Scoping the distribution of Smallmouth Bass (Micropterus dolomieu) in the Miramichi River Watershed using environmental DNA, 2021

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Irlich, U., LeBlanc, F., Bourque, D. and Akaishi, F. 2022. Scoping the distribution of Smallmouth Bass (*Micropterus dolomieu*) in the Miramichi River Watershed using environmental DNA, 2021. Can. Data Rep. Fish. Aquat. Sci. 1353: v + 9 p.

Following the report of a Smallmouth Bass (*Micropterus dolomieu*) in the Southwest Miramichi River in August 2019, an assessment of the Smallmouth Bass distribution in the Miramichi River Watershed was conducted in 2019 and 2020 using environmental DNA (eDNA) (LeBlanc et al., 2021). Characterization of the Smallmouth Bass distribution in the Miramichi River Watershed using eDNA was continued in 2021. A total of 46 sites were sampled in 2021, including 16 new sites added to better understand the results from previous years and to investigate some anecdotal reports of Smallmouth Bass observations by anglers. Sampling efforts were focused on the area downstream of Slate Island, approximately 9 km downstream of the area referred to as the "McKiel stretch". Results classified as suspected were obtained in the lower portion of McKiel Bogan, the east branch of Lake Brook and approximately 200 m downstream of McKiel Pond. Results classified as inconclusive were obtained at four sites downstream of the "McKiel stretch". Additionally, one site near Blackville and one site upstream of the confluence of Lake Brook and the Southwest Miramichi River were also classified as inconclusive. All other sites, including those in McKiel Lake and McKiel Brook, as well as in the Taxis, Bartholomew, Renous and Barnaby Rivers had results classified as not detected. In conclusion, the 2021 eDNA results did not demonstrate an expansion of the detection of Smallmouth Bass DNA in the Miramichi River Watershed.

RÉSUMÉ

Irlich, U., LeBlanc, F., Bourque, D. and Akaishi, F. 2022. Scoping the distribution of Smallmouth Bass (*Micropterus dolomieu*) in the Miramichi River Watershed using environmental DNA, 2021. Can. Data Rep. Fish. Aquat. Sci. 1353: v + 9 p.

Suite au rapport d'un achigan à petite bouche (*Micropterus dolomieu*) dans la rivière Southwest Miramichi en août 2019, une évaluation de la répartition de l'achigan à petite bouche dans le bassin versant de la rivière Miramichi a été réalisée en 2019 et 2020 en utilisant l'ADN environnemental (ADNe) (LeBlanc et al., 2021). La caractérisation de la distribution de l'achigan à petite bouche dans le bassin versant de la rivière Miramichi à l'aide de l'ADNe s'est poursuivie en 2021. Au total, 46 sites ont été échantillonnés en 2021, dont 16 nouveaux sites ajoutés pour mieux comprendre les résultats des années précédentes et pour confirmer certains rapports anecdotiques d'observations d'achigan à petite bouche par des pêcheurs. Les efforts d'échantillonnage ont été concentrés sur la zone en aval de « Slate Island », à environ 9 km en aval de la zone appelée « McKiel stretch ». Des résultats classifiés comme suspectés ont été obtenus dans la partie inférieure de « McKiel Bogan », la branche Est de « Lake Brook » et à environ 200 m en aval de la zone « McKiel stretch ». Des résultats classifiés comme non concluants ont été obtenus à quatre sites en aval de la zone « McKiel strech », ainsi qu'un site près de Blackville et un site en amont de la confluence de Lake Brook et de la rivière Southwest Miramichi. Tous les autres sites, y compris ceux dans le lac McKiel et dans le ruisseau McKiel, ainsi que dans les rivières Taxis, Bartholomew, Renous et Barnaby ont eu des résultats classifiés comme non détectés. En conclusion, les résultats d'ADNe de 2021 n'ont pas démontré une expansion de la détection de l'ADN de l'achigan à petite bouche dans le bassin versant de la rivière Miramichi.

1. INTRODUCTION

In August 2019, Smallmouth Bass (*Micropterus dolomieu*) was reported and caught in the Southwest Miramichi River (SWM River), approximately 12 km downstream of Miramichi Lake. Since then, Fisheries and Oceans Canada (DFO) has been assessing the extent of the spread of Smallmouth Bass (SMB) in parts of the Miramichi River Watershed (MRW). Environmental DNA (eDNA)-based detections of SMB has been a key tool in this assessment.

