Sediment Characterization of Freshwater Mussel Habitat in the Lower Wolastoq |Saint John River Watershed

by

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Abstract

Freshwater mussels are one of the world's endangered species, which has significant importance in aquatic ecosystems. However, there has been a widespread decline in freshwater mussels due to various factors such as excessive sedimentation and poor land use practices. This report represents a comprehensive sediment characterization of freshwater mussel habitat from 34 different survey sites within the lower Wolastoq. Particle size distribution (PSD) analysis of collected sediments revealed that fine and medium sand was the most preferred habitat for freshwater mussels. Based on geospatial analysis, no significant difference was found between non-forest and forest areas (p=0.93 and p=0.22 respectively) whereas forest road density shows a statistically significant difference (p=0.01) with fineness modulus of sediments. Therefore, linking land use type and activity with sediment particle size, in aquatic systems can be useful to understand the habitat and distribution pattern of freshwater mussels.

Keywords: Sedimentation, mussels, geospatial analysis, particle size distribution

Dedication

This work is dedicated to my loving parents whose unwavering support, unconditional love, and encouragement have been the foundation of my academic journey. They have always been my constant support through thick and thin of my life. Also, to my brother and sister-in-law whom I am kindly grateful for having in my life as they have always loved me unconditionally and provided the right direction to achieve everything that I aspire to achieve.

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Introduction

Freshwater mussels are one of the world's endangered species, which has significant importance in the aquatic ecosystem (Ethan et al., 2000). They are found in a variety of freshwater habitats, including rivers, streams, lakes, and ponds of various sizes belonging to the order Unionid and are represented by two families: the Margartiferidae and Unionidae in North America (Martin, 1997). The greatest diversity of freshwater mussels is found within North America with nearly 300 species (Ethan et al., 2000), but due to various factors such as sediment pollution, habitat degradation, or introduced species, there has been a widespread decline in freshwater mussels. Among the recorded 55 species of freshwater mussels in Canada, 11 species have been recorded in Saint John River Watershed (Martel et al., 2010) which includes Eastern Elliptio *[Elliptio complanate (Lightfoot, 1786)],* Eastern Pearlshell *[Margaritifera margaritifera, (Linnaeus, 1758)]*, Triangle Floater *[Alasmidonta undulata, (Say, 1817)],* Alewife Floater *[Anodonta implicata, (Say, 1829)],* Yellow Lampmussel *[Lampsilis cariosa, (Say, 1817)],* Eastern Lampmussel *[Lampsilis radiata, (Gmelin, 1791)],* Tidewater Mucket *[Leptodea ochracea, (Say, 1817)]*, Eastern Floater *[Pyganodon cataracta, (Say, 1817)]*, Brook Floater *[Alasmidonta varicose,(Lamarck, 1819)]* and Creeper *[Strophitus undulatus, (Say, 1817)].* Yellow Lampmussel and Brook Floater are listed as species of special concern according to the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2004, 2009). Along with the freshwater mussels, the Wolastoq | Saint John River also supports many species of freshwater fish suggesting a greater number of mussel species (Martel et al., 2010). Therefore, the persistence of freshwater mussels in the long term is reliant on the conservation of native freshwater fishes, as they play a crucial role in hosting the reproductive and dispersal stages of mussel larvae (Martel et al., 2010).

The diversity of freshwater mussels in a river system is typically high due to several factors including the presence of older and geologically stable habitats, heterogeneous habitats, varying substrates, and a greater diversity of host species (Martel et al., 2010). Various factors like stream size and water velocity also influence the distribution and abundance of freshwater mussels (Strayer & Ralley, 1993). As the habitat composition of rivers is affected by different factors like water depth, channel width, channel slope, sediment load, and sediment grain size (Leopold et al., 1964), changes in sediment composition and load directly impact the aquatic habitats (Hauer et al., 2018). Usually, different species of freshwater mussels often exhibit a preference for some sections within the river or watershed, where some species prefer the upstream section of the watershed or river, and other mussels prefer the downstream section (Martel et al., 2010). Most preferred habitats for unionid mussels are found in the middle and lower sections of the river or watershed (Martel et al., 2010).

Changes in the amount of suspended and bed material load, as well as alterations in the composition of a riverbed, due to the increase in sediment production and runoff, can have direct effects on the mussels (Box & Mossa, 1999). Though there are different reasons behind the decline in freshwater mussels, one of the important factors is excessive sedimentation caused by poor land use practices (Box & Mossa, 1999). An excessive amount of fine sediments washed into streams not only affects the mussels but also impacts the whole aquatic ecosystem by reducing the in-stream habitat diversity as well as by influencing nutrient mechanisms (Leitner et al., 2015). Also, anthropogenic disturbances like dam construction significantly impacts the sediment regime and alters the downstream flux of water by capturing the sediment on the upper stream of the rivers as well as causing a barrier to the movement of organisms between upstream and downstream of the rivers (Poff & Hart, 2002). Freshwater mussels exhibit species-specific preferences for different types of substrates, in which certain species prefer coarser substrates such as gravel whereas some others favour fine sediments such as sand, silty sand, silt, or clay (Clarke, 1918).

As freshwater mussels are highly sensitive to changes in land use practices and stream habitat conditions (Box & Mossa, 1999; Hopkins, 2009), sedimentation from different land-based activities like forest operations and agricultural practices may result in an increased deposition in riverine habitats that may affect the flow regimes of the river (Gordon et al., 2004). Also, the clearing of forested land along the upstream has a direct impact on the sediment dynamics and flow regime in the downstream river (Pandolfi et al., 2022).

The presence of dense and thriving riparian vegetation, particularly in small to moderate-sized streams or rivers, is crucial for certain mussel species like *Margaritifera margaritifera* as it provides the necessary shelter and shaded areas required for their hosts such as Brook Trout (*Salvelinus fontinalis)* and Atlantic Salmon (*Salmo salar linnaeus*) (Martel et al., 2010). Freshwater mussels are one of the important components of an aquatic ecosystem that provide different ecological functions such as increasing water clarity, nutrient recycling (Dascher et al., 2018), and enhancing the growth of organisms like benthic algae (Atkinson & Vaugh, 2014). Therefore, understanding the sediment dynamics at the river scale is important for managing the aquatic invertebrates and conserving the endangered and special concern species like freshwater mussels.

Objectives

This project aims to compare the sediment composition across 34 freshwater mussel survey sites to characterize each site's suitability for freshwater mussels found in New Brunswick. The objectives were thus:

- To identify what sediment/habitat type each species from the existing mussel community in New Brunswick occupies.
- To produce particle size summaries for each site and relate the results to habitat suitability for individual mussel species.
- To conduct a literature review of existing research that has used sediment to characterize habitats for mussels and other aquatic biota.
- To produce a geospatial analysis of freshwater mussels' occurrence.

Methods

The study was conducted in the lower Wolastoq | Saint John River Watershed. The Wolastoq flows from Northern Maine to western New Brunswick to the Atlantic Ocean Bay of Fundy and is the longest river in Eastern Canada (Esrock, 2018). Sediment from 34 freshwater mussel survey sites (Figure 1) was collected to characterize each site's suitability for freshwater mussels.

