The Use of Freshwater Environmental DNA (eDNA) for Rapid and Cost-Effective Detection of Native and Non-Native Salmonid Species in Cape Breton

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THE USE OF FRESHWATER ENVIRONMENTAL DNA (eDNA) FOR RAPID AND COST-EFFECTIVE DETECTION OF NATIVE AND NON-NATIVE SALMONID SPECIES IN CAPE BRETON

by

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ABSTRACT

Wringe, B.F., Jeffery, N.W., LeBlanc, F., Steeves, R., Moreau, D.T.R., Raab, D., Hardie, D., Gagne, N. 2023. The use of freshwater environmental DNA (eDNA) for rapid and cost effective detection of native and non-native salmonid species in Cape Breton. Can. Tech. Rep. Fish. Aquat. Sci. 3527: vii + 28 p.

The recovery of endangered Atlantic Salmon (*Salmo salar*) populations is hampered by multiple stressors, including competition with non-native salmonids. Native Atlantic Salmon of the Eastern Cape Breton Designatable Unit (DU) face competition from introduced Brown Trout (*Salmo trutta*) and introduced and aquaculture escapee Rainbow Trout (*Oncorhynchus mykiss*). The degree to which this competition impacts the DU as a whole largely depends on the extent of the spatial and temporal co-occurrence of salmon populations with either, or both, non-native trout species. To refine our understanding of the overlap between Eastern Cape Breton Atlantic Salmon and introduced Brown and Rainbow trout, we undertook a survey of rivers using environmental DNA (eDNA).

The utility of eDNA for the detection of native and introduced species has been well proven. Rivers were selected for geographic coverage of Bras d'Or Lake and based on current or historic presence of Atlantic Salmon, Rainbow, and Brown trout. Atlantic Salmon DNA was detected in 11 of 15 rivers sampled. Brown Trout DNA was detected in 10 rivers, and co-occurred with Atlantic Salmon in eight of these. Rainbow Trout DNA was detected in two sampled rivers, and co-occurred with both Brown Trout and Atlantic Salmon DNA in both sites. These results suggest that co-occurrence of Atlantic Salmon with introduced salmonid competitors may be widespread, and thus could have implications for the recovery of Eastern Cape Breton Designatable Unit Atlantic Salmon.

RÉSUMÉ

Wringe, B.F., Jeffery, N.W., LeBlanc, F., Steeves, R., Moreau, D.T.R., Raab, D., Hardie, D., Gagne, N. 2023. The use of freshwater environmental DNA (eDNA) for rapid and cost effective detection of native and non-native salmonid species in Cape Breton. Can. Tech. Rep. Fish. Aquat. Sci. 3527: vii + 28 p.

Le rétablissement des populations de saumon atlantique (*Salmo salar*) en voie de disparition est entravé par de multiples facteurs de stress, notamment la concurrence avec des salmonidés non indigènes. Le saumon atlantique indigène de l'unité désignable (UD) de l'est du Cap-Breton fait face à la concurrence de la truite brune (*Salmo trutta*) introduite et de la truite arc-en-ciel (*Oncorhynchus mykiss*) introduite et échappée de l'aquaculture. Le degré d'impact de cette compétition sur l'UD dans son ensemble dépend en grande partie de l'étendue de la cooccurrence spatiale et temporelle des populations de saumon avec l'une ou l'autre, ou les deux, des espèces de truites non indigènes. Pour affiner notre compréhension du chevauchement entre le saumon atlantique de l'est du Cap-Breton et les truites brunes et arc-en-ciel introduites, nous avons entrepris une étude des rivières à l'aide de l'ADN environnemental (ADNe).

L'utilité de l'ADNe pour la détection d'espèces indigènes et introduites a été bien prouvée. Les rivières ont été sélectionnées en fonction de la couverture géographique du lac Bras d'Or et en fonction de la présence actuelle ou historique du saumon atlantique, de la truite arc-en-ciel et de la truite brune. L'ADN du saumon atlantique a été détecté dans 11 des 15 rivières échantillonnées. L'ADN de la truite brune a été détecté dans 10 rivières et était concomitant avec le saumon de l'Atlantique dans huit d'entre elles. L'ADN de la truite arc-en-ciel a été détecté dans deux rivières échantillonnées et était concomitant avec l'ADN de la truite brune et du saumon de l'Atlantique dans les deux sites. Ces résultats suggèrent que la cooccurrence du saumon atlantique avec des concurrents salmonidés introduits pourrait être généralisée et pourrait donc avoir des répercussions sur le rétablissement du saumon atlantique de l'unité désignable de l'est du Cap-Breton.

INTRODUCTION

The competitive impacts of non-native species on local populations can be greatest when species are ecologically similar, especially when the non-native species is more aggressive (Houde et al. 2016; Sanches et al. 2012). Introduced species are often successful in a new environment due to the release from natural constraints, such as predators and parasites found in their native range (Mills et al. 2004). Interspecific competition with native species for food, habitat, nesting grounds, or other resources can lead to habitat partitioning or potential local extinction of native fishes (Hearn and Kynard 1986). In such cases, the non-native species may be better at acquiring resources, displace the native species from optimal microhabitats, and reduce growth and performance of the native species. In cases where the native species is already at low abundance (e.g. species at risk), competition with non-native or invasive species may impede the recovery of the native species. Some of the best studied examples of this include species of salmonids, many of which now co-occur because of widespread introductions (Fausch 1988). For instance, the introduction of Rainbow Trout (*Oncorhynchus mykiss*) to rivers in eastern Canada not only led to direct competition between Rainbow Trout and Atlantic Salmon (*Salmo salar*), but also increased niche overlap between indigenous Brook Trout (*Salvelinus fontinalis*) and Atlantic Salmon, potentially contributing to salmon population declines (Thibault and Dodson 2013).

Wild populations of Atlantic Salmon have experienced drastic declines in the North Atlantic over the past several decades (Lehnert et al. 2019; Parrish et al. 1998). These declines are postulated to be driven by numerous and non-mutually exclusive factors, including high at-sea mortality from commercial and illegal fishing, predation, climate change impacts, and intra- and interspecific competition with aquaculture escapees and non-native species (Dadswell et al. 2022). In Nova Scotia, the Eastern Cape Breton (ECB) Designatable Unit (DU) of Atlantic Salmon is assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as Endangered (COSEWIC 2010), and is currently under review for listing under the *Species at Risk Act*. Within the area where this DU is found, Brown Trout (*Salmo trutta*) and Rainbow Trout are intentionally stocked to enhance sport fisheries, and Rainbow Trout are further introduced to the wild, primarily in the Bras d'Or Lakes, through escapes from aquaculture. The acidic conditions of rivers in mainland Nova Scotia may prevent acid-sensitive Rainbow Trout from successfully reproducing; however, Rainbow Trout are more common in the Bras d'Or lakes watersheds where they are farmed and can reproduce in the wild (Madden et al. 2010). Feral populations of Brown (Robichaud-LeBlanc and Amiro 2004) and Rainbow trout (Madden et al. 2010) have been documented in rivers of ECB, including a number that enter Bras d'Or Lake. Competition with non-native salmonid juveniles can impact the native Atlantic Salmon populations. This has led to interaction and competition with non-native salmonids being identified as threats to the recovery of wild populations in the Eastern Cape Breton DU Recovery Potential Assessment (DFO 2013).

As juveniles in freshwater, Rainbow Trout and Atlantic Salmon live in sympatry in some regions and tend to occupy similar microhabitats (Hearn and Kynard 1986). Salmonids are generally territorial while developing in streams; however, Rainbow Trout juveniles are generally more aggressive than Atlantic Salmon, outcompete them, and displace salmon juveniles to suboptimal microhabitats, resulting in reduced performance and fitness (Houde et al. 2016). Similarly, Atlantic Salmon and Brown Trout show considerable spatial niche overlap (Armstrong et al. 2003; Heggenes et al. 1999), albeit potentially less so than salmon and Rainbow Trout (Heggenes and Dokk 2001). Brown Trout also outcompete Atlantic Salmon, as indicated by Brown Trout having greater survival and growth when the two species are in competition

(Houde et al. 2017). As such, co-occurrence with Rainbow and/or Brown Trout has the potential to impact Atlantic Salmon populations, and where salmon populations are already at low levels, may impede their recovery (DFO 2013).

Assessing the distribution of Atlantic Salmon, Rainbow and Brown trout and determining their co-occurrence is a key first step in understanding the potential impact of non-native salmonids on ECB salmon. Traditionally, salmonids are surveyed in ECB using electrofishing for juveniles or swim-through counts for adults, processes that are both time and labour intensive. Recent advances have brought to the forefront environmental DNA (eDNA) for the detection of invasive species (LeBlanc et al. 2020). Every animal sheds DNA in the environment, which contains species-specific sequences, and the collection of this DNA and molecular detection using quantitative PCR (PCR) and/or DNA metabarcoding is a relatively non-invasive, sensitive and cost-efficient approach, from which a species' presence can be inferred (Biggs et al. 2015; Jerde 2021; Sigsgaard et al. 2015; Wood et al. 2021). Where population estimates are not required, the collection of eDNA (water) samples is faster and less-invasive than electrofishing, and thus more locations can be surveyed in a given amount of time. Species detection by eDNA surveys have been shown to have high congruence with traditional capture-based survey methods, however the overlap in the species detected is not perfect (He et al. 2022). eDNA can be more sensitive and suggest the presence of more species than capture based survey methods – including electrofishing (Jerde 2021) – in fresh (Shaw et al. 2016) and saltwater (He et al. 2022). However, the ability to detect single or small numbers of individuals using eDNA can be lower than with electrofishing (Penaluna et al. 2021), and care is required during study and marker design (Shaw et al. 2016).

The aims of this study were two-fold: first to utilize eDNA to rapidly determine the presence of Atlantic Salmon, Rainbow Trout, and Brown Trout; and second to determine the co-occurrence of Atlantic Salmon, Rainbow Trout, and Brown Trout in selected rivers in the ECB region. Determining the presence of Atlantic Salmon provides information critical to the proper management of the species. Similarly, understanding the co-occurrence of Atlantic Salmon and Rainbow and Brown trouts provides information that can be used to scale the predicted impacts on salmon populations, or in formulating mitigation plans.

MATERIALS AND METHODS

TARGET SPECIES

Atlantic Salmon (*Salmo salar*) are native to the North American and European North Atlantic, with an evolutionarily distinct population being present in the Eastern Cape Breton region (ECB designatable unit; COSEWIC 2010). Within the ECB, salmon are thought to be currently present, or to have existed in 46 rivers (DFO 2013).

Rainbow Trout (*Oncorhynchus mykiss*) are native to the rivers and streams flowing into the North Pacific Ocean. Their natural range extends from the Kamchatka Peninsula in Russia, eastward along the Aleutian Island chain to southwest Alaska, and then along the North American coast as far south as Baja Mexico.

Brown Trout (*Salmo trutta*) are native to Europe, northern Africa, and western Asia; however, through a long history of introductions, both Rainbow and Brown trout have become established on all continents except Antarctica (MacCrimmon 1971; MacCrimmon and Marshall 1968).

Rainbow Trout were first introduced to Nova Scotia in 1899 (Scott and Crossman 1973) and Brown Trout were first introduced in 1925 (Alexander et al. 1986). Where introduced, both Brown and Rainbow trout can have negative impacts on native Atlantic Salmon (Houde et al. 2015a; Houde et al. 2016), and interaction and competition with them is identified as likely threats to the recovery of wild salmon populations in the Eastern Cape Breton DU (DFO 2013).

