Relating eDNA concentrations to Brook Trout abundance and biomass across varying spatial scales in Northern Ontario streams

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Abstract

Environmental DNA (eDNA) detection has been proposed as a potential method for determining the presence, abundance, and biomass of species within aquatic habitats. However, it is unclear what spatial scale(s) eDNA detections represent in lotic systems due to uncertainties surrounding its production and persistence. This study had two objectives: (1) to determine at what spatial scale(s) (length of stream segments) could eDNA concentration be related to the abundance and/or biomass of Brook Trout (*Salvelinus fontinalis)*, and (2) to determine if the relationship between eDNA concentration and Brook Trout abundance/biomass could be improved by accounting for the contribution of eDNA originating upstream of investigated stream segments. Concentrations of eDNA were related to the abundance of Brook Trout at the 25m ($R^2 = 0.22$), 50m ($R^2 = 0.18$), and 100m ($R^2 = 0.42$) spatial scales, and to the biomass of Brook Trout at the 25m spatial scale ($R^2 = 0.06$), suggesting that eDNA concentrations can represent Brook Trout abundance across numerous spatial scales. Variability observed in these relationships across spatial scales was likely due to (1) the presumably heterogeneous distribution of Brook Trout within stream segments causing different concentrations of eDNA to be detected for similar abundances and biomasses, and (2) the contribution of eDNA from upstream of examined stream segments to the detected eDNA concentrations. Concentrations of eDNA were calibrated to 25m stream segments by accounting for the contribution of upstream eDNA assuming either the constant or conditional deposition of eDNA. Both calibration methods improved the relation of eDNA concentrations to the abundance and biomass of Brook Trout within the 25m segments (abundance, $R^2 = 0.327$ and $R^2 = 0.336$ for constant and conditional calibration respectively). However, both methods incorrectly estimated the persistence and contribution of upstream eDNA on occasion, suggesting that additional investigation is required before these methods can be reliably and confidently implemented to assess the abundance and biomass of Brook Trout in lotic environments.

Lay Summary

It may be possible to monitor the status of Brook Trout in Northern Ontario streams by detecting molecules of environmental DNA (eDNA) that they release into aquatic environments. The concentration of eDNA in water samples has previously been related to the number (abundance) and the mass (biomass) of fish in aquatic environments. However, this may not always be possible in streams since the downstream movement of water allows eDNA to be transported potentially long distances from its source, making the exact length of stream or the stream segment represented by eDNA concentrations unclear. This study examined the relationship between eDNA concentrations and the abundance and biomass of Brook Trout within various sized stream segments by sampling eDNA every 25m of seven 200m stream segments then catching, counting, and weighing Brook Trout within the segments. Concentrations of eDNA were related to the abundance of Brook Trout within 25m, 50m, and 100m of sampling locations, and the biomass of Brook Trout within 25m, but all relationships had a large amount of unexplained variability. Some of this variability likely comes from: (1) the positioning of Brook Trout within stream segments likely caused different concentrations of eDNA to be detected for similar abundances and biomasses of Brook Trout, and (2) eDNA from Brook Trout above the investigated stream segments likely added to eDNA concentrations causing them to represent larger lengths of streams than the considered abundances and biomasses. This was supported by eDNA concentrations at the base of 25m stream segments being related to the concentrations sampled at the top of the segments. To account for upstream eDNA, eDNA concentrations were calibrated to the 25m stream segments using two methods. The first method assumed that upstream eDNA settled out of streams at a constant rate, whereas the second method, assumed that the settling of upstream eDNA depended on the speed of water in a segment. Both calibration methods improved the relation of eDNA concentrations to the abundance and biomass of Brook Trout but require further investigation before they can be used reliably.

Acknowledgments

This project has felt more like a journey than a study and subsequent write-up. Like any good journey, it took longer than expected, was wrought with mental turmoil, and was only accomplishable with the support of faithful companions. If I were to thank everyone who helped, encouraged, or uplifted me throughout this process to the extent that they deserve, I fear this section would be longer than all subsequent sections. Nonetheless, I'll do my best.

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Yours,

Mark Schutte (Fish Poo Water Boy, MSc)

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Chapter 1. General Introduction

Proper management and conservation of species and ecosystems requires thorough understanding of the occupation and status of species throughout their proposed ranges (Hewitt et al. 2009). Typically, species habitat occupancy and population status can be represented by their presence, abundance (number of individuals) and biomass (total mass of the individuals) within environments. In streams, these are often determined using traditional surveys such as electrofishing, which can be invasive to the targeted species and ecosystems and may not always be viable depending on environmental conditions (Jerde et al. 2011; Wilson and Wright 2013). Recently, environmental DNA (eDNA) has been proposed as a cost, effort, and time-efficient method for the detection (Ficetola et al. 2008) and estimation of the abundance and biomass (Pilliod et al. 2014; Takahara et al. 2012) of target species within aquatic systems. The targeted detection of eDNA amplifies species-specific sequences of nucleotides in water samples (Ficetola et al. 2008), and the concentrations of these sequences within samples have been related to the abundance and biomass of the target species within aquatic habitats (Thomsen et al. 2011; Takahara et al. 2012; Pilliod et al. 2013;Wilcox et al. 2016; Baldigo et al. 2017). While eDNA has been shown to reliably indicate the presence of aquatic species (Jerde et al. 2011), and has been occasionally proposed to be able to estimate species abundance (Klymus et al. 2015; Baldigo et al. 2017) and biomass (Baldigo et al. 2017), uncertainties surrounding its production (Maruyama et al. 2014) and transport (Jane et al. 2015; Laramie et al. 2015; Fukomoto et al. 2015; Wilcox et al. 2016) complicate reliable interpretations of eDNA results, especially in lotic systems. Therefore, it is likely that better understanding of eDNA these factors is required before species abundance and biomass in streams can be reliably estimated by sampling eDNA.

Environmental DNA is added into aquatic systems from cells (typically epithelial; Klymus et al. 2015) shed or excreted from living or dead organisms of a target species (Ficetola et al. 2008). The production of eDNA is expected to scale with the size of individuals (Maruyama

et al. 2014), or more specifically, with the surface areas, metabolic rates, and excretory rates of individuals (Yates et al. 2021a). These factors scale with body size, but the magnitude of this scaling decreases as size increases (Yates et al. 2021a). As a result, eDNA production likely scales allometrically with body size such that eDNA production increases but decreases per unit mass as individual size increases (Spear et al. 2015; Yates et al. 2021a). Additional factors such as the age (Maruyama et al. 2014), behaviour (Spear et al. 2015), activity level (Jo et al. 2019), and breeding readiness (Spear et al. 2015) of individuals, food availability within habitats (Klymus et al. 2015), time of year (Laramie et al. 2015), water temperature (Jo et al. 2019) and water velocity (Jane et al. 2015) are expected to influence the production of eDNA. Consequently, the concentration of eDNA initially produced and released into lotic systems likely varies depending on the investigated species and the environmental conditions of the sampled habitat.

Following its release into lotic systems, eDNA is transported downstream. Substantial uncertainty exists surrounding the transport and persistence of eDNA in lotic systems, but concentrations of eDNA are expected to decrease as they are transported downstream from their source (Wilson and Wright 2013; Jane et al. 2015; Wilcox et al. 2016) due to degradation, deposition, and diffusion (Barnes and Turner 2016). Degradation is the digestion of eDNA by nucleases, ultraviolet radiation, and microbes (Shapiro 2008) and causes the species-specific sequence of nucleotides to become unrecognizable in polymerase chain reactions (Barnes and Turner 2016). Deposition is the settling of eDNA out of water columns onto channel beds. Depending on the state eDNA exists in (dissolved, bound to suspended particles, or encased in tissues or cells; Mauvisseau et al. 2022), eDNA may behave similarly to fine particulate organic matter (FPOM). Two opposing theories of FPOM deposition in lotic systems exist: constant and conditional deposition. Constant deposition assumes that particles deposit at a constant rate independent of hydrological conditions (Cushing et al. 1993), whereas conditional deposition

assumes that whether particles are suspended or deposited depends on the hydrological conditions it is exposed to (Leopold and Maddock 1953; Einstein and Krone 1961). Diffusion is the dispersal of eDNA throughout water columns to its lowest possible concentration. The extent that these processes influence eDNA concentrations are unknown, and as a result, the distance that eDNA can be transported is unclear, leading to the uncertainty in the length of streams (spatial scale) that is represented by eDNA concentrations.

A species whose monitoring may be facilitated by eDNA sampling is Brook Trout (*Salvelinus fontinalis)*. Brook Trout are a species of interest throughout portions of Ontario, Quebec, and the northeastern United States (Scott and Crossman 1973) due to their importance for recreational fishing (OMNRF 2017), and their presence indicating good quality habitat (Steedman 1988). They inhabit small to larger rivers, ponds, and lakes, but require cold, clean, well-oxygenated water for survival (Mackay 1963; Scott and Crossman 1973). Over several decades, Brook Trout have been undergoing substantial population declines and extirpation throughout much of their historical range in Southern Ontario (Scott and Crossman 1973; OMNRF 2017) because of the degradation and loss of suitable habitat from agriculture, deforestation, pollution, and urbanization (Nyman 1970). However, their status in Northern Ontario remains relatively undocumented (OMNRF 2017), partially due to the difficulty of surveying regions of suspected Brook Trout occupancy using traditional survey methods. Because of its proposed ease of use (Jerde et al. 2011), it may be possible to monitor Brook Trout populations in Northern Ontario streams by sampling eDNA. However, for this to be possible, eDNA concentrations must first be consistently and reliably relatable to the abundance and biomass of Brook Trout across known lengths (segments) of streams.

The goal of this study was to clarify the length of stream represented in eDNA samples by examining the relationship between eDNA concentrations and Brook Trout occupancy at multiple spatial scales (i.e., stream segment lengths). The objectives were (1) to determine at

which spatial scale(s) (length of stream segments) eDNA concentrations were related to the abundance and/or biomass of Brook Trout, and (2) to determine if the relationships between eDNA concentrations and Brook Trout abundance and biomass could be improved at the 25m spatial scale by accounting for the contribution of eDNA originating upstream of the examined 25m stream segments.

Chapter 2: The relation of eDNA concentrations to the abundance, biomass, and allometrically scaled mass of Brook Trout across various spatial scales in Northern Ontario streams

Abstract

A potential method for estimating the abundance and biomass of Brook Trout in Northern Ontario streams is the use of environmental DNA (eDNA), however, eDNA's viability is limited by uncertainties surrounding the distance it represents in lotic systems. This study attempted to determine (1) if eDNA concentrations can be related to the abundance and biomass of Brook Trout occurring within 25, 50, 100, and 200m of eDNA sampling locations, (2) whether eDNA concentrations were better related to the abundance or the biomass of Brook Trout, and (3) if allometrically scaling Brook Trout mass using previously developed exponential scaling coefficients of 0.36 and 0.73 improved the relation of eDNA concentrations to biomass. Samples of eDNA were collected every 25m from seven 200m stream segments, and the segments were triple-pass electrofished to determine Brook Trout abundance and biomass. Concentrations of eDNA were related to the abundance, biomass, and biomass allometrically scaled to the exponent 0.73 within 25m of the sampling location (R^2 = 0.22, 0.06, and 0.09 respectively), abundance within 50m (R^2 = 0.18), and the abundance and biomass allometrically scaled to the exponent 0.73 within 100m (R^2 = 0.42 and 0.11 respectively). At all spatial scales, eDNA concentrations were more closely related to Brook Trout abundance than biomass, and allometrically scaling biomass by 0.73 improved the relation of eDNA concentrations to biomass whereas the scaling coefficient of 0.36 did not. Variability in these relationships likely originated from two spatial scale dependent factors: (1) the contribution of eDNA from upstream of segments causing more eDNA to be detected than was produced by Brook Trout within segments, and (2) the distribution of Brook Trout within segments causing different concentrations of eDNA to be detected for similar abundances and biomass of Brook Trout.

Introduction

Quick, accurate and cost-effective methods of determining the presence and abundance/biomass of species are essential for the proper management and conservation of species and ecosystems (Hewitt et al. 2009). There is great need to determine the distributions of species defined as at risk (Thomsen et al. 2012), non-indigenous (Klymus et al. 2015), and those that are indicators of habitat quality (Steedman 1988). Traditional methods of aquatic species detection and density/biomass estimation entail physical surveys such as electrofishing; methods that are not only invasive to the ecosystems and species they target, but often require significant cost, time, and effort to conduct properly (Wilson and Wright 2013). If a species is present in low numbers or in difficult to assess habitat, detection via traditional methods may be unfeasible (Jerde et al. 2011). Recently, environmental DNA (eDNA) detection has been proposed as a potential alternative to traditional surveys, by which species-specific sequences of nucleotides are detected from water samples to determine the presence, abundance and/or biomass of species within sampled habitats (Ficetola et al. 2008; Klymus et al. 2015). In the past, relationships have been found between detected concentrations of eDNA and the abundance and/or biomass of target species within surveyed habitats (Thomsen et al. 2011; Takahara et al. 2012; Pilliod et al. 2013;Wilcox et al. 2016; Baldigo et al. 2017), leading to the suggesting that abundance and biomass may be able to be estimated from eDNA concentrations (Klymus et al. 2015; Baldigo et al. 2017). Despite its potential, many factors surrounding the interpretation of eDNA results remain unclear, requiring further investigation before eDNA can be confidently used for conservation and management purposes.

Brook Trout (*Salvelinus fontinalis*) are a cold-water dwelling salmonid indigenous to, and widely distributed throughout parts of Ontario, Quebec, and the northeastern United States (Scott and Crossman 1973; OMNRF 2017). They are a species of interest throughout Ontario due to their importance for recreational fishing, in addition to being an indicator of good habitat quality

(Steedman 1988) because of their requirement of cold, clean, well-oxygenated water (Mackay1963; Scott and Crossman 1973). Stressors such as climate change, introduced species, and anthropogenic activity have and continue to contribute to the loss of suitable Brook Trout habitat (Nyman 1970), and as a result Brook Trout are experiencing wide-spread population decline and extirpation throughout portions of their range (OMNRF 2017). The distribution of Brook Trout has been greatly reduced over the past century in Southern Ontario but remains relatively undocumented in Northern Ontario due to the difficulty of accessing and conducting traditional surveys in streams with suspected Brook Trout occupancy (Browne 2007). Due to its proposed ease of use (Jerde et al. 2011), if Brook Trout abundance and biomass can be accurately estimated from eDNA concentrations, it may be possible to quantitatively assess the status of Brook Trout in Northern Ontario streams by sampling eDNA.

Environmental DNA is added to lotic systems from cells released from living or dead individuals of target species (Ficetola et al. 2008). The amount of eDNA that is produced and released within sampled habitats has previously been related to the abundance (number of individuals) and biomass (total mass of individuals) of the target species within the habitats (Pilliod et al. 2014; Takahara et al. 2012). However, the production and release of eDNA is expected to not only be influenced by the size of individuals (Maruyama et al. 2014) but also their surface areas, and metabolic and excretory rates, which scale allometrically with body size (Yates et al. 2021a). Consequently, larger individuals are expected to produce less eDNA per unit mass than smaller individuals, such that applying an exponential scaling coefficient (<1) to the mass of individuals may improve the relation of biomass to the concentration of eDNA that is initially produced (Yates et al. 2021a; Yates et al. 2021b). Two exponential scaling coefficients for the production of Brook Trout eDNA have previously been calculated; 0.73 from a lake study ($\Sigma mass^{0.73}$; Yates et al. 2021a) and 0.36 from a stream study ($\Sigma mass^{0.36}$; Yates et al. 2021b). In addition to body size, biotic and abiotic factors such as age, life stage (Maruyama et al. 2014),

water temperature (Jo et al. 2019), and water velocity (Jane et al. 2015) likely influence the shedding and excretion of eDNA.

Following its release into a lotic system, eDNA will be degraded, deposited out of suspension, and diffused throughout the water column (Barnes and Turner 2016) as it is transported downstream to a sampling location. The extent that these processes influence the distance that eDNA can be transported, its persistence, and its concentration throughout streams is unclear. However, it is expected that eDNA concentrations decrease as the distance from their sources increase (Wilson and Wright 2013; Jane et al. 2015; Wilcox et al. 2016), and that the distance that eDNA can be transported will increase with the discharge of a system (Wilcox et al. 2016). Consequently, the specific length of stream segments or the spatial scale that eDNA represents is unknown and it is uncertain if eDNA results correspond to individuals in close proximity, far away, or across a range of distances. It is likely that the hydrologic and environmental conditions of the sampled habitat (Pilliod et al. 2013) such as discharge (Jerde et al. 2011; Wilcox et al. 2016), velocity (Jane et al. 2015) and temperature (Jo et al. 2019; Minamoto et al. 2020) influence this range.

The current study had three objectives; First (1), to determine if eDNA concentrations can be related to the abundance and biomass of Brook Trout (*Salvelinus fontinalis*) in stream segments of various lengths (spatial scales; 25, 50, 100, and 200m) when abundance and biomass are and are not standardized by the surface area of the investigated stream segment. Second (2), to determine if eDNA concentrations are better related to the abundance or to the biomass of Brook Trout across spatial scales. Third (3), to determine if allometrically scaling mass using the previously developed exponential scaling coefficients of 0.36 (Yates et al. 2021b) and 0.73 (Yates et al. 2021a) improves the relation of biomass to eDNA concentrations. To test this, eDNA samples were collected every 25m of seven 200m stream segments, the stream segments were electrofished, and eDNA concentrations were compared to the abundance, biomass and

allometrically scaled mass of Brook Trout within 25, 50, 100, and 200m of the eDNA sampling locations.

Methods

Five Lake Superior tributary streams in the Mackenzie and Wolf River watersheds located within the historical territory of the Fort William First Nation were selected based on the detection of Brook Trout in a previous study (Wiebe 2023). Four 200m stream segments in the Mackenzie watershed were branches of the Mackenzie River (Denise, Kevin, Rod, Frank; Appendix A.1) and a single 600m stream segment in the Wolf River watershed was subdivided into three sequential 200m segments (Dave, Richardson, Rico; Appendix A.1) generating a total of seven 200m stream segments across both watersheds (Table 2.1).

Beginning at the base of the 200m stream segments and working upstream, eDNA was sampled in triplicate across transects spaced every 25m (0m, 25m, 50m, 75m, 100m, 125m, 150m, 175m, 200m, Fig 2.1). Samples were collected from the surface of the stream at 0.25, 0.5, and 0.75 of the channel width sequentially from the left bank to the right (A-C respectively, Fig 2.1) by passing 1L of stream water through 1.2µm pore sized filters (Whatman GF/C) using a peristaltic pump (Geopump Peristaltic Pump, Geotech 2017) according to the protocol for collecting environmental DNA samples from structures (Carim et al. 2016). Filters were removed and stored completely submerged in Longmire solution to minimize the degradation of eDNA (Longmire et al. 1997). Negative field controls were not collected as the protocol suggested that field controls are ineffective in detecting contamination originating in sample collection and that the equipment cleaning protocols are sufficient to prevent contamination origination from field equipment (Carim et al. 2016). After the last sample was collected at a transect, a blocking net was set separating the stream into 25m stream segments and preventing the movement of Brook Trout into or out of the segments. This was repeated until eDNA was sampled at 9 transects and the 200m stream segments were separated into eight 25m segments by blocking nets (Fig 2.1).

This allowed the combination of the results from sequential 25m stream segments into the results of larger stream segments (50m, 100m, and 200m). Wetted width was measured every 5m and the surface area (SA) of stream segments of each spatial scale were calculated by multiplying the average width by segment length (25, 50, 100, and 200m).

