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Pre-COSEWIC Review of Anadromous Atlantic Salmon (*Salmo salar*) in Canada, Part 1: Designatable Units

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Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) recognizes 16 designatable units (DUs) of Atlantic Salmon (*Salmo salar*) with 15 of those DUs representing extant anadromous populations. Last assessed by COSEWIC in 2010, this species is currently up for reassessment. As a primary generator and archivist of data related to Atlantic Salmon, Fisheries and Oceans Canada (DFO) is responsible for compiling and reviewing information on the species to help inform the upcoming reassessment. Here, as Part 1 of the pre-COSEWIC review of Atlantic Salmon, we focus on re-evaluating the DU structure. Over the last decade, new genetic and genomic data have become available that can be used to improve our understanding of the DU structure. COSEWIC's definition requires that a DU represents a discrete and evolutionarily significant unit of the species; therefore, we develop a framework using a weight of evidence approach to ensure that all DUs proposed here meet criteria for both discreteness and significance. Our approach incorporates genetic and genomic datasets, as well as life history and climate information. Our approach led to the subdivision of four of the previously defined COSEWIC DUs into multiple units, including the subdivision of Labrador, South Newfoundland, Gaspé-Southern Gulf of St. Lawrence, and Nova Scotia Southern Upland. In addition, based on a weight of evidence, we determined that some DUs required re-evaluations of their boundaries, which led to changes of the previously recognized DU boundaries in Quebec (between Western North Shore and Inner St. Lawrence) and in Newfoundland (between Northwest and Northeast Newfoundland). Re-evaluation of boundaries also supported that southern Gulf populations were not discrete from eastern Cape Breton populations, and thus these populations were combined into a single DU. Further, we identified two populations that belong in adjacent DUs, which would result in non-contiguous boundaries. This included de la Corneille River in Quebec (physically located in Western North Shore DU but groups with Eastern North Shore DU) and Gaspereau River in the Bay of Fundy (physically located in Inner Bay of Fundy DU but groups with Outer Bay of Fundy DU). Overall, using newly available data, we propose that there are 19 DUs of extant anadromous Atlantic Salmon that are supported by evidence of discreteness and significance, and we propose new names and numbering for these 19 putative DUs.

INTRODUCTION

PURPOSE

Under the support of the Species at Risk Act (SARA), the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) is conducting an independent status assessment of anadromous Atlantic Salmon (*Salmo salar*) in Canada. The Atlantic Salmon designatable units (DUs) were last assessed by COSEWIC in 2010, and the status reports for each species are updated every 10 years. As a primary generator and archivist of data related to Atlantic Salmon, Fisheries and Oceans Canada (DFO) is responsible for compiling and reviewing information held by the Department prior to making it available to COSEWIC, in the form of a pre-COSEWIC review. In this document, we review the data that was part of the 2010 assessment, and the updated information on Atlantic Salmon populations that has been collected and produced in the intervening period to evaluate the structure of the DUs. Over the last 10 years, new genetic and genomic data have become available that can be used to improve our current understanding of the discreteness and evolutionary significance of salmon populations in Canada, which is essential to defining appropriate units for conservation.

ATLANTIC SALMON: WILDLIFE SPECIES INFORMATION

The Atlantic Salmon is a ray-finned fish that belongs to the family Salmonidae. Atlantic Salmon has a fusiform body shape and this species shows extensive variability in size-at-maturity, which can range from 10 to 100+ cm. Atlantic Salmon, like other salmonids, are characterized by their diversity in life history strategies, which can result in multiple reproductive and migratory phenotypes within a population. This can include both freshwater resident (Ouananiche or landlocked salmon) and oceanic migrant (anadromous) forms. All Atlantic Salmon forms reproduce in fresh water. The anadromous form is the best-known phenotype and is the only form considered in this report. Therefore, we do not evaluate the extinct, potamodromous Lake Ontario population (Guiry et al. 2016) here, which has been subject to recent reintroduction efforts with source populations originating from outside of Lake Ontario (Dimond and Smitka 2005). In anadromous populations, Atlantic Salmon juveniles can spend one to eight years in fresh water before migrating to the marine environment (North Atlantic Ocean) where they subsequently live for a further one to four years before their first maturation. When returning to fresh water to reproduce, Atlantic Salmon generally show high levels of natal philopatry, resulting in low levels of straying among populations (<10%). Natal philopatry reduces gene flow between populations, allowing for local adaptation and causing Atlantic Salmon populations to be highly genetically structured across multiple spatial scales, with the deepest genetic split occurring between European and North American populations. Due to the low levels of straying, genetic structure is also found within North America, where populations can be differentiated at the regional scale, and in some cases the river scale. Genetic differences within watersheds can also occur, but evidence for this is generally limited. Across the Canadian range of Atlantic Salmon, a total of 16 DUs were recognized by COSEWIC in 2010 (see Figure 1) based on genetic data and broad patterns in life history variation, environmental variables, and geographic separation (COSEWIC 2010). A total of 15 of these 16 DUs represent extant, anadromous populations of Atlantic Salmon. Using newly available data, we re-evaluate the structure of these 15 DUs and based on the weight of evidence, propose revised DUs for the species.

DEFINING DESIGNATABLE UNITS OF ANADROMOUS ATLANTIC SALMON

Definition of Designatable Units

COSEWIC's definition of a DU indicates that the DU should represent a discrete and evolutionarily significant unit of the species. COSEWIC provides various guidelines for how these criteria can be met and these are summarized here.

Discreteness

The populations within the DU should be discrete compared to other populations of the species. Criteria for discreteness can be met based on genetic evidence which can include, but are not limited to, differences associated with heritable traits (i.e., phenology, migration routes, life history) and various genetic markers. Discreteness can also be supported when populations are naturally disjunct from other populations in the species range, which is expected to limit gene flow between these populations. In addition, discreteness can also be inferred when populations occupy different eco-geographic regions that are relevant to the species and reflect historical or genetic differences.

Evolutionary Significance

If criteria for discreteness are met, then the next step is to evaluate evolutionary significance of the unit using multiple types of criteria. Significance can result from either a significant period of isolation that is expected to generate an independent evolutionary history and/or the presence of specific adaptive, heritable traits that may develop over a shorter timeframe. Criteria for significance can be met by showing strong differences in characteristics that reveal deep intraspecific phylogenetic divergence. These can include significant differences in functional genes, genetic-environmental associations, behaviour, or differences in slowly evolving genetic markers. Ecological conditions can also support evidence of significance where a selective regime is likely to have led to DU-wide adaptation. Evidence of significance can also be met if the populations represent the only naturally occurring populations of the species within the native range. In addition, significance can be supported if there is evidence that the loss of the discrete population or populations under consideration would result in a large disjunction in the species' range. These above guidelines help provide support for significance; however, other criteria can also be considered.

Incorporating Genetic and Genomic Data into Designations

Advances in DNA sequencing technology are providing unprecedented amounts of genomic data for non-model species and are directly applicable to both discreteness and significance criteria. However, the use of large-scale genomic data to inform COSEWIC DU structure has been rarely attempted in other species to date. Yet, it is likely that as more genomic data become available, many assessments will begin to incorporate genomic data into their DU analyses. It is thus necessary to carefully consider how genetic and genomic data can be incorporated into the process of DU identification. For Atlantic Salmon, one of the most studied fish species of the world, various genetic and genomic datasets exist that comprise data for many populations in Canada. Here, we review the types of available data and provide clear guidelines for how to incorporate these into a decision framework for evaluating DUs (see Criteria for defining Atlantic Salmon DUs: Decision tree). Each of these datasets has inherent spatial and genomic resolution limitations and these are discussed below. As evidence supporting each criterion can come from a variety of genetic or genomic data types, a weight of evidence approach is used, where each line of evidence for the relevant criterion is evaluated and then we evaluate the full body of evidence together for discreteness and significance.

Microsatellites

Microsatellites generally behave as neutral genetic markers and segregate by Mendelian inheritance patterns. Microsatellites occur throughout the genome and are represented by short tandem repeats of DNA sequences (e.g., ATATATATAT). Generally, microsatellites are characterized by higher mutation rates relative to other genomic regions, thus enabling alleles to evolve rapidly and exhibit high levels of genetic diversity. In addition, because microsatellites are generally non-functional and behave neutrally (i.e., not under selection), genetic drift can lead to differences in the frequency of alleles between populations that are physically or reproductively isolated from one another or between populations that experience low gene flow. However, as 10s of loci are usually examined, the genomic coverage of microsatellite loci is generally low in many studies, often limited to a few markers per chromosome. As such, differences in allele frequencies at microsatellite loci can be used to determine the level of genetic discreteness between the populations. However, given that these markers do not usually influence phenotype and have low genomic coverage, these markers are generally not informative for significance criterion.

For Atlantic Salmon in Canada, there are two microsatellite datasets that are applicable. The first dataset is comprised of 15 microsatellite loci (see Bradbury et al. 2016) that have been genotyped for almost 200 locations in Canada and thus provides high geographic coverage within the recognized salmon DUs (Figure 2A; Appendix Table A1). Locations or sites are often referred to as rivers; however, in some cases multiple tributaries were sampled within some larger river systems. For this dataset, the sample size, location, year of sampling, and life stage sampled are provided in Appendix Table A1. The second dataset includes 101 genome wide microsatellite loci which is described in detail in Bradbury et al. (2018). The geographic coverage for this dataset is low across many regions, but there is high geographic coverage within specific DUs, and thus this dataset can be useful to infer discreteness within some geographic regions. The genomic coverage of this dataset is also low, but on average, this dataset includes 3.4 loci per chromosome (range 1–7 loci) (Bradbury et al. 2018).

Single Nucleotide Polymorphisms

A single nucleotide polymorphism (SNP) is represented by a change in a single base pair (i.e., A, G, C, or T) in the DNA sequence. For example, for a specific position in the genome, a population can be made up of individuals that carry copies of two different nucleotides (alleles), such as individuals that carry copies of the ‘A’ allele and those that carry copies of the ‘T’ allele, as well as individuals that carry a copy of both alleles (heterozygotes). In this case, differences in frequency of A and T alleles between populations can be used to quantify differences between these populations. Given that SNPs are bi-allelic (only two alleles), they provide less information on a per locus basis than microsatellites, but the genomic coverage provided by SNPs is generally greater, as methods enable the genotyping of hundreds to millions of SNPs across the genome. As genomic coverage increases, there is also greater potential that SNPs are located within or close to parts of the genome that directly influence phenotype. Accordingly, SNPs can provide information about both neutral differences between populations, as well as adaptive differences (Barson et al. 2015; Sylvester et al. 2018; Lehnert et al. 2019a; Lehnert et al. 2019b), allowing SNP datasets to be used to infer both discreteness and evolutionary significance.

For Atlantic Salmon in Canada, there are three SNP datasets that are applicable. The first dataset includes 96 SNPs and was developed as a range wide baseline panel for genetic stock identification (GSI) (see Jeffery et al. 2018). These 96 loci were selected to be highly informative for differentiating North American regional groups of Atlantic Salmon (Jeffery et al. 2018; Bradbury et al. 2021). The genomic coverage of this dataset is relatively low, as we would expect an average of 3 loci per chromosome. However, the geographic coverage of this panel is

high, with over 200 locations genotyped in Canada (Figure 2B; Appendix Table A2). As indicated above, locations or sites are often referred to as rivers. For this dataset, the sample size, location, year of sampling, and life stage sampled are provided in Appendix Table A2. The second SNP dataset is based on a 220,000 SNP array developed using a targeted, bi-allelic SNP Affymetrix Axiom array by the Centre for Integrative Genetics (CIGENE, Ås, Norway). The genomic coverage of this dataset is high, with an average of 7521.5 loci per chromosome, with many SNPs located within or near gene coding regions. The geographic coverage of the 220,000 SNP array is medium with over 100 locations genotyped across Atlantic Canada (Figure 2C; Appendix Table A3). Again, locations (or sites) are often referred to as rivers, but in some cases multiple tributaries were sampled within some larger river systems, such as the Miramichi, Restigouche, and Margaree Rivers. For this dataset, the sample size, location, year of sampling, and life stage sampled are provided in Appendix Table A3. This dataset has been used to identify adaptive differences between individuals and populations of Atlantic Salmon (Barson et al. 2015; Sylvester et al. 2018; Lehnert et al. 2019a; Lehnert et al. 2019b). Finally, there is also a whole genome re-sequencing dataset (unpublished data) that generally has low geographic coverage in North America, as genotyped locations are primarily located within Quebec (Figure 2C; Appendix Table A3). This dataset includes over two million SNPs genome-wide, and thus has high genomic coverage. Given the low geographic coverage of this data, it is not informative for many of the DUs; however, in some cases data from this dataset and the 220,000 SNP array can be combined to improve geographic coverage for genomic analyses.

Criteria for Defining Atlantic Salmon DUs: Decision Tree

To define DUs for anadromous Atlantic Salmon in Canada, we use criteria presented in the decision tree in Figure 3. This process generally assumes that the previously recognized COSEWIC DUs are equivalent to at least one DU. We make this assumption as the previous assessment provided support for discreteness and significance to define DUs (COSEWIC 2010). While this may seem constraining, there have been several studies that continue to largely show support the discreteness of DUs based on their prior boundaries (see below; Bradbury et al. 2014, 2021; Moore et al. 2014; Jeffery et al. 2018). Moreover, this assumption was critically evaluated (see below) and in several cases, DU boundaries are modified where required following larger scale analysis.

Over the last decade, the discreteness of Atlantic Salmon COSEWIC DUs has been largely supported by both microsatellite and SNP datasets. Moore et al. (2014) identified a total of 29 discrete genetic clusters for Atlantic Salmon using unsupervised analysis of microsatellite data with 149 sampling locations, with some genetic clusters containing only single rivers. Moore et al. (2014) concluded that there were 11 major regional genetic groups of Atlantic Salmon in Canada. Clustering supported discreteness of the majority of DUs identified by COSEWIC (2010). Some discrepancies included differences in the locations of boundaries between DUs (e.g., in Quebec) and potential splitting of the DUs due to evidence of discreteness (e.g., Gaspe and Southern Gulf of St. Lawrence) (Moore et al. 2014). Notably, at the time, the only DUs that had little support for discreteness were those located in Newfoundland (4 DUs), Eastern North Shore Quebec (1 DU), Eastern Cape Breton (1 DU), and outer Bay of Fundy (1 DU). Rivers in Eastern Cape Breton clustered with the Southern Gulf of St. Lawrence rivers, suggesting discreteness criteria have not been met here. In addition, although there are four recognized DUs in Newfoundland, discreteness for all of these DUs was not supported. Nonetheless, it is worth noting that the analysis revealed high levels of structure in Newfoundland, as there were many discrete genetic clusters that contained only a single river. Additional work using microsatellites and finer-scale sampling supported at least four genetic clusters within Newfoundland (Bradbury et al. 2014). Further, using SNPs, Moore et al. (2014) detected

additional genetic clusters providing support for some of the DUs in Newfoundland and the Eastern North Shore Quebec DU.

Similarly, using SNP data (Jeffery et al. 2018; Bradbury et al. 2021), there was support for discreteness among the 2010 Atlantic Salmon COSEWIC DUs. Bradbury et al. (2021) identified 20 discrete genetic groups in Canada, each encompassing multiple rivers. The majority of clusters identified in Moore et al. (2014) were supported, but with additional evidence of discreteness among rivers within some recognized DUs (e.g., Labrador) (Bradbury et al. 2021). Unlike the data from Moore et al. (2014), SNP data revealed seven genetic groups in Newfoundland, supporting discreteness of the recognized COSEWIC DUs, with potential for additional splitting of these DUs. SNP data also supported the discreteness of the outer Bay of Fundy DU. The only DUs that lacked evidence of discreteness from nearby DUs was again the Eastern Cape Breton DU (see above) and two DUs along the north shore of Quebec which contrasted microsatellite data.

Overall, these studies continue to support the discreteness of COSEWIC (2010) DUs with few exceptions. Given these exceptions, we also incorporated the possibility to re-evaluate DU boundaries within the decision tree framework. Here, we review the decision tree framework and discuss how these data support evidence of discreteness and significance. Our decision tree highlights the various paths that can lead to changes or no changes to the current DU structure (see Figure 3).

Discreteness

The first step in the decision tree was to examine evidence of discreteness within the previously recognized DU. Within each COSEWIC DU, genetic data were first assessed to determine if re-evaluation of previous DU boundaries is needed. Support for re-evaluation generally included evidence of discrete genetic groups near the DU boundary, existing evidence of genetic similarities among sites in adjacent DUs, and/or previous suggestion of ambiguity in DU boundary. To re-evaluate boundaries, sites from adjacent DUs were combined and analysis of discreteness was conducted following the decision tree, where we next evaluated whether a single genetic group or multiple genetic groups was/were present.

The data used for these analyses included both microsatellite and SNP data with high geographic coverage. We used two microsatellite datasets, which included

1. 15 microsatellite panel (Bradbury et al. 2016), and
2. 101 microsatellite panel (Bradbury et al. 2018).

Within each DU, the microsatellite panel that provided the greatest geographic coverage was used for the analysis. For the SNP dataset, we used the 96 SNP baseline dataset (Jeffery et al. 2018; Bradbury et al. 2021). We considered criteria for multiple genetics groups (discrete units) met if analysis of one or both datasets (microsatellites and/or SNPs) identified multiple genetic groups. Based on these criteria, only one of the datasets must show evidence of multiple genetic groups. We assume that if one dataset shows genetic clusters and the other one does not, this does not indicate the absence of genetic structure. Instead, it is more likely that alleles or loci present in only one of the datasets are important for discriminating between the populations, which warrants further investigation of evolutionarily significant differences between the discrete genetic groups.

To evaluate the presence of ‘multiple genetic clusters’, we relied on clustering analysis using the program STRUCTURE (Pritchard et al. 2000). STRUCTURE uses a Bayesian clustering approach where samples are put into groups (genetic clusters) based on shared similarity in genetic variation. Independent Markov chain Monte Carlo (MCMC) runs were performed using STRUCTURE v 2.3.4 and implemented through the R package *parallelstructure* (Besnier and

Glover 2013). For each run, a burn-in of 100,000 and 500,000 iterations were performed and this was replicated three times for each value of K (genetic clusters, which varied by DU). To determine support for the number of genetic clusters (K), here the optimal number of genetic clusters (K) was determined based on the ΔK statistic (Evanno et al. 2005). However, this statistic can be unreliable in complex evolutionary scenarios (Janes et al. 2017), which can often be the case for salmonids. Therefore, using STRUCTURE HARVESTER (Earl and vonHoldt 2012), we considered the ΔK statistic but we also examined the plateau in mean LnPr(*X*|*K*) estimates to assess support for the number of genetic clusters (Janes et al. 2017). All STRUCTURE results were inspected visually to confirm the presence of genetic structure. Overall, this STRUCTURE analysis allows us to investigate multiple levels of structure (i.e., hierarchical structure), where we are primarily focused on larger-scale geographic differences in genetics within the recognized DU.

If only a single genetic group was determined to be present, we did not consider any changes to the previously recognized DU (Figure 3; Path 4). This is because we do not expect significant changes to life history information or climate data within the DU since the last assessment, and thus without new evidence of genetic discreteness, evidence of evolutionary significance was not pursued. We acknowledge that in the absence of genetic discreteness, there may still be single genes of large effect that can lead to significant differences in adaptive phenotypes between individuals within the DU. Examples of large effect genes in salmonids can include those that influence age at maturity in Atlantic Salmon (*vgll3*) (Barson et al. 2015) and those that influence migration timing in Pacific salmon (*GREB1L*) (Prince et al. 2017). If criteria for discreteness are not met, then these alleles are freely segregating in the population. While these alleles can contribute to substantial differences in phenotype, we do not think that this represents evidence of discrete and evolutionarily significant units based on COSEWIC's current criteria. For example, while the *vgll3* gene can contribute to >39% of the variation in age at maturity in Atlantic Salmon, we would not consider salmon of different ages (e.g., one-sea-winter and two-sea-winter) to be discrete and evolutionarily significant units within an interbreeding population at this time.

In the case of Atlantic Salmon, spatial genetic structure has repeatedly been shown to be hierarchical with large genetic differentiation spanning the North Atlantic Ocean (Lehnert et al. 2019a; Lehnert et al. 2020), moderate regional differentiation (Moore et al. 2014; Jeffery et al. 2018; Bradbury et al. 2021), and even clear evidence of significant structuring among individual rivers (e.g., Bradbury et al. 2018). Here for the case of discreteness, as indicated above, we are primarily focusing on larger geographic breaks or regional groups. Although it is possible that individual rivers could meet criteria of discreteness, in most cases the available data are insufficient to provide evidence of 'significance' (see next section) at the level of individual rivers. Nonetheless, we acknowledge that many salmon rivers may possibly represent discrete and evolutionarily significant units, and this could likely become more apparent as more data become available in the future.

Evolutionary Significance

If multiple genetic groups were determined to be present, the next step in the decision tree relies on using high-density genomic data to identify adaptive differences between the genetic groups. Here we use the genomic dataset compiled using a 220,000 SNP array and/or whole genome re-sequencing. If both datasets were available for the DU, the two datasets were combined based on overlapping loci, otherwise, only one dataset was used (primarily the 220,000 SNP array). Adaptive differences between groups based on genomic data were first examined using the R package *pcadapt* (Luu et al. 2017), which is a principal component analysis (PCA) based method used to detect loci under selection. Based on this analysis, we can determine which loci contribute to differences between the discrete groups. To meet criteria of 'significance' based on this genomic analysis we require evidence to show that the loci

contributing to differences are associated with adaptation. There are several lines of evidence that can be used to support the link between identified loci and adaptation, and these can include:

1. **Loci are located within known structural variants that are associated with adaptation in Atlantic Salmon:** With advances in genomics, it is becoming clear that structural variants like chromosomal rearrangements often underlie complex phenotypes (Wellenreuther and Bernatchez 2018). For example in salmonids, a chromosomal rearrangement influences the migratory ecotypes (Rainbow Trout vs. Steelhead) in *Oncorhynchus mykiss* (Pearse et al. 2019). Known chromosomal rearrangements in Atlantic Salmon include chromosomal fusions, translocations, and inversions. These variants result in changes in chromosome structure, influence the order and position of genes, and can suppress recombination. There are three known chromosomal rearrangements that have been associated with adaptation in Atlantic Salmon. Differences in a chromosomal translocation between Atlantic Salmon chromosomes Ssa01 and Ssa23 is associated with historical European introgression in North American populations (Lehnert et al. 2019a) and evidence suggests that this translocation is under selection and associated with climate adaptation (Watson et al. 2022). Variation in a chromosomal fusion between Ssa08 and Ssa29 has also been identified across North American populations (Lehnert et al. 2019a), and this fusion has been associated with climate variation (Wellband et al. 2019). Finally, a putative chromosomal rearrangement on Ssa18 is highly associated with smolt age and climate across North America (Lehnert et al. in prep[1]).
2. **Loci are located within gene(s) with known role in adaptation and/or that are associated with climate:** Several genes have been associated with adaptation in Atlantic Salmon. These include (but are not limited to) *vgll3* that influences age at maturity (Barson et al. 2015), six which is associated with age and size at maturity, river catchment size, and run timing (Cauwelier et al. 2018; Pritchard et al. 2018; Sinclair-Waters et al. 2020), and major histocompatibility (MHC) genes which are associated with immune function and temperature (Dionne et al. 2007). Other genes that are associated with adaptive phenotypes include growth rate (Gutierrez et al. 2015), immune function (Kjærner-Semb et al. 2016), and carotenoid pigmentation (Helgeland et al. 2019). In addition, genetic markers associated with climate adaptation have also been identified in Atlantic Salmon, and generally these associations are found to be polygenic (Jeffery et al. 2017; Sylvester et al. 2018). Genes associated with known functional traits and adaptation in other salmonids may also provide insight in Atlantic Salmon, as recent evidence suggests a role for the same gene influencing the same trait across Pacific and Atlantic Salmon species (Waters et al. 2021).
3. **Loci are found within/near genes and this set of genes is associated with over-represented biological processes:** As indicated above, loci that contribute to differences between groups or populations may be located within or near genes with putative functions. In many studies, biological processes associated with this set of genes are examined using gene ontology (GO) term enrichment. This approach can help determine what types of biological processes are over-represented by the set of genes (associated with outlier SNPs) relative to the genomic background. In Atlantic Salmon studies, GO term enrichment analysis has been used to help understand functional

[1] Lehnert, S.J., Kess, T., Layton, K.K.S., Bentzen, P., Paterson, I.G., Barson, N.J., et al. In prep. Divergent supergene explains age of seaward migration in multiple lineages of Atlantic salmon.

differences between groups that may contribute to adaptation (Wringe et al. 2018; Wellband et al. 2019; Lehnert et al. 2020).

The above lines of evidence support a role for local adaptation in salmon. In addition to our analyses, genomic information from published literature will be included and considered here when available for the DUs.

If genomic evidence of adaptation is met, then we also examine additional evidence to support significance. Life history differences and/or climate-linked differences are also incorporated into this decision (see below). We require evidence for two of these three criteria to be met to support significance (Figure 3; Path 1 or 2):

1. genomic evidence of adaptation,
2. life history differences, and
3. climate-linked differences likely to give rise to local adaptation.

In the absence of high-density genomic data or where adaptive differences associated with genomic data were not found, we will rely on life history and climate-linked variation between the discrete groups to provide evidence of significance (Figure 3; Path 2). In Atlantic Salmon, the first part of life is spent in fresh water, where individuals may spend as few as one year to as many as eight years before migrating to the ocean (Klemetsen et al. 2003). Therefore adaptations to conditions experienced during this early life stage can reflect local and regional conditions, which can include, but are not limited to, temperature, precipitation, river gradient, length of growing season, size of the river, bacterial community, fish assemblages, and pH. Additional adaptive variation can relate to age at maturation, including the proportion of the population that matures precociously as male parr, or as one-sea-winter or multi-sea-winter salmon. Other sources of adaptive variation can result from life in the marine environment, which can include differences in migration routes to feeding grounds and differences in pathogen communities. Much of the known life history variation in Atlantic Salmon have been summarized in several studies (Hutchings and Jones 1998; Chaput et al. 2006; DFO and MNRF 2008; COSEWIC 2010), and thus we rely on these data to inform life history differences between the discrete groups, unless new data are available.

In addition to life history data, we also use climate variation to inform significance, as climate can be important for shaping life history variation and local adaptation (Schaffer and Elson, 1975; Metcalfe and Thorpe, 1990; King et al. 2001; Klemetsen et al. 2003). To quantitatively assess differences in climate between discrete groups, we extracted 19 bioclimatic variables from WorldClim (Fick and Hijmans 2017) for known salmon rivers based on geographic coordinates from the North Atlantic Salmon Conservation Organization (NASCO) river database. Rivers were split into groups (representing putative DUs) based on evidence of genetic discreteness. Redundancy analysis (RDA) was used to identify climate variables associated with groups using the R package *vegan* (Oksanen et al. 2017). A significant model would indicate climate differences between the groups, which we infer as evidence of local adaptation. In this case, support for splitting the previously recognized DU into multiple DUs would be sufficient. We note that this analysis can only be accomplished if multiple rivers are located within each putative DU, and therefore this analysis will not be attempted if only a single location meets criteria of discreteness. Instead other information (both life history and genomic data) is needed to inform significance.

DUs must be discrete and evolutionarily significant. Therefore, if there is no evidence for at least two of our three significance criteria (differences in genomic adaptation, differences in life history, or climate-linked differences) associated with the detected discrete genetic groups, then the previously recognized DU will remain classified as a single DU.

RESULTS

OVERVIEW

Using a decision tree (Figure 3), we have reviewed each of the 15 anadromous Atlantic Salmon DUs. We have incorporated new genetic and genomic information as well as available data on life history and climate to evaluate whether the previously recognized DUs require subdivisions or changes. Overall, we identified four existing DUs that require subdivisions. In addition, based on a weight of evidence, we re-evaluated boundaries between some previously recognized DUs, and deemed these to require changes. Based on our evaluation, we propose that anadromous Atlantic Salmon is represented by a total of 19 DUs in Canada (see Table 1 and 2). Analyses for all the previously recognized DUs and changes to their structure are outlined in this section. For each subheading we provide the previous DU numbers and names based on the last assessment (COSEWIC 2010), along with proposed changes to these DUs. Note that within the text, we refer to the numbers of the COSEWIC DUs defined in 2010, and the proposed new DU names and numbers are only provided in Tables 1 and 2 to avoid confusion.

CHANGES TO DESIGNATABLE UNITS OF ANADROMOUS ATLANTIC SALMON

DU 1 Nunavik (previous): Unchanged

This DU extends from the tip of Labrador (approximately 60°29’ N, 64°40’ W) west along Ungava Bay to the western extent of the species’ range. The most northerly known Atlantic Salmon populations in North America are found in this DU, and these populations are geographically disjunct from salmon populations in the neighbouring DU (Labrador) by approximately 650 km of coastline (limited survey work and Aboriginal traditional knowledge suggest there are no self-sustaining populations between DU 1 and DU 2). In Ungava Bay, some portions of the populations appear to have local migratory patterns (Power, 1969; Robitaille et al. 1986), while others range broadly (Power et al. 1987).

At the time of the last COSEWIC assessment, genetic data suggested that these populations were distinct from populations in Labrador and there was little genetic evidence of straying between Ungava and other regions (Fontaine et al. 1997; Dionne et al. 2008). Additional genetic studies continue to support the discreteness of this DU from other regions (Moore et al. 2014; Jeffery et al. 2018).

There are five known salmon rivers in the Nunavik DU (COSEWIC 2010), and our genetic datasets include three of these populations, including Koksoak, George, and Aux Feuilles. Using 15 microsatellite markers, clustering in STRUCTURE separated Koksoak and George from Aux Feuilles (Appendix Figure A1); however, using the 96 SNP dataset, there was no evidence of genetic structure within this DU (Appendix Figure A2). Based on these analyses and our decision tree, criteria for multiple genetic clusters (discreteness) within DU 1 are met, where Aux Feuilles is considered discrete from Koksoak and George.

To evaluate evidence of evolutionary significance, we rely on high-density genomic data, life history, and climate data. High-density genomic data (220,000 SNP array or whole-genome resequencing) were not available for DU 1. Life history data are limited for populations in Ungava, and available data for four populations are summarized in Hutchings and Jones (1998). Data used here are from 1986 and earlier. While it is possible that life history characteristics may have changed since these studies were conducted, unpublished data from the Ministère des Forêts de la Faune et des Parcs du Québec suggest they have not. For many life history traits with available data, values for Aux Feuilles fall between those of Koksoak and George (sea age, two-sea-winter [2-SW] length, proportion of grilse). Therefore, the available data do

not support that life history characteristics within Aux Feuilles are different from the rest of these populations. Climate-linked differences were not assessed here as only a single river met criteria for discreteness, and thus climate data cannot be reliably compared statistically. Our analyses suggest that salmon from Aux Feuilles do not meet the criteria of a discrete and evolutionarily significant unit. Based on current data, the Nunavik DU (DU 1) should remain as a single designatable unit (Table 1).

DU 2 Labrador (previous): Three Proposed DUs - Northern Labrador, Lake Melville, and Southern Labrador

This DU extends from the northern tip of Labrador (approximately 60°29’ N, 64°40’ W) south along the coast of Labrador to the Napitipi River in Quebec. Given the large size of this geographic region the last COSEWIC assessment suggested that there was substantial potential for smaller regional groupings within the DU, particularly in the Lake Melville area. However, at that time, the available information only supported a clear separation from other regions at the southern portion of the DU. Further, life history data showed variation in life history characteristics within the recognized DU, but with no clear geographic pattern; however, clear differences exist between Labrador and neighbouring DUs (Chaput et al. 2006). Genetic data also supported significant divergence of Labrador populations from populations in other nearby DUs in Quebec and Newfoundland (Adams 2007; Dionne et al. 2008).

At the time of the last COSEWIC assessment, genetic data suggested reasonable potential for gene flow throughout much of the southern portion of the Labrador DU (King et al. 2001; Verspoor 2005; Adams 2007; Dionne et al. 2008). At that time, there was evidence from tagging studies that individuals from the southern portion of the DU did not migrate north of Lake Melville (Anderson 1985; Reddin and Lear 1990). However, the limited genetic data available generally did not support differences between southern and northern Labrador (King et al. 2001; Verspoor 2005). There was a significant genetic difference between Lake Melville and other Labrador samples; however, only one small sample of parr from Lake Melville (Cape Caribou) was available at that time and thus was not enough data to justify separation of Lake Melville.

Recent genetic and genomic studies support the distinctiveness of the Lake Melville system from the rest of Labrador (Jeffery et al. 2018; Sylvester et al. 2018), as well as differences between populations north and south of Lake Melville (Bradbury et al. 2021). While previous studies suggested generally weak genetic structure in Labrador, more recent work has demonstrated fine scale differences between populations using microsatellites (Bradbury et al. 2018), where the majority of individual rivers can be considered discrete units.

There are 91 known salmon rivers in DU 2 (COSEWIC 2010), and our genetic datasets include samples from 34 (microsatellites) and 40 (SNPs) locations. Using 101 microsatellite markers, the optimal number of genetic clusters (K) was 2, but further structure of up to 10 groups was supported (Appendix Figure A3). At K=2, sites in Lake Melville were clearly separated from other sites in Labrador. Higher values of genetic clustering (K) continued to separate groups of populations. At K=10, approximately five clusters were present south of Lake Melville, where sites generally clustered by geography. Sites north of Lake Melville clustered into three distinct groups. Sites within Lake Melville remained distinct with some forming a separate distinct cluster (Main Brook, Mulligan, Sebaskachu). Using 96 SNPs, the optimal number of genetic clusters (K) was also 2, which separated sites south of Lake Melville from the rest of Labrador (Appendix Figure A4). Further structure was supported, where at K=3, sites were further separated into three clusters generally corresponding to south Labrador, Lake Melville, and north Labrador. Additional clustering of individual rivers and geographic region was apparent at higher values of K and structure was supported beyond K=10, consistent with microsatellites (Appendix Figure A4).

Based on these analyses and our decision tree, criteria for multiple genetic clusters (discreteness) within Labrador (previously recognized DU 2) are met. There is clear evidence of the discreteness of Lake Melville from the rest of Labrador, as well as discreteness between populations north and south of Lake Melville (see Figure 4). In addition to these three main genetic clusters, additional structure was observed that separated individual rivers and/or geographic regions within these clusters.

To evaluate evidence of evolutionary significance, we rely on high-density genomic data, life history, and/or climate data. Within Labrador, high-density genomic data (220,000 SNP array) were available for 19 sites spanning all regions of DU 2. Using *pcadapt*, populations within Lake Melville were clearly separated from coastal sites along the first PC axis (Appendix Figure A5). Additional separation on PC axis 2 further separated some sites within the Lake Melville system (Main Brook, Mulligan, Sebaskachu) from other sites in Labrador, including other sites in Lake Melville. A total of 314 loci significantly contributed to the differentiation on both PC axes (adjusted *p*-value [*q*-value] <0.05) and these loci were distributed across 27 chromosomes (out of 29). Over-representation of biological processes associated with the genes located near the outlier loci were examined using topGO (Alexa and Rahnenfuhrer 2016). A total of 86 biological processes were significantly ($p<0.05$) over-represented based on the outlier dataset, with a large proportion of processes related to 'fatty acid homeostasis' (Appendix Figure A6).

In addition, recent genomic studies have found genetic differences associated with environment between Lake Melville and coastal sites (Sylvester et al. 2018). Using SNPs and microsatellite datasets, Sylvester et al. (2018) found evidence that exposure to warmer temperatures and wide temperature ranges may strongly influence the isolation of Lake Melville populations from adjacent coastal Labrador sites. SNPs associated with the genetic/environmental split between Lake Melville and coastal sites were associated with a wide variety of molecular processes, including regulation of gene expression, immune response, and cell development and differentiation. Lehnert et al. (2019a) found differences in a chromosomal rearrangement (translocation of Ssa01 and Ssa23) associated with European introgression in Labrador populations and reported that sites within Lake Melville (n=10 sites; Peter's River excluded due to potential inclusion of Ouananiche salmon in the sample) had a higher frequency (2X greater) of the 'European' type chromosomal arrangement (non-translocated chromosome) compared to coastal Labrador sites (n=6 sites). The average frequency of the European type was 32% in Lake Melville compared to 15% in coastal sites. Although there was variation in the frequency of this chromosomal rearrangement within each group, 60% of sampled sites in Lake Melville had 30% or higher frequency of the European type arrangement, whereas only one site in coastal Labrador had a frequency as high as 30% (Lehnert et al. 2019a). This supports genetic differences associated with large structural changes in chromosomes as well as higher rates of historical European introgression in Lake Melville populations. These large genetic differences may contribute to adaptive differences, as this genomic region associated with the translocation contains over 250 genes and is under selection (Lehnert et al. 2019a), and recent work indicates that this chromosomal translocation is associated with climate variation (Watson et al. 2022).

Based on genetic and genomic data, there is clear support for discreteness and evolutionary significance of the Lake Melville system. Based on our decision tree, we also examined evidence of life history and climate-linked differences within Labrador. While genomic data supports significance of the Lake Melville system from other regions, we used life history and climate-linked differences to support further splitting of Labrador (previously DU 2) based on three discrete genetic groups (southern Labrador, Lake Melville, and northern Labrador).

DFO and MNRF (2008) suggest that these three genetic groups represent separate conservation units (CUs) of Atlantic Salmon, which are defined as "*groups of individuals likely exhibiting unique adaptations that are largely reproductively isolated from other groups, and that*

may represent important components of species biodiversity" (DFO 2008). We note that the CU report also separates the most southern portion of the DU (at Labrador-Quebec border) into another CU, although the support for this separate CU was limited in the report, thus we focus on the three main groups identified here. Aside from genetic differences identified in the CU report, life history differences are also present including differences in the incidence of maturation after one winter at sea (DFO and MNRF 2008). DFO and MNRF (2008) suggest that the incidence of maturation after 1 winter at sea is higher in Lake Melville and southern Labrador relative to northern Labrador. Differences in run timing are also reported, where run timing is earliest in Lake Melville, followed by southern Labrador, and with later run timing reported for northern Labrador (DFO and MNRF 2008). Differences in migration routes are also reported (DFO and MNRF 2008), which likely reflect different distances to feeding grounds. Other sources reporting life history variation in Labrador include Hutchings and Jones (1998), which included four populations in southern Labrador and one population northern Labrador. The mean sea age for the northern Labrador population (Hunt) was 1.75 years, which contrasted the lower sea ages reported for southern Labrador (range 1.03–1.16 years). Similarly, the only other data reported included size (length) of 1-SW and 2-SW salmon, which was larger for the northern Labrador population (57.8 and 76.6 cm, respectively) compared to the southern populations (53.2–54.4 cm and 72.9–74.7 cm). Consistent with this, recent data from the Labrador Food, Social, and Ceremonial (FSC) fishery (2017-2019), suggest younger virgin sea age in southern Labrador, followed by Lake Melville, with the older salmon in northern Labrador, potentially suggesting some differences in virgin sea age between DUs with an increase across latitude (Kelly et al. in prep[2]). Other life history data were also available for other populations in southern and northern Labrador, although no clear differences between regions were apparent based on smolt age or body size (see Appendix 1 and 2 in Caput et al. 2006). However, Kelly et al. (in prep[2]) provide some evidence to support that Lake Melville has younger smolts compared to coastal Labrador. Overall, while information on life history variation in Labrador salmon is sparse, it does support differences between the three regions.

In addition to life history, ecological differences between the three regions were also reported by DFO and MNRF (2008). Ecological differences include differences in salmonid communities, with northern rivers dominated by Arctic Charr (*Salvelinus alpinus*), whereas mainly Atlantic Salmon and sea-run Brook Trout (*Salvelinus fontinalis*) are found in Lake Melville (DFO and MNRF 2008). Conversely, all three of these species are represented equally in southern Labrador populations (DFO and MNRF 2008). In addition, differences in river gradients between the three regions were found, with Lake Melville having the lowest gradient, followed by southern Labrador, with the highest found in the north (DFO and MNRF 2008). While these factors are not directly included in our decision tree, these variables, such as gradient or elevation (Pritchard et al. 2018; Wellband et al. 2019), are relevant to influencing adaptive variation in salmon populations and are thus reported here.

Climate data for Labrador also supports differences between the three genetic clusters (Appendix Figure A7). RDA was performed using 19 bioclimatic variables (see Appendix Table A4) for all rivers in DU 2 as the response and putative DU groups (three genetic clusters) as the constraining variable. ANOVA on the RDA showed the model to be significant ($p < 0.001$) with an adjusted R^2 of 0.61. RDA axis 1 explained 74.8% of the variance in the model, while RDA axis 2 explained 25.2% of the model variance. The RDA plot clearly shows support for the splitting of Labrador populations into three separate DUs (Appendix Figure A7). RDA axis 1 mostly separates southern Labrador from Lake Melville and northern Labrador. This difference

[2] Kelly, N.I., Burke, C., Lancaster, D., Lehnert, S., Loughlin, K., Van Leeuwen, T., Dempson, B., Poole, R., Robertson, M., and Bradbury, I. In prep. Updated information on Atlantic Salmon (*Salmo salar*) populations in Labrador of relevance to the COSEWIC status report. DFO Can. Sci. Advis. Sec. Res. Doc.

is driven by variation in temperature (minimum temperature, temperature in coldest quarter, temperature seasonality, annual temperature range) and precipitation (precipitation in coldest quarter, precipitation in driest quarter, precipitation seasonality). Based on these analyses, temperatures were generally higher and less variable in southern Labrador compared to other regions. In addition, precipitation was higher in southern Labrador, although variation in precipitation was lower. RDA axis 2 separates Lake Melville from northern Labrador, which is primarily driven by the temperature (mean temperature of the driest quarter, maximum temperature, and mean temperature of warmest quarter) and precipitation in the wettest month. Generally, temperature and precipitation were higher in Lake Melville relative to northern Labrador although, temperature during the driest quarter was warmer in northern Labrador than Lake Melville. These results support clear differences in climate that are linked to the three genetic groups which can lead to local adaptation.

Overall, our analyses suggest that there are three discrete and evolutionarily significant units (DUs) within Labrador (previously DU 2), which include:

1. northern Labrador,
2. Lake Melville, and
3. southern Labrador.

Discreteness of these three DUs is supported by genetic data, and evolutionary significance is supported by genomic evidence of adaptation, life history differences, climate-linked differences, as well as ecological differences. A map of rivers (Figure 5) and a list of all rivers in this region and their proposed DUs are provided (Appendix Table A5) to highlight the boundaries between these proposed DUs.

DU 3 Northeast Newfoundland (previous): Revised Boundary

This DU extends from the northern tip of Newfoundland (approximately 51°37’ N, 55°25’ W) south and east along the northeast coast of the Island to the southeast tip of the Avalon Peninsula (approximately 46°38’ N, 53°10’ W). Previous data suggested life history variation in this DU was distinct from other nearby DUs (Chaput et al. 2006). For example, it was previously reported that mean smolt age in this DU is intermediate between Labrador and the rest of the island of Newfoundland (three to five years versus five to seven in Labrador and two to four in southern Newfoundland DUs). In addition, a high proportion of grilse are relatively small one-sea-winter (1-SW) females, and there is a high incidence of repeat spawners in this area of the Canadian range. The juvenile salmon within in this DU make extensive use of lacustrine habitat for rearing (Hutchings 1986).

At the time of the last COSEWIC assessment, genetic work suggested that salmon of the northeast coast of Newfoundland are unique in North America, in that they appear to have genetic profiles intermediate to European and North American salmon (King et al. 2000). However, recent genetic work suggests that many other populations in North America (particularly in Newfoundland and Labrador) show introgression from European salmon (Bradbury et al. 2015; Lehnert et al. 2019a). Nonetheless, at the time of the 2010 assessment, other genetic work supported distinct differences between salmon populations in northeast Newfoundland (DU 3) and salmon populations in both Labrador, and southern and western Newfoundland (Verspoor 2005, Adams 2007, Palstra et al. 2007).

Recent genetic and genomic studies support differences between this DU and nearby populations on the Avalon Peninsula (Moore et al. 2014; Jeffery et al. 2018) and populations on the south and west coast (Bradbury et al. 2021). However, it is worth noting that genetic work suggests that some populations along the northern peninsula within DU 3 may be distinct from other populations in DU 3 and may instead be genetically similar to other populations on the

northern peninsula that are in the northwest Newfoundland DU 6 (Moore et al. 2014; Jeffery et al. 2018; Bradbury et al. 2021). Additionally, some studies have identified similarities between geographically isolated populations in DU 3 and the south coast (Moore et al. 2014; Jeffery et al. 2018; Bradbury et al. 2021); however, we hypothesize that these genetic similarities represent historical colonization of Newfoundland. This is because while the mouths of these rivers are geographically separated, some tributaries of these rivers may come into close contact in interior regions of the island, which may suggest historical connectivity. It is unlikely that salmon from these rivers continue to exchange genetic variants today, and instead, these genetic signals represent historical signals that have yet to be erased from the genome.

There are 127 known salmon rivers in DU 3 (COSEWIC 2010), and our genetic datasets include 9 locations (96 SNPs) and 13 locations (microsatellites), some of which are within the same river system. Using 15 microsatellite markers, the optimal number of genetic clusters (K) was 5 (Appendix Figure A8). Beaver Brook (BVB) was clearly separated from other sites, with less structure observed among other sites. Some differences between Sop's Arm-Main River (MNR) were observed at higher values of K. Using the 96 SNP dataset, there was again evidence of two distinct genetic clusters, where BVB and MNR (westernmost sites) clustered separately from other sites at K=2 and these two sites could be separated into their own distinct clusters at K=4, beyond which there was no additional structure (Appendix Figure A9).

Based on these analyses and our decision tree, criteria for multiple genetic clusters (discreteness) within DU 3 are met. A consistent signal from the datasets is a clear genetic difference between westernmost sites (MNR and BVB) from other sites in the DU, as well as from each other. This difference in consistent with a recent genetic study which groups Beaver Brook with northern sites in northwest Newfoundland (such as Western Arm Brook) on the northern peninsula, although Main River groups with populations on the southwest coast, and all other sites in DU 3 group together and separate from other regions (Bradbury et al. 2021). Similarly, Moore et al. (2014) found that sites on the northern peninsula were distinct from other regions of Newfoundland using SNPs.

Based on evidence of discreteness and other recent studies, there appears to be evidence that westernmost sites in our data (BVB and MNR) may belong in the adjacent DU (Northwest Newfoundland; previously recognized as DU 6). This is consistent with other genetic studies grouping sites in this region of the northern peninsula (Moore et al. 2014; Jeffery et al. 2018; Bradbury et al. 2021). Therefore, given multiple lines of evidence, we have re-evaluated the boundary between Northwest and Northeast Newfoundland DUs (DU 3 and 6).

Re-assessing the boundary between DU 3 and DU 6

To re-evaluate the DU boundary between DU 3 (northeast NL) and DU 6 (northwest NL), we ran STRUCTURE for the 96 SNPs and 15 microsatellite datasets with sites from both DUs. For both datasets, genetic differences were consistent with a revised boundary between DU 3 and 6 (Figures 6 and 7). When two genetic clusters (K=2) were examined, Beaver Brook (site currently found within DU 3) clustered more closely with sites in DU 6, and Main River (within DU 3) showed signals of admixture between these two DUs. However, at higher values of genetic clustering (K), Main River groups more closely with rivers in the northeast (DU 3), but Beaver Brook consistently groups more closely with rivers in the northwest (DU 6). Based on these analyses, a revised DU boundary near Beaver Brook is supported by discreteness (Figures 6 and 7).

We also evaluated whether this new boundary could meet criteria of evolutionary significance. High-density genomic data (220,000 SNP array or whole-genome resequencing) were available for seven populations in DU 3 and 6. Using *pcadapt*, sites in the putative new DU 3 separated from sites in DU 6 along the first PC axis (Appendix Figure A10). These DU 3 sites (including Campbellton, Terra Nova, and Great Rattling Brook - Exploits) clustered very tightly together,

whereas Beaver Brook (previously in DU 3) clustered more closely with DU 6 sites, including Trout River and Western Arm Brook, on the first PC axis, as well as PC 2. The closer grouping of Beaver Brook with Trout River and Western Arm Brook is supportive of the new DU boundary. For example, Trout River is located near the other (southern) boundary of DU 6, suggesting genetic similarity between these DU 6 sites despite extensive geographic separation (>300 km). Another site in DU 6, Big East River, clearly separated from all sites on PC 1. A total of 1189 loci significantly contributed to the differentiation between sites on PC axis 1 and 2 (adjusted *p*-value [*q*-value] <0.05) and these loci were distributed across all chromosomes (Appendix Figure A10). Over-representation of biological processes associated with the genes located near the outlier loci were examined using topGO (Alexa and Rahnenfuhrer 2016). A total of 200 biological processes were significantly ($p<0.05$) over-represented based on the outlier dataset, with a large proportion of processes related to 'lateral motor column neuron migration' and 'N-terminal protein myristoylation' (Appendix Figure A11). Lateral motor column neurons are motor neurons that innervate the limb muscles (Luria and Laufer 2007). Protein myristoylation can regulate cell signaling pathways associated with different biological processes, including immune function (Udenwobele et al. 2017).

In addition to evidence of genomic differences between the putative new DUs, there are also some life history differences between these regions. Smolt ages (based on small salmon category) tend to be higher for populations in the region of the northern peninsula, this includes Salmon Brook, Western Arm Brook, and St. Genevieve (Chaput et al. 2006). These populations have a high proportion of four year old smolts (>60%). These similarities in smolt age are consistent with similar genetic signals among these populations. Populations in the northeast tend to have younger smolt ages compared to the northern peninsula; however, younger smolts have also been reported for other populations in DU-6 (Chaput et al. 2006). Overall, Kelly et al. (in prep[3]) suggest slightly older smolts on average in the northwest DU 6 compared to northeast DU 3. Kelly et al. (in prep[3]) also found that the northeast Newfoundland DU 3 had a lower proportion of maiden spawners (based on small salmon) with a mean of 89% (range 81–95%), which differed from the neighboring northwest Newfoundland DU 6 (mean:98%; range 93–100%). This suggests there are a higher proportion of small repeat spawners in northeast DU compared to the northwest DU based on the proposed boundary (Kelly et al. in prep[3]).

Climate data for DU 3 and 6 also supports differences between the new putative DUs (Appendix Figure A12). RDA was performed using 19 bioclimatic variables (see Appendix Table A4) for all rivers in DU 3 and DU 6 as the response and putative DU groups (based on revised boundary) as the constraining variable. The revised boundary shifts 11 rivers from DU 3 into DU 6 (based on NASCO river database). ANOVA on the RDA showed the model to be significant ($p<0.001$) with an adjusted R^2 of 0.23, and RDA axis 1 explained 23.2% of the variance in the model. The RDA plot shows support for the revised boundary between DU 3 and DU 6 (Appendix Figure A12). This difference is driven by temperature with mean temperature, isothermality, temperature of the coldest quarter, temperature of the driest quarter, temperature of the warmest quarter, and minimum temperature loading highly on the axis. The RDA suggests that DU 3 experiences generally warmer temperatures than DU 6. In addition, the new boundary proposed would align with a major geological break (Honsberger et al. 2019), where the proposed DU 6 would be characterized by geology that is generally unique from DU 3 and most other parts of Newfoundland.

[3] Kelly, N.I., Burke, C., Lancaster, D., Lehnert, S., Loughlin, K., Van Leeuwen, T., Dempson, B., Poole, R., Robertson, M., and Bradbury, I. In prep. Updated information on Atlantic Salmon (*Salmo salar*) populations in insular Newfoundland of relevance to the COSEWIC status report. DFO Can. Sci. Advis. Sec. Res. Doc.

Overall, our analyses support one discrete and evolutionarily significant unit (DU) within DU 3; however, the boundary of this unit with DU 6 should be revised. The boundary of DU 3 should extend from the southeast tip of the Avalon Peninsula to a new proposed boundary near Beaver Brook. Based on this boundary, criteria for discreteness and significance of Northeast Newfoundland (DU 3) from neighbouring Northwest Newfoundland (DU 6) were met. We discuss below how our analyses provides more support for a boundary between DU 6 and DU 3 compared to the previous COSEWIC report.

Support for proposed versus previous boundary location

Here, we review the evidence to support the previous boundary and the new proposed boundary between northeast and northwest Newfoundland (DU 3 and DU 6). The evidence is summarized in Table 3. Based on the previous COSEWIC 2010 report, it does not appear that there was particular support for the specific location of the boundary between DU 3 and DU 6. Previously, it was suggested that DU 3 was genetically unique compared to other Canadian populations due to salmon in this region having profiles intermediate to European and North American salmon (COSEWIC 2010). However, recent work has found that other areas on the island of Newfoundland also show these profiles (Bradbury et al. 2015; Lehnert et al. 2019a). There is evidence of European-type mitochondrial DNA in many populations in Newfoundland including in the northeast and northwest (Bradbury et al. 2015). The previous COSEWIC assessment also suggested that other genetic data provided evidence that salmon in northeast Newfoundland DU 3 were genetically different from salmon in western Newfoundland DU 6. However, DU 6 was deemed data deficient based on these genetic studies, suggesting genetic differences between populations in DU 6 and DU 3 were not fully evaluated. One of these previous studies cited by COSEWIC (2010) was based on allozyme data (Verspoor 2005). However, only one site was sampled in the northwest (DU 6) and this site did not appear to show strong differences from all sites in the northeast (DU 3) for most allozymes. Although notably, some sites on the northern peninsula in previously recognized DU 3 (Northeast Roddington and Main River) were more divergent from other sites in the rest of DU 3 at a few markers. Additional genetic data cited in the previous report did not have enough sample locations to support a specific boundary location on the northern peninsula (Palstra, O'Connell, and Ruzzante 2007). Therefore, our genetic data provide better support for the revised boundary. In our analyses, both microsatellites and 96 SNP datasets support that a river on the east side of the northern peninsula (Beaver Brook) is more genetically similar to populations in the northwest DU compared to the northeast DU. This suggests strong evidence of genetic discreteness between the two regions (proposed DUs) based on a revised boundary. Based on this revised boundary, there appears to be lower genetic differentiation within the northeast DU 3 compared to the northwest DU 6 where there is extensive genetic structure among rivers in the region. Overall, we have strong support for the revised boundary based on discreteness.

The previous COSEWIC assessment also suggested differences in life history between the DU regions. However, we would argue that this did not support the specific location of the boundary (tip of the northern peninsula), but general differences between the northeast and northwest regions of Newfoundland overall. Data suggested that smoltification in the northeast (DU 3) was different from the rest of Newfoundland (COSEWIC 2010). Data compiled by Chaput et al. (2006) includes sites on the east side of the northern peninsula and suggests that salmon populations on the northern peninsula (east and west) have older smolt ages compared to those in the northeast and more southern populations in the northwest region. Smolt ages (based on small salmon category) tend to be higher for populations in the region of the northern peninsula, which includes Salmon Brook, Western Arm Brook, St. Genevieve, as well as Main River (Chaput et al. 2006). Other populations in the northeast have lower proportion of four year old smolts, ranging from 25–57% (Chaput et al. 2006). However, we note that other sites in northwest (DU 6) including Torrent and Lomond had younger smolt ages (proportion of four year

old smolt range: 4% to 13%), although data were not available for other systems. Based on compiled smolt age data from DFO (Appendix Table A6), Lomond and Torrent appear to have younger smolts compared to other rivers in the northwest. Next, we examined life history data from rivers sampled during multiple time periods (pre-1980, 1980–99, and post 2000). For rivers with >100 individual samples for specified time periods (see Appendix Table A6), mean river age ranged from 3.34–3.79 years across time periods (full range: 2.99–4.13) in the northwest DU 6, and this was slightly higher than in the neighbouring northeast DU 3, where mean river age ranged from 3.45–3.58 years across time periods (full range: 3.16–3.95) (Kelly et al. in prep[3]). No data are available for the east side of the northern peninsula, providing no support for or against the previous or new boundary.

In addition, the previous COSEWIC report suggested that the northeast region of Newfoundland has the highest incidence of repeat spawners. In the northeast DU, there does appear to be a higher proportion of repeat spawners in sampled rivers with >50 individuals. The range in proportion of repeat spawners for large salmon is 66% (Exploits; 1980–99) to 100% (Campbellton; 1980–99) (Appendix Table A7). For the northwest (DU 6), few rivers were sampled, but the range is 34% (Lomond River; 1980–99) to 92% (West River; post-2000) (Appendix Table A7). For small salmon, the proportion ranged from 2% (Ragged Harbour River; pre-1980) to 87% (Campbellton; 1980–99) in the northeast, and in the northwest, the proportion ranged from 0% (Castors and St. Genevieve; 1980–99) to 68% (West River; post-2000) in the northwest (Appendix Table A8). Again, no data are available for rivers on the east side of the northern peninsula near the proposed boundary, and proportion of repeat spawners appears to be high in some areas of the northwest and low in some areas of the northeast, therefore the specific boundary on the tip of the northern peninsula is not highly supported based on these data. Nonetheless, the lack of data for the east side of the northern peninsula does not provide support for or against a new revised boundary either.

Additional support for previously differentiating the northwest (DU 6) was that this area had a small multi-sea-winter (MSW) component; however, data from DFO suggests that rivers in northeast (DU 3) also have a small MSW component. For example, in DU 6 for rivers which there are more than 100 individuals sampled, the percentage of MSW (based on maiden salmon; large and small) ranges from 0 (St. Genevieve; 1980–99) to 6% (Lomond; 1980–99) (Appendix Table A9). For rivers in the northeast (DU 3), this value ranges from 0 (Middle Brook and Northwest River; 1980–99) to 4% (Terra Nova; post-2000) (Appendix Table A9). Therefore, there does appear to be a small MSW component in rivers in the northeast and northwest, and thus this does not provide strong support for or against a specific boundary between these regions, and again, there is no data for the eastern side of the Northern Peninsula.

Differences in habitat were also previously suggested between the northeast and northwest DUs, where the northwest (DU 6) habitat was suggested to be significantly more alkaline than the rest of the island of Newfoundland due to the limestone geology (COSEWIC 2010). However, based on the geology of the region (Honsberger et al. 2019), we would expect many rivers on the northern peninsula (east and west sides) to have similar water chemistry that may differ from other parts of the northeast. Thus, this does not provide strong support for the specific boundary on the tip of the northern peninsula, and instead would support the revised boundary proposed here.

In addition, the proposed boundary is also supported by climate-linked differences and genomic-based differences (as discussed in detail above). This information was not available in COSEWIC (2010); however, it provides additional support for evolutionary significant differences between DU 3 and DU 6 based on the revised boundary.

While there was limited support for the specific location of the previous boundary between DU 3 and 6, our data provide better support for a specific boundary. Our proposed boundary is

supported by genetic data (microsatellite and 96 SNP) confirming discreteness between the revised regions. In addition, we show strong support for genomic differences, climate-linked differences, and differences between the geology of the regions. Overall, we argue that the new data presented here provides stronger support for differences between the northeast and northwest regions (see Table 3 for summary).

DU 4 South Newfoundland (previous): Two Proposed DUs – South Newfoundland (East) and South Newfoundland (West)

This DU extends from Mistaken Point (approximately 46°38’ N, 53°10’ W) at the southeast tip of the Avalon Peninsula, westward along the south coast of Newfoundland to Cape Ray (approximately 47°37’ N, 59°19’ W). In this DU, freshwater habitats generally have lower pH values (5.0–6.0) compared to the neighbouring DU in northeast Newfoundland. The conditions experienced in the ocean are also generally distinct from nearby DUs, as salmon in south Newfoundland encounter ocean conditions influenced by the Gulf Stream instead of the Labrador Current. At the time of the last COSEWIC assessment, population size trends for south Newfoundland rivers differed from trends in other areas of insular Newfoundland. On the south coast of the island, there is variation in life history including run timing, smolt age, the proportion of female grilse, and migration routes along the coast; however, previous reports suggest no clear geographic pattern within the DU (Chaput et al. 2006).

