

# **Estimation of Atlantic Salmon Populations in Liverpool County Rivers**

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## **Disclaimer**

Intended Use and Technical Limitations of Estimation of Atlantic Salmon Populations in Liverpool County Rivers. The sole purpose of this report is to: assess what is the current state of Atlantic salmon in the Gold, Mersey, Medway, and Roseway rivers, Nova Scotia. The information contained herein does not necessarily represent the opinion of UNBSJ and/or CRI.

## Abstract

Assessing the presence, distribution, and freshwater production of Atlantic salmon (*Salmo salar*) in low-abundance systems is challenging using capture-based methods alone. This study applied an integrated survey approach combining backpack electrofishing and environmental DNA (eDNA) analysis to evaluate juvenile abundance and determine presence or apparent absence of Atlantic salmon across four Nova Scotia rivers: the Gold, Mersey, Medway, and Roseway. Electrofishing surveys were conducted at 17 sites between 14 and 30 September 2025 using a combination of closed (multi-pass) and open (single-pass) sampling designs, covering a total area of 4,664 m<sup>2</sup>. Environmental DNA sampling was conducted concurrently at 17 sites, with duplicate water samples collected per site and analyzed using quantitative PCR targeting Atlantic salmon DNA. Surveys were conducted under extremely low water conditions, which constrained site availability and influenced detection probability. Only one juvenile Atlantic salmon was captured during electrofishing surveys, occurring in the Medway River, indicating highly limited and spatially restricted freshwater production. In contrast, eDNA analysis detected Atlantic salmon DNA in all four rivers, reflecting the presence of salmon genetic material from one or more life stages. The Medway River showed the strongest concordance between methods, with multiple positive eDNA detections and a juvenile capture. In the Roseway River, eDNA detections were limited to downstream of a dam near the river mouth, with no detections upstream. The Gold River exhibited a single positive eDNA detection without juvenile captures, suggesting extremely low abundance or restricted spatial use. In the Mersey River, multiple positive eDNA detections were most consistent with landlocked Atlantic salmon associated with lakes, headponds, and tributary spawning rather than anadromous returns. Interpretation of results was constrained by exceptionally low water levels during the survey period, which limited habitat availability and likely reduced detection probability for juvenile salmon while influencing eDNA transport and retention. Overall, findings indicate that Atlantic salmon are not broadly distributed or abundant across the four rivers. The integrated application of electrofishing and eDNA provides a robust and complementary framework for assessing salmon presence and distribution in low-abundance, barrier-affected systems and supports continued use of combined methodologies for monitoring and management.

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## Introduction

Electrofishing is a widely used method for assessing population abundance of freshwater fishes, particularly salmonids, in wadeable rivers. A portable power source generates an electrical field between a submerged anode and cathode; when fish encounter a sufficient voltage gradient, the induced galvanotaxis causes them to orient toward the anode, enabling efficient capture. Electrofishing can support several abundance-estimation approaches, including multi-pass successive-removal and single-pass catch-per-unit-effort (CPUE) sampling (Matson et al., 2018). Multi-pass methods typically require barrier nets and greater field effort but can produce accurate local population estimates (Carle & Strub, 1978; Seber & Whale, 1970; Zippin, 1958). Single-pass methods are less resource intensive and allow broader spatial coverage but must be calibrated against methods that produce reliable population estimates (Dauphin et al., 2009; Dauphin et al., 2019; Robinson, 2024). At watershed scales, combining multi-pass and single-pass sampling increases efficiency while maintaining robust population assessment (Hanks et al., 2018; Myvold & Kennedy, 2017; Robinson, 2024).

Atlantic salmon (*Salmo salar*) populations in Nova Scotia rivers exhibit considerable variability in life-history expression, including both anadromous and landlocked forms. In some river systems, particularly those containing lakes and barriers to migration, landlocked salmon populations may persist independently of anadromous returns. This distinction is important when interpreting eDNA detections, as the presence of salmon DNA does not necessarily indicate the presence of anadromous juveniles or active sea-run populations.

Environmental DNA (eDNA) provides a complementary, non-invasive tool for detecting rare or low-abundance species and is increasingly used alongside traditional capture-based methods in population assessments (Mauvisseau et al., 2020). Populations at extremely low abundance may be difficult or impossible to detect through physical sampling alone (Gu & Swihart, 2004), which can undermine status assessments and management decisions. While both electrofishing and eDNA rely on knowledge of species distribution and life history, eDNA offers increased sensitivity to rare taxa (Jerde et al., 2011; Laramie et al., 2015) and strengthens overall survey confidence, particularly in systems where juvenile salmon may be present at very low densities or functionally extirpated.

