Identifying Habitat of the Brook Floater (*Alasmidonta varicosa*) in the Shediac Bay Watershed

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1. Introduction

The Shediac Bay Watershed Association (SBWA) is a not-for-profit organization located in Shediac, New Brunswick. The SBWA was founded in 1999 as a result of growing concerns among residents from various local communities over the ecological health of the Shediac Bay. A Board of Directors, representing the various communities found within the 420 km^2 watershed boundaries of the Shediac Bay, oversees its activities. The Association deals with issues related to water quality and habitat integrity.

According to the Canadian Wildlife Federation (CWF), freshwater mussels are now among the most endangered freshwater invertebrates in the world. According to the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), 13 out of the 55 species of unionids in Canada are endangered, two are threatened and three are of Special Concern. The Brook Floater (*Alasmidonta varicosa*), is a medium-sized freshwater mussel that was found in scattered regions of New Brunswick, Nova Scotia and certain regions of the East Coast of USA. The population of this mussel was not abundant, representing only 1-5% of total freshwater mussel populations in the areas where it was found. In 2009, it was given the status of Special Concern by COSEWIC when the species disappeared from 2 historical sites and approximately half of its known locations in the USA, leaving the Canadian populations to represent the majority of the remaining global populations of the Brook Floater. In 2013, it was added to the Species At Risk Act, Schedule 1 (SARA).

The present report outlines the work on freshwater mussel conducted by the SBWA in the two main river systems in the Shediac Bay watershed; Shediac River, Scoudouc River and their tributaries. The Shediac River is divided in two major water arms; the northern water arm is created by the convergence of the McQuade, Weisner and Calhoun Brooks, and the southern water arm is the continuation of the Bateman Brook. The water velocity in both rivers is weak due to the gentle regional elevation (Henderson, G. 1999).

In 2005, the SBWA conducted a study on freshwater mussels within the boundaries of its watershed. The main objective was to establish the biodiversity and population status of freshwater mussels in the Shediac Bay watershed. During the surveys in 2005, a total of 122 Brook Floaters mussels were reported to have been found throughout the Shediac River, Scoudouc River and their tributaries.

Given the need for updated information on the Brook Floater population for the reassessment of its status with the Committee on the Endangered Wildlife in Canada (COSEWIC) and the Species At Risk Act (*Sara Schedule 1*) with the Department of Fisheries and Oceans Canada, the SBWA relaunched the project in 2014. The project contained the following objectives:

- Confirm the presence of the Brook Floater in the watershed;
- Assess the health of the surrounding habitat;
- Work with landowners to create a plan of protection for its habitat.

In addition to the primary focus to the species at risk, other project outcomes includes:

- Data collection on other freshwater mussel species found in the Shediac Bay watershed;
- Submission of survey data to the Atlantic Canada Conservation Data Centre (ACCDC) and other interested partners and agencies

Over the course of the program since 2014, many standardized surveys and habitat searches were conducted in high priority sites in the Shediac Bay watershed. The high priority sites include the areas that were said to contain Brook Floater mussels in the 2005 study, as cited in the report Caissie C., D. Audet, 2006 "Freshwater mussel inventories in the Shediac and Scoudouc Rivers, NB", and the areas in the lower reaches of freshwater habitats of our rivers. In addition to those historical sites, the team expanded their search by visually scanning the riverbed in all suitable habitat for the Brook Floater that was accessible.

In 2017, a partnership with the Department of Fisheries and Oceans Canada was formed to conduct Environmental DNA (e-DNA) sampling for the first time, in an attempt to detect Brook Floater mussels in select sites in the watershed. The first year was a pilot project, because e-DNA had never been attempted for freshwater mussels before. The protocol was modified after the first year and the sampling continued in 2018 and 2019.

According to the Management Plan for the Brook Floater prepared by Species at Risk Act (SARA) in 2016, the timeline for presence/absence surveys should be conducted on a 2-5 year period. The SBWA has now done extensive work to attempt to locate the species of special concern over the last 6 years, but they have unfortunately been unsuccessful.

However, the Association has done habitat assessment and restoration work for Brook Floater habitat over the course of this program. The habitat assessments looked at fish passage issues around culverts near potential Brook Floater habitat. The restoration activities focused on riparian zone reforestation and the mitigation of ATV use in river habitat. All these actions not only benefit freshwater mussel species but a wide variety of other sensitive species, such as Atlantic salmon.

The present report describes the work accomplished to find this rare Brook Floater mussel in the Shediac Bay watershed in the 2019 field season.

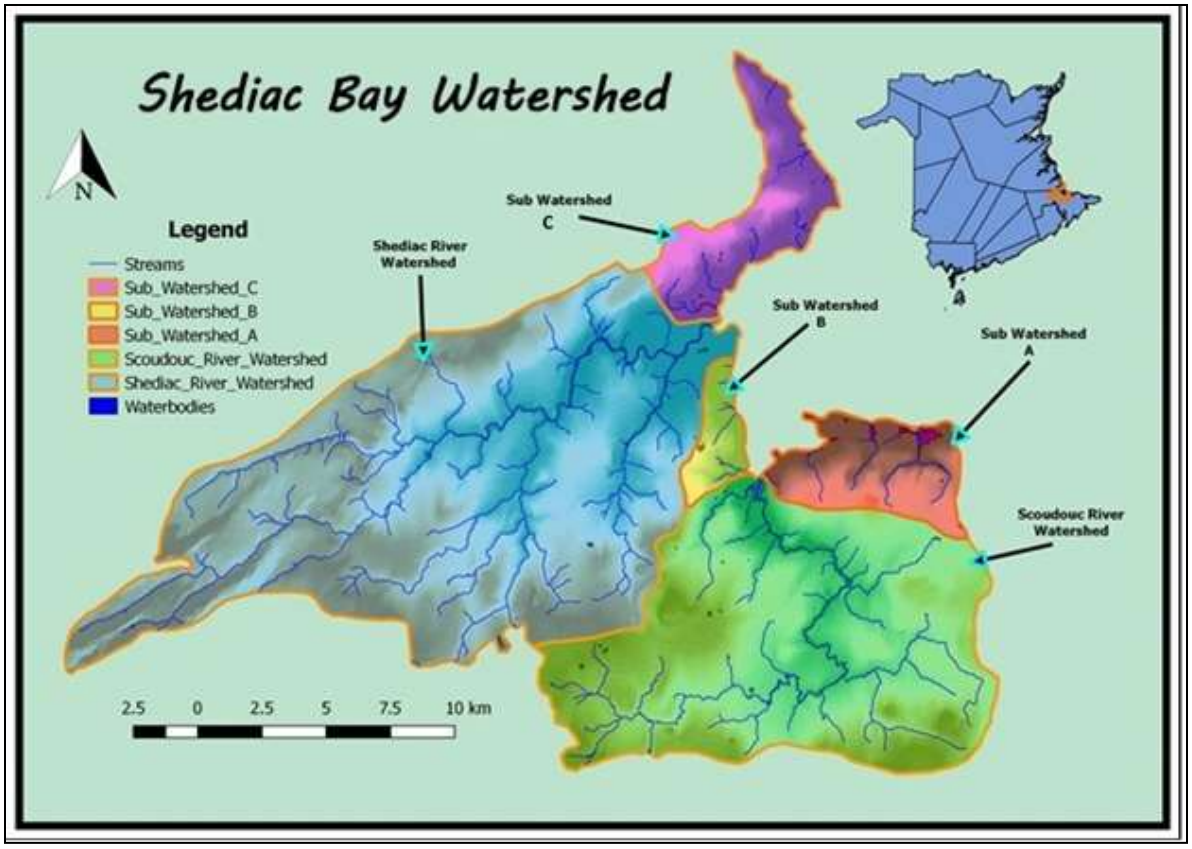

Figure 1: Map of the Shediac Bay Watershed boundaries

2. Material and Method

2.1 Sampling Protocol

The method used for the freshwater mussel inventory is the 4 hour-person Time-Search protocol, as described by Metcalfe-Smith et al. (2000) and Beaudet et al. (2002). This method consists of visually locating and counting all freshwater mussels found in a fixed and standardized amount of time. This time-based count of freshwater mussel is done on the first visit of a site only, in order to collect data that can be used for population assessment studies by other interested organizations, government, and academics.

Because the goal of this project was to locate and confirm presence/absence of the Brook Floater, all subsequent visits to the same sites are search-based only. The time-search method was not used after 2016, after having collected sufficient information on other freshwater mussel species. The priority was changed to habitat search-based surveys to cover more ground and increase the chances of finding the species at risk.

During the search-based surveys, the field team walked the rivers in search of appropriate Brook Floater habitat. All habitats deemed suitable were thoroughly searched for the presence of the rare mussel, either by seeing the mussel on the surface of the substrate or by the detection of valves indicating a burrowed mussel.

All areas searched have been recorded using GPS coordinates at the beginning and end of each sweep. For the survey data of the 2019 project, refer to Appendix A. For the collection of site photos collected over the course of the program, refer to Appendix B. The protocol and materials list for the e-DNA sampling can be found in Annex 1.

2.2 Material

The equipment used to conduct the freshwater mussel inventories are listed below:

- Underwater viewers
- Chest Waders
- Polarized sunglasses and hat
- Field data sheets, maps and clipboards
- GPS
- Digital Camera
- Stopwatch
- Water resistant notepads + pencils
- Water-condition instrument (YSI)
- Survey measuring tape (50m) and metre stick (1m)
- Reference documents (identification key)

2.3 Site Identification Codes

The site ID codes for freshwater mussel surveying in the report Caissie C., D. Audet, 2006[1] "Freshwater mussel inventories in the Shediac and Scoudouc Rivers, NB" were largely based on historical water quality monitoring sites in the Shediac Bay watershed.

When the project was relaunched in 2014, the site codes were expanded to better identify the segments of river habitat that was searched for the Brook Floater mussel. In addition to the basic station code, a numerical or alphabetical value was added depending on the direction of the survey (upstream and downstream) from the access point.

For example, the site *Shd G* in the Weisner Brook is accessed from the bridge on St-Philippe road. If a survey or simple habitat search is conducted *upstream* of the bridge, the site will be identified by an *alphabetical* value; *Shd G-A*. If the survey takes place *downstream* of that same access point, the code is given a *numerical* value; *Shd G-1*. Subsequent surveys or searches will be identified with the next numerical or alphabetical values: *Shd G-A, B, C*, etc.

