Scoping the distribution of Smallmouth Bass (Micropterus dolomieu) in the Miramichi River Watershed in 2019 and 2020 using environmental DNA

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Smallmouth Bass (*Micropterus dolomieu*), a non-native fish species in the Maritime Provinces, was first observed in Miramichi Lake (NB, Canada) in 2008. Since its discovery, Fisheries and Oceans Canada (DFO) has been leading containment, control, and monitoring activities with the support of non-government organizations and the Province of New Brunswick in an attempt to control the Smallmouth Bass (SMB) population within the lake. In August 2019, SMB was reported in the Southwest Miramichi River (SWM River), about 8 km downstream from Lake Brook, the outflow of Miramichi Lake. This led to a rapid mobilisation of resources by various partners and levels of government, including DFO, in an attempt to evaluate the spread and distribution of SMB within the SWM River system. Environmental DNA sampling in conjunction with species-specific qPCR testing was one of the methods used in both 2019 and 2020, as a means of gaining insight into the distribution of SMB. A total of 47 sites were sampled in both years and SMB DNA was found at multiple sites, with results classified as detected and suspected at sites downstream of McKiel Pond, where a total of 108 SMB were caught in 2019 and 2020. Results classified as inconclusive were also obtained upstream of Lake Brook, in McKiel Lake, and McKiel Brook, as well as a few other sites in the SWM River located between the outflow of McKiel Brook to Blackville. These inconclusive results warrant further investigation to confirm the presence of SMB in different portions of the watershed.

RÉSUMÉ

LeBlanc, F., Steeves, R., Irlich, U., Bourque, D., Akaishi, F. and Gagné, N. 2021. Scoping the distribution of Smallmouth Bass (*Micropterus dolomieu*) in the Miramichi River Watershed in 2019 and 2020 using environmental DNA. Can. Manuscr. Rep. Fish. Aquat. Sci. 3222: v + 22 p.

L'achigan à petite bouche (*Micropterus dolomieu*), une espèce de poisson non-indigène dans les Provinces Maritimes, a été observé pour la première fois dans le lac Miramichi (N.-B., Canada) en 2008. Depuis sa découverte, Pêches et Océans Canada (MPO) dirige des activités de confinement, contrôle et de surveillance avec le soutien d'organisations non gouvernementales et la Province du Nouveau-Brunswick, dans le but de contrôler la population d'achigan à petite bouche dans le lac. En août 2019, l'achigan à petite bouche a été signalé dans la rivière Southwest Miramichi (rivière SWM), à environ 8 km en aval de « Lake Brook », à la sortie du lac Miramichi. Cela a mené à une mobilisation rapide de ressources par divers partenaires et paliers de gouvernement, y compris le MPO, dans le but d'évaluer la propagation et la répartition de l'achigan à petite bouche dans le réseau hydrographique de la rivière SWM . L'échantillonnage d'ADN environnemental en conjonction avec des tests de qPCR spécifiques à l'espèce était l'une des méthodes utilisées en 2019 et en 2020, comme moyen de mieux comprendre la distribution de l'achigan à petite bouche. Un total de 47 sites ont été échantillonnés au cours des deux années. De l'ADN de l'achigan à petite bouche a été trouvé dans plusieurs sites, avec des résultats classifiés comme détectés et suspectés aux sites en aval de « McKiel Pond », où un total de 108 achigan à petite bouche ont été capturés en 2019 et 2020. Des résultats classifiés comme non concluants ont également été obtenus en amont de « Lake Brook », dans le lac McKiel et dans « McKiel Brook », ainsi que dans quelques autres sites de la rivière SWM situés entre la sortie de « McKiel Brook » à Blackville. Ces résultats non concluants justifie une enquête plus approfondie pour confirmer la présence de l'achigan à petite bouche dans différentes endroits du bassin versant.

1. INTRODUCTION

Smallmouth Bass (*Micropterus dolomieu*) is a freshwater fish species that is not native to the Maritime Provinces. It was introduced through legal and illegal transfers, as well as natural migration and now inhabits many lakes and rivers in New Brunswick (NB) and Nova Scotia (NS) (Brown, Runciman, Pollard, Grant, & Bradford, 2009). In NB, Smallmouth Bass (SMB) were introduced from Maine in about 1869 and its distribution is mainly concentrated in Bay of Fundy drainages (DFO, 2009; Scott & Crossman, 1973).

In late September 2008, SMB were discovered in Miramichi Lake, a headwater lake of the Southwest Miramichi (SWM) River (DFO, 2009). Since that time, containment, control, and monitoring activities led by Fisheries and Oceans Canada (DFO) with the support of non-government organizations and the Province of NB have been undertaken in an attempt to control the SMB population in Miramichi Lake. The Miramichi River is world renowned as an Atlantic Salmon River (*Salmo salar*), and in 2009, DFO conducted a risk assessment to identify the potential impact of SMB on local Atlantic Salmon populations. The assessment indicated that the overall risk to the aquatic ecosystem is considered high with low uncertainty in the lake environment because SMB is expected to become a dominant component of the food web and cause significant reductions in existing biota. In the riverine environment (preferably used by Atlantic Salmon), the overall risk was considered moderate with high uncertainty, because a measurable decrease in abundance of native populations is likely to occur due to the establishment of SMB (DFO, 2009).

