

EDNA-based targeted qPCR detection of freshwater mussels (*Alasmidonta varicosa*, *Margaritifera margaritifera* and *Lampsilis cariosa*) in New Brunswick from 2020 to 2024

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**Canadian Data Report of
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Canadian Data Report of Fisheries and Aquatic Sciences

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Les numéros 1 à 25 de cette série ont été publiés à titre de Records statistiques, Service des pêches et de la mer. Les numéros 26-160 ont été publiés à titre de Rapports statistiques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom de la série a été modifié à partir du numéro 161.

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ABSTRACT

Stoyel, M., Thériault, I., Pirie-Hay, D. and LeBlanc, F. 2026. eDNA-based targeted qPCR detection of freshwater mussels (*Alasmidonta varicosa*, *Margaritifera margaritifera* and *Lampsilis cariosa*) in New Brunswick from 2020 to 2024. Can. Data Rep. Fish. Aquat. Sci. 1485: v + 34 p.

The brook floater (*Alasmidonta varicosa*) is a freshwater mussel species with current known distribution in Canada confined to 13 watersheds in Nova Scotia and New Brunswick. The species is listed as Special Concern under the *Species at Risk Act* (SARA). A Management Plan for the brook floater was published by Fisheries and Oceans Canada (DFO) in 2018 to maintain a viable, self-sustaining population in Canada, and since that time work has been ongoing to assess the distribution and range of the species. Environmental DNA (eDNA) sampling combined with species-specific quantitative PCR (qPCR) testing has been one of the approaches used to assess species distribution, with method development and initial survey results from 2017 and 2018 published in 2021. This report presents further eDNA work conducted from 2020 to 2024 in various watersheds in New Brunswick (and a few in Quebec) to continue gathering distribution data for the species. A subset of the collected samples over that timespan was also opportunistically tested for the detection of two other freshwater mussel species, the eastern pearlshell (*Margaritifera margaritifera*) and yellow lampmussel (*Lampsilis cariosa*) for which qPCR assays were available in the laboratory. Brook floater DNA was detected within its known distribution in the Miramichi and Petitcodiac river watersheds, as well as for the first time in the Saint John River watershed. Brook floater DNA was not detected across northern New Brunswick or in the Richibucto River watershed despite known distribution in the nearby Kouchibouguacis and Bouctouche river watersheds. Yellow lampmussel DNA was detected within its known distribution in the lower Saint John River watershed, and was tested for but not detected in northern New Brunswick and the Miramichi watershed. Eastern pearlshell DNA was detected at all sites tested in the Miramichi River watershed, where it is known to be relatively abundant. By improving knowledge on at risk freshwater mussel species distributions, this work contributes to DFO's management plan objectives of conserving habitat where these species currently exist or are newly discovered.

RÉSUMÉ

Stoyel, M., Thériault, I., Pirie-Hay, D. and LeBlanc, F. 2026. eDNA-based targeted qPCR detection of freshwater mussels (*Alasmidonta varicosa*, *Margaritifera margaritifera* and *Lampsilis cariosa*) in New Brunswick from 2020 to 2024. Can. Data Rep. Fish. Aquat. Sci. 1485: v + 34 p.

L'alamidonte renflée (*Alasmidonta varicosa*) est une espèce de moule d'eau douce dont la répartition actuellement connue au Canada est limitée à 13 bassins versants en Nouvelle-Écosse et au Nouveau-Brunswick. L'espèce est inscrite sur la liste des espèces préoccupantes en vertu de la *Loi sur les espèces en péril* (LEP). Un plan de gestion pour l'alamidonte renflée a été publié par Pêches et Océans Canada (MPO) en 2018 avec l'objectif de maintenir une population viable et autosuffisante au Canada, et depuis ce temps des travaux sont en cours pour évaluer la distribution et l'aire de répartition de l'espèce. L'échantillonnage d'ADN environnemental (ADNe) combiné à des tests de PCR quantitative (qPCR) spécifiques de l'espèce constitue une des approches utilisées pour évaluer la répartition de l'espèce, avec le développement de méthodes et les résultats des relevés initiaux en 2017 et 2018 ayant été publiés en 2021. Ce rapport présente d'autres travaux ADNe réalisés de 2020 à 2024 dans divers bassins versants du Nouveau-Brunswick (et quelques-uns au Québec) afin de continuer à recueillir des données sur la répartition de l'espèce. Un sous-ensemble des échantillons prélevés durant cette période a aussi été testé de manière opportuniste afin de détecter deux autres espèces de moules d'eau douce, la mulette perlière de l'Est (*Margaritifera margaritifera*) et la lampsile jaune (*Lampsilis cariosa*) pour lesquels des tests qPCR étaient disponibles dans le laboratoire. L'ADN de l'alamidonte renflée a été détecté à l'intérieur de sa répartition connue dans les bassins versants des rivières Miramichi et Petitcodiac, ainsi que pour la première fois dans le bassin versant de la rivière Saint-Jean. L'ADN de l'alamidonte renflée n'a pas été détecté dans le nord du Nouveau-Brunswick ni dans le bassin versant de la rivière Richibucto, malgré une distribution connue dans les bassins versants voisins des rivières Kouchibouguacis et Bouctouche. L'ADN de la lampsile jaune a été détecté à l'intérieur de sa répartition connue dans la partie inférieure du bassin versant de la rivière Saint-Jean. L'ADN de la lampsile jaune n'a pas été détecté dans le nord du Nouveau-Brunswick ni dans le bassin versant de la rivière Miramichi. L'ADN de la mulette perlière de l'Est a été détecté à tous les sites testés dans le bassin versant de la rivière Miramichi, où l'espèce est connue d'être relativement abondante. En améliorant les connaissances sur la répartition des espèces de moules d'eau douce en péril, ces travaux contribuent aux objectifs des plans de gestion du MPO visant à conserver l'habitat où ces espèces existent actuellement ou sont découvertes.

1. INTRODUCTION

Freshwater mussels are an important indicator of watercourse health (Fisheries and Oceans Canada, 2018). As benthic filter feeders, they improve habitat conditions by stabilizing sediment and removing detritus from the water column. The brook floater is a freshwater mussel species currently assessed as Special Concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and listed as Special Concern under the *Species at Risk Act* (SARA)(COSEWIC, 2022). In Canada, its only known distribution is in New Brunswick and Nova Scotia in 13 widely scattered watersheds. A management plan was published in 2018 to identify the measures needed to conserve the species and prevent further declines, range loss or worsened status designation (Fisheries and Oceans Canada, 2018). Water sampling with environmental DNA-based methods has been utilized as one of the main tools to address the plan's research conservation measure of improving knowledge on brook floater distribution in Canada (LeBlanc et al., 2021a). Environmental DNA methods are generally accepted as being less disruptive, more sensitive, and more cost-efficient than traditional survey methods (Biggs et al., 2015; Sigsgaard, Carl, Moller, & Thomsen, 2015). The increased sensitivity of eDNA makes its use particularly applicable for threatened or endangered species which are generally found in low abundances and are sometimes cryptic in nature. In LeBlanc et al., 2021a, a targeted species-specific qPCR assay was developed for this species and initial survey work was conducted in 2017 and 2018 at a limited number of watersheds in New Brunswick.

In an effort to continue gathering important distribution and range data for the brook floater in New Brunswick, additional efforts have been undertaken to investigate more watersheds by collecting water samples for freshwater mussel eDNA testing or by testing samples already collected for other purposes, such as aquatic invasive species monitoring (LeBlanc et al., 2021a; LeBlanc et al., 2021b; Irlich et al., 2022). At the same time, opportunistic testing was conducted for other freshwater mussel species for which qPCR assays were available in house (LeBlanc et al., 2021a), i.e., the yellow lampmussel (*Lampsilis cariosa*) and eastern pearlshell (*Margaritifera margaritifera*). Yellow lampmussel is a freshwater mussel species listed as Special Concern under SARA which is found only in the Sydney River and three lakes (Blacketts Lake, Pottle Lake and Forresters Lake) in Cape Breton, Nova Scotia, and the lower Saint John River watershed below the Mactaquac Dam in New Brunswick (Fisheries and Oceans Canada, 2025). Any further data on species distribution would be of value to support monitoring and conservation efforts. The eastern pearlshell is relatively more abundant than both the brook floater and yellow lampmussel in New Brunswick and while it is not currently considered at risk, reporting on occurrence is also valuable (Baisley, 2010).

This report presents the finding of the eDNA-based detection of freshwater mussels using targeted species-specific qPCR methods, conducted from 2020 to 2024 to help advance knowledge on the distribution of the species and guide conservation approaches.

2. MATERIALS AND METHODS

Water samples collected from watersheds in New Brunswick and over the border into Quebec for eDNA-based species detection were tested for up to three species of freshwater mussels: brook floater, eastern pearlshell, and yellow lampmussel. Sampling was conducted between 2020 and 2024 by DFO, local environmental non-governmental organizations, and Indigenous groups. Some of the eDNA surveys were targeted towards freshwater mussel detection, while others had the primary purpose of monitoring for aquatic invasive species and the samples were subsequently tested for freshwater mussels.

In 2020, eDNA samples were collected from the Miramichi River watershed in September and October and tested for brook floater and eastern pearlshell (Figures 1, 2 & 3). In 2021, eDNA samples were collected from the Miramichi River watershed in June and July and tested for brook floater (Figures 1 & 4). In 2022, eDNA samples were collected from the Miramichi River watershed in July and tested for brook floater and yellow lampmussel. Additional sampling was done in the Bartibog River in October and samples were tested for brook floater, eastern pearlshell, and yellow lampmussel (Figures 1, 5 - 7). In 2023, eDNA samples were collected from the Miramichi River watershed in July and August, and from the Richibucto and Tabusintac Rivers in August and September, and tested for brook floater. Additional eDNA samples collected from the Saint John River watershed from June to October were tested for brook floater and yellow lampmussel (Figures 1, 8 & 9). In 2024, eDNA samples were collected from across New Brunswick and into Quebec from June until October and tested for brook floater and yellow lampmussel, with the exception of those sampled in the Caraquet, Pokemouche, Bartibog, Burnt Church, Big Tracadie and Little Tracadie rivers in August which were only tested for brook floater (Figures 1, 10 & 11).

Filtrations were either done in the field or water samples were kept on ice until filtered in the laboratory or a field station within 24 hours of collection. In-field filtrations were either done with OSMOS (Halltech, ON, CAN) or Citizen Scientist (Smith-Root, WA, USA) eDNA samplers. Laboratory and field station filtrations were done with a Gast™ oil-less diaphragm-type pressure/vacuum pump (Fisher Scientific, MA, USA). Either 47 mm diameter 1.5 µm Whatman 934-AH glass microfibre filters (GE Healthcare, IL, USA), 47 mm diameter 0.8 µm Whatman nylon membrane filters (GE Healthcare, IL, USA), or 1.2 µm Whatman GF/C glass microfibre filters (GE Healthcare, IL, USA) were used, depending on the sampling year. Filtration method and filter type are indicated in Table 1. Filters were preserved in microcentrifuge tubes containing 200 µl of 95% non-denatured ethanol and frozen at -20°C until DNA extraction.

DNA was extracted from half filters with the MN NucleoSpin Tissue Kit (Macherey-Nagel, PA, USA) following a modified protocol (Appendix 1). Changes were made to the protocol between years to optimize eDNA recovery. DNA extracts were stored at -20°C.

Freshwater mussel testing was done with targeted, species-specific qPCR assays using the 2x TaqMan Gene Expression Kit (Thermo Fisher Scientific, MA, USA) in 2020 and 2x TaqPath ProAmp Master Mix (Thermo Fisher Scientific, MA, USA) from 2021 to 2024. All qPCR tests were done in duplicate in 2020, and in triplicate from 2021 to 2024. Cycle threshold (Ct) values

were converted to eDNA concentration (pg/L of sample water) using standard curve equations specific to each assay.

Table 1. Summary of sample collection and filtration method for each sampling year.

Year	Target Volume (L)	Filter Type	Filtration Method	Sampling Locations
2020	1.0	0.8 µm Nylon	Laboratory	Miramichi River watershed
2021	1.0	0.8 µm Nylon 1.2 µm GF*	Laboratory	Miramichi River watershed
2022	5.0	1.2 µm GF	OSMOS	Miramichi River watershed
2023	1.0 – 5.0	1.2 µm GF	Citizen Scientist, OSMOS, Field station	Richibucto River, Tabusintac River, Saint John River watershed, Miramichi River watershed
2024	1.5 – 5.0	1.2 µm GF	Citizen Scientist, OSMOS	New Brunswick and Quebec

*Generally filtered on 0.8µm Nylon, but 1.2µm GF used when Nylon filters clogged.

For quality control purposes, an Internal Positive Control (IPC) was spiked into all samples and tested using a qPCR assay specific to the IPC, using the same parameters and reagents as the species specific qPCR assays. Samples from 2020 were spiked following DNA extraction with a linearized DNA plasmid containing a DNA sequence not found in the targeted environments. Samples from 2021 were spiked with a bacteriophage during laboratory filtration while samples from 2022 to 2024 were spiked with the bacteriophage at the beginning of the DNA extraction. The IPC was used to confirm filtration and/or extraction functioned as expected, and that PCR inhibitors were not present in the samples.

Tap water was transported and filtered as field blanks along side the water samples. When filtrations were done in the laboratory, additional tap water filtration blanks were processed for approximately every 10 samples filtered. A minimum of one extraction blank was included in each extraction batch for every 20 samples processed. DNA from any of the three freshwater mussel species was not detected in any field blanks, filtration blanks, or extraction blanks, indicating no evidence of contamination in the filtration and extraction processes.

