

# **Using eDNA to estimate juvenile salmon abundance in Fundy National Park**

by

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A Thesis Submitted in Partial Fulfillment  
of the Requirements for the Degree of

**Bachelor of Science with Honours in Biology**

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## **Abstract**

Environmental DNA (eDNA) is an emerging field that shows many benefits such as low effort and cost-effective sampling of aquatic environments. However, a variety of environmental factors influence the conservation of eDNA in water which make direct correlations between eDNA and fish abundance difficult to research. This study seeks to understand if incorporating the hydrodynamics and environmental variability of a river network will allow us to estimate juvenile salmon abundance using eDNA. We used eDNA sampling techniques and the eDITH R-Package to estimate juvenile salmon abundance throughout a river network. Based on the results of the analysis, we compared the estimated abundance results to electrofishing data to determine the comparability of our eDNA method with electrofishing. Although our results did not correlate with electrofishing abundance, producing Spearman's correlation coefficients of  $R = -0.47$ ,  $R = -0.49$ , and  $R = 0.41$ , they laid a critical foundation for future work that will adjust eDNA concentrations based on environmental variables. While abundance could not be accurately estimated and significant findings were inconsistent across trips, with adjustments, eDITH has potential to estimate relative population changes and aid in general monitoring of aquatic species.

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## **List of Symbols, Nomenclature, or Abbreviations**

C<sub>q</sub> - quantification cycle

eDITH - eDNA Integrating Transport and Hydrology

eDNA - environmental deoxyribonucleic acid

ESD - estimated salmon density

qPCR - quantitative polymerase chain reaction

USR - Upper Salmon River

## Introduction

Knowledge of fish distribution and abundance is key to managing fisheries (Jiang et al., 2024; Park et al., 2024). Sampling aquatic populations can be labour intensive and costly but recently developed environmental deoxyribonucleic acid (eDNA) measurement techniques and software can reduce sampling efforts and provide a non-invasive way to sample organisms (Evans et al., 2017). eDNA is genetic material that is shed or excreted from organisms into their environment. eDNA shed from aquatic species often stays suspended in the water column and its rate of degradation is influenced by various environmental factors. eDNA sampling and laboratory processing techniques have been used to detect the presence and absence of species (Govindarajan et al., 2023; Wang et al., 2024). In the last decade however, researchers have attempted to use eDNA to estimate abundance and distribution of fishes, but gaps remain in understanding environmental factors and the role they play in eDNA conservation. This complex relationship makes direct correlations between eDNA and species abundance or distribution difficult to ascertain (Jo, 2023; Rojahn et al., 2021).

Researchers strive to understand the relationship between eDNA, species abundance, and environmental factors. Traditionally, researchers rely on the hypothesis that the quantity of DNA in the environment is proportional to the abundance of organisms in that environment. However, this hypothesis has consistently been disproven when we try to directly relate eDNA quantities to fish abundance without considering the variability of the environment they inhabit. This discrepancy is because the input, transport, and removal of eDNA from the environment is a dynamic process that interacts with and is influenced by various environmental and biological factors. (Jo, 2023; Rojahn et al., 2021;

Carraro et al., 2024). Previous studies have examined a few factors that influence eDNA concentration but rarely incorporate the many environmental and hydrological factors into a single framework which in-turn, limits accuracy of results (Jo, 2023; Rojahn et al., 2021).

Statistical packages have been developed to relate eDNA concentrations to abundance while incorporating environmental variables. An R-package, recently developed in 2017 and first implemented in 2024, uses inputted data to model the flow of DNA throughout a river. The package, eDNA Integrating Transport and Hydrology (eDITH) creates maps showing species distribution and abundance throughout a river, while factoring in eDNA production, transport, and decay rates. (Carraro et al., 2024b). As eDITH was only recently developed, it has yet to be validated with a conventional method of estimating fish abundance such as electrofishing (Carraro et al., 2024b).

This study focuses on eDNA from Atlantic Salmon (*Salmo salar*) within New Brunswick, Canada. Atlantic Salmon are an ecologically important species present in the Bay of Fundy and surrounding rivers, holding status of being endangered and protected (Government of Canada, 2024). Due to frequent research being conducted on populations of Atlantic Salmon, eDNA, species abundance, and distribution information is easily obtainable for reference.

We seek to understand if incorporating the hydrodynamics and environmental variability of a river network will allow us to estimate juvenile salmon abundance using eDNA. We used eDNA sampling techniques and the eDITH R-Package to estimate juvenile salmon abundance throughout a river network. Based on the results of the analysis, we compared the estimated abundance results to electrofishing data to determine the comparability of our eDNA method with electrofishing.