In 2019 and 2020, water samples were collected at 47 unique sites and analyzed for SMB DNA using a targeted qPCR approach (LeBlanc et al., 2021). The presence of SMB DNA was found at multiple sites (classified as suspected and detected) in a 3.5 km stretch of the SWM River encompassing McKiel Pond, where a total of 108 SMB were caught in 2019 and 2020. Furthermore, some SMB DNA detections (classified as inconclusive) were obtained upstream of the confluence of Lake Brook and the SWM River, in McKiel Lake, McKiel Brook, as well as a few other sites in the SWM River between the outflow of McKiel Brook and Blackville (LeBlanc et al., 2021).

Efforts to characterize the SMB distribution in parts of the MRW using eDNA continued in 2021 at a selection of sites sampled in previous years in order to confirm and repeat some results, as well as at 16 new sites. This data report serves to complement the previously published 2019 and 2020 eDNA-based SMB detection data (LeBlanc et al., 2021) and adds new information to the species distribution in the MRW.

2. MATERIALS AND METHODS

Water collection for eDNA-based SMB detection was conducted in 2021 at a total of 46 sites including 16 new sites not sampled in previous years (Figure 1).The 16 new sites were added to try to confirm inconclusive results and gain additional insight about findings from previous years, and to try to corroborate anecdotal reports of SMB observations (Table 1). Sampling efforts in 2021 were focused on sites downstream of Slate Island (i.e., site 13) starting approximately 9 km downstream of the area referred to as the "McKiel stretch" (LeBlanc et al., 2021), it also included a small subset of sites upstream or in tributaries near the "McKiel stretch". The presence of SMB in the "McKiel stretch", and more precisely in McKiel Pond, is now well documented, and, as such, sampling in 2021 was mainly focused on sites outside of this area in an attempt to better delineate the SMB distribution in the MRW. All sampling was conducted during five sampling events between June 22nd and July 28th, 2021.