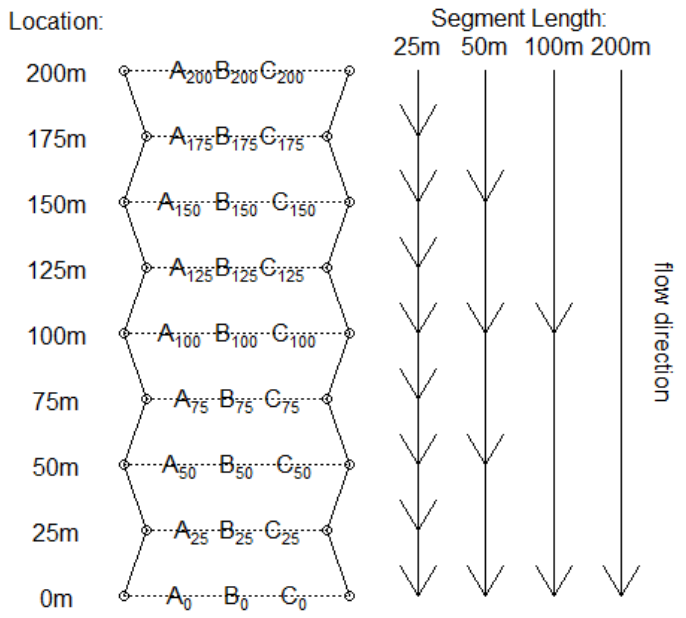


Figure 2.1. Diagram of an idealized 200m stream segment. Triplicate eDNA samples (A-C) were collected equally across the width of the channel every 25m starting at 0m and working upstream to 200m. Blocking nets were placed at each sampling location after eDNA was sampled, and the resulting 25m segments were triple pass-electrofished to estimate Brook Trout abundance and biomass. Electrofishing results of the sampled 25m segments were combined to allow the comparison of eDNA results to Brook Trout abundance and biomass across stream segments of different lengths (spatial scales) from 25-200m indicated by arrows on the right.

Following eDNA sampling, each 25m segment between eDNA sampling locations was triple-pass electrofished using an Apex backpack electrofisher (Smith-Root). After each pass, Brook Trout were counted, individually weighed to a minimum of 1g then released downstream of the blocking net. Brook Trout abundance (number of individuals captured), biomass (total mass of individuals captured), and allometrically scaled mass were calculated for each 25m segment. Allometrically scaled mass was calculated as $\sum_{i=1}^{N} mass_i^S$, where mass is the mass of individual *i*, *N* is the number of captured individuals, and *S* is the exponential scaling coefficient

(adapted from Yates et al. 2021a). The two proposed scaling coefficients were 0.36 and 0.76, calculated from a stream and a lake study respectively (Yates et al. 2021b; Yates et al. 2021a). For each stream segment length (50m, 100m, 200m), abundance, biomass, and allometrically scaled mass were summed from the contributing 25m segments. These measures were also standardized within segments by the surface area (m^2) of the examined stream segments across all spatial scales.

Preserved filters were sent to Trent University where they were extracted and amplified. The Mo Bio PowerWater DNA isolation kit was used for eDNA extraction. For each eDNA sample, the filter and 1000µL of warmed PW1 solution (Mo Bio) were added to a 5mL microcentrifuge tube, and an extraction negative control was prepared by adding 1000µL of PW1 to a 15mL Falcon tube. The tube was shaken on an orbital shaker at around 300rpm for 30 minutes, then centrifuged at 4000 RCF for 1 minute at room temperature. A minimum of 650µL of supernatant was transferred to a 2mL collection tube, then centrifuged at 13,000 RCF for 1 minute. The supernatant was transferred to a clean 2mL collection tube alongside 200µL of PW2 and was briefly vortexed then incubated at 4°C for 5 minutes. The tube was centrifuged at 13,000 RCF for 1 minute, then the supernatant was transferred to a 2mL collection tube. 650µL of PW3 solution warmed to 55°C was added to the tube then the tube was vortexed briefly. 650µL of supernatant was transferred onto a spin filter in a 2mL collection tube, then centrifuged at 13,000 RCF for 1 minute, and the flow-through liquid was discarded. This was repeated until all the supernatant had passed through the spin filter. The spin filter basket was placed into a 2mL collection tube, 650µL of vortexed PW4 was added, and the tubes were centrifuged at 13,000 RCF for 1 minute, discarding the flow-through liquid. 650µL of PW5 was added, the filter was centrifuged at 13,000 RCF for 1 minute, and flow-through liquid was discarded. The spin filter was centrifuged once more at 13,000 RCF to remove residual ethanol before being added to a 2mL collection tube alongside 100µL of 60°C PW6 (elution buffer) and left to incubate for 1

minute. The extracted DNA was then eluted by centrifuging at 13,000 RCF for 1 minute (Wilson et al. 2014).

90µL of $T_{low}E$ were added to wells 1-10 of a qPCR plate. 10µL of the 10^{10} copies/5µL control were added to the first well, which was then mixed thoroughly to create a 10^9 copies/5µL mixture. 10µL of the 10^9 copies/5µL mixture was transferred to the second well and mixed thoroughly. This process was continued to the final well, creating a series from 10^9 copies/5µL to 1 copy/5µL of which the 10^6 to 1 copies/5µL were used as positive controls in qPCR (Wilson et al. 2014).

A qPCR cocktail was made using the Environmental Master Mix (TaqMan®, Life Technologies product #4396838) with BRK2 primer (5'-3': CCACAGTGCTTCACCTTCTATTTCTA, Wilcox et al. 2013). Wells were prepared on qPCR plate containing 15µL of the qPCR cocktail and 5µL of the extracted DNA from eDNA samples, positive controls ranging from 10^6 to 1 copies/5µL, and PCR blanks. Plates were run on the qPCR thermocycler (Applied Biosystems StepOnePlus) with reaction conditions of an initial denaturation at 95°C for 10 minutes, 40 cycles at 95°C for 15s and 1 minute at 60°C (Wilson et al. 2016).

Inhibition was tested for in representative samples. In a qPCR plate with a control series ranging from 10^6 to 1 copies/µL, 15µL of the qPCR cocktail was added to wells, 5µL of eDNA samples was added to baseline testing wells, and 2.5µL of eDNA samples were added to replicate test wells. 2.5µL of 10^3 copies/5µL control samples were added to the replicate test wells. The plate was run on the qPCR thermocycler, and the copy numbers of the baseline and test wells were investigated. If the baseline had no copy number and the replicate test had a copy number less than 500 copies in the reaction, inhibition occurred, and samples required dilution (Wilson et al. 2014).

Statistical analysis:

The eDNA concentrations were examined to identify unusual values (Table 2.1). Concentrations detected at Dave 125 and Denise 175 were substantially higher than all other locations for the respective abundances and biomasses of Brook Trout captured upstream within 25m as seen in Table 2.1. Dave 125 had triplicate concentrations of 859.84, 70.19, 88.47 copies/5µL for samples A-C respectively, so sample A was removed, and a new average concentration of 79.34 copies/5µL was calculated. Denise 175 had triplicate concentrations of 499.09, 271.59 and 408.41 copies/5µL for samples A-C respectively, so the location was treated as an outlier and removed from further analyses.

To determine the relationship between eDNA concentrations and Brook Trout abundance, biomass and allometrically scaled mass across varying spatial scales, linear regressions were conducted comparing the detected concentrations of eDNA to the non-standardized and standardized abundance, biomass and allometrically scaled mass ($\Sigma mass^{0.36}$ and $\Sigma mass^{0.73}$) of Brook Trout within 25m, 50m, 100m and 200m stream segments upstream of the eDNA sampling locations. These regressions were repeated including only the three 200m stream segments from the Wolf River watershed. It should be noted that as the length of stream segments doubled (25m > 50m > 100m > 200m) sample size was halved (55 > 28 > 14 > 7). Combining the electrofishing results of the sequential 25m segments allowed the comparison of eDNA concentrations to Brook Trout abundance and biomass at the 50, 100, and 200m spatial scales. However, since larger stream segments were composed of multiple smaller stream segments, spatial scales are likely not truly independent which may have lead to error in the analysis.

Results

At the 25m spatial scale, the concentration of eDNA was positively related to the non-standardized abundance of Brook Trout, and the standardized abundance, biomass, and allometrically scaled to the exponent 0.73 mass of Brook Trout. When not standardized by surface area, abundance (Fig 2.2A) explained 9.4% of the variation in eDNA concentration (R^2 = 0.094, p = 0.013, eDNA concentration = 3.12*abundance + 57.2), but neither biomass (Fig 2.2B) nor either of the allometrically scaled masses were related to the concentration of eDNA (p > 0.05). Following standardization by surface area, abundance (Fig 2.2C) explained 22.3% of the variation in eDNA concentration (R^2 = 0.223, p < 0.0005, eDNA concentration = 544*abundance + 51.3), biomass (Fig 2.2D) explained 6.4% of the variation in eDNA concentration (R^2 = 0.063, p = 0.035, eDNA concentration = 18.2*biomass + 64.1), allometrically scaled to the exponent 0.73 mass (Fig 2.2F) explained 9.4% of the variation in eDNA concentration (R^2 = 0.094, p = 0.013, eDNA concentration = 53.2*$\Sigma mass^{0.73}$ + 60.7), but allometrically scaled to the exponent 0.36 mass (Fig 2.2E) was not related to eDNA concentrations (p > 0.05). For all the significant relationships at the 25m spatial scale, the y-intercept (eDNA concentration when Brook Trout absent) was greater than zero, indicating that even when Brook Trout were not present within 25m of the sampling location, eDNA was still being detected.

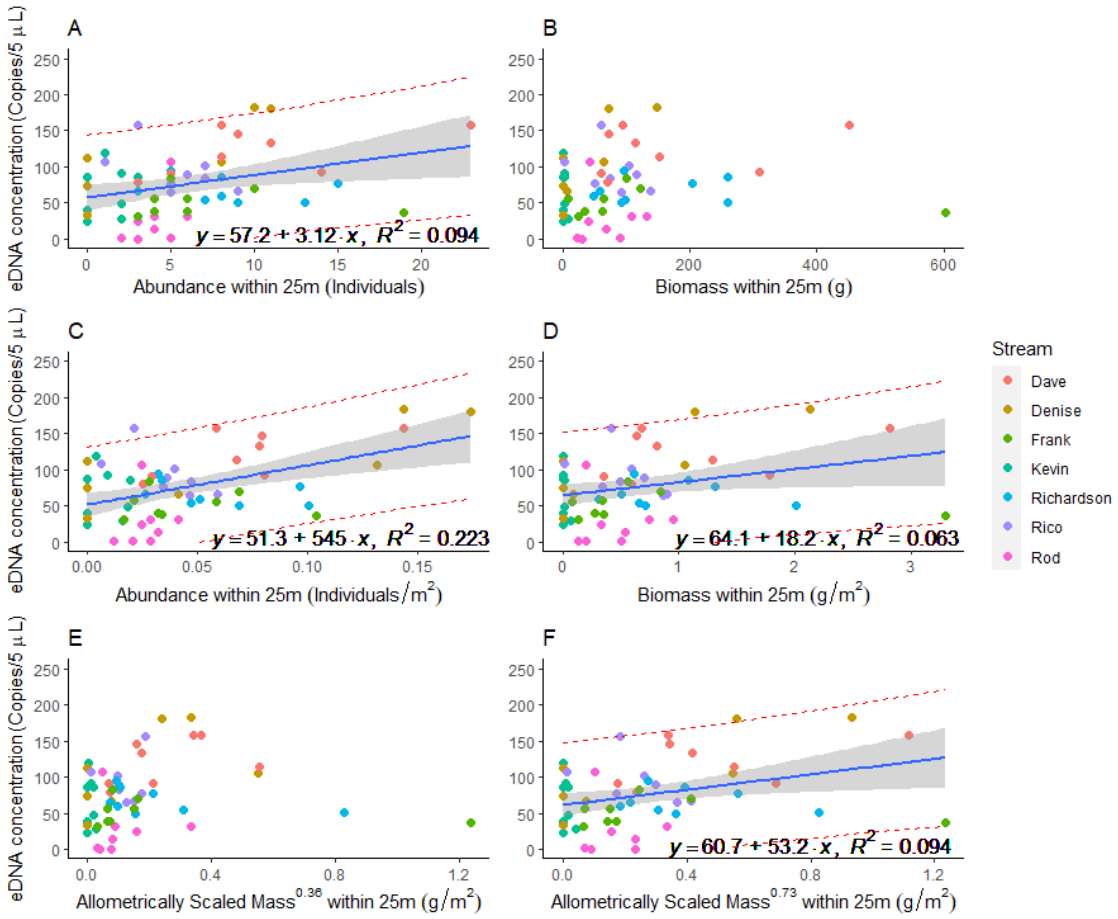


Figure 2.2. eDNA concentrations vs Brook Trout abundance, biomass, and allometrically scaled mass at the 25m spatial scale. Blue lines represent the line of the regression, the gray shade surrounding them the associated standard error, and the red dotted lines the 95% prediction interval. Panels A-F include different describors of Brook Trout occupation: A; non-standardized abundance, B; non-standardized biomass, C; standardized abundance, D; standardized biomass, E; standardized allometrically scaled to the exponent 0.36 mass, and F; standardized allometrically scaled to the exponent 0.73 mass.

At the 50m spatial scale, only the abundance of Brook Trout standardized by surface area was significantly (and positively) related to the concentration of eDNA. When not standardized by surface area, none of abundance (Fig 2.3A), biomass (Fig 2.3B), or allometrically scaled mass (to the exponent 0.36 or 0.73) of Brook Trout were related to the concentration of eDNA ($p > 0.05$). When standardized by surface area, abundance (Fig 2.3C) explained 18.1% of the variation in the concentration of eDNA ($R^2 = 0.181$, $p = 0.014$, eDNA concentration = 576.9*abundance + 47.16), allometrically scaled to the exponent 0.73 mass (Fig 2.3F) was almost significantly related to the concentration of eDNA ($p = 0.07$), but neither biomass (Fig 2.3D) nor

allometrically scaled to the exponent 0.36 mass (Fig 2.3E) were related to the concentration of eDNA ($p < 0.05$). The y-intercept (eDNA concentration when Brook Trout absent) for the standardized abundance model was positive suggesting that eDNA was still being detected when no Brook Trout were present within 50m of the sampling location but to a lesser extent than the 25m spatial scale.

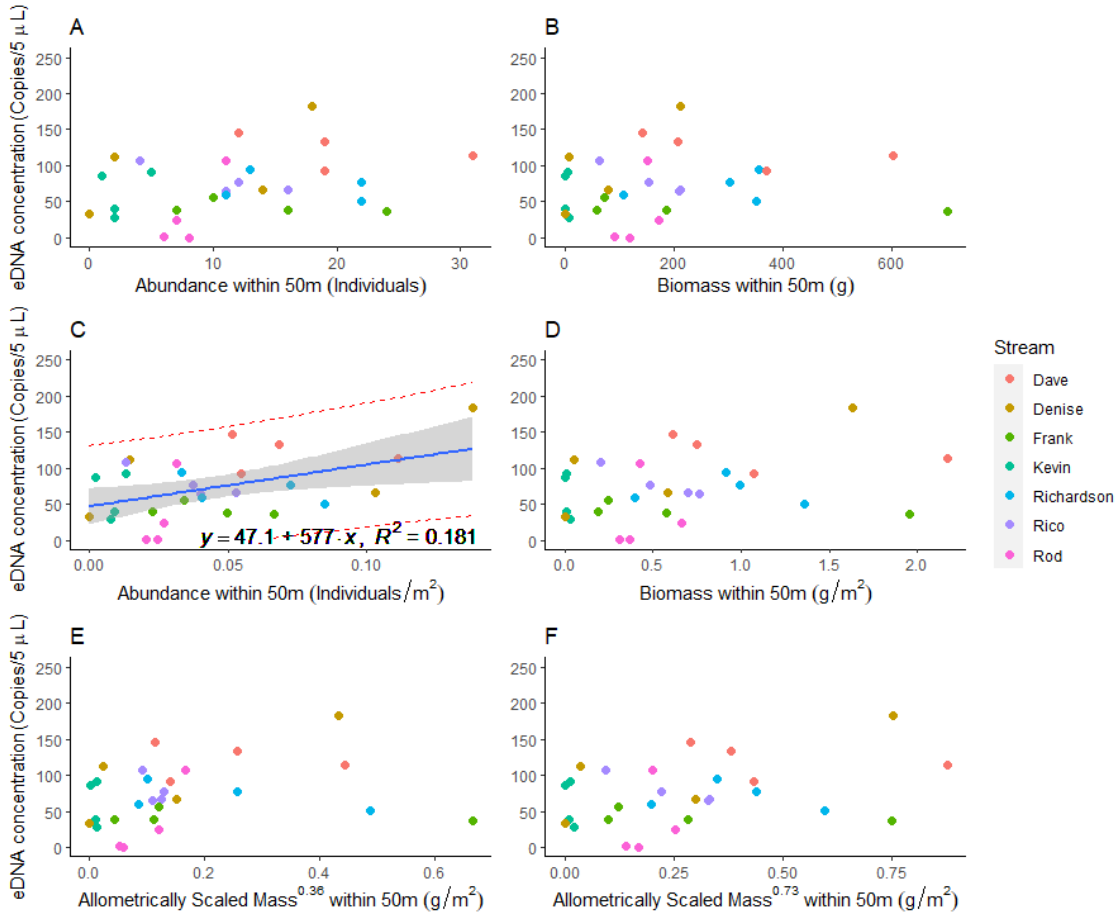


Figure 2.3. eDNA concentrations vs Brook Trout abundance, biomass, and allometrically scaled mass at the 50m spatial scale. Blue lines represent the line of the regression,the gray shade surrounding them the associated standard error, and the red dotted lines the 95% prediction interval. Panels A-F include different describors of Brook Trout occupation: A; non-standardized abundance, B; non-standardized biomass, C; standardized abundance, D; standardized biomass, E; standardized allometrically scaled to the exponent 0.36 mass, and F; standardized allometrically scaled to the exponent 0.73 mass.

At the 100m spatial scale, the abundance and allometrically scaled to the exponent 0.73 mass of Brook Trout standardized by surface area were significantly related to the concentration of eDNA. When not standardized by surface area, abundance (Fig 2.4A), biomass (Fig 2.4B), and

allometrically scaled mass (both exponential scaling coefficients) were not related to the concentration of eDNA. When standardized by surface area, abundance (Fig 2.4C) explained 42.0% of the variation in eDNA concentration ($R^2 = 0.420$, $p = 0.007$, eDNA concentration = 1038*abundance + 35.1), allometrically scaled to the exponent 0.73 mass (Fig 2.4F) explained 25.5% of the variation in the eDNA concentration ($R^2 = 0.255$, $p = 0.038$, eDNA concentration = $140*\Sigma mass^{0.73} + 41.7$), biomass (Fig 2.4D) was marginally related to the eDNA concentration ($p = 0.053$), but allometrically scaled to the exponent 0.36 mass (Fig 2.4E) was not related to the concentration of eDNA ($p = 0.133$).

