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SPECIAL ISSUE ARTICLE

Including environmental covariates clarifies the relationship between endangered Atlantic salmon (*Salmo salar*) abundance and environmental DNA

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Abstract

Collecting environmental DNA (eDNA) as a nonlethal sampling approach has been valuable in detecting the presence/absence of many imperiled taxa; however, its application to indicate species abundance poses many challenges. A deeper understanding of eDNA dynamics in aquatic systems is required to better interpret the substantial variability often associated with eDNA samples. Our sampling design 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), covering 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, we asked whether accounting for environmental variables strengthened the relationship between eDNA and salmon abundance by sampling eDNA during their spring seaward migration. Second, we asked how environmental variables affected eDNA dynamics during the summer as the parr abundance remained relatively constant. Spring eDNA samples were collected over a 6-week period (12 times) near a rotary screw trap that captured approximately 18.6% of migrating smolts, whereas summer sampling occurred (i) at three distinct salmon habitats (9 times) and (ii) along the full 9 km (3 times). We modeled eDNA concentration as a product of fish abundance and environmental variables, demonstrating 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. Our 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.

KEYWORDS
Atlantic salmon, environmental DNA, population abundance, qPCR, *Salmo salar*, stream eDNA

1 | INTRODUCTION

Noninvasive sampling approaches, such as environmental DNA (eDNA), are valuable in monitoring already threatened fishes (Mauvisseau et al., 2020). Monitoring populations at risk of further decline due to climatological or anthropogenic stressors (i.e., dams) traditionally requires physical capture of the species of interest (Dempson & Stansbury, 1991; Dolan & Miranda, 2004). Not only can this risk inadvertent injury or mortality in an already vulnerable population (Miranda & Kidwell, 2010), but abundance estimates may be inaccurate (Gu & Swihart, 2004) and may negatively impact status assessments and management decisions of imperiled 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 monitored ecosystem, the lower cost and time associated with eDNA methods allow greater accessibility and sensitivity for rare target species (Jerde et al., 2011; Laramie et al., 2015). Noninvasive 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.

Across freshwater taxa, eDNA has successfully detected the presence of individuals (Beng & Corlett, 2020); however, its application as an indicator of species abundance poses many challenges. Understanding how spatial and temporal variation of 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, and mucous; Barnes & 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; Wood et al., 2020; Yates et al., 2019). 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), there is growing evidence that such relationships become substantially more complex in natural systems (Levi et al., 2019; Sepulveda et al., 2021; Tillotson et al., 2018). The typically unaccounted and interacting dynamics of variables influencing eDNA ecology in many natural systems could lead to wide variation in the precision and accuracy of species quantification (Mahon et al., 2013; Pilliod et al., 2013; Pochardt et al., 2020).

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 optimize 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 (Fremier et al., 2019; Jerde et al., 2016; Pont et al., 2018; Wilcox et al., 2015). In addition to stream-specific hydrology, eDNA persistence can be affected by additional abiotic factors such as temperature (Caza-Allard et al., 2021). For example, although increased shedding rates have been observed in higher water temperatures (Jo et al., 2019; Lacoursière-Roussel et al., 2016) 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 both directly affecting its degradation and sequestration as well as indirectly affecting its molecular detection (i.e., polymerase chain reactions (PCR) inhibition; Barnes & Turner, 2016). Finally, there may be a significant seasonal or life stage component influencing eDNA shedding and dynamics, particularly given the large role that seasonality and life stage play in fish behavior and metabolic rate (Rourke et al., 2021).

The main goal of this study was to elucidate the complex relationships among eDNA concentration, juvenile Atlantic salmon (*Salmo salar*) population abundance, and seasonal environmental conditions in a natural stream environment to refine noninvasive eDNA sampling approaches and models for this commonly threatened species. 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 their complex life history as an anadromous species, migrating great distances between freshwater and marine environments with the potential to spawn multiple years (Koch & Narum, 2021). As such, Atlantic salmon have been the subject of significant eDNA development (e.g., Balasingham et al., 2017; Fossøy et al., 2020; Williams et al., 2019; Wood et al., 2021). Designated as Endangered in 2001, the inner Bay of Fundy populations of Atlantic salmon located in southeast New Brunswick, Canada, have been monitored at each life stage using electrofishing and trapping methods for the past two decades (Fundy Salmon Recovery, 2022). Sampling juvenile salmon along the entirety of Fundy rivers is difficult due to their high gradients and deeply incised river valleys, often requiring remote access to sampling sites. In addition to the physical constraints, electrofishing surveys require a team of three to five people for a period of approximately 4 weeks to sample 12 permanent sample plots. In contrast, collecting water eDNA samples has the potential to improve accessibility for juvenile salmon monitoring by reducing time and effort

associated with electrofishing while also reducing habitat disruption and species mortality; therefore, developing reliable, nonlethal, and indirect population monitoring is crucial to supplement conventional population assessments. Given the intricate ecology of eDNA, it is still unclear how the seasonal changes in environmental factors and life stages affect wild salmon eDNA.

Here, we examined salmon eDNA concentration as a function of fish abundance and environmental variables. First, we asked whether accounting for environmental variables would clarify the relationship between eDNA and fluctuating 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 long-term annual population assessments, giving a comprehensive and precise understanding of the life stages (parr and smolt) present at a given time.

2 | MATERIALS AND 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 1) 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 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 several deep, narrow, incised gorge-like banks and cliffs (Monk et al., 2023). 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 2 (up to 4) years before migrating to sea from May to 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, and PP; Figure 1), 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*; Parks Canada, unpublished data). American Eel (*Anguilla rostrata*) is the only other diadromous fish to reside in the USR. Over several decades of electrofishing surveys, no other salmonids have been documented in the river apart from Atlantic salmon and brook trout, which is present in relatively small numbers along with aforementioned resident fishes (Parks Canada, unpublished data).