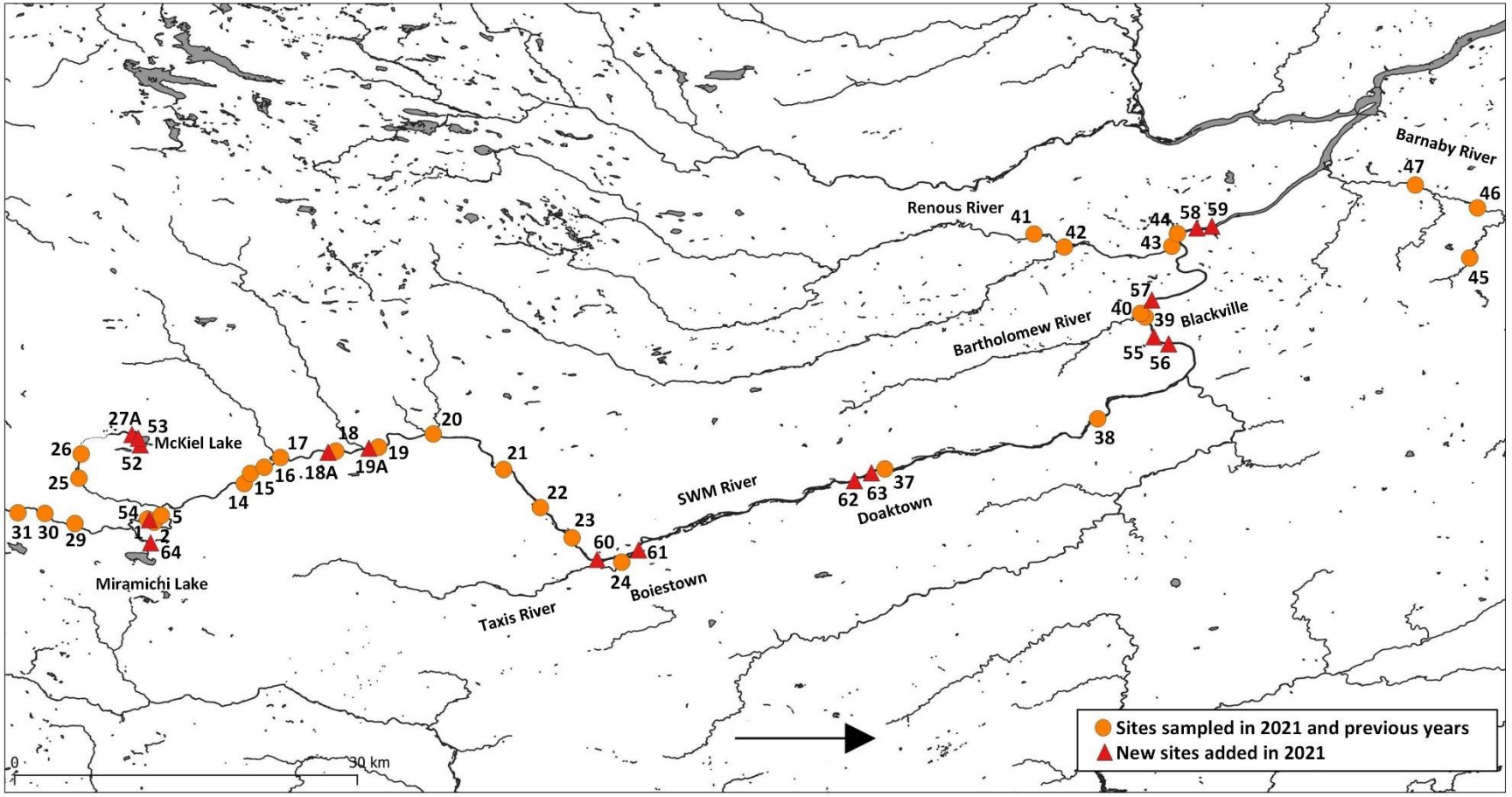

Figure 1: Map of sites surveyed in 2021. The arrow indicates the direction of the water flow.

Site 5, which is situated immediately downstream of McKiel Pond, was used as a reference site and was sampled on multiple sampling events that took place in the vicinity. This site was also used as a reference site in 2019 and 2020, and was selected for this purpose due to the known presence of SMB in McKiel Pond (i.e., removal of a total of 108 fish in 2019 and 2020, as well as 4 young of the year in 2021).

Sites sampled upstream or in tributaries in the vicinity of the "McKiel stretch" included three sites in McKiel Bogan (site 1, 2 and 54; Figure 1), and one site in the east branch of Lake Brook (site 64). Sampling was also conducted in McKiel Lake (site 27A, 52 and 53) and McKiel Brook (site 25 and 26). An additional three sites were samples in the main stem of the SWM River upstream of the confluence of Lake Brook and the SWM River (site 29, 30 and 31).

For sites downstream of Slate Island, sampling was done from site 14 to approximately 4 km downstream of Blackville in the SWM River and also included sites in the Taxis (site 24), Bartholomew (site 40), Renous (site 41, 42, 43 and 44) and the Barnaby (site 45, 46 and 47) Rivers, which were added to the sampling plan in 2020 following the capture of a SMB in the DFO index trap net near Millerton in the same year.

Table 1: List of new sites with a brief description of why they were sampled.

New Site	Close to Site	Reason for adding
Site 18A	Upstream of site 18	- Inconclusive result at site 18 in October 2019
Site 19A	Upstream of site 19	- Inconclusive result at site 19 in September 2020
Site 27A, 52 and 53	McKiel Lake	- Collection of eDNA by boat in different parts of the lake due to an inconclusive results at site 27 in September 2019 and August 2020
Site 54	Middle stretch of McKiel Bogan	- SMB eDNA has been detected at site 2, the lower reaches of the Bogan but not the upper reaches (site 1). Site 54 was selected to increase resolution
Sites 55, 56 and 57	Upstream and downstream of site 39 near Blackville	- Unconfirmed report of a SMB caught in June 2016 near Blackville and an inconclusive result in September 2020 at site 39
Site 58 and 59	Downstream of site 44 near Doyles Brook	- Unconfirmed report of an angler having caught a SMB in May 2021 near Doyles Brook
Site 60 and 61	In the main steam of the SWM River, downstream of site 23 near Boiestown	- Observation of SMB was reported in September 2020 - An angler reported to have caught a SMB in September 2016 in the Taxis River near Boiestown - No sites had previously been sampled in the main stem of the SWM River between sites 23 and 37
Site 62 and 63	Upstream of site 37 in Doaktown	- Inconclusive result in September 2020
Site 64	East branch of Lake Brook	- No previous eDNA was taken in this tributary and no knowledge of possible spread of SMB up this side arm of Lake Brook is available

At each site, water sampling consisted of collecting three x 1 L at approximately elbow depth using a lateral transect approach, when possible.