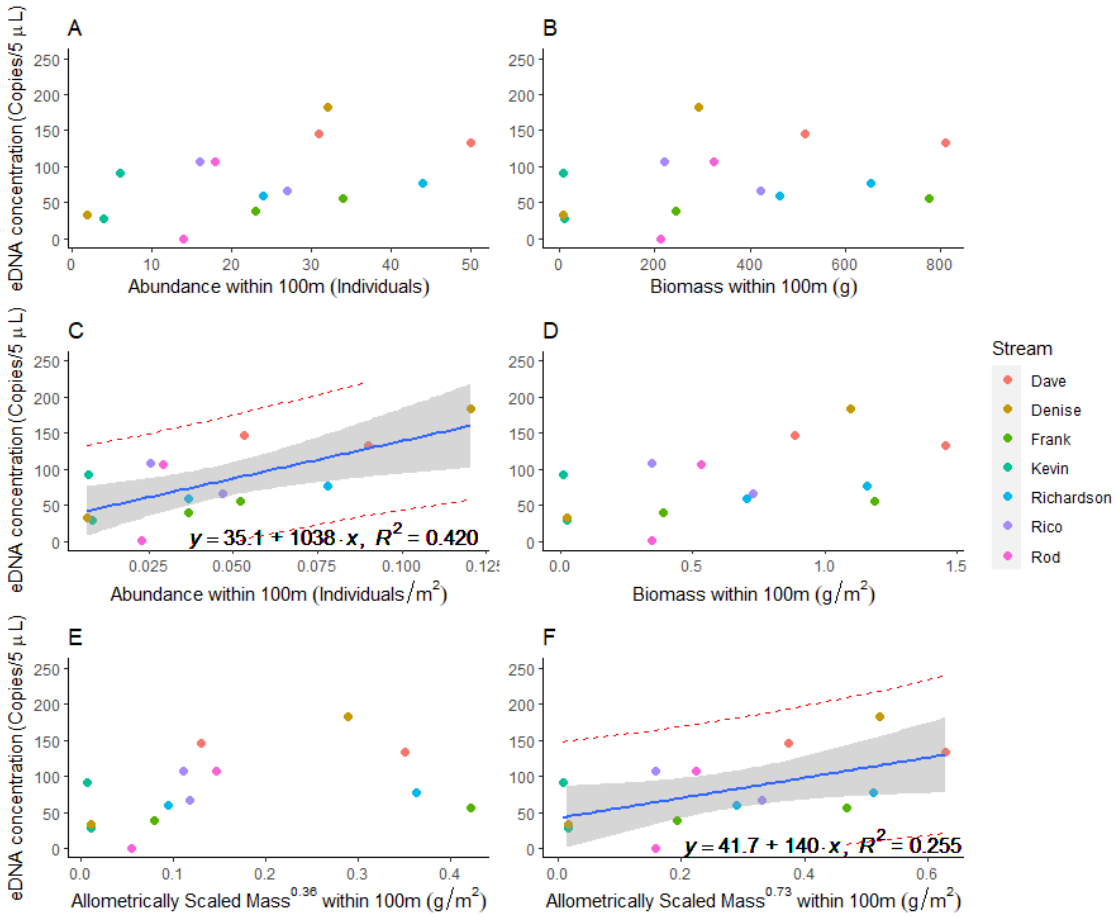


Figure 2.4. eDNA concentrations vs Brook Trout abundance, biomass, and allometrically scaled mass at the 100m spatial scale. Blue lines represent the line of the regression,the gray shade surrounding them the associated standard error, and the red dotted lines the 95% prediction interval. Panels A-F include different describors of Brook Trout

occupation: A; non-standardized abundance, B; non-standardized biomass, C; standardized abundance, D; standardized biomass, E; standardized allometrically scaled to the exponent 0.36 mass, and F; standardized allometrically scaled to the exponent 0.73 mass.

At the 200m spatial scale, the concentration of eDNA was not related to the either the standardized or non-standardized by surface area abundance, biomass or allometrically scaled mass of Brook Trout ($p > 0.05$, Fig 2.5).

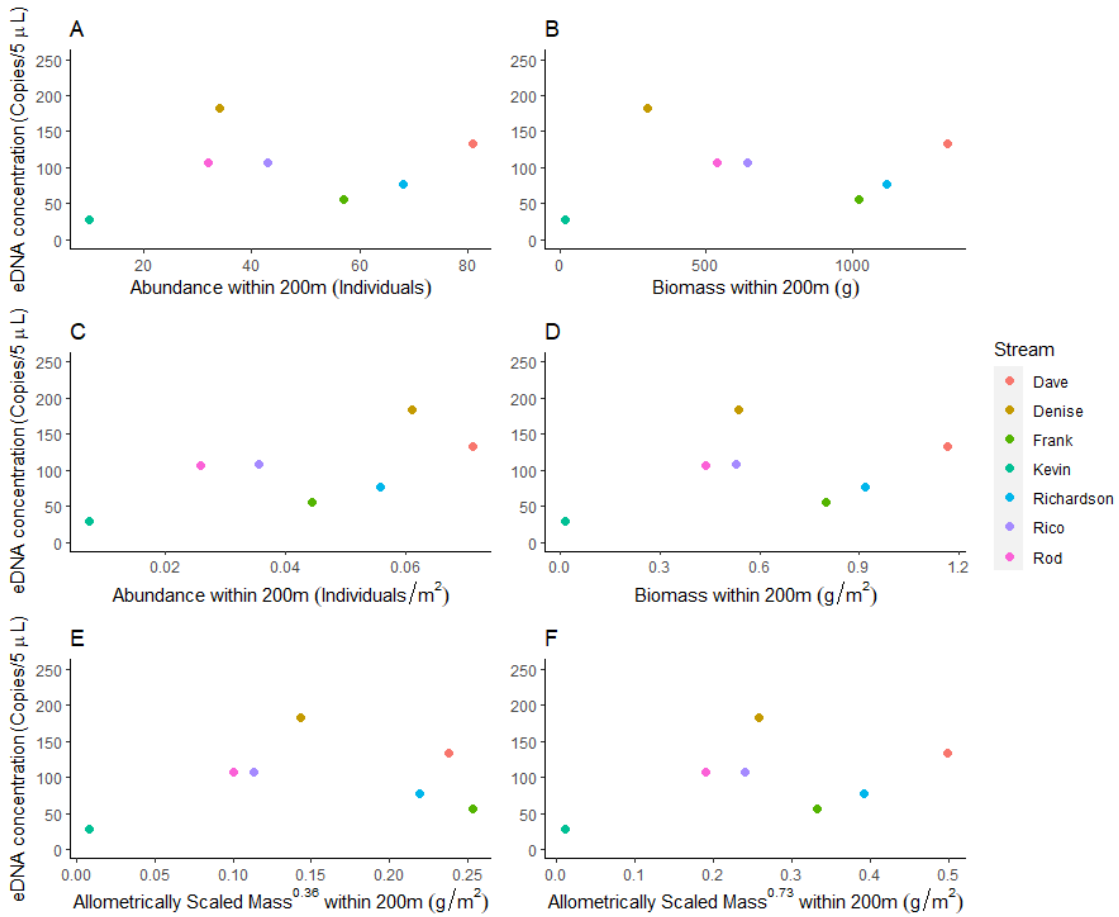


Figure 2.5. eDNA concentrations vs Brook Trout abundance, biomass, and allometrically scaled mass at the 200m spatial scale. Panels A-F include different describors of Brook Trout occupation: Panels A-F include different describors of Brook Trout occupation: A; non-standardized abundance, B; non-standardized biomass, C; standardized abundance, D; standardized biomass, E; standardized allometrically scaled to the exponent 0.36 mass, and F; standardized allometrically scaled to the exponent 0.73 mass.

In the stream segments of the Wolf River watershed (Dave, Richardson, and Rico), no relationship between eDNA concentrations and standardized abundance at the 25m ($p = 0.38$), 50m ($p = 0.84$), 100m ($p = 0.56$), and 200m ($p = 0.75$) spatial scale or between eDNA concentrations and standardized biomass at the 25m ($p = 0.52$), 50m ($p = 0.92$), 100m ($p = 0.57$) and 200m ($p = 0.78$) spatial scales.

Discussion

Environmental DNA (eDNA) concentrations were related to the abundance and biomass of Brook Trout across numerous stream segment lengths (spatial scales) when abundance and biomass were standardized by the surface area of the respective segments, but only to the abundance of Brook Trout within 25m when abundance and biomass were not standardized. At all investigated spatial scales with significant relationships, eDNA concentrations were more closely related to the abundance of Brook Trout than the biomass of Brook Trout, and allometrically scaling mass using the exponential scaling coefficient of 0.73 (Yates et al. 2021a) improved the relation of eDNA concentrations to biomass whereas using the scaling coefficient of 0.36 (Yates et al. 2021b) did not. The relation of eDNA concentrations to Brook Trout abundance and biomass across all spatial scales likely experienced variability from two factors: individuals upstream of the examined stream segments contributing to the detected eDNA concentrations, and the distribution of Brook Trout within segments causing different concentrations of eDNA to be detected for similar abundances and biomasses of Brook Trout.

A potential complication to the results and interpretations of this study is the contamination of eDNA samples. Despite the protocols used in sample collection being designed and having a high success rate of preventing sample contamination from equipment (Carim et al. 2016), the possibility of contamination cannot be completely ruled out due to the absence of field controls. The following is all under the assumption that contamination did not occur, and future studies should likely collect negative field controls to properly account for contamination from field equipment.

Individuals upstream of stream segments likely contributed to the concentration of eDNA in samples, confusing the relation of eDNA concentrations to Brook Trout abundance and biomass across all spatial scales. In the past, particles of eDNA have been detected up to 12km downstream of their source (Deiner and Altermatt, 2014), thus, eDNA samples likely contained

eDNA from individuals upstream of investigated stream segments (Wilcox et al. 2016). This may explain the positive y-intercepts of the relationships (Fig 2.3A, 2.4A, and 2.5A) as the contribution of upstream eDNA likely influenced the slope of the relationships causing the linear models to predict that eDNA was present even in the absence of Brook Trout. As spatial scale increased, the distance that upstream eDNA travelled to sampling locations increased, reducing its concentration (Wilson and Wright 2013; Wilcox et al. 2016) and contribution to eDNA samples, likely resulting in the improved relation of eDNA concentrations to Brook Trout abundance (up to the 100m spatial scale). The possibility of upstream eDNA severely complicates the interpretation of eDNA results, as it is unclear what portion of detected eDNA concentrations originate within investigated segments. Consequently, to improve the relation of eDNA concentrations to Brook Trout abundance/biomass within specific stream segments, the contribution of eDNA from individuals within segments must be distinguished from that of upstream individuals or this upstream contribution must be accounted for.

The unknown but presumably heterogenous distribution of Brook Trout within stream segments likely caused different concentrations of eDNA to be detected for similar abundances and biomasses of Brook Trout, limiting the relation of eDNA concentrations to Brook Trout abundance and biomass. Since eDNA concentrations have been found to decrease as the distance from their source increases (Wilson and Wright 2013; Jane et al. 2015; Wilcox et al. 2016), the distance of individuals from the sampling location will influence the concentration of eDNA in samples. As a result, substantially different concentrations of eDNA could be detected for the same abundances and biomasses of Brook Trout depending on the distribution of individuals throughout segments. Furthermore, as spatial scale increases, the maximum distance of individuals from the sampling location increases, resulting in greater possible differences in eDNA concentrations for similar abundances and biomasses of Brook Trout and increased variability in the eDNA-abundance/biomass relationships. Some of this variability was likely

mitigated by standardizing abundance and biomass by stream segment surface area. Since both surface area and eDNA persistence are related to stream segment length (Wilson and Wright 2013; Wilcox et al. 2016), dividing by surface area likely reduced the abundance and biomass of Brook Trout by a factor related to the decrease in eDNA concentration from production to detection. This likely better aligned the abundance and biomass of Brook Trout to the detected concentrations of eDNA, improving the eDNA-abundance/biomass relationships; especially at larger spatial scales where eDNA persistence is expected to decrease and the potential difference between produced and detected eDNA concentrations increase.

Therefore, variability in the relation of eDNA concentrations to Brook Trout abundance and biomass is likely determined by a spatial scale dependent trade-off between the influences of upstream eDNA and Brook Trout distribution. At small spatial scales, eDNA persistence should be relatively high (Wilson and Wright 2013; Wilcox et al. 2016), resulting in larger contributions of upstream eDNA as well as a smaller influence of Brook Trout distribution within segments on the detected eDNA concentrations (likely further reduced by standardizing abundance and biomass). As spatial scale increased, (upstream) eDNA persistence should decrease (Wilson and Wright 2013; Wilcox et al. 2016), resulting in smaller contributions of upstream eDNA and larger variability in eDNA concentrations depending on Brook Trout distribution. Thus, at smaller spatial scales, the eDNA-abundance/biomass relationships are likely predominately influenced by upstream eDNA, but as spatial scale increases, the influence of Brook Trout distribution becomes more prevalent. This likely also explains the differing strength of the standardized eDNA-abundance relationship (biomass will be addressed later) across spatial scales. However, to assume that eDNA persistence is determined solely by eDNA's distance from its source is an oversimplification. Assuming that eDNA behaves similarly to other fine particulate organic matter, stream discharge (Minshall et al. 2000; Jane et al. 2015; Laramie et al. 2015; Fukomoto et al. 2015; Wilcox et al. 2016) and other hydrological conditions (Leopold and Maddock 1953;

Einstein and Krone 1961; Pilliod et al. 2013) will determine the persistence of eDNA, suggesting that the trade-off between the influences of upstream eDNA and Brook Trout distribution will depend on not only stream segment length but also the hydrological conditions throughout segments.

It should be noted that the influence of upstream eDNA and Brook Trout distribution are unlikely to be the only factors that caused variability in the relationships between detected eDNA concentrations and Brook Trout abundance and biomass. Stream temperature (Jo et al. 2019; Minamoto et al. 2020) and velocity (Jane et al. 2015) likely influenced not only the amount of eDNA initially produced within segments but also the persistence of eDNA to the sampling location. Additionally, factors specific to the individuals such as age (Maruyama et al. 2014), breading readiness (Spear et al. 2015), and metabolism (Jo et al. 2019) all influence the amount of eDNA produced and released within habitats, requiring further investigation to improve the relation of eDNA concentrations and Brook Trout abundance/biomass.

Concentrations of eDNA were more closely related to the abundance than the biomass (including allometrically scaled mass) of Brook Trout at every spatial scale (with significant relationships), which has been observed in previous stream studies (Baldigo et al. 2017; Yates et al. 2021b). This does not indicate that abundance was more closely related to the concentrations of eDNA initially produced within segments but that it was more closely related to the concentrations that persisted to and were detected at sampling locations. The persistence of eDNA to sampling locations is likely incomplete, and as result, eDNA concentrations likely decreased between production and detection. This decrease likely influenced the relation of eDNA concentrations to abundance and biomass differently. Abundance does not consider individual size, and since eDNA production is expected to increase with size (Maruyama et al. 2014; Yates et al., 2021a), various concentrations of eDNA can be produced by the same abundance of Brook Trout depending on their sizes. Thus, abundance likely represents a range of

eDNA concentrations depending on fish size, whereas biomass, which does consider size, represents only a single concentration. As a result, following the decrease in concentration from production to detection (Wilson and Wright, 2013; Jane et al. 2015; Laramie et al. 2015; Fukomoto et al. 2015; Wilcox et al. 2016), a detected eDNA concentration will likely be closer to the minimum concentration of the range produced by a given abundance than the concentration initially produced by the respective biomass. Therefore, the eDNA-abundance relationship is likely more resilient than the eDNA-biomass relationship to decreases in eDNA concentrations from production to detection, allowing the eDNA-abundance relationship to exist at numerous spatial scales (25m, 50m, 100m), whereas the eDNA-biomass relationship only existed at the 25m spatial scale; the spatial scale where the decrease in eDNA concentrations was likely the smallest in this study.

Allometrically scaling Brook Trout mass using the coefficient of 0.73 ($\Sigma mass^{0.73}$) from Yates' lake study (2021a) improved the relationship between biomass and eDNA concentrations, whereas using the scaling coefficient of 0.36 ($\Sigma mass^{0.36}$) from the stream study (Yates et al. 2021b) impeded the relationship. Since both coefficients were calculated assuming that detected concentrations of eDNA were equal to initially produced concentrations, the varying success of the coefficients is likely due to how each study accounted for the difference between the produced and detected eDNA concentrations. In the stream study, eDNA was collected at the base of a 100m stream segment, Brook Trout were captured via triple-pass electrofishing and individual Brook Trout over 1g were counted and weighed (Wilcox et al. 2016; Yates et al. 2021b). The distribution of Brook Trout and the persistence of eDNA throughout the segment were unknown, and as a result, the detected concentration of eDNA was likely smaller than what was initially produced. Consequently, the scaling coefficient of 0.37 is likely incorrectly small, leading to the lack of relation between eDNA concentrations and allometrically scaled to the exponent 0.37 mass in this study. In the lake study, the detected eDNA concentration was

averaged from samples spread throughout the lake, and the abundance and biomass of Brook Trout were estimated by mark-recapture surveys then standardized by dividing by lake surface area (Yates et al., 2021a). It is likely that averaging the detected concentration of eDNA, and standardizing Brook Trout abundance/biomass partially accounted for some of the influence of Brook Trout distribution and eDNA persistence, aligning the detected and produced concentration of eDNA, and improving the accuracy of the calculated scaling coefficient of 0.73. This is supported by the improvement of the eDNA-biomass relationship following the allometric scaling of mass to the exponent 0.73 in this study. However, despite likely being better aligned than in the stream study, it is unlikely that the detected and produced eDNA concentrations were truly equal in the lake study, and it may be beneficial to calculate scaling coefficients under stable laboratory conditions where no difference exists between the detected and initially produced eDNA concentrations.

In conclusion, despite existing at numerous spatial scales, the relationship between eDNA concentrations and Brook Trout abundance and biomass was relatively weak (highest $R^2 = 0.42$), suggesting that with its current methodology, eDNA sampling can provide some information on the occupancy of Brook Trout in Northern Ontario streams, but not always to the same detail or accuracy as electrofishing surveys. Variability from upstream eDNA and the distribution of Brook Trout within segments likely severely obfuscate the relation of eDNA to abundance and biomass in lotic systems; with the influence of upstream eDNA being greatest at smaller spatial scales and the influence of Brook Trout distribution being greatest at larger spatial scales (despite likely being partially accounted for by standardizing abundance and biomass by surface area). Both the influence of upstream eDNA and Brook Trout distribution are dependent on the persistence of eDNA and thus cannot be accurately accounted for until eDNA persistence and the factors influencing it are better understood. Consequently, the specific length of stream segments

represented in eDNA samples remains largely unknown, requiring further understanding of the transport and persistence of eDNA from its production to its detection.