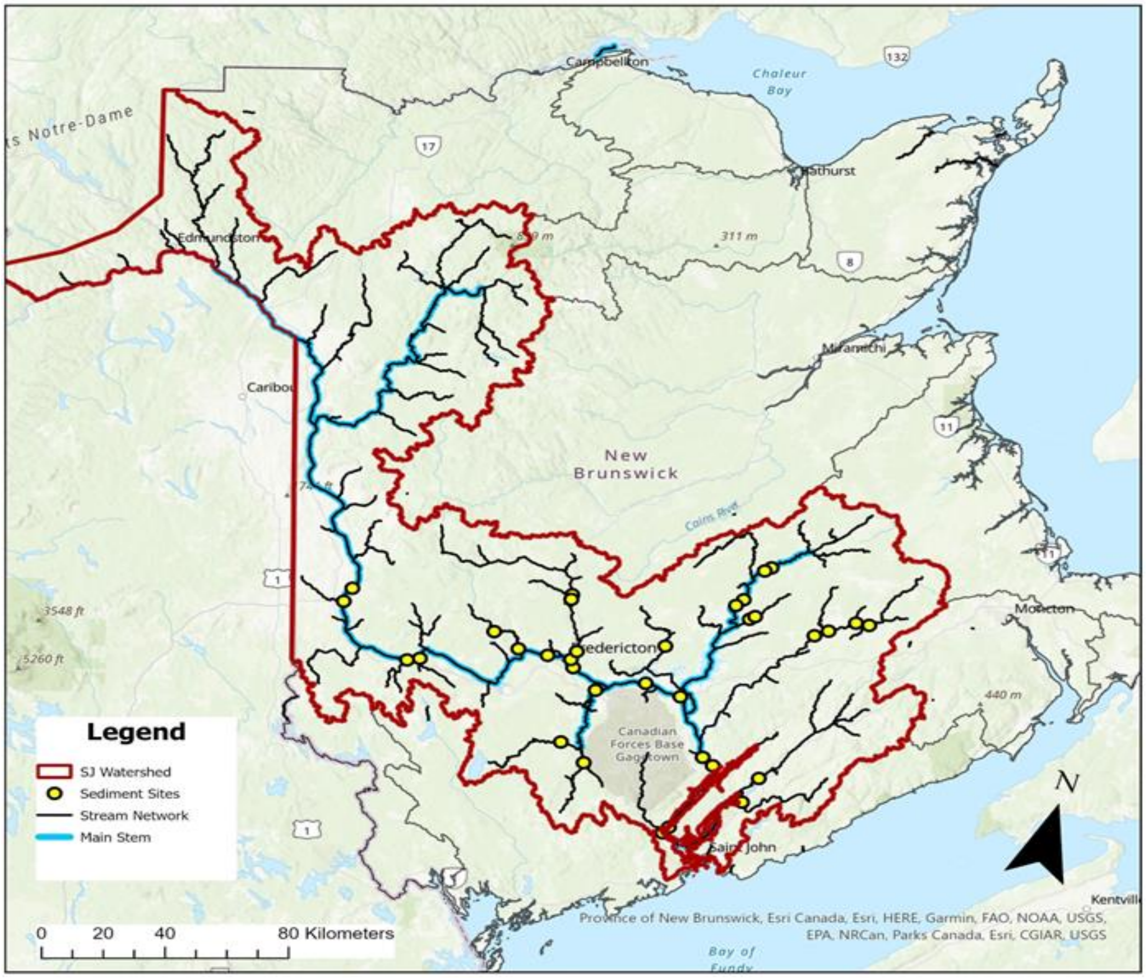

Figure 1. Map of the study area indicating sites where sediments were collected from the Wolastoq.

Sediment Sample Collection

A bulk sediment sample was collected using a metal scoop working from the downstream section of a given survey area to the upstream area during the summer season (July-August) in 2022. The representative sample was collected from the survey site from the top 5cm of the riverbed substrate and the aggregated sediment was placed into a pre-sanitized tote. To collect this sample, 2 people were involved; one for scooping and the other one following with the tote.

Laboratory Methods

Sediment samples were dried in an oven for 24 hours at a temperature of (110+/-5) [°C]. The sediment particle analysis followed the American System of Testing and Material (ASTM) procedures. The sieve analysis method was used for the measurement of particle size with different mesh sizes simply by shaking dried sediment samples for 10 minutes when the amount retained becomes constant. For the particle size distribution (PSD) analysis of the sediment sample, eight sieves measuring 4.75mm, 2mm, 0.85mm, 0.425mm, 0.25mm, 0.15mm, 0.105mm, and 0.075mm, were used. These sieves were stacked up in such a way that sieves with larger openings are placed on top and put in a mechanical sieve shaker to obtain the retained sediment in each sieve as shown in (Figure 2). Each sieve has a squared-shaped mesh with their respective sizes which separates the larger particles from the smaller particles. Along with the sieves, a pan was also used to collect those sediment particles that pass through the last sieve which were very fine particles (<0.075mm). The size fractions from each sieve were weighed to the nearest 0.1g using a calibrated balance.

Figure 2. Typical set-up of stacked sieves on a mechanical shaker (Source: Prof. Susan Burns, Georgia Tech University, Department of Civil and Environmental Engineering)

Data Analysis and Management

After the completion of the sieve analysis procedure in the lab, the percentage of sediment retained on each sieve from the mass of sediment retained was calculated, based on the cumulative percentage of sediment retained. Then the finer percentage of sediment was obtained by subtracting the cumulative percentage from 100%. The percentage of sample loss during the sieving was calculated by subtracting the total final weight from the total initial weight. The aim was to have any sample loss below 2%, else the sample was redried and resifted. All the necessary calculations followed the workflow and formulas (Table 1).

Table 1. Typical grain size analysis data sheet (Source: Step-by Step Guide for Grain Size Analysis / Geoengineer.org)

Sieve #	Opening Diameter (mm)	Weight of Empty Sieve (g)	Weight of Sieve + Soil Retained (g)	Soil Retained (g)	Percent Retained (%)	Cumulative retained (%)	Percent Passing (%)
		A	B	C	D	E	F
				$C_i = B_i - A_i$	$D_i = \frac{C_i}{Total\ Weight} * 100$	$E_i = D_i + E_{i-1}$	$F_i = 100 - E_i$
4	4,75						
10	2						
20	0,85						
40	0,425						
60	0,25						
100	0,15						
140	0,105						
200	0,075						
Pan							
			Total Weight =				

Uniformity Coefficient (Cu)

A uniformity coefficient (Cu) which expresses the variety in particle size of sediment was calculated for each sediment sample and is defined as the ratio of D_{60} to D_{10}. D-values are used to describe the particle size where D_{60} is the grain diameter at which 60% of sediment particles are finer and 40% of sediment particles are coarser, while D_{10} is the grain diameter where 10% of particles are finer and 90% of the particles are coarser. The uniformity coefficient provides a measure of the grading of sediment. When Cu is > 4, the sediment is classified as *well-graded*, whereas when Cu is < 4, the sediment is classified as *poorly graded/uniformly graded* (Geoengineer, n.d.).

According to ASTM/Unified Soil Classification System, sediments were classified based on the particle size ranges as classified in (Table 2). Following the classification system, sediments were divided into four types (fine gravel, coarse sand, medium sand, and fine sand) with their respective particle size in (Table 2).

Table 2. Soil classification based on particle size.

Sediment Type	Particle Size (mm)
Fine Gravel	2 - 4.75
Coarse Sand	0.425 - 2
Medium Sand	0.25 - 0.425
Fine Sand	0.075 - 0.25

Fineness modulus of sediments

The fineness modulus of sediments of each site gives the average particle size of sediments and is calculated by performing the sieve analysis with standard sieves. After going through the shaking of sediments in the mechanical shaker, the soil retained on each sieve was calculated and the cumulative weight of retained particles, as well as the cumulative percent retained on each sieve, was calculated. As a result, the cumulative percentage retained on each sieve was added and divided by 100 to get the fineness modulus of sediments (Vashisth, 2018). The fineness modulus of each sediment sites was calculated and categorised them in which sieve the particle lies (the size of particle is known when counted from lower sieve size to higher order sieve) and is categorized as shown in the (Table 2). The fineness modulus of sediments provides the information on distribution of particle sizes in sediment samples and the categorization of the fineness and coarser aggregates of sediments in different survey sites. As fine aggregates range from a fineness modulus of 2-6 and coarse aggregates from 6.5 to 8 (WBDG, 1980).

Geospatial Analysis

The Geographic Information System (GIS) program ArcGIS (Version 10.8) was used to analyze the geospatial data. The data was collected from GeoNB and several layers were used to determine the watershed area, forest coverage, non-forest area, and length of forest roads along the stream. The analysis was performed within the watershed area from where the sediment samples were collected. Using flow direction and flow accumulation, the watershed was delineated from the pour point data (which is the site from which sediments were collected). The total sub-watershed area, forest area, non-forest area, and forest road density for each sub-watershed were determined. Also, linear regression analysis using MS-Excel was done to determine if there were any significant relationships between variables with the dependent variable fineness modulus of sediments that were collected from the mussel survey sites. Alpha was set at 0.05 for determining statistical significance.

Results

Abundance of freshwater mussels within the lower Wolastoq basin.

The surveys conducted at 34 sites within the Wolastoq basin found a total of 13,114 mussels (Cusack. S, unpublished data). Overall, eight species of freshwater mussels were identified which were Yellow Lampmussel (YLM), Triangle Floater (TF), Eastern Elliptio

(EE), Eastern Floater (EF), Eastern Lampmussel (EL), Eastern Pearlshell (EP), Tidewater Mucket (TM), and Alewife Floater (AF). Out of the 34 sites, 7 sites had no mussels present (Figure 3).

