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ORIGINAL ARTICLE

Monitoring and predicting the presence and abundance of juvenile Atlantic salmon in tributaries according to habitat characteristics using environmental DNA

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Abstract

Conservation of the Atlantic salmon *Salmo salar* requires to monitor the spatial distribution and abundance of juveniles at a local scale in tributaries. However, tributaries are rarely accounted for in monitoring programs despite their importance for juvenile life stages. This is mainly because inventories of young salmon populations in tributaries can be technically challenging with traditional methods, as the number of tributaries in a watershed can be important and their access limited compared to the main stem. In this study, we tested the use of environmental DNA (eDNA) to quantify the abundance of juvenile Atlantic salmon in tributaries. We successfully detected eDNA of juvenile Atlantic salmon in 19 tributaries of three main rivers of the Gaspé Peninsula (Québec, Canada) using quantitative real-time PCR analyses. By comparing the eDNA approach with electrofishing surveys conducted in parallel to water sampling, we found that eDNA concentrations positively correlated with juvenile abundance, total biomass, and body surface area. The use of the allometrically scaled mass (ASM) instead of abundance improved the correlation. Furthermore, we demonstrated that the levels of eDNA molecules detected for juvenile Atlantic salmon were also correlated with water temperature and canopy cover measured in each tributary. Finally, we tested if eDNA concentrations measured in a tributary could be used as a reliable indicator of juvenile abundance or biomass in that tributary. We found that our models slightly better predicted juvenile biomass than juvenile abundance. The use of ASM did not improve model prediction, suggesting that further refinement would be required in the future. Our method will facilitate the implementation of conservation practices appropriate to the ecology of juvenile Atlantic salmon in tributaries.

KEYWORDS
environmental DNA, juvenile Atlantic salmon, tributaries

†Deceased: Louis Bernatchez.

Chloé Suzanne Berger and Sabrina Gagnon contributed equally to this work.

1 | INTRODUCTION

The Atlantic salmon *Salmo salar* has a special place in human society as a culturally valuable fish (Verspoor et al., 2008). It historically supports commercial, sustenance, and recreational fishery activities, while also bearing an important value for indigenous peoples for food, social, and ceremonial purposes (Thorstad et al., 2021). Considerable efforts have been dedicated to its conservation and management in North America where Atlantic salmon populations have been decreasing since the early 90s (Verspoor et al., 2008). In the USA and Canada, Atlantic salmon is listed as endangered under the US Endangered Species Act and Canada's Species at Risk Act (Thorstad et al., 2021). This decline has been concomitant with physical and biological changes in Northwest Atlantic ecosystems. For example, shift in Atlantic salmon populations appeared to have been triggered by sequential changes in the North Atlantic Oscillation (NAO) and in salinity after 1988, which subsequently affected lower trophic-level biological characteristics (Mills et al., 2013).

Most populations of Atlantic salmon are anadromous, with a juvenile phase in freshwater, followed by smoltification and a long migration to the ocean for feeding and growth, and a return migration to freshwater to spawn (Aas et al., 2010). It has been suggested that human activities could explain stock declines, including dam construction, pollution, or marine overexploitation (Dadswell et al., 2022). In its juvenile freshwater phase, the species is highly sensitive to anthropic disruptions in its habitat quality and quantity, induced by water regulation with dams, channelization, intensive agriculture, forestry, or activities causing substrate removal or sedimentation (Thorstad et al., 2021). Finally, it has been proposed that climate change and water warming might directly and indirectly influence the abundance and productivity of North American Atlantic salmon populations (both in their marine adult and freshwater juvenile phases), for example, by inducing changes in prey availability (Mills et al., 2013).

Social–ecological measures have been adopted to counteract declines in stocks of Atlantic salmon populations, such as mitigation measures in recreational fisheries and commercial fishing closures, and stocking to supplement early life stages (Arlinghaus et al., 2007). Although these measures facilitated recovery in some Atlantic salmon stocks, certain populations remain endangered (Cote et al., 2021). While fisheries and global warming were assumed as main drivers of the decline of fish population, the influence of environmental factors at a more local scale may be greater than previously expected (Nicola et al., 2018; Otero et al., 2011). As such, it has been suggested that Atlantic salmon population monitoring should take into account local environmental fluctuations in rivers, as it could be a key to the maintenance of the species (Friedland et al., 2009; Jonsson & Jonsson, 2017; Nicola et al., 2018).

At a local scale, river tributaries are critical for juvenile stages as they can provide better feeding conditions than the main rivers (Shustov et al., 2012), refuges against higher water temperature and flow (Erkinaro et al., 1998; Sutton et al., 2007), as well as reduced predation risks (Crabbe, 2000). Despite the importance of tributaries for juveniles, they are rarely considered in monitoring programs. This can be explained by the fact that inventory efforts are often concentrated on large freshwater and marine sites, for which recreational and commercial fishing activities are important (Dempson et al., 2004). Also, performing inventories of young salmon populations in tributaries can be technically challenging with traditional methods (such as electrofishing or seine), as the number of tributaries in a watershed can be important and their access limited compared to the main stem, particularly for small tributaries at the head of the basin. Furthermore, these methods are invasive, time-limited (e.g., dependent on hatching of eggs and season), and time-consuming (Rees et al., 2014). In this context, there is a need for a noninvasive method that would allow to counteract all these challenges, and to monitor the spatial distribution and abundance of juvenile Atlantic salmon at a local scale in tributaries.

The development of environmental DNA (eDNA) offers new possibilities to monitor species in ecosystems (Taberlet et al., 2018). This technology refers to the detection of molecules released by organisms in their environment soil (Roh et al., 2006), air (Lynggaard et al., 2022), or water (Ficetola et al., 2008), from skin cells, mucus, metabolic waste, or gonads (Lodge et al., 2012). Using specific quantitative real-time PCR (qPCR) primers and probe, it is possible to assess the presence or absence of a targeted species (Taberlet et al., 2018). eDNA can also sometimes positively correlate with species abundance and/or biomass (Coulter et al., 2019; Gaudet-Boulay et al., 2023; Lacoursière-Roussel, Côté, et al., 2016; Lacoursière-Roussel, Rosabal, et al., 2016; Pilliod et al., 2013; Yates et al., 2019). This method was successfully applied in river systems to detect many fish species (reviewed in Rourke et al., 2022). For Atlantic salmon, the eDNA qPCR approach was previously used to detect its occurrence in marine waters (Shea et al., 2020, 2022) and in rivers (Fossøy et al., 2020). In rivers, it has been shown that eDNA concentrations of adult Atlantic salmon were markedly higher below migration barriers, reflecting the expected higher biomass/abundance of fish at these places (Fossøy et al., 2020). For juvenile Atlantic salmon, cage experiments in streams (Wood et al., 2020, 2021) showed that juvenile eDNA exhibited predictable plume dynamics downstream from sources, with eDNA being initially concentrated and transported in the midstream, but eventually accumulating in stream margins with time and distance (Wood et al., 2021). Furthermore, a recent study demonstrated that eDNA of juvenile Atlantic salmon could be detected in a single river, that eDNA was highly correlated with spring smolt abundance, and that environmental covariates affected eDNA concentrations (Morrison et al., 2023). To our knowledge, the qPCR method has not been used yet to detect juvenile Atlantic salmon eDNA in small tributaries of rivers.

The objective of this study was to evaluate at a local scale the efficiency of the eDNA method to detect juvenile Atlantic salmon presence and abundance in river tributaries. The study was conducted in 19 tributaries of three main rivers of the Gaspé Peninsula, Québec, Canada: The Dartmouth, the York, and the St-Jean rivers.

The three rivers are popular for their Atlantic salmon recreational fishing activities. The Dartmouth River is the smallest of the three rivers (63 km long). The York River is 98 km long, while the St-Jean River is 121 km long with 44 km opened to angling. First, we tested the efficiency of the qPCR eDNA method to quantify the abundance of juvenile Atlantic salmon in tributaries. Second, the results of the eDNA analysis were compared to abundances obtained by electrofishing in parallel to water sampling. We aimed at determining if eDNA concentrations correlated with biotic metrics (juvenile abundance, total biomass, or body surface area). Third, we tested if site-specific environmental characteristics could influence the levels of eDNA molecules we detected. Finally, we tested if eDNA concentrations measured in one tributary could be used as a reliable indicator of juvenile abundance or biomass (Figure 1).

2 | MATERIALS AND METHODS

2.1 | Collection of water samples

Water samples were collected in the tributaries of three river networks: the Dartmouth River (48° 52′ 51″ N, 64° 33′ 20″ O), the York River (49° 00′ 38″ N, 65° 26′ 07″ O), and the St-Jean River (48° 54′ 27″ N, 65° 33′ 13″ O) in Gaspé, Québec, Canada. A total of 19 tributaries from these three rivers were sampled over 2 years: Six tributaries were sampled in July and August of 2019, while 13 tributaries were sampled in July and August of 2020 (Figure 2, Table 1). Each year, sampling was performed after the expected period of fry emergence from the river bed (Bernatchez & Giroux, 2000). We therefore expected sampling sites to be potentially occupied by fry and parr juveniles, which are known to stay between two and 5 years in rivers before performing their first migration to saltwater (Bernatchez & Giroux, 2000). In each tributary, six bottles of 2 L water were collected on the tributary as close as possible from its confluence with the main river (Figure 1). At each location, water temperature, and pH were recorded just after water sampling using a Hanna HI 98130 pH meter. Bottles were stored (<12 h) in a refrigerated backpack with ice away from light to avoid DNA degradation. Water filtration using a vacuum pump with 1.2 µm GF/C glass microfiber filter (Whatman, 47 mm) was performed within 12 h directly in the field. In addition, a field negative control using demineralized treated water was processed in the same way as the real samples for each filtration batch, for a total of 19 field negative controls. Bottles and all filtration instruments had been previously bleached with 10% chlorine during 30 min, rinsed with demineralized treated water, and UV-treated.