In August 2019, SMB was reported after sightings in the SWM River approximately 12 km downstream of Miramichi Lake, which led to the rapid mobilization of resources to better understand the extent of SMB presence in the SWM River system and to guide potential management actions. A total of 108 SMB were subsequently captured in the SWM River at a site known as McKiel Pond (approximately 10 km downstream of Miramichi Lake), and 16 in Lake Brook, in 2019 and 2020. In addition, DFO received reports of SMB observations in the SWM River near Blackville and Boiestown (D. Bourque pers.comm.), including a specimen caught in a DFO index trap near Millerton in September 2020, approximately 150 km downstream of Miramichi Lake. It is worth noting that the report and capture of SMB in the SWM River in 2019 follows a 2017 environmental DNA (eDNA) survey conducted in a portion of the SWM River that detected SMB DNA at two sites approximately 3 and 4.5 km downstream of Lake Brook, suggesting that SMB have been in the SWM River since at least 2017 (O'Sullivan et al., 2020). The use of environmental DNA (eDNA) for SMB detection was one of the tools adopted early as part of this response in 2019. Environmental DNA collection in conjunction with species-specific DNA detection using molecular assays is a powerful emerging tool for the detection of aquatic species (Ficetola, Miaud, Pompanon, & Taberlet, 2008), including aquatic invasive species (AIS) (Adrian-Kalchhauser & Burkhardt-Holm, 2016; Balasingham, Walter, Mandrak, &

Heath, 2018; Gingera et al., 2016). Organisms, including fish, release genetic material into the environment through various sources (e.g., feces, skin, mucus and gametes) and the detection of this DNA using molecular methods, such as species-specific qPCR assays, is considered a relatively cost-effective and sensitive method from which species presence can be inferred (Biggs et al., 2015; Sigsgaard, Carl, Moller, & Thomsen, 2015). The use of eDNA for SMB detection has been previously reported in the United States (Franklin et al., 2018; Rubenson & Olden, 2020) and here in Canada in the Miramichi River system (O'Sullivan et al., 2020).

This report presents the finding of the eDNA-based detection of SMB using a species-specific qPCR approach undertaken in 2019 and 2020 to scope the extent of the spread of SMB in parts of the Miramichi River Watershed and to guide resource allocation and potential control and eradication measures.

2. MATERIALS AND METHODS

2.1 Field sampling

Water collection for eDNA-based SMB detection was conducted in 2019 and 2020 at a total of 47 unique sites. In 2019, 28 sites were sampled and 46 were sampled in 2020. All sites sampled in 2019 were resampled in 2020, with the exception of site 12 due to access issues. Most targeted sites were located in the SWM River from upstream of the Lake Brook outlet to Boiestown. Sampling was also conducted in McKiel Lake and McKiel Brook, which drains from McKiel Lake into the SWM River downstream of the section of river known as the "McKiel stretch". In 2020 only, sampling was also conducted at a few sites further upstream towards Juniper, as well as downstream of Boiestown, extending all the way to Millerton, as well as a few sites in the Bartholomew, Northwest Miramichi (NWM), Barnaby and Renous Rivers (Figure 1). Some of these sites were added in September 2020, following the discovery of a SMB in a DFO index trap net near Millerton. Two sites near the outflow of Argyle Lake were also sampled exclusively in 2020. When eDNA results came back as inconclusive for a site, an attempt was made to resample that site in the same season to verify result.

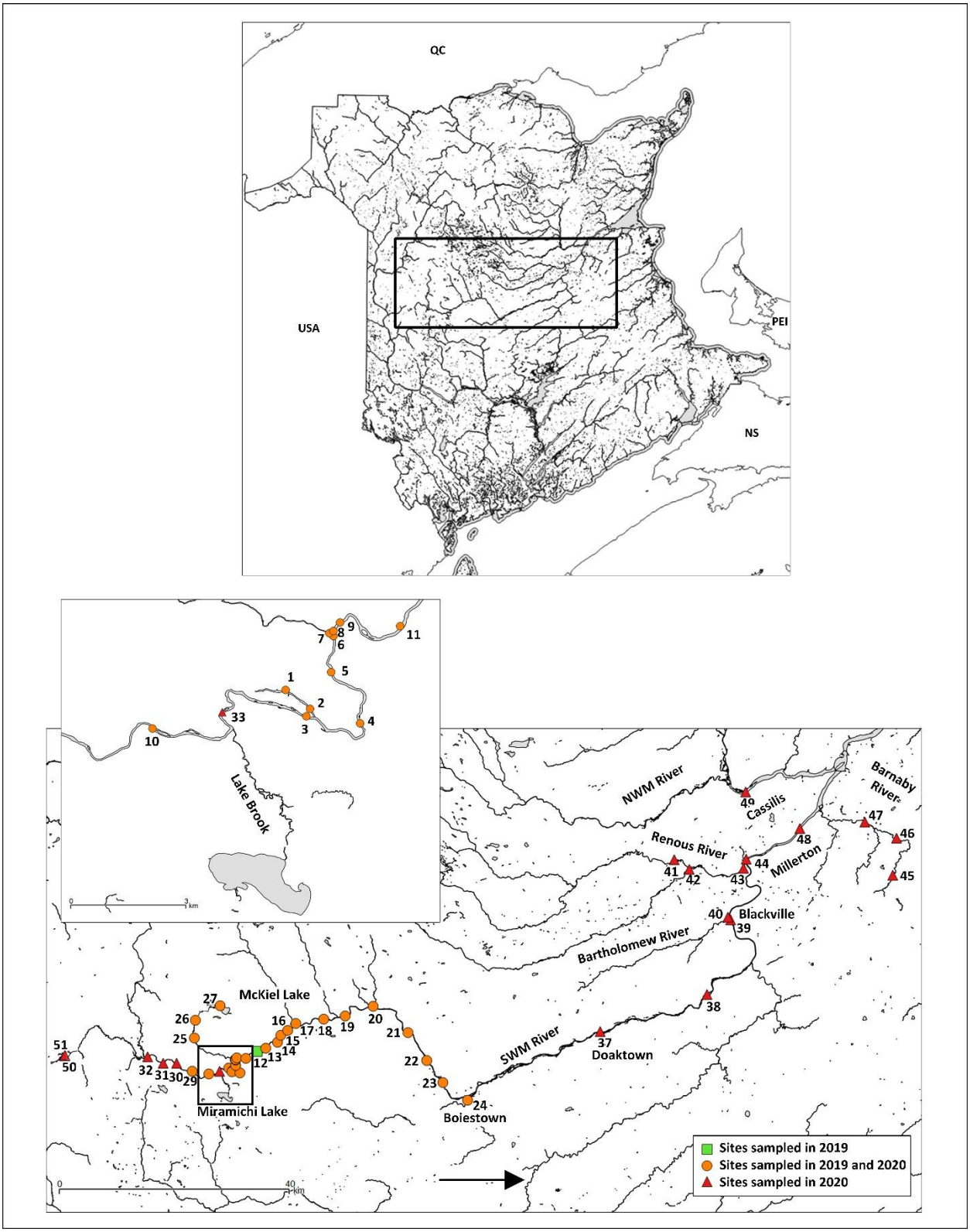

Figure 1: Map of sites surveyed in 2019 and 2020. The arrow indicates the direction of the water flow

As a reference site, Site 5, which is situated immediately downstream of McKiel Pond where a total of 108 fish were removed in 2019 and 2020, was sampled on multiple sampling trips that took place in the vicinity.