## **Methods**

### **Study Species and Location**

The Upper Salmon River (USR) in Fundy National Park, New Brunswick, Canada is habitat to Atlantic Salmon. It is a high-gradient, dynamic river with low-levels of nutrients and a bed composed of large rocks and boulders with little sediment. The USR has multiple pools with beds of pebble substrate providing ideal breeding grounds for salmon (Monk et al., 2023). The USR flows from the confluence of two rivers, the Broad River and Forty-Five River. Each of these rivers have a waterfall barrier approximately 1600 and 700 meters upstream of where they join to form the USR. Due to the barrier, upstream of these waterfalls is inaccessible to Atlantic Salmon. The USR flows into the Bay of Fundy where Salmon travel continue their life cycle as smolts.

Atlantic Salmon are an anadromous species who spend most of their life in marine ecosystems and migrate to freshwater ecosystems to breed (DFO, 2019). Breeding occurs in rivers and streams during late fall where eggs are laid in a nest dug in the gravel of the riverbed. When eggs hatch in early spring, alevins emerge and start their lives within the riverbed gravel. Alevins begin to develop and become fry, 5 to 8 cm in length, who live within the water column. As fry mature, they become parr. Depending on resource availability and environmental conditions, parr remain in freshwater for 1-6 years before they develop into smolts and prepare for life as adults in marine ecosystems (DFO, 2019).

### **Electrofishing**

Electrofishing surveys were performed on the USR by members of Parks Canada on July 22-23, 2024, and August 22, 2024, using open sampling methods and permanent sample plot electrofishing protocols (FNP, 2020). 15 sites were sampled between the

mouth of the river (+45.609, -64.958) and the most upstream extent of the river accessible to salmon (+45.657, -64.978). Data was collected in the form of counts. Robinson (2024) then estimated juvenile salmon density for each site as well as the total river.

### eDNA Field Sampling

The entire river, accessible by salmon, was sampled three times throughout the summer. Sampling occurred on July 17 and 18 (Trip A), August 7 and 8 (Trip B), and August 28 and 29 (Trip C), 2024. The full transect, approximately 8 kilometers in length, consisted of 22 sample sites spaced ~400 meters apart (Figure 1). Three tributaries leading into the USR were sampled with one additional tributary (+45.632, -64.979), dry during sampling and no information was obtained from it. During the period of sampling, fry and parr, both juvenile forms of salmon are present in the river as adults have yet to return for

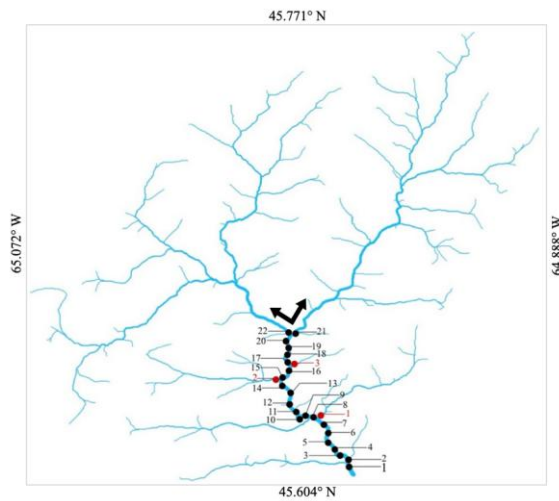


Figure 1. Map of the Upper Salmon River in Fundy National Park, New Brunswick, Canada, showing sampling sites 1 through 22 (black markers) and main tributaries 1 through 3 (red markers). Arrows indicate the main fork in the river, with the left branch representing the Broad River and the right branch representing the Forty-Five River.

breeding in late autumn. Due to the migration of salmon happening in late fall, abundance of salmon populations remained relatively constant during sampling.

Sampling consisted of using an OSMOS eDNA backpack to filter water in the field at each site. Sterile filter-housings compatible with the OSMOS eDNA backpack were disassembled and cleaned prior to use in the field by first rinsing with tap water to remove visible debris, soaked in a 15% bleach (Old Dutch, 4% w/w sodium hypochlorite) solution for 15 minutes, rinsed 5 times with distilled water, set to air dry, then lubricated with silicone grease prior to assembly. A sterile filter-housing was used at each site, assembled with 0.45 $\mu$ m sized filter (Cellulose Nitrate Membrane Filters, Whatman™) the day before field sampling. Filtration was performed in the midstream of the river by either extending the pole where water was too deep or wading downstream of where the sample was collected. Sterile practices were used and eDNA collection with the backpack was taken prior to environmental variable field sampling to ensure contamination was minimized. Samples were also taken starting downstream and moving upstream to minimize the introduction of exogenous DNA to downstream sites. This excludes site 1 as the river was crossed upstream of the site due to limitations in accessible locations. Samples were preserved in 95% ethanol over ice in the field then placed in a -20 freezer once returned to the lab.