All sample processing, including filtration, DNA extraction and qPCR testing was completed as outlined in LeBlanc et al. (2021), with the exception of the qPCR performed on some samples using the TaqPath™ ProAmp™ Master Mix (Thermo Fisher Scientific, MA, USA) instead of the 2x TaqMan Gene Expression Kit (Thermo Fisher Scientific, MA, USA). Both qPCR kits performed similarly, with similar limit of detection (LOD) (data not shown).

For quality control purposes, field and lab filtration blanks (tap water) were included and processed (ratio of one of each for every ten field samples from each sampling event) alongside field samples, as well as DNA extraction blanks and qPCR negative controls. Sanger sequencing was also performed on ~30 % of samples with detectable SMB DNA (i.e., six out of 20) for confirmatory purposes.

3. RESULTS

No contaminations were observed in any of the blanks or negative controls, and Sanger sequencing performed on six field samples classified as either suspected or inconclusive confirmed the amplified product to be SMB DNA. All results from the 2021 sampling events are depicted in Figure 2 and listed in Table 2. Results from samples collected at site 5 (the positive reference site), were classified as "suspected" on all three sampling dates.

A small subset of sites were sampled in tributaries near the "McKiel stretch". In McKiel Bogan, site 2, at the lower end of the Bogan, was classified as suspected while the two sites in the upstream portion of the Bogan were both not detected (site 1 and 54). The site sampled in the east branch of Lake Brook (site 64) was classified as suspected, while the sites sampled in McKiel Brook (site 25 and 26) and McKiel Lake (site 27A, 52 and 53) were all classified as not detected. In addition, three sites sampled further upstream of the "McKiel stretch" towards Juniper had the following results: site 29 was classified as inconclusive on July 7th and as not detected when resampled on July 19th. Sites 30 and 31 were sampled only once in 2021 and were both not detected.

Downstream of Slate Island, sites 14, 15, 16 and 18 were all classified as inconclusive. Site 17, 18A (nearly 700m upstream of site 18) and all sites downstream of site 18 were not detected, with the exception of site 55 near Blackville, which was classified as inconclusive.

All sites sampled in the Taxis (site 24), Bartholomew (site 40), Renous (site 41, 42, 43 and 44) and the Barnaby (site 45, 46 and 47) Rivers were not detected.