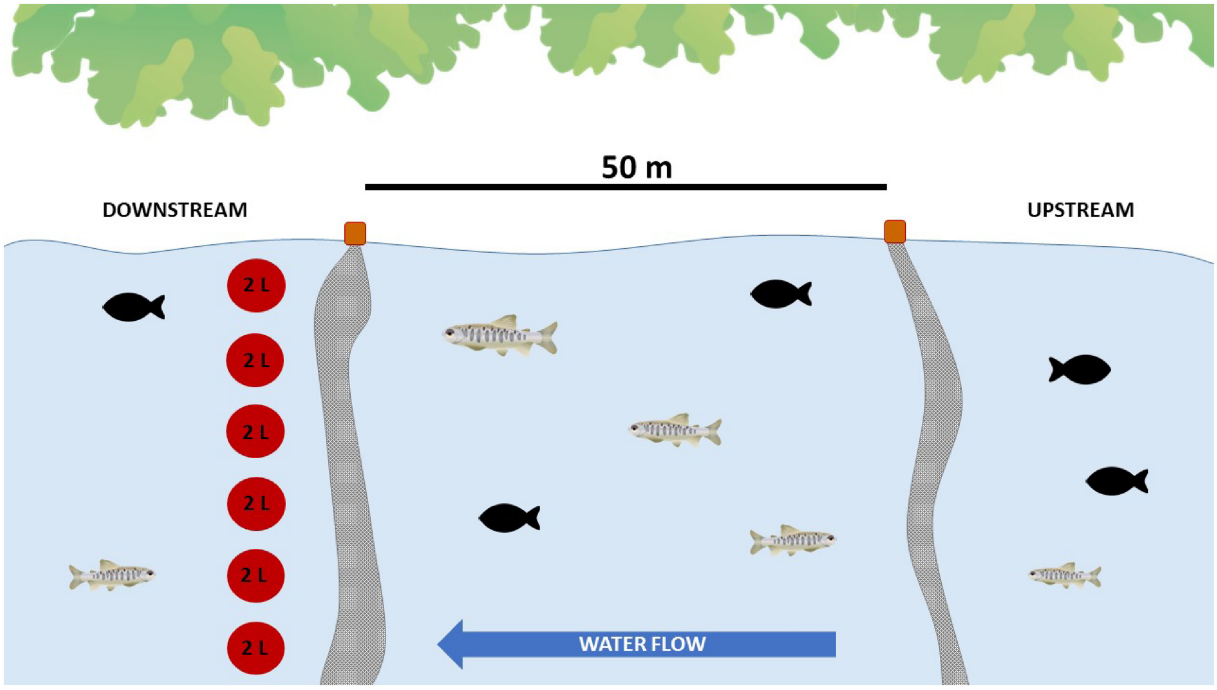

FIGURE 1 Sampling design applied to 19 tributaries of three rivers in Gaspé (Québec, Canada) to explore four research objectives: (1) Testing the efficiency of the qPCR eDNA method to quantify the abundance of juvenile Atlantic salmon in tributaries. (2) Determining if eDNA concentrations correlated with juvenile abundance, total biomass or body surface area. (3) Testing if site-specific environmental characteristics influenced the levels of eDNA detected molecules. (4) Testing if we could predict juvenile abundance or biomass from eDNA measured in tributary. In each tributary, six bottles of two liters of water were sampled for eDNA analyses (red circles) at a transect located close the confluence with the main river. After water sampling, electrofishing was immediately conducted in a 50 m-long × wetted width-wide closed-plot located just upstream from the eDNA sampling location. Fishing was performed three times, with a 15 minute pause between each pass. Images were taken from IAN Symbol libraries (references for images *Salmo salar*: Claire Sbardella, Tree: Tracey Saxby, Integration and Application Network, https://ian.umces.edu/media-library/).

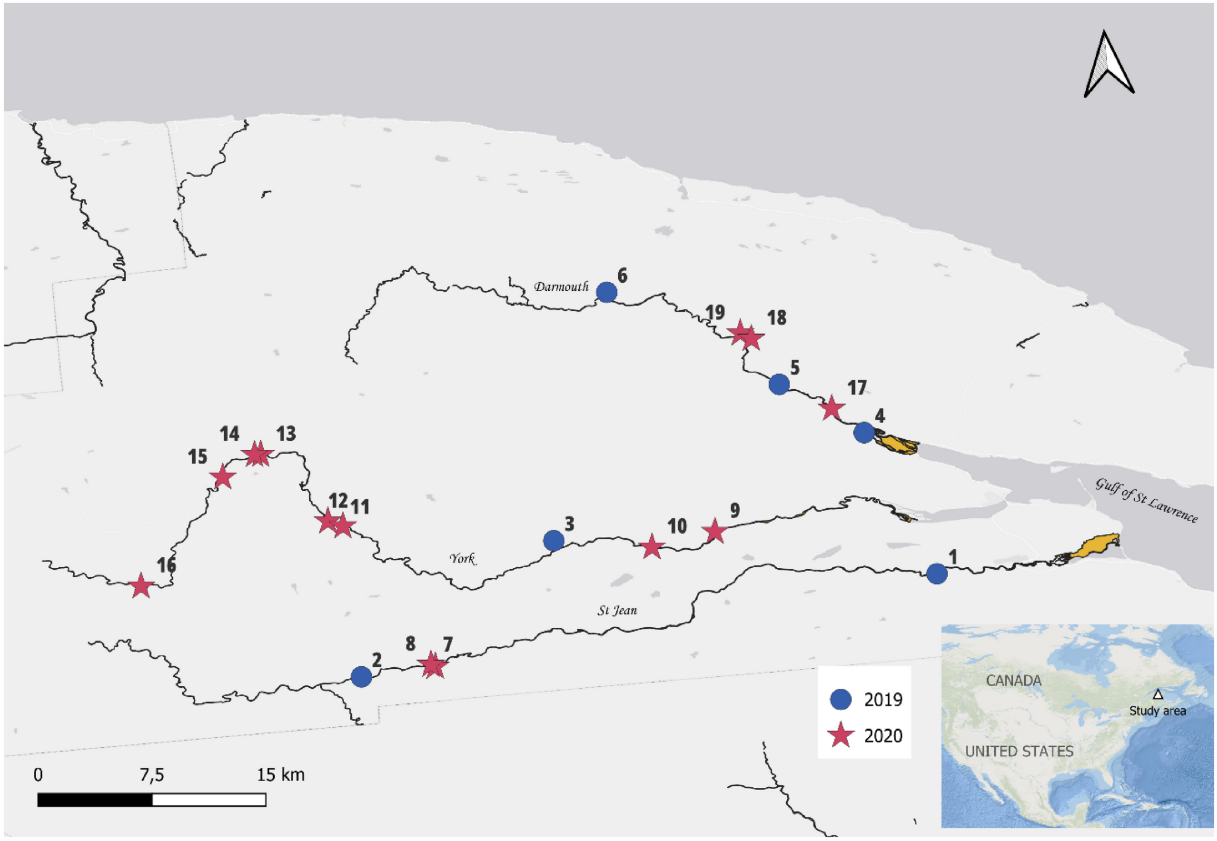

FIGURE 2 Location of the 19 tributaries sampled by eDNA and electrofishing in 2019 and 2020 in the Gaspé area (Québec, Canada). Six tributaries were sampled in 2019 and 13 tributaries were sampled in 2020. Number of each tributary refers to the ID of Table 1. The connection of the river with the Gulf of St Lawrence is indicated in yellow.

In total, 133 filters were stored individually in a 5 mL tube and frozen at −20°C until DNA extraction.

2.2 | Electrofishing surveys

Immediately after eDNA sampling, a three-pass electrofishing (Apex Backpack Electrofisher, Smith-Root Inc.) removal strategy was used to estimate juvenile salmon abundance in a 50 m-long × wetted width-wide closed-plot located just upstream from the eDNA sampling location (Figure 1). Juvenile salmon caught in each pass were used to estimate abundance using the Leslie depletion method (Leslie & Davis, 1939). After each pass, water temperature was measured in the center of the plot, juvenile Atlantic salmon were counted, and individuals were measured to the nearest 0.01 cm (fork length) and weighed to the nearest 0.01 g. All fish were then released in the tributary, outside of the sampled plot.

2.3 | Habitat characteristics of sampled plots

After fish sampling was completed and blocking nets removed, physical habitat characteristics were measured at each plot. Water depth D (m) ($n = 50$) and mean flow velocity V (m/s) ($n = 30$) were measured at a regular grid spanning the length and width of the plot, with a meter stick and an electromagnetic flow velocity meter (Marsh-McBirney Flo-mate), respectively. Median bed particle size D50 (cm) was obtained from a minimum of 100 particles sampled using the Wolman pebble count method (Wolman, 1954). Relative submergence D/D50 was calculated from mean water depth and D50 values. Mean width W was obtained from five equally spaced measures of wetted width.

Canopy cover was visually estimated to the nearest 10% by two observers standing alongside the plot (Watz et al., 2019). Four classes of shading cover percentage above the tributary (Table 2) served as a proxy to estimate the potential impacts of UV lights on eDNA presence into the water.

The length (m) of salmon habitat upstream of each plot was calculated as the distance measured along the tributary between the location of the plot and the upstream limit of salmon dispersal identified on each tributary from electrofishing surveys conducted by our team in 2018 and 2019 to identify such limit. This variable was added to account for a possible contribution of upstream salmon population to the level of eDNA measured at the downstream end of the tributary. It has indeed been reported in the literature that eDNA can be detected as far as 12–60 km from its source (Deiner & Altermatt, 2014; Pont et al., 2018).

TABLE 1 List of the 19 tributaries that were sampled with eDNA and electrofishing in Gaspé (Québec, Canada) during summer 2019 and 2020.