Six negative controls were taken each trip, three on each day of sampling. Ultra-pure (Milli-Q®) water was carried in 1 L Nalgene® bottles cleaned in the lab prior to sampling by rinsing 3 times with a 15% bleach solution (Old Dutch, 4% w/w sodium hypochlorite) then 5 times with distilled water. In the field, the ultra-pure water was then transferred to a plastic bag at the site for filtration with the Osmos apparatus. Controls were

taken at the first, middle, and last site of each sampling day at the shore of the site following same OSMOS backpack settings as filtering samples.

### **Environmental Variable Field Sampling**

Environmental variables were collected at each site after eDNA sampling. Water quality sampling was recorded using the ProDSS Multiparameter Digital Water Quality Meter (Yellow Springs Instruments (YSI), Xylem Inc.) which measured river pH, conductivity ( $\mu\text{S cm}^{-1}$ ), temperature ( $^{\circ}\text{C}$ ), and chlorophyll  $\alpha$  concentrations ( $\mu\text{g L}^{-1}$ ). The instrument was calibrated in lab the day before each trip following the ProDSS Multiparameter Digital Water Quality Meter calibration protocol with no deviations.

Average water velocity and river width and depth was taken at each site. Velocity was measured using Global Water Flow Probe (Yellow Springs Instruments (YSI), Xylem Inc.) in the midstream of the river over the course of 10 seconds. For depths  $>0.6\text{m}$ , velocity was taken at 80% depth and 20% depth then averaged. For depths  $<0.6\text{m}$ , velocity was taken at 60% depth, following eDNA sampling procedure outlined in (NPS, 2018). River depth was measured using the meter stick on the flow probe while wetted river width was taken using a retractable 100 ft measuring tape by extending the tape perpendicular to the river flow direction.

River discharge was measured 6 times each trip at the start, middle, and end of each day. At each of the sites, the river was divided into sections perpendicular to the flow of water, no greater than 2 meters across with similar depths and flow (NPS, 2018). In the center of each cell, width and depth was measured, and average velocity was recorded over a period of 10 seconds for each section. Discharge was then calculated for each cell and summed across cells.

### **eDNA Extraction and qPCR assay**

The full eDNA extraction and quantitative polymerase chain reaction (qPCR) is outlined in Wood et al. (2021). To summarize, eDNA extraction was performed less than 2 months from original collection date using half of each filter. The Macherey-Nagel NucleoSpin Tissue Kit (Macherey-Nagel, PA, United States) protocol was then followed with 1 negative control ran alongside extractions consisting of 1-20 samples and 2 negative controls ran alongside extractions consisting of 20-22 samples. The resulting extracts were stored at  $-20^{\circ}\text{C}$  with the remaining half of the filter stored as backup in case of contamination and re-extraction is required.

Quantitative polymerase chain reaction (qPCR) was performed on extracts less than 2 months from original extraction date. qPCR was processed on 96-well plates with all samples, a negative control, and a standard curve ran in triplicates using TaqMan Gene Expression Kit (Thermo Fisher Scientific, MA, United States). Each well contained 3  $\mu\text{L}$  of extracted eDNA and 22  $\mu\text{L}$  of a solution consisting of 6.8  $\mu\text{L}$  of sterile  $\text{H}_2\text{O}$ , 12.5  $\mu\text{L}$  of 2X TaqMan Gene Expression Master Mix, 0.6  $\mu\text{L}$  of [20  $\mu\text{M}$ ] forward primer, 0.6  $\mu\text{L}$  of [20  $\mu\text{M}$ ] reverse primer, 1  $\mu\text{L}$  of 1% bovine serum albumin (BSA), and 0.5  $\mu\text{L}$  of [10  $\mu\text{M}$ ] probe [Forward primer COI\_82F\_Ss: 5'-TGGCGCCCTTCTGGGA; reverse primer COI\_276R\_Ss: 5'-AAGGAGGGAGGGAGAAG TCAAAA; and probe COI\_194P\_Ss: FAM -ATTAATTCCTCTTATAATCGGG - MGB] (Morrison et al., 2023). To assess potential inhibition in qPCR, each sample and negative control had 2 $\mu\text{L}$  an exogenous internal positive control (IPC) added. This control consisted of a linearized plasmid containing a 140 bp fragment of DNA from the Giant Panda (*Ailuropoda melanoleuca*). Reaction conditions remained the same as the Salmon assay above, aside for a new primer