2.2 | eDNA field sampling

To prevent contamination from exogenous DNA, 1 L Nalgene® bottles were decontaminated in the laboratory by rinsing three times with 10% (v/v) bleach solution (Clorox Javex® 12, 10.3% sodium hypochlorite; Boivin-Delisle et al., 2020) followed by five times with distilled water. The bottles were also vigorously shaken with river water three times to remove any residual bleach prior to collecting triplicate 1 L samples of surface water (i.e., fully submerging the bottles right below the surface). Water samples for eDNA detection were collected from July 15 to September 8, 2020, and from May 4 to June 10, 2021. Samples were collected midstream by wading or using an extendable pole from the river bank when flow was too hazardous in 2021. Waders and pole sampler were both dedicated for eDNA work. Sampling commenced at the most downstream station, moving upward to avoid contaminating downstream samples. Station is defined as a consistent repeated sampling location. Field negative controls consisted of ultrapure water (Milli-Q®; Merck) 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 environmental 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 6 weeks during smolt migration (May 4–June 10, 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 1). Smolt abundance has been monitored in Fundy National Park since 2002, and smolts have been collected consistently using the same protocol in the same location each year. The average smolt run across all years (2002–2022) is 3462, ranging from a low of 36 in 2017 to a high of 20,979 in 2010. For this specific year (2021), the smolt wheel had an 18.6% efficiency and estimated a population size of 4911 (95% CI = [3411, 7027]) smolts migrating to sea (Parks Canada, unpublished data). Smolt wheel efficiency and population size were based on the total capture of 733 smolts and conducting a mark/recapture study whereby 145 smolts were collected throughout the entirety of the smolt run, marked with a caudal fin clip, and released upriver. Of the 145 smolts marked, a total of 27 were recaptured. A hierarchical Bayesian model was used to estimate the smolt population size (Rivot & Prévost, 2002; Parks Canada, unpublished data). eDNA 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).

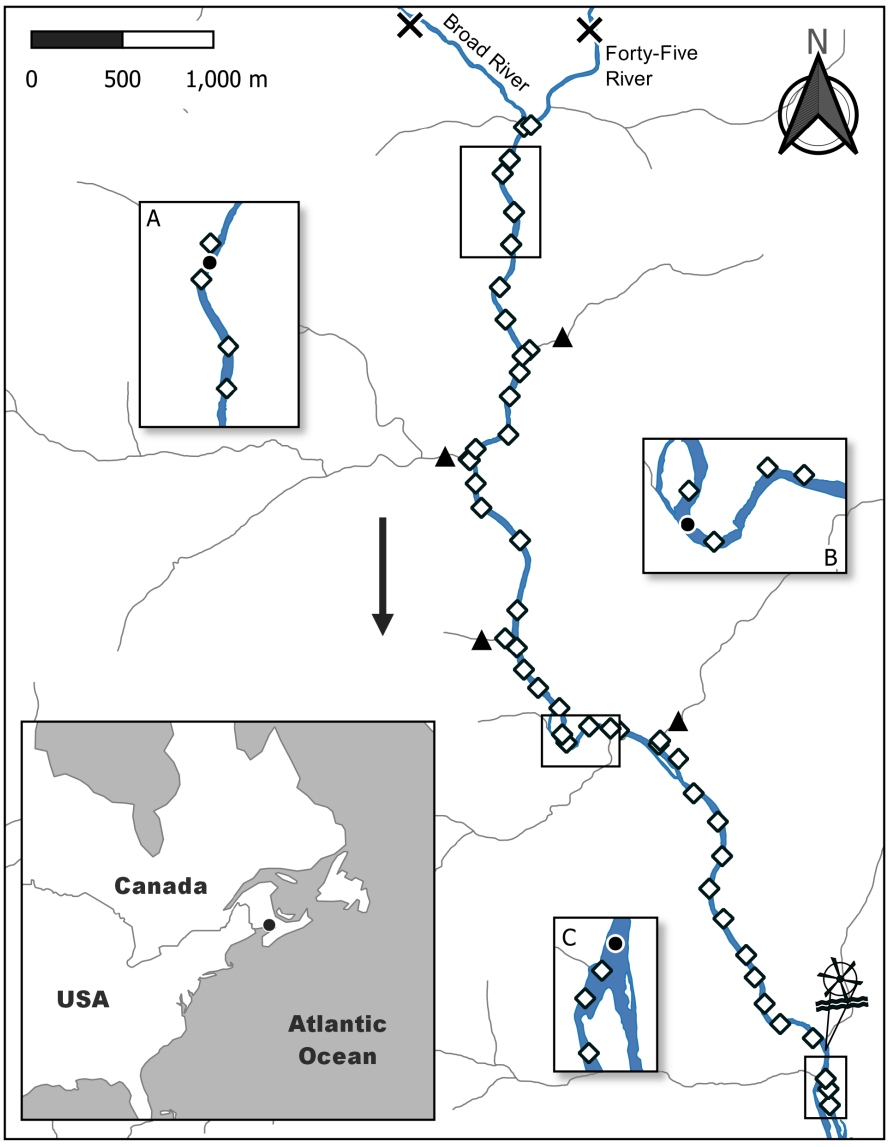

FIGURE 1 Water environmental DNA sample sites (diamonds) in Upper Salmon River, Fundy National Park, Canada (45°37′05″ N, 64°57′56″ W). Main map denotes full transect with tributary sampling locations (triangles) and flow direction (arrow). Insets include optimal Atlantic salmon habitat with primary pool (circles) in (a): The Forks, (b): Black Hole, and (c): Pumphouse (PP), and weekly upstream and downstream sample sites. The spring rotary screw trap survey was located just upstream of PP. Crosses upstream of the confluence depict physical waterfall barriers.

2.2.2 | Parr survey

Temporal fluctuations in eDNA concentration due to changing environmental conditions were explored during the period of the year when the number of parr in the river was assumed to remain relatively constant. Parr presence in USR was confirmed through snorkel surveys along the entire river on July 21 and August 20, 2020, while parr density was obtained from open and closed electrofishing surveys at each of the three main reaches (open – August 10, 11: PP; closed – August 12, 13: TF, August 19: BH; Parks Canada, unpublished data). Two different eDNA sampling designs were executed from July 15 to September 8, 2020 to assess differences in eDNA distribution along the river. The primary survey consisted of sampling three to four stations within each of the three main reaches ($n=11$ stations) to explore eDNA distribution at distances relative to the main pool in each reach (i.e., site): TF, BH, and PP (Figure 1). Sampling occurred as described above once a week for 9 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 (July 15, August 27, and September 8, 2020) that included transecting the full salmon-accessible length of USR to examine how seasonal changes affected eDNA quantification along the entire river. 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 1). One side tributary was completely dry during the first sampling event (15B, July 15), and thus we sampled that tributary further upstream on the last two sampling events. Sampling also occurred upstream of the confluence to verify that no salmon were present upstream of each physical waterfall barrier (July 15, $n=64$; August 27, $n=70$; September 8, $n=68$; 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 potential quantitative polymerase chain reactions (qPCR) inhibition (chlorophyll a; reviewed in Barnes & Turner, 2016). Conditions were measured at each sample station using a YSI® ProDSS Digital Sampling System Water Quality Meter (YSI Incorporated) immediately following sample collection, at the same location and depth. Note that for the parr survey, collection of YSI measurements began July 29, 2020; furthermore, measurements could not be obtained on August 27 and September 8, 2020, for approximately half of the stations due to equipment accessibility.

