Investigating how seasonal changes alter wild juvenile Atlantic Salmon (*Salmo salar*) environmental DNA quantification in New Brunswick, Canada

by

Melissa K. Morrison

Bachelor of Science (Honours), University of Northern British Columbia, 2013

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

Master of Science

in the Graduate Academic Unit of Biological Sciences

Supervisors: Scott Pavey, Ph.D., Department of Biological Sciences, Canadian Rivers Institute, and Canada Research Chair, UNB Saint John
Anaïs Lacoursière-Roussel, Ph.D., Research Scientist, Fisheries and Oceans Canada

Examining Board: Kurt Samways, Ph.D., Department of Biological Sciences, Canadian Rivers Institute, and Parks Canada Research Chair, UNB Saint John
Jeff Houlahan, Ph.D., Department of Biological Sciences, UNB Saint John
Margaret Docker, Ph.D., Department of Biological Sciences, University of Manitoba

This thesis is accepted by the
Dean of Graduate Studies

THE UNIVERSITY OF NEW BRUNSWICK

April, 2022

ABSTRACT

Environmental DNA (eDNA) analysis is valuable to monitor imperilled taxa; however, unexplained observed eDNA variation impedes the development of population abundance models. To address unknown seasonal impacts on the quantitative relationship between eDNA concentration and abundance, I studied seasonal eDNA dynamics of Atlantic Salmon (*Salmo salar*) along 9 km of a river in New Brunswick, Canada. During spring smolt emigration, I tested the eDNA-abundance relationship and the relevance of environmental factors in population models. Environmental variables were also tested in summer with relatively constant parr abundance at two different spatial scales. Environmental covariates significantly altered eDNA concentration in both periods and their inclusion within eDNA models was crucial to assess smolt and parr populations. This study discusses mechanisms behind the seasonal effect of a local environment on eDNA and provides recommendations to develop salmon eDNA monitoring programs using empirical-based evidence of the relative importance of environmental interactions.

ACKNOWLEDGEMENTS

I would like to acknowledge Fisheries and Oceans Canada, University of New Brunswick (Saint John), Canada Research Chairs Program, and the Bud and Peggy Bird Scholarship for funding this research project. I would also like to acknowledge Parks Canada for their support. To my supervisors, Drs. Scott Pavey and Anaïs Lacoursière-Roussel, thank you for the opportunity to fly across the country. Your confidence and guidance have allowed me to gain valuable skills, including how to conduct research during a global pandemic. I am grateful for the insightful feedback from my supervisory committee, Jeff Houlahan and Kurt Samways, whom I further thank for connecting me with his students: John Robinson, for sharing his data and articles to estimate population size, as well as Jack Nason and Lauren Cyr, without whom I would have been unable to complete my field work. Field work would also not have been possible without the help of Mark Dodds and equipment from the biology department technicians. Thank you to Zachary Wood at the University of Maine for having the patience to guide me through the complex modelling and statistics in my project. I am also grateful for the statistics, lab work, and presentation advice provided by those past and present in the Pavey Lab: Larissa Roehl, Faith Penny, Shawn Kroetsch, Nathalie LeBlanc, Ty Gormley, Howie Huynh, Jagger Watters-Grey, Rina Guxholli, Jake Reicker, and Jillian Fraser.

Finally, the moral support and encouragement from my family and close friends both here and in different time zones has powered me immensely throughout this entire venture.

Table of Contents

List of Tables

List of Figures

List of Symbols, Nomenclature or Abbreviations

bp – Base pair
°C – Degrees Celsius
cm – Centimetre
D_{50} – Median diameter
L - Litre
m – Metre
mm – Millimetre
km – Kilometre
pg – Picogram
psu – Practical salinity units
µg – Microgram
µS – Microsiemens
Biological replicate – Water sample
BSA – Bovine serum albumin
C_q – Quantification cycle
DNA – Deoxyribonucleic acid
eDNA – Environmental DNA
IPC – Internal positive control
LOQ – Limit of quantification
MGB – Minor groove binding
PCR – Polymerase chain reaction
qPCR – Quantitative PCR
UV – Ultraviolet
AICc – Akaike information criterion for small sample size
ANOVA – Analysis of variance
CI – Confidence interval
CV – Coefficient of variation
df – Degrees of freedom
GLM – Generalised linear model
IQR – Interquartile range
SE – Standard error
Tukey HSD – Tukey honestly significant difference test

Chapter 1: General Introduction

Aquatic habitats have undergone dramatic transformations in recent decades due to direct and indirect human activities (e.g., energy production, species overexploitation, introduction of invasive alien species), affecting a wide range of taxa (WWF, 2020). Freshwater ecosystems are of particular concern as they are a vital resource for humans and contain extraordinarily rich biotas, housing nearly 10% of the world's known species yet only comprising about 0.01% of the world's water sources (Dodds and Whiles, 2010; Strayer and Dudgeon, 2010). These habitats are experiencing increasingly rapid declines in fish populations due to cumulative effects from multiple stressors (Nelson *et al.*, 2016).

Current biodiversity management systems employ conventional monitoring techniques such as electrofishing (Dolan and Miranda, 2004) and active netting (Lyons, 1986). These have limitations as they require physical capture of the target species. Traditional fish surveys often require teams of highly trained personnel and are not only time-consuming, but unintentionally disturbing habitats to capture individuals may increase risk of inadvertent injury or death to target and non-target species (Miranda and Kidwell, 2010). The spatial and temporal limitations of physical capture may also render inaccurate species abundance estimations if sampling remote or large areas and targeting cryptic or rare species (Gu and Swihart, 2004).

Indirect monitoring approaches using molecular techniques have rapidly developed over the last decade. Detecting and quantifying macro-organism DNA

from the environment is a revolutionary indirect approach derived from the fields of ancient DNA (Higuchi,1984), forensics (Vincent *et al.*, 2000), and microbiology (DeFlaun *et al.*, 1987). Having first detected DNA from the environment with UV spectroscopy (Pillai and Ganguly, 1977), Polymerase Chain Reaction (PCR) methods have been developed to detect minute quantities of environmental DNA (eDNA) found in biological materials shed from the species of interest (Figure 1). Quantitative PCR (qPCR) is a robust approach as specificity for the target species is attained by designing short, species-specific DNA fragments (i.e., primers and probes) that anneal to regions of DNA known as “barcodes” (Furlan *et al*., 2016).

Barcodes within the mitochondrial genome are typically preferred in quantitative eDNA methods. Due to the greater number of mitochondria in a cell compared to its single nucleus, amplifying short fragments within mitochondrial eDNA barcodes often improves detection rates and quantification variability (Rees *et al*., 2014). One common example of a “universal barcode” is the mitochondrial cytochrome *c* oxidase subunit I (COI) gene (Hebert, Ratnasingham and deWaard, 2003). Across most animal groups, this segment of the mitochondrial genome is highly conserved within species yet generally exhibits high divergence among those that are closely related (Saccone *et al.*, 1999; Hebert, Cywinska *et al*., 2003). This divergence enables a single species to be distinguished in an environmental sample.

Due to the widespread success in species detection (e.g., Ficetola *et al.*, 2008; Dejean *et al*., 2011), research is increasingly exploring what factors

impede accurate estimates of species abundance from eDNA (e.g., Lacoursière-Roussel, Rosabal and Bernatchez, 2016; Shogren *et al.*, 2019; Yates, Cristescu and Derry, 2021). Directly relating eDNA concentration to abundance may result in misinterpreting species presence or absence due to variation in eDNA in natural lotic environments caused by factors other than abundance (Goldberg *et al*., 2016; Rice *et al.*, 2018). The different states in which eDNA exists (i.e., from whole cells, cell aggregates, extracellular DNA; Pietramellara *et al*., 2009) likely contribute to some of the observed stochasticity in its quantification (Barnes and Turner, 2016).

There has been growing evidence of the complexity of eDNA shedding and degradation among different fish life stages and lotic environments. Maruyama *et al.* (2014) found higher eDNA release rates in juvenile Bluegill Sunfish (*Lepomis macrochirus*) than adults, likely due to higher metabolism in early development. Tillotson *et al*. (2018) also found increased eDNA released from spawning Sockeye Salmon (*Oncorhynchus nerka*), which may be influenced by the release of gametes. Increased shedding rates have also been observed in higher water temperatures (Lacoursière-Roussel, Rosabal and Bernatchez, 2016; Jo *et al.*, 2019); however, while higher water temperatures may increase eDNA shedding through increased metabolism, it has also been seen to increase eDNA degradation rates (Pilliod *et al*., 2014). Environmental DNA is often subject to higher degradation (i.e., from increased temperature, acidity, UV) when it is no longer within the highly controlled environment provided by the cell to maintain stability (Strickler *et al.*, 2015).

Environmental DNA distribution in lotic systems is further impacted by different stream morphologies and bed substrates that ultimately affect its downstream transport (Howard, 1994; Dejean *et al.*, 2011; Deiner and Altermatt, 2014). Increased water volume and velocity (e.g., following a rain event) has been observed to dilute eDNA and contribute to variability in concentration among samples. Moreover, the rate of deposition in the riverbed and resuspension in the water column is impacted by the size of substrate (Jane *et al.*, 2015). Sediments, for example, contain adsorbent humic compounds which may entrap nucleic acids, allowing eDNA to persist longer than in areas with less sediment (Nielsen *et al.*, 2007).

While the dynamics of eDNA shedding, persistence, and transport have been studied at length in controlled environments, combined seasonal factors (i.e., temperature, discharge) in natural river systems likely convolute these relationships. The origin of eDNA may be impacted indirectly through cued changes in animal life stage and behaviour. For migratory fishes such as Atlantic Salmon (*Salmo salar*), changes in photoperiod, temperature, and water flow have been seen to trigger smolt emigration in the spring (McCormick *et al*., 1998). Although the onset of migration may increase the amount of shed eDNA due to both increased temperature and fish density, its quantification may be affected due to presumed dilution with increased spring runoff. As such, a better understanding of how these intricate seasonal dynamics influence eDNA production and transport is critical to improve quantitative eDNA-abundance relationships.

As an anadromous species, Atlantic Salmon are subject to an elaborate life history with specific habitat requirements. The opposing osmolarities that Atlantic Salmon experience dictate complex physiological shifts as they migrate between fresh and saltwater to complete their life cycle. In anticipation of these impending osmotic changes, a process known as smoltification prepares most juvenile salmon (parr) for maturation at sea (McCormick *et al*., 1998); however, a small fraction of male parr may remain in their natal river to mature (precocial or resident parr; Koch and Narum, 2021). Designated as Endangered in 2001, the inner Bay of Fundy (iBoF) populations of Atlantic Salmon located in southeast New Brunswick, Canada continue to experience sharp declines (COSEWIC, 2011) and are monitored at each life stage as part of a national recovery strategy: Fundy Salmon Recovery Project (Fundy Salmon Recovery, 2022). Reducing habitat disruption and species mortality to preserve these populations is essential; therefore, developing reliable, non-lethal, and indirect population monitoring is crucial to supplement conventional population assessments.

The overall objective of this thesis was to understand the temporal and spatial changes in eDNA concentration in a highly monitored river with multiple Atlantic Salmon juvenile cohorts. I assessed the complex relationships among seasonal fluctuations in spatial eDNA distribution, juvenile population abundance, and stream environmental conditions. Specifically, I predicted that:

1) the inclusion of environmental covariates in quantitative eDNA population models would clarify the relationship between eDNA and fluctuating spring smolt abundance, and

2) the seasonal environmental changes would significantly increase variation in eDNA concentration of the relatively constant parr abundance through the summer.

To my knowledge, this study is one of few to explore how environmental factors impact salmon eDNA quantification and its relationship with population abundance in a natural river. Rather than assessing eDNA during adult salmon migration and spawning (Tillotson *et al*., 2018; Levi *et al*., 2019), this research addressed seasonal changes in juvenile salmon eDNA. The knowledge gained from this study will help inform future quantitative eDNA population assessments by validating aspects of eDNA behaviour recently observed in riverine systems and providing targeted advice based on empirical data.

Chapter 2: Including environmental covariates clarifies the relationship between endangered Atlantic Salmon (*Salmo salar*) abundance and environmental DNA

Abstract

Collecting environmental DNA (eDNA) as a non-lethal sampling approach has been valuable in detecting the presence/absence of many imperilled taxa; however, its application to indicate species abundance poses technical challenges. A deeper understanding of eDNA dynamics in freshwater systems is required to better interpret the substantial variability often associated with eDNA samples. Here I took advantage of natural variation in juvenile Atlantic Salmon (*Salmo salar*) distribution and abundance along 9 km of a single river in the Province of New Brunswick (Canada). My sampling design covered different spatial and temporal scales to address the unknown seasonal impacts of environmental variables on the quantitative relationship between eDNA concentration and species abundance. First, I asked whether accounting for environmental variables strengthened the relationship between eDNA and salmon abundance by sampling eDNA during their spring seaward migration. Environmental DNA samples were collected over a six-week period (12 times) near a rotary screw trap that captured about 18.6% of migrating smolts. Second, I asked how environmental variables affected eDNA dynamics during the summer as the parr abundance remained relatively constant. Sampling occurred (i) at three distinct salmon habitats (9 times) and (ii) along the full 9 km (3 times).

I modelled eDNA concentration as a product of fish abundance and environmental variables, many of which have been rarely measured in previous eDNA studies. I present that (1) with inclusion of abundance and environmental covariates, eDNA was highly correlated with spring smolt abundance; and (2) the relationships among environmental covariates and eDNA were affected by seasonal variation with relatively constant parr abundance in summer. My findings underscore that with appropriate study design that accounts for seasonal environmental variation and life history phenology, eDNA salmon population assessments may have the potential to evaluate abundance fluctuations in spring and summer.

1 Introduction

Non-invasive sampling approaches, such as environmental DNA (eDNA), are valuable in monitoring already threatened fishes such as Atlantic Salmon (*Salmo salar*) that have critically low numbers in many rivers (ICES, 2021). Monitoring populations at risk of further decline due to climatological or anthropogenic stressors (e.g., dams) traditionally requires physical capture of the species of interest (Dempson and Stansbury, 1991; Dolan and Miranda, 2004). Not only can this risk inadvertent injury or mortality in an already vulnerable population (Miranda and Kidwell, 2010), abundance estimates may be inaccurate (Gu and Swihart, 2004) and may negatively impact status assessments and management decisions of imperilled taxa. While effective physical capture and eDNA sampling both require extensive prior knowledge of fish life history and location (e.g., migration and local distribution) in the specific ecosystem, the

lower cost and time associated with eDNA methods allow greater accessibility and sensitivity for rare target species (Jerde *et al*., 2011). Non-invasive sampling approaches can enhance biomonitoring by reducing efforts and expanding to areas that are impractical for large-scale conventional surveys (Villacorta-Rath *et al*., 2021). Finally, effective quantitative population assessments are also essential to evaluate post-intervention monitoring. The main goal of this study was to elucidate the complex relationships among eDNA concentration, juvenile Atlantic Salmon population abundance, and seasonal environmental conditions in a natural stream environment to refine non-invasive eDNA sampling approaches and models for this commonly threatened species.