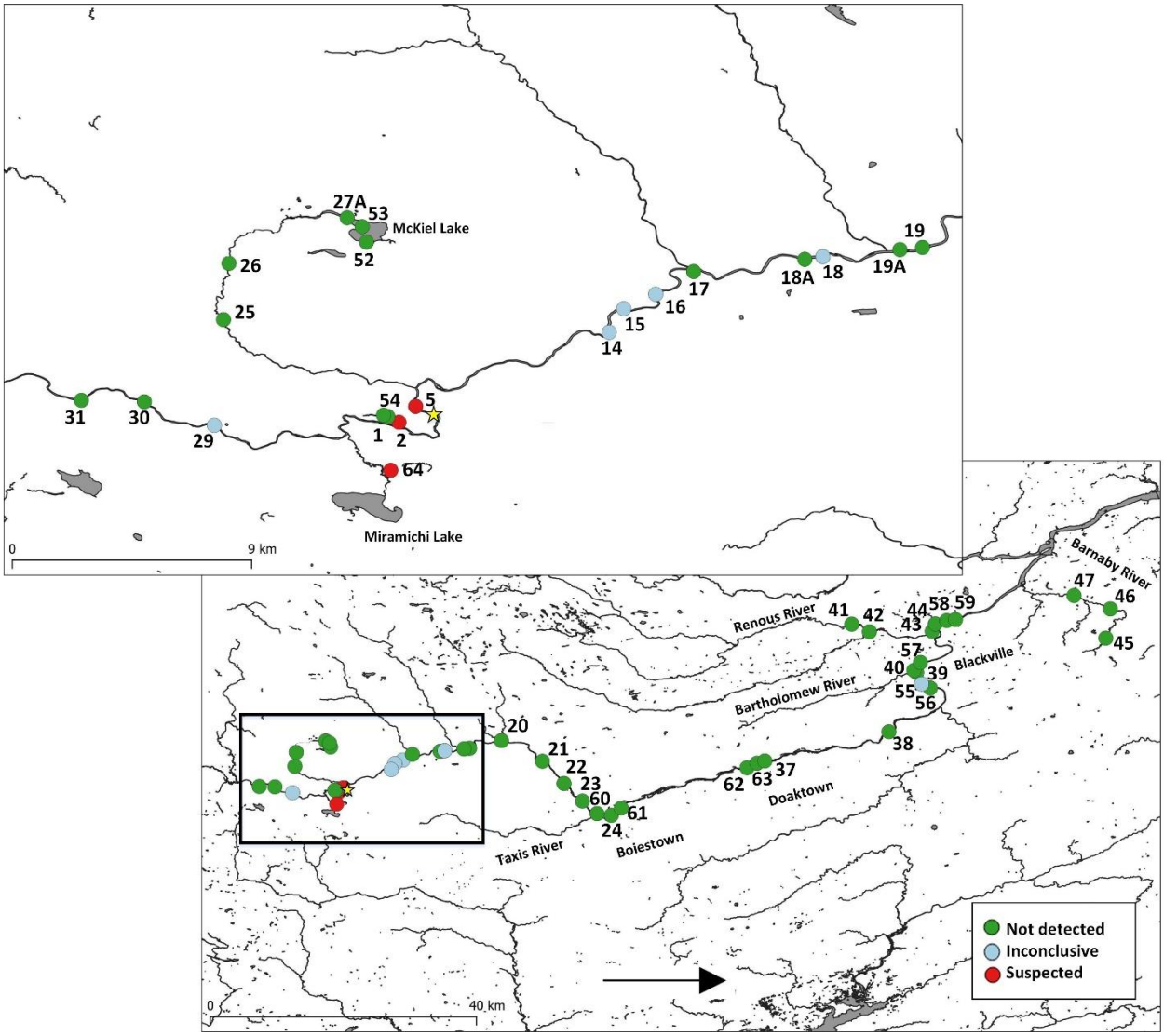

Figure 2: SMB eDNA results in 2021. The location of McKiel Pond where a total of 108 SMB have been caught in 2019 and 2020, as well as four young of the year in 2021 is denoted by a yellow star. The arrow indicates the direction of the water flow. The classification of results is similar to LeBlanc et al. (2021).

Table 2: List of sites and sampling events in 2021 and SMB qPCR results.