To evaluate the effect of river flow 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 station using a YSI® Flow Probe (YSI Incorporated), and precipitation data were obtained through the Community Collaborative Rain, Hail & Snow Network (CoCoRaHS, 2021). The nearest reporting 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 stations on July 15, August 27, and September 8, 2020, due to equipment accessibility.

2.4 | Sample filtration

Water samples were kept on ice and then at 4°C, avoiding light exposure to prevent eDNA degradation until filtration in a dedicated eDNA filtration laboratory. In general, filtration for all surveys occurred within 28 h of collection (smolt: 5–8 h; parr: 22–25 h; full transect: 22–28 h; Curtis, Larson, & Davis, 2021). 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) 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 stations to reduce contamination, while one pair was used among replicates of a single station; likewise, a single pair of forceps was used among replicates of a single station, sanitizing between stations. Sample filters did not experience any clogging, and each filtered within an average of 9.6 min. Filters were preserved in 1.7 mL microcentrifuge tubes with 900 μL 95% ethanol before storing at −20°C until DNA extraction. All reusable equipment (e.g., vacuum flasks) 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) following LeBlanc et al. (2020). The resulting DNA extracts were stored at −20°C, and the second half of the filter was kept as a back-up.

Atlantic salmon eDNA was quantified using the TaqMan™ minor groove binding (MGB) assay published in Wood et al. (2021), which targets a 195 base pairs (bp) region of the Atlantic salmon cytochrome *c* oxidase subunit 1 (COI) gene [Forward primer COI_82F_Ss: 5′-TGGCGCCCTTCTGGGA; reverse primer COI_276R_ Ss: 5′-AAGGAGGGAGGGAGAAGTCAAAAA; and probe COI_194P_Ss: FAM – ATTAATTCCTCTTATAATCGGG – MGB]. Triplicate qPCR were conducted on each extract and negative control using a QuantStudio™ 3 thermocycler (Thermo Fisher Scientific).

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-labeled fluorescent hydrolysis probe, 3 μL template DNA, and 1 μL bovine serum albumin (1%). Thermocycling parameters consisted of an initial hold at 50°C for 2 min followed by 95°C for 10 min 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 linearized 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′-GTCAATTTCCAAAGCCTCCGATC; 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–June 1: 50 m downstream; June 8: 0 m; Parr survey–July 16: PP 50 m downstream, TF 500 m downstream; September 8: 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 (10^0–10^{-7}) of tissue-derived Atlantic salmon genomic DNA prepared in a separate room from the eDNA qPCR assay. Amplification efficiency was 92.7% and $R^2 > 0.99$. Quantification cycle values were converted to

target eDNA concentrations (pg L^{-1}) using the standard curve slope-intercept equation:

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

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) with package "ggplot2" v. 3.3.5 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.

Pearson correlation coefficients were used to test environmental covariates for collinearity. Correlation matrices of all continuous variables (e.g., temperature, conductivity, and eDNA concentration) were produced using *corPlot* in the package "psych" v. 2.1.9 (Revelle, 2021). 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, these data were not normally distributed and were therefore natural log-transformed to reduce skewness of the data. The daily mean temperature among all stations was used as a proxy as temperature generally increased from beginning to end of each sampling event (average daily increase of 0.5 and 4.4°C in smolt and parr surveys, respectively).

2.6.1 | Smolt eDNA and abundance relationship

Ambient eDNA concentration was hypothesized to fluctuate as a function of smolt migration and environmental effects. Linear mixed models (LMM) were executed using the *lmer* function in the R package "lme4" v 1.1–31 (Bates et al., 2015) to model the relationship between smolt abundance and eDNA concentration. The initial LMM was built with continuous fixed effects to evaluate the influence on eDNA concentration from the six biologically relevant parameters: chlorophyll a concentration, conductivity, 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 nonlinearly 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). The final LMM was a reduced model containing the covariates chlorophyll a concentration, mean water temperature and its quadratic, and both smolt abundance metrics as the reduced model resulted in a lower Akaike's Information Criterion for small sample size (AICc <3) than the full model.

Nested random effects were included to account for the nonindependence of replicates: sample day, sample station, the interaction of sample day and station (i.e., sampling occasion), and field replicates nested within sampling occasion. The day random effect accounted for correlation of samples taken on the same sample day, the station random effect accounted for correlation of field replicates at the same station, the sampling occasion random effect accounted for correlation among field replicates taken at the same site on the same day, and the field replicate random effect accounted for correlation among qPCR technical replicates within the same field replicate. Residual variation is assumed to be due to stochastic variation across technical replicates.

To standardize the magnitude of each variable's effect on eDNA variability, all variables were centred around a mean of 0 and scaled to a standard deviation of 1 (Baguley, 2009). The quadratic temperature term was calculated prior to it being scaled and centred. Average effect sizes of each environmental and abundance covariate were calculated as standardized LMM coefficients with corresponding 95% confidence intervals.

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 hypothesized to be driven by environmental covariates. To model parr eDNA as a function of environmental variability, a LMM was developed with the same nested random effects as above and continuous fixed effects of distance from each primary pool and environmental variables as described above: chlorophyll a concentration, conductivity, pH, total precipitation between sampling events, water velocity, mean water temperature, and its quadratic term. Pool-riffle-run habitat (site = TF, BH, and PP) was included as a categorical fixed effect. All field samples that were missing environmental covariates due to equipment accessibility (34% of samples) were omitted from the LMM, which was scaled as above to directly compare the magnitude of effect on eDNA concentration from environmental variables. Finally, a LMM with planned contrasts was performed for the full transect eDNA concentrations to explore the difference in concentration between the beginning and end of the summer sampling period (July 15 compared to each August 27 and September 8).

3 | RESULTS

3.1 | eDNA quantification

We detected Atlantic salmon eDNA in nearly all replicates in the smolt survey (90%, $n=426$), with concentrations ranging from no detections to 53.0 pg L^{-1} (Table 1). The median concentration was 5.3 pg L^{-1} (interquartile range [IQR] = 2.7–8.5), and three values were outside of 1.5 times the 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 station 50 m downstream of the smolt wheel (50.0, 43.5, and 47.4 pg L^{-1}, respectively). These outliers were excluded from subsequent analyses as model results were no different with and without outliers (results not shown).

Nearly all qPCR replicates in the parr survey contained Atlantic salmon eDNA (83%, $n=783$), with concentrations ranging from no detections to 1131.6 pg L^{-1} (Table 1). The median concentration was 8.32 pg L^{-1} (IQR = 2.1–19.7). We removed three outliers as above; these values happened to be the three technical replicates for one field sample (1131.6, 1011.1, and 929.3 pg L^{-1}; site = TF, distance = 250 m, Figure 1). We 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 = 21.2 and 25.9 pg L^{-1}, respectively). Although we infer there was minimal cross-contamination throughout the sampling period as these two negative controls were from the same day. As contamination origin was unknown, all samples from this day (August 7, 2020; $n=99$) were excluded.