At the time of the last COSEWIC assessment, genetic data suggested that populations along this coast have reduced gene flow among local rivers (between south coast river), as well as between these rivers and other regions of Newfoundland (Palstra et al. 2007). Various studies suggested high levels of population structure within southern Newfoundland compared to other parts of the island (Verspoor 2005; Adams 2007; Palstra et al. 2007), and while available data did not support it, the status report suggested the potential for future subdivision of the south Newfoundland DU (COSEWIC 2010). Recent genomic and genetic studies continue to support the high level of population structure in south Newfoundland. Various studies support the genetic differences between sites in the east and west (Moore et al. 2014; Bradbury et al. 2015), with recent studies supporting up to three or four genetically distinct groups within south Newfoundland (Jeffery et al. 2018; Bradbury et al. 2021).

There are 104 known salmon rivers in south Newfoundland DU 4 (COSEWIC 2010), and our genetic datasets comprise 46 and 35 locations (96 SNPs and microsatellites, respectively). Using 15 microsatellite markers, the optimal number of genetic clusters (K) was 2; however, there was also support for more than 10 genetic clusters (Appendix Figure A13). At K=2, Northeast Brook Trepassey is clustered separately from other sites. At K=3, additional clustering separates sites east and west of the Burin Peninsula, where sites from Garnish eastward form a separate genetic cluster from those westward. Further clustering begins to separate sites in Fortune Bay and Bay D’Espoir from other regions, and at higher values of K, many rivers form their own genetic clusters. Using the 96 SNP dataset, there was evidence of two distinct genetic clusters (K=2) separating sites east and west of the Burin Peninsula with the break occurring near Garnish (Appendix Figure A14). Further structure was supported and three main genetic clusters were apparent at higher values of K, which included:

1. sites west of Garnish,
2. sites from Garnish eastward to the Avalon, and
3. sites on the Avalon.

These groupings were apparent at K=3 with some populations deviating from this general pattern. Higher values of genetic clustering (K) appeared to generally separate specific populations into discrete clusters.

Based on these analyses and our decision tree, criteria for multiple genetic clusters (discreteness) within south Newfoundland are met. There is clear evidence of the discreteness between the east and west with a genetic break at Garnish, as well as some additional evidence of discreteness within the Avalon Peninsula and west of Bay d'Espoir (see Figure 8). Further, additional structure was observed that separated individual rivers and/or geographic regions within these clusters.

To evaluate evidence of evolutionary significance, we rely on high-density genomic data, life history, and/or climate data. Within south Newfoundland DU 4, high-density genomic data (220,000 SNP array) were available for 33 sites spanning all regions, although many sites are located from Conne River eastward, with only two sites west of Conne River. Using *pcadapt*, populations were separated across both PC axes, where populations appeared to be separated between the east and west of Placentia Bay as well as between north and south within Placentia Bay (Appendix Figure A15). A total of 1582 loci significantly contributed to the differentiation on both PC axes (adjusted *p*-value [*q*-value] <0.05) and these loci were distributed across 28 of the 29 chromosomes (Appendix Figure A15). Notably, over 70% of these outliers were located on Ssa01 and Ssa23, which are involved in a known chromosomal rearrangement (chromosomal translocation) with variation that exists between individuals and is associated with European introgression in North America (Lehnert et al. 2019a) (see Appendix Figure A16 for schematic diagram describing these chromosome differences).

We further explored variation in this translocation within south Newfoundland using PCA (*pcadapt*) with loci from the translocated region on Ssa01 and Ssa23. Similar to previous work (Lehnert et al. 2019a; Watson et al. 2022), the first PC axis separated the three genotype groups representing different arrangements (karyotypes) of the Ssa01/23 translocation (see Appendix Figure A17 for details). The frequency of the European type chromosome (Ssa01/23 non-translocated; standard European karyotype) was low west of the Burin Peninsula-indicating low historical European introgression in this region of the genome in these rivers. For instance, Conne River had the lowest frequency (<2%), and other sites west of the Burin Peninsula had <15% frequency. The frequency of the European type increases at the Burin Peninsula (and eastward) with the greatest frequency occurring in the eastern part of Placentia Bay - indicating high historical European introgression (in this region of the genome) in this geographic region. Sites including Ship Harbour (SHI), Little Barasway Brook (LBB), Little Barasway Brook (GBW), Little Salmonier (LSR), Branch (BRA), South Placentia River (SPR), and Lance (LAN) had a frequency of >70% of the European chromosome type within the population. While this rearrangement has clear associations with European secondary contact with evidence of selection acting on this genomic region (Lehnert et al. 2019a), this rearrangement is also associated with climate variation in southern Newfoundland (Watson et al. 2022). This increase in translocation frequency at the Burin Peninsula is also consistent with an increase in a European mitochondrial haplotype in the same geographic region (Bradbury et al. 2015).

Overall, the presence of large-scale differences in the frequency this chromosomal rearrangement, which is linked to European introgression, selection, and climate, between the eastern and western part of south Newfoundland DU 4 shows strong support for differences in genomic adaptation and supports criteria of evolutionarily significant differences between the east and west of south Newfoundland. Based on our decision tree, we also examined evidence of life history and climate-linked differences within the DU.

DFO and MNRF (2008) suggest that south Newfoundland is represented by two CUs of Atlantic Salmon, which are separated at the Burin Peninsula consistent with our genetic and genomic evidence. The report indicates potential differences in modal smolt age between the regions (DFO and MNRF 2009). However, based on compiled data for small salmon (majority of returns), sites east and west of the Burin show similar ranges in mean smolt age (east: 2.93–3.57 years; west: 2.92–3.51 years) (Chaput et al. 2006). DFO and MNRF (2008) suggest

variation in run timing, with some rivers in the west having earlier run timing compared to some rivers in the east having run timing comparable to southern Labrador populations, which is consistent with Dempson et al. (2017). For instance, Conne River (in the west) has the earliest run timing across sampled populations in Newfoundland and Labrador and was similar to another nearby river (Little River), whereas a river in the east (Northeast Brook Trepassey) has the latest run timing, which was approximately five weeks later, although no other rivers were sampled in the east (Dempson et al. 2017). DFO and MNRF (2008) suggest sites west of the Burin are characterized by smaller-sized grilse, whereas sites east of the Burin are characterized by stocks with small grilse as well as larger-sized grilse. Data also suggests there is a higher proportion of small repeat spawners in the east (mean ~13%) compared to the west (mean ~5%) (see Kelly et al. in prep[3] for details). Overall, the compiled data suggest some differences in life history between the east and west of the south Newfoundland DU; however, generally, limited life history data exists for rivers in both of these regions (Hutchings and Jones 1998).

In addition to life history, ecological differences between the two regions were also reported by DFO and MNRF (2008). Ecological differences include differences in alkalinity, with rivers west of the Burin Peninsula having low mean alkalinities with average pH values often <5.5 and thus less than rivers found to the east of the Burin Peninsula (DFO and MNRF 2008). In addition, differences in river size was reported, where rivers east of the Burin Peninsula have relatively small drainage areas (<300 km^2) with only a few >400 km^2 in size; whereas west of the Burin Peninsula, river drainage area range from moderate (1,000 to 2,500 km^2) to small (<300 km^2). While these factors are not directly included in our decision tree, these variables, such as river catchment size and water chemistry (Bradbury et al. 2014; Pritchard et al. 2018), are relevant to influencing adaptive variation in salmon populations and are thus reported here.

Climate data for south Newfoundland also support differences between the two main genetic clusters that are split near Garnish River (Appendix Figure A18). RDA was performed using 19 bioclimatic variables (see Appendix Table A1) for all rivers in south Newfoundland as the response and putative DU groups (two genetic clusters) as the constraining variable. ANOVA on the RDA showed the model to be significant ($p<0.001$) with an adjusted R^2 of 0.40. RDA axis 1 explained 38.4% of the variance in the model and clearly shows the split between the putative new DUs (rivers east and west of Garnish). This difference on RDA axis 1 is driven by variation in temperature (annual temperature range, minimum temperature, mean temperature of coldest quarter, and variables associated with temperature variation) and precipitation (precipitation of the wettest quarter). Generally, there was greater precipitation, more temperature variability, and colder winter temperatures west of Garnish. Some additional separation on PCA axis 2 resulted in separation of sites in the putative western DU. Altogether, these results support clear differences in climate that are linked to the two main genetic groups.

Overall, our analyses suggest that there are two discrete and evolutionarily significant units (DUs) within south Newfoundland (previously DU 4), which are separated along the Burin Peninsula and include:

1. sites from Garnish River (inclusive) eastward, and
2. sites west of Garnish River.

Discreteness of these two proposed DUs is supported by genetic data, and evolutionary significance is supported by genomic evidence of adaptation, climate-linked differences, habitat, and to some extent life history differences.

Some support for additional DUs (sites on the Avalon and west of Bay d'Espoir) were evident that may warrant separation in the future when more data are available. It is worth noting that the current eastern boundary in south Newfoundland is at the southeast tip of the Avalon

Peninsula near Cape Race, but sampling is limited beyond this boundary (i.e., northern portion of the Avalon). It is possible that the Avalon Peninsula as a whole (including the northern portion) may represent its own DU in the future given its unique underlying geology and higher incidence of European ancestry, as well as populations with divergent life history characteristics. However, at this time we do not have data (including genetic/genomic data and life history data) from rivers in other parts of the Avalon Peninsula (i.e., northern portion) thus limiting our ability to make inferences here. At this time, we suggest that there is not enough data to designate the Avalon Peninsula as its own DU.

In addition, we recognize that Northeast Brook Trepassey represents a special case where this river could potentially be characterized as its own DU. Many genetic studies have identified the genetic uniqueness and discreteness of this river from other systems in Newfoundland (Palstra et al. 2007; Bradbury et al. 2014; Bradbury et al. 2015). Pairwise F_{ST} values at 15 microsatellite loci suggest it is highly divergent from all other sites in south Newfoundland (F_{ST}>0.091). This population is characterized by a small run of anadromous salmon (Robertson et al. 2013), and suspected to have a high proportion of precocious male parr which is similar to other populations in this region (Dalley et al. 1983; Johnstone et al. 2013). This river is characterized by the highest proportion of European mitochondrial haplotype in the region based on a SNP that differentiates European and North American salmon (Bradbury et al. 2015). However, this river is not characterized by a high proportion of European ancestry based on the chromosomal rearrangement (Ssa01/23) examined here (see Appendix Figure A17). More data are needed to better understand the European ancestry of this population. While microsatellite data show that this population is genetically unique, genome-wide data fail to detect differences between Northeast Brook Trepassey and nearby rivers (Bradbury et al. 2015), suggesting that genetic differences may be due to genetic drift and small population size rather than adaptive differences. Indeed, we re-evaluated our PCA analysis (*pcadapt*) without the inclusion of Ssa01/23 SNPs that were responsible for driving differences in south NL. Based on this analysis, we did not find that Northeast Brook Trepassey was differentiated from nearby rivers, again suggesting limited differences in genomic-based adaptation. The lack of differences based on genomic data agrees with previous work using other genomic datasets including RAD-seq and a 6,000 SNP array (Bradbury et al. 2015). Life history characteristics that make this population evolutionarily significant include later run timing compared to other populations in the region (B. Dempson, personal communication), although these differences are not statistically significant (Dempson et al. 2017). In addition, for the small salmon category, mean smolt age for this river was 3.57 years, which was the highest within this region (range for other sites east of Garnish [10 rivers]: 2.93–3.33 years) (Chaput et al. 2006). For large and small salmon combined, proportion of four year old smolts was 0.489 (the dominant smolt age year class for this river), whereas for other sites in the region the proportion was <0.354 and the majority of smolts in these rivers were three years old (Chaput et al. 2006). Habitat characteristics are generally similar between Northeast Brook Trepassey and other south coast rivers (i.e., temperature, turbidity, pH, precipitation, and other variables), with the exception of watershed size, as Northeast Brook Trepassey is a very small river (Bradbury et al. 2014). While this population is genetically discrete, there is not enough data to support the evolutionary significance of this population at this time.

DU 5 Southwest Newfoundland (previous): Unchanged

This DU extends from Cape Ray (approximately 47°37’ N, 59°19’ W) northwards along the west coast of Newfoundland to approximately 49°24’ N, 58°15’ W. This is the only region within the island of Newfoundland with a significant number of multi sea-winter (MSW) salmon (Dempson and Clarke 2001) and limited lacustrine habitat. In addition, this DU also has the youngest mean smolt ages (three years) and lower proportion of female grilse on the island of Newfoundland.

At the time of the last COSEWIC assessment, genetic comparisons suggested DU 5 was genetically distinct from other populations on the Island, and populations within DU 5 appeared to have higher rates of gene flow relative to populations within DU 3 and within DU 4 (Verspoor 2005; Palstra et al. 2007). Recent genetic and genomic studies continue to support the genetic distinctiveness of this DU (Bradbury et al. 2014; Jeffery et al. 2018; Bradbury et al. 2021).

There are 40 known salmon rivers in DU 5 (COSEWIC 2010), and our genetic datasets include seven locations (microsatellites) and five locations (96 SNPs). Using 15 microsatellite markers, the optimal number of genetic clusters (K) was 2; however, additional structure could be observed up to K=7, which generally supported each site representing a separate cluster with evidence of admixture among many of the sites suggesting most clusters were not clearly distinct (Appendix Figure A19). Using the 96 SNP dataset, the optimal number of clusters (K) was 2, which separated Pinchgut (Harry's River) from other sites. Additional structure was observed up to K=4, which began to separate most sites, although clusters were generally not clearly distinct (Appendix Figure A20). Based on these analyses and our decision tree, criteria for multiple genetic clusters (discreteness) within DU 5 are met. However, we note that while some sites showed genetic differentiation from other sites in the DU across different analyses, there was no consistent genetic break associated with geography. Nonetheless, to ensure the current boundaries of the DU were appropriate, we re-evaluated the boundary with DU 6 (northwest Newfoundland), as there has been some evidence that one river in DU 6 (Lomond) near the boundary may show genetic affinity to rivers in DU 5 (Jeffery et al. 2018; Bradbury et al. 2021). We ran STRUCTURE with microsatellites and 96 SNPs for all locations in DU 6 and DU 5. For the microsatellites, the optimal number of genetic clusters (K) was 8 suggesting high levels of genetic structure on this coast, and demonstrating that sites in DU 6 were distinct from DU 5 (Appendix Figure A21). At lower values of genetic clustering (K), there were some genetic similarities among some sites in DU 6 and DU 5. However, these sites were not located at the boundary of these DUs and genetic differences were seen at higher levels of K. For the 96 SNPs, the optimal number of genetic clusters (K) was 2, which separated the majority of sites in DU 6 from those in DU 5 (Appendix Figure A22), including sites located closest to the boundary between these DUs. One site in DU 5 (Harry's River-Pinchgut) showed genetic affinity to sites in DU 6, although genetic differences between these regions were found at higher values of genetic clustering (K=6). While these results suggest some heterogeneity in genetic signals along the west coast of Newfoundland, they do not provide strong support for changing the boundary. For both datasets, samples from sites nearest the boundary (Lomond and Humber Rivers) show genetic differences. In addition, the boundary between DU 6 and DU 5 represents a region where an important break point in migration phenotype occurs. In this region near the boundary, adults salmon from populations in DU 6 migrate from the north through the Strait of Belle Isle to rivers; whereas adults from populations in DU 5 migrate to rivers from the south (Pippy 1982). Smolts are expected to follow similar migrations paths. While the exact break point is unknown, these large-scale differences in ocean migration influence the conditions encountered at sea and represent an evolutionarily significant difference between these regions that supports the current boundary and provides support for the criteria of discreteness between DU 6 and DU 5. Additional support for the significance of DU 5 from other DUs include that this DU has the highest proportion of large maiden spawners (range 19–87%) compared to other Newfoundland DUs, consistent with a higher proportion of large MSW salmon in this region (Kelly et al. in prep[3]). Further, as indicated above, this DU also has the youngest smolt ages and lower proportions of female 1-SW (or small) salmon on the island of Newfoundland (COSEWIC 2010; Kelly et al. in prep[3]).

Therefore, given support for discreteness and significance of DU 5, we next focus on differences within DU 5. Within DU 5, there were some genetic differences among sites but with no consistent genetic break associated with geography. To evaluate evidence of evolutionary significance, we rely on high-density genomic data and/or life history and climate data.

High-density genomic data (220,000 SNP array or whole-genome resequencing) were only available for three sites within DU 5, and thus we did not have high enough geographic coverage to assess these data. Life history data were generally limited for populations in DU 5. There was evidence for older smolt ages at higher latitudes within the DU, with a range of 2.7–3.8 years (Hutchings and Jones 1998; Chaput et al. 2006). Mean sea age among populations ranged from 1.02–1.47 years, although few rivers were included making inferences difficult (Hutchings and Jones 1998). Given the lack of a clear genetic break associated with geography within DU 5, we did not pursue evaluation of climate differences, as clear geographic groups were not defined.

Overall, our analyses support one discrete and evolutionarily significant unit (DU) within DU 5. While criteria for discreteness were met for individual rivers there was no consistent genetic break associated with geography, and data to support significance were lacking.

DU 6 Northwest Newfoundland (previous): Revised Boundary

This DU extends northward from approximately 49°24’ N, 58°15’ W along the west coast of Newfoundland to the tip of the Great Northern Peninsula (approximately 51°37’ N, 55°25’ W). For populations in this DU, smolt migration is expected to occur northward through the Strait of Belle Isle (COSEWIC 2010). There is variation in life histories within this DU, which are generally intermediate between Labrador (previously DU 2) and Southwest Newfoundland (previously DU 5) (Chaput et al. 2006). Within DU 6, the freshwater habitat is significantly more alkaline than the rest of insular Newfoundland, due to the prevalence of limestone in the region. There are several populations within this DU that have a MSW component, including Big East, St. Genevieve, and River of Ponds.

At the time of the last COSEWIC assessment, genetic data for this DU were sparse. Recent genetic and genomic studies support a distinct genetic group on the northern peninsula, although one site in the southern portion of DU 6 (Lomond River) grouped with DU 5 (Bradbury et al. 2014; Bradbury et al. 2021). However, few rivers have been sampled between Lomond River and more northern regions (River of Ponds). Further, this genetic group of the northern peninsula also included one site from DU 3 (Beaver Brook) (Bradbury et al. 2014; Bradbury et al. 2021). Jeffery et al. (2018) reported similar population clusters, with further subdivision of northern sites (Western Arm Brook and St. Genevieve) from other sites in DU 6.

Based on our evaluation of Northeast Newfoundland (DU 3), we have re-assessed and revised the boundary between Northeast and Northwest Newfoundland DUs, as described in detail above (see *Re-assessing the boundary between DU 3 and 6*). Overall, our analyses support one discrete and evolutionarily significant unit (DU) within DU 6; however, based on the re-assessment of the DU boundary (see above for details), we have revised the boundary for Northwest Newfoundland to incorporate sites from Northeast Newfoundland (along the northern peninsula) (Figures 6 and 7). The support for discreteness and evolutionary significance of these two DUs with revised boundaries are reported in detail above. Within the proposed Northwest Newfoundland DU, the northern boundary now extends to sites near Beaver Brook. Data supporting the revision to the boundary are detailed above and summarized in Table 3, and include genetic and genomic differences, climate-linked differences, life history differences, and differences between the geology of the regions. In addition, we also re-evaluated the boundary between DU 6 and DU 5; however, no changes were made to this boundary (see above: *DU 5 Southwest Newfoundland (previous): Unchanged*).

DU 7 Quebec Eastern North Shore (previous): One River Added

This DU extends from the Napitipi River (not inclusive) westward along the north shore of the St. Lawrence to the Kegaska River (inclusive) in the west. Previously, Dionne et al. (2008) used

microsatellite markers, temperature, difficulty of river ascension (migration), and the percentage of fish that mature as 1-SW to differentiate among regions of the North Shore. This DU is characterized by populations with higher proportions of 1-SW salmon and rivers with lower temperature regimes relative to the neighbouring North Shore DU (recognized as DU 8). Populations in this DU are generally characterized by a shorter generation time (five years) compared to nearby populations in the Southern Labrador DU (six years) due to differences in age of smoltification (April et al. 2023).

At the time of the last COSEWIC assessment, the genetic data also suggested these populations have lower levels of gene flow within the DU than within other areas of the North Shore (Dionne et al. 2008) (mean F_{ST}=0.037 versus 0.027 in DU 8). Recent genetic studies confirm the distinctness of this DU from Labrador (DU 2), but these studies did not identify differences between this DU and the neighbouring North Shore DU (DU 8) (Jeffery et al. 2018; Bradbury et al. 2021). Nonetheless, differences between DU 8 and DU 7 are supported by a larger SNP dataset (Moore et al. 2014).

There are 20 known salmon rivers in DU 7 (COSEWIC 2010), and our genetic datasets include: five locations (96 SNPs) and three locations (microsatellites). Using 15 microsatellite markers, the optimal number of genetic clusters (K) was 2, and no additional structure was observed beyond this (Appendix Figure A23). At K=2, the most western site, Musquaro (MUQ), clustered separately from the two other sites (Etamamiou, ET; Gros Mecatina, MEC). Using 96 SNPs, the optimal number of genetic clusters (K) was 4 (Appendix Figure A24). Clustering separated the most western sites, Musquanousse (MUS) and Musquaro (MUQ), from other sites as well as from each other, but clustering patterns showed populations were not clearly distinct in this DU. Based on these analyses and our decision tree, criteria for multiple genetic clusters (discreteness) within DU 7 are met.

To evaluate evidence of evolutionary significance, we rely on high-density genomic data and/or life history and climate data. High-density genomic data (220,000 SNP array or whole-genome resequencing) were not available for any sites in DU 7 based on COSEWIC (2010) boundaries. Life history data was generally limited for populations in DU 7. Data from three populations (Gros Mecatina, Etamamiou, and Olomane) showed similarities in smolt ages (3.43–3.66 years) with some differences in sea age (1.08–1.93 years) (Hutchings and Jones 1998). Dionne et al. (2008) reported differences among the Quebec DUs in the proportion of 1-sea-winter (1-SW) salmon based on data from Ministère des Ressources Naturelles et de la Faune du Québec (MRNF) from 2004 (Caron et al. 2005). This dataset includes 16 rivers within DU 7 and while it shows variability in proportion of 1-SW salmon among rivers, no clear geographic pattern is present (Caron et al. 2005). Overall, clear evidence of life history differences within DU 7 are lacking. Given the lack of a clear genetically associated geographic break within DU 7, we did not pursue evaluation of climate differences (but see section below on *Re-assessing the boundary between DU 7, 8, and 10*).

Overall, our analyses support one discrete and evolutionarily significant unit (DU) within the recognized DU 7. While criteria for discreteness were met for some rivers, available data did not support evidence of significance. Therefore, we suggest that DU 7 should remain a single DU. In addition, based on our analysis and those of previous studies (Dionne et al. 2008), we suggest that Corneille (currently in DU 8) should be moved into DU 7, resulting in a non-contiguous boundary (see section below on *Re-assessing the boundary between DU 7, 8, and 10* for more details).

DU 8 Quebec Western North Shore (previous): Revised Boundary

This DU extends eastward from the Natashquan River (inclusive) along the Quebec North Shore to the Escoumins River in the west (inclusive). The salmon of DU 8 have the highest

proportion of MSW salmon relative to the populations in the other Quebec DUs (COSEWIC 2010; April et al. 2023).

At the time of the last COSEWIC assessment, genetic data from microsatellite as well as habitat and life history data separated this region of the North Shore from DUs 7 and 10 (Dionne et al. 2008). The eastern and western edge of the DU appeared to be a transitional area to DU 7 and DU 10, respectively (Dionne et al. 2008), and did not have a clear geographic feature as a boundary. Recent genetic studies show support that sites in DU 8 and DU 7 (Quebec eastern north shore) are one genetic group (Jeffery et al. 2018; Bradbury et al. 2021), although other studies suggest genetic differences between DU 8 and DU 7 sites (Dionne et al. 2008; Moore et al. 2014). In addition, two sites at the western edge of DU 8 (i.e., Laval and Escoumins) often show affinity to DU 10 or represent their own genetic cluster (Moore et al. 2014; Jeffery et al. 2018; Bradbury et al. 2021). Overall, some ambiguity to the boundaries of DU 8 is evident.

There are 25 known salmon rivers in DU 8 (COSEWIC 2010), and our genetic datasets include 11 (microsatellites) and 12 (96 SNPs) sites. Using 15 microsatellite markers, the optimal number of genetic clusters (K) was 3, however additional structure was supported up to K=6 (Appendix Figure A25). At K=3, Laval, Aux Anglais, and Corneille started to separate from each other as well as other sites, although admixture signals were present. These sites remained the main source of separation at higher values of K. Some differences could be seen between east and west sites; however, sites showed high levels of admixture. Using 96 SNPs, the optimal number of genetic clusters (K) was 2, however additional structure was supported up to K=8 (Appendix Figure A26). Here the westernmost sites (Escoumins and Laval) clustered together and separately from other sites. Further, similar to microsatellites, Corneille and Aux Anglais clustered separately from each other and other sites. Sites east and west of Corneille formed separate clusters with admixed signals, but additional structure beyond this was not as clear. Based on these analyses and our decision tree, criteria for multiple genetic clusters (discreteness) within DU 8 are met.

Based on evidence of discreteness and recent genetic studies (Bradbury et al. 2021), there appears to be evidence that westernmost sites (Laval and Escoumins) may belong in DU 10. Therefore, we have re-evaluated the boundaries of DU 8. Additional support for pursuing this analysis also comes from the previous COSEWIC assessment, which indicated that DU 8 did not have clear geographic features at the boundaries with DU 7 and DU 10, and thus these were somewhat ambiguous (COSEWIC 2010).

Re-assessing the boundary between DUs 7, 8, and 10

To re-evaluate the DU boundary between DU 7, 8, and 10, we ran STRUCTURE separately for the 96 SNPs and 15 microsatellite datasets with sites from all three previously recognized DUs using K=3. For both datasets, genetic differences were consistent with a revised boundary between DU 10 and 8, but no changes to the boundary between DU 7 and 8 were justified (Figures 9 and 10). Both Laval and Escoumins clustered more closely with sites in DU 10 (Inner St. Lawrence). In addition, we note that one site in DU 8, Corneille, was genetically similar to sites in DU 7 (as discussed above). Corneille was not distinct from DU 7 sites until much higher values of genetic clustering (K=6 for both datasets). Based on these analyses, a revised DU boundary between DU 8 and 10 is supported by discreteness, and there was support to move Corneille into DU 7.

We also evaluated whether these changes could meet criteria of evolutionary significance. High-density genomic data (220,000 SNP array or whole-genome resequencing) was available for eight populations overall in DU 8 and 10, but no sites in DU 7 based on COSEWIC (2010) boundaries. Using *pcadapt*, Corneille was clearly separated from all other sites along the first PC axis, and other sites in DU 8 (revised boundary) were clustered closely but separately from DU 10 (revised) sites on PC 2 (Appendix Figure A27). Given that Corneille appears to be

genetically similar to sites in DU 7, this analysis further supports the division of the three DUs along the north shore of Quebec (DUs 7, 8, and 10) and supports the placement of Corneille into DU 7. To better evaluate the differences between DU 8 and DU 10, we removed Corneille and re-ran the analysis. Without Corneille, differences between the revised DU 8 and DU 10 were clear as populations were separated along the first PC axis (Appendix Figure A28). Further separation of sites in DU 8 occurred along PC 2, with sites in DU 10 generally clustering closely on both PC axes. A total of 222 loci significantly contributed to the differentiation on PC 1 thus differentiating the two DUs based on the revised boundaries (adjusted *p*-value [*q*-value] <0.05) and these loci were distributed across all chromosomes (Appendix Figure A28). Over-representation of biological processes associated with the genes located near the outlier loci were examined using topGO (Alexa and Rahnenfuhrer 2016). A total of 79 biological processes were significantly ($p<0.05$) over-represented based on the outlier dataset, with a large proportion of processes related to 'regulation of secondary metabolite biosynthesis' and 'maternal determination of anterior/posterior axis, embryo' (Appendix Figure A29). Secondary metabolites are produced by plants and microorganisms and can affect fish nutrition, thus this GO term may relate to metabolism in fish (Vera et al. 2017). Maternal determination of anterior/posterior axis relates to embryonic development guided by maternally expressed genes.

For DU 8 and DU 10, life history data from 13 rivers (large salmon category) and nine rivers (small salmon category) showed an increase in smolt age from the west to the east (Chaput et al. 2006). For example, the westernmost sites (Betsiamites, Laval, and Escoumins) had >40% and >60% two year old smolts for large and small salmon categories, respectively (Chaput et al. 2006); whereas, the percentage of two year old smolts in other populations (east of Betsiamites) was <34% (range 0–34%) and <29% (range 0–29%) for large and small salmon categories, respectively (Chaput et al. 2006). Mean sea age was variable with no clear pattern across geography based on 17 rivers for which data were available (Hutchings and Jones 1998). Dionne et al. (2008) reported differences between DU 8 and DU 10 in the proportion of one-sea-winter (1-SW) salmon; however, this difference was not significant. Overall, evidence of earlier smolting in westernmost site supports life history differences between DU 8 and DU 10 based on revised boundaries.

Based on genetic, genomic, and life history differences, we suggest that the boundary between DU 8 and 10 should be moved eastward. To evaluate climate differences between the revised DUs with new boundaries, we suggest that Betsiamites, Laval, and Escoumins should be moved into DU 10 for the analysis. Climate data for DU 8 and DU 10 also supports differences using the revised DU boundaries (Appendix Figure A30). RDA was performed using 19 bioclimatic variables (see Appendix Table A1) for all rivers in DU 8 and 10 as the response and putative DU groups (two groups–based on revised boundary) as the constraining variable. ANOVA on the RDA showed the model to be significant ($p<0.001$) with an adjusted R^2 of 0.22. RDA axis 1 explained 24.3% of the variance in the model and clearly shows support for moving Escoumins, Laval, and Betsiamites from DU 8 into DU 10 (Appendix Figure A30). This difference on RDA axis 1 is driven by temperature during the warmest times of the year, with maximum temperature, mean temperature of the quarter, mean temperature of the wettest quarter and temperature seasonality loading highly on the RDA axis. This indicates that summer temperatures are warmer in DU 10 compared to DU 8, which is consistent with previous assessment of these DUs which found higher temperatures during the growing season in DU 10 compare to DU 8 (COSEWIC 2010), which may also relate to differences in smolt ages between these regions.

Overall, our analyses support one discrete and evolutionarily significant unit (DU) within the Quebec Western North Shore (DU 8); however, the boundary of this unit with Inner St. Lawrence (DU 10) should be revised. The boundary of DU 8 should extend from the

Natashquan river (inclusive) to Betsiamites (exclusive). The exact position of this boundary can only be inferred from current data, which is supported by life history differences, genetic data, and climate differences. Based on this revised boundary, criteria for discreteness and significance of DU 8 from neighbouring DU 10 were met.

In addition, based on our analysis and those of previous studies (Dionne et al. 2008), we suggest that Corneille (currently and physically in DU 8) should be moved into DU 7, resulting in a non-contiguous boundary. There is clear evidence that Corneille is genetically similar to populations in DU 7. In addition, there is evidence of genomic-based differences between Corneille and populations in DU 8 and DU 10. These differences cannot be explained by stocking between these regions, as stocking has not occurred in Corneille River (Ministère des Forêts, de la Faune et des Parcs du Québec, unpublished data). In addition, the headwater of Corneille is small and does not reach watersheds located in DU 7, suggesting no physical connectivity. Based on current data, there are no clear differences in life history of Corneille River from other nearby populations.

DU 9 Anticosti (previous): Unchanged

This DU encompasses Anticosti Island. The freshwater habitat in this DU is characterized by a lower gradient than that of nearby rivers in Quebec Eastern North Shore (previously recognized DU 7) and lower temperatures compared with several adjacent DUs (COSEWIC 2010).

At the time of the last COSEWIC assessment, genetic data available from Dionne et al. (2008) showed divergence of Anticosti populations from neighbouring DUs. These data also suggested that gene flow between Anticosti populations was high, with no significant differences in genetic differentiation among several rivers (F_{ST}=0.002). The genetic distinctness of Anticosti populations from other regions is also supported by other recent studies (Moore et al. 2014; Jeffery et al. 2018; Bradbury et al. 2021).

There are 25 known salmon rivers on Anticosti Island (COSEWIC 2010), and our genetic datasets include three of these populations, including Jupiter, Aux Saumons, and Chaloupe. More than half of the salmon in this DU are concentrated in these three populations. Using 15 microsatellite markers and the 96 SNP dataset, there was no evidence of genetic structure within the Anticosti DU (Appendix Figure A31 and A32; respectively). Based on these analyses and our decision tree, criteria for multiple genetic clusters (discreteness) within the DU are not met, and thus Anticosti should remain as a single DU.

Evidence continues to support the evolutionary significance of the Anticosti DU. For example, Bourret et al. (2013) demonstrated that geological parameters of Anticosti rivers were distinct from rivers in Quebec and Labrador. Further, the genetic divergence of Anticosti from other populations was strongly linked to these geological parameters, where analyses were performed with outlier SNPs putatively under divergent selection (Bourret et al. 2013). In addition, COSEWIC (2010) indicates that the freshwater habitat on this island is characterized by a lower gradient than that of nearby rivers and lower temperatures compared with several adjacent DUs. However, in terms of temperature, Anticosti's freshwater habitat is similar to the Quebec Eastern North Shore (based on degree days: 945 versus 938) but is cooler than other Quebec DUs (DUs 8, 10, 12) (COSEWIC 2010). Salmon from rivers on Anticosti are also smaller bodied compared to salmon from other Quebec DUs (April et al. 2023).

DU 10 Inner St. Lawrence (previous): Revised Boundary

Based on the last COSEWIC assessment, this DU extends west along the northern shore of the St. Lawrence from the Escoumins River (not included) into the lower St. Lawrence River and returns eastward along the southern shore of the St. Lawrence to the Ouelle River (included). This DU is characterized by a higher proportion of 1-SW salmon compared to the

neighbouring DU 8, and also has a lower mean age of smoltification. Recent data continue to support that smolts are younger in Inner St. Lawrence DU (mean 2.22 years across 4 rivers) compared to the rest of Quebec rivers, and the youngest reported smolts (mean smolt age) in Quebec are found in Riviere Jacques-Cartier (2.00 years old) within this DU (April et al. 2023). This contrast both neighbouring DUs where smolt are older (Gaspe mean 3.15 years; Western North Shore mean 3.08 years) (April et al. 2023). Consistent with younger smolts and higher proportion of 1-SW salmon, this DU has the shortest generation time (four years) compared to other Quebec DUs (April et al. 2023). This DU is also characterized by freshwater habitats that are the warmest along the Quebec North Shore. This DU encompasses four CUs; however, evidence to separate these CUs was based on preliminary genetic data and not life history or ecological differences (DFO and MNRF 2008).

At the time of the last COSEWIC assessment (COSEWIC 2010), genetic data from Dionne et al. (2008) suggested that gene flow was limited between this DU and both neighbouring DUs (DU 8 and 12), and differences in temperature between the regions existed. Recent genetic studies agree with differences between this DU and the neighbouring DU in Gaspé (Moore et al. 2014; Jeffery et al. 2018; Bradbury et al. 2021). However, it was recognized that the boundary between DU 10 and DU 8 was not clearly defined (COSEWIC 2010). Based on our evaluation of DU 8 and recent genetic studies (Bradbury et al. 2021), we have re-assessed and revised the boundary between DU 8 and 10, as described in detail above (see *Re-assessing the boundary between DU 7, 8, and 10*). Based on above analysis of DU 8 and DU 10 together, we have revised the boundary of this DU to encompass three more rivers. These rivers include Escoumins, Laval, and Betsiamites, thus shifting the boundary between DU 10 and DU 8 eastward (Figure 9 and 10).

DU 12 Gaspé-Southern Gulf of St. Lawrence (previous): Two Proposed DUs – Gaspé and Southern Gulf of St. Lawrence-Cape Breton

This DU extends from the Ouelle River (excluded) in the western Gaspé to the northern tip of Cape Breton (approximately 47°02’ N, 60°35’ W). At the time of the last COSEWIC assessment, genetic data from Dionne et al. (2008) suggested that the Gaspé and northeastern New Brunswick represent a regional grouping with high levels of gene flow (mean F_{ST}=0.011). However, the study only included one river system (Miramichi) south of the Restigouche (Dionne et al. 2008), and thus almost all samples were from the Gaspé region. Nonetheless, at the time of the previous assessment, there was no evidence that the southeastern Gulf displayed genetic or life history divergence from the western Gulf of St. Lawrence. There was some evidence based on neutral genetic markers that rivers of western Cape Breton were potentially divergent from the western Gulf, however more data were needed to support this claim. Other genetic work supported little evidence of divergence within the region based on allozymes (Verspoor 2005), and thus the southeastern Gulf rivers were included with Gaspé in this DU. No genetic data were available for populations on Prince Edward Island (PEI). Many larger streams in PEI had been heavily stocked, and the life history characteristics of salmon in these streams were generally similar to those found elsewhere in the southeastern Gulf (Cairns et al. 2010), thus PEI salmon populations were placed within DU 12.

Recent genetic studies suggest that sites in Gaspé are genetically differentiated from sites in the southern Gulf (Moore et al. 2014; Jeffery et al. 2018; Bradbury et al. 2021). Genetic differences among other regions in the southern Gulf have not been reported in these studies.

There are 78 known salmon rivers in previously recognized DU 12 (COSEWIC 2010), and our genetic datasets include 47 (microsatellites) and 41 (96 SNPs) sites, some of which are within the same river systems. Using 15 microsatellite markers, the optimal number of genetic clusters (K) was 2, which separated sites in Gaspé (inclusive of Restigouche) from sites in the southern Gulf (Appendix Figure A33). Limited structure was supported beyond K=2, except for some

differentiation observed in a couple PEI populations at K=3 (Appendix Figure A33). Using 96 SNPs, the optimal number of genetic clusters (K) was 2, and no additional structure was observed beyond K=2 (Appendix Figure A34). At K=2, sites in Gaspé (inclusive of Restigouche) clustered separately from sites in the southern Gulf. Based on these analyses and our decision tree, criteria for multiple genetic clusters (discreteness) within DU 12 are met (see Figure 11).

To evaluate evidence of evolutionary significance, we rely on high-density genomic data and/or life history and climate data. High-density genomic data (combined 220,000 SNP array and whole-genome resequencing) were available for 23 sites within DU 12. This included sites that cover all portions of the DU including sites in NB, PEI, NS (including western Cape Breton), and Quebec (Gaspé). *Pcadapt* clearly separates Gaspé from all southward locations in DU 12 along the first principal component (PC) axis using genome-wide SNPs (n=29,695 SNPs-combined genomic datasets). One population in PEI (NEP–Northeast Complex) was separated from other sites along PC axis 2. A total of 44 loci significantly contributed to the differentiation on PC axis 1 contributing to differences between Gaspé and the rest of the DU (adjusted *p*-value [*q*-value]<0.05) and these loci were distributed across 9 chromosomes (out of 29) (Appendix Figure A35). Over-representation of biological processes associated with the genes located near the outlier loci were examined using topGO (Alexa and Rahnenfuhrer 2016). A total of 100 biological processes were significantly ($p<0.05$) over-represented based on the outlier dataset, with a large proportion of processes related to 'nitric oxide mediated signal transduction' (Appendix Figure A36). Nitric oxide can play a role in the function of the brain, neurons, cardiovascular physiology, immune response, and development in fishes (Eddy 2005). Overall, there is support for adaptive genomic differences between Gaspé and southern Gulf sites and thus this supports evidence of evolutionary significance. Based on our decision tree, we also examined evidence of life history and climate-linked differences within the DU.

DFO and MNRF (2008) suggest that the recognized DU 12 is represented by seven CUs of Atlantic Salmon (CUs 9, 10, 11, 12, 18, 19, part of 20). However, evidence to separate these regions into seven CUs was limited, and only based on some differences in ocean migration and preliminary genetic analyses. There were no ecological or life history differences reported between these seven CUs (DFO and MNRF 2008). Nonetheless, data on smolt age from Chaput et al. (2006) demonstrate differences between Gaspé and southern Gulf populations. Generally, Gaspé populations exhibit later mean smolt ages (small salmon: 2.81–3.34 years; large salmon: 2.78–3.42 years) compared to populations in southern Gulf populations (small: 2.11–2.86 years; large: 2.09–2.65 years). This was consistent with recent data that suggests a Gaspé population (Restigouche) is dominated by three year old smolts (90–100%) and low proportion of two year old smolts (2–4%) (Dauphin 2022). Similarly, data from 14 other rivers in Gaspe (in the Quebec jurisdiction) suggests a predominance of three year old smolts, with an average smolt age of 3.15 years (range 2.56–3.51 years) (April et al. 2023). This generally contrasts rivers in the Gulf of St. Lawrence where smolts are primarily two and three years old (Cairns et al. in prep[4]; Daigle 2023; Douglas et al. 2023). In addition, while data were limited, mean sea age was often higher for populations in Gaspé (1.67–2.16 years) compared to the southern Gulf region (1.29–2.05 years) (Hutchings and Jones 1998). Overall, evidence based on smolt age, and to some extent sea age, suggests differences in life history between the two genetically discrete groups.

Climate differences between Gaspé and southern Gulf sites were also supported (Appendix Figure A37). RDA was performed using 19 bioclimatic variables (see Appendix Table A1) for all rivers in previously recognized DU 12 as the response and putative DU groups (two genetic

[4] Cairns, D.K., S.D. Roloson, R.E. MacFarlane, and D.L. Guignion. In prep. Atlantic salmon life history, population indicators, habitat, and threats on Prince Edward Island (SFA 17). DFO Can. Sci. Advis. Sec. Res. Doc.

clusters: Gaspé and southern Gulf) as the constraining variable. ANOVA on the RDA showed the model to be significant ($p<0.001$) with an adjusted R^2 of 0.18. RDA axis 1 explained 18% of the variance in the model and clearly separated rivers in the putative new DUs (Gaspé and southern Gulf). This difference on RDA axis 1 was driven by variation in temperature, including mean temperature, mean temperature of warmest quarter, maximum temperature, temperature of coldest quarter and temperature of driest quarter. These results suggest warmer temperatures in southern Gulf compared to Gaspé. Altogether, these results support clear differences in climate that are linked to the two main genetic groups. In addition, there are differences in the underlying geology between these two regions (Tremblay and Pinet 2016).

Overall, our analyses suggest that there are two discrete and evolutionarily significant units (DUs) within previously recognized DU 12, which separate Gaspé (Restigouche inclusive) from sites in the southern Gulf of St. Lawrence. Discreteness of these two DUs is supported by genetic data (see Figure 11), and evolutionary significance is supported by genomic evidence of adaptation, climate-linked differences, differences in underlying geology, and some life history differences. We note here that additional changes were made to the proposed southern Gulf DU based on analyses in the next section (see DU 13 Eastern Cape Breton).

DU 13 Eastern Cape Breton (previous): Proposed DU (merged) – Southern Gulf of St. Lawrence-Cape Breton

This DU extends from the northern tip of Cape Breton Island (approximately 47°02’ N, 60°35’ W) to northeastern Nova Scotia (approximately 45°39’N, 61°25’ W). Previously, it was recognized that within this DU, there is substantial variation in life history between the rivers on the Atlantic coast and those that drain into Bras d’Or Lake. It was also reported that there was a higher proportion of one-sea-winter (1-SW) fish in Atlantic rivers compared to Bras D’Or rivers. In addition, there are differences in river gradient between these regions, as well as differences in demographic trends. Therefore, during the last assessment, it was suggested that some structuring may exist within the DU, however genetic sampling was too sparse at the time to support any geographic pattern in structuring.

At the time of the last COSEWIC assessment (COSEWIC 2010), genetic data supported the distinctiveness of eastern Cape Breton DU populations from the neighbouring populations in the Nova Scotia Southern Upland DU (previously DU 14) (Verspoor 2005). The difference between these two regions is further supported by recent genetic and genomic studies (Moore et al. 2014; Jeffery et al. 2018; Bradbury et al. 2021). However, studies have failed to identify genetic differences between sites in the southern Gulf of St. Lawrence and those in eastern Cape Breton (Moore et al. 2014; Jeffery et al. 2018; Bradbury et al. 2021), suggesting potential changes to DU 13 may be needed.

There are 30 known salmon rivers in DU 13 (COSEWIC 2010), and our genetic datasets include six (microsatellites) and three (96 SNPs) sites. Using 15 microsatellite markers and 96 SNPs we did not find evidence to support the subdivision of this DU (Appendix Figure A38 and A39). However, we also note that previous work suggests that eastern Cape Breton sites group with sites in the southern Gulf, and the previous COSEWIC assessment provided limited support for separating sites in these two regions. Therefore, based on our decision tree, we re-evaluate the evidence for genetic discreteness between the southern Gulf (newly proposed DU) and eastern Cape Breton (previously recognized DU 13). Using STRUCTURE with two genetic clusters (K=2) for all sites in eastern Cape Breton and the southern Gulf, we found no support for genetic discreteness between these two regions based on microsatellites or the 96 SNP dataset (Appendix Figure A40). Therefore, genetic data suggests that these populations do not meet criteria of discreteness and we propose that these sites (southern Gulf and eastern Cape Breton) should be grouped together as a single DU.

Life history data supports similarities between eastern Cape Breton populations and those in the southern Gulf. Smolts are primarily two and three years old in these populations (Cairns et al. in prep[4]; Daigle 2023; Douglas et al. 2023; Taylor et al. in prep[5]), which generally contrasts older smolts in the adjacent Gaspe DU populations (April et al. 2023; Dauphin 2022). Further, rivers in eastern Cape Breton and the southern Gulf have a higher proportion of multi-sea-winter (MSW) fish (Cairns et al. in prep[4]; Daigle 2023; Douglas et al. 2023; Taylor et al. in prep[5]) compared to neighbouring populations in the southern upland (Raab et al. in prep[6]). While previous reports suggest a higher incidence of 1-SW fish in some eastern Cape Breton (COSEWIC 2010), recent data suggests that for many rivers (Baddeck River, Middle River, North River), the majority of salmon spend two winters at sea prior to spawning (Taylor et al. in prep[5]). Data from Clyburn river also suggests a higher proportion of large compared to small salmon, although sea age was not reported (Taylor et al. in prep[5]). The only monitored river in eastern Cape Breton with predominately 1-SW salmon was Grand River (Taylor et al. in prep[5]). Similarities in smolt age and sea age suggest similar generation time for populations (generally >five years) in southern Gulf and eastern Cape Breton (Cairns et al. in prep[4]; Daigle 2023; Douglas et al. 2023; Taylor et al. in prep[5]), which is longer than neighboring southern upland populations (Raab et al. in prep[6]). The geology within this DU is also similar (extensive coal deposits). Overall, these data further support combining southern Gulf and eastern Cape Breton into a single DU.

DU 14 Nova Scotia Southern Upland (previous): Two Proposed DUs – NS Southern Upland (West) and NS Southern Upland (East)

This DU extends from northeastern mainland Nova Scotia (approximately 45°39'N, 61°25' W) southward and into the Bay of Fundy to Cape Split (approximately 45°20' N, 64°30' W). This DU encompasses only a single CU (DFO and MNRF 2008). The freshwater habitat in this DU is often characterized by relatively low pH. This DU is also characterized by a lower proportion of multi-sea-winter (MSW) salmon compared to the neighbouring eastern Cape Breton and Southern Gulf of St Lawrence DU (Raab et al. in prep[6]). Within this DU, the more southern populations exhibit some of the youngest smolt ages within the Canadian range of Atlantic Salmon (Chaput et al. 2006). Younger smolt age and sea age support a shorter generation time (4.3–4.4 years for sampled populations; Raab et al. in prep[6]) compared to populations in eastern Cape Breton and Southern Gulf of St. Lawrence, where generation time is generally >five years (Cairns et al. in prep[4]; Daigle 2023; Douglas et al. 2023; Taylor et al. in prep[5]). Adults salmon within this DU return to rivers throughout the spring (May–June) and summer (July–August) months, which differs from neighbouring Inner Bay of Fundy DU (Raab et al. in prep[6]).

At the time of the last COSEWIC assessment, both mitochondrial DNA and microsatellite data suggested that gene flow was minimal between this DU and the neighbouring DUs (DU 15 Inner Bay of Fundy and DU 13 Eastern Cape Breton) (DFO and MNRF 2008). Recent genomic and genetic studies continue to support that the populations in this DU are genetically distinct from these neighbouring DUs (Moore et al. 2014; Jeffery et al. 2018; Bradbury et al. 2021). Another genetic study using microsatellites and focusing on rivers in the Southern Upland has reported two genetic clusters in the Southern Upland DU that were generally divided near Halifax, Nova

[5] Taylor, A.D., Raab, D., Hardie, D.C., and Brunsdon, E.B. In prep. Updated DFO Science information for Atlantic Salmon (*Salmo salar*) populations in the Eastern Cape Breton region of Nova Scotia. DFO Can. Sci. Advis. Sec. Res. Doc.

[6] Raab, D., Taylor, A.D., Hardie, D.C., and Brunsdon, E.B. In prep. Updated DFO Science information for Atlantic Salmon (*Salmo salar*) populations in the Southern Upland region of Nova Scotia (SFAs 20 and 21) of relevance to the COSEWIC status report. DFO Can. Sci. Advis. Sec. Res. Doc.

Scotia, consistent with the boundaries of the Salmon Fishing Areas (SFAs) in the region (O'Reilly et al. 2012).

There are 31 known salmon rivers in Nova Scotia Southern Upland DU 14 (COSEWIC 2010), and our genetic datasets include samples from 13 (microsatellites) and 9 (96 SNPs) sites. Using 15 microsatellite markers, the optimal number of genetic clusters (K) was 2, and additional structure was supported beyond this up to K=10 (Appendix Figure A41). At K=2, a clear genetic break occurred between sites east and west of Musquodoboit, and at K=3, this clustering pattern remained but with Round Hill (ROH) forming its own clearly distinct cluster. Higher values of K separated many sites into their own clusters. Using 96 SNPs, the optimal number of genetic clusters (K) was 3 (Appendix Figure A42) and additional structure was evident up to K=4 (Appendix Figure A42). At K=3, Round Hill clustered separately from other sites and at K=4, Musquodoboit clustered separately from other sites; however, we note that clustering patterns were not clearly distinct, except for Round Hill. Based on these datasets, there is evidence for discrete clusters east and west of Musquodoboit within the Nova Scotia Southern Upland DU, with Musquodoboit grouping with southern/western sites in this region, and thus criteria for multiple genetic groups (discreteness) have been met (see Figure 12). This difference was more pronounced in our microsatellite dataset, and this split is similar to a split between SFAs 21 and 20, which occurs near Halifax, NS. Previous genetic work has supported genetic differences between these two SFAs based on microsatellite data (O'Reilly et al. 2012), and is thus consistent with differences reported here.

In addition, Round Hill was clearly discrete from all other sites. While Round Hill has been grouped with Gaspereau (located in the inner Bay of Fundy) in some SNP-based studies (Jeffery et al. 2018; Bradbury et al. 2021), other studies suggest that these sites are genetically distinct from each other (O'Reilly et al. 2012; Moore et al. 2014). In our microsatellite dataset, Gaspereau and Round Hill are highly distinct from each other (F_{ST}=0.112). Rivers within the Southern Upland DU and the inner Bay of Fundy DU are also geographically separated from each other (>70 km), thus we do not expect the boundary between these DUs to require re-assessment at this time. We acknowledge that no other locations were sampled near Round Hill, as the closest sites in the datasets were Tusket and Salmon River (Digby). According to the NASCO river database, there are 9 salmon rivers between Round Hill and Salmon River (Digby), although the status of many of these populations is unknown or lost, suggesting there may be a limited number of salmon bearing rivers in this area. Overall, Round Hill appears to be unique among rivers in the Southern Upland DU, as well as in the Bay of Fundy (O'Reilly et al. 2012; Moore et al. 2014). Round Hill is highly differentiated from all sites in our microsatellite dataset (F_{ST}>0.1036), and thus there is no evidence to suggest that Round Hill would belong in any nearby DU. The high genetic divergence of Round Hill may be due to rapid recent drift and not the degree of long-term reproductive isolation based on low amounts of genetic variation, and possible genetic bottlenecks (O'Reilly et al. 2012).

To evaluate evidence of evolutionary significance for splitting DU 14, we rely on high-density genomic data and/or life history and climate data. High-density genomic data (220,000 SNP array or whole-genome resequencing) were only available for two sites in DU 14, and therefore geographic coverage was limited. Analyses using one population from the east and one from the west of the Southern Upland DU revealed genomic differences, with hundreds of SNPs contributing to differences (Figure Appendix A43). Gene ontology analyses revealed that the outlier loci contributing to these differences were associated with various biological processes, particularly with 'endoplasmic reticulum localization', as well as processes related to pigmentation and vision (i.e., 'melanin biosynthesis' and 'optic nerve structural organization'). While genomic evidence support differences between the east and west, we acknowledge that these differences may reflect population level differences rather than DU level differences.

Life history data were somewhat limited for populations in DU 14, although previous work suggested that the more southern populations exhibit some of the youngest smolt ages across the Canadian range (Chaput et al. 2006). Smolt age data were available for four (small salmon) to six (large salmon) populations (Chaput et al. 2006). A site in the southwestern portion of the DU (Tusket River) had the highest proportion of one year old smolts (34.7%) observed in Canada (Chaput et al. 2006). However, other sites in the DU generally had <4% one year old smolts, thus Tusket River may be unique within this DU and may not reflect geographic differences between the eastern and western part of this recognized DU. For other sites within the previously recognized DU 14, mean smolt age ranged between 2.02–2.40 years (depending on the salmon grouping) with no clear geographic pattern (Chaput et al. 2006), suggesting limited differences between east and west in the Southern Upland. The proportion of small salmon that are female was lower from Musquodoboit westward (range 0.2–0.46), and slightly higher for sites eastward (0.59–0.63) based on five rivers with available data. In addition, mean sea age was available for four rivers within the DU, but generally showed limited range (1.08–1.29 years). Overall, there is some evidence of life history differences in smolt age and the proportion of female salmon between the east and west of DU 14.

Climate differences between sites in the east and west in the Southern Upland (i.e., east and west of Musquodoboit) were also supported (Appendix Figure A44). RDA was performed using 19 bioclimatic variables (see Appendix Table A1) for all rivers in the previously recognized DU 14 as the response and putative DU groups (two genetic clusters: east and west) as the constraining variable. ANOVA on the RDA showed the model to be significant ($p<0.001$) with an adjusted R^2 of 0.25. RDA axis 1 explained 25.5% of the variance in the model and clearly separated rivers in the putative new DUs (east and west with split at Musquodoboit). This difference on RDA axis 1 was driven by summer precipitation, with precipitation of the warmest quarter, precipitation of the driest quarter, and precipitation of the driest month loading highly on the axis, supporting higher levels of summer precipitation in the east compared to the west. Mean temperature also contributed to differences, and was higher in the west compared to the east. Altogether, these results support clear differences in climate that are linked to the two main genetic groups.

In addition, clustering analysis based on environmental variation for 72 rivers in DU 14 identified three main clusters (see Figure 15 in DFO 2013). Sites west of Musquodoboit form two clusters that are more similar, whereas sites east of Musquodoboit form a separate cluster. This pattern further supports the genetic groups identified here are associated with differences in watershed characteristics that could drive local adaptation (DFO 2013). One particular environmental variable that is different between these watersheds includes acidification (Bowlby et al. 2014). Rivers west of Musquodoboit tend to have lower pH, where over 60% rivers are classified as class 1 (pH<4.7) or class 2 (pH 4.8–5.0); whereas, for rivers east of Musquodoboit, approximately 60% are categorized as class 3 (pH 5.1–5.4) or class 4 (pH>5.4) (Bowlby et al. 2014). Finally, in many marine species, a major biogeographic break occurs near Halifax, NS, and aligns with a gradient in ocean temperature (Stanley et al. 2018). This genetic break is found in five species from various taxa, including Sea Scallop (*Placopecten magellanicus*), European Green Crab (*Carcinus maenas*), Atlantic Cod (*Gadus morhua*), American Lobster (*Homarus americanus*), and Northern Shrimp (*Pandalus borealis*). These differences were found to be associated with winter bottom temperature and spring sea surface temperature (Stanley et al. 2018). Differences in spring sea surface temperature between the two genetic groups would suggest that smolts migrating to the marine environment would experience different surface temperatures. Generally, this suggests differences between these two genetic groups in Nova Scotia's Southern Upland may be driven by local adaptation to various environmental factors encountered in the freshwater and marine environment. Similarly, environmental differences in marine and freshwater habitats (i.e., joint adaptative zones) have

been used to delineate DUs of Pacific Salmon, supporting evidence of discreteness and evolutionary significance (Holtby and Ciruna 2007; COSEWIC 2018).

Overall, our analyses suggest that there are two discrete and evolutionarily significant units (DUs) within Nova Scotia's Southern Upland, which separate eastern rivers (east of Musquodoboit) from western rivers (Musquodoboit [inclusive] westward) (see Figure 12). Discreteness of these two DUs is supported by genetic data, and evolutionary significance is supported by many environmental differences, genomic differences, and some evidence of life history differences.

DU 15 Inner Bay of Fundy (previous): One River Removed

This DU extends from Cape Split (approximately 45°20' N, 64°30' W) around the Inner Bay of Fundy to a point just east of the Saint John River estuary (approximately 45°12' N, 65°57'). Extensive stocking has occurred in this DU, with recent stocking consisting of brood stock derived from the inner Bay of Fundy populations (Gibson et al. 2003). Much of the recent stocking has occurred as part of DFO's Live Gene Bank program, which is thought to have helped prevent the extinction of salmon within this DU (Gibson et al. 2008).

At the time of the last COSEWIC assessment, genetic data suggested strong genetic differences between this DU and neighbouring DUs (COSEWIC 2010). In addition, salmon within this DU appear to exhibit unique migratory behaviour (generally constrained within the Bay of Fundy/Gulf of Maine) (COSEWIC 2010). Recent genetic studies support the distinctiveness of rivers in this DU from other nearby DUs (Moore et al. 2014; Jeffery et al. 2018; Bradbury et al. 2021). However, we note that within this DU, one site (North River) often groups with sites in the Gulf region (Jeffery et al. 2018; Bradbury et al. 2021). In addition, another site (Gaspereau River) is often found to be unique (Moore et al. 2014), and is sometimes clustered with Round Hill in DU 14 (Jeffery et al. 2018; Bradbury et al. 2021). Indeed, Moore et al. (2014) suggests many sites are unique within this DU. In addition, Moore et al. (2014) suggest based on microsatellite data there are two genetic clusters within the inner Bay of Fundy DU which are separated by Cape Chignecto (i.e., separating sites in Chignecto Bay and Minas Basin). This is consistent with earlier genetic work suggesting that these two regions reflect distinct evolutionary lineages (Verspoor et al. 2002; Vandersteen Tymchuk et al. 2010). However, based on a larger SNP dataset, Moore et al. (2014) found that sites within this DU appear to either represent their own unique individual cluster or group with Gulf populations.

There are 17 known salmon rivers in DU 15 (COSEWIC 2010), and our genetic datasets each include 7 sites. Using 15 microsatellite markers, the optimal number of genetic clusters (K) was 6 although additional structure was supported up to K=7 (Appendix Figure A45). At K=2, a genetic break that occurred between Minas Basin and Chignecto Bay was evident, but with Gaspereau grouping with Chignecto Bay. However, at higher values of K, Gaspereau and other sites formed their own distinct clusters on their own or clustered with nearby sites (Appendix Figure A45). Using 96 SNPs, the optimal number of genetic clusters (K) was 2 (Appendix Figure A46). Additional structure was supported up to K=7 (Appendix Figure A46). At K=2, the geographic pattern was not as clear as with the microsatellite dataset, as some sites in Minas Basin (Gaspereau and North River) grouped with Chignecto Bay sites (Point Wolfe and Big Salmon River), whereas other Minas Basin sites formed their own cluster. At higher values of K, sites could eventually be mostly separated into their own clusters. Based on both datasets, there is evidence for discrete genetic clusters within the inner Bay of Fundy, with some evidence to support a geographic split between Chignecto Bay and Minas Basin. This difference was more apparent in the microsatellites, and some populations deviated from this in the 96 SNPs (i.e., Gaspereau and North River not fully separated from Chignecto Bay sites until higher values of K). It is important to note that the majority of these samples were collected in the early-2000s (2000–02), and thus we expect that the signals in our dataset reflect the genetic

signals of the wild populations prior to any potential changes associated with the Live Gene Bank program.