3. Results and Observations 2019

During the summer of 2019, 7.3 km of habitat was searched for the presence of the Brook Floater mussel the Shediac Bay watershed. In partnership with the *Department of Fisheries and Oceans Canada*, 20 environmental DNA (e-DNA) samples were collected in the highest priority locations, in an attempt to detect traces of genetic material of the Brook Floater mussel that has not been visually located. The results of the e-DNA sampling can be found in Section 4, Table 4.

3.1 Freshwater Mussel Surveys and e-DNA sampling in the Shediac River 2019 - Site Description

In the Shediac River, a total of 13 sites were searched for the Brook Floater in 2019. A total of approximately 7.3 km of the river and its tributaries were scanned for habitats containing freshwater mussels, and these habitats were thoroughly searched for Brook Floater mussels. Site descriptions and information are described below.

Given that the Shediac River is the most important river where Brook Floaters were reported in the 2006 report[1], the majority of the work was done within the main branch and the tributaries of the Shediac River. All areas where the Brook Floater was reported to have been found, at six sites in the Shediac River, are considered as the high priority sites.

3.1.1 Shd I

Located in the lowest freshwater reach in the main branch of the Shediac River. It begins a short distance away from the highest tidal zone, where the common Eastern Pearlshell mussels begin to appear. This site used to be accessed by walking down an ATV trail connecting to the Shediac River Rd, but this property has been purchased and a new home was built. Attempts were made to contact the new landowners but they were unsuccessful. The next property was approached and the landowners gave the SBWA permission to park in their driveway and access the river.

This section of the Shediac River has excellent habitat for freshwater mussels: medium to low water levels, and the substrate consists of rocks, rubble, gravel and sand. The site Shd I was reported to have 4 Brook Floaters mussels in the 2005 surveys[1]. This section of the river has been searched multiple times each year since the beginning of the project in 2014.

In 2019, the entire section of the site Shd I (A, B and C) was swept again. A total of 830 m was searched and one e-DNA sample was collected (see Section 4.2, Table 3). The total surface area searched for Shd I; 830 m with an average stream width of 15 metres, is 12,450 m^2. For more detailed information on the freshwater mussel surveys, refer to Appendix A. For the site photos, see Appendix B.

Figure 2: Map of site of the total search area for Shd I, 2019

3.1.1 Shd E

This site is located in the main branch of the Shediac River, accessed at the covered bridge on Shediac River Rd. This area is a priority because of the substrate/habitat type, and that it is located in the lower reaches of the freshwater habitat of the Shediac River, even though no Brook Floaters were reported here in the 2006 report[1]. The habitat at the covered bridge going upstream (Shd E-A, B, C, etc) is excellent for freshwater mussels; rubble, gravel and sandy substrate; clear water of an average depth of 35 cm; low water velocity and very wide (average width of 12 m). The riverbanks were also searched for Brook Floater shells during the return trips back to the covered bridge.

The substrate downstream of the covered bridge towards the Shediac River rd. bridge is mainly bedrock and boulders. Therefore, starting in 2016, the site Shd E-1 begins downstream of the Shediac River Road's bridge.

In 2019, the total surface area searched for Shd E-A; 300 m with an average stream width of 12 metres is 3,600 m^2. One e-DNA sample was collected at the covered bridge (see Section 4.2, Table 3). For more detailed information on the freshwater mussel surveys, refer to Appendix A. For the site photos, see Appendix B.

Figure 3: Figure 4: Map of site of the search area and e-DNA sampling for Shd E-A, 2019

3.1.2 Shd E-2

Along the section of Shd E-2 downstream of the covered bridge, there is the convergence of the Weisner brook to the Shediac River. This section at the lowest point of the Weisner Brook was identified as Shd E-2A, because the site Shd E-2 is used as an access point. A second access point to this section of the lower Weisner Brook is by walking down the ATV trails below the power lines, accessed off the Shediac River Road. That access is the beginning of the site Shd E-2B, which is the section that was searched in 2019.

This area is densely forested and has great canopy coverage. The water of this brook is significantly cooler, even in the heights of the summer months. This area is an excellent source of cold water, creating an important habitat for fish looking to migrate to avoid thermal stress. The riparian zones have healthy buffer zone but have some areas with high bank erosion. The substrate along ShdE-2A & B consists of rock, rubble, gravel and sand, which are suitable characteristics for freshwater mussels.

In 2019, the total surface area searched for Shd E-2B; 565 m with an average stream width of 8 metres is 4,520 m^2. For more detailed information on the freshwater mussel surveys, refer to Appendix A. For the site photos, see Appendix B.

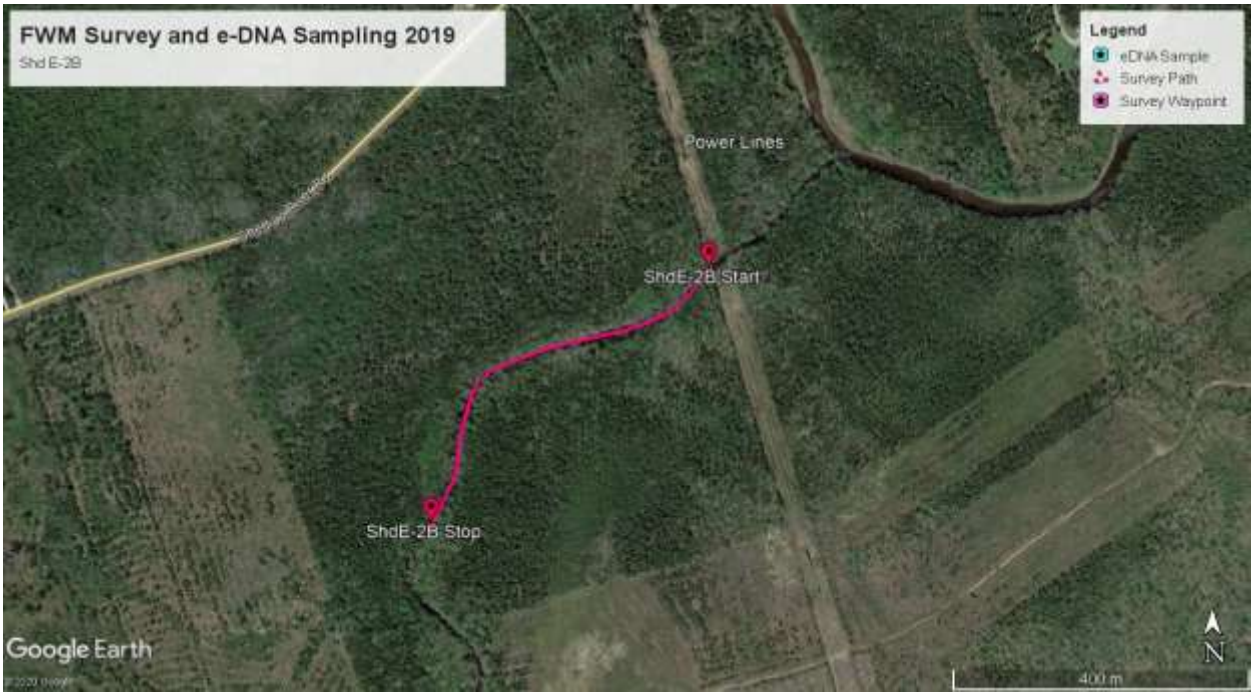

Figure 5: Map of site of the search area for Shd E-2B, 2019

3.1.3 Shd D

This site was accessed by walking down a trail located behind a private property (986 Shediac River Road), for which we received land owner permission to use. This area can also be accessed via an ATV trail connected to the Shediac River Rd. (N46°13'49.12" W64°41'48.19"), further upstream. ShdD is located upstream from site ShdE; the area searched here connects down to the end point of ShdE-C.

The habitat upstream from the access of the private property (Shd D-A), is a beautiful site for freshwater mussels; a mix of mostly rubble, gravel and sand. Water levels ranged from 25-50 cm in depth, and is perfectly clear giving great visibility of the riverbed. There are multiple sand bars along Shd D-A, which provides habitat for the Brook Floater. This area was of high priority; 30 brook floater mussels were reported to have been found in this area in the 2006 report[1]. After searching this site repeatedly since the beginning of the project in 2014 and multiple e-DNA samplings in 2017 and 2018, only one additional e-DNA sample was collected in 2019 (see Section 4.2, Table 3).

Figure 6: Map of site of the e-DNA sample for Shd D, 2019

3.1.4 Shd N

This site is further upstream of site Shd D, and it is a more remote and difficult place to access due to the lack of roads nearby. There are two access points that were widely used over the course of this project; a public ATV trail accessed off Shediac River Road (N46°13'49.21" W64°41'48.17"), and a private path (N46°13'57.24" W64°42'19.58"), behind the house at 846 Shediac River rd. depicted on the site map. The landowners were very nice to let us park in their driveway and use the path to the river in 2016. This year, 2 more landowners were approached for permission to access the river from their property and it was granted. This contact with the landowners was a great way to perform some direct outreach on the project and discuss the potential issues in land use that can harm the freshwater habitat.

The site ShdN-A has good freshwater mussel habitat; the substrate is composed of rocks, rubble, gravel and sand. Even though there is good habitat for freshwater mussels, the population of common Eastern Pearlshell mussels is less dense than in the lower reaches of the Shediac River. There are some areas were natural erosion occurs and one area impacted by beaver activity.

In 2018, an e-DNA sample collected in this area showed a result of "inconclusive". This does not mean a positive or a negative, therefore, this area was the larger focus for the e-DNA sampling this year. In 2019, the total surface area searched for Shd N; 3,495 m with an average stream width of 8 metres is 27,960 m^2. Nine e-DNA samples were collected along the site (see Section 4.2, Table 3). For more detailed information on the freshwater mussel surveys, refer to Appendix A. For the site photos, see Appendix B.

Figure 7: Map of site of the total search area for Shd N, 2019

3.1.5 Shd C

This site is located in the main branch of the Shediac River, at the crossing of the MacLean Crossroad Rd and Shediac River Road.

This site does not contain any freshwater mussel habitat upstream or downstream; the substrate is comprised primarily of bare bedrock. Surveys have been conducted in both directions in the past (2014 and 2016) to assess the habitat, presence of freshwater mussels, and to collect data of the conditions of the river's habitat. There were no reported Brook Floater mussels in this area in the 2006[1] report.

