The genetic population structure of Brown Trout (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*) in New Brunswick river systems (New Brunswick, Canada)

by

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ABSTRACT

Brown Trout (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*), native to Europe and western North America respectively, now populate New Brunswick rivers after their introduction in the 1900s. Studying the population genetics of these salmonids will help to understand how these introductions took place in New Brunswick and help inform management practices. The objective of this study was to analyze the mitochondrial DNA control region of Brown and Rainbow Trout to determine the genetic structuring of these species. One Brown Trout population revealed substantial genetic structuring; however, low genetic variation in other Brown Trout populations left any interpopulation reproductive interactions unresolved. Rainbow Trout genetic structure supported panmixia, besides two populations above a man-made barrier that displayed significant structuring. This study is the first to explore the population genetics of Brown and Rainbow Trout in New Brunswick, providing a foundation for future work on the genetic population structure of these species.

ACKNOWLEDGEMENTS

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STATEMENT OF RESEARCH CONTRIBUTION

This project began in the year 2015, when sampling collection efforts by Mark Gautreau and Lab followed up until 2018. These samples were sent to the CRI Genomics Lab at the University of New Brunswick Saint John. I had no participation in field sampling efforts. Beginning in the summer months of July to August 2019, I, along with colleagues of the CRI Genomics lab, performed all the laboratory processing of samples, including but not limited to sample digestion, DNA isolation and amplification. Sanger sequencing techniques were carried out by Genome Québec Innovation Centre at McGill University in Montreal, Québec in August 2019. Further sequence processing was performed by me in August of 2019. From September to December, I, along with guiding words of my supervisor, Dr. Scott Pavey, performed the data analysis and interpretation of results.

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List of Symbols, Nomenclature or Abbreviations

BB: Belleisle Bay

BEC: Becaguimec Stream

bp: Base pair

DNA: Deoxyribonucleic acid

F_{ST}: Fixation index

GIB: Gibson Brook

LR: Little River

MAC: Mactaquac

MED: Meduxnekeag River

MIS: Mispec River

mtDNA: Mitochondrial deoxyribonucleic acid

MUN: Muniac Stream

PCR: Polymerase chain reaction

SAL: Salmon River

SHI: Shiktehawk Stream

SNP: Single nucleotide polymorphism

1. Introduction

1.1 Brown Trout and Rainbow Trout naturalization

Brown Trout (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*) are cold-water salmonid species that have been introduced to waters on all continents except Antarctica. Brown Trout, native to Europe (Elliott, 1989), and Rainbow Trout, native to the rivers that drain to the Pacific Ocean (McCusker et al., 2000), are an important recreational and commercial resource across their native range. The value of these fish in the recreational and commercial fisheries has led to their widespread artificial introduction into suitable environments globally (Welcomme, 1992). Through means of artificial propagation and stocking programs, Brown and Rainbow Trout now populate many waters across the world and have negatively impacted native fish species (Gupta & Everard, 2019; Kitano, 2004).

Brown Trout were first introduced and stocked in New Brunswick for the recreational fishery in the year 1920. Stocking continued across most of the Province until the year 1980 (M. Gautreau, personal communication, October 18, 2019). As a result of artificial introductions, Brown Trout have developed self-sustaining populations in New Brunswick, a process known as naturalization. The Little River and Mispec River, which both drain into the Bay of Fundy near the city of Saint John, are known to be inhabited by Brown Trout (M. Gautreau, personal communication, October 18, 2019). The Belleisle Bay, a lacustrine section of the Saint John River Basin near the city of Saint John, also drains to the Bay of Fundy and has recently been recognized by the Department of Fisheries and Oceans to be populated by Brown Trout (M. Gautreau, personal communication, October 18, 2019). The Meduxnekeag River, which drains to the Saint John River above the Mactaquac Dam near the town of Woodstock, is also populated

by Brown Trout but is isolated from the rivers previously mentioned by the Mactaquac Dam (M. Gautreau, personal communication, October 18, 2019).

In the year 1900, Rainbow Trout were introduced and stocked in New Brunswick rivers for the first time. In the 1970s, stocking of Rainbow Trout ceased as New Brunswick began to focus more on the aquaculture of this species (Carr & Felice, 2006; Chadwick, 2015). As a direct result of stocking efforts, Rainbow Trout inevitably developed populations in New Brunswick, yet these populations were restricted to the south-eastern end of the province (Carr & Felice, 2006). More recently, it has been recognized that Rainbow Trout have expanded their population range in the Province due to accidental introduction in the Saint John River (Carr & Felice, 2006; Chadwick, 2015). In 2004, a large flood of Whitemarsh Creek near the town of Florenceville-Bristol had caused the release of an estimated one-hundred-thousand Rainbow Trout and Brook Trout from a nearby aquaculture facility (Carr & Felice, 2006; Chadwick, 2015). The escaped Rainbow Trout were suspected to disperse through the Saint John River, to which Whitemarsh Creek drains. As a result, Rainbow Trout are self-sustaining in the Saint John River (Carr & Felice, 2006; Chadwick, 2015). Rainbow Trout populations established by the aquaculture escapees are currently confined to the portion of the Saint John River Basin down-river to the Grand Falls Dam and up-river to the Mactaquac Dam. The only known barrier to Rainbow Trout movement along this portion of the Saint John River is the Beechwood Dam located up-river to the mouth of Whitemarsh Creek. This dam, however, has a fishway that allows Rainbow Trout movement but likely restricts it to some degree.

The naturalization and range expansion of Brown Trout and Rainbow Trout in the Province, while desired by anglers, is nevertheless a threat to the native fish species of New Brunswick. Studies documenting the adverse effects that Brown and Rainbow Trout invasion can

have on native fish populations is a call for concern (Gupta & Everard, 2019; Kitano, 2004). Most importantly in New Brunswick, Brown and Rainbow Trout represent a threat towards native salmonids, such as the Brook Trout and Atlantic Salmon (Chadwick, 2015; van Zwol et al., 2012). Brown and Rainbow Trout can be more aggressive in comparison to Brook Trout and Atlantic Salmon when ecological niches overlap (Fausch & White, 1981; Houde et al., 2016). The competition that these species share for habitat and food resources could lead to negative effects on native salmonid populations (Chadwick, 2015; Fausch & White, 1981; Houde et al., 2015; van Zwol et al., 2012). A risk assessment for the introduction of Rainbow Trout in New Brunswick has concluded that through competition for habitat, food resources and predation pressure, native salmonids are expected to suffer a moderate impact (Chadwick, 2015).

Measures to protect native salmonid species from competitive interactions with non-native salmonids is valued in New Brunswick. Brook Trout are New Brunswick's most angled fish along the Saint John River Basin (Chadwick, 2015; Kidd et al., 2011), and Atlantic Salmon are classified as an endangered species (Kidd et al., 2011). Revisions to the New Brunswick Rainbow Trout aquaculture policy have recently arisen (Province of New Brunswick, 2016) to reduce the risk of further release and range expansion of Rainbow Trout in New Brunswick (Chadwick, 2015). Further measures for both Brown and Rainbow Trout population management are thus desirable to help protect the native fish populations that inhabit New Brunswick rivers.

A common tool in fish population management, which helps in assessing and determining the presence of distinct population units, is the genetic population structure (Laikre et al., 2005). Species are generally not genetically homogenous, but rather structure into variably isolated groups of individuals, referred to as populations (Laikre et al., 2005). The limited exchange of genetic material between two populations that are reproductively isolated from one

another, and the subsequent genetic drift, results in genetic differences between them (Laikre et al., 2005). This resulting pattern of genetic differences is known as the genetic population structure. My study will provide an understanding of the genetic population structure of Brown Trout and Rainbow Trout in New Brunswick river systems and develop a foundation for informing population management of these species. Because this is the first genetic population assessment of Brown Trout and Rainbow Trout in New Brunswick, this study will also provide a baseline of these species population genetics that can be built upon by further studies.

