NOVA SCOTIA SALMON ASSOCIATION

Characterizing water chemistry and the distribution of Atlantic Salmon on Nova Scotia's eastern shore based on environmental DNA (eDNA)

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EXECUTIVE SUMMARY

Atlantic Salmon are an iconic species as indicators of ecosystem health and for their importance to Indigenous Peoples and recreational anglers. Yet, Atlantic Salmon populations have declined across North America, particularly over the past 30 years. In 2019, the Nova Scotia Salmon Association, with support from Oceans North, conducted a survey of 30 locations on Nova Scotia's eastern shore using environmental DNA (eDNA) to determine the contemporary distribution of Atlantic Salmon, including at former Fisheries and Oceans Canada (hereafter DFO) electrofishing sites last surveyed in 2008/2009, and to characterize the quality of habitat via comprehensive water quality analyses. Our results suggest that the distribution of Atlantic Salmon has remained relatively unchanged over the last 20 years, and salmon eDNA was detected at 14 of 30 sites overall. Further, we presented evidence that water quality and the presence of Atlantic Salmon appear linked, with the legacy of acid rain continuing to reduce salmon survival. Our general recommendations from this research are:

- Invest in efforts to increase the abundance of salmon populations in rivers with high-quality salmon habitats that contain salmon (e.g. Kent Brook (Musquodoboit River), Indian Harbour Lakes, and Country Harbour River).
- Invest in research to identify the limiting factors for salmon survival in rivers with high-quality salmon habitats, where we did not find evidence of salmon (e.g. Salmon River [Lake Major]).
- Continue to operate and expand upon existing liming programs to neutralize river acidity and restore the productive capacity of Atlantic Salmon habitat.
- Invest in efforts to quantify change in salmon abundance through additional traditional sampling measures (e.g. electrofishing) to confirm or refute eDNA surveys in newly identified rivers (e.g. Kent Brook and McCaffrey Brook).
- Ensure the future sampling programs are widespread and recurrent (more frequently than every 10 years) so as to capture trends in distributions earlier.
- Invest in immediate recovery efforts to maximize the number of existing populations protected and to maintain genetic diversity of salmon on the eastern shore.

Atlantic Salmon populations have not been lost in this region. The likelihood of recovering Atlantic Salmon populations declines with delays in the implementation of stewardship and restoration activities. Thus, should Canadians wish for salmon to remain a part of our coastal landscape, efforts to improve the quality of freshwater habitat must be supported. Restoring Atlantic Salmon habitat will ultimately improve the overall ecosystem functioning, biodiversity and health of Nova Scotia's eastern shore.

INTRODUCTION

Understanding the distribution of fish is a critical component of freshwater restoration planning and can help identify where recovery action is likely to be most impactful. In northeastern North America, a species of high conservation importance is the Atlantic Salmon, *Salmo salar*. Salmon populations have declined dramatically in the past 30 years, particularly in areas at the southern extent of their range, such as New England and Canada's Maritime provinces. Freshwater productivity has declined since the 1970s, resulting in fewer salmon migrating to sea. In addition, marine survival has greatly declined since 1990, meaning that fewer salmon survive and are able to return to freshwater to spawn.

In Nova Scotia's eastern shore, many factors affect salmon population declines. However, the legacy of decades of acid rain and poor land-use practices have been linked to reduced freshwater survival rates[1,2] and presumably to reduced marine survival rates.[3,4] Through the 1970s and 1980s, almost half of all rivers in Nova Scotia were severely affected by acid rain.[5] Acidification has drastically altered soil and water chemistry, leading to reduced nutrients. The result is forest soils that are poorly buffered, which has increased the export of ionic aluminum (Al_i) and iron (Fe) into streams and lakes. Streams, rivers, and lakes exhibit low pH as a result of acid rain and increasing organic acids from forest soils. Together, these changes have made freshwater conditions unfavourable for many aquatic organisms. For Atlantic Salmon, acidification has reduced freshwater productivity and decreased salmon survival in some locations by as much as 90%.[5] Other impacts to freshwater productivity include in-stream barriers that reduce connectivity of streams (e.g. culverts and dams), poor land-use practices (including poor agricultural practices) and widespread commercial forestry (including the legacy impacts of log-driving activity), all of which have altered stream hydrology and structure.

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[1] Lacroix, G.L., & Townsend, D.R. (1987) Responses of juvenile Atlantic Salmon (*Salmo salar*) to episodic increases in acidity of Nova Scotia rivers. *Canadian Journal of Fisheries and Aquatic Sciences*, 44, 1475–1484.

[2] Farmer, G.J. (2000). Effects of low environmental pH on Atlantic salmon (*Salmo salar L.*) in Nova Scotia. *Canadian Science Advisory Secretariat Research Document,* 2000/050. Available from: http://www.dfo-mpo.gc.ca/csas-sccs/publications/resdocs-docrech/2000/2000_050-eng.htm.

[3] Staurnes, M., Hansen, L.P., Fugelli, K. & Haraldstad, Ø. (1996). Short-term exposure to acid water impairs osmoregulation, seawater tolerance, and subsequent marine survival of smolts of Atlantic Salmon *(Salmo salar L.). Canadian Journal of Fisheries and Aquatic Sciences*, 53, 1695–1704.

[4] Kroglund, F. & Finstad, B. (2003). Low concentrations of inorganic monomeric aluminum impair physiological status and marine survival of Atlantic salmon. *Aquaculture*, 222, 119–133.

[5] Watt, W.D. (1987). A summary of the impact of acid rain on Atlantic Salmon (*Salmo salar*) in Canada. *Water Air Soil Pollution*, 35, 27–35. https://doi.org/10.1007/BF00183841

It has been estimated that it could take 50 to 70 more years for Nova Scotia's freshwater systems to recover from acid rain naturally.[6] However, scientists estimate that most Atlantic Salmon populations that comprise Nova Scotia's Southern Upland designatable unit (which contains the eastern shore of Nova Scotia) are likely to become extinct in less than 70 years.[7] Even a 50% improvement in freshwater productivity could potentially eliminate the likelihood that Atlantic Salmon populations become extinct, underscoring the need for immediate restoration of Nova Scotia's lakes, streams, and river systems.[7]

One of the first steps to designing and implementing an effective recovery strategy is to define the status of Atlantic Salmon along Nova Scotia's eastern shore. The most recent widespread electrofishing surveys for Atlantic Salmon were conducted by DFO and spanned the years 2008 and 2009. These DFO surveys suggested that total juvenile density decreased substantially in many locations relative to the 1980s, and fisheries managers speculate that populations have declined further since.

