

The influence of post-glacial recolonization and
contemporary stream network on the evolution of genetic
diversity within species: an examination of microsatellite
DNA variation in rainbow trout (*Oncorhynchus mykiss*)

by

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Abstract

Understanding the relative influence of historical and contemporary factors shaping patterns of genetic diversity is a major challenge in population and conservation biology. Patterns of genetic diversity were examined in rainbow trout throughout British Columbia to address the roles of historical isolation, postglacial dispersal, and local contemporary geomorphic features in structuring patterns of genetic variation and differentiation observed in nature.

Microsatellite DNA was useful in detecting patterns of historical isolation and post glacial recolonization, showing clines of genetic variation as populations were studied in an orderly manner moving away from the putative refugia. Patterns of isolation by distance were observed among drainages closest to each refuge and were absent among populations at the periphery of the species' range suggesting the existence of clines in migration drift equilibrium. Clines in genetic variation and isolation by distance were not observed in coastal populations and may result from ongoing gene flow which would prevent the detection of such trends.

On a broad geographic scale, rainbow trout populations were structured into major regions and further structured within each major region into smaller watersheds and drainages based on hydrological topography. The influences of elevation, number of connections between streams, fluvial distance, migration

barriers and stream/lake order were important in shaping observed patterns of genetic diversity among rainbow trout populations. Anadromous and fluvial populations generally displayed higher levels of genetic variation and lower levels of differentiation than lacustrine populations. Generally, within drainage, high levels of dispersal and gene flow were observed between geographically proximate and contiguous lakes. Stream/lake order was a better predictor of genetic variation than lake surface area and perimeter. Although founder events and postglacial dispersal likely played large roles in determining the broad scale patterns of genetic diversity in rainbow trout, the results suggest that contemporary factors can strongly modulate historical patterns. To properly plan for the conservation of such species, it is necessary to understand the nested nature of variation exhibited by rainbow trout populations and how important stream hydrology is because it demonstrates the importance of dispersal corridors in much the same way as land formations do for terrestrial vertebrates.

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CHAPTER 1: General introduction

Documenting how geographic variation in species richness, phenotype, behaviour and genetic diversity are influenced by the environment has a long history in studies of ecology and evolution. Such information allows us to understand how organisms survive, adapt, and ultimately evolve. From an evolutionary perspective, emphasis has been placed on understanding the forces that help shape genetic diversity, because the evolutionary potential and fitness attributes of a species may be defined, in part, in terms of its genetic diversity (Frankel and Soule 1981).

Genetic diversity (genetic variation within and differentiation among populations) within species often reflects processes that occurred during historical environmental changes (Avice 1994 and references therein; Hewitt 1996; Lees et al. 1996; Bernatchez and Wilson 1998; Avice 2000). The ability of species to respond and survive during future changes (evolutionary potential) likely depends on the availability of this same genetic diversity (Carvalho 1993; Avice and Hamrick 1996; Bouzat et al. 1998). In the United States (US) and Canada, genetic diversity is recognized as an important aspect of conservation biology and management of fish resources (Ryman and Utter 1987; COSEWIC 2005). Descriptive studies of molecular genetic diversity within and among populations in threatened species have come to dominate much of the conservation genetic

literature (Lande and Barrowclough 1987; Lacy 1992; Avise and Hamrick 1996; Caughley and Gunn 1996; Moran 2002).

In species composed of mobile individuals, environmental forces such as barriers to dispersal imposed by landscape features are particularly important in determining the different levels of gene flow (exchange of genetic material between demes) and drift (random loss of genetic variation) and, consequently, the genetic diversity and structure of populations. A major challenge in population and conservation biology is not only to identify such environmental forces, but also to predict their relative influence on shaping intraspecific genetic diversity (Wright 1931, 1978; Frankel and Soule 1981; Sork et al. 1999).

Large geographic scale: Pleistocene Glaciations

Pleistocene glaciations are the most significant historical events to have occurred during the evolutionary life span of most Holarctic species. An estimated 20 glacial events, (glacial advances, stabilization and retreat) (Martinson et al. 1987 referenced in Bernatchez and Wilson 1998) each spanning ~100 000 years with interglacial periods lasting 10 000-12 000 years, occurred during the Pleistocene (Dawson 1992). These continental scale events buried large areas of the world's landmasses repeatedly with sheets of ice resulting in advances and retreats of range limits, and extinctions of species, populations, and their associated genotypes (Pielou 1991; Jansson and Dynesius 2002).

Aquatic species were particularly affected as opportunities for dispersal were limited to direct water connections at the leading edges of the advancing ice. Their habitats were also altered through both the destruction of old environments and the creation of new lakes and rivers. As the glaciers retreated, the melt-water formed large proglacial lakes which facilitated the dispersal of freshwater species. For aquatic species able to gain access to them, the proglacial lakes provided opportunities for dispersal over large geographical areas (McPhail and Lindsey 1986). The North American ice sheets were particularly large, exceeding the combined coverage of the European and Asian glaciers (Dawson 1992) and the last glaciation event occurred between 75 000 and 12 000 years ago (McPhail and Lindsey 1970, 1986; Hewitt 1996; Ibrahim et al. 1996).

Undoubtedly, the repeated expansion and contraction of populations, isolation in glacial refugia, and changes in dispersal routes influenced interspecific patterns of genetic diversity among aquatic organisms in North America (Hewitt 1996; Bernatchez and Wilson 1998).

Small geographic scale: Geomorphic Features

On a local scale, aquatic distribution was not only influenced by proglacial lakes and rivers, but also by the surrounding geography. The formation of natural barriers, isostatic rebound of land masses following glacial retreat, the connection and separation of waterways, and changes in water levels have likely influenced not only faunal distribution, but also genetic diversity of North American species (McPhail and Lindsey 1986, Hewitt 1996, Bernatchez and

Wilson 1998). Even today, local geomorphic features such as mountain ranges, valleys, and forests have been found to limit or promote the dispersal of aquatic organisms (Shaw et al. 1991; Rogers and Curry 2004). Smaller geographic features such as waterfalls (Knudsen et al. 2002), drainage pattern (Hansen and Mansberg, 1998), elevation, stream size (Shaw et al. 1994), habitat size (Castric et al. 2001; Heath et al. 2001), and geographic distance (Saitoh et al. 2001) have also been known to influence dispersal among populations and, consequently, patterns of genetic diversity within species (i.e. Shaw et al. 1994; Angers et al. 1995, 1999; Avise and Felley 1997; Carlsson and Nilsson 2000; Castric et al. 2001; Michels et al. 2001; Costello et al. 2003; Taylor et al. 2003).

Molecular genetics and geography

Historically, discriminating populations was based on information from morphological characteristics; however, the causes of such differentiation remain obscure as most morphological traits have a polygenic basis and are heavily influenced by environmental factors (Allendorf et al. 1987). With the advent of molecular markers, inferences about dispersal, numbers and locations of glacial refugia, and patterns of genetic variation and differentiation among natural populations have been examined more powerfully (Avise 1994). The mechanisms of inheritance of molecular markers such as mitochondrial DNA (mtDNA) and microsatellite DNA are, for the most part, clearly understood (Avise 1994; Jarne and Lagoda 1996; Estoup and Angers 1998). In particular, allele frequency variation at microsatellite DNA loci has provided biologists with a

powerful tool for the genetic characterization of populations and has helped to revolutionize conservation and evolutionary genetics (Avice 1994; Jarne and Lagoda 1996; Estoup and Angers 1998; Parker et al. 1998).

Much work investigating the influence of large-scale environmental forces (glaciation and post glacial recolonization) on patterns of genetic diversity has been conducted among a wide array of organisms (e.g. Taberlet 1998; Bernatchez and Wilson 1998; Bos and Sites 2001; Beheregaray et al. 2003; Stamford and Taylor 2004). The investigation of the influence of local geomorphic features on population genetic structure is, however, still in its infancy (Angers et al. 1999; Castric et al. 2001; Costello et al. 2003). Even fewer studies have simultaneously compared the influences of historical geographic changes and local contemporary geographic features in shaping the current patterns of intraspecific genetic diversity (e.g. Castric et al. 2001; Costello et al. 2003). Because genetic differences among populations can result both from ongoing processes and deeper historical divergence, the most powerful analysis of geographic variation should include a phylogenetic perspective to differentiate between the effects of present and historical events (Templeton et al. 1990).

The combination of molecular genetics and geography would permit the investigation of the relative influence of large and local scale environmental forces on extant genetic variation and differentiation. This has implications for

questions related to evolutionary change and has become an important consideration in terms of conservation and management.

The salmonids (Salmonidae; salmon, trout and char) are among the most intensively studied fishes because of their great commercial, recreational and cultural importance. Their dependence upon waterways for dispersal, the diversity of habitats they occupy, variation in timing of spawning, behaviour, life histories, morphology, and habitat specificity (Groot and Margolis 1991; Taylor 1991; Moran et al. 1995; Small et al. 1998) make these groups of fishes interesting to study in terms of population structure. Factors that promote reproductive isolation among local populations, such as homing and the hierarchical presence of geomorphic features (e.g. stream networks, lakes, geographic barriers), minimize the potential for gene flow to constrain genetic changes caused by genetic drift and natural selection (Meffe and Vrijenhoek 1988; Riginos and Nachman 2001). These factors, particularly geomorphic features, have rarely been investigated in the organization of genetic diversity among highly mobile individuals such as salmonids. Identifying the factors that influence the patterns and levels of genetic diversity would not only assist in the classification of distinct demes for management and conservation, but may also help understand the evolutionary potential of species (Heath et al. 2002).

Rainbow trout (*Oncorhynchus mykiss*)

Life history and distribution

Oncorhynchus mykiss, a salmonid native to the North Pacific Ocean, occurs in a variety of life history types; including an anadromous form known as steelhead trout, and the non-anadromous resident rainbow trout. The native distribution of the species is restricted to the coastal drainages of North America in the Pacific Ocean from Alaska to Mexico, lakes and streams west of the continental divide in North America (except the upper Peace River), and from the Sea of Okhotsk to the Kamchatkan Peninsula in the western Pacific (Behnke 1992; Nielsen et al. 1994). Both life history types exhibit extensive ecotypic and adaptive variation across this range (Taylor 1991). Steelhead trout spawn in streams or rivers with access to the ocean, and the resulting juveniles reside in freshwater habitats for 1 to 3 years before migrating to the sea. Following another 1 to 3 years the adult steelhead return to freshwater to spawn (Scott and Crossman 1973; Wilson et al. 1984). Rainbow trout reside in freshwater throughout their life cycle. Resident rainbow trout can be found in a wide range of habitat types, including headwater streams, large rivers, various sized lakes, as well as habitats above migration barriers (e.g. water falls). Steelhead trout and resident rainbow trout are semelparous; that is, they can survive to reproduce more than once. Populations of steelhead trout in British Columbia (BC), however, typically have very low rates of repeat spawning (e.g. 8.11%-11.6% in Keogh River steelhead population, Ward and Slaney 1988; Quinn 1993). Opportunities for migration for resident populations are often restricted by patterns of hydrogeographic networks

but dispersal among anadromous populations is mainly restricted by a strong tendency to home to natal streams (Ward et al. 1994).

Genetic variability in rainbow trout populations

The large amount of phenotypic variation observed in natural populations coupled with the lack of information regarding the genetic basis for this variation has led to some difficulties in understanding the genetic structure of rainbow trout. In part, these difficulties arose from the reliance on phenotypic variability to define the biological subunits or populations (reviewed in Swain and Foote 1999). Phenotypic variability by itself does not provide much genetic information about natural populations because the relative influences of adaptations to local environments and phenotypic plasticity are typically unquantified (Lewontin 1984; Taylor 1991).

A number of studies have described the genetic structure of *Oncorhynchus mykiss* populations using various molecular genetic markers including microsatellite DNA (Beacham et al. 1999; Nielsen et al. 1999; Heath et al. 2001; Hendry et al. 2002; Taylor et al. 2003), minisatellite DNA (Taylor 1995), mitochondrial DNA (McCusker et al. 2000), and allozyme markers (Parkinson 1984, Currens et al. 1990; Knudsen et al. 2002). Analysis of genetic data on a macrogeographic scale among different markers reveal major genetic subdivision among rainbow trout populations between those located on the east side of the Cascade Mountains and the west side (mtDNA, McCusker et al. 2000;

allozymes, Okazaki 1984, Parkinson 1984; minisatellite DNA, Taylor 1995).

These genetic analyses support the existence of a major difference in natural populations based on whether they inhabit coastal or inland freshwater systems.

Other studies have also suggested that steelhead populations exchange very few migrants and are essentially independent, both genetically and demographically. For example, considerable genetic differences have been documented between steelhead populations from: tributaries of the Columbia River, coastal rivers in Washington, Oregon and California (Reisenbichler et al. 1992); coastal rivers in southern California (Nielsen et al. 1997); coastal rivers in BC (Parkinson 1984, Heath et al. 2001); coastal rivers in BC and Alaska (Taylor 1995); and coastal rivers in BC, Washington, and the Columbia River (Beacham et al. 1999). While this provides information of the existence of major population units, genetic variation and differentiation at smaller microgeographic scales were not consistently and clearly demonstrated until more variable markers with greater resolution such as microsatellite DNA variation were used (e.g. Morris et al. 1996; Nielsen et al. 1997; O'Connell et al. 1997; Bagley and Gall 1998; Nielsen and Fountain 1999; Taylor and McLean 1999; Taylor and Tamkee 2001; Heath et al. 2002; Hendry et al. 2002; Taylor 2002). Many of these studies have demonstrated the varying levels and patterns of genetic variation and differentiation among rainbow trout (mainly steelhead) populations at a microgeographic scale; however, the pattern of genetic variation and differentiation are still often non-congruent among different areas. For example,

Reisenbichler and Phelps (1989) did not find significant genetic differentiation among populations on a local scale from the north coast of Washington, whereas Parkinson (1984) found significant differentiation between populations in adjacent streams in BC. Also, Beacham et al. (2000) and Heath et al. (2001) found that genetic differences between the Nass River and Skeena River watersheds (BC) were less than among tributaries within those watersheds. Among tributaries within larger rivers, some steelhead populations appear to be genetically distinct whereas others do not (Currans et al. 1990; Beacham et al. 1999; Beacham et al. 2000; Heath et al. 2001).

The general picture of population structure of *O. mykiss*, at least in steelhead, is that it is roughly similar to that exhibited by other anadromous Pacific salmonids. There are typically large differences among regions and the degree of differentiation decreases as one continues to compare populations at smaller geographic scales. By contrast, and unlike other salmonid species that almost always show genetic differentiation among tributaries within river systems (e.g. brook trout, *Salvelinus fontinalis* Angers and Bernatchez 1998; sockeye salmon, *Oncorhynchus nerka* Nelson et al. 1998; coho salmon, *Oncorhynchus kisutch* Small et al. 1998), the degree of differentiation in steelhead is highly dependant on the specific location (Hendry et al. 2002).

Thesis objectives

Genetic population structure in species frequently forms along broad-scale geographic gradients because of historic patterns of dispersal, drift and gene flow (Avice 1994 and references therein; Hewitt 1996; Lees et al. 1996; Bernatchez and Wilson 1998; Avice 2000). Patterns of genotypic variation can be measured on multiple scales that demonstrate a number of different spatial and temporal subdivisions (Nielsen et al. 1997). In my thesis, microsatellite allelic diversity and genetic distance measures were used to test hypotheses concerning how glaciation and post-glacial recolonization have shaped patterns of genetic diversity in *Oncorhynchus mykiss* throughout BC. On a smaller geographic scale, the influence of local geography, e.g. habitat size, elevation, fluvial distance and degree of connectivity, in shaping population genetic structure was also investigated. There were two reasons why these particular issues were chosen. First, there appears to be some disagreement in the field of molecular ecology over the use of microsatellite DNA to depict phylogeography of a species. Clearly, the usefulness of such novel markers combined with data from traditional phylogeographic markers may provide further support or at least provide more information about the demography of a species at various spatial scales. In molecular ecology, the approach of combining data from microsatellite and mitochondrial DNA simultaneously over a large portion of a species' distribution have been shown to be demographically informative and useful for the management and conservation of populations (e.g. Taylor and Hass 1996; Page and Scribner 2004; Turgeon and Bernatchez 2001; Beheregaray et al.

2003; Costello et al. 2003; Spruell et al. 2003; Stamford and Taylor 2004), but has rarely been applied, particularly in salmonids. Second, local geography is known to influence fish movement, particularly fish restricted to freshwater habitats. Little is known, however, of the relative influence of various hydrogeographic variables on dispersal and gene flow, and consequently the contemporary determinants of genetic structure of fish populations.

In my second chapter, I address the usefulness of microsatellite DNA to detect and describe the relative roles of historical factors, particularly postglacial recolonization, in structuring genetic variation in rainbow trout throughout BC. In my third chapter, I present results from microsatellite DNA polymorphism, and address how specific geomorphic features influence observed patterns of genetic diversity and yield insight on how contemporary geomorphic factors may help explain patterns of genetic variation observed today. I conclude with a final discussion and synthesis chapter.

CHAPTER 2: Effects of Post Glacial Recolonization on Patterns of Genetic Diversity

The various Pleistocene glaciations have had significant impacts on the genetic composition of many species especially when comparing intraspecific genetic diversity between glaciated and non-glaciated regions (Bernatchez and Wilson 1998). During the Pleistocene, northern species that were widely distributed became fragmented by the advancing and receding glaciers. As a result, divergent genotypes evolved in multiple isolated glacial refugia (i.e. Hewitt 1996; Wilson and Hebert 1998; McPhail and Taylor 1999; Stamford and Taylor 2004). The widespread disturbances and founder-flush cycles caused by multiple glacial expansions and retreats have profoundly influenced patterns of intraspecific genetic diversity (Bernatchez and Wilson 1998).

Genetic consequences of range expansion

Patterns of genetic diversity

Within each isolated glacial refuge, the impact of bottlenecks, founder events, differential selection pressures and genetic drift initiated unique evolutionary trajectories (Avice 1994; Hewitt 1996; Schluter 1996) evidenced by variability in levels of genetic variation as measured with neutral genetic markers as well as by the evolution of divergent lineages (Sage and Wolf 1986; Merila et al. 1996; Bernatchez and Wilson 1998; Wilson and Hebert 1998; Stamford and Taylor 2004). In some North Temperate species, a general pattern of highest genetic

variation in regions located nearest to putative glacial refugia is present (Sage and Wolf 1986; Hewitt 1996; Merila et al. 1996; Paekau et al. 1998; Costello et al. 2003). A progressive decline in genetic variation with geographical distance away from the putative refugia is a commonly observed signature of postglacial range expansion (Sage and Wolff 1986; Hewitt 1996; Ibrahim et al. 1996; Merila et al. 1996; Turgeon and Bernatchez 2001; Costello et al. 2003).

Recently colonized areas often demonstrate less spatial genetic divergence and a lower degree of genetic subdivision within phylogeographic groups (e.g., Costello et al. 2003). The colonization of novel habitats from a panmictic gene pool and short time scale since colonization should result in populations demonstrating little genetic divergence. Over longer periods of time, differences may accumulate due to drift and selection pressures, resulting in the detection of genetic differences between demes. Such large-scale spatial genetic divergence is studied in the field of phylogeography (Avice 2000). The general pattern emerging from this field conforms to the prediction that within lineages, the genetic divergence among gene pools is greater for lineages that have experienced little influence from glaciations (i.e. long term, minimal catastrophic disturbances; Jansson and Dynesuis 2002) than areas that have been influenced by repeated glaciations (Bernatchez and Wilson 1998).

Migration-drift equilibrium

The last glacial event in BC occurred between 75000 and 10000 to 12000 years ago (McPhail and Lindsey 1970, 1986; Hewitt 1996; Ibrahim et al. 1996). As the glaciers retreated, individuals were able to occupy novel habitats that were once covered with ice. As time progressed, individuals at the range periphery occupied novel habitats as the ice margins retreated while individuals closest to the heart of the species distribution were able to maintain themselves under presumably more ideal conditions. Extant populations from the heart of a species' distribution have been established longer than recently colonized populations. The time since establishment and the degree to which dispersal is inhibited between populations among regions affects the genetic similarity between populations within these regions (Minkoff 1983; Hartl and Clark 1989; Carvalho 1993; Slatkin 1993; Hutchison and Templeton 1999; Avise 2000; Malone et al. 2003). Contiguous populations that have been established for very long periods of time are expected to be in migration-drift equilibrium, and exhibit isolation-by-distance (IBD, Slatkin 1993; Hutchison and Templeton 1999). The IBD model, introduced by Sewall Wright (1943) is based on the expectation of population genetic divergence being driven primarily by isolation as a function of geographic distance. That is, the further two populations are from each other, the greater the expected genetic distance between them. The relative strength of IBD may differ between geographic regions depending on how far constituent populations are from drift-migration equilibrium (Slatkin 1993; Hutchison and Templeton 1999). Recently founded populations may exhibit interrelationships

that are more a function of post-glacial recolonization (e.g. high gene flow and straying rates) than of a current balance between gene flow and drift (Sork et al. 1999). Therefore, greater departures of migration-drift equilibrium as reflected by a decreasing significance in IBD, is expected with increasing geographic distance from putative refugia (Hutchison and Templeton 1999).

Mitochondrial DNA and Phylogeography

Among vertebrates, fishes have perhaps been the most intensely studied group for local and regional genetic structure largely due to sustained long-term interest in the geographical structure of intraspecific diversity for fisheries management (e.g. Moritz et al. 1995; Beacham et al. 1999; Taylor and Tamkee 2001; Hendry et al. 2002; Taylor 2002). Although much of this work has been conducted for applications to resource management, a large amount of phylogeographic data have also been collected (e.g. Bernatchez and Wilson 1998 and references therein; Wilson and Hebert 1998; McCusker et al. 2000; Stamford and Taylor 2004). Phylogeographic studies have had an impact on fish biology by confirming major intraspecific subdivisions hypothesized from other evidence (e.g. Florida peninsula serving as a barrier for gene exchange in sunfish species; Bermingham and Avise 1986) and highlighting unexpected genetic structuring within species (Bernatchez and Wilson 1998). The most significant contribution, however, has been to provide a historical dimension for evolutionary, ecological, and applied studies. Phylogeographic studies have consistently revealed the dominant influence of historical biogeographic and demographic events in

shaping existing patterns of mtDNA variation. By doing so, phylogeographic studies have enabled us to retrace the movements, events and histories that have helped shaped the modern-day genetic and geographical structure of fish species (e.g. Hansen et al. 1999; McCusker et al. 2000; Turgeon and Bernatchez 2001; Stamford and Taylor 2004).

Mitochondrial DNA has been a very useful genetic marker for studies of phylogeny and intraspecific phylogeography (Avice 1994; reviewed in Avice 2000) and has been used to identify major phylogenetic assemblages within species that were often undetected by allozymes and other genetic methods. This marker has unique attributes such as uniparental and non-recombining mode of inheritance, simplicity of genome organization, and relatively high point mutation rates compared to most nuclear loci (Avice 1994). However, mtDNA is not without its limitations. Commonly, mitochondrial studies are based on a small number of genes and on one independently segregating locus. It provides phylogenetic information of a single tree, which may not accurately reflect a population tree under several demographic conditions. For example, when there are major phylopatric discrepancies between sexes, potentially erroneous phylogeographic inferences can be made as mtDNA reflects only maternal lineages in most taxa (Angers and Bernatchez 1998; Koskinen et al. 2002 and references therein; Zhang and Hewitt 2003). Also, the detection of population structure using mtDNA on a local geographic scale is often limited due lack of variability as compared to other more recently developed molecular markers

such as microsatellite DNA (e.g. Angers et al. 1995; Angers and Bernatchez 1998).

Microsatellite DNA markers and their use for phylogeographic studies

Microsatellite DNA markers are becoming recognized as a useful tool to help investigate the role of historical events and demographic processes in shaping the genetic compositions and structure of extant populations (Angers and Bernatchez 1997; Angers and Bernatchez 1998; Hansen et al. 1999; Beebee and Rowe 2000; Koskinen et al. 2002; Beheregaray et al. 2003; Williams et al. 2003). The application of microsatellite markers to phylogenetic and phylogeographical studies, however, has been less common because it is usually not possible to establish the phylogeny of alleles and because of unresolved problems regarding the exact mutation process and mutation rates (Goldstein and Pollock 1997; Hansen et al. 1999). Intraspecific phylogenetic relationships inferred from microsatellite DNA polymorphisms, however, are often congruent with those obtained from other approaches (e.g., Estoup et al. 1995; Angers and Bernatchez 1998; Beheregaray et al. 2003; Spruell et al. 2003; Williams et al. 2003), and in some cases, provided more accurate geographic clustering of closely related populations (e.g. Bowcock et al. 1994). For instance, Angers and Bernatchez (1998) have addressed the usefulness of microsatellite DNA in providing greater resolution than mtDNA for the inference of evolutionary history of closely related and geographically proximate populations in brook charr (*Salvelinus fontinalis*). They concluded that 'microsatellite polymorphism holds

the potential for the analysis of the evolutionary history of populations over short temporal and geographic scales' (c.f. Angers and Bernatchez 1998).

Objective

To draw robust phylogeographic conclusions regarding the evolutionary history of *Oncorhynchus mykiss* in BC, it would be important to study the genetic relationships of this species utilizing nuclear DNA loci and compare results previously collected from mtDNA (McCusker et al. 2000). Furthermore, as the evolutionary information provided by McCusker and her colleagues was limited, assessment of highly polymorphic nuclear markers could provide additional resolution for studying *O. mykiss* population structure at finer geographic scales.

In this chapter I examined genetic variation at ten microsatellite loci to study the phylogeography of rainbow trout across a large part of its natural range. In addition, I compared my results using microsatellite DNA for phylogeographic mapping with those obtained by McCusker et al. (2000) who suggested, with data collected from mtDNA, that the Queen Charlotte Islands (Haida Gwaii) and the lower Columbia River were refugia for *O. mykiss* and provided sources for postglacial dispersal to BC. I collected genetic variation and differentiation data among rainbow trout populations in BC to test the following hypotheses: First, I tested the idea that groups of rainbow trout were differentially affected by major historical events resulting in a predictable cline in genetic variation from the heart of their range and areas nearest putative southern refugia to the northern range

periphery. That is, the general pattern of genetic variation should decrease from south to north within the interior regions of BC. Likewise, samples along the coast should display higher levels of genetic variation than populations further away from the proposed refuge near the Queen Charlotte Islands.

I also tested the hypothesis that populations closest to the heart of refugia will be in or nearest to migration-drift equilibrium compared to populations at the range periphery given the longer time period that has elapsed since postglacial colonization in the former populations.

Materials and methods

Sample collection

During 1999-2002, *Oncorhynchus mykiss* tissue samples were collected from 69 locations throughout BC (Table 2.1 and 2.2, Figure 2.1). Sample locations were chosen to investigate the genetic structure of native rainbow trout populations within BC and to test the usefulness of microsatellite DNA in detecting historical isolation and postglacial recolonization previously reported by McCusker et al. (2000) (Figure 2.2). The localities in this study ranged from multiple contiguous to non-contiguous localities from the same watershed, to localities from different watersheds (Table 2.1 and 2.2, Figure 2.1). A total of 2867 fish were collected both from lakes and rivers. Samples were collected from sites that contained only native and non-stocked rainbow trout (BC Ministry of Water, Land and Air Protection (MWALP), stocking records unpublished data). A combination of angling, electro-shocking, minnow trapping, and gill netting was used to collect fish. To avoid biases inherent when sampling individual families (Hansen et al. 1997), fish of various sizes were collected when possible from each site until desired sample size was reached. Target sample size was 32 or more; however, some sample sizes were smaller but were still included owing to the importance of the population to the data set (Table 2.1 and 2.2). Samples consisted of fin tissues preserved in 95% ethanol upon collection.

DNA extraction

Total genomic DNA was extracted from tissue samples using PUREGENE animal tissue protocol salt extraction kit (Gentra Systems) with Proteinase K digestion. The DNA precipitate was resuspended in TE buffer, diluted to 100ng/μl, and stored at -20 °C.