To evaluate evidence of evolutionary significance, we rely on high-density genomic data and/or life history and climate data. High-density genomic data (220,000 SNP array or whole-genome resequencing) were only available for four sites in DU 15. While geographic coverage is quite limited thus making inferences difficult, we have included this analysis here with the caveat that there are likely not enough data to fully meet criteria of significance. Using *pcadapt*, Gaspereau clustered separately from other sites in DU 15 along the first PC axis (Appendix Figure A47). Other sites were separated along PC 2, with North River showing greater differentiation from the other sites. A total of 441 loci significantly contributed to the differentiation on both PC axes (adjusted *p*-value [*q*-value] <0.05) and these loci were distributed across 28 chromosomes (out of 29) (Appendix Figure A47). Over-representation of biological processes associated with the genes located near the outlier loci were examined using topGO (Alexa and Rahnenfuhrer 2016). A total of 89 biological processes were significantly ($p<0.05$) over-represented based on the outlier dataset, with a large proportion of processes related to 'positive regulation of mesenchymal cell proliferation involved in ureter development' (Appendix Figure A48), which relates to the embryonic development of the connection between the kidneys and urinary bladder in fishes. Overall, while the PCA analysis supports the strong genomic divergence of Gaspereau River, the PCA does not support evolutionarily significant differences between Chignecto Bay and Minas Basin, as Big Salmon River (Chignecto Bay) and Stewiacke (Minas Basin) clustered separately but most closely together in PCA space. Nonetheless, we acknowledge that with only one site from Chignecto Bay, finding support for evolutionarily significant differences may be difficult here.

For life history, previous reports suggest that salmon populations within the inner Bay of Fundy have similar life histories, which differ from those of the outer Bay of Fundy, with the exception of Gaspereau River (DFO 2010). Gaspereau River salmon exhibit different marine migratory patterns and their life history traits are more similar to salmon in the outer Bay of Fundy (DFO 2010). Therefore, life history differences between Chigencto Bay and Minas Basin are not supported here.

Climate differences between sites separated by Chignecto Cape (i.e., separating sites from Chignecto Bay and Minas Basin) were supported (Appendix Figure A49). In this case, one putative DU covers sites in Minas Basin from Cornwallis to Fox (based on NASCO river database), and the other putative DU covers sites from Chignecto Bay to the end of the DU boundary (from Apple to Mispec). RDA was performed using 19 bioclimatic variables (see Appendix Table A1) for all rivers in DU 15 as the response and putative DU groups (Minas Basin and Chignecto Bay) as the constraining variable. ANOVA on the RDA showed the model to be significant ($p=0.001$) with an adjusted R^2 of 0.15. RDA axis 1 explained 16.9% of the variance in the model and clearly separated rivers in the putative new DUs (separated at Cape Chignecto). This difference on RDA axis 1 was driven primarily by temperature variables with temperature of the wettest quarter, temperature of the coldest quarter, mean temperature, and minimum temperature loading highly on the axis. These variables had higher values for Minas Basin, indicating warmer temperatures in this region compared to Chignecto Bay. Altogether, these results support differences in climate that are linked to the two main genetic groups. In addition, the estuary environment of Minas Basin is sandy, whereas Chignecto Bay is characterized as a muddy estuary (Amos et al. 1991). In Minas Basin, the sandy habitat with intermediate wave action and strong currents leads to lower levels of sedimentation (Shepherd et al. 1995). Whereas, the muddy habitat of Chignecto Bay with higher exposure to ocean swells and more wave action can lead to higher levels of erosion, and thus higher levels of suspended sediment concentrations in Chignecto Bay than Minas Basin (Shepherd et al. 1995).

In addition to these differences, previous studies suggest that these two regions reflect distinct evolutionary lineages (Verspoor et al. 2002; Vandersteen Tymchuk et al. 2010). We have reviewed these data to determine if they further support evidence of significance. Verspoor et al. (2002) identified a unique mitochondrial haplotype that was prevalent in Minas Basin populations (present in >35% of individuals), but absent in Chignecto Bay populations. Despite their close proximity, this supports different colonization histories between these regions likely due to different glacial histories, and suggests that gene flow has been restricted since colonization (Verspoor et al. 2002). These results support the case of discreteness; however, this may not help meet criteria of significance. However, other work has been conducted on iBoF populations examined differences in gene expression which could provide support for local adaptation (Vandersteen Tymchuk et al. 2010). Vandersteen Tymchuk et al. (2010) found more genes were differentially expressed between rivers in Chignecto Bay and Minas Basin (164 differentially expressed genes) compared to among rivers within these regions (29 genes in Chignecto Bay and 46 genes in Minas Basin). Environmental conditions between the regions include differences in sediment type and quantity, which could potentially lead to different levels of contaminant exposure between the iBoF populations in Chignecto Bay and Minas Basin (Vandersteen Tymchuk et al. 2010). Indeed, genes that were differentially expressed between the iBoF regions include those related to contaminant exposure, including fatty acid binding proteins, other lipid transport genes, and oxidative stress related genes (Vandersteen Tymchuk et al. 2010). Nonetheless, the study acknowledges that differences between the regions were not as strongly supported as in Verspoor et al. (2002) and are confounded by experimental conditions.

Overall, evidence for significance between Minas Basin and Chignecto Bay is reflected primarily in climate and habitat data here, and significance is not supported by life history or genomic data. Therefore, only one out of three criteria for significance is met, and thus splitting of Chignecto Bay and Minas Basin populations is not supported at this time.

While splitting this DU is not supported, we do find strong support for the genetic uniqueness and discreteness of Gaspereau from other iBoF systems, consistent with other genetic studies (Moore et al. 2014). Pairwise F_{ST} values at 15 microsatellite loci suggest Gaspereau is highly divergent from all other sites in the iBoF (F_{ST}>0.071). We also find support for strong genomic differences between Gaspereau and all other sampled sites in the iBoF (Appendix Figure A47). Further, as indicated above, life history characteristics of salmon in the Gaspereau River are distinct from other populations in the iBoF. Gaspereau River salmon exhibit different marine migratory patterns compared to other iBoF populations and their life history traits are more similar to salmon in the oBoF (DFO 2010). Gaspereau River has similar proportion of MSW salmon compared to other oBoF rivers, such as Upper St. John River and Nashwaak (Reader et al. in prep[7,8]). The proportion of MSW salmon (>36% 2-SW) in Gaspereau differs from that of other iBoF rivers, such as Big Salmon River which has >98% 1-SW salmon (Reader et al. in prep[8]). Gaspereau river also has much earlier adult run timing (early-May/June) compared to other iBoF populations which usually return in late summer and fall (Reader et al. in prep[8]). Earlier run timing also occurs in some oBoF populations, such as in upper St. John River, where it's reported that the majority of salmon return in July (Reader et al. in prep[7]). Gaspereau River salmon also undertake marine migration to distant regions in the North Atlantic Ocean similar to

[7] Reader, J.M., Hardie, D.C., McWilliam, S. Brunsdon, E., and Gautreau, M. In prep. Updated information on Atlantic Salmon (*Salmo salar*) populations in southwest New Brunswick (outer portion of SFA 23) of relevance to the COSEWIC status report. DFO Can. Sci. Advis. Sec. Res. Doc.

[8] Reader, J.M., Hardie, D.C., McWilliam, S., Brunsdon, E., Notte, D., and Gautreau, M. In prep. Updated information on Atlantic Salmon (*Salmo salar*) Inner Bay of Fundy populations (IBoF; part of SFAs 22 and 23) of relevance to the COSEWIC status report. DFO Can. Sci. Advis. Sec. Res. Doc.

oBoF populations, which differs from the local marine migration undertaken by iBoF salmon (Reader et al. in prep[8]). Based on this information, we evaluated whether Gaspereau River may show more genetic similarities with sites in oBoF. Using STRUCTURE with two genetic clusters (K=2) for all sites in the iBoF and oBoF, we found support for Gaspereau showing greater genetic affinity to oBoF rather than the iBoF in both the microsatellite and 96 SNP datasets (Appendix Figure A50). Therefore, we propose that rather than classifying Gaspereau River as its own DU, this population should be moved into DU 16.

DU 16 Outer Bay of Fundy (previous): One River Added

This DU extends westwards from just east of the Saint John River estuary (approximately 45°12' N, 65°57') to the border with the United States of America. Within this DU, there is a higher proportion of multi-sea-winter (MSW) salmon that migrate to the North Atlantic compared to the neighbouring inner Bay of Fundy DU (Amiro 2003). One boundary of this DU occurs at the United States border, which reflects the scope of this report, and genetic relationships between the outer Bay of Fundy populations and the US populations were not examined. At least one river within the outer Bay of Fundy DU (Serpentine River) exhibits unique life history characteristics with a run of salmon that return late in the fall to the estuary and spawn the following year (Saunders 1981).

At the time of the last COSEWIC assessment, genetic data suggested minimal gene flow between the outer Bay of Fundy and nearby populations in Nova Scotia's Southern Upland and the inner Bay of Fundy (King et al. 2000, Verspoor et al. 2002 and Verspoor 2005). Recent genetic studies continue to support the distinctiveness of the outer Bay of Fundy populations from other regions (Jeffery et al. 2018).

This previously recognized DU has 17 known salmon rivers, and our datasets include samples from two tributaries within the Saint John River system (Tobique and Nashwaak), as well as Gaspereau River, which we propose belongs in this DU (see above). Using 15 microsatellite markers and the 96 SNP dataset, the optimal number of genetic clusters (K) was 2. In both cases, Gaspereau represented its own distinct cluster, whereas Tobique and Nashwaak clustered together (Appendix Figure A51 and A52; respectively). In addition, using the microsatellite dataset, we detected some substructure within the Tobique River at K=3, where some individuals clustered separately from other individuals in Tobique and Nashwaak River. While we have placed Gaspereau within the outer Bay of Fundy DU (previously recognized DU 16), it is clear that it is a genetically unique population, as demonstrated in other genetic studies (Moore et al. 2014). However, Gaspereau shows greater genetic affinity for oBoF compared to the iBoF based on our STRUCTURE analysis (see Appendix Figure A50). Similarly, for the microsatellite dataset, genetic divergence is lower between Gaspereau and oBoF sites (F_{ST}<0.063) compared to its divergence with iBoF sites (F_{ST}>0.071). In addition, Gaspereau salmon display life history characteristics and migration patterns that are different from the iBoF but more similar to the oBoF populations. For instance, Gaspereau River has similar proportion of MSW salmon compared to other oBoF rivers, such as Upper St. John River and Nashwaak (Reader et al. in prep[7,8]). The proportion of MSW salmon (>36% 2-SW) in Gaspereau differs from that of other iBoF rivers, which generally have low numbers of MSW (Reader et al. in prep[8]). Gaspereau river also has much earlier adult run timing (early-May/June) compared to iBoF populations which usually return in late summer and fall (Reader et al. in prep[8]). Earlier run timing also occurs in some oBoF populations, such as in upper St. John River, where it's reported that the majority of salmon return in July (Reader et al. in prep[7]). These life history differences support similarities among oBoF populations (including Gaspereau), and generally contrast iBoF populations.

At this time, we do not have enough data to support Gaspereau as its own distinct unit, as we have limited genomic data for sites within the oBoF (only Gaspereau and Nashwaak) and no

reported differences in life history. Therefore, within the oBoF DU (previously DU 16), Gaspereau does not meet criteria of significance as its own DU (require two out of three significance criteria to be met), but this may change as more data become available in the future. Based on these analyses and our decision tree, the oBoF DU should remain as a single unit that includes Gaspereau River.

Potential for Rescue Outside of Canada:

Salmon populations outside of Canada that could provide potential for rescue exist in Greenland, USA, and St. Pierre and Miquelon (France). The Greenland salmon population is most closely situated to populations in northern Labrador. However, Greenland harbours only one salmon river and genetic samples from this river suggest it is more genetically similar to European than North American populations (Arnekleiv et al. 2019), thus given large genomic differences between European and North American salmon (Lehnert et al. 2020), it is not a potential candidate for rescue. Populations in Maine, USA could also provide rescue to populations in Canada, as the USA borders the boundary of the outer Bay of Fundy DU. We do not have genetic samples from populations that are closest to the USA-Canada border; however, we do have samples from three rivers in Maine, including Penobscot, Narraguagus, and Sheepscot. These populations have been shown to be genetically discrete from populations in Canada (Jeffery et al. 2018; Bradbury et al. 2021). Here, we ran STRUCTURE using both the 15 microsatellite and 96 SNP datasets, and both datasets revealed that outer Bay of Fundy (with or without Gaspereau River included) and Maine populations were genetically discrete from each other (K=2) (Appendix Figure A53, 54). While many populations in Maine are endangered potentially preventing their use as rescue populations, more data are needed to assess whether any other populations in Maine could provide rescue to the outer Bay of Fundy rivers. In addition, St. Pierre and Miquelon is located close to populations in southern Newfoundland. Only one river in the archipelago, Belle-Rivière, has a resident salmon stock but its status is currently unknown (NASCO 2019). The genetic characteristics of this population are unknown; however, provided the residual nature of this stock it is unlikely that it would provide a source of rescue for southern Newfoundland populations.

CONCLUDING REMARKS

At the time of the last COSEWIC assessment, a total of 16 designatable units were recognized for Atlantic Salmon with 15 of these DUs representing anadromous extant populations that we re-evaluated here (COSEWIC 2010). Since the previous assessment, extensive amounts of genetic and genomic data have become available for Atlantic Salmon populations in Canada. We incorporated these various datasets to help inform Atlantic Salmon DUs for the upcoming COSEWIC re-assessment. We proposed and used a weight of evidence approach to re-evaluate the DU structure in eastern Canada to ensure that all proposed DUs show support for COSEWIC's criteria of discreteness and significance (see Table 1 and 2). This approach led to the subdivision of four previously recognized DUs into multiple units. This includes the subdivision of the Labrador DU into three DUs and the subdivision of the south Newfoundland DU into two DUs. In addition, Nova Scotia's Southern Upland DU was subdivided into two DUs, as well as the subdivision of sites in Gaspé and the Southern Gulf of St. Lawrence. In addition, based on a weight of evidence, we determined that some DUs required re-evaluations of their boundaries, which led to changes of DU boundaries in Quebec (Western North Shore and Inner St. Lawrence) and in Newfoundland (Northeast and Northwest Newfoundland). Re-evaluation of boundaries also suggested that southern Gulf populations are not discrete from eastern Cape Breton populations, and thus these populations were collapsed into a single DU. Further, we identified two populations that belong in adjacent DUs, which would result in non-contiguous boundaries. This included Corneille River in Quebec (physically located in Western North Shore

but groups with Eastern North Shore) and Gaspereau River in the Bay of Fundy (physically located in inner Bay of Fundy but groups with outer Bay of Fundy). Therefore, we recommend that these rivers be placed in their adjacent DUs. Overall, using newly available data, we propose that there are 19 DUs of extant anadromous Atlantic Salmon that are supported by evidence of discreteness and significance in Canada (see Table 1, 2 and Figure 13 for proposed structure). Given that Atlantic Salmon populations can be genetically structured at multiple scales, including at the level of individual rivers in some cases, we recognize the complexity of our analysis to revise the DU structure in this species. We expect that as more data and technologies become available in the future, changes to the DUs proposed here will be likely as we learn more about the underlying genetic and adaptive differences of populations at finer spatial scales. Nonetheless, the framework developed here has guided important revisions to the DUs of Atlantic Salmon, and the novelty and power of our approach will be valuable for defining COSEWIC DUs of various species in the future.

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TABLES

Table 1. Proposed designatable units (DUs) of Atlantic salmon. We propose that there are 19 DUs of extant, anadromous Atlantic Salmon, and an additional extinct non-anadramous DU was previously recognized (DU 11) and not assessed here. See Table 2 for details to support proposed DUs.

Proposed DU	COSEWIC DUs (2010)	Overall changes to previously recognized DU	Path in decision tree
DU 1 Nunavik	DU 1	Unchanged	Path 3
DU 2 Northern Labrador	DU 2	Subdivision of Labrador DU	Path 1
DU 3 Lake Melville	DU 2	Subdivision of Labrador DU	Path 1
DU 4 Southern Labrador	DU 2	Subdivision of Labrador DU	Path 1
DU 5 Northeast Newfoundland	DU 3	Revised boundary with Northwest Newfoundland DU	Re-assessed boundary; Path 1
DU 6 South Newfoundland – East	DU 4	Subdivision of South Newfoundland DU	Path 1
DU 7 South Newfoundland – West	DU 4	Subdivision of South Newfoundland DU	Path 1
DU 8 Southwest Newfoundland	DU 5	Unchanged	Path 3
DU 9 Northwest Newfoundland	DU 6	Revised boundary with Northeast Newfoundland DU	Re-assessed boundary; Path 1
DU 10 Quebec Eastern North Shore	DU 7	Added one river (Corneille) from Quebec Western North Shore DU to this DU	Path 3
DU 11 Lake Ontario	DU 11	Unchanged – DU is extinct (not assessed here; non-anadramous)	-
DU 12 Quebec Western North shore	DU 8	Revised boundary with Inner St. Lawrence DU Moved one river (Corneille) from this DU into Quebec Eastern North Shore DU	Re-assessed boundary; Path 1
DU 13 Anticosti	DU 9	Unchanged	Path 4
DU 14 Inner St. Lawrence	DU 10	Revised boundary with Quebec Western North shore DU	Re-assessed boundary; Path 1
DU 15 Gaspé	DU 12	Subdivision of Gaspé-Southern Gulf of St. Lawrence DU	Path 1
DU 16 Southern Gulf of St. Lawrence and Cape Breton	DU 12, DU 13	Subdivision of Gaspé-Southern Gulf of St. Lawrence DU Merged with eastern Cape Breton DU	Path 1 to split Gaspe and Gulf; then re-assessed boundary with eastern Cape Breton - Path 4
DU 17 Nova Scotia Southern Upland - East	DU 14	Subdivision of Nova Scotia Southern Upland DU	Path 1
DU 18 – Nova Scotia Southern Upland - West	DU 14	Subdivision of Nova Scotia Southern Upland DU	Path 1
DU 19 – Inner Bay of Fundy	DU 15	Moved one river (Gaspereau) from this DU into Outer Bay of Fundy DU	Path 3, except Gaspereau (Path 1)
DU 20 - Outer Bay of Fundy and Gaspereau	DU 15, DU 16	Added one river (Gaspereau) from Inner Bay of Fundy DU into this DU	Path 3

Table 2. Proposed DUs (names and numbers) for Atlantic Salmon. Support for the discreteness and significance of the DU are provided, with some DUs including reasons for original designation (2010) and other including updated data identified in this report. Note that DU 11 is not included as this DU is extinct and not assessed here.

Proposed DU	COSEWIC DUs (2010)	Overall changes from previous DU	Support for DU (based on prior evidence or new evidence resulting in changes)	
			Discreteness	Significance
DU 1 Nunavik	DU 1	Unchanged	Limited gene flow with other DUs based on microsatellites and SNPs	**Previous support:** Evidence of local migratory routes Disjunct from the rest of the species distribution (~650 km of coastline) At the northern extreme of the species' range in Canada, Arctic-like conditions
DU 2 Northern Labrador	DU 2	Subdivision of Labrador DU	Microsatellites separate coastal Labrador (north and south) from Lake Melville at K=2 96 SNP dataset separates northern Labrador from other regions in Labrador at K=3	**Genomic evidence of adaptation:** PCA separates coastal Labrador from Lake Melville Genomic differences associated with fatty acid homeostasis Genetic-environment associations delineating coastal Labrador from Lake Melville Lower frequency of European type Ssa01/Ssa23 chromosomal rearrangement in coastal Labrador compared to Lake Melville **Life history:** Later run timing compared to other regions of Labrador Lower incidence of maturation after 1-SW compared to Lake Melville Differences in migration routes Potentially older sea age and size at maturity than southern Labrador Older smolts in coastal Labrador compared to Lake Melville **Climate-linked differences:** Differences in temperature and precipitation from other regions in Labrador **Additional factors:** Differences in fish communities - Northern Labrador populations dominated by Arctic Charr Highest river gradients in the Labrador region
DU 3 Lake Melville	DU 2	Subdivision of Labrador DU	Microsatellites separate coastal Labrador (north and south) from Lake Melville at K=2 96 SNP dataset separates Lake Melville from other regions in Labrador at K=3	**Genomic evidence of adaptation:** PCA separates coastal Labrador from Lake Melville Genomic differences associated with fatty acid homeostasis Genetic-environment associations delineating coastal Labrador from Lake Melville Higher frequency of European type Ssa01/Ssa23 chromosomal rearrangement in Lake Melville compared to coastal Labrador

Proposed DU	COSEWIC DUs (2010)	Overall changes from previous DU	Support for DU (based on prior evidence or new evidence resulting in changes)	
			Discreteness	**Significance**
				Life history: Earlier run timing compared to other regions of Labrador Differences in incidence of maturation after 1-SW compared to coastal Labrador Differences in migration routes Younger smolts than other regions of Labrador **Climate-linked differences:** Differences in temperature and precipitation from other regions in Labrador **Additional factors:** Differences in fish communities – Lake Melville rivers generally have Atlantic Salmon and sea-run Brook Trout Lowest river gradients in this region
DU 4 Southern Labrador	DU 2	Subdivision of Labrador DU	Microsatellites separate coastal Labrador (north and south) from Lake Melville at K=2 96 SNP dataset separates southern Labrador from other regions in Labrador at K=3	**Genomic evidence of adaptation:** PCA separates coastal Labrador from Lake Melville Genomic differences associated with fatty acid homeostasis Genetic-environment associations delineating coastal Labrador from Lake Melville Lower frequency of European type Ssa01/Ssa23 chromosomal rearrangement in coastal Labrador compared to Lake Melville **Life history:** Run timing is intermediate compared to other regions of Labrador Lower incidence of maturation after 1-SW compared to Lake Melville and Northern Labrador Differences in migration routes Potentially younger sea age and smaller size at maturity than northern Labrador **Climate-linked differences:** Differences in temperature and precipitation from other regions in Labrador **Additional factors:** Differences in fish communities – Brook Trout, Arctic Charr, and Atlantic Salmon are represented more equally in southern Labrador than other regions of Labrador Intermediate river gradients in this region compared to other parts of Labrador
DU 5 Northeast Newfoundland	DU 3	Revised boundary with Northwest	Based on revised boundary, evidence of genetic discreteness between	**Genomic evidence of adaptation:** PCA separates sites based on the revised boundary between DU 5 and DU 9

Proposed DU	COSEWIC DUs (2010)	Overall changes from previous DU	Support for DU (based on prior evidence or new evidence resulting in changes)	
			Discreteness	**Significance**
		Newfoundland DU (proposed DU 9)	proposed DUs 5 and 9 (K=2) for both microsatellites and 96 SNP datasets	Genomic differences associated with development and regulation of cell signaling pathways **Life history differences:** Some differences in smolt age between regions based on new boundary (proposed DU 5 and proposed DU 9), with generally younger smolt ages compared to northern populations in proposed DU 9 Higher proportion of repeat spawners in sampled rivers in DU 5 compared to DU 9 Slightly lower MSW component in rivers in the DU 5 (up to 4%) and DU 9 (up to 6%) **Climate-linked differences:** Higher temperatures within this region compared to neighbouring DU (proposed DU 9) **Additional factors**: New boundary aligns with a major geological break
DU 6 South Newfoundland – East	DU 4	Subdivision of South Newfoundland DU	Microsatellites show that at K=3, sites west of Garnish separate from sites eastward. Northeast Brook Trepassey forms its own cluster Based on 96 SNPs, at K=3, sites west of Garnish separate from sites eastward. Sites on the Avalon Peninsula separate from other sites on south coast	**Genomic evidence of adaptation:** Higher frequency of European type Ssa01/Ssa23 chromosomal rearrangement in east compared to west on south coast. Evidence suggests rearrangement is under selection and linked to climate **Life history differences:** Later run timing compared to rivers westward on south coast Rivers west of the Burin are characterized by smaller-sized grilse, whereas sites east of the Burin are characterized by stocks with small grilse as well as larger-sized grilse Higher proportion of small repeat spawners in east (mean ~13%) compared to west (mean ~5%) **Climate-linked differences:** Evidence of lower precipitation, less temperature variability, and warmer winter temperatures in east compared to west on south coast **Additional factors**: Higher pH within river compared to rivers westward on south coast Smaller drainage areas (<300 km^2) with only a few >400 km^2 in size compared to rivers westward on south coast
DU 7 South Newfoundland – West	DU 4	Subdivision of South	Microsatellites show that at K=3, sites west of Garnish separate from sites eastward.	**Genomic evidence of adaptation:** Lower frequency of European type Ssa01/Ssa23 chromosomal rearrangement in

Proposed DU	COSEWIC DUs (2010)	Overall changes from previous DU	Support for DU (based on prior evidence or new evidence resulting in changes)	
			Discreteness	**Significance**
		Newfoundland DU	Based on 96 SNPs, at K=3, sites west of Garnish separate from sites eastward. Sites on the Avalon Peninsula separate from other sites on south coast	west compared to east on south coast. Evidence suggests rearrangement is under selection and linked to climate **Life history differences:** Earlier run timing compared to rivers eastward on south coast Rivers west of the Burin are characterized by smaller-sized grilse, whereas sites east of the Burin are characterized by stocks with small grilse as well as larger-sized grilse Lower proportion of small repeat spawners in west (mean ~5%) compared to in east (mean ~13%) **Climate-linked differences:** Evidence of higher precipitation, more temperature variability, and colder winter temperatures in west compared to east on the south coast of Newfoundland **Additional factors**: Lower pH within river compared to rivers eastward on the south coast of Newfoundland River drainage area range from moderate (1,000 to 2,500 km^2) to small (<300 km^2), which differs from rivers eastward on the south coast of Newfoundland
DU 8 Southwest Newfoundland	DU 5	Unchanged	Evidence of higher rates of gene flow within this DU than among adjacent DUs and within other DUs. Some heterogeneity in genetic signals were noted between DU 8 and DU 9, but sites located near the boundary in each DU showed clear genetic differences. Changes to boundary with Northwest NL (DU 9) were not supported.	**Previous support:** Earliest ages of smoltification on the Island. Only DU on insular Newfoundland with a substantial MSW component Migration route is different from Northwest NL DU Rivers empty in the Cabot Strait and Gulf of St. Lawrence. Many low gradient streams, limited lacustrine habitat
DU 9 Northwest Newfoundland	DU 6	Revised boundary with Northeast Newfoundland DU	Based on revised boundary, evidence of genetic discreteness between proposed DUs 5 and 9 (K=2) for both microsatellites and 96 SNP datasets.	**Genomic evidence of adaptation:** PCA separates sites based on the revised boundary between DU 9 and DU 5 Genomic differences associated with development and regulation of cell signaling pathways **Life history differences:** Some differences in smolt age between regions based on new boundary (proposed DU 5 and

Proposed DU	COSEWIC DUs (2010)	Overall changes from previous DU	Support for DU (based on prior evidence or new evidence resulting in changes)	
			Discreteness	**Significance**
			Some heterogeneity in genetic signals were noted between DU 8 and DU 9, but sites located near the boundary in each DU showed clear genetic differences. Changes to boundary with Southwest NL (DU 8) were not supported.	proposed DU 9), with generally younger smolt ages compared to northern populations in proposed DU 9 Higher proportion of repeat spawners in sampled rivers in DU 5 compared to DU 9 Slightly lower MSW component in rivers in the DU 5 (up to 4%) and DU 9 (up to 6%) Migration route is different from Southwest NL DU **Climate-linked differences:** Lower temperatures within this region compared to neighbouring DU (proposed DU 5) **Additional factors**: New boundary aligns with a major geological break
DU 10 Quebec Eastern North Shore	DU 7	Added one river (Corneille) from Quebec Western North Shore DU to this DU	Neutral markers suggest higher gene flow within this region than among adjacent DUs. Previous suggestion that the boundary of this DU with Quebec Western North Shore DU was ambiguous, but we found support for the discreteness of this DU from the neighbouring DU using both SNPs and microsatellites. One site (Corneille) from neighbouring DU (proposed DU 11) was genetically similar to sites in this DU, and was thus moved into this DU.	**Previous support:** Characterized by populations with high proportions of 1-SW salmon compared to neighboring Quebec DU Rivers with lower temperature regimes than neighbouring DU (Quebec Western North Shore, proposed DU 12) **Genomic evidence of adaptation**: PCA separates sites based on the revised boundary and changes between proposed DU 10, DU 12 and DU 14 – although only one site had genomic data within this DU
DU 12 Quebec Western North Shore	DU 8	Revised boundary with Inner St. Lawrence DU at Betsiamites River (exclusive) Moved one river	Previous suggestion that the boundary of this DU with nearby DUs was ambiguous. We found support for the discreteness of this DU based on revised boundaries with DU 14 (Inner St Lawrence DU)	**Previous support:** Higher gradient rivers than nearby DUs Highest proportion of MSW salmon by a significant margin relative to the other DUs of the North Shore **Genomic evidence of adaptation:** PCA separates sites based on the revised boundary with proposed DU 14 Genomic differences between DUs based on revised boundary are associated with metabolism and development

Proposed DU	COSEWIC DUs (2010)	Overall changes from previous DU	Support for DU (based on prior evidence or new evidence resulting in changes)	
			Discreteness	**Significance**
		(Corneille) from this DU into Quebec Eastern North Shore DU	using both SNPs and microsatellites. One site (Corneille) physically located in this DU was genetically similar to sites in neighbouring North shore DU, and was thus moved into proposed DU 10 (Quebec Eastern North Shore)	**Life history differences:** Older smolt ages compared to neighbouring DU (Inner St. Lawrence; proposed DU 14) **Climate-linked differences:** Cooler summer temperatures compared to neighbouring DU (Inner St. Lawrence; proposed DU 14)
DU 13 Anticosti	DU 9	Unchanged	Low levels of distinction among some rivers within the DU, but clearly divergent from mainland	**Previous support:** Higher proportion of 1-SW salmon than many nearby DUs Distinct island system in the Gulf of St. Lawrence Lower gradient rivers Lower temperatures compared with several adjacent DUs (proposed DUs 12, 14, 15) **Additional support:** Geological parameters of Anticosti rivers are distinct from rivers in Quebec and Labrador. Genomic divergence of Anticosti from other populations was strongly linked to these geological parameters, where analyses were performed with outlier SNPs putatively under divergent selection (Bourret et al. 2013) Adult salmon from rivers on Anticosti are also smaller bodied compared to salmon from other Quebec DUs
DU 14 Inner St. Lawrence	DU 10	Revised boundary with Quebec Western North Shore DU at Betsiamites River (inclusive)	Previous suggestion that the boundary of this DU with North Shore DU was ambiguous. We found support for the discreteness of this DU based on revised boundaries using both SNPs and microsatellites.	**Genomic evidence of adaptation:** PCA separates sites based on the revised boundary with proposed DU 12 Genomic differences between DUs based on revised boundary are associated with metabolism and development **Life history differences:** Younger smolt ages compared to neighbouring DUs (Quebec Western North Shore proposed DU 12 and Gaspe proposed DU 15) **Climate-linked differences:** Warmer summer temperatures compared to neighbouring DU (Quebec Western North Shore; proposed DU 12)
DU 15 Gaspé	DU 12	Subdivision of Gaspé-Southern Gulf	Based on microsatellites and 96 SNPs, Gaspé sites are discrete from rivers in the	**Genomic evidence of adaptation:** PCA separates sites from Gaspé and those from Southern Gulf of St. Lawrence

Proposed DU	COSEWIC DUs (2010)	Overall changes from previous DU	Support for DU (based on prior evidence or new evidence resulting in changes)	
			Discreteness	**Significance**
		of St. Lawrence DU	Southern Gulf of St. Lawrence (at K=2)	Genomic differences between Gaspé and Southern Gulf of St. Lawrence relate to 'nitric oxide mediated signal transduction'. Nitric oxide can play a role in function of the brain, neurons, cardiovascular physiology, immune response, and development in fishes **Life history differences:** Evidence of later smolt ages and later sea age in Gaspé compared to Southern Gulf Older smolts compared to neighbouring Quebec DU (proposed DU 12) **Climate-linked differences:** Colder temperatures in Gaspé compared to Southern Gulf **Additional factors**: Differences in underlying geology between Gaspé and Southern Gulf-Cape Breton
DU 16 Southern Gulf of St. Lawrence and Cape Breton	DU 12, DU 13	Subdivision of Gaspé-Southern Gulf of St. Lawrence DU Merged with eastern Cape Breton DU	Based on microsatellites and 96 SNPs, Gaspé sites are discrete from rivers in the Southern Gulf of St. Lawrence (at K=2). Further, based on limited genetic differences between Southern Gulf and Eastern Cape Breton – these sites were re-evaluated for discreteness. No evidence of discreteness between these two regions were found and thus sites in these regions were merged into one DU	**Genomic evidence of adaptation:** PCA separates sites from Gaspé and those from Southern Gulf of St. Lawrence Genomic differences between Gaspé and Southern Gulf of St. Lawrence relate to 'nitric oxide mediated signal transduction'. Nitric oxide can play a role in function of the brain, neurons, cardiovascular physiology, immune response, and development in fishes **Life history differences:** Some evidence of later smolt ages and later sea age in Gaspé compared to Southern Gulf and Eastern Cape Breton Higher proportions of MSW fish compared to neighbouring DU populations in Southern Uplands Older smolt age and sea age in this DU compared to neighbouring Southern Upland populations results in longer generation time (generally >5 years) **Climate-linked differences:** Warmer temperatures in Southern Gulf compared to Gaspé **Additional factors**: Differences in underlying geology between Gaspé and Southern Gulf-Cape Breton Underlying geology of Southern Gulf and Eastern Cape Breton are similar (extensive coal deposits) supporting their merging

Proposed DU	COSEWIC DUs (2010)	Overall changes from previous DU	Support for DU (based on prior evidence or new evidence resulting in changes)	
			Discreteness	Significance
DU 17 Nova Scotia Southern Upland - East	DU 14	Subdivision of Nova Scotia Southern Upland DU	Using microsatellites, evidence of discreteness between east and west of Southern Upland (K=2) – exception is Round Hill (located in proposed DU 18). Using 96 SNPs, some separation between east and west of Southern Upland can be observed, but not as clearly as in microsatellites (K=4)	**Previous support:** Lower proportions of MSW fish compared to northern neighbouring DU (Southern Gulf and Eastern Cape Breton) **Genomic evidence of adaptation:** Limited geographic coverage but PCA separates populations in the east and west Genomic differences between east and west relate to ‘‘endoplasmic reticulum localization’, as well as processes related to pigmentation and vision (i.e., ‘melanin biosynthesis’ and ‘optic nerve structural organization’) based on gene ontology analyses **Life history differences:** Some differences in smolt age with older smolts in east of Southern Upland compared to west. Some differences in proportion of females - Higher proportion of female small salmon in east compared to west of Southern Upland (between proposed DUs 17 and 18) Younger smolt age and sea age in Southern Upland populations results in shorter generation time compared to populations in the neighbouring DU (Southern Gulf and Eastern Cape Breton) **Climate-linked differences:** Higher levels of summer precipitation and lower temperatures in the east compared to the west of Southern Upland **Additional factors**: Differences in river pH between proposed DUs of Southern Upland – higher pH in east than west The two proposed DUs of Southern Upland are delineated by differences in watershed characteristics that could drive local adaptation The delineation between DUs in east and west of Southern Upland (proposed DUs 17 and 18) is associated with a major biogeographic break in five marine species that aligns with a gradient in ocean temperature, including spring sea surface temperature which may indicate differences marine temperature experienced by smolts
DU 18 Nova Scotia Southern Upland - West	DU 14	Subdivision of Nova Scotia Southern Upland DU	Using microsatellites, evidence of discreteness between east and west of Southern Upland (K=2) – exception is Round Hill.	**Genomic evidence of adaptation:** Limited geographic coverage but PCA separates populations in the east and west Genomic differences between east and west relate to ‘‘endoplasmic reticulum localization’, as well as processes related to pigmentation and vision (i.e., ‘melanin biosynthesis’

Proposed DU	COSEWIC DUs (2010)	Overall changes from previous DU	Support for DU (based on prior evidence or new evidence resulting in changes)	
			Discreteness	**Significance**
			Using 96 SNPs, some separation between east and west of Southern Upland can be observed, but not as clearly as in microsatellites (K=4) We note that within this DU Round Hill was clearly genetically discrete from all other sites, consistent with other studies. This river does not appear to be more similar to rivers in neighbouring DUs. It has been suggested that this river lacks genetic diversity, but no other data on this river is available at this time.	and 'optic nerve structural organization') based on gene ontology analyses **Life history differences:** Some differences in smolt age with younger smolts in west of Southern Upland compared to east. At least one site in this new proposed DU has highest proportion of 1-year old smolts in Canada (Tusket River) Some differences in proportion of females - Lower proportion of female small salmon in west compared to east of Southern Upland (between proposed DUs 17 and 18) Most adults return to Southern Upland rivers throughout the spring (May/June) and summer (July/August) which differs from the iBoF populations which return in late summer to fall **Climate-linked differences:** Lower levels of summer precipitation and higher temperatures in the west (this DU) compared to the east (DU 17) of Southern Upland **Additional factors**: Differences in river pH between proposed DUs of Southern Upland – lower pH in west than east The two proposed DUs of Southern Upland are delineated by differences in watershed characteristics that could drive local adaptation The delineation between DUs in east and west of Southern Upland (proposed DUs 17 and 18) is associated with a major biogeographic break in five marine species that aligns with a gradient in ocean temperature, including spring sea surface temperature which may indicate differences marine temperature experienced by smolts
DU 19 Inner Bay of Fundy	DU 15	Moved one river (Gaspereau) from this DU into Outer Bay of Fundy DU	Some evidence for discreteness within the DU between Chignecto Bay and Minas Basin based on microsatellites and 96 SNPs – evidence of significance was evaluated but did not meet criteria (see main text). Gaspereau was divergent from other sites (F_{ST} >0.071) in Inner Bay of Fundy. Gaspereau was moved into the	**Previous support:** Salmon in this DU exhibit unique migratory behaviour Unique Bay of Fundy tidal system **Genomic evidence of adaptation:** Some genomic differences between sites in the Inner Bay of Fundy. Most sites separate in PCA, with Gaspereau (moved into oBoF DU) being the most divergent from other sites

Proposed DU	COSEWIC DUs (2010)	Overall changes from previous DU	Support for DU (based on prior evidence or new evidence resulting in changes)	
			Discreteness	**Significance**
			outer Bay of Fundy DU based on genetic affinity and similar life history variation and migration patterns to outer Bay of Fundy	**Life history differences:** Within the iBoF, salmon have similar life history and migratory pattern that are different from the oBoF, with the exception of salmon from Gaspereau River. Salmon from Gaspereau River exhibit different marine migratory patterns and their life history traits are more similar to salmon in the Outer Bay of Fundy (moved into oBoF DU). This includes a higher proportion of MSW, earlier run timing, and distant marine migration (to North Atlantic) for Gaspereau and oBoF salmon compared to iBoF. iBoF populations are characterized by primarily 1-SW salmon, later run timing, and local marine migration in the bay. Most adults return to rivers in late summer to fall, which differs from Southern Upland populations which return in spring (May/June) and summer (July/August)
DU 20 Outer Bay of Fundy and Gaspereau	DU 15, DU 16	Added one river (Gaspereau) from Inner Bay of Fundy DU into this DU	Gaspereau was divergent from other sites (F_{ST} >0.071) in Inner Bay of Fundy. Genetic divergence was lower between Gaspereau and oBoF sites (F_{ST} <0.063). Using microsatellites and SNPs, we found support for Gaspereau showing greater genetic affinity to oBoF rather than the iBoF. We note that while Gaspereau shows genetic affinity to oBoF, it still represents a genetically unique population that may warrant its own designation in the future	**Previous support:** This DU has a higher proportion of MSW salmon migrating to the North Atlantic than neighbouring inner Bay of Fundy DU Several systems with unusual run timing. **Life history differences:** Consistent with this DU, Gaspereau shows similar life history variation and migration patterns to sites within this DU, and thus has been moved into this DU due to these similarities and its genetic affinity to sites within this DU. This includes a higher proportion of MSW, earlier run timing, and distant marine migration (to North Atlantic) for Gaspereau and oBoF salmon compared to iBoF.

Table 3. Review of evidence (previous and new) to support boundary between Northeast Newfoundland (DU 3) and Northwest Newfoundland (DU 6). We indicate whether current data provide greater support for the boundary proposed here compared to the previous boundary.

Data	Proposed differences between DU 3 and DU 6 (COSEWIC 2010)	Review of Evidence	Greater support for revised boundary location?
Genetic	DU 3 is genetically unique based on: 1) Intermediate genetic profiles to European and North American salmon 2) Genetic divergence from western Newfoundland (DU 6)	Not unique in terms of European introgression: other areas of NL show these profiles (Bradbury et al. 2015; Lehnert et al. 2019a) The number and geographic extent of sampled populations for which data were available for COSEWIC (2010) were limited: Genetic differences between populations in DU 6 and DU 3 were not fully evaluated Recent data from both microsatellites and 96 SNP datasets support that a river on the east side of the northern peninsula (Beaver Brook) is genetically similar to populations in the northwest compared to the northeast, supporting a revised boundary	Yes
Smolt age	Age of smoltification in the northeast (DU 3) was different from the rest of NL	Older smolt ages have been reported for populations on the Northern Peninsula (east and west). Slightly older smolt age in DU 6 than DU 3 (Kelly et al. in prep[3])	No support for or against due to lack of sampling near proposed boundary
Repeat spawners	This portion of the Canadian range (Northeast DU 3) has the highest incidence of repeat spawners	There is a higher proportion of repeat spawners in sampled rivers in DU 3 compared to DU 6.	No support for or against due to lack of sampling near proposed boundary
Multi-sea-winter (MSW) salmon	Some rivers in northwest DU 6 have a small multi-sea-winter (MSW) component	There is a small MSW component in rivers in the northeast (up to 4%) and northwest (up to 6%)	Does not provide strong support due to lack of clear difference between DUs
Geology	Northwest DU 6 habitat is suggested to be significantly more alkaline than the rest of the island of Newfoundland due to the limestone geology	The new proposed boundary would align with a major geological break (Honsberger et al. 2019), where the proposed DU 6 would be characterized by geology that is generally unique from DU 3 and most other parts of Newfoundland	Yes
Genomic	N/A: No genomic data were included in COSEWIC (2010)	Genomic differences support the revised boundary, where Beaver Brook clusters with DU 6 sites and separate from DU 3 sites	Yes
Climate	N/A: Climate data were not included in COSEWIC (2010)	Significant differences in climate between rivers in DU 3 and DU 6 based on the revised boundary. Redundancy analysis suggests that DU 3 experiences generally warmer temperatures than DU 6	Yes

FIGURES

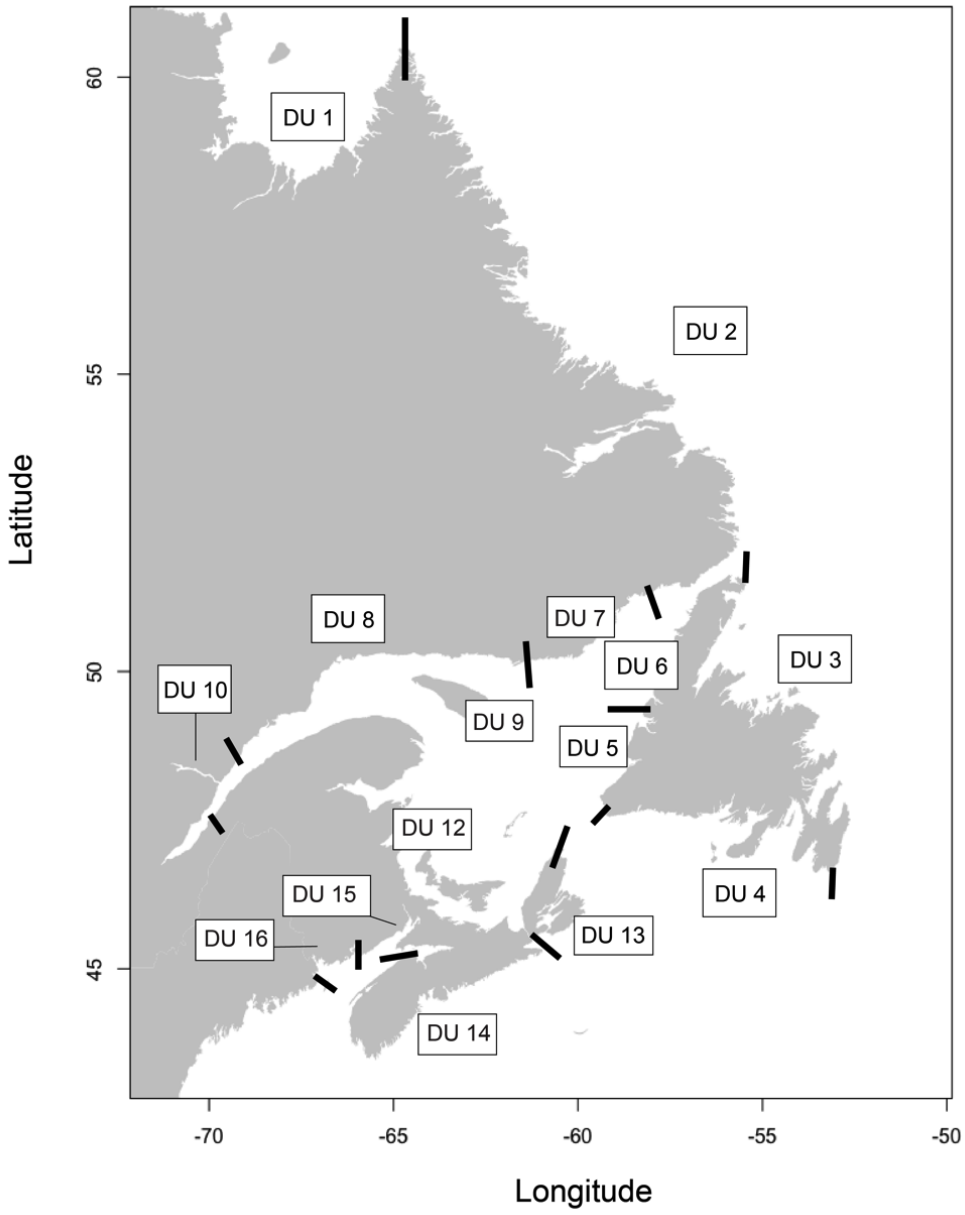


Figure 1. Map of 16 recognized Atlantic Salmon designatable units (DUs) based on the last COSEWIC assessment in 2010. Note that DU 11 (Lake Ontario) is an extinct non-andromous population that was not considered in our analysis and is not shown on the map.

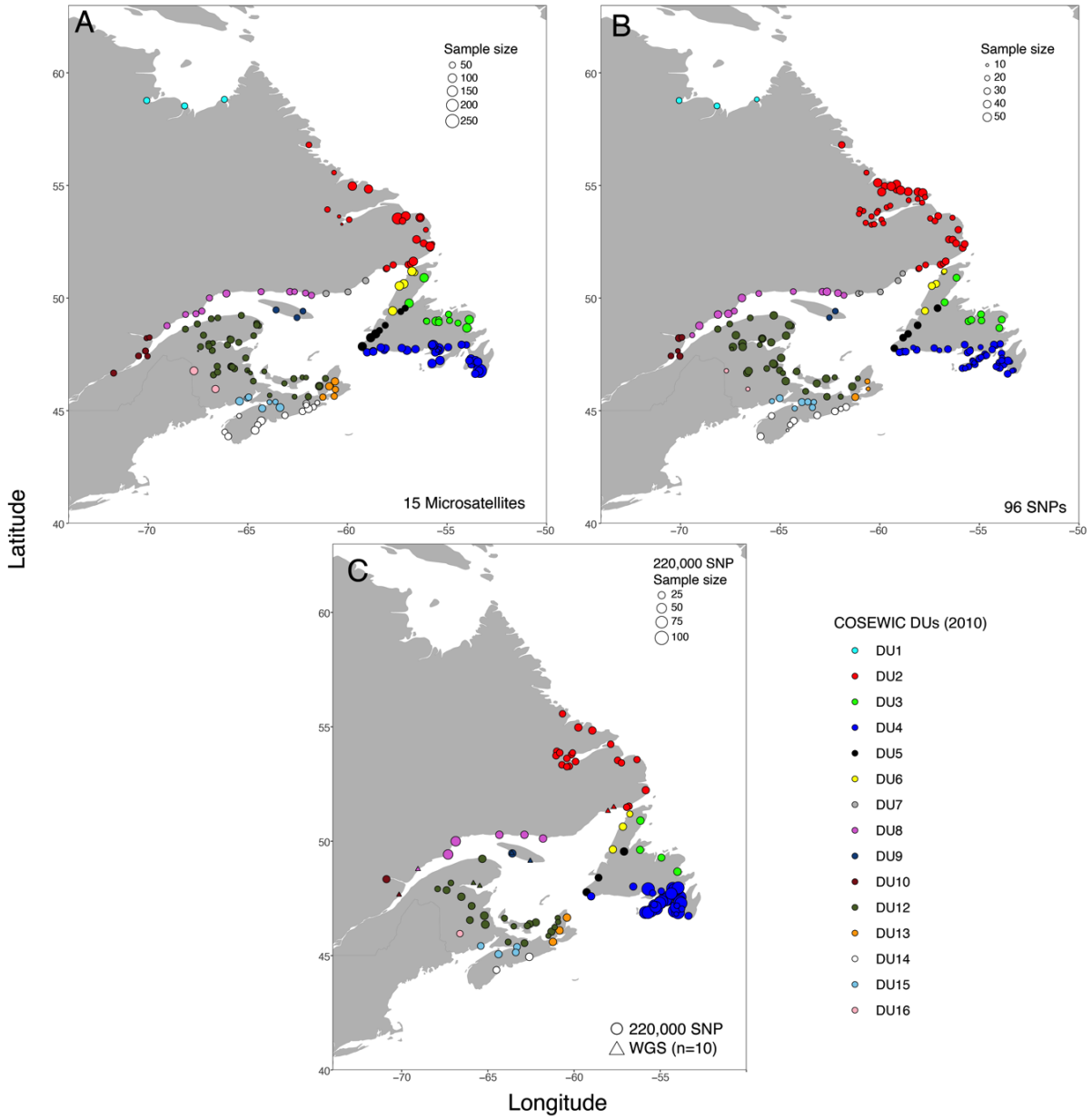


Figure 2. Map of sampling locations for the genetic and genomic datasets with locations coloured by the recognized Atlantic Salmon designatable units (DUs) based on the last COSEWIC assessment in 2010. Datasets include (A) 15 microsatellites, (B) 96 SNPs, and (C) 220,000 SNP and whole genome sequencing (WGS). Size of points represents relative sample size for that location. Additional sampling details are provided in Appendix Tables A1-3.

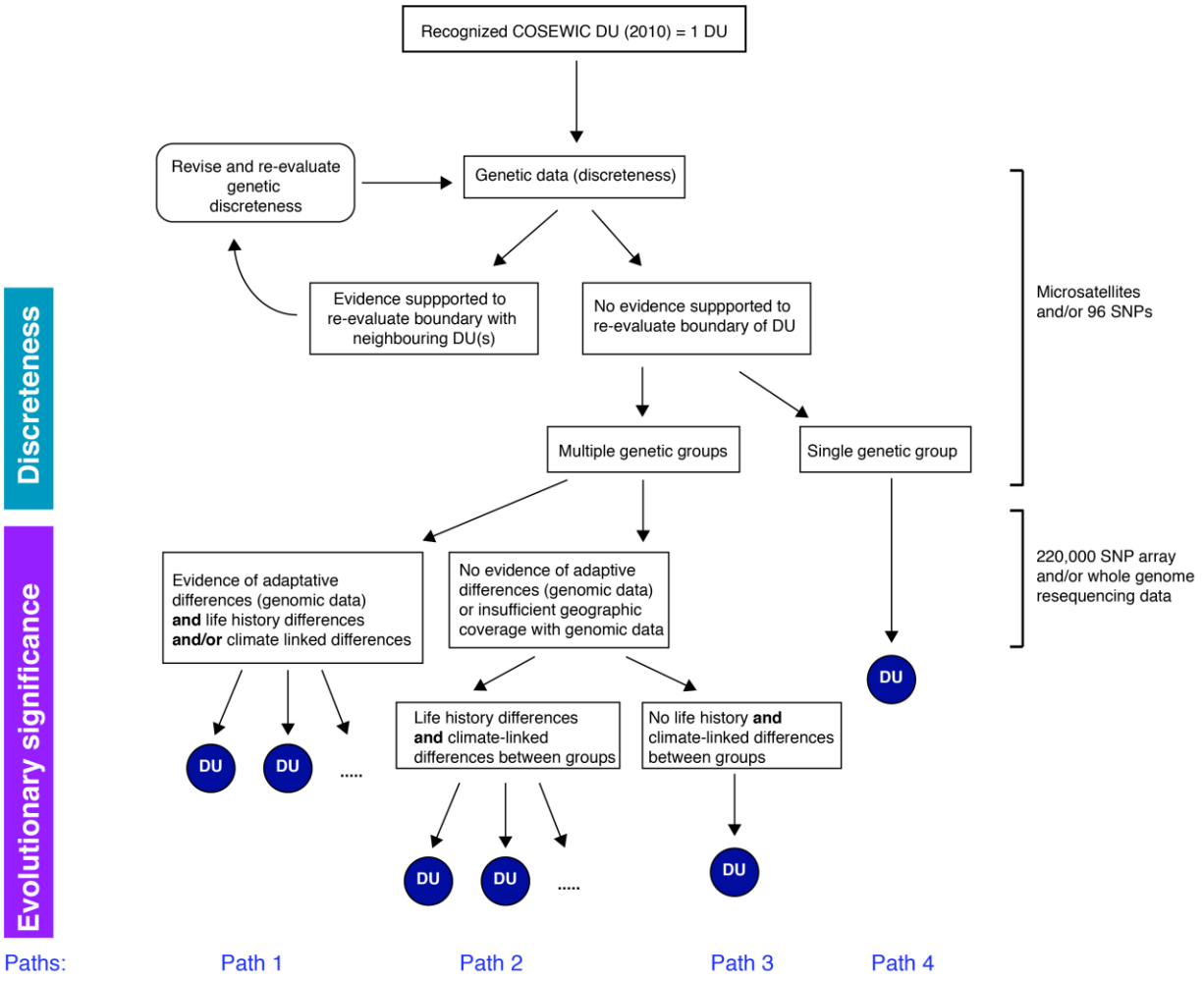


Figure 3. Decision tree used to evaluate discreteness and evolutionary significance of Atlantic Salmon populations. See text for details on how the tree is applied in our analysis.

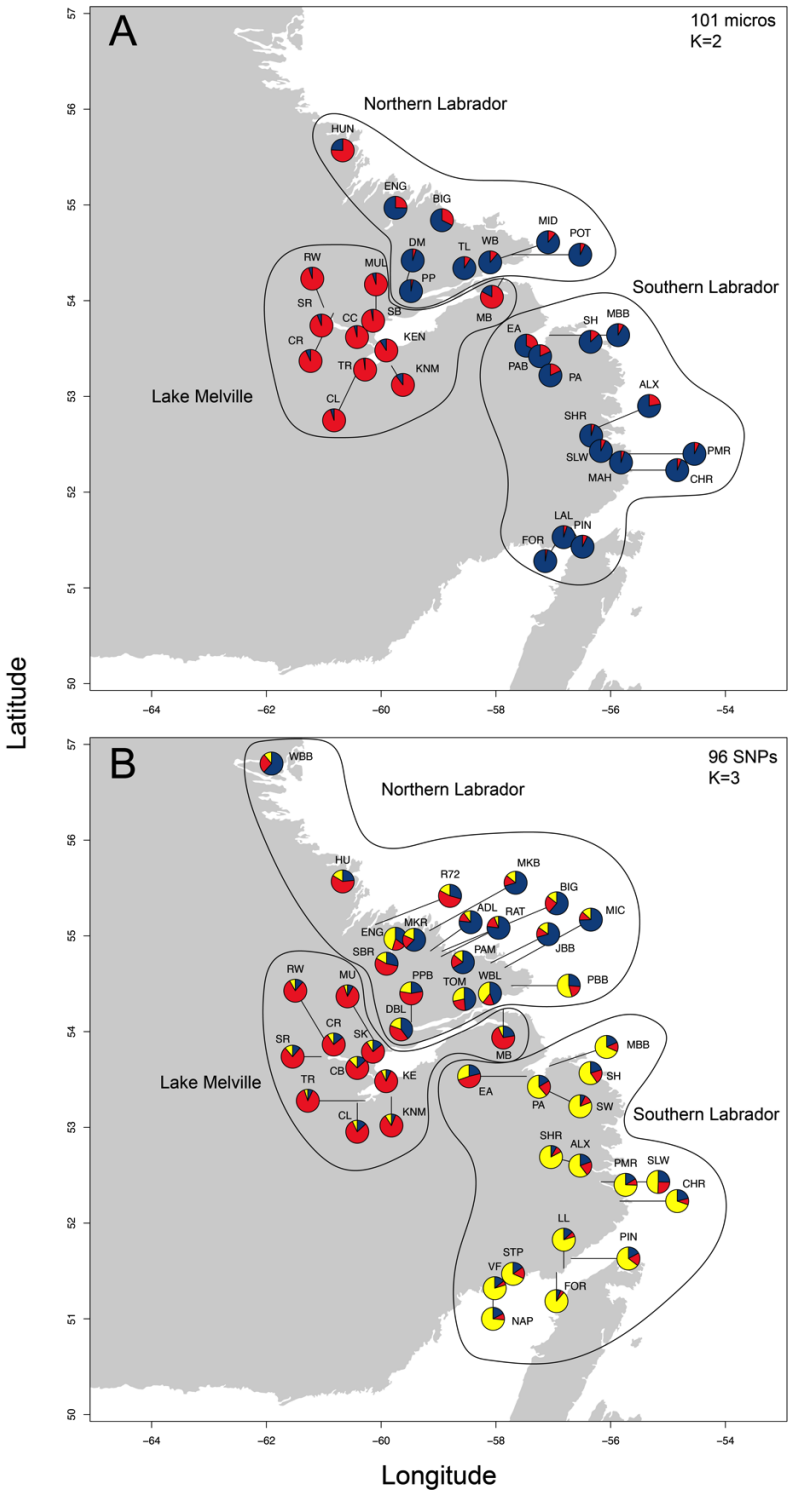


Figure 4. Maps of STRUCTURE results based on (A) 101 microsatellites and (B) 96 SNPs for Atlantic Salmon rivers in DU 2. Pie charts show proportion of membership for each population to the (A) two genetic clusters (K=2) for microsatellites and (B) three genetic clusters (K=3) for SNPs. Outline around sites indicate which populations fall within the boundaries of the three new proposed DUs. River abbreviations and sampling information can be found in Bradbury et al. (2018) for microsatellites and Appendix Table A2 for the 96 SNPs.