This report describes a project, led by the Nova Scotia Salmon Association and supported by Oceans North, that was designed to better understand the current distribution of Atlantic Salmon and characterize the quality of habitat along the eastern shore of Nova Scotia. To determine whether the distribution of Atlantic Salmon has changed, we observed the change in status (present/absent) from past electrofishing surveys (up to 2009) to current environmental DNA (eDNA) surveys in 2019. The goals of this project were: (a) to update surveys in freshwater habitat to confirm whether the distribution (number and location of occupied rivers) of Atlantic Salmon in the eastern shore of Nova Scotia has changed since 2009 and (b) to characterize water quality in some eastern shore rivers and link the distribution of Atlantic Salmon to water quality. By doing so, these actions can inform stewardship and restoration activities that support Atlantic Salmon recovery and improve the long-term functioning and health of Nova Scotia's freshwater ecosystems. A more complete scientific manuscript describing this study will be submitted for peer review.

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[6] Clair, T.A., Dennis, I.F., Amiro, P.G., & Cosby, B.J. (2004). Past and future chemistry changes in acidified Nova Scotian Atlantic salmon (*Salmo salar*) rivers: A dynamic modeling approach. *Canadian Journal of Fisheries and Aquatic Sciences*, 61(10), 1965–1975.

[7] Gibson, A.J.F., & Bowlby, H.D. (2013). Recovery potential assessment for Southern Upland Atlantic Salmon: Population dynamics and viability. *DFO Canadian Science Advisory Secretariat Research Document,* 2012/142. iv + 129 p.

METHODS

In 2019, eDNA samples were collected at 30 sites across Nova Scotia's eastern shore (Figure 1). Environmental DNA is a rapid, cost-efficient survey approach that involves filtering small samples of river water and analyzing it for DNA released by animals in the watercourse. Environmental DNA surveys have become increasingly popular for rare fish like Atlantic Salmon as the analytical tools are highly sensitive and can identify species in low abundances that may otherwise go undetected with traditional gear. Nineteen of the 30 sites were selected based on their downstream proximity to historical electrofishing sites monitored by DFO in 2008/2009 (Appendix 1 and 2 in Bowlby et al., 2013[8]), and 11 were selected based on their relevance to ongoing and proposed conservation initiatives.

Each site was sampled on three dates (late May, early June, late June), timed to overlap with the outmigration of Atlantic Salmon smolt. This is a period where smolt migrate downstream from headwater nurseries to the lowermost portions of their natal watersheds, thus leaving genetic material, which could be detected. Because resources dictated that we sampled only one site per watershed (or sub-watershed), this timing was selected to maximize our ability to predict Atlantic Salmon habitat use upstream.

During eDNA collection, staff also collected field measurements of stream pH and temperature using a YSIPro10 field meter calibrated that day. A subset of 19 sites was also sampled for detailed water chemistry. Water samples were collected during all three sampling occasions at each site; however, only samples from two sampling occasions were viable, at Dollar Lake Brook and Kent Brook in the Musquodoboit watershed, due to a technical malfunction. In total, 17 environmental variables were measured (abbreviations and units in parenthesis): alkalinity ($CaCO_3$ $mg{\cdot}L^{-1}$), conductivity (µs/cm), total dissolved solids (TDS, $mg{\cdot}L^{-1}$), total organic carbon (TOC, $mg{\cdot}L^{-1}$), turbidity (NTU), pH, exchangeable inorganic aluminum (Al_i), fluoride (F), phosphate (PO_4^{3-}), calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na), potassium (K), phosphorus (P), and zinc (Zn) (ions in $\mu g{\cdot}L^{-1}$), across 19 of the 30 sites where eDNA were sampled (Table A3 and Table A4). Differences in water chemistry across all 19 sites were analyzed statistically using summary statistics to describe the general status of each site based on single variable measurements. While single variables can indicate general water quality and predict the occurrence of salmon, it is more likely that a suite of parameters and their interactions play an important role in the quality of freshwater habitat for salmon and other aquatic organisms. To address this, we also conducted a principal component

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[8] Bowlby, H.D., Gibson, A.J.F., & Levy, A. (2013). Recovery potential assessment for Southern Upland Atlantic Salmon: Status, past and present abundance, life history and trends. *DFO Canadian Science Advisory Secretariat Research Documents*, 2013/005. v + 72 p.

analysis which permits comparison between all sites based on multiple water-chemistry parameters

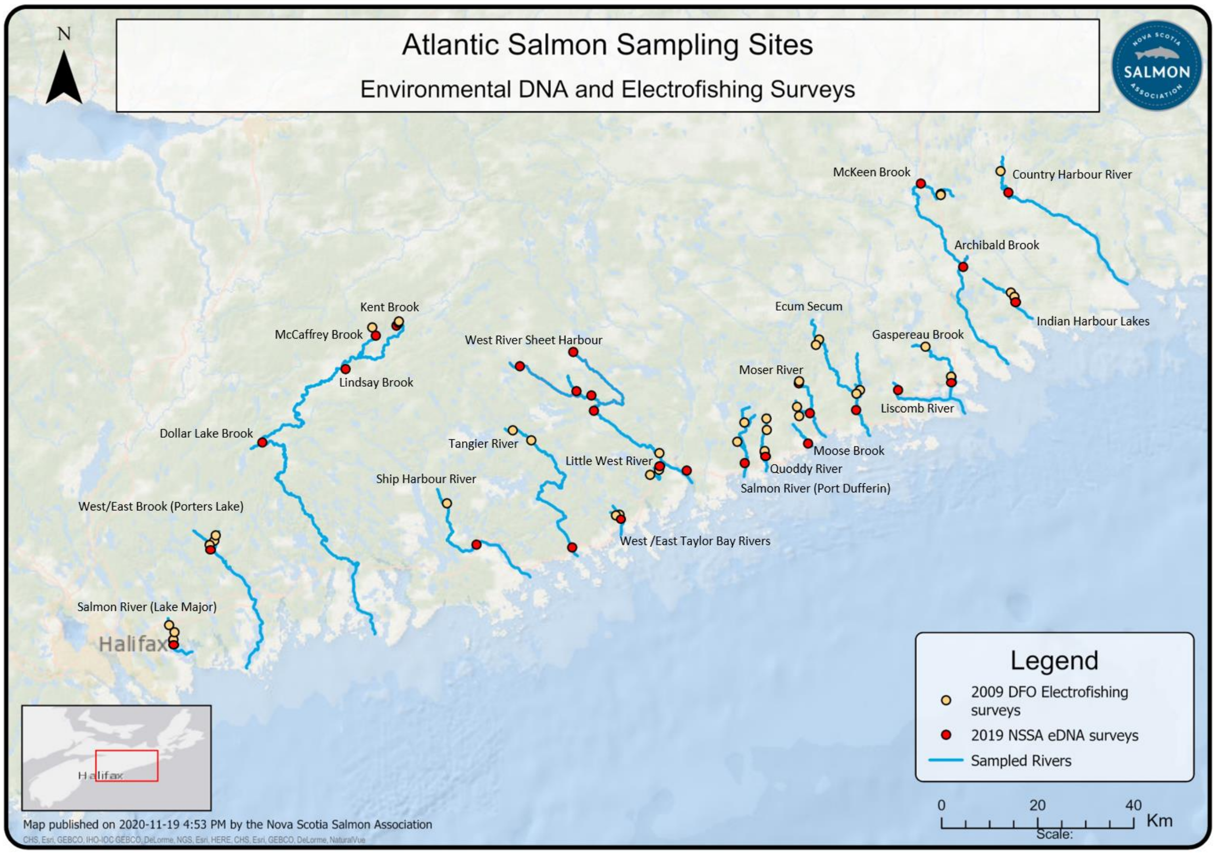

Figure 1 – Atlantic Salmon sampling sites along Nova Scotia's eastern shore; DFO-led electrofishing surveys from 2009 (yellow) and environmental DNA surveys (eDNA; red) from 2019.