1.2 Using mitochondrial DNA to assess Brown Trout and Rainbow Trout genetic structure

Assessing genetic structure requires the use of a genetic marker, which is a location in deoxyribonucleic acid (DNA) that can be used to compare population genetic variation (Gyllensten, 1985). A genetic marker successfully used to delineate the population genetics of both Brown Trout and Rainbow Trout is the mitochondrial DNA (mtDNA) control region. Studies using the mtDNA control region have been able to detect isolated Brown Trout (Aurelle & Berrebi, 2001; Simonović et al., 2017) and Rainbow Trout (Colihueque et al., 2019) stocks, the change in Brown Trout genetic structure after stocking events (Kohout et al., 2012), as well as the genetic structure and likely genetic origin and of naturalized Rainbow Trout populations (Colihueque et al., 2019), to name a few. In this study, I chose to use the mtDNA control region for the following reasons. First, the mtDNA is a haploid, maternally inherited genome (Brown, 2008) that is passed down from mother to offspring from generation to generation. This makes the mtDNA a conservative marker that is unaltered by any recombination that occurs in the bi-parentally inherited nuclear genome. Second, the control region of the mtDNA has the highest mutation rate of the mitochondrial genome (Brown, 2008). This increases the possibility of

detecting multiple genetic lineages within and between populations of the same species for genetic comparison. It should be noted, however, that the number of genetic lineages detected in Brown and Rainbow Trout populations in my study will depend to some extent on stocking practices. Lastly, the control region is considered to be selectively neutral in most cases (Brown, 2008), therefore, natural selection is assumed to not act as a factor that will influence control region variation in populations of Brown and Rainbow Trout. For these reasons, the mtDNA control region is an appropriate genetic marker to assess the genetic structure of Brown Trout and Rainbow Trout populations in New Brunswick river systems.

1.3 The processes influencing Brown Trout and Rainbow Trout genetic structure

There are four factors that can potentially influence genetic patterns in natural populations: gene flow, selection, genetic drift, and mutation. In relation to this study, using the selectively neutral mtDNA control region (Brown, 2008), it is assumed that selection will not influence haplotype variation within and between populations. Mutation can also be excluded as Brown and Rainbow Trout populations had just established in the province within the last century (Carr & Felice, 2006; Chadwick, 2015), and mutation is a process that affects haplotype variation at a slow rate (approximately 1 base pair [bp] mutation per 100,000 generations; Froufe et al., 2003). Therefore, gene flow and genetic drift are assumed to be the factors that will influence genetic variation in populations of Brown and Rainbow Trout in this study.

Gene flow is the sharing of genetic material between populations by means of migration that will maintain genetic similarity between populations. Studies assessing Brown Trout and Rainbow Trout population genetics have elicited the effects that gene flow, or lack thereof, can have on the genetic structure of these species. Even on a microgeographic scale, significant

genetic structuring has been described in Brown Trout (Estoup et al., 1998; Stelkens et al., 2012) and Rainbow Trout (Deiner et al., 2007) populations because of geographical barriers to gene flow and possibly ecological factors that modulate migration behaviours in these fish (Sanz et al., 2019). Because the extent to which gene flow occurs depends at least in part on geographical factors (Berrebi et al., 2019; Deiner et al., 2007; Sanz et al., 2019), a common practice is to map the genetic patterns of populations to help identify barriers to gene flow that may be reproductively isolating populations (Carlsson et al., 1999; Deiner et al., 2007; Stelkens et al., 2012). Physical barriers to migration, such as man-made dams or drainage partitioning, will decrease gene flow and genetic similarity between populations of Brown and Rainbow Trout (Carlsson et al., 1999; Deiner et al., 2007; Stelkens et al., 2012). However, findings on the effects of physical barriers appear to be variable and relative to individual watersheds. Gene flow may be influenced by multiple factors that contribute towards a complex and unpredictable genetic structure for Brown and Rainbow Trout (Sanz et al., 2019). Nevertheless, the unpredictability of gene flow between populations of Brown and Rainbow Trout creates the need for a genetic population structure analysis.

The other factor to consider, genetic drift, is a process with an opposing effect to gene flow. Genetic drift is the random fluctuation and eventual fixation of alleles within a population and can be perceived as the random sampling of individuals in a population that contribute towards the next generation of offspring (Vera et al., 2019). Genetic drift, in the absence of gene flow, will decrease the genetic similarity between populations (Carlsson et al., 1999; Deiner et al., 2007; Vera et al., 2019). Drift occurs at a rate that is inversely proportional to the effective population size, which in the mtDNA is one-quarter that of the nuclear genome (Hurst & Jiggins, 2005). Therefore, genetic drift will heavily influence genetic variation in small populations

(Deiner et al., 2007). The forces of gene flow and genetic drift, despite influencing genetic variation in different manners, will nevertheless influence the haplotype variation in populations of Brown and Rainbow Trout in New Brunswick river systems.

1.4 Objective

This study aims to assess the population genetics of both Brown and Rainbow Trout across their known or suspected population range in New Brunswick. The objective of this study is to analyze the mtDNA control region of Brown Trout and Rainbow Trout to determine the genetic population structure of these species inhabiting the rivers that drain into the Saint John River and surrounding rivers. This will allow me to determine whether gene flow is occurring among populations of Brown or Rainbow Trout. Genetic impacts of physical barriers, such as dams, will also be determined through geographical mapping of genetic population structure. Furthermore, this study will be the first to assess the population genetics of Brown and Rainbow Trout in New Brunswick, providing a foundation for further work that is crucial for the management of these species.

2. Methods

2.1 Sample Sites

Sample collection and site selection was led by Mark Gautreau. A total of 205 Brown Trout samples were collected through means of electrofishing in the years 2016-2018, with sampling sites (Figure 1) comprising the known population range of Brown Trout in the Province. Fin clips were taken and placed in 95% ethanol for storage and preservation. Sampled Brown Trout populations, grouped for genetic structure analysis, include the Meduxnekeag River (MED, n = 39), Belleisle Bay (BB, n = 101), Little River (LR, n = 31), and Mispec River (MIS, n = 34) populations (Figure 1).

A total of 137 Rainbow Trout were collected by means of electrofishing from the years 2015-2017. Sampling sites (Figure 2) mirrored the suspected population range after the recent (2004) accidental release and introduction of Rainbow Trout into the Saint John River from an aquaculture facility near Whitemarsh Creek. Fin clips were taken from the collected fish and placed in 95% ethanol for storage and sample preservation. Populations of Rainbow Trout were grouped based on proximity and drainage distance for genetic structure analysis and include the Salmon River (SAL, n = 35), Little River (LR, n = 10), Muniac Stream (MUN, n = 16), Shiktehawk Stream (SHI, n = 40), Becaguimec Stream (BEC, n = 14), Meduxnekeag River (MED, n = 8), Gibson Brook (GIB, n = 6), and Mactaquac (MAC, n = 8) populations (Figure 2).

2.2 Laboratory processing

Whole genomic DNA was isolated from Brown and Rainbow Trout fin tissue using the nexttec Genomic DNA Isolation Kit from Fish Tissue, following the protocol outlined by the