At each site, water sampling consisted of collecting 3 x 1 L at ~ elbow depth using a lateral transect approach when possible (i.e., 1 L on each bank and 1 L in the middle of the river), with a few exceptions where 1, 2, or 4 field replicates were collected for logistical reasons. A transect approach was used to account for possible heterogeneity in DNA signal based on the location of SMB and hydraulic flows. However, for a few sites water samples were collected on 1 bank in close proximity to one another (i.e., within 10 m) due to safety concerns from high water levels. Water samples were kept on ice, and then at 4°C until filtration was completed within 24 hours of collection. All filtrations were done with 47 mm diameter 0.8 µm Whatman nylon membrane filters (GE Healthcare, IL, USA) and a Gast™ Oil-less Diaphragm-type Pressure/Vacuum Pumps (Fisher Scientific, MA, USA). Field blanks (tap water) were brought in the field during water sample collection and processed alongside field samples for each sampling event. A minimum of one field blank was included for each day of sampling. Lab filtration blanks, DNA extraction blanks and qPCR negative controls were also included during the processing and testing of samples. Furthermore, all reusable equipment (e.g., mason jars, forceps, vacuum flasks) was soaked in a 1% bleach solution (i.e., 1 in 10 dilution prepared from 10 % commercial bleach) for a minimum of 1 hour and thoroughly rinsed with non-purified drinkable tap water prior to use.

2.2 Smallmouth Bass qPCR assay optimization

A published SMB species-specific qPCR assay (Brandl et al., 2015) targeting the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene was used. To ensure that the published qPCR assay was applicable for use in the Miramichi River Watershed, an *in silico* assessment was done by retrieving SMB CO1 DNA sequences found in the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/) and the Barcode of Life Data System (BOLD; http://www.boldsystems.org/), and sequences from closely related species (≥ 80 % genetic similarity) and other fish species found in the studied geographical area (Table 1). Sequences were aligned in Geneious (version 9.1.4) and minor modifications were made to the primers and probe to amplify a 90 bp product (Table 1). For *in vitro* validation of the assay, genomic DNA extracted from the fin clip of a local SMB was quantified using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, MA, USA) and serial genomic DNA dilutions were done to determine the efficiency ($E = 10^{[-1/slope]}$) and calculate the theoretical limit of detection (LOD). Three serial dilutions from 10^0 to 10^{-8} were made and each serial dilution was tested in duplicate for a total of 6 qPCR threshold cycle (Ct) values. The theoretical LOD was determined as the lowest dilution for which 6 of 6 qPCR reactions were positive (i.e., detection in 100 % of the qPCR reactions). Non-target DNA normalized to 5 ng/µL was used as a background when

preparing the serial dilutions. This was done to assess the efficiency of the assays under conditions similar to its prescribed usage.

The specificity of the qPCR assay was also tested *in vitro* using DNA from 17 fish species known to be present in the Miramichi River Watershed (Table 1) (Biron, 2018; Hayward, Sheasgreen, Douglas, & Reid, 2014).

Table 1: Species-specific qPCR assay used for Smallmouth Bass detection

Target species	Primer name	Sequence 5'-3'[a]	Amplicon (bp)	Efficiency (%)	Equation	LOD pg[b]	Specificity testing *in vitro*
M. dolomieu	COI_421F_Md COI_510R-Md COI-465P-Md	CATCCTAGGGGCCATCAATTTT GACCAAACAAACAGGGGTGTCTG (6-FAM)- AACCCCCAGCTATTTC-MGB	90	95.6	-3.434(log(x))+21.569	0.265	*Perca flavescens, Castostomus commersonii, Morone americana, Semotilus corporalis, Semotilus atromaculatus, Alosa pseudoharengus, Alosa aestivalis, Notemigonus crysoleucas, Ameiurus nebulosus, Fundulus diaphanous, Anguilla rostrata, Salvelinus fontinalis, Couesius plumbeus, Petromyzon marinus, Salmo salar, Margariscus margarita, Morone saxatilis*

[a] Primers and probe were modified from Brandl et al. (2015).

[b] The LOD was determined from serial dilutions done using genomic DNA from the targeted species.

2.3 DNA extraction and qPCR testing

DNA extraction from filters was conducted using half of each filter with the MN NucleoSpin Tissue Kit (Macherey-Nagel, PA, USA) following a modified protocol (LeBlanc et al. 2020). The resulting DNA extracts were stored at -20 °C and the second half of the filter was kept as a back-up.

qPCR testing was done with the species-specific qPCR assay using the 2x TaqMan Gene Expression Kit (Thermo Fisher Scientific, MA, USA). Briefly, 3 µL of template DNA, 480 nM of each primer, 200 nM of the probe, 1 µL of 1 % BSA, as well as 12.5 µL of master mix were used in 25 µL reactions. All qPCR tests were done in triplicate in 2019 and in duplicate in 2020 on a StepOnePlus™ qPCR platform (Thermo Fisher Scientific, MA, USA) using the following cycling parameters: initial hold at 50 °C for 2 min, 95 °C for 10 min, followed by 50 cycles at 95 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, with fluorescence reading at the end of each elongation cycle.