ENVIRONMENTAL DNA COLLECTION

Fifteen rivers (Table 1) were selected for sampling based on a combination of: 1) reported current or historic presence of Atlantic Salmon (Gibson et al. 2014); 2) reported current or historic presence of Rainbow Trout (DFO 1998; Gibson et al. 2014; Levy and Gibson 2014; Madden et al. 2010; Robichaud-LeBlanc and Amiro 2004); 3) reported current or historic presence of Brown Trout (Levy and Gibson 2014); and 4) geographic coverage of the Bras d'Or Lake watershed (Figure 1). Rivers in which Rainbow or Brown trout were historically known to exist were sampled to confirm the utility of eDNA to detect these species. The Framboise River was sampled to obtain updated information on the presence of Atlantic Salmon in this river, which was last assessed in 2016 (D. Raab, Pers. Comm.). Where feasible for better replication, we sampled two locations per river: one near the river mouth to maximize the amount of upstream river volume the sample represented, and one further upstream (Table 1). Precise sampling locations were chosen based on ease of public access. For some rivers due to uncertainties in land ownership, difficulty of access, or short river length, only a single sampling location was visited (Table 1).

At each sampling location, three 1 L water samples were collected. In most sampling locations, this was done as a transect, with one sample taken on each bank of the river, and the third taken in the middle. However, in some cases, because of river depth, lack of public access, or uncertainty in land ownership, all three samples were taken on the same side of the river. For some locations, the sample was filtered on site using a Halltech OSMOS backpack eDNA sampler (Halltech Environmental and Aquatic Research Inc., Guelph, ON, Canada), while at others the samples were collected in sterilized 1 L opaque Nalgene bottles and filtered using an electric vacuum pump (Gast Oil-less diaphragm-type; Fisher Scientific) later in the same day (Table 1). All samples were filtered through 47 mm diameter 0.8 µm pore size Whatman nylon filters (Fisher Scientific). Filters were preserved in 95% non-denatured ethanol and stored at -20 °C until DNA extraction.

To minimize temporal variability (e.g. Lacoursiere-Roussel et al. 2016; Levi et al. 2019; Tillotson et al. 2018), the majority of rivers were sampled over the course of three days in October 2020 (October 18-20); the Framboise River was sampled earlier on September 21, 2020. Field blanks were collected at least daily, and were prepared by pouring deionized water into a sterilized 1 L opaque Nalgene bottle at the field site.

DNA EXTRACTION AND QPCR TESTING

Each filter was cut in half, and DNA was extracted from one half, with the other kept as a back-up. In addition to the field samples and blanks, laboratory DNA extraction blanks were also included (1 for every 20 samples) for quality control purposes. The DNA extraction was conducted with the MN NucleoSpin Tissue Kit (Macherey-Nagel, PA, USA) following a modified protocol (LeBlanc et al. 2020), and extracts were stored at -20 °C.

The qPCR assays targeting the Rainbow Trout cytochrome b (CytB) and Brown Trout cytochrome c oxidase subunit 1 (COI) were obtained from the literature (Hernandez et al. 2020), however the Brown Trout TaqMan probe was designed in-house (Table 2). The primers and probe for the Atlantic Salmon-specific COI qPCR assay have been published in Wood et al.

(2021). The specificity, efficiency, limit of detection (LOD) and limit of quantification (LOQ) of these assays obtained in-house are reported in Table 2. The amount of DNA (pg) required to achieve the LOD and LOQ are also reported in Table 2.

Quantitative PCR was performed using the 2x TaqPath™ ProAmp™ MasterMix (Thermo Fisher Scientific, MA, USA). Briefly, 3 µL of template DNA, 480 nM of each primer, 200 nM of the probe, 1 µL of 1 % BSA, as well as 12.5 µL of master mix were used in 25 µL reactions. All qPCR tests were done in triplicate on a StepOnePlus™ qPCR platform (Thermo Fisher Scientific, MA, USA) using the following cycling parameters: initial hold at 95 °C for 10 min, followed by 50 cycles at 95 °C for 30 s and 60 °C for 60 s, with fluorescence reading at the end of each elongation cycle.

To evaluate if PCR inhibitors were present in field samples, which could lead to potential false negative results, all samples (including blank controls) were spiked with an exogenous internal positive control (IPC) (linearized DNA plasmid containing a DNA sequence not found in the targeted environments) and tested using a qPCR assay specific to that IPC. Inhibition was considered present if a difference of more than 2 Ct was observed between environmental samples and field blanks. The IPC qPCR assay was done using the same parameters and reagents used for the other qPCR assays found above.

RESULTS

Target DNA was not detected in any of the field blanks, however some laboratory DNA extraction blanks showed evidence of contamination. As such, the affected samples were re-extracted using the back-up half filter and the qPCR was redone. After re-extraction, no evidence of contamination was apparent in the associated extraction blanks.

DNA detection at a single site within a river was considered sufficient evidence for inferring species presence, and this criteria was used to examine species co-occurrences. Following the criteria of LeBlanc et al. (2020; Table 3), Atlantic Salmon DNA was detected in 11 of 15 rivers examined across Cape Breton Island, and was suspected (detection obtained in 2/3 or 3/3 of the qPCR technical replicates, but the value was below the LOD; LeBlanc et al. (2020)) in another two (Figure 2; Table 4). Brown Trout DNA was detected in a minimum of one location in 10 of 15 rivers, and DNA was suspected in another two. Rainbow Trout DNA was detected in two rivers, while in four other rivers the results were classified as suspected (Figure 2; Table 4).

Based on DNA detections, Brown Trout were found to co-occur with Atlantic Salmon in eight rivers, and they are suspected of co-occurring in a further three (Figure 2; Table 4). Rainbow Trout were found to co-occur with Atlantic Salmon in two rivers, and they are suspected of co-occurring in four others (Figure 2; Table 4). Of note, in the two rivers where Atlantic Salmon and Rainbow Trout were found to co-occur, Brown Trout DNA was also detected (Figure 2; Table 4). Similarly, of the four rivers in which the results showed that Atlantic Salmon DNA was detected, and Rainbow Trout DNA was suspected of being present, Brown Trout DNA was detected in three, and their DNA was suspected to be present in the other.

Previously published occurrences of Atlantic Salmon corresponded well with eDNA-based detections (Table 4). Similarly, Rainbow Trout DNA was only found in rivers in which they had been previously observed (Table 4); Rainbow Trout DNA was detected in two rivers and suspected in four. However, this study revealed the presence of Brown Trout DNA in seven rivers for which we were unable to find published records of them having been detected using other means (Table 4). Where Brown Trout had been previously reported, the eDNA-based

detections in this study were generally congruent, with the exception that our results indicated only suspected DNA presence in two of five rivers with previous physical detections (Table 4).

DISCUSSION

In this study we sampled 15 rivers on Cape Breton Island, within the area that contains Eastern Cape Breton Designatable Unit salmon populations, with the goal of determining the presence of Atlantic Salmon, as well as their introduced salmonid competitors, in rivers with known or historical presence. Using eDNA in conjunction with a targeted qPCR approach, and based on the criteria of LeBlanc et al. (2020), Atlantic Salmon DNA was detected in 11 of the 15 rivers examined, was suspected to be present in two rivers, and not detected in two rivers. This result is consistent with previous reports of Atlantic Salmon presence in the ECB DU rivers (DFO 2013, 2020; Levy and Gibson 2014; MacMillan et al. 2008). Based on DNA detections, in six of these rivers, salmon would be in competition with Brown Trout alone, and with both Brown and Rainbow trout in a further two. Anecdotal observations during fall dive counts on Middle, Baddeck, North, and Skye rivers in the past five years have confirmed the presence of Rainbow Trout in all four rivers, and Brown Trout in Middle, Baddeck, and North rivers; however, the trout population size, and the spatial extent of occupancy within these rivers and temporal variability of their occupancy is not known (Dustin Raab, pers. obs.).

It must be borne in mind while interpreting the results of both eDNA and electrofishing surveys that only positive detections can be confirmed (with eDNA there is also a risk of false positive; Jerde 2021). Thus, while eDNA is a sensitive tool capable of detecting even a single salmonid at considerable distance from the sampling location (Wood et al. 2021), a survey may fail to detect the target species if the concentration of eDNA is below the LOD. For example, failure to detect the target species' DNA when it is present may occur when samples are collected too far from the physical location of the target individuals, or when the dilution is too great (Penaluna et al. 2021; Wood et al. 2021). This may account for failure to conclusively detect a species' DNA in rivers in which they have been previously observed (Table 4), but because we have no information on population size, or spatial or temporal occupation, we cannot make conclusive statements about the adequacy of our sampling locations for any river. Considering that we sampled a single time-point on relatively few locations in each watershed and used a conservative classification of species presence, our detections of salmonid species should be treated as a conservative/minimal estimate of co-occurrence.

While only a subset of rivers in the ECB DU that may contain Atlantic Salmon were investigated (DFO 2013), and the rivers were not chosen randomly, it is still worrying that over 70% of detected Atlantic Salmon populations were found to be in competition with one or more introduced salmonid species. Expansion of this work to include more rivers would be valuable to the understanding of the true extent of the potential impact of non-native salmonid species on ECB Atlantic Salmon. This would be bolstered by investigating the river-scale abundance of these salmonid species alongside their finer-scale distributions, through for instance, targeted electrofishing.

Competition with introduced salmonids is a threat to the persistence and recovery of endangered ECB DU Atlantic Salmon (DFO 2013). Juvenile Rainbow and Brown trout have been shown to outcompete juvenile Atlantic Salmon, leading to reductions in their fitness (Houde et al. 2017; Houde et al. 2016; Thibault and Dodson 2013; Van Zwol et al. 2012a). Studies in semi-natural stream channels found that the impacts of Brown Trout are greater than those of Rainbow Trout (Van Zwol et al. 2012a, 2012b) and there is growing evidence that early

life history impacts, such as reduced growth, can have carry-over effects into later life history in the marine environment leading to reduced at-sea survival (Russell et al. 2012). For instance, lower juvenile growth rate, which could be caused by competition with non-native salmonids (van Zwol et al. 2012b), has been shown to result in female Atlantic Salmon producing fewer, but larger eggs (Jonsson et al. 1996; Thorpe et al. 1984). While larger eggs generally result in greater offspring survival, at least during very early life history stages, stabilizing selection on egg and offspring size has been detected, suggesting changes in egg size may impact the overall reproductive output (Einum and Fleming 2000). Through direct and indirect impacts of competition, where Atlantic Salmon co-occur with Rainbow or Brown trout, even if the trout are at relatively low densities (Thibault and Dodson 2013), negative outcomes for the salmon populations are possible. Moreover, the effects on Atlantic Salmon populations are likely greater when both Rainbow and Brown Trout are present because the impacts of competing with both species are greater than with either of them alone (Houde et al. 2015a).

Rainbow trout were first introduced to Nova Scotia in 1899 (Scott and Crossman 1973) and Brown Trout were first introduced in 1925 (Alexander et al. 1986). Rainbow and Brown trout are stocked into the Bras d'Or lakes and surrounding area by the Nova Scotia government, and have previously been stocked by the federal government (e.g. Alexander 1975). In addition to stocking, the abundance of Rainbow Trout in Bras d'Or Lake has been further bolstered through escapes from aquaculture (Anon. 2005; Madden et al. 2010). Madden et al. (2010) detected naturally reproducing populations of Rainbow Trout in two rivers that enter the Bras d'Or Lake, and observations during annual DFO assessment activities are consistent with the continued wild reproduction of Brown Trout in Middle, Baddeck, and North rivers, and Rainbow Trout in Middle River. Given the well acknowledged propensity for Brown Trout (MacCrimmon and Marshall 1968; Westley and Fleming 2011) and Rainbow Trout (MacCrimmon 1971) to invade areas and establish self-sustaining populations, expanded surveys aimed at detecting and potentially quantifying reproducing populations is warranted.

eDNA-based targeted qPCR species-specific detection has been shown to complement electrofishing for salmonids and represents a low-effort survey strategy. In over half of the streams sampled by Penaluna et al. (2021), Cutthroat Trout eDNA was detected further upstream than the last fish detected by electrofishing, and conversely, electrofishing extended the upper stream detections relative to eDNA in approximately one quarter of streams. Three replicate samples was adequate to capture and amplify trout eDNA when it is present (Penaluna et al. 2021). Our study underscores the already demonstrated utility of eDNA for quickly and cost-effectively evaluating the presence of aquatic species (Jerde 2021; Lacoursiere-Roussel et al. 2016). Over the course of three days eDNA samples were obtained from a total of 25 sites across 14 different rivers. Two additional sites on the Framboise River were sampled on a different day, during the course of another project which illustrates another benefit of eDNA: the ability to get opportunistic samples, with minimal equipment and training.