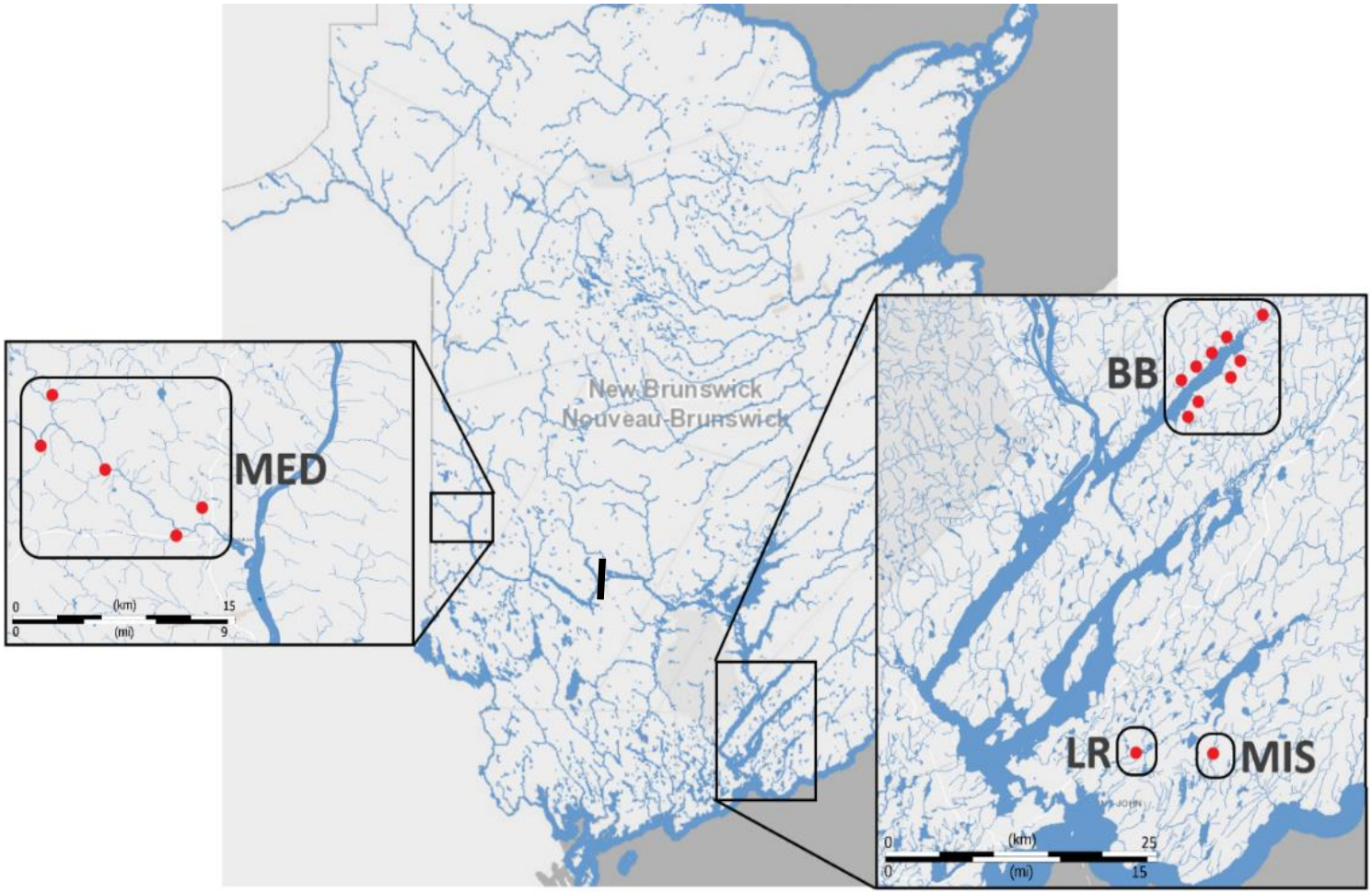

Figure 1 – Map showing Brown Trout sample sites, represented by red dots, and groupings of populations for genetic structure analysis, represented by rounded squares/rectangles. The black dash represents the location of the Mactaquac Dam, which isolates the Meduxnekeag River from the remaining populations. Populations are grouped based on proximity and drainage distance and include the Meduxnekeag River (MED, n = 39), Belleisle Bay (BB, n = 101), Little River (LR, n = 31), and Mispec River (MIS, n = 34) populations.

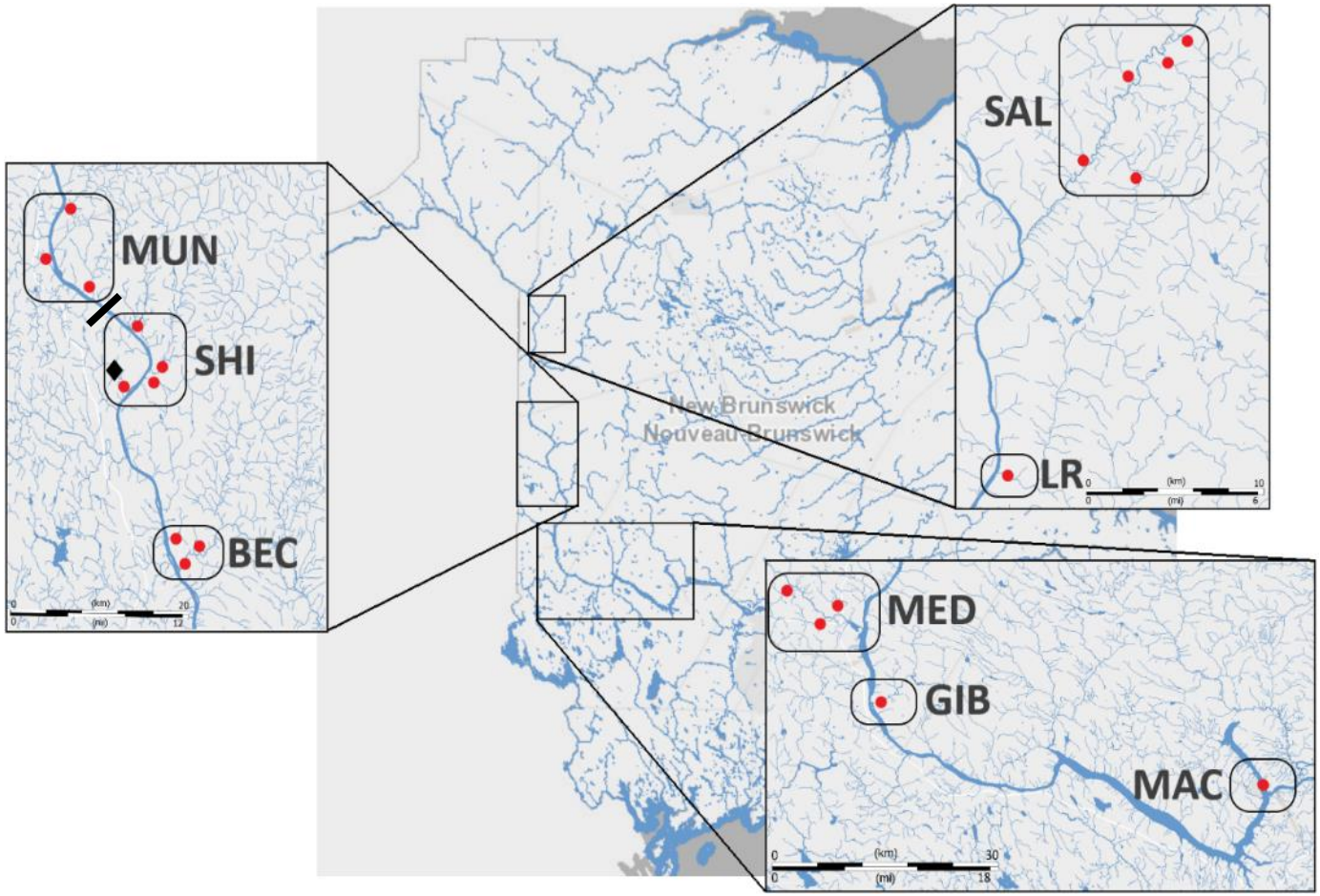

Figure 2 – Map showing Rainbow Trout sample sites, represented by red dots, and groupings of populations for genetic structure analysis, represented by rounded squares/rectangles. The black diamond represents the location of Whitemarsh Creek and the black dash represents the location of the Beechwood Dam, which has a fishway for fish passage. Each population is grouped based on proximity and drainage distance and includes the Salmon River (SAL, n = 35), Little River (LR, n = 10), Muniac Stream (MUN, n = 16), Shiktehawk Stream (SHI, n = 40), Becaguimec Stream (BEC, n = 14), Meduxnekeag River (MED, n = 8), Gibson Brook (GIB, n = 6), and Mactaquac (MAC, n = 8) populations.

manufacturer. Amplification of the Brown Trout mtDNA control region, which is around 1000 bp in length, was performed using control region specific primers (Bernatchez & Danzmann, 1993):

HN20 5' – GTGTTATGCTTTAGTTAAGC – 3'
LN20 5' – ACCACTAGCACCCAAAGCTA – 3'