The qPCR results of all field replicates were classified as either 1) not detected, 2) inconclusive, 3) suspected or 4) detected (Table 2)(LeBlanc et al., 2020)

Table 2: Criteria's used for classification of results used in this work[a]

Classification	Criteria's
Not detected	No detection in any of the qPCR technical replicates
Inconclusive	Detection obtained in 1/2 or 1/3 of the qPCR technical replicates
Suspected	Detection obtained in 2/2, 2/3 or 3/3 of the qPCR technical replicates, but the value was below the LOD (i.e., < 17.65 pg/L)
Detected	Detection obtained in 2/2 or 3/3 of the qPCR technical replicates performed and the value was above the LOD (i.e., > 17.65 pg/L)

[a] Based on classifications found in (LeBlanc et al., 2020).

To evaluate if PCR inhibitors were present in environmental samples, which could lead to potential false negative results, all samples (including blank controls) were spiked with an exogenous internal positive control (IPC) (linearized DNA plasmid containing a DNA sequence not found in the targeted environments) and tested using a qPCR assay specific to that IPC. Inhibition was considered present if a difference of more than 2 Ct was observed between environmental samples and field blanks. The IPC qPCR assay was done using the same parameters and reagents used for the species-specific Smallmouth Bass qPCR assay.

2.4 Confirmatory DNA sequencing

To confirm the specificity of the qPCR assay on field eDNA samples, a subset (< 5 %) of the positive results were sequenced. PCR and Sanger sequencing was done by using the same primers and parameters used for the species-specific qPCR assays. Samples were amplified using the AmpliTaq Gold 360 PCR Master Mix (Thermo Fisher Scientific, MA, USA) and PCR products (90 bp) were visualized on a 1.5 % agarose gel followed by PCR product cleanup using ExoSAP-IT (Affymetrix, CA, USA) and Sanger sequencing at the Centre d'expertise et de services Génome Québec. Sequence identity was confirmed by using NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and by performing an alignment with SMB CO1 reference sequences found in BOLD and NCBI.

3. RESULTS

3.1 qPCR assay performance

The Smallmouth Bass species-specific qPCR assay showed good sensitivity with a theoretical LOD of 0.265 pg of gDNA per reaction and an assay efficiency of 95.6 % (Table 1). Specificity testing using DNA from 17 fish species found in the Miramichi River Watershed showed no cross-amplification.

3.2 Smallmouth Bass eDNA findings

Sampling for SMB eDNA in August 2019 was mainly focused on an area (referred to herein as the "McKiel stretch") which extends from ~ 2km upstream of where Lake Brook flows into the SWM River to just downstream of McKiel Brook following the report of SMB in that area (Figure 1). Sites 5, 6, 8 and 9 which were all situated downstream of McKiel Pond, all had detectable SMB DNA with results classified as suspected (Figure 2 and Table 3). Site 4, located upstream of McKiel Pond was also classified as suspected. Sampling in September and October was extended further downstream with sites targeted all the way to Boiestown (site 24), including an additional site further upstream of Lake Brook (site 10) as well as in McKiel Lake (site 27) and McKiel brook (site 25 and 26) which drains into the SWM River near site 8. From the September sampling events, site 11 was also classified as "suspected" and site 13 as inconclusive with 1 of 3 field replicates collected having detectable DNA in 1 technical qPCR replicate. An inconclusive result was also obtained for sites 26 (upper reaches of McKiel Brook) and site 27 (McKiel Lake). Results from the October sampling events, showed a repeat inconclusive at site 13 and an inconclusive result at site 18, while no SMB DNA was detected on October 3rd and 10th at site 5, which is located immediately downstream (~200 m) from McKiel Pond.