3.2 | Spring environmental effects on eDNA concentration

In 2021, the smolt outmigration occurred between April 29 and June 6. On May 18, 2021, a total of 117 smolts were captured, representing the first peak in smolt outmigration, with a second peak on May 24 where 235 smolts were captured. Smolt captures throughout the rest of the smolt outmigration ranged between 1 and 50 smolts; only May 1 and 3 had no smolts captured (Parks Canada, unpublished data).

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 6-week spring smolt survey, the increase in mean temperature was associated with increased conductivity [$r_{(421)}=0.91$, $p<0.001$] and pH [$r_{(421)}=0.46$, $p<0.001$]. With increasing temperature came a decrease in rainfall [$r_{(421)}=-0.56$, $p<0.001$], and thus velocity [$r_{(421)}=-0.18$, $p<0.001$; Table 3].

Including the environmental covariates in the LMM substantially improved the eDNA-abundance relationship by explaining approximately half of the day-to-day variation in eDNA concentration as described by the random effect of sample day ($\sigma^2_{uni}=0.10$, $\sigma^2_{multi}=0.05$; Table S1). Scaling and centring the variables to a mean of 0 and a standard deviation of 1 allowed for direct comparison

TABLE 1 Summary statistics of environmental conditions and raw environmental DNA (eDNA) concentration for each smolt and parr surveys (mean $\mu \pm 1$ standard deviation σ).

	Smolt survey				Parr survey			
Parameter	Mean	Median	Min	Max	Mean	Median	Min	Max
eDNA concentration (pg L^{-1})	6.8 ± 6.8	5.3	0.0	53.0	16.1 ± 64.1	8.3	0.00	1131.6
Chlorophyll a (μg L^{-1})	0.7 ± 0.5	0.6	0.2	3.4	0.5 ± 0.2	0.3	0.1	1.1
Conductivity (μS cm^{-1})	20.7 ± 4.5	20.8	12.4	28.2	35.5 ± 7.4	37.4	11.1	44.8
pH (units)	7.2 ± 0.3	7.3	6.5	7.9	7.4 ± 0.2	7.5	6.7	7.8
Precipitation (mm)	10.8 ± 7.9	10.8	0.5	21.2	13.9 ± 10.8	10.9	0.0	33.6
Mean temperature (°C)	9.7 ± 3.4	9.4	5.1	16.2	18.4 ± 3.2	17.7	8.0	23.7
Velocity (m s^{-1})	0.4 ± 0.7	0.3	0.1	8.2	0.3 ± 0.5	0.2	0.0	0.9

TABLE 2 Summary statistics of mean river temperature and environmental DNA (eDNA) concentration adjusted with linear mixed models for both smolt and parr sampling periods (mean $\mu \pm 1$ standard deviation σ).

	Smolt survey				Parr survey			
Parameter	Mean	Median	Min	Max	Mean	Median	Min	Max
Adjusted eDNA concentration (pg L^{-1})	10.8 ± 14.6	5.9	0.2	118.8	12.0 ± 11.2	7.7	0.7	67.4
Mean temperature (°C)	9.7 ± 3.4	9.4	5.4	15.9	17.9 ± 2.9	17.0	13.5	21.9

TABLE 3 Pearson correlation coefficients for smolt abundance metrics, environmental DNA (eDNA) concentration, and environmental covariates in spring ($n = 423$).

	Variable	1	2	3	4	5	6	7	8	9	10	11
1	Sample day											
2	eDNA concentration	**−0.21**										
3	Chlorophyll	−0.07	−0.09									
4	Conductivity	**0.88**	**−0.23**	**−0.20**								
5	Distance	0.00	0.06	0.01	**0.25**							
6	Mean temperature	**0.97**	**−0.25**	−0.10	**0.91**	0.01						
7	pH	**0.45**	**−0.16**	−0.04	**0.64**	**0.45**	**0.46**					
8	Precipitation	**−0.52**	−0.01	**0.45**	**−0.51**	−0.01	**−0.56**	−0.07				
9	Velocity	**−0.17**	0.00	0.06	**−0.16**	−0.07	**−0.18**	**−0.17**	**0.20**	**0.17**		
10	Removed smolts	−0.04	**0.15**	**−0.21**	−0.01	0.00	0.01	**0.14**	**−0.36**	**−0.15**	**−0.11**	
11	Total smolts	**−0.95**	**0.29**	0.04	**−0.83**	0.00	**−0.91**	**−0.57**	**0.36**	**0.22**	**0.16**	0.07

Note: Bold values indicate a significant correlation ($p < 0.05$).

of the effect each covariate had on eDNA. Total smolt abundance was found to have the greatest effect on eDNA concentration ($\beta = 1.34$, 95% CI = [0.47, 2.21]), followed closely by mean temperature ($\beta = 1.18$, 95% CI = [0.23, 2.12]; Figure 2a). The temperature effect on eDNA concentration was nonlinear, presenting an initial increase in eDNA concentration with rising temperature; however, the rate of concentration increase slowed as temperature continued to rise ($\beta = -0.67$, 95% CI = [−1.12, −0.21]; Table S2, Figure 2a). Mean eDNA concentration was 0.9 pg L^{-1} at the minimum temperature and increased steadily with temperature until reaching the peak average of 15.8 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 10.2 pg L^{-1} (Figure 2c).

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 9-week parr survey, high temperatures were associated with an increase in chlorophyll [$r_{(514)} = 0.37$, $p < 0.001$] and a decrease in conductivity [$r_{(514)} = -0.21$, $p < 0.001$] and pH [$r_{(514)} = -0.39$, $p < 0.001$]; however, mean temperature decreased throughout the sampling period from 21.9 to 13.5°C (Table 2). An increase in rainfall as temperatures declined [$r_{(514)} = -0.58$, $p < 0.001$] was associated with increased river velocity [$r_{(514)} = 0.19$, $p < 0.001$; Table 4].