RESULTS AND DISCUSSION

DISTRIBUTION OF ATLANTIC SALMON

The following section describes the change in Atlantic Salmon distribution between past electrofishing surveys (2008/2009) and current eDNA surveys (2019). Over half of the sampled locations (63%) showed no change in Atlantic Salmon distribution since the last region-wide sampling in Nova Scotia in 2009.

At six sites, Atlantic Salmon were captured in historical electrofishing surveys and detected in recent eDNA surveys, suggesting that salmon continue to persist in those rivers (Figure 2). These six sites were: Ship Harbour River, Salmon River (Port Dufferin), Mill Brook (Moser River), McKeen

Brook (St. Mary's River), Country Harbour River, and Indian Harbour Lakes. On the other hand, both electrofishing and eDNA surveys were unable to find Atlantic Salmon in the following six rivers: Salmon River (Lake Major), East Brook (Porters Lake), West Brook (Porters Lake), Tangier River, and East Taylor Bay River and West Taylor Bay River (Figure 2), suggesting that these locations have not supported an appreciable population of Atlantic Salmon for some time. While eDNA does not indicate whether densities or abundances have declined, these eDNA results do suggest that the distribution of populations has not changed substantially since the last federal electrofishing surveys in 2009.

Six sites showed differences between eDNA and historical electrofishing results (Figure 2), suggesting the distribution of salmon among these rivers has shifted over time. Atlantic Salmon DNA was detected at four sites where historical electrofishing surveys had failed to capture them; Kent Brook (Musquodoboit River), McCaffrey Brook (Musquodoboit River), Little West River and Moser River (Figure 2A–B). The results of positive DNA in these four rivers provide strong evidence that either: (1) Atlantic Salmon have recently started using these rivers as part of their life cycle or (2) eDNA can identify Atlantic Salmon in rivers where their abundance is low and it is therefore difficult to detect individuals with traditional sampling techniques. Follow-up sampling to identify salmon presence in these rivers, specifically Kent Brook and McCaffrey Brook, will help confirm their importance in supporting salmon populations.

There were only two sites (Quoddy River and Gaspereau Brook) where Atlantic Salmon DNA was not detected despite Atlantic Salmon being previously captured by historical electrofishing surveys upstream (Figure 2C–D). Failure to identify salmon at these locations may suggest that (1) salmon populations are possibly lost in these rivers; (2) salmon smolt may have left the system prior to sampling; or (3) eDNA surveys reported false negatives (i.e. failed to detect smolt when smolt were indeed present).

Lastly, eDNA samples were taken at five tributaries along the West River Sheet Harbour, two tributaries of the Musquodoboit, one tributary of the St. Mary's River and Smelt Brook—a total of nine locations where the DFO had not previously sampled (Figure 2). Atlantic Salmon DNA was present at five sites (Dollar Lake Brook, Lindsay Brook, Rocky Brook, Upper Killag River, Archibald Brook and absent at four sites (Jack Lowe Brook, Keef Brook, Tent Brook—all tributaries of West River Sheet Harbour, plus Smelt Brook), expanding our knowledge of Atlantic Salmon distribution in this region. These results show that future sampling programs should be: (1) widespread, for a more comprehensive understanding of salmon habitat across the eastern shore, and (2) recurrent (more frequent than every 10 years), to capture trends in distributions earlier and to effectively protect, manage, and restore salmon habitat in the face of climate change and other threats.