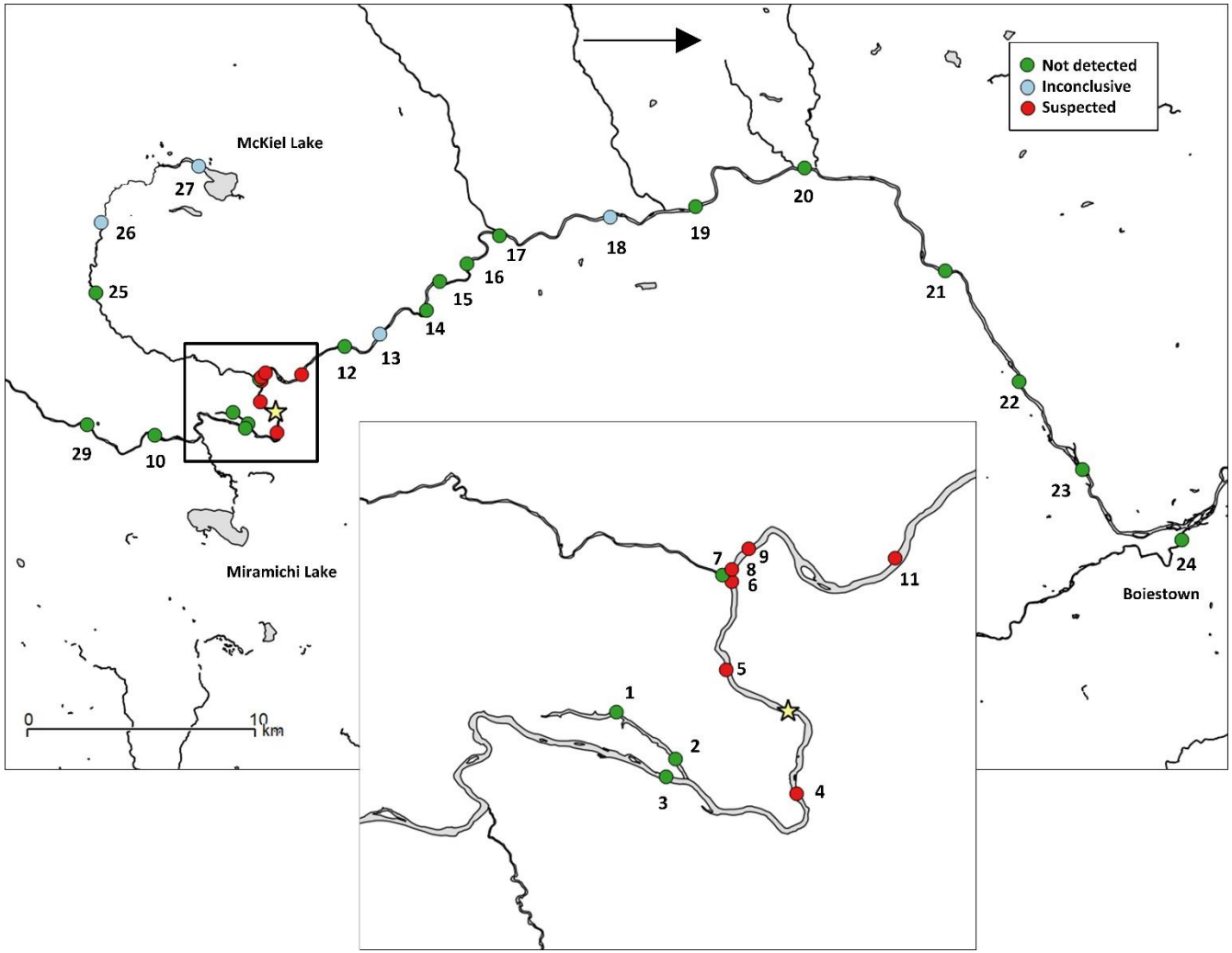

Figure 2: SMB eDNA results in 2019. The location of McKiel Pond where a total of 108 SMB have been caught in 2019 and 2020 is denoted by a yellow star. The arrow indicates the direction of the water flow. The classification of results is based on a decision framework found in LeBlanc et al., 2020

Table 3: List of sites and sampling events in 2019 and 2020 and SMB qPCR results

Site ID	Water body	Latitude (°N)	Longitude (°W)	Year	Sampling date	SMB qPCR results [a]
Site 1	McKiel Bogan	46.50335	-66.96510	2019	August 27	Not detected (0/3)
				2020	August 20	Not detected (0/3)
Site 2	McKiel Bogan	46.49891	-66.95677	2019	August 27	Not detected (0/3)
				2020	August 20	Suspected (3/3)
Site 3	SWM River	46.49718	-66.95809	2019	August 27	Not detected (0/3)
				2020	August 20	Inconclusive (2/3)
Site 4	SWM River	46.49564	-66.93989	2019	August 27	Suspected (2/3)
				2020	August 20	Suspected (2/3)
Site 5	SWM River	46.50754	-66.94977	2019	August 27	Suspected (3/3)
					September 12	Suspected (3/3)
					October 3	Not detected (0/3)
					October 10	Not detected (0/3)
				2020	August 20	Suspected (3/3)
					August 26	Suspected (3/3)
					September 1	Suspected (2/3)
					September 10	Detected (3/3)
Site 6	SWM River	46.51599	-66.94910	2019	August 27	Suspected (3/3)
				2020	September 10	Suspected (3/3)
Site 7	McKiel Brook	46.51664	-66.95040	2019	August 27	Not detected (0/3)
				2020	September 10	Not detected (0/3)
Site 8	SWM River	46.517199	-66.94911	2019	August 27	Suspected (1/3) / Inconclusive (1/3)
					September 12	Suspected (2/3)
				2020	September 10	Suspected (2/3)
Site 9	SWM River	46.51917	-66.94681	2019	August 27	Suspected (3/3)
				2020	September 10	Suspected (2/3)
Site 10	SWM River	46.49411	-67.01010	2019	August 27	Not detected (0/3)
				2020	August 20	Inconclusive (1/3)
Site 11	SWM River	46.51838	-66.92639	2019	September 12	Suspected (1/4) / Inconclusive (1/4)
				2020	September 10	Inconclusive (1/3)
Site 12	SWM River	46.52954	-66.90152	2019	October 10	Not detected (0/3)
Site 13	SWM River	46.53477	-66.88146	2019	September 12	Inconclusive (1/4)
					October 3	Inconclusive (1/3)
				2020	August 26	Inconclusive (2/3)
					September 10	Inconclusive (1/3)
Site 14	SWM River	46.54391	-66.85485	2019	August 28	Not detected (0/1)
					October 10	Not detected (0/3)
				2020	August 26	Inconclusive (1/3)
Site 15	SWM River	46.55547	-66.84771	2019	October 10	Not detected (0/3)
				2020	August 26	Not detected (0/3)
Site 16	SWM River	46.56257	-66.832	2019	October 10	Not detected (0/3)
				2020	August 26	Not detected (0/3)
Site 17	SWM River	46.5737	-66.81342	2019	October 10	Not detected (0/3)
				2020	September 1	Not detected (0/3)
Site 18	SWM River	46.58102	-66.75007	2019	October 10	Inconclusive (1/3)
				2020	September 1	Not detected (0/3)
Site 19	SWM River	46.58554	-66.70118	2019	September 12	Not detected (0/3)
				2020	September 2	Inconclusive (1/3)
Site 20	SWM River	46.60063	-66.63863	2019	September 12	Not detected (0/3)
				2020	September 2	Not detected (0/3)
Site 21	SWM River			2019	September 12	Not detected (0/3)