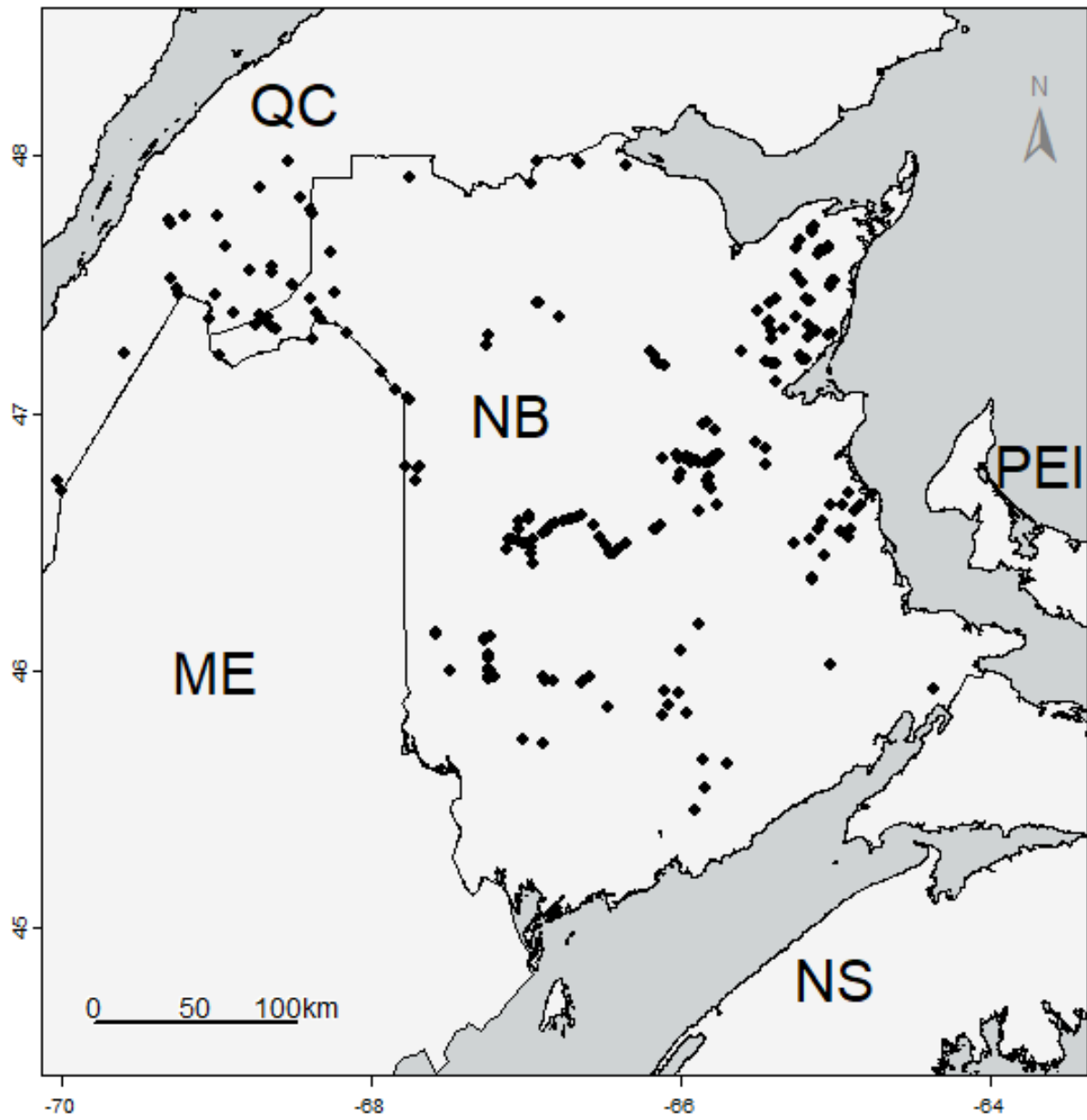


Figure 1. Map of eDNA sampling locations from 2020 to 2024.

3. RESULTS

A sample is considered detected if the target species DNA is amplified in all qPCR technical replicates, inconclusive if DNA is amplified in only some of the technical replicates, and not detected if no DNA amplification is observed in any technical replicate. A sampling site is also identified as detected, inconclusive, or not detected based on the results of the samples from that site, where a detected site has at least one detected sample or two or more inconclusive samples, an inconclusive site has only one inconclusive sample, and a not detected site has all not detected samples.

In 2020, water samples were collected from 18 sites in the Northwest Miramichi, Renous and Dungarvon rivers and tested for brook floater and eastern pearlshell DNA (Table 2). Three sites in the Renous River (AH, AJ, AK) were identified as inconclusive for brook floater (Figure 2). Eastern pearlshell was detected at all sites (Figure 3). Testing was not done for yellow lampmussel.

In 2021, water samples were collected and tested for brook floater DNA from 46 sites in the Southwest Miramichi River and tributaries, with three sampling events at site AX and two at BB (Table 3). Brook floater DNA was detected in the Southwest Miramichi River at sites AX, AY, and 11 sites between Doaktown (CJ) and Renous (BV), as well as at two sites in the Barnaby River (BX and BW) (Figure 4). Five sites were identified as inconclusive, including three in the Southwest Miramichi River between BE and CE, one in the Taxis River (CA) and one in the Renous River (BS), near their confluences with the Southwest Miramichi River. Testing was not done for eastern pearlshell or yellow lampmussel.

In 2022, water samples were collected and tested for brook floater and yellow lampmussel DNA from 26 sites in the Southwest Miramichi River and tributaries, with two sampling events at site AX, as well as from two sites in the Bartibog River (Table 4). Brook floater DNA was detected at seven sites in the Southwest Miramichi River between Doaktown (CJ) and Blackville (BP) (Figure 5). Only the two sites in the Bartibog River were tested for eastern pearlshell DNA, both of which were detected (CM and CN) (Figure 6). Yellow lampmussel DNA was tested for but not detected at any of the sampling sites (Figure 7).

In 2023, water samples were collected and tested for brook floater DNA from 61 sites, including 31 in the Southwest Miramichi River and its tributaries, 15 sites in the Richibucto River watershed, 15 sites in the Tabusintac River and its tributaries. Additional water samples were collected and tested for brook floater and yellow lampmussel DNA from 51 sites in the Saint John River watershed, with two sampling events at sites EP, FR, FS and FU (Table 5). Brook floater DNA was detected in the Southwest Miramichi River at site AY as well as at 11 sites between Boiestown (CH) and Renous (BV), and was inconclusive at sites CQ and BA (Figure 8). In the Saint John River watershed, brook floater was detected in the Kennebecasis River at Hampton (EU) and inconclusive in the Jemseg River (ER). Samples from the Saint John River watershed were tested for yellow lampmussel DNA, which was detected in Grand Lake (EK) (Figure 9). Testing was not done for eastern pearlshell.

In 2024, water samples were collected and tested for brook floater DNA from 121 sites across New Brunswick and Quebec (Table 6). Sites GF, FB and FI were each sampled twice. Brook floater DNA was detected at site HM in the Petitcodiac River at Salisbury, New Brunswick, as well as at six sites in the Miramichi River watershed, including the Northwest Miramichi River (IF), Renous River (BS and BQ), Barnaby River (BW), Bartholomew River (IJ), and Cains River (IH) (Figure 10). Two sites in the Dungarvon River (II) and the Little Southwest Miramichi River (IG) were inconclusive. Ninety-one sites, all except those on the Acadian Peninsula, were tested for yellow lampmussel DNA. Sites FI in the Saint John River and EU in the Kennebecasis River were identified as detected and site ES in Washademoak Lake was identified as inconclusive (Figure 11). Testing was not done for eastern pearlshell.

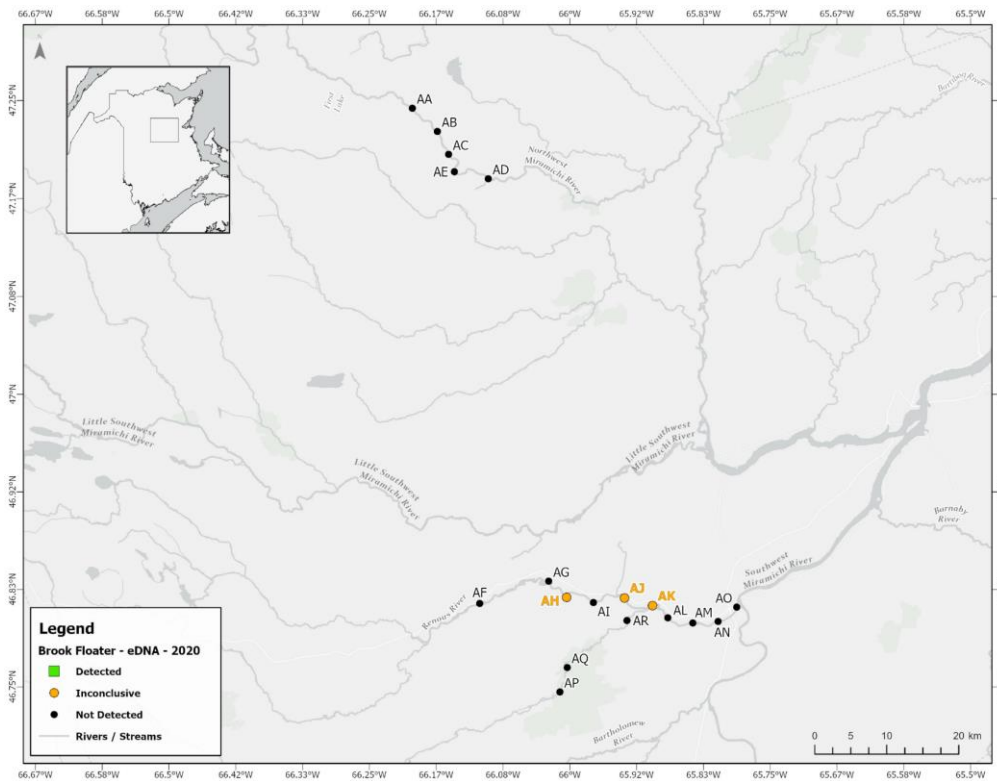


Figure 2. Map of occurrence results for sampling sites tested for brook floater eDNA 2020.

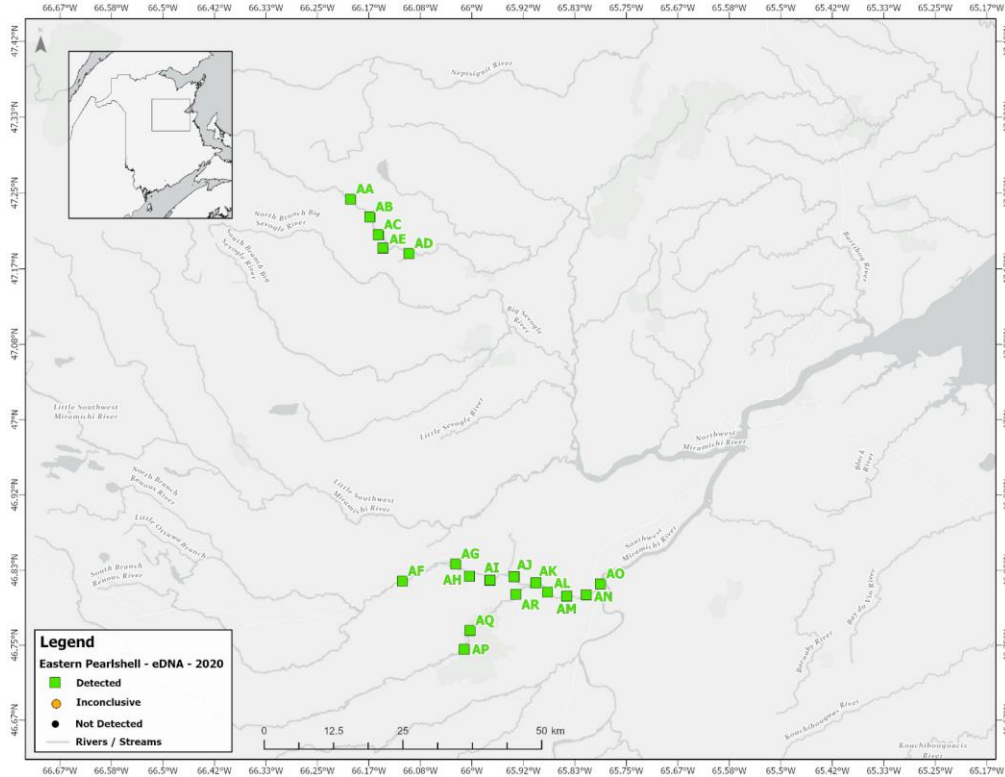


Figure 3. Map of occurrence results for sampling sites tested for eastern pearlshell eDNA in 2020.