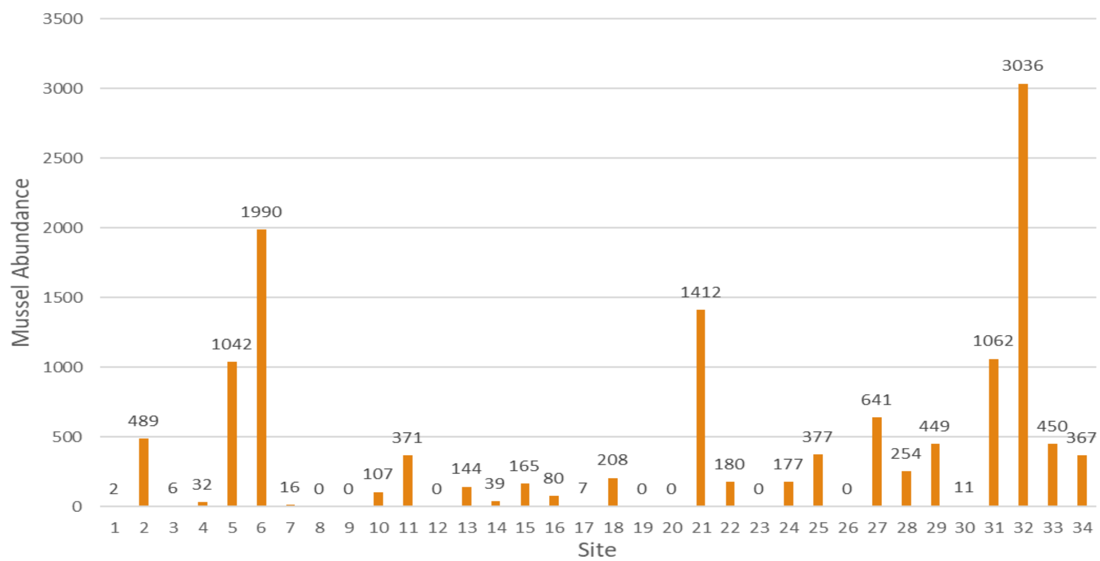

Figure 3. Total mussel abundance at each survey site where sediments were collected within the Wolastoq.

EE (n=9,499) were found across many sediment sites followed by EL (n=1231), AF (n=918), EF (n=732), EP (n=653), TM (n=71), TF (n=7). Very few numbers of special concern species YLM (n=3) were found across the sediment sites (Figure 4). Also, the fact that only three individuals of YLM were found across the sediment sites indicate that it is a very rare species.

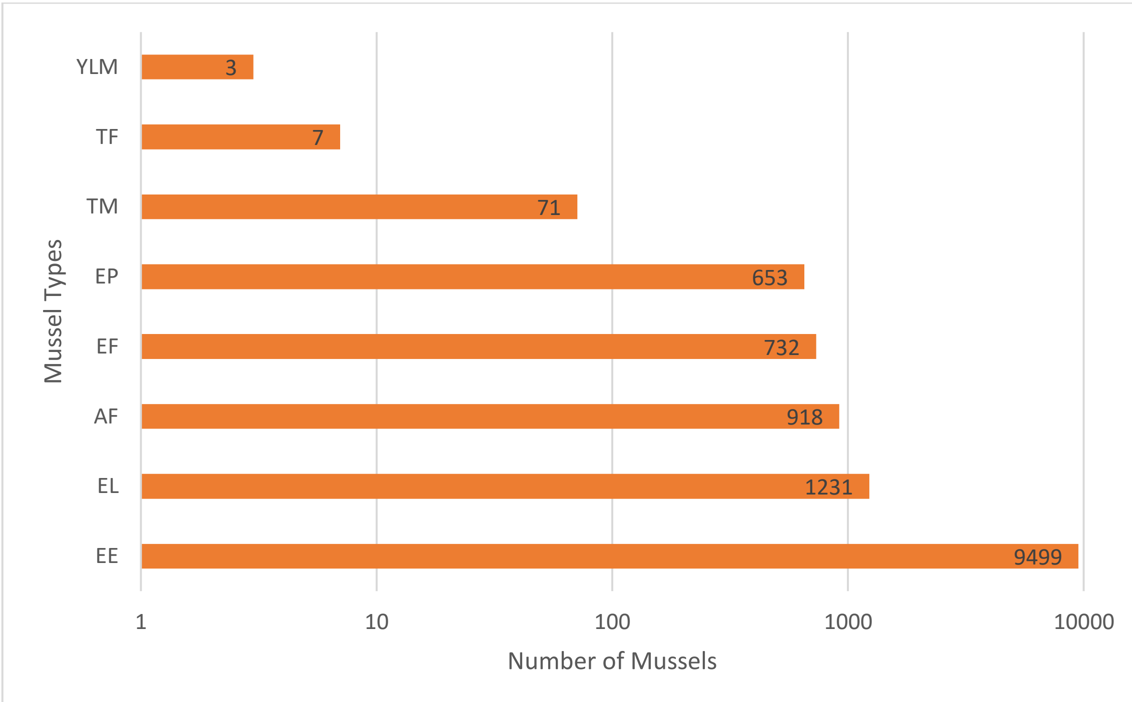

Figure 4. Mussel abundances by species across all study sites.

The study sites were divided into three study reaches (Mainstem River (n=9), Side River (n=21), and Islands (n=4) and the abundances of each mussel along the study reaches (Figure 5). In general, AF, EE, and EL have higher abundances in island study sites than in mainstem river and side river study sites. TF and YLM have lower abundances across each sediment sites. EE was found in the greatest numbers at Island sites. Whereas EP and EF were found more in mainstem river.

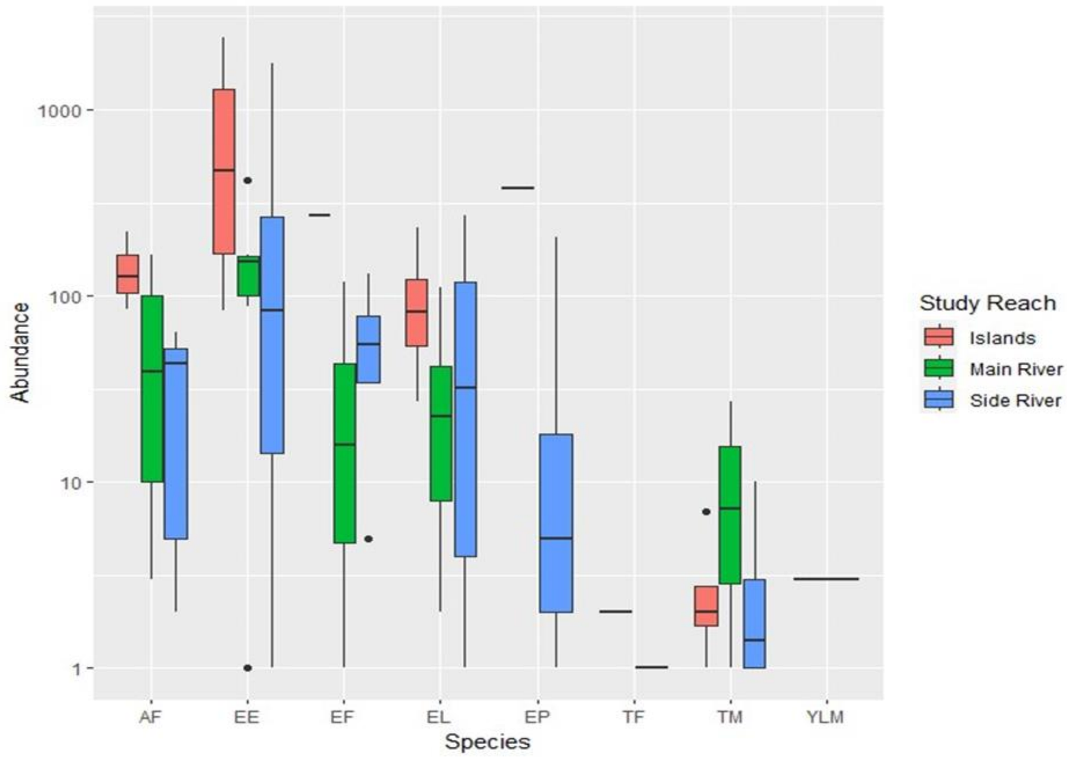

Figure 5. Abundance of mussels by species grouped by study reach type.

