APPENDIX C

Monitoring Plan

Monitoring Plan

Overview: The monitoring plan consists of four components associated with the proposed eradication of SMB from the Miramichi Watershed: (1) rotenone treatment monitoring (2) rotenone deactivation monitoring, (3) short-term and long-term SMB eradication monitoring, and (4) ecological recovery monitoring.

Recovery objectives for fishes that will be monitored are described in the Re-establishment Strategy (Appendix D).

Roles: NSMDC intends to have Indigenous technicians and biologists lead the long-term monitoring program (Component 4), with support as required from project partners and federal/provincial governments. Given that in the absence of the eradication project we are putting forward, DFO would normally be conducting a control and containment program at Miramichi Lake, we expect DFO to continue its program for 5 years post-treatment and fund Indigenous technicians to help lead the program. This includes operating the barrier fence, and sampling for fish using the electrofishing boat, backpack electrofisher, fyke nets, gill nets, beach seine, angling, and additional components such as eDNA assessment in the lake, Lake Brook, and the SW Miramichi River. This expectation is reasonable given DFO's responsibility to manage AIS and its past experience and expertise in carrying out the control and containment program.

<u>Component 1</u> - Rotenone Treatment Monitoring

Overview: Rotenone treatment monitoring consists of measuring (1) responses of sentinel fish to rotenone and (2) collecting samples for rotenone analysis in Miramichi Lake, Lake Brook and the SW Miramichi River.

Objectives: (1) Allow for adjustments to the treatment strategy during application and provide a record of efficacy throughout the treatment area; (2) Provide an analytical record of rotenone levels in Miramichi Lake, Lake Brook and the SW Miramichi River during treatment and the breakdown of rotenone over time in the lake; (3) Ensure that SMB are eliminated from the area.

Monitoring Sites (see Figure C1 and Table C1)

- One site per inlet is located on the four tributaries to Miramichi Lake immediately upstream of their confluence with the lake (Inlet1 to Inlet4)
- Six monitoring sites are located at various depths in Miramichi Lake:
    - two sites 10 m from the shoreline (ML1 & ML2)
    - two sites at mid-depth (ML3 & ML4)
    - two sites at the maximum depth (ML5 & ML6)
- Two sites are located on Lake Brook, at the upper end near the lake (LB1), and at the lower end just upstream of its confluence with the SW Miramichi River (LB2), and two sites are located on the East Branch Lake Brook placed 1 km upstream of the confluence with Lake Brook (LB3) and 320 m upstream of the confluence with Lake Brook (LB4).

- The 15 km treatment reach of the SW Miramichi River will have five to ten rotenone injection sites, locations depending on flow at the time of treatment. Each injection site will have sentinel fish and rotenone levels will be monitored at SW1, SW3, and SW5.
- The deactivation site is located at the downstream extent of the treatment area at Moose Call on the SW Miramichi River (SW5)