Chapter 2 Tables

*Table 2.1. Results of eDNA sampling and electrofishing at the 25m spatial scale. *Denise 175 was treated as an outlier and removed from analyses. **Dave 125 had an abnormally high triplicate removed*

Stream Segment	Main Stream	Location	Mean eDNA Concentration (Copies/5µL) ± Standard Deviation	Abundance (individuals)	Biomass (g)	$\Sigma Mass^{0.36}$ (g)	$\Sigma mass^{0.73}$ (g)	Surface Area (m^2)
Kevin	Mackenzie	175	118.52 ± 77.03	1	1	1	1	262.63
Kevin	Mackenzie	150	86.46 ± 51.93	0	0	0	0	207.00
Kevin	Mackenzie	125	85.24 ± 79.69	3	3	3	3	156.85
Kevin	Mackenzie	100	91.98 ± 65.95	2	2	2	2	224.50
Kevin	Mackenzie	75	48.24 ± 35.71	2	2	2	2	110.75
Kevin	Mackenzie	50	39.38 ± 25.64	0	0	0	0	110.00
Kevin	Mackenzie	25	23.57 ± 18.03	0	0	0	0	134.03
Kevin	Mackenzie	0	28.77 ± 18.88	2	9	3.11	5.56	126.28
Denise	Mackenzie	175	391.03* ± 107.73	2	7	3.13	4.98	62.20
Denise	Mackenzie	150	111.71 ± 29.52	0	0	0	0	74.85
Denise	Mackenzie	125	74.28 ± 38.57	0	0	0	0	77.60
Denise	Mackenzie	100	33.25 ± 21.41	0	0	0	0	76.75
Denise	Mackenzie	75	180.19 ± 103.77	11	72	15.10	35.37	63.05
Denise	Mackenzie	50	66.28 ± 45.39	3	7	5.46	5.46	72.50
Denise	Mackenzie	25	106.20 ± 52.21	8	64	33.35	33.35	60.60
Denise	Mackenzie	0	183.10 ± 108.81	10	148	23.04	64.72	69.45
Rod	Mackenzie	175	13.80 ± 16.11	4	68	10.11	29.49	125.00
Rod	Mackenzie	150	1.13 ± 1.34	2	23	4.81	11.87	166.43
Rod	Mackenzie	125	0.73 ± 1.25	5	90	13.46	41.68	176.60
Rod	Mackenzie	100	0.54 ± 0.77	3	30	5.56	13.75	148.03
Rod	Mackenzie	75	30.98 ± 27.73	4	133	12.28	47.14	139.38
Rod	Mackenzie	50	24.01 ± 34.84	3	41	19.12	19.12	122.10
Rod	Mackenzie	25	31.14 ± 31.47	6	109	48.95	48.95	146.20
Rod	Mackenzie	0	106.22 ± 57.68	5	42	9.59	21.55	205.65
Rico	Wolf	175	88.78 ± 24.77	6	117	16.48	50.27	167.50
Rico	Wolf	150	65.21 ± 23.46	5	93	13.36	39.97	107.68
Rico	Wolf	125	83.35 ± 67.82	7	74	15.18	37.10	149.63
Rico	Wolf	100	66.37± 29.72	9	138	22.67	63.07	152.63
Rico	Wolf	75	101.88 ± 54.73	7	105	16.72	46.58	176.40
Rico	Wolf	50	77.52 ± 46.69	5	50	24.88	24.88	144.75

Rico	Wolf	25	157.03 ± 82.25	3	61	26.71	26.71	144.28
Rico	Wolf	0	107.71 ± 52.64	1	3	1.49	2.23	166.33
Richardson	Wolf	175	86.00 ± 20.92	8	260	24.96	93.84	238.85
Richardson	Wolf	150	94.76 ± 13.94	5	96	14.35	42.88	155.55
Richardson	Wolf	125	65.77 ± 11.79	3	58	8.29	25.11	115.35
Richardson	Wolf	100	59.07 ± 24.46	8	49	14.69	28.93	155.95
Richardson	Wolf	75	49.96 ± 14.36	9	93	20	47.78	130.75
Richardson	Wolf	50	50.52 ± 10.79	13	259	106.73	106.73	128.85
Richardson	Wolf	25	53.80 ± 16.19	7	98	46.11	46.11	149.35
Richardson	Wolf	0	76.41 ± 16.21	15	204	32.35	87.62	154.98
Dave	Wolf	175	90.79 ± 26.37	5	61	11.95	30.39	172.90
Dave	Wolf	150	92.08 ± 25.92	14	310	36.81	119.63	174.13
Dave	Wolf	125	79.34** ± 15.51	3	71	8.74	28.50	119.58
Dave	Wolf	100	146.21 ± 33.82	9	72	17.89	39.07	113.80
Dave	Wolf	75	158.24 ± 49.03	23	451	58.29	178.85	160.20
Dave	Wolf	50	114.10 ± 44.17	8	152	65.10	65.10	117.63
Dave	Wolf	25	157.33 ± 47.42	8	94	47.04	47.04	137.28
Dave	Wolf	0	133.38 ± 38.80	11	114	24.64	58.96	141.05
Frank	Mackenzie	175	69.65 ± 63.53	10	123	23.40	59.76	144.73
Frank	Mackenzie	150	38.54 ± 10.22	6	63	12.60	30.83	176.70
Frank	Mackenzie	125	31.25 ± 8.33	3	25	5.56	12.55	182.63
Frank	Mackenzie	100	39.02 ± 9.12	4	34	7.96	17.77	124.45
Frank	Mackenzie	75	83.26 ± 29.32	5	100	14.41	43.79	177.40
Frank	Mackenzie	50	36.37 ±11.68	19	603	225.67	225.67	182.65
Frank	Mackenzie	25	56.51 ± 11.84	4	65	28.62	28.62	189.48
Frank	Mackenzie	0	56.02 ± 15.13	6	8	6.57	7.32	102.05

Table 2.2. Results of eDNA sampling and electrofishing at the 50m spatial scale.

Stream Segment	Location	Mean eDNA Concentration (Copies/5µL) ± Standard Deviation	Abundance (individuals)	Biomass (g)	$\Sigma Mass^{0.36}$ (g)	$\Sigma Mass^{0.73}$ (g)	Surface Area (m^2)
Kevin	150	86.46 ± 51.93	1	1	1	1	469.63
Kevin	100	91.98 ± 65.95	5	5	5	5	381.35
Kevin	50	39.38 ± 25.64	2	2	2	2	220.75
Kevin	0	28.77 ± 18.88	2	9	3.11	5.56	260.30
Denise	150	111.71 ± 29.52	2	7	3.13	4.98	137.05
Denise	100	33.25 ± 21.41	0	0	0	0	154.35
Denise	50	66.28 ± 45.39	14	79	20.56	40.83	135.55
Denise	0	183.10 ± 108.81	18	212	56.38	98.07	130.05
Rod	150	1.13 ± 1.34	6	91	14.92	41.36	291.43
Rod	100	0.54 ± 0.77	8	120	19.01	55.43	324.63

Rod	50	24.01 ± 34.84	7	174	31.40	66.26	261.48
Rod	0	106.22 ± 57.68	11	151	58.54	70.49	351.85
Rico	150	65.21 ± 23.46	11	210	29.85	90.23	275.18
Rico	100	66.37 ± 29.72	16	212	37.84	100.17	302.25
Rico	50	77.52 ± 46.69	12	155	41.60	71.46	321.15
Rico	0	107.71 ± 52.64	4	64	28.20	28.94	310.60
Richardson	150	94.76 ± 13.94	13	356	39.31	136.72	390.34
Richardson	100	59.07 ± 24.46	11	107	22.98	54.05	271.30
Richardson	50	50.52 ± 10.79	22	352	126.73	154.51	259.60
Richardson	0	76.41 ± 16.21	22	302	78.45	133.73	304.33
Dave	150	92.08 ± 25.92	19	371	48.76	150.02	347.03
Dave	100	146.21 ± 33.82	12	143	26.63	67.56	233.38
Dave	50	114.10 ± 44.17	31	603	123.39	243.95	277.83
Dave	0	133.38 ± 38.80	19	208	71.68	106.01	278.33
Frank	150	38.54 ± 10.22	16	186	36	90.59	321.43
Frank	100	39.02 ± 9.12	7	59	13.52	30.32	307.08
Frank	50	36.37 ±11.68	24	703	240.08	269.47	360.05
Frank	0	56.02 ± 15.13	10	73	35.19	35.94	291.53

Table 2.3. Results of eDNA sampling and electrofishing at the 100m spatial scale.

Stream Segment	Location	Mean eDNA Concentration (Copies/5µL) ± Standard Deviation	Abundance (individuals)	Biomass (g)	$\Sigma Mass^{0.36}$ (g)	$\Sigma Mass^{0.73}$ (g)	Surface Area (m^2)
Kevin	100	91.98 ± 65.95	6	6	6	6	850.98
Kevin	0	28.77 ± 18.88	4	11	5.11	7.56	481.05
Denise	100	33.25 ± 21.41	2	7	3.13	4.98	291.40
Denise	0	183.10 ± 108.81	32	291	76.94	138.90	265.60
Rod	100	0.54 ± 0.77	14	211	33.93	96.79	616.05
Rod	0	106.22 ± 57.68	18	325	89.94	136.75	613.33
Rico	100	66.37 ± 29.72	27	422	67.69	190.40	577.43
Rico	0	107.71 ± 52.64	16	219	69.79	100.40	631.75
Richardson	100	59.07 ± 24.46	24	463	62.29	190.76	656.42
Richardson	0	76.41 ± 16.21	44	654	205.18	288.24	563.93
Dave	100	146.21 ± 33.82	31	514	75.39	217.59	580.40
Dave	0	133.38 ± 38.80	50	811	195.07	349.95	556.15
Frank	100	39.02 ± 9.12	23	245	49.52	120.91	628.50
Frank	0	56.02 ± 15.13	34	776	275.27	305.40	651.58

Table 2.4. Results of eDNA sampling and electrofishing at the 200m spatial scale.

Stream Segment	Location	Mean eDNA Concentration (Copies/5µL) ± Standard Deviation	Abundance (Individuals)	Biomass (g)	$\Sigma Mass^{0.36}$ (g)	$\Sigma Mass^{0.73}$ (g)	Surface Area (m^2)
Kevin	0	28.77 ± 18.88	10	17	11.11	13.56	1332.03
Denise	0	183.10 ± 108.81	34	298	80.07	143.88	557.00
Rod	0	106.22 ± 57.68	32	536	123.87	233.54	1229.38
Rico	0	107.71 ± 52.64	43	641	137.48	290.81	1209.18
Richardson	0	76.41 ± 16.21	68	1117	267.47	479.00	1218.59
Dave	0	133.38 ± 38.80	81	1325	270.46	567.54	1136.55
Frank	0	56.02 ± 15.13	57	1021	324.79	426.31	1280.08

Chapter 3. Calibrating eDNA results to 25m stream segments assuming the constant or conditional deposition of upstream eDNA

Abstract

Concentrations of eDNA (species-specific nucleotides detected in water samples) have previously been related to the abundance and biomass of fish within aquatic habitats., Due to uncertainties relating to eDNA transport and persistence in lotic systems, however, it is difficult to compare the results of eDNA and electrofishing surveys because eDNA samples may represent larger stream segments than electrofishing. It may be possible to calibrate eDNA concentrations to the same length of stream segments as electrofishing surveys by accounting for the contribution of eDNA from upstream of examined segments. Two methods of calibration were investigated in this study: constant deposition, assuming upstream eDNA deposits at a constant rate in streams and conditional deposition, assuming upstream eDNA deposition is dependant on stream velocity. Samples of eDNA were collected at the base and top of 54 stream segments, each 25m in length, where each segment was triple-pass electrofished to determine the abundance and biomass of Brook Trout (*Salvelinus fontinalis)*. Concentrations of eDNA at the base of stream segments were significantly related to the concentrations at segment tops ($R^2 = 0.342$) suggesting that upstream eDNA contributed to detected eDNA concentrations. Calibrating eDNA concentrations using both methods improved the relation of eDNA concentrations to Brook Trout abundance and biomass (for abundance: prior to calibration $R^2 = 0.223$, constant deposition $R^2 = 0.327$, conditional deposition $R^2 = 0.336$). However, both methods likely incorrectly estimated the contribution of upstream eDNA on occasion; constant deposition likely overestimated the persistence of upstream eDNA whereas conditional deposition likely under or overestimated persistence depending on the accuracy and resolution of stream segment measurements. Further investigation into both models is required, but calibrating eDNA to specific stream segments shows promise for better relating eDNA concentrations to the abundance and biomass of Brook Trout in streams.

Introduction

Environmental DNA (eDNA) sampling has been proposed as a cost, time, and effort efficient alternative to traditional surveys for determining the presence, absence, and abundance/biomass of aquatic species (Jerde et al. 2011; Wilcox et al. 2016). Sampling eDNA entails the detection of species-specific sequences of nucleotides from shed or excreted cells of living or dead individuals of a target species (Ficetola et al. 2008). In some cases, eDNA concentrations have been related to the abundance and/or biomass of target species within surveyed habitats (Takahara et al. 2012; Pilliod et al. 2014; Klymus et al. 2015). However, the downstream transport and persistence of eDNA in lotic systems (such as streams and rivers) is poorly understood, and as a result, the specific length of a stream segment that is represented in an eDNA sample is generally unknown. It is therefore challenging to compare eDNA concentrations from lotic environments to the abundance and biomass measured using other methods such as electrofishing because they may represent different spatial scales. Samples of eDNA have been found to contain eDNA that originated upstream of an investigated stream segment (Wilcox et al. 2016) suggesting that eDNA results from lotic environments may represent a greater spatial scale, and therefore a different density of fish, than a fixed stream length sampled by electrofishing. Consequently, to accurately relate eDNA concentrations to segment-based measurements of abundance and biomass, it may be necessary to first calibrate eDNA concentrations to stream segments by accounting for contributions of eDNA originating upstream of the segments.

The concentration of eDNA that is produced and released within aquatic habitats has previously been found to scale with the abundance and biomass of the target species (Maruyama et al. 2014; Klymus et al. 2015; Wilcox et al. 2016). However, recent evidence suggests that despite increasing with body size (Maruyama et al. 2014), eDNA production scales allometrically with biomass such that larger organisms have lower mass-specific production rates (Yates et al.

2021a). Additionally, the production of eDNA is likely also influenced by the age, life stage (Maruyama et al. 2014), and breeding readiness of individuals (Spear et al. 2015), as well as food availability (Klymus et al. 2015), temperature (Jo et al. 2019), and water velocity (Jane et al. 2015) of the environment they inhabit. As such, eDNA production may vary substantially not only between individuals of different species but also between individuals of the same species under different environmental conditions.

The upstream contribution of eDNA is the concentration of eDNA from individuals upstream of a stream segment that persists to and is sampled at the base of the segment. The persistence of eDNA is determined by degradation, diffusion, and deposition (Barnes and Turner 2016). DNA degradation occurs when the phosphodiester bonds linking nucleotides in the sugar-phosphate backbones of DNA molecules are severed by the actions of nucleases, ultraviolet light, and/or microorganisms (Shapiro 2008), or the species-specific sequence of nucleotides is rearranged or interrupted so that it can no longer be recognized in qPCR (Barnes and Turner 2016). Much of eDNA exists contained within tissue remnants and cells (Mauvisseau et al. 2022), and for eDNA in this state to be digested the cells it is contained in must first be degraded. The impact of degradation on eDNA persistence has been suggested to be less than that of diffusion and deposition in lotic systems (Pilliod et al. 2014) and may be negligible if stream segment length is small or water velocity is high. Diffusion occurs when eDNA is dispersed from regions of higher concentrations to regions of lower concentrations and has previously been associated with stream discharge (Jane et al. 2015; Wilcox et al. 2016) but is likely coupled with deposition. Deposition is the settling of particles from suspension onto the channel bed. Typically, eDNA exists in four states: dissolved in the water column, bound to suspended particles, or encapsulated in a cell or organelle (Mauvisseau et al. 2022) and as a result, in most of its states it may behave similarly to fine particulate organic matter (FPOM).

Two opposing theories of FPOM deposition in lotic systems have been developed in the literature and will be referred to as constant deposition (Cushing et al. 1993) and conditional deposition (Leopold and Maddock 1953; Einstein and Krone 1961). Constant deposition assumes that particles are deposited at a constant rate determined by their size and density but independent of channel hydrology (Cushing et al. 1993). Two assumptions are made to validate constant deposition in lotic systems. Firstly, it is assumed that the velocity of water and the dispersion of particles is consistent throughout the entirety of a system, as increases in cross-sectional area occur to accommodate downstream increases in discharge. Secondly, it is assumed that deposition is unimpeded by water velocity, causing depositional velocities of similarly sized particles to be the same in streams (v_{dep}) as under stagnant conditions (termed fall velocity, v_{fall}; Cushing et al. 1993). Conditional deposition on the other hand postulates that the deposition of particles in lotic systems is limited primarily by the shear stress experienced by the particles, with no deposition occurring when shear stress is sufficient to prevent the fixation of particles to channel beds (Leopold and Maddock 1953; Einstein and Krone 1961). In contrast to constant deposition, conditional deposition assumes that lotic systems have location-specific capacities to suspend particles dependent on the velocity of water and the resultant shear stress experienced at the channel bed, and that deposition occurs when the amount of suspended particles is greater than the ability of a stream to suspend at a specific location or transect (Leopold and Maddock 1953; Einstein and Krone 1961). Constant deposition has been used to estimate the persistence of eDNA through known stream segments and conditions (Wilcox et al. 2016), but conditional deposition has yet to be developed or implemented for practical use.

I hypothesize (1), that if Brook Trout upstream of stream segments contribute to eDNA concentrations at segment bases, correlation will exist between eDNA concentrations detected at the base and top of 25m stream segments; (2) that calibrating eDNA concentrations to the 25m stream segments by accounting for the contribution of upstream eDNA will improve the relationship between eDNA concentrations to the abundance and biomass of Brook Trout within

segments; and (3) that calibration assuming the conditional deposition of upstream eDNA will improve the eDNA-abundance/biomass relationships more than calibration assuming the constant deposition of upstream eDNA because it takes into affect stream hydrology. To test these hypotheses, I sampled eDNA at the base and top of 54 stream segments of 25m, then triple-pass electrofished the segments to determine the abundance and biomass of Brook Trout (*Salvelinus fontinalis)* within each segment.

Methods

The same seven 200m stream segments located within the historical territory of the Fort William First Nation people investigated in the previous chapter were examined. Triplicate eDNA samples were collected every 25m starting at the base of the 200m at approximately 0.25, 0.5, and 0.75 of the channel width (labelled A, B, and C respectively, Fig 3.2) by drawing 1L of water through a 1.2um filter (Whatman GF/C) in accordance with the protocol for collecting eDNA samples from streams (Carim et al. 2016). Negative field controls were not collected as the sampling protocol was designed to minimize the risk of field contamination and the cleaning protocol to effectively remove all DNA from sampling equipment (Carim et al. 2016). Following filtration, filters were submerged in Longmire solution to prevent the degradation of eDNA (Longmire et al. 1997), then underwent qPCR at Trent University to determine the concentration of eDNA within samples (copies/5µL) as described in Chapter 2. After eDNA samples were collected, blocking nets were set across the channel width at each sampling location to separate the 200m into eight 25m segments and prevent the movement of Brook Trout between segments. This allowed for the eDNA concentration at a location to serve as the top of one segment and the bottom of the sequential segment (i.e., eDNA concentration at 25m is the top of segment 1 and the base of segment 2, Fig 3.1)

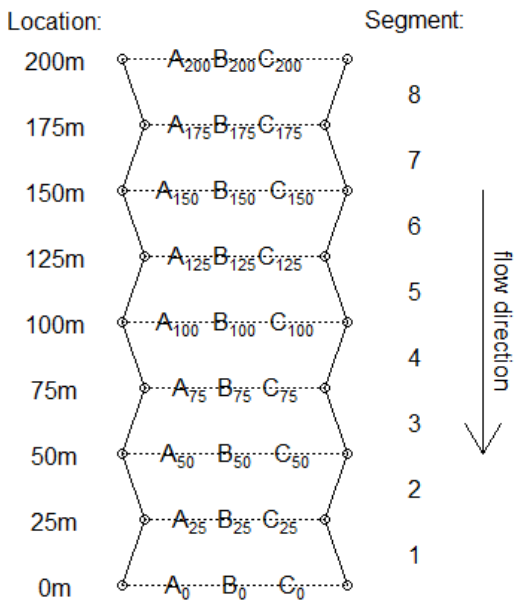


Figure 3.1. Diagram of an idealized 200m stream segment separated into eight 25m segments. Triplicate eDNA samples were collected every 25m at 0.25, 0.5, and 0.75 of the channel width (labelled A-C respectively) starting at the base (0m) and working upstream to 200m. Blocking nets were placed at eDNA sampling locations, and the resulting 25m segments were triple-pass electrofished to estimate Brook Trout abundance and biomass. The concentrations detected at each location served as the top of one segment ($eDNA_{input}$) and the base of the upstream segment ($eDNA_{base}$).