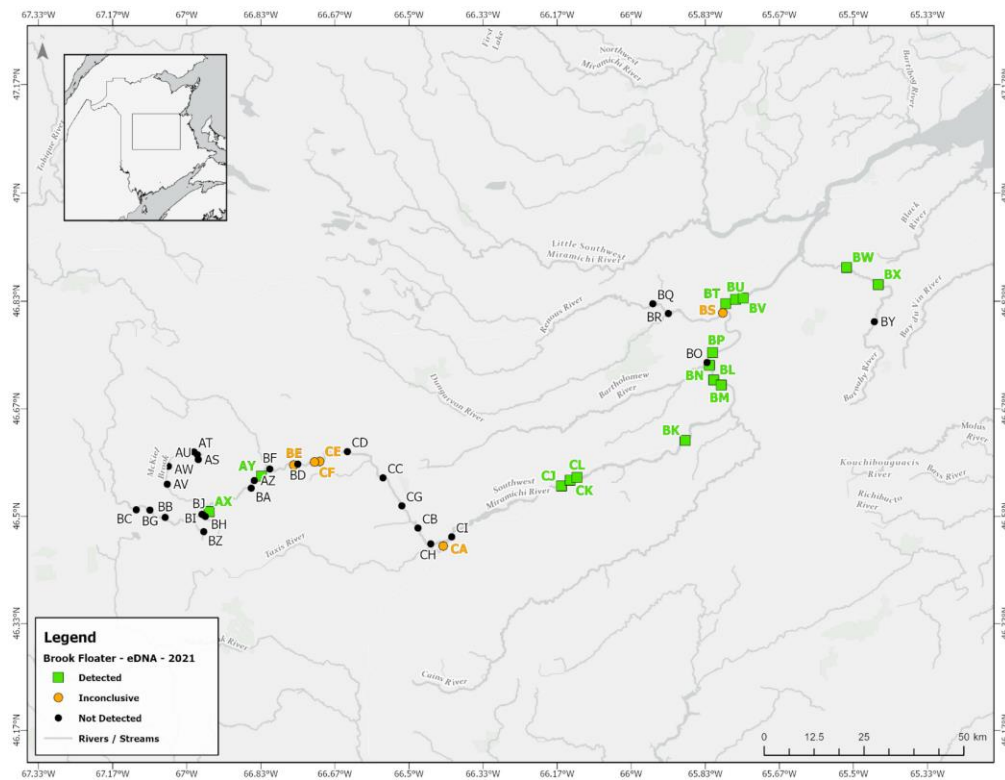


Figure 4. Map of occurrence results for sampling sites tested for brook floater eDNA in 2021.

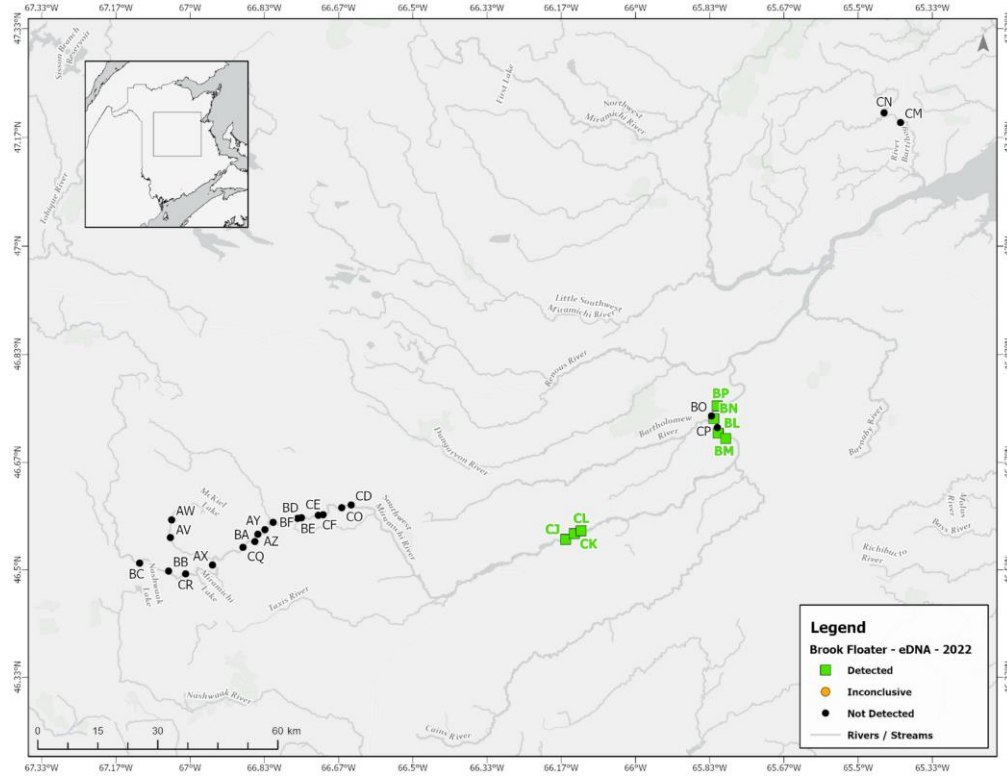


Figure 5. Map of occurrence results for sampling sites tested for brook floater eDNA in 2022.

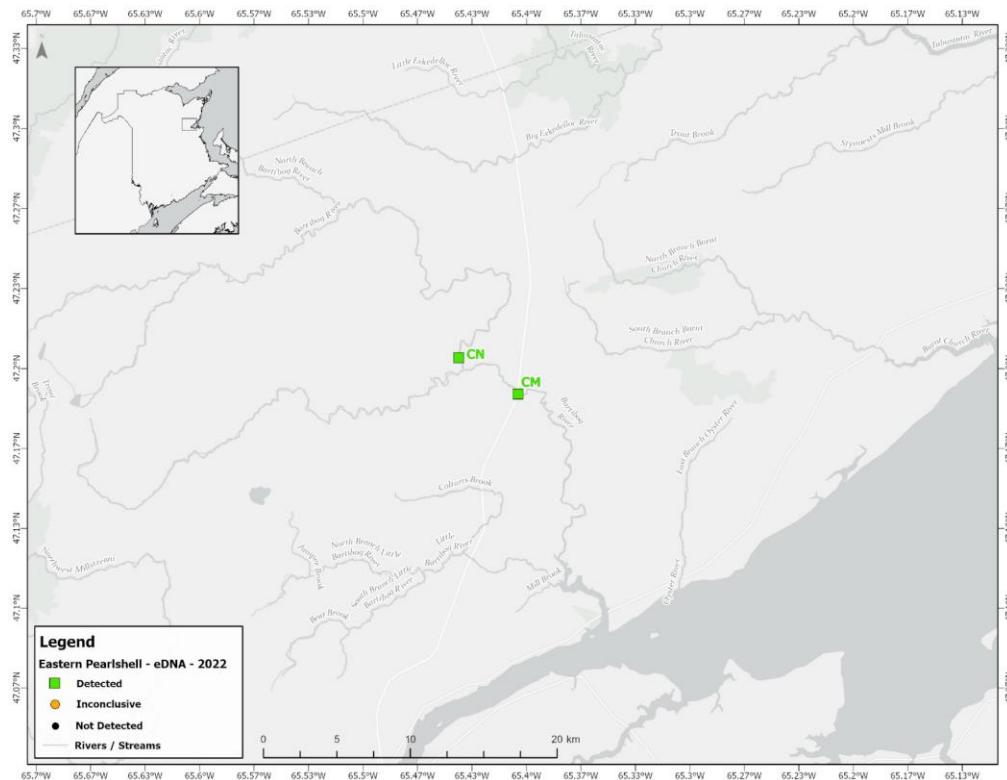


Figure 6. Map of occurrence results for sampling sites tested for eastern pearlshell eDNA in 2022.

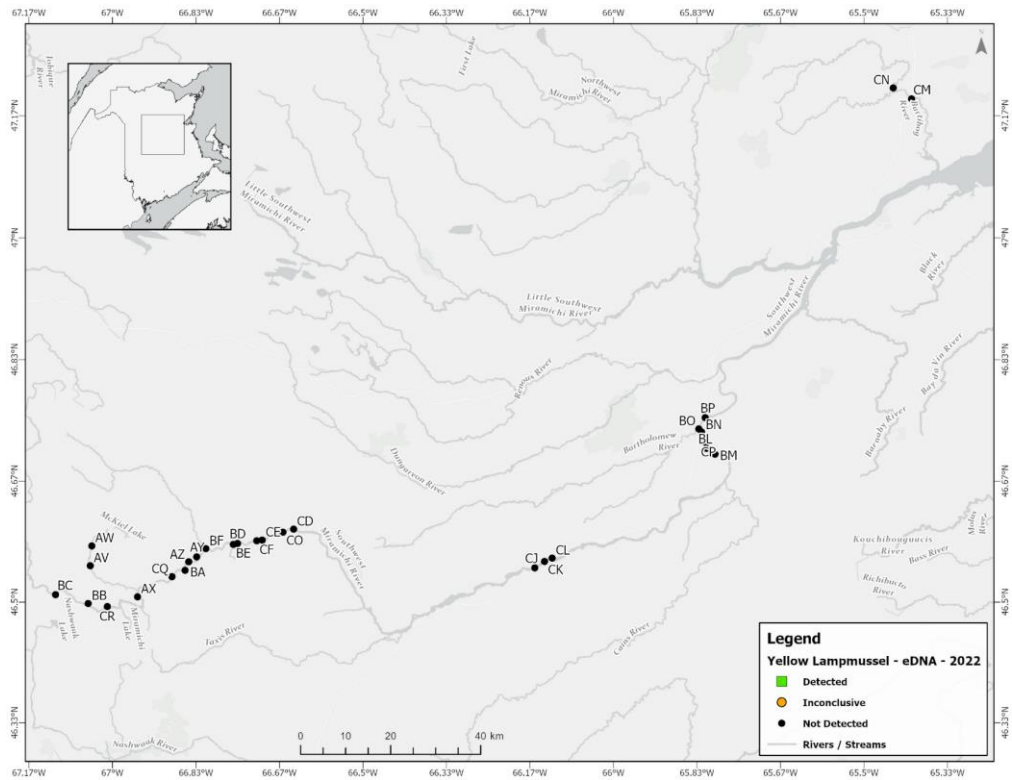


Figure 7. Map of occurrence results for sampling sites tested for yellow lampmussel eDNA in 2022.

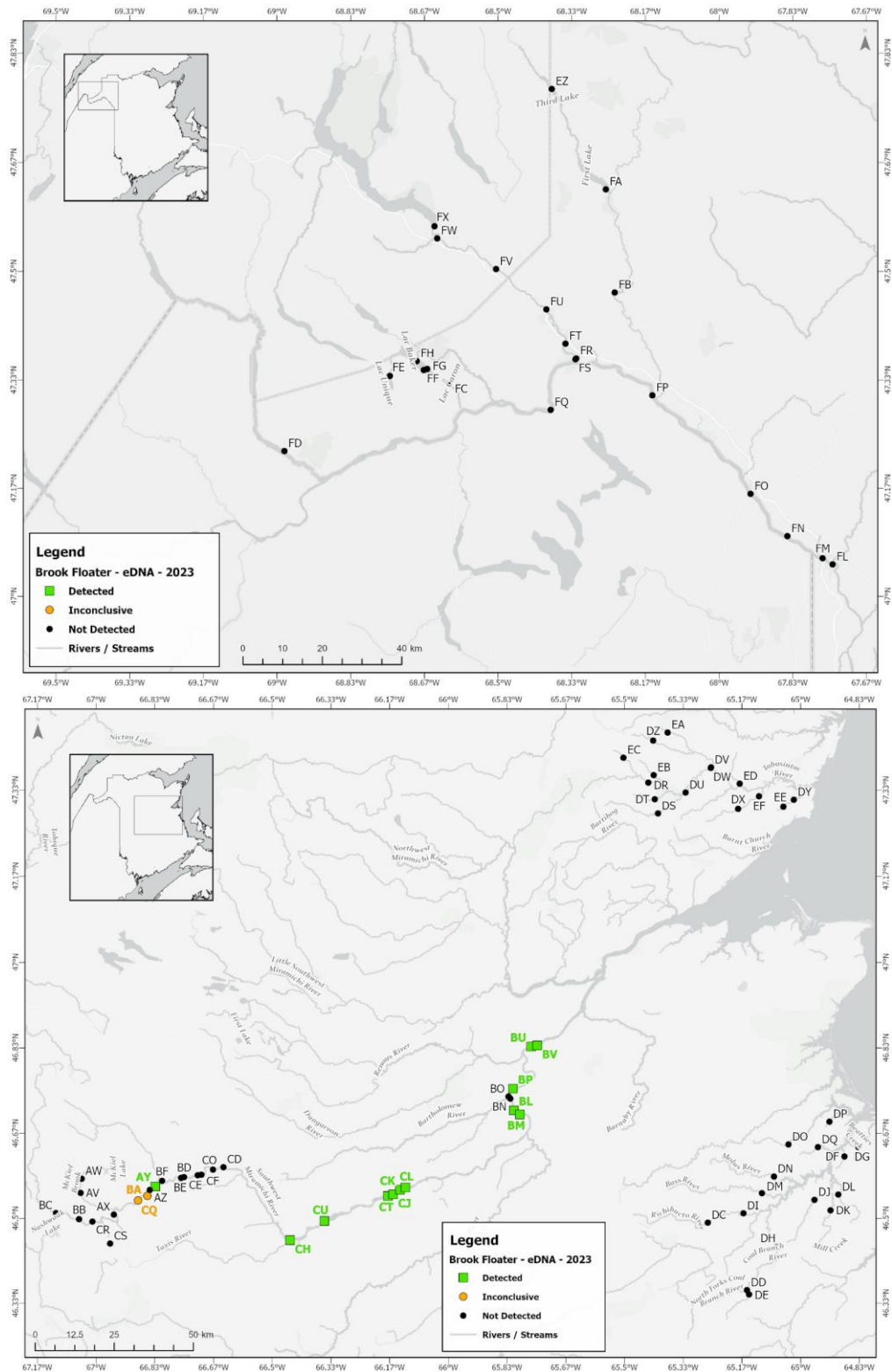


Figure 8. Maps of occurrence results for sampling sites tested for brook floater eDNA in 2023. (*Figure continued on next page.*)