Microsatellite DNA analysis

Polymorphic microsatellite loci used in this study were located by testing published primers which amplify loci found in rainbow trout and other salmonids. Tests for polymorphism and further optimization of PCR conditions were carried out using P³² forward end-labelled primers and running the PCR products on a 7% polyacrylamide gel. The gel was blotted onto filter paper, dried and exposed to film (Kodak Biomax MS). The tests for polymorphism were carried out on 15 rainbow trout that were selected from a wide geographic range assuming distinct regions were more likely to show different alleles. Loci that were polymorphic were further optimized and used for this study.

A total of 18 published primers were screened, eight of which did not amplify successfully with PCR: *Oneu2* (*Oncorhynchus nerka*, Scribner et al. 1996), *Ots4* (*O. tshawytscha*, Banks et al. 1999), *Ots101* and *Ots107* (*O. tshawytscha*, Nelson and Beacham 1999), *Ogo2* (*O. gorbuscha*, Olsen et al. 1998), *Ssa311* (*Salmo salar*, Slettan et al. 1995), *Sfo8* and *Sfo18* (*Salvelinus fontinalis*, Angers et al. 1995). To increase efficiency and minimize cost, the ten working

microsatellite markers were run in paired PCRs (i.e. "diplexes"). The diplexes were as follows: *Oneu14* and *Ssa197*, *Oneu8* and *Ssa85*, *Ssa456* and *Omy77*, *Ots3* and *Okia3*, and *Ots100* and *Ots103* (Table 2.3).

PCR protocol

PCR reactions for each optimized microsatellite diplex were carried out by first labelling the 5' end of the forward primer in a 1 µl reaction volume containing: 0.25 units of T4 polynucleotide kinase (PNK, New England BioLabs), 1X PNK buffer (70 mM Tris-HCl, 10mM MgCl₂, 5M DTT, pH 7.6), 0.5 µM forward primer, and 9.25 kBq γ^{32} P-dATP for each primers separately. The PCR reaction solution was in 10µl volumes containing: 100ng DNA template, 10x reaction buffer (Gibco/BRL), 0.4mM dNTP, 0.5µM reverse (both) primers, 0.25µM forward (both) primers, 1.5mM MgCl₂, 0.5 units of taq polymerase, and 0.05µM of γ^{32} P labeled forward (both) primers. PCR amplification was performed using PTC-100 (MJ Research) thermal-cycler under optimal annealing conditions for each diplex. Each PCR diplex profile consisted of [5X (95°C / 1 min, T_A/ 1 min, 72°C / 1 min), 30X (94°C / 1 min, T_A/ 1 min, 72°C / 1 min), and 1X (72°C / 5min)], where T_A is the annealing temperature(s) respectively (Table 2.3). Prior to running the PCR products on a 7% polyacrylamide gel loading the gel, 7µl loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF) was added to the PCR product and denatured for 5 to 15 minutes before 5µl PCR product mix was loaded onto a 7% polyacrylamide gel in 1.2X Tris-Borate-EDTA (TBE) buffer. To determine accurate measurements of alleles, an M13

control DNA sequencing ladder (T7 Sequenase v2.0, USB) was electrophoresed with all the samples. Some samples which were accurately measured were also electrophoresed with other samples with/without the sequencing ladder for reference. The product gel was blotted, dried and exposed to film before scoring. Sample sizes varied slightly among loci due to variability in PCR amplification efficiency. Any individuals that failed to produce clear bands were re-amplified under the same conditions and if amplification was not possible in the second PCR reaction the sample(s) were abandoned.

Genetic data analysis

Genetic variation

Descriptive statistics of microsatellite loci included expected heterozygosity (H_e), observed heterozygosity (H_o), number of alleles (A) and average number of alleles per locus and were compiled using TFPGA version 3.2 (Miller 1997). Allelic richness (A_r) was also calculated, using the statistical program Fstat version 2.93 (Goudet 1995). Allelic richness is a measure of the number of alleles independent of sample size and, therefore, allows comparison of the number of alleles between samples of different sizes.

Tests for deviations from Hardy-Weinberg equilibrium were performed for each locus-population combination using an exact test in which P-values were estimated using a Markov chain method performed using GENEPOP ver. 3.1 (Raymond and Rousset 1995). Tests for genotypic linkage disequilibrium for all combinations of locus pairs within populations were also made using a Markov chain method with GENEPOP default values. Tests for population differentiation between all pairs of populations was performed over all loci combined using log-likelihood (G)–based exact tests (Goudet et al. 1996) with GENEPOP default values. All critical significance levels for simultaneous tests were evaluated using sequential Bonferroni adjustment (Rice 1989) with an initial α level of 0.05. Other standard statistical tests, notably correlation and regression, were performed using the JMPin software package.

To determine how genetic variation was partitioned, allele frequency data from microsatellite loci were imported into the program ARLEQUIN (Schneider et al. 1997) which estimated the hierarchical nesting of genetic diversity using the Analysis of Molecular Variance (AMOVA) approach of Excoffier et al. (1992). The percentage of the total genetic variation explained by allele frequency variation within populations (V_{ip}), among populations within groups (V_{ig}), and by differences between groups (V_{bg}) was calculated under a variety of grouping hypotheses. For example, the divisions into coastal and inland populations, and anadromous and resident life history forms were tested to determine if they represented distinct biological groupings sufficient to explain the patterns of variation observed over a large geographic scale.

McCusker et al. (2000) identified two major phylogeographic lineages (interior and coastal) of rainbow trout found within BC. Within each phylogeographic lineage, I organized populations by latitude, longitude and straight geographic distance from putative glacial refugia and regressed these variables against measures of genetic variation to determine the presence of any spatial trends in genetic variation outwards from each putative refuge. Genetic variation as measured by expected heterozygosity (H_e), number of alleles per loci (A) and allelic richness (A_r) were used. Regression analysis was conducted using JMPin (version 3.2.1).

Genetic differentiation

A variety of mutation-based and drift-based genetic distance algorithms are available for the calculation of population subdivision and genetic distances among samples. I considered drift-based methods rather than mutation-based measures of genetic subdivision to be the most appropriate, particularly Weir and Cockerham's (1984) estimator (θ) of Wright's F_{st} for two reasons. First, the postglacial origin of the freshwater populations in our study areas set their maximum age at about 12 000 years (McPhail and Lindsey 1986; Hewitt 1996; Ibrahim et al. 1996). Over such a short evolutionary time frame, particularly when population histories may have involved large changes in population sizes, demographic processes probably overwhelm post-colonization mutation based differentiation patterns (Goldstein and Pollock 1997; Ruzzante 1998; Taylor et al. 2001; Kalinowski 2002). Recent empirical studies found that measures assuming drift- based methods were appropriate for late Pleistocene divergence (Paetkau et al. 1997; Beebee and Rowe 2000). Second, over such a short evolutionary time period, drift based allele frequency models (e.g., theta, θ) tend to outperform alternatives based on mutation allele size (e.g. R_{st}) models (Paetkau et al. 1997) especially when relatively small sample sizes and numbers of loci are used (Gaggiotti et al. 1999). Gaggiotti et al. (1999) and Ruzzante (1998) used simulations to demonstrate that R_{st} performs better than F_{st} when there are large sample sizes ($n > 50$) and many loci ($n > 20$) involved, while F_{st} -based estimates out performs R_{st} when samples sizes and the number of loci used are moderate or small. Several analyses of population structure have also

reached the conclusion that many microsatellite loci do not fit the stepwise mutation process, which R_{st} assumes (Estoup et al. 1995; Angers and Bernatchez 1997; Orti et al. 1997; Paetkau et al. 1997; Ross et al. 1997; Estoup et al. 1998; Goodman 1998; Balloux et al. 2000). This is likely because new alleles are generated at microsatellite loci through a process that is more complex than a simple stepwise mutation model (Ellegren 2000) suggesting that the most conservative approach to estimate the degree of differentiation is to use F_{st} .

Values for the degree of differentiation between all sites (pairwise F_{st}) by all pairwise comparisons of allele frequencies were calculated by ARLEQUIN, Version 2.0 (Schneider et al. 1997). F_{st} can theoretically range from 0 (no genetic divergence) to 1 (complete fixation of alternative alleles).

Genetic distances among population pairs were estimated with Cavalli-Sforza and Edward's (1967) chord distance (CSE distance), which does not make underlying assumptions concerning the particular model of molecular evolution. The magnitude of this distance is not proportional to evolutionary time, but its use generally leads to a higher probability of depicting the correct tree topology among closely related populations under either drift-based or mutation-based model of assumptions (Takezaki and Nei 1996). Angers and Bernatchez (1998) have compared the use of CSE distance with Goldstein's $\delta\mu^2$ (Goldstein et al. 1995) distance which assumes stepwise mutation model, and found that the use

of chord distance was more reliable in resolving population tree topology using the Neighbor-Joining (N-J) algorithm in closely related brook trout populations. Beebee and Rowe (2000) compared three genetic distance measures (CSE distance, Nei's distance, and Goldstein's $\delta\mu^2$ distance) to describe the phylogeographic structure of the natterjack toad (*Bufo calamita*) and found CSE distance gave the best description of differentiation among populations and was among the best methods for recovering correct tree topologies for populations founded from a single glacial refuge. Not only did CSE distance perform well at their range of 100's to 1000's km of inter-population distances, but it also implied that drift rather than mutation has dominated population differentiation at their microsatellite loci over the past 10,000-15,000 years (Beebee and Rowe 2000).

The Cavalli-Sforza and Edward's (1967) chord distances calculated in the PHYLIP software package (Felsenstein 1993), were used to build an unrooted N-J tree to visualize the genetic relationships among localities or between phylogeographic groups. Genetic distance estimates were calculated by creating a microsatellite allele frequency matrix, replicated 1000 times with SEQBOOT and calculated for each replicate data set using GENDIST program. The N-J trees were built using the program NEIGHBOUR. Reliability of tree nodes was evaluated by generating a consensus tree from 100 bootstrap replicates of the original allele frequencies using the programs SEQBOOT and CONSENSE, and the final tree was drawn in DRAWTREE. Hence, in this study, I used the CSE

distance for tree topologies and θ as a measure of genetic distances between populations.

A principal components analysis (PCA) was conducted on allele frequency data using PCA-GEN (Goudet 1999) as a comparative method to summarise genetic differentiation among all samples. The analysis summarises all the variation across the ten loci (171 alleles) and orients samples along major axes of variation (principal components, Pimental 1979) and does not make assumptions when estimating genetic distances.

Isolation-by-distance

The significance of correlations between geographic (fluvial distance for all population chains, straight distances for throughout BC) distance and genetic distance (F_{st}) was tested to determine if the observed genetic structure could be explained by the isolation-by-distance model (IBD; Slatkin 1993). The Mantel test (Mantel 1967) option in the software program R-Package version 4.0 (Casgrain and Legendre 2001) was used and significance was determined by 999 matrix permutations (default setting). Partial Mantel test were also conducted to control for influences of elevation, node, and geographic distance effects on patterns of genetic differentiation. Fluvial distance between localities was determined using the Geographic Information System (GIS) program, ArcView (ver. 3.1, ESRI). To determine whether populations have reached drift-migration equilibrium, the approach of Hutchison and Templeton (1999) was

applied. Subsequent to a significant Mantel test result between genetic and geographic distances (significant IBD), a second Mantel test was performed using residuals from the initial fitted line (calculated using JMPin version 3.2.1) against geographic distance. The scatter of the residuals should increase with greater geographic separation as drift supersedes gene flow at larger distances. The absence of any pattern of isolation-by-distance in a species suggests that the species is far from equilibrium and may have only recently invaded the area that it now occupies (Slatkin 1993).

Results

Microsatellite variation among geographic areas

Microsatellite variation across 2836 individuals at ten microsatellite loci was assayed. The number of alleles observed across all populations ranged from two (*Ssa197*) to 38 (*Oki3a*) with an average of 17.1 alleles per locus (Table 2.4). Observed heterozygosity averaged 0.4 across all loci and populations ranged from 0.24 (*Ots103*) to 0.66 (*Oki3a*), respectively (Table 2.4).

Virtually all sample sites were in Hardy-Weinberg equilibrium with only ten out of possible 690 (10 loci X 69 localities) tests showing statistically significant heterozygote deficits. These exceptions were found at several separate loci in 10 different populations and therefore do not compromise subsequent analyses (Table 2.5). Test for linkage disequilibrium resulted in significant departures in

four out of possible 3150 tests. The statistically significant results were not concentrated on particular locus pairs or within specific populations.

Within population variation

Genetic variation within populations ranged widely. Expected heterozygosity, averaged across the ten loci, ranged from a low of 0.05 (Clearwater Creek) to highs of 0.60 – 0.68 (Genelle, China Creek, Sand Bar Eddy and Murphy Creek) (Table 2.5). Some populations displayed extremely low levels of genetic variation. Clearwater Creek rainbow trout for instance, were fixed for single alleles at eight of the ten loci. Upper Sullivan Creek fish also displayed no more than three alleles at any one locus and were often fixed for single alleles whereas samples from the Columbia River at Genelle or lower Murphy Creek displayed 8-10 alleles per locus (Table 2.5).

Cline in genetic variation

Previous studies have suggested two major lineages of rainbow trout in North America, a coastal and interior lineage (Huzyk and Tsuyuki 1974; Allendorf 1975; Okazaki 1984; Parkinson 1984; Currrens et al. 1990; Behnke 1992; Taylor 1995) specifically from the Queen Charlotte Islands and the Columbia River (McCusker et al. 2000), respectively. Among localities within the interior lineage (e.g. populations east of the coastal mountains which includes sample localities #1-59, Table 2.1) measures of genetic variation were examined to detect possible trends in genetic variation with respect to changes in latitude and longitude. The

general trend was a decrease in genetic variation with an increase with latitude (i.e. south to north) and an increase in genetic variation with an increase in longitude (i.e., west to east, Table 2.6). Averaged across all loci, relationships between patterns of genetic variation and latitude and longitude were statistically significant (Table 2.6). The relationships of straight line geographic distance between each sample locality to the most southern locality (assumed to be closest to the heart of the glacial refuge in the lower Columbia River) with number of alleles (A), allelic richness (A_r), and expected heterozygosity (H_e) were also statistically significant within the interior lineage ($r = -0.35$ $p = 0.0002$, $r = -0.46$ $p=0.007$ and $r = -0.37$ $p = 0.003$, respectively). Among coastal lineage populations (population number #60-69, Table 2.1), regression analysis between genetic variation and latitude/longitude indicated general trends of decreasing genetic variation with increasing latitude and longitude; however, results were not statistically significant (Table 2.6). There was also no statistically significant relationship ($p > 0.05$) between genetic variation and straight geographic distance from the heart of the glacial refuge (north-east tip of Queen Charlotte Island; Warner et al. 1982) expanding eastwards (Figure 2.2, Table 2.6).

Because specific populations may be differentially affected by geography which may also influence its level of genetic variation, relationships between genetic variation and latitude and longitude were also conducted by major watersheds (upper Columbia River, Thompson River, upper Fraser River, Vancouver Island, Queen Charlotte Islands, Skeena River and Stikine River). My results indicated

that among watersheds within the interior lineage, those which are closest to the south (e.g., closest to glacial refuge) tend to contain higher levels of genetic variation compared to watersheds the periphery of interior lineage (Figure 2.3). For example, populations from the Columbia River watershed had significantly higher levels of allelic richness and gene diversity than populations from the Thompson and upper Fraser rivers' watersheds ($p = 0.002$ and $p = 0.002$, respectively). Among the coastal lineage, populations from the Queen Charlotte Islands and Vancouver Island contained higher levels of allelic richness ($p = 0.04$ and $p = 0.03$, respectively) and gene diversity compared to Skeena River populations ($p = 0.04$ and $p = 0.03$, respectively). Comparisons between Vancouver Island populations with Queen Charlotte Islands, however, demonstrated no significant differences (Figure 2.3). Comparisons with populations from the Stikine River watershed were not possible due to low numbers of populations examined in the Stikine River.

Population genetic structure

Among population variation

Variation among populations in my study was extensive. There were 2346 (69 pops: $68+67+66+65...+1 = 2346$) pairwise comparisons made between populations for differences in allele frequencies summed across all ten loci. Only sixty of these pairwise comparisons were not significant (i.e., $p > 0.0008$ when adjusting for multiple comparisons). The non-significant comparisons were all between populations from lake/river chains from a single drainage with a high

degree of interconnectedness, i.e. Grizzly Lake and Blanchet Lake; China Creek and Kootenay River, Sand Bar Eddy and lower Norns Creek, and Kootenay River and Genelle; Twinkle Lake and Needle Lake; 11 pairwise comparisons in the LNTH Lake chain, 10 pairwise comparisons in the Glatheli Lake chain, and 34 pairwise comparisons in the Deadman Lake chain. The somewhat lower percentage of pairwise population differentiation at individual loci, particularly within the Deadman Lake chain, reflects the overall low level of genetic diversity at microsatellite loci as compared to the LNTH Lake chain. The Deadman populations displayed no more than 4 alleles at the most variable loci and were all fixed at five loci (*Ssa85*, *Ots3*, *Oneu14*, *Ssa197*, and *Ots100*).

The proportion of the total molecular variance in allele frequencies attributable to differences among all populations was 0.39 (95% CI 0.33 – 0.46), and within each population chain varied from 0.24 (Blanchet Lake chain; 95% CI 0.16-0.32), 0.09 (Columbia River chain; 95% CI 0.07-0.10), 0.05 (Deadman Lake chain; 95% CI 0.03-0.06), 0.05 (Glatheli Lake chain; 95% CI 0.01-0.09), 0.04 (Horseshoe Lake chain; 95% CI 0.01-0.09), 0.03 (LNTH Lake chain; 95% CI 0.02-0.05), to 0.22 (Nutli Lake chain; 95% CI 0.002- 0.58).

Genetic relationships among populations

The N-J generated tree partially corresponded with mtDNA results from McCusker et al. (2000) suggesting a coastal and interior division among all *O. mykiss* populations (Figure 2.2 and Figure 2.4). No striking distinctions with high

bootstrap support were found; however, groupings of localities into coastal and inland groups (including major watersheds) were present. Populations hypothesized to have originated from a more general coastal refuge, grouped closely together with the Queen Charlotte Island cluster of populations (Mamin River, Riley Creek, Yakoun River, and Copper River), Vancouver Island populations (Gold River and Nimpkish River) formed another cluster, and populations located in north-western BC (Eaulve Lake, Moosevale Creek, Canyon Creek) formed the remaining cluster (Figure 2.4).

Most populations grouped together by drainages and then into the expected coast (e.g. Stikine River drainage, Skeena River drainage, Queen Charlotte Islands, Vancouver Island) or interior areas (Columbia River drainage, Thompson River drainage, upper Fraser River drainage). By contrast, Khatada Lake was expected to group with the coastal populations but clustered with the interior populations such as Fry Creek (upper Columbia River drainage) and Coldwater River (Thompson River drainage) (Figure 2.4). Among the interior populations, there were a few populations, with very low bootstrap values, which were found grouping with populations from different drainages rather than their own, e.g. grouping of Fish Lake (upper Fraser River) with Murray Creek and Coldwater River (Thompson River drainage), or the grouping of the Clearwater River (Thompson River drainage) with Blackwater River (upper Fraser River drainage). Populations which were hypothesized to have originated from the interior lineage (e.g. east of the Coastal Mountains) did not group closely together to form a

single distinct cluster. The N-J tree did, however, reveal striking interior population clusters with high bootstrap values at the drainage level. These included sample site chains in the upper Thompson River (LNTH lake chains), 99%; Deadman River chain, 100%; Horseshoe Lake chain (Andrews Creek) 81%; and Blanchet Lake chain, 57%. Also, high bootstrap values were found grouping all upper Fraser River chain populations (64%), Vancouver Island populations (Gold River and Nimpkish River, 99%), and the Queen Charlotte Islands' populations (Riley Creek, Copper River, Mamin River, and Yakoun River, 80%) (Figure 2.4).

Projection of populations in principal component space (Figure 2.5) also suggested substantial differentiation among four major sets of populations: most samples from the upper Fraser River drainage (Blanchet Lake chain, Glatheli Lake chain, Fenton Lake chain, Horseshoe Lake chain, and Kuyakuz Lake), a group of coastal populations (Nimpkish River, Gold River, Mamin River, Riley Creek, Copper Creek, Yakoun River, Canyon Creek, Moosevale Creek, Eaulve Lake, and Khatada Lake), a group of populations from the Columbia River drainage (lower Murphy Creek, lower Norns Creek, Kootenay River, upper Sullivan Creek, China Creek, Sand Bar Eddy, upper Murphy Creek, Norns Creek Fan, Columbia River at Genelle, and Kinbasket Reservoir), and a heterogeneous group of population lake chains (LNTH and Deadman lakes) located in the Thompson River drainage system. Above barrier populations (e.g. upper Sullivan Creek, Clearwater Creek) were highly distinct both amongst themselves

and from all other populations. The close clustering of populations by general geographic region (coast versus interior) and by lake/river chain indicates a general association between geographic proximity and genetic similarity (discussed in chapter 3).

There were no fixed allele differences that were associated with glacial refugial groups of *Oncorhynchus mykiss* in BC although several alleles unique to specific areas were found (Table 2.7, Figure 2.6A through J). Alleles unique to the coastal populations were found among a few individuals from: Moosevale Creek (*Ssa85**105), Canyon Creek (*Ssa85**153, *Ots3**96), Nimpkish River (*Ssa456**161, *Oneu14**165), Gold River (*Ssa456**161), and Riley Creek (*Omy77**94, *Ots100**220) at very low frequencies. There were more alleles unique to the inland populations at very low frequencies found compared to the coastal populations, these included: 9 alleles from *Oneu8* (allele *150, *162, *164, *176, *178, *180, *182, *184, *192), 7 alleles from *Ssa85* (allele *97, *117, *139, *143, *145, *147, *149), 6 alleles from *Ots103* (allele *77, *79, *87, *91, *93, *97), 3 alleles from *Ots3* (allele *76, *92, *94), 8 alleles from *Omy77* (allele*96, *98, *108, *110, *114, *118, *140, *142), 1 from *Oneu14* (allele *145), 10 alleles from *Ots100* (allele*168, *172, *176, *180, *204, *206, *208, *210, *214, *222), and 22 alleles from *Okia3* (allele*112, *116, *120, *124, *130, *138, *154, *162, *166, *170, *174, *182, *186, *190, *192, *194, *196, *198, *200, *202, *204, *206).

The majority of unique alleles found among interior populations belonged to localities from the Columbia River drainage, particularly: Fry Creek, Salmo River, Kinbasket Reservoir, Lardeau River, Lower Murphy Creek, lower Norns Creek, Kootenay River, China Creek, lower Blueberry Creek, Sand Bar Eddy, upper Murphy Creek, Norns Creek Fan, and the Columbia River at Genelle. Out of a possible 63 alleles unique to interior populations (as compared to coastal populations), populations from the Columbia River possessed 54 of these alleles whereas populations further north, the Thompson River drainage populations and the upper Fraser River populations, had fewer of these unique alleles (20 and 20, respectively). Along with the presence of unique alleles for each lineage, there were also private alleles (alleles which only occurred in one population). The private alleles, however, were in very low frequencies with the exception of Canyon Creek where the allele *Ssa85*153* occurred at a frequency of 0.45 and Kuyakuz Lake where the *Ots103*79* alleles occurred at a frequency of 0.28 (Table 2.7).

Comparatively little microsatellite variation was found between major phylogeographic regions across the study area. For instance, McCusker et al. (2000) argued, based on mtDNA data, that there were at least two areas of glacial refuge where *Oncorhynchus mykiss* survived during the last glaciation which later colonized BC (Queen Charlotte Islands and lower Columbia River) and a possible third from Beringia. To test this idea, I analyzed variation after pooling all populations into groups that reflected the geographic subdivision by

these putative refugia: North Coast, South Coast, and Interior origin (Figure 2.2). This division among the three putative refugial groups represented only 4.3% of the total variation, compared to 21.9% of the microsatellite variation that was explained by differences among populations within these groups, and 59.2% within individual populations (all $P < 0.001$, Table 2.8). Interestingly, these results were very similar to those obtained when all populations were divided into only two groups: coastal populations (Skeena River watershed, Stikine River watershed, Queen Charlotte Islands, and Vancouver Island) and interior populations (all remaining populations) (4.8%, 27.4%, 67.7%, respectively, $p = 0.0088$). Consequently, no greater amount of molecular variation attributable to major refugial groupings was resolved when imposing a third, Bering refuge.

I examined variation by grouping populations into major watersheds. Similar amounts of variation were resolved among watersheds located across the study area compared to variation among multiple samples within watersheds. Among the Stikine River (one locality), Skeena River (three localities), Vancouver Island (two localities), Queen Charlotte Islands (four localities), Thompson River (21 localities), upper Fraser River (23 localities) and upper Columbia River (15 localities), similar amounts of variation was found among watershed groups to within them (18.8% versus 21.9%, both $P < 0.001$). In addition, when all samples were partitioned into the four groups suggested by the principal components analysis (Figure 2.5), variation among groups was 19.1%, among populations, 21.8% within groups, and 59.1% within populations (all $p < 0.001$).

Isolation-by-distance

Oncorhynchus mykiss displayed a pattern of isolation-by-distance (IBD) throughout its range (coastal and interior lineages combined) as well as within the interior lineage ($r = 0.18$ $p = 0.001$ and $r = 0.38$ $p = 0.001$ respectively; Figure 2.7), but residuals were not significantly correlated with geographic distance ($r = 0.000001$, $p = 0.5$ for both). No significant pattern of IBD was found among the coastal lineage populations ($r = 0.20$, and $p = 0.26$). Among all seven population chains within the interior lineage, only the Lower North Thompson (LNTH) Lake chain and the Deadman Lake chain showed significant IBD ($r = 0.61$ and $r = 0.66$, $p = 0.007$ and $p = 0.002$ respectively). Surprisingly, populations among the Columbia River chain, which was expected to be significant for IBD due to its southerly origin reflecting colonization before northerly populations, did not show IBD ($r = 0.24$ and $p = 0.14$). Within the overall pattern among the Columbia River chain there were some notable deviations. First, a cluster of comparisons which represented substantial divergence at relatively low geographic distances (7 to 30km), and another cluster of comparisons involved relatively low divergences at the highest geographic distances (75 to 95 km, Figure 2.7C). The former involved mostly comparisons between and among populations that were isolated above migration barriers. When all above barrier populations (upper Sullivan Creek, upper Murphy Creek, and Clearwater Creek) were removed, this left only populations separated by no known natural migration barriers. The removal resulted in a greatly increased significance of IBD ($r = 0.44$, $p = 0.017$; Table 2.9, Figure 2.7D); however, the residuals still showed no significant correlation with

geographic distance ($r = 0.08$, $p = 0.41$, Table 2.9). Among the LNTH and Deadman lakes chains, there were no significance between residuals and geographic distance ($r = 0.00005$, $p = 0.4$ and $r = 0.000002$, $p = 0.51$, respectively). Among all three population chains that were significant for IBD, the residuals (scatter) from standard linear regressions of pairwise F_{st} on geographic distance did not increase with increasing geographic distance suggesting that these regions have not yet reached drift-migration genetic equilibrium.

Discussion

Many studies of DNA polymorphisms have demonstrated that postglacial colonization events, such as population subdivision, range expansion, and long-distance colonization, have markedly shaped the contemporary distribution of genetic variation in nature (e.g., Hutchison and Templeton 1999; Hansen et al. 1999; Avise 2000 and references therein, Beebee and Rowe 2000; McCusker et al. 2000; Comps et al. 2001; Koskinen et al. 2002; Costello et al. 2003; Stamford and Taylor 2004). Therefore, it is reasonable to expect that those processes can be inferred from patterns of genetic variation. Several studies of molecular variation have demonstrated the influence of postglacial colonization on genetic variability in contemporary populations (e.g. Merila et al. 1996; Bernatchez and Wilson 1998; Bos and Sites 2001; Koskinen et al. 2002; Costello et al. 2003). The result is typically one of reduced levels of variation within populations, attributable in part to bottlenecks and founder events associated with dispersal into previously unoccupied areas (Nei et al. 1975; Sage and Wolff 1986).