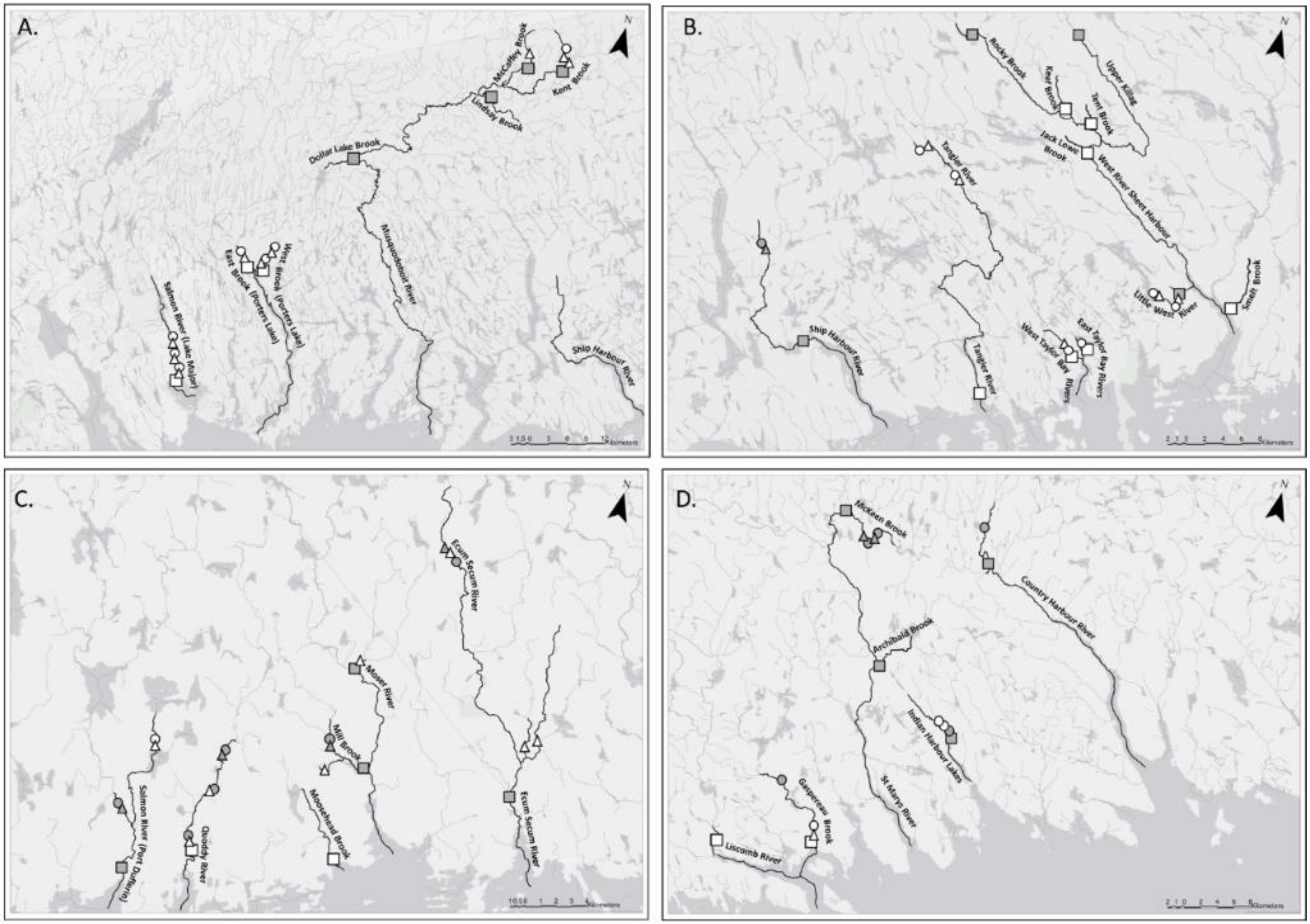

Figure 2 - Environmental DNA (eDNA; squares) and DFO-led electrofishing surveys from 2000 (circles) and 2008/2009 (triangles) along Nova Scotia's eastern shore. Salmon (individuals or eDNA) were detected (grey symbols) or not detected (white symbols) during electrofishing surveys or any of the three eDNA surveys.

WATER CHEMISTRY

The following section describes the variety of water-quality conditions across Nova Scotia's eastern shore in order to better understand how the legacy of acid rain and land use has had an impact on salmon habitat in these rivers. The main finding supports the notion that rivers in this region differ largely by acidification status, specifically by water-chemistry variables such as the pH, conductivity, alkalinity, TDS, Mg, Ca, and Na.

The impact of water chemistry on fish health is complicated, and a single parameter rarely gives a full description of water quality. However, stream pH is a reasonable measure of freshwater acidification. Healthy freshwater lakes, ponds, and streams typically have a pH between 6 and 8; the lower the pH, the more acidic the water is and the larger the impact of acidification. In this study, the average springtime pH across the 30 sites was 5.19, ranging from a highly acidic 4.38 in Tent Brook (West River Sheet Harbour) to a relatively neutral 6.49 in Kent Brook (Musquodoboit River).

As expected, we found that pH was an important indicator of the presence of Atlantic Salmon. Rivers where Atlantic Salmon DNA was not detected were very acidic and had a mean field-measured pH of 4.7, compared to a mean pH of 5.3 in rivers with Atlantic Salmon DNA. Studies from the 1980s[9] found that Atlantic Salmon were not present in Nova Scotia rivers with a pH of < 4.7. All six sites sampled in this study where mean pH was <4.7 failed to show evidence of Atlantic Salmon eDNA (Figure 3). Of the 13 sites with a mean field-measured pH between 5.0 and 5.4 (i.e. moderately impacted by acidification), salmon eDNA was present at seven sites and absent at seven sites (Figure 3). Finally, ten sites had a mean pH > 5.4, which are considered to be sites where the impacts of acidification on salmon fry and parr life stages are minimal. Here, salmon eDNA was detected in all but one site—Salmon River (Lake Major) (Figure 3).

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[9] Watt, W.D. (1987). A summary of the impact of acid rain on Atlantic salmon (*Salmo salar*) in Canada. *Water Air and Soil Pollution*, 35, 27–35.

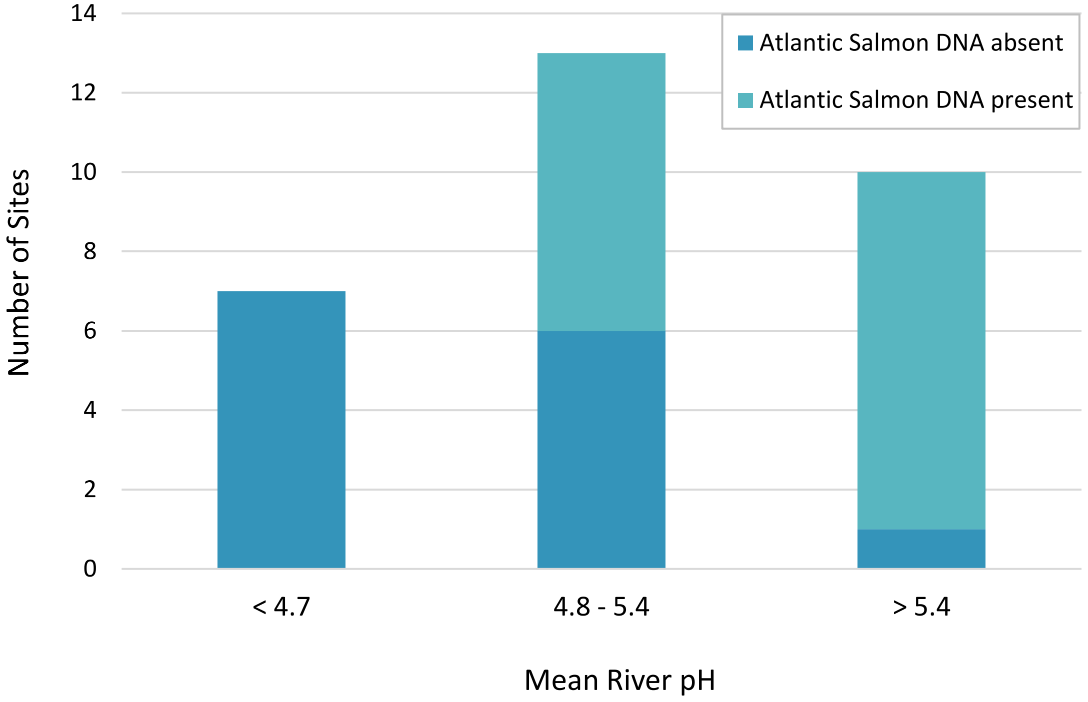

Figure 3 - Results of presence/absence eDNA assessments of 30 sites based on field-measured stream pH. Categorization of stream pH based on Watt (1987), where pH <4.7 is considered unsuitable for Atlantic Salmon, pH 4.8-5.4 is considered marginal for Atlantic Salmon, and pH >5.4 is considered acceptable for Atlantic Salmon.