Across freshwater taxa, eDNA has successfully detected the presence of individuals (Ficetola *et al.*, 2008; Jerde *et al.*, 2011; Bylemans *et al.*, 2016; Lugg *et al.*, 2018); however, its application as an indicator of species abundance poses management challenges. Understanding how spatial and temporal variation in environmental factors influence eDNA is especially crucial to deriving abundance predictions as eDNA dynamically interacts with its environment once shed from an organism (i.e., urine, feces, sloughed cells, mucus; Barnes and Turner, 2016). The ecology of eDNA, such as how it sheds, degrades, and is transported, has been broadly explored in laboratory settings and controlled streams (Shogren *et al*., 2018; Yates *et al*., 2019; Wood *et al*., 2020). While mesocosm and caged fish experiments have presented strong correlations between concentration and abundance metrics (Jo *et al.*, 2019; Shogren *et al.*, 2019; Wood *et al.*, 2021), few studies in natural systems have exhibited such relationships (Lacoursière-

Roussel, Côté *et al.*, 2016; Lacoursière-Roussel, Dubois *et al.*, 2016). The typically complex dynamics of eDNA ecology in many natural systems could lead to wide variation in the precision and accuracy of species quantification (Takahara *et al.*, 2012; Mahon *et al.*, 2013; Pilliod *et al.*, 2013).

Understanding how eDNA persists and is transported in lotic ecosystems might help to overcome these abundance estimation challenges and is increasingly seen as essential to design and optimise monitoring programs and interpret time series data (Wood *et al.*, 2020, 2021). Although flow and dilution have very predictable effects on eDNA quantification, what is increasingly clear is that eDNA distribution is more complex than a conservative tracer or monodispersed solution in riverine systems due to the various states and pools in which it resides (Jerde *et al.*, 2016; Wilcox *et al*., 2015; Pont *et al*., 2018). For example, although increased shedding rates observed in higher water temperatures (Lacoursière-Roussel, Rosabal and Bernatchez, 2016; Jo *et al.*, 2019) are likely due to metabolic rate effects on eDNA shedding, temperature has also been seen to increase eDNA degradation rates (Pilliod *et al*., 2014). The chemistry of field water, including pH and carbon compounds, also interacts with eDNA quantification by directly affecting its degradation and sequestration as well as indirectly affecting its molecular detection (i.e., PCR inhibition; Barnes and Turner, 2016). Finally, there may be a significant seasonal or life stage component to eDNA dynamics, particularly given the large role that seasonality and life stage play in fish behaviour and metabolic rate contributions to when, where, and how eDNA is shed.

North American Atlantic Salmon populations have experienced sharp declines since the late 19th century, many of which are classified as Endangered in Canada and the United States (COSEWIC, 2011; National Marine Fisheries Service, 2021). Atlantic Salmon populations are inherently vulnerable due to their complex life history as an anadromous species, migrating great distances between freshwater and marine environments with the potential to spawn multiple years (Koch and Narum, 2021). As such, Atlantic Salmon have been the subject of significant eDNA development as minimising further habitat disruption by conventional monitoring methods is essential to preserve these populations (e.g., Balasingham *et al.*, 2017; Williams *et al.*, 2019; Fossøy *et al.*, 2020; Wood *et al*., 2021). Given the intricate ecology of eDNA, it is still unclear how the seasonal changes in environmental factors and life stages affect wild salmon eDNA.

I examined salmon eDNA concentration as a function of fish abundance and environmental variables. First, I asked whether accounting for environmental variables would clarify the relationship between eDNA and smolt abundance; and second, how environmental variables affected eDNA dynamics during the summer as parr abundance remained relatively constant. Seasonal environmental effects on juvenile Atlantic Salmon eDNA quantification were intensively explored in a single river that is well documented with extensive annual population assessments over the last two decades, giving a comprehensive and precise understanding of the life stages (parr and smolts) present and their relative location at a given time.

2 Methods

2.1 Study species and location

Upper Salmon River (USR) in Fundy National Park, New Brunswick, Canada (45°37'05" N, 64°57'56" W; Figure 2) is a high-gradient, dynamic, and oligotrophic river with a bed composed of large rocks with little sediment. The river flows from the confluence of the Broad and 45 River tributaries, each of which has a physical waterfall barrier approximately 1600 m and 700 m upstream of the confluence, respectively; thus, salmon are absent upstream of these waterfalls. Primarily fed by groundwater, the length accessible by salmon flows 9 km through a mixed wood forest (Wilken *et al.*, 2011) with deep, narrow, incised gorge-like banks and cliffs (Monk *et al*., 2022). Five small adjoining tributaries drain into the USR before emptying into the Bay of Fundy; however, these side streams were largely dry during part of this study period when USR experienced the lowest water level since recording began in 2011 (Government of Canada, 2020).

USR can be divided into three main reaches where Atlantic Salmon occur, each consisting of riffles, runs, and pools. Juvenile Atlantic Salmon (parr) in this river typically rear in these habitats for two (up to four) years before migrating to sea from May through June as smolts. The three main regions along USR are in the upper, middle, and lower reaches of the river known as The Forks, Black Hole, and Pumphouse, respectively (TF, BH, PP; Figure 2), named for the primary pool within each reach. In addition to Atlantic Salmon, USR resident fishes include Brook Trout (*Salvelinus fontinalis*), Blacknose Dace (*Rhinichthys*

atratulus), and although rare, Northern Redbelly Dace (*Phoxinus eos*; K. Samways, personal comm., 09 Jul 2020). American Eel (*Anguilla rostrata*) is the only other diadromous fish to reside in the USR. Over several decades of electrofishing surveys, no other salmon species have been documented in the river and resident fishes are present in relatively small numbers (K. Samways, personal comm., 09 Jul 2020).

2.2 eDNA field sampling

Water samples for eDNA detection were collected from 15 Jul to 08 Sep 2020 and from 04 May to 10 Jun 2021. Samples were collected midstream following recommendations of Wood *et al.* (2021). To ensure no contamination from exogenous DNA, 1 L Nalgene® bottles were shaken with 10% (v/v) bleach solution (Clorox Javex® 12, 10.3% sodium hypochlorite) three times followed by five times with distilled water. The bottles were also vigorously shaken with river water three times prior to collecting triplicate 1 L samples of surface water (i.e., fully submerging the bottles right below the surface). Field negative controls were obtained using ultrapure water (Milli-Q®; Merck, Darmstadt, Germany) poured into a bleached and rinsed 1 L Nalgene® bottle every five to 10 samples.

2.2.1 Smolt survey

To assess the relationships among fluctuating population abundance, spring seasonal conditions, and the resulting eDNA concentration, water samples for DNA analysis were collected during and after the spring smolt migration to sea. Sampling occurred twice a week for six weeks during smolt migration (04

May through 10 Jun 2021). Smolts migrating to the ocean were captured by a rotary screw trap (i.e., smolt wheel) situated approximately 300 m upstream of the head of tide on the USR (Figure 2). For this specific year, the smolt wheel had an 18.6% efficiency and estimated a population size of 4911 (95% CI = [3411, 7027]) smolts migrating to sea (J. Robinson, Parks Canada). Environmental DNA samples were collected as described above at distances 50 m upstream of the smolt wheel, beside the smolt wheel, and downstream of the smolt wheel at 50 and 150 m (50 m upstream; 0, 50, 150 m downstream; 12 days, n = 48, triplicates).

2.2.2 Parr survey

Temporal fluctuations in eDNA concentration due to seasonal environmental conditions were explored during the period of the year when the number of parr in the river remained relatively constant. Two different sampling designs were executed from 15 Jul through 08 Sep 2020 to assess differences in eDNA distribution at a local habitat and full river scale. The primary survey consisted of sampling three to four sites (n = 11 sites) within each of the three main reaches to explore eDNA distribution at a local scale. As each reach contained adequate habitat for spawning adult Atlantic Salmon (K. Samways, personal comm., 09 Jul 2020), it was assumed that parr would remain in close proximity to the pool-riffle-run habitats in each reach. Samples were collected as described above at distances relative to the main pool in each reach: The Forks (TF), Black Hole (BH), and Pumphouse (PP; Figure 2). Sampling occurred once a week for nine weeks, at upstream and downstream distances for TF and BH

(50 m upstream; 50, 250, 500 m downstream; 9 days, *n* = 72, triplicates). Note that for PP, sampling only occurred downstream of the primary pool (50, 100, and 250 m downstream; 9 days, *n* = 27, triplicates) and the 500 m downstream distance was omitted as the river becomes tidal, which was beyond the scope of this study.

The second survey consisted of three sampling events (15 Jul, 27 Aug, 08 Sep 2020) that included transecting the full salmon-accessible length of USR to examine how seasonal changes affected eDNA quantification at the river scale. A secondary goal of this survey was to explore the possible occurrence of downstream eDNA accumulation. Samples were collected at intervals of 40 – 500 m (average 200 m) along the river corridor. Additional samples were obtained from four adjoining tributaries along the river to test if salmon were distributed in tributaries and potentially contributing eDNA (Figure 2). Sampling also occurred upstream of the confluence to verify that no salmon were present upstream of each physical waterfall barrier (15 Jul, *n* = 64; 27 Aug, *n* = 70; 08 Sep, *n* = 68; no replicates).

2.3 Environmental monitoring

Water quality variables were obtained to examine their effect on eDNA quantification. Environmental conditions were chosen based on potential interactions with eDNA molecules directly (conductivity, pH, salinity, and temperature) and indirectly, through quantification inhibition (chlorophyll α and turbidity; reviewed in Barnes and Turner, 2016). Environmental variables were measured at each sample site using a YSI® ProDSS Digital Sampling System

Water Quality Meter (YSI Incorporated, Yellow Springs, OH, USA). Note that for the parr survey, collection of YSI measurements began 29 Jul 2020; furthermore, measurements could not be obtained on 27 Aug and 08 Sep 2020 for approximately half of the sites due to lack of equipment accessibility.

To evaluate the effect of river discharge on downstream eDNA transport and its potential dilution (Jane *et al*., 2015), respectively, water velocity and total precipitation data were obtained for both smolt and parr sampling periods. Flow rate (velocity) was measured at each sample site using a YSI® Flow Probe (YSI Incorporated, Yellow Springs, OH, USA) and precipitation data were obtained through the Community Collaborative Rain, Hail & Snow Network (CoCoRaHS, 2021). The nearest station at Waterside, New Brunswick about 10 km from USR was used to infer the total precipitation that occurred between each sampling event. As with YSI measurements, velocity could not be measured for approximately half of the sites on 15 Jul, 27 Aug, and 08 Sep 2020 due to equipment inaccessibility.

2.4 Sample filtration

Water samples (1 L, triplicates) were kept on ice and then at 4 °C until filtration, which was performed in a dedicated eDNA filtration laboratory within 27 h of collection. Field samples and controls were filtered through 47 mm diameter, 0.8 µm pore nylon membrane filters (Whatman™). One sterile pair of forceps was used to place filters on a 3-place 500 mL Sentino™ Magnetic Filter Funnel manifold (Pall® Life Sciences, Ann Arbor, MI, USA) for simultaneous filtering of three samples. The outside of each bottle was rinsed with bleach and distilled

water before pouring into the corresponding filter funnel. Separate pairs of gloves were used among sites to reduce contamination, while one pair was used among replicates of a single site. Sample filters did not experience any clogging, and each filtered within an average of 9.6 minutes. Filters were preserved with 900 µL 95% ethanol before storing at -20 °C until DNA extraction. All reusable equipment (e.g., forceps, vacuum flasks, and bottles) was thoroughly cleaned between samples with bleach three times followed by rinsing with distilled water five times. The benchtop was cleaned with bleach and 70% ethanol before the subsequent three samples were filtered. One filtration control of distilled water was filtered every nine or 10 samples.

2.5 eDNA extraction and qPCR assay

Details of eDNA extraction and amplification are provided in Wood *et al*. (2021). In brief, half of each filter was extracted using a Macherey-Nagel® NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany) following LeBlanc *et al.* (2020). The second half of the filter was kept as a back-up and stored at -20 °C along with the resulting DNA extracts.

Atlantic Salmon eDNA was quantified using the TaqMan™ minor groove binding (MGB) assay published in Wood *et al.* (2021), which targets a 195 base pair (bp) region of the Atlantic Salmon cytochrome *c* oxidase subunit 1 (COI) gene. Triplicate quantitative polymerase chain reactions (qPCR) were conducted on each extraction and negative control using a QuantStudio™ 3 thermocycler (ThermoFisher Scientific, MA, USA) on a 96-well plate [Forward primer COI_82F_Ss: 5’-TGGCGCCCTTC TGGGA; reverse primer COI_276R_ Ss: 5’-

AAGGAGGGAGGGAGAAGTCAAAA A; and probe COI_194P_Ss: FAM – ATTAATTCCTCTTATAATCGGG – MGB] as recommended by Bustin *et al.* (2009).

The total reaction volume of 25 µL contained 12.5 µL 2x TaqMan™ Gene Expression Master Mix, 20 µM each forward and reverse primers, 10 µM FAM-labelled fluorescent hydrolysis probe, 3 µL template DNA, and 1 µL bovine serum albumin (BSA; 1%). Thermocycling parameters consisted of an initial hold at 50 °C for 2 minutes followed by 95 °C for 10 minutes and 50 cycles of: 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, with fluorescence read at the end of each elongation cycle. To confirm species specificity of the qPCR assay, unpurified PCR products of a subset of samples (n = 8) were sent to the Centre d'expertise et de services Génome Québec (Montréal, QC, Canada) for Sanger sequencing.

Each sample and negative control was spiked with an exogenous internal positive control (IPC) of a linearised DNA plasmid containing a 140 bp segment of Giant Panda (*Ailuropoda melanoleuca*) DNA to detect potential qPCR inhibition [Forward primer 64F: 5'-GCCTGGAGCTCTGTTAGGAGATGAC; reverse primer 207R: 5'-GTCAATTTCCAAAGCCT CCGATC; and probe inset 26bp_V2: 5'-VIC-CTAAAGCTTACGTAGATCTGT-MGB]. The IPC assay was run under the same conditions as described for salmon amplification. A sample was considered inhibited if there was an increase in quantification cycle of three or more ($C_q \geq 3$) relative to a positive control of pure panda DNA (Hartman *et al.*, 2005). All three technical replicates from five field samples either did not amplify or amplified with $C_q \geq 3$ and were omitted from statistical analyses (Smolt survey

– 01 Jun: 50 m downstream; 08 Jun: 0 m; Parr survey - 16 Jul: PP 50 m downstream, TF 500 m downstream; 08 Sep: BH 500 m downstream). Finally, three replicates of qPCR negative controls were included per PCR plate to test for contamination.

Target eDNA concentrations were calculated from an eight-point standard curve consisting of a 1:8 serial dilution (10^0 to 10^{-7}) of tissue-derived Atlantic Salmon genomic DNA prepared in a separate room from the eDNA qPCR assay. Amplification efficiency was 92.7% and $R^2 > .99$. Quantification cycle values were converted to target eDNA concentrations (pg L^{-1}) using the standard curve slope-intercept equation:

$$-3.511[\log(x)] + 23.822 \quad (1)$$

where x = C_q value of each replicate, reported in a log-scale by the thermocycler software.