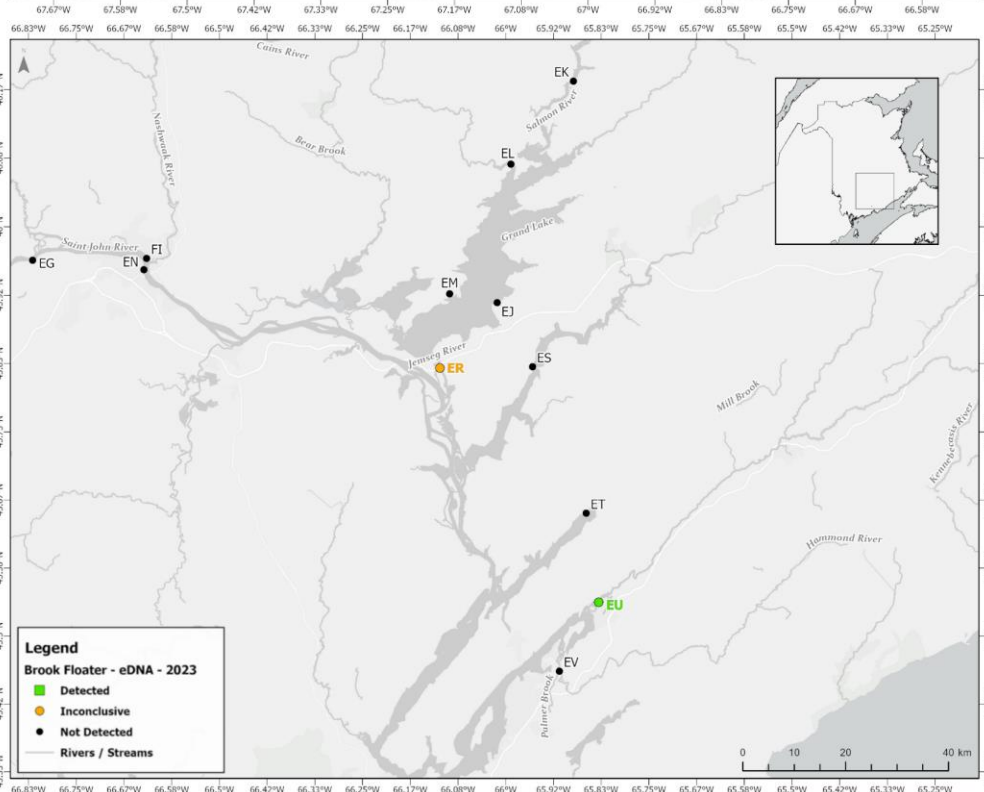
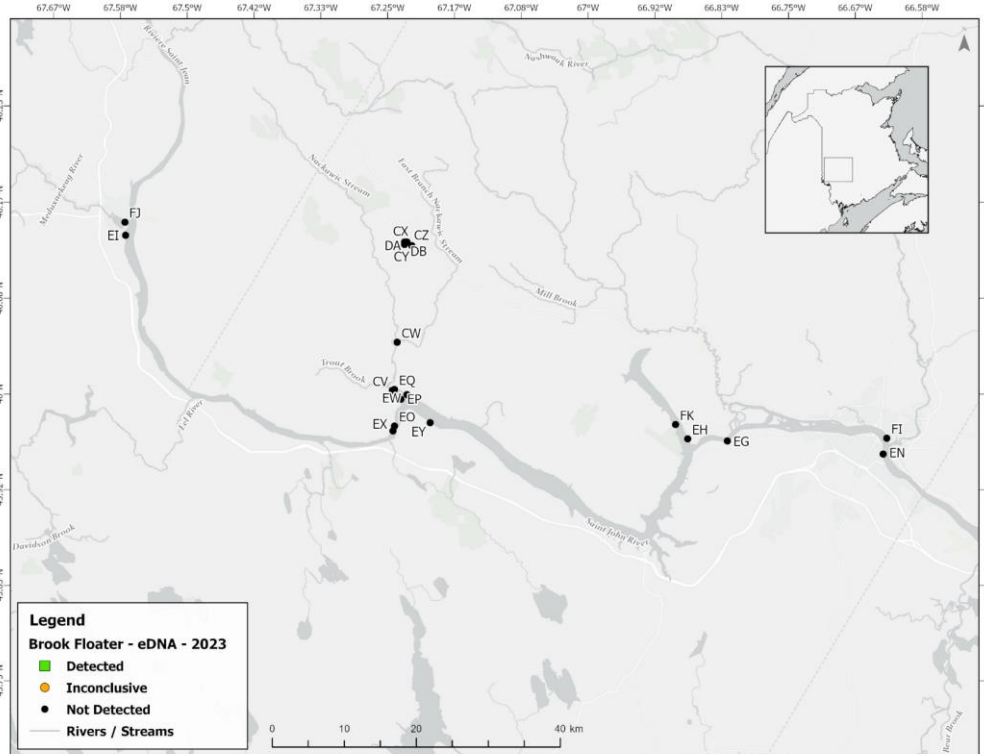


Figure 8 continued. Maps of occurrence results for sampling sites tested for brook floater eDNA in 2023.

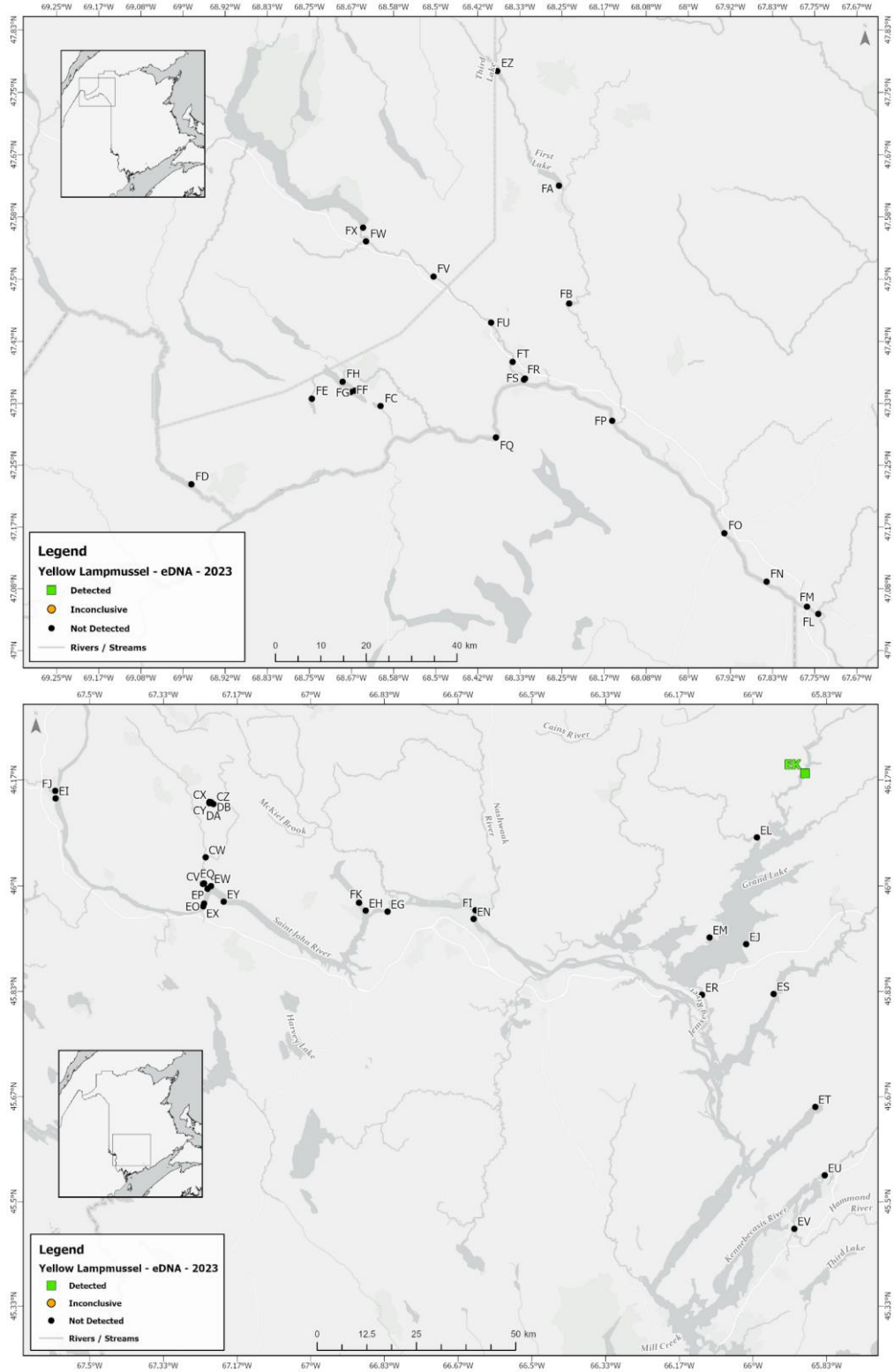


Figure 9. Maps of occurrence results for sampling sites tested for yellow lampmussel eDNA in 2023.

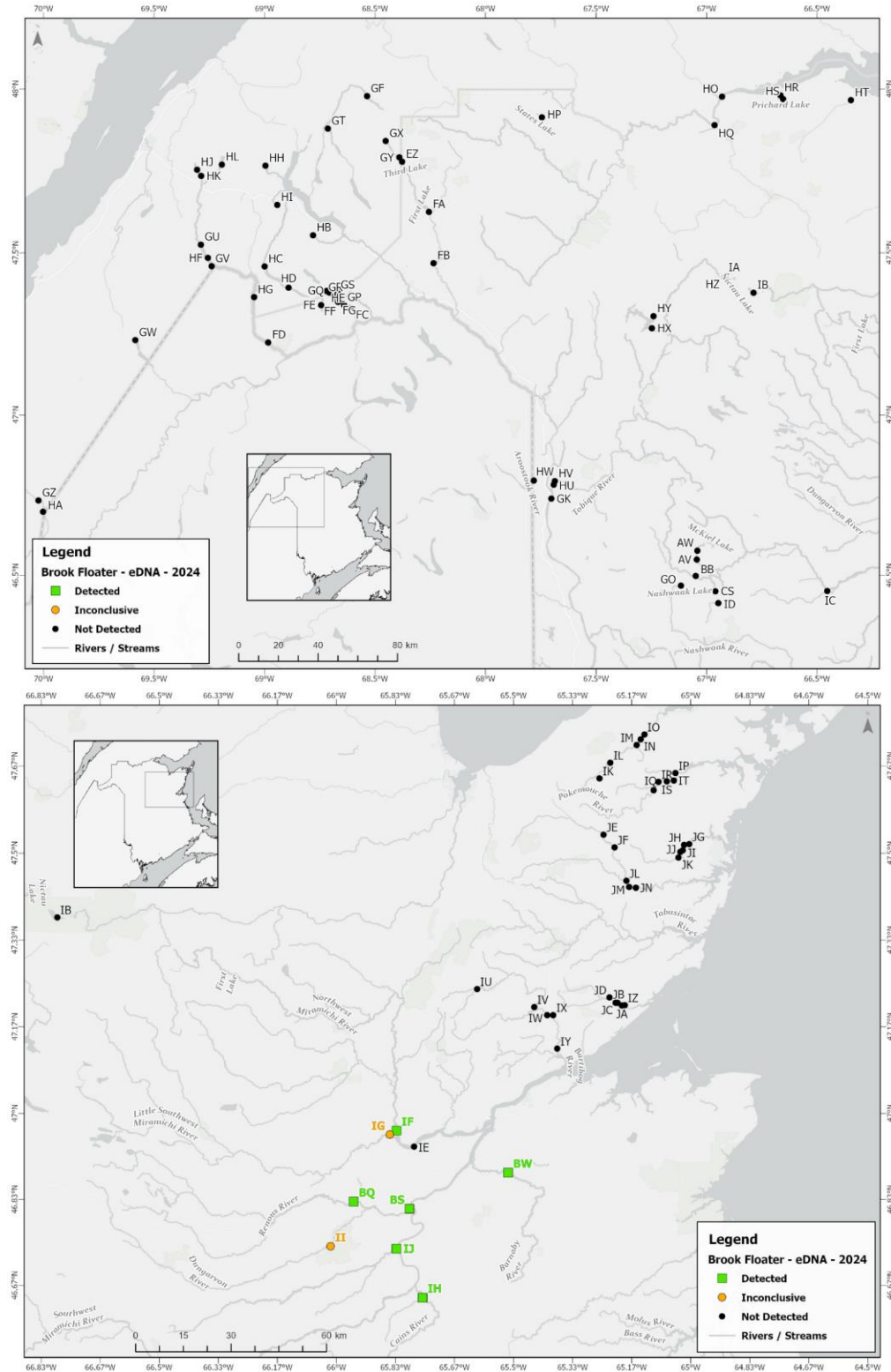


Figure 10. Maps of occurrence results for sampling sites tested for brook floater eDNA in 2024. (Figure continued on next page.)