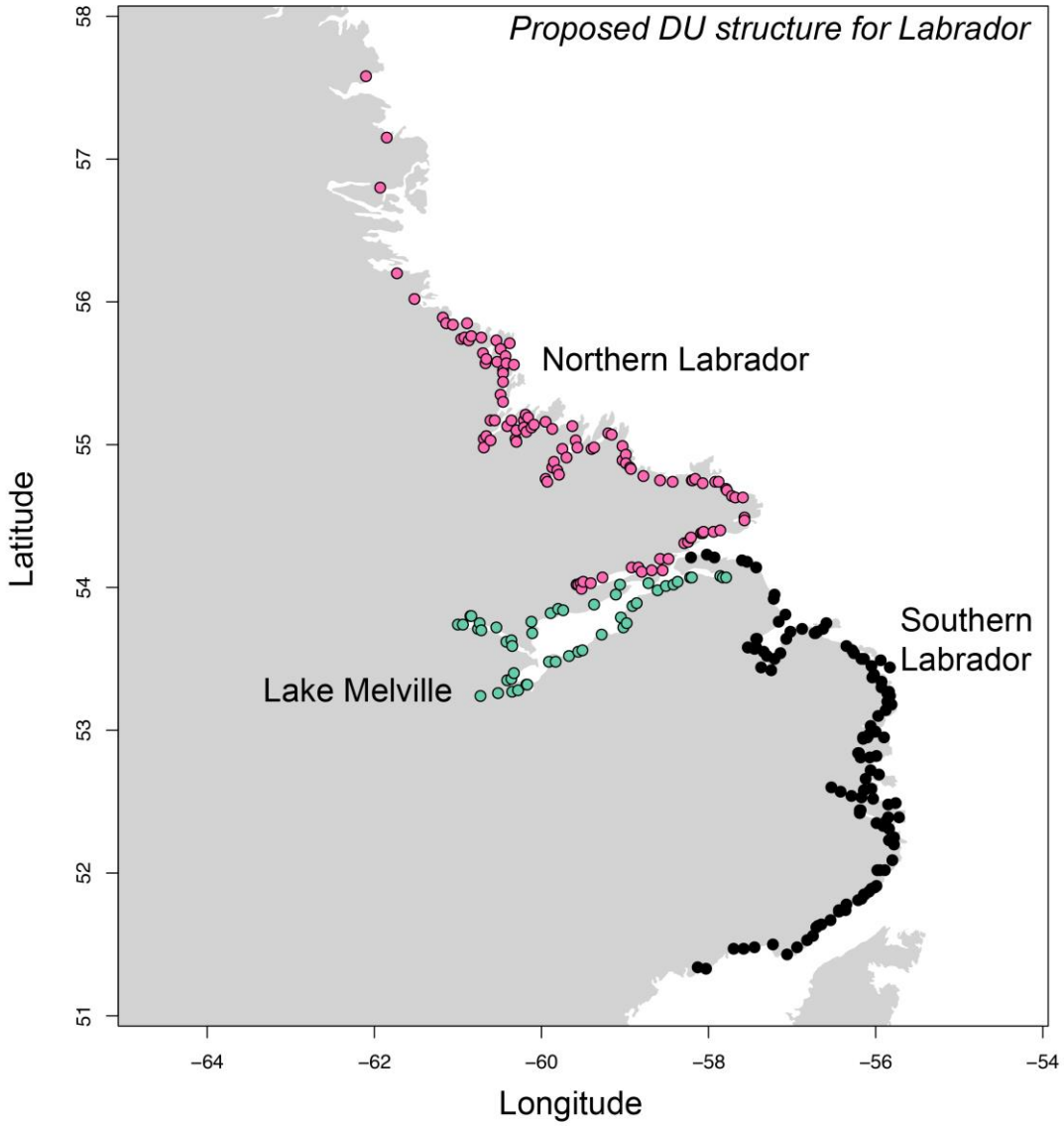


Figure 5. Map of proposed DU structure for Labrador region. Each point represents a river from the North Atlantic Salmon Conservation Organization (NASCO) river database and is coloured based on the proposed DU. See Appendix Table A5 for river names and further information.

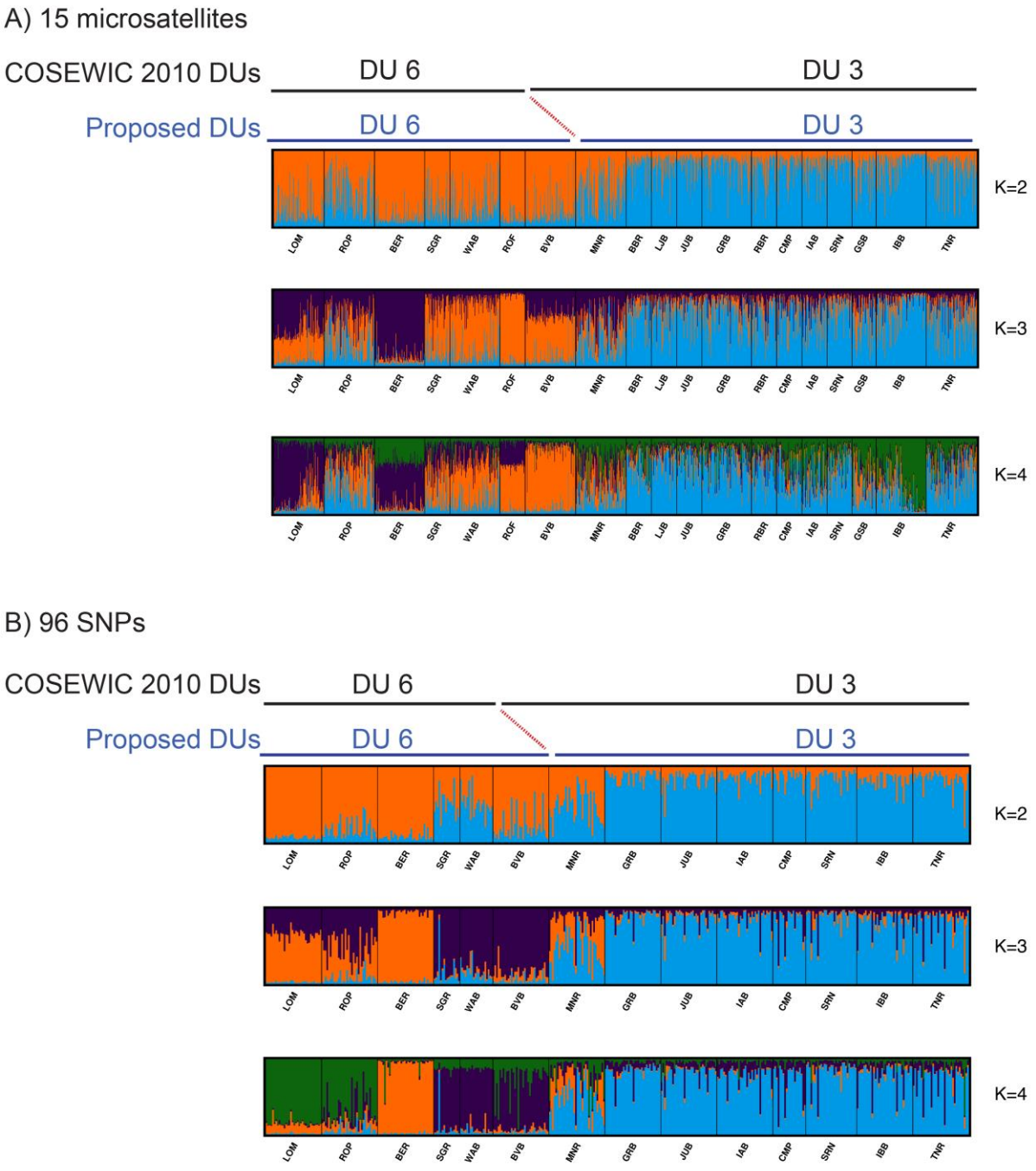


Figure 6. STRUCTURE plots based on (A) 15 microsatellites and (B) 96 SNPs for Atlantic Salmon rivers in DU 6 (northwest Newfoundland) and DU 3 (northeast Newfoundland). Colours indicate the proportion of membership to the genetic clusters (K=2–4) for each individual. Black lines above plots indicate previous assignment of rivers to DU 3 and DU 6, with revised boundaries indicated below with blue lines. River abbreviations and sampling data for the 15 microsatellite and 96 SNP datasets can be found in Appendix Tables A1 and A2 respectively.

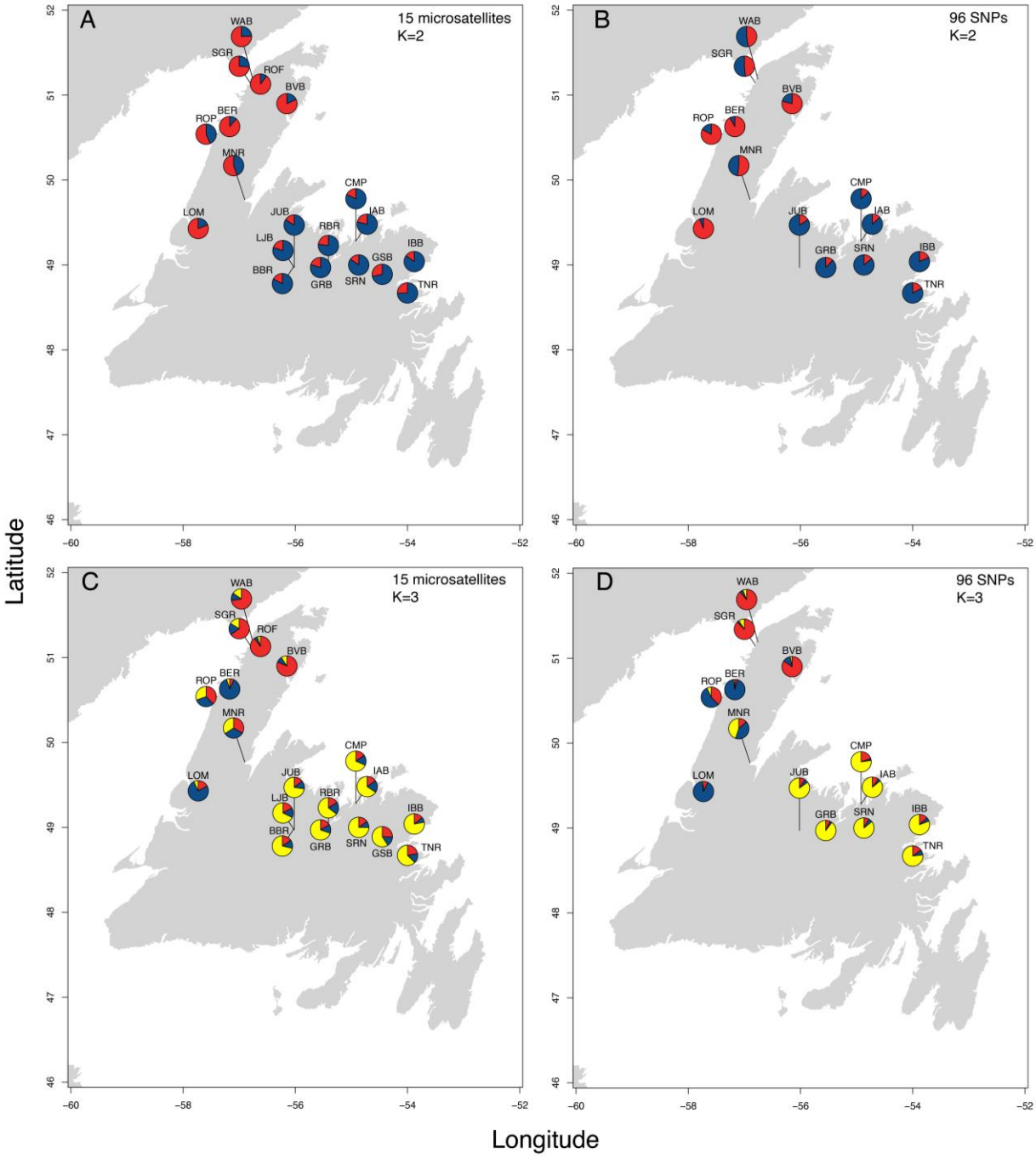


Figure 7. Maps of STRUCTURE results based on (A, C) 15 microsatellites and (B, D) 96 SNPs for Atlantic Salmon rivers in DU 3 and DU 6. Pie charts show proportion of membership to the (A, B) two genetic clusters (K=2) and (C, D) three genetic clusters (K=3) for each population. River abbreviations and sampling data for the 15 microsatellite and 96 SNP datasets can be found in Appendix Tables A1 and A2 respectively.

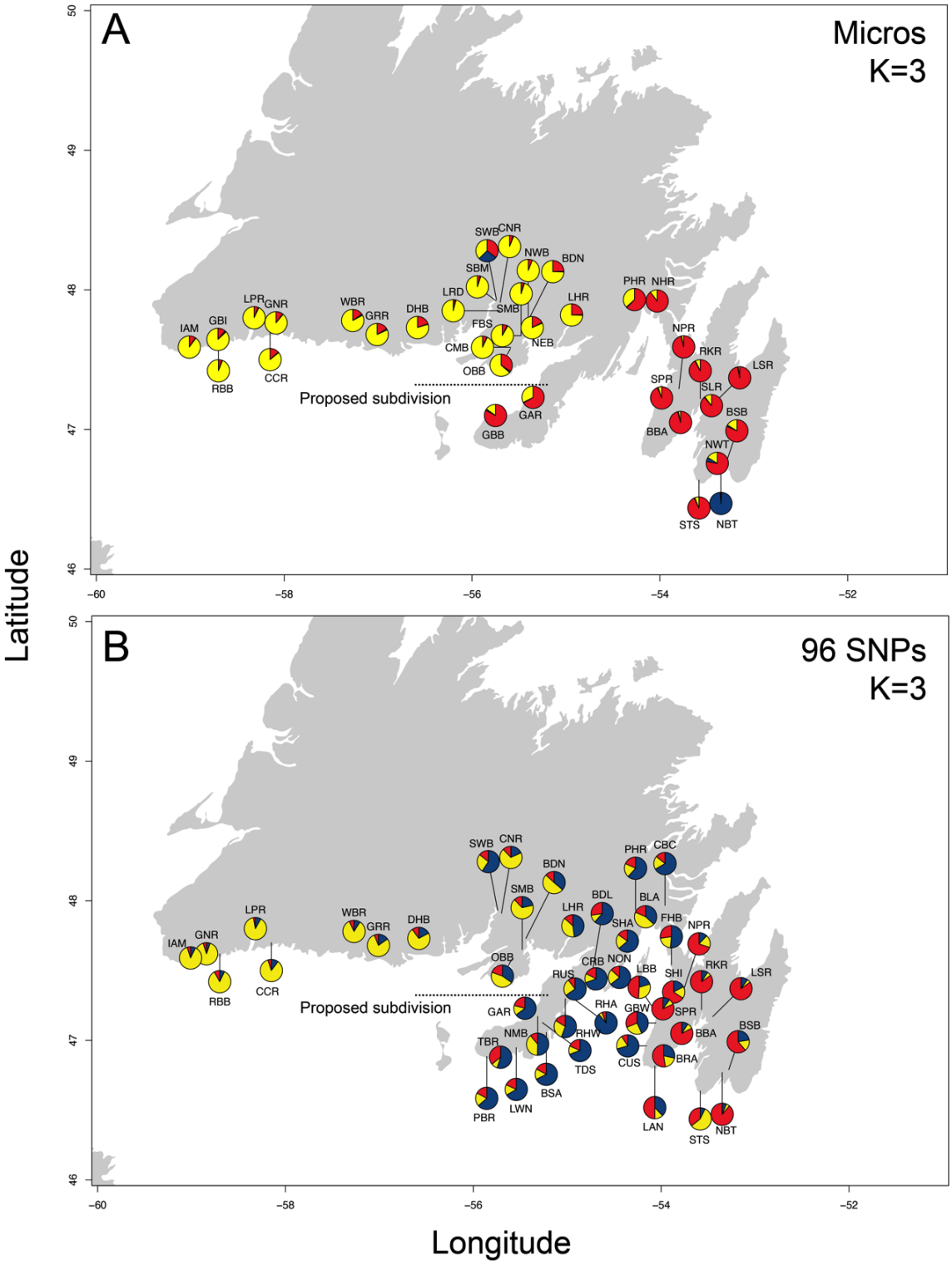


Figure 8. Maps of STRUCTURE results based on (A) 15 microsatellites and (B) 96 SNPs for Atlantic Salmon rivers in DU 4. Pie charts show proportion of membership for each population to three genetic clusters (K=3). The new proposed subdivision of the DU near Garnish River (GAR) is indicated by a dotted line. River abbreviations and sampling data for the 15 microsatellite and 96 SNP data sets can be found in Appendix Tables A1 and A2 respectively.

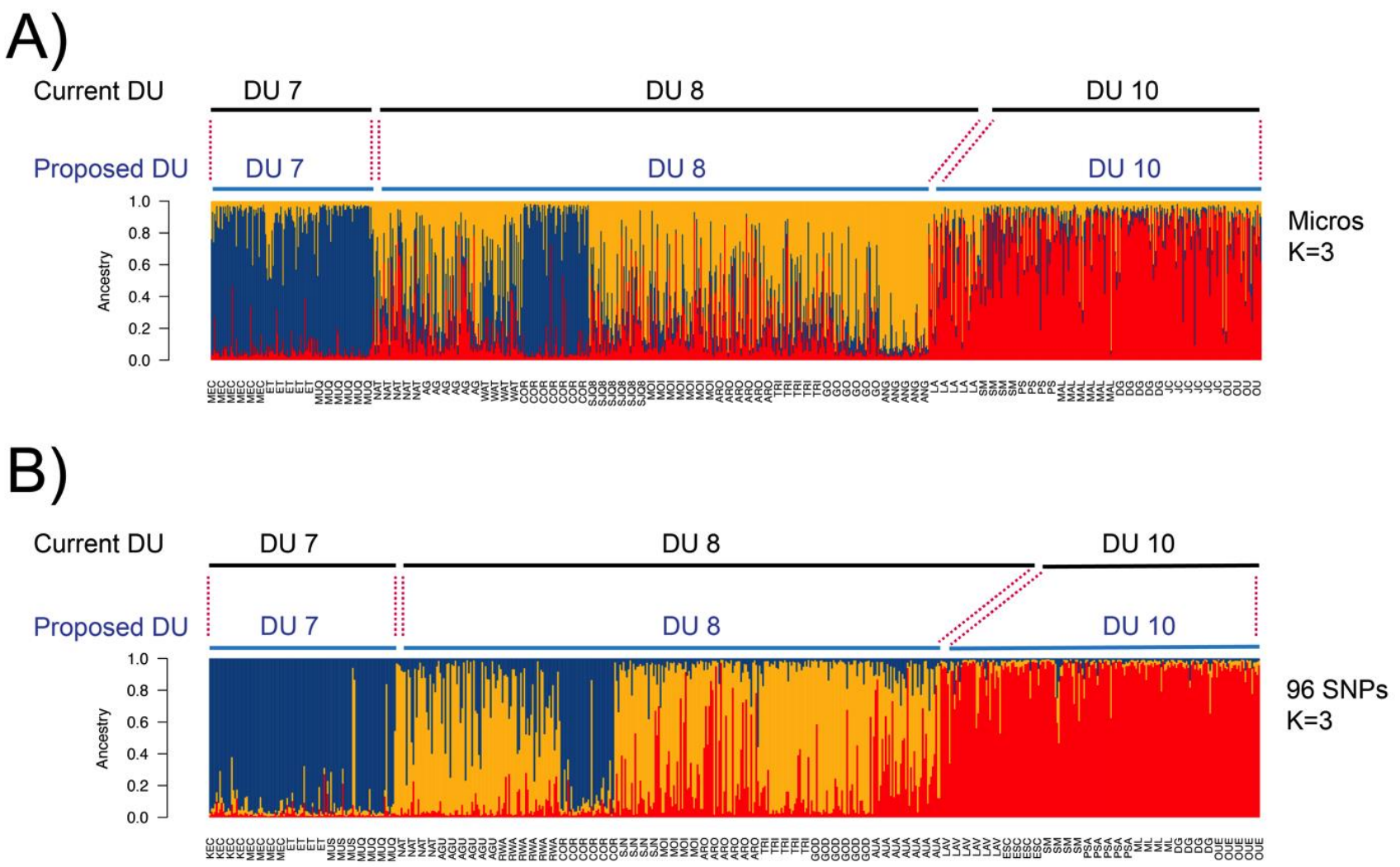


Figure 9. STRUCTURE plots based on (A) 15 microsatellites and (B) 96 SNPs for Atlantic Salmon rivers in DU 7, DU 8, and DU 10. Colours indicate the proportion of membership to the three genetic clusters (K=3) for each individual. Black lines above plots indicate previous assignment of rivers to each DU, with revised boundaries indicated below with blue lines. River abbreviations and sampling data for the 15 microsatellite and 96 SNP datasets can be found in Appendix Tables A1 and A2 respectively.

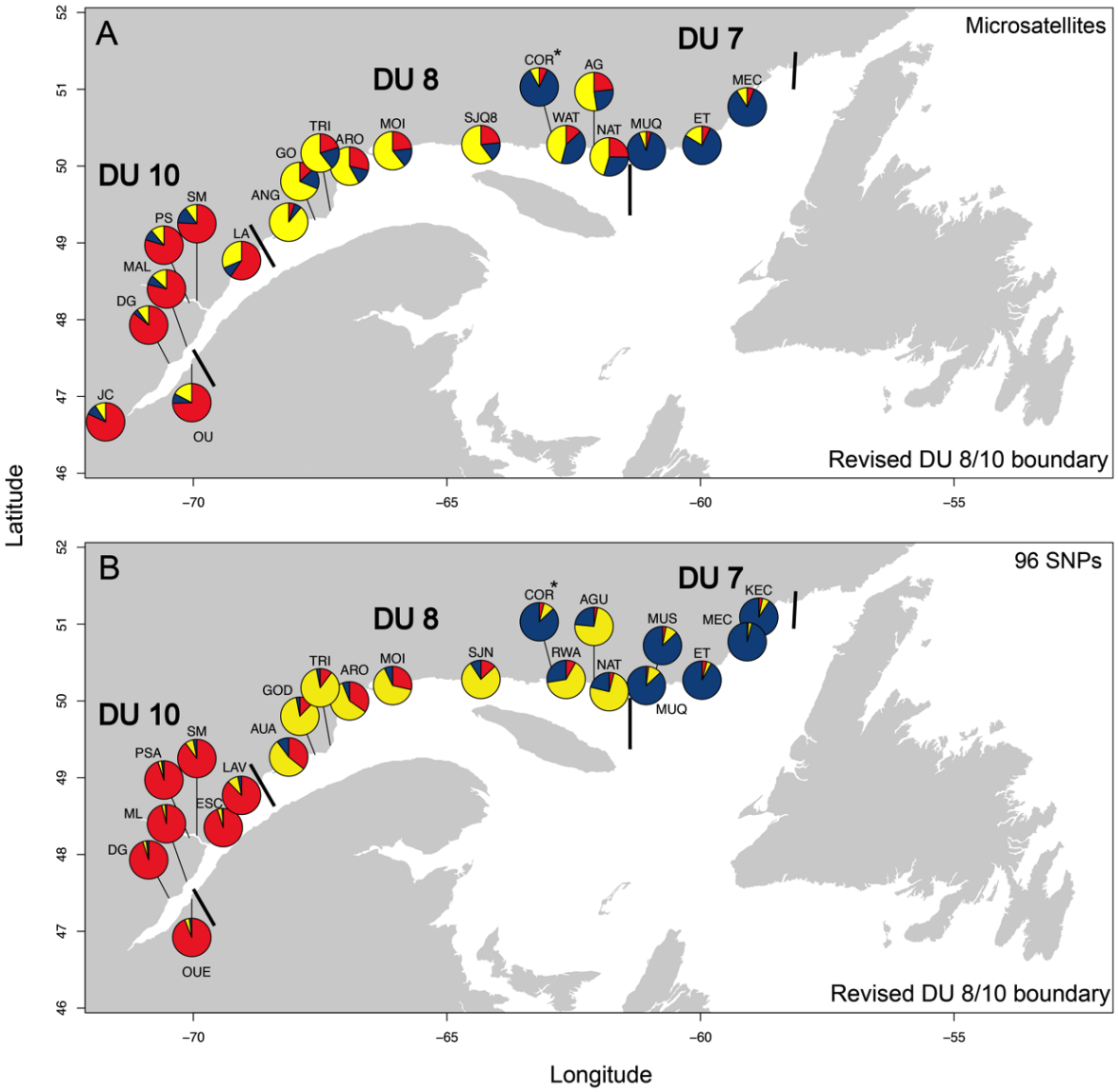


Figure 10. Maps of STRUCTURE results based on (A) 15 microsatellites and (B) 96 SNPs for Atlantic Salmon rivers in DU 7, DU 8, and DU 10. Pie charts show proportion of membership to the three genetic clusters (K=3) for each population. The revised boundary between DU 10 and DU 8 is indicated east of Laval (LA). The asterisk (*) above one population, Corneille (COR), indicates that this population has a genetic signature associated with the neighbouring DU and we propose that this site should be moved into DU 7. River abbreviations and sampling data for the 15 microsatellite and 96 SNP datasets can be found in Appendix Tables A1 and A2 respectively.

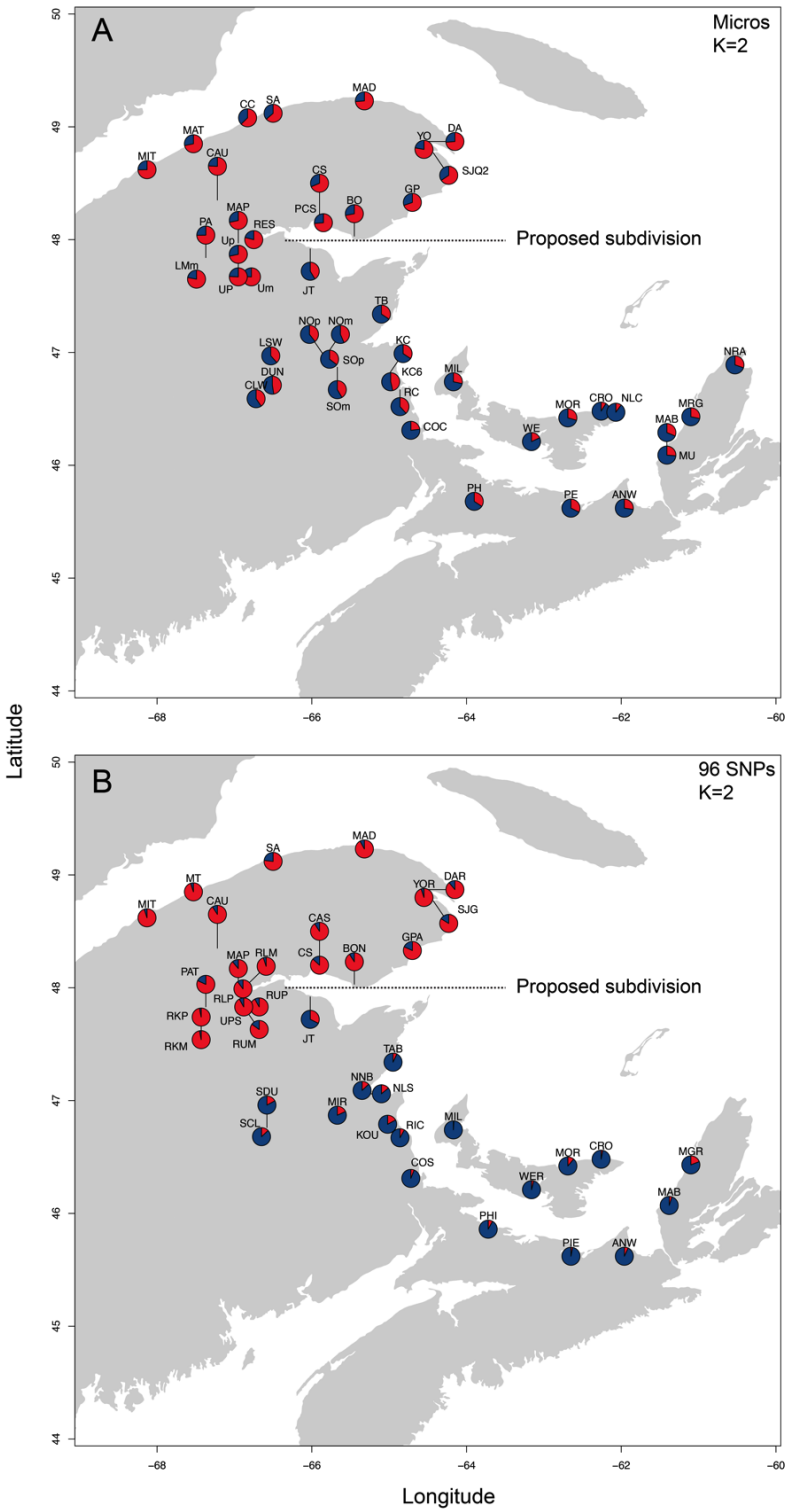


Figure 11. Maps of STRUCTURE results based on (A) 15 microsatellites and (B) 96 SNPs for Atlantic Salmon rivers in DU 12. Pie charts show proportion of membership for each population to two genetic clusters (K=2). The new proposed subdivision of the DU dividing Gaspé and Gulf regions is indicated by a dotted line near Restigouche River. River abbreviations and sampling data for the 15 microsatellite and 96 SNP datasets can be found in Appendix Tables A1 and A2 respectively.

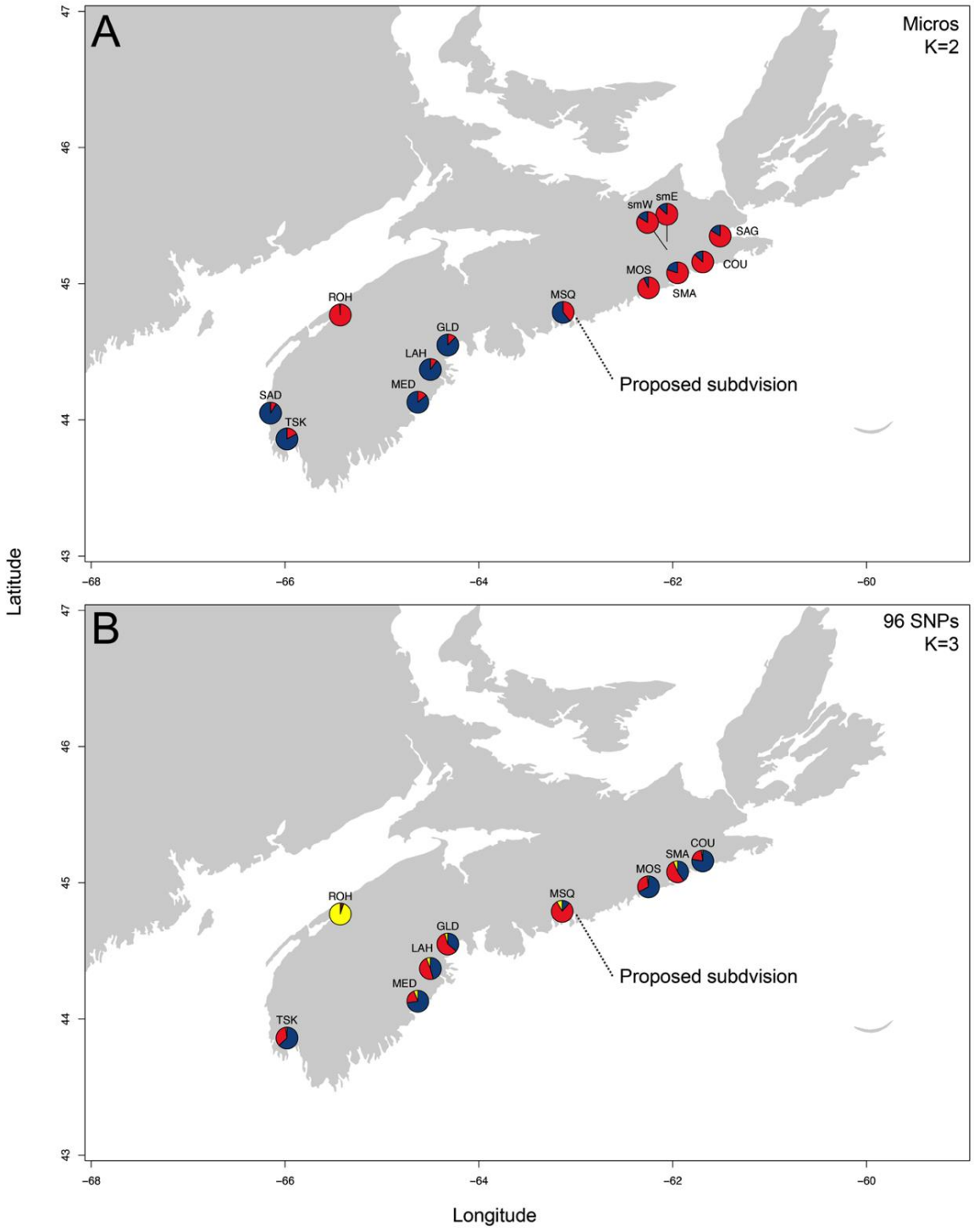


Figure 12. Maps of STRUCTURE results based on (A) 15 microsatellites and (B) 96 SNPs for Atlantic Salmon rivers in DU 14. Pie charts show proportion of membership for each population to the (A) two genetic clusters (K=2) for microsatellites and (B) three genetic clusters (K=3) for SNPs. The proposed subdivision of the DU is indicated by the dotted line near Musquodoboit River (MSQ), which is more clearly supported by (A) microsatellite data. River abbreviations and sampling data for the 15 microsatellite and 96 SNP datasets can be found in Appendix Tables A1 and A2 respectively.

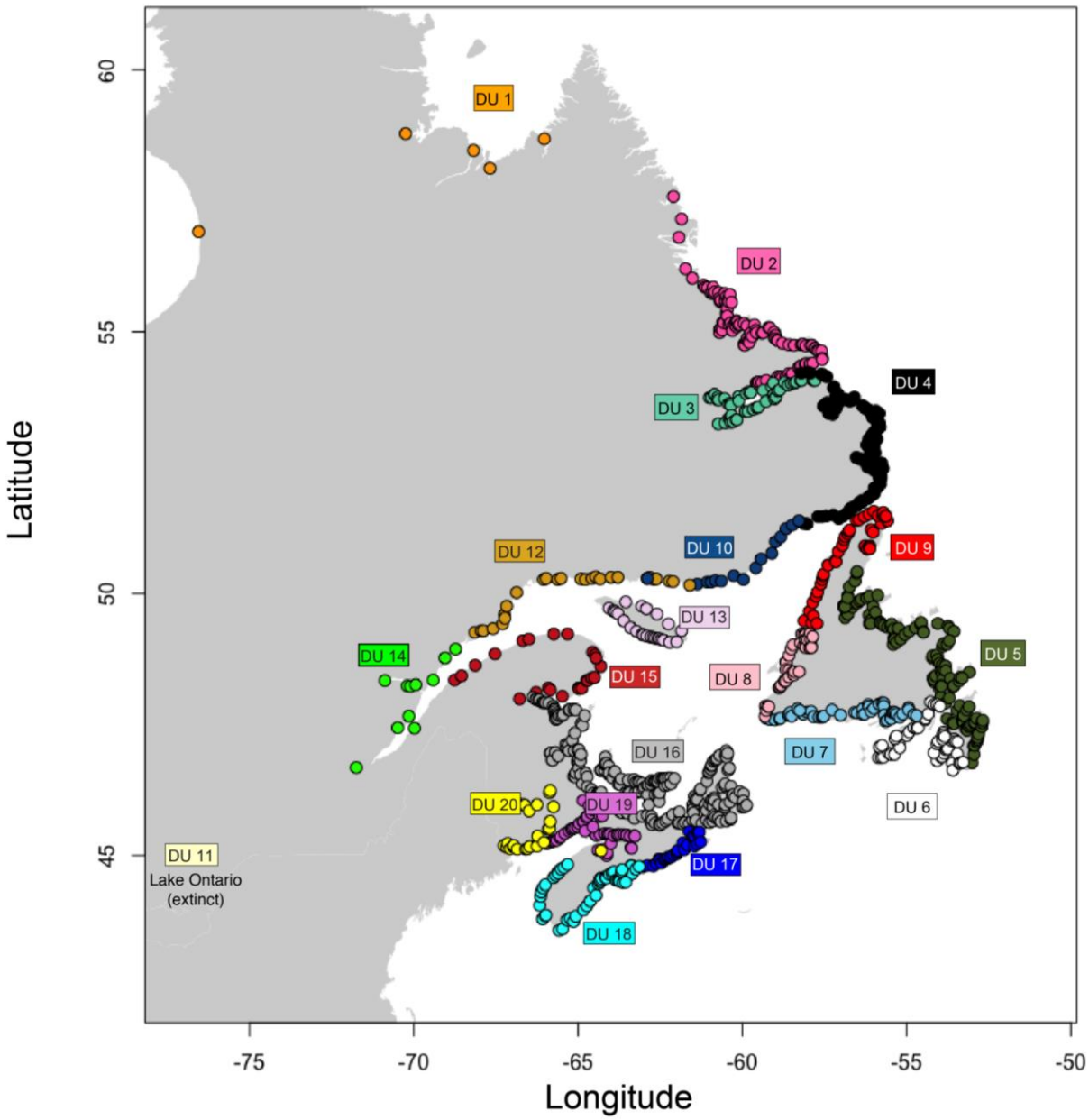


Figure 13. Map of proposed 19 DUs for anadromous Atlantic Salmon in Canada. All salmon rivers based on the NASCO river database are coloured by their proposed DU. Note that two individual rivers were placed in adjacent DUs (Corneille and Gaspereau), resulting in non-contiguous boundaries of the DUs. For support of these DUs see Table 1 and 2, as well as main text.

APPENDIX

Appendix Table A1. Sampling locations for 15 microsatellites dataset for rivers located in Atlantic Salmon designatable units (DUs). Location coordinates and sample size are provided, as well as sample year and life stage when data were available.

DU	River Name	Code	Longitude	Latitude	Sample Size	Sample Year	Life Stage
1	George	GE	-66.17	58.82	48	2004	adults
1	Aux Feuilles	AF	-70.07	58.77	50	2004	adults
1	Koksoak	KO	-68.17	58.53	50	2004	adults
2	Webbs Brook	WBB	-61.93	56.80	43	2011	parr
2	Hunt River	HUR	-60.67	55.57	28	2009	parr & smolt
2	English River	ENG	-59.75	54.97	99	2010	parr
2	Big River	BIG	-58.94	54.84	94	2009	parr
2	Red Wine River	RWR	-61.00	53.93	40	2009	parr
2	Muddy Bay Brook	MBB	-57.07	53.64	106	2011 & 2004	parr & adults
2	Cape Caribou	CAC	-60.42	53.62	76	2011	parr
2	Sandhill River	SAN	-56.35	53.57	99	2010	parr
2	Sandhill River	SHR	-56.35	53.57	50	2004	adults
2	Eagle	EAG	-57.47	53.53	176	2011	adults
2	Kenamu River	KEN	-59.91	53.48	41	2009	parr
2	Southwest Brook	PRB	-57.23	53.42	42	2011	parr
2	South Feeder	PRF	-57.23	53.42	40	2011	parr
2	South West Brook	SW	-57.23	53.42	57	2004	adults
2	Traverspine River	TSP	-60.28	53.28	10	2011	parr
2	Hawke River	HWK	-56.06	53.03	31	2011	parr
2	Alexis River	ALX	-56.53	52.60	81	2009	parr
2	Shinny's River	SHINNY	-56.34	52.59	65	2011	parr
2	St. Lewis River	SLW	-56.17	52.43	64	2011	parr
2	Port Marum	PMR	-55.74	52.40	33	2011	parr
2	Mary's Harbour	MYH	-55.82	52.31	69	2011	parr
2	St. Mary's River	SMR	-55.85	52.30	100	2010	parr
2	St. Charles' River	CHR	-55.84	52.23	60	2011	parr

DU	River Name	Code	Longitude	Latitude	Sample Size	Sample Year	Life Stage
2	Pinware River	PIN	-56.69	51.63	100	2010	parr
2	L'Anse au Loup	LAL	-56.82	51.53	61	2011	parr
2	Forteau	FOR	-56.94	51.48	58	2011	parr
2	Saint-Paul	SPQ	-57.70	51.47	53	2004	adults
2	Vieux Fort	VF	-58.02	51.32	49	2004	adults
2	Napetipi	NAP	-58.05	51.30	50	2004	adults
3	Beaver Brook	BVB	-56.15	50.90	100	2009	parr
3	Main River	MNR	-56.90	49.77	100	2010	parr
3	Campbellton River - Campbellton River Watershed	CMP	-54.92	49.28	50	2009	parr
3	Indian Arm Brook - Campbellton River Watershed	IAB	-54.92	49.28	50	2009	parr
3	Indian Bay Brook	IBB	-53.88	49.04	99	2009	parr
3	Rocky Brook - Exploits River Watershed	RBR	-55.41	49.03	50	2009	parr
3	Salmon River - Gander River Watershed	SRN	-54.87	49.00	49	2009	parr
3	Badger Brook - Exploits River Watershed	BBR	-56.03	48.98	50	2009	parr
3	Great Rattling Brook - Exploits River Watershed	GRB	-55.55	48.97	98	2009	parr
3	Junction Brook - Exploits River Watershed	JUB	-56.02	48.97	50	2009	parr
3	Little Junction Brook - Exploits River Watershed	LJB	-56.02	48.97	50	2009	parr
3	Gander River / Soulis Brook - Gander River Watershed	GSB	-54.45	48.89	48	2009	parr
3	Terra Nova River - Terra Nova River Watershed	TNR	-54.00	48.67	100	2009	parr
4	Pipers Hole River	PHR	-54.27	47.93	70	2009	parr
4	Southwest Brook	SWB	-55.74	47.93	76	2002 & 2011	parr
4	Southeast Brook	SBM	-55.74	47.92	76	2011	parr
4	North Harbour River	NHR	-54.03	47.92	57	2011	parr
4	Conne River	CNR	-55.70	47.91	137	1987 & 1988 & 2010	parr
4	Little River	LRD	-55.70	47.85	82	2011	parr

DU	River Name	Code	Longitude	Latitude	Sample Size	Sample Year	Life Stage
4	Long Harbour River	LHR	-54.94	47.82	68	2008	parr
4	LaPoile River	LPR	-58.32	47.80	81	2008	parr
4	White Bear River	WBR	-57.27	47.78	79	2008	parr
4	Grandy River	GNR	-58.09	47.76	77	2011	parr
4	Northwest Brook	NWB	-55.40	47.74	22	2002	parr
4	Bay Du Nord River	BDN	-55.44	47.73	188	2002, 2008 & 2011	parr
4	Dollard Bk / Hare Bay Bk	DHB	-56.58	47.73	78	2011	parr
4	Northeast Brook	NEB	-55.36	47.73	24	2002	parr
4	Cinq Cerf River	CCR	-58.15	47.70	80	2011	parr
4	Grey River	GRR	-57.01	47.68	95	2008	parr
4	Simm Brook	FBS	-55.48	47.67	73	2011	parr
4	Simms Brook	SMB	-55.48	47.67	20	2002	parr
4	Grady Burnt Island (actually 'Great Burnt Island')	GBI	-58.71	47.64	75	2011	parr
4	Rose Blanch Brook	RBB	-58.70	47.62	75	2011	parr
4	Isle aux Morte	IAM	-59.01	47.59	75	2011	parr
4	Conne Mill Brook	CMB	-55.59	47.59	77	2011	parr
4	Old Bay Brook	OBB	-55.59	47.58	69	2011	parr
4	North Placentia River	NPR	-53.80	47.29	101	2011	parr
4	Garnish River	GAR	-55.35	47.23	100	2009	parr
4	South Placentia River	SPR	-53.88	47.23	73	2011	parr
4	Rocky River	RKR	-53.57	47.22	100	2010	parr
4	Little Salmonier River	LSR	-53.45	47.17	75	2011	parr
4	Salmonier River	SLR	-53.45	47.17	92	2008	parr
4	Grand Bank Brook	GBB	-55.75	47.10	100	2009	parr
4	Big Barachois River	BBA	-53.78	47.05	68	2011	parr
4	Big Barachois River	BSB	-53.28	46.79	73	2011	parr
4	Northeast Brook-Trepassey	NBT	-53.35	46.77	261	2010 & 2011	parr
4	Northwest Trepassey	NWT	-53.39	46.76	88	2011	parr
4	St. Shotts	STS	-53.58	46.64	75	2011	parr

DU	River Name	Code	Longitude	Latitude	Sample Size	Sample Year	Life Stage
5	Taylors Brook - Humber River Watershed	TYB	-57.10	49.55	50	2009	parr
5	Dead Water Brook - Humber River Watershed	DWB	-57.32	49.40	50	2009	parr
5	Pinchgut Brook - Harry's River Watershed	PGB	-58.10	48.79	50	2009	parr
5	Black Duck Brook - Harry's River Watershed	BDB	-58.39	48.56	50	2009	parr
5	Flat Bay	FLB	-58.58	48.41	96	2009	parr
5	Mid Barachois	MBA	-58.83	48.24	98	2009	parr
5	Grand Codroy Brook Watershed	COD	-59.25	47.85	96	2009	parr
6	Western Arm Brook	WAB	-56.76	51.19	99	2009	parr
6	St. Genvieve River - Main Stem - St. Genevieve River Watershed	SGR	-56.80	51.14	50	2009	parr
6	Roses Feeder - St. Genevieve River Watershed	ROF	-56.62	51.13	50	2009	parr
6	Big East River	BER	-57.17	50.63	99	2009	parr
6	River of Ponds	ROP	-57.39	50.54	100	2009	parr
6	Lomond River	LOM	-57.73	49.43	100	2009	parr
7	Gros Mecatina	MEC	-59.08	50.77	50	2004	adults
7	Etamamiou	ET	-59.97	50.27	48	2004	adults
7	Musquaro	MUQ	-61.07	50.20	50	2004	adults
8	Corneille	COR	-62.88	50.28	60	2004	adults
8	Saint-Jean (North Shore)	SJQ8	-64.33	50.28	50	2004	adults
8	Watshishou	WAT	-62.65	50.28	42	2004	adults
8	Aganus	AG	-62.10	50.22	48	2004	adults
8	Moisie	MOI	-66.07	50.20	68	2004	adults
8	Natashquan	NAT	-61.80	50.12	50	2004	adults
8	Aux Rochers	ARO	-66.92	50.00	50	2004	adults
8	Trinite	TRI	-67.30	49.42	50	2004	adults
8	Godbout	GO	-67.60	49.30	50	2004	adults
8	Aux Anglais	ANG	-68.12	49.27	45	2004	adults
8	Laval	LA	-69.05	48.77	50	2004	adults

DU	River Name	Code	Longitude	Latitude	Sample Size	Sample Year	Life Stage
9	Jupiter	JU	-63.58	49.47	50	2004	adults
9	Aux Saumons	SU	-62.23	49.42	44	2004	adults
9	Chaloupe	CH	-62.53	49.13	46	2004	adults
10	Sainte-Marguerite	SM	-69.93	48.25	36	2004	adults
10	Petit Saguenay	PS	-70.08	48.22	34	2004	adults
10	Malbaie	MAL	-70.13	47.65	50	2004	adults
10	Du Gouffre	DG	-70.48	47.43	48	2004	adults
10	Ouelle	OU	-70.03	47.42	39	2004	adults
10	Jacques Cartier	JC	-71.73	46.67	50	2004	adults
12	Madeleine	MAD	-65.32	49.23	49	2004	adults
12	Sainte-Anne	SA	-66.50	49.12	44	2004	adults
12	Cap Chat	CC	-66.83	49.08	46	2004	adults
12	Dartmouth	DA	-64.55	48.87	50	2004	adults
12	Matane	MAT	-67.53	48.85	50	2004	adults
12	York	YO	-64.55	48.80	50	2004	adults
12	Saint-Jean (Gaspesie)	SJQ2	-64.43	48.77	35	2004	adults
12	Mitis	MIT	-68.13	48.62	49	2004	adults
12	Causapscal1	CAU	-67.22	48.35	50	2004	adults
12	Grand Pabos	GP	-64.70	48.33	44	2004	adults
12	Grande Cascapedia	CS	-65.90	48.20	38	2004	adults
12	Petite Cascapedia	PCS	-65.85	48.15	67	2004	adults
12	Bonaventure	BO	-65.45	48.03	50	2004	adults
12	Restigouche	RES	-66.75	48.00	34	2004	adults
12	Matapedia1	MAP	-66.95	47.97	50	2004	adults
12	Jacquet	JT	-66.02	47.92	50	2010	adults
12	Upsalquitch 1*1	UP	-66.95	47.87	50	2004	adults
12	Upsalquitch 2*1	Up	-66.95	47.87	37	2004	adults
12	Patapedia1	PA	-67.37	47.84	47	2004	adults
12	Restigouche	RKRKED	-67.51	47.67	58	2004	adults
12	Restigouche	Um	-66.78	47.67	49	-	-

DU	River Name	Code	Longitude	Latitude	Sample Size	Sample Year	Life Stage
12	Little Main1	LMm	-67.49	47.65	8	2004	adults
12	Little Main1	LMp	-67.49	47.65	50	2004	adults
12	Tabusintac	TB	-65.10	47.34	50	2010	adults
12	Miramichi (N.W. Barrier) 2	MIRNWB	-66.24	47.25	50	2010	adults
12	Miramichi (Little South West) 2	LSW	-66.53	46.97	50	2010	adults
12	Miramichi	NOm	-65.83	46.96	63	-	-
12	Miramichi	NOp	-65.83	46.96	46	-	-
12	Miramichi (N.W.) 2	SOp	-65.77	46.94	51	2010	adults
12	Miramichi (S.W.) 2	SOm	-65.67	46.87	57	2010	adults
12	Kouchibouguac	KC	-65.02	46.79	33	2010	parr
12	Kouchibouguacis	KC6	-64.98	46.74	9	2010	adults
12	Mill	MIL	-64.17	46.74	32	2010	parr
12	Miramichi (Dungarvon) 2	DUN	-66.51	46.71	50	2010	adults
12	Richibouctou	RC	-64.86	46.67	20	2010	adults
12	Miramichi (Clearwater) 2	CLW	-66.72	46.59	50	2010	adults
12	Cross	CRO	-62.26	46.48	30	2010	parr
12	North Lake Creek	NLC	-62.07	46.47	29	2010	parr
12	Margaree	MRG	-61.10	46.43	49	2001	parr
12	Morell	MOR	-62.69	46.42	50	2010	parr
12	Cocagne	COC	-64.72	46.31	44	2010	parr
12	West	WE	-63.16	46.21	37	2010	parr
12	Mabou	MAB	-61.41	46.09	80	2006	parr
12	Mabou 1*	MU	-61.41	46.09	50	2010	parr
12	Phillip	PH	-63.90	45.68	27	2010	adults
12	Antigonish West	ANW	-61.96	45.62	50	2010	parr
12	Pictou East	PE	-62.65	45.62	31	2010	parr
13	North Aspy	NRA	-60.53	46.89	44	2006	parr
13	North: Victoria Co.	NRV	-60.62	46.30	73	2006	parr
13	Baddeck	BAD	-60.84	46.10	52	2010	parr
13	Middle: Victoria Co.	MDV	-60.91	46.08	73	2006	parr

DU	River Name	Code	Longitude	Latitude	Sample Size	Sample Year	Life Stage
13	Indian River (Eskasoni)	ESK	-60.60	45.94	52	2007	parr
13	Grand	GRA	-60.66	45.64	53	2010	parr
13	Inhabitants	INH	-61.23	45.60	53	2010	parr
14	Salmon: Guysborough Co.	SAG	-61.51	45.35	30	2009	parr
14	St. Mary's East	smE	-62.06	45.31	59	2007	parr
14	St. Mary's West	smW	-62.06	45.25	41	2007	parr
14	Country Harbour	COU	-61.69	45.16	42	2000	parr
14	Saint Mary's	SMA	-61.95	45.08	78	2000	parr
14	Moser	MOS	-62.25	44.97	58	2000	parr
14	Musquodoboit	MSQ	-63.13	44.79	53	2000	parr
14	Round Hill	ROH	-65.43	44.77	28	2000	parr
14	Gold	GLD	-64.32	44.55	84	2001	parr
14	LaHave	LAH	-64.50	44.37	49	2000	parr
14	Medway	MED	-64.63	44.13	83	2001	parr
14	Salmon: Digby Co.	SAD	-66.15	44.05	44	2000	parr
14	Tusket	TSK	-65.98	43.86	60	1999	parr
15	Upper Salmon River (NB)	USR	-64.95	45.60	55	2001	parr
15	Pointe Wolfe	PWF	-65.02	45.55	46	2002	parr or smolt
15	Big Salmon	BSR	-65.41	45.42	81	2001	parr
15	Great Village	GRV	-63.61	45.39	37	2001	parr
15	Economy	ECO	-63.91	45.38	30	2001	parr
15	Stewiacke	STW	-63.38	45.14	82	2001	parr
15	Gaspereau: Kings Co.	GAK	-64.27	45.10	66	2002	parr
16	Tobique	TOB	-67.70	46.77	84	2000 & 2001	parr
16	Nashwaak	NSH	-66.62	45.96	70	2000	parr
USA	Narraguagus River (Maine)	NGR	-67.92	44.60	119	2012 & 2013	-
USA	Penobscot (USA)	PEN	-68.80	44.52	100	2000 & 2001	adults
USA	Sheepscot River (Maine)	SHP	-69.69	43.80	119	2012 & 2013	-

Appendix Table A2. Sampling locations for 96 SNP dataset for rivers located in Atlantic Salmon designatable units (DUs). Location coordinates, data source, and sample size are provided, as well as sample year and life stage when data were available.