The last glacial event (ending 10-12,000 years ago) had a major impact upon BC's freshwater fish fauna including effects on patterns of genetic variation within and between populations. Glacial advances and associated destruction of habitats presumably reduced genetic diversity and limited distribution to relatively small ice-free regions called glacial refugia. Several refugia have been hypothesized in and around BC that may have supported rainbow trout and other species. These include the Columbia River, the Chehalis River at the south

margin of the Olympic Peninsula, the Brooks Peninsula on north-western Vancouver Island, the Queen Charlotte Islands, Beringia (Yukon River valley and the exposed portion of the Bering Strait area), and the Nahanni River region in the Northwest Territories and north-eastern BC (Behnke 1992; McPhail and Carveth 1992). McCusker et al. (2000) addressed the role of glaciation and post glacial recolonization, its impact on the distribution of rainbow trout genetic diversity, and the refugial origin of rainbow trout found throughout BC. Their results suggested that there were at least two glacial refugia that supported rainbow trout that subsequently colonized BC following the last glacial event. These two regions were a portion of the Queen Charlotte Islands and the lower Columbia River, and populations in these areas had relatively high mitochondrial haplotype diversity and sequence divergence (McCusker et al. 2000). Consistent differences in allozyme allele frequencies and morphology also distinguished two geographical groups of rainbow trout in BC, an inland group and a coastal group, east and west of the Cascade-Coast Mountains (Huzyk and Tsuyuki 1974; Allendorf 1975; Okazaki 1984; Parkinson 1984; Currens et al. 1990; Behnke 1992; Taylor 1995). Other non-genetic data further supports the Columbia River as a glacial refuge for freshwater fish species which later colonized most of BC, i.e., high faunal similarity between the Columbia River and major northern watersheds including the Fraser River, Skeena River, Nass River, and Stikine River (faunal similarity 84%, 77%, 80%, and 71%, respectively, McPhail and Lindsey 1986).

Zoogeography of microsatellite DNA variation among populations

My results detected genetic patterns indicative of post glacial colonization and provided further support for coastal and interior lineages of rainbow trout within BC. After analysing the N-J tree based on CSE distance and PCA of allele frequencies, my results demonstrated a strong broader grouping of coastal and interior populations as well as groupings based on watersheds within these regions. The aggregation of populations into interior and coastal groups is consistent with many genetic and non-genetic studies that have demonstrated similar results within *Oncorhynchus mykiss* (Huzyk and Tsuyuki 1974; Allendorf 1975; Okazaki 1984; Parkinson 1984; Currens et al. 1990; Behnke 1992; Taylor 1995). Groupings based on major watersheds within coastal and interior lineages were evident and were supported with relatively high bootstrap values. Other studies using microsatellite DNA to infer historical evolution of genetic diversity found similar results, suggesting that microsatellite DNA is useful in detecting major lineages and further supports results collected from mtDNA (brook char, *Salvelinus fontinalis*, Angers and Bernatchez 1998; European grayling, *Thymallus thymallus*, Koskinen et al. 2001, 2002; and lake herring, *Coregonus artedii*, Turgeon and Bernatchez 2001).

An analysis of molecular variance (AMOVA) demonstrated that groupings of coastal and interior lineages explained a small, but significant fraction of the genetic variation observed compared to groups based on major watersheds suggesting that variables other than glacial history may explain more of the

observed variation. Similarly, Knudsen et al. (2002) found that 15.5% of the molecular variation in microsatellites was explained by differences between watersheds for rainbow trout populations in the Kootenay River. Consequently, these analyses both across broad and narrower geographic scales suggest that: (i) there is considerable demographic independence among these watersheds since time of colonization, and (ii) more contemporary geographic features or geographic variables across the riverscape may influence historical patterns of genetic variation.

Interior lineage: Southern Columbia River

The genetic signature of postglacial colonization was also evident from my analyses as demonstrated by a significant relationship between latitude, longitude, and geographic distance with measures of genetic variation (H_e , A , and A_r). Within the interior lineage, mapping the changes in genetic variation with geography suggested that postglacial colonization occurred west of the continental divide (from the Columbia River vicinity) and may have expanded north-west, rather than in a strictly south-north manner. As rainbow trout dispersed north-west the cline in genetic variation persisted up to just east of the Coast Mountain Range and west of the Rockies, through the tributaries of the upper Fraser River. Populations at the periphery of the range in interior rainbow trout were expected to possess lower levels of genetic variation compared to populations at or closest to its glacial refuge (Avice 1994; Hewitt 1996; reviewed in Bernatchez and Wilson 1998). My results were consistent with this

expectation based on comparing genetic variation between populations within the Columbia River watershed to populations in the Thompson and upper Fraser River watersheds. Assuming that rainbow trout that colonized the Thompson and upper Fraser rivers were from the same origin as the Columbia River rainbow trout, Thompson and upper Fraser River populations demonstrated a loss of rare alleles that were present in more southern localities (e.g., populations from the upper Columbia River watershed). Similar trends can be found among other species of salmonids in BC including bull trout where more northerly populations within drainages demonstrated lower levels of genetic variation than populations residing closer to the south (Costello et al. 2003).

Furthermore, my results support previous studies that suggested that northern interior regions of BC were more recently colonized than those located farther south (McPhail and Lindsey 1986). The timing and patterns of historical colonization have affected relative levels of genetic diversity between regions. Populations at the periphery of the interior lineage such as the upper Fraser River population chains (Skinny Lake chain, Glatheli Lake chain, Nutli Lake Chain, and Blanchet Lake chain) did not show any significant pattern of isolation-by-distance (IBD). In contrast, population chains in the upper Columbia River and Thompson River (Deadman and LNTH Lake chains), which presumably have had more time to develop such a pattern, did show significant relationships of IBD. Given its more northerly location, the upper Fraser River was colonized later in deglaciation than the upper Columbia River (McPhail and Lindsey 1986)

as also demonstrated by the loss of rare alleles found among populations from the Columbia River watershed. Furthermore, the upper Columbia River was connected directly to refugial populations of rainbow trout in the south, while the upper Fraser River was recolonized more indirectly by glacial lakes between the extant Fraser and Columbia rivers (McPhail and Lindsey 1986). Twice during deglaciation ice re-advanced across the northern plateau and blocked the Fraser River's southward flow (McPhail and Lindsey 1986). These blockages created a series of glacial lakes which later allowed faunal exchange with the Columbia-Fraser-Peace-Mackenzie watersheds. Populations in the upper Fraser River may have been influenced by changes in drainage patterns and re-advances of ice. Such consequences may have involved reshuffling of individuals and the colonization of once occupied habitats at a later date compared to more southern populations.

Although the southern population chains (upper Columbia River, LNTH and Deadman Lake chain) showed significant IBD, there was no cline in the significances of IBD with increasing latitude. In fact, both LNTH and Deadman lake chains (north of the Columbia River population chain) demonstrated higher correlations between geographic distance and genetic distance compared to the Columbia River population chain which was not expected. The general pattern of deglaciation in BC particularly in the lowland areas was a rapid ice retreat northwards up major valleys and back into mountain areas (McPhail and Lindsey 1986). The rapid ice retreat may have resulted in the colonization of the upper

Columbia tributaries simultaneously or just before the colonization of the Thompson River watershed through large pro-glacial lakes, resulting in relatively similar levels of IBD equilibrium. Alternatively, local geography and life history characteristics of resident rainbow trout may reflect differences in the degree to which dispersal is limited within each region which could also influence patterns of IBD. As demonstrated earlier, populations above migration barriers have a large impact on the significance for the detection of IBD. This has also been found among other salmonid species in BC (bull trout, *Salvelinus confluentus*, Costello et al. 2003; and westslope cutthroat trout, *Oncorhynchus clarkii lewisi*, Taylor et al. 2003).

Alternatively, the differences in detection of migration-drift equilibrium may be the result of differing effective population sizes. The time to reach migration-drift equilibrium will depend on the effective population size or the inverse of migration rate, whichever is greater (c.f. Hartl and Clark 1989; Turgeon and Bernatchez 2001). The LNTH and DEAD Lake chains may have higher effective population sizes than those from the Columbia River chains and consequently would likely be more near, or in, migration-drift equilibrium.

Other geographic variables that I have not taken into consideration may also influence the degree of dispersal or migration (Bisson et al. 1988; Muhlfeld et al. 2001; Weigel and Sorensen 2001; Bramblett et al. 2002; James and Graynoth 2002) and possibly any patterns of IBD. For instance, the fluvial distance

between populations among the LNTH Lake chain and Deadman Lake chain were small relative to the Columbia River chain (0.6 to 73 km and 0.5 to 12 km versus 0.75 to 130 km, respectively). Perhaps patterns of IBD can only be detected when populations analysed are separated by large geographic distances. Likewise, the number of localities used to detect IBD, and the type of habitat may be important in affecting patterns of IBD (discussed in Chapter 3).

Coastal lineage: Queen Charlotte Islands

The north-east corner of Graham Island (Queen Charlotte Islands) is one of the few areas in BC that was ice free during the last glaciation (Warner et al. 1982). Evidence exists suggesting that the Queen Charlotte Islands was a refuge both for plants and animal species (e.g., black bear, Byun et al. 1997; black stickleback, Moodie and Reimchen 1976; plants, Calder and Taylor 1968). In *Oncorhynchus mykiss*, high mtDNA diversity was found among Queen Charlotte Island populations compared to other populations in BC (McCusker et al. 2000) which further suggests that this area was a refuge.

Results from my study using microsatellite DNA variation support the idea that the Queen Charlotte Island or at least that a more general coastal refuge was present. Based on the grouping of coastal populations from the CSE distance N-J tree and the PCA of allele frequencies, I found that there were genetic similarities among populations derived from the same refuge which would group together forming the coastal group. Coastal populations greater than 450

kilometres (Moosevale Creek and Eaulve Lake) from the Queen Charlotte Islands grouped among the other coastal populations compared to a few interior populations which were geographically more proximate to the Queen Charlotte Islands (e.g., < 350 km, Skinny Lake chain, Blanchet Lake chain). Such data suggest that regardless of geographic proximity, populations originating from a common source cluster together in a N-J tree or a PCA of allele frequencies. The only exception was Khatada Lake (Skeena River watershed) which did not group within the coastal populations. Khatada Lake is a piscivorous population characterized by low population size (Parkinson, E., BC Ministry of Water, Land, and Air Protection; Pers. Comm.) and consequently the fixation of alleles and high genetic differentiation, as compared to other nearby populations, was not surprising. The reduced variation within populations tends to exaggerate measures of interpopulation divergence such as F_{st} (Hedrick 1999) and consequently the grouping of Khatada Lake separately from most other populations may be an artefact of its extremely low intrapopulation variation. Even among the most variable loci (*Okai3*) in this study, Khatada Lake was fixed for one allele.

A genetic signature of postglacial colonization was not clearly evident from my analyses among populations within the coastal group of *O. mykiss*. In addition, no correlations were found between straight line geographic distance and measures of genetic variation. I did find, however, two populations which had very high levels of genetic variation and low levels of genetic differentiation when

compared with Queen Charlotte Islands' populations: Gold River and Nimpkish River. Both rivers were furthest away from the presumed heart of the coastal glacial refuge (Queen Charlotte Islands) and yet demonstrated lower levels of genetic differentiation from the Queen Charlotte Island populations compared to the remaining mainland-coastal populations. Removing these two Vancouver Island populations, however, did not result in significant correlations between genetic variation and latitude, longitude and straight line geographic distance.

The greater potential dispersal among populations of anadromous trout is likely to explain for the lack of relationship between measures of genetic variation and latitude, and longitude, and geographic distance. Fish species limited to dispersal by river and streams often demonstrate high genetic-population subdivision (F_{st}) compared to marine species (Gyllenstein 1985; Ward et al. 1994). Greater connectivity resulting in the exchange of individuals (gene flow) would result in reduced levels of genetic differentiation and population subdivision, but also maintain levels of genetic variation (Hartl and Clark 1989). The anadromous populations (Queen Charlotte Island and Vancouver Island populations) in my study not only demonstrated relatively higher levels of genetic variation as compared to lacustrine populations but also demonstrated low levels of genetic differentiation. Microsatellite based assays of anadromous rainbow trout show F_{st} values that are consistently low, ranging from 0.01 to 0.07, and may reflect the greater connectivity of steelhead trout promoted by their anadromous behaviour (Reisenbichler and Phelps 1989, see also for cutthroat

trout Weinburg et al. 1998; see also Bouza et al. 1999 for brown trout, *Salmo trutta*). The potential for ongoing gene flow between the Queen Charlotte Island populations and those on Vancouver Island may, therefore, make detection of historical processes like postglacial colonization difficult.

The amount of variation explained by differences between coastal and interior lineages was small relative to differences explained between populations within these groups. The time span available since deglaciation may have been too short to result in the accumulation of substantial differences between groups (e.g., mutations) (Gyllensten 1985) and consequently very little variation could be explained between the two groupings of coastal and interior lineages. One possibility is that more contemporary factors such as drainage patterns are likely to supersede the effects of historical recolonization. Costello et al. (2003) found that migration barriers to dispersal played an important role in explaining the observed patterns of genetic variation in bull trout and that contemporary hydrological features are capable of masking patterns of genetic variation from historical events.

The presence of unique alleles supports the concept of distinct inland and coastal lineages. Following the clustering of populations into the coastal and interior groups from results of CSE distance N-J tree and the PCA of allele frequencies, I further investigated the presence of unique alleles specific to each lineage and also the presence of clinal variation at each microsatellite locus.

Others have also found distinct clustering of different lineages using microsatellite DNA, and in some cases alleles unique to specific lineages were also found (Angers and Bernatchez 1998; Turgeon and Bernatchez 2001; Koskinen et al. 2001, 2002). Turgeon and Bernatchez (2001), however, also documented clinal variation in allele frequencies characteristic of lake herring from different areas of North America which further supported the existence of distinct lineages within this species, but also revealed the direction of postglacial dispersal and areas of secondary contact between lineages. In my study I also found alleles unique to each lineage; however, there was no noticeable cline on frequency of these unique alleles from the heart of the lineage's range. The lack of such clines may reflect the relatively small geographic distances involved. The maximum distance between localities within the coastal and interior lineages of rainbow trout was 1000 and 900 kilometres, respectively, whereas the study on lake herring spanned most of northern North America (Turgeon and Bernatchez 2001).

Conclusion

Relatively few studies have demonstrated the usefulness of microsatellite DNA to detect major historical events shaping a species' current distribution. Once predominately used for investigating genetic population structure on localized scales, microsatellites are now becoming utilized for more than just local scale structuring and or being used to investigate the influence of major historical events as demonstrated here. Results from my study corroborate and extend earlier studies using both molecular and non-molecular techniques and indicate that there are two major groups of rainbow trout in BC, an interior group originating from the south of BC and a coastal group. Pattern of post-glacial colonization was detected as a cline in genetic variation within the interior lineage, but not as clearly within the coastal lineage possibly because of lack of sufficient sample size or ongoing gene flow in the latter. The postglacial colonization of salmonid fishes in BC has been relatively recent and my results coupled with those from other species suggest that not enough time has passed for most populations to reach migration-drift equilibrium. Local geography has the ability to influence patterns of IBD observed in the nature by affecting migration as seen by above barrier populations in this study and other salmonid studies. As evidenced by their influences on the clustering of populations with N-J trees and PCA analysis and by its accounting for a large portion of the genetic variation observed among natural populations, the contemporary patterns of watershed interconnectedness, clearly can act to influence population structure and patterns of IBD. The influence of local geography on patterns of genetic

diversity is relatively unknown and my third chapter will address the role of local geography in shaping the genetic diversity in natural populations.

Figure 2.1 Map of rainbow trout (*Oncorhynchus mykiss*) localities examined in the study. Sample sites are identified by the circled numbers which correspond to the population numbers found in Table 2.1. Insets represent population chains.

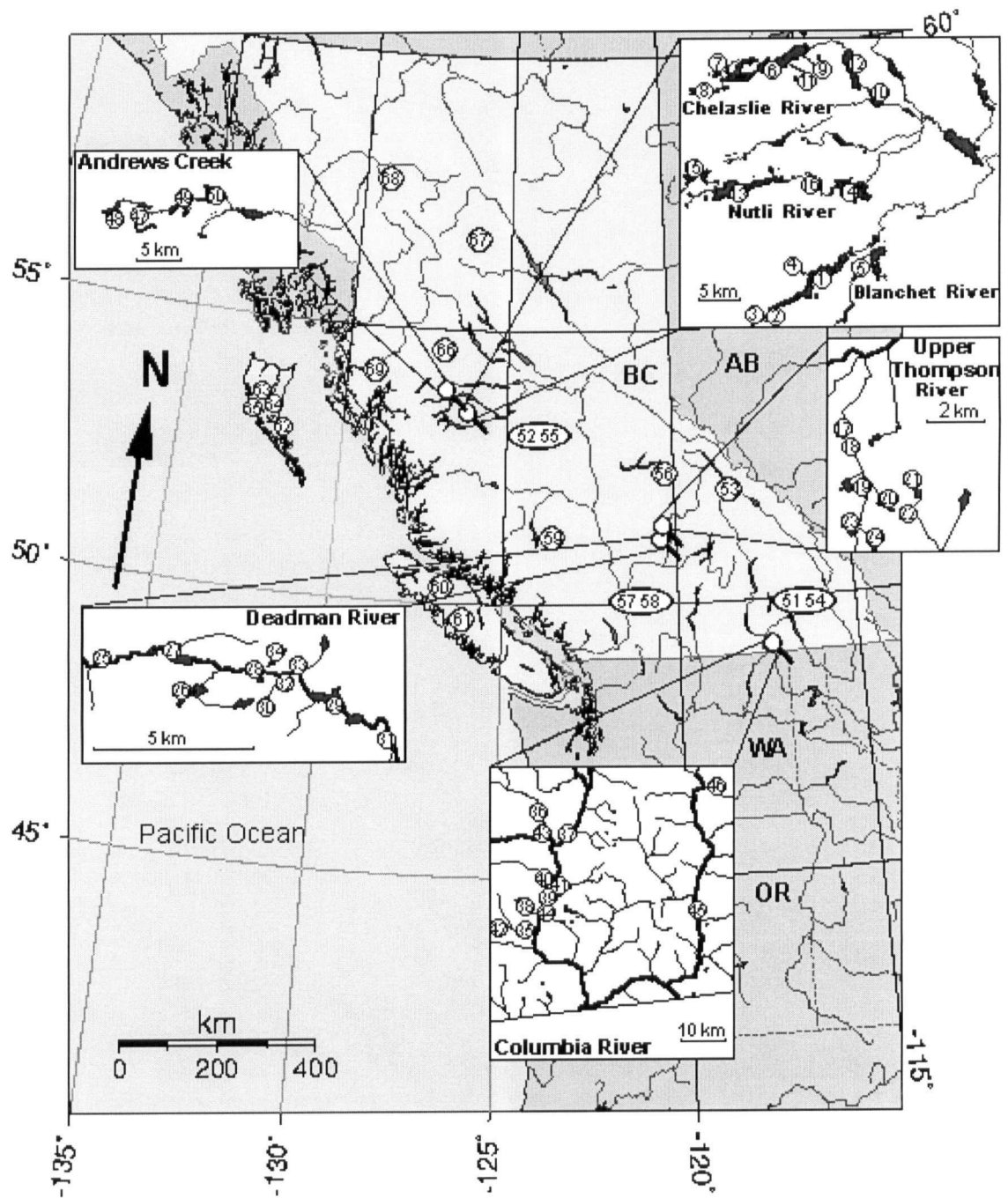


Figure 2.2 Distributions of phylogenetic groups of *Oncorhynchus mykiss* in British Columbia (BC, adapted from McCusker et al. 2000; Stamford 2002). The light shaded area shows the range of rainbow trout and steelhead in BC and dark shaded lines indicate the breaks between the geographically contiguous phylogenetic groups. Arrows show postglacial dispersal routes inferred from the geographic contiguity of each lineage and their association with the Haida Gwaii and Columbia River glacial refugia which are underlined. Arrows also show which phylogeographic groups occur together in the dark shaded areas.

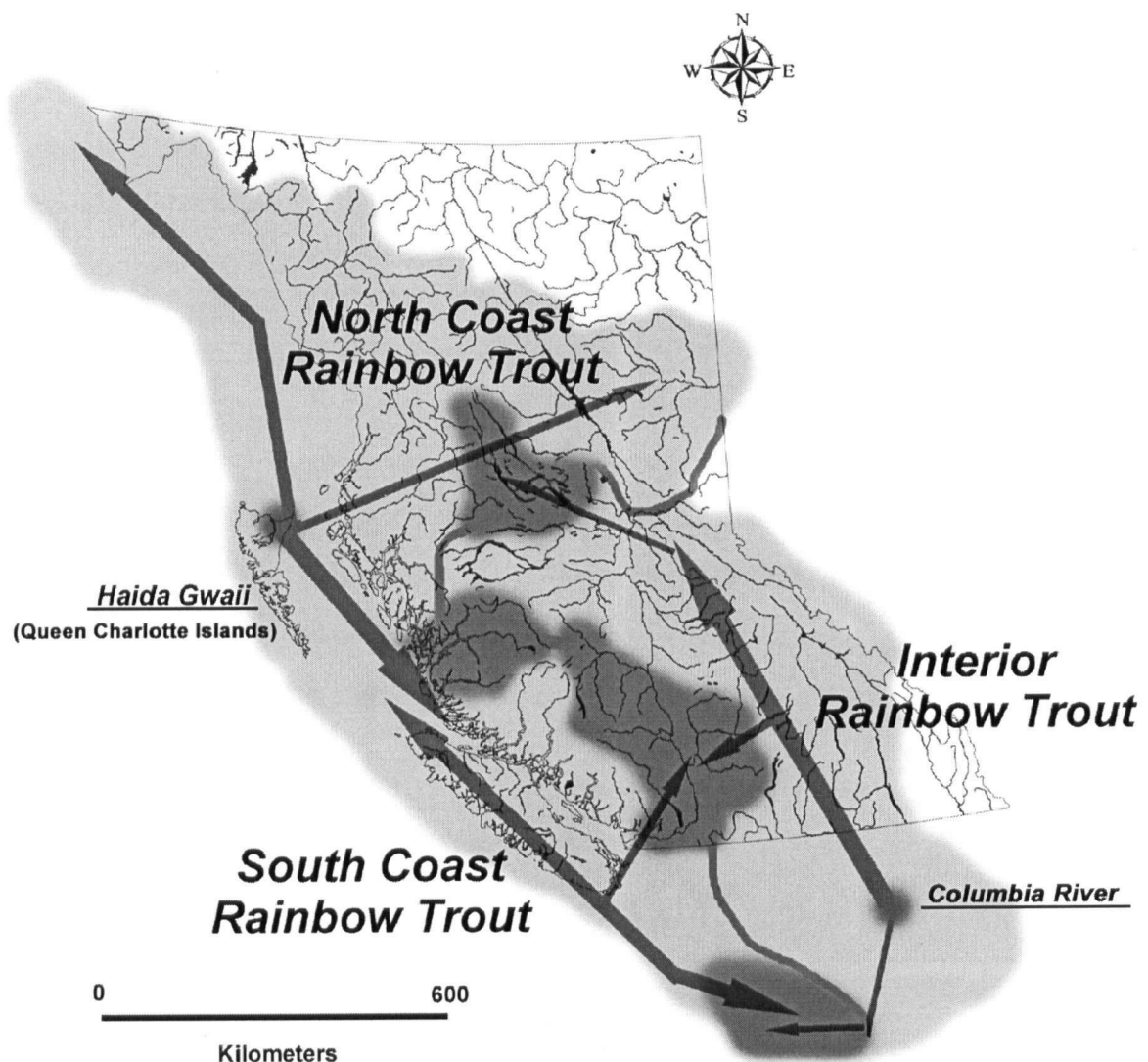
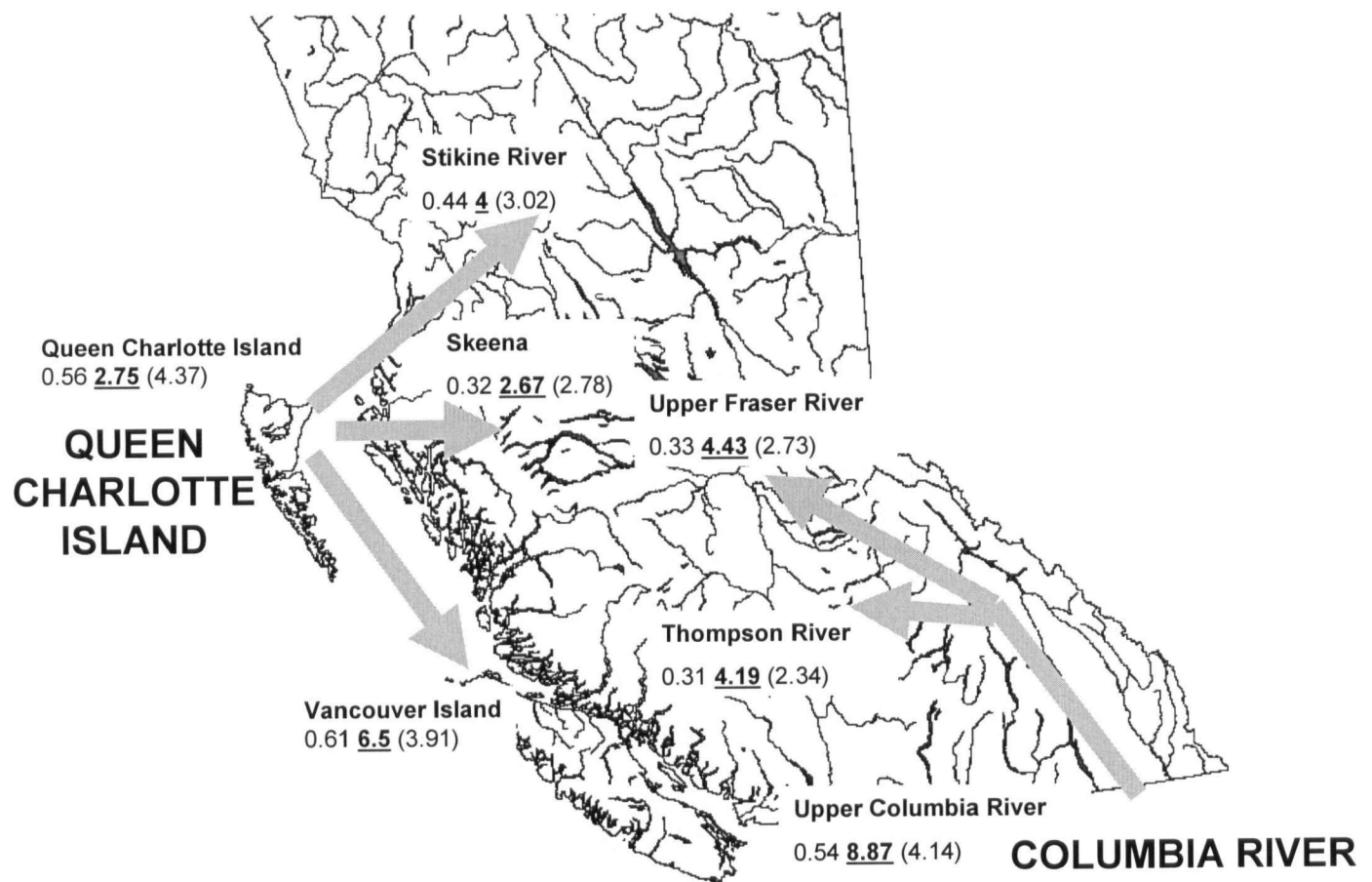


Figure 2.3 Geographic distribution of genetic variation at microsatellite loci among populations of rainbow trout (*Oncorhynchus mykiss*) from major watersheds. Expected heterozygosity, mean number of alleles per locus (in bold and underlined) and allelic richness (in parentheses) are shown. Arrows show inferred postglacial dispersal routes from glacial refugia, labelled with large font (see Figure 2.2).