It should also be noted that eDNA samples have uses that can extend beyond one study. For instance, while the current study was focused on the detection of three salmonid species through targeted qPCR, DNA metabarcoding could be applied to the same sample in the future for the detection of multiple species of virtually any taxa (Min et al. 2021) or the elucidation of ecosystem-level biodiversity (Stat et al. 2017). This means the value and utility of eDNA samples cannot always be accurately predicted based on their current use. Moreover, the utility of samples is expected to increase as more advanced and sensitive eDNA analysis techniques are developed. However, physically capturing individuals allows for the collection of tissue samples and electrofishing surveys can be used to answer questions that are as of yet unanswerable using eDNA such as population age and size structure or population genomics.

eDNA concentrations within a lotic environment are affected by changes in flow, with lower concentrations often being detected after rain events even when the number of organisms is constant (LeBlanc et al. 2021). There were three rain events in the 7 day period prior to our sampling (11.4 mm on October 11, 23.8 mm on October 14 and 10.2 mm on October 17 recorded at the Environment and Climate Change Canada Data “Sydney CS” weather station) which increased the flows of rivers in the area and likely decreased our ability to detect the target species at some sites. However, the impact of these events on the different rivers in terms of eDNA dilution, and thus our ability to detect the presence of each species’ DNA relative to its respective LOD is difficult to predict given the rivers differ in geomorphology, catchment area, and geographical location within the ECB DU (DFO 2013). That said, our sampling took place for the most part over the course of three days in an effort to minimize climatic differences and over the course of these three days of sampling, there was only 1.4 mm of precipitation, thus it is unlikely this rain event affected discharges sufficiently to affect our results.

Environmental DNA concentration, and thus probability of detection is also a function of fish density or abundance, and DNA shedding rate (Thalinger et al. 2020; Wood et al. 2021). While we did generally find a good concordance between the detection of species DNA and previous physical detections through electrofishing and swim-throughs, where this was not the case it is difficult to say for certain why our eDNA approach did not detect a species’ DNA. The target species may occupy different microhabitats, however our sampling design included samples from as close to the river mouth as feasible in an effort to sample as much of the river as possible. If a species’ area of occurrence was very far upstream and/or they were at low abundance, it is possible that dilution or degradation of DNA would result in samples being below the LOD (Pont et al. 2018; Wood et al. 2021). It is also possible that if the species was at low density, and we happened to sample very near the few individuals present, we may have failed to recover any DNA because it had not had the opportunity to fully disperse across the width and depth of the river (Wood et al. 2021). This was found to the be the case in Penaluna et al. (2021), with failure of eDNA to detect very few individuals at the upper boundary of their distribution in headwaters where their presence was confirmed by electrofishing.

While modeling work has been undertaken to link eDNA plume dynamics to fish distribution (Wood et al. 2021) as well as eDNA concentration to salmon abundance (Tillotson et al. 2018), this generally requires a good deal of ground truthing to establish the relationships. No information is available on Rainbow or Brown trout abundance in any of the rivers examined, and while some Atlantic Salmon data is available for Middle, Baddeck and North rivers (DFO 2020), it is not of the appropriate resolution to make linking DNA concentration to population abundance tractable (Tillotson et al. 2018). Additionally, given the majority of the samples taken in this study were from near the river mouth and thus potentially reflective of the majority of the river system (Laramie et al. 2015; Tillotson et al. 2018; Wood et al. 2021), determination of fine scale overlap of trout species would require systematic eDNA sampling and/or capture (e.g. electrofishing).

The results of this study indicate that co-occurrence of endangered Atlantic Salmon and introduced salmonids in the ECB DU is likely widespread. Where they co-occur, competition between Atlantic Salmon and these non-native salmonid species can result in negative outcomes for salmon (Houde et al. 2015b) and may impede their recovery (DFO 2013). Further eDNA and electrofishing studies in the ECB DU focusing on salmonids would provide valuable information on both current Atlantic Salmon population presence, as well as the extent of overlap with non-native salmonids. The eDNA samples collected as part of this expanded sampling (as well as those already collected) could inform future in-stream assessments of overall native Atlantic Salmon and invasive Rainbow and Brown trout abundance, as well as

their fine-scale distributions and co-occurrence. The collected eDNA samples could also be used to determine fish and/or overall aquatic species compositions and these could be examined to investigate potential ecosystem-level impacts of non-native salmonids.

Finally, whether the non-native salmonids are the result of stocking, natural reproduction, or in the case of Rainbow Trout escapees from aquaculture cannot be determined from eDNA data, but should be investigated. The locations and rivers to be targeted for these investigations could be rapidly and relatively cheaply informed by eDNA surveys.

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REFERENCES

Alexander, D.R. 1975. Hatchery trout production and distribution. 1974. Resource Development Branch. Resource Development Branch, Fisheries and Marine Service, Department of the Environment. Data Record Series No. MAR/D-75-9. .

Alexander, D.R., Kerekes, J.J., and Sabean, B.C. 1986. Description of selected lake characteristics and occurrence of fish species in 781 Nova Scotia Lakes. Proc. N.S. Inst. Sci. Vol. 36; Part 2, pp. 63-106.

Anon. 2005. Nova Scotia Trout Management Plan. Nova Scotia Department of Agriculture and Fisheries, Inland Fisheries Division. i + 45 pp.

Armstrong, J.D., Kemp, P.S., Kennedy, G.J.A., Ladle, M., and Milner, N.J. 2003. Habitat requirements of Atlantic salmon and brown trout in rivers and streams. Fish. Res. **62**(2): 143-170. doi:Pii S0165-7836(02)00160-1 Doi 10.1016/S0165-7836(02)00160-1.

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R.A., Foster, J., Wilkinson, J.W., Arnell, A., Brotherton, P., Williams, P., and Dunn, F. 2015. Using eDNA to develop a national citizen science-based monitoring programme for the Great Crested Newt (*Triturus cristatus*). Biol. Conserv. **183**: 19-28. doi:10.1016/j.biocon.2014.11.029.

COSEWIC. 2010. COSEWIC assessment and status report on the Atlantic Salmon *Salmo salar* (Nunavik population, Labrador population, Northeast Newfoundland population, South Newfoundland population, Southwest Newfoundland population, Northwest Newfoundland population, Quebec Eastern North Shore population, Quebec Western North Shore population, Anticosti Island population, Inner St. Lawrence population,Lake Ontario population, Gaspé-Southern Gulf of St. Lawrence population, Eastern Cape Breton population, Nova Scotia Southern Upland population, Inner Bay of Fundy population, Outer Bay of Fundy population) in Canada. Ottawa.

Dadswell, M., Spares, A., Reader, J., McLean, M., McDermott, T., Samways, K., and Lilly, J. 2022. The decline and impending collapse of the Atlantic Salmon (*Salmo salar*) population in the North Atlantic Ocean: A review of possible causes. Rev. Fish. Sci. Aquac. **30**(2): 215-258. doi:10.1080/23308249.2021.1937044.

DFO. 1998. Atlantic Salmon Cape Breton SFA 18 [part] & SFA 19. DFO Science Stock Status Report D3-09(1998).

DFO. 2013. Recovery Potential Assessment for Eastern Cape Breton Atlantic Salmon. DFO Can. Sci. Advis. Sec. Sci. Advis. Resp. 2013/072.

DFO. 2020. Stock Status Update of Atlantic Salmon (*Salmo salar*) in Salmon Fishing Areas (SFAs) 19-21 and 23. DFO Can. Sci. Advis. Sec. Sci. Resp. 2020/002.

Einum, S., and Fleming, I.A. 2000. Highly fecund mothers sacrifice offspring survival to maximize fitness. Nature **405**(6786): 565-567. doi:10.1038/35014600.

Fausch, K.D. 1988. Tests of competition between native and introduced salmonids in streams: What have we learned? Can. J. Fish. Aquat. Sci. **45**(12): 2238-2246. doi:10.1139/f88-260.

Gibson, A.J.F., Horsman, T., Ford, J., and Halfyard, E.A. 2014. Recovery Potential Assessment for Eastern Cape Breton Atlantic Salmon (*Salmo salar*): Habitat requirements and availability; and threats to populations. DFO Can. Sci. Advis. Sec. Res. Doc. 2014/071. vii + 141 p.

He, X.P., Stanley, R.R.E., Rubidge, E.M., Jeffery, N.W., Hamilton, L.C., Westfall, K.M., Gilmore, S.R., Roux, L.M.D., Gale, K.S.P., Heaslip, S.G., Steeves, R., and Abbott, C.L. 2022. Fish community surveys in eelgrass beds using both eDNA metabarcoding and seining: implications for biodiversity monitoring in the coastal zone. Can. J. Fish. Aquat. Sci. **79**(8): 1335-1346. doi:10.1139/cjfas-2021-0215.

Hearn, W.E., and Kynard, B.E. 1986. Habitat utilization and behavioral Interiction of juvenile Atlantic Salmon (*Salmo salar*) and Rainbow Trout (*S. gairdneri*) in tributaries of the White River of Vermont. Can. J. Fish. Aquat. Sci. **43**(10): 1988-1998. doi:10.1139/f86-244.

Heggenes, J., and Dokk, J.G. 2001. Contrasting temperatures, waterflows, and light: Seasonal habitat selection by young Atlantic salmon and brown trout in a boreonemoral river. Regul. Rivers: Res. Manage. **17**(6): 623-635. doi:DOI 10.1002/rrr.620.

Heggenes, J., Bagliniere, J.L., and Cunjak, R.A. 1999. Spatial niche variability for young Atlantic salmon (S*almo salar*) and brown trout (*S. trutta*) in heterogeneous streams. Ecol. Freshwat. Fish **8**(1): 1-21. doi:DOI 10.1111/j.1600-0633.1999.tb00048.x.

Hernandez, C., Bougas, B., Perreault-Payette, A., Simard, A., Côté, G., and Bernatchez, L. 2020. 60 specific eDNA qPCR assays to detect invasive, threatened, and exploited freshwater vertebrates and invertebrates in Eastern Canada. Environ. DNA **2**(3): 373-386. doi:10.1002/edn3.89.

Houde, A.L., Wilson, C.C., and Neff, B.D. 2015a. Predictability of multispecies competitive interactions in three populations of Atlantic salmon *Salmo salar*. J. Fish Biol. **86**(4): 1438-1443. doi:10.1111/jfb.12644.

Houde, A.L.S., Wilson, C.C., and Neff, B.D. 2015b. Competitive interactions among multiple non-native salmonids and two populations of Atlantic salmon. Ecol. Freshwat. Fish **24**(1): 44-55. doi:10.1111/eff.12123.

Houde, A.L.S., Wilson, C.C., and Neff, B.D. 2017. Performance of four salmonids species in competition with Atlantic salmon. J. Great Lakes Res. **43**(1): 211-215. doi:10.1016/j.jglr.2016.10.017.

Houde, A.L.S., Smith, A.D., Wilson, C.C., Peres-Neto, P.R., and Neff, B.D. 2016. Competitive effects between rainbow trout and Atlantic salmon in natural and artificial streams. Ecol. Freshwat. Fish **25**(2): 248-260. doi:10.1111/eff.12206.

Jerde, C.L. 2021. Can we manage fisheries with the inherent uncertainty from eDNA? J. Fish Biol. **98**(2): 341-353. doi:10.1111/jfb.14218.

Jonsson, N., Jonsson, B., and Fleming, I.A. 1996. Does early growth cause a phenotypically plastic response in egg production of Atlantic Salmon? Funct. Ecol. **10**(1): 89-96. doi:10.2307/2390266.

Klymus, K.E., Merkes, C.M., Allison, M.J., Goldberg, C.S., Helbing, C.C., Hunter, M.E., Jackson, C.A., Lance, R.F., Mangan, A.M., Monroe, E.M., Piaggio, A.J., Stokdyk, J.P., Wilson, C.C., and Richter, C.A. 2019. Reporting the limits of detection and quantification for environmental DNA assays. Environ. DNA **2**(3): 271-282. doi:10.1002/edn3.29.