and probe set that targeted Giant Panda DNA [Forward primer 64F: 5'-GCCTGGAGCTCTGTTAGGAGATGAC; reverse primer 207R: 5'-GTCAATTTCCAAAGCCTCCGATC; and probe inset 26bp\_V2: 5'-VIC-CTAAAGCTTACGTAGATCTGT-MGB] (Morisson et al., 2023). A sample was deemed inhibited if the quantification cycle (Cq) increased by three or more compared to the positive control containing pure panda DNA (Hartman et al., 2005). qPCR was run in QuantStudio™ 3 Thermocycler (Thermo Fisher Scientific) with the following cycling parameters: 50°C for 2 min, 95°C for 10 min, followed by 50 cycles at 95°C for 30 s, 60°C for 30 s and 72°C for 30 s with fluorescence reading at the end of each elongation cycle (Wood et al., (2021)). No inhibition was detected in any of the samples after qPCR was performed. All negative controls including field, extraction, and qPCR were free of contamination.

The Cq values obtained from qPCR were then converted into DNA concentrations in picograms per liter (pg/L) using the following equation:

$$C = 1000 \left( \left( 10^{\left( \frac{Cq - S_i}{S_s} \right)} \right) 2V_e \right) \left( \frac{1000}{V_f} \right)$$

where C is the concentration of eDNA in the sample (pg/L); Cq is the quantification cycle value obtained via qPCR; S<sub>i</sub> is the intercept of the standard curve ran alongside the sample; S<sub>s</sub> is the slope of the standard curve ran alongside the sample; V<sub>e</sub> is the total elution volume in μL; V<sub>f</sub> is the total volume filtered in the field during sampling.

For the concentrations to be used in analysis, 2/3 of the triplicate's values needed to be amplified during qPCR. Concentration values were considered 0 if they did not meet the criteria (Benoit et al., 2023).

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## **Data Analysis**

R-Package, “eDITH” was used to perform data analysis and abundance estimates (Carraro et al., 2024b). Procedure from Carraro et al (2024b) was followed to obtain estimates of relative juvenile salmon abundance. In brief, the package created a river map object from an inputted digital elevation map of the USR river obtained from the government of Canada public archives (NRC, 2019). Sampling locations were then mapped to the river object using the “rivnet” (Carraro, 2023) R-package by inputting latitudes and longitudes. The model was then run with eDNA concentration data as well as velocity, discharge, and depth for each site. Temperature, pH, and conductivity were initially considered for analysis but had to be excluded due to package limitations. The eDITH package models how these variables directly affect species distribution, rather than influencing eDNA concentrations and inferred species density. All eDNA concentrations and hydrological data from sampling sites 4, 5, 6, 7, 10, 11, 14, 16, 18, 19, and 20 were excluded from analysis because they were found to be in the same aggregate river (AG) reach as at least one other site. An AG reach is defined as a stretch of river uninterrupted by tributaries. Since the eDITH r-package allows only one data set per AG reach, this resulted in 14 unique reaches throughout the USR having measured eDNA and hydrological data. eDITH identified the USR as having a total of 230 reaches. However, the model was unable to distinguish salmon-inhabitable reaches due to the physical barriers, such as waterfalls upstream of the major fork in the USR, which made up the vast majority of the 216 reaches that went unsampled. For these 216 reaches without defined measurements, eDNA concentrations and hydrological data were predicted by the model (Carraro et al., 2024b). As a result, the model inaccurately predicted densities in regions where salmon could not realistically travel. Maps of relative taxon density were produced

by the 'run\_eDITH\_BT' function. This function uses a method called the Bayesian sampler to estimate the values and factors of the eDITH model and associates each AG reach with a DNA production rate assumed to be proportional to abundance (Carraro et al., 2024b).

$$C_j = \frac{1}{Q_j} \sum_{i \in y(j)} p_i A_{s,i} \exp\left(-\frac{L_{ij}}{\bar{v}_{ij} \tau}\right)$$

where  $j$  is the reach containing the sampling site;  $C_j$  is the DNA concentration at a sampling site;  $Q_j$  is the water discharge at  $j$ ;  $y(j)$  is the collection of reaches upstream of  $j$ ;  $p_i$  is the DNA production rate at an upstream site  $i$ ;  $A_{s,i}$  is the area of  $j$ ;  $L_{ij}$  is the distance between  $i$  and  $j$ ;  $\bar{v}_{ij}$  is the average water velocity along  $L_{ij}$ ;  $\tau$  is the decay rate of DNA in the river (Carraro et al., 2024a), estimated from the inputted eDNA data.