INTERIOR



Figure 2.5 Results of principal components analysis of allele frequency variation in *Oncorhynchus mykiss* assayed at ten microsatellite loci depicted as plots of mean component scores for each population along axes 1 and 2. Population numbers are defined in Table 2.1. Population sites are colour coded to represent the major drainage it belongs to. Groups of populations belonging to a major drainage are included within ellipses with the exception of coastal populations which were combined (green labelled). Note that for visual clarity, some populations from the upper Fraser, upper Columbia, and Thompson River drainages were not circled. Above barrier populations are underlined. Above barrier populations are underlined.

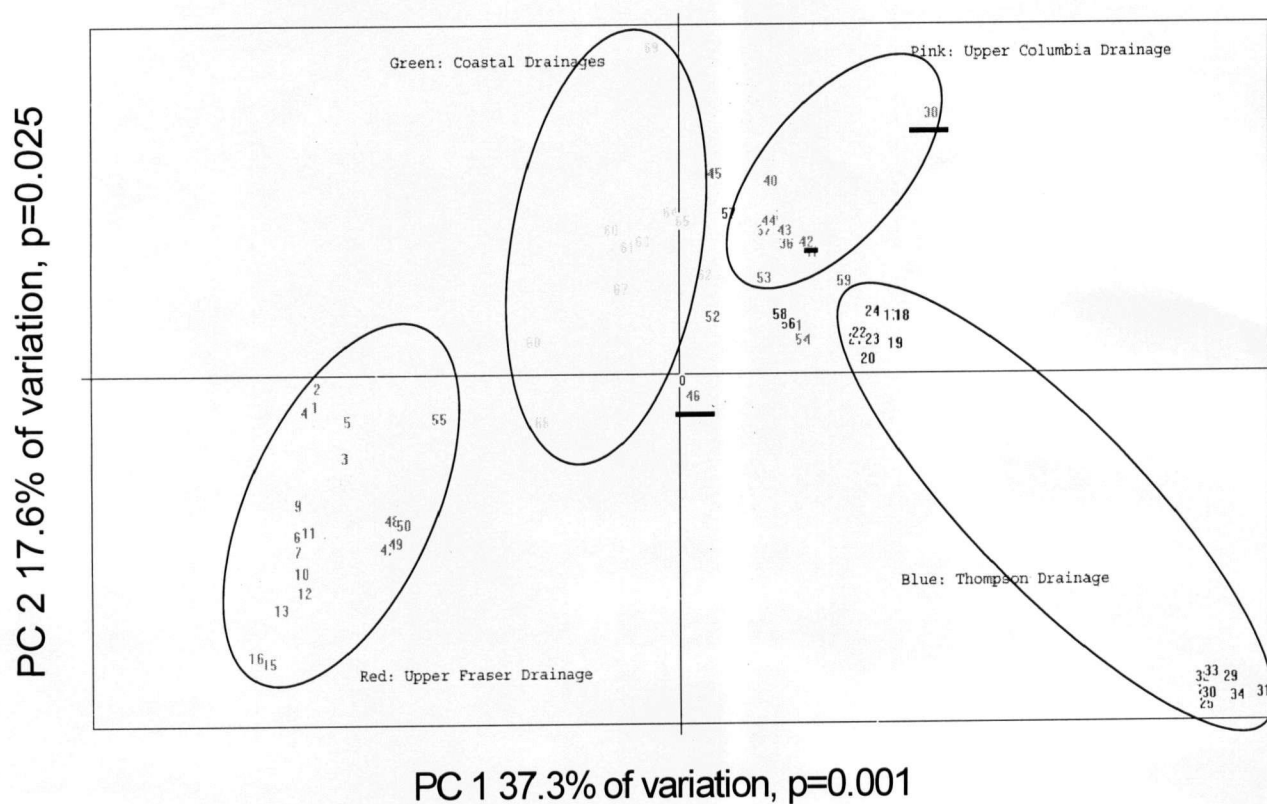
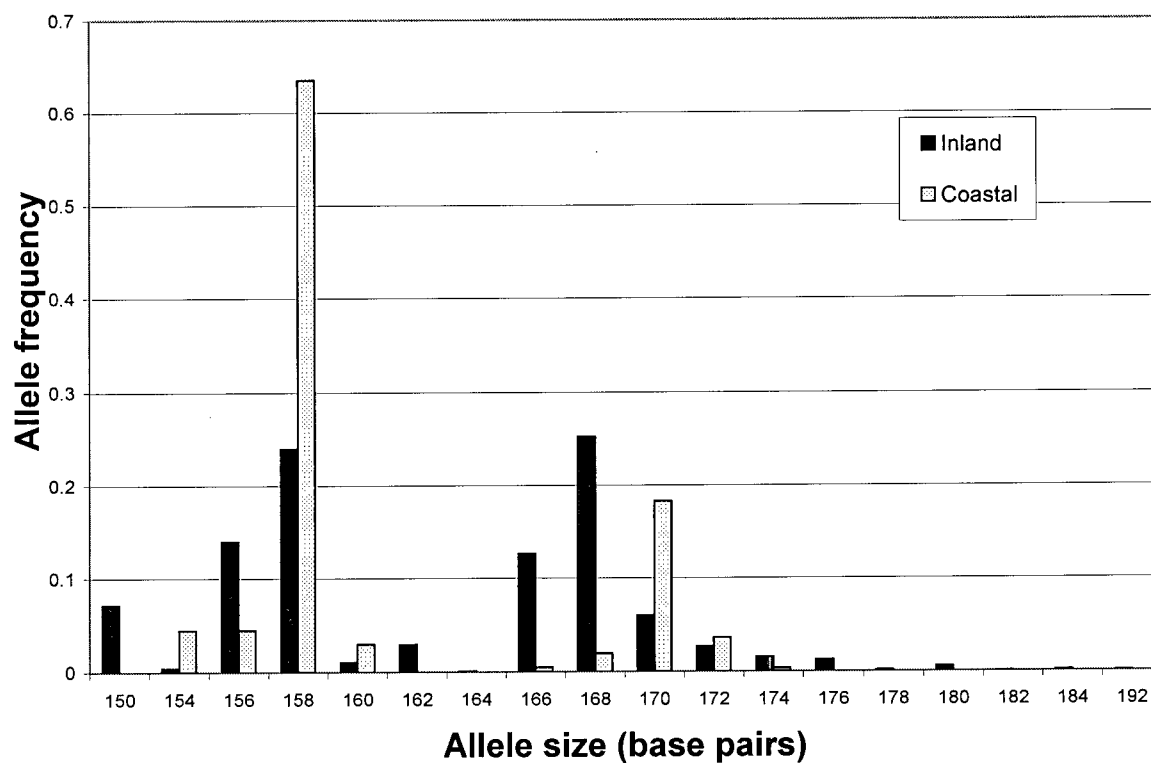
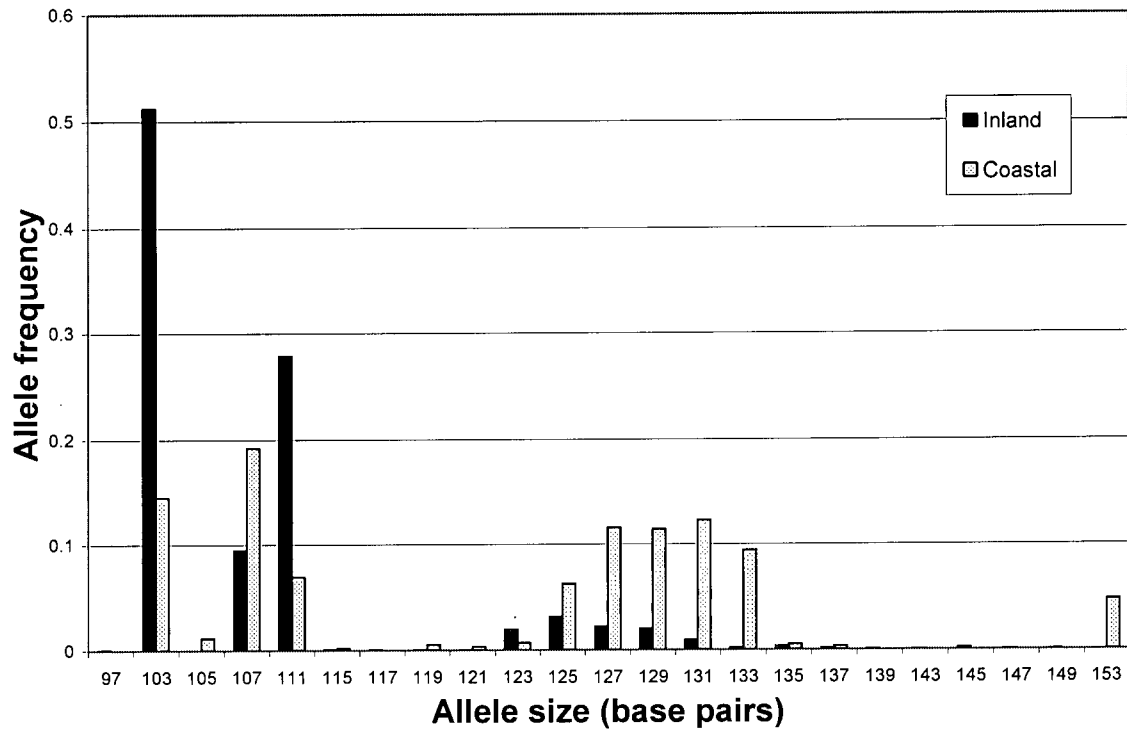


Figure 2.6 Allele frequencies at microsatellite loci found among population groups of rainbow trout (*Oncorhynchus mykiss*) from coastal or interior origin (refer to Figure 2.2, Table 2.1).

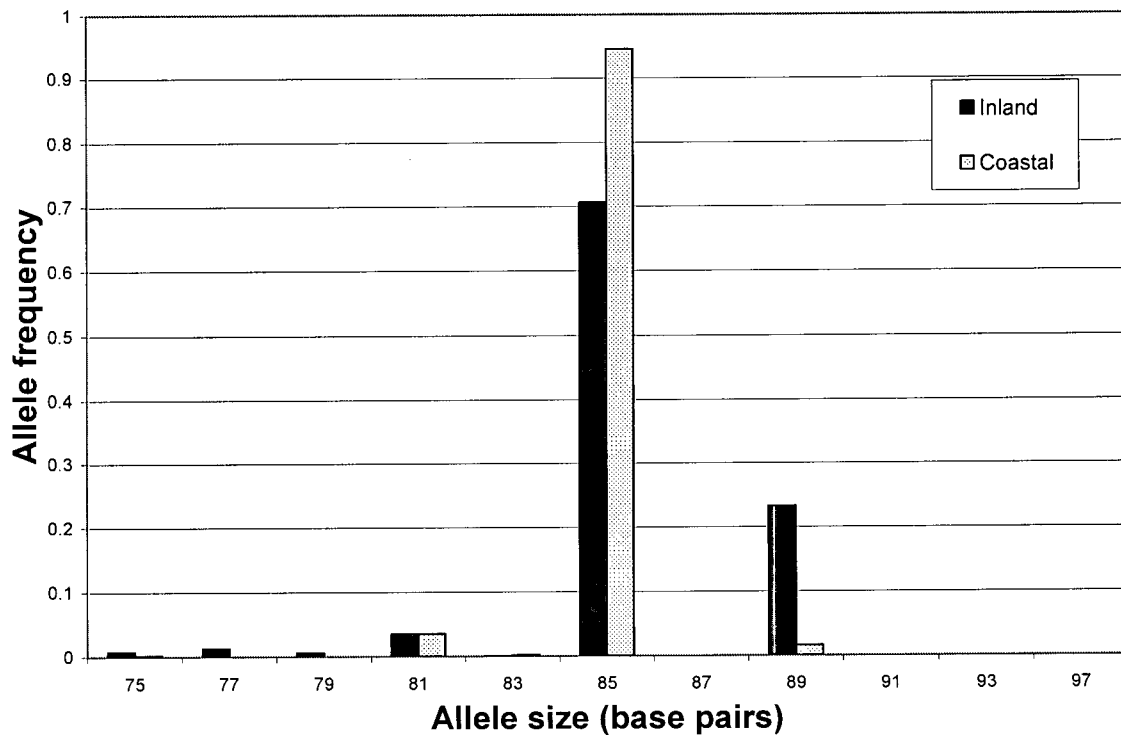
A. Allele frequencies at the Oneu8 locus among interior and coastal groups.



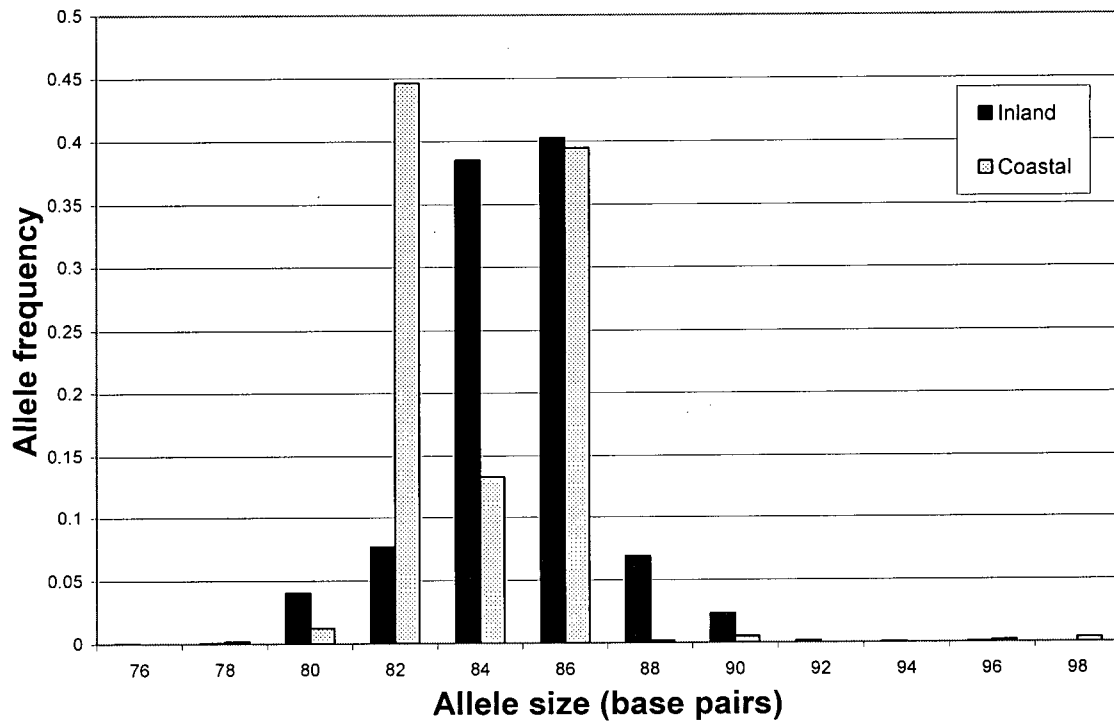
B. Allele frequencies at the Ssa85 locus among interior and coastal groups.



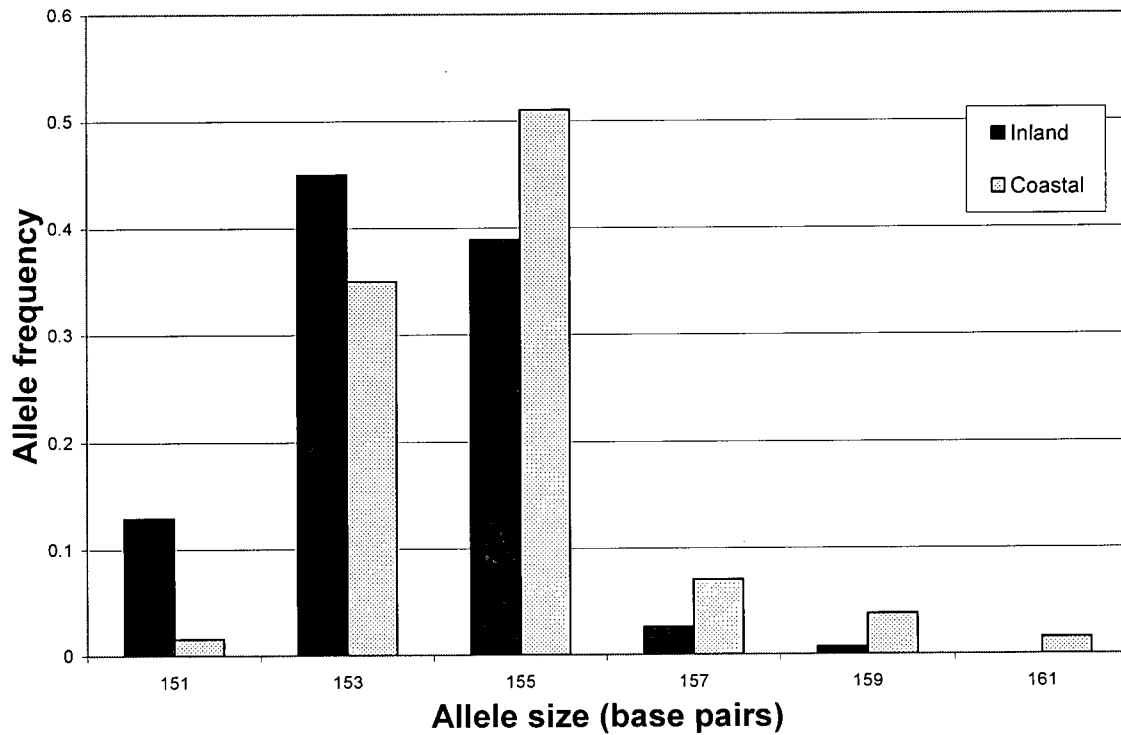
C. Allele frequencies at the Ots103 locus among interior and coastal groups.



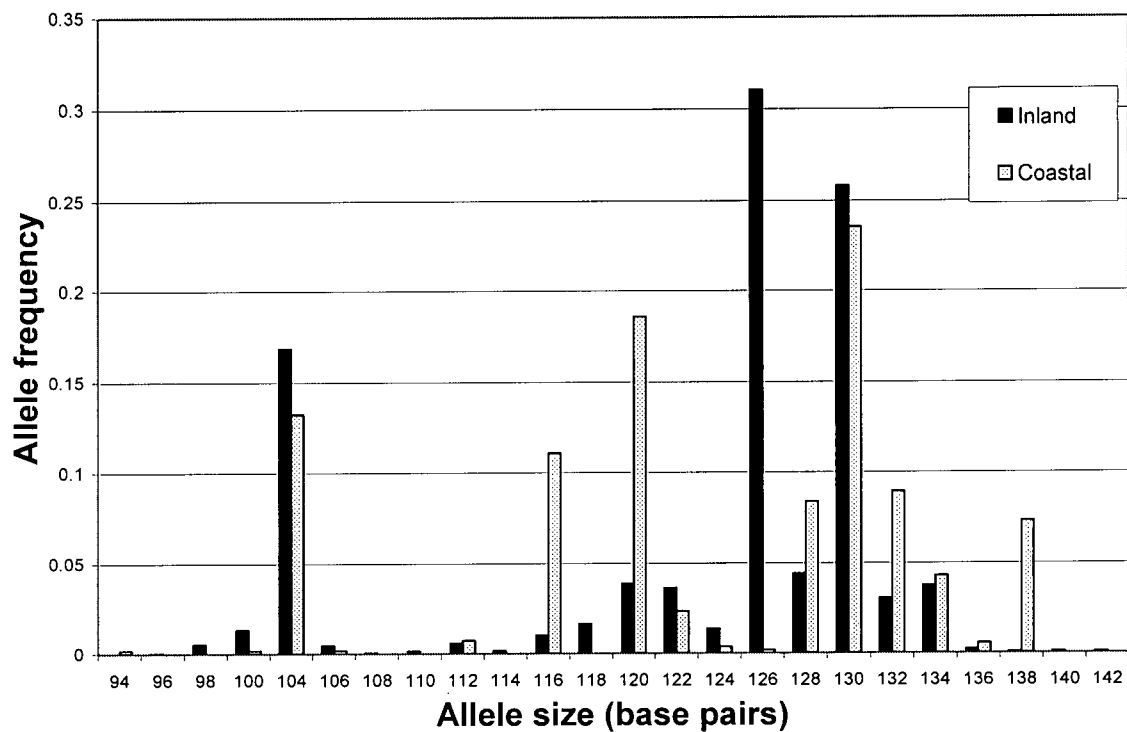
D. Allele frequencies at the Ots3 locus among interior and coastal groups.



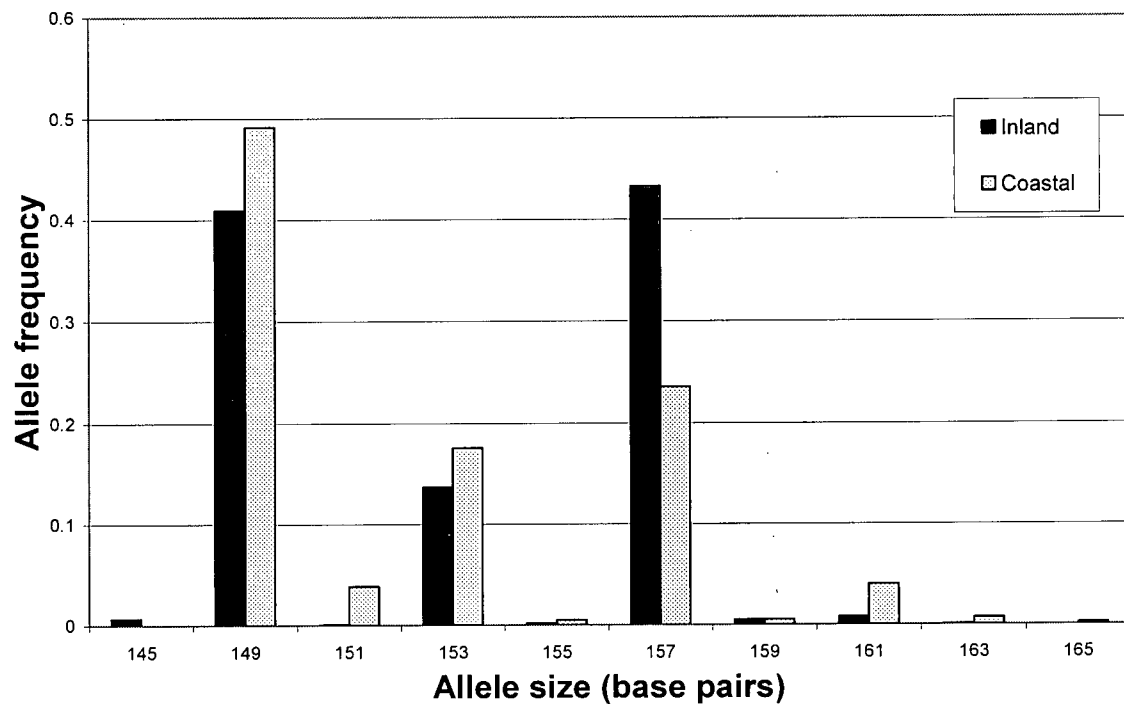
E. Allele frequencies at the Ssa456 locus among interior and coastal groups.



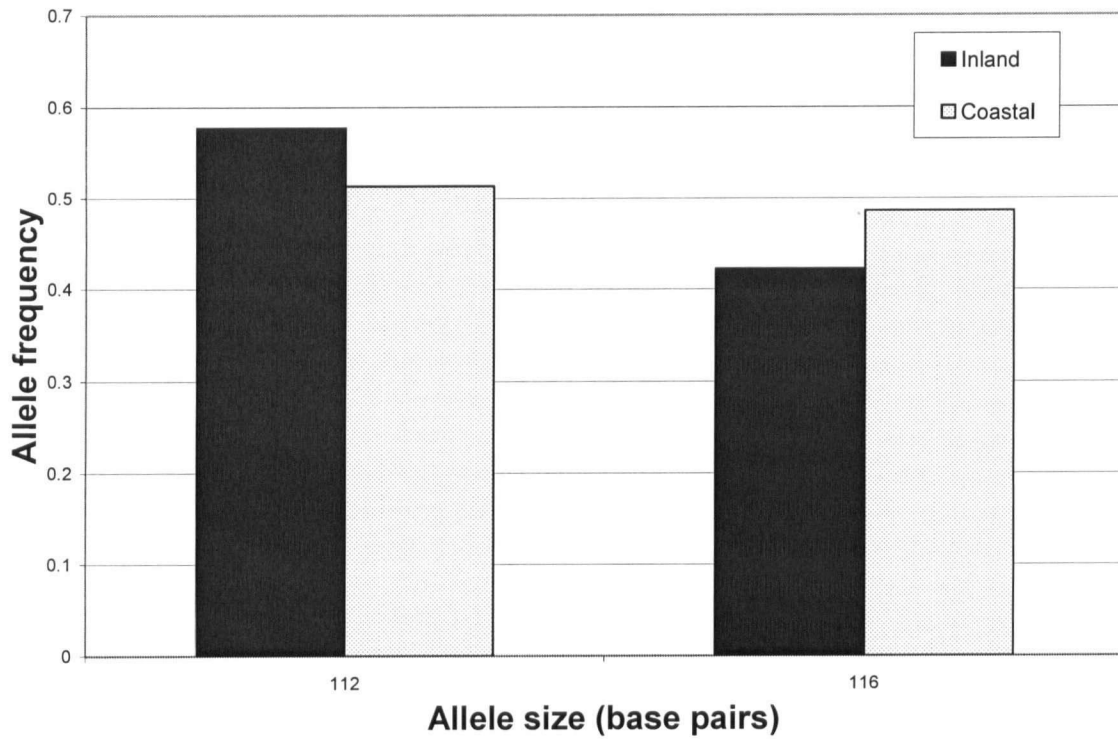
F. Allele frequencies at the Omy77 locus among interior and coastal groups.



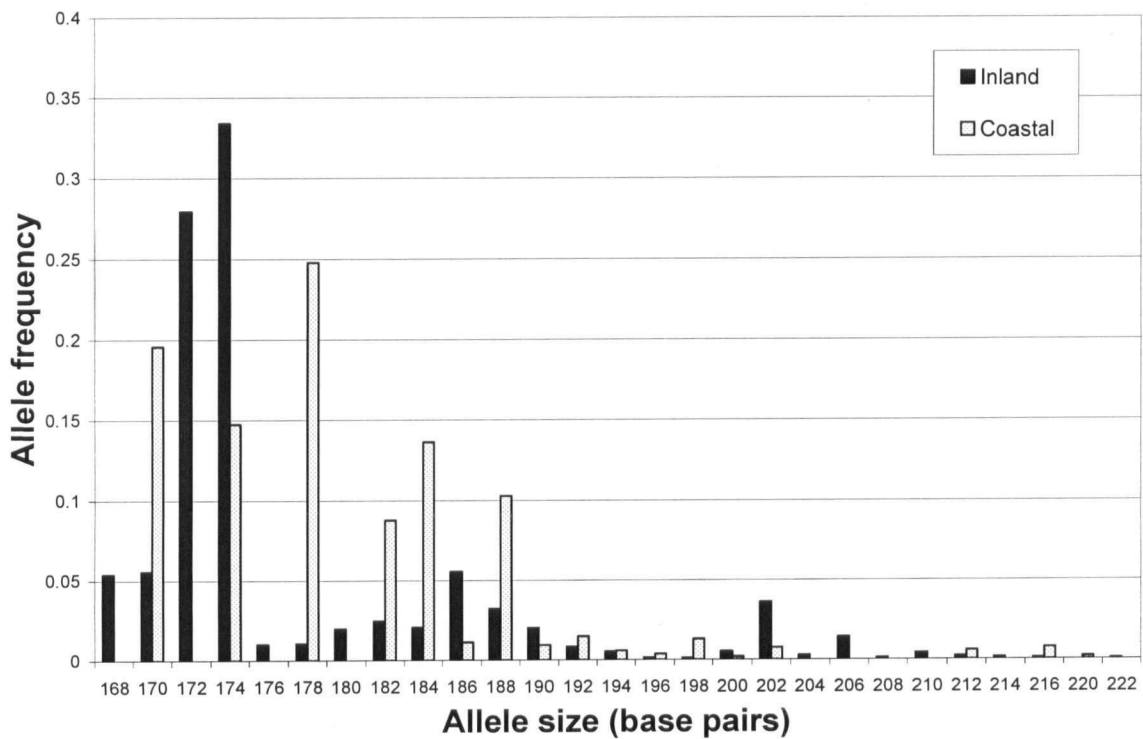
G. Allele frequencies at the Oneu14 locus among interior and coastal groups.