Lacoursiere-Roussel, A., Rosabal, M., and Bernatchez, L. 2016. Estimating fish abundance and biomass from eDNA concentrations: variability among capture methods and environmental conditions. Mol. Ecol. Res. **16**(6): 1401-1414. doi:10.1111/1755-0998.12522.

Laramie, M.B., Pilliod, D.S., and Goldberg, C.S. 2015. Characterizing the distribution of an endangered salmonid using environmental DNA analysis. Biol. Conserv. **183**: 29-37. doi:10.1016/j.biocon.2014.11.025.

LeBlanc, F., Steeves, R., Belliveau, V., Akaishi, F., and Gagne, N. 2021. Detecting the Brook Floater, a freshwater mussel species at risk, using environmental DNA. Aquat. Conserv.: Mar. Freshwat. Ecosyst. **31**(6): 1233-1244. doi:10.1002/aqc.3551.

LeBlanc, F., Belliveau, V., Watson, E., Coomber, C., Simard, N., DiBacco, C., Bernier, R., and Gagne, N. 2020. Environmental DNA (eDNA) detection of marine aquatic invasive species (AIS) in Eastern Canada using a targeted species-specific qPCR approach. Manag Biol Invasion **11**(2): 201-217. doi:10.3391/mbi.2020.11.2.03.

Lehnert, S.J., Kess, T., Bentzen, P., Kent, M.P., Lien, S., Gilbey, J., Clément, M., Jeffery, N.W., Waples, R.S., and Bradbury, I.R. 2019. Genomic signatures and correlates of widespread population declines in salmon. Nat. Commun. **10**(1): 2996. doi:10.1038/s41467-019-10972-w.

Levi, T., Allen, J.M., Bell, D., Joyce, J., Russell, J.R., Tallmon, D.A., Vulstek, S.C., Yang, C., and Yu, D.W. 2019. Environmental DNA for the enumeration and management of Pacific salmon. Mol. Ecol. Resour. **19**(3): 597-608. doi:10.1111/1755-0998.12987.

Levy, A.L., and Gibson, A.J.F. 2014. Recovery Potential Assessment for Eastern Cape Breton Atlantic Salmon (*Salmo salar*): Status, past and present abundance, life history, and trends. DFO Can. Sci. Advis. Sec. Res. Doc. 2014/099. v + 72 p.

MacCrimmon, H.R. 1971. World distribution of Rainbow Trout (*Salmo gairdneri*). J. Fish. Res. Board Can. **28**(5): 663-+. doi:DOI 10.1139/f71-098.

MacCrimmon, H.R., and Marshall, T.L. 1968. World distribution of Brown Trout, *Salmo trutta*. J. Fish. Res. Board Can. **25**(12): 2527-2548. doi:10.1139/f68-225.

MacMillan, J.L., Caissie, D., Marshall, T., and Hinks, L. 2008. Population indices of Brook Trout (*Salvelinus fontinalis*), Atlantic Salmon (*Salmo salar*), and salmonid competitors in relation to summer water temperature and habitat parameters in 100 streams in Nova Scotia. Department of Fisheries and Oceans, Gulf Region, Oceans and Science Branch, Diadromous Fish Section. P.O. Box 5030, Moncton, NB, E1C 9B6.

Madden, R.J., MacMillan, J.L., and Apaloo, J. 2010. Examining the occurrence of wild Rainbow Trout in the Bras d'Or Lakes, Nova Scotia: Using scale pattern analysis to diferentiate hatchery and wild populations *In* Wild Trout X. pp. 176-187.

Mills, M.D., Rader, R.B., and Belk, M.C. 2004. Complex interactions between native and invasive fish: the simultaneous effects of multiple negative interactions. Oecologia **141**(4): 713-721. doi:10.1007/s00442-004-1695-z.

Min, M.A., Barber, P.H., and Gold, Z. 2021. MiSebastes: An eDNA metabarcoding primer set for rockfishes (genus *Sebastes*). Conserv. Genet. Resour. **13**(4): 447-456. doi:10.1007/s12686-021-01219-2.

Parrish, D.L., Behnke, R.J., Gephard, S.R., McCormick, S.D., and Reeves, G.H. 1998. Why aren't there more Atlantic Salmon (*Salmo salar*)? Can. J. Fish. Aquat. Sci. **55**: 281-287. doi:DOI 10.1139/cjfas-55-S1-281.

Penaluna, B.E., Allen, J.M., Arismendi, I., Levi, T., Garcia, T.S., and Walter, J.K. 2021. Better boundaries: identifying the upper extent of fish distributions in forested streams using eDNA and electrofishing. Ecosphere **12**(1): e03332. doi:ARTN e03332 10.1002/ecs2.3332.

Pont, D., Rocle, M., Valentini, A., Civade, R., Jean, P., Maire, A., Roset, N., Schabuss, M., Zornig, H., and Dejean, T. 2018. Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. Sci. Rep. **8**(1): 10361. doi:10.1038/s41598-018-28424-8.

Robichaud-LeBlanc, K.A., and Amiro, P.G. 2004. Assessments of Atlantic Salmon stocks in selected rivers of Eastern Cape Breton, SFA 19, to 2003. Can. Sci. Advis. Sec. Res. Doc. 2004/017. ii + 66 p.

Russell, I.C., Aprahamian, M.W., Barry, J., Davidson, I.C., Fiske, P., Ibbotson, A.T., Kennedy, R.J., Maclean, J.C., Moore, A., Otero, J., Potter, T., and Todd, C.D. 2012. The influence of the freshwater environment and the biological characteristics of Atlantic Salmon smolts on their subsequent marine survival. ICES J. Mar. Sci. **69**(9): 1563-1573. doi:10.1093/icesjms/fsr208.

Sabean, B.C. 1983. Bras D'Or Lake rainbow fishery. Nova Scotia Department of Lands and Forests, Technical Note No. 17, 7 p.

Sanches, F.H., Miyai, C.A., Costa, T.M., Christofoletti, R.A., Volpato, G.L., and Barreto, R.E. 2012. Aggressiveness overcomes body-size effects in fights staged between invasive and native fish species with overlapping niches. PLoS One **7**(1): e29746. doi:10.1371/journal.pone.0029746.

Scott, W.B., and Crossman, E.J. 1973. Freshwater Fishes of Canada. Bulletin of the Fisheries Research Board of Canada, 184, 966 p.

Shaw, J.L.A., Clarke, L.J., Wedderburn, S.D., Barnes, T.C., Weyrich, L.S., and Cooper, A. 2016. Comparison of environmental DNA metabarcoding and conventional fish survey methods in a river system. Biol. Conserv. **197**: 131-138. doi:10.1016/j.biocon.2016.03.010.

Sigsgaard, E.E., Carl, H., Moller, P.R., and Thomsen, P.F. 2015. Monitoring the near-extinct European Weather Loach in Denmark based on environmental DNA from water samples. Biol. Conserv. **183**: 46-52. doi:10.1016/j.biocon.2014.11.023.

Stat, M., Huggett, M.J., Bernasconi, R., DiBattista, J.D., Berry, T.E., Newman, S.J., Harvey, E.S., and Bunce, M. 2017. Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment. Sci. Rep. **7**(1): 12240. doi:10.1038/s41598-017-12501-5.

Thalinger, B., Kirschner, D., Pütz, Y., Moritz, C., Schwarzenberger, R., Wanzenböck, J., and Traugott, M. 2020. Lateral and longitudinal fish environmental DNA distribution in dynamic riverine habitats. Environ. DNA **3**(1): 305-318. doi:10.1002/edn3.171.

Thibault, I., and Dodson, J. 2013. Impacts of exotic Rainbow Trout on habitat use by native juvenile salmonid species at an early invasive stage. Trans. Am. Fish. Soc. **142**(4): 1141-1150. doi:10.1080/00028487.2013.799516.

Thorpe, J.E., Miles, M.S., and Keay, D.S. 1984. Developmental rate, fecundity and egg Size in Atlantic Salmon, *Salmo Salar* L. Aquaculture **43**(1-3): 289-305. doi:Doi 10.1016/0044-8486(84)90030-9.

Tillotson, M.D., Kelly, R.P., Duda, J.J., Hoy, M., Kralj, J., and Quinn, T.P. 2018. Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. Biol. Conserv. **220**: 1-11. doi:10.1016/j.biocon.2018.01.030.

Van Zwol, J.A., Neff, B.D., and Wilson, C.C. 2012a. The effect of competition among three salmonids on dominance and growth during the juvenile life stage. Ecol. Freshwat. Fish **21**(4): 533-540. doi:10.1111/j.1600-0633.2012.00573.x.

van Zwol, J.A., Neff, B.D., and Wilson, C.C. 2012b. The effect of nonnative salmonids on social dominance and growth of juvenile Atlantic Salmon. Trans. Am. Fish. Soc. **141**(4): 907-918. doi:10.1080/00028487.2012.675899.

Westley, P.A.H., and Fleming, I.A. 2011. Landscape factors that shape a slow and persistent aquatic invasion: Brown Trout in Newfoundland 1883-2010. Divers. Distrib. **17**(3): 566-579. doi:10.1111/j.1472-4642.2011.00751.x.

Wood, Z.T., Lacoursiere-Roussel, A., LeBlanc, F., Trudel, M., Kinnison, M.T., McBrine, C.G., Pavey, S.A., and Gagne, N. 2021. Spatial heterogeneity of eDNA transport improves stream assessment of threatened salmon presence, abundance, and location [original research]. Front. Ecol. Evol. **9**(168). doi:ARTN 650717 10.3389/fevo.2021.650717.

Table 1 – Locations of the sites at which eDNA samples were collected and their respective detection classifications for Atlantic Salmon, Rainbow Trout and Brown Trout. Location refers to the relative location of the sampling Site and whether it was near the river Mouth or Upstream from the mouth. Where there was only one sampling site, the Location is left blank. Detection classification categories are from LeBlanc et al. (2020). Refer also to Figure 1 for visualization of the sites.

River	Site	Location	Atlantic Salmon	Rainbow Trout	Brown Trout	Latitude	Longitude
River Denys	1	Mouth	Detected	Not Detected	Suspected	45.8364	-61.1775
River Denys	2	Upstream	Detected	Not Detected	Suspected	45.8539	-61.2157
Pooles Brook	1	Upstream	Not Detected	Not Detected	Not Detected	45.9136	-61.1304
Pooles Brook	2	Mouth	Not Detected	Not Detected	Not Detected	45.9240	-61.1286
Skye River	1	Mouth	Detected	Detected	Detected	45.9719	-61.1319
Skye River	2	Upstream	Detected	Not Detected	Detected	46.0431	-61.1974
Middle River	1	Upstream	Detected	Not Detected	Not Detected	46.2257	-60.9277
Middle River	2	Mouth	Detected	Suspected	Suspected	46.1058	-60.9213
Baddeck River	1	Mouth	Detected	Detected	Detected	46.1237	-60.8036
Baddeck River	2	Upstream	Detected	Not Detected	Suspected	46.1991	-60.7226
North River	1	Mouth	Detected	Inconclusive	Suspected	46.3080	-60.6224
North River	2	Upstream	Detected	Not Detected	Detected	46.3157	-60.6684
Benacadie River	1	Upstream	Detected	Not Detected	Detected	45.9613	-60.7057
Benacadie River	2	Mouth	Detected	Not Detected	Detected	45.9292	-60.7275
Indian Brook			Detected	Suspected	Detected	45.9483	-60.6026
Gillis Brook	1	Mouth	Detected	Suspected	Detected	46.0261	-60.3828
Gillis Brook	2	Upstream	Not Detected	Not Detected	Not Detected	46.0521	-60.3925
Breac Brook	1	Upstream	Detected	Suspected	Detected	45.9130	-60.5131
Breac Brook	2	Mouth	Detected	Suspected	Detected	45.9139	-60.5292
Toms Brook			Suspected	Not Detected	Detected	45.7395	-60.7280
Salmon River			Not Detected	Not Detected	Detected	45.6798	-60.7809
River Tillard	1	Mouth	Suspected	Not Detected	Not Detected	45.6579	-60.9131
River Tillard	2	Upstream	Inconclusive	Not Detected	Not Detected	45.6649	-60.9834
Black River	1	Upstream	Detected	Not Detected	Detected	45.6637	-61.0945
Black River	2	Mouth	Detected	Not Detected	Suspected	45.6951	-61.1021
Framboise River	1	Mouth	Suspected	Not Detected	Not Detected	45.7342	-60.3844
Framboise River	2	Upstream	Detected	Not Detected	Not Detected	45.7402	-60.3986