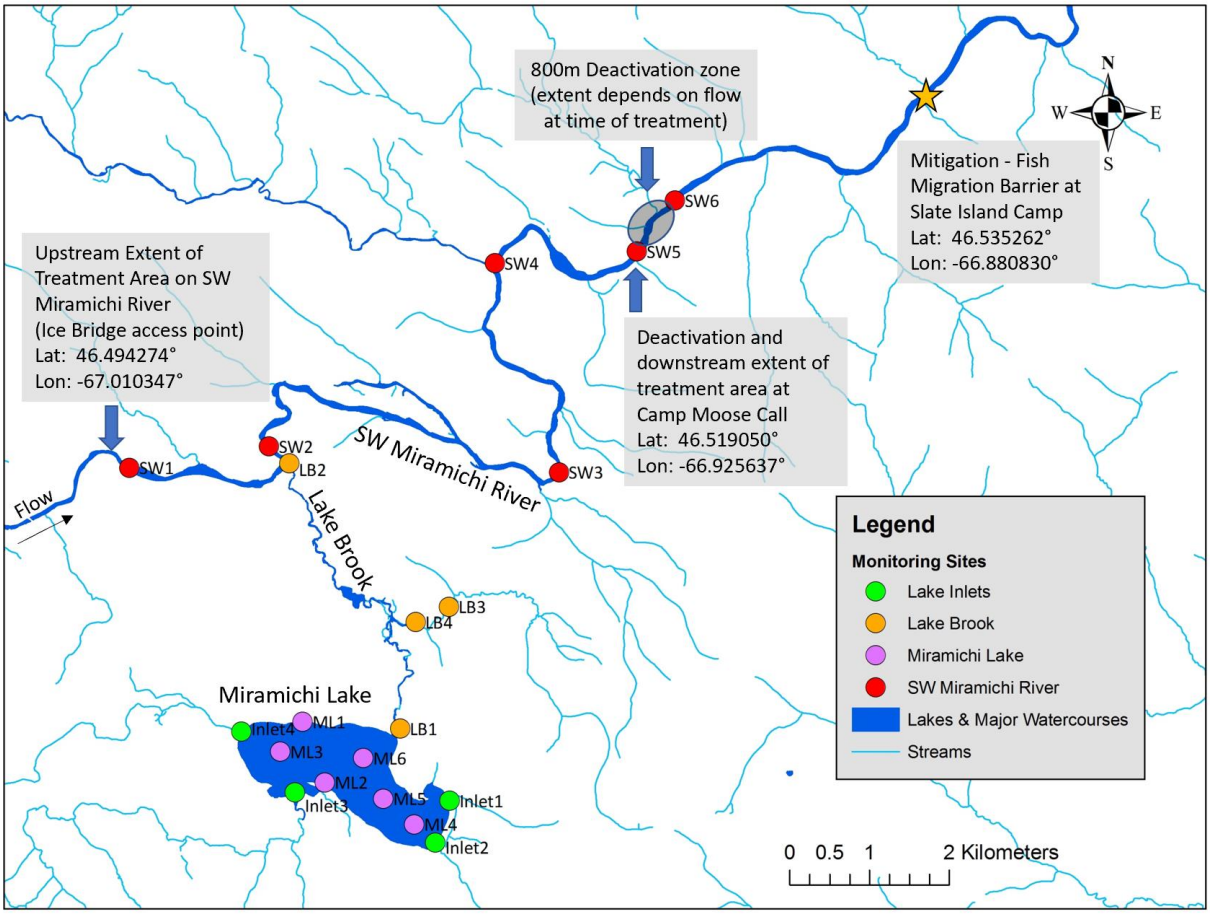

Figure C1. Monitoring sites - smallmouth bass eradication and ecological recovery.

Table C1. Coordinates of monitoring sites for smallmouth bass eradication and ecological recovery.

ID	Latitude	Longitude
Inlet1*	46.456160	-66.956251
Inlet2*	46.451433	-66.958524
Inlet3*	46.456995	-66.981503
Inlet4*	46.463819	-66.990329
ML1	46.464960	-66.980364
ML2	46.458137	-66.976645
ML3	46.461588	-66.983960
ML4	46.453477	-66.961996
ML5	46.456339	-66.967070
ML6	46.460913	-66.970390
LB1	46.464262	-66.964391
LB2	46.494170	-66.982804
LB3	46.478031	-66.956578
LB4	46.476280	-66.961977
SW1	46.493533	-67.008842
SW2	46.496040	-66.986128
SW3	46.493325	-66.938691
SW4	46.516863	-66.949367
SW5	46.518262	-66.926180
SW6**	46.524048	-66.920007

*Final placement to be determined prior to treatment based electrofishing results and SMB presence in each inlet; criteria explained in the AIS application (Appendix B).
**Final placement based on flows just prior to treatment to ensure SW6 is located 30-min water travel time downstream of the deactivation site.

Rotenone: The protocols for analyzing rotenone concentrations in water are detailed in SOP 16.1 of Finlayson et al. (2018) and utilize liquid chromatography (LC) as described by Dawson et al. (1983) or Sandvick et al. (2018) or direct injection liquid chromatography/mass spectrometry (LC/MS) as described by Vasquez et al. (2012); these analyses have a MDL of 0.001 mg/L and RL of 0.002 mg/L rotenone. Water samples are collected using a Kemmerer bottle in the lake or directly a few centimeters below the water surface in streams and shallow lake sites. Samples are put in 250-ml amber glass bottles with Teflon-lined caps, stored chilled (4 °C), and transported to the laboratory for analysis with chain-of-custody forms.

Assessment Criteria and Monitoring Timelines –

- **Sentinel Fish:** Cages containing sentinel fish (yellow perch) are placed in the lake one day before application and within two hours pretreatment at each rotenone injection site in flowing waters (4 lake inlets, Lake Brook, and the SW Miramichi River). The sentinel fish are checked in the lake 24 hours after the application is complete and are checked in the flowing sections throughout the treatment.
- **Miramichi Lake:** Verifying that (1) the dosage target of 75 ppb rotenone was obtained, (2) rotenone levels ≥ 19 ppb are present for ≥ 4 days, and (3) rotenone degrades to undetectable

levels (< 2 ppb) in ≤ 3 weeks. Samples for rotenone analysis are collected at 1, 2, 4, 7, 14 and 21 days post-treatment or until levels become undetectable.