*Table 3. Results of one-way ANOVA testing for differences in total abundance across study reaches for each freshwater mussel species. * Indicates statistically significant differences.*

Species	F-value	p-value
AF	8.782	0.001*
EE	3.894	0.031*
EF	1.659	0.207
EL	2.87	0.072
EP	0.637	0.535
TF	7.046	0.003*
TM	2.34	0.113
YLM	4.559	0.018*

There was a significant difference in species diversity between the three study reaches (F (2, 31) =4.339, *p*=0.014. Total relative abundances for four species (i.e., AF, EE, TF and YLM were significantly different among the study reaches which can be seen in (Table 3). Also, post hoc pairwise comparison showed a significant difference in abundance ($p<0.05$) between the main river and islands as well as between the side river and islands whereas no significant differences were found between the main river and the side river ($p>0.05$).

Sediment/Habitat type each freshwater mussel occupies.

The finer substrate (fine and medium sand) is mostly preferred sediments by many freshwater mussels whereas fewer mussels preferred fine gravel (Figure 6). Also, we can see that each species has mixed substrate preference, Eastern Elliptio, Eastern Lampmussel, and Alewife Floater, are the most common species found in fine to medium sand followed by coarse sand and fine gravel. Whereas Eastern Pearlshell mussels prefer fine gravel and coarse sand. Eastern Floater are found in medium sand followed by fine sand and coarse sand. Yellow Lampmussel and Triangle Floater are found within the medium sand.

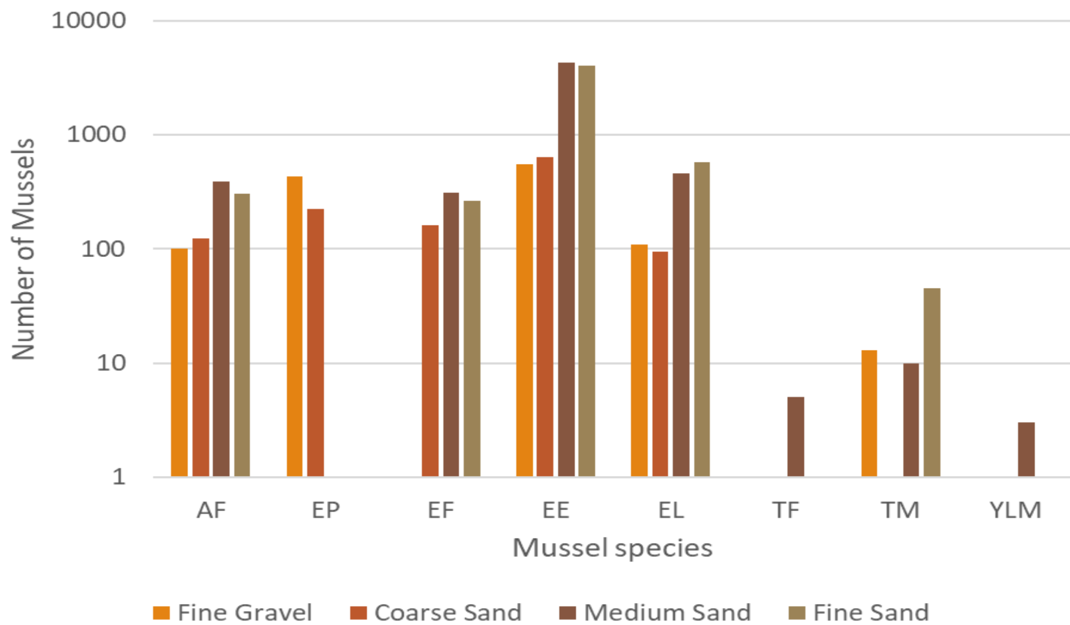

Figure 6. Sediment preference by each mussel species.

Particle size distribution graph of each sediment sites.

The particle size distribution graph for each sediment category (fine gravel, coarse sand, medium sand, and fine sand). The particle size distribution was plotted in a logarithmic graph shown in (Figure 7). In these graphs, particle diameters were plotted in log scale and percentage passing in arithmetic scale. The S-curve which is regarded as a gradation curve is a logarithmic graphical representation of the cumulative percent passing of sediment grains (Figure 7). The particle size distribution graph shows that the percentage fineness curves varied across sites. For sites 6, 7, 9, 10, 16, 27, 28, 19, and 20, the percentage finer curves have a steep gradient from 0.075mm to 0.25mm and a mild gradient in the range of 0.25mm to 5mm. This indicates that more than 60% of the sediment was in the size range of 0.1mm to 0.25mm and the remaining were larger than 0.25mm (Figure 7; top left). Similarly, in sites 8, 11, 17, and 34, the percentage of finer curves have a steep gradient from 0.25mm to 0.425 mm indicating that more than 95% of the sediment was in the range of 0.1mm to 0.425mm, whereas 5% was larger than 0.425mm (Figure 7; top right). Also, we found that a larger number of freshwater mussels were found at these sites, which indicates that, the fine sediments (fine and medium sand) are the most common habitat for freshwater mussels in the Wolastoq. Looking at the remaining sites (Figure 8; bottom left and right), the percentage finer curves have a steep slope gradient from 0.425mm to 2mm and a very mild gradient in the range of 0.075mm to 0.425mm, indicating less than 10% is finer particles and more than 90% are coarser or fine gravel.

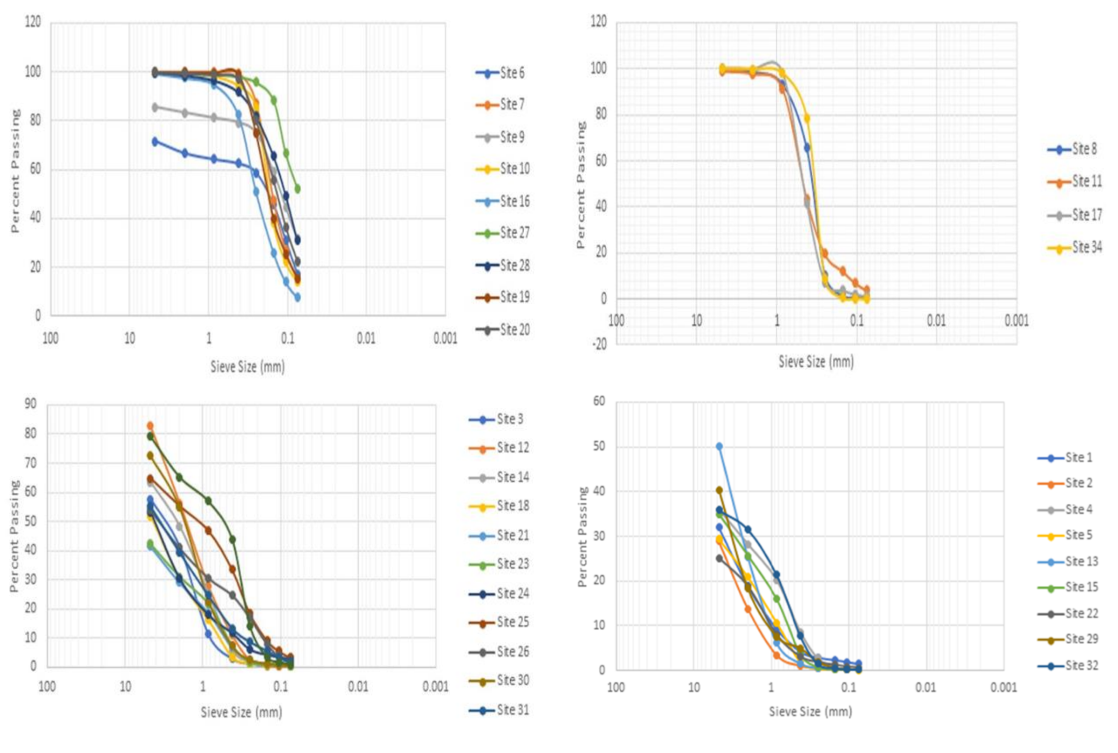

Figure 7. Particle size distribution for sites with predominantly fine sand (top left), medium sand (top right), coarse sand (bottom left), and fine gravel (bottom right).