However, this access point was used in 2019 to collect an e-DNA sample at the bridge crossing. The results were negative (see Section 4.2, Table 3).

Figure 8: Map of site of the e-DNA sample for Shd C, 2019

3.1.6 Shd G

This site is located at the bridge on St-Philippe Road, in the Weisner Brook. An extensive search was conducted upstream and downstream over the course of this project since 2014. The habitat upstream (Shd G-A) has been modified over time by the presence of beaver dams. It has higher water levels and the substrate contains a greater percentage of fine sediments over the substrate. The population of freshwater mussel is not as dense as the downstream habitat.

In 2019, the habitat downstream of the bridge (Shd G-1) was prioritized for another search. One e-DNA sample was collected at the bridge (see Section 4.2, Table 3). The total surface area searched for Shd G; 375 m with an average stream width of 4.5 metres, is 1,688 m^2. For more detailed information on the freshwater mussel surveys, refer to Appendix A. For the site photos, see Appendix B.

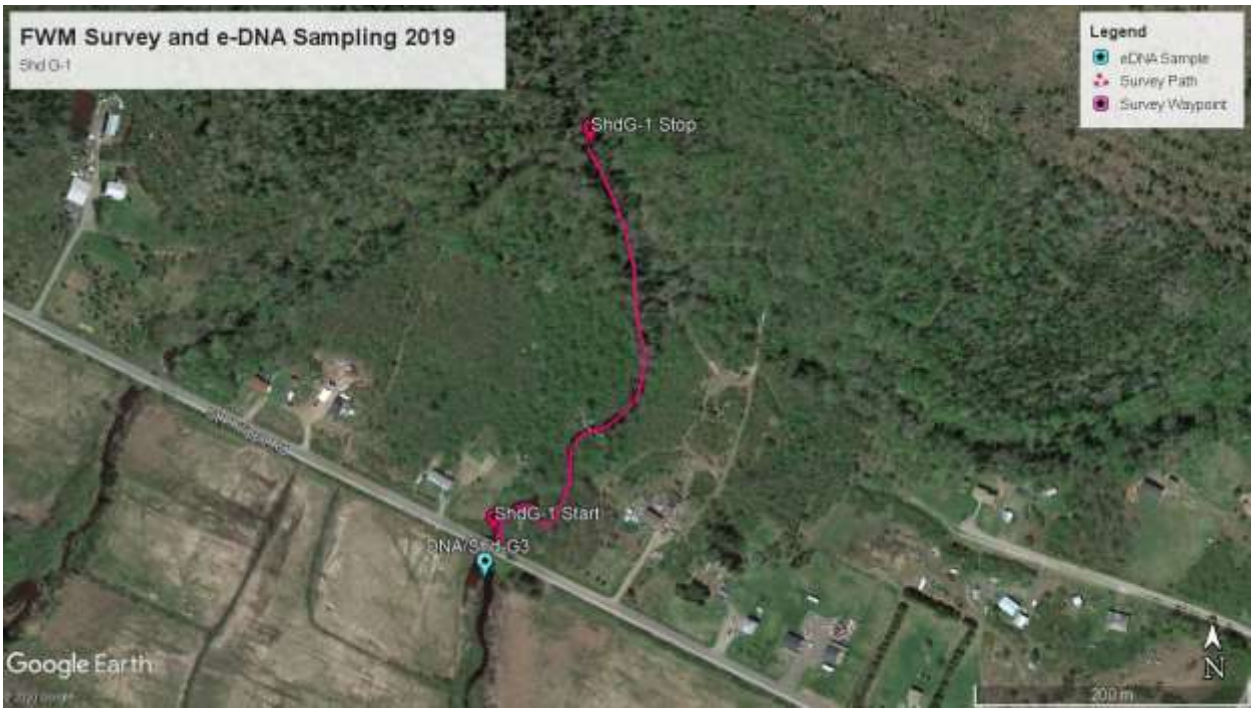

Figure 9: Map of site of the search area and e-DNA sampling for Shd G, 2019

3.1.7 Shd M

Located on Bateman Mill Rd., at the bridge where the Weisner Brook crosses the road. This area upstream of the bridge (Shd M-A), was high priority as there was 27 Brook Floaters reported in the 2006 report[1] (Shd M-A). The site was repeatedly searched during each of the 6 years of this project.

The habitat upstream (Shd M-A) contains good habitat conditions for freshwater mussels; the substrate composed of mostly rocks and sand with good vegetation cover along the banks. However, there is some erosion nearby on a riverfront property owner's land, due to a lack of vegetation. There are a lot of common eastern pearlshell mussels here.

In 2019, the habitat upstream of the culvert was prioritized for another search. One e-DNA sample was collected upstream of the culvert (see Section 4.2, Table 3). The total surface area searched for Shd M; 495 m with an average stream width of 7 metres, is 3,465 m^2. For more detailed information on the freshwater mussel surveys, refer to Appendix A. For the site photos, see Appendix B.

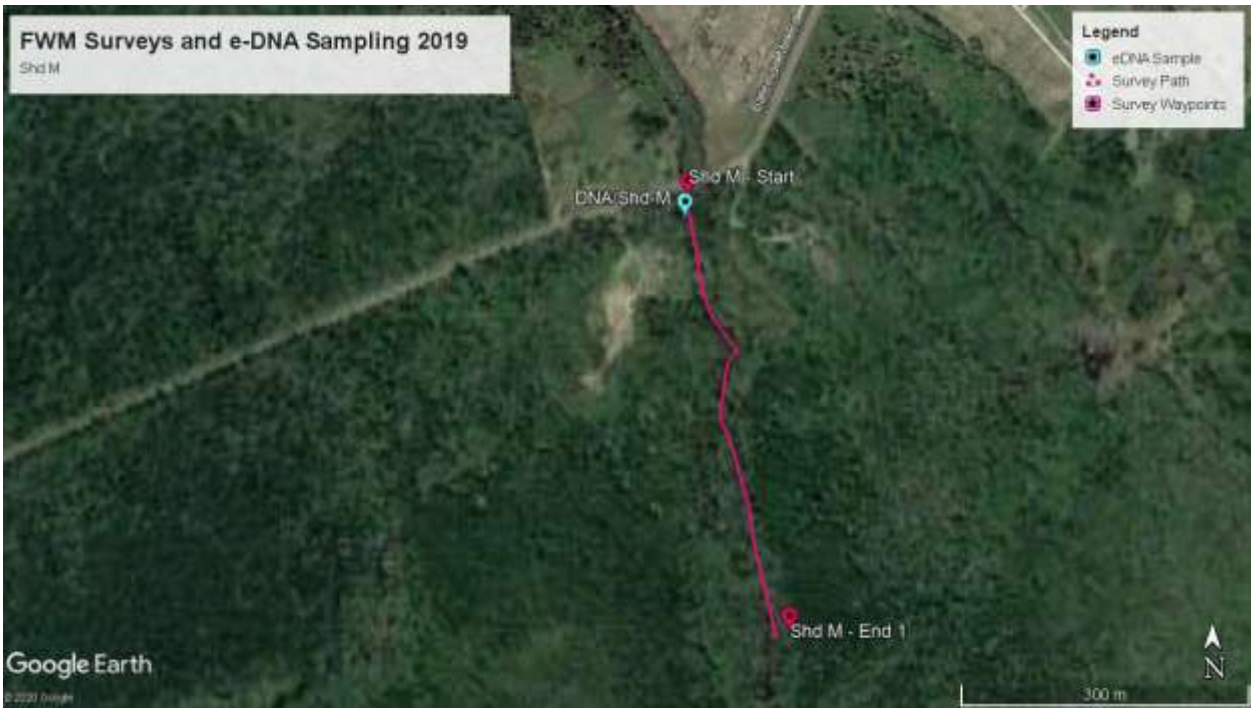

Figure 10: Map of site of the total search area and e-DNA sampling for Shd M, 2019

3.1.1 Shd L

This site is located in the Calhoun Brook, accessed on Weisner Rd., at the bridge that is nearest to St-Philippe Rd. This site was of low priority because no Brook Floaters was found here, but the stream was searched upstream due to the habitat characteristics.

Upstream of the bridge (Shd L-A), the beginning of the habitat is in a residential area with very little vegetation in the buffer zone due to lawn mowing close to the stream. There is fine sediment covering the substrate in this area, and sediment loads impacting the area immediately downstream of the bridge. Once past the residential area upstream, the habitat becomes healthier and more favourable to freshwater mussels; a substrate containing rocks, rubble, gravel, and sand, with some sections of bare bedrock. The average width of the stream was approximately 5 metres, with water depths ranging from 20-50 cm. There were some areas where erosion was noted, and also some areas that had significant green algae growth.

In 2019, the habitat upstream of the culvert was prioritized for another search. One e-DNA sample was collected upstream of the culvert (see Section 4.2, Table 3). The total surface area searched for Shd L; 990 m with an average stream width of 5 metres, is 4,950 m^2. For more detailed information on the freshwater mussel surveys, refer to Appendix A. For the site photos, see Appendix B.