All of the smolts had migrated out of USR by the time, we began the parr survey, and only a small number (8) of anadromous adults had returned by the end of the sampling period; therefore, we measured eDNA of the remaining fish in the river consisting mostly of young-of-the-year and parr (Parks Canada, unpublished data). When we first explored the univariate relationship between eDNA concentration and time, we observed a potential breakpoint halfway through the sampling period with a marked drop in concentration after August 14 (Figure 3a). After adjusting for environmental covariates with LMMs (chlorophyll a, distance from primary pool, mean temperature and its quadratic term, total precipitation between sampling events, site (i.e., TF, BH, and PP), and water velocity; Figure 3b), the breakpoint was no longer apparent. After scaling and centring the variables to a mean of 0 and a standard deviation of 1, mean temperature appeared to have the greatest association with increased eDNA concentration ($\beta = 0.54$, 95% CI = [0.38, 0.71]), with a nonlinear relationship as eDNA concentration increased faster with rising temperatures ($\beta = 0.23$, 95% CI = [0.004, 0.45]; Figure 4b). Increased precipitation was associated with reducing eDNA concentration ($\beta = -0.25$, 95% CI = [−0.40, −0.09]; Table S4, Figure 2b).

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. The average river temperature was higher at the beginning of the summer sampling period, ranging from 20.9 to 21.9°C before dropping to range from 14.4 to 17.0°C after August 17 (Figure 4d). Parr eDNA concentration on July 29 was the highest, reaching a mean high of 22.0 pg L^{-1} at 20.9°C. Once the temperature dropped during the second, cooler part of the sampling period, mean eDNA ranged from 3.1 to 4.1 pg L^{-1} (Figure 4b).

3.3.2 | Seasonal effect on eDNA spatial distribution

We explored how eDNA concentration fluctuated over the course of the summer parr survey as parr abundance remained relatively constant. There was a significant decrease in eDNA concentrations at the end of the summer (August 27, $\beta = -1.61$, 95% CI = [−1.90, −1.31]; September 8, $\beta = -1.75$, 95% CI = [−2.05, −1.46]) compared to those at the beginning of the summer sampling period (July 15, $\beta = 2.81$, 95% CI = [2.60, 3.02]; Table 5). This pattern was also observed with the presence of the initial potential breakpoint in raw eDNA concentration from the primary parr survey (Figure 3a).

eDNA 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 5). In all but one of the side tributaries (15B), eDNA was detected on at least one sampling event (25B, July 15; 33B, August 27; 29B, August 27 and September 8; Figure 5, Table S5). Finally, we confirmed Atlantic salmon to be absent upstream from both waterfall barriers with zero eDNA detected.

4 | DISCUSSION

Our study took advantage of the natural distribution and migration of wild juvenile Atlantic salmon in a single, well-characterized river to address unknown seasonal impacts on the quantitative relationship between eDNA and fish population abundance and distribution. The results of our extensive sampling effort support that the dynamics underlying variability in freshwater eDNA quantification depend on environmental factors (Shogren et al., 2019; Tillotson et al., 2018; Wood et al., 2021; Yates, Glaser, et al., 2021). Specifically, we found that (1) the inclusion of environmental covariates in quantitative eDNA abundance models explained over half of the variation in eDNA concentration during spring smolt migration and (2) the environmental variation in the summer affected the relationships among environmental covariates and parr eDNA during the period of relatively constant parr abundance.

4.1 | Effect of local environmental factors in eDNA fish population assessment model

Our work extends previous studies by modeling eDNA concentration as a function of fish population abundance and environmental factors. The relationship between Atlantic salmon smolt abundance and ambient eDNA concentration was dramatically strengthened when our models incorporated environmental variables, which explained most of the day-to-day variation. The amount of eDNA should be proportional to the average eDNA produced by the number of individuals in a source region (Lacoursière-Roussel et al., 2016); however, the noise produced by dynamic interactions between eDNA and environmental factors can muddy this relationship (Barnes & Turner, 2016; Takahara et al., 2012). 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 ($R^2 = 0.02$) prior to the inclusion of environmental covariates ($R^2 = 0.21$; 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 our top models for the smolt survey did not include velocity and the environmental characteristics in our study differ from those in Shogren et al. (2019), our findings confirm the importance of including environmental covariates, specifically that of water temperature. Our linear mixed model for the spring smolt survey included the following covariates, in order of relative importance: mean river temperature (and its quadratic), total smolt abundance (i.e., smolts distributed throughout the river), number of smolts removed from the smolt wheel daily, and chlorophyll concentration. Although we did not include turbidity in our models due to USR being oligotrophic, it may be a factor of interest or surrogate variable depending on the ecosystem, target organism, and management goal (Gray et al., 2014). We emphasize that inclusion of ecologically relevant stream-specific characteristics significantly reduces variability in eDNA quantification in quantitative population models.

We observed nonzero eDNA concentrations after the smolt migration had ended. Similarly, the mixed models produced by Sepulveda et al. (2021) demonstrated significant positive eDNA concentrations when their target salmonid abundance was zero; moreover, they were unable to differentiate whether the resulting eDNA was due to low fish abundance or from upstream eDNA sources. As our sampling ended after there were no more smolts present in the river (Parks Canada, unpublished data), our results support that the adjusted eDNA concentration remaining could be due to eDNA produced by resident parr (Figure 4a,b), which is the only cohort present in the river after the smolts leave. Since 1983, Parks Canada has been conducting electrofishing surveys throughout the USR to quantify juvenile salmon densities. These extensive surveys have been the foundation for understanding parr recruitment, movement, and habitat use and demonstrated that salmon parr are found throughout the entirety of the river from the head of tide to the upper waterfall barriers on the Broad and 45 River branches (Parks Canada, unpublished data). Therefore, although we had a strong understanding of parr abundance for the river at large, we had no prior knowledge of their distribution throughout the river, and thus upstream distance of parr eDNA from the smolt sampling locations was unknown (Parks Canada, unpublished data). Had we