COSEWIC DU 2010	River Name	Pop Code	Latitude	Longitude	Sample Size	Data Source	Year	Life Stage
DU1	George	GE	58.82	-66.17	18	Moore et al. 2014	2004	adults
DU1	Aux Feuilles	AF	58.77	-70.07	25	Moore et al. 2014	2004	adults
DU1	Koksoak	KOK	58.53	-68.17	25	Moore et al. 2014	2004	adults
DU2	Webs Brook	WBB	56.80	-61.91	31	Jeffery et al. 2018	2011	parr
DU2	Hunt River	HU	55.57	-60.67	20	Moore et al. 2014	2009	parr & smolt
DU2	River 72	R72	55.12	-60.10	50	unpublished	2017	parr
DU2	Makkovik Brook	MKB	55.05	-59.16	47	unpublished	2017	parr
DU2	English River	ENG	54.97	-59.75	33	Jeffery et al. 2018	2010	parr
DU2	Makkovik River	MKR	54.96	-59.43	50	unpublished	2017	parr
DU2	Adlavik Brook	ADL	54.84	-59.14	49	unpublished	2017	parr
DU2	Big River	BIG	54.84	-58.94	26	Jeffery et al. 2018	2009	parr
DU2	Rattling Brook	RAT	54.78	-58.95	50	unpublished	2017	parr
DU2	Pamiulik River	PAM	54.72	-58.58	46	unpublished	2017	parr
DU2	Jeanette Bay Brook	JBB	54.72	-58.09	42	unpublished	2017	parr
DU2	South Brook	SBR	54.71	-59.91	47	unpublished	2017	parr
DU2	Michael River	MIC	54.67	-57.84	50	unpublished	2017	parr
DU2	Pottle's Bay	PBB	54.48	-57.73	21	unpublished	2016	parr
DU2	West Brook	WBL	54.40	-58.10	20	unpublished	2016	parr
DU2	Tom Luscombe	TOM	54.34	-58.55	20	unpublished	2016	parr
DU2	Main Brook	MB	54.24	-57.87	21	Sylvester et al. 2018	2013 or 2014	parr
DU2	Partridge Point	PPB	54.10	-59.48	21	unpublished	2016	parr
DU2	Double Mer	DBL	54.02	-59.65	21	unpublished	2016	parr
DU2	Red Wine River	RW	53.93	-61.00	22	Sylvester et al. 2018	2013 or 2014	parr
DU2	Mulligan River	MU	53.87	-60.09	21	Sylvester et al. 2018	2013 or 2014	parr
DU2	Crooked River	CR	53.87	-60.83	21	Sylvester et al. 2018	2013 or 2014	parr
DU2	Sebaskachu River	SK	53.79	-60.14	22	Sylvester et al. 2018	2013 or 2014	parr
DU2	Susan River	SR	53.74	-61.04	22	Sylvester et al. 2018	2013 or 2014	parr
DU2	Muddy Bay Brook	MBB	53.64	-57.07	34	Jeffery et al. 2018	2011	parr

COSEWIC DU 2010	River Name	Pop Code	Latitude	Longitude	Sample Size	Data Source	Year	Life Stage
DU2	Cape Caribou	CB	53.62	-60.42	21	Sylvester et al. 2018	2013 or 2014	parr
DU2	Sand Hill River	SH	53.57	-56.35	20	Sylvester et al. 2018	-	-
DU2	Eagle River	EA	53.53	-57.47	22	Sylvester et al. 2018	2011	parr
DU2	Kenamu River	KE	53.48	-59.91	22	Sylvester et al. 2018	2013 or 2014	parr
DU2	Paradise River	PA	53.42	-57.25	20	Sylvester et al. 2018	2011	parr
DU2	Southwest Brook	SW	53.42	-57.23	25	Moore et al. 2014	2004	adults
DU2	Peters River	PR	53.34	-60.71	21	Sylvester et al. 2018	2013 or 2014	parr
DU2	Kenemich River	KNM	53.32	-59.82	20	unpublished	2016	parr
DU2	Traverspine River	TR	53.28	-60.28	22	Sylvester et al. 2018	2013 or 2014	parr
DU2	Caroline River	CL	53.25	-60.42	20	Sylvester et al. 2018	2013 or 2014	parr
DU2	Hawke River	HWK	53.03	-56.06	31	Jeffery et al. 2018	2011	parr
DU2	Alexis	ALX	52.60	-56.53	34	Jeffery et al. 2018	2009	parr
DU2	Shinnys	SHR	52.59	-56.34	34	Jeffery et al. 2018	2011	parr
DU2	St. Lewis	SLW	52.43	-56.17	34	Jeffery et al. 2018	2011	parr
DU2	Port Marum	PMR	52.40	-55.74	33	Jeffery et al. 2018	2011	parr
DU2	St Charles	CHR	52.23	-55.84	34	Jeffery et al. 2018	2011	parr
DU2	Pinware	PIN	51.63	-56.69	34	Jeffery et al. 2018	2010	parr
DU2	L'anse au Loup River	LL	51.53	-56.82	22	Sylvester et al. 2018	2011	parr
DU2	Forteau River	FOR	51.48	-56.94	34	Moore et al. 2014	2011	parr
DU2	St Paul River	STP	51.47	-57.70	25	Bourret et al. 2013	2004	-
DU2	Vieux Fort	VF	51.32	-58.02	25	Moore et al. 2014	2004	adults
DU2	Napetipi	NAP	51.30	-58.05	25	Moore et al. 2014	2004	adults
DU3	Beaver Brook	BVB	50.90	-56.15	34	Jeffery et al. 2018	2009	parr
DU3	Sops Arm Brook - Main River	MNR	49.77	-56.90	34	Jeffery et al. 2018	2010	parr
DU3	Campbellton (also IAB)	CMP	49.28	-54.92	20	Bradbury et al. 2015	2009	parr
DU3	Indian Arm Brook	IAB	49.28	-54.92	34	Jeffery et al. 2018	2009	parr
DU3	Indian Bay Brook	IBB	49.04	-53.88	34	Jeffery et al. 2018	2009	parr
DU3	Exploits River - Junction Brook	JUB	49.03	-55.41	34	Jeffery et al. 2018	2009	parr

COSEWIC DU 2010	River Name	Pop Code	Latitude	Longitude	Sample Size	Data Source	Year	Life Stage
DU3	Salmon River NL (aka Salmon Brook in Gander River watershed)	SRN	49.00	-54.89	31	Jeffery et al. 2018	2009	parr
DU3	Great Rattling Brook - Exploits	GRB	48.97	-55.55	34	Jeffery et al. 2018	2009	parr
DU3	Terra Nova River	TNR	48.67	-54.00	34	Jeffery et al. 2018	2009	parr
DU4	Come By Chance River	CBC	47.97	-53.96	30	Unpublished	2017	parr
DU4	Pipers Hole Brook	PHR	47.93	-54.27	34	Jeffery et al. 2018; unpublished	2009 & 2017	parr
DU4	Southwest Brook Milltown	SWB	47.93	-55.74	33	Jeffery et al. 2018	2011	parr
DU4	Conne	CNR	47.91	-55.70	21	Bradbury et al. 2015	2010	parr
DU4	Black River	BLA	47.89	-54.17	24	Unpublished	2017	parr
DU4	Long Harbour	LHR	47.82	-54.94	20	Bradbury et al. 2015	2008	parr
DU4	La Poile	LPR	47.80	-58.32	20	Bradbury et al. 2015	2008	parr
DU4	White Bear River	WBR	47.78	-57.27	31	Jeffery et al. 2018	2008	parr
DU4	Bay du Nord	BDN	47.73	-55.44	18	Bradbury et al. 2015	2008	parr
DU4	Dollards	DHB	47.73	-56.58	34	Bradbury et al. 2015	2011	parr
DU4	Sandy Harbour River	SHA	47.71	-54.36	30	Unpublished	2017	parr
DU4	Cinq Cerf Brook	CCR	47.70	-58.15	34	Jeffery et al. 2018	2011	parr
DU4	Grey	GRR	47.68	-57.01	20	Bradbury et al. 2015	2008	parr
DU4	Simmons Brook	SMB	47.65	-55.48	34	Jeffery et al. 2018	2002 & 2014	parr
DU4	Grandys Brook	GNR	47.62	-58.84	34	Jeffery et al. 2018	2011	parr
DU4	Rose Blanch Brook	RBB	47.62	-58.70	34	Jeffery et al. 2018	2011	parr
DU4	Isle aux Morts River	IAM	47.59	-59.01	34	Jeffery et al. 2018	2011	parr
DU4	Old Brook	OBB	47.58	-55.59	34	Jeffery et al. 2018	2011	parr
DU4	Fair Haven Brook	FHB	47.54	-53.89	30	Unpublished	2017	parr
DU4	Bay de L'Eau River	BDL	47.51	-54.73	30	Unpublished	2017	parr
DU4	Nonsuch River	NON	47.45	-54.64	28	Unpublished	2017	parr
DU4	Cape Roger Brook	CRB	47.44	-54.69	30	Unpublished	2017	parr
DU4	Rushoon River	RUS	47.37	-54.92	25	Unpublished	2017	parr

COSEWIC DU 2010	River Name	Pop Code	Latitude	Longitude	Sample Size	Data Source	Year	Life Stage
DU4	Ship Harbour Brook	SHI	47.35	-53.87	22	Unpublished	2017	parr
DU4	Red Harbour River East	RHA	47.33	-54.99	30	Unpublished	2017	parr
DU4	Red Harbour River West	RHW	47.30	-55.02	29	Unpublished	2017	parr
DU4	Northeast River Placentia	NPR	47.29	-53.80	18	Bradbury et al. 2015; unpublished	2011 & 2017	parr
DU4	Garnish	GAR	47.23	-55.35	22	Bradbury et al. 2015	2009	parr
DU4	Southeast Placentia River	SPR	47.23	-53.88	27	Unpublished	2017	parr
DU4	Rocky River	RKR	47.22	-53.57	40	Bradbury et al. 2015	2010	parr
DU4	Little Barasway Brook	LBB	47.18	-54.03	16	Unpublished	2017	parr
DU4	Northwest Brook (Mortier Bay)	NMB	47.17	-55.32	28	Unpublished	2017	parr
DU4	Salmonier	LSR	47.17	-53.45	19	Bradbury et al. 2015	2011	parr
DU4	Tides Brook	TDS	47.13	-55.26	17	Unpublished	2017	parr
DU4	Great Barasway Brook	GBW	47.12	-54.06	18	Unpublished	2017	parr
DU4	Big Salmonier Brook	BSA	47.06	-55.22	30	Unpublished	2017	parr
DU4	Big Barachois River	BBA	47.05	-53.78	34	Jeffery et al. 2018	2011	parr
DU4	Cuslett Brook	CUS	46.96	-54.16	30	Unpublished	2017	parr
DU4	Lawn River	LWN	46.95	-55.54	28	Unpublished	2017	parr
DU4	Branch River	BRA	46.89	-53.97	30	Unpublished	2017	parr
DU4	Piercey's Brook	PBR	46.88	-55.86	30	Unpublished	2017	parr
DU4	Taylor Bay Brook (Burin Penn)	TBR	46.88	-55.71	22	Unpublished	2017	parr
DU4	Lance River	LAN	46.82	-54.07	9	Unpublished	2017	parr
DU4	Biscay Bay River	BSB	46.79	-53.28	20	Bradbury et al. 2015	2011	parr
DU4	Northeast Brook Trepassey	NBT	46.77	-53.35	20	Bradbury et al. 2015	2010	parr
DU4	St Shotts River	STS	46.64	-53.58	33	Jeffery et al. 2018	2011	parr
DU5	Humber River	TYB	49.55	-57.10	34	Jeffery et al. 2018	2009	parr
DU5	Harrys River - Pinchgut	PGB	48.79	-58.10	34	Jeffery et al. 2018	2009	parr
DU5	Flat Bay Brook	FLB	48.41	-58.58	33	Jeffery et al. 2018	2009	parr

COSEWIC DU 2010	River Name	Pop Code	Latitude	Longitude	Sample Size	Data Source	Year	Life Stage
DU5	Middle Barachois River	MBA	48.24	-58.83	34	Jeffery et al. 2018	2009	parr
DU5	Little Codroy River	COD	47.77	-59.27	33	Jeffery et al. 2018	2009	parr
DU6	Western Arm	WAB	51.19	-56.76	20	Jeffery et al. 2018	-	-
DU6	St. Genevieve	SGR	51.14	-56.80	16	Jeffery et al. 2018	2009	parr
DU6	Big East River	BER	50.63	-57.17	34	Jeffery et al. 2018	2009	parr
DU6	River of Ponds	ROP	50.54	-57.39	34	Jeffery et al. 2018	2009	parr
DU6	Lomond River	LOM	49.43	-57.73	34	Jeffery et al. 2018	2009	parr
DU7	Kecarpoui	KEC	51.09	-58.85	21	Jeffery et al. 2018	-	-
DU7	Gros Mecatina	MEC	50.77	-59.08	25	Bourret et al. 2013; Moore et al. 2014	2004	-
DU7	Etamamiou	ET	50.27	-59.97	25	Moore et al. 2014	2004	adults
DU7	Musquanousse	MUS	50.22	-60.95	15	Moore et al. 2014	-	-
DU7	Musquaro	MUQ	50.20	-61.07	25	Moore et al. 2014	2004	adults
DU8	Corneille	COR	50.28	-62.88	32	Jeffery et al. 2018	2004	adults
DU8	Watshishou	RWA	50.28	-62.65	40	Jeffery et al. 2018	2004	adults
DU8	Saint Jean North Shore	SJQ8	50.28	-64.33	24	Moore et al. 2014	2004	adults
DU8	Aguanus	AGU	50.22	-62.10	33	Jeffery et al. 2018	-	-
DU8	Moisie	MOI	50.20	-66.07	25	Moore et al. 2014	2004	adults
DU8	Natashquan	NAT	50.12	-61.80	25	Moore et al. 2014	2004	adults
DU8	Aux Rochers	ARO	50.00	-66.92	40	Jeffery et al. 2018	2004	adults
DU8	Trinite	TRI	49.42	-67.30	25	Moore et al. 2014	2004	adults
DU8	Godbout	GOD	49.30	-67.60	40	Jeffery et al. 2018	2004	adults
DU8	Aux Anglais	AUA	49.27	-68.12	40	Jeffery et al. 2018	2004	adults
DU8	Laval	LAV	48.77	-69.05	39	Jeffery et al. 2018	2004	adults
DU8	Escoumins	ESC	48.35	-69.41	19	Jeffery et al. 2018	-	-
DU9	Aux Saumons	SU	49.42	-62.23	24	Moore et al. 2014	2004	adults
DU9	Chaloupe	CHA	49.13	-62.53	23	Bourret et al. 2013	2004	-
DU10	Sainte-Marguerite	SM	48.25	-69.93	25	Moore et al. 2014	2004	adults
DU10	Petit Saguenay	PSA	48.22	-70.08	34	Jeffery et al. 2018	2004	adults

COSEWIC DU 2010	River Name	Pop Code	Latitude	Longitude	Sample Size	Data Source	Year	Life Stage
DU10	Malbaie	ML	47.65	-70.13	19	Moore et al. 2014	2004	adults
DU10	Du Gouffre	DG	47.43	-70.48	25	Bourret et al. 2013; Moore et al. 2014	2004	-
DU10	Ouelle	OUE	47.42	-70.03	29	Jeffery et al. 2018	2004	adults
DU12	Madeleine	MAD	49.23	-65.32	40	Jeffery et al. 2018	2004	adults
DU12	Sainte-Anne	SA	49.12	-66.50	25	Moore et al. 2014	2004	adults
DU12	Matane	MT	48.85	-67.53	25	Moore et al. 2014	2004	adults
DU12	York	YOR	48.80	-64.55	40	Jeffery et al. 2018	2004	adults
DU12	Saint-Jean (Gaspsie)	SJQ2	48.77	-64.43	25	Moore et al. 2014	2004	adults
DU12	Mitis	MIT	48.62	-68.13	29	Jeffery et al. 2018	2004	adults
DU12	Causapscal	CAU	48.35	-67.22	40	Jeffery et al. 2018	2004	adults
DU12	Grand Pabos	GPA	48.33	-64.70	40	Jeffery et al. 2018	2004	adults
DU12	Cascapedia	CAS	48.20	-65.90	40	Jeffery et al. 2018	2004	adults
DU12	Grande Cascapedia	CS	48.20	-65.90	25	Moore et al. 2014	2004	adults
DU12	Bonaventure	BON	48.03	-65.45	37	Jeffery et al. 2018	2004	adults
DU12	Restigouche- Little Main-M	RLM	47.99	-66.89	8	Jeffery et al. 2018	2004	adults
DU12	Restigouche- Little Main-P	RLP	47.99	-66.89	40	Jeffery et al. 2018	2004	adults
DU12	Matapedia	MAP	47.97	-66.95	25	Bourret et al. 2013	2004	-
DU12	Jacquet	JT	47.92	-66.02	20	Moore et al. 2014	2010	adults
DU12	Patapedia	PAT	47.83	-67.37	40	Jeffery et al. 2018	2004	adults
DU12	Restigouche-Upsalquitch-M	RUM	47.83	-66.88	40	Jeffery et al. 2018	2004	adults
DU12	Restigouche-Upsalquitch-P	RUP	47.83	-66.88	39	Jeffery et al. 2018	2004	adults
DU12	Upsalquitch	UPS	47.83	-66.88	40	Jeffery et al. 2018	2004	adults
DU12	Restigouche-Kedgewick-M	RKM	47.74	-67.43	10	Jeffery et al. 2018	2004	adults
DU12	Restigouche-Kedgewick-P	RKP	47.74	-67.43	13	Jeffery et al. 2018	2004	adults
DU12	Tabusintac	TAB	47.34	-64.95	40	Jeffery et al. 2018	2010	adults
DU12	NWM NW Barrier	NNB	47.09	-65.35	40	Jeffery et al. 2018	2010	adults

COSEWIC DU 2010	River Name	Pop Code	Latitude	Longitude	Sample Size	Data Source	Year	Life Stage
DU12	NWM Little SW	NLS	47.06	-65.30	40	Jeffery et al. 2018	2010	adults
DU12	Miramichi	MIR	46.87	-65.67	25	Moore et al. 2014	2010	adults
DU12	Kouchibouguac	KOU	46.79	-65.02	30	Jeffery et al. 2018	2010	parr
DU12	SWM Dungarvon	SDU	46.76	-66.58	40	Jeffery et al. 2018	2010	adults
DU12	Mill River	MIL	46.74	-64.17	32	Jeffery et al. 2018	2010	parr
DU12	SWM Clearwater	SCL	46.68	-66.65	40	Jeffery et al. 2018	2010	adults
DU12	Richibucto	RIC	46.67	-64.86	20	Jeffery et al. 2018	2010	adults
DU12	Cross	CRO	46.48	-62.26	20	Moore et al. 2014	2010	parr
DU12	Margaree	MGR	46.43	-61.10	20	Moore et al. 2014	2001	parr
DU12	Morell	MOR	46.42	-62.69	40	Jeffery et al. 2018	2010	parr
DU12	Cocagne	COS	46.31	-64.72	40	Jeffery et al. 2018	2010	parr
DU12	West River	WER	46.21	-63.16	37	Jeffery et al. 2018	2010	parr
DU12	Mabou	MAB	46.07	-61.38	39	Jeffery et al. 2018	2010	parr
DU12	Phillip	PHI	45.86	-63.72	26	Jeffery et al. 2018	2010	adults
DU12	Antigonish West	ANW	45.62	-61.96	20	Moore et al. 2014	2010	parr
DU12	Pictou East River	PIE	45.62	-62.65	31	Jeffery et al. 2018	2010	parr
DU13	North	NRV	46.30	-60.62	20	Moore et al. 2014	2006	parr
DU13	Eskasoni	ESK	45.94	-60.60	14	Jeffery et al. 2018	2007	parr
DU13	Indian River	IND	45.60	-61.23	34	Jeffery et al. 2018	-	-
DU14	Country Harbour River	COU	45.16	-61.69	32	Jeffery et al. 2018	2000	parr
DU14	St Mary's (NS)	SMA	45.08	-61.95	20	Moore et al. 2014	2000	parr
DU14	Moser River	MOS	44.97	-62.25	33	Jeffery et al. 2018	2000	parr
DU14	Musquodobit River	MSQ	44.79	-63.14	32	Jeffery et al. 2018	2000	parr
DU14	Round Hill River	ROH	44.77	-65.43	28	Jeffery et al. 2018	2000	parr
DU14	Gold River	GLD	44.55	-64.32	34	Jeffery et al. 2018	2001	parr
DU14	LaHave	LAH	44.37	-64.50	22	Moore et al. 2014	2000	parr
DU14	Medway	MED	44.13	-64.63	10	Moore et al. 2014	2001	parr
DU14	Tusket River	TSK	43.86	-65.98	34	Jeffery et al. 2018	1999	parr
DU15	Pointe Wolfe River	PWF	45.55	-65.02	34	Jeffery et al. 2018	2002	parr or smolt

COSEWIC DU 2010	River Name	Pop Code	Latitude	Longitude	Sample Size	Data Source	Year	Life Stage
DU15	Big Salmon	BSR	45.42	-65.41	19	Moore et al. 2014	2000	parr
DU15	Great Village River	GRV	45.39	-63.61	28	Jeffery et al. 2018	2001	parr
DU15	Economy River	ECO	45.38	-63.91	34	Jeffery et al. 2018	2001	parr
DU15	North River NS	NRH	45.38	-63.31	22	Jeffery et al. 2018	-	-
DU15	Stewiacke	STW	45.14	-63.38	27	Moore et al. 2014	2001	parr
DU15	Gaspereau	GAK	45.10	-64.27	20	Moore et al. 2014	2001	parr
DU16	Tobique	TOB	46.77	-67.70	16	Moore et al. 2014	2000 and 2011	parr
DU16	Nashwaak	NSH	45.96	-66.62	15	Moore et al. 2014	2000	parr

Appendix Table A3. Sampling locations for the genomic datasets (220,000 SNP array and whole genome sequencing) for rivers located in Atlantic Salmon designatable units (DUs). Location coordinates and sample size are provided, as well as sample year and life stage when data were available.

DU COSEWIC 2010	River Name	Code	Latitude	Longitude	Sample size	Sample Year	Life stage
220,000 SNP array		**-**	**-**	**-**	**-**	**-**	**-**
DU2	Hunt River	HU	55.57	-60.67	20	-	-
DU2	English River	ENG	54.97	-59.75	28	2010	parr
DU2	Big River	BIG	54.84	-58.94	28	2009	parr
DU2	Main Brook	MB	54.24	-57.87	21	2013–14	parr
DU2	Red Wine River	RW	53.93	-61.00	22	2013–14	parr
DU2	Mulligan River	MU	53.87	-60.09	17	2013–14	parr
DU2	Crooked River	CR	53.87	-60.83	21	2013–14	parr
DU2	Sebaskachu River	SK	53.79	-60.14	22	2013–14	parr
DU2	Susan River	SR	53.74	-61.04	22	2013–14	parr
DU2	Cape Caribou	CB	53.62	-60.42	21	2013–14	parr
DU2	Sand Hill River	SH	53.57	-56.35	20	-	-
DU2	Eagle River	EA	53.53	-57.47	22	-	-
DU2	Kenamu River	KE	53.48	-59.91	22	2013–14	parr
DU2	Paradise River	PA	53.42	-57.25	20	2011	parr
DU2	Peters River	PR	53.34	-60.71	21	2013–14	parr

DU COSEWIC 2010	River Name	Code	Latitude	Longitude	Sample size	Sample Year	Life stage
DU2	Traverspine River	TR	53.28	-60.28	22	2013–14	parr
DU2	Caroline River	CL	53.25	-60.42	20	2013–14	parr
DU2	St Charles	CHR	52.23	-55.84	27	2011	parr
DU2	L'anse au Loup River	LL	51.53	-56.82	22	2011	parr
DU2	Forteau River	FO	51.48	-56.94	21	2011	parr
DU3	Beaver Brook	BVB	50.90	-56.15	29	2009	parr
DU3	Great Rattling Brook - Exploits	GRB	49.62	-56.17	26	2010	parr
DU3	Campbellton	CMP	49.28	-54.93	25	2009	parr
DU3	Terra Nova River	TNR	48.67	-54.00	29	2009	parr
DU4	North Brook Trepassey	NBT	46.74	-53.36	25	2010	parr
DU4	Little Salmonier	LSR	47.04	-53.75	17	2011	parr
DU4	Northeast Placentia River	NPR	47.29	-53.80	81	2017–19	parr
DU4	Ship Harbour Brook	SHI	47.35	-53.87	84	2017–19	parr
DU4	Southeast Placentia River	SPR	47.23	-53.88	97	2017–19	parr
DU4	Fair Haven Brook	FHB	47.54	-53.89	103	2017–19	parr
DU4	Come By Chance River	CBC	47.97	-53.96	79	2017–19	parr
DU4	Branch River	BRA	46.89	-53.97	92	2017–19	parr
DU4	North Harbour River	NHR	47.92	-54.03	88	2017–19	parr
DU4	Little Barasway Brook	LBB	47.18	-54.03	15	2017–19	parr
DU4	Great Barasway Brook	GBW	47.12	-54.06	89	2017–19	parr
DU4	Lance River	LAN	46.82	-54.07	9	2017–19	parr
DU4	Cuslett Brook	CUS	46.96	-54.16	99	2017–19	parr
DU4	Black River	BLA	47.89	-54.17	83	2017–19	parr
DU4	Pipers Hole River	PHR	47.93	-54.27	88	2017–19	parr
DU4	Sandy Harbour River	SHA	47.71	-54.36	74	2017–19	parr
DU4	Nonsuch River	NON	47.45	-54.64	93	2017–19	parr
DU4	Cape Roger Brook	CRB	47.44	-54.69	86	2017–19	parr
DU4	Bay de L'Eau River	BDL	47.51	-54.73	91	2017–19	parr

DU COSEWIC 2010	River Name	Code	Latitude	Longitude	Sample size	Sample Year	Life stage
DU4	Rushoon River	RUS	47.37	-54.92	85	2017–19	parr
DU4	Long Harbour	LHR	47.82	-54.94	20	2012	parr
DU4	Red Harbour River East	RHA	47.33	-54.99	91	2017–19	parr
DU4	Red Harbour River West	RHW	47.30	-55.02	78	2017–19	parr
DU4	Big Salmonier Brook	BSA	47.06	-55.22	84	2017–19	parr
DU4	Tides Brook	TDS	47.13	-55.26	69	2017–19	parr
DU4	Northwest Brook (Mortier Bay)	NMB	47.17	-55.32	87	2017–19	parr
DU4	Garnish	GAR	47.23	-55.35	22	2009	parr
DU4	Bay du Nord	BDN	47.73	-55.44	20	2008	parr
DU4	Lawn River	LWN	46.95	-55.54	81	2017–19	parr
DU4	Conne	CNR	47.91	-55.70	90	2017–19	parr
DU4	Taylor Bay Brook (Burin Penn)	TBR	46.88	-55.71	80	2017–19	parr
DU4	Piercey's Brook	PBR	46.88	-55.86	83	2017–19	parr
DU4	Dollards Brook	DLR	48.02	-56.57	26	2016	parr
DU4	Isle aux Morts River	IAM	47.59	-59.01	28	2011	parr
DU5	Humber River	TYB	49.55	-57.10	29	2009	parr
DU5	Flat Bay Brook	FLB	48.41	-58.58	24	2009	parr
DU5	Little Codroy River	COD	47.77	-59.27	28	2009	parr
DU6	Western Arm	WAB	51.19	-56.76	18	2016	adults
DU6	Big East	BER	50.63	-57.17	27	2009	parr
DU6	Trout River	TRE, TRF, TRN, TRW	49.64	-57.75	27	2019	parr
DU8	Corneille	COR	50.28	-62.88	28	2018	adult
DU8	Saint-Jean (NorthShore) SJQ8	SJQ	50.28	-64.33	28	2018	adult
DU8	Natashquan	NAT	50.12	-61.80	28	2018	adult
DU8	Riviere Aux Rochers	ARO	50.00	-66.86	48	2012	adult
DU8	Riviere de la Trinite	TRI	49.42	-67.30	49	2012	adult

DU COSEWIC 2010	River Name	Code	Latitude	Longitude	Sample size	Sample Year	Life stage
DU9	Jupiter	JUP	49.47	-63.58	28	2018	adult
DU10	A mars	aMars	48.34	-70.88	26	2018	adult
DU12	Madeleine	MAD	49.23	-65.32	28	2018	adult
DU12	Matapedia	MAT	48.18	-67.14	15	2018	parr
DU12	Kedgwick	KED	47.91	-67.91	15	2018	parr
DU12	Patapedia	PAT	47.86	-67.39	24	2018	parr
DU12	Upsalquitch	UPS	47.57	-66.54	28	2018	parr
DU12	Miramichi-Upper Northwest	MUN	47.17	-65.94	24	2016	parr
DU12	Kouchibouguac	KOU	46.74	-65.20	31	2018	parr
DU12	Cheticamp River	CHT	46.64	-60.95	12	2018	parr
DU12	Northwest Complex (PEI)	NWP	46.63	-64.04	17	2018	parr
DU12	Miramichi-Upper Southwest	MSW	46.55	-66.04	23	2016	parr
DU12	Northeast Margaree	MNE	46.47	-60.92	12	2018	parr
DU12	Northeast Complex-1 (PEI)	NEP	46.45	-62.21	27	2018	parr
DU12	Northeast Complex-2 (PEI)	NET	46.38	-62.57	24	2018	parr
DU12	Richibucto	RIC	46.36	-65.15	31	2018	parr
DU12	Morells	MOR	46.30	-62.71	18	2018	parr
DU12	South Central PEI	SCP	46.28	-63.49	14	2018	parr
DU12	Southwest Margaree	MRS	46.24	-61.12	14	2018	parr
DU12	Mabou River	MAB	46.04	-61.31	27	2018	parr
DU12	Graham River	JGC	45.86	-61.49	11	2018	parr
DU12	River Philip	RPH	45.59	-63.82	17	2018	parr
DU12	East River Pictou	PIE	45.54	-62.88	23	2018	parr
DU13	Clyburn	CLY	46.66	-60.41	28	2019	-
DU13	Baddeck	BAD	46.10	-60.84	28	2016	parr
DU13	Inhabitants River	INH	45.60	-61.23	28	2016	parr
DU14	Sheet Harbour West River	WES	44.95	-62.59	28	2019	smolt

DU COSEWIC 2010	River Name	Code	Latitude	Longitude	Sample size	Sample Year	Life stage
DU14	LaHave	LAH	44.37	-64.50	22	-	-
DU15	Big Salmon	BSR	45.42	-65.41	22	2014	-
DU15	North River NS	NRH	45.38	-63.31	22	-	-
DU15	Stewiacke	STW	45.14	-63.38	22	2014	-
DU15	Gaspereau River	GAK	45.06	-64.38	26	2016	-
DU16	Nashwaak	NSH	45.96	-66.62	20	2006–09	-
Whole genome sequencing		-	-	-	-	-	-
DU2	Du Vieux Fort	VF	51.32	-58.03	10	-	-
DU2	Saint-Paul	SP	51.49	-57.69	10	-	-
DU8	Laval	LA	48.77	-69.05	10	-	-
DU9	De la Chaloupe	CH	49.14	-62.54	10	-	-
DU9	Jupiter	JU	49.48	-63.61	10	-	-
DU10	Malbaie (Charlevoix)	MA	47.66	-70.15	10	-	-
DU12	Bonaventure	BO	48.04	-65.47	10	-	-
DU12	Petite riviere Cascapedia	PC	48.16	-65.84	10	-	-

Appendix Table A4. Bioclimatic variables downloaded from WorldClim (Fick and Hijmans 2017) using the R package sdmpredictors (Bosch et al. 2018). The bioclimatic variables were standardized to a mean of 0 and a standard deviation of 1 for analyses.

BioClim Variable	Description
WC_bio1_stand	Annual mean temperature
WC_bio2_stand	Mean diurnal temperature range - Mean of the monthly (maximum temperature - minimum temperature)
WC_bio3_stand	Isothermality - Mean diurnal temperature range (bio2) / Annual temperature range (bio7)
WC_bio4_stand	Temperature seasonality - Standard deviation of the annual mean temperature
WC_bio5_stand	Maximum temperature - Maximum temperature of the warmest month
WC_bio6_stand	Minimum temperature - Minimum temperature of the coldest month
WC_bio7_stand	Annual temperature range - Maximum temperature (bio5) - minimum temperature (bio6)
WC_bio8_stand	Mean temperature of wettest quarter
WC_bio9_stand	Mean temperature of driest quarter
WC_bio10_stand	Mean temperature of warmest quarter
WC_bio11_stand	Mean temperature of coldest quarter
WC_bio12_stand	Annual precipitation
WC_bio13_stand	Precipitation of wettest month
WC_bio14_stand	Precipitation of driest month
WC_bio15_stand	Precipitation seasonality - Coefficient of variation of the monthly precipitation
WC_bio16_stand	Precipitation of wettest quarter
WC_bio17_stand	Precipitation of driest quarter
WC_bio18_stand	Precipitation of warmest quarter
WC_bio19_stand	Precipitation of coldest quarter

Appendix Table A5. List of rivers from North Atlantic Salmon Conservation Organization (NASCO) river database for proposed designable units (DUs) in Labrador (previously DU 2). Information on salmon fishing areas (SFAs) or Q as well as river coordinates and river code were extracted from the NASCO database. The proposed DU to which each river belongs is indicated.

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA01A	DFO-NL	Siugak Brook	-62.1	57.58	R1	North Labrador
SFA01A	DFO-NL	Webb Brook	-61.93	56.8	R2	North Labrador
SFA01A	DFO-NL	Avakutak River	-61.85	57.15	R3	North Labrador
SFA01A	DFO-NL	Kogaluk River	-61.73	56.2	R4	North Labrador
SFA01A	DFO-NL	Notakwanon River	-61.52	56.02	R5	North Labrador
SFA01A	DFO-NL	Sango Brook	-61.18	55.89	R6	North Labrador
SFA01A	DFO-NL	Unnamed River 933	-61.14	55.85	R7	North Labrador
SFA01A	DFO-NL	River 80	-61.06	55.84	R8	North Labrador
SFA01A	DFO-NL	River 81	-61.06	55.84	R9	North Labrador
SFA01A	DFO-NL	Flowers River	-60.96	55.74	R10	North Labrador
SFA01A	DFO-NL	Unnamed River 930	-60.92	55.75	R11	North Labrador
SFA01A	DFO-NL	Unnamed River 932	-60.89	55.85	R12	North Labrador
SFA01A	DFO-NL	Unnamed River 929	-60.87	55.73	R13	North Labrador
SFA01A	DFO-NL	Unnamed River 931	-60.84	55.76	R14	North Labrador
SFA01A	DFO-NL	Unnamed River 928	-60.72	55.75	R15	North Labrador
SFA01A	DFO-NL	River 78	-60.7	55.64	R16	North Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA01A	DFO-NL	Adlatok (Ugjoktok and Adlatok Bay) River	-60.69	55.04	R17	North Labrador
SFA01A	DFO-NL	River 75	-60.69	54.98	R18	North Labrador
SFA01A	DFO-NL	Hunt River	-60.67	55.57	R19	North Labrador
SFA01A	DFO-NL	Unnamed River 927	-60.66	55.6	R20	North Labrador
SFA01A	DFO-NL	Unnamed River 912	-60.66	55.06	R21	North Labrador
SFA01A	DFO-NL	Unnamed River 914	-60.61	55.17	R22	North Labrador
SFA01A	DFO-NL	Unnamed River 911	-60.61	55.03	R23	North Labrador
SFA01A	DFO-NL	Unnamed River 913	-60.56	55.17	R24	North Labrador
SFA01A	DFO-NL	Unnamed River 926	-60.54	55.73	R25	North Labrador
SFA01A	DFO-NL	Unnamed River 923	-60.53	55.58	R26	North Labrador
SFA01A	DFO-NL	Unnamed River 924	-60.49	55.67	R27	North Labrador
SFA01A	DFO-NL	Unnamed River 916	-60.49	55.35	R28	North Labrador
SFA01A	DFO-NL	Unnamed River 920	-60.46	55.52	R29	North Labrador
SFA01A	DFO-NL	Unnamed River 919	-60.46	55.5	R30	North Labrador
SFA01A	DFO-NL	Unnamed River 917	-60.46	55.44	R31	North Labrador
SFA01A	DFO-NL	Unnamed River 915	-60.46	55.3	R32	North Labrador
SFA01A	DFO-NL	Unnamed River 922	-60.43	55.62	R33	North Labrador
SFA01A	DFO-NL	Unnamed River 921	-60.42	55.57	R34	North Labrador
SFA01A	DFO-NL	Unnamed River 910 (Rapids)	-60.41	55.13	R35	North Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA01A	DFO-NL	Unnamed River 925	-60.38	55.71	R36	North Labrador
SFA01A	DFO-NL	Unnamed River 909	-60.36	55.17	R37	North Labrador
SFA01A	DFO-NL	Unnamed River 918	-60.33	55.56	R38	North Labrador
SFA01A	DFO-NL	Unnamed River 904	-60.31	55.04	R39	North Labrador
SFA01A	DFO-NL	Little Bay River	-60.3	55.1	R40	North Labrador
SFA01A	DFO-NL	Kanairiktok River	-60.3	55.02	R41	North Labrador
SFA01A	DFO-NL	Unnamed River 907	-60.21	55.17	R42	North Labrador
SFA01A	DFO-NL	Unnamed River 905 (Falls)	-60.21	55.12	R43	North Labrador
SFA01A	DFO-NL	Unnamed River 908	-60.19	55.21	R44	North Labrador
SFA01A	DFO-NL	Unnamed River 903	-60.18	55.09	R45	North Labrador
SFA01A	DFO-NL	Unnamed River 906	-60.16	55.19	R46	North Labrador
SFA01A	DFO-NL	River 72	-60.12	55.12	R47	North Labrador
SFA01A	DFO-NL	Unnamed River 902	-60.09	55.14	R48	North Labrador
SFA01A	DFO-NL	Unnamed River 901	-59.95	55.16	R49	North Labrador
SFA01A	DFO-NL	Kaipokok River	-59.95	54.76	R50	North Labrador
SFA01A	DFO-NL	South Brook	-59.93	54.74	R51	North Labrador
SFA01A	DFO-NL	Southeast Brook (Bay of Islands)	-59.87	55.11	R52	North Labrador
SFA01A	DFO-NL	Salmon Brook	-59.87	54.84	R53	North Labrador
SFA01A	DFO-NL	Little River	-59.85	54.88	R54	North Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA01A	DFO-NL	Unnamed Brook	-59.81	54.82	R55	North Labrador
SFA01A	DFO-NL	Beaver Brook	-59.79	54.79	R56	North Labrador
SFA01A	DFO-NL	English River	-59.75	54.97	R57	North Labrador
SFA01A	DFO-NL	Gouru Brook	-59.7	54.91	R58	North Labrador
SFA01A	DFO-NL	East Brook (Bay of Islands)	-59.63	55.13	R59	North Labrador
SFA01A	DFO-NL	Libbies Brook	-59.59	55.03	R60	North Labrador
SFA01A	DFO-NL	Alkami Brook	-59.57	54.98	R61	North Labrador
SFA01A	DFO-NL	Makkovik River	-59.4	54.97	R62	North Labrador
SFA01A	DFO-NL	Southeast Brook (Makkovik Bay)	-59.37	54.98	R63	North Labrador
SFA01A	DFO-NL	Big Island Brook	-59.2	55.08	R64	North Labrador
SFA01A	DFO-NL	Makkovik Brook	-59.16	55.07	R65	North Labrador
SFA01A	DFO-NL	Big Bight Brook	-59.03	54.99	R66	North Labrador
SFA01A	DFO-NL	Muskrat Pond Brook (River 65)	-59.03	54.89	R67	North Labrador
SFA01A	DFO-NL	Meshers Harbour Brook (River 66)	-58.99	54.93	R68	North Labrador
SFA01A	DFO-NL	Adlavik Brook	-58.99	54.87	R69	North Labrador
SFA01A	DFO-NL	Big River	-58.94	54.84	R70	North Labrador
SFA01A	DFO-NL	Rattling Brook	-58.93	54.83	R71	North Labrador
SFA01A	DFO-NL	Stag Bay Brook	-58.78	54.78	R72	North Labrador
SFA01A	DFO-NL	Pamiulik River	-58.58	54.75	R73	North Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA01A	DFO-NL	Tukialik River	-58.43	54.74	R74	North Labrador
SFA01A	DFO-NL	Unnamed Brook (River 58)	-58.2	54.75	R75	North Labrador
SFA01A	DFO-NL	Tilt Cove Pond Brook	-58.19	54.75	R76	North Labrador
SFA01A	DFO-NL	Unnamed Brook (Jeanette Bay_bottom)	-58.16	54.76	R77	North Labrador
SFA01A	DFO-NL	Jeanette Bay Brook	-58.07	54.73	R78	North Labrador
SFA01A	DFO-NL	Unnamed Brook (Jeanette Bay_mouth)	-57.92	54.74	R79	North Labrador
SFA01A	DFO-NL	Bobs Brook	-57.88	54.74	R80	North Labrador
SFA01A	DFO-NL	Big Brook (Michaels River)	-57.79	54.69	R81	North Labrador
SFA01A	DFO-NL	Tooktashina Brook	-57.78	54.68	R82	North Labrador
SFA01A	DFO-NL	Unnamed Brook 2 (Byron Bay)	-57.72	54.64	R83	North Labrador
SFA01A	DFO-NL	Unnamed Brook 1 (Byron Bay)	-57.68	54.63	R84	North Labrador
SFA01A	DFO-NL	Cape Rouge Brook (River 55)	-57.59	54.63	R85	North Labrador
SFA01B	DFO-NL	Double Mer	-59.58	54.02	R114	North Labrador
SFA01B	DFO-NL	Rattling Brook (Double Mer)	-59.56	54.02	R115	North Labrador
SFA01B	DFO-NL	Mocassin Brook	-59.53	54.03	R117	North Labrador
SFA01B	DFO-NL	Coleys Brook	-59.52	53.99	R118	North Labrador
SFA01B	DFO-NL	Partridge Point Brook (River 49)	-59.5	54.04	R120	North Labrador
SFA01B	DFO-NL	Long Point Brook	-59.41	54.03	R121	North Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA01B	DFO-NL	Goose Pt Brook	-59.27	54.07	R124	North Labrador
SFA01B	DFO-NL	Big Brook (Double Mer)	-58.92	54.14	R130	North Labrador
SFA01B	DFO-NL	Campbells Point Brook	-58.84	54.14	R133	North Labrador
SFA01B	DFO-NL	Pompey Brook	-58.8	54.11	R134	North Labrador
SFA01B	DFO-NL	Saltwater Pond Brook	-58.68	54.12	R136	North Labrador
SFA01B	DFO-NL	Dennys Pond Brook	-58.58	54.2	R138	North Labrador
SFA01B	DFO-NL	Moliak Brook	-58.55	54.12	R139	North Labrador
SFA01B	DFO-NL	Unnamed Brook	-58.48	54.2	R141	North Labrador
SFA01B	DFO-NL	Goose Brook	-58.29	54.31	R144	North Labrador
SFA01B	DFO-NL	Pottles Bay River 53	-58.24446	54.3187	R145	North Labrador
SFA01B	DFO-NL	Pottles Bay River 54	-58.21456	54.34664	R147	North Labrador
SFA01B	DFO-NL	Tom Luscombe Brook	-58.21	54.35	R148	North Labrador
SFA01B	DFO-NL	West (Fox Cove) Brook	-58.09	54.38	R151	North Labrador
SFA01B	DFO-NL	Middle Brook (Fox Cove)	-58.07	54.38	R152	North Labrador
SFA01B	DFO-NL	Corner Brook	-58.06	54.39	R153	North Labrador
SFA01B	DFO-NL	Jules Head Brook	-57.94	54.39	R155	North Labrador
SFA01B	DFO-NL	Trouting Brook	-57.86	54.4	R157	North Labrador
SFA01B	DFO-NL	Northwest Brook (River 54)	-57.57	54.49	R162	North Labrador
SFA01B	DFO-NL	Aerial Pond Brook	-57.57	54.47	R163	North Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA01B	DFO-NL	Susan River	-61	53.74	R86	Lake Melville, Labrador
SFA01B	DFO-NL	Beaver River	-60.94	53.74	R87	Lake Melville, Labrador
SFA01B	DFO-NL	Naskaupi River	-60.85	53.8	R88	Lake Melville, Labrador
SFA01B	DFO-NL	Crooked River	-60.84	53.8	R89	Lake Melville, Labrador
SFA01B	DFO-NL	Unnamed River 3 (Grand Lake)	-60.76	53.71	R90	Lake Melville, Labrador
SFA01B	DFO-NL	Unnamed River 4 (Grand Lake)	-60.74	53.75	R91	Lake Melville, Labrador
SFA01B	DFO-NL	McKenzie River	-60.73	53.24	R92	Lake Melville, Labrador
SFA01B	DFO-NL	Unnamed River 2 (Grand Lake)	-60.72	53.7	R93	Lake Melville, Labrador
SFA01B	DFO-NL	Unnamed River 5 (Grand Lake)	-60.54	53.72	R94	Lake Melville, Labrador
SFA01B	DFO-NL	Caroline Brook	-60.52	53.26	R95	Lake Melville, Labrador
SFA01B	DFO-NL	Cape Caribou River	-60.42	53.62	R96	Lake Melville, Labrador
SFA01B	DFO-NL	Otter Creek	-60.41	53.35	R97	Lake Melville, Labrador
SFA01B	DFO-NL	Ten Mile Brook (Grand Lake)	-60.36	53.63	R98	Lake Melville, Labrador
SFA01B	DFO-NL	Goose River	-60.36	53.36	R99	Lake Melville, Labrador
SFA01B	DFO-NL	Unnamed River 1 (Grand Lake)	-60.35	53.59	R100	Lake Melville, Labrador
SFA01B	DFO-NL	Peter Jackies Brook	-60.35	53.27	R101	Lake Melville, Labrador
SFA01B	DFO-NL	Gosling Brook	-60.33	53.4	R102	Lake Melville, Labrador
SFA01B	DFO-NL	Traverspine River	-60.28	53.28	R103	Lake Melville, Labrador
SFA01B	DFO-NL	Churchill River (Hamilton)	-60.18	53.32	R104	Lake Melville, Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA01B	DFO-NL	Mud Lake & tribs	-60.17	53.32	R105	Lake Melville, Labrador
SFA01B	DFO-NL	Sebaskachu River	-60.12	53.76	R106	Lake Melville, Labrador
SFA01B	DFO-NL	Woody Is Brook	-60.11	53.68	R107	Lake Melville, Labrador
SFA01B	DFO-NL	Kenamu River	-59.91	53.48	R108	Lake Melville, Labrador
SFA01B	DFO-NL	Mulligan River	-59.89	53.82	R109	Lake Melville, Labrador
SFA01B	DFO-NL	Kenemich River	-59.83	53.48	R110	Lake Melville, Labrador
SFA01B	DFO-NL	Pearl River	-59.8	53.85	R111	Lake Melville, Labrador
SFA01B	DFO-NL	Black Pt Brook	-59.74	53.84	R112	Lake Melville, Labrador
SFA01B	DFO-NL	Big River	-59.67	53.52	R113	Lake Melville, Labrador
SFA01B	DFO-NL	Rabbit Pt Brook	-59.56	53.55	R116	Lake Melville, Labrador
SFA01B	DFO-NL	Unamed River (L. Melville)	-59.51	53.56	R119	Lake Melville, Labrador
SFA01B	DFO-NL	Lowland Barren Brook	-59.37	53.88	R122	Lake Melville, Labrador
SFA01B	DFO-NL	Shoal River	-59.28	53.67	R123	Lake Melville, Labrador
SFA01B	DFO-NL	Charley Cove Brook	-59.11	53.95	R125	Lake Melville, Labrador
SFA01B	DFO-NL	Vallies Brook	-59.06	54.02	R126	Lake Melville, Labrador
SFA01B	DFO-NL	Etagaulet Point Brook	-59.05	53.79	R127	Lake Melville, Labrador
SFA01B	DFO-NL	Etagaulet River	-59.02	53.72	R128	Lake Melville, Labrador
SFA01B	DFO-NL	Swallow Hr Brook	-58.98	53.75	R129	Lake Melville, Labrador
SFA01B	DFO-NL	Frenchman Point Brook	-58.91	53.87	R131	Lake Melville, Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA01B	DFO-NL	English River	-58.86	53.89	R132	Lake Melville, Labrador
SFA01B	DFO-NL	Dinner Brook	-58.72	54.03	R135	Lake Melville, Labrador
SFA01B	DFO-NL	Peter Lucys Brook	-58.61	53.98	R137	Lake Melville, Labrador
SFA01B	DFO-NL	Grants Brook	-58.51	54.01	R140	Lake Melville, Labrador
SFA01B	DFO-NL	Pease Brook	-58.42	54.02	R142	Lake Melville, Labrador
SFA01B	DFO-NL	Longue Point Brook	-58.37	54.04	R143	Lake Melville, Labrador
SFA01B	DFO-NL	Mackenzies Brook	-58.22	54.07	R146	Lake Melville, Labrador
SFA01B	DFO-NL	Berry Brook	-58.2	54.07	R150	Lake Melville, Labrador
SFA01B	DFO-NL	Main Brook	-57.86	54.08	R158	Lake Melville, Labrador
SFA01B	DFO-NL	Mild Brook	-57.83	54.07	R159	Lake Melville, Labrador
SFA01B	DFO-NL	River of Sticks Brook	-57.79	54.07	R160	Lake Melville, Labrador
Q09	Quebec	Napetipi	-58.13	51.34	R1049	South Labrador
Q09	Quebec	Du Vieux Fort	-58.03	51.33	R1050	South Labrador
Q09	Quebec	Saint-Paul	-57.7	51.47	R1051	South Labrador
Q09	Quebec	Ruisseau au Saumon	-57.58	51.47	R1052	South Labrador
Q09	Quebec	Ruisseau des Belles Amours	-57.45	51.48	R1053	South Labrador
Q09	Quebec	Brador Est	-57.23	51.5	R1054	South Labrador
SFA01B	DFO-NL	Cunninghams Brook	-58.21	54.21	R149	South Labrador
SFA01B	DFO-NL	Nats Brook	-58.02	54.23	R154	South Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA01B	DFO-NL	Cranford Head Brook	-57.93	54.21	R156	South Labrador
SFA01B	DFO-NL	Flatwater Brook	-57.6	54.19	R161	South Labrador
SFA01B	DFO-NL	Broomfields Brook	-57.54	54.18	R164	South Labrador
SFA02	DFO-NL	Southwest Brook	-57.53	53.58	R165	South Labrador
SFA02	DFO-NL	White Bear River	-57.53	53.58	R166	South Labrador
SFA02	DFO-NL	Eagle River	-57.45	53.57	R167	South Labrador
SFA02	DFO-NL	Plances Brook	-57.43	54.14	R168	South Labrador
SFA02	DFO-NL	Dove Brook	-57.43	53.64	R169	South Labrador
SFA02	DFO-NL	Martins Brook	-57.43	53.58	R170	South Labrador
SFA02	DFO-NL	Bob 'n Joyce Brook	-57.42	53.64	R171	South Labrador
SFA02	DFO-NL	River Sticks	-57.37	53.44	R172	South Labrador
SFA02	DFO-NL	Saddle Island Brook	-57.34	53.55	R173	South Labrador
SFA02	DFO-NL	Red Island Brook	-57.3	53.52	R174	South Labrador
SFA02	DFO-NL	Paradise River	-57.25	53.42	R175	South Labrador
SFA02	DFO-NL	Big Brook	-57.22	53.92	R176	South Labrador
SFA02	DFO-NL	Wolfreys Brook	-57.21	53.95	R177	South Labrador
SFA02	DFO-NL	Duck Island Brook	-57.21	53.5	R178	South Labrador
SFA02	DFO-NL	Fancies Brook	-57.16	53.76	R179	South Labrador
SFA02	DFO-NL	Coombes Brook	-57.14	53.54	R180	South Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA02	DFO-NL	North River	-57.08	53.81	R181	South Labrador
SFA02	DFO-NL	Muddy Bay Brook (Dykes River)	-57.07	53.64	R182	South Labrador
SFA02	DFO-NL	Burdetts Brook (2)	-57.02	53.69	R183	South Labrador
SFA02	DFO-NL	Goose Cove Brook	-56.88	53.71	R184	South Labrador
SFA02	DFO-NL	Burdetts Brook (1)	-56.73	53.68	R185	South Labrador
SFA02	DFO-NL	Table Bay Brook	-56.72	53.68	R186	South Labrador
SFA02	DFO-NL	Old Womans Brook	-56.7	53.69	R187	South Labrador
SFA02	DFO-NL	Isthmus Bay Brook	-56.63	53.71	R188	South Labrador
SFA02	DFO-NL	Southeast Brook	-56.59	53.75	R189	South Labrador
SFA02	DFO-NL	Alexis River	-56.53	52.6	R190	South Labrador
SFA02	DFO-NL	Bobbys Brook	-56.42	52.57	R191	South Labrador
SFA02	DFO-NL	Sand Hill River	-56.35	53.59	R192	South Labrador
SFA02	DFO-NL	Black Water Brook	-56.29	52.54	R193	South Labrador
SFA02	DFO-NL	Salt Pond Brook	-56.28	53.56	R194	South Labrador
SFA02	DFO-NL	Roaches Brook	-56.26	53.54	R195	South Labrador
SFA02	DFO-NL	Unamed Brook (White Bear Arm)	-56.21	52.84	R196	South Labrador
SFA02	DFO-NL	White Bear Arm River	-56.2	52.84	R197	South Labrador
SFA02	DFO-NL	St. Lewis River	-56.19	52.44	R198	South Labrador
SFA02	DFO-NL	South Brook (St. Lewis Inlet)	-56.19	52.42	R199	South Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA02	DFO-NL	Southwest Brook (River 14) Michaels	-56.18	52.81	R200	South Labrador
SFA02	DFO-NL	North Brook (St. Lewis Inlet)	-56.18	52.44	R201	South Labrador
SFA02	DFO-NL	Bills Brook	-56.17	53.5	R202	South Labrador
SFA02	DFO-NL	Notleys Brook	-56.17	52.53	R203	South Labrador
SFA02	DFO-NL	Cushes Brook (Southarm)	-56.15	52.95	R204	South Labrador
SFA02	DFO-NL	Tackers Brook (Southarm)	-56.15	52.94	R205	South Labrador
SFA02	DFO-NL	Meshers Brook	-56.14	53.5	R206	South Labrador
SFA02	DFO-NL	North Brook (PHS)	-56.14	52.58	R207	South Labrador
SFA02	DFO-NL	West Brook (PHS)	-56.14	52.58	R208	South Labrador
SFA02	DFO-NL	Pumbley Brook (Southarm)	-56.13	52.95	R209	South Labrador
SFA02	DFO-NL	Gilbert River	-56.12	52.66	R210	South Labrador
SFA02	DFO-NL	Trout Pond Brook	-56.1	52.95	R211	South Labrador
SFA02	DFO-NL	South Brook (Backwater Arm)	-56.08	52.97	R212	South Labrador
SFA02	DFO-NL	Peters Brook (River 16)	-56.07	52.81	R213	South Labrador
SFA02	DFO-NL	Hawke River	-56.06	53.03	R214	South Labrador
SFA02	DFO-NL	Birchy Narrows Brook (St. Michael's Bay)	-56.06	52.72	R215	South Labrador
SFA02	DFO-NL	Reeds Pond Brook	-56.05	53.45	R216	South Labrador
SFA02	DFO-NL	Shinneys Waters	-56.05	52.59	R217	South Labrador
SFA02	DFO-NL	Porcupine Harbour River	-56.04	53.37	R218	South Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA02	DFO-NL	Mungo Run Brook	-56.03	52.52	R219	South Labrador
SFA02	DFO-NL	Mussell Brook (River 26)	-56.02	53.39	R220	South Labrador
SFA02	DFO-NL	Southern Harbour Brook	-56	52.99	R221	South Labrador
SFA02	DFO-NL	Blubber Island Brook	-55.99	52.82	R222	South Labrador
SFA02	DFO-NL	Hoop Pole Brook	-55.99	52.35	R223	South Labrador
SFA02	DFO-NL	Caplin Bay Brook	-55.97	53.1	R224	South Labrador
SFA02	DFO-NL	Green Cove Brook	-55.96	52.69	R225	South Labrador
SFA02	DFO-NL	Chair Brook	-55.94	53.49	R226	South Labrador
SFA02	DFO-NL	Open Bay Brook	-55.93	53.34	R227	South Labrador
SFA02	DFO-NL	Black Bear River	-55.93	53.3	R228	South Labrador
SFA02	DFO-NL	Effingham Brook	-55.91	52.33	R229	South Labrador
SFA02	DFO-NL	Trout Cove Brook	-55.9	52.95	R230	South Labrador
SFA02	DFO-NL	Long Pond Brook	-55.88	53.14	R231	South Labrador
SFA02	DFO-NL	Long Harbour Brook	-55.88	52.36	R232	South Labrador
SFA02	DFO-NL	Shoal Bay Brook (Pollo Brook)	-55.86	53.26	R233	South Labrador
SFA02	DFO-NL	Smarts Brook (River 22)	-55.86	53.26	R234	South Labrador
SFA02	DFO-NL	Partridge Bay Brook	-55.86	53.2	R235	South Labrador
SFA02	DFO-NL	Ship Harbour Brook	-55.85	52.48	R236	South Labrador
SFA02	DFO-NL	Deer Harbour Brook	-55.85	52.39	R237	South Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA02	DFO-NL	Unamed Brook (Shoal Bay)	-55.84	53.27	R238	South Labrador
SFA02	DFO-NL	St. Mary's River	-55.84	52.31	R239	South Labrador
SFA02	DFO-NL	Rabbit Brook	-55.84	52.23	R240	South Labrador
SFA02	DFO-NL	St. Charles River	-55.84	52.23	R241	South Labrador
SFA02	DFO-NL	Trout Brook	-55.83	53.44	R242	South Labrador
SFA02	DFO-NL	Edridges Brook (River 20)	-55.83	53.24	R243	South Labrador
SFA02	DFO-NL	Pallows Cove Brook	-55.81	53.18	R244	South Labrador
SFA02	DFO-NL	Salt Brook	-55.78	52.25	R245	South Labrador
SFA02	DFO-NL	Mungo Brook	-55.76	52.49	R246	South Labrador
SFA02	DFO-NL	Port Marnham Brook	-55.72	52.39	R247	South Labrador
SFA14B	DFO-NL	L'ance au Clair Brook	-57.06	51.43	R248	South Labrador
SFA14B	DFO-NL	Forteau Brook	-56.94	51.48	R249	South Labrador
SFA14B	DFO-NL	Lance au Loup Brook	-56.82	51.53	R250	South Labrador
SFA14B	DFO-NL	Lance au Diable Brook	-56.75	51.56	R251	South Labrador
SFA14B	DFO-NL	Pinware Bay Brook	-56.71	51.62	R252	South Labrador
SFA14B	DFO-NL	Pinware River	-56.69	51.63	R253	South Labrador
SFA14B	DFO-NL	Lilly Island Brook	-56.65	51.64	R254	South Labrador
SFA14B	DFO-NL	Skipper Neds Brook	-56.54	51.67	R255	South Labrador
SFA14B	DFO-NL	North Brook (Red Bay)	-56.44	51.74	R256	South Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
SFA14B	DFO-NL	Southwest Brook(Red Bay)	-56.44	51.73	R257	South Labrador
SFA14B	DFO-NL	Wiseman Brook	-56.36	51.74	R258	South Labrador
SFA14B	DFO-NL	Black Bay Brook	-56.35	51.78	R259	South Labrador
SFA14B	DFO-NL	Barge Bay Brook	-56.21	51.81	R260	South Labrador
SFA14B	DFO-NL	Unnamed Brook 1	-56.17	51.82	R261	South Labrador
SFA14B	DFO-NL	South Green Bay Brook	-56.14	51.85	R262	South Labrador
SFA14B	DFO-NL	North Green Bay Brook	-56.13	51.85	R263	South Labrador
SFA14B	DFO-NL	Unamed Brook 2	-56.08	51.87	R264	South Labrador
SFA14B	DFO-NL	Woody Cove Brook	-56.05	51.89	R265	South Labrador
SFA14B	DFO-NL	Twin Brook 1st entrance	-56.01	51.9	R266	South Labrador
SFA14B	DFO-NL	Twin Brook 2nd entrance	-55.99	51.91	R267	South Labrador
SFA14B	DFO-NL	Temple Brook	-55.98	52.02	R268	South Labrador
SFA14B	DFO-NL	Barry Barns Brook	-55.95	52.02	R269	South Labrador
SFA14B	DFO-NL	Pitts Harbour Brook	-55.89	52.02	R270	South Labrador
SFA14B	DFO-NL	St. Peters River	-55.8	52.09	R271	South Labrador
SFA14B	DFO-NL	Sound Brook	-55.78	52.2	R272	South Labrador
Q09	Quebec	Napetipi	-58.13	51.34	R1049	South Labrador
Q09	Quebec	Du Vieux Fort	-58.03	51.33	R1050	South Labrador
Q09	Quebec	Saint-Paul	-57.7	51.47	R1051	South Labrador

SFA	Jurisdiction	River Name	Longitude	Latitude	Code	Proposed DU
Q09	Quebec	Ruisseau au Saumon	-57.58	51.47	R1052	South Labrador
Q09	Quebec	Ruisseau des Belles Amours	-57.45	51.48	R1053	South Labrador
Q09	Quebec	Brador Est	-57.23	51.5	R1054	South Labrador

Appendix Table A6. Mean smolt age for rivers in DU 3 and DU 6 based on data provided from DFO's Salmonid Section in the Newfoundland and Labrador region. Data were divided into three time periods (pre-1980, 1980–1999, and post-2000). Sample size for each time period and river are provided, and those with sample sizes >100 individuals are highlighted in gray. Blank cells indicate no samples.

-	-	-	-	-	Pre-1980		1980–1999		Post-2000	
Proposed DU	SFA	River Name	Latitude	Longitude	Mean Smolt Age	N	Mean Smolt Age	N	Mean Smolt Age	N
NortheastNL	SFA04	Exploits River	49.03	-55.41	3.37	4,986	3.39	24,154	3.38	4,788
NortheastNL	SFA04	Campbellton	49.28	-54.92	3.16	100	3.37	3,890	3.43	5,347
NortheastNL	SFA04	Dog Bay River	49.45	-54.56	-	-	3.50	6	-	-
NortheastNL	SFA04	Gander River	49.26	-54.49	3.77	114	3.70	4,190	3.69	1,211
NortheastNL	SFA04	Ragged Harbour River	49.43	-54.05	3.36	117	3.42	90	-	-
NortheastNL	SFA04	Anchor Brook	49.34	-53.70	3.09	11	4.00	1	-	-
NortheastNL	SFA04	Deadman's Brook	49.38	-53.74	-	-	3.28	36		-
NortheastNL	SFA04	Windmill Brook	49.28	-53.56	3.44	66	-	-	-	-
NortheastNL	SFA05	Southwest Brook	49.10	-53.70	-	-	-	-	4.00	2
NortheastNL	SFA05	Indian Bay Brook	49.04	-53.88	3.35	95	3.63	195	-	-
NortheastNL	SFA05	Traverse Brook	48.83	-54.08	-	-	3.39	23	-	-
NortheastNL	SFA05	Middle Brook	48.81	-54.21	3.64	42	3.53	1,132	3.52	435
NortheastNL	SFA05	Gambo Brook	48.77	-54.22	3.26	54	3.36	11	-	-
NortheastNL	SFA05	Terra Nova River	48.67	-54.00	3.43	205	3.47	3,380	3.53	685
NortheastNL	SFA05	Wings Brook	48.63	-53.92	-	-	3.69	16	-	-
NortheastNL	SFA05	Bread Cove Brook	48.48	-53.92	-	-	-	-	3.95	172
NortheastNL	SFA05	Northwest River	48.39	-54.20	3.31	239	3.73	649	3.19	69
NortheastNL	SFA05	Salmon Brook	48.39	-54.20	-	-	4.00	1	-	-

-	-	-	-	-	Pre-1980		1980–1999		Post-2000	
Proposed DU	**SFA**	**River Name**	**Latitude**	**Longitude**	**Mean Smolt Age**	**N**	**Mean Smolt Age**	**N**	**Mean Smolt Age**	**N**
NortheastNL	SFA06	Salmon Cove River	48.39	-53.31	3.51	37	3.75	8	-	-
NortheastNL	SFA06	Trouty River	48.33	-53.40	3.25	53	-	-	-	-
NortheastNL	SFA06	Popes Harbour River	48.24	-53.56	-	-	3.12	17	-	-
NortheastNL	SFA07	North River	47.55	-53.28	-	-	-	-	3.00	1
NortheastNL	SFA08	Renews River	46.93	-52.95	2.94	49	-	-	-	-
NorthwestNL	SFA14A	Lomond River	49.43	-57.73	2.91	66	2.99	683	3.19	21
NorthwestNL	SFA14A	Parsons Pond River	50.03	-57.71	-	-	3.50	2	-	-
NorthwestNL	SFA14A	Portland Creek	50.18	-57.61	3.00	1	3.09	46	-	-
NorthwestNL	SFA14A	River of Ponds	50.54	-57.39	3.36	130	3.46	50	4.00	1
NorthwestNL	SFA14A	Little Brook	50.55	-57.39	3.20	40	4.00	2	-	-
NorthwestNL	SFA14A	Torrent River	50.61	-57.15	3.58	74	3.26	2,349	3.15	447
NorthwestNL	SFA14A	East River	50.63	-57.17	3.80	209	3.45	29	3.00	1
NorthwestNL	SFA14A	Castors River	50.92	-56.95	3.56	9	3.83	117	-	-
NorthwestNL	SFA14A	Ste. Genevieve River	51.14	-56.79	4.13	166	3.83	382	-	-
NorthwestNL	SFA14A	West River	51.19	-56.76	3.85	2,782	3.72	9,502	3.52	7,058
NorthwestNL	SFA14A	East River	51.21	-56.74	-	-	4.00	7	-	-
NorthwestNL	SFA14A	Big Brook	51.52	-56.15	-	-	4.00	1	-	-

Appendix Table A7. Proportion of repeat spawners in the large salmon category for rivers in DU 3 and DU 6 based on data provided from DFO's Salmonid Section in the Newfoundland and Labrador region. Data were divided into three time periods (pre-1980, 1980-1999, and post-2000). Sample size for each time period and river are provided, and those with sample sizes >50 individuals are highlighted in gray. Blank cells indicate no samples.

-	-	-	Pre-1980		1980–1999		Post-2000	
River Name	**SFA**	**Proposed DU**	**% Repeat**	**N**	**% Repeat**	**N**	**% Repeat**	**N**
Campbellton River	4	NortheastNL	-	-	100.0	121	95.9	244
Exploits River	4	NortheastNL	70	60	66.4	265	80.9	341
Gander River	4	NortheastNL	100	1	87.0	177	46.8	47
Middle Brook (Gambo)	5	NortheastNL	-	-	77.8	9	85.0	20

-	-	-	Pre-1980		1980–1999		Post-2000	
River Name	**SFA**	**Proposed DU**	**% Repeat**	**N**	**% Repeat**	**N**	**% Repeat**	**N**
Northwest River	5	NortheastNL	100	2	100.0	14	-	-
Ragged Harbour River (New Pond)	4	NortheastNL	100	1	-	-	-	-
Terra Nova River	5	NortheastNL	-	-	79.2	144	80.4	143
Castors River	14A	NorthwestNL	-	-	0.0	1	-	-
East River	14A	NorthwestNL	0	11	-	-	-	-
Lomond River	14A	NorthwestNL	25	4	34.0	53	-	-
Portland Creek	14A	NorthwestNL	-	-	28.6	7	-	-
River of Ponds	14A	NorthwestNL	0	1	100.0	1	-	-
St Genevieve River	14A	NorthwestNL	-	-	50.0	2	-	-
Torrent River	14A	NorthwestNL	-	-	66.4	208	100.0	2
West River	14A	NorthwestNL	100	4	85.8	134	92.4	397

Appendix Table A8. Proportion of repeat spawners in the small salmon category for rivers in DU 3 and DU 6 based on data provided from DFO's Salmonid Section in the Newfoundland and Labrador region. Data were divided into three time periods (pre-1980, 1980–1999, and post-2000). Sample size for each time period and river are provided, and those with sample sizes >50 individuals are highlighted in gray. Blank cells indicate no samples.