		46.56006	-66.55818	2020	September 2	Not detected (0/3)
Site 22	SWM River	46.51649	-66.5159	2019	September 12	Not detected (0/3)
				2020	September 2	Not detected (0/2)
Site 23	SWM River	46.48188	-66.47978	2019	September 12	Not detected (0/3)
				2020	September 2	Not detected (0/3)
Site 24	Taxis River	46.45406	-66.42301	2019	September 12	Not detected (0/3)
				2020	September 1	Not detected (0/3)
Site 25	McKiel Brook	46.55011	-67.04400	2019	September 6	Not detected (0/3)
					September 12	Not detected (0/2)
				2020	August 19	Not detected (0/2)
Site 26	McKiel Brook	46.57803	-67.04152	2019	September 6	Inconclusive (1/3)
					October 3	Not detected (0/3)
					October 10	Not detected (0/3)
				2020	August 19	Not detected (0/2)
Site 27	McKiel Lake	46.60062	-66.9858	2019	September 6	Inconclusive (1/3)
					October 10	Not detected (0/3)
				2020	August 19	Inconclusive (1/1)
Site 29	SWM River	46.49828	-67.0485	2019	October 10	Not detected (0/3)
				2020	August 19	Not detected (0/3)
Site 30	SWM River	46.51033	-67.08379	2020	August 19	Not detected (0/3)
Site 31	SWM River	46.51054	-67.11370	2020	August 19	Not detected (0/3)
					August 26	Not detected (0/3)
					September 1	Not detected (0/3)
Site 32	SWM River	46.51984	-67.14979	2020	August 19	Not detected (0/3)
Site 33	SWM River	46.49801	-66.98650	2020	August 20	Inconclusive (1/2)
Site 37	SWM River	46.56064	-66.12210	2020	September 2	Inconclusive (1/3)
					September 21	Not detected (0/3)
Site 38	SWM River	46.61789	-65.87861	2020	September 2	Not detected (0/2)
Site 39	SWM River	46.73431	-65.82416	2020	September 2	Not detected (0/3)
					September 21	Inconclusive (1/3)
Site 40	Bartholomew River	46.73844	-65.82952	2020	September 2	Not detected (0/3)
					September 21	Not detected (0/3)
Site 41	Renous River	46.82914	-65.95141	2020	September 21	Not detected (0/3)
Site 42	Renous River	46.81429	-65.91673	2020	September 21	Not detected (0/3)
Site 43	Renous River	46.81524	-65.79379	2020	September 21	Not detected (0/3)
Site 44	Renous River	46.82956	-65.78744	2020	September 21	Not detected (0/3)
Site 45	Barnaby River	46.80176	-65.45304	2020	September 21	Not detected (0/3)
Site 46	Barnaby River	46.85900	-65.44416	2020	September 21	Not detected (0/3)
Site 47	Barnaby River	46.88523	-65.51533	2020	September 21	Not detected (0/3)
Site 48	SWM River	46.87626	-65.66438	2020	September 21	Not detected (0/6)
Site 49	NWM River	46.93450	-65.78671	2020	September 21	Not detected (0/6)
Site 50	Argyle Lake	46.52097	-67.33674	2020	August 26	Not detected (0/3)
Site 51	Argyle Lake	46.52128	-67.33706	2020	August 26	Not detected (0/3)

Environmental DNA sampling was again performed in 2020, from August 19th to September 21st, at the 28 sites sampled in 2019, with the exception of site 12 which wasn't sampled because of access issues. Overall, the results were very similar between the two years for the "McKiel stretch" (i.e., sites 4, 5, 6, 8 and 9), with all classified as suspected or detected (Figure 3 and Table 3). The reference site, site 5, downstream of McKiel Pond was sampled on 4 separate dates, with similar results (i.e. suspected or detected) for all sampling events. Site 2 in the lower reaches of McKiel Bogan was suspected, while sites 33 and 3 were inconclusive. Site 10, sampled upstream of Lake Brook and site 27 (McKiel Lake) gave an inconclusive result. Sampling further downstream to Boiestown, showed an inconclusive result at site 13 (similar to 2019), as well as site 14 and site 19. Following reports of SMB sightings near Boiestown and Blackville, as well as a SMB caught in the DFO index trap net in Millerton in 2020, sites were surveyed as far as Millerton, as well as in the Bartholomew, Northwest Miramichi (NWM), Renous, and Barnaby Rivers. Two sites, site 37 (Doaktown) and site 39 (in the SWM River in Blackville) produced an inconclusive result at 1 of the 2 sampling events conducted in September. All other sites were not detected.