Table 2 – Species-specific qPCR assays. LOD is the theoretical limit of detection, LOQ is the theoretical limit of quantification and efficiency relates to how closely the qPCR assay matches the theoretical doubling of DNA with each PCR cycle. Refer to material and methods for their quantification and calculation.

qPCR Assay	Locus targeted[a]	Primer/Probe name	Sequence 5'-3'	Amplicon size (bp)	Efficiency (%)	LOD (pg)[d]	LOQ (pg)[d]
Rainbow Trout[b]	CytB	ONMY_CYTBF	CCTCCCGTGAGGACAAATATCA	125	83	0.10	0.55
		ONMY_CYTBR	TGGCGTTGTCAACGGAGAAG				
		ONMY_CYTB_Probe	FAM - TACGTAGGAGGCGCCCT - MGB				
Brown Trout[b]	COI	SATR_COIF	GCTTCTGACTCCTCCCTCCG	248	89	0.34	0.88
		SATR_COIR	AAGTGGAGTTTGATATTGGGAGATG				
		Salmot_COI_Probe*	FAM - CTAGCCTCGTCTGGAGTT - MGB				
Atlantic Salmon[c]	COI	COI_82F_Ss	TGGCGCCCTTCTGGGA	195	87	0.26	1.05
		COI_276R_Ss	AAGGAGGGAGGGAGAAGTCAAAAA				
		COI_194P_Ss	FAM – ATTAATTCCTCTTATAATCGGG - MGB				

[a] CytB is cytochrome B and COI is cytochrome c oxidase subunit 1
[b] Primers and probes found in Hernandez et al. (2020), with the exception of the Brown Trout Probe which was designed in-house
[c] Primers and probe published in Wood et al. (2021)
[d] LOD and LOQ were determined from 3 serial dilutions prepared from genomic DNA from the targeted species and tested in qPCR duplicate for a total of 6 values per dilution. The R script found in Klymus et al. (2019) was used for the data analysis.

Table 3 – Criteria used for classification of results used in this study.

Classification	Criteria
Not detected	No detection in any of the qPCR technical replicates
Inconclusive	Detection obtained in 1/3 of the qPCR technical replicates
Suspected	Detection obtained in 2/3 or 3/3 of the qPCR technical replicates, but the value was below the LOD
Detected	Detection obtained in 3/3 of the qPCR technical replicates performed and the value was above the LOD

[a] Based on classifications found in (LeBlanc et al. 2020).

Table 4 – River detection classifications for the 15 Eastern Cape Breton rivers sampled in this study, and previously published records of detection. Where more than one site was sampled in a river, in this study, and where the eDNA-based detection classification differed between sites, the higher of the two classifications is reported. eDNA detection classification categories are from LeBlanc et al. (2020), and as in LeBlanc et al. (2020), the strongest category is reported. References for published studies are noted in the footnote for the table.

	Atlantic Salmon		Rainbow Trout		Brown Trout	
River	**This study**	**Published**	**This study**	**Published**	**This study**	**Published**
River Denys	Detected	a, b, c	Not Detected		Suspected	a
Pooles Brook	Not Detected		Not Detected		Not Detected	
Skye River	Detected	c	Detected	e, g	Detected	
Middle River	Detected	a, b, d	Suspected	f, g	Suspected	g
Baddeck River	Detected	a, c, d	Detected	g	Detected	g
North River	Detected	b, c, d	Inconclusive	f, g	Detected	g
Benacadie River	Detected	c	Not Detected		Detected	
Indian Brook	Detected	b	Suspected	b	Detected	b
Gillis Brook	Detected	c	Suspected	e	Detected	
Breac Brook	Detected	c	Suspected	e	Detected	
Toms Brook	Suspected	c	Not Detected		Detected	
Salmon River	Not Detected		Not Detected		Detected	
River Tillard	Suspected	c	Not Detected		Not Detected	
Black River	Detected	b	Not Detected		Detected	
Framboise River	Detected	c	Not Detected		Not Detected	

a MacMillan et al. (2008); b Levy and Gibson (2014); c DFO (2013); d DFO (2020); e Sabean (1983); f Robichaud-LeBlanc and Amiro (2004); g D. Raab, pers. obs.

Figure 1 – eDNA Sampling locations in Eastern Cape Breton.

Figure 2 – Detections of Atlantic Salmon, Brown Trout and Rainbow Trout DNA in the 15 Eastern Cape Breton rivers sampled. A detection is indicated if at least one sampling location in a river meets the definition of detection as defined in LeBlanc et al. (2020). Refer to Table 3 for LeBlanc et al. (2020) definitions, and Table 1 for sampling site specific detection classifications.

APPENDICES

*Supplemental Table 1 – Cycle threshold (Ct) values qPCRs undertaken in this study, and subsequent determination of sample- and site-specific DNA detection. For each river site sample (River Replicate), three replicate samples were taken (Sample Replicate), and these were each in turn subjected to three replicate qPCRs. Where target DNA copy number exceeded the LOD, CTs are reported separately for each qPCR replicate (*Species *Ct-1,* Species *Ct-2,* Species *Ct-3). Based on the qPCR replicate Ct values, sample-specific detection results are reported (*Species *Result). The replicate-specific detections are summed into river sample-specific results (*Species *Site Result). These classifications are from LeBlanc et al. (2020), and definitions are given in Table 3. The location of the river sample replicates are given in the Latitude and Longitude columns, their position either near the river Mouth or Upstream from the river mouth is indicated and the relative position of each replicate sample within the river is in the Replicate Description column. Where there was only one sampling site, the Location is left blank*

Results consistent with contamination during the extraction phase were observed during this study. For the samples potentially affected, extractions were conducted again using the back-up filter halves, and the qPCRs were repeated. Following re-extraction, the extraction blanks showed no signs of contamination, and thus the RETEST values were used. For transparency, all results are reported.

For ease of interpretation, the River, River Replicate, Sample Replicate, Replicate Description, Latitude and Longitude columns are repeated for each species.

A – Atlantic Salmon

River	River Replicate	Sample Replicate	Replicate Relative Location	Replicate Description	Latitude	Longitude	Atlantic salmon Ct - 1	Atlantic Salmon Ct - 2	Atlantic Salmon Ct - 3	Atlantic salmon Result	Atlantic salmon Site Result	RETEST Atlantic salmon Ct - 1	RETEST Atlantic Salmon Ct - 2	RETEST Atlantic Salmon Ct - 3	RETEST Atlantic salmon Result	RETEST Atlantic salmon Site Result
River Denys	1	1	Mouth	Mid stream, below bridge	45.836	-61.177	36.18	35.90	34.29	Detected	Detected	34.34	34.32	33.65	Detected	Detected
River Denys	1	2	Mouth	Left bank, above bridge	45.836	-61.177	34.65	35.28	36.35	Detected		35.07	34.57	35.26	Detected	
River Denys	1	3	Mouth	Left bank, below bridge	45.836	-61.177	34.71	34.80	33.96	Detected		34.60	35.49	35.89	Detected	
River Denys	2	2	Upstream	Mid stream, below bridge	45.854	-61.216	33.86	35.82	34.76	Detected	Detected					Detected
River Denys	2	4	Upstream	Left bank, below bridge	45.854	-61.216	No Ct	No Ct	No Ct	Not detected						
River Denys	2	5	Upstream	Right bank, below bridge	45.854	-61.216	34.71	35.17	36.33	Detected		35.04	35.20	35.93	Detected	
River Denys	2	6		BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
Pooles Brook	1	1	Upstream	Right bank, downstream of bridge	45.914	-61.130	37.47	37.27	36.91	Suspected	Suspected	No Ct	No Ct	No Ct	Not detected	Not detected
Pooles Brook	1	2	Upstream	Left bank, downstream of bridge	45.914	-61.130	No Ct	No Ct	No Ct	Not detected						
Pooles Brook	1	3	Upstream	Mid stream, downstream of bridge	45.914	-61.130	No Ct	No Ct	No Ct	Not detected						
Pooles Brook	2	2	Mouth	Left bank, downstream of bridge	45.924	-61.129	No Ct	No Ct	No Ct	Not detected	Not detected	No Ct	No Ct	No Ct	Not detected	Not detected
Pooles Brook	2	3	Mouth	Mid stream, downstream of bridge	45.924	-61.129	No Ct	No Ct	No Ct	Not detected						
Pooles Brook	2	4	Mouth	Right bank, downstream of bridge	45.924	-61.129	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	No Ct	Not detected	
Skye River	1	1	Mouth	Rep 1 - left bank	45.972	-61.132	33.17	33.67	32.86	Detected	Detected					
Skye River	1	2	Mouth	Rep 2 - left bank	45.972	-61.132	34.39	33.41	33.78	Detected						
Skye River	1	3	Mouth	Rep 3 - left bank	45.972	-61.132	33.44	33.17	33.06	Detected						
Skye River	2	3	Upstream	Left bank, upstream of bridge	46.043	-61.197	34.71	36.69	34.65	Detected	Detected					
Skye River	2	4	Upstream	Mid stream, upstream of bridge	46.043	-61.197	33.90	33.99	34.79	Detected						
Skye River	2	5	Upstream	Right bank, upstream of bridge	46.043	-61.197	36.70	35.63	35.65	Detected						
Skye River	2	6		BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
Middle River	1	1	Upstream	Right bank, upstream of bridge	46.226	-60.928	31.47	31.56	31.69	Detected	Detected	31.92	31.34	31.42	Detected	Detected
Middle River	1	3	Upstream	Left bank, upstream of bridge	46.226	-60.928	32.25	32.24	32.27	Detected						
Middle River	1	5	Upstream	Mid stream, upstream of bridge	46.226	-60.928	32.15	31.58	31.84	Detected						
Middle River	2	1	Mouth	Rep 1 - right bank	46.106	-60.921	32.76	32.45	33.24	Detected	Detected	32.57	32.84	32.57	Detected	Detected
Middle River	2	2	Mouth	Rep 2 - right bank	46.106	-60.921	32.49	32.36	33.37	Detected		32.25	32.76	33.40	Detected	
Middle River	2	3	Mouth	Rep 3 - right bank	46.106	-60.921	33.01	33.56	33.00	Detected						
Baddeck River	1	1	Mouth	Mid stream	46.124	-60.804	32.17	32.73	32.60	Detected	Detected	32.14	31.83	32.18	Detected	Detected
Baddeck River	1	2	Mouth	Left bank, upstream of bridge	46.124	-60.804	32.94	32.03	32.44	Detected						
Baddeck River	1	5	Mouth	Right bank, upstream of bridge	46.124	-60.804	32.99	32.40	32.65	Detected						
Baddeck River	1	6	Mouth	BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
Baddeck River	2	3	Upstream	Right bank, upstream of bridge	46.199	-60.723	32.33	32.36	32.19	Detected	Detected	32.54	32.17	32.29	Detected	Detected
Baddeck River	2	4	Upstream	Left bank, upstream of bridge	46.199	-60.723	31.94	31.58	31.91	Detected						
Baddeck River	2	5	Upstream	Left bank, downstream of bridge	46.199	-60.723	32.74	32.65	32.26	Detected		31.81	31.81	31.67	Detected	
North River	1	2	Mouth	Mid stream, upstream of bridge	46.308	-60.622	31.76	31.75	31.60	Detected	Detected	30.66	30.74	31.37	Detected	Detected
North River	1	3	Mouth	Left bank, upstream of bridge	46.308	-60.622	31.72	31.8	31.59	Detected		31.18	31.37	31.62	Detected	
North River	1	5	Mouth	Right bank, upstream of bridge	46.308	-60.622	31.61	31.92	31.85	Detected						