Spearman's correlations were performed comparing the estimated juvenile density produced by eDITH with juvenile estimated abundances for the electrofishing survey of the USR (Robinson, 2024). For correlations, electrofishing sampling sites and AG reaches were aligned using coordinates. In the case where multiple electrofishing sampling sites fell within the same AG reach, electrofishing densities were summed. This resulted 9/14 eDNA sampled reaches corresponding with electrofishing data. Data analyses, correlations, and figures were created using the R v. 4.4.2 (R Core Team, 2024) with package "rivnet" and "eDITH" (Wickham, 2016; Carraro et al., 2024b).

## Results

### eDNA Quantification

Average water temperatures and the number of sampling sites with positive eDNA detection decreased from Trip A to Trip C. During Trip A, the average river temperature was 20.68°C, and the average tributary temperature was 15.43°C. Of the 25 sampling locations, 22 (88%) had quantifiable eDNA, where "quantifiable" is defined as amplification in 2/3 of the triplicates after 50 cycles of qPCR. However, only 11 of the 14 AG reaches (79%) showed quantifiable eDNA. The highest eDNA concentrations were recorded at Site 1 (28.66 pg/L) and Site 22 (28.41 pg/L), while Site 21 and Tributaries 1 and 3 showed no detectable eDNA (Figure 2a).

In Trip B, the average river temperature was 19.19°C, and the average tributary temperature was 13.80°C. Of the 25 sampling locations, 17 (68%) had quantifiable eDNA, and 8 of the 14 AG reaches (57%) had detectable concentrations. The highest eDNA concentration in Trip B was found at Site 22 (11.40 pg/L). Sites 10, 13-15, 21, and Tributaries 1-3 had no detectable eDNA (Figure 2b).

For Trip C, the average river temperature was 16.49°C, and the average tributary temperature was 12.80°C. Of the 25 sampling locations, 12 (48%) had quantifiable eDNA, and 7 of the 14 AG reaches (50%) showed detectable concentrations. Tributary 1 had the highest eDNA concentration at 23.92 pg/L. Sites 3-5, 8-11, 14-15, 20-21, and Tributaries 2 and 3 had no detectable eDNA (Figure 2c). Note the difference in eDNA concentration scales across the figures.

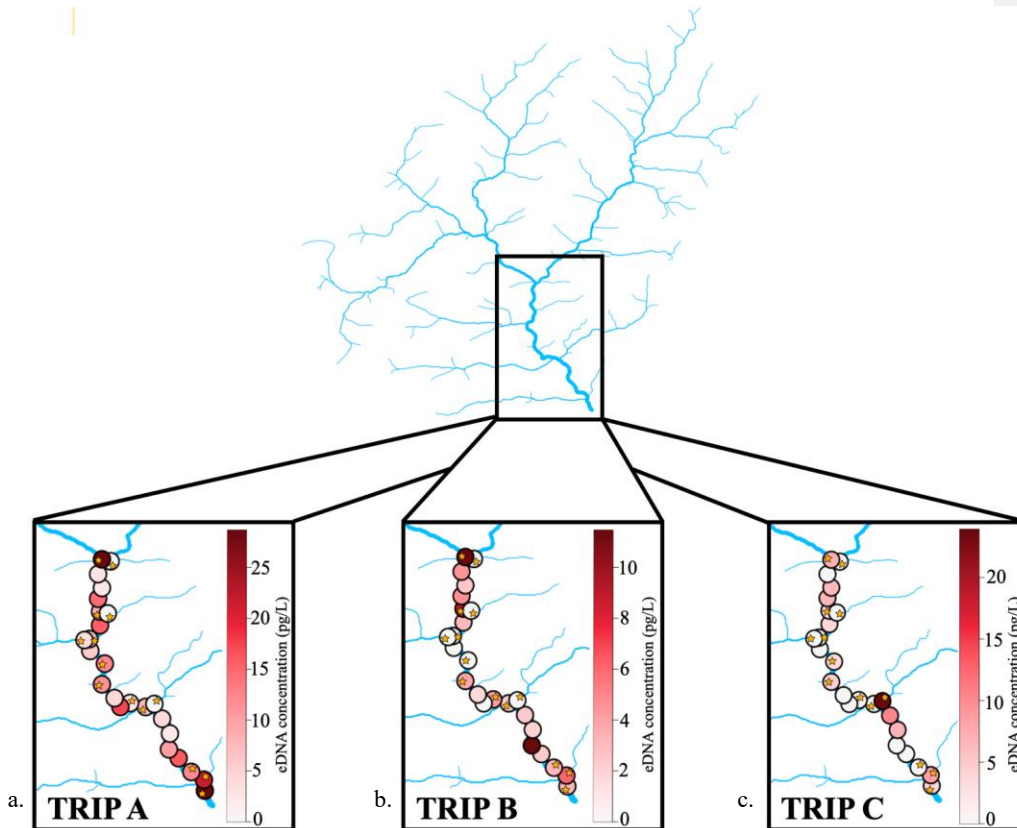


Figure 2. Map of the Upper Salmon River in Fundy National Park, New Brunswick, Canada, showing eDNA concentrations (pg/L) sampled on July 17-18, 2024 (Trip A), August 7-8, 2024 (Trip B), and August 28-29, 2024 (Trip C) at 22 sites and 3 tributaries. Stars indicate data from unique reaches used in the analysis. Note the different scales among Trips A-C.