2.6 Statistical analyses

All statistical analyses and graphics were created using R v. 4.1.2 (R Core Team, 2021). The package 'ggplot2' v. 3.3.5 was used for the graphics (Wickham, 2016). QGIS v. 3.16 (QGIS.org, 2021) was used to create the map graphics using public watershed data (Service New Brunswick, 2020). All uninhibited qPCR replicates (including negative detections and positive detections with $C_q > 40$) were used in analyses; eDNA concentrations for all technical and biological replicates were averaged among sample sites (n = 186 samples).

Measured eDNA concentrations tend to be variable due to the unevenly dispersed and degraded state of eDNA from various sources (Shogren *et al.*, 2017). To examine this variability within and between field (i.e., water samples) sites and qPCR technical replicates for each smolt and parr survey, a linear mixed model with nested random effects of field replicate and technical replicate was performed using 'lme4' v. 1.1-27.1 library (Bates *et al.*, 2015) with the equation:

$$\ln(C + 0.1) = \beta_0 + \sigma_S^2 + \sigma_{S\times B}^2 + \varepsilon^2 \quad (2)$$

where C = eDNA concentration; β_0 = fixed intercept; σ_S^2 = sample location; $\sigma_{S\times B}^2$ = sample, nested within location; ε^2 = residual variation (σ^2 indicates random effect). Residual variation is assumed to be due to stochastic variation across technical replicates.

Pearson correlation coefficients were used to test environmental covariates for collinearity. Correlation matrices of all continuous variables (e.g., temperature, conductivity, eDNA concentration) were produced using the package 'psych' v. 2.1.9 (Revelle, 2021). As turbidity and conductivity were highly correlated, subsequent analysis retained conductivity for both sampling periods due to its greater ecological relevance; USR is not generally turbid due to lack of sediments in the streambed (MM, personal observation). Furthermore, salinity was omitted from subsequent analysis due to its lack of variation within both sampling periods (0.01 – 0.02 ± 0.003 psu for both smolt and parr surveys).

Univariate relationships between covariates were first produced to explore biological relevance and linearity among covariates in each smolt and parr

survey. Due to the prevalence of small and zero values in eDNA concentration and water velocity, the data were not normally distributed and were therefore natural log-transformed to reduce skewness of the data. The daily mean temperature among all sites was used as a proxy as temperature generally increased from beginning to end of each sampling event (average daily increase of 0.49 °C and 4.44 °C in smolt and parr surveys, respectively).

2.6.1 Smolt eDNA and abundance relationship

Ambient eDNA concentration was hypothesised to fluctuate as a function of smolt migration and environmental effects. Generalised linear models (GLMs) for log-normally distributed eDNA concentrations were executed with an identity link function using the 'MCMCglmm' v. 2.32 package (Hadfield, 2010). GLMs were appropriate due to violations of homoscedasticity and linear relationship assumptions. A global GLM for a was built with continuous fixed effects to evaluate the influence on eDNA concentration from the seven biologically relevant parameters: chlorophyll *α* concentration, conductivity, distance from the smolt wheel, mean water temperature, pH, total precipitation between sampling events, and velocity. A quadratic term was included in the model for temperature (mean temperature^2) in addition to the first-order temperature term as eDNA is known to relate non-linearly with temperature (Strickler *et al*., 2015). Two metrics of smolt abundance were included as additional fixed effects: total remaining number of smolts presumably distributed throughout the full accessible river area (total smolt abundance) and number of smolts caught and removed from the smolt wheel on the given sampling day (removed smolts). For each sampling

day, the estimated total smolt abundance was calculated in two steps. First, the number of smolts removed from the river between sampling days was divided by the smolt wheel efficiency (i.e., 18.6%) to estimate the number of smolts having left the river. Second, the total abundance remaining in the river on any given sampling day was calculated by subtracting the number of smolts having migrated from the sum of the total smolt abundance at the beginning of the season.

Rather than choosing a single best model (i.e., hypothesis) based on an arbitrary probability threshold (e.g., $p < .05$), a model averaging approach was used to evaluate the relative support of environmental covariates from a set of top models (i.e., biological hypotheses; Burnham and Anderson, 2002). This multimodel inference is an information-theoretic approach automated using the *dredge* function in the R package 'MuMIn' v. 1.44.3 (Burnham and Anderson, 2002). Model selection was based on model weight calculated using Akaike's Information Criterion for small sample size (AICc) resulting in a set of top candidate models with $\Delta AICc < 2$ from which model averaging was performed. In all cases, none of the top models had a model weight greater than 0.9 ω, indicating that averaging across models was appropriate in this analysis (Grueber *et al.*, 2011). Scaling each covariate to have a mean of 0 and standard deviation of 1 obtained a standardised measure of effect on eDNA variability (Baguley, 2011). From the top model set, average effect sizes of each environmental and abundance covariate were calculated as standardised GLM coefficients with corresponding 95% confidence intervals. Cox and Snell

maximum likelihood pseudo-R^2 was calculated for each GLM to reflect the improvement of the full model over the linear model, using the function *nagelkerke* in the 'rcompanion' v. 2.4.1 library (Mangiafico, 2016).

To visualise the relationships between eDNA and variables which had a significant effect, an "adjusted" eDNA concentration was calculated using the averaged GLM coefficients. The coefficients were multiplied by the values for each variable of interest while multiplying by the means of each remaining variable, thereby removing variation due to the remaining variables. This allowed for a clear conceptualisation of the residual variation due to a single variable by holding all other variables constant.

2.6.2 Parr eDNA distribution relationship with environmental covariates

As parr abundance was assumed to be relatively constant throughout the sampling period, temporal eDNA variability was hypothesised to be driven by environmental covariates. To model parr eDNA as a function of environmental variability, a global log-normally distributed GLM with an identity link function was developed as described above with continuous fixed effects of distance from each primary pool and environmental variables: chlorophyll *α* concentration, conductivity, pH, total precipitation between sampling events, water velocity, mean water temperature and its quadratic term. Pool-riffle-run habitats (site = TF, BH, PP) were included as categorical fixed effects. Model selection (ΔAICc < 2) and averaging was performed as described above. All field samples that were missing environmental covariates due to equipment accessibility (34% of

samples) were omitted from the GLMs. Adjusted eDNA values were calculated as described above for visualisation.

As a secondary objective, a Tukey honestly significant difference (HSD) test for multiple comparisons was performed following an analysis of variance (ANOVA) to explore the significant difference in total river eDNA concentration among the three full transect sampling events.

3 Results

3.1 eDNA quantification

I detected Atlantic Salmon eDNA in nearly all replicates in the smolt survey (90%, n = 426), with concentrations ranging from no detections to 53.02 pg L^{-1} (Table 1). The median concentration was 5.29 pg L^{-1} (IQR = 2.66 – 8.51), and three values were outside of 1.5 times the interquartile range (IQR) and had model residuals greater than three standard deviations from the mean. Two values were technical replicates from the same water sample, and the third was from a different day but the same sample site 50 m downstream of the smolt wheel (50.02, 43.51, and 47.35 pg L^{-1}, respectively). These outliers were excluded from subsequent analyses as model results were no different with and without outliers (results not shown).

Table 1. Summary statistics of environmental conditions and raw environmental DNA (eDNA) concentration for each smolt and parr surveys (mean μ ± 1 standard deviation σ).

	Smolt Survey				Parr Survey			
Parameter	Mean	Median	Min	Max	Mean	Median	Min	Max
eDNA Concentration (pg L^{-1})	6.77 ± 6.77	5.29	0.00	53.02	16.10 ± 64.13	8.32	0.00	1131.57
Chlorophyll *α* (µg L^{-1})	0.71 ± 0.47	0.63	0.18	3.37	0.35 ± 0.20	0.30	0.14	1.12
Conductivity (µS cm^{-1})	20.71 ± 4.50	20.80	12.40	28.20	35.57 ± 7.43	37.40	11.10	44.80
pH (units)	7.22 ± 0.28	7.26	6.49	7.87	7.41 ± 0.24	7.47	6.65	7.76
Precipitation (mm)	10.80 ± 7.85	10.80	0.50	21.20	13.89 ± 10.79	10.90	0.00	33.60
Mean Temperature (°C)	9.67 ± 3.35	9.40	5.10	16.20	18.36 ± 3.18	17.70	8.00	23.70
Velocity (m s^{-1})	0.42 ± 0.70	0.27	0.06	8.18	0.27 ± 0.50	0.20	0.00	0.90

Nearly all qPCR replicates in the parr survey contained Atlantic Salmon eDNA (83%, *n* = 783), with concentrations ranging from no detections to 1131.57 pg L^{-1} (Table 1). The median concentration was 8.32 pg L^{-1} (IQR = 2.05 – 19.69). I removed three outliers similar to above; these values happened to be the three technical replicates for one field sample (1131.57, 1011.12, and 929.34 pg L^{-1}; site = TF, distance = 250 m, Figure 2). I also confirmed that model results were not different with and without the outliers (results not shown). One of 39 field blanks and one of 37 laboratory filtration blanks from the parr survey amplified Atlantic Salmon eDNA in all technical replicates (mean concentration = 21.23 and 25.87 pg L^{-1}, respectively). Although I infer there was minimal cross-contamination throughout the sampling period as these two negative controls

were from the same day, all samples from this day (07 Aug 2020, *n* = 99) were excluded as contamination origin was unknown.

I tested for nested random effects of the eDNA residuals to see if most of the variation resided within or among field sites and qPCR technical replicates for each smolt and parr sampling period. For both surveys, strong evidence suggests that there was greater variation among qPCR technical replicates than within field replicates as the 95% confidence interval bounds for each of the two standard deviation estimates do not overlap with each other's mean standard deviation (Table A1, A2). Additional variation was observed among sample sites in the parr survey, with strong support of this measure contributing the greatest to eDNA variation as the 95% confidence interval bounds for this standard deviation estimate does not overlap with that of within field replicates or between technical replicates (Table A2).

3.2 Spring environmental effects on eDNA concentration

Environmental factors were significantly correlated among each other during the smolt sampling period. Mean river temperature generally increased with each sampling day, ranging from 5.4 to 15.9 °C (Table 2). During the six-week spring smolt survey, the increase in mean temperature was associated with increased conductivity and pH [$r(46) = .91, .45$]. With increasing temperature came a decrease in rainfall, and thus declines in velocity and turbidity [$r(46) = -.55, -.20, -.18$; Table 3].

Table 2. Summary statistics of mean river temperature and environmental DNA (eDNA) concentration adjusted with generalised linear models for both smolt and parr sampling periods (mean μ ± 1 standard deviation σ).

	Smolt Survey				Parr Survey			
Parameter	Mean	Median	Min	Max	Mean	Median	Min	Max
Adjusted eDNA Concentration (pg L^{-1})	11.54 ± 16.11	5.63	0.16	123.41	11.87 ± 11.08	7.68	0.67	65.54
Mean Temperature (°C)	9.67 ± 3.36	9.38	5.35	15.90	17.89 ± 2.88	16.95	13.50	21.91

Table 3. Pearson correlation coefficients for smolt abundance metrics, environmental DNA concentration, and environmental covariates in spring. Parameters defined: distance from smolt wheel, mean river temperature, total precipitation between sampling days, number of smolts removed from trap daily, and total smolt abundance remaining in river (n = 48).

	Variable	1	2	3	4	5	6	7	8	9	10	11
1	Sample Day											
2	eDNA Concentration	-.33										
3	Chlorophyll	-.07	-.14									
4	Conductivity	.88	-.37	-.20								
5	Distance	.00	.10	.01	.24							
6	Mean Temperature	.97	-.40	-.10	.91	.00						
7	pH	.45	-.23	-.04	.64	.45	.46					
8	Precipitation	-.51	-.03	.45	-.51	-.00	-.55	-.05				
9	Turbidity	-.16	.10	.14	-.29	-.19	-.18	-.34	.03			
10	Velocity	-.19	.00	.06	-.17	-.07	-.20	-.18	.20	.18		
11	Removed Smolts	-.04	.24	-.21	-.02	.00	.00	.15	-.35	-.15	-.11	
12	Total Smolts	-.95	.44	.04	-.83	.00	-.91	-.57	.34	.22	.18	.07

First, the simple linear model relating measured eDNA particle concentration and total Atlantic Salmon smolt abundance demonstrated a weak positive relationship (linear model, R^2 = .19; Table 4, Figure 3A).

Table 4. Parameter coefficients for (A) the simple linear model of smolt abundance related to environmental DNA concentration, and (B) the average of three top generalised linear models examining the effects of spring environmental variables and smolt abundance on environmental DNA concentration. Parameters defined: distance from smolt wheel, mean river temperature and its quadratic term, smolts removed from trap daily, and total precipitation between sampling days (n = 48).

Variable	Estimate	*SE*	R^2 and AICc
A. Simplest model summary: eDNA ~ smolt abundance			
Intercept	1.464	0.105	R^2 = .19
Total smolt abundance	0.0001	0.00004	AICc = 72.53
B. Average model summary: eDNA ~ parameters			
Intercept	-5.287	2.850	
Distance	0.0001	0.0005	
Mean temperature	1.099	0.451	
(Mean temperature) ^{2}	-0.044	0.017	
Removed smolts	-0.006	0.005	R^2 = .41
Total smolt abundance	0.0005	0.0002	AICc = 65.76

My model averaging approach resulted in a set of three top models which included the variables: distance from smolt wheel, mean temperature and its quadratic, smolts removed daily from smolt wheel, and total smolt abundance (Table 4). Including these covariates substantially improved the relationship between smolt abundance and eDNA concentration, explaining more than twice the variation in eDNA concentration (GLM, pseudo-R^2 = .41; Table 4, Figure 3B). The most parsimonious model (ω = .53) in the set of three models contained total abundance, smolts removed daily from the smolt wheel, mean temperature, and its quadratic; all of which were present in all three models (Table 4, 5).

Table 5. Top candidate generalised linear models (ΔAICc < 2) and weight of each (ω) explaining the variance of environmental DNA concentration for both the smolt and parr surveys. Smolt model parameters defined: mean river temperature and its quadratic term, total smolt abundance, smolts removed from trap daily, and distance from trap. Parr model parameters defined: total precipitation between sampling dates, distance from primary pool in each reach (Site = The Forks, Black Hole, Pumphouse), mean river temperature and its quadratic term, water velocity, and chlorophyll α concentration.

Smolt Survey	AICc	ΔAICc	ω
Temp + $Temp^2$ + Total + Removed	66.59	0.00	.53
Temp + $Temp^2$ + Total	67.88	1.29	.28
Temp + $Temp^2$ + Total + Removed + Distance	68.56	1.96	.20
Parr Survey			
Rain + Site + Temp + $Temp^2$	91.51	0.00	.22
Rain + Site + Temp + $Temp^2$ + Distance	91.63	0.12	.21
Rain + Site + Temp + $Temp^2$ + Distance + Velocity	91.72	0.21	.20
Rain + Site + Temp + $Temp^2$ + Velocity	92.51	1.00	.13
Rain + Site + $Temp^2$ + Distance	93.44	1.93	.08
Rain + Site + $Temp^2$	93.47	1.96	.08
Rain + Site + Temp + $Temp^2$ + Chlorophyll	93.48	1.97	.08

Scaling the coefficients to a mean of 0 and a standard deviation of 1 allowed for direct comparison of the effect each covariate had on eDNA. Total smolt abundance was found to have the greatest effect on eDNA concentration (β = 1.83, 95% CI = [0.69, 2.98]), followed closely by mean temperature (β = 1.55, 95% CI = [0.31, 2.78]; Figure 4A). The temperature effect on eDNA concentration was non-linear, presenting an initial increase in eDNA concentration with rising temperature; however, the rate of concentration increase slowed as temperature continued to rise (β = -0.93, 95% CI = [-1.55, -0.31]; Table 6). Mean eDNA concentration was 0.88 pg L^{-1} at the minimum temperature and increased steadily with temperature until reaching the peak average of 15.75 pg L^{-1} at 13.2 °C when concentration began to decline. When

the river reached its maximum mean temperature of 15.9 °C, mean eDNA concentration had decreased to 10.15 pg L^{-1} (Figure 5A).