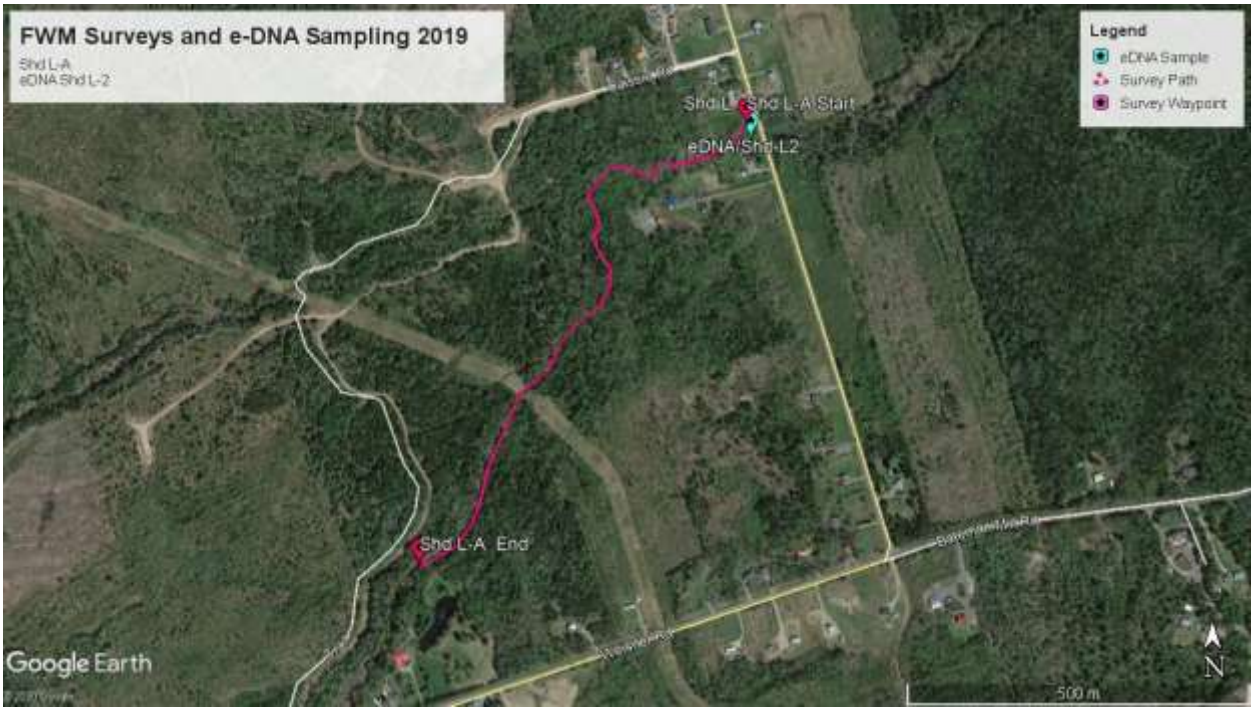

Figure 11: Map of site of the total search area and e-DNA sampling for Shd L, 2019

3.2 Freshwater Mussel Inventory in Scoudouc River 2019 – Site Description

In the Scoudouc River, only one area was searched for the Brook Floater mussel in 2019. Environmental DNA samples were collected at 4 accessible locations.

The Scoudouc River is of lower priority for the Brook Floater mussel; only 19 Brook Floaters were reported to have been found in 2005, in a higher section of the river (Scd I=1, and Scd A=18). Those sites are difficult to access, requiring an ATV. In 2014 and 2015, the site Scd I was surveyed twice. The site Scd A, a small tributary adjacent to Scd I, was attempted to be surveyed in 2015, but it was discovered to be difficult to survey. The site has been modified by beaver activity over the last 10 years. Beaver dams were observed and the site photos comparing the habitat to the one described and shown in the 2006 report[1] were much different. The conditions for conducting freshwater mussel surveys in this tributary are no longer favourable; low riverbed visibility due to concentration of tannins in the water; fine sediments covering the substrate, creating clouds of suspended particles; an abundance of large aquatic plants that effectively hides freshwater mussels. It is possible that in 2005, the brook was in a better condition for freshwater mussel habitat and for surveying, and the team was able to discover 18 Brook Floaters. No additional surveys were performed in this unnamed tributary, but an e-DNA sample was collected in 2019 and came back negative.

Another problem with the Scoudouc River is a lack of access points to suitable freshwater mussel habitat. Therefore, the team focused their efforts in the lower reaches of the river, where there are some access points to freshwater mussel habitat.

Figure 12: Photos of Eastern Elliptio (*Elliptio complanata*) mussels found in the Scoudouc River

3.2.1 Scd D

This site is located where freshwater habitat begins in the Scoudouc River. It was accessed by Red Bridge Road off Route NB-132 (Scoudouc Road). Once at the end of the paved road, it transitions to a dirt road that follows the power lines down to the river.

The habitat suitable for freshwater mussels begins a short distance away from the access point. The substrate is comprised of a mix of rocks, rubble, and sand, with some sections of bedrock. The riparian zone is healthy without any sign of severe erosion (mild natural erosion), and has good mixed forest vegetation on both sides. In addition to the common Eastern Pearlshell mussel (*Maragaritifera margaritifera*), there is a second species commonly found in the Scoudouc River; the Eastern Elliptio mussel (*Elliptio complanata*).

The river is very wide here, and is very rich with 2 species of freshwater mussels. Therefore, a lot of time is needed to conduct a survey when a species count is needed, as each mussel needs to be examined so that they were properly identified. The two species looks very similar and require training to note the differences the characteristics of the shells.

In 2019, the total surface area searched for Scd D; 265 m with an average stream width of 16 metres, is 4,240m^2. One e-DNA sample was collected (see Section 4.2, Table 3). For more detailed information on the freshwater mussel surveys, refer to Appendix A. For the site photos, see Appendix B.

Figure 13: Map of site of the total search area and e-DNA sampling for Scd D, 2019

3.2.2 Scd F

This site is located upstream of Scd D in the main branch of the Scoudouc River. It was accessed by taking Pellerin Rd., a dirt road off Lino rd. in Shediac. On Google maps, this road is labelled as Sackville rd. Approximately 15 min down this road, there is an ATV trail (N46°11'5.02" W64°30'27.83") that goes down to the river, which is about a 5 min walk.

The habitat here, going downstream (Scd F-1) was surveyed in 2016 and was found not to be suitable for freshwater mussels; the substrate is comprised mostly of large flat rocks and boulders. There were no mussels seen during that survey, and at the time, the water was too deep for wading upstream (Scd F-A). In September of 2017, water levels were low enough to allow the team to walk upstream and observed freshwater mussels with underwater viewers. The water was still too deep to be able to pick up and identify the freshwater mussels.

In 2019, an e-DNA samples were collected at this location in the hopes that it would detect a positive signal from Brook Floater mussels upstream. No additional searches were done considering the difficulties and safety conditions from previous years.

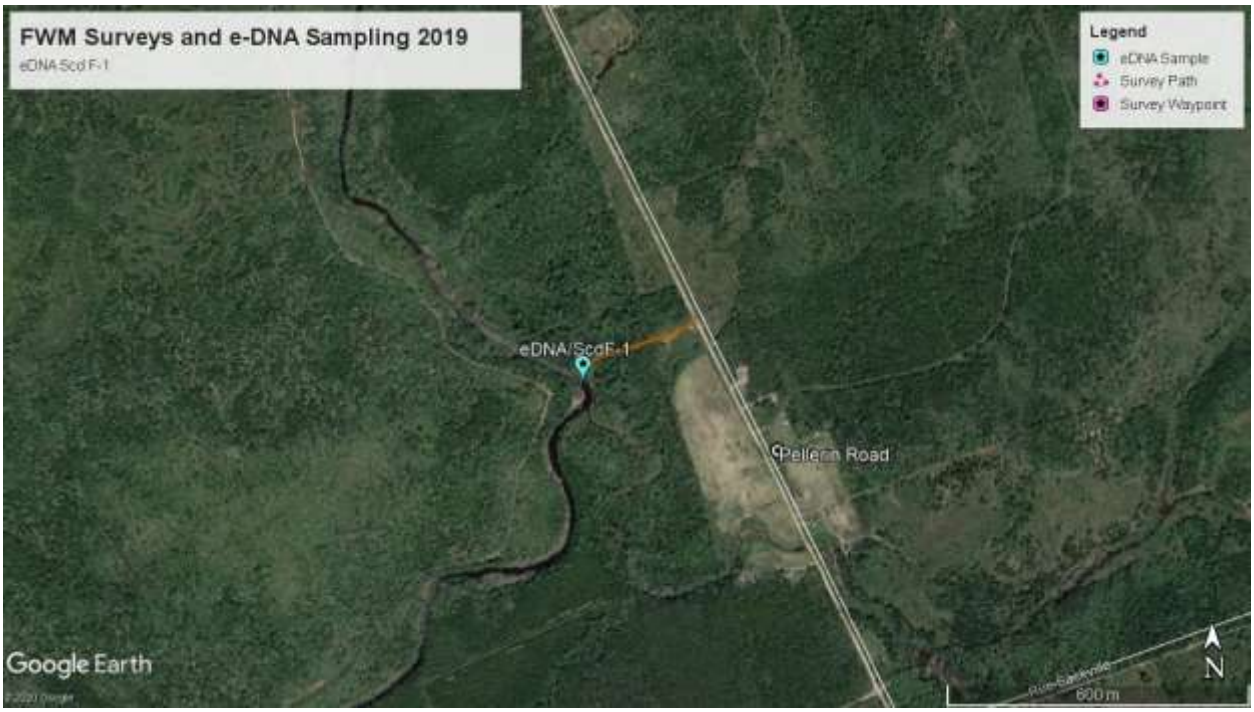

Figure 14: Map of site of the e-DNA sampling for Scd F, 2019

3.2.3 Scd I

This site is located in the mid to higher reaches of the Scoudouc River. It is a site that is more difficult to access, as the dirt road requires an ATV or 4x4 vehicle to get there. The dirt road name is Hanikoff Rd, at the end of Marcelin Rd, off Malakoff Rd in Scoudouc.

The habitat at this site is in good condition with forested riparian zones. The substrate is a mix of rock, rubble and sand, with some extended sections of bedrock. The site contains freshwater mussel habitat with the presence of Eastern Pearlshell mussels. Surveys have been performed here in 2014 and 2015. In 2019, only an eDNA sample was collected (see Section 4.2, Table 3).

Figure 15: Map of site of the e-DNA sampling for Scd I, 2019

3.2.4 Scd A

This site is located in an unnamed tributary of the Scoudouc River. This brook merges with the main branch where the site Scd I is located. As described in section 3.2, this site was said to have 18 Brook Floaters found in 2005.

This site was attempted to be surveyed in 2015, but it was discovered to be difficult to search for freshwater mussels. The site has been modified by beaver activity over the last 10 years. Beaver dams were observed and the site photos comparing the habitat to the one described and shown in the 2006 report1 were much different. The conditions for conducting freshwater mussel surveys in this tributary are no longer favourable; low riverbed visibility due to concentration of tannins in the water; fine sediments covering the substrate, creating clouds of suspended particles; an abundance of large aquatic plants that effectively hides freshwater mussels. No Brook Floater mussels were found, but this doesn't mean they are actually absent.

Therefore, an e-DNA sample was collected here in 2019 in an attempt to detect the species. The results were negative (see Section 4.2, Table 3).