ID	Tributary	Main river	Latitude	Longitude	Year	Catch (*n*)			Total estimated abundance (*n*)
						Fry	Parr	Total	
1	Chesnaye	St Jean	48.76864	−64.546461	2019	0	27	27	33
2	Sirois	St Jean	48.721898	−65.066143	2019	42	63	105	157
3	Mississippi	York	48.827761	−64.884594	2019	0	3	3	4
4	Watering	Dartmouth	48.899766	−64.601813	2019	0	37	37	59
5	Salmon Hole	Dartmouth	48.948991	−64.672034	2019	24	107	131	209
6	Jean Louis	Dartmouth	49.055640	−64.814334	2019	18	49	67	95
7	Island	St Jean	48.725429	−64.999709	2020	0	29	29	34
8	OS3d_os3_1	St Jean	48.727587	−65.001866	2020	120	4	124	235
9	La Petite Fourche rfp	York	48.823288	−64.740582	2020	14	94	108	131
10	Baillargeon	York	48.814389	−64.797827	2020	7	57	64	70
11	Pine Hill	York	48.859033	−65.078222	2020	0	14	14	30
12	OS5_os2_11	York	48.868713	−65.087574	2020	0	8	8	8
13	OS5_os2_15	York	48.926749	−65.138362	2020	0	22	22	26
14	Du Basque	York	48.927366	−65.143953	2020	0	35	35	42
15	OS3f_os3_1	York	48.909404	−65.172985	2020	0	53	53	55
16	Oatcake	York	48.819499	−65.255625	2020	221	67	288	530
17	La Petite Fourche pf	Dartmouth	48.923508	−64.627268	2020	23	67	90	145
18	OS5_os1_15	Dartmouth	48.991582	−64.693228	2020	54	33	87	90
19	Bechervais	Dartmouth	48.997346	−64.702725	2020	1	3	4	4

Note: For each tributary is indicated its main river, GPS coordinates and sampling year. The number of catch (fry, parr, and total) as well as the total estimated abundance is indicated for each tributary.

TABLE 2 Definition of four classes to estimate the level (%) of shade above each tributary.

Class	Shade on the tributary (%)	Potential impact of UV lights on eDNA detection
0	0	Very high
1	1–25	High
2	26–50	Moderate
3	51–75	Low
4	76–100	None

Note: Shade on each tributary was determined visually with the help of two observers according to a method developed by Watz et al., 2019.

2.4 | eDNA extraction

eDNA extraction was performed using a QIAshredder and DNeasy Blood and Tissue Kit (Qiagen) according to a previously developed protocol (Goldberg et al., 2011). To avoid external DNA contamination, extractions were performed under a UV hood with the surface washed with DNA away and exposed to UV light during 30 min before each extraction batch. Instruments were sterilized with 10% sodium hypochlorite and exposed to UV light for 30 min before use. The extracted eDNA was stored at −20°C until amplification by the qPCR method. For each extraction batch, an extraction negative laboratory control was added to account for possible contamination.

2.5 | qPCR amplification

To analyze samples, we used specific primers and probe that targeted a 205 base pair sequence within the cytochrome oxidase I mitochondrial DNA (COI mtDNA) region that is specific to the Atlantic salmon *Salmo salar* (Supp Table 1). Primers and probe specificity and efficiency had been previously validated (Hernandez et al., 2020). To quantify Atlantic salmon DNA in each sample, we used the TaqMan qPCR method with the addition of SPUD to the reaction. SPUD is a universal system to control for the presence of inhibitors in the qPCR reaction (Nolan et al., 2006). The amplification was performed in a final volume reaction of 20 µL including 10 µL of Environmental Master Mix 2.0 (Life Technologies), 1.8 µL of each Atlantic salmon primer (10 µM), 0.5 µL of Atlantic salmon probe (10 µM), 1.2 µL of each SPUD primer (10 µM), 0.5 µL of SPUD probe, 1 µL of SPUD template, and 2 µL of extracted DNA following these conditions: 2 min at 50°C, 10 min at 95°C, 50 cycles of 15 s at 95°C, and 60 s at 60°C. A standard curve of known DNA quantities was added on each plate, which allowed us to quantify positive PCR amplification in number of

molecules. For each sample as well as field and extraction controls, the DNA presence of the Atlantic salmon was tested on six technical replicates. Following amplification, all negative values were removed from further analyses.

2.6 | Statistical analyses

All statistical analyses were conducted with the R software version 4.2.2.Potential differences between tributaries based on local environmental conditions at the time of sampling were assessed using a principal component analysis (PCA) biplot of individuals (nine tributaries) and explanatory variables (temperature (°C), water depth (m), flow velocity (m/s), plot area (m^2), sediment size D50 (cm), vegetation cover (classes defined in Table 2), pH, tributary width (m), relative submergence (m), and the length of the tributary known to be used by salmons upstream of the sampled plot (m)).

The juvenile Atlantic salmon total biomass (g) in each tributary was obtained by calculating the weight sum of all salmon juveniles caught in the plot. The mean body surface area (cm^2) of captured juveniles in each tributary was calculated using the formula: $S = 0.72\,L^{1.88}$ with L = fork length (O'Shea et al., 2006). We used the nlme package to create linear mixed models and test if the eDNA levels detected in each tributary correlated with (1) salmon abundance (Leslie) (2) total biomass (g) (3) mean body surface area (cm^2) and (4) ASM or allometrically scaled mass (ASM) of juveniles. The last metric was developed by Yates, Glaser et al. (2021) to account for the relative decline in mass-specific eDNA production rates typically observed as individual organismal mass increases. We wanted to test if ASM could improve our model of abundance and eDNA concentrations. For ASM, we used the formula: Σ(Juvenile mass measured in one tributary $(g)^{0.73}$)/tributary width (m). We created four distinct lme models, using either "salmon abundance," "total biomass," "mean body surface area," or "ASM" as a fixed factor, and "tributary" and "bottle" as random effects. For each analysis, we checked homoscedasticity by plotting residuals and fitted data. We checked for homogeneity of variances using a Levene Test. We tested normality of residuals using graphical assessment (QQ-plot and normal distribution histogram) and a Shapiro–Wilk test. eDNA data were log transformed to fit assumptions.

To test for the potential effects of environmental factors on detected eDNA concentrations, we performed model selection with the help of the Akaike's information criterion (AIC). Using the AICcmodavg package, we first included all environmental variables, then removed redundant variables and tested a subset of models. We ran lme analyses using the parameters identified during model selection. eDNA data were log transformed to fit assumptions as previously.

To evaluate the performance of the model(s) selected, we measured how well the eDNA level predictions made by the model(s) matched the eDNA levels detected. We used the caret package with the leave-one-out cross-validation (LOOCV) method.

Finally, we determined to which extent the selected model(s) could be used to predict fish abundance or biomass, based on known eDNA concentrations. We also tested if the model(s) could more accurately predict ASM compared to fish abundance. We first generated predicted values (respectively for abundance, biomass, and ASM) based on the model(s) previously selected using the ggeffects package from R. We then checked the correlation between predicted and observed values in this study using a Spearman Correlation.

All data generated are available in article supplementary material.

3 | RESULTS

3.1 | Electrofishing and abundance estimates

The number of juvenile salmon caught by electrofishing was highly variable between tributaries, with a mean of 68 juveniles/site across sites, a minimum of three fish at site Mississippi (ID: 3, York River) and a maximum of 288 fish at site Oatcake (ID: 16, York River) (Table 1). Overall, parr ($n = 772$) were more abundant than fry ($n = 524$). At least one parr was caught at each site compared to fry being caught in only 10 of the 19 sites. At three sites, fry represented a higher proportion of fish caught than parr: 97% at site OS3d_os3_1 (ID:8, St-Jean River), 77% at site Oatcake (ID:16, York River), and 62% at site OS5_os1_15 (ID: 18, Dartmouth River).

Abundance estimates obtained with the Leslie method ranged from 4 to 530 juveniles/site with a mean of 103 juveniles/site (Table 1).

3.2 | Negative controls

All field and laboratory extraction negative controls showed no positive amplification indicating the absence of contamination during sample preparation, extraction, and amplification. During qPCR, SPUD controls confirmed the absence of inhibitors in nucleic acid preparations, except for one sample (ruisseau Bechervais bottle 5) that was removed. The results could therefore be taken with confidence for further analyses.

3.3 | Assay sensitivity

To evaluate assay sensitivity, a standard curve experiment was previously performed for *Salmo salar* (Hernandez et al., 2020). Briefly, a synthetic DNA template (Integrated DNA Technologies Inc.) including the target amplicon sequence was designed from the COI, and used to estimate by serial dilution the detection limit of the primer pair SASA_COIF and SASA_COIR, that is, until the fluorescence signal corresponding to one molecule is reached (Forootan et al., 2017). From the stock, diluted at 1.00E + 10 copies/µL, a nine-level dilution series (2000, 1000, 500, 100, 20, 8,

4, 2, and 1 copies per reaction) was prepared in a sterile yeast tRNA (10 μg/μL) solution. Ten replicates of each dilution were run to determine, for the pair of primers and probe, the amplification efficiency and the limit of detection (LOD) defined as the lowest standard concentration at which 95% of technical replicates amplify (Bustin et al., 2009; Klymus et al., 2020). This standard curve approach demonstrated that the amplification efficiency for *Salmo salar* assay was 98.7% and that the LOD was two copies by reaction with a R^2 of 0.969 (Hernandez et al., 2020).

To confirm the LOD threshold, we applied on our dataset the statistical method developed by Klymus et al., 2020, using the Ct (Cycle threshold) and SQ (expected standard concentration) values of all the standard curves we generated. The R Script used is available at: https://github.com/cmerkes/ (Klymus et al., 2020). The concentration ranged between 100,000 and 10 copies per reaction, with three replicate standards per concentration. Analysis was performed with the curve-fitting modeling approach, and the specific criteria for LOD probability of detection was applied at 95. Using this approach, we found that the LOD was <10 copies by reaction, which corresponds to the lowest concentration tested. In accordance with previous finding, the LOD value was set at two copies by reaction. In our dataset, two sample eDNA values were below the LOD: mississipi-2 (Mississippi tributary, York River, bottle 2) at 0.4 copies and Bechervais-4 (Bechervais tributary, Dartmouth River, bottle 4) at 0.9 copies. For these two samples, there is less than a 95% chance of detecting the target DNA sequence even if it is present at this low concentration. We therefore decided to keep these two samples for qualitative analysis (presence/absence) of *Salmo truta*, but to remove them from all quantitative, statistical analyses, as suggested by Klymus et al., 2020.