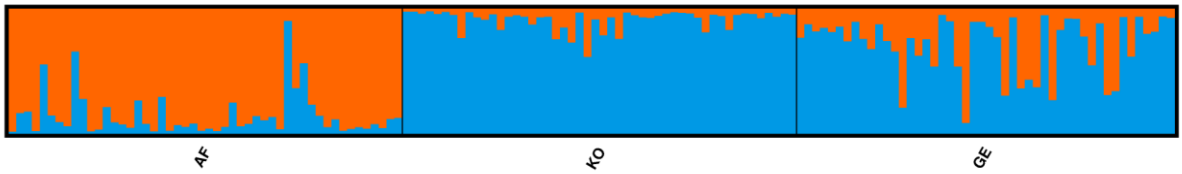
-	-	-	Pre-1980		1980–1999		Post-2000	
River Name	**SFA**	**Proposed DU**	**% Repeat**	**N**	**% Repeat**	**N**	**% Repeat**	**N**
Anchor Brook	4	NortheastNL	-	-	0%	1	-	-
Bread Cove Brook	5	NortheastNL	-	-	-	-	35%	173
Campbellton River	4	NortheastNL	-	-	87%	2,124	51%	1,348
Deadman's Brook	4	NortheastNL	-	-	0%	36	-	-
Dog Bay River	4	NortheastNL	-	-	33%	6	-	-
Exploits River	4	NortheastNL	7%	5,232	4%	17,460	8%	2,508
Gambo River (North Pond)	5	NortheastNL	-	-	0%	11	-	-
Gander River	4	NortheastNL	9%	77	10%	3,160	7%	1,125
Indian Bay Brook	5	NortheastNL	32%	19	6%	101	-	-
Middle Brook (Gambo)	5	NortheastNL	0%	11	9%	1,060	6%	426
North River	7	NortheastNL	-	-	-	-	0%	1
Northwest River	5	NortheastNL	8%	39	11%	639	25%	52

-	-	-	Pre-1980		1980–1999		Post-2000	
River Name	**SFA**	**Proposed DU**	**% Repeat**	**N**	**% Repeat**	**N**	**% Repeat**	**N**
Popes Harbour River	6	NortheastNL	-	-	6%	17	-	-
Ragged Harbour River (New Pond)	4	NortheastNL	2%	51	0%	31	-	-
Renews River	8	NortheastNL	22%	27	-	-	-	-
Salmon Brook (Port Blandford)	5	NortheastNL	-	-	0%	1	-	-
Salmon Cove River	6	NortheastNL	0%	3	13%	8	-	-
Southwest Arm Brook	5	NortheastNL	-	-	-	-	50%	2
Terra Nova River	5	NortheastNL	11%	121	18%	3,390	18%	586
Traverse Brook	5	NortheastNL	-	-	0%	23	-	-
Windmill Brook	4	NortheastNL	3%	29	-	-	-	-
Big Brook	14A	NorthwestNL	-	-	0%	1	-	-
Castors River	14A	NorthwestNL	-	-	0%	115	-	-
East River	14A	NorthwestNL	0%	42	3%	29	0%	1
East River	14A	NorthwestNL	-	-	0%	7	-	-
Little Brook Ponds	14A	NorthwestNL	0%	8	0%	2	-	-
Lomond River	14A	NorthwestNL	4%	52	3%	542	5%	22
Parsons Pond River (Western Brk)	14A	NorthwestNL	-	-	0%	2	-	-
Portland Creek	14A	NorthwestNL	0%	1	3%	38	-	-
River of Ponds	14A	NorthwestNL	8%	12	0%	45	0%	1
St Genevieve River	14A	NorthwestNL	0%	38	0%	379	-	-
Torrent River	14A	NorthwestNL	0%	4	7%	1,731	4%	230
Watts Bight Brook (Watsons Brk)	14A	NorthwestNL	-	-	0%	3	-	-
West River	14A	NorthwestNL	41%	1,225	60%	5,712	68%	3,435

Appendix Table A9. Proportion of multi-sea-winter salmon (maiden; small and large) for rivers in DU 3 and DU 6 based on data provided from DFO's Salmonid Section in the Newfoundland and Labrador region. Data were divided into three time periods (pre-1980, 1980–1999, and post-2000). Sample size for each time period and river are provided, and those with sample sizes >100 individuals are highlighted in gray. Blank cells indicate no samples.

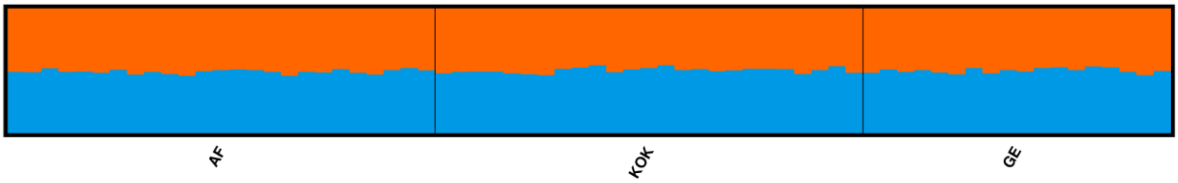
			Pre-1980		1980–1999		Post-2000	
River Name	**SFA**	**Proposed DU**	**% MSW**	**N**	**% MSW**	**N**	**% MSW**	**N**
Exploits River	SFA04	NortheastNL	0.1%	4,897	0.3%	16,693	2.3%	2,343
Gander River	SFA04	NortheastNL	0.0%	70	0.2%	2,870	0.2%	1,069
Ragged Harbour River	SFA04	NortheastNL	0.0%	50	0.0%	31	-	-
Windmill Brook	SFA04	NortheastNL	0.0%	28	-	-	-	-
Indian Bay Brook	SFA05	NortheastNL	0.0%	13	0.0%	93	-	-
Middle Brook	SFA05	NortheastNL	0.0%	11	0.0%	968	0.2%	404
Terra Nova River	SFA05	NortheastNL	0.9%	108	0.5%	2,801	4.2%	500
Northwest River	SFA05	NortheastNL	2.8%	36	0.0%	563	0.0%	38
Salmon Cove River	SFA06	NortheastNL	0.0%	3	0.0%	7	-	-
Renews River	SFA08	NortheastNL	0.0%	21	-	-	-	-
Lomond River	SFA14A	NorthwestNL	3.8%	53	6.1%	559	0.0%	21
Portland Creek	SFA14A	NorthwestNL	0.0%	1	11.9%	42	-	-
River of Ponds	SFA14A	NorthwestNL	8.3%	12	0.0%	45	0.0%	1
Little Brook	SFA14A	NorthwestNL	0.0%	8	0.0%	2	-	-
Torrent River	SFA14A	NorthwestNL	0.0%	4	4.1%	1,675	0.5%	221
East River	SFA14A	NorthwestNL	20.8%	53	0.0%	28	0.0%	1
Ste. Genevieve River	SFA14A	NorthwestNL	0.0%	38	0.0%	380	-	-
West River	SFA14A	NorthwestNL	0.3%	721	0.5%	2,290	1.1%	1,134

K=2

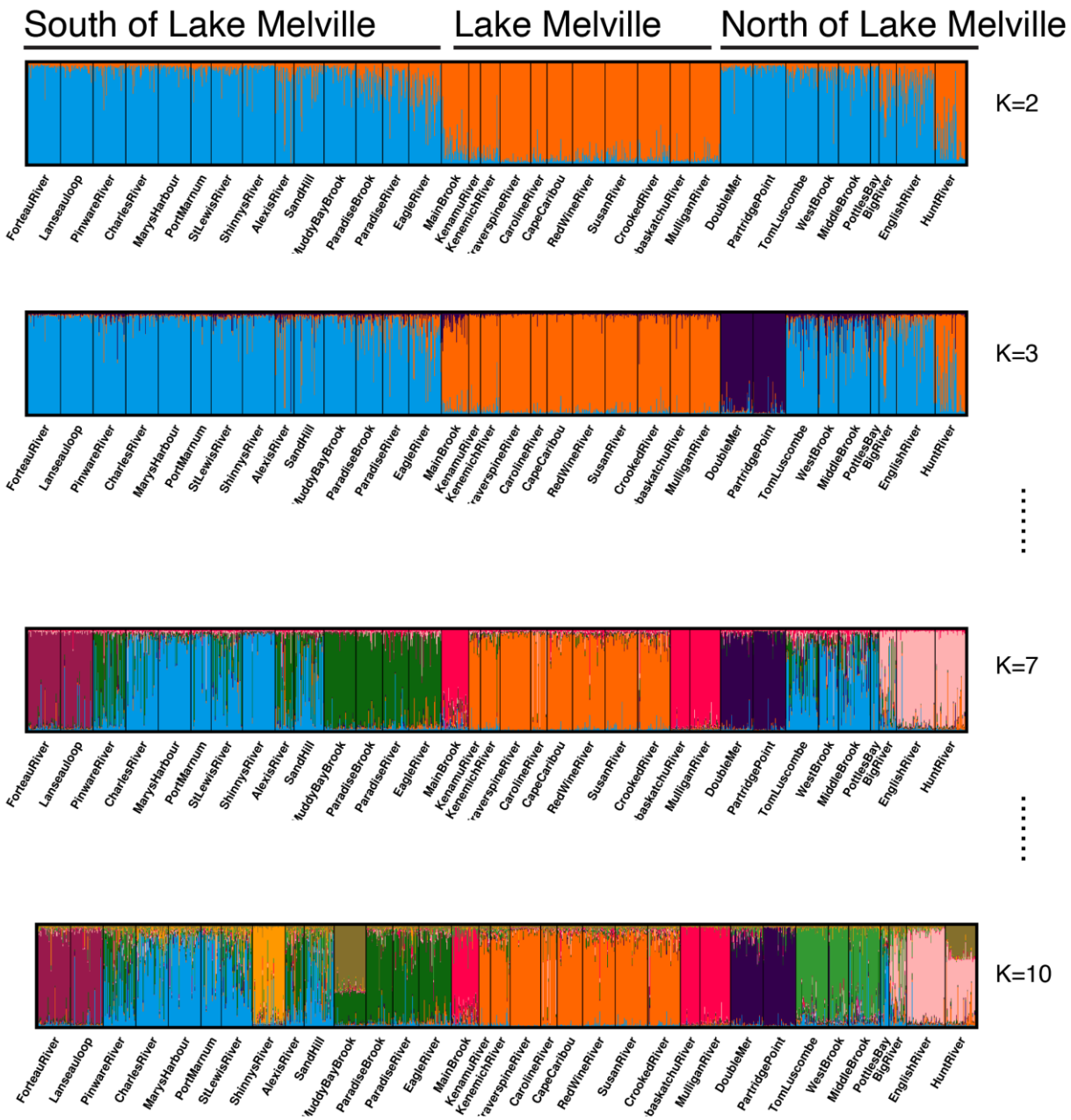


Appendix Figure A1. Results from STRUCTURE for DU 1 (Nunavik) using the 15 microsatellite dataset showing genetic clusters K=2. We tested values of K ranging from 1 to 3, and best supported K in STRUCTURE was 2. Clustering separated KO and GE from AF to some extent.

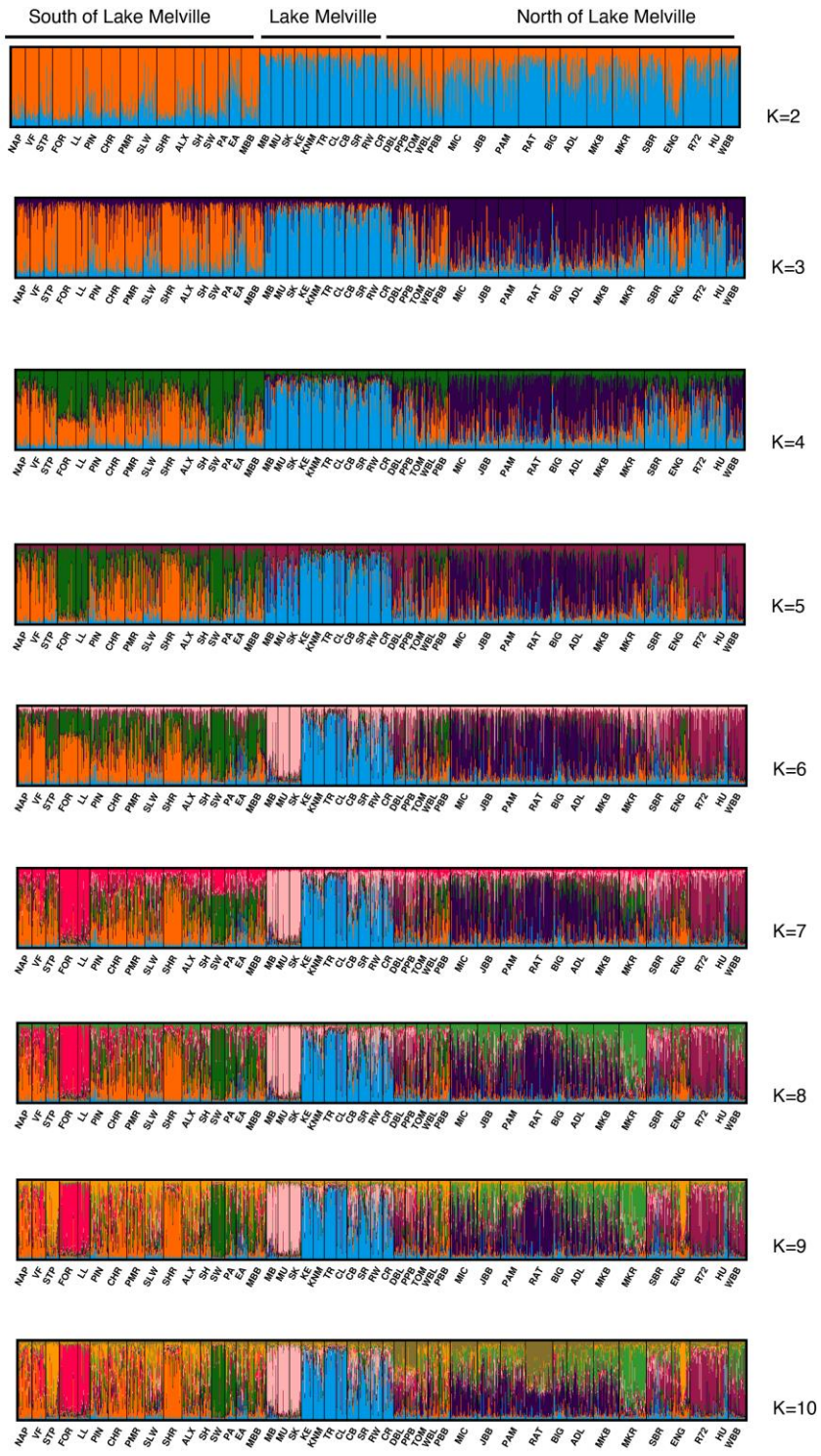
K=2



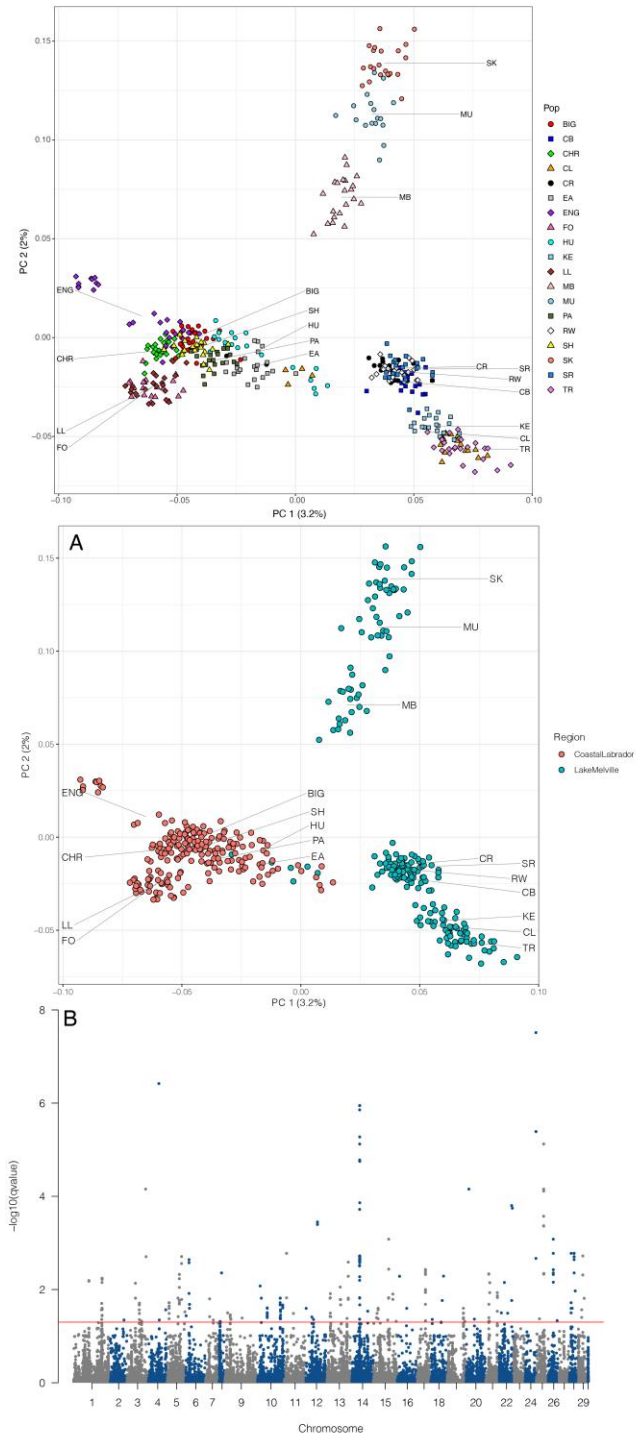
Appendix Figure A2. Results from STRUCTURE for DU 1 (Nunavik) using the 96 SNP baseline with genetic clusters K=2. Best K in STRUCTURE was 2, and no structuring was observed at K=2 or higher values. We tested values of K ranging from 1 to 3.



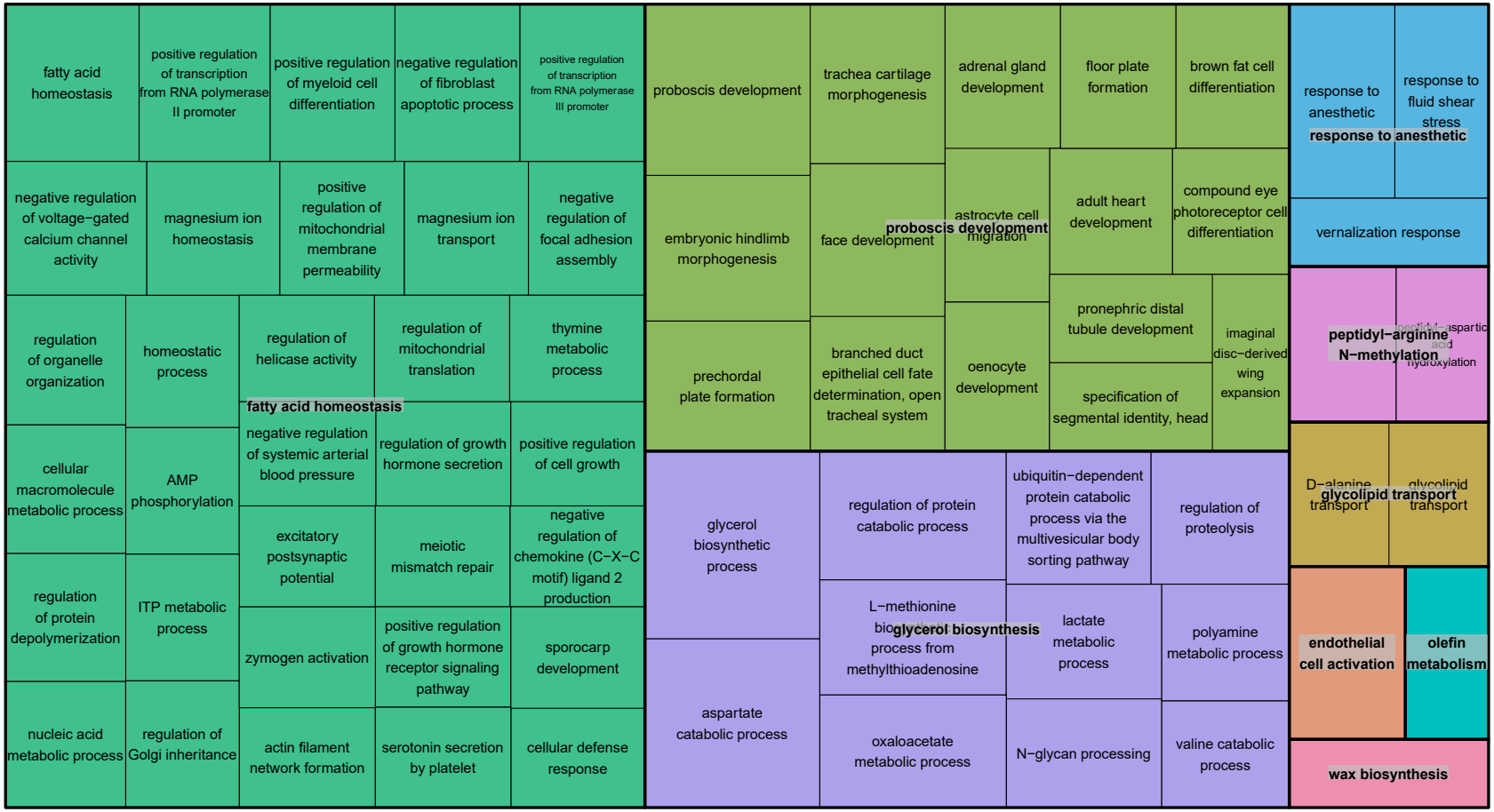
Appendix Figure A3. Results from STRUCTURE for DU 2 using the 101 microsatellites showing genetic clusters K=2 to K=10. We tested values of K ranging from 1 to 10. Best K in STRUCTURE was 2, but K values beyond K=2 were supported, and additional structuring was observed beyond K=10. At K=2, Lake Melville sites were clearly separated from other sites in Labrador. At higher values of K, various rivers or geographic regions formed their own clusters.



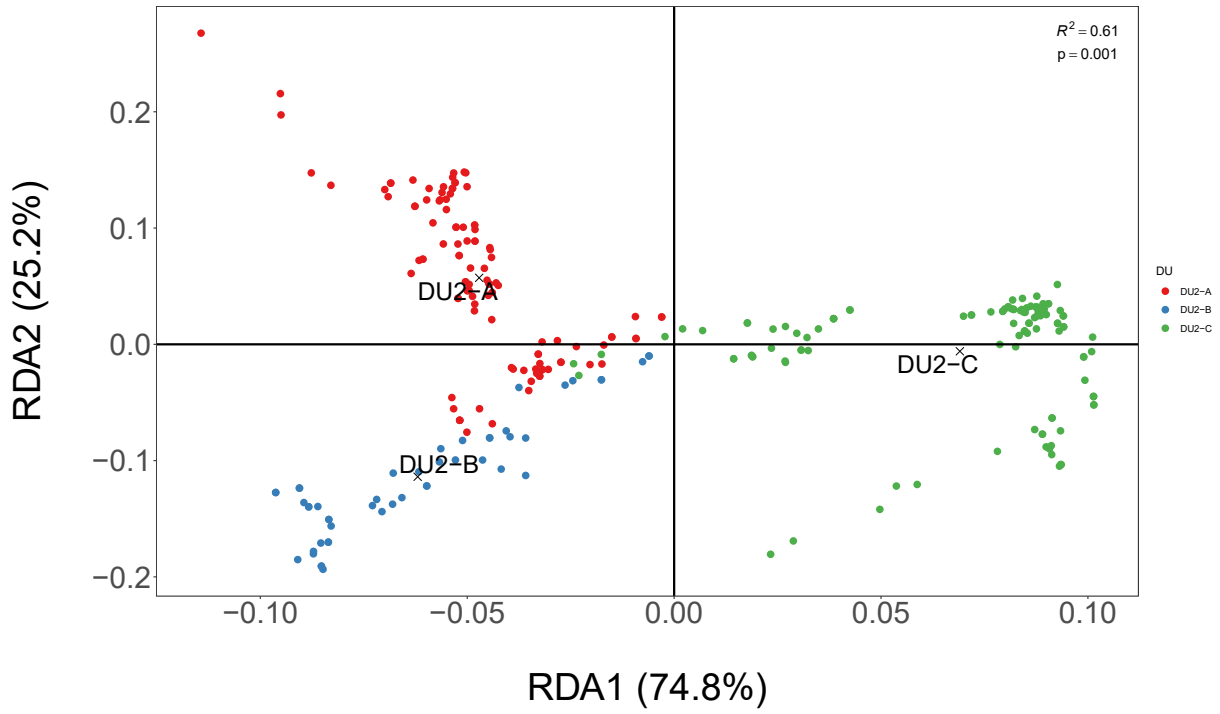
Appendix Figure A4. Results from STRUCTURE using the 96 SNP baseline with genetic clusters K=2 to K=10. We tested values of K ranging from 1 to 10. Best K in STRUCTURE was 2, but K values beyond K=2 were supported, and additional structuring was observed. Sites south of Lake Melville generally clustered separately from sites from Lake Melville and those northward at K=2. At K=3, the DU was separated into three clusters (south Labrador, Lake Melville, and north Labrador). Further clustering of individual rivers and geographic region was apparent at higher values of K.



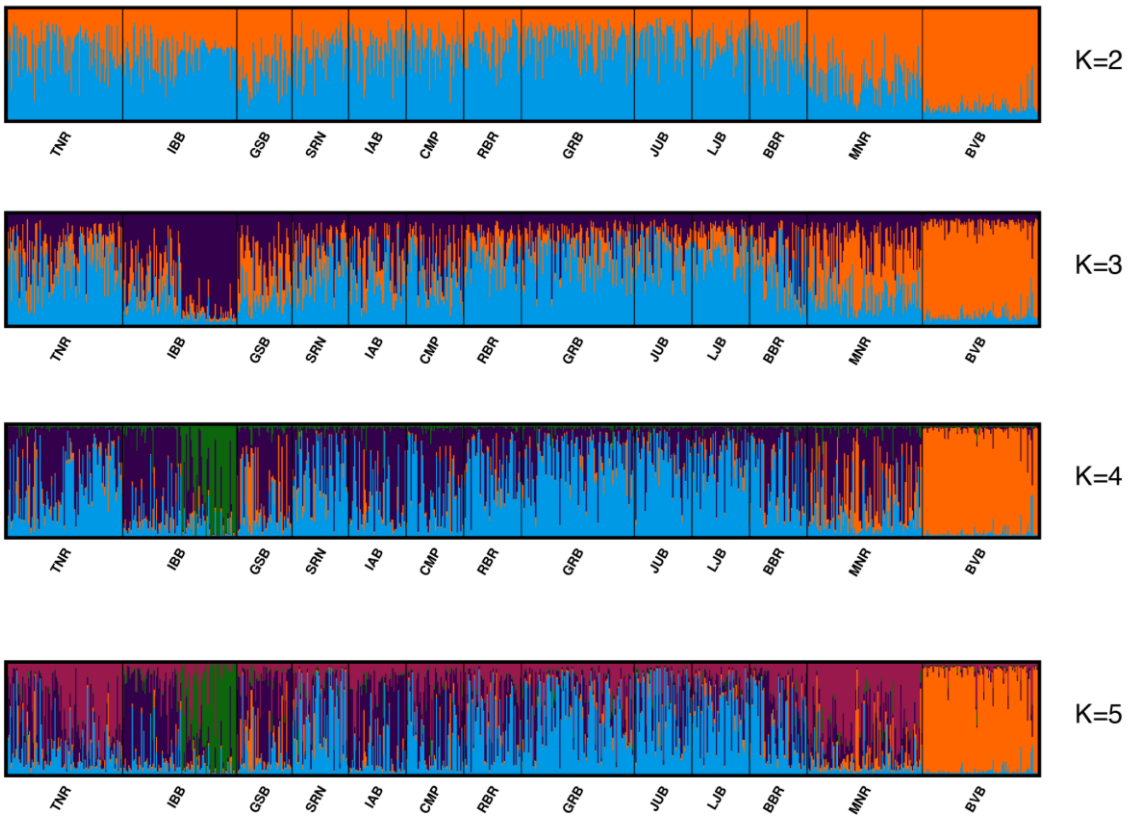
Appendix Figure A5. (A, B) In DU 2, Pcadapt separates populations in Lake Melville from those along the coast of Labrador on the first PC axis using 85,745 SNPs (MAF>0.05). Panel (A) highlights individual populations whereas panel (B) highlights Lake Melville and coastal Labrador locations. The second axis further separates populations within Lake Melville. The mean PC 1 and PC2 values for each population are indicated by lines. (B) A total of 314 loci significantly contributed to the differentiation on both PC axes (adjusted p-value or q-value <0.05) and these loci were distributed across 27 chromosomes (out of 29). Outlier loci are indicated by those above the red line.



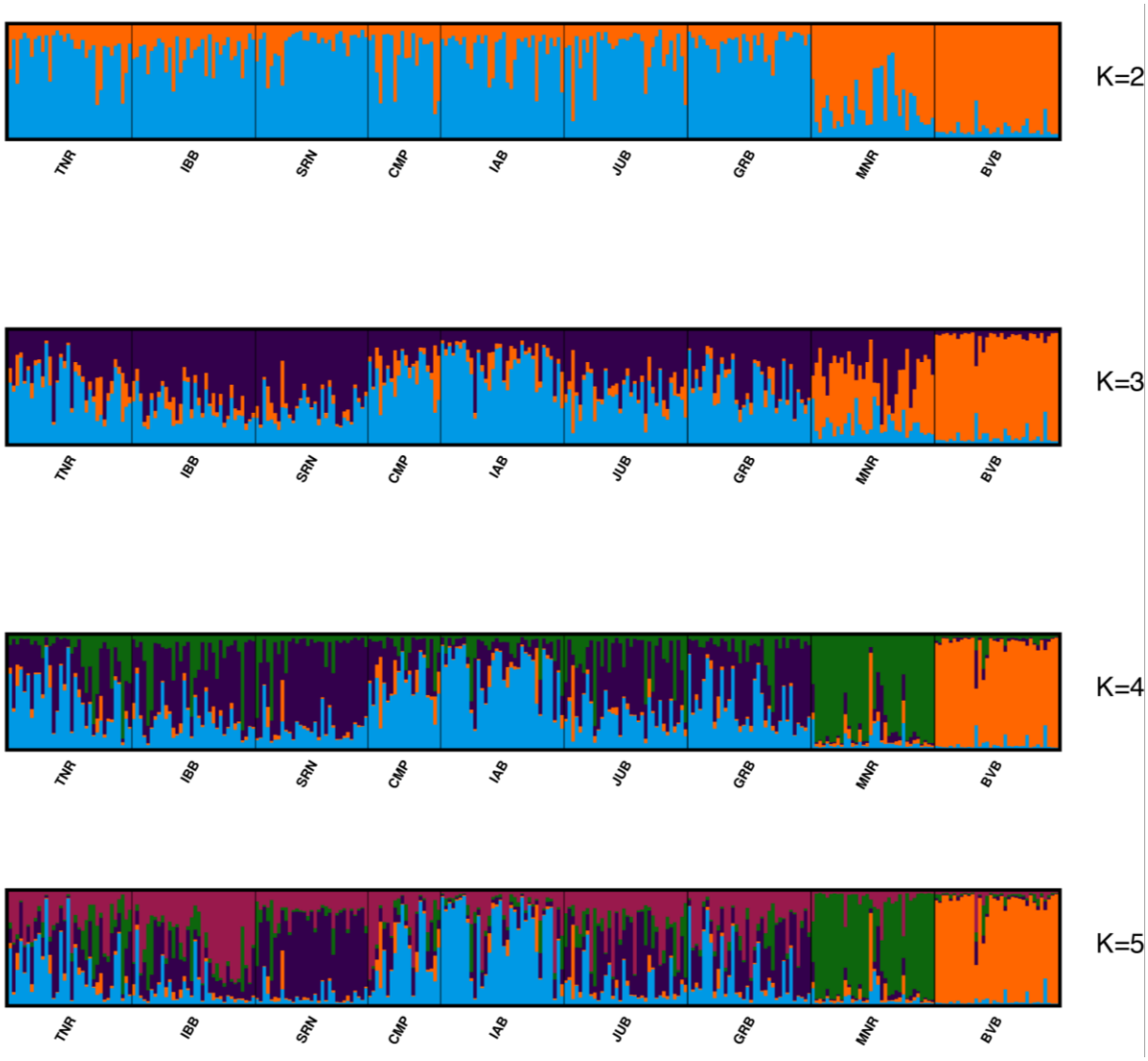
Appendix Figure A6. Results of gene ontology analysis based on biological processes that were significantly overrepresented in the outlier data for DU 2. These processes were associated with genes located within 10,000 bp of outlier SNPs (314 SNPs based on K=2 in pcadapt). Outliers are those that differentiate Lake Melville sites from other sites in DU 2. Higher level processes overrepresented in the analysis are indicated by different colour squares in the REVIGO treemap.



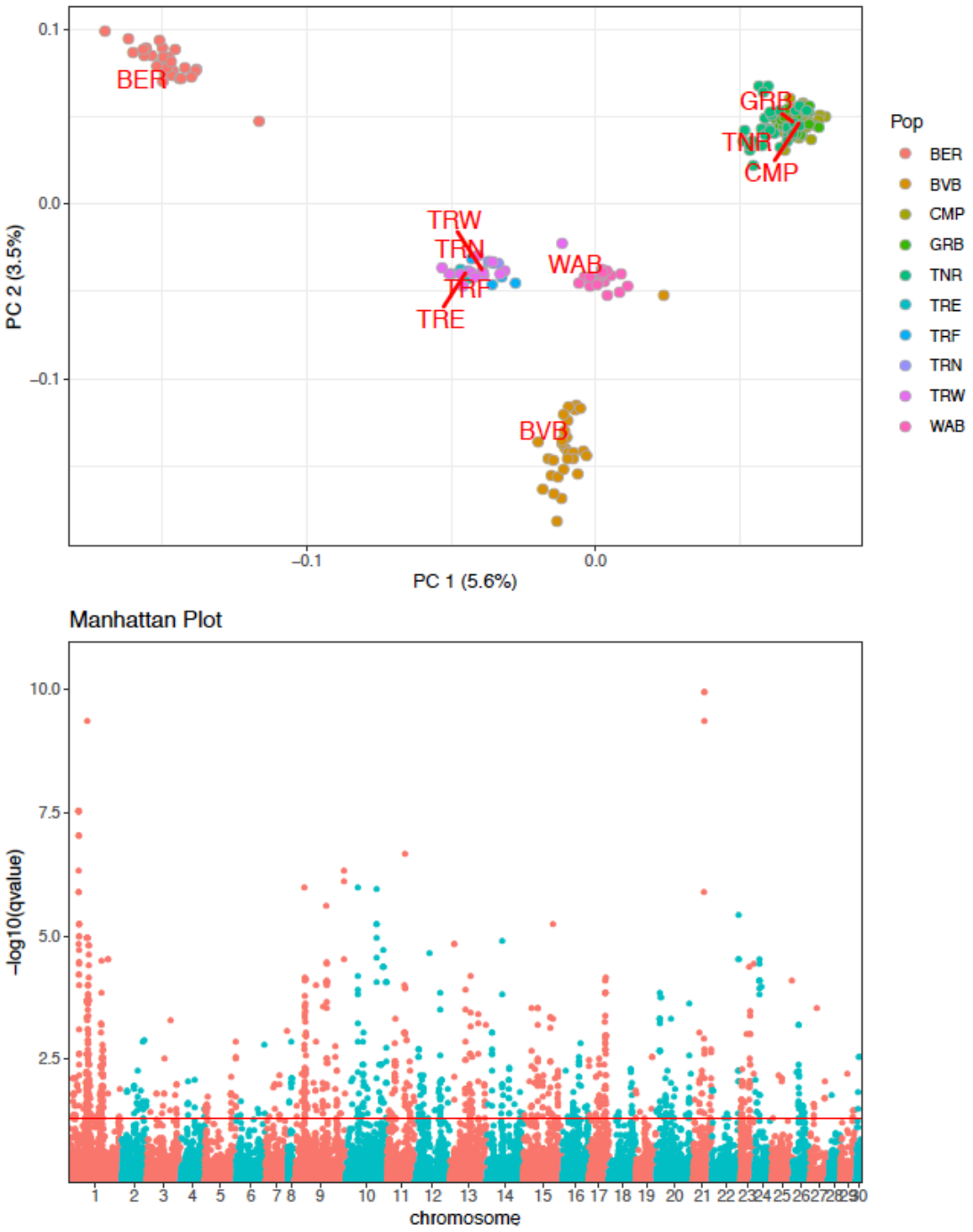
Appendix Figure A7. Redundancy analysis (RDA) using bioclimatic data for all rivers in DU 2 as the response and putative DU groups (three genetic clusters) as the constraining variable. The three putative new DUs include: northern Labrador (DU2-A; red), rivers draining into Lake Melville (DU2-B; blue), and southern Labrador (DU2-C; green). Centroids of DU groups are indicated by text, with point representing each river. ANOVA on RDA showed the model to be significant (p <0.001) with an adjusted R^2 of 0.61. RDA axis 1 explained 74.8% of the variance explained by the model, while RDA axis 2 explained 25.2% of the model variance. The RDA plot clearly shows support for the splitting of DU2 into 3 separate DUs.



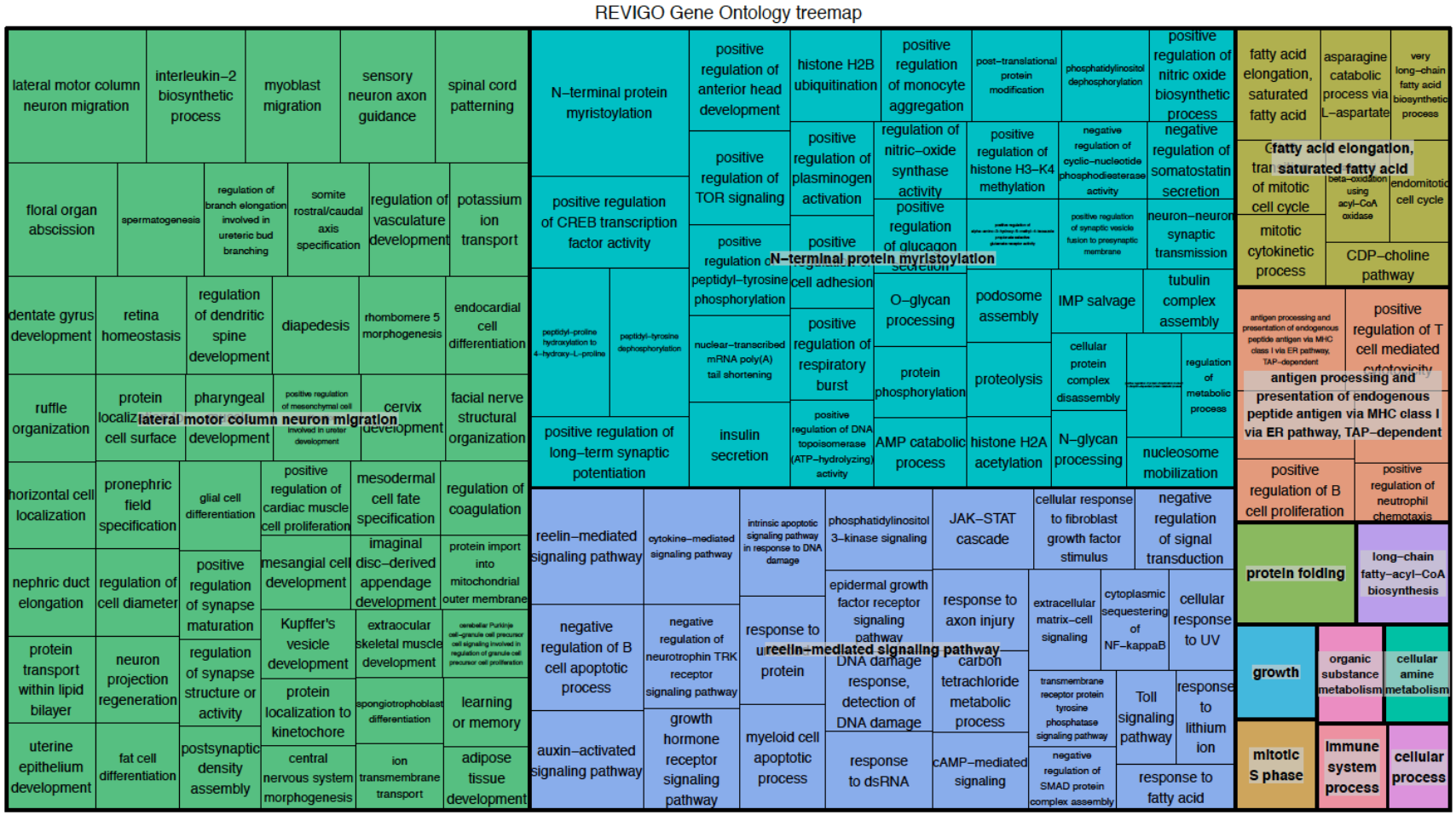
Appendix Figure A8. Results from STRUCTURE for DU 3 using the microsatellite dataset showing genetic clusters K=2-5. We tested values of K ranging from 1 to 13, and best K in STRUCTURE was 5. BVB showed clear differences from other sites, and genetic structure was generally more limited among other locations, except for at higher values of K.



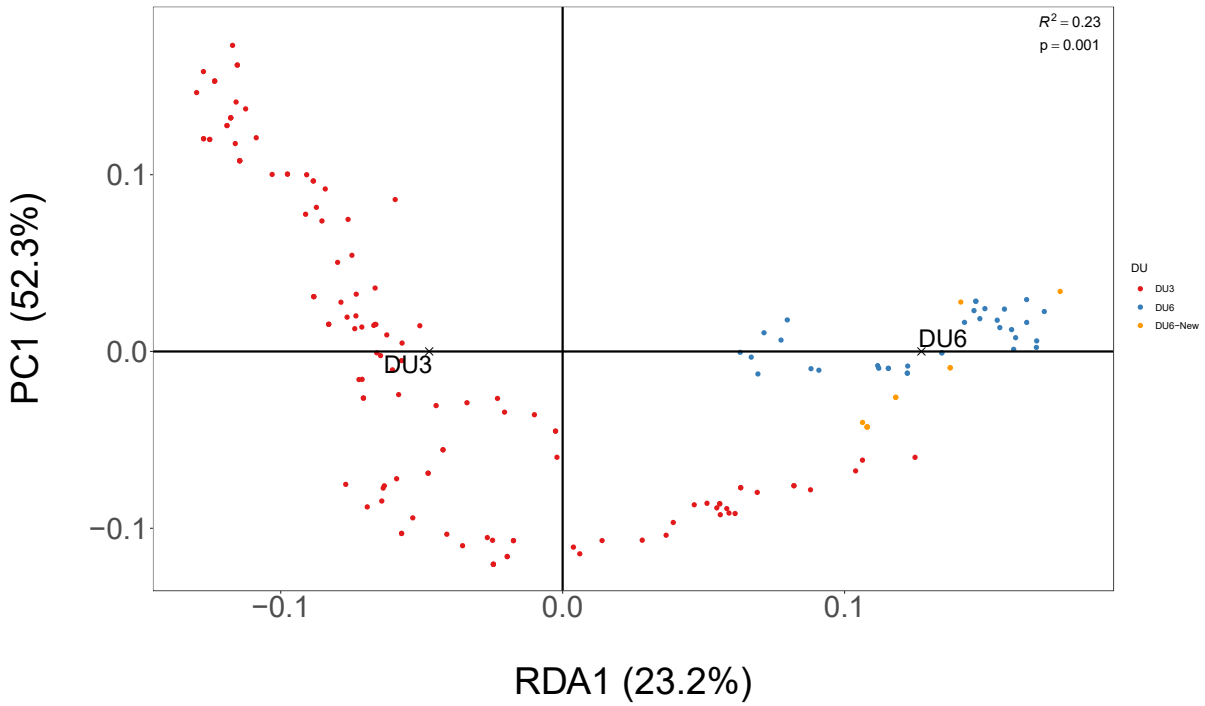
Appendix Figure A9. Results from STRUCTURE for DU 3 using the 96 SNP baseline for DU 3 with genetic clusters K=2-5. Best K in STRUCTURE was 2 based on Evanno's delta K. We tested values of K ranging from 1 to 9. At K=2, Main River (MNR) and Beaver Brook (BVB) were clustered separately from other sites in DU 3, and these sites were separated by K=4. Further structure was supported (K=5; mean LnPr(X|K)), although clearly distinct clusters beyond K=3 were not evident.



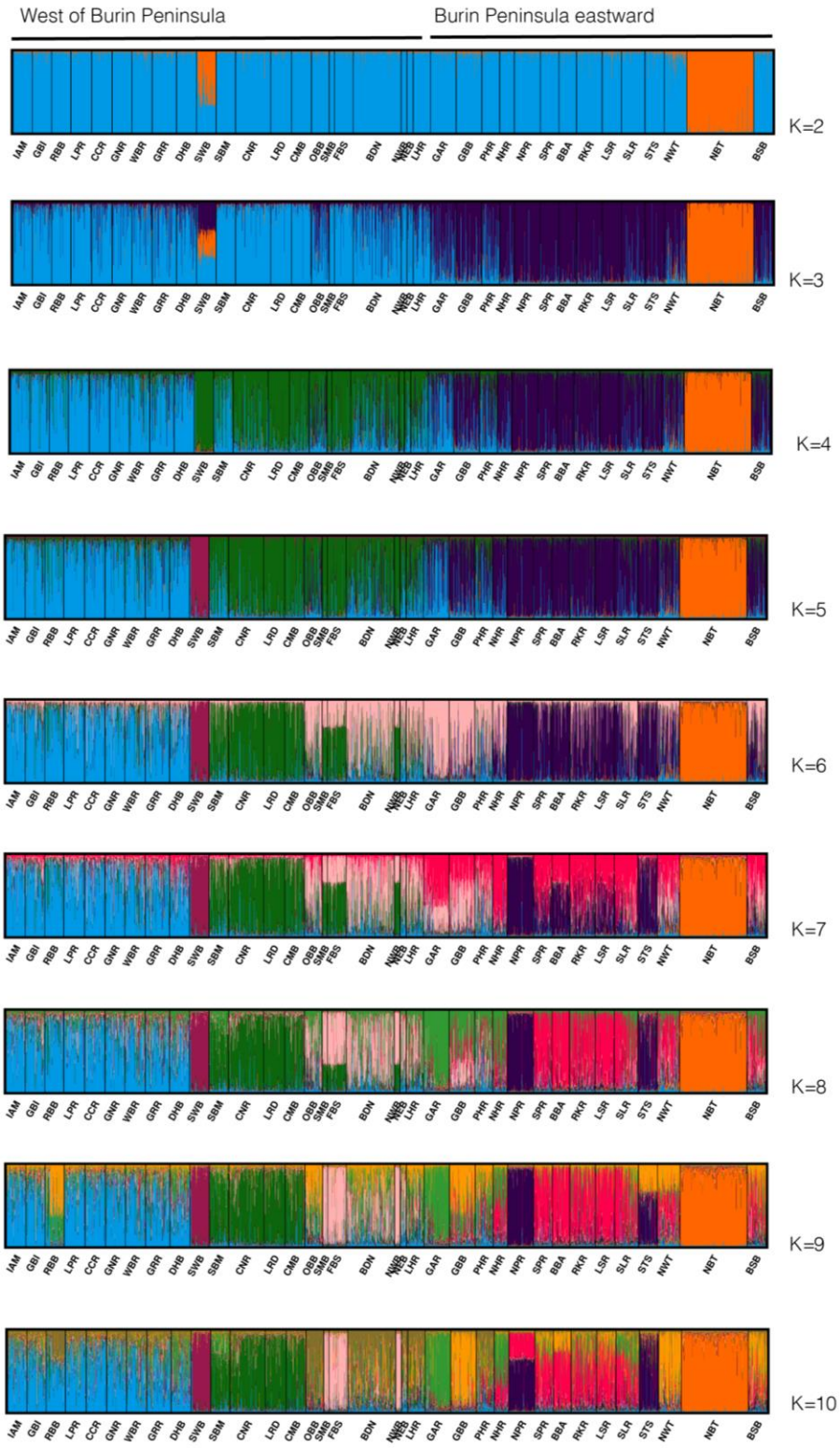
Appendix Figure A10. (A) In DU 3 and 6 using 90,548 SNPs (MAF>0.05), pcadapt separated sites in the putative new DU 3 separated from sites in DU 6 along the first PC axis. These DU 3 sites clustered very tightly together (GRB, TNR, and CMP), whereas Beaver Brook (BVB) (previously in DU 3) clustered more closely with DU 6 sites, including Trout River sites (TRE, TRN, TRF, TRW) and Western Arm Brook (WAB), on the first PC axis, as well as PC 2. Another site in DU 6, Big East River (BER), clearly separated from all sites on PC 1. (B) A total of 1,189 loci significantly contributed to the differentiation on PC axis 1 and 2 (adjusted p-value or q-value <0.05) and these loci were distributed across 29 chromosomes (out of 29). Outlier loci are indicated by those above the red line.



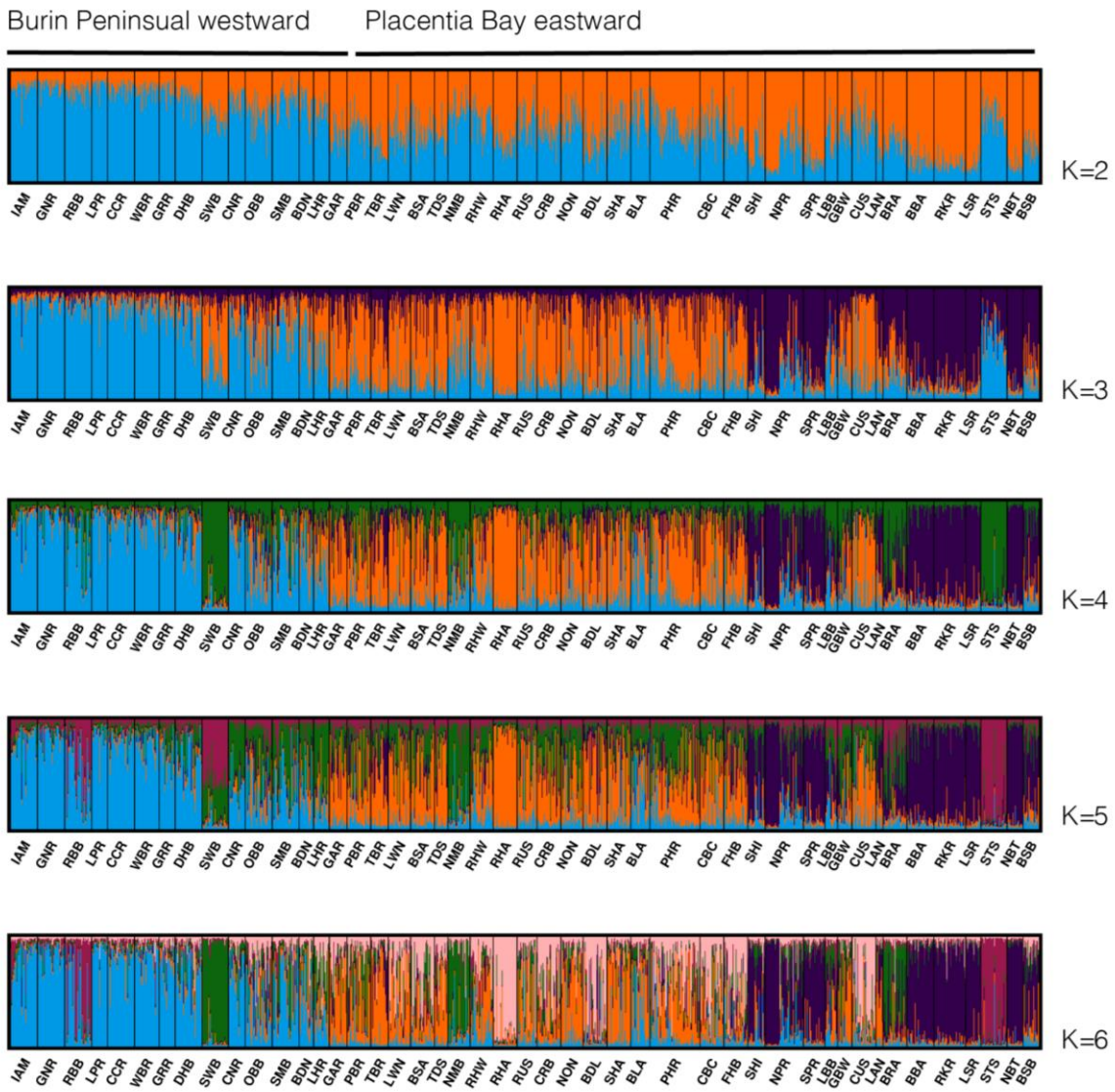
Appendix Figure A11. Results of gene ontology analysis based on biological processes that were significantly overrepresented in the outlier data for DU 3 and 6. These processes were associated with genes located within 10,000 bp of outlier SNPs (1,189 SNPs based on K=2 in pcadapt). Outliers are those that differentiate sites in DU 3 and 6. Higher level processes overrepresented in the analysis are indicated by different colour squares in the REVIGO treemap.



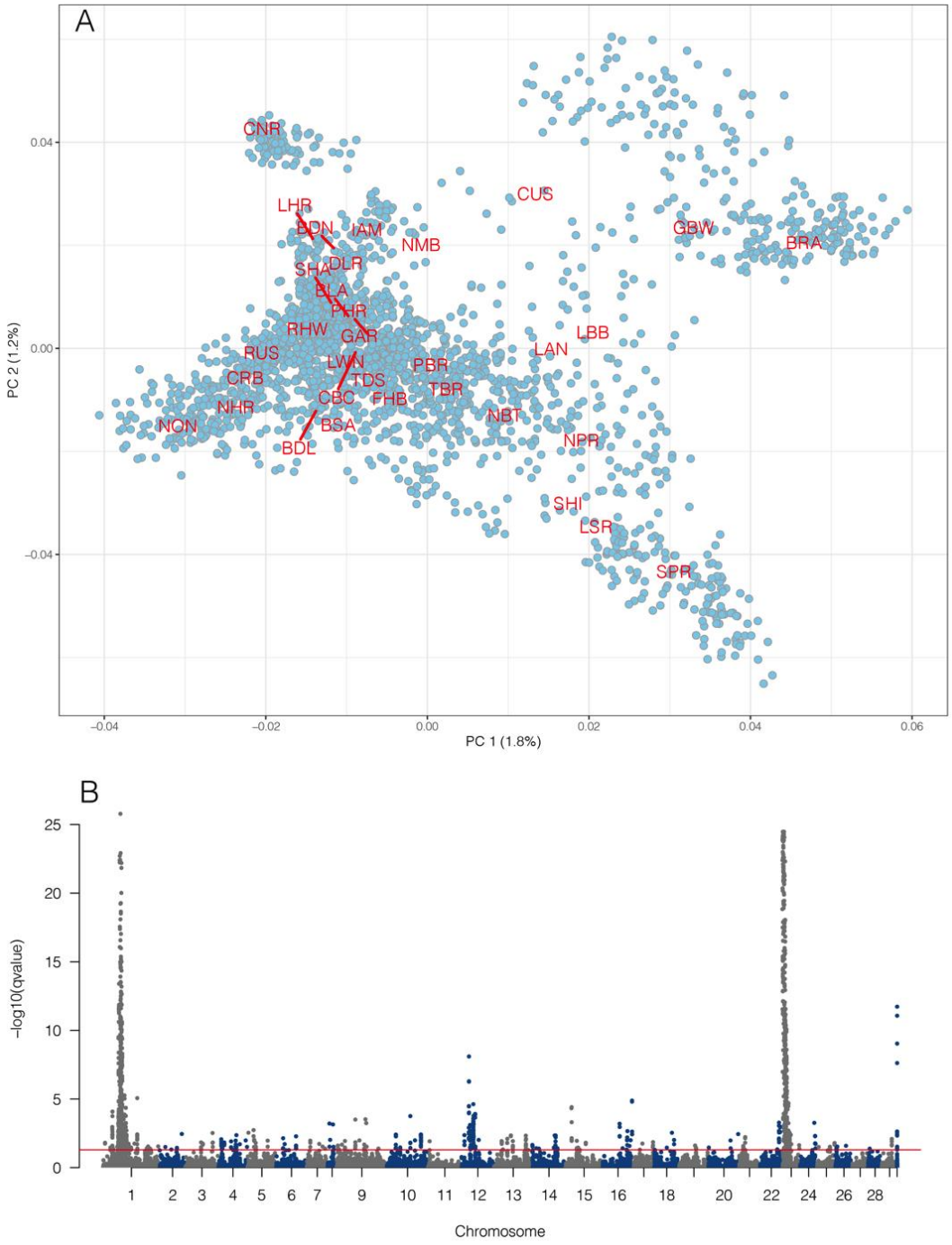
Appendix Figure A12. Redundancy analysis (RDA) using bioclimatic data for all rivers in DU 3 and DU 6 as the response and putative DU groups (two genetic clusters) as the constraining variable. Centroids of DU groups are indicated by text, with point representing each river. The proposed rivers to be moved into DU 6 are indicated by yellow. ANOVA on RDA showed the model to be significant (p <0.001) with an adjusted R^2 of 0.23. RDA axis 1 explained 23.2% of the variance explained by the model. The RDA plot clearly shows support for the splitting the DUs based on the new boundaries.



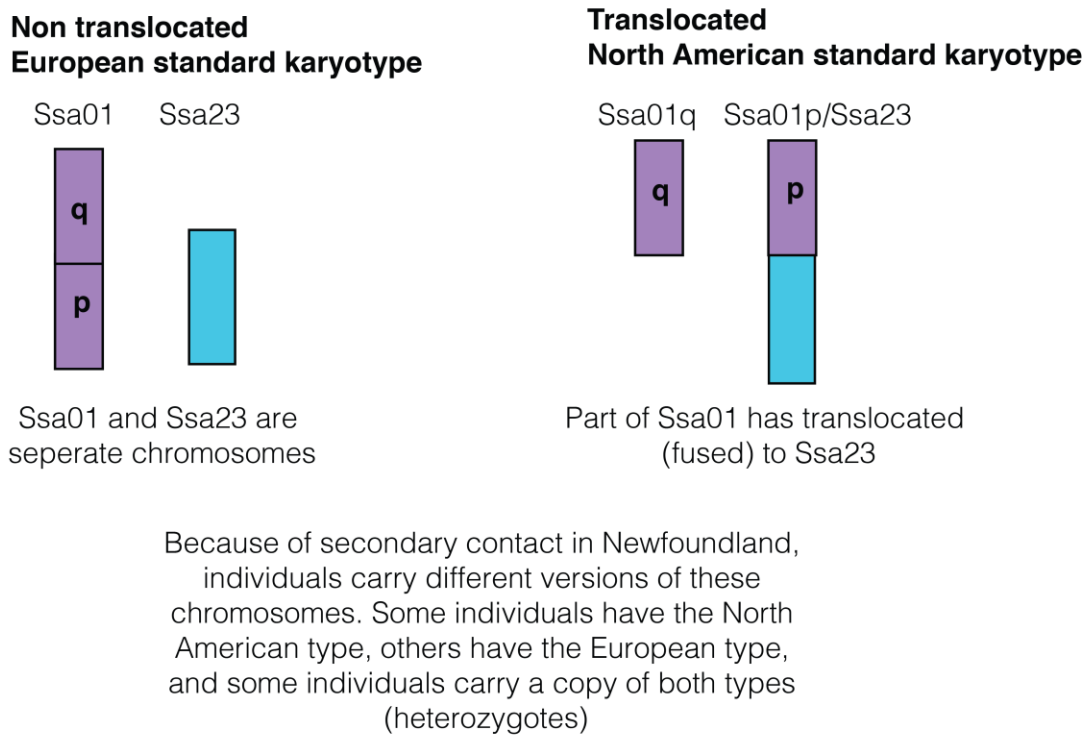
Appendix Figure A13. Results from STRUCTURE for DU 4 using the microsatellite dataset showing genetic clusters K=2 and K=10. We tested values of K ranging from 1 to 10, and best K in STRUCTURE was 2, although clear structure was observed beyond K=2. Clustering at K=3 separated populations east and west of Garnish River.