The entire Rainbow Trout mtDNA control region, also 1000 bp in length, was amplified using Rainbow Trout control region specific primers (Khalaf et al., 2014):

Om F 5' – CCCAAAGCTAAGATTCTAAAT – 3'
Om R 5' – GCTTTAGTTAAGCTACGT – 3'

Polymerase chain reaction (PCR) amplifications were performed in an Agilent SureCycler 8800 thermal cycler with a 25 µL reaction volume. Brown Trout reaction volumes contained 3 µL template DNA, 1X PCR Buffer, 2 mM $MgSO_4$, 0.2 mM dNTPs, 2 µM of each primer, and 1U of Taq DNA polymerase (*Bio Basic*). Rainbow Trout reaction volumes contained 1.5 µL template DNA, 1X PCR Buffer, 2 mM $MgSO_4$, 0.2 mM dNTPs, 2 µM of each primer, and 1U of Taq DNA polymerase (*Bio Basic*). Thermal cycling conditions for PCR amplification of Brown and Rainbow Trout mtDNA control regions were according to previous studies (Bernatchez et al., 1992; Khalaf et al., 2014). Aliquots of the amplified DNA fragments were run on a 1% agarose gel to verify amplification efficiency and specificity. The amplified products were then sent to the Genome Québec Innovation Centre at McGill University in Montreal, Québec for Sanger sequencing, which determined the bp sequence of each sample's mtDNA control region.

2.3 Sequence and genetic structure analysis

Sequence trimming and analyses were carried out using the software Molecular Evolutionary Genetics Analysis (MEGA X; Kumar et al., 2018). Any sequences containing base

calls which were low quality or ambiguous in nature were trimmed manually to obtain a shortened but higher quality sequence for haplotype identity and genetic structure analysis. After manual trimming, Brown Trout control region sequences in a length of 492 bp, and Rainbow Trout control region sequences in a length of 503 bp, were grouped into their corresponding sample population (Figure 1; Figure 2) for genetic structure analysis. Genetic structuring of Brown Trout and Rainbow Trout populations was assessed using Arlequin 3.5.2.2 (Excoffier & Lischer, 2010). To measure population differentiation due to genetic structure, Arlequinn 3.5.2.2 computed population pairwise fixation indexes (F_{ST}). F_{ST} values range from 0 to 1 and can give insight into the population genetics of any two populations. At one extreme, an F_{ST} value of 0, which would result in the case of identical haplotype structure between two populations, implies complete panmixia; the two populations are sharing gene flow freely and can be considered a single breeding population. At the other extreme, an F_{ST} of 1 would result in the absence of shared haplotype structure. This can only be explained by the absence of gene flow and the subsequent genetic drift causing the divergence in haplotype diversity between two populations. If an F_{ST} of 1 is observed, it can be concluded that the two populations in question are distinct breeding populations (Figure 3).

To test whether F_{ST} values in this study indicate significant differentiation in haplotype structure in populations of Brown or Rainbow Trout, haplotypes were permuted 1000 times between the two populations, and an F_{ST} value was calculated per permutation to construct a null distribution. The F_{ST} value observed was then tested for significance against the null distribution at $\alpha = 0.05$. Haplotype frequency maps were constructed to compare the genetic population structure against a geographical landscape to ascertain whether genetic structuring may be explained by geographical factors, such as the proximity of two populations from one another or

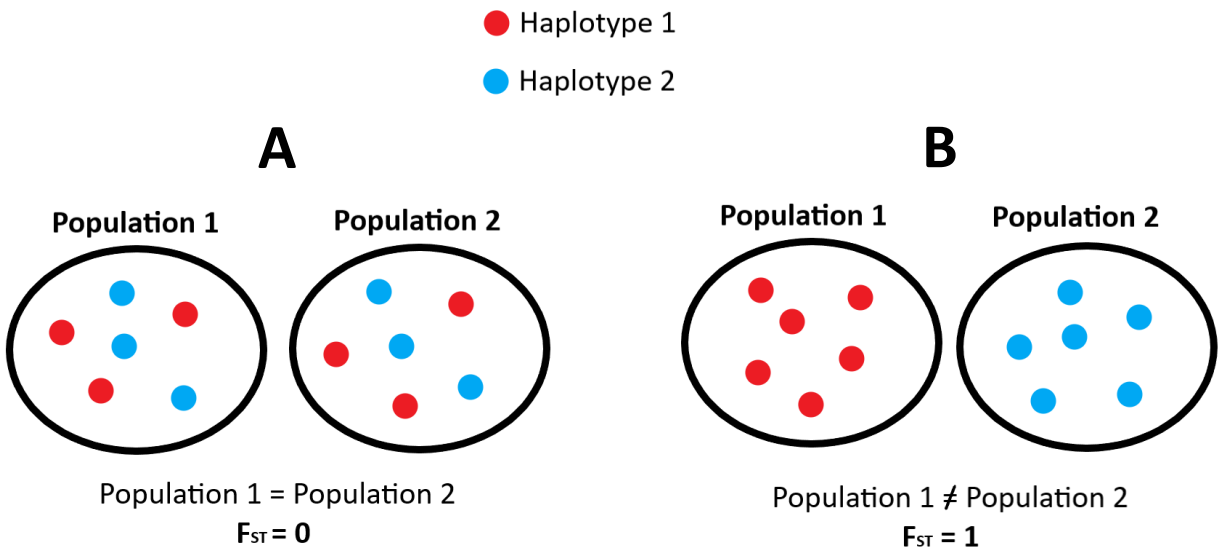

Figure 3 – A simplified illustration of genetic structure and the meaning of an F_{ST} statistic. In the scenario of identical frequencies of haplotypes between two populations (scenario A), F_{ST} will be equal to zero. In the opposite scenario, when haplotypes are not shared between two populations (scenario B), F_{ST} will be equal to one.

geographical barriers (e.g. the Mactaquac or Beechwood Dam).

3. Results

3.1 Sequence results

A total of six haplotypes, referred to here as BRT1, BRT2, BRT3, BRT4, BRT5, and BRT6 were resolved among 205 Brown Trout mtDNA control region sequences. Brown Trout haplotypes were distinguishable by six variable bp positions (Table 1), three of which were transitions and three of which were transversions. Of the 137 Rainbow Trout sequences, a total of four haplotypes categorized as RBT1, RBT2, RBT3, and RBT4 were resolved. Three of the five-hundred and three bp positions in the sequences were variable (Table 1), two of which were transitions and one of which a transversion.

Table 1 – Variable base pair positions among Brown Trout and Rainbow Trout haplotypes. Numbers refer to the variable base pair position and the letters T, G, C and A refer to the nucleotide identified at each base pair position.

Brown Trout

Sequence haplotype	**Variable bp position**					
	45	57	166	167	420	461
BRT1	T	C	A	T	T	T
BRT2	T	T	A	T	T	T
BRT3	T	C	A	T	T	C
BRT4	T	C	G	G	T	T
BRT5	G	C	G	G	T	T
BRT6	G	C	G	G	G	T

Rainbow Trout

Sequence haplotype	**Variable bp position**		
	57	210	290
RBT1	T	T	G
RBT2	A	C	G
RBT3	G	C	G
RBT4	T	T	A

3.2 Haplotype distribution

Brown Trout samples were predominantly comprised of the BRT1 haplotype, which had an overall frequency of 89.3% among samples. BRT1 was the most abundant haplotype in every population except for the Little River, with a frequency of 97.1% to 100% in these populations (Figure 4; Appendix I). BRT4 was the dominant haplotype in the Little River, being found in 45.2% of the samples (Appendix I). BRT1 was the only ubiquitous haplotype in this study; the BRT2 haplotype was unique to the Belleisle Bay, and haplotypes BRT3, BRT4, BRT5, and BRT6 were unique to the Little River (Figure 4), making Little River the most genetically diverse population of Brown Trout with respect to the mtDNA control region. The other two populations, Meduxnekeag River and Mispec River, were fixed for the BRT1 haplotype.