3.4 | Habitat characteristics

Using PCA biplot, we found significant differences between tributaries of each sampled river (Dartmouth, York and St-Jean) based on local environmental conditions at the time of sampling (Figure 3). Based on water temperature, pH, water flow, and vegetation cover, the tributaries from the Dartmouth River tended to cluster apart from the tributaries of the York and Saint-Jean rivers, which were more similar to each other. Generally, tributaries from the Dartmouth River were characterized by warmer water temperature and lower pH levels at the time of sampling, as opposed to tributaries of the York and St-Jean rivers. Sirois tributary (ID: 2, St-Jean River) was mostly characterized by higher flow velocities at the time of sampling, which were the fastest measured in this study. OS5_os1_15 tributary (ID: 18, Dartmouth River) was mostly defined by its abundant vegetation cover. All other environmental variables (water depth, plot area, sediment size, tributary width, relative submergence, and upstream length of salmon habitat) did not contribute significantly to site differentiation.

3.5 | Detection of juvenile Atlantic salmon using eDNA in tributaries

Using qPCR, we detected eDNA molecules from juvenile Atlantic salmon in all tributaries sampled (Figure 4, Supp Table 2). The highest detection was measured in 2020 in the Dartmouth River, at the La Petite Fourche pf tributary (ID: 17, Dartmouth River), while the lowest detection was measured in 2019 in the York River, at the Mississippi tributary (ID: 3, York River). Tributaries for which high levels of eDNA molecules were detected tended to geographically cluster: Island (ID: 7, St-Jean River) with OS3d_os3_1 (ID: 8, St-Jean River) sampled in the St Jean River in 2020, La Petite Fourche rfp (ID: 9, York River) with Baillargeon (ID: 10, York River) sampled in the York River in 2020, and La Petite Fourche pf (ID: 17, Dartmouth River) with OS5_os1_15 (ID: 18, Dartmouth River) sampled in the Dartmouth River in 2020.

Using four distinct lme models, we found that eDNA of juvenile Atlantic salmon positively correlated with fish abundance (Leslie Method) ($R = 0.65$, $p = 0.04$*), fish total biomass (g) ($R = 0.82$, $p = 0.002$**), body surface area (cm^2) ($R = 0.81$, $p = 0.007$**), and ASM or allometrically scaled mass (ASM) ($R = 0.88$, $p = 0.009$**) (Figure 5). The weakest, but still significant, positive relationship was detected between eDNA concentrations and abundance (Figure 5a), while a strong positive relationship was detected between eDNA concentrations and juvenile biomass (Figure 5b) and surface area (Figure 5c). Using ASM allowed improving the model fit for abundance and eDNA concentrations. As such, the strongest positive relationship was observed between eDNA concentrations and ASM (Figure 5d).

3.6 | Influence of environmental factors on the levels of eDNA molecules detected

Following model selection with the AIC criterion, we found that two models were best-fitted, carrying, respectively, 52% and 31% of the cumulative model weight. The first model included "ASM," "temperature," and "vegetation" as fixed factors, and "tributary" and "bottle" as random effects. The second model included "biomass," "temperature," and "vegetation" as fixed factors, and "tributary" and "bottle" as random effects (Table 3). Using lme analyses, results from the first model suggested that detected eDNA concentrations were mostly influenced by temperature ($p = 0.03$*) (Table 3 model 1), while results from the second model highlighted the influence of both temperature ($p = 0.049$*) and vegetation cover ($p = 0.01$*) on detected eDNA concentrations (Table 3 model 2). For all models, temperature and vegetation cover both positively influenced the levels of eDNA molecules detected.

We evaluated the performance of the two selected models by measuring how well the eDNA level predictions made by the models matched the eDNA levels detected using a leave-one-out cross-validation approach. For each model, we found a good correlation between the eDNA predictions made by the model and the

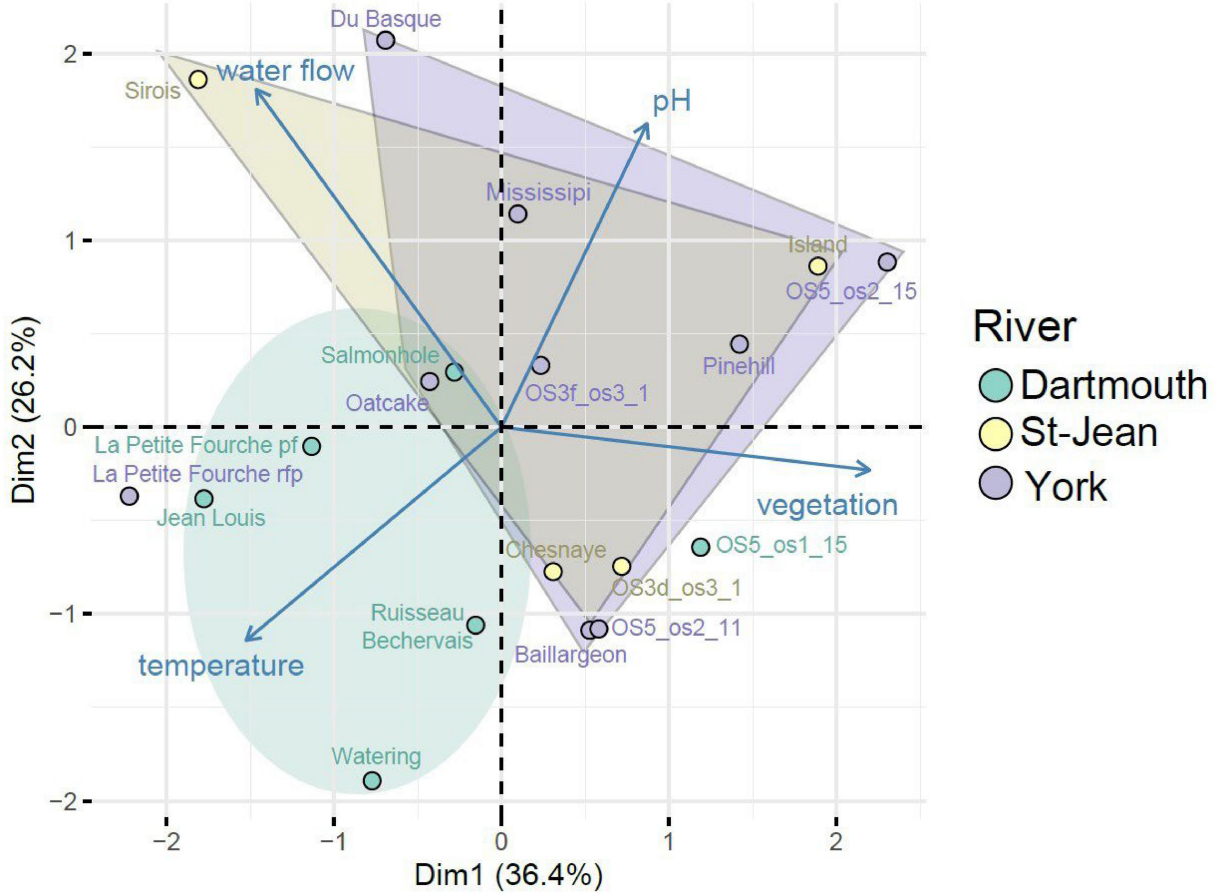

FIGURE 3 Tributaries from the Dartmouth River tended to cluster apart from the tributaries of the York and St-Jean rivers. They were characterized by warm water and low pH at the time of sampling, as opposed to the tributaries of the St-Jean and York rivers. Principal component analysis (PCA) biplot was performed using individuals (nine tributaries) and four explanatory variables (temperature (°C), pH, water flow (m/s), and vegetation cover (four classes)) that best explained differences between sampled tributaries. All other environmental variables did not contribute significantly to site differentiation.

actual eDNA observations (model 1: $R^2=0.4$, root mean squared error RMSE = 1.57 and mean absolute error MAE = 1.27; model 2: $R^2=0.54$, RMSE = 1.38 and MAE = 1.07).

Finally, we tested if the sampling year could have affected the eDNA concentrations measured. To do so, we included year as an additional parameter in the two selected models. For both models, we found that year did not influence the levels of eDNA molecules detected (model 1: $p=0.32$, model 2: $p=0.50$).

3.7 | Prediction of juvenile abundance, biomass, and allometrically scaled mass

Based on the models previously selected (accounting for "temperature" and "vegetation" as fixed effects and "tributary" and "bottle" as random effects), we tested if juvenile abundance (Leslie), juvenile biomass (g), and allometrically scaled mass (ASM) could be predicted based on known eDNA concentrations. For each of these three variables, we generated a range of predicted values depending on various concentrations of eDNA molecules (Figure Supp 1). We then tested for each variable the correlation between predicted values and observed values. We found that our models slightly better predicted juvenile biomass (Figure 6b, $R=0.59$, $p=0.009^{**}$) then juvenile abundance (Figure 6a, $R=0.56$, $p=0.013^{*}$) and ASM (Figure 6c, $R=0.46$, $p=0.05$).

4 | DISCUSSION

The use of environmental DNA to document the spatial distribution and abundance of juvenile Atlantic salmon in tributaries has not been tested yet. Here, we evaluated at a local scale the efficiency of the eDNA method to detect the presence and abundance of juvenile Atlantic salmon in 19 tributaries of three main rivers of the Gaspé Peninsula, Québec, Canada.