### Distribution Amongst Sampling Trips

The heat maps of salmon abundance produced for each sampling trip (Figure 3a-c) show inconsistent estimated salmon density (ESD) for all reaches along the river. The package is unable to account for physical barriers that inhibit distribution of salmon such as the waterfalls past the fork of the Upper Salmon River. This invalidates the ESD results as the model predicts high densities in upstream, unsampled regions that likely are

influencing the ESD of downstream sampled reaches. Based on eDNA production rates, trip A (Figure 3a) displays low ESD throughout the river until the river forks, where the left side then shows relatively higher ESD compared to the right. Trip B shows low to moderate ESD throughout the river compared to where ESD increase slightly after the forks. Trip C (Figure 3c) displays relatively high ESD at the outlet and after the fork of the river compared to other reaches of that trip. One consistent finding among trips is the left branch of the fork, shows relatively higher ESD compared to the rest of the river across all three trips. Note the difference in scales among trips.

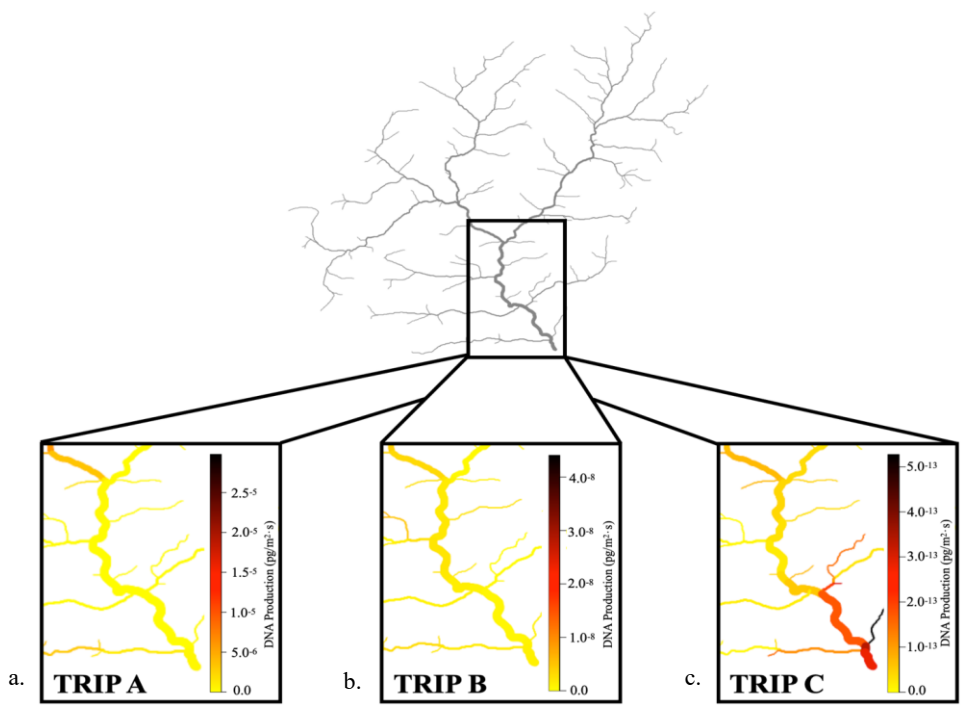


Figure 3. Map of the Upper Salmon River in Fundy National Park, New Brunswick Canada, displaying estimated salmon (*Salmo salar*) density ( $\text{pg}/\text{m}^2\cdot\text{s}$ ) sampled on July 17-18, 2024 (Trip A), August 7 and 8, 2024 (Trip B), and August 28 and 29, 2024 (Trip C) for individual reaches throughout the USR. Note different scales among trips A-C.