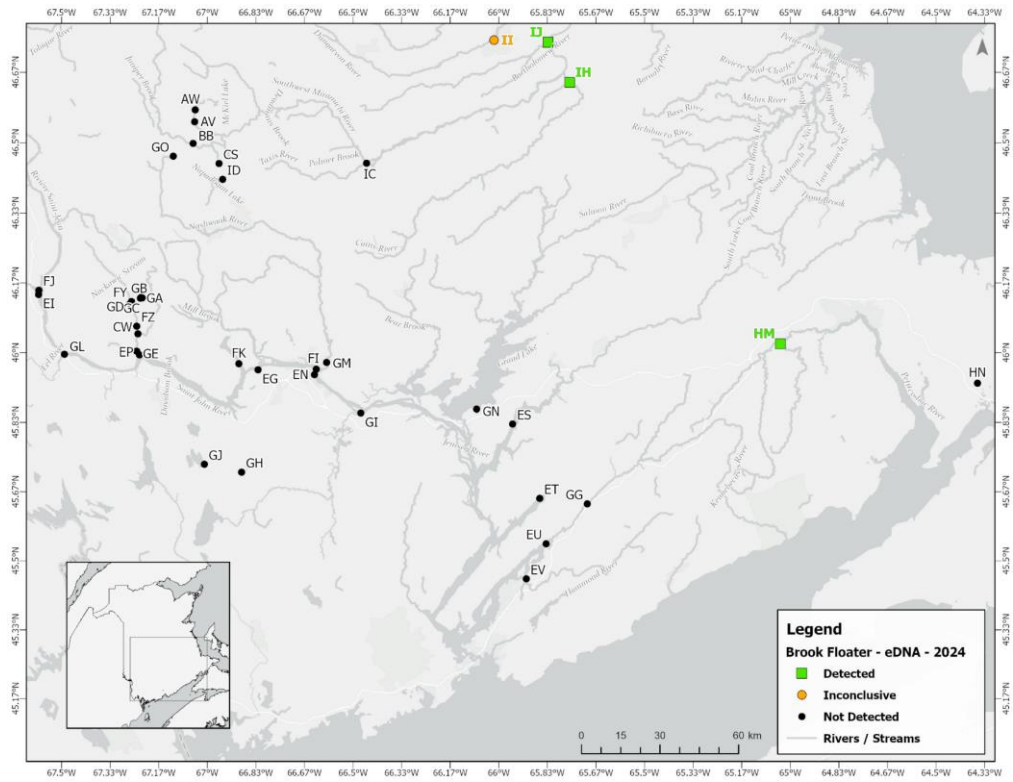


Figure 10 *continued*. Maps of occurrence results for sampling sites tested for brook floater eDNA in 2024.

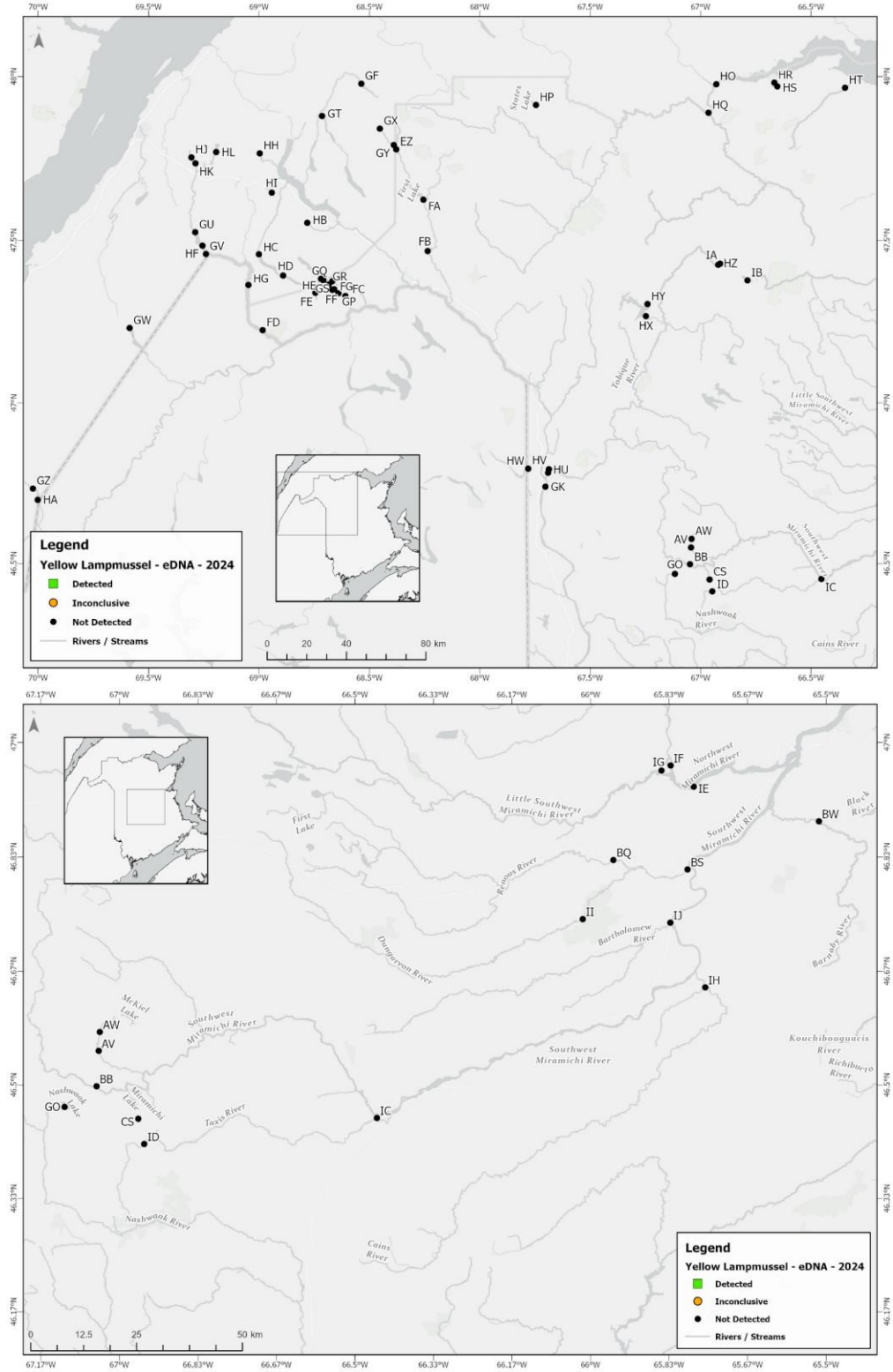


Figure 11. Maps of occurrence results for sampling sites tested for yellow lampmussel eDNA in 2024.
(Figure continued on next page.)

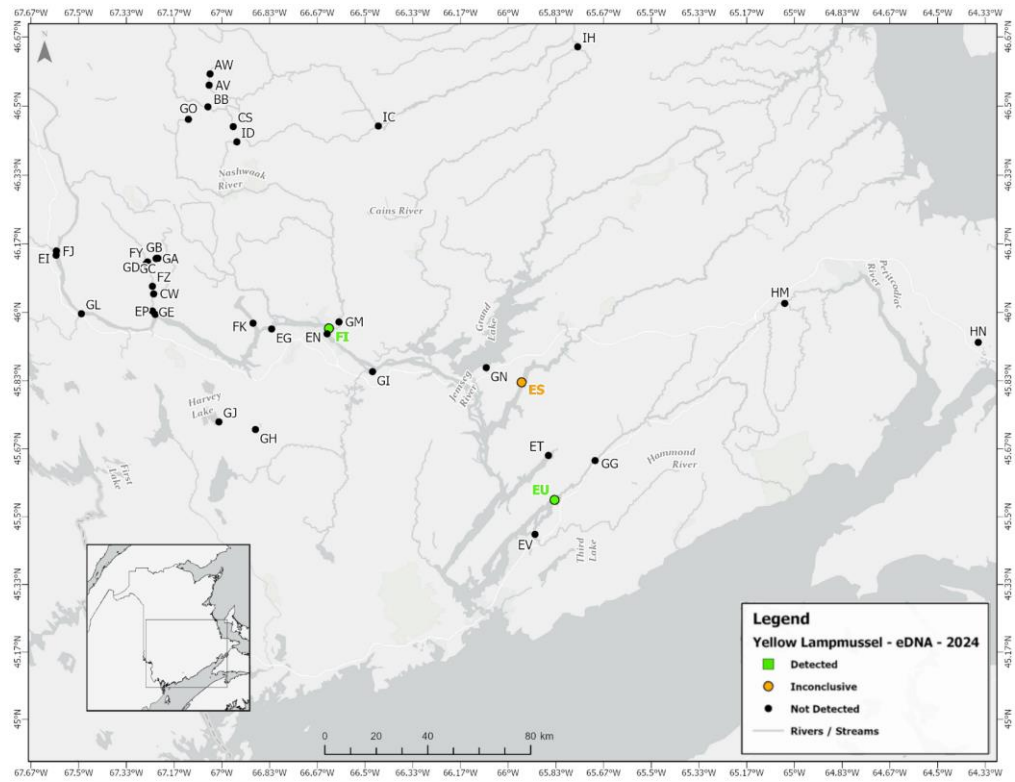


Figure 11 *continued*. Maps of occurrence results for sampling sites tested for yellow lampmussel eDNA in 2024.

Table 2. Date, location, sample number (n), and freshwater mussel eDNA occurrence for sampling events in 2020

Date	Waterbody	n	Site Code	Latitude	Longitude	Brook Floater Site Result	Eastern Pearlshell Site Result	Yellow Lampmussel Site Result
2020-09-17	Northwest Miramichi River	3	AA	47.2434	-66.1963	Not Detected	Detected	NA
2020-09-17	Northwest Miramichi River	3	AB	47.2238	-66.1651	Not Detected	Detected	NA
2020-09-17	Northwest Miramichi River	3	AC	47.2042	-66.1512	Not Detected	Detected	NA
2020-09-21	Northwest Miramichi River	3	AD	47.1835	-66.1017	Not Detected	Detected	NA
2020-09-21	Northwest Miramichi River	3	AE	47.1894	-66.1439	Not Detected	Detected	NA
2020-09-25	Renous River	3	AF	46.8213	-66.1125	Not Detected	Detected	NA
2020-09-25	Renous River	3	AG	46.8403	-66.0262	Not Detected	Detected	NA
2020-09-25	Renous River	3	AH	46.8267	-66.0038	Inconclusive	Detected	NA
2020-09-25	Renous River	3	AI	46.8222	-65.9706	Not Detected	Detected	NA
2020-09-25	Renous River	3	AJ	46.8261	-65.9317	Inconclusive	Detected	NA
2020-09-29	Renous River	3	AK	46.8194	-65.8965	Inconclusive	Detected	NA
2020-09-29	Renous River	3	AL	46.8090	-65.8775	Not Detected	Detected	NA
2020-09-29	Renous River	3	AM	46.8046	-65.8466	Not Detected	Detected	NA
2020-09-29	Renous River	3	AN	46.8060	-65.8148	Not Detected	Detected	NA
2020-09-29	Renous River	3	AO	46.8182	-65.7918	Not Detected	Detected	NA
2020-10-01	Dungarvon River	3	AP	46.7455	-66.0125	Not Detected	Detected	NA
2020-10-01	Dungarvon River	3	AQ	46.7665	-66.0031	Not Detected	Detected	NA
2020-10-01	Dungarvon River	3	AR	46.8066	-65.9288	Not Detected	Detected	NA

Table 3. Date, location, sample number (n), and freshwater mussel eDNA occurrence for sampling events in 2021

Date	Waterbody	n	Site Code	Latitude	Longitude	Brook Floater Site Result	Eastern Pearlshell Site Result	Yellow Lampmussel Site Result
2021-06-22	McKiel Lake	3	AS	46.5882	-66.9739	Not Detected	NA	NA
2021-06-22	McKiel Lake	3	AT	46.6001	-66.9833	Not Detected	NA	NA
2021-06-22	McKiel Lake	3	AU	46.5957	-66.9760	Not Detected	NA	NA
2021-06-22	McKiel Brook	3	AV	46.5501	-67.0440	Not Detected	NA	NA
2021-06-22	McKiel Brook	3	AW	46.5777	-67.0413	Not Detected	NA	NA
2021-06-22	Southwest Miramichi River	3	AX	46.5075	-66.9498	Not Detected	NA	NA
2021-07-07	Southwest Miramichi River	3	AY	46.5626	-66.8320	Detected	NA	NA
2021-07-07	Southwest Miramichi River	3	AZ	46.5555	-66.8477	Not Detected	NA	NA
2021-07-07	Southwest Miramichi River	3	BA	46.5439	-66.8549	Not Detected	NA	NA
2021-07-07	Southwest Miramichi River	3	AX	46.5075	-66.9498	Not Detected	NA	NA
2021-07-07	Southwest Miramichi River	3	BB	46.4983	-67.0485	Not Detected	NA	NA
2021-07-07	Southwest Miramichi River	3	BC	46.5105	-67.1137	Not Detected	NA	NA
2021-07-19	Southwest Miramichi River	3	BD	46.5810	-66.7501	Not Detected	NA	NA
2021-07-19	Southwest Miramichi River	3	BE	46.5797	-66.7589	Inconclusive	NA	NA
2021-07-19	Southwest Miramichi River	3	BF	46.5737	-66.8134	Not Detected	NA	NA
2021-07-19	Southwest Miramichi River	3	BB	46.4983	-67.0485	Not Detected	NA	NA
2021-07-19	Southwest Miramichi River	3	BG	46.5098	-67.0828	Not Detected	NA	NA
2021-07-19	Southwest Miramichi River	3	AX	46.5075	-66.9498	Detected	NA	NA
2021-07-19	McKiel Bogan	3	BH	46.4998	-66.9579	Not Detected	NA	NA
2021-07-19	McKiel Bogan	3	BI	46.5026	-66.9634	Not Detected	NA	NA
2021-07-19	McKiel Bogan	3	BJ	46.5033	-66.9655	Not Detected	NA	NA
2021-07-20	Southwest Miramichi River	3	BK	46.6179	-65.8786	Detected	NA	NA
2021-07-20	Southwest Miramichi River	3	BL	46.7117	-65.8143	Detected	NA	NA
2021-07-20	Southwest Miramichi River	3	BM	46.7036	-65.7974	Detected	NA	NA
2021-07-20	Southwest Miramichi River	3	BN	46.7343	-65.8242	Detected	NA	NA
2021-07-20	Bartholomew River	3	BO	46.7384	-65.8295	Not Detected	NA	NA
2021-07-20	Southwest Miramichi River	3	BP	46.7538	-65.8167	Detected	NA	NA