The qPCR approach was able to detect eDNA from juvenile Atlantic salmon in all tributaries sampled. In accordance with their geographical proximity, PCA biplot analyses showed that tributaries from the York and St-Jean rivers shared similar environmental conditions that were notably characterized at the time of sampling

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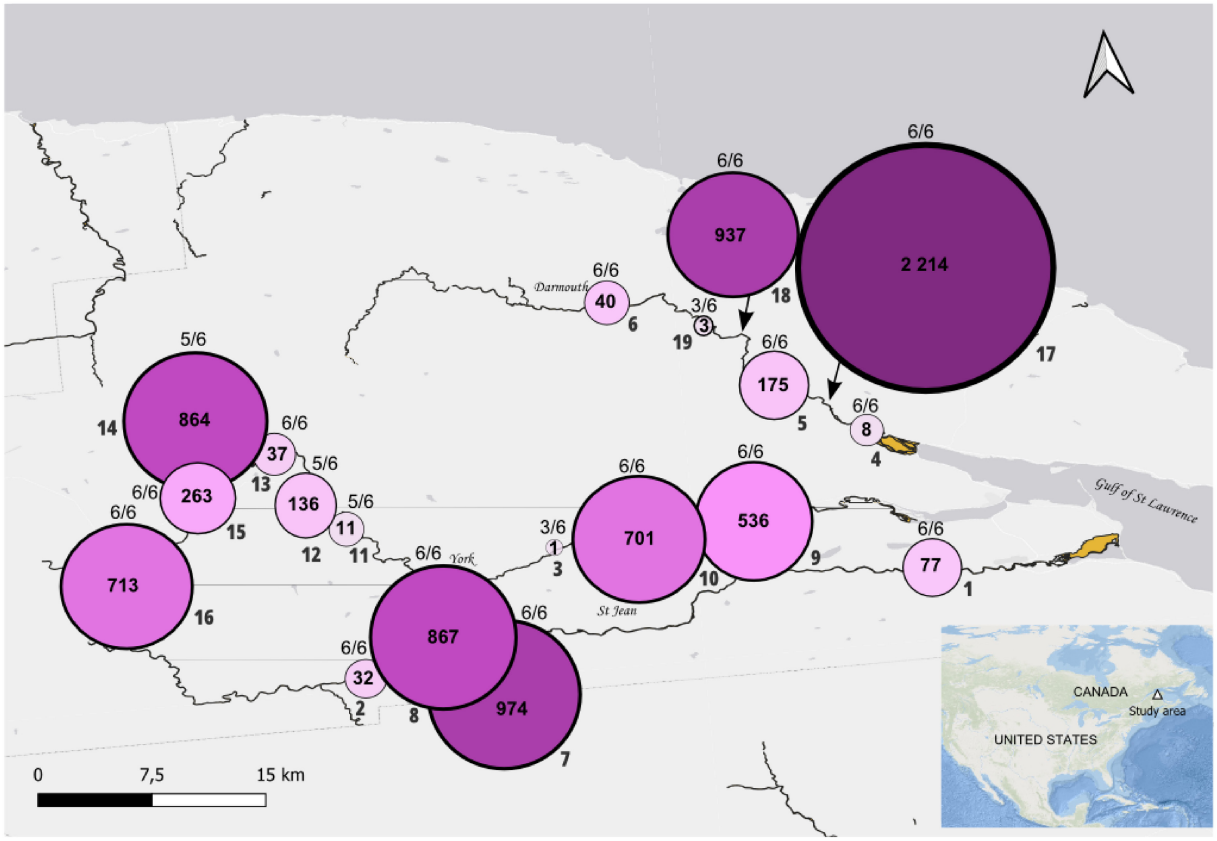

FIGURE 4 eDNA molecules from juvenile Atlantic salmon were detected in all the sampled tributaries of the Gaspé area. For each tributary, the number outside the circle refers to the ID of Table 1. Inside each circle, the total number of eDNA molecules detected by qPCR at that tributary is indicated. Size of the circle is relative to the number of eDNA molecules detected. Above each circle, the number of bottles for which eDNA-positive amplification was detected by qPCR at that tributary is indicated (maximum of six bottles sampled per tributary).

by lower temperature and higher pH levels compared to the Dartmouth River. In the St-Jean River, the lowest levels of eDNA molecules were detected in the Sirois tributary (ID: 2) despite the high abundance of fish estimated with the Leslie method at that tributary (157 juveniles). This tributary was reported to exhibit the highest flow velocities measured in this study. Previous studies highlighted two potential effects of flow velocities on eDNA detection in lotic environments (Curtis et al., 2021). On one hand, fast velocities could transport eDNA farther from its source, potentially increasing detectability (Milhau et al., 2019; Shogren et al., 2018). On the other hand, fast velocities could dilute eDNA molecules, reducing concentrations and detection (Klymus et al., 2015; Shogren et al., 2019). Our study gives additional support to the latter hypothesis given the high abundance of fish estimated at the Sirois tributary. In the York River, the Mississippi tributary (ID: 3) exhibited the lowest eDNA levels of juvenile Atlantic salmon detected in our analyses, with only one molecule reported (this sample was not included in the statistical analyses as it was below the LOD). At this tributary, the estimated abundance of fish was very low (four juveniles), which could explain the very low levels of eDNA molecules detected. Also, we found very low levels of eDNA molecules in two tributaries of the Dartmouth River: the Bechervais tributary (ID: 19) with three molecules detected and the Watering tributary (ID: 4) with eight molecules detected. In the Watering tributary, we estimated a relatively high level of fish abundance with the Leslie method (59 juveniles). In the Bechervais tributary, the presence of only four fish was estimated, which could explain the low levels of eDNA molecules detected. Another possibility is that high temperature, which was mainly associated to the Bechervais and Watering tributaries according to PCA biplot analyses, might have contributed to the low levels of eDNA reported in these tributaries through active degradation of the molecules (specifically for the Watering tributary with higher abundance estimation). As a matter of fact, previous studies including experimental investigations in laboratory conditions reported that the susceptibility of eDNA to degradation increased with temperature (Caza-Allard et al., 2022; Kasai et al., 2020). Highest detections of juvenile Atlantic salmon eDNA occurred in the Dartmouth River at La Petite Fourche pf (ID: 17) and at the OS5_os1_15 (ID: 18) tributaries, as well as in the St-Jean River at the Island tributary (ID: 7). These tributaries were associated with high estimates of juvenile abundance (respectively 148 99 and 34 juveniles). Furthermore, the OS5_os1_15 tributary was mostly defined by its abundant vegetation cover according to PCA biplot analyses. In the same way, the Island tributary was

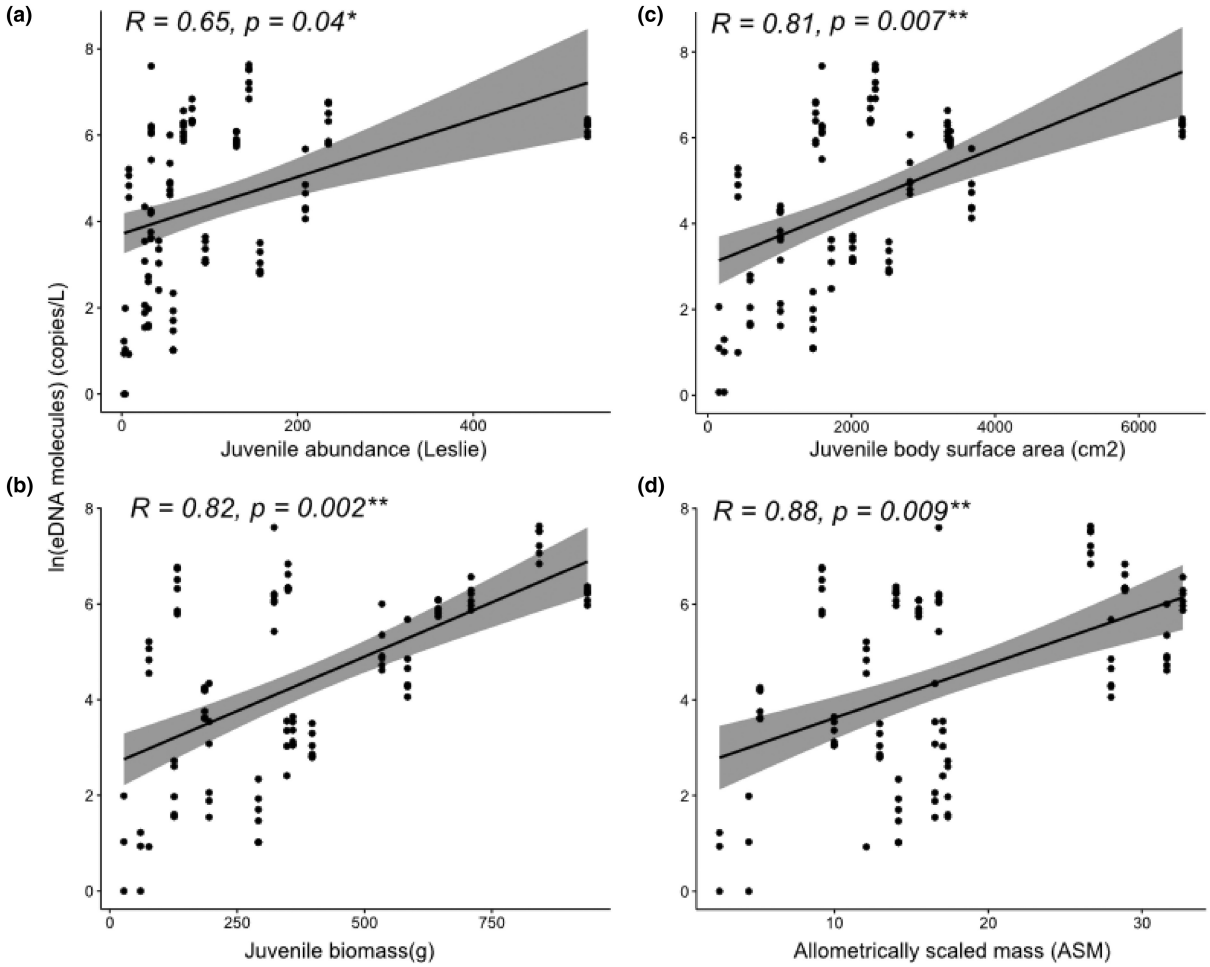

FIGURE 5 eDNA of juvenile Atlantic salmon detected in 19 tributaries of the Gaspé area positively correlated with (a) fish abundance (Leslie Method) ($R=0.65$, $p=0.04^{*}$), (b) fish total biomass (g) ($R=0.82$, $p=0.002^{**}$), (c) body surface area (cm^2) ($R=0.81$, $p=0.007^{**}$) and (d) allometrically scaled mass (ASM) ($R=0.88$, $p=0.009^{**}$). For each correlation, a linear mixed model with tributary" and "bottle" as random effects was performed. Each sample is represented by a dot, with the number of eDNA molecules detected in that sample expressed per liter of water.

classified in class #4 (76–100% shade on the tributary) using our visual estimation method of vegetation cover. Vegetation cover has been suggested to reduce light penetration (Kazanjian et al., 2018), and consequently eDNA degradation that is highly susceptible to UV (Kessler et al., 2020; Strickler et al., 2015). We hypothesize that the strong vegetation cover we measured at these tributaries could have contributed to reduce eDNA degradation, therefore promoting eDNA accumulation.