The most abundant haplotypes among Rainbow Trout, RBT1 and RBT2, each comprised 29.2% among Rainbow Trout samples. Haplotypes RBT3 and RBT4 comprised 21.2% and 20.4% of the samples, respectively, with RBT3 being the only haplotype found in every population (Figure 5; Appendix II). While RBT1 and RBT2 were found to be the most abundant haplotypes, the two displayed differing geographical distributions (Figure 5). In populations above the Beechwood Dam (Salmon River, Little River, and Muniac Stream), RBT1 had an overall frequency of 47.5%, whereas RBT2 was only found to be in 9.8% of samples. Notably, the population in this group nearest to the Beechwood Dam (Muniac Stream) was dominated by the RBT1 haplotype at a frequency of 87.5% (Appendix II; Figure 5). In populations below the Beechwood Dam (Shiktehawk Stream, Becaguimec Stream, Meduxnekeag River, Gibson Brook, and Mactaquac), RBT1 only comprised 14.5% of the samples, and RBT2 had an overall frequency of 44.7%.

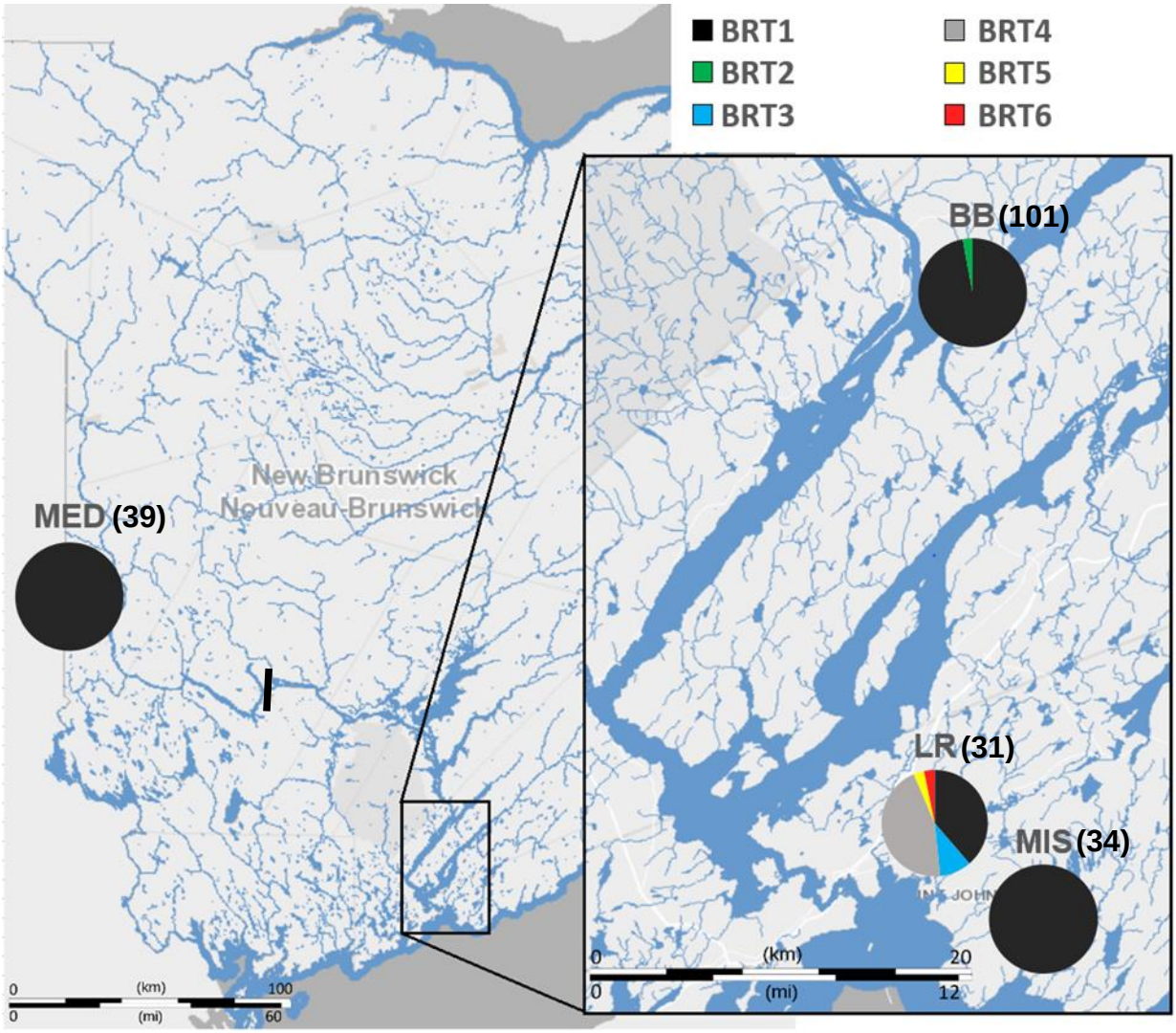

Figure 4 – Map representing the frequencies of haplotypes among Brown Trout populations. The black dash represents the location of the Mactaquac Dam. Population names are abbreviated above pie charts as follows: Meduxnekeag River (MED), Belleisle Bay (BB), Little River (LR), and Mispec River (MIS). Sample sizes are reported in parentheses above pie charts.

