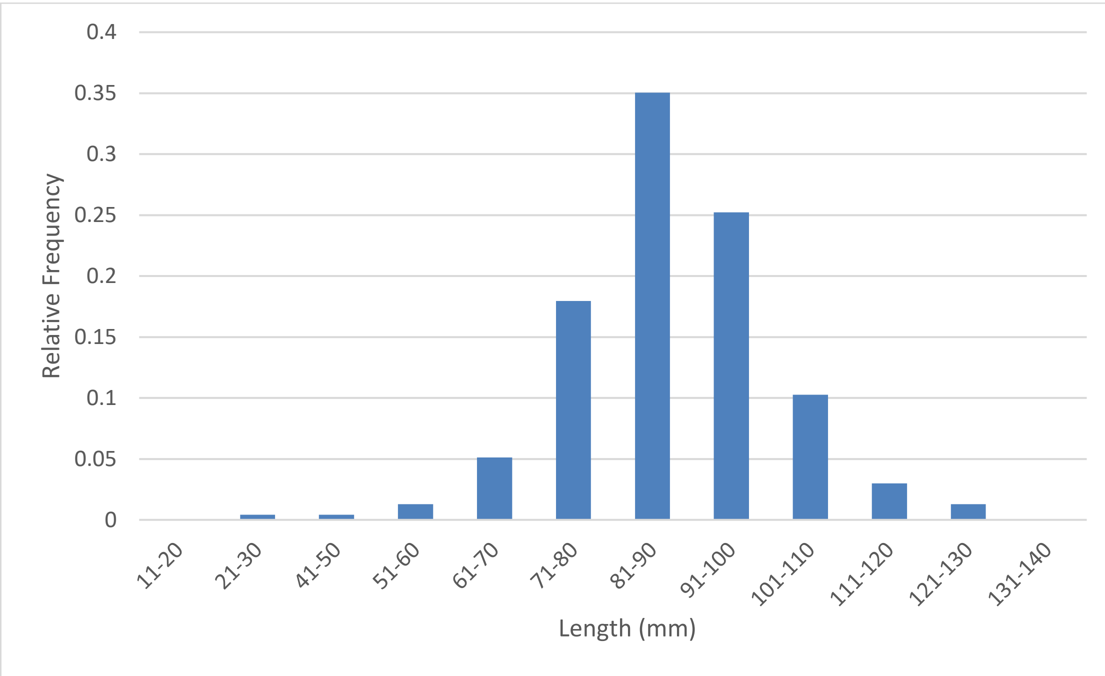
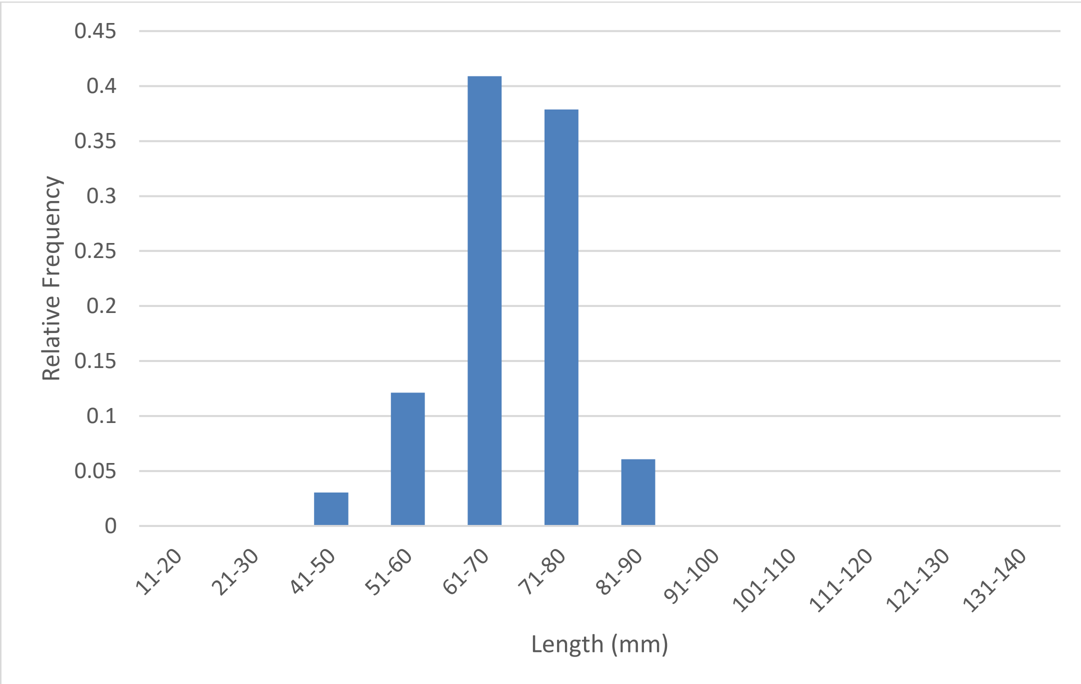