H. Allele frequencies at the Ssa197 locus among interior and coastal groups.



I. Allele frequencies at the Ots100 locus among interior and coastal groups.



J. Allele frequencies at the Okia3 locus among interior and coastal groups.

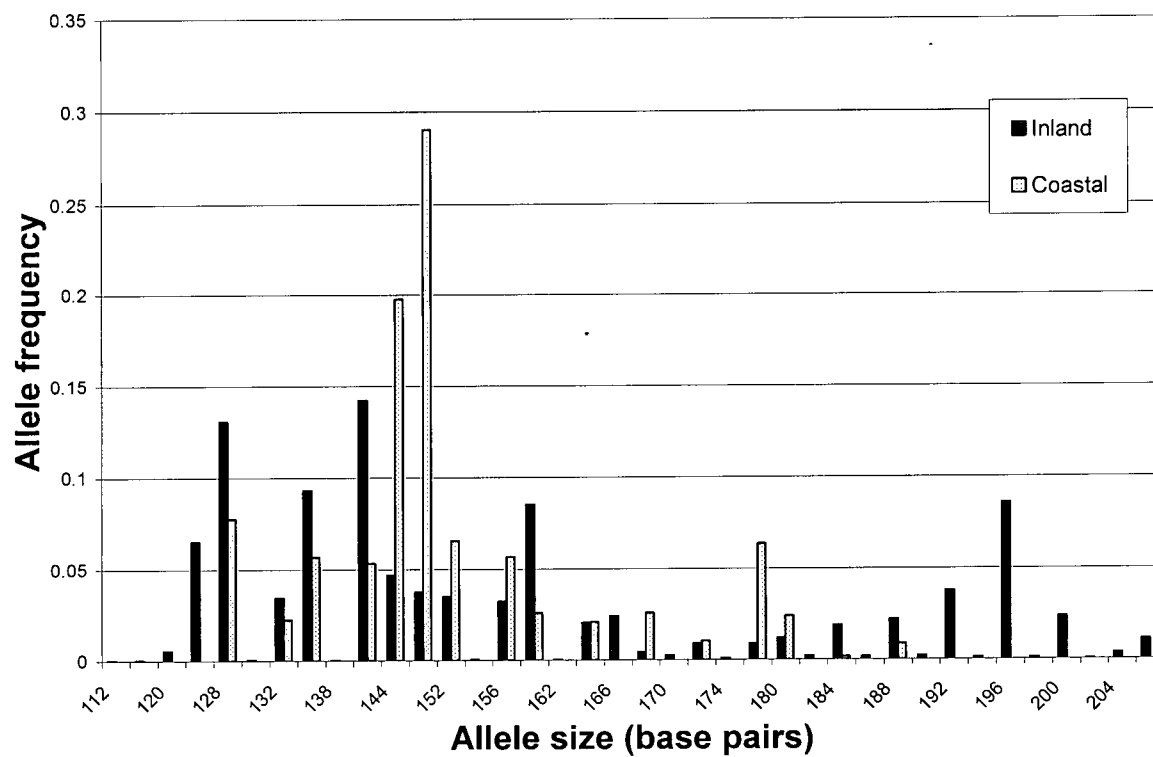
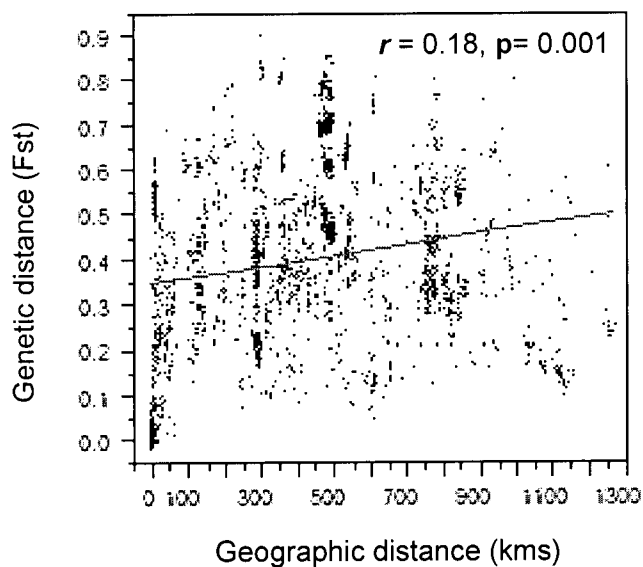
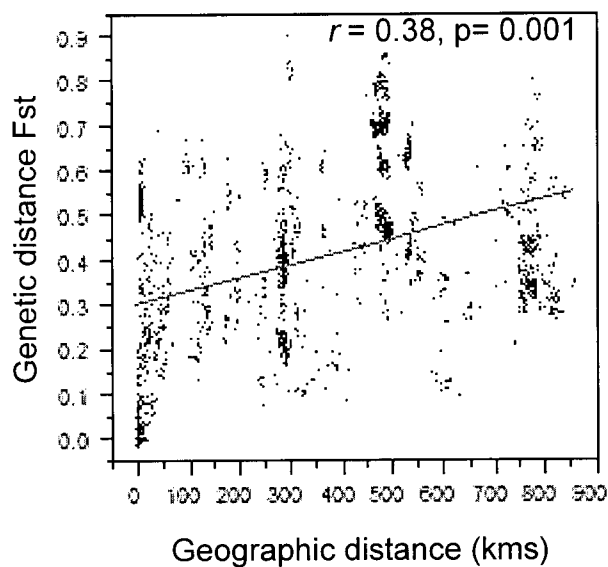


Figure 2.7 Isolation by distance analyses (IBD) for *Oncorhynchus mykiss* populations throughout BC (A), throughout populations from the Columbia lineage (B), and population chains in the interior lineage (C-F) assayed at ten microsatellite loci. Pairwise F_{st} (θ) distances (y-axis) are plotted against pair-wise geographic distances (x-axis) for all populations.

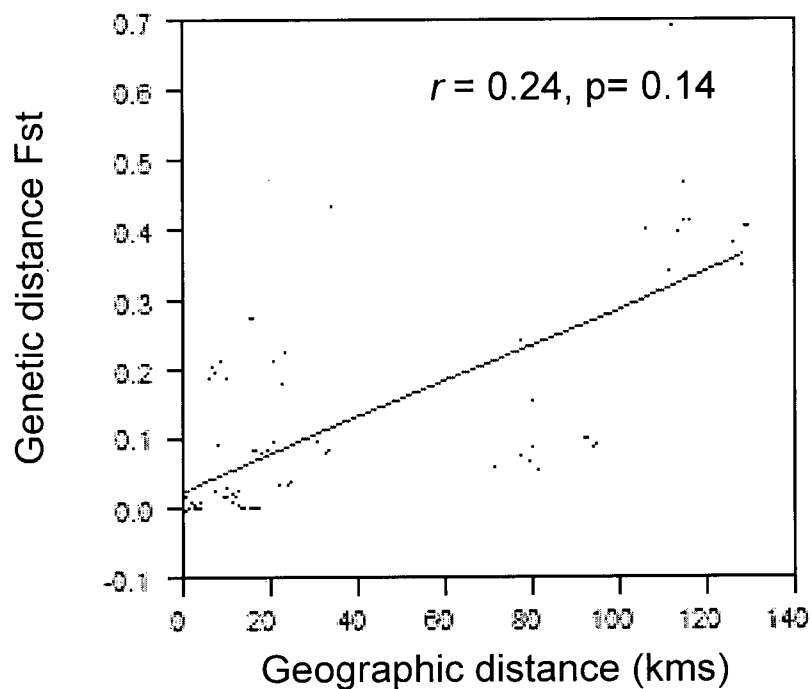
A. All populations (N=69)



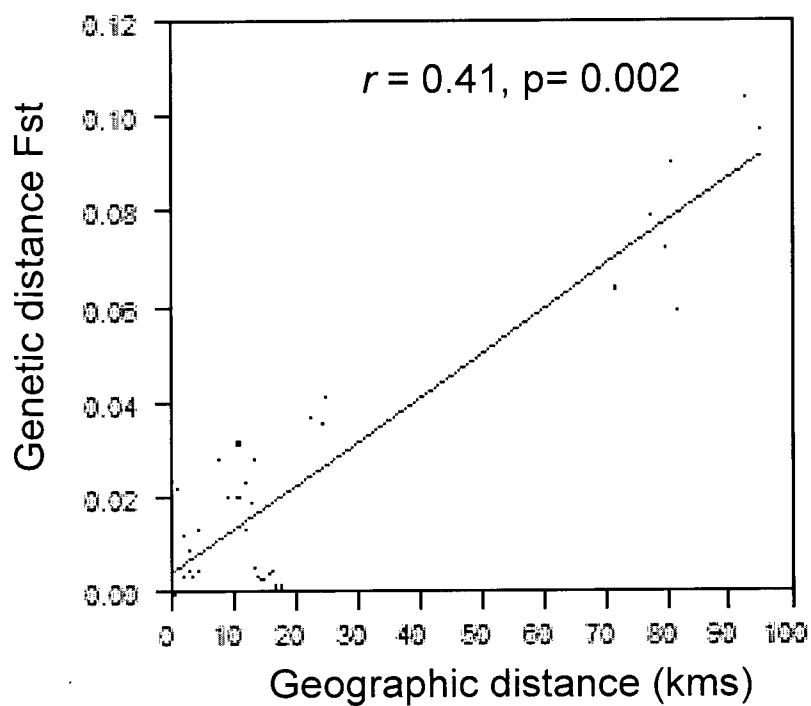
B. Within interior lineage (N=59)



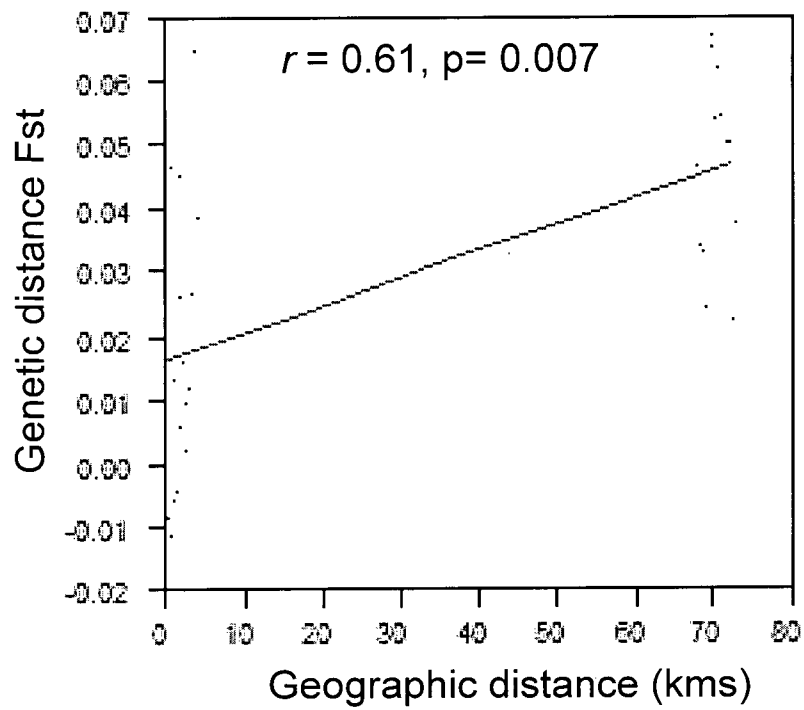
C. Within Columbia River population chain (N=12)



D. Within Columbia River population chain without above barrier populations (N=9)



E. Within lower North Thompson River lake chain (N=8)



F. Within Deadman River lake chain (N=10)

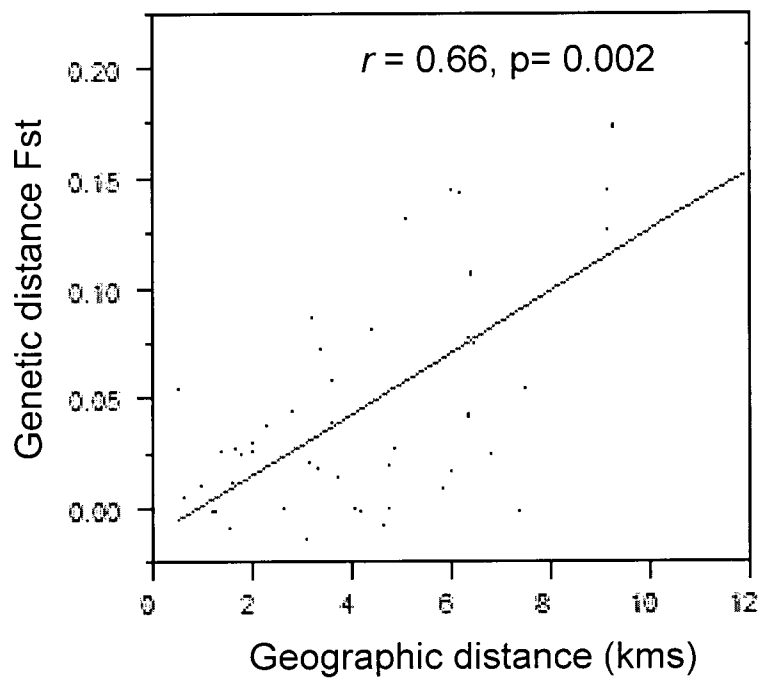


Table 2.1 Name, sample size, latitude and longitude, and drainage (watershed) location of the sample locality of rainbow trout (*Oncorhynchus mykiss*) examined. Populations located above migration barriers are in bold face type.

Population number	Sample locality	Sample size	Latitude	Longitude	Drainage
1	Blanchet Lake	50	53.39577	-126.31658	upper Fraser River
2	Blanchet 2 Lake	50	53.35926	-126.39587	upper Fraser River
3	Blanchet 3 Lake	50	53.3516	-126.4193	upper Fraser River
4	Grizzly Lake	50	53.40581	-126.3857	upper Fraser River
5	Tlutlias Lake	50	53.40541	-126.24645	upper Fraser River
6	Glathele Lake	32	53.61611	-126.40918	upper Fraser River
7	Unnamed 1 Lake	32	53.61667	-126.49397	upper Fraser River
8	Michel Lake	32	53.5957	-126.52203	upper Fraser River
9	Unnamed 2 Lake	32	53.61558	-126.31898	upper Fraser River
10	Theleteban Lake	32	53.58764	-126.21465	upper Fraser River
11	Unnamed 3 Lake	32	53.6071	-126.33541	upper Fraser River
12	Ghitzeli Lake	32	53.61852	-126.25256	upper Fraser River
13	Fenton Lake	32	53.4869	-126.46224	upper Fraser River
14	Nutli Lake	32	53.49087	-126.26358	upper Fraser River
15	Goodrich Lake	32	53.50428	-126.53842	upper Fraser River
16	Morgan Lake	32	53.49574	-126.32609	upper Fraser River
17	01157LNTH Lake	21	51.18843	-120.4163	Thompson River
18	01166LNTH Lake	22	51.18294	-120.41102	Thompson River
19	01179LNTH Lake	32	51.17198	-120.40801	Thompson River
20	01184LNTH Lake	32	51.16724	-120.39827	Thompson River
21	01176LNTH Lake	32	51.17128	-120.38379	Thompson River
22	01189LNTH Lake	32	51.16397	-120.388	Thompson River
23	01193LNTH Lake	32	51.1615	-120.41332	Thompson River
24	01201LNTH Lake	32	51.15649	-120.40487	Thompson River
25	00376DEAD Lake	32	51.11033	-120.56183	Thompson River
26	00422DEAD Lake	32	51.10017	-120.52824	Thompson River
27	00357DEAD Lake	32	51.11114	-120.53096	Thompson River
28	00409DEAD Lake	32	51.10372	-120.49249	Thompson River
29	00439DEAD Lake	32	51.0937	-120.46036	Thompson River
30	00447DEAD Lake	32	51.09571	-120.49127	Thompson River
31	00466DEAD Lake	32	51.08257	-120.43624	Thompson River
32	00416DEAD Lake	32	51.10169	-120.48212	Thompson River
33	00410DEAD Lake	32	51.10357	-120.47443	Thompson River
34	00369DEAD Lake	32	51.10811	-120.48483	Thompson River
35	Lower Murphy Creek	50	49.15788	-117.73803	upper Columbia River
36	Lower Norns Creek	47	49.38224	-117.68362	upper Columbia River
37	Kootenay River	52	49.3338	-117.6126	upper Columbia River

Table 2.1 Continued.

Population number	Sample locality	Sample size	Latitude	Longitude	Drainage
38	Upper Sullivan Creek	38	49.19982	-117.74045	upper Columbia River
39	China Creek	49	49.2182	-117.67775	upper Columbia River
40	Lower Blueberry Creek	49	49.25266	-117.67332	upper Columbia River
41	Sand Bar Eddy	43	49.23077	-117.66585	upper Columbia River
42	Upper Murphy Creek	50	49.18468	-117.83038	upper Columbia River
43	Norns Creek Fan	175	49.33417	-117.66215	upper Columbia River
44	Columbia River at Genelle	162	49.20454	-117.69613	upper Columbia River
45	Salmo River	60	49.1656	-117.26481	upper Columbia River
46	Clearwater Creek	27	49.39091	-117.1816	upper Columbia River
47	Skinny Lake	50	53.8229	-126.92226	upper Fraser River
48	Needle Lake	50	53.81335	-127.00779	upper Fraser River
49	Twinkle Lake	50	53.81141	-127.04696	upper Fraser River
50	Horseshoe Lake	32	53.83342	-126.87572	upper Fraser River
51	Fry Creek	46	50.06495	-116.73011	upper Columbia River
52	Blackwater River	50	53.21832	-123.55017	upper Fraser River
53	Kinbasket Reservoir	14	52.12698	-118.45007	upper Columbia River
54	Lardeau River	40	50.38187	-117.08272	upper Columbia River
55	Kuyakuz Lake	50	53.14677	-124.60286	upper Fraser River
56	Clearwater River	54	52.36161	-120.16162	Thompson River
57	Murray Creek	38	50.41762	-121.362	Thompson River
58	Coldwater River	35	49.9509	-120.89458	Thompson River
59	Fish Lake	50	51.45132	-123.61051	upper Fraser River
60	Nimkish River	35	50.23296	-126.64342	Vancouver Island
61	Gold River	35	49.76782	-126.09592	Vancouver Island
62	Cooper Creek	21	53.13205	-131.80562	Queen Charlotte Islands
63	Mamin River	31	53.60258	-132.29136	Queen Charlotte Islands
64	Yakoun River	20	53.43521	-132.27361	Queen Charlotte Islands
65	Riley Creek	30	53.37143	-132.45154	Queen Charlotte Islands
66	Canyon Creek	32	54.82376	-127.15114	Skeena River
67	Moosevale Creek	32	56.69227	-126.63124	Skeena River
68	Ealue Lake	32	57.77233	-129.82921	Stikine River
69	Khtada Lake	50	54.13146	-129.46866	Skeena River

Table 2.2 The seven defined (population number, site name, samples size, and origin of drainage) population chains of rainbow trout (*Oncorhynchus mykiss*) used in the study. Population chains are represented by populations within a watershed which are geographically proximate and potentially contiguous.

Population chain names are in italics.

Population number	Sample locality	Sample size	Drainage
<i>Blanchet Lake chain</i>			
1	Blanchet Lake	50	upper Fraser River
2	Blanchet 2 Lake	50	upper Fraser River
3	Blanchet 3 Lake	50	upper Fraser River
4	Grizzly Lake	50	upper Fraser River
5	Tluttias Lake	50	upper Fraser River
<i>Glattheli Lake chain</i>			
6	Glattheli Lake	32	upper Fraser River
7	Unnamed 1 Lake	32	upper Fraser River
8	Michel Lake	32	upper Fraser River
9	Unnamed 2 Lake	32	upper Fraser River
10	Theleteban Lake	32	upper Fraser River
11	Unnamed 3 Lake	32	upper Fraser River
12	Ghitzeli Lake	32	upper Fraser River
<i>Fenton Lake chain</i>			
13	Fenton Lake	32	upper Fraser River
14	Nutli Lake	32	upper Fraser River
15	Goodrich Lake	32	upper Fraser River
16	Morgan Lake	32	upper Fraser River
<i>Skinny Lake chain</i>			
47	Skinny Lake	50	upper Fraser River
48	Twinkle Lake	50	upper Fraser River
49	Needle Lake	50	upper Fraser River
50	Horseshoe Lake	32	upper Fraser River
<i>Lower North Thompson chain</i>			
17	01157LNTH Lake	21	Thompson River
18	01166LNTH Lake	22	Thompson River
19	01179LNTH Lake	32	Thompson River
20	01184LNTH Lake	32	Thompson River
21	01176LNTH Lake	32	Thompson River
22	01189LNTH Lake	32	Thompson River
23	01193LNTH Lake	32	Thompson River
24	01201LNTH Lake	32	Thompson River
<i>Deadman chain</i>			
25	00376DEAD Lake	32	Thompson River
26	00422DEAD Lake	32	Thompson River
27	00357DEAD Lake	32	Thompson River
28	00409DEAD Lake	32	Thompson River
29	00439DEAD Lake	32	Thompson River
30	00447DEAD Lake	32	Thompson River
31	00466DEAD Lake	32	Thompson River
32	00416DEAD Lake	32	Thompson River
33	00410DEAD Lake	32	Thompson River
34	00369DEAD Lake	32	Thompson River
<i>Columbia River chain</i>			
35	Lower Murphy Creek	50	upper Columbia River
36	Lower Nors Creek	47	upper Columbia River
37	Kootenay River	52	upper Columbia River
38	Upper Sullivan Creek	38	upper Columbia River
39	China Creek	49	upper Columbia River
40	Lower Blueberry Creek	49	upper Columbia River
41	Sand Bar Eddy	43	upper Columbia River
42	Upper Murphy Creek	50	upper Columbia River
43	Nors Creek Fan	175	upper Columbia River
44	Columbia River at Genelle	162	upper Columbia River
45	Salmo River	60	upper Columbia River
46	Clearwater Creek	27	upper Columbia River

Table 2.3 Five diplexed PCR reactions with microsatellite locus names, references, annealing temperatures (T_A , the temperature on the left indicates the annealing temperature during the first five cycles while the temperature on the right indicates the annealing temperature for the remaining cycles), and size range of alleles in base pairs.

Diplex	Locus	Source species	Reference	T_A (C)	Range (bp)
1	Oneu14	<i>Oncorhynchus nerka</i>	Scribner et al. (1996)	62/60	145-165
	Ssa197	<i>Salmo salar</i>	O'Reilly et al. (1996)		112-116
2	Oneu8	<i>Oncorhynchus nerka</i>	Scribner et al. (1996)	58/56	150-192
	Ssa85	<i>Salmo salar</i>	O'Reilly et al. (1996)		97-153
3	Ssa456	<i>Salmo salar</i>	Slettan et al. (1995)	56/55	149-161
	Omy77	<i>Oncorhynchus mykiss</i>	Morris et al. (1996)		94-142
4	Ots3	<i>Oncorhynchus tshawytscha</i>	Banks et al. (1999)	52/50	74-98
	Okia3	<i>Oncorhynchus kistuch</i>	P. Bentzen, Dalhousie U.		112-206
5	Ots100	<i>Oncorhynchus tshawytscha</i>	Nelson et al. (1998)	59/57	168-22
	Ots103	<i>Oncorhynchus tshawytscha</i>	Beacham et al. (1998)		75-97

Table 2.4 Individual and averaged values for observed and expected heterozygosity (H_o and H_e), and number of alleles per locus (N) over all 69 rainbow trout (*Oncorhynchus mykiss*) localities.

	H_e	H_o	N
Oneu8	0.827	0.568	18
Ssa85	0.695	0.399	23
Ots103	0.419	0.242	11
Ots3	0.692	0.402	12
Ssa456	0.631	0.247	6
Omy77	0.82	0.524	24
Oneu14	0.636	0.268	10
Ssa197	0.49	0.277	2
Ots100	0.821	0.424	27
Okia3	0.928	0.661	38
Average	0.696	0.401	17.1

Table 2.5 Summary of allelic variation at ten microsatellite loci in rainbow trout (*Oncorhynchus mykiss*). Populations are grouped into major drainages in italics (*upper Fraser River, Thompson River, upper Columbia River, South Coast British Columbia, North Coast British Columbia, Stikine River, and Skeena River*). Number of alleles per locus (A), expected heterozygosity (He), observed heterozygosity (Ho), allelic richness (Ar), and the number of genotyped individuals (N) are given for each loci per population. Significant departures from Hardy Weinberg equilibrium are denoted by "*" (using Bonferroni corrections for 69 populations; $\alpha = 0.05/69 = 0.00072$).