This project was commissioned by Perennia Food & Agriculture Corporation to estimate juvenile Atlantic salmon abundance in four Nova Scotia rivers: the Gold, Mersey, Medway, and Roseway. The objectives were to: (1) generate credible, science-based estimates of juvenile Atlantic salmon (fry and parr) abundance where present; and (2) determine presence or absence of Atlantic salmon in sites where physical capture may not be feasible. Using a combined electrofishing and eDNA protocol represents a modern, recommended approach for assessing populations in extremely low-abundance or historically depleted systems. Where juvenile salmon are detected, the methods employed here provide a robust assessment framework recognized by Fisheries and Oceans Canada (DFO) and the North Atlantic Salmon Conservation Organization (NASCO).

## **Methods**

### **Site Selection**

A total of 18 sites were surveyed between September 14<sup>th</sup> to 30<sup>th</sup>, 2025, across the Gold River in Lunenburg County, Mersey and Medway rivers in Queens County and Roseway River in Shelburne County, Nova Scotia. There were to be 3 sites on the Gold River (which include the two previously established DFO electrofishing sites and one additional site), six sites on the Mersey River (one site below each of the dams and of these, five were previously established DFO), five sites on the Medway River (all previously established DFO), and four sites on the Roseway River (two sites above and two sites below the Roseway River Dam, three of which were previously established by DFO). Sites consisted of either riffle or run habitats. Riffle type habitats are characterized by shallow, fast moving water with a broken flow over the substrate, while run habitats, often found at the end of pools, have fast flow but more uniform with no broken water surface, often slightly deeper. However, due to extreme low water conditions, many of the previously established DFO electrofishing sites were dry (Figure 1) and therefore alternative sites were chosen post hoc based suitable habitat availability.



Figure 1: Examples of previously established DFO electrofishing sites that due to extreme drought conditions, were dry.

### Electrofishing Surveys

Backpack electrofishing surveys were conducted to estimate Atlantic salmon fry and parr abundance (Figure 2). Closed site electrofishing took place at seven different sites across the four rivers, with one closed electrofishing site on Gold River (Figure 3), two on the Mersey River (Figure 4), two on the Medway River (Figure 5), and two on the Roseway River (Figure 6). When sampling closed sites or multiple pass sites, a 25 meter-long block or barrier nets was installed perpendicular to the river banks at the lower and upper boundaries of the site in order to ‘close’ the site for the duration of the sampling and prevent fish movement from or to the site. Nets are made of nylon with a 7 mm mesh size, allowing the water to flow but no fish of interest to swim through them. In order, to maintain the fish population as close as possible to its state prior the installation of the

nets, the lower net was installed first to minimize the number of fish escaping the site during net installation, as disturbed fish tend to primarily follow the current (personal observation). Rocks from the riverbed were used to anchor the net bottom, while wooden stakes were used to elevate the net top out of the water and seal the site. The size of the site was characterized by two length measurements (one on each side) and two width measurements (upstream, downstream), forming a sampling area.

The general methodology was to electrofish each square meter of the site to sample all fish present. Following the first pass through the site, all fish were brought to the processing area where fish were placed in live wells waiting to be processed. In compliance with DFO permitting, all fish species are collected and recorded. Fish were kept outside of the sampling area until all the passes were completed. The second pass started no less than 10 minutes after the precedent one ended to let the remaining fish settle back in their habitat. Three to six passes were conducted at each site with the objective of observing a depletion (i.e., less and less fish captured after each pass). However, in the event that no salmon were detected in the first pass, additional passes were not conducted as it was deemed no salmon to be present. Electrofishing surveys were conducted by a four-person crew, which includes a certified operator, two crew members using a ~1.5 m collection lip seine downstream of the electrofisher operator, and one dip netter. Sampling passes were conducted from the downstream to upstream end of the site to mimic how open sites are sampled (see below). Power was standardized across sites and voltage was systematically adjusted to ensure an ~80-watt output, using a SmithRoot LR24 backpack electrofisher, set to 30Hz, 12% duty cycle, and pulsed DC.

The remaining nine electrofishing sites were surveyed using the single-pass method (open sites) method. The protocol is similar to the multi-pass site protocol, except no barrier nets were installed and only one pass was conducted. The lower operational requirements in comparison to the closed site method allowed for a larger number of sites to be sampled throughout the catchments over the assessment period. The single-pass method does require calibration or pairing with variance data from the multi-pass removal sites in order to estimate population abundance. When estimating population abundance at the river-scale, a combination of multi- and single-pass methods is ideal, as single-pass sites can increase area coverage, while the multi-pass depletion sites allow collection of more intensive data.

Data collected at the sampling site consisted of the sampling effort, number of passes, fish captures per pass (including species), standard morphometric data for salmon only (including but not limited to sex, length, and weight), site area, and GPS location. In addition, a scale sample was collected from each salmon for future stable isotope analysis, as well as a caudal fin clip was collected from each salmon, stored in ethanol for future DNA analysis. These samples will be provided to CMAR.