Part of the variability in the relationship between eDNA levels and fish abundance estimates could possibly be explained by the relatively short length (50 m) of the electrofishing sampling plots. Indeed, habitats located upstream from the sampling plot may exhibit larger or smaller fish abundances than at the sampling plot itself, which would contribute to blur the eDNA level-fish abundance relationship. As sampling over a longer river stretch was too labor intensive and time consuming to be considered, a variable describing the length of salmon habitat upstream from the sampling plot was measured and included in the analysis. However, this variable did not come out as significant in our model, suggesting that fish abundance in the sampling plots captured most of the variability in eDNA abundance.

Using linear mixed models, we found that detected eDNA of juvenile Atlantic salmon positively correlated with all biotic metrics measured in this study: fish abundance, fish total biomass, body surface area, and allometrically scaled mass (ASM). Before correcting abundance with ASM, the strongest positive relationship was found between detected eDNA concentrations and juvenile biomass, in accordance with previous studies conducted in rivers that demonstrated that eDNA levels detected from Atlantic salmon at the adult stage (Fossøy et al., 2020) and at the juvenile stage (Morrison et al., 2023) reflected fish biomass. To the best of our knowledge, our study is the first to demonstrate such relationship for juvenile Atlantic salmon in tributaries. A strong positive correlation was also observed between detected eDNA levels and juvenile body surface area. Body surface area is generally less utilized in eDNA analyses despite its potential importance in shedding rates, as the total exposed body surface in direct contact with the environment is expected to be larger for a group of small fish than

for a single big fish of the same total biomass (Hansen et al., 2018). Our finding highlights the importance of taking this metrics into consideration and is consistent with a recent metabarcoding study in fish that demonstrated that read numbers were significantly correlated to the total surface area (Skelton et al., 2023). Finally, we found a weaker but still significant correlation between eDNA levels detected and juvenile abundance. While laboratory studies previously demonstrated a strong correlation between eDNA concentration and abundance (Eichmiller et al., 2016), studies in nature have generally found weaker correlations (Yates et al., 2019). This can be partially explained by the complexity of the environmental and biological processes in nature that influence the rates of eDNA released by the organisms (Yates, Glaser et al., 2021). In order to take into account this complexity in our system, we replaced abundance with ASM as suggested by Yates, Glaser et al., 2021. The use of ASM greatly improved positive correlation between abundance and detected eDNA concentration of juveniles. This density metric allowed to account for the relative decline in mass-specific eDNA production or excretion rates typically observed as individual organismal mass increases (Yates, Glaser et al., 2021). It is important to note that the allometric scaling coefficient was set at 0.73 (which was the value used by Yates, Glaser et al., 2021 for brook trout *Salvelinus fontinalis*) as no data on allometric scaling in eDNA production or excretory rates was available for Atlantic salmon. Furthermore, inference of the scaling coefficient would have required to estimate the population size in each tributary ($\hat{N}$) with repeated mark-recapture experiments based on the method of Schnabel (Chapman & Overton, 1966). Therefore, it would be important to adjust the allometric scaling coefficient in future projects interested in tracking juvenile Atlantic salmon with eDNA, by accounting for both the production and excretion rates of this species and the population size sampled.

In our study, there was some variability in the proportion of fry versus parr caught by electrofishing. We took into account this size distribution of fry and parr by including body surface area into our analyses, which can be considered as a proxy of life stage. As we found that eDNA concentrations positively correlated with body surface area, we can extrapolate that eDNA concentrations were likely positively influenced by life stage. We were not able to confirm it with eDNA as this technology cannot discriminate between life stages. However, the emergence of a new discipline, environmental RNA (eRNA) could offer promising opportunities as eRNA is based on transcriptomic data that allows to discriminate between life stages depending on the qPCR primers used (Yates, Derry et al., 2021). In the future, using eRNA instead of eDNA with two pairs of primers, one specific to fry and one specific to parr, would allow to validate that the levels of molecules detected is on accordance with the proportion of fry and/or parr detected.

TABLE 3 Results of lme analyses testing the effects of environmental variables on the levels of eDNA molecules detected for juvenile Atlantic salmon in the Gaspé area.

	Value	Std. error	DF	*t*-value	*p*-value
Model 1 (52% Cum.Wt)					
(Intercept)	−2.54	2.02	85	−1.26	0.21
ASM	0.1	0.05	15	2.15	0.048*
Temperature	0.32	0.13	15	2.44	0.03*
Vegetation	0.32	0.35	15	0.93	0.37
Model 2 (31% Cum. Wt)					
(Intercept)	−2.41	1.67	85	−1.44	0.15
Biomass	0.005	0.001	15	3.69	0.002**
Temperature	0.24	0.11	15	2.14	0.049*
Vegetation	0.76	0.26	15	2.92	0.01*

Note: Results are presented for the first two best-fitted models. Model one included "ASM," "temperature," and "vegetation" as fixed factors, and "tributary" and "bottle" as random effects. Model 2 included the same parameters as model 1, except for the "ASM" factor that was replaced by "biomass." Cum.Wt: sum of the AIC weight. * *p*-value below 0.05; ** *p*-value below 0.005.

Abbreviation: DF, degrees of freedom.

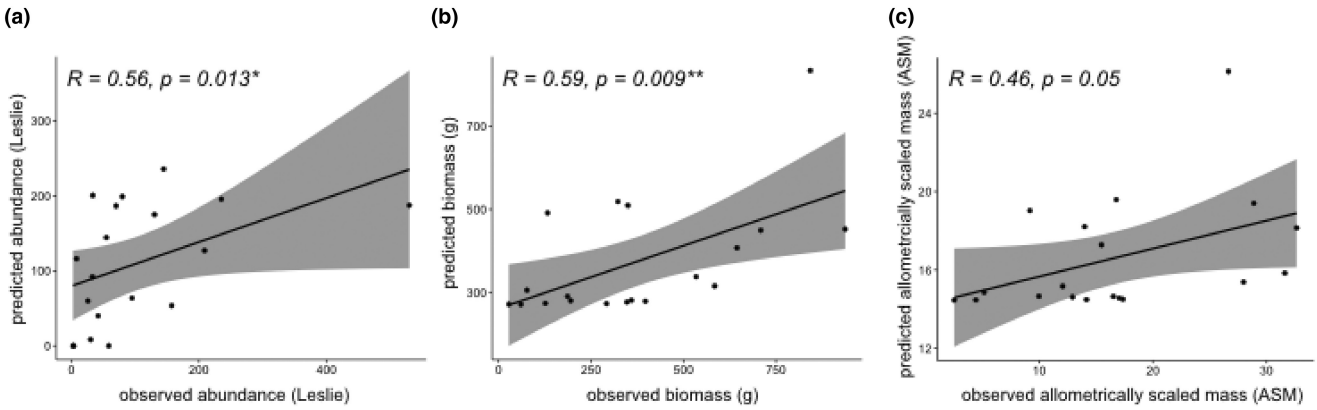

FIGURE 6 Spearman correlations (Wickham, 2009) between values predicted with the models used in this study and observed values for (a) juvenile abundance (Leslie method) ($R=0.56$, $p=0.013$*), (b) juvenile total biomass (g) ($R=0.59$, $p=0.009$**) and (c) allometrically scaled mass (ASM) ($R=0.46$, $p=0.05$).

According to the environmental conditions measured in each tributary, we demonstrated that the levels of eDNA molecules detected for juvenile Atlantic salmon were mostly influenced by temperature and vegetation cover. As discussed previously, the impact of temperature and vegetation cover – which itself influences exposure to UV – on eDNA degradation rates has been extensively studied in laboratory and/or natural conditions, with temperature and UV exposure favoring the degradation of eDNA molecules as they increase (e.g., for temperature (Jo et al., 2019; Tsuji et al., 2017) and UV (Pilliod et al., 2014; Strickler et al., 2015)). In our system, we found that an increase in temperature and vegetation cover both positively influenced the levels of eDNA molecules detected. First, greater vegetation cover probably contributed to protect eDNA molecules from UV exposure and degradation. However, the impact of UV radiation on the levels of eDNA molecules detected in a natural environment is still debated. For example, it was suggested that natural levels of UV radiation is not sufficient alone to affect eDNA detection rates (Mächler et al., 2018). During our analyses, we used vegetation cover defined by class as a proxy of UV exposure. Future work would require more precise measures, for example, using a solarmeter to more accurately estimate the impact of UV on the detection of juvenile Atlantic salmon eDNA. Furthermore, our study was performed at a small temporal scale (in July and August), and conducting sampling across seasons (e.g., summer vs winter) would allow to better understand the role of the vegetal cover in protecting eDNA from UV exposure and degradation. Second, the positive correlation reported between temperature and eDNA levels could be explained by an increase in the metabolism of juveniles with temperature. As eDNA shedding rates depend on the metabolism and physiological activity of organisms, higher temperatures might have promoted higher metabolism rates, and ultimately higher shedding rates of eDNA (Jo et al., 2019). Furthermore, experiments conducted in controlled conditions previously demonstrated that water temperature only induces the degradation of fish eDNA molecules at high levels (24°C), with an accumulation of molecules observed at 15°C (Caza-Allard et al., 2022). As water temperature measured in all tributaries was below the threshold of 24°C and close to 15°C (maximum of 21°C measured in the Petite Fourche rfp tributary, mean of 14°C for all tributaries), we suggest that water temperature was not elevated enough to significantly degrade eDNA molecules.