- **Lake Brook:** Verifying that the dosage target of 75 ppb rotenone was obtained for 6 h in Lake Brook. Samples for rotenone analysis are collected at 2 and 4 h after treatment begins in the treatment area (LB1, LB2, LB3, LB4). The timing of sample collection is dependent on the timing of the treatment in the area adjacent to the individual monitoring locations.
- **SW Miramichi River:** Verifying that the dosage target of 75 ppb rotenone was obtained for 6 h in the treatment reach of the SW Miramichi River. Samples for rotenone analysis are collected at 2 and 4 h after treatment begins at the upper, middle, and lower reaches of the treatment area (SW1, SW3, SW5).

<u>Component 2</u> - Deactivation Monitoring in the SW Miramichi River

Overview: Deactivation monitoring consists of (1) real-time responses of sentinel fish upstream and downstream of the deactivation station, (2) collecting samples for rotenone analysis above and below the deactivation station, and (3) collecting and analyzing samples for $KMnO_4$ residual below the deactivation station.

Objectives: (1) Allow for adjustments to the deactivation strategy of applying $KMnO_4$ by monitoring the response of sentinel fish and $KMnO_4$ residues; (2) Provide an analytical record of rotenone concentrations upstream and downstream of the deactivation station during and following the treatment; (3) Maintain a 1 ppm $KMnO_4$ residual and an analytical record of $KMnO_4$ levels at 30 min water travel time downstream of the deactivation station; (4) Ensure that rotenone concentrations are <2 ppb below the deactivation zone.

Deactivation Monitoring Sites (see Figure C1)

- SW5 – immediately upstream of the deactivation site located at Moose Call
- SW6 – located 30 min water travel time downstream of the deactivation site (location varies depending on discharge and water velocity at the time of treatment; for planning purposes the assumption is a water velocity of 1.6 km/h meaning the 30 min contact zone extends 800m below the deactivation site)

Rotenone: See Component 1

Potassium Permanganate: The protocols for the on-site analysis of potassium permanganate concentrations in water upstream and downstream of the deactivation station are detailed in SOP 7.1 of Finlayson et al. (2018) and utilize either direct (Standard Method 4500-$KMnO_4$ B; American Public Health Association 1998) or indirect (USEPA DPD Method 8167 for Total Chlorine) colorimetry.

Assessment Criteria and Monitoring Timelines –

- **Rotenone:** Verifying that rotenone is oxidized to <2 ppb at least 30-minutes water travel time downstream of the deactivation station by collecting and analyzing samples for rotenone

upstream (SW5) and downstream (SW6) of the deactivation station every 2 h during treatment and for 24.75 h afterwards.

- **$KMnO_4$:** Verifying that $KMnO_4$ residual is maintained at 1 ppm at SW6 by collecting and analyzing samples for $KMnO_4$ analysis every 30 minutes downstream of the deactivation station and relaying the results to the deactivation station (SW5) for adjustment of $KMnO_4$ input.
- **Criteria for Beginning Deactivation:** Deactivation begins at a minimum of several hours before the rotenone treatment to reduce the $KMnO_4$ demand of the streambed in the 30-minute section below the deactivation station. Injecting $KMnO_4$ until residues stabilize will ensure that the streambed is fully oxidized prior to contact with rotenone. This will be done for each of the two treatment in the river. Deactivation begins concurrently with the rotenone treatment, and the reaction of sentinel fish signals the arrival of rotenone at the deactivation station.
- **Criteria for Terminating:** The survival of sentinel fish upstream of the deactivation site for 4 h signals the lack of need for deactivation and the termination of $KMnO_4$ input.
- **Effectiveness:** Measured by (1) maintaining a 1 ppm $KMnO_4$ residual and (2) sentinel fish survival 30 minutes water travel time downstream of the deactivation site, and (3) rotenone concentrations of < 2 ppb downstream of the 30-minute deactivation zone (SW6).

<u>Component 3</u> - Short-Term & Long-Term SMB Eradication Monitoring

Overview: Monitoring of sentinel fish during and immediately following treatment are the first indicators of eradication success and continued monitoring using eDNA, electrofishing, and netting techniques for 5 years post-treatment are longer term evidence of SMB eradication.

***Objectives*:** (1) Provide a report on the treatment that contains short-term evidence that SMB were eliminated from the treatment area using sentinel fish and rotenone analysis results and treatment statistics; (2) Provide annual post-treatment assessment reports using monitoring results that demonstrate SMB are absent.