### **Density Estimates**

Juvenile salmon density estimates would have been calculated using the methodologies developed by John Robinson (see Robinson 2024), in collaboration with Parks Canada, DFO, and the University of New Brunswick. Population estimate methodology is recognized by DFO and NASCO.

In general, for any given year  $t$  and river  $k$ , the total abundance of an Atlantic salmon at life stage  $l$  in site  $i$  is drawn from a Poisson distribution:

Equation 1.  $N_{t,k,i,l}^{tot} | \lambda_{t,k,i,l}^N \sim \text{Poisson}(\lambda_{t,k,i,l}^N)$

where  $\lambda_{t,k,i,l}$  is the product of the density  $d_{t,k,i,l}$  (in fish of lifestage  $l$  per  $\text{m}^2$ ) multiplied by the area  $A_{t,k,i}$ , of the sites (in  $\text{m}^2$ ) and  $\lambda_{t,k,i,l} = d_{t,k,i,l} \times A_{t,k,i}$ .

### **eDNA Sampling**

For additional confirmation of the presence/absence of Atlantic salmon in the Gold (Figure 3), Mersey (Figure 4), Medway (Figure 5), and/or Roseway (Figure 6) rivers, 34 eDNA samples will be collected. Water samples were collected in duplicate from one site on the Gold River, nine sites on the Medway River, five sites on the Mersey Rive, and two sites on the Roseway River. In addition, seven field blank samples were collected from each of the rivers to test for contamination in the sampling procedure.

Field sampling followed the protocol established by Dr. Scott Pavey, Director of the CRI Genomics Laboratory at the University of New Brunswick Saint John. Forceps, cellulose nitrate membrane filters (Whatman—47 mm diameter/0.45  $\mu\text{l}$ ), and a filter funnel were packaged in a sterile laboratory prior to field sampling. At each site, we pumped 1 L of water through a filter using a GeoPump 2 Peristaltic Pump while wearing disposable gloves. Upon completion of filtration, samples were placed in vials and topped with ethanol. Samples were stored in an ice-filled cooler and moved within 24 hrs to a  $-20^\circ\text{C}$  freezer. Three field blanks (one from each river) were collected to test for contamination. This requires filtering 1 L of deionized water using the same techniques and equipment outlined above. Samples were taken to the University of New Brunswick Saint John campus and stored at  $-20^\circ\text{C}$  until DNA analysis. Samples underwent quantitative PCR (qPCR) analysis for the detection of any salmon DNA.