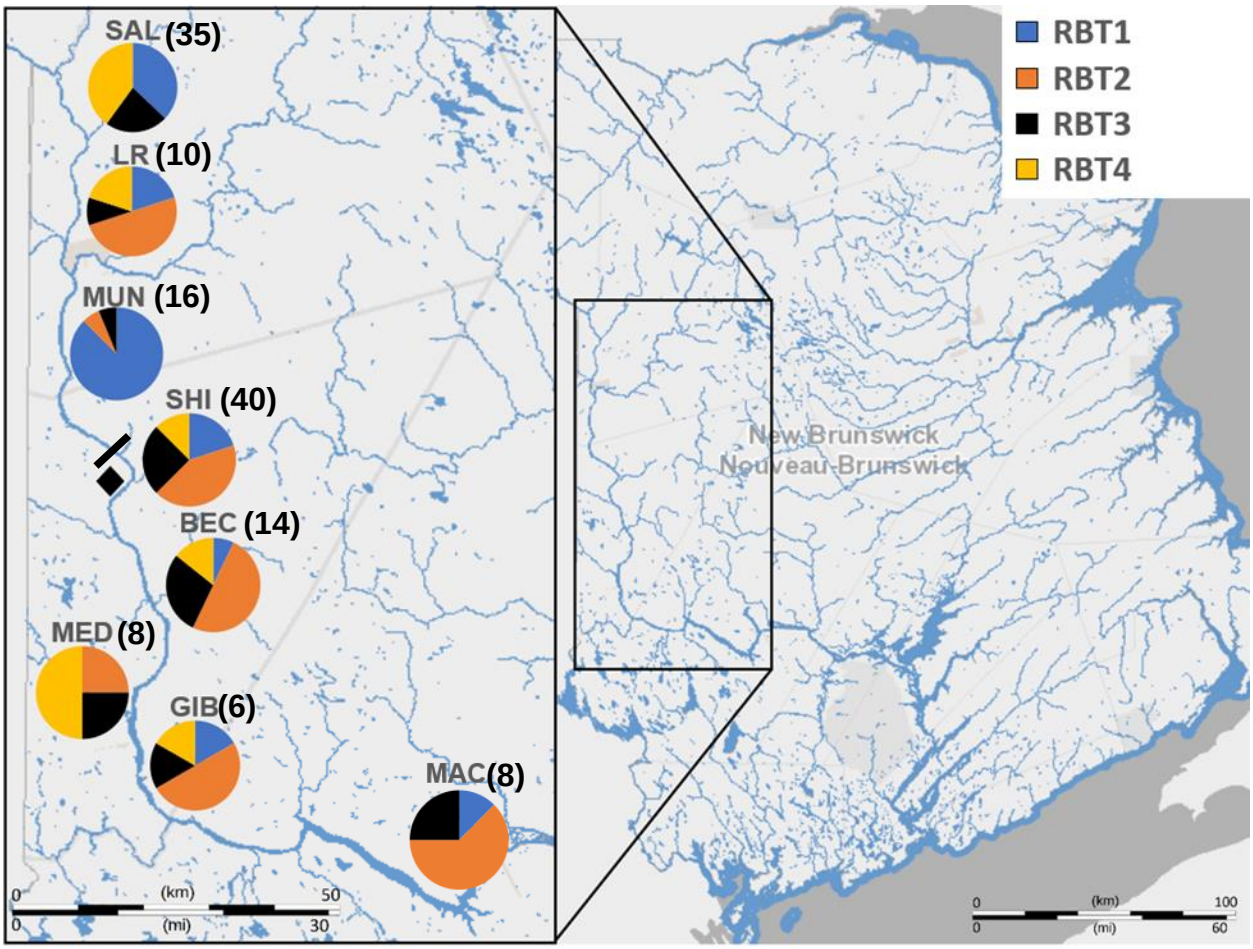

Figure 5 – Map representing the frequencies of haplotypes among Rainbow Trout populations. The black diamond represents the location of Whitemarsh Creek and the black dash represents the Beechwood Dam. Population names are abbreviated above pie charts as follows: Salmon River (SAL), Little River (LR), Muniac Stream (MUN), Shiktehawk Stream (SHI), Becaguimec Stream (BEC), Meduxnekeag River (MED), Gibson Brook (GIB), and Mactaquac (MAC). Sample sizes are reported in parentheses above pie charts.

3.3 Genetic structure

Brown Trout population pairwise F_{ST} analyses (Table 2) revealed limited and non-significant ($P = 0.9910$) structuring of the Meduxnekeag River, Belleisle Bay and Mispec River populations. Little River exhibited significant structuring from all other populations ($F_{ST} \geq 0.4410$; $P < 0.0001$), owing to the diversity of haplotypes of this population (Figure 4). The high F_{ST} values of the Little River supports the notion that Little River differs in its genetic structure in comparison to all other populations. The population genetics of the Meduxnekeag River, Belleisle Bay, and Mispec River, while seemingly panmictic, is difficult to ascertain due to the low genetic variation in these three populations. Nonetheless, the significant structuring of Little River in comparison to all other populations (Table 2) provides support for limited gene flow between Little River and the Meduxnekeag River, Belleisle Bay, and Mispec River populations.

All F_{ST} analyses resulted in non-significant Rainbow Trout population structure, aside from the Salmon River and Muniac Stream populations (Table 3). Salmon River differed significantly in genetic structure ($F_{ST} \geq 0.1288$; $P \leq 0.0270$) in comparison to all populations aside from the Meduxnekeag River ($F_{ST} = 0.0362$; $P = 0.2793$). Both the Salmon River and Meduxnekeag River shared an abundance of the RBT4 haplotype, with Salmon River having a frequency of 40% and Meduxnekeag River having a frequency of 50% (Appendix II). The Muniac Stream differed significantly in genetic structure in comparison to all populations ($F_{ST} \geq 0.1288$; $P \leq 0.0090$), which can be owed to the high frequency of the RBT1 haplotype in this population (Table 3; Figure 5). Interestingly, the Muniac Stream and Shiktehawk Stream populations, which are separated by roughly 10 river kilometers and the Beechwood Dam, exhibited structuring ($F_{ST} = 0.3141$; $P = <0.0001$) despite their proximity (Table 3; Figure 5). The Meduxnekeag River was found to differ moderately in genetic structure with Mactaquac

Table 2 – Population pairwise F_{ST} values between Brown Trout populations. F_{ST} values are reported below the diagonal and *P*-values are reported above the diagonal. Negative F_{ST} values can be interpreted as zero. Populations are abbreviated as follows: Meduxnekeag River (MED), Belleisle Bay (BB), Little River (LR), and Mispec River (MIS).

Populations	MED	BB	LR	MIS
MED	-	0.9910	**<0.0001**	0.9910
BB	-0.0111	-	**<0.0001**	0.9910
LR	0.4612	0.6146	-	**<0.0001**
MIS	0	-0.0133	0.4410	-

Statistically significant *P*-values ($P < 0.05$) are represented in bold.

Table 3 – Population pairwise F_{ST} values between Rainbow Trout populations. F_{ST} values are reported below the diagonal and *P*-values are reported above the diagonal. Negative F_{ST} values can be interpreted as zero. Populations are abbreviated as follows: Salmon River (SAL), Little River (LR), Muniac Stream (MUN), Shiktehawk Stream (SHI), Becaguimec Stream (BEC), Meduxnekeag River (MED), Gibson Brook (GIB), and Mactaquac (MAC).

Populations	SAL	LR	MUN	SHI	BEC	MED	GIB	MAC
SAL	-	**0.0270**	**0.0090**	**<0.0001**	**<0.0001**	0.2793	**0.0090**	**<0.0001**
LR	0.1879	-	**0.0090**	0.7207	0.5045	0.5045	0.9910	0.3423
MUN	0.1288	0.3167	-	**<0.0001**	**<0.0001**	**0.0090**	**<0.0001**	**0.0090**
SHI	0.2510	-0.0439	0.3141	-	0.8018	0.1261	0.9910	0.3874
BEC	0.3245	-0.0351	0.4630	-0.0326	-	0.1081	0.9910	0.8018
MED	0.0362	-0.0251	0.3097	0.0629	0.0831	-	0.4324	0.0631
GIB	0.2140	-0.1454	0.3894	-0.0988	-0.1088	-0.0403	-	0.5586
MAC	0.4355	0.0262	0.6176	0.0004	-0.0667	0.2104	-0.0599	-

Statistically significant *P*-values ($P < 0.05$) are represented in bold.

(F_{ST} = 0.2104), however was determined to be just above the significance threshold (P = 0.0631; Table 3). Nevertheless, all populations aside from Salmon River and Muniac Stream displayed low F_{ST} values (Table 3), supporting that the Little River, Shiktehawk Stream, Becaguimec Stream, Meduxnekeag River, Gibson Brook, and Mactaquac populations share similar genetic structure. The Muniac Stream and Salmon River were shown to have high F_{ST} values, supporting that the Muniac Stream and Salmon River populations differ in their genetic structure from other Rainbow Trout populations.

4. Discussion

Analyses of the population genetics of Brown Trout and Rainbow Trout successfully addressed questions regarding the genetic population structure of these species in New Brunswick. I found that Rainbow Trout populations inhabiting the Saint John River and its tributaries differed non-significantly in genetic structure, aside from two populations located in the northern end of this study's sampling range. Brown Trout populations, aside from the Little River, displayed non-significant structuring as well. The presence of a single dominating haplotype leaves the reproductive interactions of these Brown Trout populations unresolved.

4.1 Brown Trout population genetics

The extremely low genetic variability observed in this study among all Brown Trout populations aside from the Little River (Figure 4; Table 2) contrast with the high genetic variability observed across Brown Trout in their native range. One study in southeast Europe resolved eighteen mtDNA control region haplotypes among three-hundred and ninety-nine individual Brown Trout, with nine out of the eleven populations sampled possessing at least three haplotypes (Simonović et al., 2017). Another study in southeast Europe resolved fifteen mtDNA control region haplotypes among only one-hundred and one individual Brown Trout, with each population possessing at minimum three haplotypes, and maximum ten haplotypes (Marić et al., 2006). It should be noted, however, that this high genetic variability is due to thousands of years of evolution of the mitochondrial genome in native populations of Brown Trout and is non-representative of purely naturalized populations. In the case of Brown Trout inhabiting New Brunswick, the genetic variability of these species depends on the stocking practices during introduction. The low genetic variability in the Meduxnekeag River, Belleisle

Bay, and Mispec River is likely due to the stocking of many fish from a single or few genetic origins. The Little River, which is much more genetically diverse, likely experienced its own founder effect with stocking of fish from multiple genetic origins. Stocking of hatchery reared Brown Trout in native Brown Trout populations has been shown to decrease natural genetic variation (Berrebi et al., 2019; Kohout et al., 2012), thus is a possible explanation for why genetic variability was so low among most Brown Trout populations here. However, unlike Rainbow Trout, the history of Brown Trout stocking is not as well characterized. Consequently, genetic origin is unknown, but the results of my study support that only one or few maternal lineages were used as the major source of Brown Trout stocking in New Brunswick.