Based on the uniformity coefficient value, the effectiveness of sediments as well as grading of sediments calculated for each sediment sample. It is found that eleven sites had a lower uniformity coefficient (Cu), indicating narrow range of sediments with a narrow particle size distribution, where particles are of almost the same size. Twenty-three sites have a higher Cu which indicates well graded sediments and contain a wide range of particle size distribution (Figure 8). Compared with the total number of mussels found in each site, a greater number of mussels were found in uniformly graded sediments with narrow size distribution rather than more heterogenous sediments.

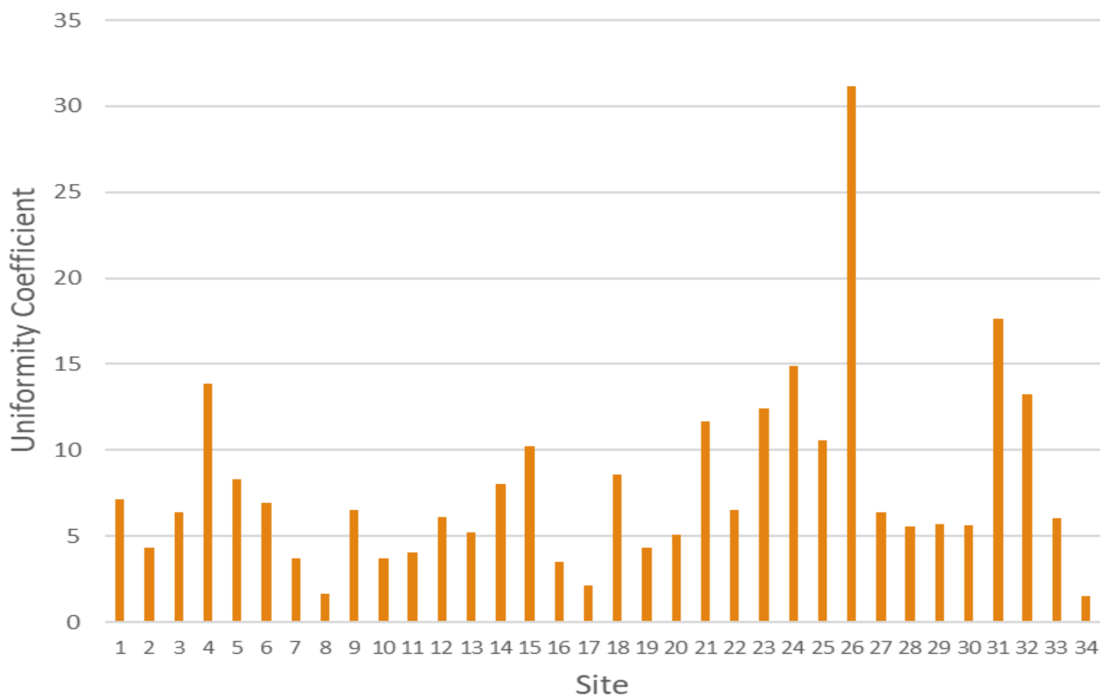

Figure 8. Uniformity coefficient of sediments collected at each site.

The graphical representation of the association between the number of mussels and the fineness modulus of sediments is shown in (Figure 9). The number of mussels is high in between the fineness modulus of sediments (between 2-6) that represents the finer substrate (fine sand and medium sand), which implies these substrates are highly preferred by the mussels.

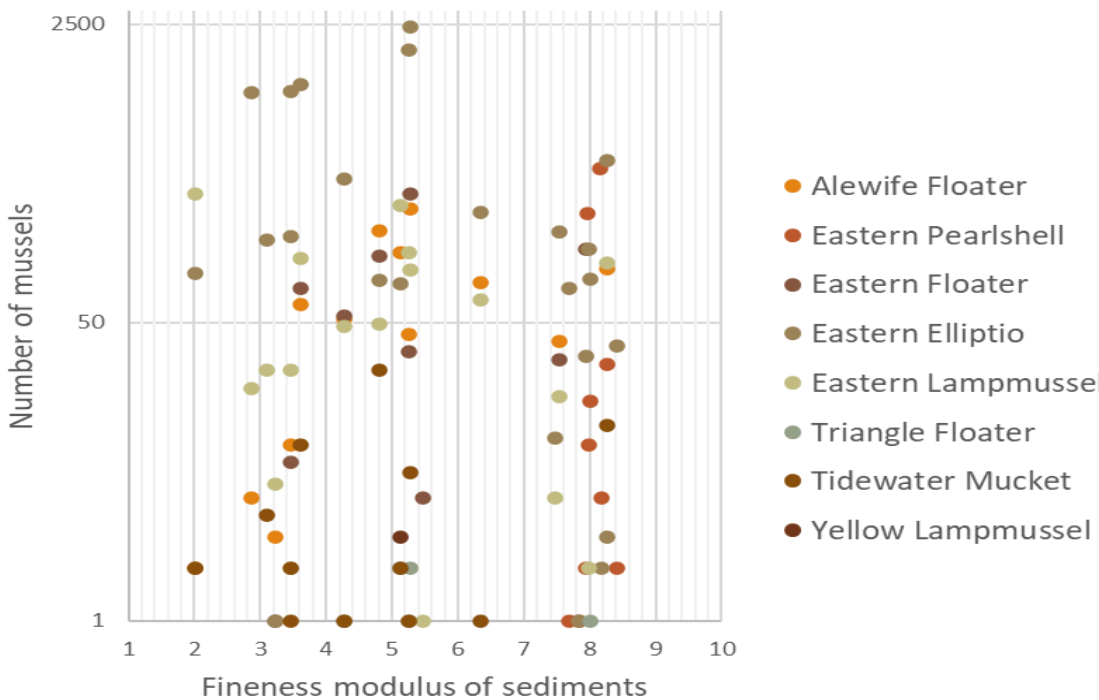

Figure 9. Association between the number of mussels and fineness modulus of sediments.

Research using sediment to characterize habitats for freshwater mussels and macroinvertebrates.

Several studies were identified and reviewed that have used sediment to characterize habitats for mussels and other macroinvertebrates. Overall, land use change, substrate composition, and bed material composition are the major factors influencing macroinvertebrates' habitat (Box & Mossa, 1999). Despite various research efforts to establish an association between freshwater mussels and sediments, ongoing uncertainties and debate persists regarding the effects of sediments on freshwater mussels (Box &

Mossa, 1999). The nature of the substrate composition was one of the important habitat descriptors when assessing the distribution pattern of mussel species (Bonmark & Malmqvist, 1982), whereas the research conducted on fine sediments and macroinvertebrate assemblage in Appalachian streams (Angradi, 1999), indicated a weak correlation between fine sediments and macroinvertebrates. Neves & Widlak (1987) conducted research on the habitat ecology of juvenile freshwater mussels in a headwater stream in Virginia. Their research found that coarse gravel, pebble, and cobble were mostly preferred by juvenile mussels. The research conducted by the (Libois & Hallet-Libois, 1987; Sickel et al., 2007) found that the gravel substrate is preferred by the mussels. Our findings suggest that fine and medium sand were preferred by most of the freshwater mussels encountered at sites in the Wolastoq basin. Also, Strayer & Ralley (1993) examined the microhabitat use by six different mussel species including two rare Alasmidonta species in the Neversink River, New York. Their research considered different factors like water depth, the current speed of the river, bottom roughness, extent of patches of fine sediments, and sediment granulometry. They found that Brook Floater (*Alasmidonta heterodon)* was found in patches of fine sediment whereas the Dwarf Wedgemussel (*Alasmidonta varicosa)* occurred more in the medium sand. They concluded that geomorphological descriptors of streambeds can be useful indicators to determine mussel distribution (Strayer & Ralley, 1993).

In our study, eight different freshwater mussels were identified, and they preferred different habitats based on riverbed morphology and most of the mussels preferred the finer sediments as compared to coarser sediments, like pebble and gravel within the lower Wolastoq basin. A recent study conducted by Wegscheider et al. (2018) on freshwater mussel abundance and species composition upstream and downstream of a large hydroelectric generating station on the Wolastoq, found that the substrate composition was dominated by cobble, small gravel, and silt in the upstream areas whereas sand was in the downstream areas. They divided the research area into five different study reaches (tailrace, side channel, islands, main channel, and depositional zone) and found that there were no significant differences in species diversity between the study reaches. Also, their research found that habitat use curves demonstrate a consistent trend across all species, showing a decrease in relative abundance as water velocity and depth increase while indicating a preference for small-sized substrates.