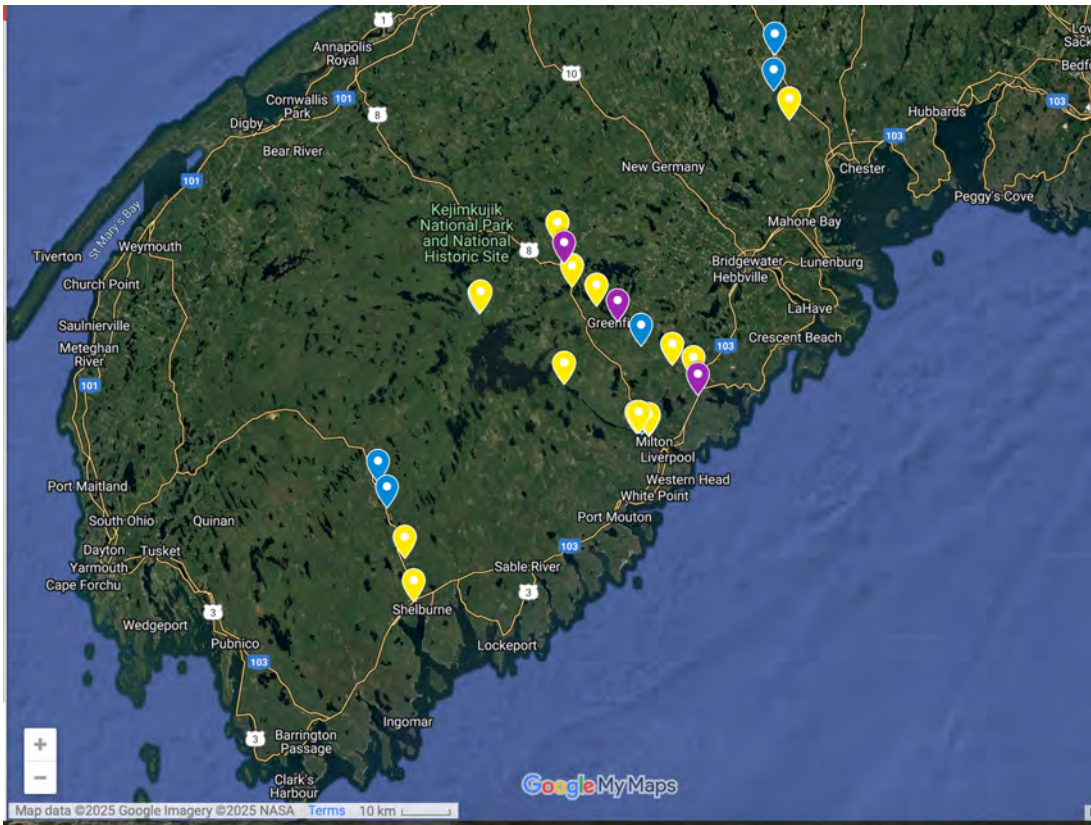


Figure 2: Map of all electrofishing and eDNA sites across the Gold, Mersey, Medway, and Roseway rivers, NS. Balloons represent electrofishing (yellow) and eDNA collection (blue and purple) sites. It should be noted that eDNA was collected at many of the same locations as electrofishing occurred (see Table 1).



Figure 3: Map of electrofishing and eDNA sites in the Gold River, NS. Balloons represent electrofishing (yellow) and eDNA collection (blue and purple) sites. It should be noted that eDNA was collected at many of the same locations as electrofishing occurred (see Table 1).

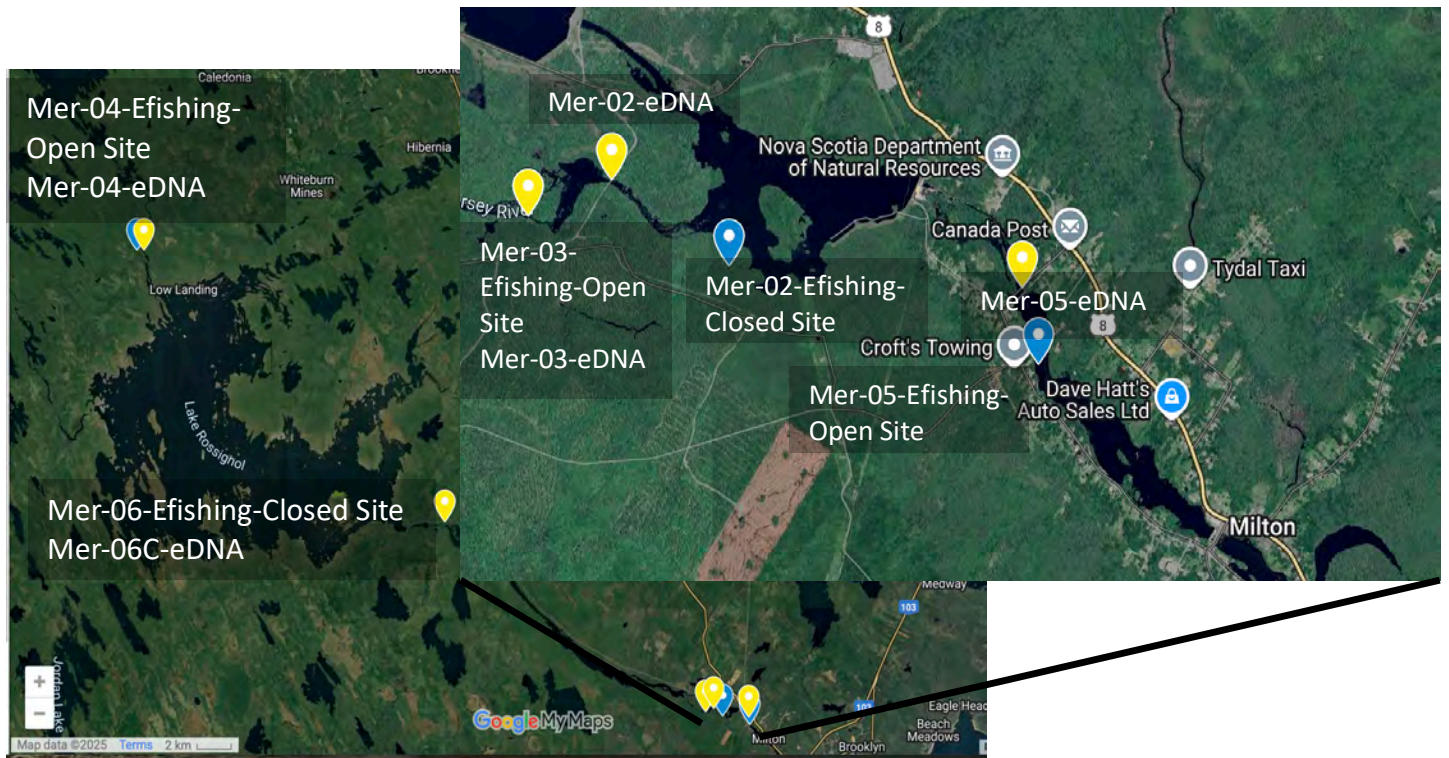


Figure 4: Map of electrofishing and eDNA sites in the Mersey River, NS. Balloons represent electrofishing (yellow) and eDNA collection (blue and purple) sites. It should be noted that eDNA was collected at many of the same locations as electrofishing occurred (see Table 1).