Short-Term Eradication Monitoring –

- **Sentinel Fish & Rotenone Concentrations** - The effectiveness of the treatment is documented using a combination of caged sentinel fish (Yellow or White Perch) results and rotenone concentrations in the treatment area described in Component 1.
- **Application Statistics:**
    - The lake is divided into quadrants, volume of each quadrant calculated using computer assisted bathymetry, rotenone dosage calculated for each quadrant based on volume, and an applicator boat assigned to each quadrant. The application from boats is monitored using GPS tracking and recording the volume of rotenone applied to each quadrant. Sprayers from boats will spray the shoreline areas and the emergent weed beds around the lake and its use also GPS tracked. It is expected that the application will require one to two days to complete.
    - The flowing water segments are treated using drip stations in low flow areas such as the inlets to Miramichi Lake and Lake Brook and using peristaltic pumps in higher flow areas

such as the SW Miramichi River. Flows at these sites are taken immediately prior to treatment to ensure the correct rotenone dose. The applications from these devises are monitored continually throughout application period of 6 h. The volumes discharged from the cans are checked every 30 minutes using a volumetric cylinder and stopwatch while the volumes from the peristaltic pumps are checked every 30 minutes by reading the attached flowmeter. All measurements are recorded for use in treatment assessment.

Long-Term Eradication Monitoring – A combination of eDNA, netting techniques (fyke, seine, gillnet), boat electrofishing, backpack electrofishing, and angling for a 5-year post-treatment period will provide data for evaluating the success of eradicating SMB from Miramichi Lake, Lake Brook and the SW Miramichi River. The techniques and sampling locations employed by DFO (e.g., Biron 2015) in the control and monitoring program for the lake will be used. Using this approach will serve the dual purpose of eradication monitoring and long-term recovery monitoring (see Component 4 below). It also enables comparison with pre-treatment catch-per-unit-effort and community composition data.

The distribution of SMB in both the lake and the river are well known and these areas will be a focus. Samples for eDNA analysis will be collected from areas of known SMB inhabitation in the lake and at intervals in the SW Miramichi River according to the DFO sampling locations established in the 2019 and 2020 eDNA surveys. The absence of SMB from manual fish collection techniques or the lack of SMB eDNA in water are by themselves not conclusive evidence of their absence, but the two techniques used together increases the level of certainty that SMB are absent.

<u>Component 4</u> - Ecological Recovery Monitoring

Overview: Long-term monitoring of the treatment area will begin prior to rotenone application for pre-treatment conditions. Monitoring will be carried out for 5 years post-treatment to document the recovery of the aquatic community, including zooplankton, benthic macroinvertebrates, mussels, and fishes. The study will employ a before-after-control-impact (BAACI) sample design which is well suited to detecting changes due to rotenone treatments. Sampling will be restricted to the treatment area both before and after treatment.

Objectives: (1) Monitor for 5 years and provide an annual report on the recovery of the aquatic community in Miramichi Lake Lake Brook, and the 15 km reach of the SW Miramichi River, including zooplankton, benthic macroinvertebrates, mussels, and fishes; (2) Compare pre-treatment conditions with annual post-treatment results; (2) Monitor for presence of adults and juveniles of each fish species to inform triggers of transplantation for non-migratory fishes at the 2-year and 5-year post-treatment mark (as set out in the Re-establishment Strategy in Appendix D).

Sampling Intervals: Sampling will be carried out beginning during the spring and summer field season prior to treatment, one-week post-treatment, and annually in spring, summer, and fall post-treatment for

5 years. The fish sampling will be conducted during summer and into fall similar to DFO's protocol that has been employed at the lake since 2009 to ensure comparability.

***Sample Parameters*:** Water quality, zooplankton, benthic macroinvertebrates, mussels and fishes

Water Quality: Turbidity, water clarity (lake only), temperature, pH, total dissolved solids and conductivity are recorded at each sampling event. Water clarity or transparency is measured using a Secchi disk (30-cm), turbidity is measured using a turbidity meter, and conductivity, pH, total dissolved solids and water temperature are measured *in-situ* using a portable multi-parameter probe.

Zooplankton: Zooplankton are collected in Miramichi Lake by vertical tows of plankton net from a depth of 3m with 4 tows per sampling event. The net has a 20 cm opening with a 30 cm reduction collar and a mesh size of 64 μm. The samples are preserved in 70% ethanol. Zooplankton are identified to the most practical level. Zooplankton are quantified by counting abundances in five 10-mL subsamples using an inverted light microscope at 100× and 200× magnification from each site and the mean value is recorded. Based on previous studies of rotenone treatments in lakes (e.g., McGann 2018), we anticipate that zooplankton diversity and abundance will have recovered to at least pre-treatment levels within 1-year post-treatment.