Poor land-use practices have been implicated in increased sedimentation in aquatic systems, which is one of the most common and detrimental factors affecting mussel populations (Box & Mossa, 1999). Van der Schalie (1938) studied the contributing factors for the depletion of *Naides* (aquatic oligochaete worms) in the Eastern United States and found that the area along the Apalachicola River basin has experienced significant silting from intensive farming practices, as well as the removal of ground cover leading to catastrophic flooding conditions, which may affect the resident mussel population. Ellis (1936) found mortality rates of freshwater mussels reached up to 90% for all species when the silt layer starts to cover the sand or gravel, as mussel valves get closed for 75-95% of the time in the muddy water as compared to the mussels residing in silt-free water. The current state of understanding from existing research is that there is a variety of habitat preference for different freshwater mussel species, and which is closely linked to sedimentation. Focused research on other factors like water depth, velocity, channel morphology, and changes in land use, is useful to characterize suitable habitat for freshwater mussels (Box & Mossa, 1999). From these various studies it can be concluded that sediment load, disturbances in habitat connectivity like construction of dams, substrate compositions, and poor land use practices can impact mussel habitat.

Geospatial analysis of freshwater mussel occurrence.

Geospatial analysis with the combination of statistical methods using ArcGIS was performed to analyze the relationship between the dependent variable's fineness modulus of sediment and independent variables like forest area, non-forest area, and forest road density along the Saint John River watershed. Changes in the surrounding landscape has a significant impact on the abiotic conditions of streams, which in turn influence the structure and function of stream biota (Allan, 2004), highlighting the potential for organisms within the stream to be affected by alterations resulting from landscape modifications. The mean forest percentage across the lower Wolastoq is 68% and forest road density is 9.94 m/ha. The forest coverage, non-forest coverage, and forest road density along the study area with the major rivers and tributaries (i.e., side rivers) from where the sediment samples were collected are shown in the (Table 4).

Table 4. Summary of %forest, forest road density, sub-watershed area for all sites grouped by reach type.

Site Number (#)	Study reach type	Total mussels	Sub-watershed area (ha;)	% Forest	Forest road density(m/ha)
SIDE RIVER					
12	Side River	0	60291.2	76.4	11.97
1	Side River	2	17396.4	69.7	8.79
8	Side River	0	107122	89.4	14.19
9	Side River	0	22904.7	90.5	10.96
21	Side River	1412	1781.2	73.8	8.93
6	Side River	1990	14917.9	30.1	12.52
18	Side River	0	36897.6	25.3	13.41
17	Side River	7	108368	86.5	9.59
2	Side River	489	800.667	79.4	6.36
3	Side River	6	23438.7	88.6	8.59
18	Side River	208	2076.63	73.4	10.71
13	Side River	144	64695.8	82.1	9.83
14	Side River	39	28481.9	87.6	11.30
15	Side River	165	19847.5	87.2	11.54
16	Side River	80	14829.7	46.4	14.93
11	Side River	371	124288	70.3	9.45
5	Side River	1042	137666	64.1	10.53
4	Side River	32	48589.6	66.4	9.94
20	Side River	0	38930	88.6	10.67
7	Side River	16	1768.06	46.4	3.49
10	Side River	107	39778.8	86	10.65
MEAN		**291**		**71.8**	**10.40**

MAIN RIVER					
27	Main River	641	150655	71.2	10.82
29	Main River	449	8933.19	45.7	5.32
30	Main River	11	1020990	45.6	16.31
24	Main River	177	65221.7	82.4	8.11
25	Main River	377	150661	70.7	10.32
28	Main River	254	23229.7	76.6	9.78
22	Main River	180	6636.86	62.8	6.13
23	Main River	0	70228.6	69.7	7.66
26	Main River	0	51111.4	76.4	8.63
MEAN		**232**		**66.8**	**9.23**
ISLANDS					
32	Islands	3036	14297.2	48.5	9.57
31	Islands	1062	283079	66.2	9.35
34	Islands	367	2799.98	33.5	7.24
33	Islands	450	14671.8	53.8	10.07
MEAN		**1229**		**50.5**	**9.06**

The mussel survey sites were divided into three study reaches (main river, side river, and islands) and we found that higher abundance of mussels at the side river study reaches, with an associated mean forest percentage of 71.8%. This indicates that the side river with high forest area along the stream highlighting the higher potential of an organism. The average forest road density was 10.40m/h (Table 4). Whereas less abundances of mussels (n=2089) were found as compared to side rivers (n=6110) and Islands (n=4915). The mean forest coverage on the main river and islands was 66.8 % and 50.5% respectively as well as the mean forest road density along the main river and the island was 9.23m/ha and 9.06m/ha respectively.

We found a positive linear relationship between the fineness modulus of sediments and forest area (Figure 10) and forest road density (Figure 11), and conversely a negative relationship between the non-forest coverage and fineness modulus of sediments (Figure 12). Forest road density was the only variable that was useful in predicting the fineness modulus of sediments ($p = 0.01$). The other independent variables of forest (p=0.93) and non-forest areas (p=0.22) showed no significant differences.

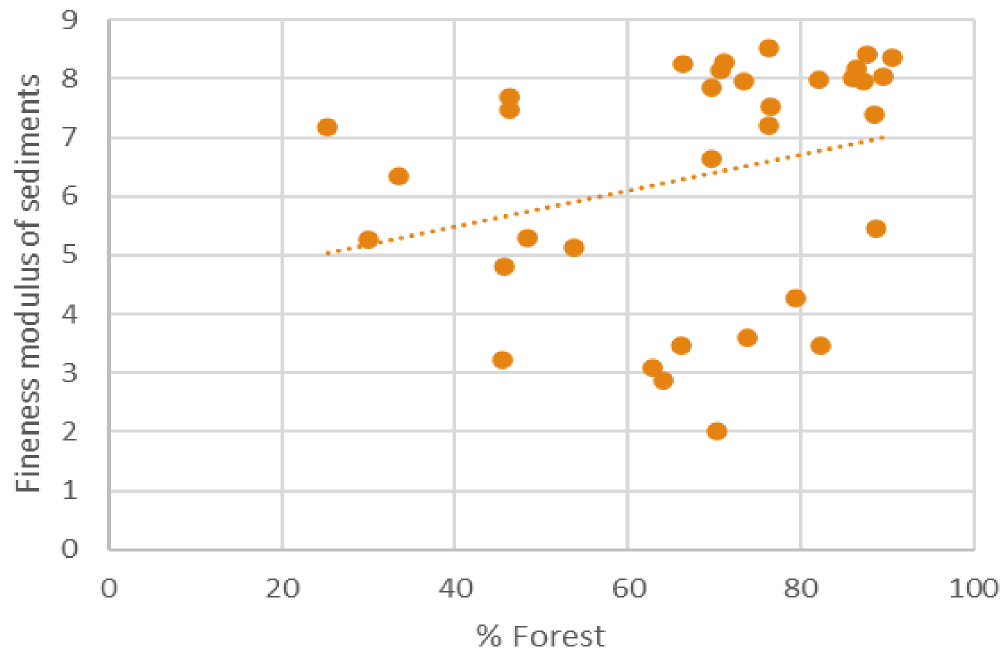

Figure 10. Relationship between fineness modulus of sediments and forest percentage.