Figure 5: Map of electrofishing and eDNA sites in the Medway River, NS. Balloons represent electrofishing (yellow) and eDNA collection (blue and purple) sites. It should be noted that eDNA was collected at many of the same locations as electrofishing occurred (see Table 1).



Figure 6: Map of electrofishing and eDNA sites in the Roseway River, NS. Balloons represent electrofishing (yellow) and eDNA collection (blue and purple) sites. It should be noted that eDNA was collected at many of the same locations as electrofishing occurred (see Table 1).

## Results

### Electrofishing Surveys

Electrofishing surveys were conducted in the Gold, Mersey, Medway, and Roseway rivers between September 14 and 30, 2025 (Table 1). A total of 17 sites were surveyed, including seven closed sites and ten open sites. Closed sites included one site in the Gold River, three sites in the Medway River, two sites in the Mersey River, and one site in the Roseway River. Open sites included two sites in the Gold River, three sites in the Medway River, three sites in the Mersey River, and two sites in the Roseway River (Table 2).

Across all sites, a total area of 4,664.01 m<sup>2</sup> was sampled. By river, surveyed areas totaled 872.46 m<sup>2</sup> in the Gold River, 1,660.70 m<sup>2</sup> in the Mersey River, 1,392.91 m<sup>2</sup> in the Medway River, and 737.95 m<sup>2</sup> in the Roseway River (Table 1). Individual site areas ranged from 132.60 m<sup>2</sup> in the Roseway River to 501.75 m<sup>2</sup> in the Mersey River. Shocking time per site ranged from 746 to 1,867 seconds, resulting in a cumulative shocking time of 20,794 seconds (5.78 hours) across all sites (Table 2).

Only one Atlantic salmon (*Salmo salar*) was captured during electrofishing surveys, occurring at site Med-03 on the Medway River (Table 3). Across all sites, a total of 554 fish representing 12 species were captured. American eel (*Anguilla rostrata*) was the most frequently encountered species, occurring at 14 of the 17 sites, with a total of 303 individuals captured (Table 2).

Invasive species were detected at multiple sites. Chain pickerel (*Esox niger*) were captured at three sites, including Roseway-03 and two sites on the Mersey River (Mer-04

and Mer-06) (Table 2; Figure 4). Brown trout (*Salmo trutta*) were captured at two sites on the Gold River (GR-02 and GR-03) (Table 2; Figure 3). Smallmouth bass (*Micropterus dolomieu*) were captured at all sampled sites in the Gold and Medway rivers and at one site in the Mersey River (Mer-03) (Table 2; Figures 3-5).

### **eDNA**

Environmental DNA samples were collected from the Gold, Mersey, Medway, and Roseway rivers in September 2025. A total of 34 samples were analyzed for the presence of Atlantic salmon (*Salmo salar*) DNA using quantitative PCR (qPCR) at the CRI Genomics Laboratory (Table 4). At each site, water samples were collected in duplicate, and each field sample was analyzed using four qPCR technical replicates, resulting in up to eight qPCR replicates per site. When target DNA concentrations are low, variability among qPCR replicates is expected. Consequently, sites for which no qPCR replicates amplified Atlantic salmon DNA do not necessarily indicate species absence, but rather that any eDNA present was below the detection capability of the assay. Environmental and biological factors known to influence eDNA detection include sunlight exposure, water temperature, species abundance, and the presence of organic material, particularly leaf litter.

There were three types of negative controls (field, extraction, and quantitative polymerase chain reaction) used throughout the process to detect if contamination occurred at any step. No DNA from any target species was detected in any of these negative controls, which means that no contamination occurred, and positive detections are a result of the target species' DNA being present at these sites. An internal positive

control was also used on all the samples to test whether PCR inhibition was present and preventing target species DNA from amplifying. This control found no inhibition present.

Positive eDNA detections for Atlantic salmon were observed at least one site in each of the four rivers sampled: Gold, Mersey, Medway, and Roseway (Table 4). In the Medway River, four of nine sites yielded positive detections. One site (Med-01) exhibited a low-level signal, with amplification observed in a single qPCR replicate from one of the two field samples. In contrast, the remaining three Medway sites showed strong eDNA signals, with six to eight positive amplifications out of eight total qPCR replicates, indicating a robust presence of Atlantic salmon eDNA at those locations. In the Roseway River, Atlantic salmon eDNA was detected at only one site, located downstream of the dam. No detections were observed upstream of the structure, which is considered a migration barrier to Atlantic salmon. In the Mersey River, positive eDNA detections were observed at four of the five sites sampled (Table 4). This result was unexpected, as the dams within the Mersey River system are considered to lack functional upstream fish passage and are generally regarded as migration barriers for Atlantic salmon. Notably, sites with positive detections were typically associated with lakes and/or dam headponds.