Of the sampled water-quality parameters, sodium was the highest concentrated metal ion, ranging from 2126.3 μg·L^{-1} in Tent Brook (West River Sheet Harbour) to 8395.7 μg·L^{-1} in Jack Lowe Brook (West River Sheet Harbour), which is approximately 3 km downstream (Table A3 and Figure 4). This concentration of Na is in a healthy range for salmon, as they require a minimum 1 mg·L^{-1} of Na and have an optimum around 8 mg. Calcium showed a large variation in sampled concentrations among sites, ranging from 268.83 μg·L^{-1} in Salmon River (Porters Lake) to 1560.33 μg L^{-1} in East Taylor Bay River (Table A3 and Figure 4A). Contrary to Na levels across sites, all Ca levels collected were below the assumed minimum threshold of 4 mg·L^{-1} for good salmonid production. This is important, as Ca and Na levels above the assumed threshold would buffer the effects of acidity and aluminum, making salmon populations more resilient to acidification.

Concentrations of exchangeable aluminum were as low as 2.1 μg·L^{-1} in Country Harbour River and as high as 59.0 μg·L^{-1} in Mill Brook (Moser River) (Table A3 and Figure 4). Sixty-five percent of sites (n = 13) exceeded the presumed toxic aluminum threshold of 15 μg·L^{-1}, suggesting that these high-aluminum sites would no longer support salmon survival in freshwater.[10] However, it has been suggested that an aluminum threshold as low as 5-12 $\mu g \cdot L^{-1}$ is necessary to maintain the number of adults returning from the marine phase,[11] at which point 85% of sites would exceed this threshold. Although the data collected may not represent the most extreme conditions that occur in the sampled streams (i.e. those that occur during the first spring freshet), the values that we report here are comparable between sites and offer insight on general water quality of these habitats.

Overall, higher levels of water conductivity and higher concentrations of alkaline earth (Ca and Mg) and alkali metal (Na and K) cations indicate which rivers are good-quality habitat for salmon and have been only mildly affected by acidification. Sites with these qualities where salmon DNA were also found include Kent Brook (Musquodoboit River), Indian Harbour Lake and Country Harbour River. Efforts to increase the abundance of these salmon populations would be a worthwhile investment, given the quality of habitat in these rivers. Salmon River (Lake Major) also shares similar habitat qualities as those sites and would be considered good-quality salmon habitat. However, both electrofishing and eDNA surveys have failed to find evidence of salmon in this river. The amount of accessible habitat is limited in this area, but further research should be done to identify the factors limiting Atlantic Salmon in this river, given its fine water quality.

Several mitigation techniques are currently implemented in Nova Scotia to combat the impacts of acidification on water quality and Atlantic Salmon populations. Techniques such as dolomite lime ($CaCO_3$ / $MgCO_3$) treatments through land-based lime dosers and helicopter catchment liming are used at several sites within the West River, Sheet Harbour watershed. These treatments have resulted in increases in soil and water pH and returns of adult Atlantic Salmon.[12] Results from the water-chemistry analysis emphasize that acid rain continues to have widespread impacts on the rivers of Nova Scotia's eastern shore. Samples indicate low alkalinity, low base cations, and elevated levels of toxic aluminum. Coupled with the fact that rivers with Atlantic Salmon DNA had significantly higher mean pH, this study supports the continuation and expansion of liming programs to neutralize river acidity and restore the productive capacity of Atlantic Salmon habitat.

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[10] Sterling, S.M., MacLeod, S., Rotteveel, L., Hart, K., Clair, T.A., Halfyard, E.A., & O'Brien, N.L. (2020). Ionic aluminium concentrations exceed thresholds for aquatic health in Nova Scotian rivers, even during conditions of high dissolved organic carbon and low flow. *Hydrology and Earth System Sciences*, 24(10), 4763–4775.

[11] Kroglund, F., Rosseland, B.O., Teien, H.C., Salbu, B., Kristensen, T., & Finstad, B. (2008). Water quality limits for Atlantic salmon (*Salmo salar L.*) exposed to short term reductions in pH and increased aluminum simulating episodes. *Hydrology and Earth System Sciences*, 12, 491–507.

[12] Nova Scotia Salmon Association, unpublished data.