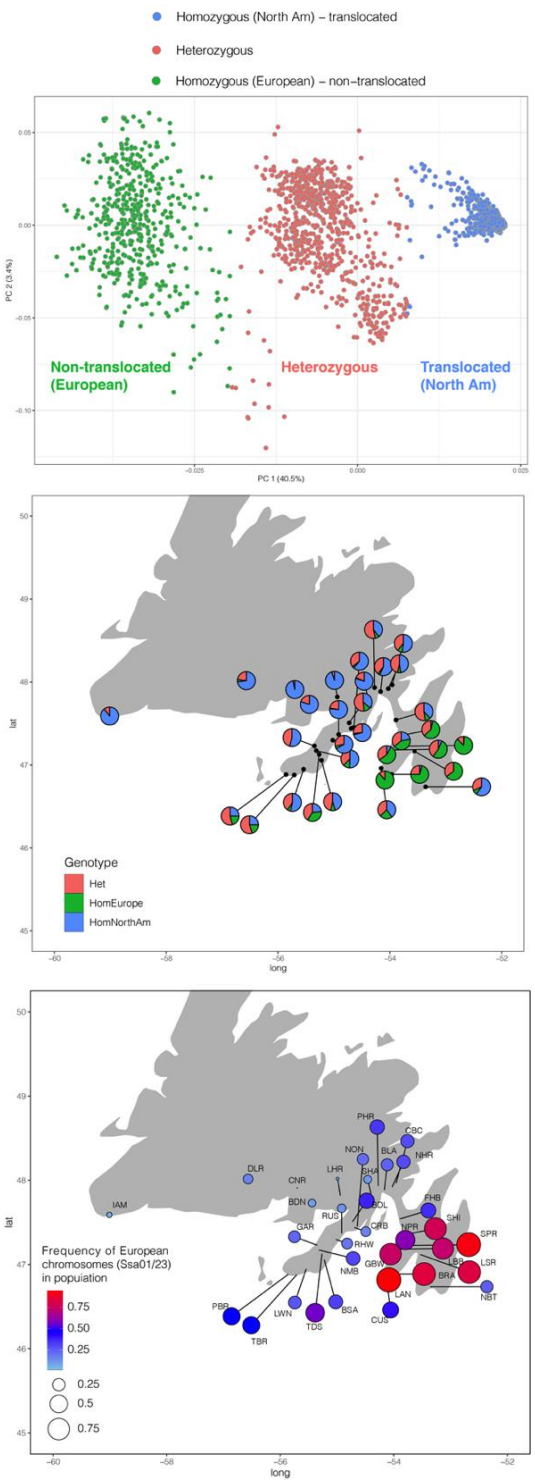
Appendix Figure A14. Results from STRUCTURE for DU 4 using the 96 SNP baseline with genetic clusters K=2. Best K in STRUCTURE was 2, but additional structuring was observed beyond K=2. We tested values of K ranging from 1 to 10. Genetic clusters appear to separate populations in the west (IAM to LHR), and those on the Burin Peninsula and in Placentia Bay (GAR to FHB), and those in the eastern portion of Placentia Bay and eastward (SHI to BSB). Some populations deviated from this general pattern.



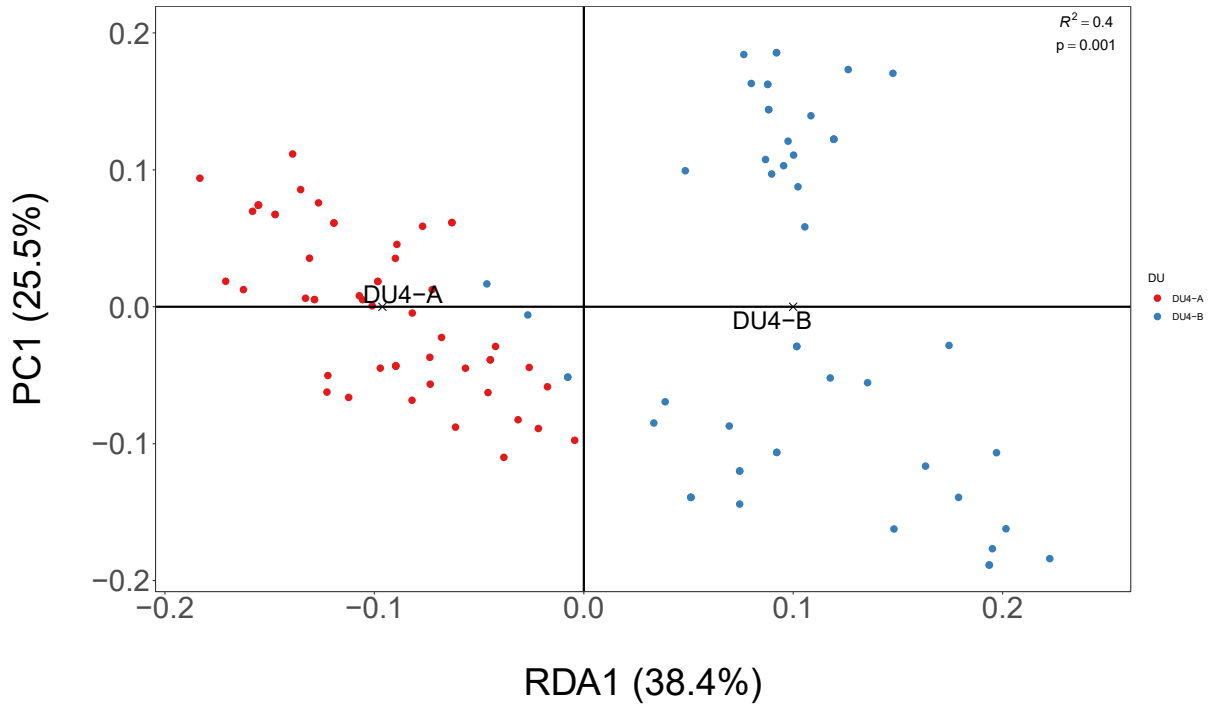
Appendix Figure A15. (A) In DU 4 using 92,009 SNPs (MAF>0.05), Pcadapt separated populations across both axes, where populations appeared to be separated between the east and west of Placentia Bay as well as between north and south within Placentia Bay. The mean PC 1 and PC2 values for each population are indicated by lines. (B) A total of 1,582 loci significantly contributed to the differentiation on both PC axes (adjusted p-value or q-value <0.05) and these loci were distributed across 28 chromosomes (out of 29). Outlier loci are indicated by those above the red line. Over 70% of these outliers were located on Ssa01 and Ssa23, which are involved in a known chromosomal rearrangement that exists between individuals (chromosomal translocation). This rearrangement was explored further here.



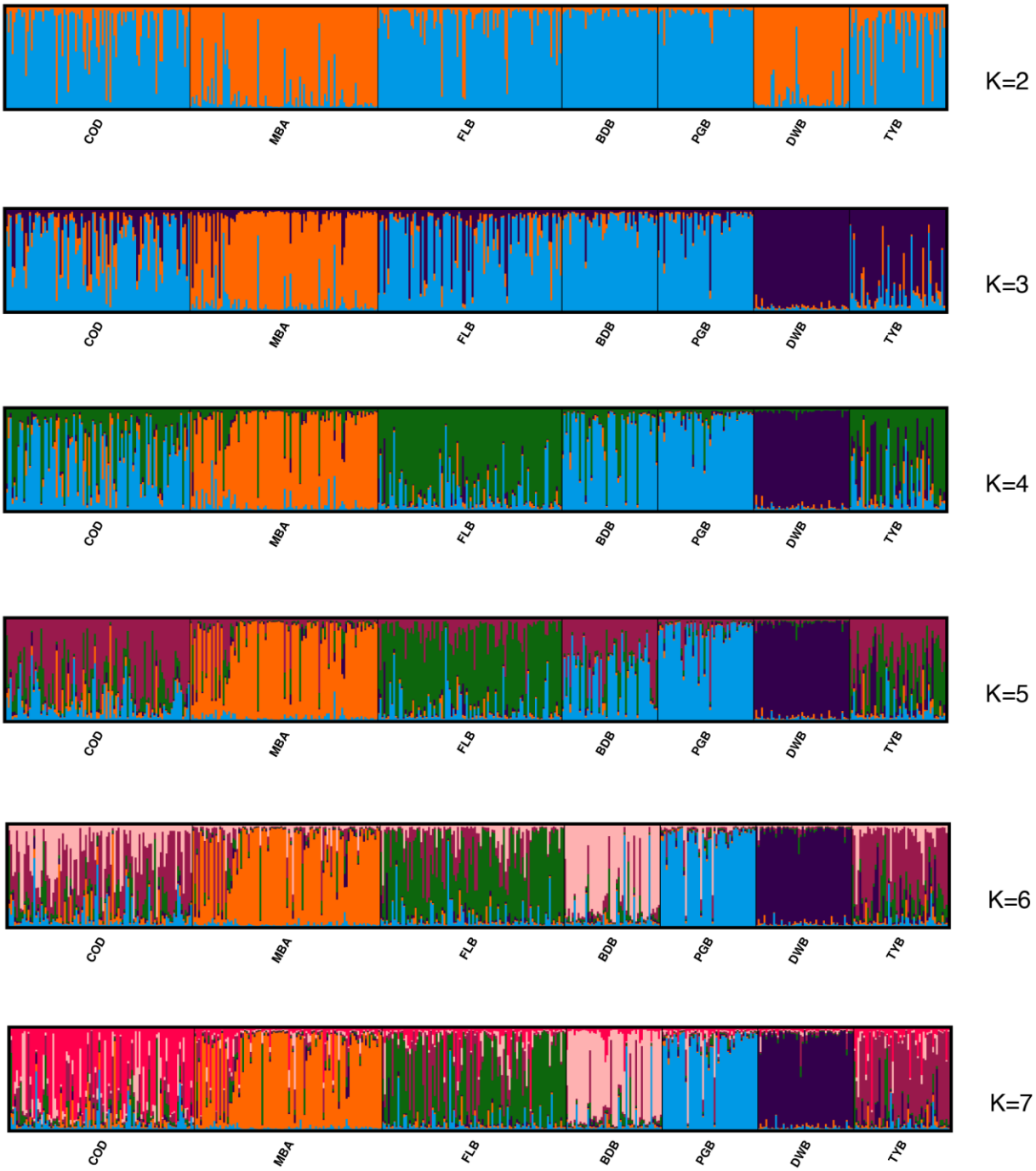
Appendix Figure A16. Schematic showing the chromosomal differences in Europe and North America for Ssa01 and Ssa23. In Europe, the standard karyotype is two separate chromosomes for Ssa01 and Ssa23. In North America, the standard karyotype includes a translocation, where part of Ssa01 has attached (fused) with Ssa23, resulting in a chromosome rearrangement compared to Europe. In some parts of North America, including southern Newfoundland (DU2), different configurations of these chromosomes exist because of secondary contact from Europe. See additional figure below.



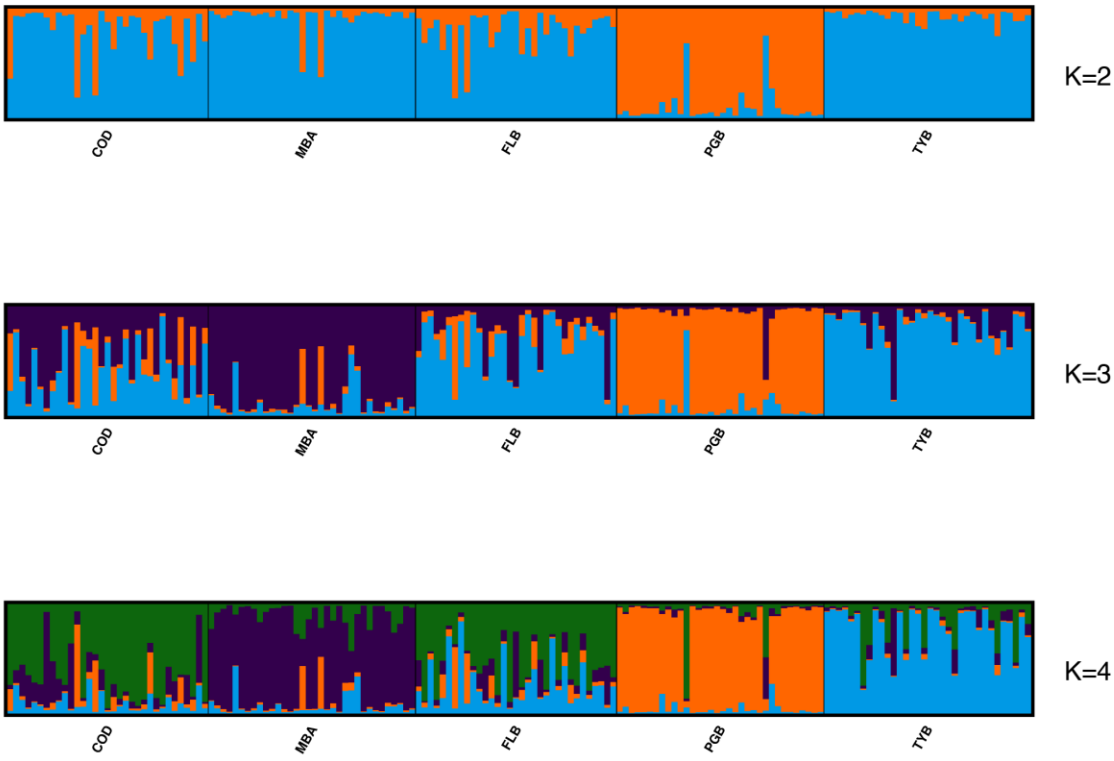
Appendix Figure A17. (A) Principal component analysis (PCA) of chromosome Ssa01 and Ssa23 translocated region. The first PC axis separates the three genotypes. (B) Map with proportion of each genotype in each population. (C) Map showing the frequency of European type chromosomes (Ssa01 and Ssa23) in the population. This frequency was calculated by determining total number of European type chromosomes in the population (i.e., 2 copies in homozygotes European, and 1 copy in heterozygotes, 0 copies in homozygotes North American) out of all chromosomes (2 copies per individual).



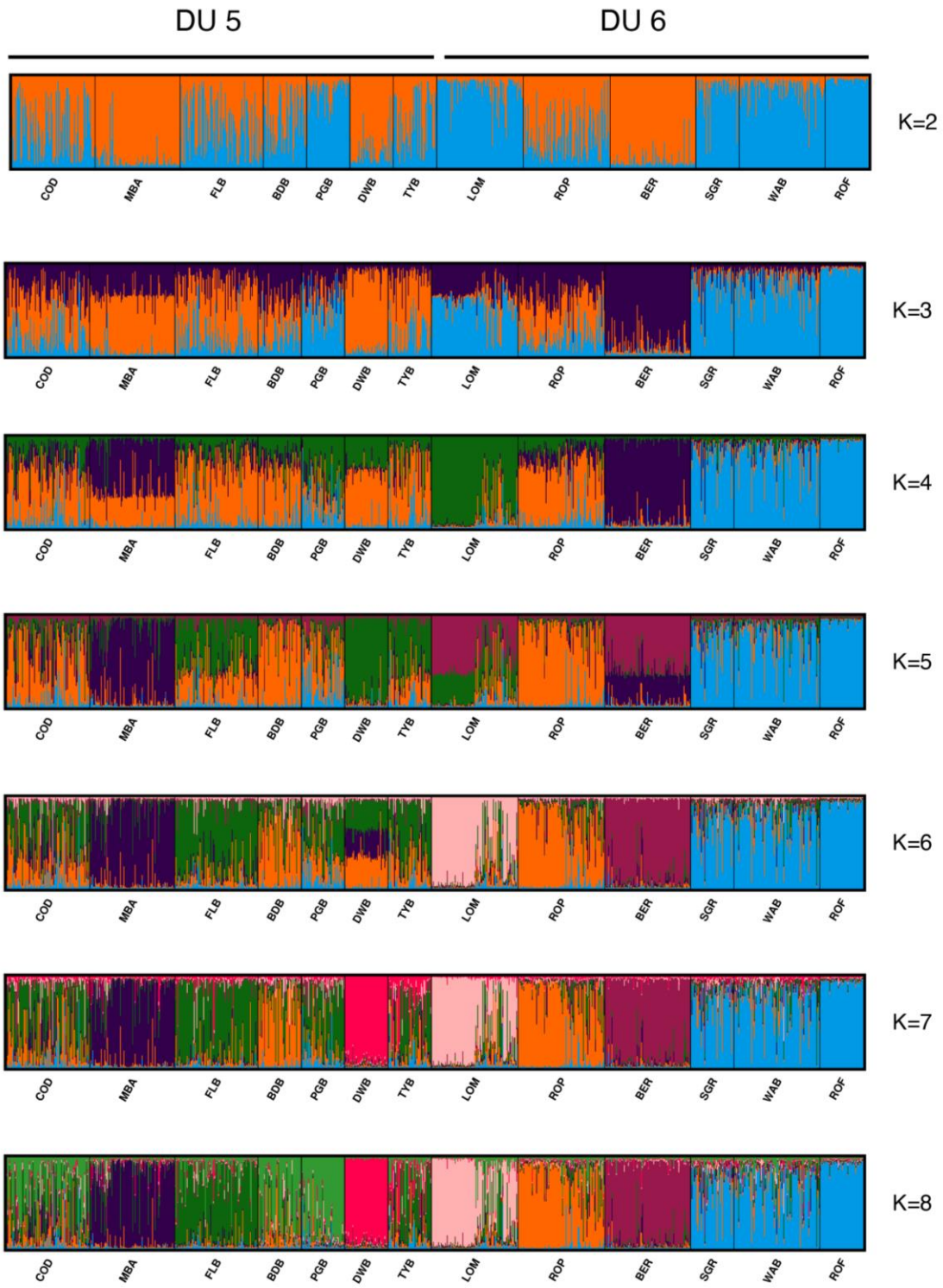
Appendix Figure A18. Redundancy analysis (RDA) using bioclimatic data for all rivers in DU 4 as the response and putative DU groups (two main genetic clusters) as the constraining variable. The two putative new DUs include: rivers from Garnish eastward (DU4-A; red) and rivers west of Garnish (DU4-B; blue). Centroids of DU groups are indicated by text, with point representing each river. ANOVA on the RDA showed the model to be significant ($p < 0.001$) with an adjusted R^2 of 0.40. RDA axis 1 explained 38.4% of the variance in the model and clearly shows the split between the putative new DUs, thus supporting the splitting of DU 4.



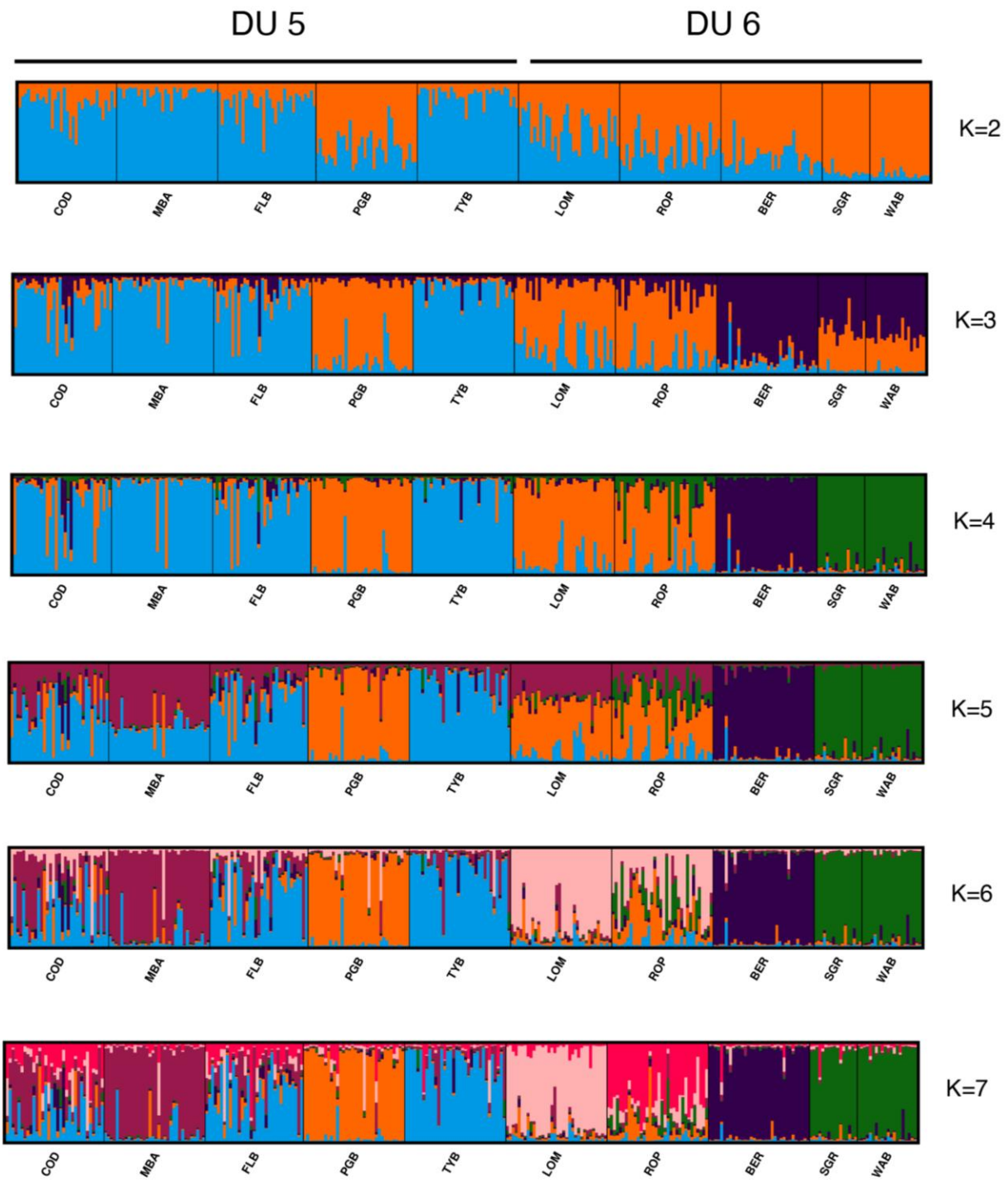
Appendix Figure A19. Results from STRUCTURE for DU 5 using the microsatellite dataset showing genetic clusters K=2 and K=7. We tested values of K ranging from 1 to 7, and best K in STRUCTURE was 2, although clear structure was observed beyond K=2, where each population could be separated into their own cluster at K=7.



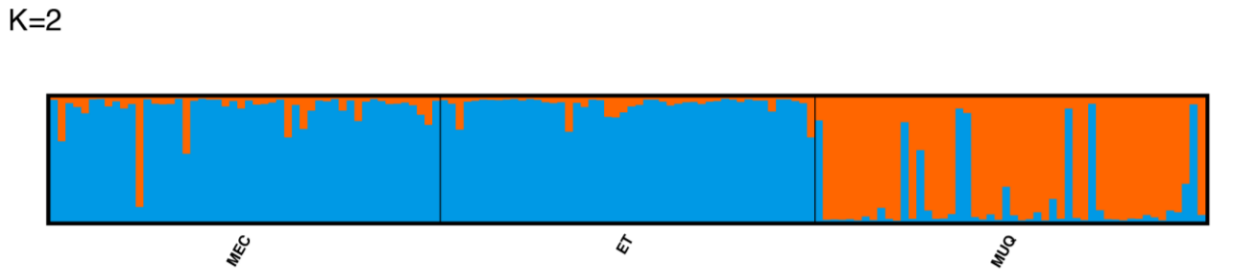
Appendix Figure A20. Results from STRUCTURE for DU 5 using the 96 SNP baseline with genetic clusters K=2. Best K in STRUCTURE was 2, and little additional structuring was observed beyond K=2. We tested values of K ranging from 1 to 5. At K=2, Pinchgut formed a separate cluster from other sites.



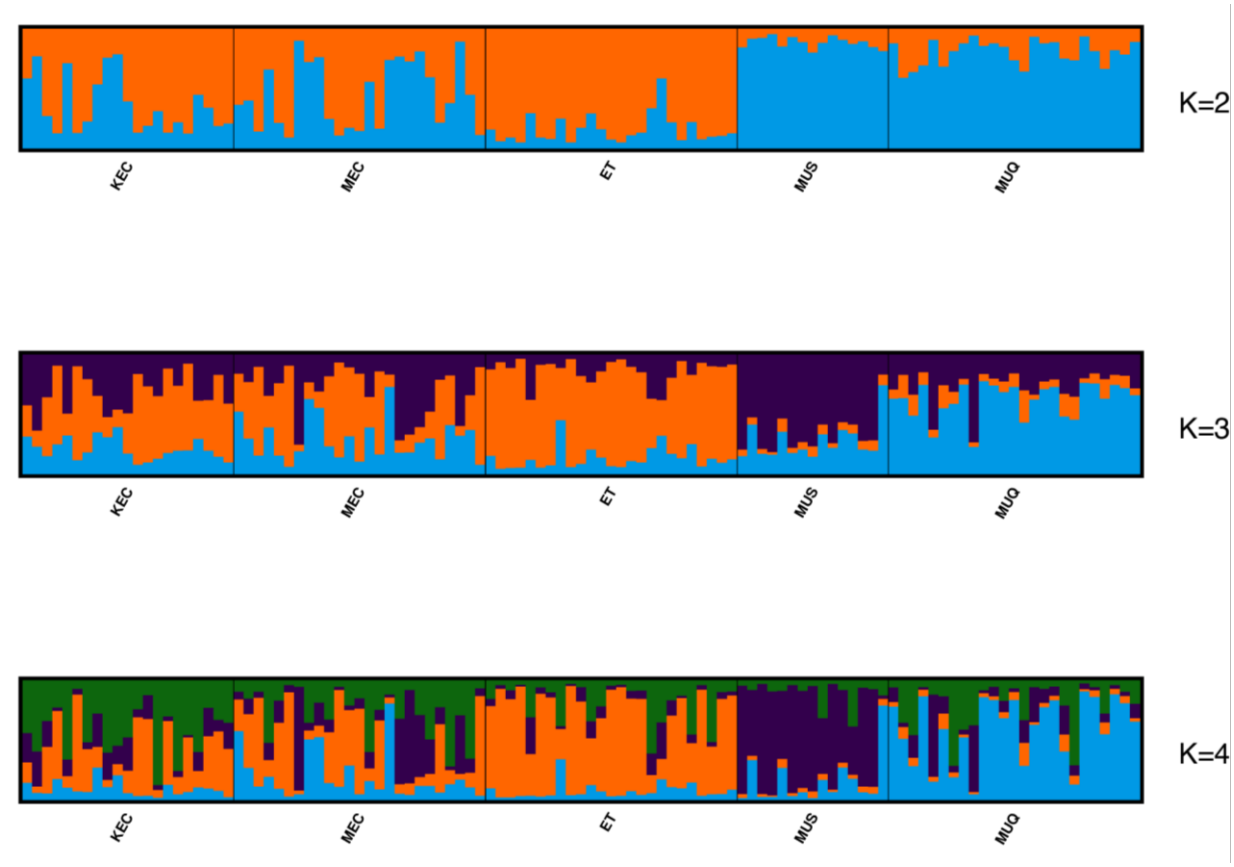
Appendix Figure A21. Results from STRUCTURE for DU 5 and DU 6 using the microsatellite dataset showing genetic clusters K=2 and K=8. We tested values of K ranging from 1 to 13, and the optimal number of genetic clusters (K) in STRUCTURE was 8.



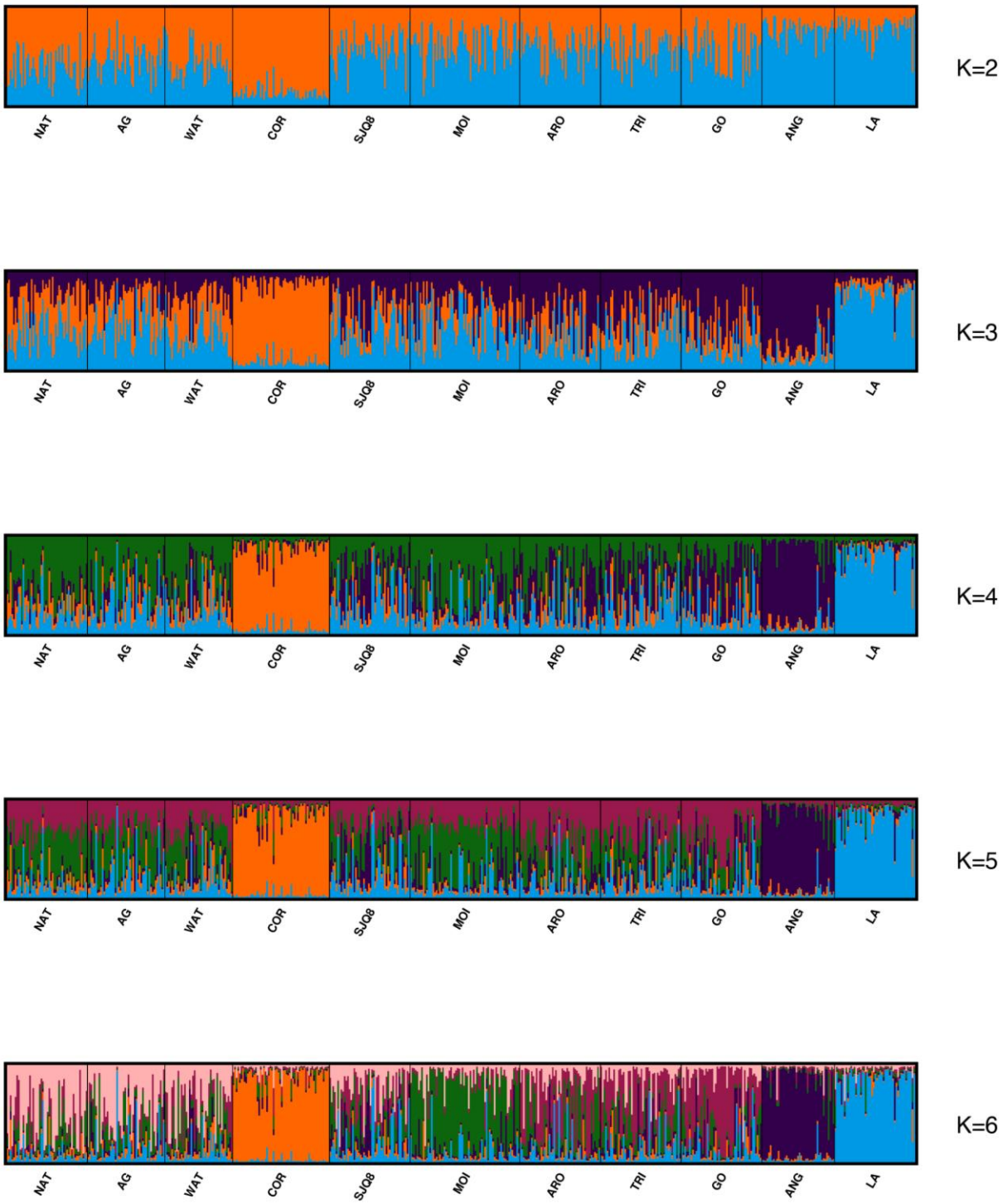
Appendix A22. Results from STRUCTURE for DU 5 and DU 6 using the 96 SNP baseline with genetic clusters K=2 to K=7. Best K in STRUCTURE was 2, but additional structure was observed beyond K=2.



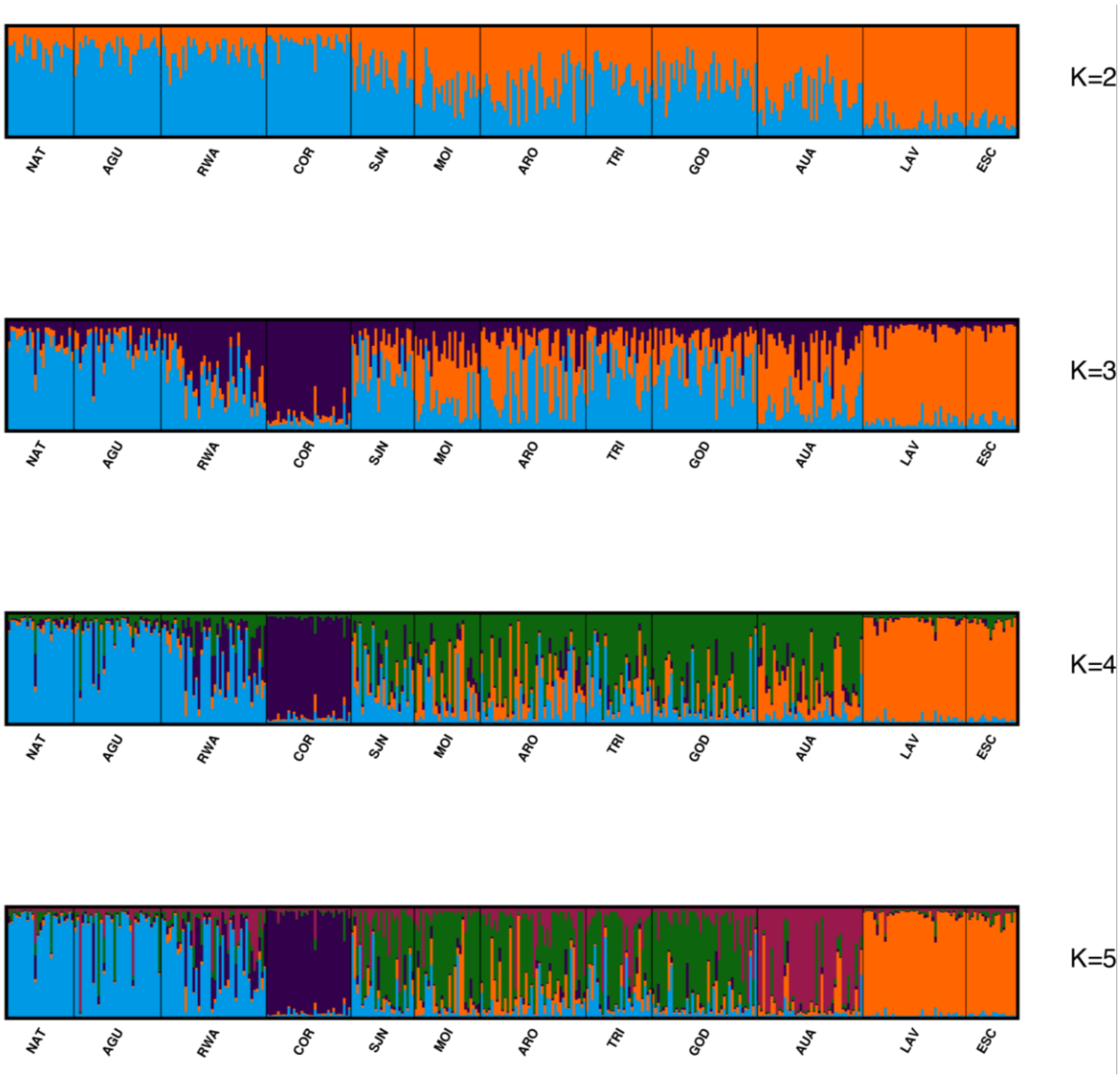
Appendix Figure A23. Results from STRUCTURE for DU 7 using the microsatellite dataset showing genetic clusters K=2. We tested values of K ranging from 1 to 3, and best K in STRUCTURE was 2, and no additional structure was observed at K=3. At K=2, MUQ clustered separately from MEC and ET.



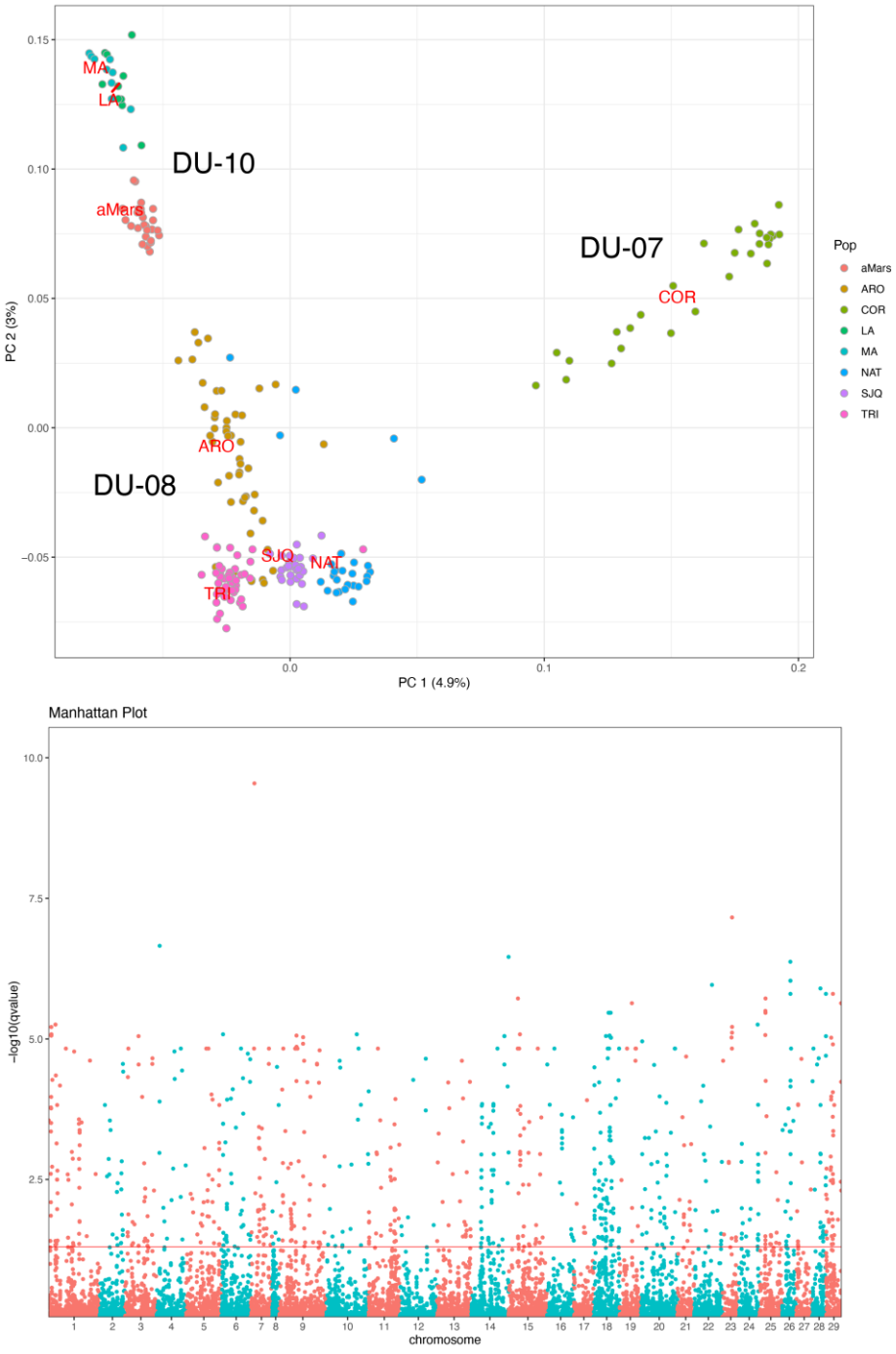
Appendix Figure A24. Results from STRUCTURE for DU 7 using the 96 SNP baseline with genetic clusters K=2 to K=4. Best K in STRUCTURE was 4. We tested values of K ranging from 1 to 5. Clustering separated MUS and MUQ from other sites as well as from each other, but clustering patterns showed populations were not clearly distinct.



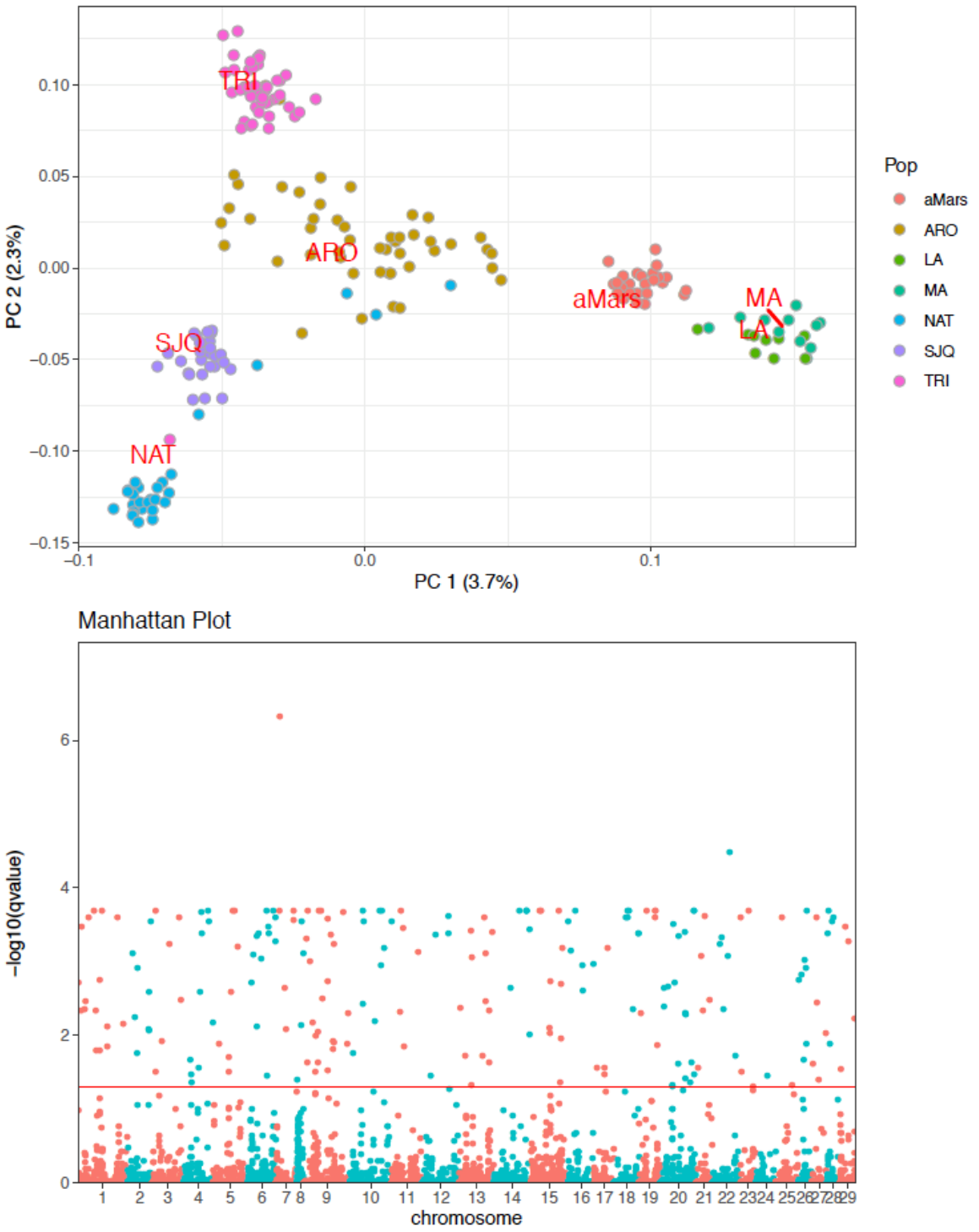
Appendix Figure A25. Results from STRUCTURE for DU 8 using the microsatellite dataset showing genetic clusters K=2 and K=6. We tested values of K ranging from 1 to 11, and best K in STRUCTURE was 3, although some additional structure was observed beyond K=3. Three populations (COR, ANG, and LA) each formed separate clusters, whereas other sites generally clustered together with some differentiation observed at higher values of K.



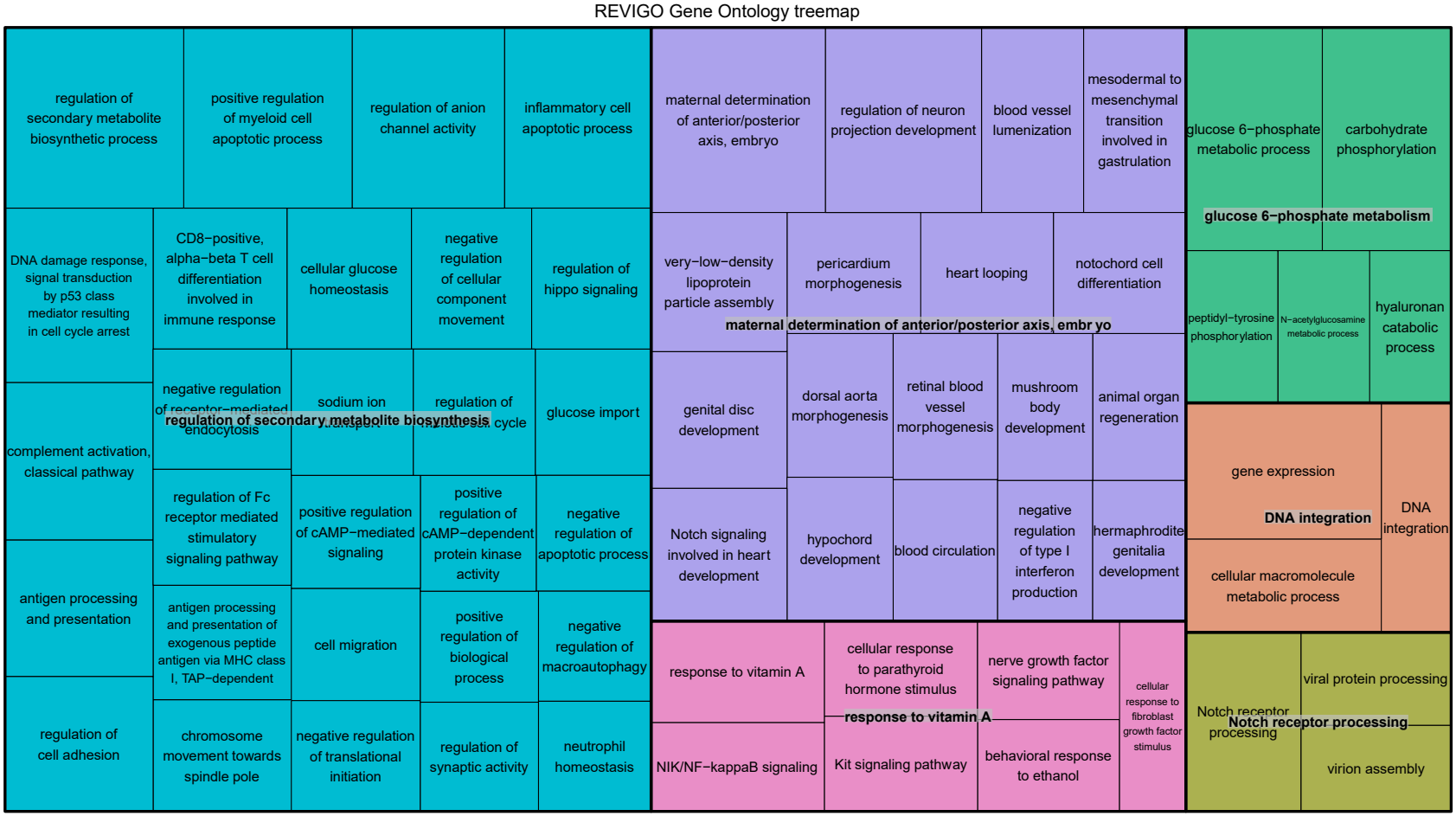
Appendix Figure A26. Results from STRUCTURE for DU 8 using the 96 SNP baseline with genetic clusters K=2 to 5. Best K in STRUCTURE was 2, but some additional structuring was observed beyond K=2. We tested values of K ranging from 1 to 12. Clustering patterns appeared to follow geography with sites in the east, west, mid portion of the DU forming separate clusters at higher values of K. One exception was COR which clustered separately from nearby sites at K=3 and higher.



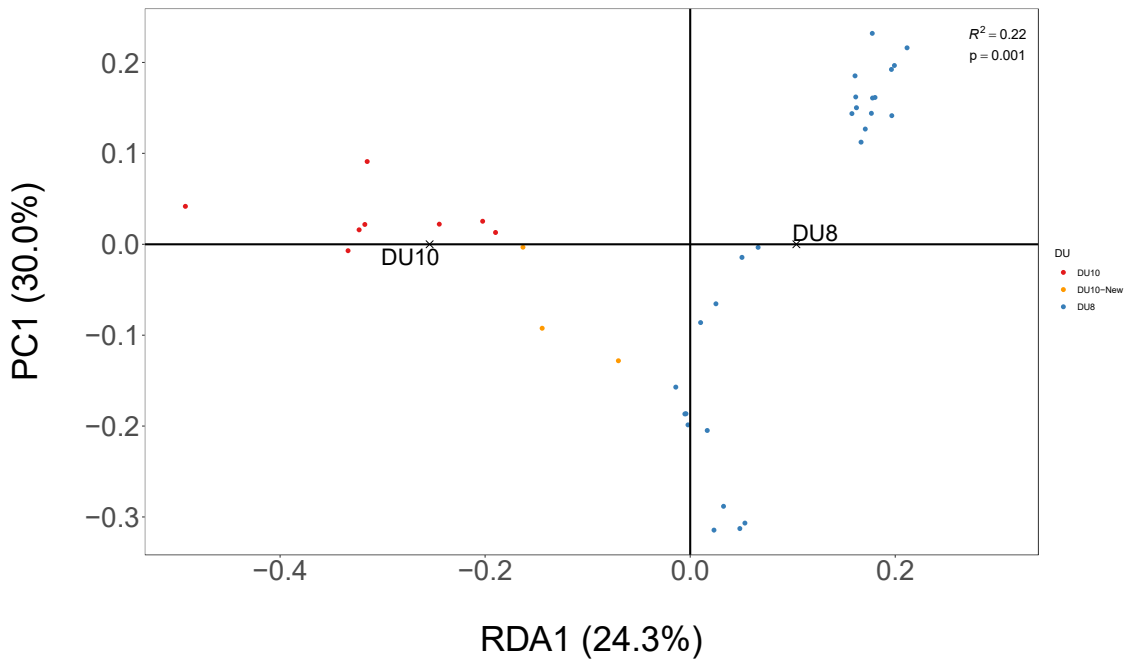
Appendix Figure A27. Analysis for populations in DU 8 and DU 10 using genome-wide SNPs. Pcadapt clearly separated Corneille (COR – now placed in DU 7) from all other locations in DU 8 and DU 10 along the first principal component (PC) axis supporting its placement into DU 7. Sites in DU 8 and DU 10 (based on revised boundary) are separated along PC 2. The mean PC 1 and PC2 values for each population are indicated by lines. Bottom panel shows a total of 864 loci (out of 31,900 SNPs) significantly contributed to the differentiation on PC axis 1 and 2 thus differentiating the three revised DUs (adjusted p-value [q-value] <0.05) and these loci were across all chromosomes (out of 29). Outlier loci are indicated by those above the horizontal red line.



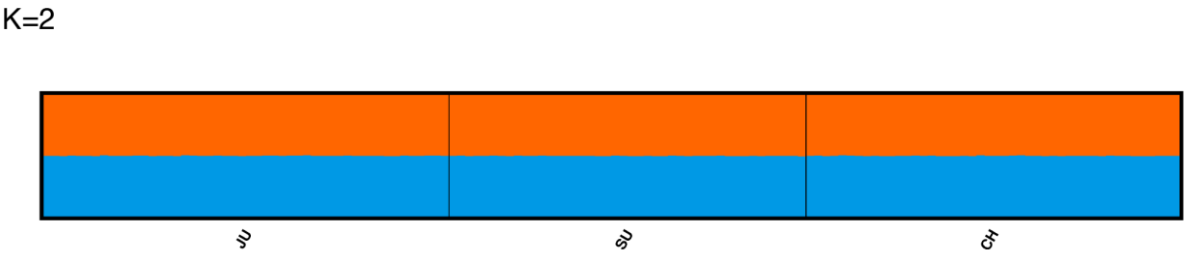
Appendix Figure A28. (A) Revised analysis using populations in DU 8 and 10 without the inclusion of Corneille. Pcadapt separated populations from the revised DU 8 and DU 10 along the first PC axis. Further separation of sites in DU 8 occurred along PC 2, with sites in DU 10 generally clustering closely on both PC axes. The mean PC 1 and PC2 values for each population are indicated by lines. (B) A total of 222 loci significantly contributed to the differentiation on PC axis 1 thus differentiating the two revised DUs (adjusted p-value [q-value] <0.05) and these loci were across all chromosomes (out of 29). Outlier loci are indicated by those above the red line.



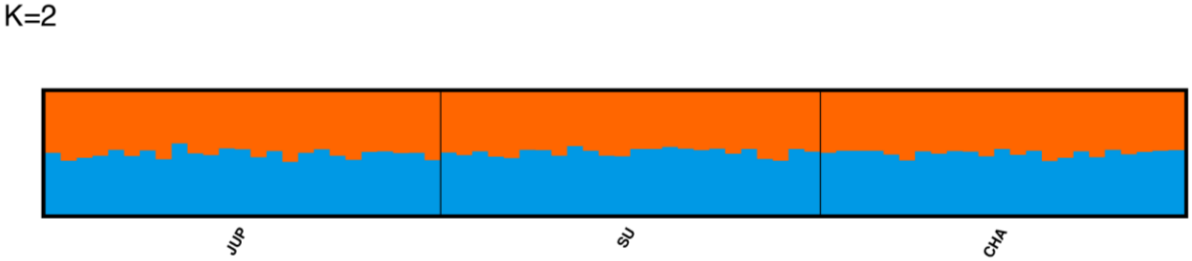
Appendix Figure A29. Results of gene ontology analysis for DU 8 and 10 (revised boundary) based on biological processes that were significantly overrepresented in the outlier data. A total of 79 processes were over-represented. These processes were associated with genes located within 10,000 bp of outlier SNPs (222 SNPs based on pcadapt, K=1). Outliers are those that differentiate sites along PC 1, which separated sites in DU 10 from sites in DU 8 (with revised boundaries). Higher level processes overrepresented in the analysis are indicated by different colour squares in the REVIGO treemap.



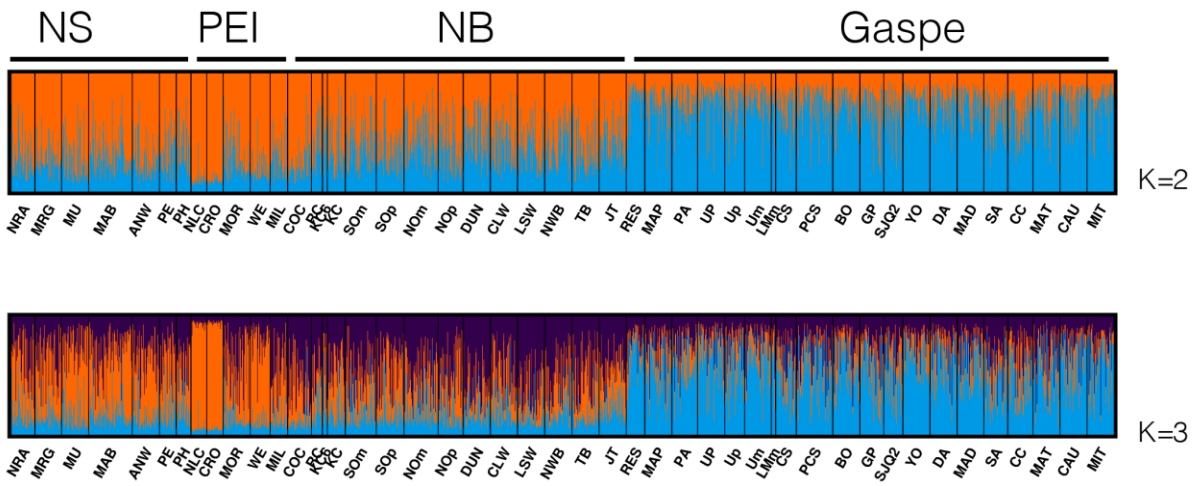
Appendix Figure A30. Redundancy analysis (RDA) using bioclimatic data for all rivers in DU 8 and DU 10 as the response and putative DU groups (two genetic clusters) as the constraining variable. Centroids of DU groups are indicated by text, with point representing each river. The proposed rivers to be moved into DU 10 are indicated by orange, and include three rivers: Betsiamites, Laval, and Escoumins. ANOVA on RDA showed the model to be significant (p <0.001) with an adjusted R^2 of 0.22. RDA axis 1 explained 24.3% of the variance explained by the model. The RDA plot clearly shows support for the splitting the DUs based on the new boundaries.



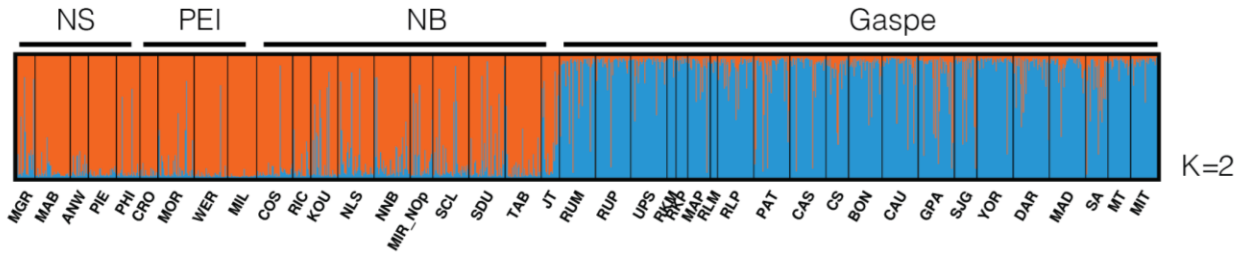
Appendix Figure A31. Results from STRUCTURE for DU 9 (Anticosti) using the microsatellite dataset showing genetic clusters K=2. We tested values of K ranging from 1 to 3, and best K in STRUCTURE was 2, although no clear structure could be observed in DU 9.



Appendix Figure A32. Results from STRUCTURE for DU 9 (Anticosti) using the 96 SNP baseline with genetic clusters K=2. Best K in STRUCTURE was 2. We tested values of K ranging from 1 to 3. No genetic structure was observed in DU 9.