Table 1: Summary of electrofishing surveys conducted in September 2025.

River	Site ID	Electrofishing		eDNA		Electrofishing Site Dimensions				
		Latitude	Longitude	Latitude	Longitude	Upstream Width (m)	Downstream Width (m)	RR Length (m)	RL Length (m)	Size (m2)
Gold River	GR-01	44.626614	-64.419565	44.626614	-64.419565	6.2	5.6	27.8	31.1	173.76
Gold River	GR-02	44.67617	-64.45674	NA	NA	8.7	15.9	27.4	25.3	324.11
Gold River	GR-03	44.73932	-64.45355	NA	NA	3.1	8.2	66.3	66.3	374.60
Medway River	Med-01	44.29829	-64.89413	44.29829	-64.89413	5.6	9.7	53.3	21.6	286.49
Medway River	Med-02	44.33454	-64.9545	44.33268	-64.95325	5.7	4.1	37.3	38.7	186.20
Medway River	Med-03	44.40816	-64.98835	44.40807	-64.98874	6.3	6.3	25.4	29.7	173.57
Medway River	Med-04	44.19395	-64.70542	44.19395	-64.70542	9.7	2.7	36.8	29.5	205.53
Medway River	Med-05	44.17046	-64.65577	44.17074	-64.6545	7.5	8.9	38.2	38.2	313.24
Medway River	Med-06	44.22747	-64.78407	44.22747	-64.78407	17.7	21.1	24	27.1	495.67
Medway River	Med-A	NA	NA	44.14044	-64.6433					
Medway River	Med-B	NA	NA	44.27131	-64.84149					
Medway River	Med-C	NA	NA	44.37272	-64.97271					
Mersey River	Mer-02	44.07092	-64.78194	44.07434	-64.78874	3.6	6.2	30.5	28.7	145.04
Mersey River	Mer-03	44.07292	44.07292	44.07292	-64.79365	13.9	15.1	16.3	16.3	236.35
Mersey River	Mer-04	44.28529	-65.18312	44.28559	-65.17867	9.8	12.7	44.6	44.6	501.75
Mersey River	Mer-05	44.06705	-64.76384	44.07009	-64.76473	16.5	15.2	23.5	21.1	353.46
Mersey River	Mer-06	44.16049	-64.97267	44.16049	-64.97266	10.5	4.9	17.3	23.3	156.31
Roseway River	RR-01	43.852446	-65.365021	43.852446	-65.365021	5.7	6.8	25.3	25.5	158.75
Roseway River	RR-02	43.98436	-65.43208	NA	NA	4.2	17.8	42.7	38.5	446.60
Roseway River	RR-03	43.94193	-65.40909	NA	NA	3	3	44.2	44.2	132.60
Roseway River	RR-LWR	NA	NA	43.77425	-65.34446					

Table 2: Summary of fish species captured during electrofishing surveys conducted in September 2025.

River	Site ID	Size	Effort (s)	Site Type (Open/Closed)	Water Temp (°C)	Atlantic Salmon	Creek Chub	Brown Bullhead	Blacknose Dace	White Sucker	Banded Killifish	Smallmouth Bass	Brown Trout	Yellow Perch	American Eel	Sea Lamprey	Chain Pickerel	Other	
		(m2)			Date	(°C)													
Gold River	GR-01	173.76	15-09-2025	1057	Closed	13	0	4	11	2	39	3	1	0	0	5	0	0	0
Gold River	GR-02	324.11	15-09-2025	1213	Open	21	0	0	0	0	0	0	28	1	0	6	0	0	0
Gold River	GR-03	374.60	15-09-2025	1537	Open	20	0	1	1	0	3	0	34	1	0	1	0	0	0
Medway River	Med-01	286.49	17-09-2025	1867	Closed	16	0	0	3	0	0	0	3	0	22	7	0	0	0
Medway River	Med-02	186.20	20-09-2025	1426	Open	11	0	0	0	0	3	0	14	0	0	5	0	0	0
Medway River	Med-03	173.57	17-09-2025	857	Closed	15	0	0	1	0	0	0	3	0	1	0	1	0	0
Medway River	Med-04	205.53	18-09-2025	1345	Closed	17	0	0	0	0	0	0	2	0	0	66	0	0	0
Medway River	Med-05	313.24	20-09-2025	1199	Open	19	0	0	0	0	0	0	1	0	0	19	18	0	0
Medway River	Med-06	495.67	24-09-2025	1720	Open	16	0	0	1	0	0	0	4	0	0	47	9	0	0
Mersey River	Mer-02	145.04	22-09-2025	1291	Closed	NA	0	0	0	0	0	0	0	0	0	3	0	0	0
Mersey River	Mer-03	236.35	22-09-2025	1192	Open	18.5	0	0	0	0	0	0	4	0	0	13	0	0	0
Mersey River	Mer-04	501.75	19-09-2025	1066	Open	21	0	0	0	0	0	0	0	0	3	0	0	5	0
Mersey River	Mer-05	353.46	23-09-2025	1480	Open	18	0	0	0	0	0	0	0	0	0	73	0	0	0
Mersey River	Mer-06	156.31	19-09-2025	746	Closed	19	0	0	0	0	1	0	0	0	5	1	0	1	0
Roseway River	RR-01	158.75	16-09-2025	810	Closed	17	0	0	0	0	0	0	0	0	0	10	0	0	0
Roseway River	RR-02	446.60	16-09-2025	1017	Open	19.5	0	0	0	0	0	0	0	0	0	35	0	0	0
Roseway River	RR-03	132.60	18-09-2025	971	Open	19.5	0	0	0	0	0	0	0	0	4	12	0	3	0