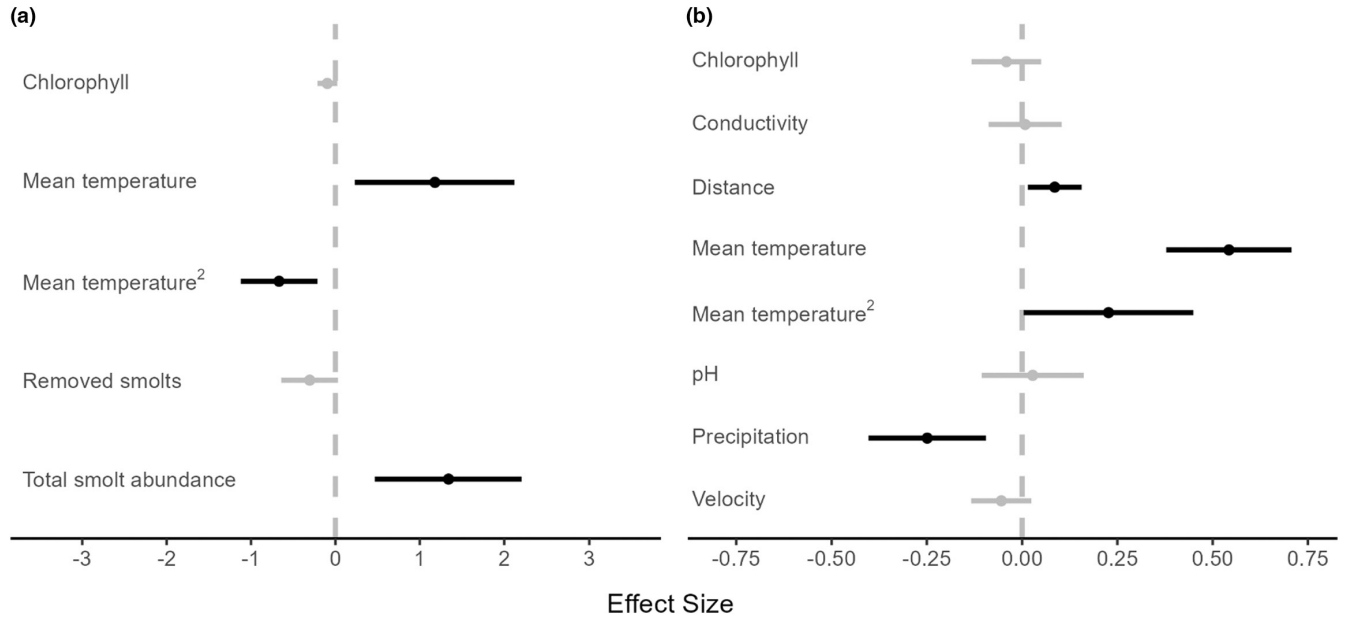

FIGURE 2 Effect sizes and 95% confidence intervals for scaled and centred ($\mu = 0$, $\sigma = 1$) covariates present in linear mixed models for each (a) smolt survey and (b) parr survey. Covariates in black depict significant positive or negative effect on environmental DNA concentration.

TABLE 4 Pearson correlation coefficients for summer environmental covariates and parr environmental DNA (eDNA) concentration ($n = 516$).

	Variable	1	2	3	4	5	6	7	8	9
1	Sample day									
2	eDNA concentration	**−0.66**								
3	Chlorophyll	**−0.37**	**0.27**							
4	Conductivity	**0.29**	**−0.17**	**0.14**						
5	Distance	−0.02	0.08	−0.02	**−0.09**					
6	Mean temperature	**−0.88**	**0.74**	**0.37**	**−0.21**	0.01				
7	pH	**0.40**	**−0.28**	−0.24	**0.64**	0.05	**−0.39**			
8	Precipitation	**0.60**	**−0.60**	−0.07	0.00	−0.01	**−0.58**	0.13		
9	Velocity	**−0.12**	−0.04	**0.28**	−0.06	**0.18**	0.07	**−0.16**	**0.19**	−0.05

Note: Bold values indicate a significant correlation ($p < 0.05$).

greater resolution of parr abundance at specific locations upstream of the smolt wheel, adding an additional parr abundance term to our model may have accounted for the residual eDNA.

As reviewed in Yates, Cristescu, and Derry (2021), appropriate parameterization of eDNA models is crucial to progress the integration of indirect eDNA methods in quantitative population models. Emerging research has been exploring allometric scaling to improve abundance relationships, which considers physiological changes proportional to body size in fishes (Stoeckle et al., 2021; Yates, Glaser, 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 1 and 2 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 for the USR.

4.2 | Multiple life-stage analyses: Seasonal effect on eDNA quantification

4.2.1 | Spring

Standardizing each LMM variable 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 behavioral change in fishes (Person-Le Ruyet et al., 2004). Although increased temperature can degrade eDNA faster, several studies have demonstrated increased eDNA shedding

at warmer temperatures (e.g., Jo et al., 2019; Klymus et al., 2015; Maruyama et al., 2014). For example, Lacoursière-Roussel et al. (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 (Jo et al., 2019; Klymus et al., 2015), 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 may slough more eDNA from 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). These myriad environmental and physiological factors may affect the relationships between eDNA and temperature 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 (Parks Canada, unpublished data). 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 & 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.

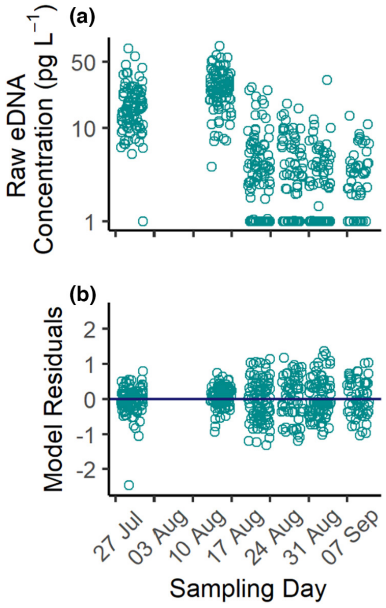

FIGURE 3 Relationship between raw parr environmental DNA (eDNA) concentration and sampling day presented from (a) univariate linear model (eDNA concentration ~ sampling day), and (b) model residuals of the linear mixed model with inclusion of environmental covariates (chlorophyll, conductivity, distance from primary pool in each reach, mean temperature and its quadratic, total precipitation between sampling events, site [The Forks, Black Hole, Pumphouse], and velocity; $n = 516$). Potential breakpoint in (a) is no longer present in model residuals (b).

4.2.2 | Summer

During the parr survey, higher eDNA concentrations were associated with increased temperature in the warmer, earlier part of the