Figure 16: Map of site of the e-DNA sampling for Scd A, 2019

4. Project Summary 2019

4.1 Survey Results

The following tables summarizes the total distances searched for the Brook Floater mussel and its habitat over the 2019 field sessions, adding up to approximately 7.3 km. Table and the e-DNA sampling results. All of those locations have been visited several times over the past 6 years, since the beginning of this project in 2014.

Table 1: Results of Habitat-Search freshwater mussels' surveys in the Shediac and Scoudouc Rivers, 2019 with GPS coordinates

Site ID	Distance (m)	Start		Stop	
		Latitude	Longitude	Latitude	Longitude
Shd M-A	495	N46°12'27.22"	W64°40'20.96"	N46°12'12.24"	W64°40'15.95"
Shd E-A	300	N 46º 14' 42.72"	W 64º39'53.22"	N 46º 14' 41.7"	W 64º 40' 05.7"
Shd E-2B	565	N 46°14'24.84"	W 64°39'45.57"	N 46°14'13.39"	W 64°40'3.28"
Shd G-1	375	N 46°12'53.99"	W 64°40'29.39"	N 46°13'3.26"	W 64°40'26.03"
Shd L-A	990	N 46°12'32.96"	W 64°41'38.27"	N 46°12'12.30"	W 64°42'0.40"
Shd I-A	275	N 46º 14' 42.89"	W 64º 39' 52.36"	N 46°14'34.21"	W 64°39'16.89"
Shd I-B	255	N 46°14'34.21"	W 64°39'16.89"	N 46°14'27.59"	W 64°39'22.95"
Shd I-C	300	N 46°14'27.59"	W 64°39'22.95"	N 46°14'30.29"	W 64°39'36.30"
Shd N-A	225	N 46°14'16.90"	W 64°42'28.80"	N 46°14'16.60"	W 46°14'16.60"
Shd N-B	135	N 46°14'16.70"	W 64°42'38.20"	N 46°14'13.60"	W 46°14'13.60"
Shd N-C	625	N 46°14'13.60"	W 64°42'42.30"	N 46°14'0.71"	W 64°43'3.10"
Shd N-D	1,330	N 46°14'0.71"	W 64°43'3.10"	N 46°13'40.23"	W 64°43'22.71"
Shd N-E	1,180	N 46°13'40.23"	W 64°43'22.71"	N 46°13'20.60"	W 64°43'50.61"
Total Shediac River	**7,050 m**				
Scd D-A	265	N 46º 11' 39.12"	W 64º 31' 25.68"	N 46º 11'38.41"	W 64º 31'13.44"
Total Scoudouc River	**265 m**				
Total distance surveyed	**7, 315 m**				

Table 2: Total surface area (m^2) searched for the Brook Floater in the Shediac and Scoudouc rivers and their tributaries in 2019

Site ID	Distances Searched (m)	Average Width (m)	Total Surface Area (m^2)
ShdM-A	495	7	3,465
ShdE-A	300	12	3,600
ShdE-2B	565	8	4,520
ShdG-1	375	4.5	1,688
ShdL-A	990	5	4,950
ShdI-A	275	15	4,125
ShdI-B	255	15	3,825
ShdI-C	300	15	4,500
ShdN-A	225	8	1,800
ShdN-B	135	8	1,080
ShdN-C	625	8	5,000
ShdN-D	1,330	8	10,640
ShdN-E	1,180	8	9,440
ScdD-A	265m	16	4,240
Total	**7, 315 m**		**62, 873 m^2**

Figure 17: Map of the total search area in the Shediac River, 2019

Figure 18: Map of the total search area in the Scoudouc River, 2019

4.2 e-DNA Results

In partnership with the *Department of Fisheries and Oceans Canada*, the sampling to detect Brook Floater with *Environmental DNA* sampling was continued in 2019. The purpose of this sampling is to attempt to detect the species at risk by traces of its DNA in the river systems, coming from its reproductive materials (sperm, glochidia), excrements, etc.

A total of 20 e-DNA samples were collected in 2019. Two rounds of sampling were done earlier in 2019, in order to receive results while the field season is ongoing. If a positive signal would have been detected, intensive surveys would have been carried out immediately. The first 10 samples were collected and analysed early in July, and the same process was repeated with the other 10 samples at the end of the month of July. All samples came back negative, with no detection of the species at risk. The protocol can be found in Annex 1. The following tables provides the sample location coordinates and results for the e-DNA sampling of 2019.

In 2018, an e-DNA sample along the site Shd N came back as "Inconclusive", indicating a possible presence. Therefore, in 2019, this site was the focus of the e-DNA sampling. Unfortunately, there were no positive signals indicating the presence of Brook Floater mussels.

Table 3: e-DNA site information and sampling results 2019, *Department of Fisheries and Oceans Canada*

Collection Date	Tube ID	Site ID	Location	Latitude	Longitude	qPCR Brook Floater (A. varicosa)
2019-06-11	SAR 19-01	Weisner Brook	Shd M-1	N 46°12'27.22"	W 64°40'21.11"	Not detected
2019-07-03	SAR 19-02	Shediac River	Shd E-1	N 46°14'42.22"	W 64°39'53.23"	Not detected
2019-07-03	SAR 19-03	Shediac River	Shd N1	N 46°14'11.74"	W 64°42'13.41"	Not detected
2019-07-03	SAR 19-04	Shediac River	Shd D-1	N 46°14'15.85"	W 64°41'22.41"	Not detected
2019-07-03	SAR 19-05	Shediac River	Shd I-2	N 46°14'33.79"	W 64°39'17.53"	Not detected
2019-07-04	SAR 19-06	Calhoun Brook	Shd L-2	N 46°12'32.30"	W 64°41'37.95"	Not detected
2019-07-04	SAR 19-07	Scoudouc River tributary	Scd F	N 46°11'1.38"	W 64°30'37.83"	Not detected
2019-07-04	SAR 19-08	Shediac River	Shd C	N 46°12'32.90"	W 64°44'33.27"	Not detected
2019-07-04	SAR 19-09	Scoudouc River	Scd D-1	N 46°11'38.78"	W 64°31'24.19"	Not detected
2019-07-04	SAR 19-10	Weisner Brook	Shd G-3	N 46°12'52.93"	W 64°40'29.71"	Not detected
2019-07-17	SAR 19-11	Scoudouc River tributary	Scd A	N 46° 8'58.47"	W 64°30'48.14"	Not detected
2019-07-17	SAR 19-12	Scoudouc River	Scd I	N 46° 8'59.26"	W 64°30'49.89"	Not detected
2019-07-26	SAR 19-13	Shediac River	Shd N2	N 46°14'0.31"	W 64°43'3.22"	Not detected
2019-07-26	SAR 19-14	Shediac River	Shd N3	N 46°13'50.30"	W 64°43'10.37"	Not detected
2019-07-26	SAR 19-15	Shediac River	Shd N4	N 46°13'46.51"	W 64°43'16.70"	Not detected
2019-07-26	SAR 19-16	Shediac River	Shd N5	N 46°13'43.34"	W 64°43'13.51"	Not detected
2019-07-26	SAR 19-17	Shediac River	Shd N6	N 46°13'39.25"	W 64°43'24.09"	Not detected
2019-07-26	SAR 19-18	Shediac River	Shd N7	N 46°13'33.71"	W 64°43'32.45"	Not detected
2019-07-26	SAR 19-19	Shediac River	Shd N8	N 46°13'26.33"	W 64°43'37.94"	Not detected
2019-07-26	SAR 19-20	Shediac River	Shd N9	N 46°13'23.03"	W 64°43'49.66"	Not detected

Figure 19: Map of the e-DNA samples collected in the Shediac River, 2019

Figure 20: Map of the e-DNA samples collected in the Scoudouc River, 2019

5. Habitat and Water Quality Enhancement

Habitat restoration and water quality enhancement is a major initiative of the SBWA. Areas where bank erosion occurs causes an excess of sediment in the watercourse. Sedimentation can cause various issues for aquatic ecosystems; it can suffocate fish and fish eggs, bury aquatic insects, destroy freshwater mussel habitat, can carry harmful pollutants such as heavy metals and excessive nutrients that can further worsen conditions of the ecosystem, etc.

Blockage to fish migration are both naturally occurring and man-made, like debris jams, hanging culverts, and man-made dams. When these barriers occur in lower areas of a watershed, it can close off a large amount of suitable spawning grounds for important migratory fish species like the Atlantic salmon. Because freshwater mussels rely on host fish in their reproductive cycle, fish migration is an important element in assessing the watershed for threats to rare species like the Brook Floater.

Two sites were selected for habitat enhancement; the continuation of the efforts at "Edna's pond" along the Scoudouc River, and the restoration of a culvert crossing in the Scotch Settlement area.

5.1 Edna's pond

In 2017, an area in the Scoudouc River surrounding a precious salmon habitat, was selected for major restoration efforts intended to reduce sediment runoff from a sloped ATV trail and to halt the river bank erosion. Ongoing efforts are being carried out every year to maintain the previous restoration work of the area.

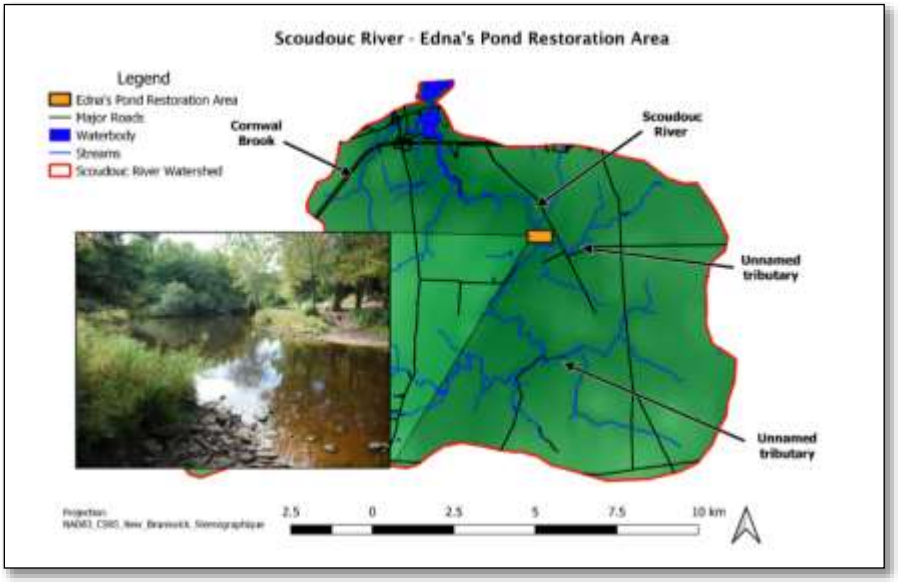

Figure 21: Map of E-DNA's pond restoration area

The five sediment deflectors in the area require constant maintenance every year. They capture sediments from runoff which causes a sediment buildup on the logs. In the spring of 2019, small trenches were dug by shovel to eliminate the sediment build up on the stabilizers and also to help direct the runoff towards the forested areas.