Figure 3 shows the length-frequency histogram for the elliptio in the study area (sample size of 264). From this figure we can see that the distribution is generally bell-shaped and that most size classes are represented in the study area. Juvenile recruitment appears to be occurring as evidenced by the presence of the smallest size class. The number of mussels in this size class is typically low, likely in part due to the difficulty in finding very small individuals. The presence of a few individuals in the larger size classes indicates that some individuals are in the older age classes as well.

Figure 4 shows the length-frequency histogram for creeper mussels documented in the study area (sample size of 66). This species also shows the bell-shaped curve see in Figure 3, but only five age classes are present. This histogram is shifted to the left compared to the elliptio histogram because this species does not grow as large as the elliptio. Also, no mussels in the lower size classes for this species were detected. This may be a factor of sampling methodology rather than an actual lack of younger individuals. Younger mussels more frequently reside below the surface of the sediment and can only be reliably detected when the sediment is excavated. Excavation of the sediment occurs during the Quantitative Plot sampling but not during the timed searches. Since this species was relatively rare in the study area, there were no individuals that occurred within the Quantitative Plots and therefore no areas excavated that contained creeper mussels.

Figure 4 Length-Frequency Histogram of Strophitus undulatus (n=66)



The above data provides a point in time analysis of the density of mussel beds and abundance of mussels in the study area. It is difficult to make conclusive statements about trends in mussel populations without reliable historic data to act as a baseline. There have been no previous quantitative surveys of mussel beds in the upper Missisquoi to act as baseline data and to provide a point of comparison for the current study. However, there are three sources of information that can shed some light on overall trends and provide some regional context for the data presented above.

The first source of historical information provides data on diversity of mussel species. In 1995, an inventory of freshwater mussels in Vermont was conducted (Fichtel and Smith 1995). That inventory included a single sampling location in the study area located at the confluence of the

Missisquoi and Trout Rivers. Three mussel species were documented during that inventory: elliptio, creeper and triangle floater. As noted above, all three of these species were documented during the current inventory with the elliptio being common, the triangle floater very rare (relative abundance of 0.3%) and the creeper also rare (relative abundance of 5.0%). The 1995 inventory did not report on the abundance of mussels, only the presence/absence. We do not, therefore, know if these species have become more rare since the 1995 study. However, we can conclude that the diversity of species in this stretch of the Missisquoi River has not changed since the 1995 inventory.

Another species, the creek heelsplitter (*Lasmigona compressa*), was documented upstream of the current study area along an unnamed tributary of the Missisquoi in the 1995 inventory. This same species was recently documented just downstream of the current study area and was found to be very rare which likely explains its absence in this study (Nedeau 2020).

The second data source that is available for somewhat limited comparison is mussel abundance data from lower in the Missisquoi River and in similar rivers nearby. In 1999, a study was conducted in the lower Missisquoi that included quantitative evaluation of mussel beds (Marangelo 1999). Comparison between the two studies should be made cautiously; the lower Missisquoi studied by Marangelo in 1999 has been impacted by numerous dams (Swanton and Highgate) and the riverine habitats present are structurally very different than in the unobstructed upper Missisquoi. Dams alter the habitats by creating impoundments which result in lower flows, deeper pools and runs and a build-up of finer sediments. One commonality between the two reaches is that elliptio is the most abundant mussel species in both areas. During the 1999 study, most of the mussel beds measured had around 2 elliptio per square meter (median = 2.76). This is very similar to the density of elliptio measured in the current study in the quantitative plots. However, in the lower Missisquoi study, the two most dense beds had 86 and 140 elliptio per square meter, far greater than the maximum 26 elliptio mussels per square meter documented in the current study. Given the differences in habitat, it is unknown if the upper Missisquoi ever had mussels with such high abundance.

A third data source for comparison looks at a recent study on the Lamoille River. In terms of riverine habitat, the upper Missisquoi is fairly similar to the middle reaches of the Lamoille River: there is a lack of large impoundments and the habitats are dominated by shallow riffles and runs with coarser substrates with occasional deeper runs and pools with finer sediments. In 2020, data from a study conducted on the Lamoille showed a total of 6.23 mussels per square meter, which is within the range of abundance documented during the current inventory (Lew-Smith and Marangelo 2020). In addition, CPUE data in the areas inventoried were very similar to the abundance of mussels documented in the current inventory.