Upper Fraser River

	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
Blanchet Lake											
A	3.00	2.00	2.00	4.00	2.00	2.00	1.00	2.00	1.00	10.00	2.90
He	0.6136	0.4592	0.095	0.4703	0.1993	0.4998	0	0.4991	0	0.7346	0.36
Ho	0.52	0.4286	0.1	0.4545	0.1837	0.6122	0	0.4583	0	0.7391	0.35
Ar	2.998	2	1.72	3.624	1.949	2	1	2	1	6.416	2.47
N	50.00	49.00	50.00	44.00	49.00	49.00	47.00	48.00	50.00	46.00	48.20
Blanchet 2 Lake											
A	3.00	2.00	2.00	4.00	2.00	2.00	1.00	2.00	1.00	7.00	2.60
He	0.61	0.4082	0.1638	0.51	0.255	0.347	0	0.4928	0	0.7232	0.35
Ho	0.58	0.449	0.1	0.5745	0.3	0.3191	0	0.44	0	0.76	0.35
Ar	2.977	2	1.904	3.16	1.983	1.998	1	2	1	5.355	2.34
N	50.00	49.00	50.00	47.00	50.00	47.00	50.00	50.00	50.00	50.00	49.30
Blanchet 3 Lake											
A	3.00	3.00	2.00	3.00	1.00	2.00	1.00	2.00	4.00	5.00	2.60
He	0.41	0.2326	0.0594	0.4654	0	0.0968	0	0.0202	0.3782	0.4478	0.21
Ho	0.4082	0.1429	0.0204	0.4694	0	0.102	0	0.0204	0.4082	0.44	0.20
Ar	2.643	2.186	1.538	2.538	1	1.728	1	1.224	3.399	3.698	2.10
N	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	50.00	49.10
Grizzly Lake											
A	3.00	2.00	2.00	4.00	2.00	2.00	1.00	2.00	1.00	8.00	2.70
He	0.6314	0.3648	0.095	0.4873	0.1866	0.5	0	0.4992	0	0.7878	0.36
Ho	0.5918	0.44	0.1	0.4694	0.1667	0.5833	0	0.44	0	0.78	0.36
Ar	2.997	1.999	1.72	3.566	1.936	2	1	2	1	6.192	2.44
N	49.00	50.00	50.00	49.00	48.00	48.00	50.00	50.00	50.00	50.00	49.40
Tlutlias Lake											
A	3.00	2.00	2.00	4.00	2.00	2.00	1.00	2.00	1.00	6.00	2.50
He	0.4058	0.2408	0.4758	0.6229	0.1638	0.18	0	0.3418	0	0.6718	0.31
Ho	0.34	0.28	0.58	0.7143	0.14	0.16	0	0.3125	0	0.5918	0.31
Ar	2.922	1.977	2	3.213	1.904	1.927	1	1.998	1	4.341	2.23
N	50.00	50.00	50.00	49.00	50.00	50.00	47.00	48.00	50.00	49.00	49.30
Blackwater River											
A	9.00	5.00	3.00	5.00	3.00	8.00	3.00	2.00	9.00	12.00	5.90
He	0.7655	0.5822	0.1352	0.5905	0.3027	0.8126	0.5059	0.498	0.778	0.8901	0.59
Ho	0.6596	0.5417	0.1429	0.413	0.2708	0.9149	0.6383	0.5106	0.7333	0.8043	0.56
Ar	5.773	3.486	2.183	4.285	2.628	6.663	2.234	2	6.211	9.469	4.49
N	47.00	48.00	49.00	46.00	48.00	47.00	47.00	47.00	45.00	46.00	47.00

Table 2.5 Continued

Upper Fraser River

Gialtheli Lake	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	6.00	4.00	1.00	3.00	2.00	4.00	1.00	2.00	2.00	6.00	3.10
He	0.6873	0.3543	0	0.5466	0.0328	0.5351	0	0.4688	0.0666	0.7819	0.35
Ho	0.7097	0.3548	0	0.5185	0.0333	0.5	0	0.4375	0.069	0.4815	0.31
Ar	4.296	3.168	1	2.8	1.367	3.799	1	2	1.619	5.734	2.68
N	31.00	31.00	31.00	27.00	30.00	28.00	32.00	32.00	29.00	27.00	29.80
Unnamed 1 Lake											
A	5.00	3.00	1.00	3.00	1.00	5.00	1.00	2.00	1.00	7.00	2.90
He	0.5744	0.3044	0	0.5192	0	0.6677	0	0.4813	0	0.7877	0.33
Ho	0.5161	0.3548	0	0.4	0	0.6071	0	0.5484	0	0.7308	0.32
Ar	3.672	2.716	1	2.44	1	4.346	1	2	1	6.349	2.55
N	31.00	31.00	32.00	25.00	28.00	28.00	32.00	31.00	30.00	26.00	29.40
Michel Lake											
A	6.00	4.00	1.00	3.00	1.00	4.00	1.00	2.00	2.00	9.00	3.30
He	0.6694	0.3883	0	0.5189	0	0.6828	0	0.4824	0.0308	0.7902	0.36
Ho	0.6333	0.4667	0	0.4348	0	0.6	0	0.4375	0.0313	0.7826	0.34
Ar	4.563	3.279	1	2.478	1	3.941	1	2	1.344	7.031	2.76
N	30.00	30.00	32.00	23.00	30.00	30.00	32.00	32.00	32.00	23.00	29.40
Unnamed 2 Lake											
A	5.00	3.00	1.00	2.00	1.00	4.00	1.00	2.00	2.00	9.00	3.00
He	0.6172	0.2983	0	0.4786	0	0.5447	0	0.4956	0.1483	0.8297	0.34
Ho	0.5938	0.3125	0	0.4483	0	0.5806	0	0.5313	0.1613	0.5	0.31
Ar	3.682	2.78	1	2	1	3.424	1	2	1.898	7.594	2.64
N	32.00	32.00	32.00	29.00	31.00	31.00	32.00	32.00	31.00	28.00	31.00
Theleteban Lake											
A	6.00	4.00	1.00	5.00	1.00	4.00	1.00	2.00	1.00	9.00	3.40
He	0.6202	0.5416	0	0.5905	0	0.6167	0	0.2417	0	0.7487*	0.34
Ho	0.5484	0.6129	0	0.6667	0	0.7813	0	0.2188	0	0.4286	0.33
Ar	4.669	3.676	1	3.78	1	3.529	1	1.984	1	6.643	2.83
N	31.00	31.00	32.00	27.00	32.00	32.00	32.00	32.00	32.00	28.00	30.90
Fenton Lake											
A	2.00	2.00	2.00	3.00	1.00	3.00	1.00	1.00	1.00	9.00	2.50
He	0.2919	0.0308	0.144	0.3313	0	0.063	0	0	0	0.8231	0.17
Ho	0.2258	0.0313	0.1563	0.3333	0	0.0645	0	0	0	0.7419	0.16
Ar	1.995	1.344	1.888	2.405	1	1.71	1	1	1	7.266	2.06
N	31.00	32.00	32.00	27.00	31.00	31.00	32.00	32.00	32.00	31.00	31.10

Table 2.5 Continued

Upper Fraser River

Nutli Lake	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	4.00	2.00	2.00	2.00	2.00	2.00	1.00	1.00	1.00	8.00	2.50
He	0.2315	0.0894	0.1699	0.1948	0.0605	0.1483	0	0	0	0.8111	0.17
Ho	0.2581	0.0938	0.1875	0.2188	0	0.1613	0	0	0	0.7742	0.17
Ar	2.647	1.724	1.93	1.957	1.573	1.898	1	1	1	6.823	2.16
N	31.00	32.00	32.00	32.00	32.00	31.00	32.00	32.00	32.00	31.00	31.70
Unnamed 3 Lake											
A	6.00	3.00	1.00	3.00	2.00	4.00	1.00	2.00	2.00	6.00	3.00
He	0.6767	0.3594	0	0.5899	0.0317	0.6939	0	0.4745	0.095	0.682*	0.36
Ho	0.6333	0.3333	0	0.4286	0.0323	0.6333	0	0.3871	0.1	0.6538	0.32
Ar	4.333	2.838	1	2.959	1.355	3.964	1	2	1.753	5.673	2.69
N	30.00	30.00	30.00	28.00	31.00	30.00	31.00	31.00	30.00	26.00	29.7
Ghitzeli Lake											
A	4.00	4.00	1.00	5.00	1.00	4.00	1.00	2.00	2.00	7.00	3.10
He	0.5913	0.5981	0	0.6461	0	0.4606	0	0.2003	0.0308	0.7206*	0.32
Ho	0.625	0.5	0	0.7037	0	0.4	0	0.1613	0.0313	0.3793	0.28
Ar	3.613	3.818	1	3.81	1	2.969	1	1.962	1.344	6.116	2.66
N	32.00	32.00	32.00	27.00	32.00	30.00	31.00	31.00	32.00	29.00	30.80
Goodrich Lake											
A	2.00	1.00	2.00	2.00	2.00	2.00	1.00	1.00	1.00	7.00	2.10
He	0.2847	0	0.1244	0.2482	0.3314	0.0317	0	0	0	0.8417	0.19
Ho	0.3438	0	0.1333	0.2903	0.2903	0.0323	0	0	0	0.7	0.18
Ar	1.994	1	1.849	1.987	1.999	1.355	1	1	1	6.55	1.97
N	32.00	32.00	30.00	31.00	31.00	31.00	32.00	32.00	31.00	30.00	3.12
Morgan Lake											
A	3.00	2.00	2.00	2.00	2.00	2.00	1.00	1.00	1.00	8.00	2.40
He	0.3179	0.0624	0.2311	0.2003	0.0624	0.0317	0	0	0	0.7716	0.17
Ho	0.3871	0.0645	0.2	0.1613	0.0645	0.0323	0	0	0	0.7419	0.17
Ar	2.35	1.588	1.981	1.962	1.588	1.355	1	1	1	6.592	2.04
N	31.00	31.00	30.00	31.00	31.00	31.00	31.00	31.00	30.00	31.00	30.80
Kuyakuz Lake											
A	5.00	4.00	3.00	5.00	1.00	7.00	1.00	2.00	5.00	9.00	4.20
He	0.736	0.3698	0.643	0.6504	0	0.724	0	0.0605	0.5527	0.8174	0.46
Ho	0.8	0.3878	0.5833	0.62	0	0.82	0	0.0625	0.4792	0.7755	0.45
Ar	4.208	3.034	2.999	4.176	1	5.331	1	1.546	3.844	6.811	3.39
N	50.00	49.00	48.00	50.00	50.00	50.00	48.00	48.00	48.00	49.00	49.00

Table 2.5 Continued

Upper Fraser River

Fish Lake	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	4.00	2.00	2.00	3.00	2.00	2.00	2.00	1.00	4.00	7.00	2.90
He	0.4915	0.1499	0.0416	0.5787	0.2593	0.4248	0.04	0	0.5575	0.7196	0.33
Ho	0.4286	0.1633	0.0426	0.4694	0.3061	0.3673	0.0408	0	0.5957	0.6875	0.31
Ar	3.265	1.88	1.415	2.909	1.984	2	1.4	1	3.175	5.56	2.46
N	49.00	49.00	47.00	49.00	49.00	49.00	49.00	49.00	47.00	48.00	48.50

Skinny Lake

A	5.00	3.00	2.00	4.00	1.00	5.00	1.00	2.00	3.00	10.00	3.60
He	0.6954	0.5346	0.3944*	0.5477	0	0.6536	0	0.1128	0.5649	0.7094	0.42128
Ho	0.56	0.42	0.1081	0.6522	0	0.5435	0	0.12	0.4167	0.75	0.35705
Ar	4.358	2.988	2	3.228	1	3.798	1	1.785	2.849	5.452	2.85
N	50.00	50.00	37.00	46.00	50.00	46.00	50.00	50.00	48.00	48.00	47.50

Twinkle Lake

A	5.00	3.00	3.00	3.00	1.00	5.00	1.00	2.00	3.00	8.00	3.40
He	0.5312	0.6304	0.1347	0.4327	0	0.6421	0	0.1866	0.6042	0.4872	0.36
Ho	4.335	2.994	2.128	2.949	1	3.756	1	1.936	2.979	4.643	2.77
Ar	0.4681	0.7234	0.1429	0.413	0	0.4167	0	0.1667	0.7609	0.4375	0.35
N	47.00	47.00	49.00	46.00	48.00	48.00	48.00	48.00	46.00	48.00	47.5

Needle Lake

A	5.00	3.00	3.00	3.00	1.00	6.00	1.00	2.00	3.00	8.00	3.50
He	0.6378	0.633	0.1908	0.4672	0	0.5425	0	0.0768	0.5186	0.6102	0.37
Ho	0.5435	0.6522	0.1316	0.4375	0	0.4792	0	0.08	0.4894	0.617	0.34
Ar	4.226	2.994	2.208	2.967	1	4.095	1	1.636	2.415	5.14	2.77
N	46.00	46.00	38.00	48.00	48.00	48.00	50.00	50.00	47.00	47.00	46.8

Horseshoe Lake

A	5.00	4.00	3.00	4.00	1.00	7.00	1.00	2.00	3.00	7.00	3.70
He	0.71	0.66	0.40	0.47	0	0.679	0	0.22	0.58	0.81	0.45
Ho	0.7813	0.7188	0.3871	0.5	0	0.6129	0	0.1875	0.625	0.6897	0.45
Ar	4.54	3.82	2.737	3.655	1	5.673	1	1.973	2.957	6.244	3.3599
N	32.00	32.00	31.00	30.00	32.00	31.00	32.00	32.00	32.00	29.00	31.3

*Thompson River***01157LNTH Lake**

A	8.00	2.00	4.00	3.00	2.00	7.00	2.00	2.00	5.00	5.00	4.00
He	0.7902	0.1723	0.5613	0.3698	0.2098	0.6745	0.4898	0.0907	0.6298	0.5201	0.45
Ho	0.7143	0.1905	0.45	0.1538	0.2381	0.6842	0.5714	0.0952	0.5294	0.5556	0.42
Ar	6.628	1.957	3.354	2.846	1.982	6.144	2	1.779	4.283	4.444	3.54
N	21.00	21.00	20.00	13.00	21.00	19.00	21.00	21.00	17.00	18.00	19.20

Table 2.5 Continued

Thompson River

01166LNTH Lake	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	7.00	2.00	4.00	3.00	2.00	7.00	2.00	2.00	5.00	4.00	3.80
He	0.7727	0.1271	0.56	0.3873	0.3628	0.6678	0.4339	0.0444	0.6348	0.5227	0.45
Ho	0.7727	0.0455	0.6	0.3889	0.0952	0.619	0.4545	0.0455	0.625	0.5455	0.42
Ar	6.293	1.884	3.871	2.611	2	5.628	2	1.5	4.369	3.855	3.40
N	22.00	22.00	15.00	18.00	21.00	21.00	22.00	22.00	16.00	22.00	20.10
01179LNTH Lake											
A	8.00	2.00	3.00	3.00	2.00	5.00	3.00	2.00	7.00	4.00	3.90
He	0.8179	0.0894	0.4917	0.3823	0.2482	0.6894	0.4946	0.0894	0.6235	0.4331	0.44
Ho	0.8125	0.0938	0.4828	0.3125	0.1613	0.6774	0.6875	0.0938	0.5938	0.3125	0.42
Ar	6.576	1.724	2.379	2.343	1.987	4.552	2.344	1.724	4.263	3.067	3.10
N	32.00	32.00	29.00	32.00	31.00	31.00	32.00	32.00	32.00	32.00	31.50
01184LNTH Lake											
A	8.00	2.00	2.00	2.00	2.00	5.00	2.00	2.00	5.00	3.00	3.30
He	0.8101	0.0894	0.4933	0.3496	0.1483	0.6889	0.4978	0.0644	0.5525	0.5135	0.42
Ho	0.8387	0.0938	0.5	0.1935	0.0968	0.7742	0.5333	0.0667	0.6452	0.4516	0.42
Ar	6.227	1.724	2	1.999	1.898	4.186	2	1.603	3.448	2.898	2.80
N	31.00	32.00	26.00	31.00	31.00	31.00	30.00	30.00	31.00	31.00	30.40
01176LNTH Lake											
A	5.00	2.00	4.00	2.00	2.00	4.00	2.00	2.00	5.00	4.00	3.20
He	0.6267	0.1748	0.6811	0.3829	0.2248	0.5411	0.498	0.0605	0.6196	0.6467	0.45
Ho	0.6667	0.1935	0.7667	0.2581	0.1935	0.5161	0.4375	0.0625	0.5938	0.6129	0.43
Ar	3.722	1.938	3.907	2	1.977	3.094	2	1.573	3.684	3.352	2.72
N	30.00	31.00	30.00	31.00	31.00	31.00	32.00	32.00	32.00	31.00	31.10
01189LNTH Lake											
A	4.00	2.00	4.00	2.00	2.00	3.00	2.00	2.00	4.00	3.00	2.80
He	0.5518	0.095	0.6953	0.4592	0.1014	0.5425	0.4841	0.0666	0.644	0.6145	0.43
Ho	0.3846	0.1	0.6667	0.5	0.1071	0.5	0.25	0.069	0.6296	0.6296	0.38
Ar	3.368	1.753	3.96	2	1.784	2.724	2	1.619	3.946	2.99	2.61
N	26.00	30.00	24.00	28.00	28.00	32.00	28.00	29.00	27.00	27.00	27.90
01193LNTH Lake											
A	5.00	1.00	4.00	3.00	2.00	5.00	3.00	2.00	5.00	4.00	3.40
He	0.7622	0	0.5416	0.3738	0.1207	0.6618	0.5483	0.144	0.5801	0.5386	0.43
Ho	0.5938	0	0.7391	0.2222	0.129	0.8387	0.5938	0.1563	0.4839	0.5625	0.43
Ar	4.918	1	2.957	2.407	1.836	3.707	2.888	1.888	3.681	3.316	2.86
N	32.00	32.00	23.00	27.00	31.00	31.00	32.00	32.00	31.00	32.00	30.30

Table 2.5 Continued

Thompson River

01201LNTH Lake	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	4.00	2.00	5.00	2.00	2.00	5.00	2.00	2.00	4.00	3.00	3.10
He	0.5823	0.1014	0.7106	0.498	0.1528	0.6036	0.4043	0.1948	0.6658	0.4005	0.43
Ho	0.4444	0.1071	0.5926	0.5625	0.1667	0.6538	0.3125	0.2188	0.7917	0.2857	0.41
Ar	3.294	1.784	4.378	2	1.908	3.911	2	1.957	3.458	2.87	2.76
N	27.00	28.00	27.00	32.00	30.00	26.00	32.00	32.00	24.00	28.00	28.60
00376DEAD Lake											
A	2.00	1.00	2.00	1.00	2.00	2.00	1.00	1.00	1.00	3.00	1.60
He	0.3901	0	0.3394	0	0.0894	0.0605	0	0	0	0.6616	0.15
Ho	0.2813	0	0.3667	0	0.0938	0.0625	0	0	0	0.75	0.16
Ar	2	1	1.999	1	1.724	1.573	1	1	1	3	1.53
N	32.00	32.00	30.00	28.00	32.00	32.00	32.00	32.00	32.00	32.00	31.40
00422DEAD Lake											
A	2.00	1.00	2.00	1.00	2.00	2.00	1.00	1.00	1.00	4.00	1.70
He	0.4878	0	0.4483	0	0.0921	0.0308	0	0	0	0.6162	0.17
Ho	0.4063	0	0.3214	0	0.0968	0.0313	0	0	0	0.5625	0.14
Ar	2	1	2	1	1.739	1.344	1	1	1	3.338	1.54
N	32.00	32.00	28.00	28.00	31.00	32.00	32.00	32.00	32.00	32.00	31.10
00357DEAD Lake											
A	2.00	1.00	2.00	1.00	2.00	2.00	1.00	1.00	1.00	4.00	1.70
He	0.4688	0	0.3237	0	0.2188	0.0605	0	0	0	0.647	0.17
Ho	0.4375	0	0.4063	0	0.25	0.0625	0	0	0	0.6875	0.18
Ar	2	1	1.998	1	1.973	1.573	1	1	1	3.34	1.59
N	32.00	32.00	32.00	29.00	32.00	32.00	32.00	32.00	31.00	32.00	31.60
00409DEAD Lake											
A	2.00	1.00	2.00	1.00	2.00	2.00	1.00	1.00	1.00	3.00	1.60
He	0.4688	0	0.4144	0	0.0317	0.0308	0	0	0	0.5968	0.15
Ho	0.5625	0	0.3793	0	0.0323	0.0313	0	0	0	0.6774	0.17
Ar	2	1	2	1	1.355	1.344	1	1	1	2.992	1.47
N	32.00	32.00	29.00	30.00	31.00	32.00	32.00	32.00	31.00	31.00	31.20
00439DEAD Lake											
A	2.00	1.00	2.00	1.00	2.00	1.00	1.00	1.00	1.00	4.00	1.60
He	0.4813	0	0.1626	0	0.3282	0	0	0	0	0.4294	0.14
Ho	0.4194	0	0.1786	0	0.3448	0	0	0	0	0.3333	0.13
Ar	2	1	1.927	1	1.999	1	1	1	1	3.3	1.52
N	31.00	30.00	28.00	29.00	29.00	29.00	31.00	31.00	30.00	30.00	29.80

Table 2.5 Continued

Thompson River

00447DEAD Lake	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	2.00	1.00	2.00	1.00	2.00	2.00	1.00	1.00	1.00	4.00	1.70
He	0.4604	0	0.3829	0	0.0894	0.0308	0	0	0	0.6235	0.16
Ho	0.3438	0	0.4516	0	0.0938	0.0313	0	0	0	0.6875	0.16
Ar	2	1	2	1	1.724	1.344	1	1	1	3.342	1.54
N	32.00	32.00	31.00	30.00	32.00	32.00	32.00	32.00	31.00	32.00	31.60
00466DEAD Lake											
A	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	2.00	1.20
He	0.4745	0	0	0	0	0	0	0	0	0.255	0.07
Ho	0.3871	0	0	0	0	0	0	0	0	0.1667	0.06
Ar	2	1	1	1	1	1	1	1	1	1.989	1.20
N	31.00	31.00	26.00	24.00	29.00	28.00	31.00	31.00	29.00	30.00	29.00
00416DEAD Lake											
A	2.00	1.00	2.00	1.00	2.00	2.00	1.00	1.00	1.00	3.00	1.60
He	0.4979	0	0.3995	0	0.2637	0.0308	0	0	0	0.6108	0.18
Ho	0.5484	0	0.4138	0	0.3125	0.0313	0	0	0	0.625	0.19
Ar	2	1	2	1	1.99	1.344	1	1	1	2.997	1.53
N	31.00	32.00	29.00	32.00	32.00	32.00	32.00	32.00	32.00	32.00	31.60
00410DEAD Lake											
A	2.00	1.00	2.00	1.00	2.00	3.00	1.00	1.00	1.00	3.00	1.70
He	0.4979	0	0.2854	0	0.3122	0.155	0	0	0	0.576	0.18
Ho	0.6129	0	0.2759	0	0.3226	0.1	0	0	0	0.6452	0.20
Ar	2	1	1.995	1	1.997	2.215	1	1	1	2.986	1.62
N	31.00	31.00	29.00	26.00	31.00	30.00	31.00	31.00	29.00	31.00	30.00
00369DEAD Lake											
A	3.00	1.00	2.00	1.00	2.00	1.00	1.00	2.00	1.00	3.00	1.70
He	0.5254	0	0.0364	0	0.1699	0	0	0.0308	0	0.6128	0.14
Ho	0.5	0	0.037	0	0.0625	0	0	0.0313	0	0.5	0.11
Ar	2.573	1	1.407	1	1.93	1	1	1.344	1	2.994	1.52
N	32.00	32.00	27.00	30.00	32.00	32.00	32.00	32.00	31.00	32.00	31.20
Murray Creek											
A	4.00	3.00	1.00	2.00	2.00	4.00	2.00	2.00	3.00	5.00	2.80
He	0.6155	0.269	0	0.4996	0.2137	0.588*	0.3343	0.2188	0.4862	0.5669	0.38
Ho	0.6765	0.2857	0	0.4167	0.1892	0.1379	0.2424	0.25	0.5588	0.6571	0.34
Ar	3.322	2.298	1	2	1.967	3.297	1.999	1.973	2.963	4.174	2.50
N	34.00	35.00	32.00	36.00	37.00	29.00	33.00	32.00	34.00	35.00	33.70

Table 2.5 Continued

Thompson River

Coldwater River	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	6.00	4.00	3.00	4.00	2.00	8.00	4.00	2.00	8.00	11.00	5.20
He	0.715	0.3559	0.3114	0.6706	0.382	0.8498	0.302	0.4853	0.5804	0.776	0.54
Ho	0.7941	0.2571	0.3429	0.6571	0.3429	0.8	0.2857	0.4857	0.5429	0.7647	0.53
Ar	4.62	2.988	2.522	3.314	2	7.249	3.227	2	5.904	7.337	4.12
N	34.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	34.00	34.80

Clearwater River

A	8.00	4.00	3.00	2.00	3.00	8.00	4.00	2.00	8.00	10.00	5.20
He	0.7994	0.5475	0.2627	0.0187	0.339	0.6938	0.4825	0.3352	0.6552	0.7522	0.49
Ho	0.7037	0.4444	0.1852	0.0189	0.3148	0.6667	0.4259	0.3148	0.5926	0.6226	0.43
Ar	6.202	2.891	2.663	1.208	2.2	5.654	3.085	1.997	4.651	6.58	3.71
N	54.00	54.00	54.00	53.00	54.00	54.00	54.00	54.00	54.00	53.00	53.80

Upper Columbia River

Fry Creek

A	6.00	8.00	1.00	2.00	2.00	4.00	2.00	2.00	3.00	5.00	3.50
He	0.7639	0.6182	0	0.3688	0.4024	0.7225	0.4362	0.2449	0.4378	0.6757	0.47
Ho	0.6744	0.4524	0	0.3415	0.4186	0.6905	0.5	0.2381	0.525	0.6667	0.45
Ar	5.251	5.361	1	1.999	2	3.979	2	1.981	2.808	4.73	3.11
N	43.00	42.00	43.00	41.00	43.00	42.00	42.00	42.00	40.00	42.00	42.00

Lower Murphy Creek

A	13.00	10.00	3.00	4.00	4.00	13.00	3.00	2.00	12.00	16.00	8.00
He	0.7694	0.7698	0.4365	0.5471	0.6766	0.8762	0.585	0.4118	0.8663	0.9002	0.68
Ho	0.74	0.7	0.3265	0.6735	0.6	0.78	0.56	0.42	0.7959	0.94	0.65
Ar	7.971	6.737	2.4	3.128	3.904	8.939	2.927	2	8.788	10.545	5.73
N	50.00	50.00	49.00	49.00	50.00	50.00	50.00	50.00	49.00	50.00	49.70

Lower Norns Creek

A	9.00	7.00	4.00	6.00	4.00	12.00	6.00	2.00	12.00	14.00	7.60
He	0.7402	0.5704	0.4697	0.5457	0.4638	0.7506	0.5774	0.4919	0.7795	0.8266	0.62
Ho	0.7234	0.4783	0.4651	0.5556	0.4255	0.6222	0.4468	0.3617	0.7333	0.7872	0.56
Ar	6.337	5.134	2.512	3.93	2.896	6.602	3.447	2	7.461	8.357	4.87
N	47.00	46.00	43.00	45.00	47.00	45.00	47.00	47.00	45.00	47.00	45.90

Kootenay River

A	9.00	8.00	4.00	5.00	3.00	12.00	6.00	2.00	12.00	11.00	7.20
He	0.7219	0.5939	0.4649	0.4196	0.5187	0.7859	0.6084*	0.4933	0.7793	0.813	0.62
Ho	0.6923	0.5962	0.3556	0.4286	0.5	0.6364	0.4038	0.4231	0.6531	0.84	0.55
Ar	6.158	5.017	2.676	3.709	2.754	7.361	3.998	2	6.813	7.329	4.78
N	52.00	52.00	45.00	49.00	46.00	44.00	52.00	52.00	49.00	50.00	49.10

Table 2.5 Continued

Upper Columbia River

Upper Sullivan Creek	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	2.00	2.00	2.00	2.00	1.00	3.00	2.00	1.00	3.00	2.00	2.00
He	0.4581	0.4654	0.4321	0.4986	0	0.5942	0.0997	0	0.519	0.3999	0.35
Ho	0.4474	0.5263	0.4211	0.5263	0	0.7632	0.0526	0	0.5789	0.5526	0.39
Ar	2	2	2	2	1	2.997	1.754	1	2.981	2	1.97
N	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00

China Creek

A	11.00	7.00	3.00	5.00	4.00	13.00	6.00	2.00	16.00	12.00	7.90
He	0.7485	0.6962	0.38	0.4714	0.5071	0.8036	0.6023	0.498	0.8392	0.8744	0.64
Ho	0.7083	0.6042	0.25	0.4375	0.3469	0.8163	0.5957	0.4255	0.8333	0.8571	0.59
Ar	6.817	5.459	2.228	2.687	3.358	7.717	3.939	2	9.038	9.132	5.24
N	48.00	48.00	48.00	48.00	49.00	49.00	47.00	47.00	48.00	49.00	48.10

Lower Blueberry Creek

A	12.00	10.00	3.00	5.00	3.00	9.00	5.00	2.00	15.00	12.00	7.60
He	0.7276	0.6483	0.2611	0.5614	0.5506	0.8481	0.5777	0.4167	0.866	0.8419	0.63
Ho	0.6735	0.6122	0.2609	0.5435	0.5227	0.8298	0.4792	0.3878	0.766	0.7959	0.59
Ar	6.868	5.952	2.218	3.551	2.953	7.378	3.345	2	9.235	8.147	5.16
N	49.00	49.00	46.00	46.00	44.00	47.00	48.00	49.00	47.00	49.00	47.40

Sand Bar Eddy

A	10.00	7.00	2.00	7.00	4.00	12.00	6.00	2.00	14.00	13.00	7.70
He	0.7633	0.4538	0.4688	0.6147	0.56	0.824	0.6228	0.4997	0.8033	0.8574	0.65
Ho	0.8095	0.4524	0.5	0.5641	0.5581	0.6047	0.4419	0.4651	0.7857	0.881	0.61
Ar	6.83	4.573	2	4.323	3.14	7.972	4.289	2	7.9	8.638	5.17
N	42.00	42.00	32.00	39.00	43.00	43.00	43.00	43.00	42.00	42.00	41.10

Upper Murphy Creek

A	8.00	7.00	4.00	4.00	2.00	8.00	3.00	2.00	5.00	9.00	5.20
He	0.4386	0.6576	0.4198	0.5196	0.4884	0.7974	0.5024	0.5	0.3288	0.7134	0.54
Ho	0.4	0.8	0.4103	0.48	0.4565	0.6122	0.5417	0.3333	0.381	0.66	0.51
Ar	4.033	4.736	3.404	2.44	2	6.013	2.654	2	3.334	5.919	3.65
N	50.00	50.00	39.00	50.00	46.00	49.00	48.00	48.00	42.00	50.00	47.20