After blocking nets were set, channel widths (*w)* and depths (*h*) were measured every 5m working from the base (0m) to the top of the 200m segments. Channel depth was measured at 0.25, 0.5, and 0.75 of the channel width (labelled A, B, and C respectively, Fig 3.2), and average depth was calculated by summing the three depth measurements then dividing by four to account for the assumed trapezoidal shape of the channel and the depths of 0m at the banks. Cross-sectional area at each 5m transect (A_n) was calculated by multiplying the average depth by the wetted width of the transect, and the average cross-sectional area of each of the 25m segments was calculated by averaging the cross-sectional areas of the six transects within segments. Surface area (m^2) was calculated for each 25m segment by multiplying the average width by the length of the segment (25m, with the exception of the Richardson segment 8 which was 33m).

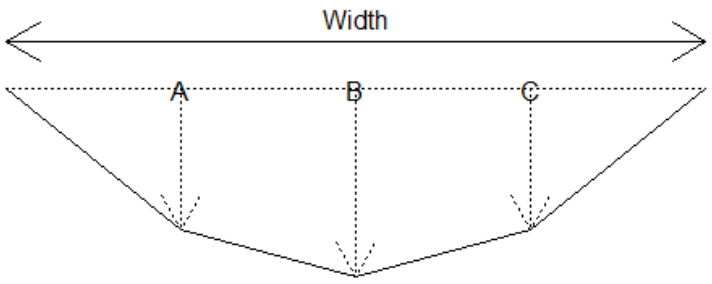


Figure 3.2. Diagram of the cross-sectional measurements taken every 5m of the stream segments. Depth was measured at points A-C. All transects were assumed to have a trapezoidal shape with depths of 0m at its banks.

Stream discharge was surveyed at each eDNA sampling location by measuring the width of the channel, and the depth and water velocity at equal distances across the wetted-width. Velocity was measured using by suspending an electromagnetic flow meter (MF pro, OTT) parallel to flow direction at 0.6 of the stream depth. The number of measurements was based on transect widths, with 10, 15, or 20 measurements for transects less than 1m, greater than 1m but less than 2m and greater than 2m respectively. The discharge at each sampling location was calculated by multiplying the wetted width by the average velocity and the average depth of the cross-section (Discharge = Width*Average Velocity*Average Depth; Chow 1959). Since discharge should be constant within stream segments if no additional inputs or outputs exist (Chow 1959), the 9 discharge measurements within the 200m stream segments were averaged to give a single discharge value for each 200m segment. The average velocity of each 25m stream segment was calculated by dividing discharge by the average cross-sectional area of the segment. Temperature was measured at three equally spread-out points across a streams cross-section every 5m by pressing a thermal probe (ThermoWorks) to the substrate. Average cross-sectional temperature was calculated from the three measurements. Temperature loggers (Hoboware, Onset) were placed into the stream substrate every 5m days prior to eDNA sampling and measured substrate temperature every 30 minutes. Average cross-sectional temperature at the time of eDNA sampling was calculated by dividing the average cross-sectional temperature by the logged temperature at the time of the temperature survey then multiplying by the logged

temperature at the time of eDNA sampling. Segment average temperature at time of sampling was calculated by averaging the cross-sectional temperatures throughout the stream segment.Following eDNA sampling and channel measurements, each 25m segment was triple-pass electrofished using an Apex Backpack Electrofisher (Smith-Root). After each pass, Brook Trout were counted, individually weighed to the nearest 1g, then released downstream of the blocking net. The abundance (number of individuals) and biomass (total mass of individuals) were summed for each 25m segment then standardized by dividing by the surface area of the segment.

To spatially-calibrate eDNA concentrations to the electrofished 25m segments ($eDNA_{segment}$), the contributions of eDNA originating upstream of the segments ($eDNA_{upstream}$) were subtracted from the concentrations of eDNA detected at the base of the segments ($eDNA_{base}$). The contribution of upstream eDNA was determined by multiplying the concentration of eDNA detected at the top of a segment ($eDNA_{input}$) by the longitudinal persistence of eDNA/FPOM through the segment ($P_{segment}$), using equation 1:

$$eDNA_{segment} = eDNA_{base} - eDNA_{input}(P_{segment}) \qquad \text{(equation 1)}$$

Two methods were used to estimate $P_{segment}$; one method assumed the constant deposition of eDNA and the other assumed the conditional deposition of eDNA.

Constant deposition assumes that the concentration of fine particulate organic matter (FPOM) suspended within a lotic system decreases longitudinally at an exponential rate, as represented by equation 2 (modified from Cushing et al. 1993):

$$C_{(x)} = C_{(x+a)}\exp(-k_p a) \qquad \text{(equation 2)}$$

Where, $C_{(x)}$ is the concentration of particles that persists to location x, a is the distance upstream of location x of the input of upstream particles, $C_{(x+a)}$ is the concentration of particles at the location of upstream input, and k_p (m^{-1}) is a 1st order longitudinal loss rate (Cushing et al. 1993;

Minshall et al. 2000). The result of $exp(-k_p a)$ is the persistence of particles (P_a) a distance downstream of their source. From Wilcox et al. (2016), the persistence of eDNA 100m from its source was 0.63, which results in equation 3a:

$$P_{(a)} = \exp(-k_p a) \text{ or } 0.63 = \exp(-k_p * 100) \quad \text{(equation 3a)}$$

This can be rearranged to solve for the longitudinal loss rate (k_p).

$$k_p = -\ln(0.63)/100 \quad \text{(equation 3b)}$$

Since constant deposition assumes that similarly sized particles will deposit out of suspension at the same velocity independent of hydrological conditions (Cushing et al. 1993), the longitudinal loss rate from Wilcox et al. (2016) can be used to calculate a depositional velocity (v_{dep}) of eDNA applicable in this study:

$$v_{dep} = uhk_p \quad \text{(equation 4a)}$$

Where, u is the average velocity of water (m/s), and h is the average depth (m) throughout the segment. Parameters u and h are not reported for the stream segment Wilcox et al. (2016) used to calculate persistence, but the discharge (Q in m^3/s) and average width (w in m) are known ($0.065 m^3/s$ and 3m respectively) allowing the value of uh to be calculated using the equation for discharge (Chow 1959):

$$Q = uhw \quad \text{(equation 5)}$$

$$\text{Rearranged, } uh = Q/w \qquad uh = 0.065 m^3 s^{-1}/3m$$

Substituting uh into equation 4a, v_{dep} is calculated as $4.35 \times 10^{-5} m/s$ from Wilcox et al. (2016). Equation 4a can be rearranged to calculate the specific longitudinal loss rate (k_p) of eDNA for each 25m segment of this study, since the average velocity (u) and depth (h) of each segment are known:

$$k_p = v_{dep}/\mathit{u}h \quad \text{(equation 4b)}$$

These segment specific longitudinal loss rates (k_p) can be inserted into equation 3a to calculate the persistence of eDNA through the segment ($P_{segment}$), which can then be inserted into equation 1 to calibrate the concentration of eDNA detected at the base of a segment to the electrofished 25m segment assuming the constant deposition of upstream eDNA:

$$eDNA_{segment} = eDNA_{base} - eDNA_{input} * \exp(-k_p 25) \quad \text{(equation 6)}$$

Conditional deposition assumes that the concentration of a particle will decrease longitudinally due to locational variations in the capacity of a channel to suspend particles (Leopold and Maddock 1953). This can be expanded into an approach to calculate eDNA/FPOM persistence, referred to as the Suspension Water Area Gradient (SWAG) method hereafter, which assumes that the shear stress experienced by particles at the channel bed is proportional to the velocity at that location (transect), and that if discharge is constant throughout a stream segment, velocity will decrease proportionally to an increase in cross-sectional area. The SWAG method allows for the deposition of eDNA/FPOM whenever a longitudinal increase in cross-sectional area occurs, and suggests that following deposition, the remaining suspended eDNA/FPOM will be dispersed equally throughout the cross-section, resulting in a reduction to the concentration of eDNA/FPOM that is inversely proportional to the increase in cross-sectional area. If multiple changes in cross-sectional area occur, the reduction to the concentration of eDNA/FPOM will be inversely proportional to the product of the positive changes in cross-sectional area, as expressed by the product operated decline:

$$C_{(x)} = C_{(x+a)} \prod_{n=x}^{x+a-b} \frac{A_{n+b}}{A_n} \quad \text{(Equation 7)}$$

And in cases where $A_{n+b} > A_n$, $\frac{A_{n+b}}{A_n} = 1$

where, $C_{(x)}$ is the concentration of eDNA/FPOM that persists to location x, $C_{(x+a)}$ is the concentration of the upstream eDNA/FPOM at the input point a distance upstream of location x, b is the distance between sequential transects, A_{n+b} is the cross-sectional area of a transect at location n+b, A_n is the cross-sectional area of a transect at location n, and n ranges between x and $x+a-b$ in increments of b (as illustrated in Figure 3.3).

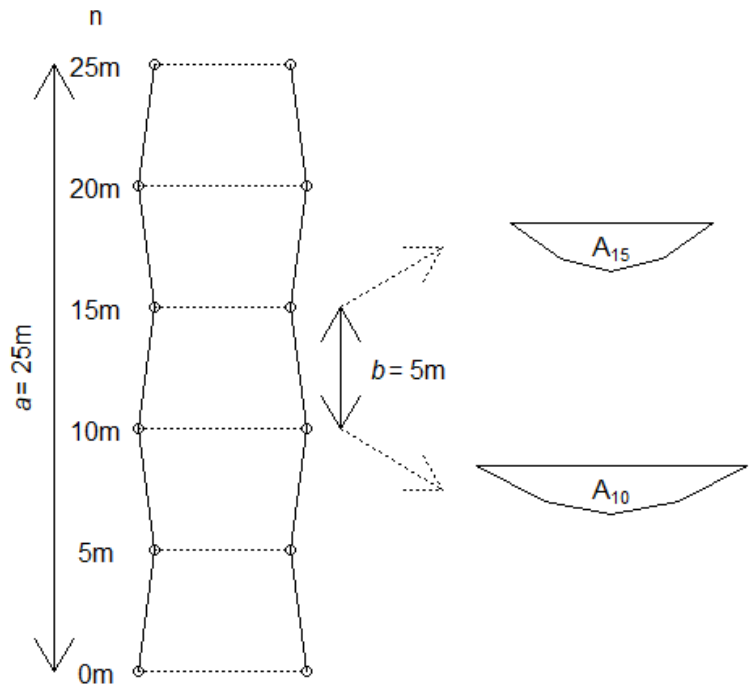


Figure 3.3. Diagram of an idealized 25m stream segment. Where (as specified in equations 7 and 8) a is the length of the segment, n is the location of measured transects, b is the distance between measured transects and A_n is the cross-sectional area of the transect at location n.

A_{n+b}/A_n is the persistence of upstream eDNA/FPOM between sequential transects b distance apart (it can be replaced with u_n/u_{n+b} where u is the average velocity at the transect). When A_{n+b} is larger than A_n ($A_{n+b} > A_n$), the persistence of upstream eDNA should be assigned a value of 1, despite A_{n+b}/A_n being greater than one. This is because a decrease in cross-sectional area (and consequent increase in velocity) will neither decrease the amount of water that eDNA/FPOM is suspended in, nor increase the amount of suspended upstream eDNA/FPOM since no additional upstream eDNA/FPOM is considered to exist at these locations. Assuming the

conditional deposition of upstream eDNA (SWAG method), the contribution of upstream eDNA (Equation 7) can be substituted into equation 1 to calibrate the concentrations of eDNA to the electrofished 25m stream segments ($eDNA_{segment}$). Since the input point of upstream eDNA in this study is 25m above the sampling location and sequential transects are 5m apart, *a* and *b* are 25 and 5 meters respectively, resulting in:

$$eDNA_{segment} = eDNA_{base} - eDNA_{input}\prod_{n=x}^{x+20}\frac{A_{n+5}}{A_n} \qquad \text{(equation 8)}$$

And in cases where $A_{n+5} > A_n$, $\frac{A_{n+5}}{A_n} = 1$

Statistical Analysis:

As in chapter 2, the eDNA concentrations at Dave 125 and Denise 175 were abnormally high for the respective abundances and biomasses of Brook Trout captured within 25m upstream of sampling locations. An abnormally high sample concentration (859.84 copies/5µL) was removed from Dave 125 and the average concentration of the remaining 2 samples was used for that location. Denise 175 was treated as an outlier and because it was included as the top of segment 7 and the bottom of segment 8 (150-175m), segments 7 and 8 of Denise were excluded from future analyses.

To determine if eDNA originating upstream of stream segments contributed to detected concentrations of eDNA, a linear regression was conducted comparing the concentrations of eDNA detected at the bases ($eDNA_{base}$) and tops ($eDNA_{input}$) of 25m stream segments.

To determine if calibrating the concentrations of eDNA to the 25m stream segments improved the relation of eDNA concentrations to Brook Trout abundance and biomass, linear regressions were conducted comparing the detected ($eDNA_{base}$) and spatially calibrated concentrations of eDNA assuming the constant ($eDNA_{constant}$) or conditional ($eDNA_{SWAG}$) deposition of upstream eDNA to the abundance and biomass of Brook Trout within segments. It

should be noted that 25m segments within the same 200m stream segments are not truly independent because the top of one 25m segment is the base of the subsequent segment. However, this non-independence is likely mitigated by attempting to account for the contribution of eDNA originating upstream of considered segments.

Mixed effect models where generated comparing the calibrated eDNA concentrations to the interacting effects of Brook Trout biomass, segment average velocity and segment average temperature, and the random effect of stream (calibrated eDNA = Biomass*Velocity* Temperature +(1|Stream) using lmerTest package in R (4.2.0). Non-significant interacting effects were removed then added as additive effects until all considered effects were significant.

Results

Concentrations of eDNA at the top of the 25m segments ($eDNA_{input}$) were found to account for 34% of the variation in the concentrations at the base of the segments ($eDNA_{base}$) ($R^2 = 0.324$, $p < 0.001$, Fig 3.4), suggesting that concentrations of eDNA at the base of the segments were not solely comprised of eDNA from Brook Trout within the segments but also from Brook Trout upstream of the segments, indicating that eDNA at the site of sampling represented a larger spatial scale than the electrofished 25m segments.

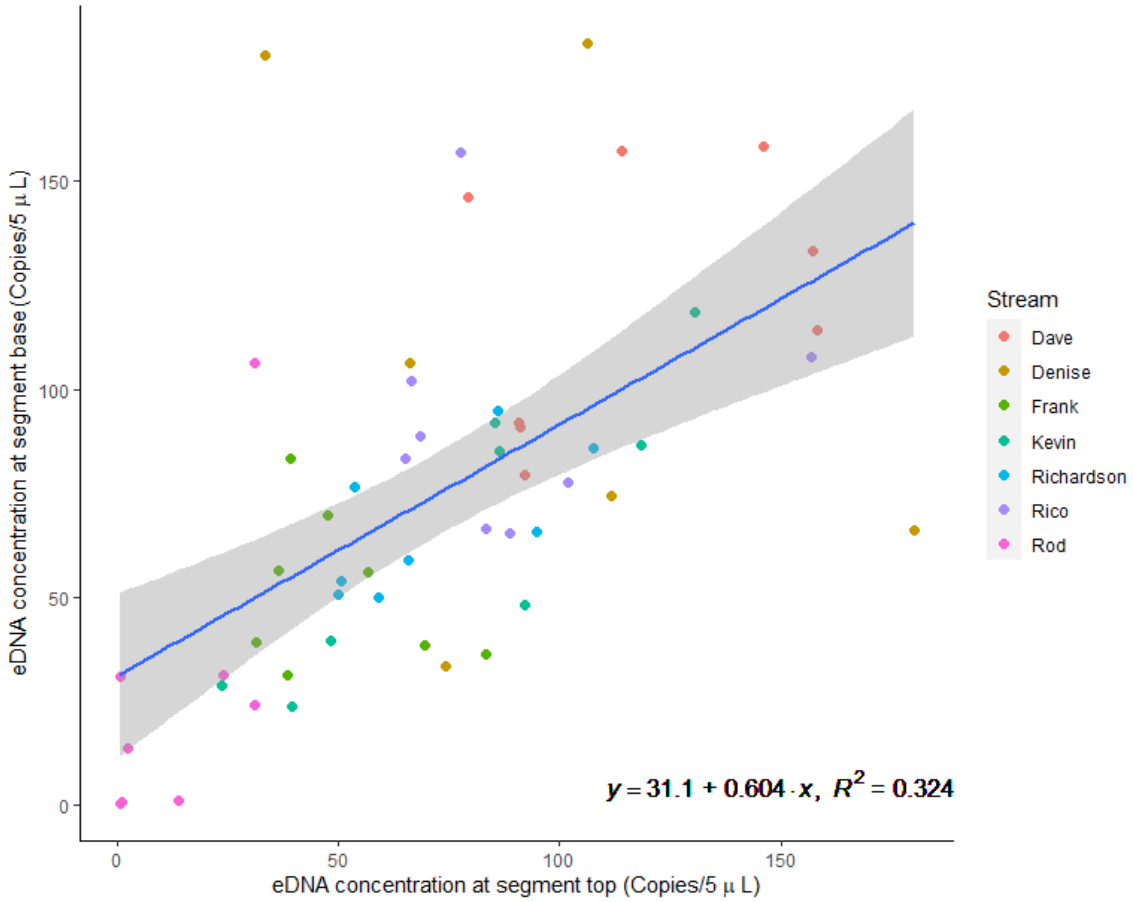


Figure 3.4. Relationship between eDNA concentrations at the base and top of 25m stream segments. The blue line represents the linear regression and the grey shade the associated standard error.

Following the calibration of eDNA concentrations to the 25m segments assuming the constant deposition of upstream eDNA, 18 predicted concentrations using equation 6 were negative ($eDNA_{constant}$, table 3.1). Of these segments, 5 had no Brook Trout captured by electrofishing so the calibrated eDNA concentrations should be zero; this suggests that calibration assuming constant deposition predicts that more upstream eDNA persists to the base of the segments than actually occurs, resulting in negative eDNA concentrations. Following the calibration of eDNA concentrations to the 25m segments assuming the conditional deposition of upstream eDNA, 5 predicted concentrations using equation 8 were negative ($eDNA_{SWAG}$, Table 3.1). Of these segments, 4 had Brook Trout and 1 did not, indicating that calibrating eDNA

results assuming conditional deposition overestimated the persistence of upstream eDNA under some conditions, but less frequently and/or less intensely than constant deposition. However, following calibration, 4 concentrations of eDNA were positive despite the absence of Brook Trout within the respective stream segments ($eDNA_{SWAG}$, Table 3.1), suggesting that under some conditions calibration assuming conditional deposition underestimated the persistence of upstream eDNA and its contribution to eDNA samples.

The abundance of Brook Trout within the 25m stream segments accounted for 22.3% of the variation in the eDNA concentrations prior to calibration ($R^2 = 0.223$, $p < 0.001$, Fig 3.5), 32.7% of the variation in the eDNA concentration calibrated assuming constant deposition ($R^2 = 0.327$, $p < 0.001$, Fig 3.5) and 33.6% of the variation in the eDNA concentration calibrated assuming conditional deposition ($R^2 = 0.336$, $p < 0.001$, Fig 3.5). The models including non-calibrated and conditionally calibrated eDNA concentrations had y-intercepts (predicted eDNA concentrations when abundance was zero) greater than zero (48.7 and 21.7 respectively, $p < 0.05$) suggesting that the contribution of upstream eDNA had not been completely accounted for on average. The model including eDNA concentrations calibrated assuming constant deposition had a non-significant y-intercept ($p = 0.825$), indicating it was not significantly different from zero and therefore the relationship can be assumed to pass through the origin.