River	River Replicate	Sample Replicate	Replicate Relative Location	Replicate Description	Latitude	Longitude	Atlantic salmon Ct - 1	Atlantic Salmon Ct - 2	Atlantic Salmon Ct - 3	Atlantic salmon Result	Atlantic salmon Site Result	RETEST Atlantic salmon Ct - 1	RETEST Atlantic Salmon Ct - 2	RETEST Atlantic Salmon Ct - 3	RETEST Atlantic salmon Result	RETEST Atlantic salmon Site Result
North River	1	6	Mouth	BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
North River	2	1	Upstream	Right bank, rep 1	46.316	-60.668	30.64	30.82	30.87	Detected	Detected					
North River	2	2	Upstream	Left bank, rep1	46.316	-60.668	31.11	31.44	31.10	Detected						
North River	2	4	Upstream	Mid stream	46.316	-60.668	31.15	31.42	31.23	Detected						
Benacadie River	1	1	Upstream	Mid stream, upstream of bridge	45.961	-60.706	35.17	35.73	34.61	Detected	Detected					
Benacadie River	1	2	Upstream	Right bank, upstream of bridge	45.961	-60.706	35.76	37.07	33.65	Detected						
Benacadie River	1	3	Upstream	Left bank, upstream of bridge	45.961	-60.706	37.21	34.43	33.74	Detected						
Benacadie River	2	3	Mouth	Right bank, downstream of bridge	45.929	-60.727	31.17	31.45	31.04	Detected	Detected					
Benacadie River	2	4	Mouth	Left bank, downstream of bridge	45.929	-60.727	30.79	30.89	31.27	Detected						
Benacadie River	2	5	Mouth	Right bank, downstream of bridge	45.929	-60.727	30.87	31.13	30.83	Detected						
Indian Brook	1	1		Rep 1	45.948	-60.603	34.87	34.51	34.81	Detected	Detected					Detected
Indian Brook	1	2		Rep 2	45.948	-60.603	33.97	35.16	34.55	Detected		33.55	34.56	35.02	Detected	
Indian Brook	1	3		Rep 3	45.948	-60.603	33.62	33.84	33.79	Detected		34.39	33.95	33.24	Detected	
Gillis Brook	1	2	Mouth	Mid stream, upstream of bridge	46.026	-60.383	33.61	34.84	34.44	Detected	Detected					Detected
Gillis Brook	1	3	Mouth	Right bank, upstream of bridge	46.026	-60.383	34.77	33.96	33.23	Detected		34.65	33.61	33.94	Detected	
Gillis Brook	1	5	Mouth	Left bank, upstream of bridge	46.026	-60.383	33.93	33.90	33.17	Detected		33.62	34.51	33.48	Detected	
Gillis Brook	2	1	Upstream	Mid stream	46.052	-60.393	No Ct	No Ct	No Ct	Not detected	Not detected					
Gillis Brook	2	2	Upstream	Left bank, rep 1	46.052	-60.393	No Ct	No Ct	No Ct	Not detected						
Gillis Brook	2	5	Upstream	Right bank, rep 1	46.052	-60.393	No Ct	No Ct	No Ct	Not detected						
Breac Brook	1	1	Upstream	Mid stream, downstream of bridge	45.913	-60.513	33.42	32.73	33.62	Detected	Detected	31.65	32.61	32.24	Detected	Detected
Breac Brook	1	2	Upstream	Right bank, downstream of bridge	45.913	-60.513	33.25	33.22	33.50	Detected		32.54	33.12	32.76	Detected	
Breac Brook	1	3	Upstream	Left bank, downstream of bridge	45.913	-60.513	33.18	32.98	32.62	Detected						
Breac Brook				BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
Breac Brook	2	3	Mouth	Left bank, downstream of bridge	45.914	-60.529	35.00	33.89	33.16	Detected	Detected	32.88	32.74	33.20	Detected	Detected
Breac Brook	2	4	Mouth	Right bank, downstream of bridge	45.914	-60.529	33.75	33.27	33.99	Detected		33.97	34.04	34.04	Detected	
Breac Brook	2	5	Mouth	Mid stream, downstream of bridge	45.914	-60.529	34.48	34.29	33.86	Detected		33.35	33.75	33.28	Detected	
Toms Brook	1	2		Left bank, downstream of bridge	45.740	-60.728	38.18	37.96	36.96	Suspected	Suspected					Suspected
Toms Brook	1	3		Mid stream, downstream of bridge	45.740	-60.728	37.06	36.02	No Ct	Suspected		35.13	No Ct	No Ct	Inconclusive	
Toms Brook	1	4		Right bank, downstream of bridge	45.740	-60.728	No Ct	No Ct	35.96	Inconclusive		34.98	No Ct	35.79	Suspected	
Salmon River	1	1		Samples taken around bridge	45.680	-60.781	No Ct	No Ct	No Ct	Not detected	Not detected					
Salmon River	1	2		Samples taken around bridge	45.680	-60.781	No Ct	No Ct	No Ct	Not detected						
Salmon River	1	3		Samples taken around bridge	45.680	-60.781	No Ct	No Ct	No Ct	Not detected						
River Tillard	1	2	Mouth	Left bank, upstream of bridge	45.658	-60.913	37.84	37.37	38.00	Suspected	Suspected					Suspected
River Tillard	1	4	Mouth	Mid stream, upstream of bridge	45.658	-60.913	No Ct	35.43	36.96	Suspected		No Ct	37.06	37.34	Suspected	
River Tillard	1	5	Mouth	Right bank, upstream of bridge	45.658	-60.913	35.39	36.96	34.78	Suspected						
River Tillard	2	1	Upstream	Right bank, upstream of bridge	45.665	-60.983	36.92	No Ct	No Ct	Inconclusive	Inconclusive	No Ct	No Ct	35.99	Inconclusive	Inconlusive
River Tillard	2	2	Upstream	Left bank, upstream of bridge	45.665	-60.983	No Ct	38.01	No Ct	Inconclusive		No Ct	44.14	No Ct	Inconclusive	

River	River Replicate	Sample Replicate	Replicate Relative Location	Replicate Description	Latitude	Longitude	Atlantic salmon Ct - 1	Atlantic Salmon Ct - 2	Atlantic Salmon Ct - 3	Atlantic salmon Result	Atlantic salmon Site Result	RETEST Atlantic salmon Ct - 1	RETEST Atlantic Salmon Ct - 2	RETEST Atlantic Salmon Ct - 3	RETEST Atlantic salmon Result	RETEST Atlantic salmon Site Result
River Tillard	2	3	Upstream	Left bank, downstream of bridge	45.665	-60.983	No Ct	No Ct	37.96	Inconclusive		No Ct	No Ct	37.28	Inconclusive	
Black River	1	1	Upstream	Right bank, downstream of bridge	45.664	-61.095	33.64	33.62	34.05	Detected	Detected					
Black River	1	2	Upstream	Left bank, upstream of bridge	45.664	-61.095	34.54	35.35	34.56	Detected						
Black River	1	3	Upstream	Left bank, downstream of bridge	45.664	-61.095	33.60	34.17	34.61	Detected						
Black River	2	1	Mouth	Black River side - rep 1	45.695	-61.102	34.54	35.37	34.11	Detected	Detected					
Black River	2	2	Mouth	Black River Side - rep 2	45.695	-61.102	33.43	35.61	35.52	Detected						
Black River	2	3	Mouth	Ballams Pond - rep 1	45.695	-61.102	34.28	33.28	33.70	Detected						
Framboise				BLANK - distilled water	45.734	-60.384	No Ct	No Ct	No Ct	Not detected	Suspected					
Framboise	1	1	Mouth		45.734	-60.384	37.14	No Ct	38.61	Suspected						
Framboise	1	2	Mouth	Mid stream	45.734	-60.384	37.12	No Ct	38.06	Suspected						
Framboise	1	3	Mouth		45.734	-60.384	35.99	36.98	38.81	Suspected						
Framboise	2	1	Upstream		45.740	-60.399	34.06	35.39	33.96	Detected	Detected					
Framboise	2	2	Upstream	Mid stream	45.740	-60.399	33.50	33.87	33.71	Detected						
Framboise	2	3	Upstream		45.740	-60.399	33.35	34.30	33.78	Detected						

B – Brown Trout

River	River Replicate	Sample Replicate	Replicate Relative Location	Replicate Description	Latitude	Longitude	Brown trout Ct - 1	Brown trout Ct - 2	Brown trout Ct - 3	Brown trout Result	Brown trout Site Result	RETEST Brown trout Ct - 1	RETEST Brown trout Ct - 2	RETEST Brown trout Ct - 3	RETEST Brown trout Result	RETEST Brown trout Site Result
River Denys	1	1	Mouth	Mid stream, below bridge	45.836	-61.177	No Ct	35.19	No Ct	Inconclusive	Inconclusive	No Ct	No Ct	37.03	Inconclusive	Suspected
River Denys	1	2	Mouth	Left bank, above bridge	45.836	-61.177	No Ct	36.13	No Ct	Inconclusive		No Ct	No Ct	No Ct	Not detected	
River Denys	1	3	Mouth	Left bank, below bridge	45.836	-61.177	37.07	No Ct	No Ct	Inconclusive		37.27	36.86	No Ct	Suspected	
River Denys	2	2	Upstream	Mid stream, below bridge	45.854	-61.216	35.43	33.34	34.99	Suspected	Suspected	No Ct	35.59	37.24	Suspected	Suspected
River Denys	2	4	Upstream	Left bank, below bridge	45.854	-61.216	No Ct	No Ct	No Ct	Not detected						
River Denys	2	5	Upstream	Right bank, below bridge	45.854	-61.216	No Ct	No Ct	37.28	Inconclusive						
River Denys	2	6		BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
Pooles Brook	1	1	Upstream	Right bank, downstream of bridge	45.914	-61.130	36.16	No Ct	35.55	Suspected	Suspected	No Ct	No Ct	No Ct	Not detected	Not detected
Pooles Brook	1	2	Upstream	Left bank, downstream of bridge	45.914	-61.130	No Ct	No Ct	No Ct	Not detected						
Pooles Brook	1	3	Upstream	Mid stream, downstream of bridge	45.914	-61.130	No Ct	No Ct	No Ct	Not detected						
Pooles Brook	2	2	Mouth	Left bank, downstream of bridge	45.924	-61.129	No Ct	No Ct	No Ct	Not detected	Suspected	No Ct	No Ct	No Ct	Not detected	Not detected
Pooles Brook	2	3	Mouth	Mid stream, downstream of bridge	45.924	-61.129	No Ct	No Ct	No Ct	Not detected						
Pooles Brook	2	4	Mouth	Right bank, downstream of bridge	45.924	-61.129	35.24	No Ct	35.98	Suspected		No Ct	No Ct	No Ct	Not detected	
Skye River	1	1	Mouth	Rep 1 - left bank	45.972	-61.132	28.93	28.84	28.92	Detected	Detected					
Skye River	1	2	Mouth	Rep 2 - left bank	45.972	-61.132	28.65	28.62	28.69	Detected						
Skye River	1	3	Mouth	Rep 3 - left bank	45.972	-61.132	28.28	28.65	28.58	Detected						
Skye River	2	3	Upstream	Left bank, upstream of bridge	46.043	-61.197	29.76	29.78	29.80	Detected	Detected					
Skye River	2	4	Upstream	Mid stream, upstream of bridge	46.043	-61.197	29.80	29.44	29.43	Detected						
Skye River	2	5	Upstream	Right bank, upstream of bridge	46.043	-61.197	29.75	30.03	29.80	Detected						
Skye River	2	6		BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
Middle River	1	1	Upstream	Right bank, upstream of bridge	46.226	-60.928	No Ct	36.12	No Ct	Inconclusive	Inconclusive	No Ct	No Ct	No Ct	Not detected	Not detected
Middle River	1	3	Upstream	Left bank, upstream of bridge	46.226	-60.928	No Ct	No Ct	No Ct	Not detected						
Middle River	1	5	Upstream	Mid stream, upstream of bridge	46.226	-60.928	No Ct	No Ct	No Ct	Not detected						
Middle River	2	1	Mouth	Rep 1 - right bank	46.106	-60.921	No Ct	No Ct	35.71	Inconclusive	Suspected	34.78	No Ct	35.51	Suspected	Suspected
Middle River	2	2	Mouth	Rep 2 - right bank	46.106	-60.921	No Ct	36.38	34.66	Suspected		No Ct	No Ct	No Ct	Not detected	
Middle River	2	3	Mouth	Rep 3 - right bank	46.106	-60.921	No Ct	No Ct	No Ct	Not detected						
Baddeck River	1	1	Mouth	Mid stream	46.124	-60.804	32.58	33.62	33.12	Detected	Detected	33.25	32.31	33.15	Detected	Detected
Baddeck River	1	2	Mouth	Left bank, upstream of bridge	46.124	-60.804	32.73	32.95	33.63	Detected						
Baddeck River	1	5	Mouth	Right bank, upstream of bridge	46.124	-60.804	32.71	33.56	33.76	Detected						
Baddeck River	1	6	Mouth	BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
Baddeck River	2	3	Upstream	Right bank, upstream of bridge	46.199	-60.723	36.29	No Ct	No Ct	Inconclusive	Suspected	35.31	34.46	36.71	Suspected	Suspected
Baddeck River	2	4	Upstream	Left bank, upstream of bridge	46.199	-60.723	35.39	36.31	33.90	Suspected						
Baddeck River	2	5	Upstream	Left bank, downstream of bridge	46.199	-60.723	No Ct	36.29	35.76	Suspected		37.45	35.31	37.07	Suspected	