It is important to take into account the impact of sampling year when measuring eDNA levels of salmonids in nature. As a matter of fact, migratory behavior and tributary occupancy of fish might be affected by biological (e.g., warmer or cooler seasons) and physical factors (e.g., anthropic constructions such as culverts or dams), which might ultimately result in variations of eDNA detection probability (De Souza et al., 2016). Our study was conducted over two consecutive years, but on different tributaries for each year, which reduces the relevancy of statistically testing if inter-annual effects have impacts on eDNA detection/concentration levels. We nevertheless included sampling year in the selected models and did not find any significant effect of this variable on eDNA levels. Our results are consistent with a recent study that notably tested the impact of year on eDNA detection (Morrison et al., 2023). Using several models including various environmental covariates, the authors demonstrated that water temperature was the main driver of eDNA concentration variability, as we found in our study. While the year itself did not affect eDNA detection, the authors underlined the importance of sampling at the same times and locations each year to reduce potential confounding variables (Morrison et al., 2023).

Based on the models selected in this study, we tested if eDNA concentrations measured in one tributary could be used as a reliable indicator of juvenile biomass, juvenile abundance, and/or ASM. We found that our models slightly better predicted juvenile biomass, then juvenile abundance. Surprisingly, incorporating allometric scaling coefficient with ASM did not improve model prediction. Therefore, we remain cautious with the use of our selected models to predict juvenile ASM. We propose that further biotic parameters should be taken into account to improve model prediction. For ASM calculation and prediction, it would be important as discussed previously to estimate the population size per tributary ($\hat{N}$) with mark-recapture essays in order to determine the scaling coefficient for juvenile Atlantic salmon. Here, we used the scaling coefficient of brook trout, which could partially explain the low prediction power for ASM. For biomass calculation and prediction, the number of fry versus parr juveniles in each tributary would be important to take into account, as developmental stage has been reported to affect eDNA shedding rates and detection (Maruyama et al., 2014). Furthermore, other ecological processes such as eDNA transport should be considered, which is all the more critical in a system where there is connectivity between tributaries and their main stems. This could be achieved for example by sampling eDNA the main river at the mouth of the tributary, then at different distances from the river mouth in each tributary.

To summarize, our results demonstrate that it is possible to detect juvenile Atlantic salmon in tributaries using qPCR eDNA, and that we can link eDNA concentrations with biotic parameters (juvenile abundance, total biomass, and body surface area) as well as with environmental conditions (temperature and vegetation cover). We hope that our method will complement traditional capture methods and facilitate the implementation of conservation practices that will be appropriate for monitoring juvenile Atlantic salmon in tributaries.

AUTHOR CONTRIBUTIONS

L.B., N.B., and S.G. designed the study. S.G. performed field sampling. S.G. performed the laboratory work. C.S.B. analyzed the results. C.S.B. wrote the manuscript with collaboration from L.B. and N.B.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data generated are available in article supplementary material.

DEDICATION

This article is dedicated to the memory of Louis Bernatchez.

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REFERENCES

Aas, Ø., Klemetsen, A., Einum, S., & Skurdal, J. (2010). *Atlantic salmon ecology*. John Wiley & Sons.

Arlinghaus, R., Cooke, S. J., Lyman, J., Policansky, D., Schwab, A., Suski, C., Sutton, S. G., & Thorstad, E. B. (2007). Understanding the complexity of catch-and-release in recreational fishing: An integrative synthesis of global knowledge from historical, ethical, social, and biological perspectives. *Reviews in Fisheries Science*, *15*(1–2), 75–167.

Bernatchez, L., & Giroux, M. (2000). *Les poissons d'eau douce du Québec et leur distribution dans l'est du Canada*. Éditions Broquet Inc.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L. & Vandesompele, J. (2009). *The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments*. Oxford University Press.

Caza-Allard, I., Laporte, M., Côté, G., April, J., & Bernatchez, L. (2022). Effect of biotic and abiotic factors on the production and degradation of fish environmental DNA: An experimental evaluation. *Environmental DNA*, *4*(2), 453–468.

Chapman, D., & Overton, W. (1966). Estimating and testing differences between population levels by the Schnabel estimation method. *The Journal of Wildlife Management*, *30*, 173–180.

Cote, D., Van Leeuwen, T., Bath, A., Gonzales, E., & Cote, A. (2021). Social–ecological management results in sustained recovery of an imperiled salmon population. *Restoration Ecology*, *29*(5), e13401.

Coulter, D. P., Wang, P., Coulter, A. A., Van Susteren, G. E., Eichmiller, J. J., Garvey, J. E., & Sorensen, P. W. (2019). Nonlinear relationship between silver carp density and their eDNA concentration in a large river. *PLoS One*, *14*(6), e0218823.

Crabbe, S. J. (2000). *Use of small streams by juvenile Atlantic salmon, Salmo salar, and brown trout, Salmo trutta, in the Ponoi River, Russia*. University of Guelph.

Curtis, A. N., Tiemann, J. S., Douglass, S. A., Davis, M. A., & Larson, E. R. (2021). High stream flows dilute environmental DNA (eDNA) concentrations and reduce detectability. *Diversity and Distributions*, *27*(10), 1918–1931.

Dadswell, M., Spares, A., Reader, J., McLean, M., McDermott, T., Samways, K., & Lilly, J. (2022). The decline and impending collapse of the Atlantic salmon (*Salmo salar*) population in the North Atlantic Ocean: A review of possible causes. *Reviews in Fisheries Science & Aquaculture*, *30*(2), 215–258.

De Souza, L. S., Godwin, J. C., Renshaw, M. A., & Larson, E. (2016). Environmental DNA (eDNA) detection probability is influenced by seasonal activity of organisms. *PLoS One*, *11*(10), e0165273.

Deiner, K., & Altermatt, F. (2014). Transport distance of invertebrate environmental DNA in a natural river. *PLoS One*, *9*(2), e88786.

Dempson, J., O'Connell, M., & Schwarz, C. (2004). Spatial and temporal trends in abundance of Atlantic salmon, *Salmo salar*, in Newfoundland with emphasis on impacts of the 1992 closure of the commercial fishery. *Fisheries Management and Ecology*, *11*(6), 387–402.

Eichmiller, J. J., Miller, L. M., & Sorensen, P. W. (2016). Optimizing techniques to capture and extract environmental DNA for detection and quantification of fish. *Molecular Ecology Resources*, *16*(1), 56–68.

Erkinaro, J., Julkunen, M., & Niemelä, E. (1998). Migration of juvenile Atlantic salmon *Salmo salar* in small tributaries of the subarctic river Teno, northern Finland. *Aquaculture*, *168*(1–4), 105–119.

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

Forootan, A., Sjöback, R., Björkman, J., Sjögreen, B., Linz, L., & Kubista, M. (2017). Methods to determine limit of detection and limit of quantification in quantitative real-time PCR (qPCR). *Biomolecular Detection and Quantification*, *12*, 1–6.

Fossøy, F., Brandsegg, H., Sivertsgård, R., Pettersen, O., Sandercock, B. K., Solem, Ø., Hindar, K. & Mo, T. A. (2020). Monitoring presence and abundance of two gyrodactylid ectoparasites and their salmonid hosts using environmental DNA. *Environmental DNA*, *2*(1), 53–62.

Friedland, K. D., MacLean, J. C., Hansen, L. P., Peyronnet, A. J., Karlsson, L., Reddin, D. G., Ó Maoiléidigh, N., & McCarthy, J. L. (2009). The recruitment of Atlantic salmon in Europe. *ICES Journal of Marine Science*, *66*(2), 289–304.

Gaudet-Boulay, M., García-Machado, E., Laporte, M., Yates, M., Bougas, B., Hernandez, C., Côté, G., Gilbert, A. & Bernatchez, L. (2023). Relationship between brook charr (*Salvelinus fontinalis*) eDNA concentration and angling data in structured wildlife areas. *Environmental DNA*, *5*, 861–879.

Goldberg, C. S., Pilliod, D. S., Arkle, R. S., & Waits, L. P. (2011). Molecular detection of vertebrates in stream water: A demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. *PLoS One*, *6*(7), e22746.

Hansen, B. K., Bekkevold, D., Clausen, L. W., & Nielsen, E. E. (2018). The sceptical optimist: Challenges and perspectives for the application of environmental DNA in marine fisheries. *Fish and Fisheries*, *19*(5), 751–768.

Hernandez, C., Bougas, B., Perreault-Payette, A., Simard, A., Côté, G., & Bernatchez, L. (2020). 60 specific eDNA qPCR assays to detect invasive, threatened, and exploited freshwater vertebrates and invertebrates in eastern Canada. *Environmental DNA*, *2*(3), 373–386.

Jo, T., Murakami, H., Yamamoto, S., Masuda, R., & Minamoto, T. (2019). Effect of water temperature and fish biomass on environmental DNA shedding, degradation, and size distribution. *Ecology and Evolution*, *9*(3), 1135–1146.

Jonsson, B., & Jonsson, N. (2017). Fecundity and water flow influence the dynamics of Atlantic salmon. *Ecology of Freshwater Fish*, *26*(3), 497–502.

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

Kazanjian, G., Flury, S., Attermeyer, K., Kalettka, T., Kleeberg, A., Premke, K., Köhler, J., & Hilt, S. (2018). Primary production in nutrient-rich kettle holes and consequences for nutrient and carbon cycling. *Hydrobiologia*, *806*, 77–93.