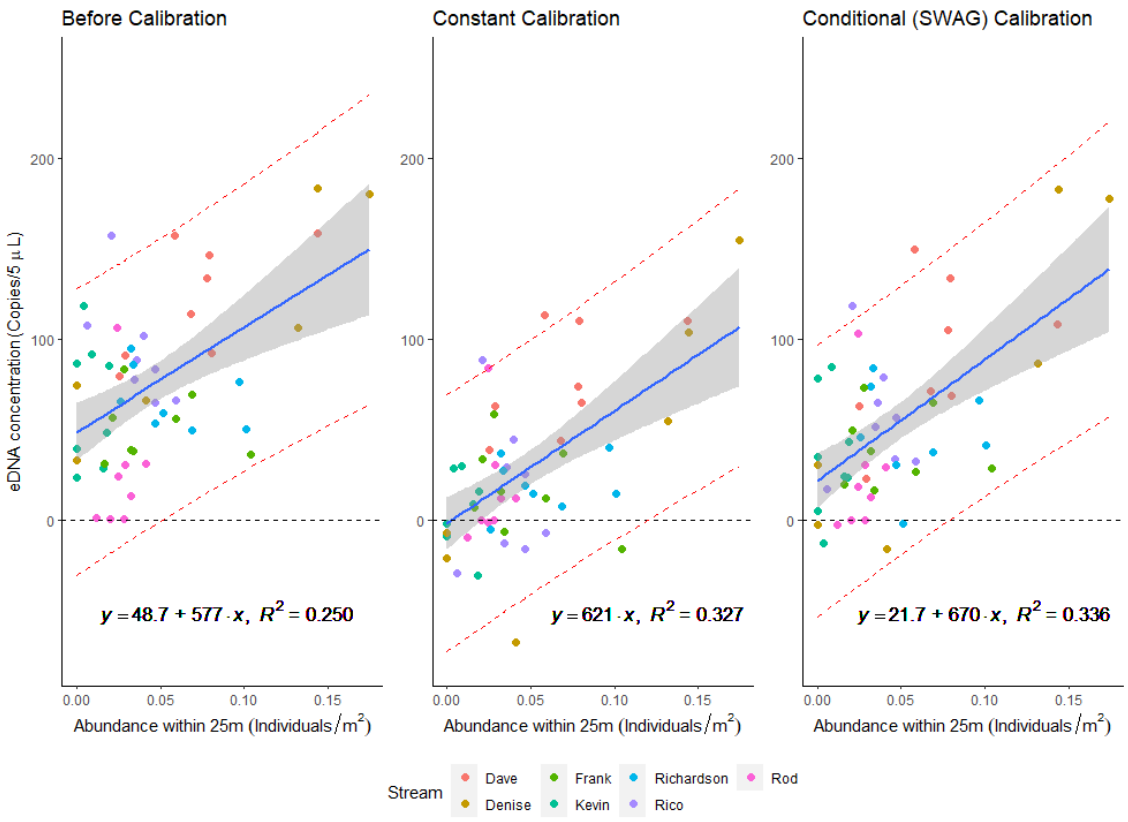


Figure 3.5. Uncalibrated and calibrated eDNA concentrations vs Brook Trout abundance. Left to right, eDNA concentrations prior to calibration, following calibration assuming constant deposition, and following calibration assuming conditional deposition. Blue lines are linear regressions, the surrounding grey shade is the associated error, red lines are the 95% prediction interval, and black lines show 0 copies/5µL.

The biomass of Brook Trout within stream segments accounted for 6.4% of the variation in the non-calibrated eDNA concentration ($R^2 = 0.064$, $p = 0.035$, Fig 3.6), 12.4% of the variation in the constantly calibrated eDNA concentration ($R^2 = 0.124$, $p = 0.006$, Fig 3.6), and 14.3% of the variation in the conditionally calibrated eDNA concentration ($R^2 = 0.143$, $p = 0.003$, Fig 3.6). The uncalibrated and conditionally calibrated models had y-intercepts greater than zero (62.4 and 35.5 respectively, $p < 0.05$) suggesting that the contribution of upstream eDNA had not been completely accounted for by calibration assuming conditional deposition. The model calibrated assuming the constant deposition of upstream eDNA did not have a significant y-intercept ($p = 0.11$), indicating that it passed through the origin.

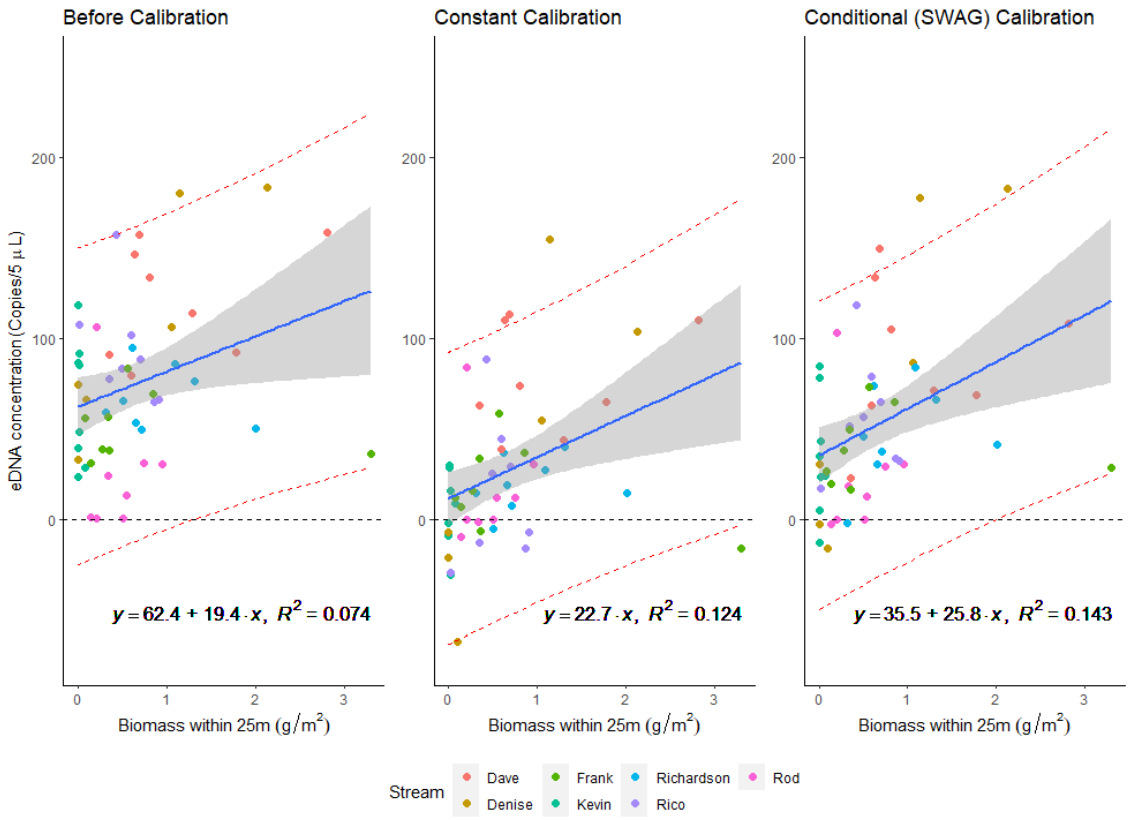


Figure 3.6. Uncalibrated and calibrated eDNA concentrations vs Brook Trout biomass. Left to right, eDNA concentrations prior to calibration, following calibration assuming constant deposition, and following calibration assuming conditional deposition. Blue lines are linear regressions, the surrounding grey shade is the associated error, and red lines are the 95% prediction interval.

The biomass of Brook Trout, average velocity, and average temperature at the time of eDNA sampling had significant interacting effects ($p < 0.05$) on the calibrated concentration of eDNA assuming the conditional deposition of upstream eDNA (Table 3.3). The biomass of Brook Trout and average temperature at time of eDNA sampling had significant interacting effects, and velocity had a significant additive affect ($p < 0.05$) on the calibrated concentration of eDNA assuming the constant deposition of upstream eDNA (Table 3.4). This suggests that stream temperature and velocity influence the amount of eDNA that is produced within segments.

Discussion

Environmental eDNA from individuals upstream of considered stream segments contribute to the concentrations in samples collected at segment bases but this contribution can likely be accounted for. Calibrating eDNA results to the electrofished 25m stream segments improved the relation of eDNA concentrations to the abundance and biomass of Brook Trout, suggesting that to best relate eDNA results to species density at known spatial scales in lotic systems, the contribution of upstream eDNA should be considered. Both methods of calibration (constant and conditional deposition) resulted in closer relationships between eDNA concentrations and Brook Trout abundance/biomass, with conditional deposition resulting in marginally better fitting models, and constant deposition resulting in models that passed through the origin. However, following calibration, some concentrations of eDNA were negative whereas others remained relatively high for the abundance and biomass of Brook Trout within their respective segments, indicating that unaccounted factors likely confounded the relation of eDNA concentrations to species density. These factors are likely: (1) production rates of eDNA varying between stream segments, (2) the incomplete persistence of eDNA produced by individuals within segments to sampling locations, (3) inaccurate calculation of the contributions of upstream eDNA due to the inaccurate estimation of eDNA persistence throughout segments assuming constant and conditional deposition, and (4) the resuspension of eDNA stored in sediment.

It is unlikely that eDNA production rates were equal between stream segments or amongst individuals within segments. Previous studies have suggested or shown that abiotic and biotic factors such as temperature (Jo et al. 2019), velocity (Jane et al. 2015), food availability (Klymus et al. 2015), organism age (Maruyama et al. 2014), metabolic rate and activity (Jo et al. 2019) influence the production of eDNA relative to the biomass of the target species. Thus, variation in environmental conditions between segments likely caused the production rate of

eDNA to differ between segments resulting in different amounts of eDNA being produced by similar abundances/biomasses of Brook Trout. This was further supported by mixed effect models in this study indicating that water velocity and temperature interacted with the relation of Brook Trout biomass to the calibrated eDNA concentrations (Tables 3.3 and 3.4). Future studies should investigate how environmental factors, notably temperature and velocity, influence eDNA production relative to biomass in controlled settings to facilitate the relation of eDNA concentrations to biomass in natural settings.

Attempting to relate the concentration of eDNA calibrated to stream segments ($eDNA_{segment}$) to Brook Trout abundance and biomass assumes that all the eDNA produced by Brook Trout within segments persists to and is detected at sampling locations. This is unlikely to be the case. Similarly to eDNA originating upstream of stream segments, eDNA originating from individuals within segments likely undergoes degradation, diffusion, and deposition prior to the point of being collected. Consequently, $eDNA_{segment}$ is likely not only a factor of Brook Trout abundance/biomass and eDNA production, but also eDNA persistence and Brook Trout distribution within segments. Since eDNA persistence varies with the distance of eDNA from its source (Wilson and Wright, 2013; Jane et al. 2015; Laramie et al. 2015; Fukomoto et al. 2015; Wilcox et al. 2016), the distribution of individuals and biomass throughout segments will influence the concentration of eDNA that persists to sampling locations, causing the concentrations that are detected to differ from the concentrations that are initially produced. Thus, a large amount of variability in eDNA concentrations is likely due to the unknown distribution of Brook Trout throughout stream segments.

The potential magnitude of variability associated with distribution patterns was higher when relating eDNA concentrations to biomass than to abundance, as the potential heterogeneity of distribution was smaller for abundance than biomass; abundance assigned values of 1 to all individuals, but biomass values varied substantially between individuals from 1 to 118g. The

greater heterogeneity of biomass than abundance potentially explains why abundance accounted for more variation in eDNA concentrations than biomass in this and previous studies (Baldigo et al. 2017; Yates et al. 2021b). This may appear surprising because biomass considers individual size and thus should be more closely related to the initially produced concentration of eDNA (Maruyama et al. 2014), however, it may be that in lotic systems, the influence of the distribution of individuals and biomass on the persistence of eDNA outweighs the factors influencing its production. Because large differences will occur in eDNA concentrations that persist to the sampling locations depending on the distribution of individuals, the presumably heterogeneous distribution of individuals within stream segments will likely always result in unaccounted variability in the relationship between eDNA concentrations and Brook Trout abundance/biomass. The magnitude of this variability is likely related to eDNA persistence, and as persistence increases, the influence of distribution will decrease, as the concentrations of eDNA that persist to and are detected at sampling locations approach the concentrations initially produced within segments.

The inaccurate estimation of upstream eDNA persistence and contribution when eDNA concentrations were calibrated assuming constant deposition may be attributed to two possibilities: first, that inaccurate measurements of segment average depth and velocity resulted in incorrect calculations of longitudinal loss rates (k_p) of eDNA, or second, that eDNA does not deposit at a constant rate in lotic systems. Collecting three depth measurements for each of the six transects in the 25m segments may have been insufficient to accurately calculate the average depth and velocity of the segments. If the calculated depth or velocity of a segment were inaccurate, the longitudinal loss rate of upstream eDNA would be incorrectly estimated, resulting in the under- or overestimation of eDNA persistence depending on whether calculated depth and velocity values were lesser or greater than the actual values. However, averaging the transect depths over the 25m segments should have reduced some of the inaccuracy caused by the small

number of measurements for each transect, better aligning the calculated and actual average depths and velocities.

Alternatively, the incorrect estimation of upstream eDNA persistence may be because particles do not deposit at a constant rate in streams. Fundamental flaws exist in two of the assumptions made to validate the constant deposition of eDNA and fine particulate organic matter (FPOM). First, the assumption that no longitudinal change in velocity occur within streams because longitudinal increases in cross-sectional area compensate for increases in discharge (Cushing et al. 1993) was neither the case in this study, where cross-sectional area varied substantially within segments (25 and 200m segments), nor in the majority of naturally occurring streams that have significant longitudinal diversity in channel morphology (Fischer et al. 1979). Second, the assumption that the deposition of particles is not influenced by water velocity was disproved in 1961 when sufficiently high velocities of water prevented the deposition and fixation of particles to channel substrates (Einstein and Krone 1961). Additionally, if particles did deposit at a constant rate, depositional and fall velocities of FPOM should be universal for all streams, which has not been the case as discrepancies have occurred between depositional and fall velocities both within and between studies (e.g., Minshall et al. 2000; Paul and Hall 2002). These flawed assumptions and inconsistent depositional velocities among studies, may suggest that eDNA (and other FPOM) does not deposit at a constant rate within streams and that assuming the constant deposition of upstream eDNA incorrectly estimated its persistence in this study. However, since the calibrated concentrations of eDNA assuming the constant deposition of upstream eDNA was more closely related to Brook Trout abundance/biomass than the uncalibrated concentrations and passes through the origin of the relationship with abundance and biomass, constant deposition cannot be confidently discredited. Future studies should re-evaluate constant deposition under stable and well-understood stream conditions to determine if it can accurately predict the persistence of eDNA and FPOM despite its incorrect assumptions.

Conditional deposition (the SWAG method) likely incorrectly estimated the persistence of upstream eDNA through segments due to the low-resolution of the cross-sectional area measurements in this study. The SWAG method would incorrectly estimate persistence if cross-sectional area increased between measured transects, or if differences between the calculated and actual cross-sectional areas of transects caused calculated longitudinal increases in cross-sectional area to be greater or less than what actually occurred. An unmeasured increase in cross-sectional area between measured transects would decrease the capacity of a channel to suspend eDNA, causing the deposition and dispersal of eDNA and overestimation of eDNA persistence by the SWAG method. If calculated increases in cross-sectional area were greater than the actual increases in cross-sectional area, the SWAG method would underestimate eDNA persistence, as more deposition/dispersal would be calculated than had actually occurred. Similarly, if calculated increases in cross-sectional area were less than the actual increases in cross-sectional area, the SWAG method would overestimate eDNA persistence as less deposition/dispersal would be calculated than had occurred. These errors would be reduced if more detailed cross-sectional measurements were collected at each transect (i.e., more depth measurements), and the distance between sequential transects was shortened (b in equation 7). Because it is novel to this study, the assumptions of the SWAG method remain largely untested and require further investigation before it can confidently calculate eDNA persistence and account for the contribution of upstream eDNA.

The transient storage of eDNA in streams is another factor that may have contributed to the high level of variability in the relation of eDNA concentrations to Brook Trout abundance and biomass in this study. Transient storage occurs when particles are transferred from regions of main flow to regions of stagnant storage, where they can remain for indeterminate amounts of time (Bencala 1983; Hart 1995; Morrice et al. 1997). It is likely that eDNA is exchanged between the main flow and stagnant storage depending on, and altering, the concentration of eDNA in

both regions (Bencala 1983; Hart 1995;). Thus, transient storage would influence the persistence of eDNA and may serve to stabilize it temporally (Paul and Hall 2002). In most regions of stagnant storage, eDNA can be assumed to deposit according to Stokes' Law as there is not sufficient shear stress to keep it suspended (Einstein and Krone 1961; Dietrich 1982). Additionally, since regions of transient storage such as back eddies (Hart 1995) are often favoured places for Brook Trout to inhabit (OMNRF 2017), it is likely that many Brook Trout occupy regions of transient storage, rendering the fate of eDNA from these individuals uncertain. A detailed understanding of flow dynamics within streams, as well as the transfer of particles between main flow and stagnant storage is required before transient storage can be accounted for in the longitudinal transport and persistence of eDNA.

The resuspension of sedimentary eDNA may explain some of the unaccounted-for variation in eDNA concentrations. In streams, concentrations of eDNA within sediments have in some cases been found to be greater and degrade slower than concentrations suspended within water columns (Turner et al. 2015). Therefore, under certain conditions, sedimentary eDNA, which is likely no longer related to individuals within stream segments (at least not temporally; Turner et al. 2015), may be resuspended and contribute to sampled eDNA concentrations (Shogren et al. 2017), obfuscating the relation of eDNA concentrations to current Brook Trout abundance/biomass. The possibility of eDNA resuspension severely complicates the interpretation of eDNA results, and future studies should examine the interaction between concentrations of sedimentary and suspended eDNA in relation to channel hydrology.

Despite the relation of eDNA concentrations to Brook Trout abundance/biomass being improved by the calibration of eDNA results to the electrofished 25m stream segments, there may be a limit to the extent that eDNA concentrations can be related to the abundance and biomass of Brook Trout in streams. In streams, the amount of eDNA that is initially produced within segments is unlikely to be the same as the amount that persists in the water column and is

detected at sampling locations. The distribution of Brook Trout within segments, and the resultant persistence of their eDNA likely varies within and among stream segments, resulting in different concentrations of eDNA being detected for similar abundances/biomasses of Brook Trout. Additionally, the relation of eDNA concentrations to abundance/biomass is probably further confused by the resuspension and contribution of sedimentary eDNA to samples. Thus, sources of variability and uncertainty will likely always be present when interpreting eDNA results in lotic systems but may be diminished with an improved understanding of eDNA persistence and transportation. Both methods of calibration, constant and conditional deposition of upstream eDNA, showed potential to improve the relation of eDNA concentrations to Brook Trout abundance/biomass. However, both methods rely on untested or flawed assumptions and may not accurately predict eDNA persistence, requiring further investigation before they can be confidently implemented in future studies.