Table 6. Scaled (μ = 0, σ = 1) parameter coefficients of the average of three top generalised linear models (ΔAICc < 2) exploring the effect on environmental DNA concentration variability during the smolt survey. Parameters defined: daily captured smolts, distance from smolt wheel, mean river temperature and its quadratic term, and total precipitation between sampling events (*n* = 48).

Parameter	Estimate	*SE*	95% Confidence Interval	
			Lower Bound	Upper Bound
Intercept	**0.913**	**0.383**	**0.274**	**1.552**
Distance	0.020	0.066	-0.91	0.130
Mean temperature	**1.549**	**0.740**	**0.314**	**2.783**
(Mean temperature) ^{2}	**-0.932**	**0.372**	**-1.552**	**-0.312**
Removed smolts	-0.320	0.280	-0.785	0.145
Total smolt abundance	**1.832**	**0.688**	**0.685**	**2.979**

Note: Parameters with significant effect on eDNA in bold (i.e., 95% CI not overlapping zero). See Table 4 for the estimates from the average of the three models and Table 5 for the candidate models used.

3.3 Summer environmental effects on eDNA concentration

3.3.1 Individual covariate influence with constant abundance

Similar to the spring smolt survey, many environmental factors were significantly correlated among each other during the summer parr sampling period. During the nine-week parr survey, high temperatures were associated with an increase in turbidity and chlorophyll [$r(55)$ = .37, .78] and a decrease in conductivity and pH [$r(55)$ = -.22, -.41]; however, mean temperature decreased throughout the sampling period from 21.9 to 13.5 °C (Table 2). An increase in rainfall as temperatures declined [$r(55)$ = -.57] was associated with increased river velocity [$r(55)$ = .14] and, surprisingly, a decline in turbidity [$r(55)$ = -.26; Table 7].

Table 7. Pearson correlation coefficients for summer environmental covariates and parr environmental DNA concentration. Parameters defined: distance from primary pool in each reach (The Forks, Black Hole, Pumphouse), mean river temperature, total precipitation between sampling days, and water velocity (n = 58).

	Variable	1	2	3	4	5	6	7	8	9
1	Sample Day									
2	eDNA Concentration	-.75								
3	Chlorophyll	-.68	.68							
4	Conductivity	.29	-.19	-.17						
5	Distance	-.02	.10	-.10	-.08					
6	Mean Temperature	-.88	.85	.78	-.22	.02				
7	pH	.41	-.33	-.27	.64	.06	-.41			
8	Precipitation	.60	-.68	-.36	.00	-.02	-.57	.14		
9	Turbidity	-.31	.37	.24	-.89	.03	.37	-.57	-.26	
10	Velocity	-.15	-.00	.18	-.06	.16	.12	-.15	.14	-.04

All of the smolts had migrated out of Upper Salmon River by the time I began the parr survey, and only a small number (8) of anadromous adults had returned by the end of the sampling period; therefore, I measured eDNA of the remaining fish in the river consisting mostly of young-of-the-year and parr (K. Samways, personal comm., 27 Dec 2021). When I first explored the univariate relationship between eDNA concentration and time, I observed a potential breakpoint halfway through the sampling period with a marked drop in concentration after 14 Aug (Figure 6A). After adjusting for environmental covariates with GLMs (chlorophyll α, distance from primary pool, mean temperature and its quadratic term, total precipitation between sampling events, site, and water velocity; Figure 6B), the breakpoint was removed.

Table 8. Parameter coefficients for the average of seven top generalised linear models analysing the effects of summer environmental variables on parr eDNA concentration. Parameters defined: distance from primary pool in each reach (Site = The Forks, Black Hole, Pumphouse), mean river temperature and its quadratic term, and total precipitation between sampling events (*n* = 57).

Variable		Estimate	*SE*	R^2 and AIC
Global model summary: eDNA ~ parameters				
Site	TF	11.598	4.871	
	BH	11.145	4.852	
	PP	11.431	4.852	
Chlorophyll *α*		0.127	0.520	
Distance		0.0004	0.0004	
Mean temperature		-1.288	0.534	
(Mean temperature) ^{2}		0.042	0.014	
Precipitation		-0.030	0.007	R^2 = .84
Velocity		-0.054	0.115	AIC = 87.04

Overall, my set of top GLMs explained over 84% of the variance in parr eDNA concentration. The most parsimonious model (ω = .22) in the set of seven models contained total precipitation between sampling events, site (The Forks, Black Hole, Pumphouse), mean temperature, and its quadratic term; all of which were present in all but two of the models. The remaining two models omitted the first-order temperature term (Table 5, 8). After scaling the model coefficients to a mean of 0 and a standard deviation of 1, mean temperature appeared to have the greatest association with increased eDNA concentration (*β* = 0.54, 95% CI = [0.39, 0.70]), with a non-linear relationship as eDNA concentration increased faster with rising temperatures (*β* = 0.31, 95% CI = [0.13, 0.49]; Figure 5B). Increased precipitation was associated with reducing eDNA concentration (*β* = -0.31, 95% CI = [-0.43, -0.19]; Table 9, Figure 4B).

Table 9. Scaled (μ = 0, σ = 1) parameter coefficients of the average of seven top generalised linear models (ΔAICc < 2) exploring the relationship between parr environmental DNA concentration and summer environmental covariates. Parameters defined: distance from the primary pool in each reach (Site = The Forks, Black Hole, Pumphouse), mean river temperature and its quadratic term, total precipitation between sampling events, and water velocity (n = 57).

Parameter		Estimate	*SE*	95% Confidence Interval	
				Lower Bound	Upper Bound
Site	TF	-0.086	0.146	-0.330	0.159
	BH	**-0.495**	**0.141**	**-0.731**	**-0.258**
	PP	-0.237	0.153	-0.494	0.020
Chlorophyll *α*		0.012	0.048	-0.068	0.091
Distance		0.063	0.068	-0.050	0.176
Mean temperature		**0.543**	**0.091**	**0.391**	**0.696**
(Mean temperature)^2		**0.308**	**0.107**	**0.129**	**0.487**
Precipitation		**-0.312**	**0.073**	**-0.434**	**-0.189**
Velocity		-0.023	0.048	-0.103	0.058

Note: Parameters with significant effect on eDNA in bold (i.e., 95% CI not overlapping zero). See Table 8 for the estimates from the average of the seven models and Table 5 for the candidate models used.

Similar to the spring smolt survey, eDNA concentration during the summer parr survey increased with temperature after adjusting for environmental covariates; however, temperature did not increase with time. Mean river temperature at the beginning of the parr survey was higher, ranging from 20.9 to 21.9 °C before dropping to range from 14.4 to 17.0 °C after 17 Aug. Parr eDNA concentration on 29 Jul was the highest, reaching a mean high of 21.98 pg L^{-1} at 20.9 °C. Once the temperature dropped during the second, cooler half of the sampling period, mean eDNA ranged from 3.13 to 4.14 pg L^{-1} (Figure 5B).

3.4.2 Seasonal effect on eDNA spatial distribution

I explored how total river eDNA concentration fluctuated over the course of the summer parr survey as parr abundance remained relatively constant.

Mean river eDNA concentration was significantly higher in the first full transect sampling event at the beginning of the summer (15 Jul, 20.83 ± 13.91 pg L^{-1}) than either of the last sampling events occurring near the end of the summer sampling period (27 Aug, 4.55 ± 5.07 pg L^{-1}; 08 Sep, 4.56 ± 9.11 pg L^{-1}; Table A3). There was a significant decrease in mean eDNA concentration of 15.50 pg L^{-1} (95% CI = [11.64, 19.36] and 15.43 pg L^{-1} (95% CI = [11.55, 19.31] on 27 Aug and 08 Sep, respectively. Finally, there was no significant difference in mean eDNA concentration between the last two sampling events (95% CI = [-3.70, 3.84]; Table A4, A5). This pattern was also observed with the presence of the initial potential breakpoint (Figure 6A).

Environmental DNA concentrations did not show a predictable spatial pattern along the length of the river relative to the main pool-riffle-run habitats, though the first sampling event revealed some evidence of downstream accumulation (Figure 7). One side tributary was completely dry during the first sampling event (15B, 15 Jul), and thus I sampled that tributary further upstream and detected no eDNA for either of the last sampling events (Table A6); however, eDNA was detected in each of the remaining three tributaries for at least one sampling event (25B, 15 Jul; 33B, 27 Aug; 29B, 27 Aug and 08 Sep; Figure 7, Table A6). Finally, I confirmed Atlantic Salmon to be absent upstream from both of the waterfall barriers with zero eDNA detected.

4 Discussion

My study took advantage of the natural distribution and migration of wild juvenile Atlantic Salmon in a single, well-characterised river to address unknown

seasonal impacts on the quantitative relationship between eDNA and fish population abundance and distribution. The results of my extensive sampling effort support that the dynamics underlying variability in freshwater eDNA quantification depend on environmental factors (Tillotson *et al.*, 2018; Shogren *et al*., 2019; Wood *et al*., 2021; Yates, Glaser, *et al.*, 2021). Specifically, I found that (1) with inclusion of environmental covariates, eDNA was highly correlated with smolt abundance during spring migration in quantitative eDNA abundance models; and (2) the seasonal variation in the summer affected the relationships among environmental conditions and parr eDNA during the period of relatively constant parr abundance.

4.1 Local environmental factors affect eDNA fish population assessment models

My work extends previous studies by modelling eDNA concentration as a function of fish population abundance and environmental factors. The relationship between Atlantic Salmon smolt abundance and eDNA concentration was dramatically strengthened when environmental variables were incorporated in my models. The amount of eDNA should be proportional to the average eDNA produced by the number of individuals in a source region (Lacoursière-Roussel, Rosabal, and Bernatchez, 2016), and the noise produced by dynamic interactions between eDNA and environmental factors muddies this relationship (Takahara *et al.*, 2012; Barnes and Turner, 2016). In a study using eDNA to examine the distribution of an invertebrate species in a natural flowing stream, the relationship between eDNA and abundance was demonstrated to be weak

(R^2 = .02) prior to the inclusion of environmental covariates (R^2 = .21; Shogren *et al.*, 2019). Furthermore, Levi *et al.* (2019) found significant eDNA relationships with daily emigrating Sockeye Salmon smolts following correction for environmental variables such as flow rate. Although my top models for the smolt survey did not include velocity and the environmental characteristics in my study differ from those in Shogren *et al*. (2019), my findings confirm the importance of including environmental covariates, specifically that of water temperature. My top three multivariate models for the spring smolt survey included the following covariates, in order of relative importance: total smolt abundance (i.e., smolts throughout the river), mean temperature and its quadratic, number of smolts removed daily, and distance from the smolt wheel. Although I did not include turbidity in my models due to its correlation with and lower ecological relevance than conductivity, it may be a factor of interest or surrogate variable depending on the ecosystem, target organism, and management goal (Gray *et al*., 2014). I emphasise that inclusion of ecologically relevant stream-specific characteristics significantly reduces variability in eDNA quantification in quantitative population models.

My results support that the adjusted eDNA concentration remaining after smolt migration has ended (Figure 3B) could be due to eDNA produced by resident parr (Figure 5B), which is the only cohort present in the river after the smolts leave. I had a strong understanding of parr abundance at the whole river scale; however, I had no prior knowledge of their local distribution, and thus upstream distance of parr eDNA from the smolt sampling locations was

unknown. This limited my ability to relate eDNA concentrations to very specific local abundances, but it is known that the juvenile salmon in this river system do not move extensively among sites (K. Samways, personal comm., 04 May 2021). Therefore, temporal variation in eDNA is likely attributable to environmental factors that vary over time.

As reviewed in Yates, Cristescu, and Derry (2021), appropriate parameterisation of eDNA models is crucial to improve indirect eDNA-based quantitative population models. Emerging research has been exploring allometric scaling to improve abundance relationships, which considers physiological changes proportional to body size in fishes (Yates, Glaser, *et al*., 2021; Stoeckle *et al*., 2021). Including additional abundance terms may also describe some of the variability in measured eDNA. Levi *et al.* (2019) demonstrated the utility of an additional lagged abundance term (i.e., salmon counts from one and two days before sampling), possibly accounting for eDNA that can be caught in riverbanks and eddies. Future studies should continue to explore the addition of allometric scaling and lagged abundance terms in population models, especially in systems with robust annual population metrics as does Upper Salmon River.

4.2 Multiple life-stage analyses: seasonal effect on eDNA quantification

4.2.1 Spring

Multimodel inference and standardising each covariate revealed that temperature was strongly associated with the observed increase in Atlantic Salmon smolt eDNA. Temperature is considered a main ecological driver of physiological and behavioural change in fishes (Person-Le Ruyet *et al.*, 2004),

and several studies have demonstrated increased eDNA shedding at warmer temperatures (e.g., Maruyama *et al.*, 2014; Klymus *et al*., 2015; Jo *et al*., 2019). For example, Lacoursière-Roussel, Rosabal, and Bernatchez (2016) attributed higher shedding rates of juvenile Brook Trout at 14 °C than at 7 °C to increased metabolism. Studies have also presented increased eDNA shedding in aquaria with higher biomass (Klymus *et al.*, 2015; Jo *et al*., 2019), possibly as individuals in closer proximity slough more cells as they interact with each other. Atlantic Salmon smolts tend to emigrate in groups (McCormick *et al.*, 1998), with numbers increasing as the migration period progresses; thus, higher densities of fish would likely be interacting more and moving through more turbulent waters that would have typically been avoided. Furthermore, in conjunction with other environmental variables (e.g., increased flow rate, daylength), increasing spring temperatures (~10 °C) have been associated with the onset of smolt migration, during which smolts experience great changes in kidney function and structure to withstand the high osmolarity of saltwater (reviewed in McCormick *et al.*, 1998). Consistent with these published works, my findings provide evidence that the spring seasonal temperature effect on eDNA may work through multiple combined mechanisms. Environment-adjusted eDNA concentrations began to dramatically increase at 9 °C (Figure 4A) which corresponded to the first spike in smolt outmigration. In addition to the expected increase in eDNA with greater numbers of individual seaward smolts, increasing eDNA concentrations with time may have been associated with higher rates of exfoliation and increased urination (Barnes and Turner, 2016); therefore, more research is necessary to

explore the relationship between temperature-dependent eDNA mechanisms and increasing abundance passing through at a given point.