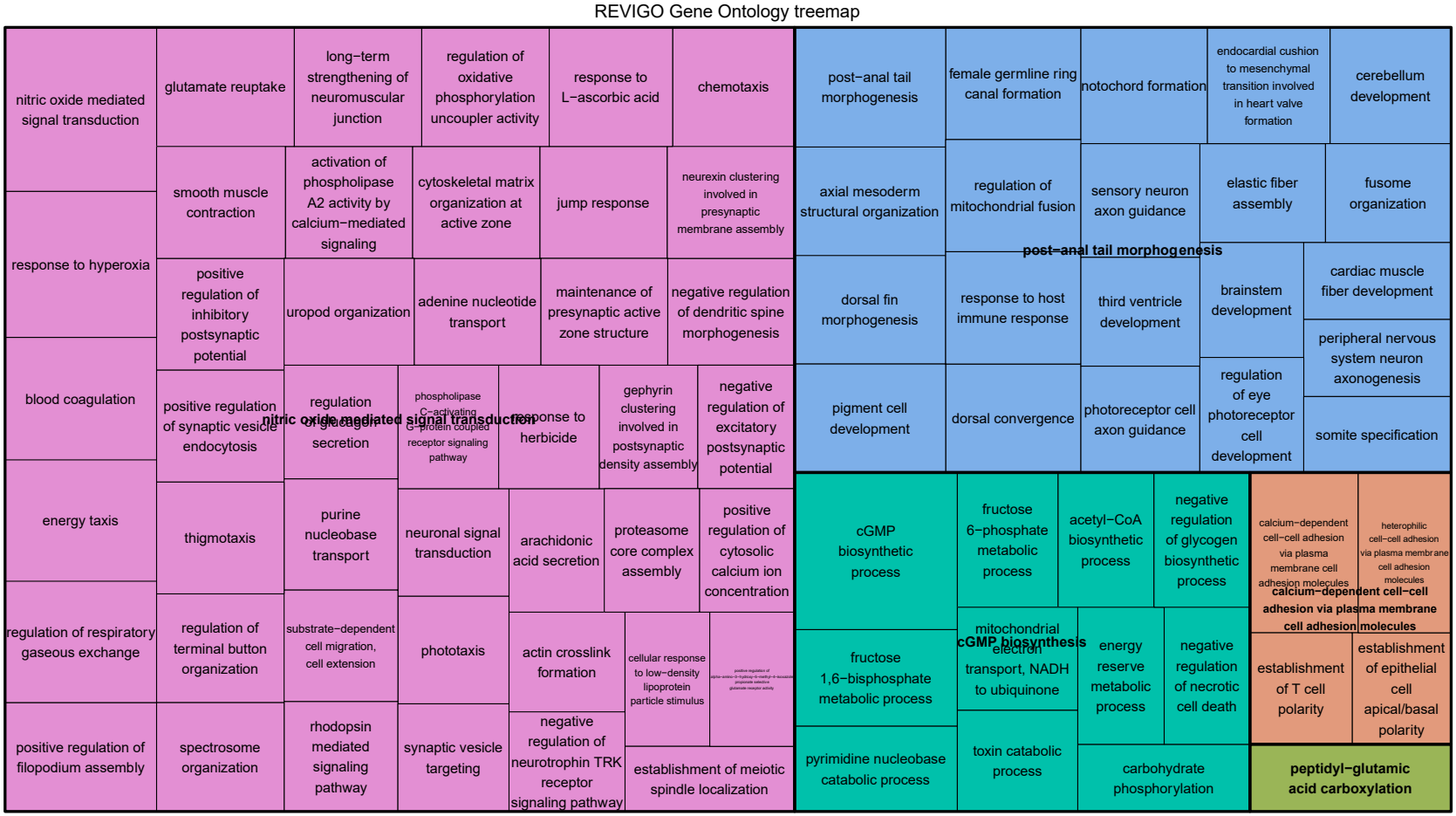


Appendix Figure A33. Results from STRUCTURE for DU 12 using the microsatellite dataset showing genetic clusters K=2 and K=3. Gaspé sites are separated from other sites in DU 12. Best K in STRUCTURE was 2, and little additional structuring was observed at K=3. We tested values of K ranging from 1 to 10.

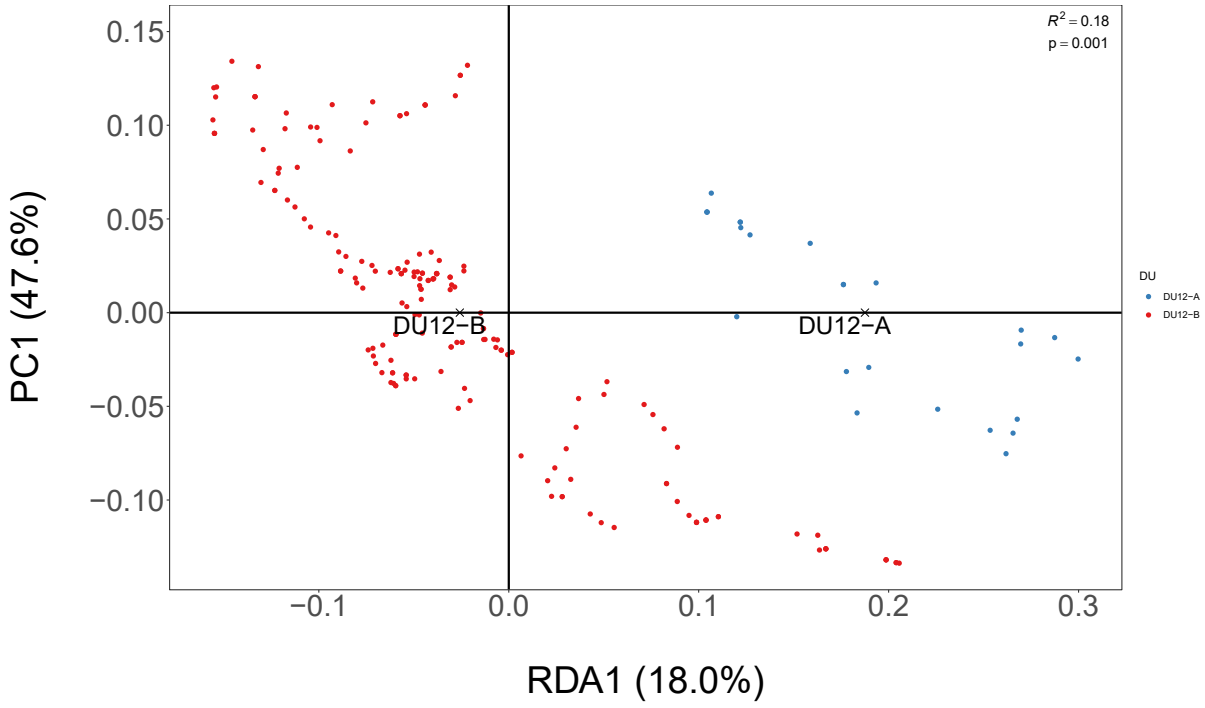


Appendix Figure A34. Results from STRUCTURE for DU 12 using the 96 SNP baseline with genetic clusters K=2. Gaspé sites are separated from other sites in DU 12. Best K in STRUCTURE was 2, and no additional structuring was observed beyond K=2. We tested values of K ranging from 1 to 10.

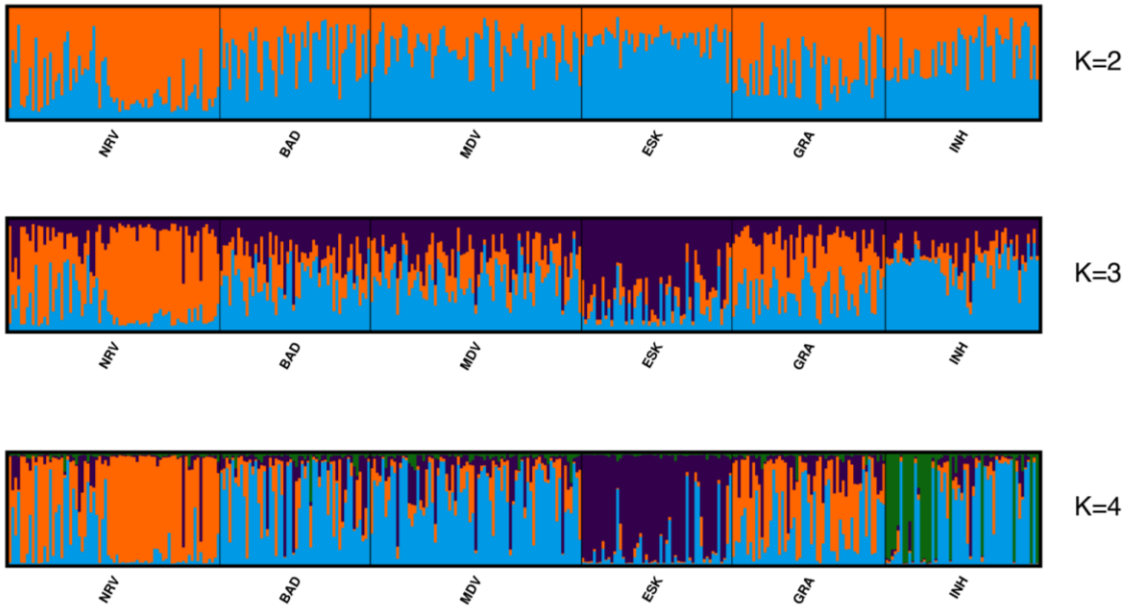
Appendix Figure A35. (A) Pcadapt clearly separates Gaspé from all southward locations in DU 12 along the first principal component (PC) axis using genome-wide SNPs (n=29,695 – combined whole genome resequencing and 220K). One population in PEI (NEP – Northeast Complex) was separated from other sites along PC axis 2. Names of each site are provided to show the mean location of data points on PC 1 and PC 2. (B) A total of 44 loci significantly contributed to the differentiation on PC axis 1 (K=1; adjusted p-value [q-value] <0.05) and these loci were distributed across 9 chromosomes (out of 29).



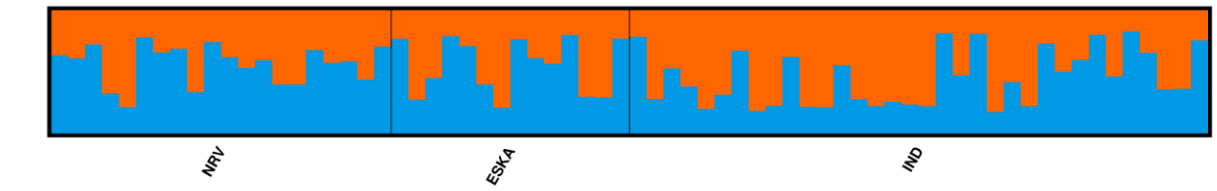
Appendix Figure A36. Results of gene ontology analysis based on biological processes that were significantly overrepresented in the outlier data. A total of 100 processes were over-represented. These processes were associated with genes located within 10,000 bp of outlier SNPs (44 SNPs based on K=1 in pcadapt). Outliers are those that differentiate Gaspé from other sites in DU 12. Higher level processes overrepresented in the analysis are indicated by different colour squares in the REVIGO treemap.



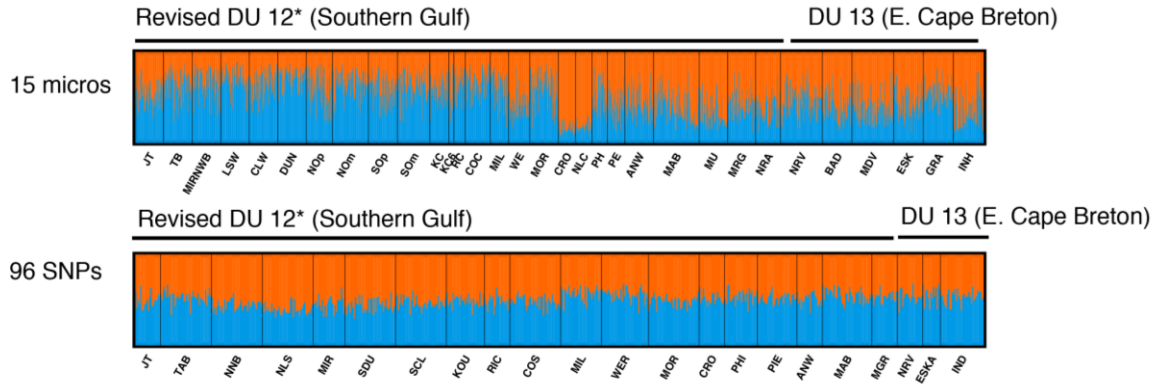
Appendix Figure A37. Redundancy analysis (RDA) using bioclimatic data for all rivers in DU 12 as the response and putative DU groups (two genetic clusters) as the constraining variable. The two putative new DUs include: Gaspé (DU2-A; blue) and southern Gulf (DU2-B; red). Centroids of DU groups are indicated by text, with point representing each river. ANOVA on RDA showed the model to be significant ($p < 0.001$) with an adjusted R^2 of 0.18. RDA axis 1 explained 18.0% of the variance explained by the model, and clearly separated the two putative DUs.



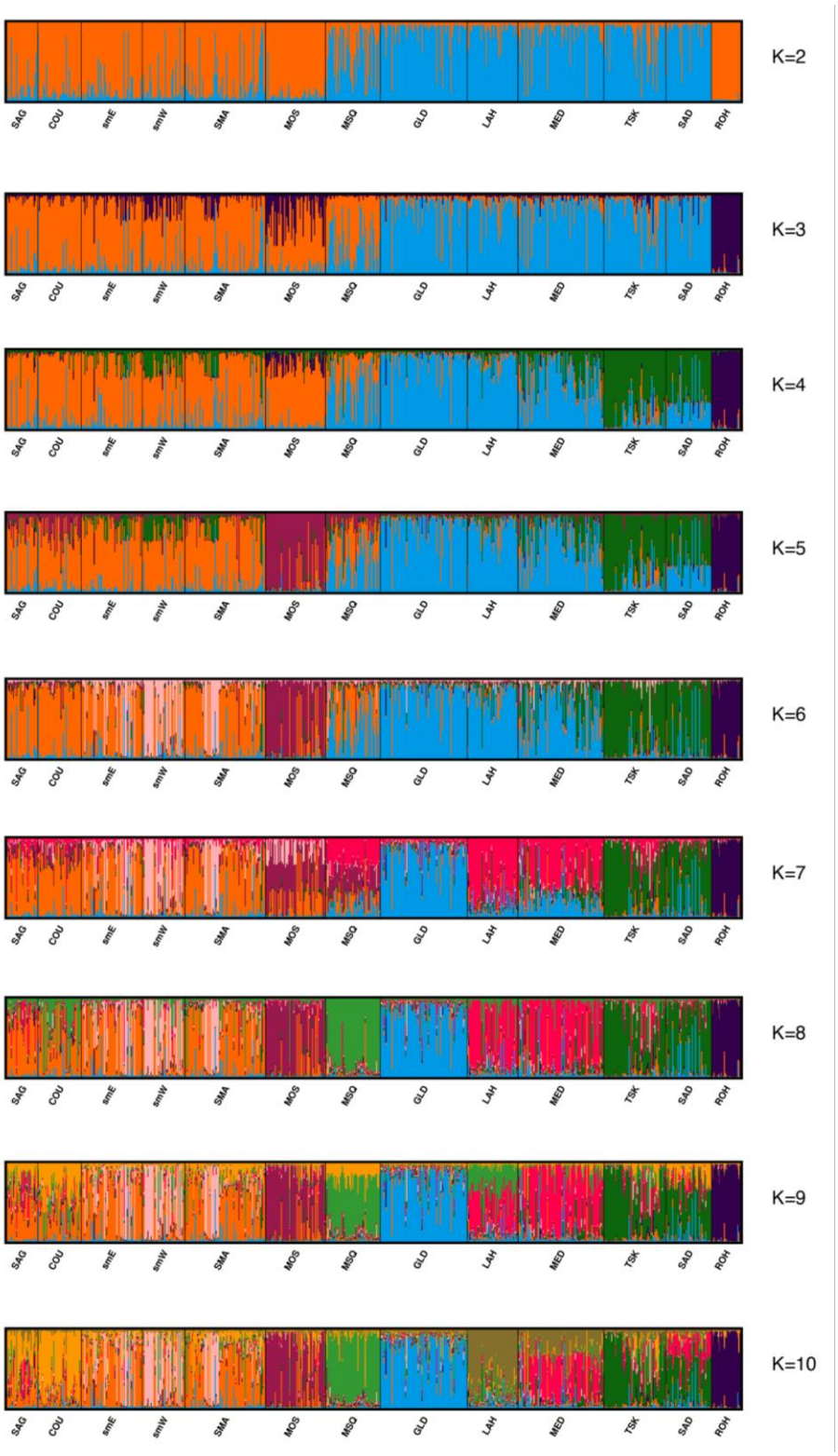
Appendix Figure A38. Results from STRUCTURE for DU 13 using the microsatellite dataset showing genetic clusters K=2 and K=4. We tested values of K ranging from 1 to 6, and best K in STRUCTURE was 4. At K=4, ESK formed its own cluster. Most other sites were not clearly differentiated into separate clusters. Although some substructure appeared to be present within INH and NRV populations.



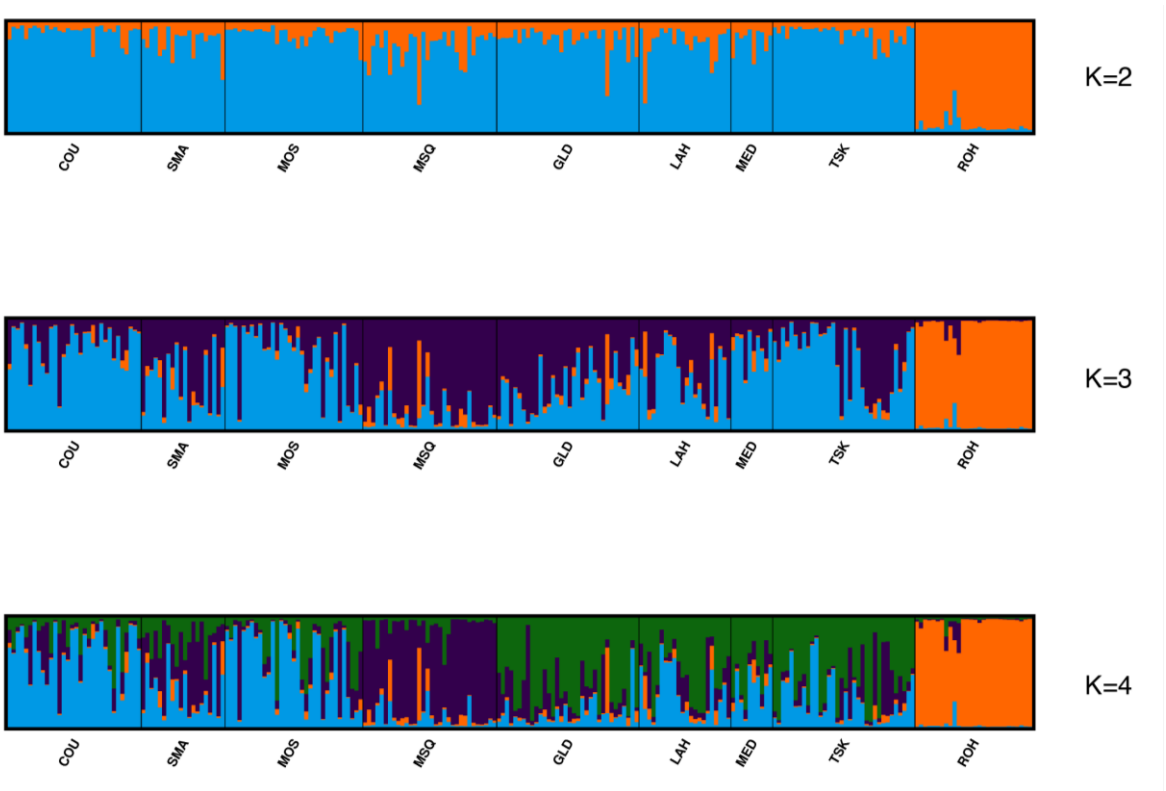
Appendix Figure A39. Results from STRUCTURE for DU 13 using the 96 SNP baseline with genetic clusters K=2. Best K in STRUCTURE was 2. We tested values of K ranging from 1 to 3. No genetic structure was observed.



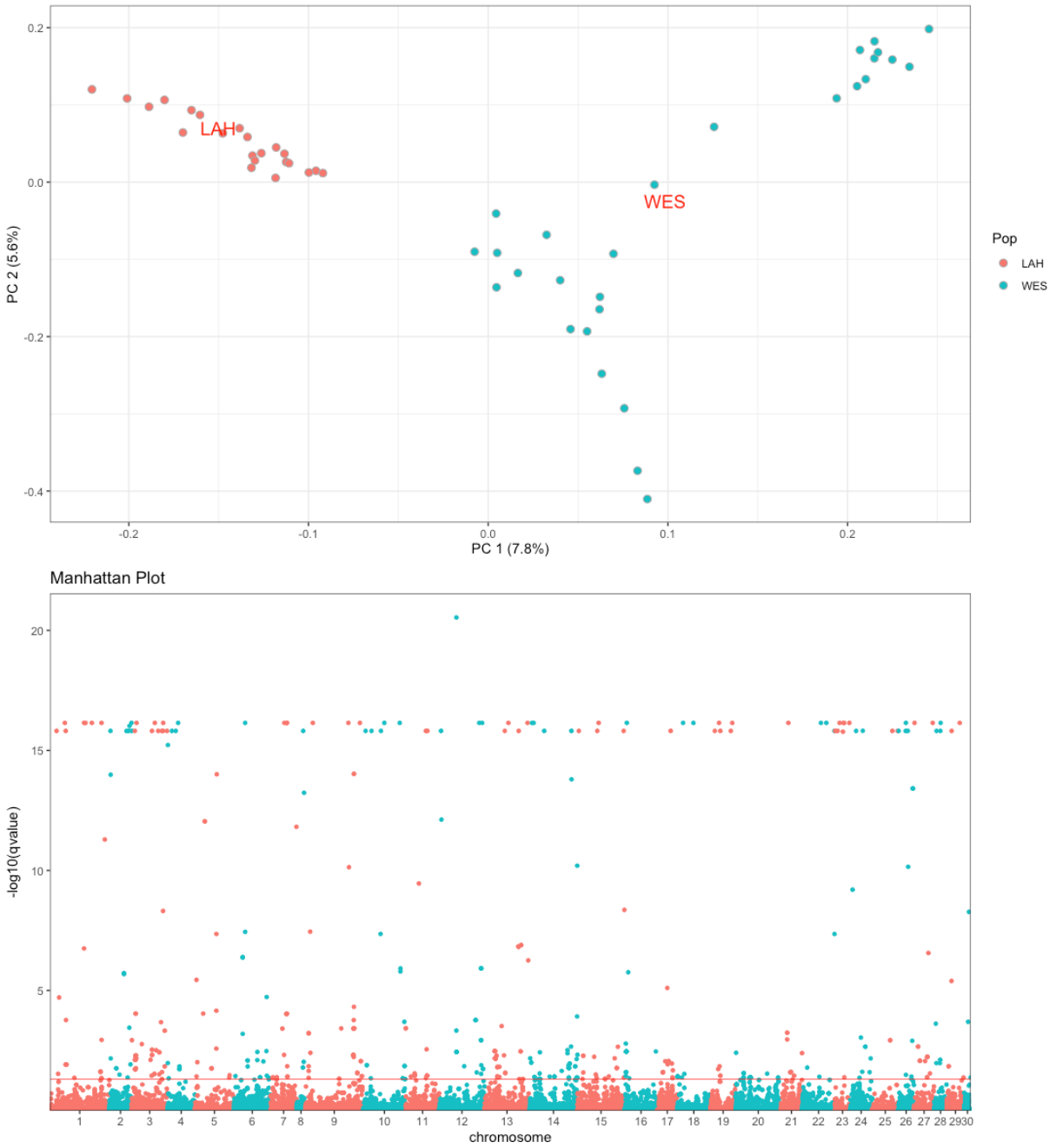
Appendix Figure A40. Results from STRUCTURE for sites in DU 12 and DU 13 using (A) microsatellite dataset and (B) 96 SNPs dataset for two genetic clusters (K=2). No genetic structure was present in either dataset, suggesting evidence for discreteness is not met. Note that sites in DU 12 include only those in the southern Gulf region based on revisions to this DU.



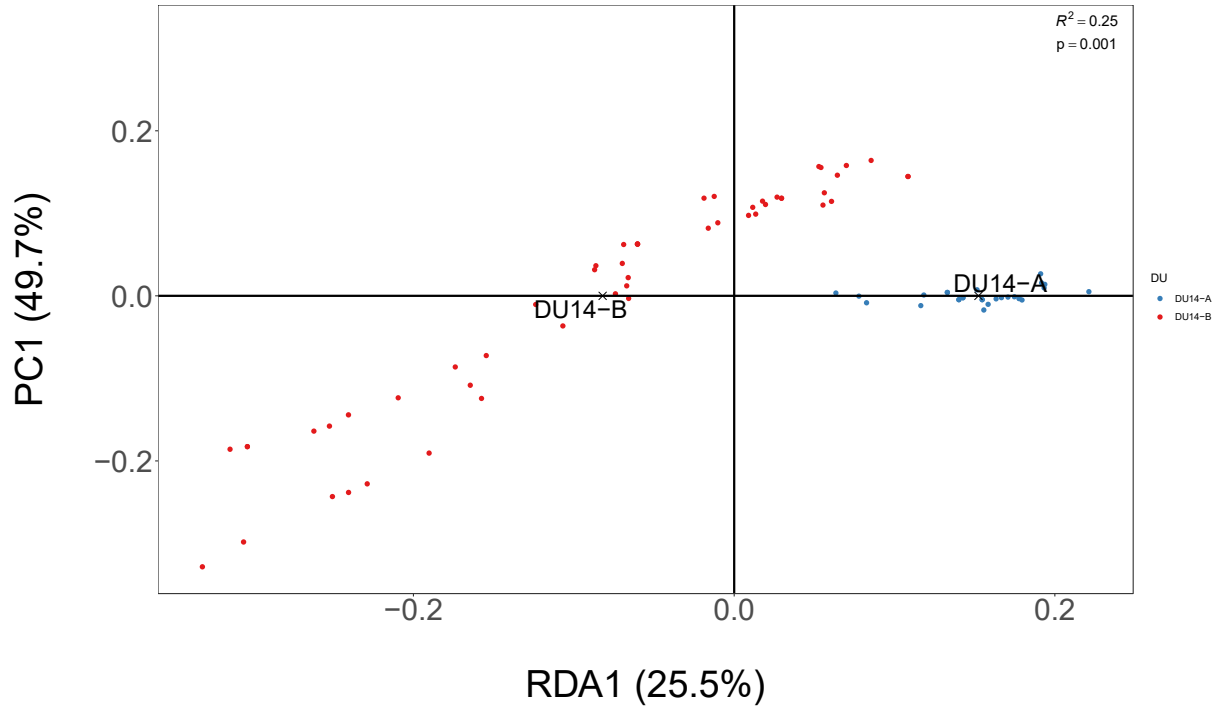
Appendix Figure A41. Results from STRUCTURE for DU 14 using the microsatellite dataset showing genetic clusters K=2 and K=10. We tested values of K ranging from 1 to 13, and best K in STRUCTURE was 2, although additional structure was observed beyond K=2. At lower values of K (K=3), sites were separated into clustered based on geography (west and east) near Musquodoboit (MSQ), with ROH forming its own cluster. Higher values of K separated many sites into their own clusters.



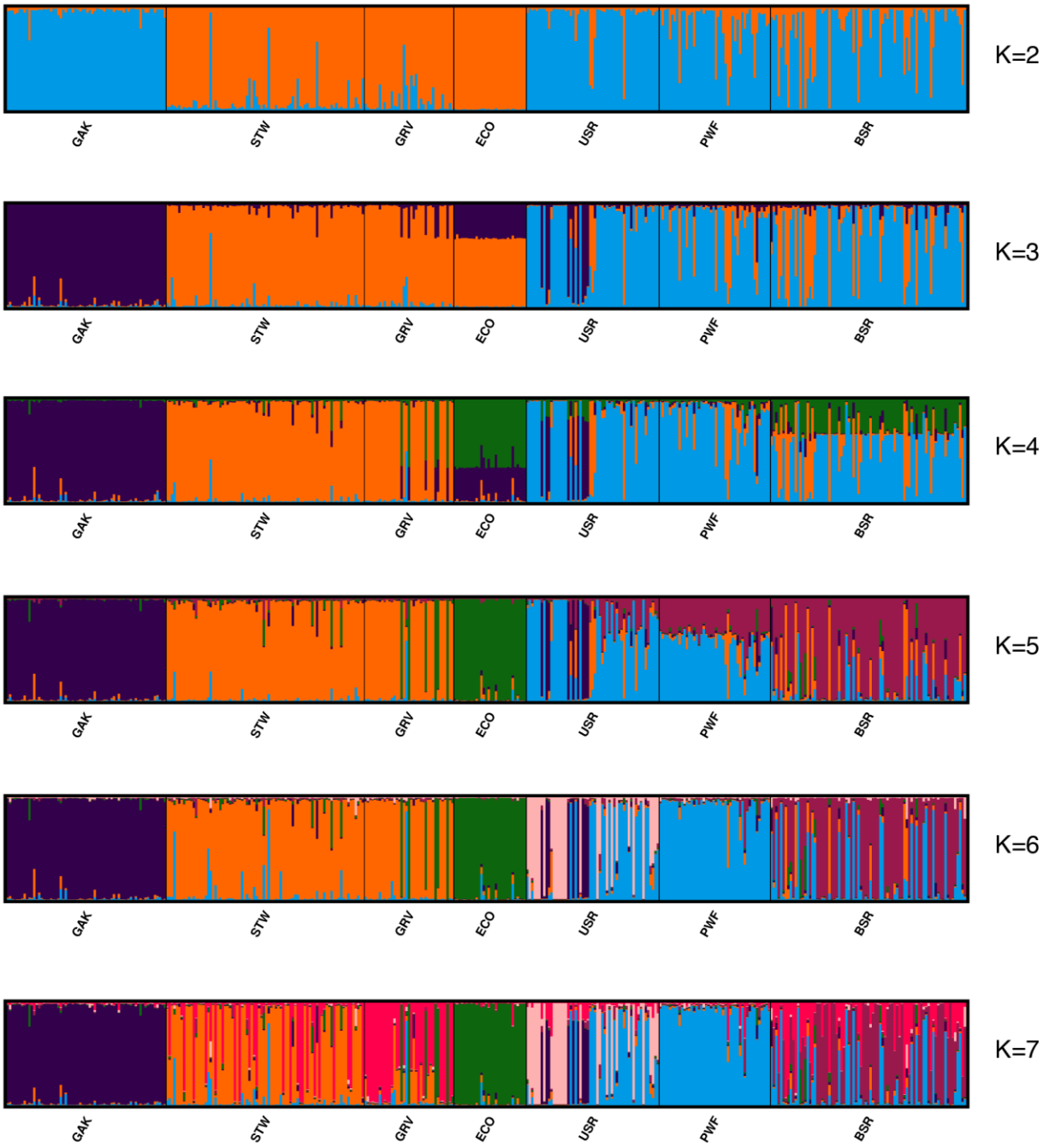
Appendix Figure A42. Results from STRUCTURE for DU 14 using the 96 SNP baseline with genetic clusters K=2 to 4. Best K in STRUCTURE was 3, but some additional structuring was observed at K=4. We tested values of K ranging from 1 to 9. ROH clustered separately from other sites. Some additional clustering was observed based on geography, where sites east and west of Musquodoboit (MSQ) showed greater membership to different clusters. MSQ also clustered separately from other sites by K=4. Nonetheless, we note that clustering patterns were not clearly distinct, except for ROH.



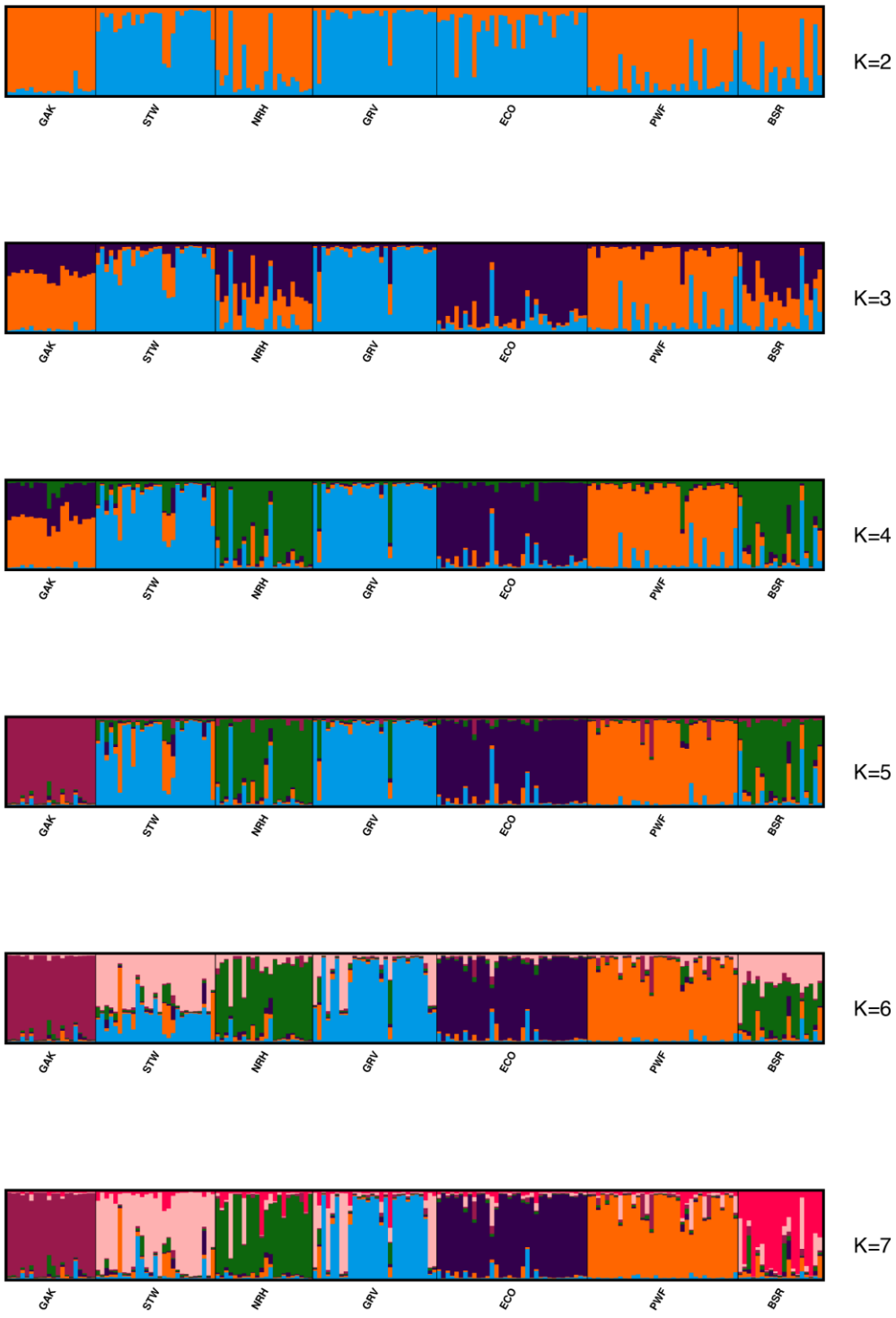
Appendix Figure A43. Upper panel: Pcadapt separates West River – Sheet Harbour (WES; blue points) in the east (DU14A) from Lahave River (LAH; red points) in the west (DU14B) along the first and second principal component (PC) axis using genome-wide SNPs (n=52,776). Names of each site are provided to show the mean location of data points on PC 1 and PC 2. Lower panel: A total of 593 loci significantly contributed to the differentiation on PC axis 1 and 2 (K=2; adjusted p-value [q-value] <0.05) and these loci were distributed across all chromosomes.



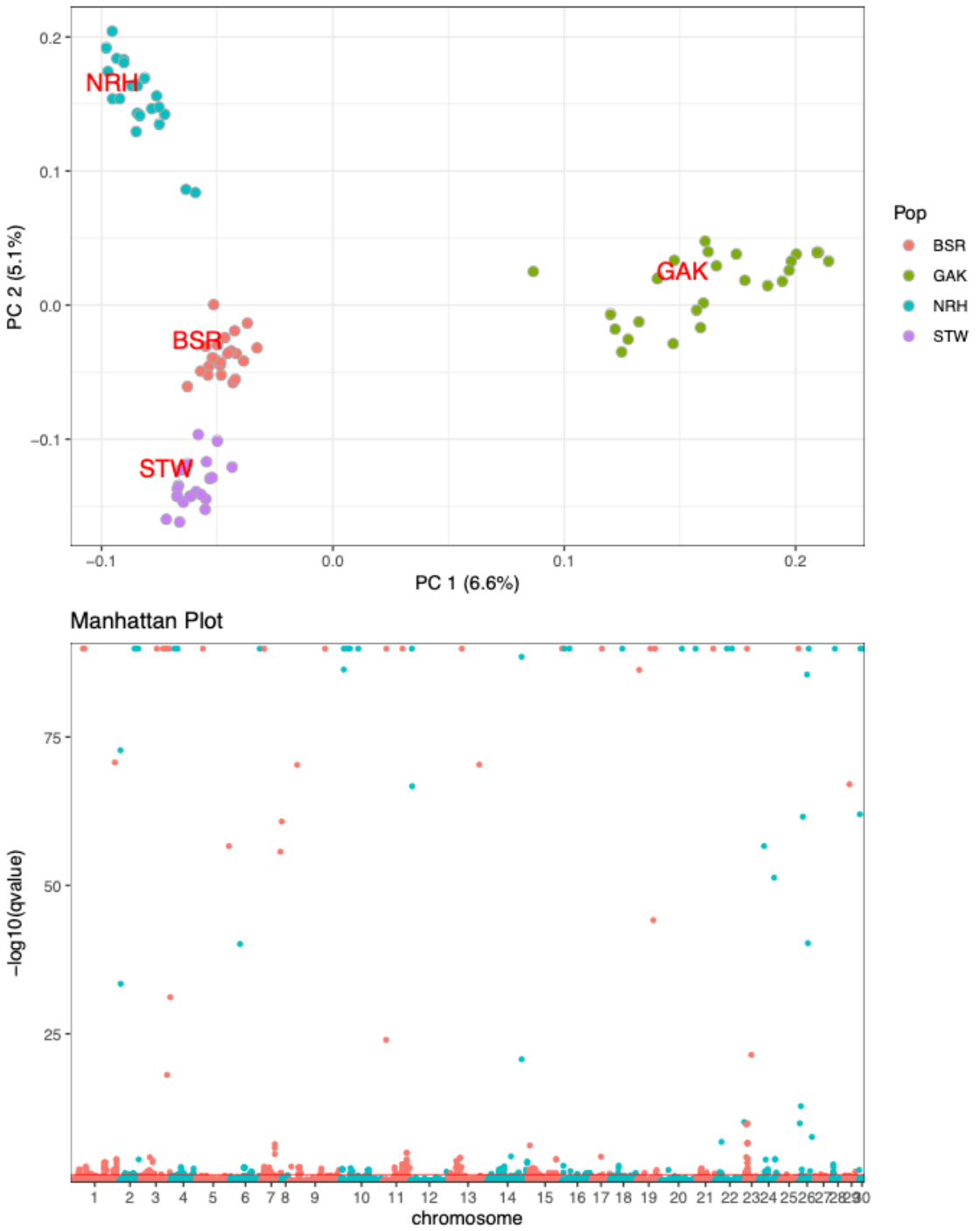
Appendix Figure A44. Redundancy analysis (RDA) using bioclimatic data for all rivers in DU 14 as the response and putative DU groups (two genetic clusters) as the constraining variable. The two putative new DUs include: sites east of Musquodoboit (DU14-A; blue) and sites west of Musquodoboit (inclusive) (DU14-B; red). Centroids of DU groups are indicated by text, with point representing each river. ANOVA on RDA showed the model to be significant ($p < 0.001$) with an adjusted R^2 of 0.25. RDA axis 1 explained 25.5% of the variance explained by the model, and clearly separated the two putative DUs.



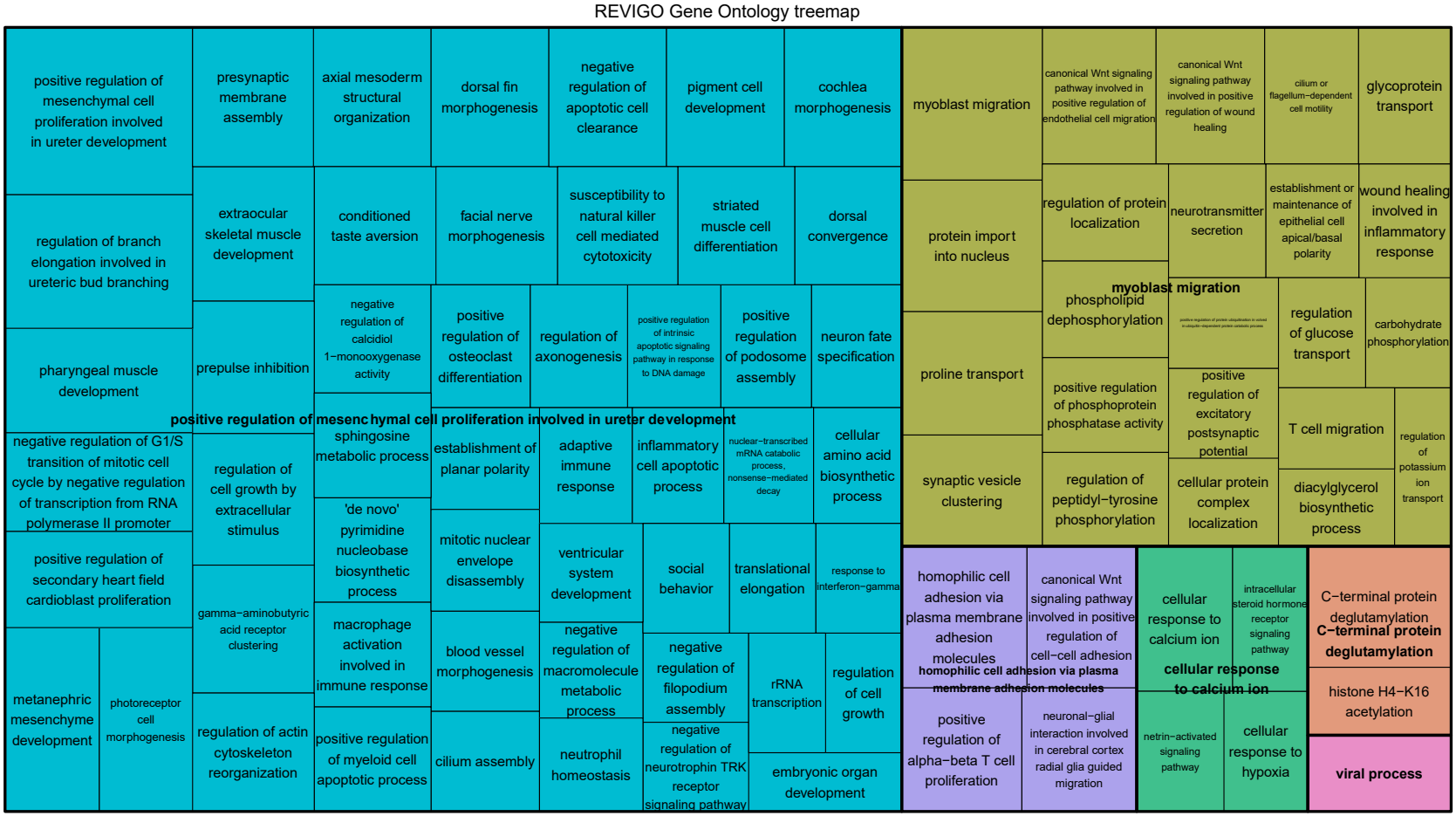
Appendix Figure A45. Results from STRUCTURE for DU 15 using the microsatellite dataset showing genetic clusters K 2 to 7. We tested values of K ranging from 1 to 7, and best K in STRUCTURE was 6. At K=2, sites in Chignecto Bay (USR, PWF, BSR) were differentiated from sites in Minas Basin (STW, GRV, ECO), with the exception of GAK (grouped with Chignecto Bay) which represented its own distinct cluster at K=3. At K=7, STRUCTURE separated many populations into separate clusters.



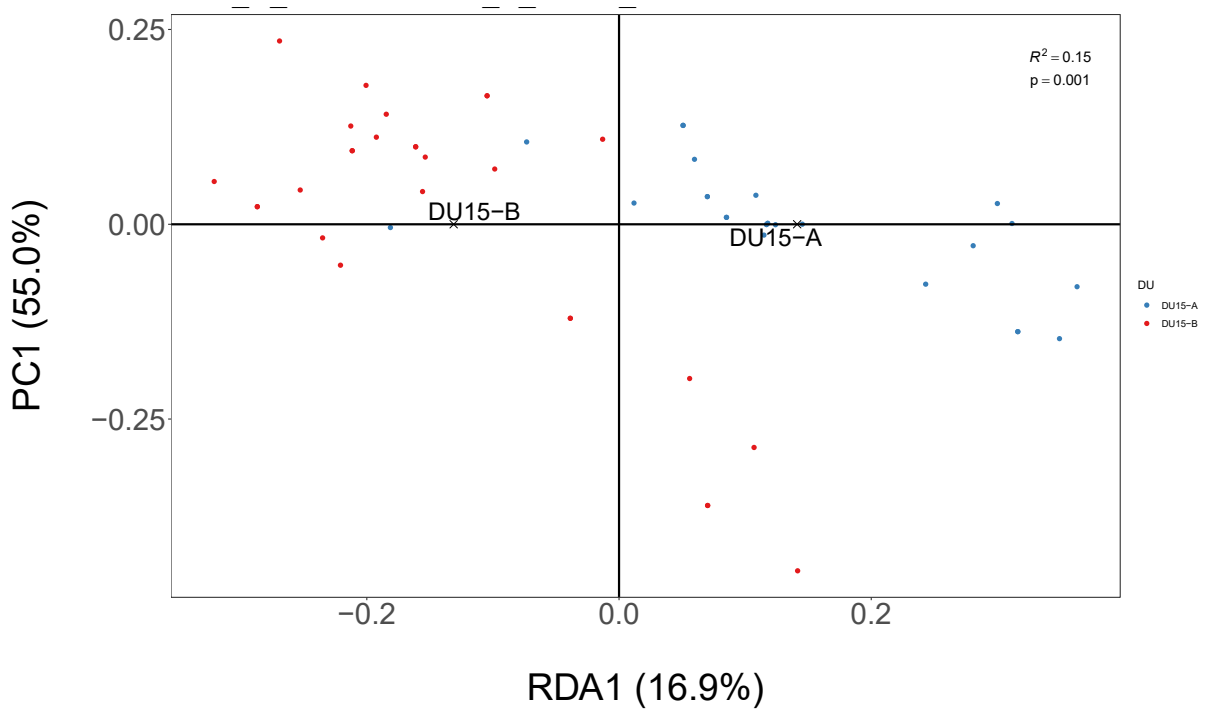
Appendix Figure A46. Results from STRUCTURE for DU 15 using the 96 SNP baseline with genetic clusters K=2 to K=7. Best K in STRUCTURE was 2, but additional structuring was observed beyond K=2. We tested values of K ranging from 1 to 7. At K=2, some differentiation was observed between sites in Chignecto Bay (PWF, BSR) and Minas Basin (STW, GRV, ECO), although other sites in Minas Basin (GAK, NRH) grouped with Chignecto Bay sites. At K=7, sites could be mostly divided into distinct clusters.



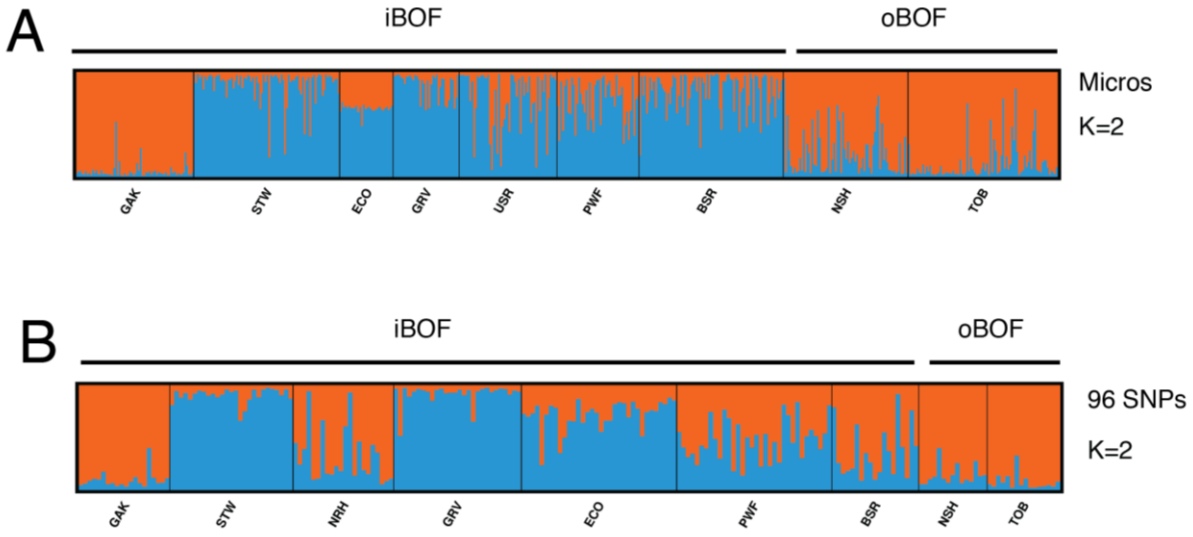
Appendix Figure A47. (A) In DU 15 using 63,509 SNPs (MAF>0.05), Pcadapt separated Gaspereau River (GAK) from other sites along the first PC axis. All other sites were separated on PC axis 2. (B) A total of 441 loci significantly contributed to the differentiation on both PC axes (adjusted p-value or q-value <0.05) and these loci were distributed across 28 chromosomes (out of 29). Outlier loci are indicated by those above the red line.



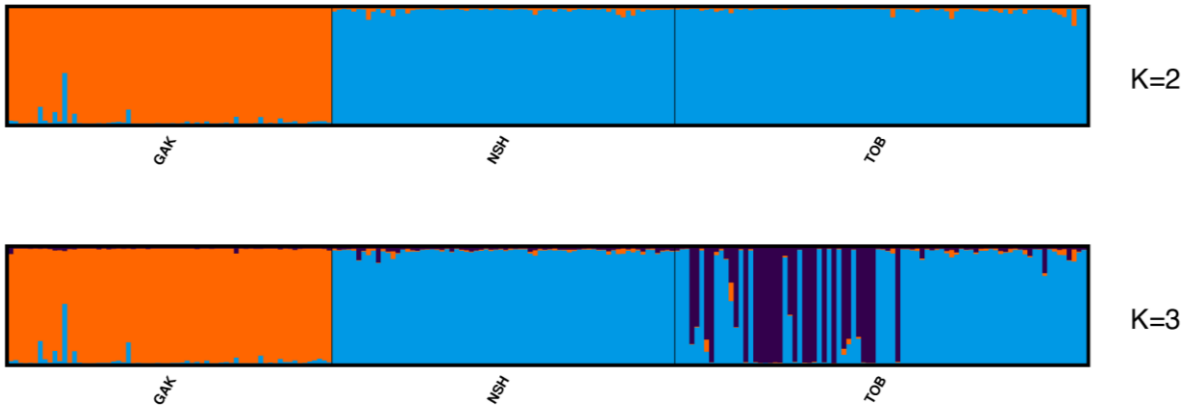
Appendix Figure A48. REVIGO treemap for DU 15 based on genes near outliers from pcadapt. Each cell represents a biological process based on the gene ontology and cells are joined into "superclusters" based on similarly related terms (same colours). The size of the cell represents the p-value of the GO term in the analysis. The most significant GO term in each supercluster is indicated in the center.



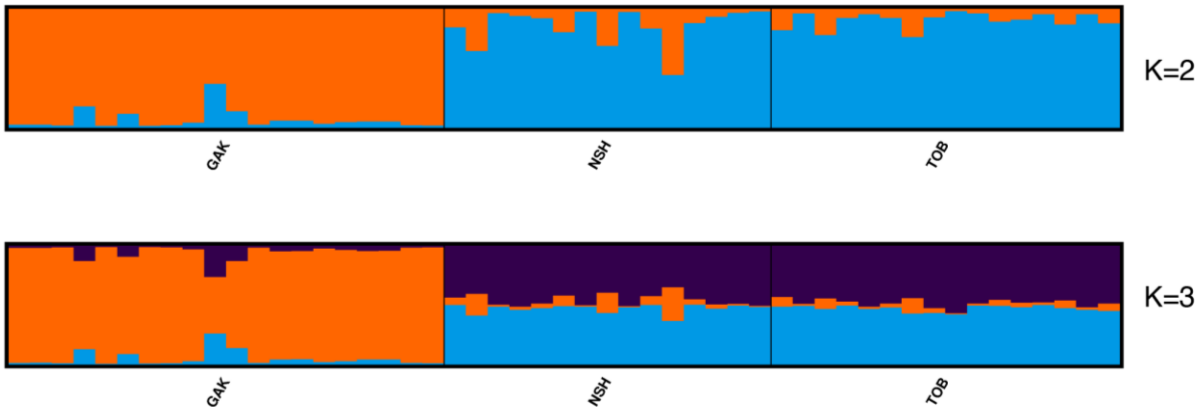
Appendix Figure A49. Redundancy analysis (RDA) using bioclimatic data for all rivers in DU 15 as the response and putative DU groups (two genetic clusters) as the constraining variable. The two putative DUs include: one covering Minas Basin from Cornwallis to Fox (DU15-A), and one covering Chignecto Bay from Apple to Mispec (DU15-B). Centroids of DU groups are indicated by text, with point representing each river. ANOVA on RDA showed the model to be significant (p = 0.001) with an adjusted R^2 of 0.15. RDA axis 1 explained 16.9% of the variance explained by the model, and clearly separated the two putative DUs.



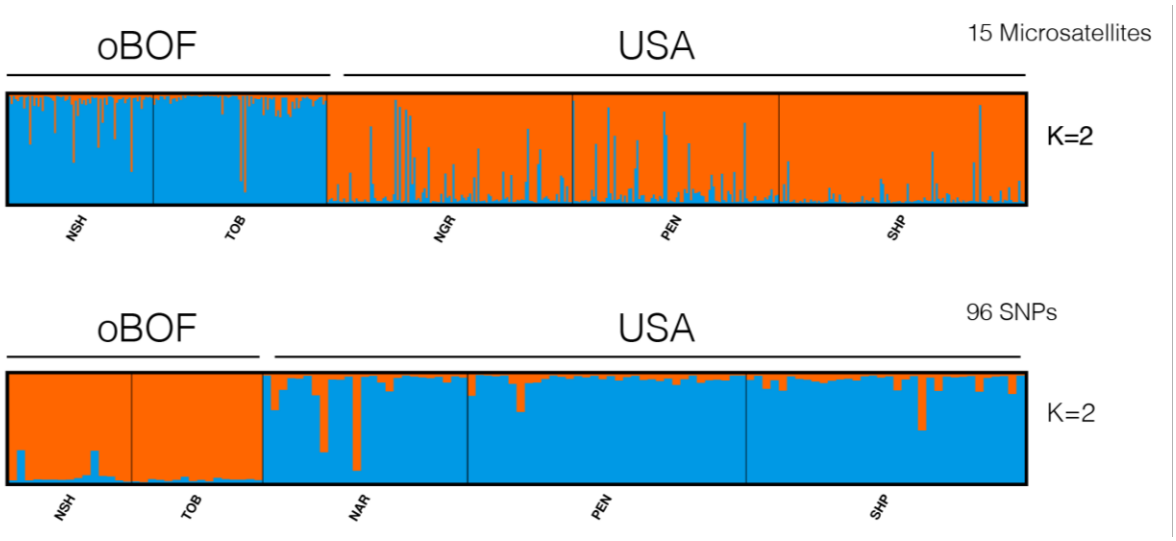
Appendix Figure A50. Results from STRUCTURE for sites in DU 16 (outer Bay of Fundy; oBoF) and DU 15 (inner Bay of Fundy; iBoF) using (A) microsatellite dataset and (B) 96 SNPs dataset for two genetic clusters (K=2). Microsatellites dataset showed that Gaspereau clustered with sites in the oBoF. Similarly, Gaspereau grouped with the oBoF in the 96 SNP data, although genetic differences between all iBoF and all oBoF was not as clear as in microsatellites.



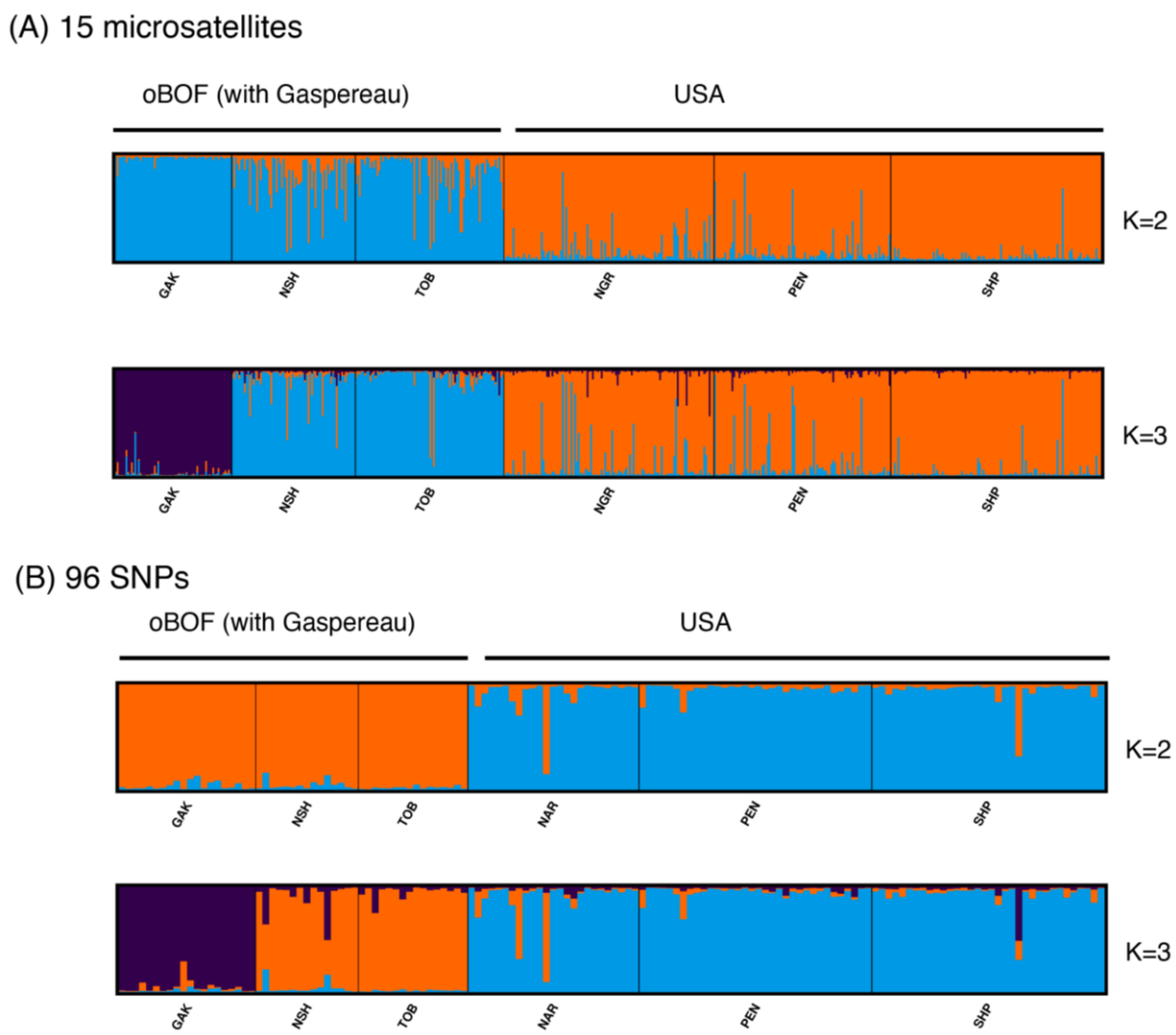
Appendix Figure A51 Results from STRUCTURE for DU 16 (including Gaspereau which was moved into DU 16) using the microsatellite dataset showing genetic clusters K=2 and K=3. We tested values of K ranging from 1 to 3, and best K in STRUCTURE was 2. Gapsereau clustered on its own and separate from NSH and TOB. At K=3, TOB and NSH still clustered together, except with some substructure in TOB.



Appendix Figure A52. Results from STRUCTURE for DU16 using the 96 SNP baseline with genetic clusters K=2. Best K in STRUCTURE was 2. We tested values of K ranging from 1 to 3. No genetic structure was observed in DU 16.



Appendix Figure A53. Results from STRUCTURE for outer Bay of Fundy (oBoF; DU 16) and USA populations in Maine using the 15 microsatellite and 96 SNP datasets with genetic clusters K=2. We tested values of K ranging from 1 to 5. Clear differences between oBoF and USA were detected. Some additional structure was detected beyond K=2 in the microsatellite dataset but not the 96 SNP dataset.



Appendix Figure A54. Results from STRUCTURE for outer Bay of Fundy (oBoF; DU 16) with Gaspereau included and USA populations in Maine using the (A) 15 microsatellite and (B) 96 SNP datasets with genetic clusters K=2 and K=3. We tested values of K ranging from 1 to 6. Clear differences between oBoF and USA were detected at K=2.