Characterizing the degree of gene flow among the Meduxnekeag River, Belleisle Bay, and Mispec River populations in the current study is indeterminable due to the lack of genetic variability. This genetic pattern is likely a result of low stocking diversity, possibly in combination with small effective population sizes and genetic drift. Gene flow to and from the Little River, however, is functionally non-existent as supported by this populations significant genetic structure ($F_{ST} \geq 0.4410$, $P < 0.0001$) and high genetic variability in comparison to other Brown Trout populations (Figure 4). Despite not being able to discriminate gene flow among the Meduxnekeag River, Belleisle Bay and Mispec River populations, it is reasonable to look towards geographical factors that may impact the reproductive interactions among these populations.

Brown Trout across their native range commonly structure into populations due to geographical factors such as isolation by distance or physical barriers (Arslan & Bardakci, 2010; Griffiths et al., 2009; Ősz et al., 2018; Sanz et al., 2019; Vera et al., 2019; Vilas et al., 2010). Physical barriers that restrict Brown Trout migration have been demonstrated to greatly

differentiate genetic structure in the populations isolated by them (Griffiths et al., 2009; Sanz et al., 2019). The Mactaquac Dam, which separates the Meduxnekeag River from the remaining Brown Trout populations, is a physical barrier that is unlikely to support gene flow to and from the Meduxnekeag River. Due to the lack of a fishway and the large fall down the dam that is likely damaging, if not fatal, the Meduxnekeag River is unlikely to share reproductive interactions with populations below the dam.

The Belleisle Bay and Mispec River populations have no known physical barriers isolating them from each other aside from a distance greater than 60 river kilometers. Therefore, gene flow between them is dependent on the migratory behaviours of the fish in these populations. Studies based on direct observations of Brown Trout movement have documented migrations up to 41 kilometers (Höjesjö et al., 2007; R. G. Young et al., 2010). However, it's important to note that these studies have obtained divergent results, and most fish have been documented to move less than 1 kilometer (Höjesjö et al., 2007; Knouft & Spotila, 2002; R. G. Young et al., 2010). Brown Trout have also shown genetic structuring on a microgeographic scale (Estoup et al., 1998), which is proposed to be the result of limited gene flow between populations, partly due to the homing instinct demonstrated by these species (Colihueque et al., 2003). The migration of Brown Trout appear to be regulated by many ecological factors (Sanz et al., 2019), thus, gene flow between the Belleisle Bay and Mispec River, while unlikely due to the distance between them, is difficult to predict given the insufficient genetic data in my study.

To delineate the reproductive interactions among most Brown Trout populations in further studies, I suggest using genetic markers that may lead to a greater resolution of these species' population genetics. Microsatellites may be a candidate marker, as their use in genetic structure assessments of Brown Trout across their native range are well characterized (Arslan &

Bardakci, 2010; Carlsson et al., 1999; Estoup et al., 1998; Vilas et al., 2010). Microsatellites have also been able to discern genetic structure in naturalized Brown Trout populations (Colihueque et al., 2003) with greater resolution than the results presented here. Nevertheless, using different genetic markers to assess the population genetics of Brown Trout in New Brunswick is an area for further work that could establish a greater understanding of the genetic structure of these species.

4.2 Rainbow Trout population genetics

Rainbow Trout populations were shown to differ non-significantly in their haplotype structure, aside from two populations in the more northern end of this study's sampling range (Figure 5; Table 3). These genetic patterns may be explained by those found in a study contrasting the genetic structure of two populations differentially impacted by Rainbow Trout aquaculture (Canales-Aguirre et al., 2018). More specifically, this study assessed the genetics of one watershed frequently impacted by escapes from aquaculture facilities in comparison to another watershed where aquaculture escapes have been reported only once. Canales-Aguirre et al. (2018) found that the populations impacted by frequent escapes from aquaculture facilities showed a lower-level of interpopulation genetic diversity. It is postulated that the high number of individuals released over consecutive aquaculture escapes led to lower interpopulation genetic variability due to reduced genetic drift (Simberloff, 2009). The population which experienced only one aquaculture escape event experienced a higher-level of interpopulation genetic variability (Canales-Aguirre et al., 2018). This is likely due to the escape being characterized by a small number of individuals released from a single source population, followed by further population sub-structuring during population establishment. It is possible that the Rainbow Trout

in my study represent an intermediary form of the two populations studied by Canales-Aguirre et al. (2018). The large number of Rainbow Trout released from the aquaculture facility near Whitemarsh Creek in 2004 would explain the low interpopulation genetic variability (i. e., the non-significant genetic structuring) due to decreased genetic drift. In contrast, the occurrence of only one release event may be the explanation for the structuring exhibited by the Salmon River, Muniac Stream, and to a lesser, non-significant degree, the Meduxnekeag River, that experienced genetic bottlenecks during population establishment.

The differentiated genetic structure of two of the three Rainbow Trout populations above the Beechwood Dam (Figure 2 & Figure 5; Table 3) may be explained by the semi-permeability of this physical barrier to Rainbow Trout gene flow. Insurmountable physical barriers have been shown to significantly differentiate salmonid populations isolated by them (Carlsson et al., 1999; Sanz et al., 2019; Winans et al., 2018) by extinction of gene flow and subsequent genetic drift. Semi-permeable barriers, however, limit gene flow but do not fully obstruct it, leading commonly to founder effects in populations above these barriers (Small et al., 2007). This is highlighted in a study assessing genetic structure of Rainbow Trout above and below man-made fast-flow culverts and waterfalls that allowed limited, but not complete impedance, of upstream movement (Small et al., 2007). More specifically, it was found that Rainbow Trout populations above these semi-permeable barriers possessed lower allelic richness but were non-significantly differentiated in their genetic structure. These findings suggest a founder effect during population establishment above the barrier and limited but significant gene flow across the barrier maintaining similarity in genetic structure (Small et al., 2007).

The findings of Small et al. (2007) may be true for the Rainbow Trout populations above the Beechwood Dam in my study. Specifically, the fishway at the Beechwood Dam may

represent a semi-permeable barrier to Rainbow Trout movement through the Saint John River, partially isolating populations above the dam. During Rainbow Trout release in 2004, limited Rainbow Trout movement through the Beechwood Dam would represent a founder effect, followed by further genetic bottle necks during population establishment. The similar genetic structure of the Little River to populations below the dam (Figure 5; Table 3) is likely the result of maintained gene flow across the dam, and/or a larger effective population size that is resisting genetic drift (Small et al., 2007). The differentiated genetic structure of the Salmon River and Muniac Stream may be the result of genetic bottle necks during population establishment, limited amounts of gene flow to and from these populations, or a smaller effective population size that is experiencing significant genetic drift (Small et al., 2007). Limited amounts of gene flow are certainly supported by significant F_{ST} values (Table 3). Similar to the results presented here, Deiner et al. (2007) found that Rainbow Trout populations above physical barriers displayed significant genetic structuring from one another, likely as a result of small effective population sizes, limited gene flow, and genetic drift. Studies estimating the effective population size of the above dam populations and the permeability of the Beechwood Dam to Rainbow Trout movement would help discriminate the likely reason for the genetic structuring observed in my study.

The similar genetic structure of populations below the Beechwood Dam (Figure 5; Table 3) supports panmixis among these populations. Similar observations have been made in naturalized populations of Rainbow Trout in Chile using the mtDNA control region (Colihueque et al., 2019). More specifically, Rainbow Trout populations within river basins lacking any known physical barriers to movement generally displayed limited structuring, supporting panmixia (Colihueque et al., 2019). However, due to small sample sizes, these findings should be

interpreted with caution (Colihueque et al., 2019). Comparable to my findings, similar genetic structure of Rainbow Trout have been observed in waters impacted by aquaculture escapees (Canales-Aguirre et al., 2018). These findings are likely due to high propagule pressure and large effective population sizes that resist genetic drift, thus maintaining genetic similarity to their source population. The findings presented by Canales-Aguirre et al. (2018) may suggest that the genetic similarity of Rainbow Trout populations in my study may be a result of recent establishment from a single source population and large effective population sizes resisting genetic drift, rather than migration and gene flow maintaining genetic similarity. Migration of Rainbow Trout has been documented to be greater than Brown Trout, yet the average distance of dispersal is only about 2 kilometers (Young et al., 1997), which is much shorter of a distance than that between populations in my study. It is possible that, due to the recent (2004) establishment of Rainbow Trout in the Saint John River and perhaps large effective population sizes, populations below the Beechwood Dam have not been reproductively isolated long enough for genetic drift to cause variations in genetic structure. Determining the effective population sizes of Rainbow Trout in the Saint John River is thus an area of study that could help address gaps in the knowledge of these species' population genetics.