4.2.2 Summer

During the parr survey, higher eDNA concentrations were associated with increasing temperature in the warmer, earlier part of the summer before dropping and staying constant in late summer along with lower temperatures. Temperature-dependent eDNA degradation (e.g., Eichmiller *et al.*, 2016; Bylemans *et al.*, 2018; Kasai *et al*., 2020) and shedding have individually been explored in controlled environments, yet few studies have investigated the two concurrently (e.g., Jo *et al*., 2019; Andruszkiewicz Allan *et al*., 2021). In one example examining eDNA degradation and shedding rates of three freshwater fishes (Brown Bullhead [*Ameiurus nebulosus*], Yellow Perch [*Perca flavescens*], and Tench [*Tinca tinca*]) at three different temperatures, Caza-Allard *et al*. (2021) observed higher eDNA concentrations at 16 °C than either 8 or 24 °C, with no difference between the latter. They suggest that, although less eDNA was produced and degraded at the lower temperature, the balance between eDNA degradation and production, and therefore shedding, may have been equal to that at the higher temperature. Although these studies have improved our understanding of eDNA shedding and degradation in controlled systems, there remains much to be learned about temperature effects in natural settings. A stronger understanding of the ecological and physiological impacts of temperature on eDNA in natural streams may allow future models to better reflect salmon abundance and distribution.

Consistent with the findings of Caza-Allard *et al*. (2021), my study encountered less eDNA at cooler temperatures; however, at the warmer summer temperatures, eDNA was present in higher concentrations suggesting that shedding rates increased more with temperature than degradation. Similarly, Takahara *et al*. (2012) demonstrated a significant positive correlation between water temperature and Common Carp (*Cyprinus carpio*) eDNA concentration in freshwater lagoons. They postulated that carp were seeking habitats closer to their optimal temperature. In addition to potentially higher metabolic-induced shedding rates in warmer waters, it is possible that changes in parr behaviour or microhabitat use might partly explain increases in eDNA shedding in the current study. Although generally preferring to occupy small home territories (McCormick *et al*., 1998), parr may move great distances (>1 km) to cooler habitats in the summer to avoid high temperatures (Corey *et al*., 2022). I measured local water temperatures as high as 23.7 °C, and as temperatures increase so do metabolic demands as thermoregulatory plasticity has been observed in salmonids in response to acute thermal stress (Corey *et al.*, 2020). Thermal responses may force parr to feed and interact more aggressively or to congregate in refugia (Petty *et al*., 2012), thereby concentrating their eDNA. However, future studies would be needed to discern the specific metabolic or behavioural drivers of greater salmon eDNA shedding with temperature.

4.3 Factors affecting eDNA variation and spatial distribution

The variation in eDNA concentration observed during the summer parr survey further underscores the importance of incorporating stream environmental

variables into eDNA population models. My top seven multivariate models for the summer parr survey included the following covariates, in order of relative importance: mean temperature and its quadratic, total precipitation between sampling events, pool-riffle-run habitat (i.e., site), distance from primary pool in each reach, water velocity, and chlorophyll. In contrast to the amount of variation explained by the multivariate models in the smolt survey (R^2 = .41, Table 4), my best environmental factor models for the summer parr survey accounted for significantly more of the parr eDNA variation (R^2 = .84; Table 8). Some of the variation among parr eDNA samples that I observed in the summer is likely due to the heterogeneity of eDNA states in nature (i.e., extracellular DNA, aggregates of cells; Pietramellara *et al.*, 2009). My models omitted three statistical outliers of high concentration eDNA samples that were collected 250 m downstream from the most upstream pool reach (TF, Figure 2). The high concentration of eDNA detected in these samples may have resulted from larger multicellular eDNA material mixed among more degraded and dispersed material. Although the "polydisperse" (Shogren *et al*., 2017) nature of eDNA presents challenges, there are several ways to mitigate its effects. Spatial variation may be reduced by increasing field replication as there is growing evidence that increasing water sample volumes and/or number of field replicates captures more eDNA fragments to further reduce stochastic noise (Takahara *et al*., 2012; Shelton *et al.*, 2019; Sepulveda *et al.*, 2021). Increasing eDNA sample volumes from 1 L to 2 L has been shown to improve eDNA detection in streams more than five times (Bedwell and Goldberg, 2020); however, while increasing water volumes can

help to reduce sample heterogeneity, it can increase potential for PCR inhibition from higher abundance of inhibitory compounds (e.g., humic acids from leaf litter) and filter saturation leading to a non-linear increase of DNA captured (Goldberg *et al*., 2016). Increasing the number of field replicates, however, offers more potential for understanding and controlling biases associated with the multistate nature of eDNA.

Much of the variation I observed in the parr survey was attributed to the eDNA variance between sample sites; however, high variation was also prevalent between qPCR technical replicates (Table A2). One method to circumvent potential low confidence in results due to high technical replicate variation is to establish a limit of quantification (LOQ). The LOQ of an eDNA assay can be determined using the coefficient of variation (CV) of multiple standard curves (e.g., lowest concentration with CV < 35% between replicates; Klymus *et al.*, 2020). Rather than being interpreted quantitatively, it is recommended that eDNA samples that have a CV greater than 35% be treated only as a positive detection as results may be misinterpreted more when concentrations are below the LOQ (Goldberg *et al*., 2016). Optimistically, wildlife managers are pushing to standardise eDNA reporting and inference; however, the utility of this stringent quality control measure is highly debated at present. The reality of developing eDNA surveys for rarer species is that very low abundance commonly results in low eDNA concentrations. Strictly adhering to the LOQ may overlook the stochastic eDNA dispersion that exists from sample collection through amplification and risks omitting samples that may be

biologically relevant. Recent studies provide evidence that the number of successful qPCR amplifications per sample can be used to estimate species abundance with confidence (e.g., where 1/3 indicates low and 3/3 indicates high abundance; Wood *et al.*, 2021). Furthermore, modelling the number of positive detections can accurately estimate detection probability (Furlan *et al.*, 2016). Rojahn *et al*. (2021) demonstrated that the percentage of positive amplifications accurately quantified the relative abundance of invasive Redfin Perch (*Perca fluviatilis*) and native Macquarie Perch (*Macquaria australasica*), consistent with their conventional monitoring results. Coherent with these published works, my results support the inclusion of all positive and non-detections in assessing eDNA dynamics in Upper Salmon River. However, more research is needed on both sides of the debate as it is clear that increasing the number of qPCR replicates may satisfy LOQ requirements while acknowledging the natural eDNA variation.

Rather than eDNA being transported similar to a monodispersed solution or conservative solute tracer in rivers (Wilcox *et al*., 2016), eDNA transport has demonstrated greater complexity in recent years (Shogren *et al*., 2017; Pont *et al*., 2018). Wood *et al*. (2020) first hypothesised that as individuals shed eDNA, a predictable high-concentration plume could be detected with midstream sampling at short downstream distances from the source (< 100 m) with concentrations decreasing farther downstream. However, although plume dynamics were apparent downstream from caged Atlantic Salmon (Wood *et al.*, 2021), eDNA transport and subsequent quantification may be obscured by factors affecting its dilution, such as higher stream flows (Curtis *et al.*, 2021) and rain events. I

detected eDNA throughout the entire 9 km length of Upper Salmon River in all three full transect sampling events during the summer; however, downstream accumulation of eDNA was not equally apparent across all three days. Upper Salmon River experienced increased rainfall over the second half of the parr sampling period, potentially contributing to the sustained lower concentrations that I observed in the final two full transect events; the significant negative effect of precipitation on eDNA for the primary parr survey supports this dilutive effect (Table 9). Furthermore, one explanation for the lack of plume dynamics in my findings is that although sampling occurred downstream of pool-riffle-run reaches, those areas were not necessarily preferred habitats for parr. As there was some evidence of eDNA increasing with downstream distance, however, it may be possible that areas with higher concentrations may be in close proximity to areas with higher parr densities (Figure 7).

The downstream transport and lateral dispersion (i.e., width-wise bank to bank mixing) of eDNA in lotic systems is dictated by stream-specific hydrology (Laporte *et al*., 2020). Longitudinally, eDNA undergoes an approximate transport spiral between the water column and benthos until it is permanently sequestered or degraded (Jerde *et al.*, 2016; Shogren *et al*., 2017). If eDNA spirals are long enough, one might predict that as sampling occurs downstream it is apt to integrate the eDNA of more and more fish leading to increased concentrations downstream. Although not observed in the present study, such a pattern might be obscured if eDNA spirals are relatively short compared to total stream length. One factor that is known to favour shorter spiralling is bed substrate.

Simultaneously injecting Common Carp eDNA with a conservative tracer into a series of experimental streams with identical flows (2 L s^{-1}) revealed that eDNA was removed from the water column faster in streams with finer “pebble” substrates (median diameter, D_{50} = 0.05 cm; Jerde *et al.*, 2016; Shogren *et al*., 2017). The Upper Salmon River’s streambed, however, is primarily composed of large rocks and boulders (D_{50} > 25.6 cm; Wentworth, 1922). As every ecosystem is unique, future studies in natural systems will need to continue investigating the influence of bed substrates and other factors on eDNA retention spirals in individual rivers.

5 Conclusions and recommendations

My study is one of few to investigate the impact of seasonal environmental conditions on eDNA persistence in natural flowing waters (de Souza *et al.*, 2016; Curtis *et al*., 2021). The variability in environmental conditions across and within seasons emphasises the value of increasing sampling effort to estimate abundance of salmon or other species from eDNA. Despite the ability of the environmental factor models to account for most of the variation outside of fish abundance, I still documented considerable differences in eDNA concentration across the spring and summer seasons (i.e., smolt and parr eDNA studies). However, my findings provide encouragement that non-invasive eDNA-based quantification may be achieved with suitable study designs and environmental measures. Along these lines, I offer the following recommendations for future eDNA salmon population assessments:

1. Increased eDNA field and qPCR replicates: To minimise the stochasticity caused by heterogeneous eDNA distribution in the environment, I recommend increasing the number of qPCR technical replicates per sample. Furthermore, collecting samples at multiple downstream and lateral sites (i.e., midstream and bankside) can also reduce variation by increasing the probability of detecting plume dynamics (Wood *et al.*, 2021).
2. Annual sampling in multiple periods: My study showcases the seasonal variation in eDNA relationships in a single river (spring smolt versus summer parr). Consequently, I recommend that monitoring programs seek to standardise sampling at the same times and locations each year to reduce potential confounding variables related to life history and environmental phenology. I also present the importance of collecting water at a high frequency to monitor smolt migration. Due to the correlation between environmental conditions and seasonality, I recommend sampling eDNA at multiple times throughout the summer to capture the seasonal variation of resident parr eDNA year-over-year.
3. Inclusion of ecologically relevant covariates: I validated the importance of including stream-specific environmental variables in eDNA population models, particularly that of water temperature. I strongly advocate integrating temperature and flow data in stream eDNA surveys and suggest that even broader sets of ecologically relevant environmental covariates are supported based on my findings. I

removed turbidity from my models due to its correlation with conductivity; however, Upper Salmon River is not generally turbid due to the lack of sediment in the rocky riverbed (MM, personal observation). Moreover, based on the clear importance of temperature to eDNA concentrations in my study, I reinforce calls by others for a need to integrate metabolism and allometric scaling in eDNA inference across life stages (Yates, Glaser *et al*., 2021).

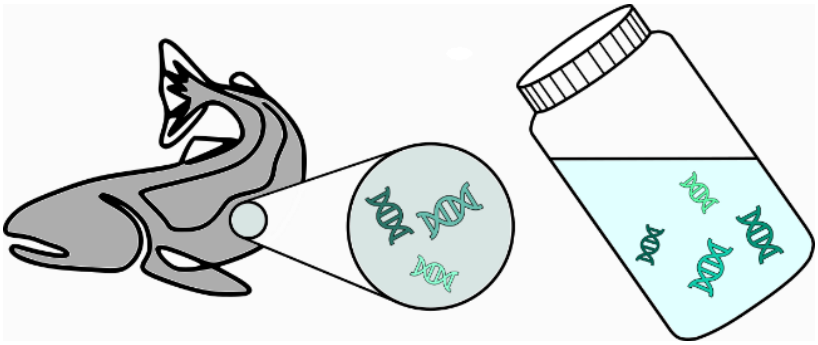

Figure 1. Water samples taken downstream of fish contain traces of environmental DNA (eDNA) from shed biological materials.

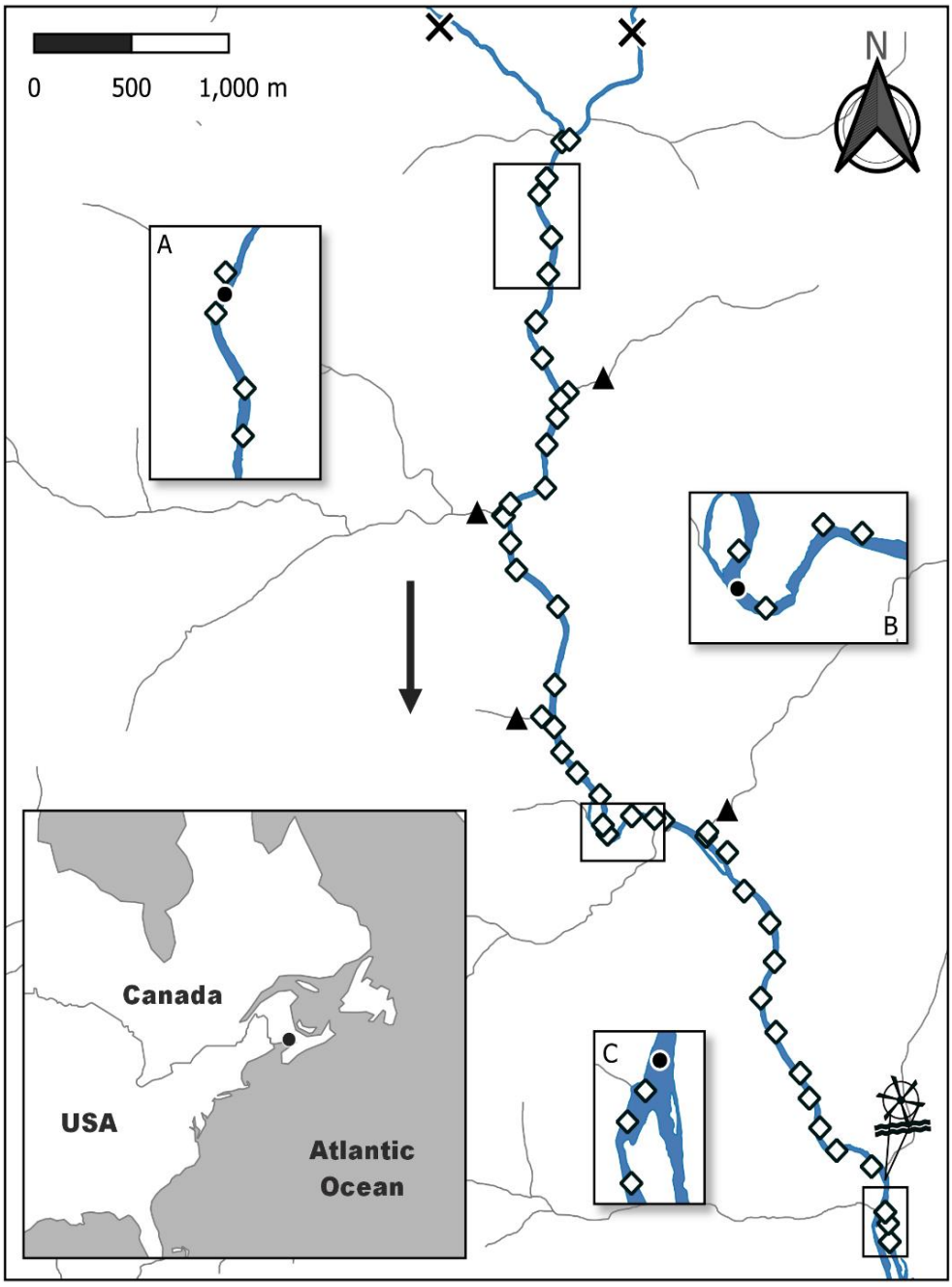

Figure 2. Water environmental DNA sample sites (diamonds) in Upper Salmon River, Fundy National Park, Canada (45°37'05" N, 64°57'56" W). Main map denotes full transect with triangles indicating tributary sampling locations and arrow indicating flow direction. Insets include optimal Atlantic Salmon habitat with primary pool (circles) in A: The Forks (TF), B: Black Hole (BH), and C: Pumphouse (PP), and weekly upstream and downstream sample sites. The spring survey rotary screw trap was located just upstream of Pumphouse. Crosses upstream of the confluence depict physical waterfall barriers.