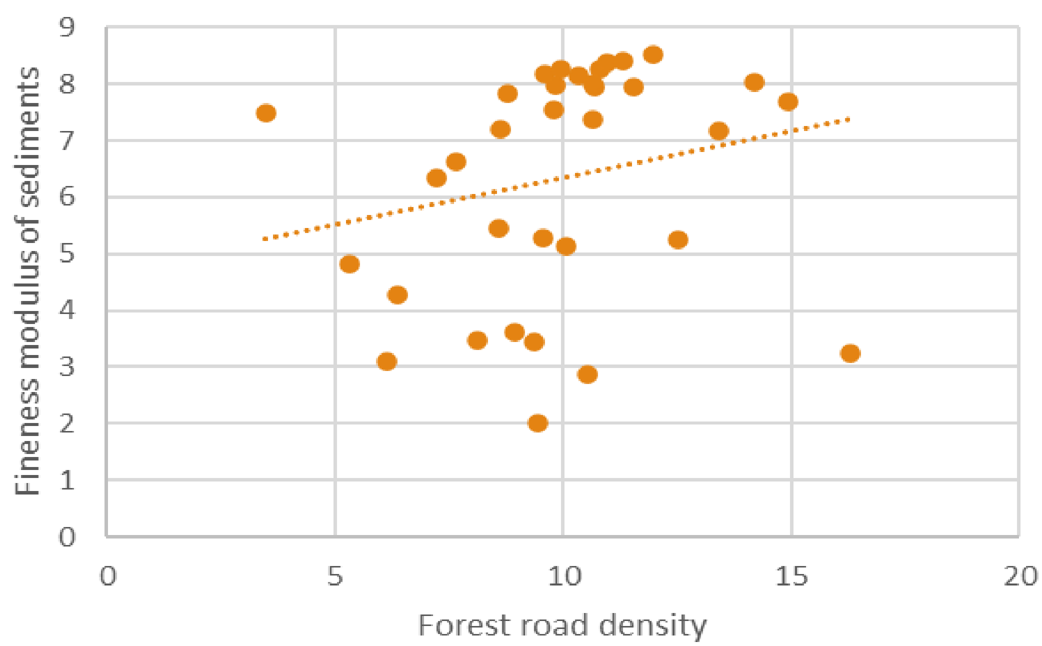

Figure 11. Relationship between the fineness modulus of sediments and forest road density (m/ha).

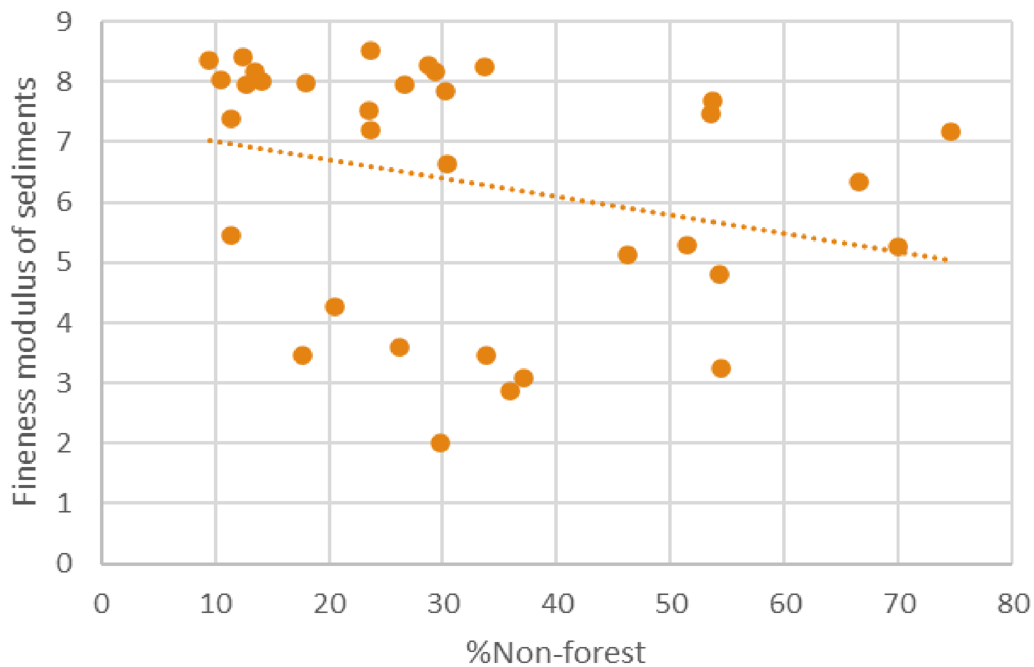

Figure 12. Relationship between the fineness modulus of sediments and non-forest percentage.

Discussion

This study characterizes the different sediment samples of freshwater mussels' habitat which were collected along the Lower Saint John River | Wolastoq Watershed and identified which sediments or substrate are preferred by resident freshwater mussels. We also examined the association between the independent variables like forest area, non-forest area, and forest road density within the sub-watershed area and freshwater mussel habitat. From our study, we found that a high number of mussels preferred the fine and medium sand which was also characterized by the uniformity coefficient (Cu). The uniformity coefficient was calculated for each sediment and found that 11 sites had uniformly graded sediments and particles are of almost the same size, whereas 23 sites had well sorted sediments and contained a wider range of particle size distribution.

In this research, we focused on laboratory work and sieve size analysis using the mechanical sieve shaker to characterize the sediment particles. For the association between freshwater mussels and substrate composition, characterizing the sediments into different size categories plays an important role. We grouped the substrate into four categories which were fine gravel (2-4.75mm), coarse sand (0.425-2mm), medium sand (0.25-0.425mm), and fine sand (0.105-0.25mm). It is necessary to make a clear distinction between coarse sediments and fine sediments which might have different impacts on freshwater mussels. Brönmark & Malmqvist (1982) showed that preference for mixed substrates was shown by individual species. Other factors to characterize river habitats such as sediment bed properties, channel morphology, floodplain, water depth, and velocity help to predict the effects of sediments (Box & Mossa, 1999).

The number of mussels was higher for sites with a fineness modulus of sediments of between 2-6, representing the fine sand and medium sand, implying a preference by most mussel species. The uniformity coefficient data also provided information on which sites are poorly sorted (little variation in particle sizes) and well sorted (broader range in sediment particle sizes present).

This study also documented the geospatial analysis of freshwater mussels' occurrence, in which eight different types of mussels were identified in different survey sites within the lower Wolastoq. Among them, Eastern Elliptio (EE) were found to be in greater number (n=9499) followed by Eastern lamp mussels, Alewife Floater, Eastern Floater, and Eastern

Pearl shell. A previous study conducted along the Wolastoq by (Wegscheider et al., 2018) also documented that Eastern Elliptio, Eastern Lampmussel, and Alewife Floater were most common throughout the study area. In our study, the abundance and diversity were greatest at the island's study reaches (Gilbert Island, Grimross Island, Spoon Island (Evandale), and Dunphy Island) compared to the other habitat types (Main River and Side River). The likely reason for increased abundance at the island sites is the availability of finer sand habitats in the deposition zones around the islands.

To explore the linkage of freshwater mussels and land use, our analysis examined three land use categories: forest area, non-forest area, and forest road density. We found no significant relationship sediment particle size and forest and non-forest area, whereas forest road density was positively related to the particle size category (fineness modulus). This finding was unexpected, as presumably unpaved forest roads would introduce finer sediments into aquatic systems with terrestrial runoff. Research conducted by Pandolfi et al. (2022), found that the frequency of mussel (*Alasmidonta raveneliana)* occurrence was not related to the land use at the watershed scale but had a significantly positive correlation to the forest coverage within the riparian zones. Direct connections between alterations in land use within a watershed or riparian area and mussel populations are difficult to detect given the many other biophysical factors that influence mussel distribution in rivers (Pandolfi et al., 2022). Increased fine sediments and associated dissolved ion inputs, as well as variation in the thermal regime, may have a significant impact on freshwater mussels (Pandolfi, 2016). Studies conducted by (Österling & Högberg, 2014) on Eastern Pearlshell found that forestry practices like clear-cuts have been the major cause for the decline of this species, whereas no effect found between the freshwater Eastern Pearlshell and forest road.

Some sites (Tracy, Nashwaak1, Nashwaak 2, Mouth of Keswick, Ripples, Morrisdale, and GB Westfield), no abundance of mussels was found this area and was coincident with the presence of dominant gravel substrates. At these sites, the fineness modulus of sediment ranged from 6.5 – 8.0, which is indicative of coarser sediments like pebble and cobble. These coarser substrates are least favourable for many freshwater mussels. Poor agricultural and forestry practices, runoff from construction sites, road building, urbanization, loss of riparian vegetation, erosion of stream banks, and changes in the hydrological patterns may all cumulatively contribute to inputs of sedimentation in streams and rivers, which may affect freshwater mussels (Box & Mossa, 1999).