River	River Replicate	Sample Replicate	Replicate Relative Location	Replicate Description	Latitude	Longitude	Brown trout Ct - 1	Brown trout Ct - 2	Brown trout Ct - 3	Brown trout Result	Brown trout Site Result	RETEST Brown trout Ct - 1	RETEST Brown trout Ct - 2	RETEST Brown trout Ct - 3	RETEST Brown trout Result	RETEST Brown trout Site Result
North River	1	2	Mouth	Mid stream, upstream of bridge	46.308	-60.622	34.48	36.01	No Ct	Suspected	Suspected	35.91	36.30	36.34	Suspected	Suspected
North River	1	3	Mouth	Left bank, upstream of bridge	46.308	-60.622	No Ct	No Ct	No Ct	Not detected		36.30	No Ct	No Ct	Inconclusive	
North River	1	5	Mouth	Right bank, upstream of bridge	46.308	-60.622	No Ct	36.92	37.60	Suspected						
North River	1	6	Mouth	BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
North River	2	1	Upstream	Right bank, rep 1	46.316	-60.668	33.67	33.77	34.44	Detected	Detected					
North River	2	2	Upstream	Left bank, rep1	46.316	-60.668	36.26	34.07	34.14	Suspected						
North River	2	4	Upstream	Mid stream	46.316	-60.668	36.26	35.45	34.99	Suspected						
Benacadie River	1	1	Upstream	Mid stream, upstream of bridge	45.961	-60.706	33.50	33.48	33.73	Detected	Detected					
Benacadie River	1	2	Upstream	Right bank, upstream of bridge	45.961	-60.706	32.83	32.81	32.49	Detected						
Benacadie River	1	3	Upstream	Left bank, upstream of bridge	45.961	-60.706	33.17	32.59	32.46	Detected						
Benacadie River	2	3	Mouth	Right bank, downstream of bridge	45.929	-60.727	29.11	29.05	29.10	Detected	Detected					
Benacadie River	2	4	Mouth	Left bank, downstream of bridge	45.929	-60.727	28.71	29.25	28.96	Detected						
Benacadie River	2	5	Mouth	Right bank, downstream of bridge	45.929	-60.727	28.74	29.29	28.70	Detected						
Indian Brook	1	1		Rep 1	45.948	-60.603	32.39	31.39	31.92	Detected	Detected					
Indian Brook	1	2		Rep 2	45.948	-60.603	31.78	31.53	32.29	Detected		31.58	31.57	31.38	Detected	Detected
Indian Brook	1	3		Rep 3	45.948	-60.603	33.54	32.91	32.78	Detected		34.62	34.08	33.34	Detected	
Gillis Brook	1	2	Mouth	Mid stream, upstream of bridge	46.026	-60.383	30.81	31.52	30.70	Detected	Detected					
Gillis Brook	1	3	Mouth	Right bank, upstream of bridge	46.026	-60.383	31.03	30.76	31.25	Detected		31.69	30.96	30.70	Detected	Detected
Gillis Brook	1	5	Mouth	Left bank, upstream of bridge	46.026	-60.383	30.73	30.84	30.62	Detected		31.28	30.71	31.21	Detected	
Gillis Brook	2	1	Upstream	Mid stream	46.052	-60.393	No Ct	No Ct	No Ct	Not detected	Not detected					
Gillis Brook	2	2	Upstream	Left bank, rep 1	46.052	-60.393	No Ct	No Ct	No Ct	Not detected						
Gillis Brook	2	5	Upstream	Right bank, rep 1	46.052	-60.393	No Ct	No Ct	No Ct	Not detected						
Breac Brook	1	1	Upstream	Mid stream, downstream of bridge	45.913	-60.513	29.74	29.73	29.98	Detected	Detected	29.15	29.12	28.87	Detected	Detected
Breac Brook	1	2	Upstream	Right bank, downstream of bridge	45.913	-60.513	29.99	30.14	29.73	Detected		30.27	29.69	29.98	Detected	
Breac Brook	1	3	Upstream	Left bank, downstream of bridge	45.913	-60.513	29.68	29.83	29.76	Detected						
Breac Brook				BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
Breac Brook	2	3	Mouth	Left bank, downstream of bridge	45.914	-60.529	31.17	31.59	31.44	Detected	Detected	31.08	30.56	30.83	Detected	Detected
Breac Brook	2	4	Mouth	Right bank, downstream of bridge	45.914	-60.529	31.85	31.81	31.54	Detected		31.38	30.82	30.95	Detected	
Breac Brook	2	5	Mouth	Mid stream, downstream of bridge	45.914	-60.529	31.66	32.34	32.79	Detected		31.19	32.10	31.37	Detected	
Toms Brook	1	2		Left bank, downstream of bridge	45.740	-60.728	27.99	27.95	27.88	Detected	Detected					
Toms Brook	1	3		Mid stream, downstream of bridge	45.740	-60.728	27.93	28.10	28.04	Detected		28.31	28.12	27.88	Detected	Detected
Toms Brook	1	4		Right bank, downstream of bridge	45.740	-60.728	28.60	28.35	28.29	Detected		28.16	28.22	28.46	Detected	
Salmon River	1	1		Samples taken around bridge	45.680	-60.781	31.59	31.46	31.57	Detected	Detected					
Salmon River	1	2		Samples taken around bridge	45.680	-60.781	33.69	33.51	33.53	Detected						
Salmon River	1	3		Samples taken around bridge	45.680	-60.781	33.87	33.49	33.77	Detected						

River	River Replicate	Sample Replicate	Replicate Relative Location	Replicate Description	Latitude	Longitude	Brown trout Ct - 1	Brown trout Ct - 2	Brown trout Ct - 3	Brown trout Result	Brown trout Site Result	RETEST Brown trout Ct - 1	RETEST Brown trout Ct - 2	RETEST Brown trout Ct - 3	RETEST Brown trout Result	RETEST Brown trout Site Result
River Tillard	1	2	Mouth	Left bank, upstream of bridge	45.658	-60.913	No Ct	No Ct	No Ct	Not detected	Not detected	No Ct	No Ct	No Ct	Not detected	Not detected
River Tillard	1	4	Mouth	Mid stream, upstream of bridge	45.658	-60.913	No Ct	No Ct	No Ct	Not detected						
River Tillard	1	5	Mouth	Right bank, upstream of bridge	45.658	-60.913	No Ct	No Ct	No Ct	Not detected						
River Tillard	2	1	Upstream	Right bank, upstream of bridge	45.665	-60.983	No Ct	No Ct	No Ct	Not detected	Not detected	No Ct	No Ct	No Ct	Not detected	Not detected
River Tillard	2	2	Upstream	Left bank, upstream of bridge	45.665	-60.983	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	No Ct	Not detected	
River Tillard	2	3	Upstream	Left bank, downstream of bridge	45.665	-60.983	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	No Ct	Not detected	
Black River	1	1	Upstream	Right bank, downstream of bridge	45.664	-61.095	32.68	33.19	32.47	Detected	Detected					
Black River	1	2	Upstream	Left bank, upstream of bridge	45.664	-61.095	31.14	31.55	31.63	Detected						
Black River	1	3	Upstream	Left bank, downstream of bridge	45.664	-61.095	33.14	32.53	32.48	Detected						
Black River	2	1	Mouth	Black River side - rep 1	45.695	-61.102	34.18	33.47	35.56	Suspected	Suspected					
Black River	2	2	Mouth	Black River Side - rep 2	45.695	-61.102	34.65	35.91	35.38	Suspected						
Black River	2	3	Mouth	Ballams Pond - rep 1	45.695	-61.102	34.59	35.70	36.52	Suspected						
Framboise				BLANK - distilled water	45.734	-60.384	No Ct	No Ct	No Ct	Not detected						
Framboise	1	1	Mouth		45.734	-60.384	No Ct	No Ct	No Ct	Not detected	Not detected					
Framboise	1	2	Mouth	Mid stream	45.734	-60.384	No Ct	No Ct	No Ct	Not detected						
Framboise	1	3	Mouth		45.734	-60.384	No Ct	No Ct	No Ct	Not detected						
Framboise	2	1	Upstream		45.740	-60.399	No Ct	No Ct	No Ct	Not detected	Not detected					
Framboise	2	2	Upstream	Mid stream	45.740	-60.399	No Ct	No Ct	No Ct	Not detected						
Framboise	2	3	Upstream		45.740	-60.399	No Ct	No Ct	No Ct	Not detected						