Table 3: Summary of Atlantic salmon collected during electrofishing surveys

River	Site ID	Date	Species	Pass	Sex	Fork length (cm)	Weight (g)	Scale envelope ID	Vial ID
Medway River	Med-03	17-09-2025	Atlantic salmon	1	Immature	11.6	17.1	1	1

Table 4: Atlantic Salmon results from samples collected in September 2025. Each sample is reported as the number of qPCR replicates that amplified each species' DNA.

Sample	Replicate	Atlantic Salmon
GR Control		0/4
GR Control 2		0/4
GR 01	A	1/4
	B	2/4
MED Control		0/4
MED Control 2		0/4
MED 01	A	0/4
	B	1/4
MED 02	A	0/4
	B	0/4
MED 03	A	4/4
	B	4/4
MED 04	A	0/4
	B	0/4
MED 05	A	0/4
	B	0/4
MED 06	A	0/4
	B	0/4
MED A	A	0/4
	B	0/4
MED B	A	4/4
	B	4/4
MED C	A	4/4
	B	2/4
MER Control		0/4
MER Control 2		0/4
MER 02	A	4/4
	B	0/4
MER 03	A	0/4
	B	0/4
MER 04	A	0/4
	B	1/4
MER 05	A	0/4
	B	3/4
MER 06C	A	4/4
	B	1/4
RR Control		0/4
RR 01	A	0/4
	B	0/4
RR LWR	A	2/4
	B	0/4

## **Discussion**

This assessment integrated electrofishing and environmental DNA (eDNA) methodologies to evaluate the presence, apparent absence, and spatial distribution of Atlantic salmon (*Salmo salar*) across four Nova Scotia rivers: the Gold, Mersey, Medway, and Roseway. The two methods provide complementary but distinct information. Electrofishing targets juvenile salmon (fry and parr) and therefore provides direct evidence of local freshwater production, whereas eDNA can detect genetic material originating from any life stage and may reflect resident, migratory, or transient individuals. Interpreted together, the results indicate that Atlantic salmon occur at low abundance across all four rivers, with marked differences in juvenile detection and inferred sources of eDNA among systems.

### **Juvenile Atlantic Salmon Distribution Based on Electrofishing**

Electrofishing surveys conducted across 17 sites and more than 4,600 m<sup>2</sup> of habitat resulted in the capture of a single juvenile Atlantic salmon at site Med-03 on the Medway River. No juvenile salmon were captured in the Gold, Mersey, or Roseway rivers. These findings suggest extremely low juvenile abundance across the study area.

Sampling in September 2025 coincided with extremely low water levels, which substantially limited the availability of wetted habitat at several previously established electrofishing sites. Many tributary and riffle habitats, typically considered optimal for juvenile Atlantic salmon, were dry at the time of sampling. As a result, several sites were selected post hoc based on remaining accessible habitat rather than predefined locations.

Under such conditions, the absence of juvenile captures cannot be attributed solely to biological absence. Low flows may reduce electrofishing effectiveness and may influence fish distribution in ways that are not readily observable. Juvenile salmon may occupy deeper or less accessible habitats during drought conditions, including main-stem reaches or residual pools that are not effectively sampled using backpack electrofishing. Consequently, the lack of juvenile detections at many sites should be interpreted cautiously and may reflect reduced detectability rather than definitive absence.

### **eDNA Detections and Life-Stage Interpretation**

Environmental DNA analysis detected Atlantic salmon DNA in all four rivers, indicating that salmon genetic material was present within each system during the sampling period. Because eDNA can originate from any life stage, detections do not directly confirm the presence of locally rearing juveniles. Instead, they provide evidence of salmon presence at the watershed scale and complement electrofishing results in these low-abundance systems.

The Medway River showed the strongest concordance between electrofishing and eDNA results. Four of nine sites yielded positive eDNA detections, three of which exhibited strong amplification across most or all qPCR replicates. When considered alongside the single juvenile captured at Med-03, these results suggest that Atlantic salmon are present within the Medway River and that limited freshwater occupancy or production may persist.