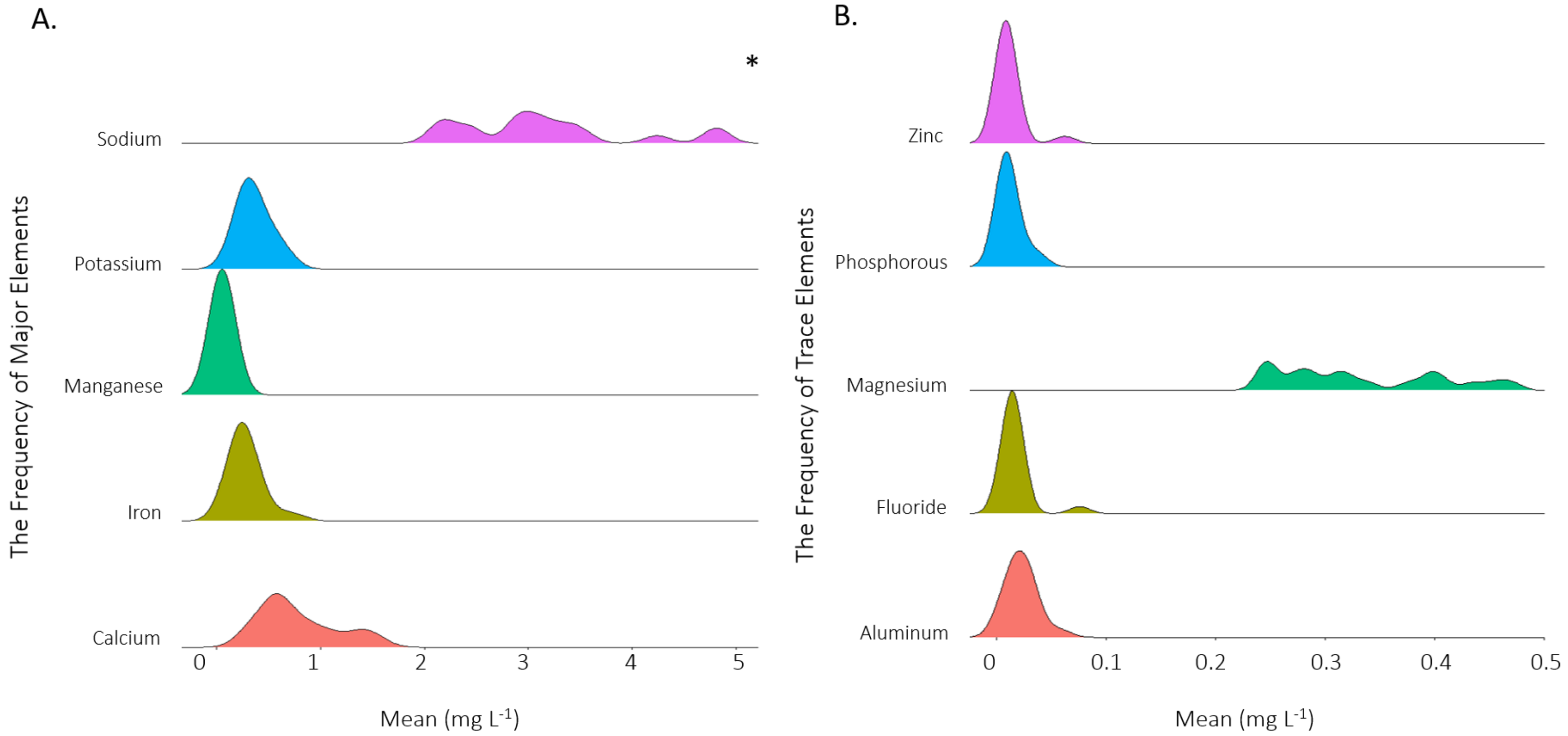

Figure 4 - The distribution frequency of metal ion concentrations for A. major elements (mg·L^{-1}) and B. trace elements (mg·L^{-1}) collected in freshwater samples from 19 rivers (three samples per river) across Nova Scotia's eastern shore. The x-axis represents the range of mean concentrations for each metal ion across the 19 rivers (e.g. calcium ranged from 0.27-1.57 mg·L^{-1}). The y-axis represents the relative frequency of each sampled concentration across the 19 rivers; the higher the peak, the more frequently the concentration was observed. * Two outliers for sodium not presented: SU_01 – 6.5 mg·L^{-1} and WR_17 – 8.4 mg·L^{-1}.

CONCLUSIONS

Acidification has had a long-lasting impact on Nova Scotia's freshwater rivers, directly affecting Atlantic Salmon populations throughout the Southern Uplands. In this study, we show that, although acidification still affects rivers throughout Nova Scotia's eastern shore, the distribution of Atlantic Salmon has remained relatively unchanged over the last 20 years. Most evidence suggests that the abundance of Atlantic Salmon in Nova Scotia's eastern shore is low[13], and while our study does not provide insights regarding salmon abundance, we demonstrate that Atlantic Salmon populations continue to persist in this region. Because these fish are genetically distinct and adapted to this landscape,[13] recovery efforts should be implemented immediately to maximize the number of populations protected and to maintain the genetic diversity of salmon on the eastern shore.

We report evidence that water quality and the distribution of Atlantic salmon appear linked. Therefore, improvements to water quality of current Atlantic Salmon habitat can play a large role in maintaining existing populations and supporting long-term viability. Additionally, we demonstrated that eDNA offers a unique opportunity to get widespread sampling coverage with minimal effort and resources, although it is limited to detecting the presence and may produce false absences. Efforts to quantify change in salmon abundance through traditional sampling measures would help to confirm or refute eDNA surveys and provide further insight into the health of populations affected by to a wide degree by acidification.

Atlantic Salmon occupy a diversity of habitats throughout their life cycle, highlighting the need for broad-based efforts to protect and restore aquatic habitats in this region. Throughout their migration, Atlantic Salmon depend on multiple habitats and effectively integrate terrestrial ecosystems, freshwater watersheds, estuaries, and coastal nearshore areas through nutrient and energy transport. Efforts to restore Atlantic Salmon habitat will therefore ultimately improve the overall ecosystem functioning, biodiversity, and health of Nova Scotia's eastern shore in freshwater, in estuaries, and on the coast.

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[13] Gibson, A. J. F., Bowlby, H. D., Hardie, D. C., & O'Reilly, P. T. (2011). Populations on the brink: Low abundance of Southern Upland Atlantic salmon in Nova Scotia, Canada. North American Journal of Fisheries Management, 31(4), 733-741.

APPENDIX

Table A1. Sites surveyed for environmental DNA (eDNA) and the corresponding upstream electrofishing sites surveyed by DFO in 2000 or 2008/2009. Sites where water-chemistry analysis was conducted indicated with an asterisk.

Site Code	Site Name	Latitude	Longitude	Upstream DFO electrofishing sites[14]
SU_30	Country Harbour River*	45.273861	-61.890767	SU61B
SU_19	Salmon River (Port Dufferin)*	44.916912	-62.384004	SU50A, SU50B
SU_20	Quoddy River*	44.931416	-62.34701	SU51A, SU51B, SU51C, SU51D
SU_21	Moosehead Brook	44.942983	-62.26549	*n/a*
SU_24	Ecum Secum River*	44.986957	-62.175677	SU54A, SU54B, SU54C, SU54D
SU_25	Liscomb River	45.013452	-62.097324	*n/a*
SU_26	Gaspereau Brook*	45.030766	-61.997462	SU56A, SU56B
MO_22	Moser River*	45.022445	-62.282753	SU52D
MO_23	Mill Brook (Moser River) *	44.983005	-62.262544	SU52A. SU52C
MU_04	Dollar Lake Brook (Musquodoboit River) *	44.944185	-63.286494	*n/a*
MU_05	Lindsay Brook (Musquodoboit River)	45.040979	-63.131121	*n/a*
MU_06	McCaffrey Brook (Musquodoboit River)	45.08531	-63.074411	SU40D
MU_07	Kent Brook (Musquodoboit River) *	45.098588	-63.03593	SU40B, SU40C
SU_01	Salmon River (Lake Major) *	44.682409	-63.453315	SU35A, SU35B, SU35C
SU_02	West Brook (Porters Lake) *	44.808782	-63.385232	SU37A
SU_03	East Brook (Porters Lake) *	44.81414	-63.376478	SU38A, SU38B
SM_27	McKeen Brook (St Mary's River)	45.285366	-62.055125	STMR854.4, STMR854.2
SM_28	Archibald Brook (St Mary's River) *	45.175953	-61.975802	*n/a*
SU_29	Indian Harbour Lakes*	45.136176	-61.880061	SU59A, SU59B, SU59C
SU_08	Ship Harbour River*	44.809041	-62.886009	SU42B
SU_09	Tangier River*	44.805283	-62.706835	SU43A, SU43B
SU_10	West Taylor Bay River	44.846851	-62.622575	SU45A
SU_11	East Taylor Bay River*	44.847674	-62.618318	SU44A
SU_12	Little West River*	44.907871	-62.544511	SU46A, SU46B
SU_18	Smelt Brook	44.907111	-62.49239	*n/a*
WR_13	Rocky Brook (West River Sheet Harbour)	45.045019	-62.805012	*n/a*
WR_14	Upper Killag (West River Sheet Harbour)	45.063727	-62.705022	*n/a*
WR_15	Keef Brook (West River Sheet Harbour)	45.011796	-62.698626	*n/a*
WR_16	Tent Brook (West River Sheet Harbour) *	45.006273	-62.670827	*n/a*
WR_17	Jack Lowe Brook*	44.985962	-62.666567	*n/a*

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[14] Bowlby, H.D., Gibson, A.J.F., & Levy, A. (2013). Recovery potential assessment for Southern Upland Atlantic Salmon: Status, past and present abundance, life history and trends. *DFO Canadian Science Advisory Secretariat Research Documents*, 2013/005. v + 72 p.