C – Rainbow Trout

River	River Replicate	Sample Replicate	Replicate Relative Location	Replicate Description	Latitude	Longitude	Rainbow trout Ct - 1	Rainbow trout Ct - 2	Rainbow trout Ct - 3	Rainbow trout Result	Rainbow trout Site Result	RETEST Rainbow trout Ct - 1	RETEST Rainbow trout Ct - 2	RETEST Rainbow trout Ct - 3	RETEST Rainbow trout Result	RETEST Rainbow trout Site Result
River Denys	1	1	Mouth	Mid stream, below bridge	45.836	-61.177	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	No Ct	Not detected	
River Denys	1	2	Mouth	Left bank, above bridge	45.836	-61.177	No Ct	No Ct	No Ct	Not detected	Not detected	No Ct	No Ct	No Ct	Not detected	Not detected
River Denys	1	3	Mouth	Left bank, below bridge	45.836	-61.177	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	No Ct	Not detected	
River Denys	2	2	Upstream	Mid stream, below bridge	45.854	-61.216	No Ct	No Ct	No Ct	Not detected						
River Denys	2	4	Upstream	Left bank, below bridge	45.854	-61.216	No Ct	No Ct	No Ct	Not detected	Not detected					Not detected
River Denys	2	5	Upstream	Right bank, below bridge	45.854	-61.216	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	No Ct	Not detected	
River Denys	2	6		BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
Pooles Brook	1	1	Upstream	Right bank, downstream of bridge	45.914	-61.130	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	No Ct	Not detected	
Pooles Brook	1	2	Upstream	Left bank, downstream of bridge	45.914	-61.130	No Ct	No Ct	No Ct	Not detected	Not detected					Not detected
Pooles Brook	1	3	Upstream	Mid stream, downstream of bridge	45.914	-61.130	No Ct	No Ct	No Ct	Not detected						
Pooles Brook	2	2	Mouth	Left bank, downstream of bridge	45.924	-61.129	No Ct	No Ct	36.42	Inconclusive		No Ct	No Ct	No Ct	Not detected	
Pooles Brook	2	3	Mouth	Mid stream, downstream of bridge	45.924	-61.129	No Ct	No Ct	No Ct	Not detected	Inconclusive					Not detected
Pooles Brook	2	4	Mouth	Right bank, downstream of bridge	45.924	-61.129	No Ct	36.31	No Ct	Inconclusive		No Ct	No Ct	No Ct	Not detected	
Skye River	1	1	Mouth	Rep 1 - left bank	45.972	-61.132	33.90	33.36	32.93	Detected						
Skye River	1	2	Mouth	Rep 2 - left bank	45.972	-61.132	33.57	34.22	32.51	Detected	Detected					
Skye River	1	3	Mouth	Rep 3 - left bank	45.972	-61.132	33.57	34.35	33.35	Detected						
Skye River	2	3	Upstream	Left bank, upstream of bridge	46.043	-61.197	No Ct	No Ct	No Ct	Not detected						
Skye River	2	4	Upstream	Mid stream, upstream of bridge	46.043	-61.197	No Ct	No Ct	No Ct	Not detected	Not detected					
Skye River	2	5	Upstream	Right bank, upstream of bridge	46.043	-61.197	No Ct	No Ct	No Ct	Not detected						
Skye River	2	6		BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
Middle River	1	1	Upstream	Right bank, upstream of bridge	46.226	-60.928	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	No Ct	Not detected	
Middle River	1	3	Upstream	Left bank, upstream of bridge	46.226	-60.928	No Ct	No Ct	No Ct	Not detected	Not detected					Not detected
Middle River	1	5	Upstream	Mid stream, upstream of bridge	46.226	-60.928	No Ct	No Ct	No Ct	Not detected						
Middle River	2	1	Mouth	Rep 1 - right bank	46.106	-60.921	34.60	35.46	36.55	Suspected		35.39	34.27	35.75	Suspected	
Middle River	2	2	Mouth	Rep 2 - right bank	46.106	-60.921	34.40	34.14	35.30	Suspected	Suspected	34.39	34.70	35.30	Suspected	Suspected
Middle River	2	3	Mouth	Rep 3 - right bank	46.106	-60.921	36.94	36.32	35.29	Suspected						
Baddeck River	1	1	Mouth	Mid stream	46.124	-60.804	35.40	36.53	No Ct	Suspected		No Ct	No Ct	No Ct	Not detected	
Baddeck River	1	2	Mouth	Left bank, upstream of bridge	46.124	-60.804	33.80	34.41	33.87	Detected	Detected					Detected
Baddeck River	1	5	Mouth	Right bank, upstream of bridge	46.124	-60.804	No Ct	No Ct	No Ct	Not detected						
Baddeck River	1	6	Mouth	BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
Baddeck River	2	3	Upstream	Right bank, upstream of bridge	46.199	-60.723	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	No Ct	Not detected	
Baddeck River	2	4	Upstream	Left bank, upstream of bridge	46.199	-60.723	No Ct	No Ct	No Ct	Not detected	Not detected					Not detected
Baddeck River	2	5	Upstream	Left bank, downstream of bridge	46.199	-60.723	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	No Ct	Not detected	
North River	1	2	Mouth	Mid stream, upstream of bridge	46.308	-60.622	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	36.34	Inconlusive	
North River	1	3	Mouth	Left bank, upstream of bridge	46.308	-60.622	No Ct	No Ct	No Ct	Not detected	Not detected	No Ct	No Ct	No Ct	Not detected	Inconclusive
North River	1	5	Mouth	Right bank, upstream of bridge	46.308	-60.622	No Ct	No Ct	No Ct	Not detected						
North River	1	6	Mouth	BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						

River	River Replicate	Sample Replicate	Replicate Relative Location	Replicate Description	Latitude	Longitude	Rainbow trout Ct - 1	Rainbow trout Ct - 2	Rainbow trout Ct - 3	Rainbow trout Result	Rainbow trout Site Result	RETEST Rainbow trout Ct - 1	RETEST Rainbow trout Ct - 2	RETEST Rainbow trout Ct - 3	RETEST Rainbow trout Result	RETEST Rainbow trout Site Result
North River	2	1	Upstream	Right bank, rep 1	46.316	-60.668	No Ct	No Ct	No Ct	Not detected						
North River	2	2	Upstream	Left bank, rep1	46.316	-60.668	No Ct	No Ct	No Ct	Not detected	Not detected					
North River	2	4	Upstream	Mid stream	46.316	-60.668	No Ct	No Ct	No Ct	Not detected						
Benacadie River	1	1	Upstream	Mid stream, upstream of bridge	45.961	-60.706	No Ct	No Ct	No Ct	Not detected						
Benacadie River	1	2	Upstream	Right bank, upstream of bridge	45.961	-60.706	No Ct	No Ct	No Ct	Not detected	Not detected					
Benacadie River	1	3	Upstream	Left bank, upstream of bridge	45.961	-60.706	No Ct	No Ct	No Ct	Not detected						
Benacadie River	2	3	Mouth	Right bank, downstream of bridge	45.929	-60.727	No Ct	No Ct	No Ct	Not detected						
Benacadie River	2	4	Mouth	Left bank, downstream of bridge	45.929	-60.727	No Ct	No Ct	No Ct	Not detected	Not detected					
Benacadie River	2	5	Mouth	Right bank, downstream of bridge	45.929	-60.727	No Ct	No Ct	No Ct	Not detected						
Indian Brook	1	1		Rep 1	45.948	-60.603	35.64	34.20	34.59	Suspected						
Indian Brook	1	2		Rep 2	45.948	-60.603	35.44	No Ct	No Ct	Inconclusive	Suspected	36.80	No Ct	35.31	Suspected	Suspected
Indian Brook	1	3		Rep 3	45.948	-60.603	35.44	No Ct	35.36	Suspected		No Ct	35.22	No Ct	Inconclusive	
Gillis Brook	1	2	Mouth	Mid stream, upstream of bridge	46.026	-60.383	35.57	35.54	34.57	Suspected						
Gillis Brook	1	3	Mouth	Right bank, upstream of bridge	46.026	-60.383	No Ct	36.45	No Ct	Inconclusive	Suspected	36.80	No Ct	35.31	Suspected	Suspected
Gillis Brook	1	5	Mouth	Left bank, upstream of bridge	46.026	-60.383	No Ct	No Ct	35.45	Inconclusive		No Ct	35.22	No Ct	Inconclusive	
Gillis Brook	2	1	Upstream	Mid stream	46.052	-60.393	No Ct	No Ct	No Ct	Not detected						
Gillis Brook	2	2	Upstream	Left bank, rep 1	46.052	-60.393	No Ct	No Ct	No Ct	Not detected	Not detected					Not detected
Gillis Brook	2	5	Upstream	Right bank, rep 1	46.052	-60.393	No Ct	No Ct	No Ct	Not detected						
Breac Brook	1	1	Upstream	Mid stream, downstream of bridge	45.913	-60.513	No Ct	No Ct	34.64	Inconclusive		33.76	No Ct	No Ct	Inconclusive	
Breac Brook	1	2	Upstream	Right bank, downstream of bridge	45.913	-60.513	35.46	No Ct	34.83	Suspected	Suspected	36.32	35.54	35.26	Suspected	Suspected
Breac Brook	1	3	Upstream	Left bank, downstream of bridge	45.913	-60.513	34.96	35.67	35.45	Suspected						
Breac Brook				BLANK - distilled water	NA	NA	No Ct	No Ct	No Ct	Not detected						
Breac Brook	2	3	Mouth	Left bank, downstream of bridge	45.914	-60.529	36.28	37.21	No Ct	Suspected		34.70	No Ct	35.39	Suspected	
Breac Brook	2	4	Mouth	Right bank, downstream of bridge	45.914	-60.529	No Ct	36.59	34.55	Suspected	Suspected	No Ct	No Ct	No Ct	Not detected	Suspected
Breac Brook	2	5	Mouth	Mid stream, downstream of bridge	45.914	-60.529	No Ct	35.25	No Ct	Inconclusive		No Ct	35.30	34.78	Suspected	
Toms Brook	1	2		Left bank, downstream of bridge	45.740	-60.728	No Ct	No Ct	No Ct	Not detected						
Toms Brook	1	3		Mid stream, downstream of bridge	45.740	-60.728	No Ct	No Ct	No Ct	Not detected	Not detected	No Ct	No Ct	No Ct	Not detected	Not detected
Toms Brook	1	4		Right bank, downstream of bridge	45.740	-60.728	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	No Ct	Not detected	
Salmon River	1	1		Samples taken around bridge	45.680	-60.781	No Ct	No Ct	No Ct	Not detected						
Salmon River	1	2		Samples taken around bridge	45.680	-60.781	No Ct	No Ct	No Ct	Not detected	Not detected					
Salmon River	1	3		Samples taken around bridge	45.680	-60.781	No Ct	No Ct	No Ct	Not detected						
River Tillard	1	2	Mouth	Left bank, upstream of bridge	45.658	-60.913	No Ct	No Ct	No Ct	Not detected						
River Tillard	1	4	Mouth	Mid stream, upstream of bridge	45.658	-60.913	No Ct	No Ct	No Ct	Not detected	Not detected	No Ct	No Ct	No Ct	Not detected	Not detected
River Tillard	1	5	Mouth	Right bank, upstream of bridge	45.658	-60.913	No Ct	No Ct	No Ct	Not detected						
River Tillard	2	1	Upstream	Right bank, upstream of bridge	45.665	-60.983	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	No Ct	Not detected	
River Tillard	2	2	Upstream	Left bank, upstream of bridge	45.665	-60.983	No Ct	No Ct	No Ct	Not detected	Not detected	No Ct	No Ct	No Ct	Not detected	Not detected
River Tillard	2	3	Upstream	Left bank, downstream of bridge	45.665	-60.983	No Ct	No Ct	No Ct	Not detected		No Ct	No Ct	No Ct	Not detected	

River	River Replicate	Sample Replicate	Replicate Relative Location	Replicate Description	Latitude	Longitude	Rainbow trout Ct - 1	Rainbow trout Ct - 2	Rainbow trout Ct - 3	Rainbow trout Result	Rainbow trout Site Result	RETEST Rainbow trout Ct - 1	RETEST Rainbow trout Ct - 2	RETEST Rainbow trout Ct - 3	RETEST Rainbow trout Result	RETEST Rainbow trout Site Result
Black River	1	1	Upstream	Right bank, downstream of bridge	45.664	-61.095	No Ct	No Ct	No Ct	Not detected						
Black River	1	2	Upstream	Left bank, upstream of bridge	45.664	-61.095	No Ct	No Ct	No Ct	Not detected	Not detected					
Black River	1	3	Upstream	Left bank, downstream of bridge	45.664	-61.095	No Ct	No Ct	No Ct	Not detected						
Black River	2	1	Mouth	Black River side - rep 1	45.695	-61.102	No Ct	No Ct	No Ct	Not detected						
Black River	2	2	Mouth	Black River Side - rep 2	45.695	-61.102	No Ct	No Ct	No Ct	Not detected	Not detected					
Black River	2	3	Mouth	Ballams Pond - rep 1	45.695	-61.102	No Ct	No Ct	No Ct	Not detected						
Framboise				BLANK - distilled water	45.734	-60.384	No Ct	No Ct	No Ct	Not detected						
Framboise	1	1	Mouth		45.734	-60.384	No Ct	No Ct	No Ct	Not detected						
Framboise	1	2	Mouth	Mid stream	45.734	-60.384	No Ct	No Ct	No Ct	Not detected	Not detected					
Framboise	1	3	Mouth		45.734	-60.384	No Ct	No Ct	No Ct	Not detected						
Framboise	2	1	Upstream		45.740	-60.399	No Ct	No Ct	No Ct	Not detected						
Framboise	2	2	Upstream	Mid stream	45.740	-60.399	No Ct	No Ct	No Ct	Not detected	Not detected					
Framboise	2	3	Upstream		45.740	-60.399	No Ct	No Ct	No Ct	Not detected						