Chapter 3 Tables

*Table 3.1. eDNA concentrations and electrofishing results for 25m segments. *$eDNA_{base}$ of Denise segment 8 and $eDNA_{input}$ of Denise segment 7 were treated as outliers due to its high concentrations of eDNA for its respectively low abundance and biomass of Brook Trout within 25m, so Denise segments 7 and 8 were removed not included in analyses **One triplicate of $eDNA_{base}$ of Dave segment 6 was removed as an outlier due to its high concentration relative to its other triplicates.*

Stream Name	Segment	Mean $eDNA_{base}$ (Copies/5µL) ± Standard Deviation	Mean $eDNA_{input}$ (Copies/5µL) ± Standard Deviation	$eDNA_{constant}$ (Copies/5µL) ± Standard Deviation	$eDNA_{SWAG}$ (Copies/5µL) ± Standard Deviation	Abundance (#/m^2)	Biomass (g/m^2)
Kevin	8	118.52 ± 77.03	130.75 ± 85.66	28.90 ± 135.73	-12.23 ± 162.69	0.004	0.004
Kevin	7	86.46 ± 51.93	118.52 ± 77.03	-1.53 ± 109.12	78.11 ± 57.36	0	0
Kevin	6	85.24 ± 79.69	86.46 ± 51.93	16.24 ± 121.13	43.21 ± 104.93	0.019	0.019
Kevin	5	91.98 ± 65.95	85.24 ± 79.69	30.27 ± 123.65	84.84 ± 72.62	0.009	0.009
Kevin	4	48.24 ± 35.71	91.98 ± 65.95	-30.19 ± 91.95	23.94 ± 53.13	0.018	0.018
Kevin	3	39.38 ± 25.64	48.24 ± 35.71	-1.80 ± 56.13	35.40 ± 28.58	0	0
Kevin	2	23.57 ± 18.03	39.38 ± 25.64	-8.90 ± 39.17	5.32 ± 29.91	0	0
Kevin	1	28.77 ± 18.88	23.57 ± 18.03	9.12 ± 33.92	24.25 ±22.34	0.016	0.071
Denise	8	391.03* ± 107.73	469.26 ± 122.73			0.032	0.113
Denise	7	111.71 ± 29.52	391.03* ± 107.73			0	0
Denise	6	74.28 ± 38.57	111.71 ± 29.52	-6.57 ± 59.93	30.50 ± 50.13	0	0
Denise	5	33.25 ± 21.41	74.28 ± 38.57	-20.70 ± 49.42	-2.56 ± 40.01	0	0
Denise	4	180.19 ± 103.77	33.25 ± 21.41	154.62 ± 120.23	177.21 ± 105.69	0.174	1.142
Denise	3	66.28 ± 45.39	180.19 ± 103.77	-66.93 ± 122.10	-15.60 ± 92.54	0.041	0.097
Denise	2	106.20 ± 52.21	66.28 ± 45.39	54.72 ± 87.47	86.92 ± 65.41	0.132	1.056
Denise	1	183.10 ± 108.81	106.20 ± 52.21	103.59 ± 147.89	182.72 ± 108.99	0.144	2.131
Rod	8	13.80 ± 16.11	2.19 ± 1.60	12.01 ± 17.41	13.05 ± 16.66	0.032	0.544
Rod	7	1.13 ± 1.34	13.80 ± 16.11	-9.42 ± 13.65	-2.14 ± 5.16	0.012	0.138
Rod	6	0.73 ± 1.25	1.13 ± 1.34	-0.12 ± 2.26	0.14 ± 1.95	0.028	0.510
Rod	5	0.54 ± 0.77	0.73 ± 1.25	-0.03 ± 1.76	0.36 ± 1.08	0.020	0.203
Rod	4	30.98 ± 27.73	0.54 ± 0.77	30.55 ± 28.35	30.71 ± 28.12	0.029	0.954
Rod	3	24.01 ± 34.84	30.98 ± 27.73	-1.42 ± 57.61	18.32 ± 39.94	0.025	0.336

Rod	2	31.14 ± 31.47	24.01 ± 34.84	12.18 ± 58.98	29.16 ± 34.34	0.041	0.746
Rod	1	106.22 ± 57.68	31.14 ± 31.47	83.89 ± 80.25	103.44 ± 60.50	0.024	0.204
Rico	8	88.78 ± 24.77	68.58 ± 39.80	29.11 ± 59.42	65.23 ± 38.45	0.036	0.699
Rico	7	65.21 ± 23.46	88.78 ± 24.77	-15.97 ± 46.13	33.58 ± 32.30	0.046	0.864
Rico	6	83.35 ± 67.82	65.21 ± 23.46	25.76 ± 88.54	56.80 ± 77.37	0.047	0.495
Rico	5	66.37± 29.72	83.35 ± 67.82	-7.05 ± 89.47	32.35 ± 57.40	0.059	0.904
Rico	4	101.88 ± 54.73	66.37 ± 29.72	44.56 ± 80.41	79.20 ± 64.89	0.040	0.595
Rico	3	77.52 ± 46.69	101.88 ± 54.73	-12.82 ± 95.22	51.87 ± 60.47	0.035	0.345
Rico	2	157.03 ± 82.25	77.52 ± 46.69	88.27 ± 123.67	118.36 ± 105.547	0.021	0.423
Rico	1	107.71 ± 52.64	157.03 ± 82.25	-29.06 ± 124.28	17.51 ± 99.89	0.006	0.018
Richardson	8	86.00 ± 20.92	107.72 ± 18.80	27.37 ± 20.92	83.80 ± 20.92	0.033	1.089
Richardson	7	94.76 ± 13.94	86.00 ± 20.92	36.88 ± 28.03	74.01 ± 18.99	0.032	0.617
Richardson	6	65.77 ± 11.79	94.76 ± 13.94	-4.86 ± 22.19	45.92 ± 14.71	0.026	0.503
Richardson	5	59.07 ± 24.46	65.77 ± 11.79	14.85 ± 32.38	-1.78 ± 35.36	0.051	0.314
Richardson	4	49.96 ± 14.36	59.07 ± 24.46	7.62 ± 31.89	37.45 ± 19.54	0.069	0.711
Richardson	3	50.52 ± 10.79	49.96 ± 14.36	14.54 ± 21.13	41.49 ± 13.39	0.101	2.010
Richardson	2	53.80 ± 16.19	50.52 ± 10.79	19.26 ± 16.92	30.91 ± 21.08	0.047	0.656
Richardson	1	76.41 ± 16.21	53.80 ± 16.19	40.15 ± 27.13	66.16 ± 19.30	0.097	1.316
Dave	8	90.79 ± 26.37	91.24 ± 20.14	63.15 ± 32.47	22.84 ± 41.36	0.029	0.353
Dave	7	92.08 ± 25.92	90.79 ± 26.37	64.81 ± 33.84	68.52 ± 32.76	0.080	1.780
Dave	6	79.34** ± 15.51	92.08 ± 25.92	39.02 ± 28.86	63.27 ± 22.03	0.025	0.594
Dave	5	146.21 ± 33.82	79.34 ± 15.51	110.06 ± 41.80	133.52 ± 36.62	0.079	0.633
Dave	4	158.24 ± 49.03	146.21 ± 33.82	109.89 ± 60.22	107.96 ± 60.66	0.144	2.815
Dave	3	114.10 ± 44.17	158.24 ± 49.03	43.88 ± 65.93	71.55 ± 57.36	0.068	1.292
Dave	2	157.33 ± 47.42	114.10 ± 44.17	113.12 ± 64.54	149.67 ± 50.39	0.058	0.685
Dave	1	133.38 ± 38.80	157.33 ± 47.42	73.99 ± 56.71	105.18 ± 47.31	0.078	0.808
Frank	8	69.65 ± 63.53	47.43 ± 39.73	36.79 ± 91.04	64.96 ± 67.45	0.069	0.850

Frank	7	38.54 ± 10.22	69.65 ± 63.53	-5.93 ± 50.79	16.76 ± 30.09	0.034	0.357
Frank	6	31.25 ± 8.33	38.54 ± 10.22	7.01 ± 14.76	19.56 ± 11.43	0.016	0.137
Frank	5	39.02 ± 9.12	31.25 ± 8.33	16.23 ± 15.18	38.20 ± 9.33	0.032	0.273
Frank	4	83.26 ± 29.32	39.02 ± 9.12	58.39 ± 35.13	73.21 ± 31.67	0.028	0.564
Frank	3	36.37 ±11.68	83.26 ± 29.32	-16.00 ± 30.13	29.05 ± 14.26	0.104	3.301
Frank	2	56.51 ± 11.84	36.37 ± 11.68	34.03 ± 19.06	49.60 ± 14.06	0.021	0.343
Frank	1	56.02 ± 15.13	56.51 ± 11.84	12.40 ± 24.27	26.64 ± 21.23	0.059	0.078

Table 3.2: The average temperature and velocity of stream segments at the time of eDNA sampling.

Stream	Segment	Temperature at Time of Sampling (°C)	Average Velocity (m/s)
Kevin	8	20.9	0.01
Kevin	7	21.1	0.02
Kevin	6	20.9	0.03
Kevin	5	20.3	0.01
Kevin	4	20.8	0.08
Kevin	3	19.6	0.05
Kevin	2	19.0	0.06
Kevin	1	18.6	0.04
Denise	8	20.7	0.06
Denise	7	19.5	0.01
Denise	6	21.0	0.05
Denise	5	18.6	0.06
Denise	4	18.5	0.04
Denise	3	18.1	0.07
Denise	2	17.5	0.06
Denise	1	16.8	0.03
Rod	8	20.6	0.05
Rod	7	20.7	0.05
Rod	6	19.3	0.03
Rod	5	18.6	0.04
Rod	4	18.1	0.05
Rod	3	24.4	0.07
Rod	2	17.0	0.02
Rod	1	15.7	0.03
Rico	8	20.9	0.04
Rico	7	21.9	0.13

Rico	6	20.8	0.09
Rico	5	20.1	0.07
Rico	4	19.5	0.08
Rico	3	19.2	0.09
Rico	2	18.8	0.07
Rico	1	18.6	0.08
Richardson	8	17.8	0.02
Richardson	7	17.9	0.04
Richardson	6	17.9	0.05
Richardson	5	17.5	0.04
Richardson	4	17.5	0.03
Richardson	3	17.3	0.02
Richardson	2	16.9	0.04
Richardson	1	16.8	0.03
Dave	8	21.1	0.01
Dave	7	21.4	0.01
Dave	6	20.3	0.01
Dave	5	18.5	0.01
Dave	4	18.4	0.01
Dave	3	18.4	0.01
Dave	2	17.2	0.01
Dave	1	16.1	0.01
Frank	8	19.9	0.03
Frank	7	19.2	0.04
Frank	6	18.1	0.02
Frank	5	16.5	0.03
Frank	4	14.6	0.01
Frank	3	14.3	0.01
Frank	2	13.6	0.02
Frank	1	13.7	0.05

*Table 3.3. Summary of the mixed effects model: conditionally calibrated eDNA concentration ~ biomass*velocity*temperature + (1|Stream)*

Fixed effects	Estimate	Standard Error	df	t value	Pr(>|t|)
Intercept	370.95	108.41	45.37	3.422	0.00133
Biomass	-215.42	78.04	43.515	-2.760	0.00842
Velocity	-7801.89	2700.08	42.97	-2.890	0.00602
Temperature	-16.25	5.68	44.54	-2.863	0.00637
Biomass:Velocity	11607.22	3917.83	45.59	2.963	0.00483
Biomass:Temperature	11.33	4.42	44.11	2.563	0.0139
Velocity:Temperature	365.42	139.22	42.34	2.625	0.0120
Biomass:Velocity:Temperature	-553.23	200.81	45.30	-2.755	0.00842

*Table 3.4. Summary of the mixed effect model: constantly calibrated eDNA concentration ~ biomass*temperature + velocity + (1|Stream). Model was originally run with all effects interacting, but interacting effects were removed until only significant effects remained.*

Fixed effects	Estimate	Standard Error	df	t value	Pr(>|t|)
Intercept	154.35	55.14	45.91	2.799	0.00747
Biomass	-134.25	50.94	48.98	-2.635	0.0112
Temperature	-6.09	3.01	45.843	-2.025	0.0487
Velocity	-676.53	228.95	18.99	-2.955	0.00814
Biomass:Temperature	8.55	2.95	48.16	2.894	0.00571

Chapter 4. General Conclusions

Environmental DNA concentrations were related to the abundance and biomass of Brook Trout in Northern Ontario streams. These relationships are likely spatial scale dependent as concentrations of eDNA were related to the abundance of Brook Trout within 25m, 50m, and 100m and the biomass of Brook Trout within 25m upstream of sampling locations. Substantial variability exists in the eDNA-abundance/biomass relationships, and it appears that the eDNA-abundance relationship is more resilient than the eDNA-biomass relationship across all spatial scales. A large portion of variability in these relationships can likely be associated with the production and persistence of eDNA, as well as then environmental factors that influence both production and persistence such as velocity (Pilliod et al. 2014; Jane et al. 2015) and temperature (Jo et al. 2019; Minamoto et al. 2020). Variability from production likely occurred similarly across all spatial scales, whereas the variability from persistence likely depended on the specific spatial scale investigated.

Since the production of eDNA is expected to scale with body size (Maruyama et al. 2014), and abundance does not consider body size, it is unlikely that variability originating from eDNA production can be accounted for in the eDNA-abundance relationships. This is not the case when considering biomass. Allometrically scaling mass by the scaling coefficient of 0.73 (Yates et al. 2021a) improved the relation of eDNA concentrations to Brook Trout biomass at the 25m and 100m spatial scales, suggesting that allometrically scaling mass accounted for some of the variability from eDNA production. This is likely because allometrically scaling mass better aligned Brook Trout mass to the concentration of eDNA it initially produced within stream segments. As of such allometrically scaling mass shows promise and should be further investigated and implemented into future studies.

Uncertainties still exist in the persistence of eDNA, but this study further developed and clarified two opposing methods for predicting eDNA persistence in lotic systems: (1) assuming

the constant deposition of eDNA (Cushing et al. 1993; Wilcox et al. 2016) and (2) assuming the deposition of eDNA conditional to stream velocity. Assuming the constant deposition of eDNA is a quick method for predicting eDNA persistence in streams, only requiring the average velocity of water within investigated stream segments. However, this quickness may be at the cost of accuracy, as calibrating assuming constant deposition resulted in 18 negative eDNA concentrations suggesting that on these occasions persistence of (upstream) eDNA was overestimated. This overestimation potentially originates from the oversimplification of stream composition in the assumptions made by the method (i.e., streams have constant cross-sectional areas and water velocities over their entire lengths and velocity does not affect deposition; Cushing et al. 1993). Despite this, the relationships between constantly calibrated eDNA concentrations and Brook Trout abundance and biomass both passed through the origin, potentially indicating that constant deposition may appropriately account for upstream eDNA on most occasions. On the other hand, assuming the conditional deposition of eDNA is a more time-consuming method for predicting eDNA persistence, as it requires in-depth understanding of stream composition (cross-sectional area) throughout investigated stream lengths. The accuracy of conditional deposition (SWAG method) likely depends upon the resolution of stream measurements taken, with accuracy decreasing whenever increases in cross-sectional area are not measured. Consequently, depending on the quality and quantity of stream measurements, conditional deposition may become an accurate method to account for upstream eDNA. Both methods require additional investigation before they can be confidently and reliably implemented in future studies. Regardless of the exact method of eDNA persistence, it likely causes variability in the eDNA-abundance/biomass relationships in two ways: Firstly, by influencing the concentration of eDNA that is detected for given abundances and biomasses of Brook Trout depending on their distribution throughout stream segments and secondly, by influencing the contribution of eDNA from Brook Trout upstream of stream segments to the detected eDNA concentrations.

Since the persistence of eDNA is expected to decrease the farther it gets from its source (Wilson and Wright, 2013; Jane et al. 2015; Laramie et al. 2015; Fukomoto et al. 2015; Wilcox et al. 2016), the distance that individual Brook Trout are from sampling locations or their distribution within stream segments will likely influence the concentrations of eDNA detected at sampling locations. The specific distribution of Brook Trout throughout stream segments is unknown (if distribution is known there is likely no reason to sample eDNA), but the minimum and maximum distances that Brook Trout can be located from sampling locations are not. The minimum distance will be directly upstream of the sampling location, and the maximum distance will be at the most upstream location of the stream segment, therefore the possible variability due to Brook Trout distribution will increase as the length of stream segments increase. For this reason, part of this variability is likely accounted for by dividing Brook Trout abundance and biomass by the surface area of the examined stream segment as it reduces abundance/biomass by a factor related to the variability in eDNA concentrations, likely better aligning them to the detected eDNA concentrations. This may be supported in this study, where dividing abundance and biomass (allometrically scaled mass included) by surface area improved the relationship between eDNA and abundance at the 25m spatial scale, allowed it to exist at the 50m and 100m spatial scales, allowed the eDNA-biomass relationship to exist at the 25m spatial scale, and allowed the eDNA-allometrically scaled mass (0.73; Yates et al. 2021a) relationship to exist at the 25 and 100m spatial scales. It is highly unlikely that dividing by surface area perfectly accounts for all of the variability from eDNA persistence and Brook Trout distribution within streams, but it may be the best option currently available, in addition to being a more appropriate way of considering abundance and biomass as an amount over a measured area rather than a general quantity.

The contribution of upstream eDNA caused substantial variability in the eDNA-abundance-biomass relationships at small spatial scales (25m). This variability can likely be

accounted for by calibrating eDNA concentrations to specific stream segments by subtracting the contribution of upstream eDNA. Both methods of calibrating eDNA concentrations (constant and conditional deposition) improved the relationship between eDNA and abundance/biomass at the 25m spatial scale. However, both methods incorrectly calculated the contribution of upstream eDNA on occasion because of their respective flaws and shortcomings mentioned previously. Nevertheless, calibrating eDNA concentrations to targeted stream segments should likely be implemented in future eDNA studies in lotic systems, and both methods would benefit from further investigation and revision.

With all this in mind, eDNA can likely be used to monitor the local occupancy of Brook Trout in Northern Ontario streams. In this study and previous others, eDNA successfully detected the presence of Brook Trout in streams (Jane et al. 2015; Wilcox et al. 2016; Baldigo et al. 2017). Specifying the length of stream occupied by Brook Trout may prove to be more challenging but can likely be accomplished by calibrating eDNA concentrations to the stream segments accounting for upstream eDNA. Quantifying this occupancy into abundance and biomass estimates is substantially more challenging but not out of the realm of possibility. It will likely require the consideration of the hydrological conditions of the stream segments and how they affect eDNA persistence alongside eDNA concentrations. If persistence is low, it is unlikely that eDNA concentrations will be representative of anything outside of individuals in close proximity to sampling locations, reducing the viability of estimating Brook Trout abundance and biomass. If eDNA persistence is high, it is likely possible to estimate Brook Trout abundance and biomass from eDNA concentrations, but eDNA concentrations will likely need to be calibrated to targeted stream segments by accounting for the contribution of upstream eDNA. It cannot yet be decisively stated whether this calibration should assume the constant or conditional deposition of eDNA, however, I predict that conditional deposition will prove to be the more promising method in the long run. Only time and additional study will tell.

References

Baldigo, B. P., Sporn, L. A., George, S. D., and Ball, J. A. (2017). Efficacy of environmental DNA to detect and quantify brook trout populations in headwater streams of the Adirondack Mountains, New York. *Transactions of the American Fisheries Society*, *146*(1), 99-111.

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, 17(1), 1-17.

Bencala, K. E. (1983). Simulation of solute transport in a mountain pool-and-riffle stream with a kinetic mass transfer model for sorption. *Water Resources Research*, 19(3), 732-738.

Browne, D. R. (2007). Freshwater Fish in Ontario's Boreal: Status, Conservation, and Potential Impacts of Development. *Wildlife Conservation Society Canada Conservation Report No. 2.*

Carim, K. J., McKelvey, K. S., Young, M. K., Wilcox, T. M., & Schwartz, M. K. (2016). *A protocol for collecting environmental DNA samples from streams* (pp. 1-18). United States, Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Chow, V. T. (1959). *Open Channel Hydraulics*. Caldwell.