In the Roseway River, eDNA detections were limited to a single site downstream of the dam located near the mouth of the river. No detections were observed upstream. This pattern is consistent with the dam functioning as a barrier to upstream migration and

suggests that Atlantic salmon presence within the Roseway River is spatially restricted. The absence of juvenile captures upstream further supports the interpretation that freshwater production above the dam was limited or absent during the survey period.

The Gold River exhibited a single positive eDNA detection without corresponding juvenile captures. This finding indicates the presence of Atlantic salmon DNA within the system but does not allow inference regarding life stage or local rearing. The absence of electrofishing detections may reflect extremely low juvenile abundance, reduced detectability under low-flow conditions, or spatial separation between occupied habitats and sampled sites.

The Mersey River produced positive eDNA detections at four of five sampled sites, despite no juvenile salmon being captured during electrofishing surveys. The Mersey River system contains multiple dams that are considered to lack functional upstream fish passage and extensive lake habitat which has been shown to support landlocked Atlantic salmon populations in other systems (Hutchings et al. 2019). Given these system characteristics, the observed eDNA detections are unlikely to reflect annual returns of anadromous Atlantic salmon.

Instead, the spatial pattern of detections, particularly their association with lakes and dam headponds, is most consistent with the presence of landlocked Atlantic salmon residing within these environments. The positive detection at site Mer-04 is similarly consistent with landlocked salmon, likely originating from spawning activity in tributaries connected to lake or headpond habitats. The absence of juvenile electrofishing detections supports the interpretation that local rearing of anadromous juveniles was not detected during the survey period.

## **Invasive Species and Potential Implications for Atlantic Salmon**

Electrofishing surveys documented the presence of several non-native fish species across the study area, including chain pickerel (*Esox niger*), brown trout (*Salmo trutta*), and smallmouth bass (*Micropterus dolomieu*). These species were detected in multiple rivers and at multiple sites, including areas where Atlantic salmon were absent or detected only through eDNA. While this study was not designed to quantify species interactions or assess ecological impacts, the distribution of invasive species provides important context for interpreting Atlantic salmon presence and apparent absence.

Chain pickerel were detected in the Mersey and Roseway rivers, including at sites upstream of dams. Brown trout were detected in the Gold River, and smallmouth bass were widespread in the Gold and Medway rivers and present at one site in the Mersey River. The occurrence of these species indicates established non-native fish assemblages in portions of all four river systems.

Non-native species may influence Atlantic salmon through multiple mechanisms, including predation on juveniles, competition for habitat and food resources, and indirect effects on community structure (Cairns 2006; Falkegård et al. 2023). These interactions may be particularly consequential in systems where Atlantic salmon populations are already at low abundance, as even modest additional mortality or habitat displacement could have disproportionate effects (Cairns 2006; Falkegård et al. 2023). However, the present study did not measure interaction rates, relative abundance, or habitat overlap at a resolution sufficient to evaluate these mechanisms directly.

Given the observational nature of the data, conclusions regarding the role of invasive species in limiting Atlantic salmon distribution cannot be drawn from this study alone. Nonetheless, their documented presence across multiple rivers suggests that

invasive species represent an additional stressor that may interact with hydrological conditions, barriers to migration, and low population abundance. Future monitoring and assessment efforts would benefit from explicitly incorporating invasive species distribution and abundance into study designs to better evaluate their potential influence on Atlantic salmon recovery.

### **Hydrological Limitations and Methodological Considerations**

Hydrological conditions during the survey period represent an important limitation affecting both electrofishing and eDNA interpretations. Extremely low flows reduced the availability of wadeable habitat and constrained site selection, potentially lowering detection probability for juvenile salmon. Low water levels may also influence eDNA dynamics by altering transport, dilution, and retention processes, particularly in main-stem reaches, lakes, and headponds.

As a result, both the absence of juvenile captures and the spatial distribution of eDNA detections should be interpreted within the context of drought-like conditions. Neither method alone provides definitive evidence of absence, particularly in systems characterized by low abundance, barriers, and complex hydrology. The combined use of electrofishing and eDNA nonetheless provides a more robust assessment framework than either approach independently.

### **Conclusions**

Collectively, the results indicate that Atlantic salmon are not broadly distributed or abundant across the four rivers surveyed. The Medway River shows the strongest evidence of ongoing freshwater occupancy, including limited juvenile presence. The Gold and Roseway rivers show molecular evidence of salmon presence without

corresponding juvenile detections, suggesting extremely low abundance or restricted spatial use. In the Mersey River, eDNA detections are most consistent with landlocked salmon populations rather than anadromous returns.

While this study was not designed to formally assess conservation status, the observed patterns are consistent with systems supporting severely reduced or fragmented Atlantic salmon populations. Continued monitoring using integrated methods will be necessary to improve confidence in presence/absence determinations and to track changes over time under varying hydrological conditions.

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