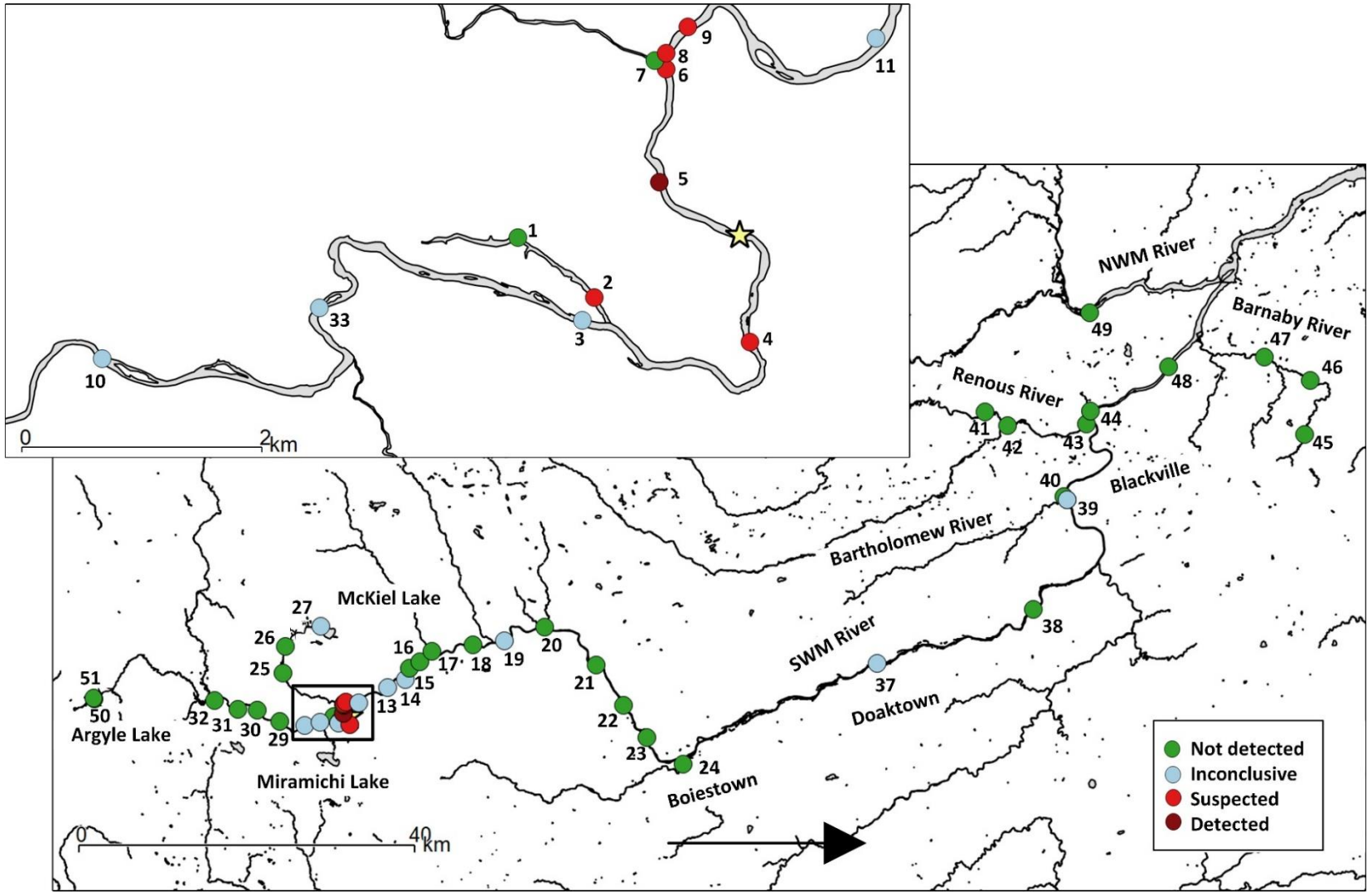

Figure 3: SMB eDNA results in 2020. The location of McKiel Pond where a total of 108 SMB have been caught in 2019 and 2020 is denoted by a yellow star. The arrow indicates the direction of the water flow. The classification of results is based on a decision framework found in LeBlanc et al., 2020

3.3 Confirmation and QC

Sanger sequencing on 4 samples done in an attempt to confirm field results was successful for 3 samples (i.e., one sample from site 5, site 19 and site 37) and showed the amplified product to be SMB CO1. No signs of inhibition which could result in false negative was observed with any samples through IPC testing. In both 2019 and 2020, no contaminations were observed in any of the lab blanks, DNA extraction blanks, or qPCR negative control. All field blanks were free from contamination, with the exception of one field blank in 2020 from the August 26 sampling event which gave an inconclusive result.

4. DISCUSSION

In August and September 2019, SMB were reported and discovered in the SWM River in an area extending approximately 12 km downstream of Miramichi Lake, which led to a rapid response investigation by DFO and various partners to evaluate and assess the extent of this threat. Environmental DNA-based detection of SMB was one of the tools used early in this response to scope the extent of the distribution of SMB in the Miramichi River Watershed and to guide resource allocations. Multiple sampling events were undertaken in both 2019 and 2020 in the SWM River, from upstream of Lake Brook to Millerton (approximatly150 km downstream), as well as in the Bartholomew, NWM, Renous and Barnaby Rivers, and in Argyle Lake. In total, 47 sites were surveyed. Detected, suspected, or inconclusive results were obtained at most sites and sampling events in the "McKiel stretch" (where a total of 108 SMB were caught and removed in 2019 and 2020). Inconclusive findings (i.e., SMB DNA found in only 1/2 or 1/3 of the qPCR technical replicates) were also obtained further downstream all the way to Blackville, and at one site upstream of Lake Brook, as well as in McKiel Brook and McKiel Lake.

The use of eDNA in conjunction with targeted species-specific qPCR testing as an indirect approach to infer species presence, as performed herein, aligns with recent eDNA work (Balasingham et al., 2018; Carim et al., 2019; Loeza-Quintana et al., 2021) which has shown this approach to be effective for AIS detections. Furthermore, DFO recently produced a Science Advisory Report on the use of targeted eDNA analysis for the management of aquatic invasive species and species at risk as guidance in response to the growing interest in this tool (Abbott et al., 2021; DFO, 2020). Because of its sensitivity and the relative ease of water sample collection compared to other survey methods, it was deemed especially valuable as part of this response due to the logistical challenges and remoteness associated with the area.

Environmental DNA sampling from 2019 in the "McKiel stretch", undertaken shortly after the report of a SMB observed in that area, showed a relatively repeatable detection of SMB DNA at sites 5, 6, 8, and 9 with results classified as suspected. All these sites are located downstream of McKiel Pond (with the closest site, site 5, situated ~ 200 m downstream) where a total of 22 SMB were subsequently captured that year. Sites 11 and 13 also had detectable SMB DNA, with results classified as suspected and inconclusive, respectively. These eDNA results downstream of McKiel Pond are expected knowing that SMB were caught in McKiel Pond, however, downstream movement of DNA in the riverine environment, which has been to shown to be 5 km or more in some lotic systems (Deiner & Altermatt, 2014; Laporte et al., 2020; Wood et al., 2021) limits the ability of this information to be used for the purpose of fine scale spatial resolution. For example, the DNA detected at site 11 could represent DNA transported downstream from SMB in McKiel Pond rather than from a SMB source closer upstream. In that same sense, the suspected result obtained for site 4, located upstream of McKiel Pond, does indicate the presence of another source of SMB upstream. While we cannot exclude that this