Norns Creek Fan

A	13.00	10.00	5.00	7.00	5.00	14.00	8.00	2.00	17.00	18.00	9.90
He	0.7214	0.5802	0.4308	0.5274	0.4656	0.8035	0.6211*	0.4996	0.7738	0.8403	0.63
Ho	0.7126	0.5057	0.4161	0.4472	0.4195	0.7778	0.5119	0.4529	0.8107	0.7706	0.58
Ar	6.32	4.898	2.268	3.655	2.872	7.1	4.003	2	7.544	7.891	4.86
N	174.00	174.00	161.00	161.00	174.00	171.00	168.00	170.00	169.00	170.00	169.20

Table 2.5 Continued

Upper Columbia River

Columbia River at Genel	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	13.00	11.00	3.00	7.00	4.00	16.00	7.00	2.00	19.00	19.00	10.10
He	0.7618	0.6017	0.4002	0.5575	0.5262	0.8054*	0.5883	0.4938	0.8101	0.8467	0.64
Ho	0.7143	0.5802	0.3968	0.4932	0.4815	0.7187	0.535	0.4658	0.719	0.8291	0.59
Ar	6.552	4.917	2.24	3.9	2.93	7.747	3.713	2	7.586	8.301	4.99
N	161.00	162.00	126.00	146.00	162.00	160.00	157.00	161.00	153.00	158.00	154.60

Clearwater Creek

A	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	3.00	1.30
He	0.3841	0	0	0	0	0	0	0	0	0.1482	0.05
Ho	0.4444	0	0	0	0	0	0	0	0	0.1579	0.06
Ar	2	1	1	1	1	1	1	1	1	2.408	1.24
N	27.00	27.00	27.00	19.00	27.00	27.00	27.00	27.00	27.00	19.00	25.40

Salmo River

A	11.00	9.00	2.00	5.00	4.00	13.00	5.00	2.00	12.00	15.00	7.80
He	0.8443	0.7818	0.0177	0.562	0.4186	0.8692*	0.6192	0.3707	0.7878	0.8369	0.61
Ho	0.9322	0.7833	0.0179	0.4681	0.3167	0.7667	0.6271	0.3898	0.6833	0.7018	0.57
Ar	7.3	6.226	1.196	3.505	3.106	8.498	3.68	1.999	6.348	7.919	4.98
N	59.00	60.00	56.00	47.00	60.00	60.00	59.00	59.00	60.00	57.00	57.70

Kinbasket Reservoir

A	9.00	3.00	2.00	3.00	2.00	5.00	3.00	2.00	4.00	9.00	4.20
He	0.7551	0.4464	0.3967	0.6224	0.477	0.6709	0.1352	0.4974	0.6893	0.8036	0.55
Ho	0.8571	0.4286	0.1818	0.5	0.3571	0.5714	0.1429	0.6429	0.6923	0.7143	0.51
Ar	7.916	2.994	2	3	2	4.779	2.571	2	3.998	8.063	3.93
N	14.00	14.00	11.00	14.00	14.00	14.00	14.00	14.00	13.00	14.00	13.60

Lardeau River

A	5.00	2.00	2.00	4.00	2.00	7.00	2.00	2.00	2.00	3.00	3.10
He	0.6137	0.2888	0.0247	0.4844	0.1387	0.7997	0.4747	0.2887	0.2337	0.5122	0.39
Ho	0.75	0.35	0.025	0.425	0.15	0.8	0.675	0.35	0.2162	0.55	0.43
Ar	4.007	1.993	1.275	3.614	1.865	5.843	2	1.993	1.978	2.275	2.68
N	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	37.00	40.00	39.70

South Coast BC

Nimpkish River

A	7.00	8.00	1.00	4.00	5.00	7.00	6.00	2.00	6.00	9.00	5.50
He	0.7535	0.7935	0	0.5286	0.5684	0.7342	0.683	0.4844	0.743	0.8556	0.61
Ho	0.6471	0.7188	0	0.6571	0.6061	0.7273	0.5294	0.4118	0.7419	0.9	0.59
Ar	5.605	6.127	1	3.253	4.39	5.161	4.849	2	5.275	7.492	4.52
N	34.00	32.00	33.00	35.00	33.00	33.00	34.00	34.00	31.00	30.00	32.90

Table 2.5 Continued

South Coast BC

Gold River	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	6.00	9.00	1.00	4.00	5.00	11.00	6.00	2.00	9.00	10.00	6.30
He	0.5265	0.7943	0	0.5753	0.5959	0.8143	0.7217	0.5	0.762	0.8774	0.62
Ho	0.6286	0.5455	0	0.5588	0.5429	0.8286	0.5938	0.4545	0.5926	0.7813	0.55
Ar	5.095	6.619	1	3.301	4.638	7.386	5.356	2	6.473	8.577	5.04
N	35.00	33.00	35.00	34.00	35.00	35.00	32.00	33.00	27.00	32.00	33.10

North Coast BC

Cooper Creek

A	5.00	7.00	2.00	6.00	4.00	10.00	7.00	2.00	6.00	11.00	6.00
He	0.2948	0.737	0.1172	0.6077	0.5987	0.7675	0.7608	0.4082	0.5675	0.8288	0.57
Ho	0.2381	0.8095	0.125	0.7143	0.65	0.7	0.8571	0.4762	0.5	0.85	0.59
Ar	3.528	6.061	1.909	4.528	3.517	7.756	5.982	2	5.073	8.77	4.91
N	21.00	21.00	16.00	21.00	20.00	20.00	21.00	21.00	20.00	20.00	20.10

Mamin River

A	2.00	9.00	2.00	3.00	3.00	8.00	5.00	2.00	5.00	12.00	5.10
He	0.2706	0.7622	0.0624	0.6041	0.5718	0.7711	0.5939	0.4953	0.7196	0.8809	0.57
Ho	0.2581	0.9032	0.0645	0.4839	0.6129	0.8	0.3	0.5806	0.7742	0.871	0.56
Ar	1.992	7.028	1.588	2.997	2.962	6.409	4.518	2	4.542	9.521	4.36
N	31.00	31.00	31.00	31.00	31.00	30.00	30.00	31.00	31.00	31.00	30.80

Yakoun River

A	3.00	7.00	2.00	3.00	3.00	9.00	4.00	2.00	4.00	12.00	4.90
He	0.1838	0.7025	0.0644	0.6094	0.4862	0.8275	0.6937	0.4988	0.4972	0.8587	0.54
Ho	0.1	0.55	0.0667	0.6316	0.35	0.65	0.5	0.35	0.5789	1	0.48
Ar	2.467	5.449	1.733	3	2.55	7.748	3.966	2	3.9	9.458	4.23
N	20.00	20.00	15.00	19.00	20.00	20.00	20.00	20.00	19.00	19.00	19.20

Riley Creek

A	1.00	5.00	1.00	5.00	3.00	9.00	5.00	2.00	6.00	10.00	4.70
He	0	0.7328	0	0.6806	0.5148	0.7977	0.7175	0.48	0.6326	0.8478	0.54
Ho	0	0.7333	0	0.7667	0.4167	0.625	0.75	0.5333	0.6207	0.8276	0.53
Ar	1	4.569	1	3.969	2.458	7.225	4.567	2	4.887	8.109	3.98
N	30.00	30.00	29.00	30.00	24.00	24.00	28.00	30.00	29.00	29.00	28.30

Table 2.5 Continued

Stikine River

Ealue Lake	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	4.00	5.00	1.00	2.00	5.00	4.00	1.00	2.00	5.00	4.00	3.30
He	0.6866	0.6948	0	0.074	0.5911	0.6291	0	0.455	0.6578	0.6048	0.44
Ho	0.6667	0.5926	0	0	0.5667	0.6364	0	0.5	0.6087	0.52	0.41
Ar	3.931	4.342	1	1.672	4.182	3.754	1	2	4.726	3.689	3.03
N	27.00	27.00	31.00	26.00	30.00	22.00	26.00	30.00	23.00	25.00	26.70

Skeena River

Khtada Lake

A	2.00	4.00	2.00	1.00	1.00	3.00	2.00	2.00	3.00	1.00	2.10
He	0.023	0.6322	0.2355	0	0	0.2383	0.2188	0.4898	0.1144	0	0.20
Ho	0.0233	0.75	0.2727	0	0	0.2647	0.1875	0.5143	0.12	0	0.21
Ar	1.256	3.614	1.981	1	1	2.668	1.973	2	2.131	1	1.86
N	43.00	40.00	33.00	29.00	34.00	34.00	32.00	35.00	25.00	44.00	34.90

Canyon Creek

A	1.00	3.00	2.00	2.00	2.00	3.00	2.00	2.00	2.00	4.00	2.30
He	0	0.5578	0.0308	0.0605	0.2188	0.5005	0.3418	0.2188	0.0605	0.1272	0.21
Ho	0	0.5484	0.0313	0.0625	0.125	0.375	0.375	0.25	0.0625	0.1333	0.20
Ar	1	2.836	1.344	1.573	1.973	2.344	1.999	1.973	1.573	2.336	1.90
N	32.00	31.00	32.00	32.00	32.00	32.00	32.00	32.00	32.00	30.00	31.70

Moosevale Creek

A	5.00	6.00	3.00	4.00	5.00	6.00	4.00	2.00	8.00	10.00	5.30
He	0.4443	0.6816	0.3907	0.6704	0.4677	0.5239*	0.4907	0.4121	0.692	0.8174	0.56
Ho	0.4516	0.5938	0.3871	0.625	0.4194	0.2667	0.5	0.5161	0.7419	0.6774	0.52
Ar	3.827	5.416	2.354	3.879	4.149	4.649	2.687	2	6.579	7.281	4.28
N	31.00	32.00	31.00	32.00	31.00	30.00	32.00	31.00	31.00	31.00	31.20

Table 2.6 The relationship between latitude and longitude with number of alleles (A), allelic richness (A_r), and expected heterozygosity (H_e) at ten microsatellite loci in populations of rainbow trout (*Oncorhynchus mykiss*) from areas of the interior (N=59) and coastal (N=10) lineages. Significant values are in bold and underlined.

A. Interior lineage (N=59 populations)

	Number of alleles				Allelic richness				Expected heterozygosity			
	Latitude		Longitude		Latitude		Longitude		Latitude		Longitude	
	r	p	r	p	r	p	r	p	r	p	r	p
Onue8	<u>-0.32</u>	<u>0.01</u>	<u>0.32</u>	<u>0.01</u>	<u>-0.28</u>	<u>0.03</u>	<u>0.33</u>	<u>0.01</u>	-0.22	0.08	<u>0.28</u>	<u>0.03</u>
Ssa85	<u>-0.35</u>	<u>0.01</u>	<u>0.28</u>	<u>0.04</u>	<u>-0.28</u>	<u>0.03</u>	<u>0.22</u>	0.09	-0.10	0.40	<u>0.02</u>	0.59
Ots103	<u>-0.26</u>	<u>0.04</u>	<u>0.28</u>	<u>0.03</u>	<u>-0.22</u>	0.08	<u>0.26</u>	<u>0.05</u>	<u>-0.30</u>	<u>0.02</u>	<u>0.33</u>	<u>0.01</u>
Ots3	<u>-0.03</u>	0.81	<u>-0.04</u>	0.70	<u>0.10</u>	0.39	<u>-0.17</u>	0.18	0.35	<u>0.40</u>	<u>-0.20</u>	0.15
Ssa456	<u>-0.42</u>	<u>0.001</u>	<u>0.40</u>	<u>0.002</u>	<u>-0.54</u>	<u><0.0001</u>	<u>0.51</u>	<u><0.0001</u>	<u>-0.55</u>	<u><0.0001</u>	<u>0.54</u>	<u><0.0001</u>
Omy77	<u>-0.41</u>	<u>0.001</u>	<u>0.37</u>	<u>0.004</u>	<u>-0.39</u>	<u>0.003</u>	<u>0.36</u>	<u>0.01</u>	-0.20	0.11	<u>0.20</u>	0.12
Oneu14	<u>-0.49</u>	<u><0.0001</u>	<u>0.45</u>	<u>0.000</u>	<u>-0.58</u>	<u><0.0001</u>	<u>0.57</u>	<u><0.0001</u>	<u>-0.57</u>	<u><0.0001</u>	<u>0.59</u>	<u><0.0001</u>
Ssa197	<u>0.04</u>	0.72	<u>-0.03</u>	0.82	<u>0.03</u>	0.78	<u>-0.03</u>	0.81	0.01	0.95	<u>-0.03</u>	0.78
Ots100	<u>-0.47</u>	<u>0.0002</u>	<u>0.41</u>	<u>0.001</u>	<u>-0.50</u>	<u><0.0001</u>	<u>0.46</u>	<u>0.0003</u>	<u>-0.44</u>	<u>0.001</u>	<u>0.44</u>	<u>0.001</u>
Okia3	<u>-0.05</u>	0.69	<u>-0.03</u>	0.84	<u>0.08</u>	0.54	<u>-0.14</u>	0.30	0.20	0.14	<u>-0.24</u>	0.07
All loci	<u>-0.48</u>	<u>0.0001</u>	<u>0.42</u>	<u>0.001</u>	<u>-0.36</u>	<u>0.01</u>	<u>0.33</u>	<u>0.01</u>	<u>-0.39</u>	<u>0.003</u>	<u>0.37</u>	<u>0.004</u>

B. Coastal lineage (N=10 populations)

	Number of alleles				Allelic richness				Expected heterozygosity			
	Latitude		Longitude		Latitude		Longitude		Latitude		Longitude	
	r	p	r	p	r	p	r	p	r	p	r	p
Onue8	-0.40	0.26	0.52	0.12	-0.36	0.30	0.52	0.12	-0.14	0.73	0.45	0.20
Ssa85	-0.61	0.06	-0.03	0.93	-0.52	0.13	-0.09	0.79	-0.62	0.06	-0.09	0.80
Ots103	0.45	0.20	-0.01	1.00	0.37	0.29	0.10	0.78	0.42	0.22	0.17	0.62
Ots3	-0.39	0.28	-0.17	0.65	-0.33	0.35	-0.17	0.61	0.40	0.25	-0.22	0.55
Ssa456	-0.10	0.78	0.37	0.29	-0.20	0.60	0.41	0.24	-0.22	0.55	-0.14	0.68
Omy77	-0.62	0.06	-0.26	0.44	-0.50	0.14	-0.46	0.18	-0.46	0.19	-0.32	0.36
Oneu14	<u>-0.73</u>	<u>0.02</u>	-0.08	0.82	<u>-0.78</u>	<u>0.01</u>	-0.20	0.58	<u>-0.73</u>	<u>0.02</u>	-0.17	0.65
Ssa197	N/A	N/A	N/A	N/A	-0.17	0.66	-0.30	0.39	-0.35	0.33	-0.32	0.38
Ots100	-0.32	0.37	0.30	0.39	-0.22	0.54	0.17	0.62	-0.24	0.49	0.02	0.95
Okia3	-0.39	0.27	-0.30	0.39	-0.45	0.20	-0.32	0.37	-0.33	0.36	-0.20	0.57
All loci	-0.57	0.09	-0.01	0.97	-0.26	0.46	-0.26	0.46	-0.41	0.23	-0.09	0.79

Table 2.7 The occurrence of private alleles among *Oncorhynchus mykiss* localities investigated in this study.

Location	Locus	Allele (base pairs)	Number of alleles observed	Sample size
Clearwater River	Oneu8	192	1 out of 108	54
Moosevale Creek	Ssa85	105	7 out of 64	32
Lower Murphy Creek	Ssa85	139	1 out of 100	50
		134	1 out of 100	50
Columbia River at Genelle	Ssa85	147	2 out of 324	162
Lower Norns Creek	Ssa85	149	1 out of 92	46
Canyon Creek	Ssa85	153	28 out of 62	31
Kuyakuz Lake	Ots103	79	27 out of 96	48
Salmo River	Ots103	87	1 out of 112	56
Lower Norns Creek	Ots103	91	1 out of 86	43
Kootenay River	Ots103	93	1 out of 90	45
01201LNTH Lake	Ots103	97	1 out of 94	47
01166LNTH Lake	Ots3	76	1 out of 36	18
Columbia River at Genelle	Ots3	94	1 out of 293	146
Canyon Creek	Ots3	98	2 out of 64	32
Riley Creek	Omy77	94	1 out of 48	24
00410DEAD Lake	Omy77	96	1 out of 60	30
Salmo River	Omy77	140	4 out of 120	60
Lower Murphy Creek	Omy77	142	3 out of 100	50
Nimpkish River	Oneu14	165	1 out of 64	34
Riley Creek	Ots100	220	1 out of 58	29
Norns Creek Fan	Ots100	222	2 out of 268	169
Salmo River	Okia3	112	1 out of 38	19
		116	1 out of 38	19
Theleteban Lake	Okia3	138	1 out of 56	28
Lower Norns Creek	Okia3	162	1 out of 94	47
Lower Murphy Creek	Okia3	202	1 out of 100	50

Table 2.8 Hierarchical analysis of the regional and subregional distribution of genetic diversity in *Oncorhynchus mykiss* populations included in this study under various hypotheses. Calculated using ARLEQUIN ver 2.0, V_{bg} represents the percentage of variation existing between groups, V_{ap} , the amount existing among populations within groups, and V_{wp} is the percentage of variation existing within populations. The stated P-value refers to the probability that the observed value for V_{bg} is equalled or exceeded by chance determined from 1000 permutations. Probability values for all observed values of V_{ap} and V_{wp} were 0.0001. Among watersheds groupings include the Stikine River, Skeena River, Queen Charlotte Islands, Vancouver Island, Thompson River, upper Fraser River, and upper Columbia River. PCA groups refer to those population groups shown in Figure 2.4.

Comparison	V_{bg}	V_{ap}	V_{wp}	P
North coast vs south coast vs. interior	4.28	35.63	60.09	0.0430
Coast vs. interior	5.41	35.07	59.52	0.0088
Among watersheds	18.87	21.94	59.19	0.0001
Among PCA groups	19.10	21.86	59.05	0.0001

Table 2.9 Results of analysis of isolation by distance (IBD) after controlling for elevation for seven rainbow trout (*Oncorhynchus mykiss*) population chains within the interior lineage assayed at ten microsatellite loci. The p-value reported is the probability of obtaining the observed correlation between pair-wise comparisons of geographic distance and: (i) F_{st} (θ), or (ii) the residuals of the pairwise F_{st} values and geographic distance by chance as determined using Mantel tests (999 permutations).

Population Chain	Average Latitude	Fst		Residual	
		<i>r</i>	p-value	<i>r</i>	p-value
Columbia River Chain	49.2546	0.052055	0.41	0.000004	0.37
Columbia Chain w/o barrier population	49.2546	0.436274	0.017	0.08	0.41
Deadman Lake Chain	51.1011	0.502373	0.024	0.000002	0.51
Lower North Thompson Chain	51.1705	0.600929	0.021	0.00005	0.4
Blanchet Lake Chain	53.3836	0.258089	0.295	-0.000007	0.63
Fenton Lake Chain	53.4944	-0.177307	0.678	-0.04	0.13
Glatheli Lake Chain	53.6082	0.324092	0.136	0.25	0.17
Skinny Lake Chain	53.8203	0.781827	0.123	0.000003	0.44
Interior populations		0.407154	0.001	<0.00000	0.52
Coastal populations		0.197354	0.262	0.000006	0.32
All Populations in total		0.220399	0.001	<0.00000	0.5

CHAPTER 3: The effects of Contemporary Stream Networks on patterns of Genetic Variation in *Oncorhynchus mykiss*

There is abundant geographic variation in both morphology and allele frequency in most species. Geographic variation is a fundamental observation in nature and the extent of geographic variation results from a balance of forces tending to produce genetic differentiation and forces tending to produce genetic homogenization (Slatkin 1987). Genetic drift, the unpredictable change in allele frequency due to chance and finite population size, generally leads to genetic differentiation whereas gene flow, genetic change due to movement of gametes between populations, will oppose differentiation and can even maintain levels of genetic variation within populations (Hartl and Clark 1989). In population genetics theory, genetic drift and gene flow play crucial roles in influencing patterns of genetic diversity (genetic variation and genetic differentiation) observed in natural populations. Understanding the forces that influence drift and gene flow are of interest because these forces may help explain the origin and persistence of genetic diversity in nature.

Geography plays an important role in the distribution of a species and consequently its genetic diversity. The overall geographic range of a species is determined to a large extent by a series of historical events (Pielou 1991; Carvalho 1993; Lees et al. 1996; Avise 2000). A species will extend its range until it is limited by barriers, which may be physical or ecological (e.g.,

competition). Phylogeography (the physical mapping of intraspecific lineages) has recently emerged as a powerful method for assessing the roles of historical events e.g. glaciation and the detection of such migration barriers. By detecting patterns of genetic variation and differentiation, phylogeographic data allow the retracing of movements, events and histories that have shaped the modern day genetic and geographic structure of a species.

Almost all organisms demonstrate some degree of genetic differentiation, which is often dependant on life-history characteristics as well as environmental factors (Avice 1994). Heterogeneity generated by fragmentation can create barriers to movement because unfavourable habitat may not permit dispersal, may not provide cover against predators, or because distances between suitable habitats are greater than the species' ability to disperse. Consequently, the dispersal abilities of animals and particularly their tendency to disperse may be affected by the local geography such as landscape corridors and fragmentation. Dispersal and gene flow are linked in that geographic features that influence dispersal can also affect the degree of genetic differentiation or population subdivision.

Landscape variables can have dramatic consequences on population differentiation because of the reduction of gene flow between populations which can lead to loss of genetic variation through drift (Hartl and Clark 1989; Frankham et al. 2002; Costello et al. 2003).

Recent studies have focused on studying how local geography can influence dispersal and consequently patterns of genetic diversity (Carlsson and Nilsson 2000; Castric et al. 2001; Costello et al. 2003; Taylor et al. 2003). General conclusions from their studies suggest a variety of geographic variables can influence patterns of intraspecific genetic diversity particularly those which may influence accessibility to dispersal corridors. Recent examples include the influence of geographic distance and fragmentation in Bank voles (*Clethrionomys glareolus*; Gerlach and Musolf 2000) and a common frog (*Rana temporaria*; Hitchings and Beebee 1997), woodland isolation and fragmentation in the winter moth (*Operophtera brumata* L.; Van Dongen et al. 1998), large open bodies of water in the bat (*Myotis myotis*; Castella et al. 2000), and connecting valleys in the giant kangaroo rat (*Dipodomys ingens*; Good et al. 1997). In addition, habitat size is thought to have influenced the levels of genetic variation observed in the wild e.g. greater prairie chicken (Bouzat et al. 1998) and steelhead trout (Heath et al. 2001).

Here, I test the influence of local geography on population structure within a fish species, rainbow trout (*Oncorhynchus mykiss*) in British Columbia (BC) using observations of genetic variation and geography to directly compare patterns of genetic diversity in natural communities. Rainbow trout in BC represent a good study organism to address the influence of local geography on population genetic structure for three reasons. First, among vertebrates, freshwater fishes are limited to dispersal via waterways. This predisposes them to geographic

isolation and consequently a degree of genetic differentiation based on their surrounding geomorphology. Geomorphic features likely to influence genetic structure of fish populations on a local scale include waterfalls, geographic proximity, drainage pattern, stream gradient, fluvial distance and elevation (i.e. Shaw et al. 1994; Angers et al. 1999; Carlsson and Nilsson 2000; Castric et al. 2001; Costello et al. 2003). The complex nature of stream or lake geomorphology should promote the development of a hierarchical pattern of subdivision at the genetic level (Avice et al. 1987). Second, BC's glacial history has provided an abundance of features which promote rapid evolutionary divergence within species such as variation in ecological conditions as well as a very heterogeneous landscape (McPhail and Lindsey 1986). In particular, the vast array of environmental variables provides good opportunities to examine their impacts on the genetic structure of natural populations. Third, rainbow trout can be found in a diversity of habitat types due to their wide range of life history traits, they are often abundant, and can be found throughout different parts of BC making widespread collection across diverse landscapes feasible.

The genetic structure of rainbow trout is likely influenced by drainage patterns (river and lake systems), often separated by mountains and hills thus providing opportunities for genetic divergence. Conversely, large river systems connecting populations may have facilitated dispersal between populations from various drainages and possibly between refugial groups (Chapter 2). The complex distribution of rainbow trout has made resolution of the species' population

structure difficult and has resulted in numerous, frequently conflicting reports regarding the level of genetic differentiation and genetic variation of the species (e.g., Nielson 1999; Heath et al. 2001; Hendry et al. 2002). Until recently, however, few studies have attempted to understand how geomorphic features, which are expected to influence dispersal, influence the observed patterns of genetic diversity (but see Angers et al. 1999; Costello et al. 2003).

Observation of genetic diversity of natural systems in the field combined with geomorphic data can be used to investigate the influence of geography on the genetic structure of rainbow trout populations. The most convincing evidence for the importance of geography comes in systems where similar observations have been made within drainages for different species (Angers et al. 1999; Costello et al. 2003). In this chapter, I combine genetic and geomorphic data to investigate how geography influences population structure in rainbow trout. I tested the hypotheses that geographic variables that restrict dispersal will increase genetic differentiation among populations, geographic variables that promote population size would maintain high levels of genetic variation, and geographic variables that promote gene flow between populations will promote high levels of genetic variation within populations. To evaluate these hypotheses, I combined three approaches. For each hypothesis, I made predictions based on previous results from other taxa.

1. I predicted that the level of genetic variation would be correlated with the size of habitat within which a population resides.

I tested for a correlation between habitat size (lake surface area and perimeter, and lake/stream order) and genetic variation for all sample localities studied throughout BC. All else being equal, the number of fish (population size) should reflect the size of habitat the population lives in. Therefore, one would expect that as habitat size increases, so will population size which may result in the maintenance of genetic variation. Heath et al. (2001) studied the relationship between habitat rearing area and genetic variation among young steelhead trout and found that there was a significant positive relationship between heterozygosity and area of juvenile rearing habitat. There are, however, other studies that observed no impact of habitat size on genetic variation in natural populations (Angers et al. 1999; Castaic et al. 2001), so further tests of the general hypothesis are needed.

2. I predicted that an increase in geographic distance between populations would promote genetic differentiation.

I tested this hypothesis by comparing geographic distance between sampled populations with the level of genetic differentiation and level of misassignment to population of origin over large and local scales (major watershed and through out BC, respectively). A few studies have addressed the impact of geographic distance on the genetic structure of steelhead trout populations over large geographic scales e.g. up to 200 kilometres (Nielson et al. 1997), and 900

kilometres (Heath et al. 2001); however, few have addressed the influence of geographic distance on genetic structure on a more local scale such as within drainages.

3. I predicted that watershed characteristics that reduce interconnectiveness between localities (e.g. presence of barriers) would promote genetic differentiation.

I pooled all available genetic and environmental data available to conduct a multiple regression analysis to test this hypothesis. This test would permit me to determine which environmental variables best explain the observed differences in genetic diversity among populations. Significant effects of migration barriers (Costello et al. 2003) and drainage patterns (Angers et al. 1999) on genetic differentiation have been demonstrated in *Salvelinus* species, but little is known about how these watershed characteristics and others influence *Oncorhynchus mykiss* genetic diversity.

Materials and methods

A total of 2836 rainbow trout were collected for microsatellite DNA analysis, from throughout BC (Table 2.1 and 2.2, Figure 2.1). Of the total, 2141 represented interconnected localities from tributaries of the upper Columbia River (upper Columbia River watershed; 12 sample sites, 802 individuals), the Deadman River (Thompson River watershed; 10 sample sites, 320 individuals), upper Thompson River (Thompson River watershed; 8 sample sites, 235 individuals), Chelaslie River (upper Fraser River watershed; 7 sample sites, 224 individuals), Nutli River (upper Fraser River watershed; 4 sample sites, 128 individuals), Blanchet River (upper Fraser River watershed; 5 sample sites, 250 individuals), and Andrews Creek (upper Fraser River watershed; 4 sample sites, 182 individuals). These interconnected habitats within watersheds are here after referred to as population "chains". The upper Columbia River population chain, the two Thompson River Lake chains, and four upper Fraser River Lake chains were sampled from several major tributaries spanning comparable within-drainage pairwise geographic distances (0.8 km to 129.7 km, 0.6 km to 12 km, 0.8 km to 73 km, 4.8 km to 27.5 km, 6.1 km to 25.4 km, 2.1 km to 22.6 km, and 3.6 km to 18.4 km, respectively). The wide range in elevation also varied among the sample sites in the Blanchet Lake chain (417 to 464 meters), in the Nutli Lake chain (399 to 408 meters), in the Chelaslie River Lake chain (Glatheli Lake chain, 306 to 334 meters), in the Andrews Creek chain (Horseshoe Lake chain, 395 to 304 meters), in the upper Thompson River Lake chain (LNTN Lake chain, 434 to 457 meters),

in the Deadman River chain (DEAD Lake chain, 445 to 484 meters), and in the upper Columbia River chain (128 to 338 meters). All fish samples were collected by electro-shocking, gillnetting, angling, or seining. Adipose or pelvic fin clips were collected and stored in 95% ethanol until DNA could be isolated from ~5mg of tissue using the PureGene DNA isolation kit (Gentra).