### Comparison of eDNA and Electrofishing Techniques

Correlations were inconsistent across the three trips. The DNA production rate ( $\text{pg}/\text{m}^2\cdot\text{s}$ ), equivalent to predicted density, derived from eDNA sampling and analysis was compared to the ESDs ( $\text{fish}/\text{m}^2$ ) obtained through electrofishing. For trips A and B (Figures 4 and 5), both showed significant weak negative correlations with values of  $R = -0.47$  and  $p = 0.04$  for trip A and  $R = -0.49$  and  $p = 0.035$  for trip B. In contrast, trip C (Figure 6) revealed an insignificant weak positive correlation ( $R = 0.41$ ) and  $p = 0.08$ . Site 22 was visually identified as an outlier for both trips A and B (Figures 4 and 5), while Site 2 was a visual outlier in trip C. After removing these outliers, Trips A and B no longer showed significant correlations with values of  $R = -0.45$  and  $p = 0.064$  for trip A and  $R = -0.45$  and  $p = 0.063$  for trip B and trip C now exhibited a significant correlation with  $R = 0.54$  and  $p = 0.02$ .

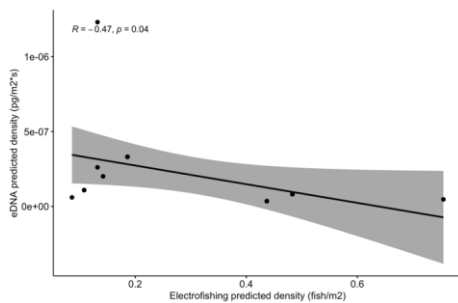


Figure 5. Scatterplot illustrating a negative correlation between electrofishing predicted density ( $\text{fish}/\text{m}^2$ ) and eDNA predicted density equivalent to DNA production rate ( $\text{pg}/\text{m}^2\cdot\text{s}$ ), with Spearman's correlation coefficient ( $R = -0.47$ ) indicating a weak, significant relationship ( $p < 0.05$ ).

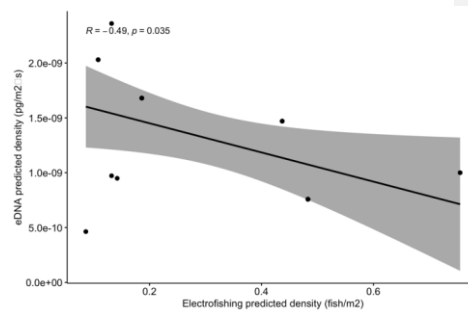


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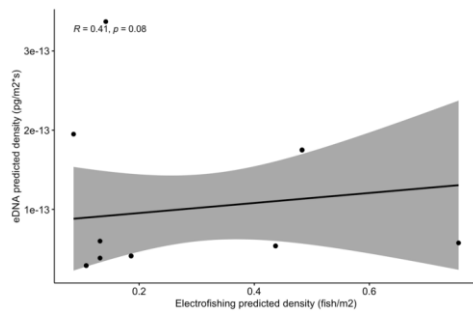


Figure 6. Scatterplot illustrating a positive correlation between electrofishing predicted density (fish/m<sup>2</sup>) and eDNA predicted density equivalent to DNA production rate (pg/m<sup>2</sup>·s), with Spearman's correlation coefficient ( $R = 0.41$ ) indicating a weak, insignificant relationship ( $p > 0.05$ ).

## Discussion

This study aimed to assess whether incorporating the hydrodynamics and environmental variability of a river network could improve estimates of juvenile salmon abundance using eDNA and the eDITH package. We compared eDNA-predicted densities to electrofishing-predicted densities at multiple points throughout a river over three different trips. Overall, we found that eDNA sampling techniques and the eDITH model were not able to accurately estimate local salmon abundance, as only weak correlations were observed. Without further refinement, using the eDITH model to estimate population abundance would likely result in inaccurate predictions. Adjusting eDNA to incorporate environmental variables may allow stronger correlations to be observed, therefore improving the application of the eDITH model (Morrison et al., 2023). Additionally, methods such as electrofishing are associated with high error rates. Testing ESD produced by eDITH against alternative techniques for measuring abundance may also improve the strength of correlations (Lyons et al., 1993; Wood et al., 2021).

One potential reason for the weak correlations observed, may be the discrepancies in sampling timing between eDNA and electrofishing surveys. eDNA sampling was conducted three times throughout the summer, while electrofishing was only performed once, split over two main trips. The first half of the river was sampled on July 22–23, and the second half was sampled on August 22. Electrofishing used open-sampling methods, which do not guarantee that all individuals in a given area are accounted for (Lyons et al., 1993). Aligning eDNA and electrofishing sampling dates more closely, electrofishing the full river more than once, and utilizing closed sampling sites for electrofishing could

improve abundance estimates, resulting in stronger correlations with eDNA data (Lyons et al., 1993).

Further investigation is needed to determine why negative correlations were observed, as the data showed that eDNA ESDs decreased when electrofishing ESDs increased. It would be expected that eDNA ESDs would increase alongside increasing electrofishing ESDs, as the amount of eDNA in the water column should be greater with a higher number of fish present in the area (Jo, 2023; Rojahn et al., 2021; Carraro et al., 2024b). However, differences in eDNA shedding rates among individuals due to allometry, as well as the effects of temperature, can play a substantial role in eDNA concentrations in the water column and could be a factor resulting in negative correlations. (Lacoursière-Roussel et al., 2016; Yates et al., 2021).