2021-07-20	Renous River	3	BQ	46.8291	-65.9514	Not Detected	NA	NA
2021-07-20	Renous River	3	BR	46.8143	-65.9167	Not Detected	NA	NA
2021-07-20	Renous River	3	BS	46.8152	-65.7938	Inconclusive	NA	NA
2021-07-20	Renous River	3	BT	46.8296	-65.7874	Detected	NA	NA
2021-07-20	Southwest Miramichi River	3	BU	46.8360	-65.7653	Detected	NA	NA
2021-07-20	Southwest Miramichi River	3	BV	46.8382	-65.7480	Detected	NA	NA
2021-07-20	Barnaby River	6	BW	46.8852	-65.5153	Detected	NA	NA
2021-07-20	Barnaby River	4	BX	46.8590	-65.4442	Detected	NA	NA
2021-07-20	Barnaby River	3	BY	46.8018	-65.4530	Not Detected	NA	NA
2021-07-20	East branch of Lake Brook	3	BZ	46.4762	-66.9620	Not Detected	NA	NA
2021-07-28	Taxis River	3	CA	46.4541	-66.4230	Inconclusive	NA	NA
2021-07-28	Southwest Miramichi River	3	CB	46.4819	-66.4798	Not Detected	NA	NA
2021-07-28	Southwest Miramichi River	3	CC	46.5601	-66.5582	Not Detected	NA	NA
2021-07-28	Southwest Miramichi River	3	CD	46.6006	-66.6386	Not Detected	NA	NA
2021-07-28	Southwest Miramichi River	3	CE	46.5855	-66.7012	Inconclusive	NA	NA
2021-07-28	Southwest Miramichi River	3	CF	46.5844	-66.7123	Inconclusive	NA	NA
2021-07-28	Southwest Miramichi River	3	CG	46.5165	-66.5161	Not Detected	NA	NA
2021-07-28	Southwest Miramichi River	3	CH	46.4573	-66.4511	Not Detected	NA	NA
2021-07-28	Southwest Miramichi River	3	CI	46.4681	-66.4037	Not Detected	NA	NA
2021-07-28	Southwest Miramichi River	3	CJ	46.5473	-66.1568	Detected	NA	NA
2021-07-28	Southwest Miramichi River	3	CK	46.5562	-66.1375	Detected	NA	NA
2021-07-28	Southwest Miramichi River	4	CL	46.5606	-66.1221	Detected	NA	NA

Table 4. Date, location, sample number (n), and freshwater mussel eDNA occurrence for sampling events in 2022

Date	Waterbody	n	Site Code	Latitude	Longitude	Brook Floater Site Result	Eastern Pearlshell Site Result	Yellow Lampmussel Site Result
2022-07-13	Southwest Miramichi River	3	CE	46.5855	-66.7012	Not Detected	NA	Not Detected
2022-07-13	Southwest Miramichi River	3	CF	46.5844	-66.7123	Not Detected	NA	Not Detected
2022-07-13	Southwest Miramichi River	3	CO	46.5965	-66.6596	Not Detected	NA	Not Detected
2022-07-13	Southwest Miramichi River	3	CD	46.6006	-66.6386	Not Detected	NA	Not Detected
2022-07-13	Southwest Miramichi River	3	CJ	46.5473	-66.1569	Detected	NA	Not Detected
2022-07-15	Southwest Miramichi River	3	CK	46.5563	-66.1375	Detected	NA	Not Detected
2022-07-15	Southwest Miramichi River	3	CL	46.5606	-66.1221	Detected	NA	Not Detected
2022-07-15	Southwest Miramichi River	3	BL	46.7117	-65.8143	Detected	NA	Not Detected
2022-07-15	Southwest Miramichi River	3	CP	46.7201	-65.8165	Not Detected	NA	Not Detected
2022-07-15	Southwest Miramichi River	3	BM	46.7036	-65.7975	Detected	NA	Not Detected
2022-07-15	Southwest Miramichi River	3	BN	46.7343	-65.8242	Detected	NA	Not Detected
2022-07-15	Bartholomew River	3	BO	46.7384	-65.8295	Not Detected	NA	Not Detected
2022-07-15	Southwest Miramichi River	4	BP	46.7538	-65.8167	Detected	NA	Not Detected
2022-07-19	McKiel Brook	3	AW	46.5777	-67.0413	Not Detected	NA	Not Detected
2022-07-19	McKiel Brook	3	AV	46.5501	-67.0440	Not Detected	NA	Not Detected
2022-07-19	Southwest Miramichi River	3	AY	46.5626	-66.8320	Not Detected	NA	Not Detected
2022-07-19	Southwest Miramichi River	3	AZ	46.5555	-66.8477	Not Detected	NA	Not Detected
2022-07-19	Southwest Miramichi River	3	BA	46.5439	-66.8549	Not Detected	NA	Not Detected
2022-07-19	Southwest Miramichi River	3	CQ	46.5350	-66.8813	Not Detected	NA	Not Detected
2022-07-19	Southwest Miramichi River	3	AX	46.5075	-66.9498	Not Detected	NA	Not Detected
2022-07-20	Southwest Miramichi River	3	BD	46.5810	-66.7501	Not Detected	NA	Not Detected
2022-07-20	Southwest Miramichi River	3	BE	46.5797	-66.7589	Not Detected	NA	Not Detected
2022-07-20	Southwest Miramichi River	3	BF	46.5737	-66.8134	Not Detected	NA	Not Detected
2022-07-20	Southwest Miramichi River	4	BC	46.5105	-67.1137	Not Detected	NA	Not Detected
2022-07-20	Southwest Miramichi River	3	BB	46.4983	-67.0485	Not Detected	NA	Not Detected
2022-07-20	Southwest Miramichi River	3	CR	46.4941	-67.0101	Not Detected	NA	Not Detected
2022-07-20	Southwest Miramichi River	4	AX	46.5075	-66.9498	Not Detected	NA	Not Detected

2022-10-17	Bartibog River	3	CM	47.1894	-65.4051	Not Detected	Detected	Not Detected
2022-10-17	Bartibog River	3	CN	47.2046	-65.4415	Not Detected	Detected	Not Detected

Table 5. Date, location, sample number (n), and freshwater mussel eDNA occurrence for sampling events in 2023

Date	Waterbody	n	Site Code	Latitude	Longitude	Brook Floater Site Result	Eastern Pearlshell Site Result	Yellow Lampmussel Site Result
2023-06-07	Nackawick Stream	6	CV	46.0030	-67.2440	Not Detected	NA	Not Detected
2023-06-07	Nackawick Stream	3	CW	46.0450	-67.2380	Not Detected	NA	Not Detected
2023-06-07	Westie Lake	3	CX	46.1320	-67.2290	Not Detected	NA	Not Detected
2023-06-08	Westie Lake	3	CY	46.1300	-67.2290	Not Detected	NA	Not Detected
2023-06-08	Big Lake	3	CZ	46.1290	-67.2200	Not Detected	NA	Not Detected
2023-06-08	Big Lake	3	DA	46.1319	-67.2258	Not Detected	NA	Not Detected
2023-06-08	Big Lake	3	DB	46.1318	-67.2270	Not Detected	NA	Not Detected
2023-06-22	Madawaska River	3	FU	47.4419	-68.3905	Not Detected	NA	Not Detected
2023-06-22	Madawaska River	3	FR	47.3668	-68.3231	Not Detected	NA	Not Detected
2023-06-22	Madawaska River	3	FS	47.3653	-68.3251	Not Detected	NA	Not Detected
2023-07-10	Saint John River	3	FL	47.0493	-67.7430	Not Detected	NA	Not Detected
2023-07-10	Saint John River	3	FM	47.0591	-67.7655	Not Detected	NA	Not Detected
2023-07-10	Saint John River	3	FN	47.0929	-67.8454	Not Detected	NA	Not Detected
2023-07-10	Saint John River	3	FO	47.1582	-67.9286	Not Detected	NA	Not Detected
2023-07-10	Saint John River	3	FP	47.3097	-68.1507	Not Detected	NA	Not Detected
2023-07-11	Saint John River	3	FQ	47.2876	-68.3808	Not Detected	NA	Not Detected
2023-07-11	Madawaska River	3	FR	47.3666	-68.3230	Not Detected	NA	Not Detected
2023-07-11	Madawaska River	3	FS	47.3652	-68.3251	Not Detected	NA	Not Detected
2023-07-11	Madawaska River	3	FT	47.3893	-68.3475	Not Detected	NA	Not Detected
2023-07-11	Madawaska River	3	FU	47.4418	-68.3905	Not Detected	NA	Not Detected
2023-07-11	Madawaska River	3	FV	47.5037	-68.5045	Not Detected	NA	Not Detected
2023-07-12	Madawaska River	3	FW	47.5507	-68.6377	Not Detected	NA	Not Detected
2023-07-12	Madawaska River	3	FX	47.5693	-68.6438	Not Detected	NA	Not Detected
2023-07-18	Southwest Miramichi River	3	BD	46.5810	-66.7501	Not Detected	NA	NA
2023-07-18	Southwest Miramichi River	3	BE	46.5795	-66.7588	Not Detected	NA	NA
2023-07-18	Southwest Miramichi River	3	BF	46.5734	-66.8129	Not Detected	NA	NA
2023-07-18	Southwest Miramichi River	3	AY	46.5626	-66.8320	Detected	NA	NA

2023-07-18	Southwest Miramichi River	3	AZ	46.5555	-66.8477	Not Detected	NA	NA
2023-07-18	Southwest Miramichi River	3	BA	46.5439	-66.8549	Inconclusive	NA	NA
2023-07-18	Southwest Miramichi River	3	CQ	46.5350	-66.8813	Inconclusive	NA	NA
2023-07-19	Southwest Miramichi River	3	CE	46.5844	-66.7120	Not Detected	NA	NA
2023-07-19	Southwest Miramichi River	3	CF	46.5857	-66.7012	Not Detected	NA	NA
2023-07-19	Southwest Miramichi River	3	CO	46.5954	-66.6681	Not Detected	NA	NA
2023-07-19	Southwest Miramichi River	3	CD	46.6005	-66.6386	Not Detected	NA	NA
2023-07-19	Southwest Miramichi River	4	BC	46.5105	-67.1137	Not Detected	NA	NA
2023-07-19	Southwest Miramichi River	3	BB	46.4983	-67.0485	Not Detected	NA	NA
2023-07-19	Southwest Miramichi River	3	CR	46.4939	-67.0102	Not Detected	NA	NA
2023-07-19	Southwest Miramichi River	3	AX	46.5075	-66.9498	Not Detected	NA	NA
2023-07-25	Southwest Miramichi River	3	CH	46.4574	-66.4497	Detected	NA	NA
2023-07-25	Southwest Miramichi River	3	CT	46.5441	-66.1718	Detected	NA	NA
2023-07-26	Southwest Miramichi River	3	CU	46.4949	-66.3521	Detected	NA	NA
2023-07-26	Southwest Miramichi River	3	CJ	46.5473	-66.1569	Detected	NA	NA
2023-07-26	Southwest Miramichi River	3	CK	46.5563	-66.1375	Detected	NA	NA
2023-07-26	Southwest Miramichi River	3	CL	46.5606	-66.1221	Detected	NA	NA
2023-07-26	Bartholomew River	3	BO	46.7384	-65.8295	Not Detected	NA	NA
2023-07-26	Southwest Miramichi River	3	BN	46.7343	-65.8242	Not Detected	NA	NA
2023-07-27	Southwest Miramichi River	3	BL	46.7117	-65.8143	Detected	NA	NA
2023-07-27	Southwest Miramichi River	3	BM	46.7036	-65.7975	Detected	NA	NA
2023-07-27	Southwest Miramichi River	3	BP	46.7538	-65.8167	Detected	NA	NA
2023-07-27	Southwest Miramichi River	3	BU	46.8361	-65.7653	Detected	NA	NA
2023-07-27	Southwest Miramichi River	3	BV	46.8382	-65.7480	Detected	NA	NA
2023-08-02	Saint John River	3	FI	45.9616	-66.6272	Not Detected	NA	Not Detected
2023-08-10	Glazier Lake	3	FD	47.2242	-68.9834	Not Detected	NA	Not Detected
2023-08-15	Lac Unique	3	FE	47.3395	-68.7449	Not Detected	NA	Not Detected
2023-08-15	Lac Baker	3	FF	47.3487	-68.6681	Not Detected	NA	Not Detected
2023-08-15	Lac Baker	3	FG	47.3505	-68.6601	Not Detected	NA	Not Detected
2023-08-17	Third Lake	3	EZ	47.7786	-68.3783	Not Detected	NA	Not Detected
2023-08-17	First Lake	3	FA	47.6256	-68.2563	Not Detected	NA	Not Detected