Cushing, C. E., Minshall, G. W., & Newbold, J. D. (1993). Transport dynamics of fine particulate organic matter in two Idaho streams. *Limnology and Oceanography*, 38(6), 1101-1115.

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

Dietrich, W. E. (1982). Settling velocity of natural particles. *Water Resources Research*, 18(6), 1615-1626.

Einstein, H. A., & Krone, R. B. (1961). Estuarial sediment transport patterns. *Journal of the Hydraulics Division*, 87(2), 51-59.

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

Fischer, H. B. (1979). Mixing in inland and coastal waters. *Academic press*.

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.

Fukumoto, S., Ushimaru, A., & Minamoto, T. (2015). A basin-scale application of environmental DNA assessment for rare endemic species and closely related exotic species in rivers: A case study of giant salamanders in Japan. *Journal of Applied Ecology*, *52*(2), 358-365.Hart, D. R. (1995). Parameter estimation and stochastic interpretation of the transient storage model for solute transport in streams. *Water Resources Research*, 31(2), 323-328.

Hewitt, C. L., Everett, R. A., & Parker, N. (2009). Examples of current international, regional and national regulatory frameworks for preventing and managing marine bioinvasions. In *Biological invasions in marine ecosystems: ecological, management, and geographic perspectives* (pp. 335-352). Berlin, Heidelberg: Springer Berlin Heidelberg. Jane, S. F., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., Letcher, & Whiteley, A. R. (2015). Distance,

flow and PCR inhibition: eDNA dynamics in two headwater streams. *Molecular Ecology Resources*, 15(1), 216-227.

Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters*, *4*(2), 150-157.

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.

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.

Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., ... & Richter, C. A. (2020). Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA*, *2*(3), 271-282.

Laramie, M. B., Pilliod, D. S., & Goldberg, C. S. (2015). Characterizing the distribution of an endangered salmonid using environmental DNA analysis. *Biological Conservation*, *183*, 29-37.Leopold, L. B., & Maddock, T. (1953). The hydraulic geometry of stream channels and some physiographic implications (Vol. 252). *US Government Printing Office.*

Longmire, J. L., Maltbie, M., & Baker, R. J. (1997). Use of "lysis buffer" in DNA isolation and its implication for museum collections. Museum of Texas Tech University. Occasional Papers, Number 163

MacKay, H. H. 1963. Fishes of Ontario. Ontario Department of Lands and Forests. *Bryant Press Ltd*., 1, 300

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.

Mauvisseau, Q., Harper, L. R., Sander, M., Hanner, R. H., Kleyer, H., & Deiner, K. (2022). The multiple states of environmental DNA and what is known about their persistence in aquatic environments. *Environmental Science & Technology*, *56*(9), 5322-5333.

Minshall, G. W., Thomas, S. A., Newbold, J. D., Monaghan, M. T., & Cushing, C. E. (2000). Physical factors influencing fine organic particle transport and deposition in streams. *Journal of the North American Benthological Society*, 19(1), 1-16.

Morrice, J. A., Valett, H. M., Dahm, C. N., & Campana, M. E. (1997). Alluvial characteristics, groundwater–surface water exchange and hydrological retention in headwater streams. *Hydrological Processes*, 11(3), 253-267.

Nyman, O. L. (1970). Ecological interaction of Brown Trout, Salmo trutta L., Brook Trout, Salvelinus fontinalis (Mitchill), in a stream. *Canadian Field Naturalist* 84, 343-350.

Ontario Ministry of Natural Resources and Forestry. (2017). The Brook Trout of Ontario Report.

Paul, M. J., & Hall Jr, R. O. (2002). Particle transport and transient storage along a stream-size gradient in the Hubbard Brook Experimental Forest. *Journal of the North American Benthological Society*, 21(2), 195-205.

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.

Scott, W. B. Crossman, E. J. (1973). Freshwater fishes of Canada. Fish. Res. Board Can. Bull., 184, 1-966.

Shapiro, B. (2008). Engineered polymerases amplify the potential of ancient DNA. *Trends in Biotechnology*, 26(6), 285-287.

Shogren, A. J., Tank, J. L., Andruszkiewicz, E., Olds, B., Mahon, A. R., Jerde, C. L., & Bolster, D. (2017). Controls on eDNA movement in streams: Transport, retention, and resuspension. *Scientific reports*, *7*(1), 5065.

Spear, S. F., Groves, J. D., Williams, L. A., & Waits, L. P. (2015). Using environmental DNA methods to improve detectability in a hellbender (*Cryptobranchus alleganiensis*) monitoring program. *Biological Conservation*, 183, 38-45.

Steedman, R. J. (1988). Modification and assessment of an index of biotic integrity to quantify stream quality in southern Ontario. *Canadian Journal of Fisheries and Aquatic Sciences*, 45(3), 492-501.

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., & Kawabata, Z. I. (2012). Estimation of fish biomass using environmental DNA. *PloS one*, 7(4), e35868.

Thomsen, P. F., Kielgast, J. O. S., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., ... & Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, 21(11), 2565-2573.

Turner, C. R., Barnes, M. A., Xu, C. C., Jones, S. E., Jerde, C. L., & Lodge, D. M. (2014). Particle size distribution and optimal capture of aqueous macrobial eDNA. *Methods in Ecology and Evolution*, 5(7), 676-684.

Turner, C. R., Uy, K. L., & Everhart, R. C. (2015). Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biological Conservation*, 183, 93-102.

Wiebe, M. (2023). Brook Trout abundance and distribution at multiple spatial scales in Lake Superior Tributaries. Lakehead University

Wilcox, T. M., McKelvey, K. S., Young, M. K., Jane, S. F., Lowe, W. H., Whiteley, A. R., & Schwartz, M. K. (2013). Robust detection of rare species using environmental DNA: the importance of primer specificity. *PloS one*, *8*(3), e59520.

Wilcox, T. M., McKelvey, K. S., Young, M. K., Sepulveda, A. J., Shepard, B. B., Jane, S. F., Whiteley, A. R., Lowe, W. H., & Schwartz, M. K. (2016). Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*. *Biological Conservation*, 194, 209-216.

Wilson, C., & Wright, E. (2013). Using Environment DNA (eDNA) as a Tool in Risk-Based Decision-Making. Ontario Ministry of Natural Resources. *Aquatic Research Series* 2013-01

Wilson, C. C., Bronnenhuber, J., Boothroyd, M., Smith, C., & Wozney, K. (2014). Environmental DNA (eDNA) monitoring and surveillance: Field and laboratory standard operating procedures. *Peterborough: Aquatic Research and Monitoring Section, Ontario Ministry of Natural Resources and Forestry*, *10*.

Wilson, C. C., Wozney, K. M., Smith, C. M., & Yu, D. (2016). Recognizing false positives: synthetic oligonucleotide controls for environmental DNA surveillance. *Methods in Ecology & Evolution*, *7*(1).

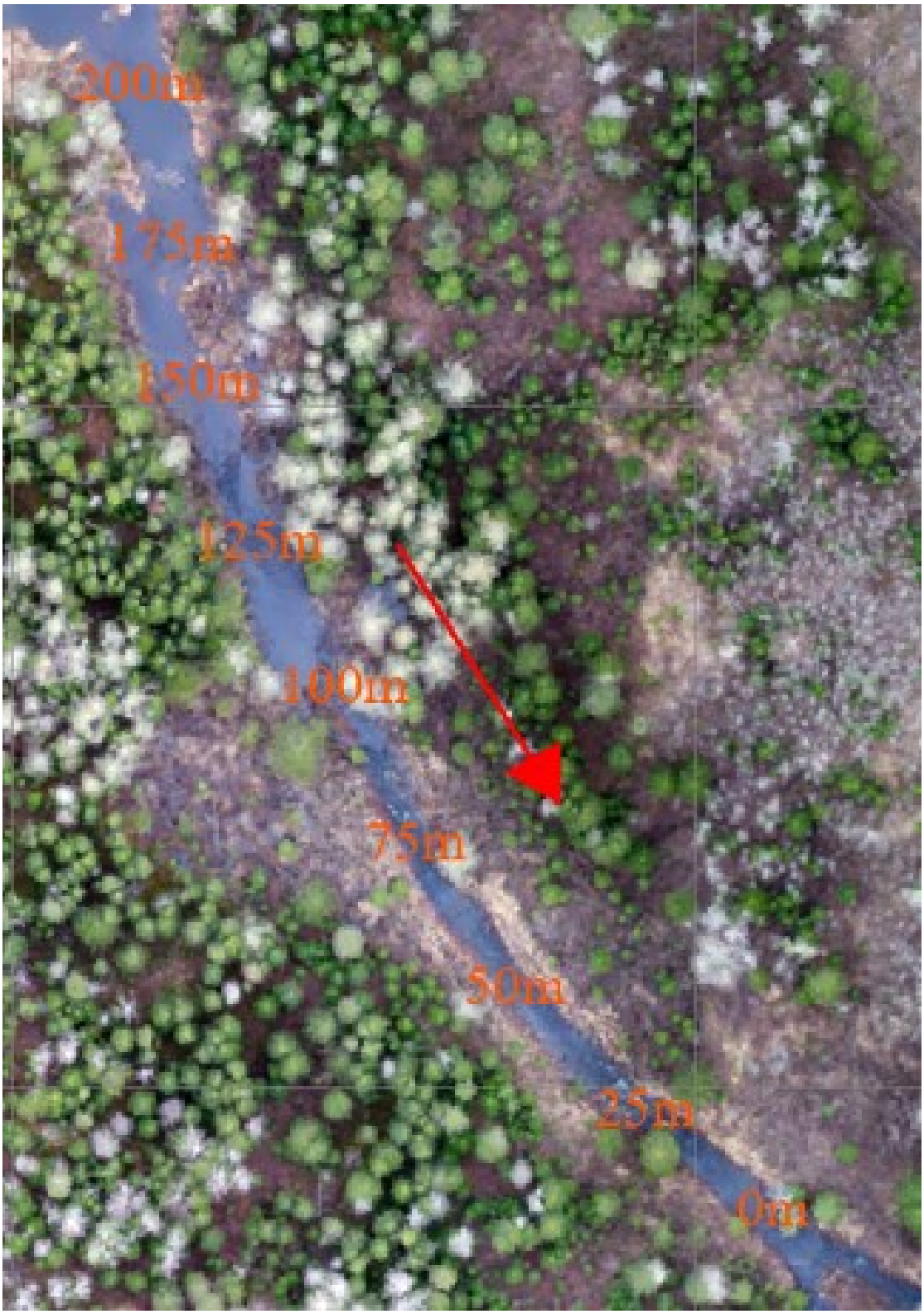
[a]Yates, M. C., Glaser, D. M., Post, J. R., Cristescu, M. E., Fraser, D. J., & Derry, A. M. (2021). The relationship between eDNA particle concentration and organism abundance in nature is strengthened by allometric scaling. *Molecular Ecology*, 30(13), 3068-3082.

[b]Yates, M. C., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., & Derry, A. M. (2021). Allometric scaling of eDNA production in stream-dwelling brook trout (Salvelinus fontinalis) inferred from population size structure. *Environmental DNA*, *3*(3), 553-560.

Appendix

Figure A.1. Map of stream segment locations.

Figure A.2. Aerial View of Denise. Red arrow indicates flow direction. Imagery was taken under different flow conditions than surveys.



Fiigure A.3. Aerial View of Kevin. Red arrow indicates flow direction. Imagery was taken under different flow conditions than surveys.

Figure A.4. Aerial View of Rod. Red arrow indicates flow direction. Imagery was taken under different flow conditions than surveys.

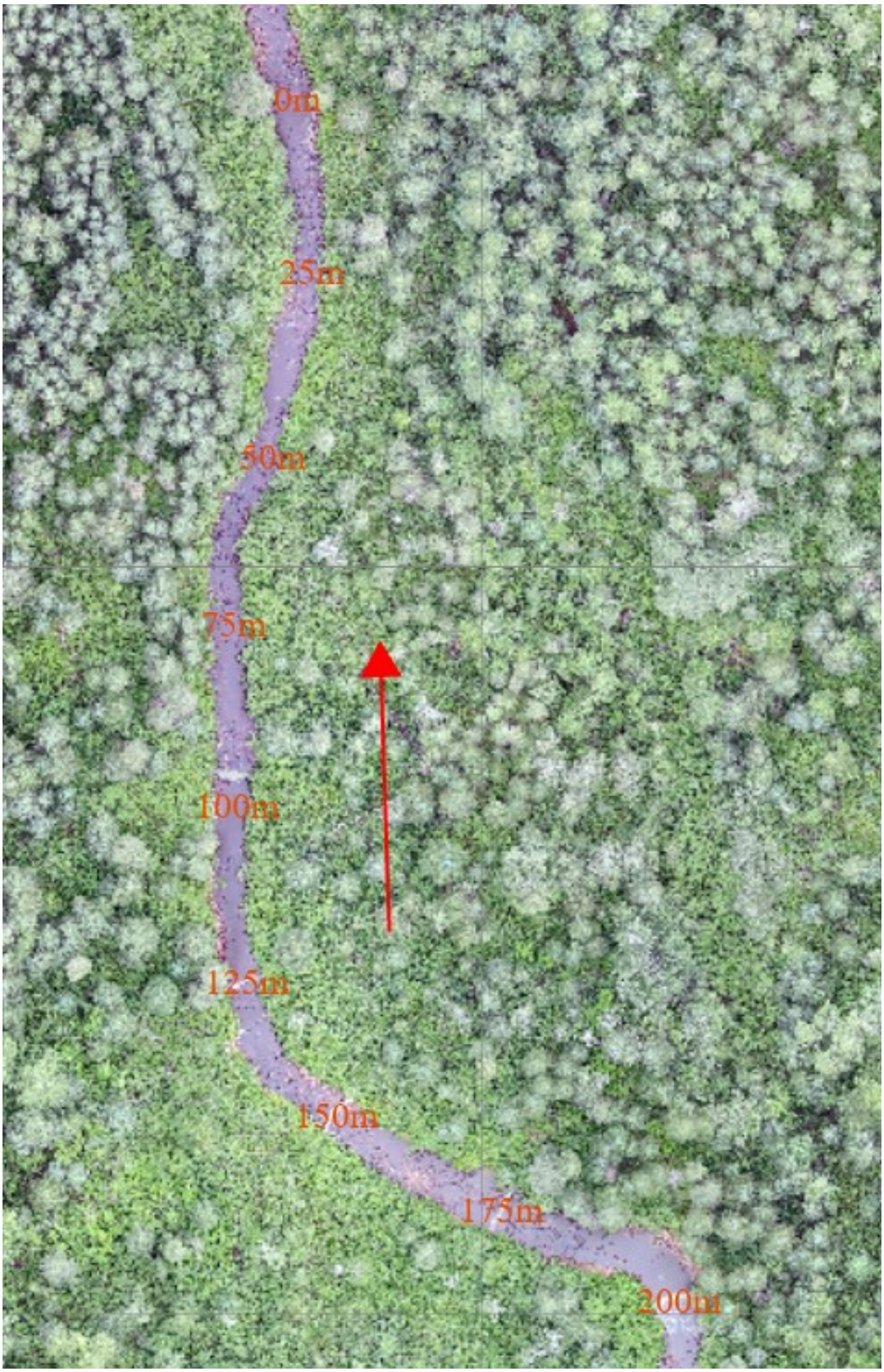


Figure A.5. Aerial View of Dave. Red arrow indicates flow direction.

Figure A.6. Aerial View of Richardson. Red arrow indicates flow direction.

Figure A.7. Aerial View of Rico. Red arrow indicates flow direction.

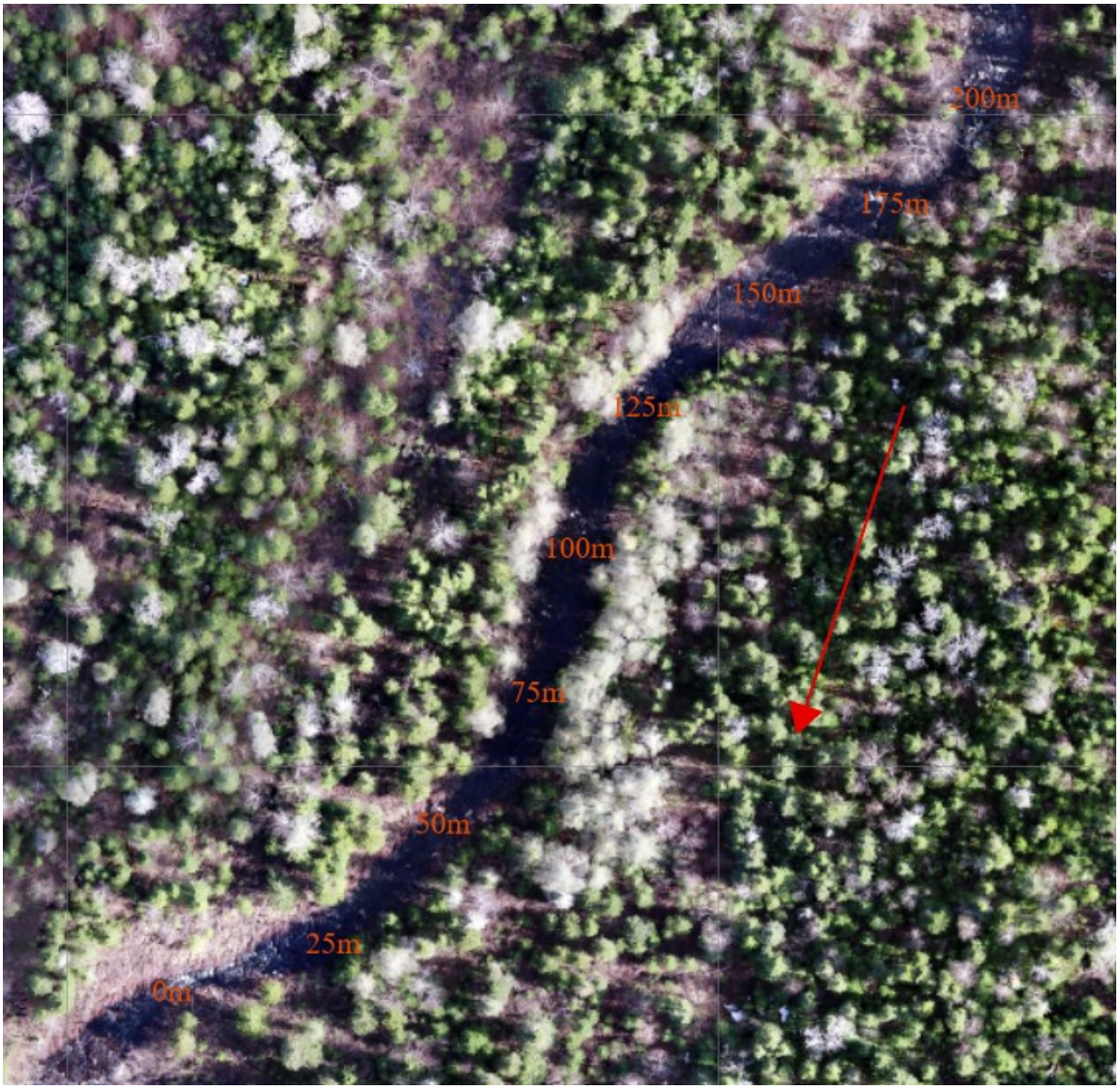


Figure A.8. Aerial View of Frank. Red arrow indicates flow direction. Imagery was taken under different flow conditions than surveys.