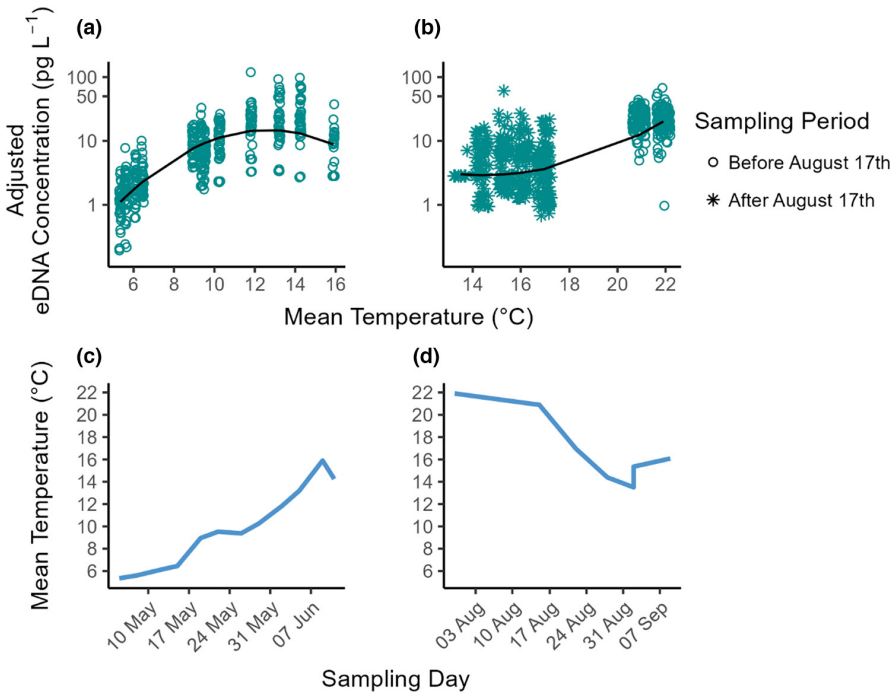

FIGURE 4 Effect of mean river temperature on environmental DNA (eDNA) concentration after inclusion of environmental variables in linear mixed models. All other variables were held constant to visualize the effect of temperature alone (a) during fluctuating smolt abundance in the spring, and (b) in the 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 summer sampling period (after August 17), while the change in mean temperature through each (c) spring, and (d) summer sampling period is depicted.

TABLE 5 Parameter estimates from linear mixed model with planned contrasts exploring the change in environmental DNA concentrations between the full transect sampling events at the beginning (July) and end of the summer sampling period (August, September; $n = 603$).

Variable				
Random effects	Variance			
Among field sites	0.58			
Within field replicates	0.15			
Among technical replicates (residual)	0.26		95% confidence interval	
Fixed effects	Estimate	Standard error	Lower bound	Upper bound
July	**2.81**	**0.13**	**2.60**	**3.02**
August	**−1.61**	**0.18**	**−1.90**	**−1.31**
September	**−1.75**	**0.18**	**−2.05**	**−1.46**

Note: July eDNA concentrations were significantly different from both August and September, indicated in bold (i.e., 95% CI not overlapping zero).

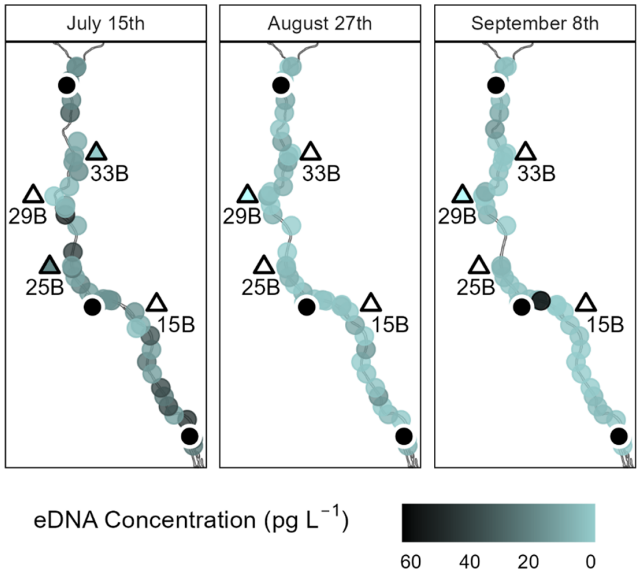

FIGURE 5 Upper Salmon River environmental DNA (eDNA) concentrations for the three summer-parr full transect sampling events. Black circles depict primary pool in each reach. Triangles indicate tributary sampling sites with relative eDNA concentrations.

summer before dropping and staying constant in late summer along with lower temperatures (Figure 4d). Takahara et al. (2012) demonstrated a significant positive correlation between water temperature and Common Carp (*Cyprinus carpio*) eDNA concentration in freshwater lagoons, postulating that carp were seeking habitats closer to their optimal temperature. It is possible that changes in parr behavior or microhabitat use might partly explain increases in eDNA concentration in the current study, in addition to potentially higher metabolic-induced shedding rates in warmer waters (Jo et al., 2019). Although generally preferring to occupy small home territories (McCormick et al., 1998), it has been noted in other systems that parr can move great distances (>1 km) to find cold water refuge habitats in the summer to avoid high temperatures (Corey et al., 2022). However, there is genetic evidence to support that parr in the USR do not move extensively among sites (Diesbourg, 2022) due in large part because the USR is primarily groundwater fed and therefore water temperatures rarely exceed 23°C (Parks Canada, unpublished data). In 2020, we measured water temperatures as high as 23.7°C during the summer parr survey, and although parr may not move great distances along USR (i.e., sites >3 km apart), metabolic demands increase with temperature as thermoregulatory plasticity has been observed in salmonids in response to acute thermal stress at 23°C (Corey et al., 2020; Dugdale et al., 2016). Thermal responses may force parr to feed and interact more aggressively or to congregate in refugia (Petty et al., 2012), thereby concentrating their eDNA further. Not only were temperatures high at the beginning of the sampling period, the low river flow and precipitation could magnify the physiological effects of temperature; thus, future studies would be needed to discern the specific metabolic or behavioral drivers of greater salmon eDNA shedding with temperature.

During the second, cooler part of the parr sampling period, increased precipitation was associated with a significant eDNA decrease. Secondary to the strong effect of temperature, Curtis, Tiemann, et al. (2021) found significantly lower concentrations of invertebrate eDNA with higher stream flows when species density was assumed to remain constant, which is congruent with the present study. Similar to Curtis, Tiemann, et al. (2021), our model supports that dilution might also explain the observed temporal reduction in eDNA. As parr abundance was assumed to remain relatively constant throughout the parr sampling period, correcting eDNA concentrations with flow rate may have explained more of the observed variation. For example, correcting for flow rate can improve correlations of eDNA and species abundance in some cases (e.g., sockeye salmon; Levi et al., 2019). The geomorphic complexity of USR limited our ability to obtain discharge measurements, though correcting our eDNA concentrations with discharge may have demonstrated more consistent eDNA concentrations throughout the summer. Nevertheless, our study emphasizes the risk that may arise in sporadic eDNA sampling to monitor a population. A stronger understanding of the ecological and physiological impacts of seasonal changes in temperature and precipitation on eDNA in natural streams is essential to avoid undesirable management actions from misinterpreting eDNA data.

Alternative hypotheses to the drop in temperature causing a sudden reduction in parr eDNA are redistribution of parr or a decline

in abundance. We feel that both of these are unlikely to explain the observed data. Parr redistribution from the main river stem is unlikely because of the patterns observed from our full river transects. The same pattern of reduced eDNA concentration was observed throughout the river for the later sampling dates, and eDNA concentrations present in the side tributaries were also low ($<1\,pg\,L^{-1}$; Table S5); as such, parr redistribution would have resulted in some sites increasing while others decreased, whereas we found a reduction at all sites and no increase in the adjoining tributaries. While we cannot categorically excluded a sudden drastic reduction in parr density (i.e., mass mortality event), we feel that it is also unlikely as declines in parr have generally been documented in association with prolonged high temperatures or anthropogenic changes to habitat (e.g., increased pollution; Wagner & Sweka, 2011). Mortality due to habitat destruction is unlikely as USR is within a nationally protected area which is less susceptible to anthropogenic impacts as non-protected areas (Le Saout et al., 2013). However, due to the lack of documentation around parr mortality, future research comparing eDNA concentrations to parr abundance would benefit from having multiple density estimates coinciding with eDNA sampling to corroborate whether changes in eDNA concentration are associated to changes in parr population size.