Conclusion

This study provided the baseline characterization of sediments of freshwater mussel habitat by analyzing sediments collected from each of 34 study sites in the lower Wolastoq basin. The abundance of mussels was higher where the substrates were characterized by fine and medium sand. Summarising the particle size distribution across the survey sites helped to group sites that had poorly/uniformly sediments ($Cu < 4$; $n = 11$), and well-graded sediments ($Cu > 4$; $n=23$). In this study, we also found a statistically significant difference in species diversity across the three areas (main river, side river, and islands) was found, with islands standing out from the main river and side rivers sites, with increased abundance and diversity. Forest road density showed a statistically significant positive relationship with the fineness modulus of sediments. Although not statistically significant, the fineness modulus of sediments showed a positive linear relation with forest coverage. Beyond measures of sedimentation, more detailed research on water depth and velocity, channel morphology, bed sediment properties, organic content, nutrient concentrations, and water quality parameters would be useful to understand more about the influences on suitable habitat for freshwater mussels. Also, it is very important to consider the effect of sediment production from terrestrial land use practices because land use changes adjacent to the river or stream may directly impact the habitat of freshwater mussels.

References

Angradi, T. R. (1999). Fine Sediment and Macroinvertebrate Assemblages in Appalachian Streams: A Field Experiment with Biomonitoring Applications. *Journal of the North American Benthological Society*, *18*(1), 49–66. https://doi.org/10.2307/1468008

Allan, J. (2004). Landscapes and Riverscapes: The Influence of Land Use on Stream Ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *35*(1), 257–284. https://doi.org/10.1146/annurev.ecolsys.35.120202.110122

Atkinson, C. L., & Vaughn, C. C. (2014). Biogeochemical hotspots: temporal and spatial scaling of the impact of freshwater mussels on ecosystem function. *Freshwater Biology*, *60*(3), 563–574. https://doi.org/10.1111/fwb.12498

Box, J. B., & Mossa, J. (1999). Sediment, Land Use, and Freshwater Mussels: Prospects and Problems. *Journal of the North American Benthological Society*, *18*(1), 99–117. https://doi.org/10.2307/1468011

Brönmark, C., & Malmqvist, B. (1982). Resource partitioning between unionid mussels in a Swedish lake outlet. *Ecography*, *5*(4), 389–395. https://doi.org/10.1111/j.1600-0587.1982.tb01053.x

Clarke, A.H. (1981). The freshwater mollusks of Canada. National Museum of Natural Sciences, National Museums of Canada, Ottawa, ON. 446 pp.

COSEWIC. (2004). COSEWIC assessment and status report on the Yellow Lampmussel *Lampsilis cariosa* in Canada. Committee on Status of Endangered Wildlife in Canada, Ottawa, ON.

COSEWIC. (2009). COSEWIC assessment and status report on the Brook Floater *Alasmidonta varicosa* in Canada. Committee on Status of Endangered Wildlife in Canada, Ottawa, ON.

Dascher, E. D., Burlakova, L. E., Karatayev, A. Y., Ford, D. F., & Schwalb, A. N. (2018). Distribution of unionid freshwater mussels and host fishes in Texas. A study of broad-scale spatial patterns across basins and a strong climate gradient. *Hydrobiologia*, *810*(1), 315–331. https://doi.org/10.1007/s10750-017-3168-5

Ethan, N., McCollough, M. A., & Swartz, B. I. (2000). The Freshwater Mussels of Maine. *Biodiversity*.

Ellis, M. D. (1936). Erosion Silt as a Factor in Aquatic Environments. *Ecology*, *17*(1), 29–42. https://doi.org/10.2307/1932951

Esrock, R. (2018). St. John River Valley. *National Geographic*. Retrieved July 13, 2023, from https://www.nationalgeographic.com/travel/national-parks/saint-john-river-valley/

Geoengineer. (n.d.). Step-by-Step Guide for Grain Size Analysis. Geoengineer. https://www.geoengineer.org/education/laboratory-testing/step-by-step-guide-for-grain-size-analysis

Gordon, N. D., McMahon, T. A., Finlayson, B. L., Gippel, C. J., & Nathan, R. J. (2004). *Stream hydrology: an introduction for ecologists*. John Wiley and Sons.

Hauer, C., Leitner, P., Unfer, G., Pulg, U., Habersack, H., & Graf, W. (2018). The Role of Sediment and Sediment Dynamics in the Aquatic Environment. *Springer eBooks*, 151–169. https://doi.org/10.1007/978-3-319-73250-3_8

Hopkins, R. (2009). Use of landscape pattern metrics and multiscale data in aquatic species distribution models: a case study of a freshwater mussel. *Landscape Ecology*, *24*(7), 943–955. https://doi.org/10.1007/s10980-009-9373-5

Leitner, P., Hauer, C., Ofenböck, T., Borgwardt, F., Schmidt-Kloiber, A., & Graf, W. (2015). Fine sediment deposition affects biodiversity and density of benthic macroinvertebrates: A case study in the freshwater pearl mussel river Waldaist (Upper Austria). *Limnologica*, *50*, 54–57. https://doi.org/10.1016/j.limno.2014.12.003

Leopold, L. B., Wolman, M. G., & Miller, J. W. (1964). Fluvial processes in geomorphology. *Journal of Hydrology*, *3*(3–4), 342. https://doi.org/10.1016/0022-1694(65)90101-0

Libois, R., & Hallet-Libois, C. (1987). The Unionid mussels (Mollusca, Bivalvia) of the Belgian upper river Meuse: an assessment of the impact of hydraulic works on the river water self-purification. *Biological Conservation, 42*, 115-132.

Martel, A.L., Mcalpine, D.F., Madill, J.B., Sabine, D.L., Paquet, A.P., Pulsifer, M.D., & Elderkin, M.F. (2010). Freshwater mussels (Bivalvia: Margaritiferidae, Unionidae) of the Atlantic Maritime Ecozone.

Martin, S. T. (1997). Freshwater Mussels (Bivalvia: Unionoida) of Maine. *Northeastern Naturalist*, *4*(1), 1. https://doi.org/10.2307/3858527

Neves, R. J., & Widlak, J. C. (1987). Habitat Ecology of Juvenile Fresh water Mussels (Bivalvia, Unionidae) in a Headwater Stream in Virginia. *American Malacological Bulletin*, *5*(1), 1–7. https://www.biodiversitylibrary.org/part/143191

Österling, M., & Högberg, J. (2014). The impact of land use on the mussel Margaritifera margaritifera and its host fish Salmo trutta. *Hydrobiologia*, *735*(1), 213–220. https://doi.org/10.1007/s10750-013-1501-1

Pandolfi, G. S. (2016). Effects of climate, land use and in-stream habitat on Appalachian elktoe (Alasmidonta raveneliana) in the Nolichucky River drainage, North Carolina. Appalachian State University.

Pandolfi, G. S., Mays, J., & Gangloff, M. M. (2022). Riparian land-use and in-stream habitat predict the distribution of a critically endangered freshwater mussel. *Hydrobiologia*, *849*(8), 1763–1776. https://doi.org/10.1007/s10750-022-04826-8

Poff, N. L., & Hart, D. D. (2002). How dams vary and why it matters for the emerging science of dam removal. *BioScience*, *52*(8), 659. https://doi.org/10.1641/0006-3568(2002)052

Sickel, J. B., Burnett, M. F., Chandler, C. J., Lewis, C. A., Blalock-Herod, H. N., & Herod, J. J. (2007). Changes in the Freshwater Mussel Community in the Kentucky Portion of Kentucky Lake, Tennessee River, since Impoundment by Kentucky Dam. *Journal of the Kentucky Academy of Science*, *68*(1), 68–80. https://doi.org/10.3101/1098-7096(2007)68

Strayer, D. L., & Ralley, J. (1993). Microhabitat Use by an Assemblage of Stream-Dwelling Unionaceans (Bivalvia), including Two Rare Species of Alasmidonta. *Journal of the North American Benthological Society*, *12*(3), 247–258. https://doi.org/10.2307/1467459

Van der Schalie, H. (1938). Contributing factors in the Depletion of Naiades in Eastern United States. *Basteria*, *3*(4), 51-57.

Vashisth, A. (2018). Determination of fineness modulus of coarse aggregates and fine aggregates. http://dx.doi.org/10.13140/RG.2.2.18131.68648

Whole Building Design Guide. (1980, March 1). CRD-C104-80 Method of Calculation of the Fineness Modulus of Aggregate. WBDG. https://www.wbdg.org/FFC/ARMYCOE/STANDARDS/crd_c104.pdf

Wegscheider, B., Maclean, H. C., Linnansaari, T., & Curry, R. A. (2019). Freshwater mussel abundance and species composition downstream of a large hydroelectric generating station. *Hydrobiologia*, *836*(1), 207–218. https://doi.org/10.1007/s10750-019-3954-3

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