4.3 Potential limitations

Several limitations associated with my study may influence my overall findings and conclusions on the genetic population structure of Brown Trout and Rainbow Trout in New Brunswick river systems. First, a limitation to my project involves the number of samples that were able to be collected during sampling efforts, as well as samples being lost during laboratory work due to unsuccessful DNA extraction, amplification, and/or sequencing. While this

limitation is less pronounced in Brown Trout, some Rainbow Trout populations consisted of small sample sizes (n = 6 in one case) that are unlikely of being representative of the source population. It was possible to group populations together to increase sample size; however, I believe the way I grouped populations for my study was more logical and allowed me to detect population differences on a smaller scale. Another limitation is the incomplete coverage of the mtDNA control region for genetic structure analysis. The control region is known to be approximately 1000 bp in length, however, only a fragment in the length of 492 or 503 bp of high-quality base reads after sequencing and trimming was obtainable. The remaining base pairs that were not analyzed in this study may have led to greater haplotype variation, and thus a greater resolution of Brown Trout and Rainbow Trout population structure. Finally, it is also possible that populations of Brown and Rainbow Trout in my study may be impacted by introductions from anthropogenic sources. Transplanting fish from one area to another or the flooding and subsequent release of fish from hobby or commercial fish ponds may influence my conclusions on the genetic structure of Brown and Rainbow Trout populations.

4.4 Conclusions and further research suggestions

My study is the first to characterize the population genetics of two naturalized species of salmonid in New Brunswick river systems. Interestingly, I found a genetically diverse and reproductively isolated population of Brown Trout among populations exhibiting extremely low genetic variation. Although not commonly observed among Brown Trout in their native range, my results support that Brown Trout stocked in New Brunswick rivers originated from a single or few genetic lineages, aside from one population that was likely stocked from multiple genetic origins. Additionally, I found that even after recent (2004) population development of Rainbow

Trout in the Saint John River, genetic structuring was still detectable up-river to a hydro-electric dam. My results support the findings of other studies that detect little genetic differentiation among populations recently established by aquaculture escapes, but also the impacts of semi-permeable barriers on gene flow and genetic drift in populations of salmonids.

This project is the first to establish a foundation of Brown Trout and Rainbow Trout population genetics in New Brunswick. Since my study is the first to characterize the genetic structure of these species in the province, similar genetic testing should be undertaken to reveal any genetic patterns that my study was unable to ascertain. One such study could be the use of different genetic markers, such as microsatellites or single nucleotide polymorphisms (SNPs) to possibly attain greater resolution into the genetic structure of these salmonids. Another suggestion is the assessment of temporal stability of the population genetics of these species, which may vary in relation to changing environmental parameters such as water level and temperature. If population structure remains stable, the identification of distinct population units will help in informing management of these species. Nevertheless, my study has successfully addressed questions regarding the previously unknown population genetics of Brown Trout and Rainbow Trout in New Brunswick, allowing further research to build upon these findings and help in distinguishing genetic patterns that may arise in the development of naturalized, invasive, or unintentionally released fish populations – a rich area of future research in the field of genetics.

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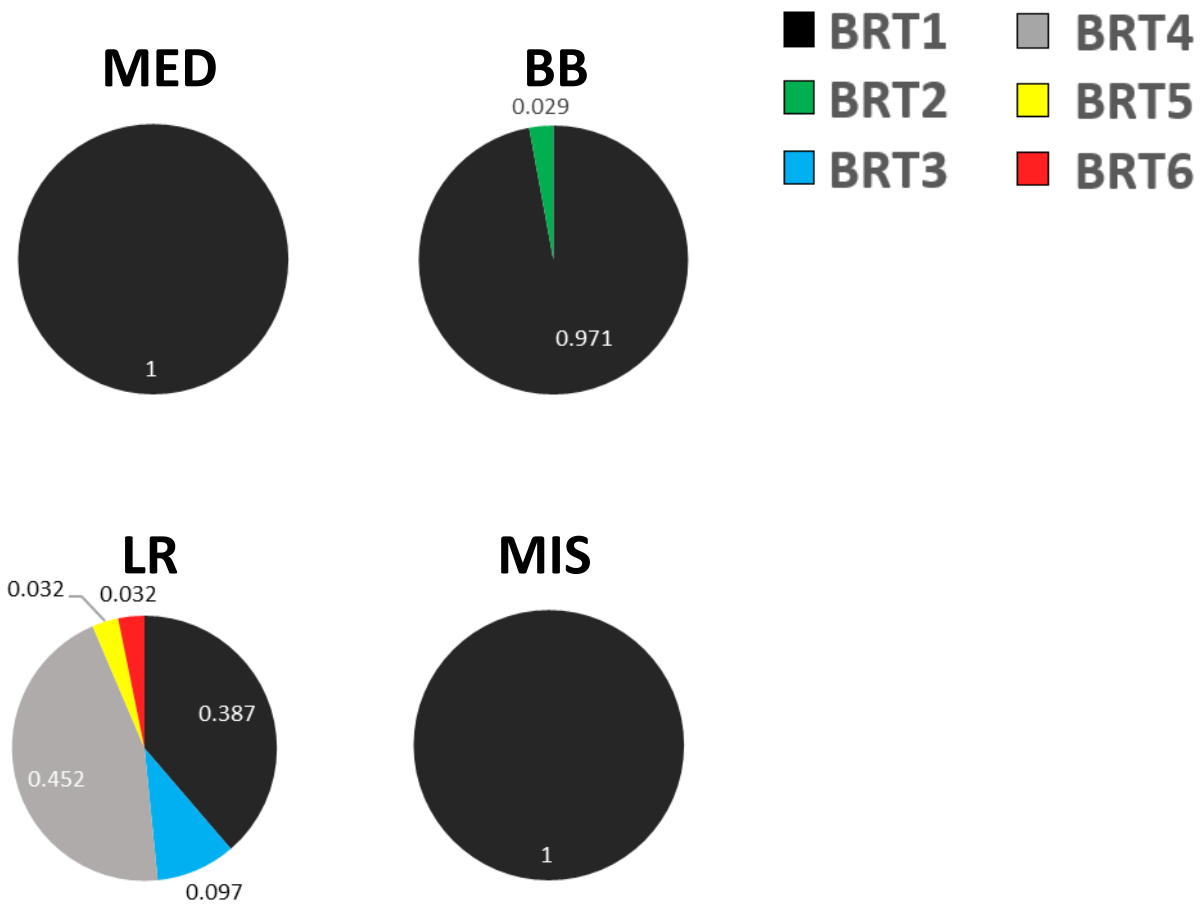
Appendix I

Enlarged pie charts representing the relative frequencies of haplotypes among populations of Brown Trout. Population names are abbreviated as follows: Meduxnekeag River (MED, n = 39), Belleisle Bay (BB, n = 101), Little River (LR, n = 31), and Mispec River (MIS, n = 34).



Appendix II

Enlarged pie charts representing the relative frequencies of haplotypes among populations of Rainbow Trout. Population names are abbreviated above pie charts as follows: Salmon River (SAL, n = 35), Little River (LR, n = 10), Muniac Stream (MUN, n = 16), Shiktehawk Stream (SHI, n = 40), Becaguimec Stream (BEC, n = 14), Meduxnekeag River (MED, n = 8), Gibson Brook (GIB, n = 6), Mactaquac (MAC, n = 8).