4.3 | eDNA spatial distribution assessment

4.3.1 | Can eDNA spatial distribution be used to define preferential population habitats?

The variation in eDNA concentration observed during the summer parr survey further underscores the importance of incorporating stream environmental variables into eDNA population models. Our model 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, chlorophyll, and conductivity. In contrast to the amount of variation explained by the multivariate models in the smolt survey ($R^2=0.60$, Table S1), our best environmental factor model for the summer parr survey accounted for substantially more of the parr eDNA variation ($R^2=0.85$; Table S3). Some of the variation among parr eDNA samples that we 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). Our model omitted three statistical outliers of very high eDNA concentration that were obtained from a single sample 250 m downstream from the most upstream pool reach (TF, Figure 1). While it is possible that this bottle captured eDNA from the six known adults that had returned from sea by this day, their distribution was unknown as their presence was determined from a passive integrated transponder (PIT) antenna at the river mouth (DFO, 2020, p. 10; Parks Canada, unpublished data). Furthermore, the amount of eDNA that they may have contributed is likely to be non-significant in contrast to the parr eDNA. 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. This 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 (Sepulveda et al., 2021; Shelton et al., 2019; Takahara et al., 2012). Increasing eDNA sample volumes from 1 to 2 L has been shown to improve eDNA detection in streams more than five times (Bedwell & Goldberg, 2020); however, although increasing water volumes can help to reduce sample heterogeneity, it can create challenges including increased 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 we observed in the parr survey was attributed to the eDNA variance between field replicates within each sampling occasion; however, high variation was also prevalent between qPCR technical replicates (Table S3). 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). 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 species 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 relative species abundance with confidence (e.g., where 1/3 indicates low and 3/3 indicates high abundance; Wood et al., 2021). Furthermore, modeling 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 fluviatulis*) and native Macquarie Perch (*Macquaria australasica*), consistent with their conventional monitoring results. Coherent with these published works, our results support the inclusion of all positive and nondetections in assessing eDNA dynamics in USR. 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.

4.3.2 | Downstream eDNA transport depends on dilution rate and proximity to source

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 (Fremier et al., 2019; Pont et al., 2018). Wood et al. (2020) first hypothesized 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. Although plume dynamics were apparent downstream from known numbers of caged Atlantic salmon (Wood et al., 2021), eDNA transport, and subsequent quantification may be obscured by hydrological factors when fish distribution is patchy or unknown. We detected eDNA throughout the entire 9 km length of USR in all three full transect sampling events during the summer; however, as observed in other studies (e.g., Laramie et al., 2015) downstream accumulation of eDNA was not equally apparent across all 3 days. The first full transect sampling event demonstrates some evidence of eDNA increasing with downstream distance, but it is unclear if this spatial pattern reflects higher parr densities in these locations (Figure 5). This pattern is not apparent in the final two sampling events. Factors such as higher stream flows can increase eDNA dilution, affecting its concentration and general detection (Curtis, Tiemann, et al., 2021). USR experienced increased rainfall over the second half of the parr sampling period, potentially contributing to the sustained lower concentrations that we observed in the final two full transect events. The significant negative effect of precipitation on eDNA for the primary parr survey supports this dilution effect, though we could not test this statistically for the full transect due to the lack of field replication at each sampling station. Finally, one explanation for the lack of plume dynamics in our findings is that although sampling occurred downstream of pool-riffle-run reaches, those areas were not necessarily preferred habitats for parr.

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 benthic zone until it is permanently sequestered or degraded (Jerde et al., 2016; Shogren et al., 2017). Based on these dynamics, 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 beyond the scope of 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 favor shorter spiraling 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 USR'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 | CONCLUSION AND RECOMMENDATIONS

Our study is one of few to investigate the impact of fluctuating seasonal environmental conditions on eDNA persistence in natural flowing waters (Curtis, Tiemann, et al., 2021; de Souza et al., 2016). The variability in environmental conditions across and within seasons emphasizes 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, we still documented considerable differences in eDNA concentration across the spring and summer seasons (i.e., smolt and parr eDNA studies). However, our findings provide encouragement that noninvasive eDNA-based quantification may be achieved with suitable study designs and environmental measures. Along these lines, we offer the following recommendations for future eDNA salmon population assessments:

1. *Increased eDNA field and qPCR replicates*: To minimize the stochasticity caused by heterogeneous eDNA distribution in the environment, we recommend increasing the number of qPCR technical replicates per sample (e.g., eight replicates). Furthermore, collecting samples at multiple downstream and lateral sites (i.e., midstream and bankside) may also reduce variation by increasing the probability of detecting plume dynamics (Wood et al., 2021).
2. *Annual sampling in multiple periods*: Our study showcases the seasonal variation in eDNA relationships in a single river (spring smolt versus summer parr). Consequently, we recommend that monitoring programs seek to standardize sampling at the same times and locations each year to reduce potential confounding variables related to life history and environmental phenology. We also present the importance of collecting water at a high frequency to monitor smolt migration. Due to the correlation between environmental conditions and seasonality, we 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*: We validated the importance of including stream-specific environmental variables in eDNA population models, particularly that of water temperature. We 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 our findings. Moreover, based on the clear importance of temperature to eDNA concentrations in our study, we reinforce calls by others for a need to integrate metabolism and allometric

scaling in eDNA inference across life stages as needed (Yates, Glaser, et al., 2021).

AUTHOR CONTRIBUTIONS

Anaïs Lacoursière-Roussel, Nellie Gagné, Francis LeBlanc, and Scott A. Pavey obtained funding and designed the study with Kurt Samways and Zachary T. Wood. Melissa K. Morrison, Anaïs Lacoursière-Roussel, Nellie Gagné, and Francis LeBlanc collected the data, while Melissa K. Morrison and Zachary T. Wood led data analysis. Melissa K. Morrison led manuscript writing, with Anaïs Lacoursière-Roussel, Scott A. Pavey, Zachary T. Wood, Marc Trudel, Kurt Samways, Nellie Gagné, Francis LeBlanc, Michael T. Kinnison, and Scott A. Pavey having made major contributions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data used in this study are available in the Dryad Data Repository.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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