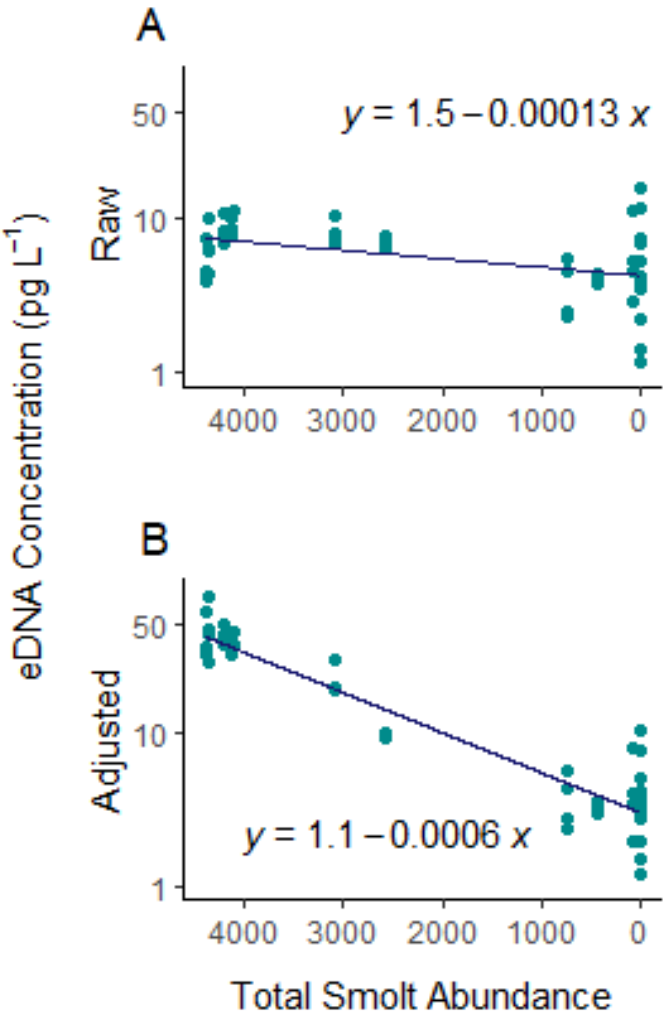

Figure 3. Relationship between environmental DNA (eDNA) concentration and decreasing smolt abundance in (A) univariate linear model (R^2 = .19), and (B) generalised linear model with inclusion of spring covariates from model-averaging the three top candidate models: distance from smolt wheel, mean river temperature and its quadratic, number of smolts removed daily from smolt wheel, and total smolt abundance (R^2 = .41; n = 48).

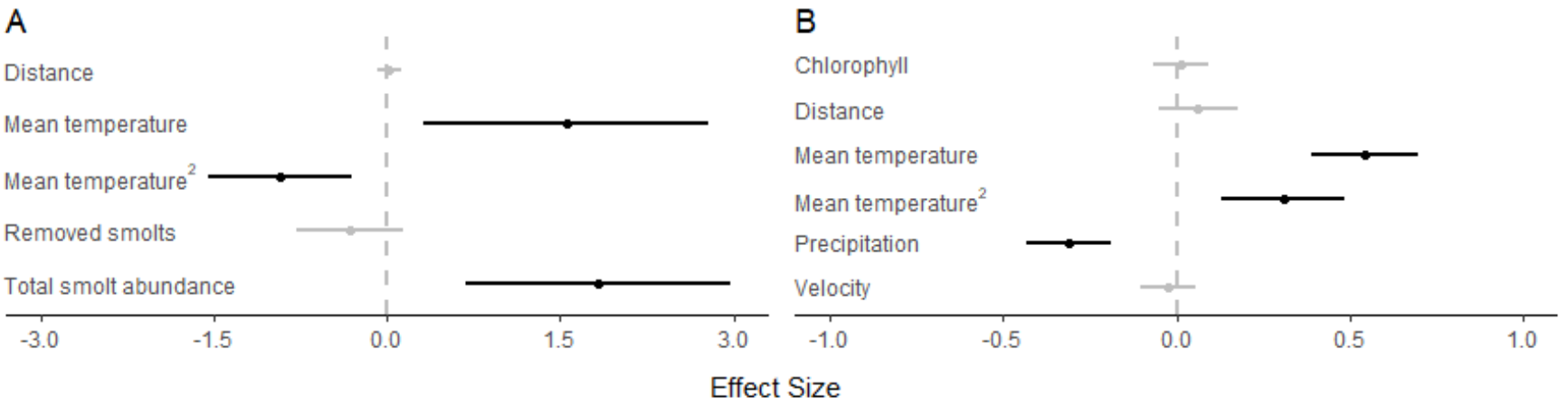
A
Distance
Mean temperature
Mean temperature^2
Removed smolts
Total smolt abundance
-3.0
-1.5
0.0
1.5
3.0
B
Chlorophyll
Distance
Mean temperature
Mean temperature^2
Precipitation
Velocity
-1.0
-0.5
0.0
0.5
1.0
Effect Size

Figure 4 (Previous page). Model-averaged (ΔAICc < 2) and scaled (μ= 0, σ = 1) coefficient effect sizes with 95% confidence intervals for the top candidate generalised linear models for each (A) smolt survey, three models; and (B) parr survey, seven models. Covariates in black depict significant effect on environmental DNA concentration.

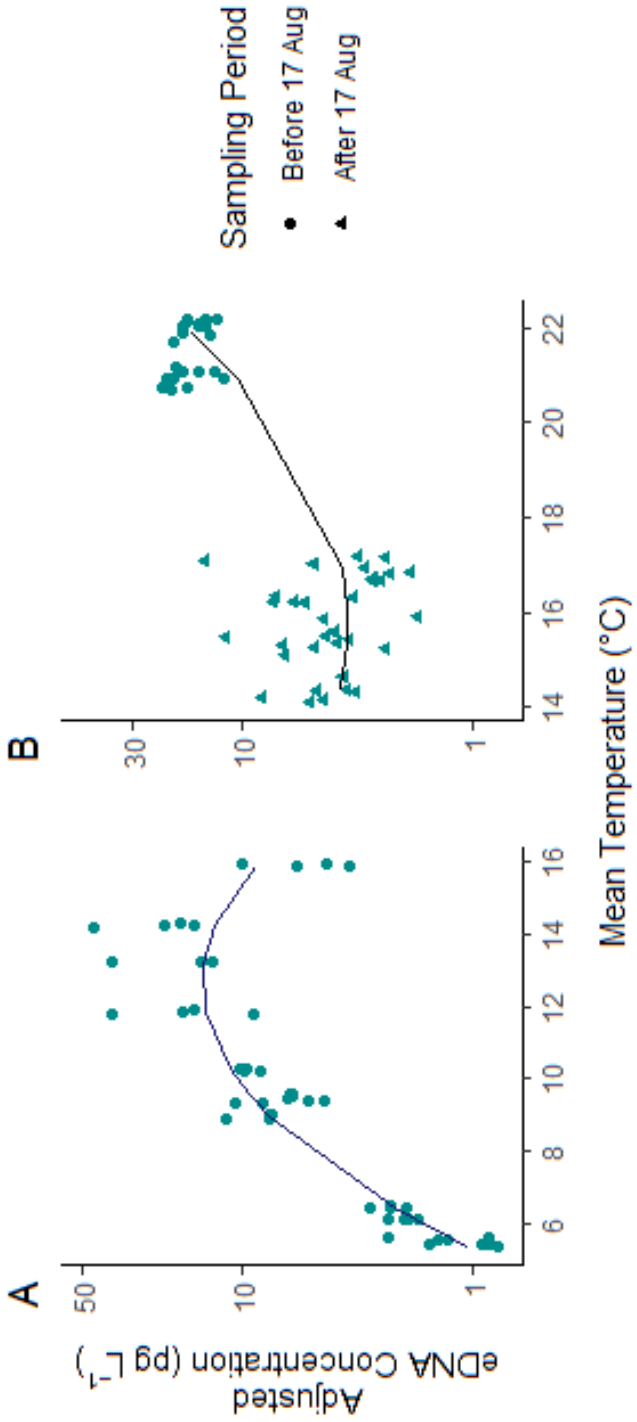

Figure 5 (Previous page). Mean river temperature and environmental DNA (eDNA) concentration relationship adjusted with inclusion of environmental covariates. (A) shows the relationship with fluctuating smolt abundance in the spring, and (B) is the relationship in the summer as the parr abundance was assumed constant. Circles and stars in (B) highlight the change in the eDNA ~ temperature relationship with lower temperature the second half of the sampling period (after 17 Aug).

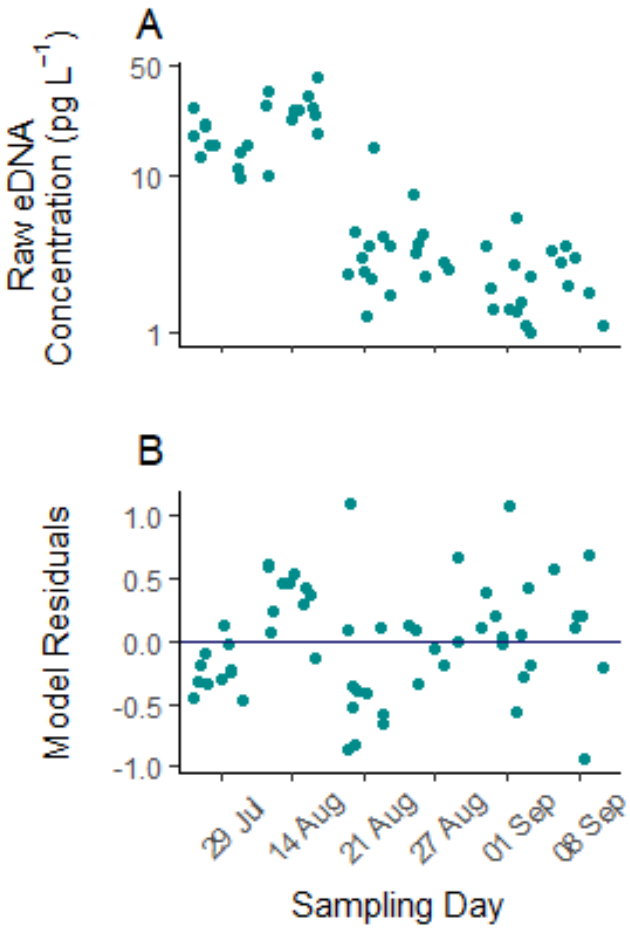

Figure 6. Relationship between parr environmental DNA (eDNA) concentration and sampling day presented from (A) univariate linear model, and (B) model residuals of top four generalised linear models with inclusion of all environmental covariates (chlorophyll, conductivity, distance from primary pool in each reach, mean temperature and its quadratic, pH, total precipitation between sampling days, site (The Forks, Black Hole, Pumphouse), and water velocity; *n* = 516). Potential breakpoint in (A) is no longer present in model residuals (B).

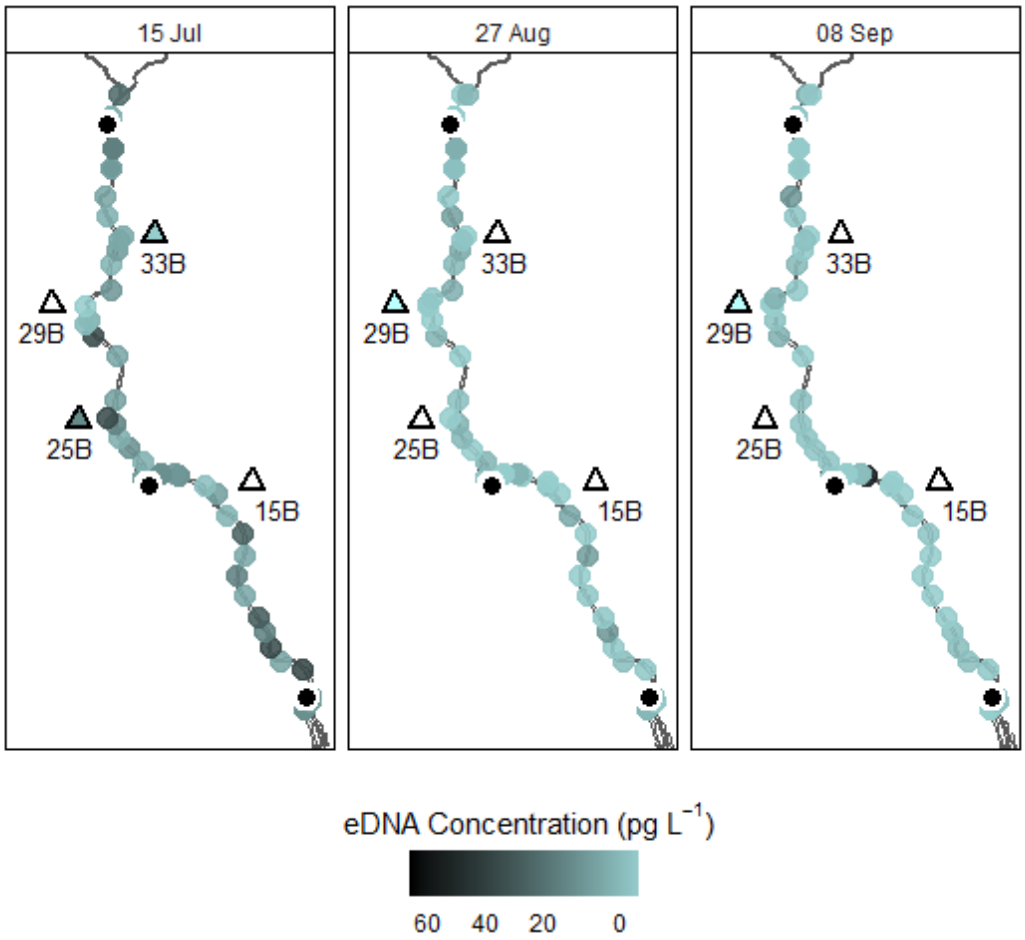

Figure 7. Upper Salmon River environmental DNA (eDNA) concentrations for the three summer full transect sampling events. Black circles depict primary pool in each reach (From top to bottom: The Forks, Black Hole, Pumphouse). Triangles indicate tributary sampling sites with relative eDNA concentrations.