2023-08-17	Etang Deuxieme-Sault (Green River)	3	FB	47.4676	-68.2359	Not Detected	NA	Not Detected
2023-08-17	Meduxnekeag River	3	FJ	46.1494	-67.5778	Not Detected	NA	Not Detected
2023-08-17	Saint John River	3	EP	45.9957	-67.2329	Not Detected	NA	Not Detected
2023-08-17	Saint John River	3	FK	45.9732	-66.8909	Not Detected	NA	Not Detected
2023-08-23	Tabusintac River	3	DR	47.3480	-65.4322	Not Detected	NA	NA
2023-08-23	Tabusintac River	3	DS	47.2881	-65.4050	Not Detected	NA	NA
2023-08-23	Tabusintac River	3	DT	47.3158	-65.4139	Not Detected	NA	NA
2023-08-23	Tabusintac River	3	DU	47.3286	-65.3269	Not Detected	NA	NA
2023-08-23	Lac Caron	3	FC	47.3297	-68.6091	Not Detected	NA	Not Detected
2023-08-24	Lac Baker	3	FH	47.3625	-68.6841	Not Detected	NA	Not Detected
2023-08-30	McKiel Brook	3	AW	46.5777	-67.0413	Not Detected	NA	NA
2023-08-30	McKiel Brook	3	AV	46.5501	-67.0440	Not Detected	NA	NA
2023-08-30	Miramichi Lake	3	CS	46.4507	-66.9600	Not Detected	NA	NA
2023-09-01	Richibucto River	3	DC	46.4915	-65.2638	Not Detected	NA	NA
2023-09-01	North Forks Coal Branch River	3	DD	46.3591	-65.1521	Not Detected	NA	NA
2023-09-01	South Forks Coal Branch River	3	DE	46.3507	-65.1460	Not Detected	NA	NA
2023-09-01	Childs Creek	3	DF	46.6214	-64.8757	Not Detected	NA	NA
2023-09-01	Beatties Creek	3	DG	46.6397	-64.8359	Not Detected	NA	NA
2023-09-04	Coal Branch River	3	DH	46.4437	-65.0642	Not Detected	NA	NA
2023-09-04	Richibucto River	3	DI	46.5099	-65.1627	Not Detected	NA	NA
2023-09-04	West Branch St. Nicholas River	3	DJ	46.5365	-64.9606	Not Detected	NA	NA
2023-09-04	South Branch St. Nicholas River	3	DK	46.5159	-64.9150	Not Detected	NA	NA
2023-09-04	East Branch St. Nicholas River	3	DL	46.5467	-64.8932	Not Detected	NA	NA
2023-09-05	Bass River	3	DM	46.5495	-65.1098	Not Detected	NA	NA
2023-09-05	Molus River	3	DN	46.5821	-65.0760	Not Detected	NA	NA
2023-09-05	Riviere Saint-Charles	3	DO	46.6449	-65.0348	Not Detected	NA	NA
2023-09-05	Petit Riviere Aldouane	3	DP	46.6893	-64.9182	Not Detected	NA	NA
2023-09-05	Mill Creek	3	DQ	46.6395	-64.9506	Not Detected	NA	NA
2023-09-07	Tabusintac River	3	DV	47.3771	-65.2547	Not Detected	NA	NA
2023-09-07	Tabusintac River	3	DW	47.3761	-65.2553	Not Detected	NA	NA
2023-09-07	Tabusintac River	3	DX	47.2971	-65.1774	Not Detected	NA	NA

2023-09-07	Tabusintac River	3	DY	47.3146	-65.0195	Not Detected	NA	NA
2023-09-07	Saint John River	3	EO	45.9722	-67.2414	Not Detected	NA	Not Detected
2023-09-07	Saint John River	3	EP	45.9955	-67.2333	Not Detected	NA	Not Detected
2023-09-07	Nackawick Stream	3	EQ	46.0040	-67.2413	Not Detected	NA	Not Detected
2023-09-07	Saint John River	3	EW	45.9995	-67.2258	Not Detected	NA	Not Detected
2023-09-07	Saint John River	3	EX	45.9678	-67.2435	Not Detected	NA	Not Detected
2023-09-07	Saint John River	3	EY	45.9752	-67.1969	Not Detected	NA	Not Detected
2023-09-08	Jemseg River	3	ER	45.8279	-66.1150	Inconclusive	NA	Not Detected
2023-09-08	Washademoak Lake	3	ES	45.8294	-65.9531	Not Detected	NA	Not Detected
2023-09-08	Saint John River	3	ET	45.6505	-65.8592	Not Detected	NA	Not Detected
2023-09-08	Kennebecasis River	3	EU	45.5417	-65.8375	Detected	NA	Not Detected
2023-09-08	Hammond River	3	EV	45.4569	-65.9064	Not Detected	NA	Not Detected
2023-09-22	Tabusintac River	3	DZ	47.4292	-65.4188	Not Detected	NA	NA
2023-09-22	Tabusintac River	3	EA	47.4445	-65.3774	Not Detected	NA	NA
2023-09-22	Tabusintac River	3	EB	47.3622	-65.4174	Not Detected	NA	NA
2023-09-22	Tabusintac River	3	EC	47.3960	-65.5029	Not Detected	NA	NA
2023-09-26	Saint John River	3	EN	45.9478	-66.6319	Not Detected	NA	Not Detected
2023-09-28	Tabusintac River	3	ED	47.3457	-65.1733	Not Detected	NA	NA
2023-09-28	Tabusintac River	3	EE	47.3012	-65.0492	Not Detected	NA	NA
2023-09-28	Tabusintac River	3	EF	47.3214	-65.1179	Not Detected	NA	NA
2023-09-29	Saint John River	3	EG	45.9592	-66.8264	Not Detected	NA	Not Detected
2023-09-29	Saint John River	3	EH	45.9610	-66.8758	Not Detected	NA	Not Detected
2023-09-29	Saint John River	3	EI	46.1378	-67.5770	Not Detected	NA	Not Detected
2023-10-04	Grand Lake	3	EJ	45.9077	-66.0151	Not Detected	NA	Not Detected
2023-10-04	Grand Lake	3	EK	46.1770	-66.8820	Not Detected	NA	Detected
2023-10-04	Newcastle	3	EL	46.0762	-65.9909	Not Detected	NA	Not Detected
2023-10-04	Grand Lake	3	EM	45.9184	-66.0981	Not Detected	NA	Not Detected

Table 6. Date, location, sample number (n), and freshwater mussel eDNA occurrence for sampling events in 2024

Date	Waterbody	n	Site Code	Latitude	Longitude	Brook Floater Site Result	Eastern Pearlshell Site Result	Yellow Lampmussel Site Result
2024-06-14	Kennebecasis River	3	GG	45.6376	-65.6967	Not Detected	NA	Not Detected
2024-07-03	Saint John River	1	EN	45.9477	-66.6320	Not Detected	NA	Not Detected
2024-07-17	Taxis River	3	IC	46.4518	-66.4535	Not Detected	NA	Not Detected
2024-07-17	Napadogan Lake	3	ID	46.4134	-66.9474	Not Detected	NA	Not Detected
2024-07-17	Southwest Miramichi River	3	BB	46.4983	-67.0485	Not Detected	NA	Not Detected
2024-07-23	Northwest Miramichi River	3	IE	46.9358	-65.7807	Not Detected	NA	Not Detected
2024-07-23	Northwest Miramichi River	4	IF	46.9665	-65.8302	Detected	NA	Not Detected
2024-07-23	Little Southwest Miramichi River	3	IG	46.9592	-65.8495	Inconclusive	NA	Not Detected
2024-07-23	Barnaby River	3	BW	46.8852	-65.5153	Detected	NA	Not Detected
2024-07-24	Cains River	3	IH	46.6429	-65.7566	Detected	NA	Not Detected
2024-07-24	Dungarvon River	3	II	46.7427	-66.0165	Inconclusive	NA	Not Detected
2024-07-24	Bartholomew River	3	IJ	46.7378	-65.8308	Detected	NA	Not Detected
2024-07-25	Renous River	3	BQ	46.8291	-65.9514	Detected	NA	Not Detected
2024-07-25	Renous River	3	BS	46.8152	-65.7938	Detected	NA	Not Detected
2024-07-30	Lac de l'Est	3	GW	47.2313	-69.5855	Not Detected	NA	Not Detected
2024-07-30	Riviere Noir Nords -Ouest	3	GZ	46.7343	-70.0233	Not Detected	NA	Not Detected
2024-07-30	Lac Frontiere	6	HA	46.6992	-70.0020	Not Detected	NA	Not Detected
2024-07-31	Petit Lac Squatec	3	GT	47.8800	-68.7144	Not Detected	NA	Not Detected
2024-07-31	Lac Biencourt	6	GF	47.9787	-68.5367	Not Detected	NA	Not Detected
2024-07-31	Lac Lavoie	3	HB	47.5539	-68.7813	Not Detected	NA	Not Detected
2024-07-31	Petitcodiac River	3	HM	46.0217	-65.0342	Detected	NA	Not Detected
2024-07-31	Silver Lake	3	HN	45.9272	-64.3583	Not Detected	NA	Not Detected
2024-08-01	Pohenegamook Lake	3	GU	47.5252	-69.2881	Not Detected	NA	Not Detected
2024-08-01	Pohenegamook Lake	3	GV	47.4589	-69.2402	Not Detected	NA	Not Detected
2024-08-01	Pohenegamook Lake	3	HF	47.4844	-69.2561	Not Detected	NA	Not Detected
2024-08-01	Lac Beau	3	HG	47.3635	-69.0479	Not Detected	NA	Not Detected

2024-08-02	Saint John River	3	FK	45.9736	-66.8916	Not Detected	NA	Not Detected
2024-08-02	Saint John River	3	GH	45.7139	-66.8821	Not Detected	NA	Not Detected
2024-08-02	Saint John River	3	EG	45.9592	-66.8263	Not Detected	NA	Not Detected
2024-08-02	Washademoak Lake	3	ES	45.8295	-65.9530	Not Detected	NA	Inconclusive
2024-08-02	Lac Sload	3	HH	47.7667	-68.9967	Not Detected	NA	Not Detected
2024-08-02	Lac Dole	3	HI	47.6467	-68.9425	Not Detected	NA	Not Detected
2024-08-02	Lac Snt-Francois	3	HJ	47.7544	-69.3057	Not Detected	NA	Not Detected
2024-08-02	Lac Snt-Francois	3	HK	47.7357	-69.2872	Not Detected	NA	Not Detected
2024-08-02	Lac De La Grande Fourche	3	HL	47.7702	-69.1934	Not Detected	NA	Not Detected
2024-08-03	Saint John River	3	GI	45.8554	-66.4737	Not Detected	NA	Not Detected
2024-08-03	Harvey Lake	3	GJ	45.7325	-67.0102	Not Detected	NA	Not Detected
2024-08-04	Lac Asselin	3	GX	47.8421	-68.4521	Not Detected	NA	Not Detected
2024-08-04	Lac Ango	3	GY	47.7918	-68.3897	Not Detected	NA	Not Detected
2024-08-05	Lac Long	3	HC	47.4577	-69.0001	Not Detected	NA	Not Detected
2024-08-05	Lac Long	3	HD	47.3924	-68.8911	Not Detected	NA	Not Detected
2024-08-05	Lac Baker	3	HE	47.3831	-68.7191	Not Detected	NA	Not Detected
2024-08-06	Restigouche River	3	HO	47.9770	-66.9296	Not Detected	NA	Not Detected
2024-08-07	States Lake	3	HP	47.9137	-67.7452	Not Detected	NA	Not Detected
2024-08-07	Restigouche River	3	HQ	47.8903	-66.9642	Not Detected	NA	Not Detected
2024-08-08	McKiel Brook	3	AW	46.5777	-67.0413	Not Detected	NA	Not Detected
2024-08-08	McKiel Brook	3	AV	46.5501	-67.0440	Not Detected	NA	Not Detected
2024-08-08	Upper Reservoir Lake	3	HR	47.9814	-66.6659	Not Detected	NA	Not Detected
2024-08-08	Prichard Lake	3	HS	47.9700	-66.6532	Not Detected	NA	Not Detected
2024-08-08	Charlo River Reservoir	3	HT	47.9661	-66.3464	Not Detected	NA	Not Detected
2024-08-12	First Lake	3	FA	47.6254	-68.2557	Not Detected	NA	Not Detected
2024-08-12	Third Lake	3	EZ	47.7786	-68.3784	Not Detected	NA	Not Detected
2024-08-13	Glazier Lake	3	FD	47.2242	-68.9834	Not Detected	NA	Not Detected
2024-08-13	Lac Unique	3	FE	47.3394	-68.7448	Not Detected	NA	Not Detected
2024-08-13	Lac Baker	3	GP	47.3380	-68.6397	Not Detected	NA	Not Detected
2024-08-13	Tobique River	3	HU	46.7835	-67.6914	Not Detected	NA	Not Detected
2024-08-13	Tobique River	3	HV	46.7945	-67.6869	Not Detected	NA	Not Detected
2024-08-13	Aroostook River	3	HW	46.7959	-67.7811	Not Detected	NA	Not Detected