Table A2. Summarized results of environmental DNA (eDNA) for the 30 sites (appendix A1) in each of the three sampling events and our assigned presence (Y)/absence (N). Sites where water-chemistry analysis was conducted indicated with an asterisk.

Site	Site Name	Salmon eDNA detected? May 21-23	June 4-6	June 17-20	Declared Occurrence
SU_30	Country Harbour River*	Y	Y	Y	Present
SU_19	Salmon River (Port Dufferin) *	Y	Y	Y	Present
SU_20	Quoddy River*	N	N	N	Absent
SU_21	Moosehead Brook	N	N	N	Absent
SU_24	Ecum Secum River*	N	Y	Y	Present
SU_25	Liscomb River	N	N	N	Absent
SU_26	Gaspereau Brook*	N	N	N	Absent
MO_22	Moser River*	Y	N	Y	Present
MO_23	Mill Brook (Moser River) *	N	Y	N	Present
MU_04	Dollar Lake Brook (Musquodoboit River) *	Y	Y	Y	Present
MU_05	Lindsay Brook (Musquodoboit River)	Y	N	N	Present
MU_06	McCaffrey Brook (Musquodoboit River)	Y	Y	Y	Present
MU_07	Kent Brook (Musquodoboit River) *	Y	Y	Y	Present
SU_01	Salmon River (Lake Major) *	N	N	N	Absent
SU_02	West Brook (Porters Lake) *	N	N	N	Absent
SU_03	East Brook (Porters Lake) *	N	N	N	Absent
SM_27	McKeen Brook (St Mary's River)	Y	N	N	Present
SM_28	Archibald Brook (St Mary's River) *	Y	Y	Y	Present
SU_29	Indian Harbour Lakes*	N	Y	N	Present
SU_08	Ship Harbour River*	N	Y	N	Present
SU_09	Tangier River*	N	N	N	Absent
SU_10	West Taylor Bay River	N	N	N	Absent
SU_11	East Taylor Bay River*	N	N	N	Absent
SU_12	Little West River*	N	Y	Y	Present
SU_18	Smelt Brook	N	N	N	Absent
WR_13	Rocky Brook (West River Sheet Harbour)	N	Y	Y	Present
WR_14	Upper Killag (West River Sheet Harbour)	Y	Y	Y	Present
WR_15	Keef Brook (West River Sheet Harbour)	N	N	N	Absent
WR_16	Tent Brook (West River Sheet Harbour) *	N	N	N	Absent
WR_17	Jack Lowe Brook*	N	N	N	Absent

Table A3a. Summary statistics (mean and standard deviation [SD]) for metal ions collected from 19 rivers throughout Nova Scotia. All concentrations are measured in $\mu g \cdot L^{-1}$. Each site sampled three times in spring 2019.

	Al_i		Ca		Fe		F^-		K		Mg	
Site Code	**Mean**	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**
MO_22	19.8	(11.7)	650.1	(181.5)	248.8	(56.9)	16.2	(3.2)	213.6	(38.6)	285.7	(60.2)
MO_23	59.0	(55.4)	595.7	(205.2)	275.5	(41.9)	12.9	(8.0)	149.7	(32.1)	313.8	(81.8)
MU_04	32.8	(0.7)	1363.0	(93.5)	309.6	(86.6)	16.1	(3.5)	235.3	(70.2)	455.6	(31.4)
MU_07	7.6	(9.4)	1172.4	(338.9)	59.2	(2.7)	15.9	(16.1)	338.0	(99.4)	397.2	(102.5)
SM_28	21.4	(14.5)	308.9	(95.9)	226.0	(13.6)	6.0	(6.8)	305.2	(83.5)	245.4	(59.6)
SU_01	3.8	(6.5)	1560.3	(155.7)	217.4	(60.0)	17.6	(6.5)	555.5	(66.4)	434.8	(31.4)
SU_02	37.9	(30.8)	562.9	(193.1)	212.1	(62.5)	15.9	(6.6)	337.7	(179.1)	246.7	(57.3)
SU_03	30.4	(36.5)	908.3	(162.8)	375.5	(93.7)	76.3	(114.5)	602.0	(299.0)	323.3	(33.0)
SU_08	23.5	(8.1)	1418.4	(1433.6)	158.7	(35.7)	20.2	(6.1)	476.1	(353.0)	395.8	(116.6)
SU_09	35.9	(38.3)	506.6	(92.1)	222.9	(31.7)	15.3	(5.2)	686.7	(274.0)	310.1	(30.8)
SU_11	13.9	(24.1)	268.8	(83.7)	144.6	(62.4)	12.7	(3.3)	353.6	(128.3)	248.9	(51.1)
SU_12	27.4	(45.4)	464.1	(131.0)	276.6	(24.0)	10.0	(5.3)	295.7	(26.9)	290.5	(64.2)
SU_19	21.2	(17.7)	751.7	(95.4)	316.8	(36.7)	16.4	(5.6)	297.3	(53.7)	375.3	(64.8)
SU_20	15.0	(13.0)	636.6	(223.5)	290.1	(38.4)	16.4	(3.3)	264.1	(61.1)	340.3	(101.7)
SU_26	13.0	(12.0)	564.3	(253.0)	746.7	(853.4)	12.4	(7.5)	280.5	(53.3)	270.4	(92.3)
SU_29	14.4	(2.6)	741.8	(202.2)	222.5	(42.6)	10.7	(10.3)	534.3	(263.7)	408.2	(55.6)
SU_30	2.1	(3.6)	909.9	(340.4)	166.4	(66.5)	14.9	(4.6)	339.9	(138.9)	470.9	(124.2)
WR_16	22.7	(7.5)	438.3	(165.8)	519.4	(96.4)	16.8	(2.6)	253.0	(86.3)	247.5	(61.0)
WR_17	29.6	(31.6)	1051.8	(344.2)	365.5	(118.7)	11.6	(6.8)	383.6	(143.1)	276.2	(83.5)
All Sites	**22.7**	**(13.5)**	**782.8**	**(377.1)**	**281.8**	**(150.5)**	**17.6**	**(14.6)**	**363.2**	**(143.1)**	**333.5**	**(75.2)**

Table A3b. Summary statistics (mean and standard deviation [SD]) for metal ions collected from 19 rivers throughout Nova Scotia. All concentrations are measured in $\mu g \cdot L^{-1}$. Each site sampled three times in spring 2019.