Chapter 3: General Conclusion

My thesis sought to understand the dynamics underlying juvenile Atlantic Salmon abundance and eDNA variability resulting from seasonal environmental changes in a natural flowing river. I predicted that 1) a strong relationship between fluctuating smolt abundance and eDNA would present after including environmental factors, and 2) that any eDNA variation would primarily be due to seasonal environmental changes as parr abundance remained relatively constant. My principal finding was that quantifying and including environmental conditions was critical to obtain significantly stronger relationships among environmental factors and eDNA concentrations in both spring smolt and summer parr surveys. Declining smolt abundance strongly related to decreasing eDNA concentration, and concentrations obtained near the beginning and the end of the summer varied significantly; however, the mechanisms behind the observed summer eDNA variation will need further exploration. Finally, the observed variation in eDNA may be further reduced by increasing the number of qPCR technical replicates per sample to better capture the stochastic nature of eDNA dispersal.

As expected, the relationship between eDNA and temperature was non-linear in both the smolt and parr surveys (Strickler *et al*., 2015). The variation in these relationships between seasons was dramatic, with the rate of eDNA concentration either decreasing or increasing with temperature throughout the spring and summer sampling periods, respectively. In regions that experience all four seasons annually, such as Atlantic Canada, temperature and time are

fundamentally interconnected; however, correlation does not necessarily indicate a causal relationship. Although temperature and photoperiod both drive many ecological processes, caution should be exercised as future studies will need to continue exploring the role that seasonality has on eDNA shedding and degradation in natural stream environments.

Another main goal of this study was to explore changes in eDNA distribution along the full 9 km salmon-accessible length of Upper Salmon River. Rather than reveal consistent spatial eDNA patterns at the river scale, my key finding from the full river transect was that eDNA concentrations throughout the river were significantly higher during the first sampling event than the either of the final two. The variation in eDNA between the warmer, earlier part of the summer and the cooler period near the end of the season underscores the importance of sampling at different times throughout the year; however, it is imperative for adequate salmon eDNA quantitative assessments to survey at the same times each year to capture the seasonal and interannual environmental variability. My study highlighted this as the parr sampling period occurred during atypical weather patterns for the area, with rivers in eastern Canada generally warming into August. Sampling periods should also coincide with the target species' life stage of interest to reduce potential confounding eDNA origins such as contributions from reproduction or hatching (Tillotson *et al*., 2018; Ostberg and Chase, 2021).

One limitation of the full transect survey was that I lacked the resources to collect replicate water samples or increase qPCR technical replicates. Some of

the stochasticity among eDNA samples may be due to the heterogeneous distribution of eDNA. Thus, it is possible that some of the variation may have been reduced by collecting more than a single water sample and increasing its three qPCR replicates. With improved quantification power, it is possible that clear spatial eDNA patterns may have emerged at the river scale, such as potential plume dynamics and/or downstream accumulation

As freshwater ecosystems continue to shift, it becomes increasingly important to ensure management accurately represents true population changes with minimal impact to such populations and habitats. Non-invasive eDNA population assessments may not only improve abundance estimates when conducted alongside conventional surveys, but they may have the ability to scale up to larger systems that may be too costly, time-, and/or labour-intensive to monitor conventionally. For example, regularly characterising the fauna of larger riverine systems such as the Wolastoq River, near Saint John, New Brunswick, may be impractical with traditional biomonitoring and thus population growth and decline may not be accurately tracked. While more research in natural flowing waters is necessary to better understand eDNA dynamics, the results from this study support that indirect eDNA-based population assessments may be successful with appropriate study design.

Literature Cited

Andruszkiewicz Allan, E., Zhang, W. G., Lavery, A. C., and Govindarajan, A. F. (2021). Environmental DNA shedding and decay rates from diverse animal forms and thermal regimes. *Environ. DNA*, *3*, 492–514.

Baguley, T. (2009). Standardized or simple effect size: What should be reported? *British J. Psychol.*, *100*, 603–617.

Balasingham, K. D., Walter, R. P., and Heath, D. D. (2017). Residual eDNA detection sensitivity assessed by quantitative real-time PCR in a river ecosystem. *Mol. Ecol. Resour.*, *17*, 523–532.

Barnes, M. A., and Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conserv. Genet.*, *17*, 1–17.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.*, *67*, 1–48. doi: 10.18637/jss.v067.i01.

Bedwell, M. E., and Goldberg, C. S. (2020). Spatial and temporal patterns of environmental DNA detection to inform sampling protocols in lentic and lotic systems. *Ecol. Evol.*, *00*, 1–11. doi: 10.1002/ece3.6014.

Burnham, K. P., and Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* (2nd ed.). Springer.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., … , and Wittwer, C. T. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.*, *55*, 611–622.

Bylemans, J., Furlan, E. M., Pearce, L., Daly, T., and Gleeson, D. M. (2016). Improving the containment of a freshwater invader using environmental DNA (eDNA) based monitoring. *Biol. Invasions*, *18*, 3081–3089.

Bylemans, J., Furlan, E. M., Gleeson, D. M., Hardy, C. M., and Duncan, R. P. (2018). Does size matter? An experimental evaluation of the relative abundance and decay rates of aquatic environmental DNA. *Environ. Sci. Technol.*, *52*, 6408–6416.

Caza-Allard, I., Laporte, M., Côté, G., April, J., and Bernatchez, L. (2021). Effect of biotic and abiotic factors on the production and degradation of fish environmental DNA: An experimental evaluation. *Environ. DNA*, *00*, 1-16. doi: 10.1002/edn3.266.

Corey, E., Linnansaari, T., Dugdale, S. J., Bergeron, N., Gendron, J.-F., Lapointe, M., and Cunjak, R. A. (2020). Comparing the behavioural thermoregulation response to heat stress by Atlantic salmon parr (*Salmo salar*) in two rivers. *Ecol. Freshw. Fish*, *29*, 50–62.

Corey, E., Linnansaari, T., O'Sullivan, A. M., Curry, R. A., and Cunjak, R. A. (2022). Quantifying the movement patterns of wild juvenile Atlantic salmon (*Salmo salar*) throughout a thermally stressful summer. *Ecology* (In Review).

COSEWIC. (2011). *COSEWIC assessment and status report on the Atlantic Salmon Salmo salar (Inner Bay of Fundy populations) in Canada.* Ottawa. p. vi + 52 pp.

Curtis, A. N., Tiemann, J. S., Douglass, S. A., Davis, M. A., and Larson, E. R. (2021). High stream flows dilute environmental DNA (eDNA) concentrations and reduce detectability. *Divers. Distrib.*, *27*, 1918–1931.

CoCoRaHS. (2021). *List daily precipitation reports*. https://www.cocorahs.org/ViewData/ListDailyPrecipReports.aspx [Accessed July 13, 2021].

de Souza, L. S., Godwin, J. C., Renshaw, M. A., and Larson, E. (2016). Environmental DNA (eDNA) detection probability is influenced by seasonal activity of organisms. *PLOS ONE*, *11*, e0165273. doi: 10.1371/journal.pone.0165273.

Deiner, K., and Altermatt, F. (2014). Transport distance of invertebrate environmental DNA in a natural river. *PLoS ONE*, *9*, e88786. doi: 10.1371/journal.pone.0088786.

Dejean, T., Valentini, A., Duparc, A., Pellier-Cuit, S., Pompanon, F., Taberlet, P., and Miaud, C. (2011). Persistence of environmental DNA in freshwater ecosystems. *PLoS ONE*, *6*, e23398. doi: 10.1371/journal.pone.0023398.

Dempson, J. B., and Stansbury, D. E. (1991). Using partial counting fences and a two-sample stratified design for mark–recapture estimation of an Atlantic salmon smolt population. *N. Am. J. Fish. Manag.*, *11*, 27–37.

Dodds, W., and Whiles, M. (2010). *Freshwater ecology: Concepts & environmental applications of limnology* (2nd ed.). Elsevier Inc.

Dolan, C. R., and Miranda, L. E. (2004). Injury and mortality of warmwater fishes immobilized by electrofishing. *N. Am. J. Fish. Manag.*, *24*, 118–127.

Eichmiller, J. J., Best, S. E., and Sorensen, P. W. (2016). Effects of temperature and trophic state on degradation of environmental DNA in lake water. *Enviro. Sci. Technol.*, *50*, 1859–1867.

Elliott, J. M., and Elliott, J. A. (2010). Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: Predicting the effects of climate change. *J. Fish Biol.*, *77*, 1793–1817.

Ficetola, G. F., Miaud, C., Pompanon, F., and Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biol. Lett.*, *4*, 423–425.

Fossøy, F., Brandsegg, H., Sivertsgård, R., Pettersen, O., Sandercock, B. K., Solem, Ø., … , and Mo, T. A. (2020). Monitoring presence and abundance of two gyrodactylid ectoparasites and their salmonid hosts using environmental DNA. *Environ. DNA*, *2*, 53–62.

Fundy Salmon Recovery. (2022). *Fundy Salmon Recovery: Changing the face of conservation*. https://www.fundysalmonrecovery.com [Accessed January 21, 2022].

Furlan, E. M., Gleeson, D., Hardy, C. M., and Duncan, R. P. (2016). A framework for estimating the sensitivity of eDNA surveys. *Mol. Ecol. Resour.*, *16*, 641–654.

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., … , and Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods Ecol. Evol.*, *7*, 1299–1307.

Government of Canada. (2020). *Daily Discharge Data for POINT WOLFE RIVER AT FUNDY NATIONAL PARK (01BV006) [NB]*. https://wateroffice.ec.gc.ca/report/historical_e.html?start_year=1850&end_year=2021&y1Max=1&y1Min=1&mean1=1&scale=normal&mode=Table&stn=01BV006&datatypee=Daily&parameterType=Flow&year=2019&page=historical [Accessed December 06, 2021].

Gray, S. M., Bieber, F. M. E., McDonnell, L. H., Chapman, L. J., and Mandrak, N. E. (2014). Experimental evidence for species-specific response to turbidity in imperilled fishes. *Aquat. Conserv., 24*, 546–560.

Grueber, C. E., Nakagawa, S., Laws, R. J., and Jamieson, I. G. (2011). Multimodel inference in ecology and evolution: Challenges and solutions. *J. Evol. Biol.*, *24*, 699–711.

Gu, W., and Swihart, R. K. (2004). Absent or undetected? Effects of non-detection of species occurrence on wildlife–habitat models. *Biol. Conserv.*, *116*, 195–203.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *J. Stat. Softw.*, *33*, 1–22. doi: 10.18637/jss.v033.i02.

Hartman, L. J., Coyne, S. R., and Norwood, D. A. (2005). Development of a novel internal positive control for Taqman® based assays. *Mol. Cel. Probes*, *19*, 51–59.

Hebert, P. D. N., Cywinska, A., Ball, S. L., and deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proc. Biol. Sci.*, *270*, 313–321.

Hebert, P. D. N., Ratnasingham, S., and deWaard, J. R. (2003). Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. Biol. Sci.*, *270 (Suppl)*, S96-99.

Higuchi, R., Bowman, B., Freiberger, M., Ryder, O. A., and Wilson, A. C. (1984). DNA sequences from the quagga, an extinct member of the horse family. *Nature*, *312*, 282–284.

Hood, J. M., Benstead, J. P., Cross, W. F., Huryn, A. D., Johnson, P. W., Gíslason, G. M., … , and Tran, C. (2018). Increased resource use efficiency amplifies positive response of aquatic primary production to experimental warming. *Glob. Chang. Biol.*, *24*, 1069–1084.

Howard, A. D. (1994). A detachment-limited model of drainage basin evolution. *Water Resour. Res.*, *30*, 2261–2285.

ICES. (2021). *Working Group on North Atlantic Salmon (WGNAS)*. ICES Scientific Reports, 3:29. p. 407.

Jane, S. F., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., … , and Whiteley, A. R. (2015). Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. *Mol. Ecol. Resour.*, *15*, 216–227.

Jerde, C. L., Mahon, A. R., Chadderton, W. L., and Lodge, D. M. (2011). “Sight-unseen” detection of rare aquatic species using environmental DNA. *Conserv. Lett.*, *4*, 150–157.

Jerde, C. L., Olds, B. P., Shogren, A. J., Andruszkiewicz, E. A., Mahon, A. R., Bolster, D., and Tank, J. L. (2016). Influence of stream bottom substrate on retention and transport of vertebrate environmental DNA. *Environ. Sci. Technol.*, *50*, 8770–8779.

Jo, T., Murakami, H., Yamamoto, S., Masuda, R., and Minamoto, T. (2019). Effect of water temperature and fish biomass on environmental DNA shedding, degradation, and size distribution. *Ecol. Evol.*, *9*, 1135–1146.

Kasai, A., Takada, S., Yamazaki, A., Masuda, R., and Yamanaka, H. (2020). The effect of temperature on environmental DNA degradation of Japanese eel. *Fish. Sci.*, *86*, 465–471.

Klymus, K. E., Richter, C. A., Chapman, D. C., and Paukert, C. (2015). Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*. *Biol. Conserv.*, *183*, 77–84.

Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., … , and Richter, C. A. (2020). Reporting the limits of detection and quantification for environmental DNA assays. *Environ. DNA*, *2*, 271–282.

Koch, I. J., and Narum, S. R. (2021). An evaluation of the potential factors affecting lifetime reproductive success in salmonids. *Evol. Appl.*, *14*, 1929–1957.

Lacoursière-Roussel, A., Côté, G., Leclerc, V., and Bernatchez, L. (2016). Quantifying relative fish abundance with eDNA: a promising tool for fisheries management. *J. Appl. Ecol.*, *53*, 1148–1157.

Lacoursière-Roussel, A., Dubois, Y., Normandeau, E., and Bernatchez, L. (2016). Improving herpetological surveys in eastern North America using the environmental DNA method. *Genome*, *59*, 991–1007.

Lacoursière-Roussel, A., Rosabal, M., and Bernatchez, L. (2016). Estimating fish abundance and biomass from eDNA concentrations: variability among capture methods and environmental conditions. *Mol. Ecol. Resour.*, *16*, 1401–1414.

Laporte, M., Bougas, B., Côté, G., Champoux, O., Paradis, Y., Morin, J., and Bernatchez, L. (2020). Caged fish experiment and hydrodynamic bidimensional modeling highlight the importance to consider 2D dispersion in fluvial environmental DNA studies. *Environ. DNA*, *2*, 362–372.

LeBlanc, F., Belliveau, V., Watson, E., Coomber, C., Simard, N., DiBacco, C., … , and Gagné, N. (2020). Environmental DNA (eDNA) detection of marine aquatic invasive species (AIS) in Eastern Canada using a targeted species-specific qPCR approach. *Manag. Biol. Invasions*, *11*, 201–217.

Levi, T., Allen, J. M., Bell, D., Joyce, J., Russell, J. R., Tallmon, D. A., … , and Yu, D. W. (2019). Environmental DNA for the enumeration and management of Pacific Salmon. *Mol. Ecol. Resour.*, *19*, 597–608.

Lugg, W. H., Griffiths, J., Rooyen, A. R. van, Weeks, A. R., and Tingley, R. (2018). Optimal survey designs for environmental DNA sampling. *Methods Ecol. Evol.*, *9*, 1049–1059.