One limitation of the current approach is the inability to directly control for or incorporate measured values for temperature, pH, and conductivity into the eDITH package. Environmental factors can be accounted for separately by adjusting eDNA concentrations before inputting them into the program (Morrison et al., 2023). Future iterations may reveal stronger correlations when these factors are considered, as previous studies have shown that environmental conditions significantly influence the preservation and decay rates of eDNA in the water column. The eDITH package uses a decay rate,  $\tau$ , which represents a pre-set characteristic time for the decay of DNA in streams (Carraro et al., 2024b). The exact value of  $\tau$  is not specified, as it is estimated by the model.

Additional limitations and a potential bias include the inability of the eDITH package (Carraro et al., 2024b) to account for physical barriers that inhibit salmon from residing in certain sections of the river. For example, the Broad and Forty-Five rivers,

upstream of the main fork in the USR, both contain waterfalls that salmon cannot pass. The eDITH model currently estimates salmon presence in these upstream areas because no data are provided to indicate that eDNA would not be present there. Future iterations will need to exclude these areas as potential eDNA sources.

Studies such as Lacoursière-Roussel et al. (2016) and Doi et al. (2016) show positive correlations between eDNA concentration and fish abundance, though they use different approaches, their methodologies provide insight for future research. Lacoursière-Roussel et al. (2016) conducted their experiments in a controlled aquarium environment, manipulating temperature to observe effects on eDNA concentrations. While this approach provides valuable data on the relationship between eDNA and environmental conditions, it does not account for the complex hydrodynamics of natural stream and river environments, which could affect eDNA transport and degradation. Our study could build on Lacoursière-Roussel et al.'s (2016) findings by incorporating these real-world environmental factors, offering a more accurate reflection of eDNA dynamics in natural habitats. On the other hand, Doi et al. (2016) compared eDNA concentrations to fish abundance estimates derived from snorkeling observations in streams. While this comparison is insightful, visual abundance estimates can be limited, especially for juvenile species like salmon that often reside in complex, substrate-rich habitats where visibility is low (Hankin et al., 1988). By focusing solely on eDNA, my research could eliminate the biases present in visual observation methods, offering a more reliable, non-invasive tool for estimating fish abundance, including those species that are difficult to observe. (Hankin et al., 1988).

The findings of this study provide important insight into using the eDITH package to estimate salmon abundance and distribution. Although our findings were inconsistent, additional refinement may allow eDNA sampling and the eDITH package to become a useful tool in monitoring relative change of a population. Future studies should manipulate eDNA concentrations to factor in all environmental variables that may influence eDNA decay and preservation in a water column before input into eDITH, as well as define areas inaccessible to a species when running eDITH.

## **Conclusion**

Measuring eDNA in nature is a recently developed but rapidly evolving area of study. Identifying the relationship between eDNA and species abundance is complex, particularly when accounting for environmental factors that influence eDNA preservation in the water column. This challenge has been tackled by many scientists (Jo, 2023; Rojahn et al., 2021). This study was an important first step in using eDITH to estimate salmon distribution in this context. Although our results did not correlate with electrofishing abundance, they laid a critical foundation for future work that will adjust eDNA concentrations based on environmental variables. While abundance could not be accurately estimated and significant findings were inconsistent across trips, with adjustments, eDITH has potential to estimate relative population changes and aid in general monitoring of aquatic species.

## Contributions to Thesis

**Dr. Scott Pavey:** Supervised the study and provided funding, insight, and guidance throughout the research

**Jagger Watters-Gray:** Conceptualized and designed the study, providing additional insight and guidance. Assisted in eDNA sample collection.

**John Whitelaw with Fundy National Park:** Granted use of park trails and accommodations.

**John Robinson with Fundy National Park:** Provided electrofishing data.

**Dr Kurt Samways:** Facilitated connections with Fundy National Park.

**Larissa Roehl:** Provided eDNA training in the field and laboratory. Assisted in eDNA sample collection.

**Nathalie LeBlanc:** Provided insight and guidance on data analysis. Assisted in eDNA sample collection.

**Laken Devost:** Assisted in eDNA sample collection.

**Suroush Bastani:** Assisted in eDNA sample collection.

**Jack Nason:** Assisted in data analysis and electrofishing sample collection.

**Ben Andrews:** Assisted in electrofishing sample collection.

**Lauren Comeau:** Assisted in electrofishing sample collection.

**Kristin Armstrong:** Assisted in electrofishing sample collection.

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