Actions have been taken this year to block two illegal river crossing access trails. Three large posts made out of a fallen birch tree was set deep in the middle of the two river access trails and cement was poured in the holes to help secure them. Along with the posts, straw bales were set in the trails. This will hopefully discourage ATV from trying to use the river access trails and also will help capture sediment from runoff. These were installed with student volunteers from Shediac Cape School.

In addition, 7 native trees and grass seeds have been planted on the slope and around the river bank to further stabilize the soil of the area. The trees used were provided by the SBWA tree nurseries.

A sign was installed at the southernmost river access indicating the trail blockages and the negative implications of crossing the river with motor vehicles to this sensitive habitat.

Figure 22: Before and after photos of ATV access blockage and sediment control (hay-bale check dam)

Figure 23: ATV access blockage and sediment control (hay-bale check dam)

Figure 24: New signage designed for *Edna's pond* restoration site, 2019

5.2 Culvert Restoration – Scotch Settlement

Aquatic connectivity is very important for the biodiversity of a watershed. Culverts modify the morphology and the hydrology of a stream, and can sometimes hinder that connectivity by creating barriers. The presence of an outflow drop, steep culvert slopes, deteriorating culverts, and the presence of beaver dams or debris blockages within the culvert, can all negatively influence fish passage. Problematic culverts in terms of passage prevent fish to access upstream habitats. Other issues includes erosion of stream banks and road washouts caused by flooding and improperly sized culverts.

In 2018, the SBWA field team received training and conducted culvert assessments within the Shediac Bay watershed. The objective of these assessments was to target culverts located on Atlantic salmon and/or Brook Floater host fish bearing streams, then classify them as either passable, partial barrier or full barrier to fish passage.

In 2019, four problematic culverts were revisited. Two of these culverts had been replaced by the *Department of Transportation and Infrastructure* during the summer. The two other culverts were now free of the debris that classified them as partial or full barriers to fish passage. Other culverts deemed to be possible fish barriers were also revisited. While stopped at a culvert known to have issues in the Scotch Settlement area, a local resident confirmed that debris frequently blocks the culvert and leads to flooding during heavy rain events. The stream is a tributary of the McQuade Brook, an important branch of the Shediac River.

The issue was caused by shrub overgrowth directly upstream of the culvert, which causes debris to build up every spring. These blockages caused impediments to water flow, leading to flooding and fish passage issues. The area surrounding the culvert showed evidence of streambank erosion and sedimentation, due to a lack of a vegetative buffer zone, flooding and agriculture activities in the surrounding farmland.

The landowner was then contacted, and permission was granted to the SBWA to clear the obstructions at the mouth of the culvert, clean the excess of woody debris up the stream and plant native shrubs along the buffer zone to enhance fish habitat.

Figure 25: Map of the Scotch Settlement stream restoration, 2019

On September 12, the team trimmed the overgrowth at the culvert using manual tools. The stream was cleaned of excessive woody debris on a distance of approximately 235 meters upstream of the culvert. The native shrubs planted were Red Osier Dogwood, for its strong roots and its capacity to stabilize stream banks and prevent erosion. A total of 28 native shrubs were planted on a distance of approximately 60 meters. The farmer did not want reforestation beyond the planting of shrubs, but he may be willing to continue in future years if the project goes well.

Figure 26: Before and after photos of the shrub thinning upstream of the culvert

6. Discussion

As previously mentioned, the purpose of this project was to re-confirm the presence of Brook Floater (*Alasmidonta varicosa*) in the Shediac Bay watershed, based on the finding of the report *Freshwater Mussel Inventory in the Shediac and Scoudouc Rivers, New Brunswick* (Caissie C., and D. Audet 2006). After thoroughly searching all sites where the rare Brook Floater mussel was reported to be found in 2005, on multiple occasion since 2014, no Brook Floater mussels nor shells were found.

All sites considered high priority areas were visited multiple times during between May and August between 2014 and 2019. The sites were visited several times to account for varying factors that may cause burrowed mussels to rise to the surface of the substrate. According to recent studies, as much as 30-80% of freshwater mussels may be buried (*COSEWIC* 2009). Schwalb and Pusch (2007) found that up to 75% of a mussel population that is buried varies with discharge volumes of the river, day length, water temperature and possibly the mussel's reproductive cycle. This is also the reason why the SBWA has been encouraged to embark on a multi-year project to confirm the presence of the Brook Floater mussel in our watershed. The timeline for undertaking presence/absence surveys is 2-5 years (*Management Plan for the Brook Floater in Canada, SARA 2016*). The Shediac Bay Watershed Association will therefore not be pursuing presence/absence surveys in 2020.

Most of the work in the past 6 years was conducted in the Shediac River for two main reasons: the large majority of the Brook Floater mussels in the 2006 report[1] were found in the Shediac River; there is more suitable freshwater mussel habitat and more access points to those suitable habitats than in the Scoudouc River. The Scoudouc River proved to be difficult to access, requiring the use of an ATV or landowner's permission of access through their properties. The Shediac River had a total 103 Brook Floater mussels found during the 2005 surveys[1], in the mid to lower reaches of the river. The Scoudouc River has a total of 19 individuals found in the higher reaches of the river.

In the Assessment and Status Report on the Brook Floater in Canada (COSEWIC 2009), the population assessment of the Brook Floater in the Shediac River was estimated at 6,100, based on the findings of these 103 mussels. This population estimate would classify the Shediac River as one of the most important rivers for the species in New Brunswick. However, the same report warns of probable errors when calculating population estimates. Populations are often overestimated, as the assumption used is that the Brook Floater would be continuously distributed throughout the occupied reaches of the river. In reality freshwater mussels are found in the sections of rivers with suitable habitats comprised of sand or sandy gravel substrate and moderate flow. Another source of error underestimates populations as surveys rely primarily on visual inventories of mussels occurring at the surface of the substrate but between 30 and 80% of mussels may be buried. Populations may also be *underestimated* using a timed search method. This protocol is commonly used in freshwater mussel surveys but does not locate all mussels present in a site or suitable habitat, only those found during the allotted amount of search time.

Another cause for concern is regarding whether or not the Brook Floater mussels reported in the 2006 report[1] were properly identified as *Alasmidonta varicosa*. No vouchers specimens were ever submitted to the NB Museum for positive identification. Very few photos from 2005 were found in the archives of the SBWA, and the only photos on a disk named "Brook Floater 2005" that were not blurry were sent to two experts (Dwayne Sabine of DFO and Dr. Donald McAlpine, curator of the NB Museum). The two photos, showing frontal and lateral view of the mussel, were inspected by Dr. McAlpine and M. Sabine. Although identification of freshwater mussels cannot be definitive by analyzing them through photographs, both their opinions were that the lateral view of the mussel confirmed that it was not a Brook Floater, but resemble the Triangle floater (*Alasmidonta undulata*).

In addition, the only other known survey in the Shediac River that reports Brook Floaters being found is in 2002 by Kate Bredin, as cited in the report, "Assessment and status report on the Brook Floater (*Alasmidonta varicosa*) in Canada, COSEWIC. 2009." The information in this report saying that Bredin found 2 Brook Floaters in the Shediac River in 2002 is also likely a mistake. The ACCDC (Atlantic Canada Conservation Data Centre) was contacted during the winter 2014-2015 for the raw data of this 2002 survey. The data received indicated that 2 mussels *resembling* the Brook Floater were found, and they were noted as "Unidentified floaters". Yet those two *unidentified floaters* were taken as proof of the existence of the Brook Floater population in the Shediac River. Not only is there the possibility of misidentification of Brook Floater mussels in the 2006 report[1], it is also likely that the mention of Eastern Elliptio (*Elliptio complanata*) in the Shediac River is wrong. The Eastern Pearlshell and Eastern Elliptio are two very similar looking mussels, frequently described as confusing species. During the surveys from 2014 to 2019, only the Scoudouc River was found to contain a population of Elliptio. The only species found in the Shediac River during those years are: the Eastern Pearshell (*Margaritifera margaritifera*), the Creeper mussel (*Strophitus undulatus*), and one other unidentified mussels, a possible Triangle Floater (*Alasmidonta undulata*) or a shell variation of a Creeper mussel.

The possible detection of Brook Floater by e-DNA in 2018 is however encouraging. The SBWA will continue to partner with DFO to conduct samplings in the Shediac and Scoudouc rivers in the coming years.

7. Communications and outreach

The absence of Brook Floaters during field surveys has delayed the outreach activity to specific landowners. The focus of the 2019 communications strategy was instead targeted to users of the rivers that have potential Brook Floater habitat.

7.1 Landowner Outreach

A new pamphlet was developed for riverfront property owners in the winter of 2018-2019. The new tool provides best practices for freshwater habitat protection. The new pamphlet was sent to 19 riverfront property owners along the Shediac River. Included in the package was a letter describing the efforts made to locate the species of special concern, and a second pamphlet on freshwater mussels that feature's the Brook Floater mussel. The letter can be found in Annex 2.

Two landowners were directly engaged after contacting them for permission to access the river through their properties. Both landowners were happy to let us use their trails to walk down to conduct our surveys and e-DNA sampling. It provided a great opportunity to discuss issues surrounding the freshwater habitat and engage the landowners by discussing the Association's ongoing projects.