	Mn		**Na**		**P**		**Phosphate**		**Zn**	
Site Code	**Mean**	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**
MO_22	56.9	(20.2)	2929.0	(1335.0)	8.7	(6.5)	8.4	(5.8)	4.3	(1.4)
MO_23	63.6	(24.6)	3316.7	(1743.5)	5.0	(0.0)	7.7	(4.7)	3.3	(1.4)
MU_04	83.8	(37.8)	2212.3	(136.3)	5.0	(0.0)	5.0	(0.0)	6.5	(4.8)
MU_07	15.0	(6.3)	2468.5	(1071.3)	8.3	(4.7)	16.9	(16.8)	8.4	(4.5)
SM_28	50.9	(2.0)	2180.7	(514.6)	7.0	(3.5)	7.4	(4.2)	10.9	(8.7)
SU_01	78.7	(12.9)	6550.0	(343.3)	10.3	(4.8)	13.0	(8.5)	12.1	(8.0)
SU_02	28.2	(3.3)	2431.7	(842.6)	9.5	(7.8)	18.7	(12.2)	7.0	(3.3)
SU_03	28.5	(3.0)	3184.3	(1238.9)	37.0	(40.4)	11.8	(5.9)	13.1	(6.9)
SU_08	34.1	(8.2)	2860.3	(681.1)	7.0	(3.5)	13.0	(8.3)	9.8	(2.2)
SU_09	37.1	(1.0)	4243.0	(1986.9)	36.0	(42.6)	13.7	(7.6)	12.5	(4.8)
SU_11	10.9	(0.3)	3536.0	(1641.1)	8.7	(6.3)	24.1	(18.8)	9.7	(4.5)
SU_12	32.5	(0.9)	3040.3	(356.9)	12.4	(7.4)	12.9	(6.9)	6.3	(2.4)
SU_19	92.2	(7.9)	3427.7	(1464.8)	16.0	(13.5)	8.2	(5.6)	7.4	(6.4)
SU_20	96.1	(26.0)	3108.3	(1062.7)	6.9	(3.3)	8.7	(6.5)	5.6	(1.3)
SU_26	74.0	(67.5)	2901.3	(403.8)	15.0	(12.2)	10.8	(5.0)	11.1	(8.4)
SU_29	88.6	(44.8)	4828.3	(1234.4)	15.9	(13.4)	7.7	(4.6)	9.1	(4.8)
SU_30	70.1	(28.3)	4802.0	(574.5)	6.8	(3.1)	7.9	(5.1)	5.6	(0.8)
WR_16	30.1	(1.5)	2126.3	(907.0)	5.0	(0.0)	10.9	(10.2)	62.5	(95.8)
WR_17	44.2	(3.9)	8395.7	(2501.8)	10.1	(5.0)	8.4	(5.9)	14.4	(9.4)
All Sites	**53.4**	**(27.1)**	**3607.5**	**(1601.2)**	**12.1**	**(9.2)**	**11.3**	**(4.6)**	**11.5**	**(12.7)**

Table A4. Summary statistics (mean and standard deviation [SD]) for field-measured pH collected from 29 rivers throughout Nova Scotia, and detailed physiochemistry variables collected from a subset of 19 rivers. Each site sampled three times in spring 2019. A thirtieth site is omitted here due to saltwater intrusion.

	Field-measured pH		**Alkalinity** ($CaCO_3$ mg·L^{-1})		**Specific Conductivity** (µs·cm^{-1})		**Total dissolved solids** (mg·L^{-1})		**Total organic carbons** (mg·L^{-1})		**Turbidity** (NTU)	
Site	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
MO_22	5.0	(0.9)	1.9	(0.8)	23.6	(0.7)	14.7	(0.6)	8.4	(1.1)	1.1	(0.4)
MO_23	4.9	(0.7)	0.8	(0.4)	27.6	(3.1)	17.6	(2.1)	11.7	(2.6)	0.7	(0.5)
MU_04	5.8	(0.3)	3.3	(0.3)	26.6	(0.3)	16.9	(0.0)	6.7	(1.1)	2.0	(1.1)
MU_05	6.5	(0.2)										
MU_06	6.0	(0.5)										
MU_07	5.6	(0.3)	5.3	(0.8)	28.6	(3.8)	19.0	(4.2)	6.2	(3.6)	0.5	(0.0)
SM_27	5.6	(0.7)										
SM_28	4.8	(0.6)	0.8	(0.7)	22.2	(0.3)	13.6	(0.8)	6.8	(0.5)	0.7	(0.1)
SU_01	5.6	(0.1)	4.0	(0.8)	58.3	(6.2)	35.3	(4.6)	5.2	(0.5)	0.9	(0.5)
SU_02	4.6	(0.2)	2.0	(0.7)	24.1	(1.6)	14.7	(1.2)	8.7	(0.7)	1.2	(0.3)
SU_03	4.7	(0.4)	2.5	(1.0)	28.6	(2.1)	19.6	(4.1)	10.7	(1.8)	1.7	(0.6)
SU_08	4.8	(0.6)	1.9	(1.6)	30.5	(0.2)	19.6	(0.3)	7.3	(2.0)	0.7	(0.2)
SU_09	4.5	(0.0)	2.2	(1.5)	28.8	(4.4)	17.8	(2.8)	8.4	(0.9)	1.0	(0.3)
SU_10	4.8	(0.9)										
SU_11	4.8	(1.1)	1.1	(1.0)	30.8	(1.3)	19.1	(0.7)	8.0	(0.7)	0.5	(0.1)
SU_12	5.7	(0.5)	1.7	(0.5)	30.0	(0.8)	17.9	(1.4)	8.3	(1.8)	0.9	(0.3)
SU_18	4.5	(0.1)										
SU_19	5.1	(0.3)	2.6	(1.2)	28.1	(1.9)	17.4	(1.0)	8.3	(2.1)	2.3	(1.0)
SU_20	4.9	(0.2)	2.0	(0.6)	28.8	(1.8)	17.8	(1.5)	9.4	(0.8)	2.0	(0.2)
SU_21	4.6	(0.3)										
SU_25	4.5	(0.1)										
SU_26	4.8	(0.4)	1.1	(1.0)	29.3	(2.4)	17.4	(1.1)	7.8	(1.2)	7.3	(10.8)
SU_29	5.6	(0.7)	2.7	(0.8)	37.6	(5.6)	24.4	(3.8)	5.7	(1.1)	0.7	(0.1)
SU_30	5.9	(0.7)	4.7	(0.6)	42.0	(1.7)	26.3	(0.8)	4.2	(0.6)	0.8	(0.3)
WR_13	5.2	(0.3)										
WR_14	5.0	(0.3)										
WR_15	4.8	(0.4)										
WR_16	4.4	(0.3)	0.0	(0.0)	22.4	(1.0)	14.3	(0.4)	14.4	(0.5)	0.8	(0.6)
WR_17	5.3	(0.3)	1.5	(0.3)	54.6	(3.9)	33.5	(2.9)	11.8	(0.9)	0.5	(0.0)
All Sites	**5.1**	**(0.5)**	**2.2**	**(1.4)**	**31.7**	**(9.9)**	**19.8**	**(6.0)**	**8.3**	**(2.5)**	**1.4**	**(1.5)**