5. Conclusions

The lower reach of the Wild and Scenic section of the Missisquoi River in Berkshire and Enosburg includes a diverse array of riverine habitats suitable for freshwater mussels. A total of 9 mussel beds were mapped and assessed within the study area, resulting in a robust data set for this reach of the river. Timed searches were conducted at each of these beds and present a picture of healthy (as indicated by a range of age classes) and relatively abundant mussels at many of these locations. Three different mussel species were documented during these timed searches: elliptio, creeper and triangle floater. Elliptio is a common species while the creeper and triangle floater are both considered uncommon (S3-ranked) species in the state, as was seen in the frequency of these species in this study. Quantitative plot data was also collected at four different mussel beds with results ranging from 2 to 26 mussels per square meter. There is limited historical data by which to analyze and compare population trends for the study area. Overall, the diversity and abundance of mussels in this reach of the river appear to be consistent with other data collected historically on the Missisquoi River and more recently on nearby rivers. This current dataset will be a valuable addition to the scientific knowledge about the Wild and Scenic Missisquoi River and can serve as a benchmark to monitor a dynamic river system over time.

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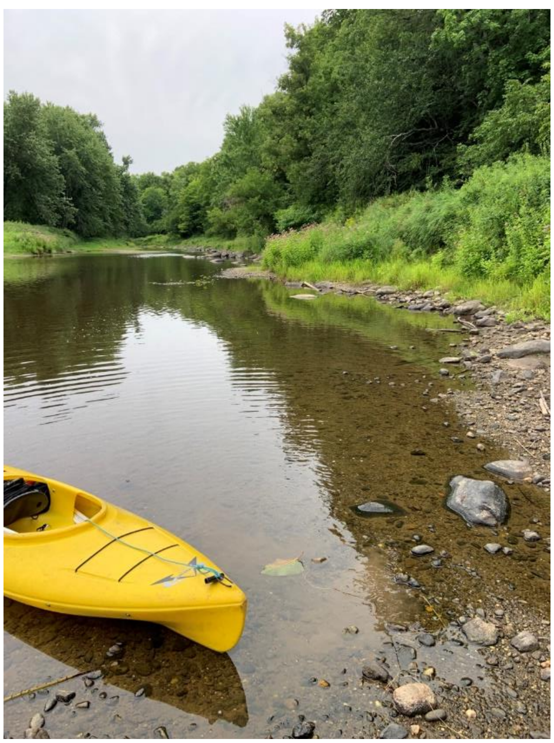
Appendix 1

Photo of each Mussel Bed Site

Mussel Bed C

7/29/2021



Mussel Bed D

7/29/2021

Mussel Bed F

8/5/2021

Mussel Bed G

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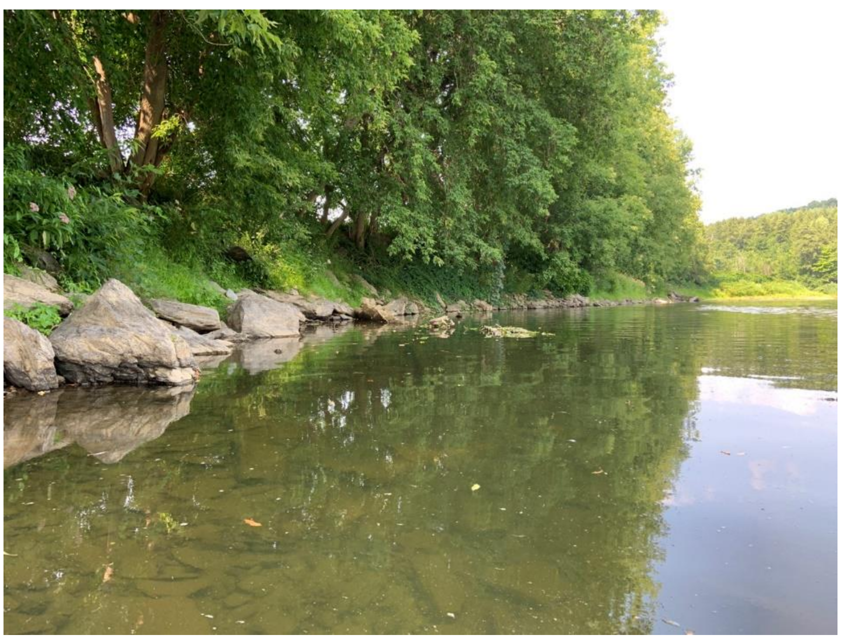
Mussel Bed I

8/25/2021



Mussel Bed J

8/25/2021

Mussel Bed L

8/25/2021

Mussel Bed N

8/25/2021

Mussel Bed P

10/6/2021