Figure 27: Riverfront property owners guide pamphlet developed in 2019, English side

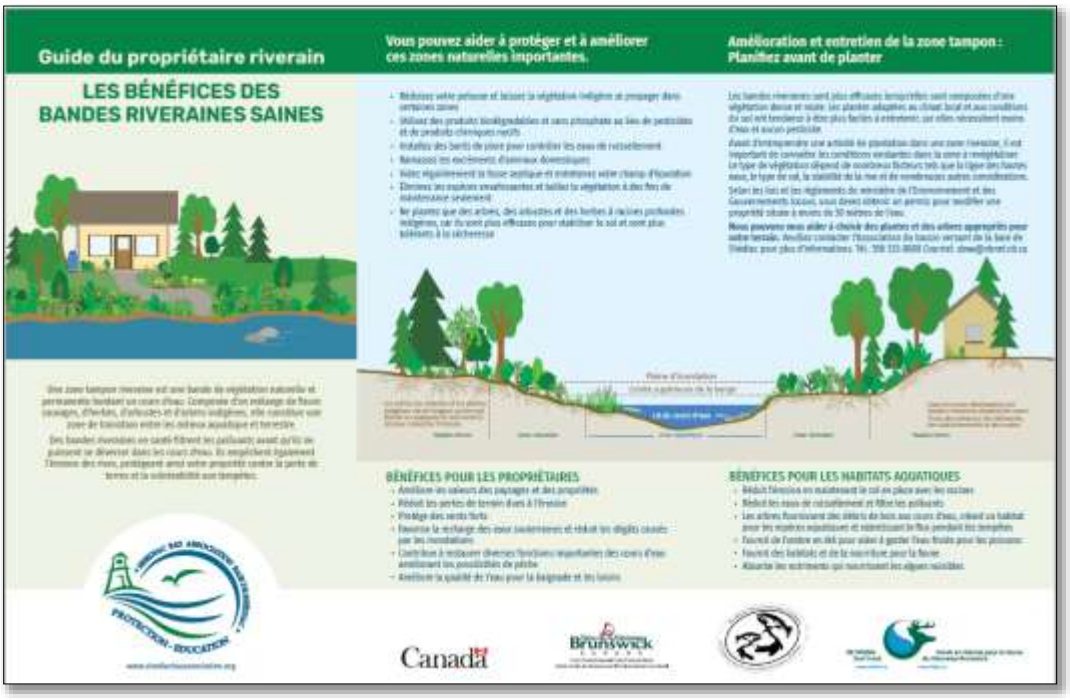

Figure 28: Riverfront property owners guide pamphlet developed in 2019, French side

7.2 Freshwater Mussel Video

A new educational video on freshwater mussels was developed in 2019, using footage collected in the field over the duration of the project. The video gives an underwater view to local freshwater mussel habitat, while providing context for their role in maintaining water quality. The bilingual video also highlights the key threats to these sensitive species. The video will be uploaded to our website and the SBWA YouTube channel.

Figure 29: Freshwater mussel video thumbnail, 2019

8. Conclusion

The Shediac Bay Watershed Association wishes to thank its funding partners the New Brunswick Wildlife Trust Fund and the federal Habitat Stewardship Program for Species at Risk for supporting this project. Although no Brook Floater was found during the past 6 years important information on the state of riparian habitat was gathered. The presence of other freshwater mussel species in our rivers are a good indications of healthy ecosystems.

The program allowed the association to build relationships with local landowners and the local ATV clubs to improve riparian zones. Several river crossings used by ATV'S were blocked and sediment mitigation measures were put in place at Edna's pond on the Scoudouc River. The SBWA also worked to restore fish passage and reforest streambanks.

The educational part of this project was successful, in that many residents were made aware of the existence of freshwater mussels. The subject comes as a surprise to many people, possibly due to the fact that freshwater mussels are not consumed like their saltwater cousins. Many riverfront property owners were also informed of the existence of a rare freshwater mussel that could possibly be found in their own backyard. The topic seemed to interest most property owners, and discussions were always in a positive note.

The Shediac Bay Watershed Association is proud of works accomplished throughout this project. Although the survey program will not continue in 2020 some research may be done in partnership with the department of Fisheries and Oceans to detect the Brook Floater with E -DNA sampling. The SBWA will continue to promote freshwater mussels and the Brook Floater as part of our presentations on biodiversity.

9. LITERATURE CITED

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APPENDIX A: Field Sheet Data 2019

APPENDIX B: Site Photos

Site Photos Shd I

Site photos of ShdI-A survey site, upstream view (left), downstream view (right)

Site Photos Shd E

Site photos of ShdE-A survey site, upstream view (left), downstream view (right)

Site photos of ShdE-B survey site, upstream view (left), downstream view (right)

Site photos of ShdE-C survey site, upstream view (left), downstream view (right)

Site photos of ShdE-1 survey site, upstream view (left), downstream view (right)

Site photos of ShdE-2 survey site, upstream view (left), downstream view (right)

Site photos of ShdE-2A survey site, upstream view (left), downstream view (right)

Site photos of ShdE-2B survey site, upstream view (left), downstream view (right)

Site Photos Shd D


Site photos of ShdD-A survey site, upstream view (left), downstream view (right)


Site photos of ShdD-B survey site, upstream view (left), downstream view (right)


Site photos of ShdD-1 survey site, upstream view (left), downstream view (right)

Site photos of ShdD-2 survey site, upstream view (left), downstream view (right)

Site Photos Shd N

Site photos of ShdN-A survey site, upstream view (left), downstream view (right)

Site photos of ShdN-B survey site, upstream view (left), downstream view (right)


Site photos of ShdN-C survey site, upstream view (left), downstream view (right)


Site photos of ShdN-1 survey site, upstream view (left), downstream view (right)


Site photos of ShdN-2 survey site, upstream view (left), downstream view (right)

Site Photos Shd C

Site photos of ShdC-A survey site, upstream view (left), downstream view (right)

Site photos of ShdC-B survey site, upstream view (left), downstream view (right)

Site photos of ShdC-C survey site, upstream view (left), downstream view (right)

Site photos of ShdC-D survey site, upstream view (left), downstream view (right)

Site Photos Shd A

Site photos for ShdA, upstream view (left), downstream view (right

Shd H (Bateman Brook)

Site photos for ShdH, upstream of bridge (left), downstream of bridge (right)

Site Photos Shd G

Site photos of ShdG-A survey site, upstream view (left), downstream view (right)

Site photos of ShdG-1 survey site, upstream view (left), downstream view (right)

Site Photos Shd M

Site photos of ShdM-A survey site, upstream view (left), downstream view (right)


Site photos of ShdM-1 survey site, upstream view (left), downstream view (right)

Site Photos Shd L


Site photos for ShdL-A, upstream view (left), downstream view (right)


Site photos for ShdL-1, upstream view (left), downstream view (right)

Site Photos Shd B

Site photos for site ShdB, upstream view (left), downstream view (right)

Site Photos Scd D

Site photos of ScdD-A survey site, upstream view (left), downstream view (right)

Site photos of ScdD-B survey site, upstream view (left), downstream view (right)

Site Photos Scd F

Site photos of access site ScdF-A survey site, upstream view (left), downstream view (right)

Site photos of ScdF-1 survey site, upstream view (left), downstream view (right)

Site Photos Scd I

Site photos for ScdI, upstream view (left), downstream view (right)

Site Photos Scd A

Figure 1: Site photos for ScdA, upstream view (left), downstream view (right)

Site Photos Scd B

Figure 2: Site Photos of site ScdB, upstream view (left), downstream view (right)

ANNEX 1: e-DNA Protocol

DFO-GULF environmental DNA (eDNA) sampling protocol (Brook Floater survey 2017)

Version 1.00

Prepared by: Francis LeBlanc

The following document provides an overview of the procedure for the collection and preservation of environmental DNA (eDNA) samples for molecular testing. Kits with the required materials will be supplied to each field group and preserved filters should be returned to the MBU lab (DFO-GULF, Moncton) for lab analysis.

Materials needed:

1) Disposable 250 mL filter funnels
2) Vacuum flask 1L
3) Tubing L/S # 15
4) Hand operated pump
5) Rubber stopper
6) 2 L water collection bottle
7) 1.5 uM glass microfiber filters (GFC)
8) 2 mL labeled filter preservation tubes (contains 0.2mL of 100% ethanol)
9) Filter forceps
10) 50 mL tubes (1 for bleach and 1 for tap water/distilled water)
11) Latex gloves
12) Filter funnel adaptor
13) eDNA sampling protocol
14) eDNA sampling information collection form
15) Black plastic bags
16) Household bleach (not provided)
17) Tap water (not provided)

Figure 1: Materials included in the e-DNA water sampling kits.

General considerations

1) Water sampling should be done from the downstream to upstream waterflow direction to minimise surface water disturbance and contamination.

2) Sample collection should be done within the stream/river and in the middle of the water column in areas where the Brook Floater is at the highest probability of being present (i.e. suitable habitat). Collection from the bank is also acceptable if collection within the stream/river is not an option.

3) Gloves should be worn when handling field sampling materials that will come in contact with the water samples (i.e. sterile filter funnel assembly/interior of the funnel, filter paper) and forceps should be used to handle filter papers. A new pair of gloves should be used at each location within a site. No gloves are needed when handling materials that are below of the filter paper (i.e. vacuum flask, rubber stopper, vacuum pump, etc.).

4) Forceps used to handle the filter papers needs to be cleaned between samples by soaking them in a 50 % household bleach solution (1 part household bleach with 1 part tap water) for at least 2 minutes followed by rinsing them a few times in tap water. The provided 50 mL tubes should be used to perform this cleaning step.

5) Individual 2 L collection bottles should be used for each sample collected and not reused in more than one site without having been thoroughly cleaned. One filter funnel adapter should also be used per sample and not reused without having been thoroughly cleaned. The 250 mL filter funnels are disposable and can be thrown away after they've been used.

6) Non-disposable materials below the filter (i.e. vacuum flask, tubing, rubber stopper) do not need to be cleaned between samples from the same site.

7) Non-disposable materials should be thoroughly cleaned between sites or at the end of the day with a 10% bleach solution (1 part household bleach with 9 part tap water) (minimum 30 min) and rinsed 2-3 times in tap water (i.e. 2 L collection bottles, vacuum flask, forceps, rubber stopper, tubing, filter funnel adapters). After bleaching and rinsing, materials should be allowed to air dry and then stored. 2L bottles should be stored with lids on once dried.

8) If water samples (500 mL) in the collection bottles are not filtered immediately it is acceptable to store them temporarily in a cooler with ice for up to 6 hours.