Site ID	Waterbody	Latitude (°N)	Longitude (°W)	Sampling date	SMB qPCR Result
Site 1	McKiel Bogan	46.50327	-66.96548	July 19	Not detected (0/3)
Site 2	McKiel Bogan	46.49976	-66.95794	July 19	Suspected (1/3) / Inconclusive (2/3)
Site 5	SWM River	46.50753	-66.94977	June 22	Suspected (2/3) / Inconclusive (1/3)
				July 7	Suspected (3/3)
				July 19	Suspected (2/3) / Inconclusive (1/3)
Site 14	SWM River	46.54391	-66.85485	July 7	Inconclusive (1/3)
Site 15	SWM River	46.55547	-66.84771	July 7	Inconclusive (2/3)
Site 16	SWM River	46.56257	-66.83200	July 7	Inconclusive (1/3)
Site 17	SWM River	46.57370	-66.81342	July 19	Not detected (0/3)
Site 18	SWM River	46.58102	-66.75007	July 19	Inconclusive (1/3)
Site 18A	SWM River	46.57970	-66.75891	July 19	Not detected (0/3)
Site 19	SWM River	46.58554	-66.70118	July 28	Not detected (0/3)
Site 19A	SWM River	46.58444	-66.71226	July 28	Not detected (0/3)
Site 20	SWM River	46.60063	-66.63863	July 28	Not detected (0/3)
Site 21	SWM River	46.56006	-66.55818	July 28	Not detected (0/3)
Site 22	SWM River	46.51649	-66.51611	July 28	Not detected (0/3)
Site 23	SWM River	46.48188	-66.47978	July 28	Not detected (0/3)
Site 24	Taxis River	46.45406	-66.42301	July 28	Not detected (0/3)
Site 25	McKiel Brook	46.55011	-67.04399	June 22	Not detected (0/3)
Site 26	McKiel Brook	46.57771	-67.04132	June 22	Not detected (0/3)
Site 27A	McKiel Lake	46.60005	-66.98332	June 22	Not detected (0/3)
Site 29	SWM River	46.49828	-67.04850	July 7	Inconclusive (1/3)
				July 19	Not detected (0/3)
Site 30	SWM River	46.50981	-67.08282	July 19	Not detected (0/3)
Site 31	SWM River	46.51053	-67.11370	July 7	Not detected (0/3)
Site 37	SWM River	46.56064	-66.12210	July 28	Not detected (0/4)
Site 38	SWM River	46.61789	-65.87861	July 20	Not detected (0/3)
Site 39	SWM River	46.73431	-65.82416	July 20	Not detected (0/3)
Site 40	Bartholomew River	46.73844	-65.82952	July 20	Not detected (0/3)
Site 41	Renous River	46.82914	-65.95141	July 20	Not detected (0/3)
Site 42	Renous River	46.81429	-65.91673	July 20	Not detected (0/3)
Site 43	Renous River	46.81524	-65.79379	July 20	Not detected (0/3)
Site 44	Renous River	46.82956	-65.78744	July 20	Not detected (0/3)
Site 45	Barnaby River	46.80176	-65.45304	July 20	Not detected (0/3)
Site 46	Barnaby River	46.85900	-65.44416	July 20	Not detected (0/3)
Site 47	Barnaby River	46.88523	-65.51533	July 20	Not detected (0/3)
Site 52	McKiel Lake	46.58815	-66.97387	June 22	Not detected (0/3)

Site ID	Waterbody	Latitude (°N)	Longitude (°W)	Sampling date	SMB qPCR Result
Site 53	McKiel Lake	46.59570	-66.97599	June 22	Not detected (0/3)
Site 54	McKiel Bogan	46.50257	-66.96341	July 19	Not detected (0/3)
Site 55	SWM River	46.71168	-65.81432	July 20	Inconclusive (1/3)
Site 56	SWM River	46.70361	-65.79745	July 20	Not detected (0/3)
Site 57	SWM River	46.75384	-65.81673	July 20	Not detected (0/3)
Site 58	SWM River	46.83605	-65.76528	July 20	Not detected (0/3)
Site 59	SWM River	46.83822	-65.74800	July 20	Not detected (0/3)
Site 60	SWM River	46.45730	-66.45112	July 28	Not detected (0/3)
Site 61	SWM River	46.46809	-66.40372	July 28	Not detected (0/3)
Site 62	SWM River	46.54727	-66.15685	July 28	Not detected (0/3)
Site 63	SWM River	46.55625	-66.13745	July 28	Not detected (0/3)
Site 64	East branch of Lake Brook	46.47623	-66.96195	July 20	Suspected (1/3)

4. DISCUSSION

The characterization of the SMB distribution in the MRW using eDNA was continued by DFO in 2021 and this report supplements the results presented in LeBlanc et al. (2021).

For the small subset of sites sampled upstream or in tributaries near the "McKiel stretch", results for sites 1, 2, 30 and 31 were similar to the results in previous years (LeBlanc et al., 2021). Site 29 was inconclusive in one of the two sampling events in 2021, but not detected in previous years. This site is less than 4 km upstream of site 10 (which was inconclusive in 2020 - see LeBlanc et al. (2021)), both of which are upstream of the confluence of Lake Brook and the SWM River, which suggests the presence of SMB above the confluence. Two new sites, site 54 located in the McKiel Bogan and site 64 located in the east branch of Lake Brook, were added in 2021 to enhance our resolution on the possible distribution of SMB in those tributaries. At site 54, as well as at site 1 in the upper reaches of McKiel Bogan, no SMB DNA was detected, suggesting that SMB have not moved up the Bogan. This was expected due to the presence of a beaver dam limiting movement upstream. At site 64, the result was classified as suspected, indicating the likely presence of SMB in the east branch of Lake Brook. Lake Brook drains Miramichi Lake into the SWM River and 16 SMB young of the year were caught in the upper reaches of the brook in 2019 and 2020.