2024-08-14	Sisson Branch Reservoir	3	HX	47.2680	-67.2476	Not Detected	NA	Not Detected
2024-08-14	Sisson Branch Reservoir	3	HY	47.3049	-67.2406	Not Detected	NA	Not Detected
2024-08-14	Nictau Lake	3	HZ	47.4255	-66.9206	Not Detected	NA	Not Detected
2024-08-14	Nictau Lake	3	IA	47.4289	-66.9126	Not Detected	NA	Not Detected
2024-08-14	Lake Bathurst	3	IB	47.3773	-66.7877	Not Detected	NA	Not Detected
2024-08-19	Etang Deuxieme-Sault (Green River)	3	FB	47.4677	-68.2359	Not Detected	NA	Not Detected
2024-08-21	Saint John River	3	GK	46.7399	-67.7030	Not Detected	NA	Not Detected
2024-08-21	Meduxnekeag River	3	FJ	46.1496	-67.5777	Not Detected	NA	Not Detected
2024-08-21	Saint John River	3	EI	46.1393	-67.5789	Not Detected	NA	Not Detected
2024-08-21	Saint John River	3	GL	45.9967	-67.4903	Not Detected	NA	Not Detected
2024-08-21	Saint John River	3	EP	45.9953	-67.2329	Not Detected	NA	Not Detected
2024-08-21	Lac Baker	3	FF	47.3486	-68.6681	Not Detected	NA	Not Detected
2024-08-21	Lac Baker	3	GQ	47.3780	-68.7083	Not Detected	NA	Not Detected
2024-08-21	Lac Baker	3	GR	47.3652	-68.6837	Not Detected	NA	Not Detected
2024-08-21	Ruisseau Baker-Brook	3	GS	47.3754	-68.6686	Not Detected	NA	Not Detected
2024-08-21	Lac Baker	3	FG	47.3505	-68.6601	Not Detected	NA	Not Detected
2024-08-21	Lac Caron	3	FC	47.3295	-68.6087	Not Detected	NA	Not Detected
2024-08-22	Saint John River	3	FI	45.9610	-66.6260	Not Detected	NA	Detected
2024-08-26	Hammond River	3	EV	45.4567	-65.9060	Not Detected	NA	Not Detected
2024-08-26	Kennebecasis River	3	EU	45.5416	-65.8375	Not Detected	NA	Detected
2024-08-26	Saint John River	3	ET	45.6505	-65.8594	Not Detected	NA	Not Detected
2024-08-27	Caraquet River	3	IK	47.6429	-65.2574	Not Detected	NA	NA
2024-08-27	Caraquet River	3	IL	47.6730	-65.2267	Not Detected	NA	NA
2024-08-27	Caraquet River	3	IM	47.7066	-65.1529	Not Detected	NA	NA
2024-08-27	Caraquet River	3	IN	47.7176	-65.1413	Not Detected	NA	NA
2024-08-27	Caraquet River	3	IO	47.7266	-65.1305	Not Detected	NA	NA
2024-08-27	Pokemouche River	3	IP	47.6532	-65.0430	Not Detected	NA	NA
2024-08-27	Pokemouche River	3	IQ	47.6202	-65.1048	Not Detected	NA	NA
2024-08-27	Pokemouche River	3	IR	47.6362	-65.0913	Not Detected	NA	NA
2024-08-27	Pokemouche River	3	IS	47.6374	-65.0673	Not Detected	NA	NA
2024-08-27	Pokemouche River	3	IT	47.6387	-65.0472	Not Detected	NA	NA

2024-08-28	Bartibog River	3	IU	47.2396	-65.6028	Not Detected	NA	NA
2024-08-28	Bartibog River	3	IV	47.2046	-65.4416	Not Detected	NA	NA
2024-08-28	Bartibog River	3	IW	47.1895	-65.4054	Not Detected	NA	NA
2024-08-28	Bartibog River	3	IX	47.1894	-65.3882	Not Detected	NA	NA
2024-08-28	Bartibog River	3	IY	47.1247	-65.3766	Not Detected	NA	NA
2024-08-28	Burnt Church River	3	IZ	47.2086	-65.1864	Not Detected	NA	NA
2024-08-28	Burnt Church River	3	JA	47.2079	-65.1944	Not Detected	NA	NA
2024-08-28	Burnt Church River	3	JB	47.2128	-65.2064	Not Detected	NA	NA
2024-08-28	Burnt Church River	3	JC	47.2129	-65.2116	Not Detected	NA	NA
2024-08-28	Burnt Church River	3	JD	47.2235	-65.2292	Not Detected	NA	NA
2024-08-29	Big tracadie River	3	JE	47.5357	-65.2461	Not Detected	NA	NA
2024-08-29	Big tracadie River	3	JF	47.5112	-65.2150	Not Detected	NA	NA
2024-08-29	Little tracadie River	3	JG	47.5173	-65.0043	Not Detected	NA	NA
2024-08-29	Little tracadie River	3	JH	47.5158	-65.0185	Not Detected	NA	NA
2024-08-29	Little tracadie River	3	JI	47.5057	-65.0227	Not Detected	NA	NA
2024-08-29	Little tracadie River	3	JJ	47.5026	-65.0294	Not Detected	NA	NA
2024-08-29	Little tracadie River	3	JK	47.4919	-65.0345	Not Detected	NA	NA
2024-08-29	Big tracadie River	3	JL	47.4472	-65.1817	Not Detected	NA	NA
2024-08-29	Big tracadie River	3	JM	47.4354	-65.1739	Not Detected	NA	NA
2024-08-29	Big tracadie River	3	JN	47.4339	-65.1551	Not Detected	NA	NA
2024-09-04	Nashwaak River	3	GM	45.9765	-66.5908	Not Detected	NA	Not Detected
2024-09-04	Saint John River	3	FI	45.9614	-66.6271	Not Detected	NA	Not Detected
2024-09-12	Grand Lake	3	GN	45.8652	-66.0765	Not Detected	NA	Not Detected
2024-09-20	Nashwaak Lake	3	GO	46.4681	-67.1163	Not Detected	NA	Not Detected
2024-09-20	Miramichi Lake	3	CS	46.4507	-66.9598	Not Detected	NA	Not Detected
2024-10-01	Big Lake Stream	3	FY	46.1227	-67.2597	Not Detected	NA	Not Detected
2024-10-01	Nackawick Stream	3	FZ	46.0638	-67.2419	Not Detected	NA	Not Detected
2024-10-01	Nackawick Stream	3	CW	46.0450	-67.2379	Not Detected	NA	Not Detected
2024-10-02	Westie lake	3	GA	46.1306	-67.2297	Not Detected	NA	Not Detected
2024-10-02	Big Lake	3	GB	46.1315	-67.2271	Not Detected	NA	Not Detected
2024-10-02	Big Lake	3	GC	46.1312	-67.2233	Not Detected	NA	Not Detected
2024-10-02	Nackawick Stream	3	GD	46.1182	-67.2643	Not Detected	NA	Not Detected

2024-10-02	Nackawick Stream	3	GE	46.0034	-67.2425	Not Detected	NA	Not Detected
2024-10-04	Etang Deuxieme-Sault (Green River)	3	FB	47.4677	-68.2359	Not Detected	NA	Not Detected
2024-10-23	Lac Biencourt	6	GF	47.9787	-68.5367	Not Detected	NA	Not Detected

4. DISCUSSION

Understanding the distribution of species at risk is necessary to identify and protect key habitats (Fisheries and Oceans Canada, 2018). As species at risk are very often found in low abundance or have a limited distribution, finding them through visual surveys can be labour intensive, costly, and time consuming. Environmental DNA offers a sensitive method of detecting species in low abundance (Sigsgaard, Carl, Moller, & Thomsen, 2015). In addition, collection of eDNA samples is non-invasive, causing little to no harm to the organism or damage to its environment, which is ideal for surveying and monitoring species at risk. While extracting and amplifying DNA requires specialized equipment, collecting the water samples (often the most labour intensive part of the monitoring process) is feasible for a layperson, requiring little equipment or training and can be done at a relatively low cost (Biggs et al., 2015). This facilitates participation of non-governmental organizations and Indigenous groups in sample collection. Furthermore, water samples collected for one purpose can be reanalyzed for additional target species, as was done in this report, where samples originally collected for aquatic invasive species monitoring were subsequently used for freshwater mussel detection.

Within New Brunswick, brook floater sub-populations have been found in the St. Croix, Magaguadavic, Petitcodiac, Miramichi, Kouchibouguacis, and Bouctouche watersheds (COSEWIC, 2022). Environmental DNA sampling in 2017 and 2018 found brook floater DNA in the Kouchibouguacis and Bouctouche rivers, and the Southwest Miramichi River and tributaries (LeBlanc et al, 2021a). A single inconclusive result was also obtained for one sample collected in the Shediac River in 2018, however significant sampling and survey efforts in the Shediac Bay watershed from 2014 to 2019 produced no evidence of brook floater, and as such, this sample and specimens found in the Shediac and Scoudouc river in 2005 are now thought to have been misidentified (Fisheries and Oceans Canada, 2018; Hébert and Donelle, 2020; LeBlanc et al 2021a; COSEWIC, 2022). In this report, sampling between 2020 and 2024 detected brook floater DNA in the Petitcodiac River and the Northwest and Southwest Miramichi rivers and tributaries, including the Renous, Barnaby, Taxis, Cains, Dungarvon, Bartholomew, and Little Southwest Miramichi rivers, where populations were previously observed or suspected (COSEWIC, 2009; COSEWIC, 2022; Baisley, 2010). Brook floater DNA was also recovered outside the species' previously reported distribution, in the Saint John River watershed at one inconclusive site in the Jemseg River and one detected site in the Kennebecasis River in 2023. Brook floater DNA was not detected in the Saint John River watershed in 2024, which could be due to differences in sampling locations between years or expected variability in eDNA recovery at low concentrations, which is even observed between sample replicates. This is the first report of brook floater detection in the Saint John River watershed since the unconfirmed collection of a specimen in the Aroostook River in 1960 (COSEWIC, 2022; COSEWIC, 2009). Brook floater DNA was not detected in samples collected from Quebec or northern New Brunswick, including the Upper Saint John River watershed, Restigouche River basin or Acadian Peninsula Composite watershed. Brook floater DNA was also not detected in the Richibucto River or its tributaries, despite known presence and previous eDNA detections in the nearby Bouctouche and Kouchibouguacis rivers (COSEWIC, 2022; LeBlanc et al, 2021a).

Samples were opportunistically tested for yellow lampmussel and eastern pearlshell DNA. Yellow lampmussel is also listed as a species of Special Concern under SARA and, in New Brunswick, is only known to be found in the lower Saint John River watershed (Fisheries and Ocean Canada, 2010; Fisheries and Ocean Canada, 2025). Yellow lampmussel DNA was recovered at four sites in the Saint John River watershed, in the Wolastoq (Saint John) and Kennebecasis rivers, and Washademoak and Grand lakes. Yellow lampmussel DNA was tested for but not detected in samples from northern New Brunswick or Quebec, or in the Miramichi watershed. Eastern pearlshell DNA was detected at every site tested for this species, namely in the Northwest Miramichi, Renous, Dungarvon and Bartibog rivers where it is known to be relatively abundant (Baisley, 2010). While eastern pearlshell is not considered a species at risk, these detections confirm that eDNA is an appropriate tool for detecting freshwater mussels.

With the work from this study and LeBlanc et al., 2021a, eDNA has now been collected and tested in most New Brunswick watersheds known to contain brook floater, with ongoing efforts to sample additional watersheds. The detection of brook floater eDNA in all of these systems demonstrates that eDNA-based methods are effective for locating sub-populations of this species. Furthermore, the use of eDNA-based methods enabled the participation of local environmental and Indigenous groups and the repurposing of samples already collected for AIS surveillance, allowing for an efficient and cost effective province-wide survey which resulted in the detection of brook floater DNA in the Saint-John River watershed, outside of its reported range. Although species at risk monitoring requires ground surveys to evaluate population dynamics (e.g., abundance, life stage, recruitment), eDNA-based methods can help guide these investigations by drastically increasing the search area for initial species detection.

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APPENDIX 1

eDNA Extraction Protocols

The following steps provides an overview of the modifications to the MN NucleoSpin Tissue Kit procedure for the extraction of DNA from filters. The protocol was modified over the years to optimize eDNA recovery from filters. Notably, in V 1.20 (used in 2020) the B3 buffer was added to the tube containing the lysis mix and filter, and the filter was removed following the 70°C incubation. In V 1.30 (used in 2021 and 2022), the filter is removed prior to adding the B3 buffer, and in V 1.40 (used in 2023 and 2024) spin baskets are used to separate the filter from the lysis mix.

- 1) Spray the exterior of all sample tubes with Bleach-Rite or bleach 0.5 %.
- 2) Spin down to collect the liquid. Remove the filter from the tube and place on a weighing boat using sterile toothpicks and scalpel blades.
- 3) Cut filter in half using a scalpel blade. Return one half to the ethanol tube (as a backup) and allow the other half to air dry until all ethanol has evaporated.
- 4) Prepare a master mix consisting of 360 µL of T1 lysis buffer (MN) and 50 µL of Proteinase K for each sample to process. Add 410 µL of this mix to each tube (V1.20 and V1.30) or spin basket (V1.40).
- 5) Once dried, fold the filters so that the surface containing the material is facing inward and place them in a 2 mL tube (V1.20 and V1.30) or spin basket (V1.40), submerging the tube in the lysis buffer.
- 6) Incubate at 56±3 °C for 3 hours to overnight with light shaking (optional) to digest the material found on the filters. The filters themselves will not digest.
- 7) Spin down the tubes. If using spin baskets (V1.40), centrifuge at 18 000g for 1 min to separate the filter from the supernatant. Discard the filter and spin basket. For V1.30, remove the filter from the supernatant either by pipetting the supernatant into a new 2 mL tube, or by removing the filter with sterile toothpicks. Squeeze the filter with the pipette tip or toothpicks to extract all the supernatant.
- 8) Add 400 µL of Buffer B3 to the tube containing the supernatant (V1.3 and 1.4) or the supernatant and filter (V1.20). Mix vigorously.
- 9) Incubate at 70±3 °C on the heat block for 10 minutes.
- 10) Spin down to collect the liquid. For V1.20 Transfer the supernatant to a new 2 mL tube. While collecting the supernatant, squeeze the filter with the pipette tip to push all the supernatant out of the material.
- 11) Add 420 µL of absolute ethanol 100 % to the tube, mix vigorously and spin down the liquid briefly.
- 12) Transfer the supernatant to a MN spin-column. You will need to transfer the supernatant two or three times (450 µL x3) in order to pass everything through the spin-column.
- 13) Centrifuge at 11 000g for 1 min. Discard the content of the collection tube, then dab the rim of the collection tube on a clean paper towel and return the spin-column to the collection tube.
Note : Repeat steps 12 and 13 as many times as needed to transfer all mix to the spin-column.
- 14) Follow the MN NucleoSpin Tissue Kit manufacturer's protocol for the rest of the extraction, including the optional dry spin step and using two rounds of elution with 50uL BE each.