9) In some streams/rivers, the filter may clog before the full water volume (500 mL) has been filtered. The filtering rate may slow to individual drips separated by several seconds. Consider setting a cutoff time or drip rate for ending filtering. Squeezing the lever of the manual hand pump repeatedly can also help filter the remaining water. For example, you might end filtering when the drip rate slows to 3 drips every 10 seconds. Just make sure to take notes of the approximate volume filtered.

Sampling procedure:

For each selected site, a total of 2 water samples will be collected as well as 1 control sample (tap water). The 2 water samples should be at different locations within the site during the survey period. For each sample collected record the sampling information in the provided sample collection form. An effort should be made to collect the water sample at the location where Brook Floaters are observed or close downstream. If Brook Floaters are observed after a water sample has been collected, please indicate the GPS location and distance from the water sampling location.

The following three scenarios should be considered for guidance purposes when planning the collection of the 2 samples on a site.

Scenario 1: If Brook Floaters are known to be present at that site and have been observed in 2016, sample water above or close downstream of where the individuals are located.

Scenario 2: If Brook Floaters were observed in previous years, but not in 2016, attempt to sample above or close downstream of where individuals have historically been detected.

Scenario 3: If Brook Floaters have never been detected on a site, collect a first water sample within the first hour of the search survey in an area where the species has a higher probability of being present (suitable habitat). If Brook Floater is not found towards the end of the time-search survey, collect the second water sample at the end of the search survey period.

Note: At each site, an effort should be made to sample above Brook Floaters if they are seen.

The control sample (500 mL of tap water) should be filtered first to ensure that the equipment is clean. The 2 water samples (500 mL collected in individual 2 L bottles) can then be filtered for that site.

The 500 mL water sample or tap water must be filtered in two steps of 250mL (maximum capacity of the filter funnel).

Step 1: Equipment assembly

1) Prepare the filtering assembly as seen in Figure 2.
2) The filter paper included in the sterile filter funnel package (0.45 uM nitrocellulose filter) must be replaced with the provided 1.5 uM glass microfiber filter (GFC). The 0.45 uM nitrocellulose filter can be disposed.
3) Remove the upper chamber of the filter funnel from the membrane collar (Figure 3) and replace the filter paper with the supplied 1.5 uM GFC filter (Figure 3).
4) Re-attach upper chamber to membrane collar.

Note: Make sure that gloves are worn when replacing the filter paper and use forceps to handle filters.

Figure 2: Water filtering equipment assembly: Taken from "eDNA protocol sample collection, Goldberg & Strickler, 2015, Washington State University".

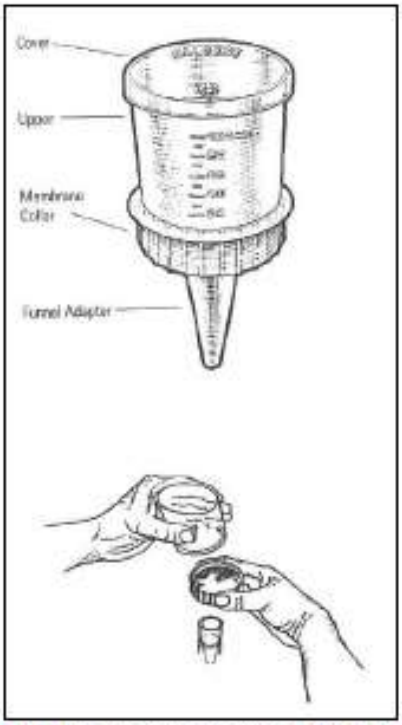

Figure 3: Filter funnel assembly and replacement/removal of filter paper.

Step 2: Water collection and filtration

1) Collect 500 mL of water from the stream/river using the provided 2 L collection bottles.

Note: Gloves do not need to be worn when collecting the water sample.

2) Pour the water sample slowly into the filter funnel up to the 250 mL mark.

Note: make sure that the filter paper has been replaced with the supplied 1.5um GFC filter.

3) Engage vacuum pump to begin filtration by squeezing the lever a few times.
4) Once the first 250mL of water has completely passed through the filter paper, slowly pour the second half (250mL) of the water sample into the funnel.

Note: The vacuum should not be to be stopped when adding the second half of the sample.

Note: Squeezing the lever of the manual hand pump a few more times can be done to help filter all the water.

5) Allow the vacuum to run for 1 min once all the water has passed through the filter to dry the filter as much as possible and keep the vacuum applied until the filter funnel has been removed from the filter funnel adapter.

Figure 4: Water filtration illustration: Taken from "eDNA protocol sample collection, Goldberg & Strickler, 2015, Washington State University".

Step 3: Filter paper removal and storage (make sure that gloves are worn for these steps)

1) Remove filter funnel from filter funnel adapter with the vacuum still applied to reduce chances of water droplets splashing from the filter funnel adaptor/vacuum flask onto the filter.
2) Remove upper chamber of the filter funnel.

Note: Some water might remain on the filter and in the membrane collar of the filter funnel once filtration is done. It is best to remove the filter from the membrane collar using the forceps,

dump the water remaining in the membrane collar and replace the filter in the membrane collar to proceed with the folding of the filter paper. Removal of as much water as possible will help with the ethanol preservation of the filter.

3) Fold filter in half once, a second time and then roll the filter so that it fits in the provided 2 mL collection tube using clean forceps (Figure 5).
4) Place filter in 2 mL collection tube containing 100% ethanol and inverse the tube a few times until the filter is completely soaked in ethanol.
5) Once at home, store the collection tubes with the ethanol preserved filters in a clean ziplock bag or box and store samples in a freezer if one is available.

Note: Ethanol preserved filters stored in a freezer can be kept for a prolonged period of time (up to 2 months).

Figure 5: Removal and folding of filter paper using forceps: Taken from "A protocol for collecting Environmental DNA Samples from Streams, Carim et al., 2016, Rocky Mountain Research Station, USDA."

Step 4: Log info in collection form and clean-up

1) Ensure that all the information requested has been captured in the provided collection form.
2) Place all non-disposable materials in the provided black plastic bag for thorough cleaning.
3) Contact Francis LeBlanc or Fabiola Akaishi to coordinate the pick-up/delivery of the samples for lab analysis.

For any question or concerns regarding this eDNA sampling protocol please contact:

Francis LeBlanc
Email: francis.leblanc@dfo-mpo.gc.ca
Phone number: (506) 851-6222

Fabiola Akaishi
Email: Fabiola.akaishi@dfo-mpo.gc.ca
Phone number: (506) 851-4381

Annex 2: Landowner Outreach - Correspondence Letter

Landowner letter - Brook Floater Mussel Project

Dear Landowner,

For over 20 years, the Shediac Bay Watershed Association (SBWA) has been working with individuals and various organizations to protect and restore the Shediac and Scoudouc Rivers. As a landowner in the Shediac Bay watershed, you can contribute to its health so that we can continue to enjoy the waterways we use and value.

The purpose of this letter is to inform you that environmental DNA testing done in the river near your property has confirmed the possible presence of a rare freshwater mussel in this area.

The Brook Floater is a species of special concern that has been reported to live in the Shediac and Scoudouc Rivers. Due to the limited range of the species and the small number of known sites, we are contacting all landowners who live or own land with potential Brook Floater populations.

Although the Shediac River is generally healthy, we need to reduce the threats to this rare mussel. The information included with this letter will help you better understand the importance of vegetation buffer zones to maintain the health of this natural habitat.

We have programs that can help you improve your riverfront, not only for the Brook Floater, but for fish and other species that need healthy rivers to thrive. These measures also protect your investment by preventing land loss due to erosion.

Your collaboration is very much appreciated. Please contact us by phone or email to discuss further.

Rémi Donelle, Manager
Shediac Bay Watershed Association
506 533-8880
sbwa@nbnet.nb.ca

Annex 3: Freshwater Mussel Pamphlet

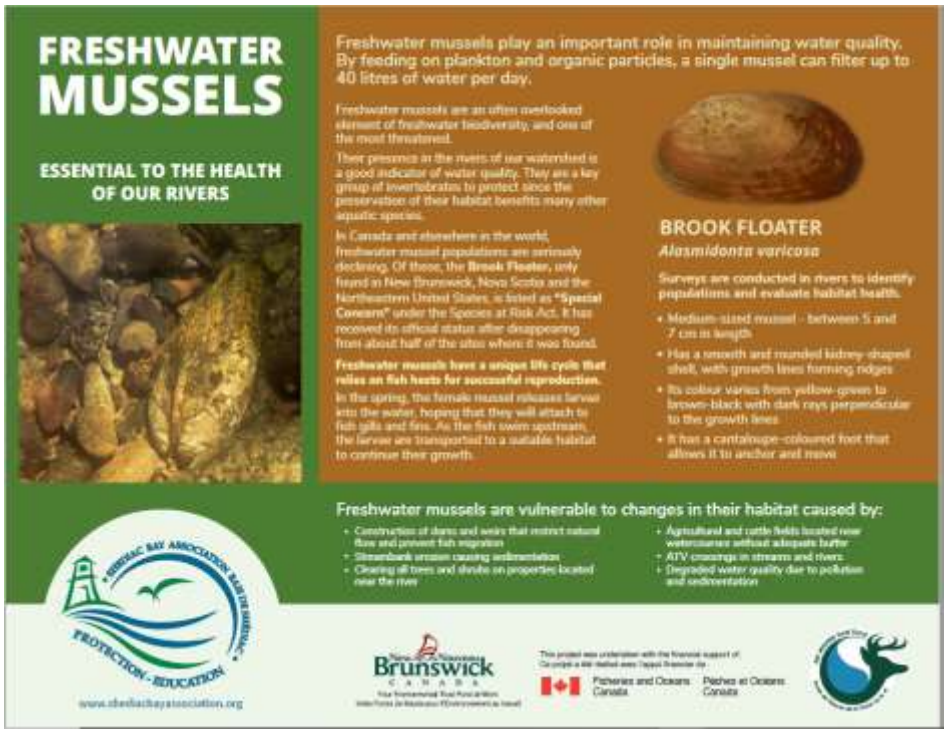

Figure 30: Freshwater mussel pamphlet; English side

Figure 31: Freshwater mussel pamphlet; French side

Annex 4: SBWA Newsletter