Within McKiel Lake, site 27A (sampled by boat) was located close to the previously sampled site 27 (sampled from the shore), which gave inconclusive results in September 2019 and August 2020. In 2021, site 27A as well as the two new sites sampled in the lake (site 52 and 53) were all not detected. Attempts to capture SMB in McKiel Lake using netting and boat electrofishing techniques was again undertaken in 2021 over the course of two days, with no SMB caught.

Results from sites sampled downstream of the "McKiel stretch" up to Boiestown were overall in agreement with results from previous years, with some slight differences observed. Sites 14, 15,

16 and 18 were all inconclusive in 2021, while in previous years sites 15 and 16 were not detected, site 14 was inconclusive in 2020, but not detected in 2019 and site 18 was not detected in 2020, but inconclusive in 2019. These minor differences highlight the potential stochasticity in eDNA detections when species are found in small numbers and low biomass, but the repeated inconclusive results between years for some sites provides increased confidence that SMB are likely present in this area. Site 14 is about 9 km from the "McKiel stretch" and site 18 about 21.5 km, with two sites not detected (site 17 and 18A) just upstream. This gap in spatial eDNA distribution, taken together with the information that eDNA detection decreases with the distance from the source population (Wood et al., 2021), suggests that the eDNA detections at these sites, and especially site 18, are unlikely resultant of SMB present in the "McKiel stretch".

When looking at results for sites in the SWM River downstream of Boiestown, site 37, in Doaktown, was inconclusive in 2020 on one of the sampling events. Hence, additional sites (site 62 and 63) were sampled in 2021 to gather more information. All three sites around Doaktown were sampled once in 2021 and they were all not detected. Another area where additional investigation was required was the main stem of the SWM River near Blackville. A report was received of a SMB being angled in this area (exact location unknown) in 2016, and, in 2020, an inconclusive result was obtained in one of the two sampling events at site 39, which is located directly in Blackville. In 2021, an inconclusive result was obtained at a new site (site 55) situated less than 3 km upstream of site 39, while site 39 itself was not detected.

As in 2020, sampling was conducted in the Renous and Barnaby Rivers (sites 41, 42, 43, 45, 46 and 47) in 2021. These sites were added in 2020 following the capture of one SMB in a DFO index trap net in Millerton (site 48). Two additional SMB were caught in the Millerton index trap net in 2021 (August 18th and September 24th). All sites in the Renous and Barnaby Rivers were not detected in both years. Site 48 located near the Millerton index trap net was not sampled in 2021 due to the expected low detection probability caused by the tidal influence at that site and the relatively large volume of water.

An important consideration in the interpretation of 2021 results, and the comparison to 2019 and 2020 results is the timing of the sampling in 2021 compared to previous years. The 2021 eDNA sampling events took place between June 22nd and July 28th, which was significantly earlier than in 2019 (between August 27th and October 10th) and 2020 (between August 20th and September 21st). Sampling earlier in the season adds considerations such as increased water levels which can dilute eDNA detection. While water levels were not measured, based on observations, they appeared higher in 2021 than in previous sampling years. Water temperatures in July 2021 were warmer than in September 2020. This should likely not negatively impact the detection probability of SMB DNA, since SMB are know to prefer and be more active at warmer temperatures (Brown et al., 2009). Additionally, warmer temperatures have been shown to be associated to higher eDNA concentrations for some species (Jo et al., 2019). In Miramichi Lake, SMB spawn in May/June and the first young of the year are usually caught mid-July (Biron, 2018). However, in 2021, the first young of year were caught at the end of June, the earliest on record since Miramichi Lake control management operations started, indicating that SMB were active during the sampling period. In conclusion, the 2021 eDNA results presented in this report

helped corroborate some previous findings and showed an overall similar distribution of SMB in the MRW as reported in previous years.

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