Benthic Macroinvertebrates: The rocky bottom of Miramichi Lake will influence the sampling gear used. The benthic macroinvertebrate data are collected in triplicate using a petite PONAR (152 × 152 mm) dredge from the monitoring sites. The samples are sieved through a 500-μm mesh and aggregated in major taxonomic groups; some samples are retained for analysis to species level. Samples are preserved in 99% ethanol. When identified to species, samples with more than 500 organisms are subsampled using a Caton gridded tray with a 500-μm wire mesh and 30 grids to expand raw samples. Based on previous studies of rotenone treatments in lakes (e.g., Eilers 2008), we anticipate that invertebrates will have recovered to at least pre-treatment levels within 1-year post-treatment. Sampling using the Canadian Aquatic Biomonitoring Network (CABIN) protocols will also be carried out in Lake Brook (LB1, LB2) and in the SW Miramichi River (SW1-SW5).

Mussels – The sampling protocol described in Appendix K will be employed to carry out mussel surveys throughout the Lake during the 5-year monitoring period, with particular focus on brook floater, a Species at Risk. Freshwater mussels generally have a high toxicity threshold to rotenone, and we anticipate mussels to survive the treatment.

Fishes – A combination of electrofishing (boat and backpack), netting methods (fyke, seine, gillnet, minnow trap), and angling will be employed over a 5-year post-treatment monitoring period to evaluate recovery of fish species in the treatment area. Fish recolonization monitoring will overlap with the long-term eradication efficiency monitoring. The diversity of methods in Miramichi Lake will ensure different size classes of the various fish species are captured. This approach will generate presence/absence and catch-per-unit-effort data to characterize fish community structure, compare to pre-treatment data, and provide relative abundance of the re-establishing fish species.

Miramichi Lake - The fish sampling protocol employed by DFO since 2009 (e.g., DFO 2013, Biron 2015) in the control and monitoring program will be used on Miramichi Lake. This approach will serve the dual purpose of eradication monitoring and long-term recovery monitoring. It also enables comparison with pre-treatment catch-per-unit-effort data. Monitoring will be carried out by Indigenous technicians under the direction of DFO staff who have worked on the control program to date; this will ensure consistency with sampling pre-treatment and comparability of the data.

Lake Brook & SW Miramichi River - In Lake Brook and the SW Miramichi River, monitoring will be carried out via backpack electrofishing using open sites and characterized by species composition and catch-per-unit-effort at sites LB1, LB2 and SW1-SW5.

Monitoring results will indicate whether fish species are successfully re-colonizing the treatment area. In Miramichi Lake, presence/absence of adults and juveniles of each fish species will inform triggers of transplantation for non-migratory fishes at the 2-year and 5-year post-treatment mark (as set out in the Re-establishment Strategy in Appendix D). Should transplantation be required after 2 years post-treatment, the remaining 3 years of the monitoring program will enable assessment of whether the transplanted species are demonstrating successful reproduction and establishment.

Special Considerations for Fish Monitoring

- Golden shiner and brown bullhead – expected to be present immediately post-treatment given their high toxicity threshold to rotenone
- Alewives (migratory/anadromous) – adult alewives at sea during treatment will be unaffected and will enter the lake and spawn the following spring after treatment (as occurs naturally). Juvenile alewives expected to be present in the lake during summer months, emigrating in July and August (as occurs naturally).
- Atlantic salmon (migratory/anadromous) – the following summer post-treatment, young of the year juvenile Atlantic salmon are expected to be present in Lake Brook and in the 15 km treatment reach of the SW Miramichi River at similar densities compared to pre-treatment since spawning adults holding below the fish migration barrier in the river during treatment are not anticipated to be impacted. Resulting juvenile densities will depend on a variety of factors that are independent of the application (e.g., adult spawner abundance, spawning conditions, overwintering conditions/survival, etc.) and will be monitored via backpack electrofishing.
- American Eel (migratory/panmictic) – juveniles arriving from sea are anticipated to be present throughout treatment area the following year after treatment.
- Sea Lamprey (migratory/anadromous) – adults at sea during treatment will be unaffected and are anticipated to enter the treatment area the following spring after treatment. During DFO SMB control efforts since 2009 in Miramichi Lake and Lake Brook, very few sea lamprey have been captured; therefore, we anticipate low catches of this species during monitoring. This is likely due to the significant distance of Miramichi Lake and Lake Brook from salt water (>100 km).

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