Lyons, J. (1986). Capture efficiency of a beach seine for seven freshwater fishes in a north-temperate lake. *N. Am. J. Fish. Manag.*, *6*, 288–289.

Mahon, A. R., Jerde, C. L., Galaska, M., Bergner, J. L., Chadderton, W. L., Lodge, D. M., … , and Nico, L. G. (2013). Validation of eDNA surveillance sensitivity for detection of Asian carps in controlled and field experiments. *PLoS ONE*, *8*, e58316. doi: 10.1371/journal.pone.0058316.

Mangiafico, S. S. (2016). Summary and analysis of extension program evaluation in R, version 1.18.8. rcompanion.org/handbook/ [Accessed December 03, 2021].

Maruyama, A., Nakamura, K., Yamanaka, H., Kondoh, M., and Minamoto, T. (2014). The release rate of environmental DNA from juvenile and adult fish. *PLoS ONE*, *9*, e114639. doi: 10.1371/journal.pone.0114639.

McCormick, S. D., Hansen, L. P., Quinn, T. P., and Saunders, R. L. (1998). Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci., 55*, 77–92.

Miranda, L. E., and Kidwell, R. H. (2010). Unintended effects of electrofishing on nongame fishes. *Trans. Am. Fish. Soc.*, *139*, 1315–1321.

Monk, W. A. Gray, M. A., McCarthy, J. H., Samways, K. M., and Curry, R. A. (2022). Atlantic coast rivers of Canada. In A. C. Benke, and C. E. Cushing (Eds.), *Rivers of North America* (2nd ed.). in Press.

National Marine Fisheries Service. (2021). *Species in the spotlight: Atlantic salmon* (Priority actions 2021-2025). National Oceanic and Atmospheric Administration. https://media.fisheries.noaa.gov/2021-04/SIS%20Action%20Plan%202021_AtlanticSalmon-FINAL%20508.pdf.

Nelson, J. S., Grande, T. C., and Wilson, M. V. H. (2016). *Fishes of the world* (5th ed). John Wiley & Sons.

Nielsen, K. M., Johnsen, P. J., Bensasson, D., and Daffonchio, D. (2007). Release and persistence of extracellular DNA in the environment. *Environ. Biosaf. Res.*, *6*, 37–53.

Person-Le Ruyet, J., Mahé, K., Le Bayon, N., and Le Delliou, H. (2004). Effects of temperature on growth and metabolism in a Mediterranean population of European sea bass, *Dicentrarchus labrax*. *Aquaculture*, *237*, 269–280.

Petty, J. T., Hansbarger, J. L., Huntsman, B. M., and Mazik, P. M. (2012). Brook Trout movement in response to temperature, flow, and thermal refugia within a complex Appalachian riverscape. *Trans. Am. Fish. Soc.*, *141*, 1060–1073.

Pietramellara, G., Ascher, J., Borgogni, F., Ceccherini, M. T., Guerri, G., and Nannipieri, P. (2009). Extracellular DNA in soil and sediment: Fate and ecological relevance. *Biol. Fertil. Soils*, *45*, 219–235.

Pillai, T. N. V., and Ganguly, A. K. (1970). Nucleic acids in the dissolved constituents of sea-water. *Curr. Sci.*, *39*, 501–504.

Pilliod, D. S., Goldberg, C. S., Arkle, R. S., and Waits, L. P. (2013). Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Can. J. Fish. Aquat. Sci.*, *70*, 1123–1130.

Pilliod, D. S., Goldberg, C. S., Arkle, R. S., and Waits, L. P. (2014). Factors influencing detection of eDNA from a stream-dwelling amphibian. *Mol. Ecol. Resour.*, *14*, 109–116.

Pont, D., Rocle, M., Valentini, A., Civade, R., Jean, P., Maire, A., … , and Dejean, T. (2018). Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. *Sci. Rep.*, *8*, 10361. doi: 10.1038/s41598-018-28424-8.

QGIS.org. (2021). *QGIS Geographic Information System*. QGIS Association. http://www.qgis.org.

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., and Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA – a review of eDNA as a survey tool in ecology. *J. Appl. Ecol.*, *51*, 1450–1459.

Revelle, W. (2021). *psych: Procedures for psychological, psychometric, and personality research*. Northwestern University, Evanston, Illinois. R package version 2.1.9, https://CRAN.R-project.org/package=psych.

Rice, C. J., Larson, E. R., and Taylor, C. A. (2018). Environmental DNA detects a rare large river crayfish but with little relation to local abundance. *Freshw. Biol.*, *63*, 443–455.

Rojahn, J., Pearce, L., Gleeson, D. M., Duncan, R. P., Gilligan, D. M., and Bylemans, J. (2021). The value of quantitative environmental DNA analyses for the management of invasive and endangered native fish. *Freshw. Biol.*, *66*, 1619–1629.

Saccone, C., De Giorgi, C., Gissi, C., Pesole, G., and Reyes, A. (1999). Evolutionary genomics in Metazoa: the mitochondrial DNA as a model system. *Gene*, *238*, 195–209.

Service New Brunswick. (2020). *New Brunswick Hydrogeographic Network (NBHN)*. http://www.snb.ca/geonb1/e/DC/NBHN.asp [Accessed November 11, 2020].

Sepulveda, A. J., Al-Chokhachy, R., Laramie, M. B., Crapster, K., Knotek, L., Miller, B., … , and Pilliod, D. S. (2021). It's complicated … environmental DNA as a predictor of trout and char abundance in streams. *Can. J. Fish. Aquat. Sci.*, *78*, 422–432.

Shelton, A. O., Kelly, R. P., O'Donnell, J. L., Park, L., Schwenke, P., Greene, C., … , and Beamer, E. M. (2019). Environmental DNA provides quantitative estimates of a threatened salmon species. *Biol. Conserv.*, *237*, 383–391.

Shogren, A. J., Tank, J. L., Andruszkiewicz, E., Olds, B., Mahon, A. R., Jerde, C. L., and Bolster, D. (2017). Controls on eDNA movement in streams: Transport, retention, and resuspension. *Sci. Rep.*, *7*, 5065. doi: 10.1038/s41598-017-05223-1.

Shogren, A. J., Tank, J. L., Egan, S. P., August, O., Rosi, E. J., Hanrahan, B. R., … , and Bolster, D. (2018). Water flow and biofilm cover influence environmental DNA detection in recirculating streams. *Env. Sci. Technol.*, *52*, 8530–8537.

Shogren, A. J., Tank, J. L., Egan, S. P., Bolster, D., and Riis, T. (2019). Riverine distribution of mussel environmental DNA reflects a balance among density, transport, and removal processes. *Freshw. Biol.*, *64*, 1467–1479.

Stoeckle, M. Y., Adolf, J., Charlop-Powers, Z., Dunton, K. J., Hinks, G., and VanMorter, S. M. (2021). Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. *ICES J. Mar. Sci.*, *78*, 293–304.

Strayer, D. L., and Dudgeon, D. (2010). Freshwater biodiversity conservation: recent progress and future challenges. *J. North Am. Benthol. Soc.*, *29*, 344–358.

Strickler, K. M., Fremier, A. K., and Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biol. Conserv.*, *183*, 85–92.

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., and Kawabata, Z. (2012). Estimation of fish biomass using environmental DNA. *PLoS ONE*, *7*, e35868. doi: 10.1371/journal.pone.0035868.

Tillotson, M. D., Kelly, R. P., Duda, J. J., Hoy, M., Kralj, J., and Quinn, T. P. (2018). Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. *Biol. Conserv.*, *220*, 1–11.

Villacorta-Rath, C., Hoskin, C. J., Strugnell, J. M., and Burrows, D. (2021). Long distance (>20 km) downstream detection of endangered stream frogs suggests an important role for eDNA in surveying for remnant amphibian populations. *PeerJ*, *9*, e12013. doi: 10.7717/peerj.12013.

Vincent, S., Vian, J. M., and Carlotti, M. P. (2000). Partial sequencing of the cytochrome oxydase b subunit gene I: a tool for the identification of European species of blow flies for postmortem interval estimation. *J. Forensic Sci.*, *45*, 820–823.

Wentworth, C. K. (1922). A scale of grade and class terms for clastic sediments. *J. Geol.*, *30*, 377–392.

Wetzel, R. G. (2001). *Limnology: Lake and river ecosystems*. Academic Press.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.

Wilcox, T. M., McKelvey, K. S., Young, M. K., Lowe, W. H., and Schwartz, M. K. (2015). Environmental DNA particle size distribution from Brook Trout (*Salvelinus fontinalis*). *Conserv. Genet. Resour.*, *7*, 639–641.

Wilcox, T. M., McKelvey, K. S., Young, M. K., Sepulveda, A. J., Shepard, B. B., Jane, S. F., …, and Schwartz, M. K. (2016). Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*. *Biol. Conserv.*, *194*, 209-216.

Wilken, E., Nava, F. J., and Griffith, G. (2011). *North American Terrestrial Ecoregions—Level III*. Commission for Environmental Cooperation, Montreal, Canada. p. 149.

Williams, M.-A., O'Grady, J., Ball, B., Carlsson, J., Eyto, E. de, McGinnity, P., … , and Parle-McDermott, A. (2019). The application of CRISPR-Cas for single species identification from environmental DNA. *Mol. Ecol. Resour.*, *19*, 1106–1114.

Wood, Z. T., Erdman, B. F., York, G., Trial, J. G., and Kinnison, M. T. (2020). Experimental assessment of optimal lotic eDNA sampling and assay multiplexing for a critically endangered fish. *Environ. DNA*, *00*, 1–11. doi: 10.1002/edn3.64.

Wood, Z. T., Lacoursière-Roussel, A., LeBlanc, F., Trudel, M., Kinnison, M. T., Garry McBrine, C., … , and Gagné, N. (2021). Spatial heterogeneity of eDNA transport improves stream assessment of threatened salmon presence, abundance, and location. *Front. Ecol. Evol.*, *9*, 650717. doi: 10.3389/fevo.2021.650717.

WWF. (2020*). Living Planet Report 2020 - Bending the curve of biodiversity loss*. Almond, R. E. A., Grooten, M., and Petersen, T. (Eds). Gland, Switzerland.

Yates, M. C., Fraser, D. J., and Derry, A. M. (2019). Meta-analysis supports further refinement of eDNA for monitoring aquatic species-specific abundance in nature. *Environ. DNA*, *1*, 5–13.

Yates, M. C., Cristescu, M. E., and Derry, A. M. (2021). Integrating physiology and environmental dynamics to operationalize environmental DNA (eDNA) as a means to monitor freshwater macro-organism abundance. *Mol. Ecol.*, *30*, 6531–6550.

Yates, M. C., Glaser, D. M., Post, J. R., Cristescu, M. E., Fraser, D. J., and Derry, A. M. (2021). The relationship between eDNA particle concentration and organism abundance in nature is strengthened by allometric scaling. *Mol. Ecol.*, *30*, 3068–3082.

Appendix

Table A1. Linear mixed model assessing field replicate (i.e., water sample) and qPCR technical replicate variation for smolt survey (*n* = 423).

Parameter	Variance	*SD*	95% Confidence Interval (*SD*)		AIC
			Lower Bound	Upper Bound	
Random effects					
Between field sites	0.187	0.432	0.322	0.558	930.3
Within field replicates	0.163	0.404	0.320	0.495	
Between technical replicates	0.340	0.583	0.545	0.626	
Fixed effects					
	Estimate	*SE*			
Intercept	1.721	0.077	1.593	1.849	

Table A2. Linear mixed model assessing field replicate (i.e., water sample) and qPCR technical replicate variation for parr survey (*n* = 516).

Parameter	Variance	*SD*	95% Confidence Interval (*SD*)		AIC
			Lower Bound	Upper Bound	
Random effects					
Between field sites	1.218	1.103	0.910	1.268	1216.63
Within field replicates	0.225	0.474	0.339	0.488	
Between technical replicates	0.310	0.556	0.525	0.595	
Fixed effects					
	Estimate	*SE*			
Intercept	1.751	0.151	1.489	1.974	

Appendix (continued)

Table A3. Summary statistics of environmental DNA concentration (pg L^{-1}; mean ± 1 standard deviation) for each full transect sampling event.

Sampling day	Mean	Median	Min	Max
15 Jul	20.83 ± 13.91	18.43	0.00	71.12
27 Aug	4.55 ± 5.07	2.80	0.00	26.68
08 Sep	4.56 ± 9.11	1.96	0.00	78.20

Table A4. ANOVA comparing mean environmental DNA concentration among each full transect sampling event (*n* = 138).

	df	Sum of Squares	Mean Square	*F*
Between Sampling Events	2	7080	3540	40.98
Within Sampling Events	135	11661	86	

Table A5. Tukey HSD multiple comparisons of mean environmental DNA concentration (pg L^{-1}) for each full transect sampling day (*n* = 138).

Sampling Day (I)	Sampling Day (J)	Mean Difference (I – J)	*SE*	95% Confidence Interval	
				Lower Bound	Upper Bound
15 Jul	**27 Aug**	**15.500**	**1.951**	**11.641**	**19.360**
	08 Sep	**15.429**	**1.961**	**11.550**	**19.308**
27 Aug	**15 Jul**	**-15.500**	**1.951**	**-19.360**	**-11.641**
	08 Sep	-0.071	1.907	-3.843	3.700
08 Sep	**15 Jul**	**-15.429**	**1.961**	**-19.308**	**-11.550**
	27 Aug	0.071	1.907	-3.700	3.843

Note: Pairwise comparisons with significant difference in bold (i.e., 95% CI not overlapping zero).

Appendix (continued)

Table A6. Side tributaries and sites upstream of the physical waterfall barriers sampled during the three full river transect events. Sampling day (2020) and environmental DNA concentrations noted when present.

Site	Sampling Day	# Positive qPCR Replicates	Mean eDNA Concentration (pg L^{-1})
15B	-	-	-
25B	15 Jul	3/3	43.88
29B	27 Aug 08 Sep	2/3 1/3	0.25 0.53
33B	15 Jul	3/3	15.17
Upstream of waterfall barriers			
40B	-	-	-
41B	-	-	-

Curriculum Vitae

Candidate's full name: Melissa K. Morrison

Universities attended:

University of Northern British Columbia
Bachelor of Science (Honours) in Biochemistry and Molecular Biology
May 2013

Presentations and Publications:

Presentations

Morrison, M. K., Pavey, S. A., and Lacoursière-Roussel, A. 2021. Including environmental covariates clarifies the relationship between endangered Atlantic Salmon (*Salmo salar*) abundance and environmental DNA. (Oral). Canadian Rivers Institute, Virtual.

Morrison, M. K., Pavey, S. A., and Lacoursière-Roussel, A. 2021. Including environmental covariates clarifies the relationship between endangered Atlantic Salmon (*Salmo salar*) abundance and environmental DNA. (Oral). American Fisheries Society, Virtual.

Publications

Morrison, M. K., Lacoursière-Roussel, A., Wood, Z. T., Trudel, M., Gagné, N., LeBlanc, F., Samways, K., Kinnison, M. T., Pavey, S. A. Including environmental covariates clarifies the relationship between endangered Atlantic Salmon (*Salmo salar*) abundance and environmental DNA. (In preparation).