Kessler, E. J., Ash, K. T., Barratt, S. N., Larson, E. R., & Davis, M. A. (2020). Radiotelemetry reveals effects of upstream biomass and UV

exposure on environmental DNA occupancy and detection for a large freshwater turtle. *Environmental DNA*, *2*(1), 13–23.

Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., Jackson, C. A., Lance, R. F., Mangan, A. M., Monroe, E. M., Piaggio, A. J., Stokdyk, J. P., Wilson, C. C., & Richter, C. A. (2020). Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA*, *2*(3), 271–282.

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

Lacoursière-Roussel, A., Côté, G., Leclerc, V., & Bernatchez, L. (2016). Quantifying relative fish abundance with eDNA: A promising tool for fisheries management. *Journal of Applied Ecology*, *53*(4), 1148–1157.

Lacoursière-Roussel, A., Rosabal, M., & Bernatchez, L. (2016). Estimating fish abundance and biomass from eDNA concentrations: Variability among capture methods and environmental conditions. *Molecular Ecology Resources*, *16*(6), 1401–1414. https://doi.org/10.1111/1755-0998.12522

Leslie, P., & Davis, D. (1939). An attempt to determine the absolute number of rats on a given area. *The Journal of Animal Ecology*, *8*, 94–113.

Lodge, D. M., Turner, C. R., Jerde, C. L., Barnes, M. A., Chadderton, L., Egan, S. P., Feder, J. L., Mahon, A. R., & Pfrender, M. E. (2012). Conservation in a cup of water: Estimating biodiversity and population abundance from environmental DNA. *Molecular Ecology*, *21*(11), 2555–2558.

Lynggaard, C., Bertelsen, M. F., Jensen, C. V., Johnson, M. S., Frøslev, T. G., Olsen, M. T., & Bohmann, K. (2022). Airborne environmental DNA for terrestrial vertebrate community monitoring. *Current Biology*, *32*(3), 701–707. e705.

Mächler, E., Osathanunkul, M., & Altermatt, F. (2018). Shedding light on eDNA: Neither natural levels of UV radiation nor the presence of a filter feeder affect eDNA-based detection of aquatic organisms. *PLoS One*, *13*(4), e0195529.

Maruyama, A., Nakamura, K., Yamanaka, H., Kondoh, M., & Minamoto, T. (2014). The release rate of environmental DNA from juvenile and adult fish. *PLoS One*, *9*(12), e114639.

Milhau, T., Valentini, A., Poulet, N., Roset, N., Jean, P., Gaboriaud, C., & Dejean, T. (2019). Seasonal dynamics of riverine fish communities using eDNA. *Journal of Fish Biology*, *98*(2), 387–398.

Mills, K. E., Pershing, A. J., Sheehan, T. F., & Mountain, D. (2013). Climate and ecosystem linkages explain widespread declines in north American Atlantic salmon populations. *Global Change Biology*, *19*(10), 3046–3061.

Morrison, M. K., Lacoursière-Roussel, A., Wood, Z. T., Trudel, M., Gagné, N., LeBlanc, F., Samways, K., Kinnison, M. T. & Pavey, S. A. (2023). Including environmental covariates clarifies the relationship between endangered Atlantic salmon (*Salmo salar*) abundance and environmental DNA. *Environmental DNA*, *5*, 987–1003.

Nicola, G. G., Elvira, B., Jonsson, B., Ayllón, D., & Almodóvar, A. (2018). Local and global climatic drivers of Atlantic salmon decline in southern Europe. *Fisheries Research*, *198*, 78–85.

Nolan, T., Hands, R. E., Ogunkolade, W., & Bustin, S. A. (2006). SPUD: A quantitative PCR assay for the detection of inhibitors in nucleic acid preparations. *Analytical Biochemistry*, *351*(2), 308–310.

O'Shea, B., Mordue-Luntz, A., Fryer, R., Pert, C., & Bricknell, I. (2006). Determination of the surface area of a fish. *Journal of Fish Diseases*, *29*(7), 437–440.

Otero, J., Jensen, A. J., L'Abée-Lund, J. H., Stenseth, N. C., Storvik, G. O., & Vøllestad, L. A. (2011). Quantifying the ocean, freshwater and human effects on year-to-year variability of one-sea-winter Atlantic salmon angled in multiple Norwegian rivers. *PLoS One*, *6*(8), e24005.

Pilliod, D. S., Goldberg, C. S., Arkle, R. S., & Waits, L. P. (2013). Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*(8), 1123–1130.

Pilliod, D. S., Goldberg, C. S., Arkle, R. S., & Waits, L. P. (2014). Factors influencing detection of eDNA from a stream-dwelling amphibian. *Molecular Ecology Resources*, *14*(1), 109–116.

Pont, D., Rocle, M., Valentini, A., Civade, R., Jean, P., Maire, A., Roset, N., Schabuss, M., Zornig, H., & Dejean, T. (2018). Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. *Scientific Reports*, *8*(1), 10361.

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R., & Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA–a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, *51*(5), 1450–1459. https://doi.org/10.1111/1365-2664.12306

Roh, C., Villatte, F., Kim, B.-G., & Schmid, R. D. (2006). Comparative study of methods for extraction and purification of environmental DNA from soil and sludge samples. *Applied Biochemistry and Biotechnology*, *134*, 97–112.

Rourke, M. L., Fowler, A. M., Hughes, J. M., Broadhurst, M. K., DiBattista, J. D., Fielder, S., Wilkes Walburn, J., & Furlan, E. M. (2022). Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. *Environmental DNA*, *4*(1), 9–33.

Shea, D., Bateman, A., Li, S., Tabata, A., Schulze, A., Mordecai, G., Ogston, L., Volpe, J. P., Neil Frazer, L., Connors, B., Miller, K. M., Short, S., & Krkošek, M. (2020). Environmental DNA from multiple pathogens is elevated near active Atlantic salmon farms. *Proceedings of the Royal Society B*, *287*(1937), 20202010.

Shea, D., Frazer, N., Wadhawan, K., Bateman, A., Li, S., Miller, K. M., Short, S., & Krkošek, M. (2022). Environmental DNA dispersal from Atlantic salmon farms. *Canadian Journal of Fisheries and Aquatic Sciences*, *79*(9), 1377–1388.

Shogren, A. J., Tank, J. L., Egan, S. P., August, O., Rosi, E. J., Hanrahan, B. R., Renshaw, M. A., Gantz, C. A., & Bolster, D. (2018). Water flow and biofilm cover influence environmental DNA detection in recirculating streams. *Environmental Science & Technology*, *52*(15), 8530–8537.

Shogren, A. J., Tank, J. L., Egan, S. P., Bolster, D., & Riis, T. (2019). Riverine distribution of mussel environmental DNA reflects a balance among density, transport, and removal processes. *Freshwater Biology*, *64*(8), 1467–1479.

Shustov, Y. A., Baryshev, I., & Belyakova, E. (2012). Specific features of the feeding of juvenile Atlantic salmon (*Salmo salar* L.) in the subarctic Varzuga River and its small tributaries (Kola peninsula). *Inland Water Biology*, *5*, 288–292.

Skelton, J., Cauvin, A., & Hunter, M. E. (2023). Environmental DNA metabarcoding read numbers and their variability predict species abundance, but weakly in non-dominant species. *Environmental DNA*, *5*, 1092–1104.

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

Sutton, R. J., Deas, M. L., Tanaka, S. K., Soto, T., & Corum, R. A. (2007). Salmonid observations at a Klamath River thermal refuge under various hydrological and meteorological conditions. *River Research and Applications*, *23*(7), 775–785.

Taberlet, P., Bonin, A., Zinger, L., & Coissac, E. (2018). *Environmental DNA: For biodiversity research and monitoring*. Oxford University Press.

Thorstad, E. B., Bliss, D., Breau, C., Damon-Randall, K., Sundt-Hansen, L. E., Hatfield, E. M., Horsburgh, G., Hansen, H., Maoiléidigh, N. Ó., Sheehan, T., & Sutton, S. G. (2021). Atlantic salmon in a rapidly changing environment—Facing the challenges of reduced marine survival and climate change. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *31*(9), 2654–2665.

Tsuji, S., Ushio, M., Sakurai, S., Minamoto, T., & Yamanaka, H. (2017). Water temperature-dependent degradation of environmental DNA and its relation to bacterial abundance. *PLoS One*, *12*(4), e0176608.

Verspoor, E., Stradmeyer, L., & Nielsen, J. L. (2008). *The Atlantic salmon: Genetics, conservation and management*. John Wiley & Sons.

Watz, J., Otsuki, Y., Nagatsuka, K., Hasegawa, K., & Koizumi, I. (2019). Temperature-dependent competition between juvenile salmonids in small streams. *Freshwater Biology*, *64*(8), 1534–1541.

Wickham, H. (2009). Elegant graphics for data analysis. *Media*, *35*(211), 10.1007.

Wolman, M. G. (1954). A method of sampling coarse river-bed material. *Eos, Transactions American Geophysical Union*, *35*(6), 951–956.

Wood, Z. T., Erdman, B. F., York, G., Trial, J. G., & Kinnison, M. T. (2020). Experimental assessment of optimal lotic eDNA sampling and assay multiplexing for a critically endangered fish. *Environmental DNA*, *2*(4), 407–417.

Wood, Z. T., Lacoursière-Roussel, A., LeBlanc, F., Trudel, M., Kinnison, M. T., Garry McBrine, C., Pavey, S. A., & Gagné, N. (2021). Spatial heterogeneity of eDNA transport improves stream assessment of threatened salmon presence, abundance, and location. *Frontiers in Ecology and Evolution*, *9*, 650717.

Yates, M. C., Derry, A. M., & Cristescu, M. E. (2021). Environmental RNA: a revolution in ecological resolution? *Trends in Ecology & Evolution*, *36*(7), 601–609.

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

Yates, M. C., Glaser, D., Post, J. R., Cristescu, M. E., Fraser, D. J., & Derry, A. M. (2021). The relationship between eDNA particle concentration and organism abundance in nature is strengthened by allometric scaling. *Molecular Ecology*, *30*(13), 3068–3082.

SUPPORTING INFORMATION

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

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