result might be from DNA moving downstream from Miramichi Lake, the lack of detections at other sites (in this case site 3) close upstream suggests that there is likely another source of SMB in the river. This same logic holds true for all other detections that are obtained downstream of one or multiple sites with non-detected results. For example, site 18, which gave an inconclusive result, was situated downstream of four non-detected sites. This inconclusive result (i.e., based on SMB DNA found in one of three field samples collected and in one of three qPCR technical replicates) suggests the potential presence of SMB nearby, but the lack of repeatability warrants further investigation through follow-up eDNA sampling and/or other species presence monitoring methods. When species are found in small numbers or low biomass in an environment, variability in eDNA qPCR results and low detection probability is quite common (Furlan, Eeson, Wisniewski, Yick, & Duncan, 2019; Jerde, 2021; LeBlanc, Steeves, Belliveau, Akaishi, & Gagne, 2021) and highlights the importance of replicates, and increased spatial and temporal sampling efforts to minimize potential false negatives. Two other inconclusive results were also obtained in Mckiel Brook and McKiel Lake during the 2019 eDNA sampling work. In the context of the current management measures being considered to deal with the threat posed by SMB in the SWM River, investigating the presence of SMB in other lakes (which SMB are known to favor over rivers), such as McKiel Lake, is important to assess re-introduction risks.

Follow-up sampling of many inconclusive sites and the reference site, site 5, in October 2019, failed to detect SMB DNA at those sites, with the exception of site 13 which was again found to be inconclusive based on the October 3rd sampling. The fact that the reference site had non-detected results on both October 3rd and October 10th could be because of a reduced detection probability at this time of year. Smallmouth Bass are known to reduce their activity when temperatures are below 10 °C (Brown et al., 2009) and the water temperature as measured in the SWM River above the Doaktown bridge on October 3rd was 8.8 °C (NB Department of Environment and Local Government) suggesting that temperatures in early October were near that 10 °C mark. Interestingly, past work on SMB eDNA detection conducted in 2017 in Miramichi Lake and a portion of the SWM River did find SMB DNA at two sites in the SWM River which were sampled in mid-October when temperatures were likely also near or below that 10 °C mark, which suggests that detections are still possible (O'Sullivan et al., 2020). Another possible reason for the non-detection of SMB DNA at the reference site on October 3rd and 10th, 2019 is the possibility that SMB might have relocated from McKiel Pond to a more suitable overwintering ground which is a known behavior of SMB (Baker, Warrenhicks, Gallagher, & Christensen, 1993; Ettinger-Dietzel, Dodd, Westhoff, & Siepker, 2016; Lyons & Kanehl, 2002)

eDNA results from 2020 in the "McKiel stretch" were mostly similar to 2019 results, with relatively strong signals detected downstream of McKiel Pond, where a total of 86 fish were captured by boat electrofishing or angling activities in 2020. Interestingly, results for the reference site (i.e., site 5) sampled on September 10th after the 86 fish had been caught and removed from McKiel Pond still produced a strong result indicating that quite a few fish were

possibly still present in that area; although DNA persistence, from fish recently removed, based on DNA decay, geochemical adsorption, and sedimentation/resuspension processes cannot be completely disregarded as a potential source of the detected DNA (Harrison, Sunday, & Rogers, 2019). Site 13, which gave inconclusive results on two sampling occasions in 2019 was again inconclusive, as was site 14. It is important to note here that one field blank collected during the August 26th sampling event gave an inconclusive result, which could put into question the validity of those results, however site 13 was again inconclusive during a subsequent sampling event. While results from site 13 and 14 could also again be from the downstream transport of DNA from the "McKiel stretch", it is worth investigating these findings further to exclude the presence of SMB nearby. The suspected and inconclusive results upstream of McKiel Pond, which include one site in McKiel Bogan (site 2) and one site upstream of Lake Brook (site 10) also suggests the presence of SMB in other areas of the SWM River, aside from McKiel Pond. Sampling in McKiel Lake was again inconclusive in 2020, following a similar result in September 2019. In an attempt to confirm if SMB inhabited this lake, netting work was conducted for 5 days at the end of October 2020; however, no SMB were caught. That said, the water temperature was likely below 10 °C, which means that SMB may move around less and are likely less susceptible to being captured by netting. Furthermore, the locations where the nets were set might not have been ideal due to the lack of bathymetric data for the lake.

Site 19, approximately 4 km downstream from site 18 (which was inconclusive in 2019, but not detected in 2020), was inconclusive in 2020 and should also be investigated further. Following the report of a SMB sighting near Boiestown and near Blackville, as well as the SMB caught in the DFO index trap net at Millerton, sampling was extended up to approximately 96 km in the SWM River and in the Bartholomew, NWM, Renous and Barnaby Rivers. From this work, inconclusive results were obtained at site 37 (Doaktown) and site 39 (Blackville), which suggests the potential presence of SMB and the need for follow-up investigation. The eDNA sampling performed in response to the SMB caught in the DFO index trap net did not detect SMB DNA at sites nearby, although this wasn't surprising considering the large size of the river in the Millerton area, the tidal effect and the fact that no other SMB has been reported or caught in that area, except for the one fish caught in the trap net.

Overall, the use of targeted eDNA analysis in 2019 and 2020 as part of this response provided valuable insight on the potential distribution of SMB in the SWM River and the results from the "McKiel stretch" were confirmed with the high number of SMB caught in McKiel Pond. The information obtained from this work, including the inconclusive results in McKiel Lake and at sites substantially downstream from the "McKiel stretch", taken together with the report near Boiestown and Blackville and the SMB caught in the DFO index trap net in Millerton points towards the possibility of extended but limited SMB spread in the SWM River, however more work would help provide increased certainty. As next steps, eDNA could be used to repeat sampling where inconclusive results were obtained, and other assessment methods (angling,

netting, etc.) could be attempted to confirm that SMB are indeed present near those sites with inconclusive results. To increase SMB DNA detection probability, we also recommend sampling at times when SMB are expected to be active and avoid sampling when water temperatures are below 10 °C. Finally, these eDNA results contribute towards one of the key elements to manage aquatic invasive species, which is to understand the distribution of the species in specific habitats. This information is important for the development of ecological risk assessment to determine the probability of a species to become established and the appropriate response level, including the actions to control the spread and the impact of the species.

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