Microsatellites

See Chapter 2 Material and methods

Genetic data analysis

Genetic variation

Descriptive statistics of microsatellite loci included expected heterozygosity (H_e), observed heterozygosity (H_o), number of alleles (A) and average number of alleles per locus and were compiled using TFGPA version 3.2 (Miller 1997). Allelic richness (A_r) was also calculated, using Fstat version 2.93 (Goudet 2001). Microsatellite allele frequencies were examined for Hardy-Weinberg equilibrium within populations and for departures from linkage disequilibrium between loci using GENEPOP (Version 3.1d, Raymond and Rousset 1995). Measures of F_{st} among populations within regions and pairwise genetic differences between populations (θ , Weir and Cockerham 1984) were determined using GENEPOP and their significance from permutation procedures in FSTAT (Version 2.8 for F_{st} within regions, Goudet 1999). All critical significance levels for simultaneous tests were evaluated using sequential Bonferroni adjustment (Rice 1989) with an

initial α level of 0.05. Other standard statistical tests, notably correlation and regression, were performed using the JMPin software package.

Analysis of molecular variance (AMOVA) between regions, and within and among populations were determined using ARLEQUIN (version 2.0; Schneider et al. 1997). In particular, I tested if the distribution of genetic variation was best explained by separation into above and below barrier populations, collectively, and then individually with each barrier forming a distinct group. I also tested to see if the distribution of genetic variation was best explained by separation into the different life history types and major watersheds of rainbow trout.

Genetic differentiation

The calculation of genetic distances, cluster analysis and principal components analysis were conducted among all samples as described in Chapter 2. The program STRUCTURE (Pritchard et al. 2000) was used to estimate the most likely number of genetic populations among all localities. STRUCTURE employs a Bayesian model-based clustering method for inferring population structure from genotypic data. It makes use of Markov chain Monte Carlo methods that cluster individuals into populations to minimize Hardy-Weinberg disequilibrium and linkage disequilibrium between loci within these populations. STRUCTURE was run a total of 10 times, each with a 'burn-in' period of 50,000 simulations, to minimize the dependence of subsequent parameter estimates on starting values, followed by parameter estimation after a further 450,000 simulations. Localities

that were not significantly differentiated from each other were typically geographically close to one another and probably represent individuals from the same genetic population, but were sampled when they were utilizing different habitats. Consequently, these localities were pooled based on results from STRUCTURE and a second set of analyses were conducted to ensure pooled sample sites were in Hardy-Weinberg and linkage (gametic phase) equilibrium. Consequently, for all subsequent analyses I present two sets of results. The first set is based on examining all 69 of the original localities while the second set utilized the 42 populations defined of the use of STRUCTURE as described above and subsequent pooling of some localities.

As an alternative measure of distinctiveness among populations, I conducted an assignment test to assess the accuracy with which individual fish could be classified to their known sample population based on their composite ten locus microsatellite genotypes (Hansen et al. 2001). This analysis includes two methods for assigning individuals to populations: likelihood-based methods and genetic distance-based methods. In the first method, individuals are assigned to the population in which the likelihood of their genotype is highest. In the second, individuals are assigned to the closest (genetically) population. The second analysis involves the calculation of a genetic distance between the individual being classified and the average of all of the individuals of the possible source populations. In earlier analyses (e.g. Taylor and McLean 1999) the maximum-likelihood based analyses showed superior performance in assigning individual

trout to their known sample of origin. Consequently, I adopted the maximum-likelihood approach for the current analysis, although results using genetic distance methods were very similar. The assignment test is a likelihood-based technique that calculates population allele frequencies, computes the likelihood of an individual multi-locus genotype belonging to candidate set of populations, and assigns that individual to the population where the likelihood of its genotype is the highest. Unlike F_{st} and other traditional measures of interpopulation divergence, assignment tests are based on individual multilocus genotypes rather than population wide descriptors (Paetkau et al. 1995; Waser and Strobeck 1998; Cornuet et al 1999; Hansen et al. 2001). Individuals are assigned to the candidate population in which the likelihood of their genotype occurring is highest. I used the software program GENECLASS version 5.1 (Cornuet et al. 1999) to assign individuals according to Rannala and Mountain's (1997) Bayesian method. To avoid biasing likelihoods, the individual being tested was excluded from its sample population when estimating allele frequencies by employing the 'leave on out' option. Assignment tests were calculated with a Bayesian method using a simulation procedure with 10 000 randomly generated genotypes. I chose the Bayesian method because it has performed better in computer simulations than other assignments tests (Cornuet et al. 1999), it takes into account the sampling error associated with estimating allele frequencies and considers differences in genetic diversity between populations (Rannala and Mountain 1997). Rainbow trout with a <5% likelihood of belonging to their sampled population where not assigned to that locality. To

be conservative, individuals who were assigned with data from fewer than seven loci were also not used due to the possible lack of correct assignment (Bernatchez and Duchesne 2000).

Isolation-by-distance

The test to determine if observed genetic structure was consistent with the Isolation-by-Distance model (Wright 1943; Slatkin 1993), analyses were conducted as described in Chapter 2. The influences of local geographic features including number of nodes and elevation were taken into consideration using partial Mantel tests (Mantel 1967) with the software program *r*-Package version 4.0 (Casgrain and Legendre 2001). Nodes were located at each directional turn as one traced a path (as a dispersing fish might) moving from the root (the root being defined as the common branching point for all populations) to each locality (e.g. Figure 3.1 and Figure 3.2). I also examined the effect of geographic distance on genetic structure using the assignment index. The proportion of “misassigned” genotypes at each site was compared with the geographic distance between sites. Anadromous trout often stray (Quinn 1993), compared to non-anadromous trout, and this may influence possible trends observed with misassignment. Consequently, assignment analysis were conducted with and without coastal anadromous populations. A Mantel test could not be used for this comparison because the proportion of the misassignments was not asymmetrical between sites (i.e., the number of misassignments differ from site A assigned to site B as compared to the

misassignment from site B assigned to site A). I therefore tested statistical significance using a regression analysis in the program JMP (version 3.2.1).

Spatial autocorrelation analysis

Spatial autocorrelation analysis was conducted to assess the spatial structure of *Oncorhynchus mykiss* throughout BC. Specifically, I used Mantel tests (Mantel 1967) to assess associations between the significance level for test of population differentiation and spatial location using fluvial distance between sites.

Correlograms were developed from simple Mantel test results (Legendre and Fortin 1989). The number of distance classes for correlograms was determined at each spatial scale in order to exceed 12 site x site comparisons within each distance category (Hitt et al. 2003). Significance of correlations was assessed with a Bonferroni corrected error rate, $\alpha = 0.05/N$ (N = number of distance classes). All simple Mantel tests were conducted with *r*-Package version 4.0.

Environmental analysis

A variety of geomorphic variables at watershed to site-specific scales were gathered to determine the extent to which aspects of the contemporary physical environment could be related to the patterns of microsatellite variation in rainbow trout in BC. To assess the relative importance of these factors, the program CANOCO (ter Braak 1988) was used to perform a canonical correspondence analysis (CCA). CCA incorporates both ordination and multiple regression techniques for direct analysis of the relationships between tables of multivariate

data. It is one of the most efficient tools for relating species composition to different predictive variables (ter Braak 1988; Magnan et al. 1995) and has recently been applied to describing the relationships between environmental variables and genetic diversity (e.g. Angers et al. 1999; Costello et al. 2003; Brouat et al. 2004).

In CCA, the number of alleles, expected heterozygosity, allelic richness and allele frequencies for ten microsatellite loci act as dependent variables and are related separately to two sets of independent variables: drainage pattern and a set of environmental variables. The drainage pattern matrix represents the spatial organization of populations in terms of their connectivity through the hydrographic network (cf. Magnan et al. 1995; Angers et al. 1999; Costello et al. 2003) (see Figure 3.1 and Figure 3.2). Nodes were numbered so each population could be coded by the pattern of nodes traversed from each locality to the root. Reported migration barriers existed in only one region (upper Columbia River population chain). These barriers were coded as though they were distinct nodes in the drainage network (Figure 3.1 and Figure 3.2, Table 2.1 and 2.2).

For the environmental matrix, I included variables that are hypothesized to influence rainbow trout demography (principally through population size effects) in terms of natural processes operating over longer evolutionary time frames. The environmental matrix components included elevation (Elev), number of fish species present (Spp), stream order (Stream order), lake surface area (Area),

lake perimeter (Perimeter), latitude (Alb X), longitude (Alb Y), and number of nodes from the root of all localities (Nodes).

Both matrices were related independently to the genetic data, and for each, the variables most able to account for the distribution of genetic variation were extracted using the forward selection procedure available in CANOCO. The forward selection procedure helps reduce the number of variables in the analysis to those which may significantly explain for the variation observed in the species variables. Specifically, the forward selection procedure adds environmental variables one at a time until no other variables significantly explain residual variation genetic variation. Selection of a particular drainage node, for example, would suggest that it was a significant factor in explaining differences in genetic variation or genetic divergence between populations located upstream and downstream of that node. The forward selected variables were then used to construct regression models whose contribution to explaining the genetic data and statistical significance was determined from the sum of canonical eigenvalues and p-values estimated using a permutation process.

As many environmental variables exhibit some type of spatial heterogeneity, the variation explained by environmental and spatial (drainage) models may, in fact, be correlated and therefore partly redundant. To determine whether the selected environmental variables still explain a significant proportion of the variation once spatial trends are removed, I used the method of variation partitioning suggested

by Bocard et al. (1992). I calculated the 'pure' environmental component of variation after removing the effects of drainage pattern using partial CCA. I similarly calculated the 'pure' drainage component by removing the effects of environmental variables and the component 'shared' between environmental and spatial variables. This allowed me to determine the relative influence of purely spatial and environmental factors in structuring the genetic variation that I observed. The statistical significance of the pure components was assessed in CANOCO by permuting the sum of all eigenvalues ($N=1000$) and applying Bonferroni corrections (initial $\alpha = 0.05/13$ in the upper Columbia River and Thompson River drainage, and $0.05/12$ in the upper Fraser River drainage).

Results

Genetic structure and subsequent pooling of localities

Among the 2346 pairwise comparisons made between localities for differences in allele frequencies summed across all ten loci, 60 were not significant (discussed in Chapter 2). Localities that were not significantly different from each other were pooled and tested for deviations from Hardy-Weinberg equilibrium, genotypic linkage disequilibrium and significance for population differentiation (Table 3.1 and Figure 3.2).

Following pooling of localities, virtually all were in Hardy-Weinberg equilibrium with 19 out of possible 420 (10 loci X 42 sample sites) tests showing statistically significant heterozygote deficits. These exceptions were found at several separate loci in 14 different localities and therefore do not compromise subsequent analyses (Table 3.2). Tests for linkage disequilibrium resulted in significant departures in five out of a possible 1953 tests. The statistically significant results were not concentrated on particular locus pairs or within specific localities.

Microsatellite variation among geographic areas

Reanalyzing pooled localities produced similar microsatellite variation results as when using the original sample set of 69 localities. The observed heterozygosity averaged 0.42 across all loci and localities ranged from 0.42 (*Ots103*) to 0.93 (*Okia3*), respectively. Genetic variation within sample sites ranged widely.

Expected heterozygosity, averaged across the ten loci, ranged from a low of 0.05 (Clearwater Creek) to highs of 0.62 – 0.68 (Gold River, China Creek, Kootenay River and lower Murphy Creek; Table 3.2).

Comparatively little microsatellite variation was found between lake and river/stream localities across the study area. Among lakes and river/stream localities within the coastal and interior lineages and among all populations, the percentages of variation existing between lake and stream/river groups were 9%, 10.3%, and 8.6% respectively (Table 3.3). A hierarchical analysis of the distribution of genetic diversity revealed that the grouping of populations that explained the greatest amount of variation across the sampling area was the comparison between above and below barrier populations and the comparison between different regions (Coast, upper Fraser River, upper Columbia River, and Thompson River; Table 3.3). In the above and below barrier comparison, 17.8% of the total variation was due to differences between them, 1.8% due to differences existing within groups and 80.4% of the genetic diversity was found to reside within populations themselves (Table 3.3). The distribution of genetic diversity between coastal (Vancouver Island, Skeena River, Stikine River) and interior (upper Fraser River, upper Columbia River, and Thompson River) regions also had similar levels of variation across the sampling area. Among localities, 17.7% of the total variation was due to differences between the regions, 22.9% due to differences existing within regional groups, and 59.4% was found to reside within populations themselves (Table 3.3). Variation across different life history

categories (lake resident, stream resident and anadromous) demonstrated a low, but significant amount of variation across the sampling area with, 3.3% of the total variation due to differences between the life history categories, 7.1% due to differences existing within groups, and 89.5% of the variation was found to reside within populations themselves (Table 3.3).

The large percentage of variation due to differences within groups in the various grouping strategies above, suggests that further regional and subregional population structure may exist. With nearly as much genetic variability observed within geographical regional groups as between them (Table 3.3), I chose to perform similar hierarchical analyses for individual regions. Hierarchical analyses were performed on the major regions sampled in this study which included coastal drainages, upper Fraser drainage, Thompson drainage, and the Columbia drainage separately (Table 3.4). I tested whether the distribution of genetic variability within each major watershed was best explained by grouping populations by drainages. In both the coastal region and upper Columbia River, the groupings by major tributary explained little of the observed genetic variation (0.27 to 4.4%) while pooling by major tributary in the upper Fraser and Thompson drainages explained the greatest amount of variation between groups (26.4 and 50.0 %, respectively Table 3.4).

Genetic variation and habitat characteristics

I then assessed the influence of habitat size and the level of genetic variation observed among all sample sites ($n=69$). Measures of habitat size characteristics, particularly area and perimeter, showed few very weak significantly positive relationships with genetic variation (Table 3.5). Those that were statistically significant had relatively low r values ranging from 0.22 to 0.32 suggesting that these particular habitat size characteristics may not be the most ideal predictors of genetic variation in my study. Stream/lake order, however, was an important force influencing the level of genetic variation found among natural populations; all tests were positive and statistically significant with stronger relationships with lake surface area and parameter (Table 3.5).

When the Columbia River sample sites were grouped into those residing above migration barriers ($N = 3$) and those below barriers ($N = 9$), permutation tests demonstrated that above barrier sites had significantly lower allelic richness (2.3 versus 5.1, respectively) and expected heterozygosity (0.365 versus 0.585, respectively), but significantly greater pairwise F_{st} (0.458 versus 0.025, respectively) than populations sampled from below upstream migration barriers (all $p < 0.05$).

There was a significant association between elevation and genetic variation (allelic richness, average number of alleles per locus, and expected heterozygosity) among all sample sites in BC ($r = -0.67$, $p < 0.0001$; $r = -0.71$,

$p < 0.0001$; $r = -0.66$, $p < 0.0001$, respectively). Within each drainage encompassing lake/stream chain localities there was significant relationship between elevation and the level of genetic variation observed in the upper Fraser River lake chains and the upper Columbia River localities; however, there was no significant relationship observed within the Thompson River lake chain localities (Table 3.6).

Genetic divergence among localities

Following the pooling of localities, the Neighbour-Joining (N-J) generated tree grouped populations by drainages and then into major geographic region, the coast (e.g. Stikine drainage, Skeena drainage, Queen Charlotte Island, Vancouver Island) or the interior (Columbia drainage, Thompson drainage, upper Fraser drainage) (Figure 3.4). The only exceptions were Khatada Lake and upper Sullivan Creek which clustered with localities that were from different drainages and was not proximally contiguous. Grouping of localities based on the Neighbour-Joining (N-J) generated tree and projection of populations in principal component space were very similar to previous analysis in Chapter 2 (Figure 2.4 and Figure 3.4; Figure 2.5 and Figure 3.3). Both demonstrated groupings of samples sites based on inland or coastal origin, and then by major drainages which were consistent with the AMOVA results.

Pairwise F_{st} estimates ranged from lows of 0.0 (between 01157 LNTH and 01166 LNTH, and between Glatheli Lake and Unamed 301 Lake) to highs of 0.21

(between 00466DEAD and Blanchet Lake 2, and between upper Sullivan Creek and Morgan Lake) before pooling of localities (Appendix A). After pooling, estimates for pairwise F_{st} ranged from 0.004 (between Blanchet Lake and Blanchet2 Lake), to highs of 0.3 (between Nutli Lake and upper Sullivan Creek) (Appendix B). Within lineages and regions, populations that were most divergent were those from geographically isolated sites, e.g. located above a migration barrier or an isolated lake. Populations that had the lowest levels of genetic differentiation were those that were contiguous and geographically proximate to one another, e.g. most population lake chains.

The assignment index indicated little differentiation among localities within the upper Fraser River, Columbia River and Thompson River chains (Table 3.7). In upper Fraser River lake chain, only 413 of 784 trout (52.7%) were assigned to the locality from which they were sampled (ranging from 11.7% to 93.8% of trout at each locality). Among Columbia River localities, only 354 of 817 trout (43.3%) were assigned to the locality from which they were sampled (ranging from 14.8% to 100% of trout at each locality). Among Thompson River chain localities, only 108 of 551 trout (19.6%) were assigned to the locality from which they were sampled (ranging from 0% to 80.1% of trout at each locality). Following the pooling and the reduction of localities from $N=69$ to $N=42$, the assignment index indicated increased differentiation with higher assignment scores between localities (Table 3.8). Among the upper Fraser River lake chains, 653 of 784 trout (83.3%) were assigned to the locality from which they were sampled

(ranging from 62% to 96% of trout at each locality). Among Columbia River localities, only 421 of 817 trout (51.5%) were assigned to the locality from which they were sampled (ranging from 22.4% to 100% of trout at each locality). Among Thompson River lake chain localities, 506 of 551 trout (91.8%) were assigned to the locality from which they were sampled (ranging from 78.1% to 100% of trout at each locality). Among coastal island populations (anadromous populations e.g. Queen Charlotte Island and Vancouver Island), 116 of 172 trout (67.4%) were assigned to the locality from which they were sampled (ranging from 40% to 80% of trout at each locality).

The relationships between fluvial distance and the level of misassignment, and with the significance for population differentiation were tested. Regression analysis indicated that there were statistically significant, negative relationships between fluvial distances separating localities and the level of misassignment within the upper Fraser River lake chains, upper Columbia River chains, Thompson lake chains, among the coastal populations, and on a larger scale, all sample sites throughout BC (Table 3.9). Following the pooling of sample sites there was still a significantly negative relationship between misassignment with increasing geographic distance among the Columbia River localities, among the coastal localities and among all localities (Table 3.10). Upon analyzing the data points of misassignment and geographic distance, there was an obvious skewness of the distribution of data points especially after plotting the residuals. Consequently, to meet the assumption of normality, the Y-variable

(misassignment scores) was log-transformed and I retested the relationship between misassignment and geographic distance. The relationship between log-transformed misassignment and geographic distance was stronger but the X-axis was still skewed following tests for normality. Following the log-transformation of the x and y axis and regression analysis, there was a stronger relationship between misassignment scores and geographic distances ($r=-0.54$, $p<0.0001$). When the analysis included anadromous localities, however, the results indicated that there was no statistically significant relationship between geographic distance and level of misassignment ($r=-0.17$, $p=0.16$).

Regression analysis between geographic distance and the significance (i.e. p-value) for population differentiation was tested to determine if geographic distance influences the level of population differentiation. There was a significantly positive, but weak relationship ($r=-0.19$, $p<0.0001$). Plotting the residuals from the regression analysis, data showed that these were not evenly distributed along the residual line and consequently a log transformation of the X and Y-axis was conducted and tested again with geographic distance. Following log transformation of both axes, the relationship was stronger ($r=-0.47$, $p<0.001$). There were noticeable numbers of pairwise comparisons which were non-significant between samples sites separated by eight or fewer kilometres. Thereafter, a sharp decline in the number of non-significant pairwise comparisons was documented with increasing geographic distance.

Binary measures of significance for population differentiation showed no statistically significant trend of spatial autocorrelation with significance for population differentiation and geographic distance following the correction for multiple tests (Rice 1989) at $\alpha = 0.05/5$ (five distance classes) = 0.01. At each of the five distance classes (0.6 km to 5 km, 5 to 10 km, 10 to 14 km, 14 to 28 km, and 28 to 60 km) the significance of pairwise tests for population differentiation did not demonstrate any significant trend ($r = -0.25$, $p = 0.02$; $r = -0.41$, $p = 0.01$; $r = 0.11$, $p = 0.16$; $r = -0.12$, $p = 0.18$; $r = 0.05$, $p = 0.42$ respectively) following Bonferroni corrections ($\alpha = 0.05/5$). When all localities were separated from each other by more than 20 km they were always significantly different from one another.

Significant associations between geographic and genetic distance (θ) among localities within the Deadman Lake chain ($r = 0.66$, $p = 0.002$), LNTH Lake chain ($r = 0.61$, $p = 0.007$), Glatheli Lake chain ($r = 0.5$, $p = 0.04$) as well as the Columbia River chain ($r = 0.41$, $p = 0.03$; without above barrier localities), were found prior to pooling of localities (from 69 to 42, Table 3.11). When the influence of elevation was partialled out, the relationship between geographic and genetic distance was significant only among the Deadman and LNTH Lake chains ($r = 0.5$ and 0.6 , $p = 0.2$ and 0.2 , respectively) as well as the Columbia River chain ($r = 0.44$, $p = 0.02$; excluding above barrier localities). Within the Columbia River localities, however, there was no significant association between geographic and genetic distance when above barrier populations were included before and after taking into account elevation effects (Table 3.6). When the influence of nodes was

partialled out, the relationship between geographic and genetic distance was significant among the Deadman, LNTH, and Blanchet Lake chains ($r=0.7$, 0.6 and 0.53 , $p=0.001$, 0.02 and 0.03 , respectively) as well as the Columbia River chain ($r=0.71$ $p=0.02$; including above barrier localities).

Significant associations between the number of nodes separating localities and genetic distance (θ), following the partialling out of elevation effects, within the Deadman Lake chain ($r=0.35$, $p=0.02$) and LNTH Lake chain ($r=0.43$, $p=0.01$) were found prior to pooling of localities (from 69 to 42, Table 3.11). When the influence of geographic distance was partialled out, the relationship between nodes and genetic distance was significant only among the LNTH Lake chain ($r=0.32$, $p=0.05$) and Columbia River chain ($r=0.29$, $p=0.05$; excluding above barrier localities).

Significant associations between elevation and genetic distance (θ) among localities within the Deadman Lake chain ($r=0.76$, $p=0.01$), LNTH Lake chain ($r=0.38$, $p=0.04$), Glatheli Lake chain ($r=0.61$, $p=0.008$) as well as the Columbia River chain ($r=0.62$, $p=0.03$; including above barrier localities), were found prior to pooling of localities (from 69 to 42, Table 3.11).

When the effects of geographic distances were partialled out, there was still a significant association between elevation and genetic distance among all Columbia River localities ($r=0.6$, $p=0.03$), Deadman, LNTH, and Glatheli Lake

chains ($r=0.65$, 0.36 and 0.5 ; $p=0.04$, 0.05 and 0.04 , respectively (Table 3.11). When the effects of nodes were partialled out, there was still a significant association between elevation and genetic distance among all Columbia River localities ($r=0.45$, $p=0.04$), Deadman, LNTH, and Glatheli Lake chains ($r=0.79$, 0.45 and 0.6 ; $p=0.003$, 0.02 and 0.02 , respectively (Table 3.11) including Columbia River localities except above barrier populations ($r=0.92$; $p=0.003$).

The association between genetic distance and geographic distance, elevation, and hydrographic network nodes was tested following the pooling of localities. Due to the large reduction of localities among the upper Fraser River lake chains, sample sites were pooled (Blanchet, Glatheli, Fenton, Skinny Lake chains all analyzed collectively) (Table 3.1, Figure 3.2). Among the Fraser River lake chain localities, geographic distance was not observed to influence genetic differentiation after controlling for the effects of elevation and the number of nodes (Table 3.12). Both elevation and presence of nodes did, however, impact the genetic structure of Fraser River lake chain localities following controlling for geographic distance (Table 3.12). Within the Columbia River chain localities, the presence of stream nodes had a limited influence on genetic distance between populations, whereas the influence of geographic distance and elevation were found to have profound effects (Table 3.12). When above barrier localities were removed, there was an even stronger relationship between geographic distance and genetic differentiation with the presence of nodes and elevation having limited effects on genetic differentiation. Test results from the Thompson Lake

chains were omitted due to the low post-pooling sample size ($n=2$). Across all 42 localities, there was strong signature of isolation-by-distance effect ($r=0.23$, $p=0.001$; Figure 3.5).

Canonical correspondence analysis

The canonical correspondence analyses for the upper Fraser, Columbia, and Thompson localities did not reveal statistically significant influences of spatial or environmental variables on the distribution of genetic diversity (Table 3.13). Using forward selection of variables, however, showed the environmental and drainage variables that were most likely to explain the distribution of genetic variation. In the upper Fraser River, UF2 was a major node which was forward selected as being predictor of genetic diversity. Selection of nodes UF4 and UF6, however, also occurred often. After the pooling of localities, however, only node UF2 and to some extent UF3 continued to be forward selected. The environmental variables which were continually forward selected before and after pooling of localities included Nodes, Elevation, and Species (Spp) for the upper Fraser River lake chains.

Among the drainage components for the upper Columbia River localities, only the nodes to above barrier sample sites (labelled as upper Murphy and upper Sullivan, and Clearwater Creek) and node CL2 were commonly selected as being predictors of genetic diversity (Table 3.13). The environmental variables which were commonly forward selected included Barriers, Elevation, latitude (Alb

X), and longitude (Alb Y). Following the pooling of localities, the forward selected drainage and environmental variables included the same variables except node CL2 (Table 3.13, 3.14).

Within the Thompson River lake chains, node TR2 was selected as being a predictor of genetic diversity 100% of the time (across all loci). The importance of TR2 is in agreement with tests for population differentiation that suggests a genetic discontinuity between localities from the LNTH Lake chains (upper north Thompson River) and the DEAD Lake chains (Deadman River). Among the environmental variables Alb Y and Elevation were consistently selected also (Table 3.13, 3.14).

Of the eight environmental variables and two drainage variables, elevation, number of nodes, and specific nodes (which indicated genetic discontinuity between drainages) were consistently selected as predictor variables in all the analyses. Barrier variables were also observed, but only among the Columbia River localities since no other drainage contained known above barrier populations (Table 3.13, 3.14). The influence of migration barriers in the upper Columbia River chain is in agreement with my AMOVA and regression results that demonstrated significant genetic discontinuities between populations located upstream and downstream of barriers.

Discussion

Patterns of genetic variation among populations can provide insights into the populations' life history and degree of evolutionary isolation. Among salmonids, the rainbow trout has one of the greatest measures of average heterozygosity revealed using microsatellite DNA (e.g. Beacham et al. 2000, Heath et al. 2002, Hendry et al. 2002). Although most of the variation found in studies over a broad geographic scale is found within populations (96%, Beacham et al. 2000; 96%, 75%, Nielsen and Fountain 1999; Heath et al. 2001), the remaining variation reflects substantial subdivision. Consequently, it is at the within-population level that we may understand how particular evolutionary events such as local geomorphology have influenced patterns of genetic variation.

Habitat size, genetic variation and differentiation

Genetic variation is the raw material for evolutionary change within populations and allows populations to evolve in response to environmental change. Maintenance of genetic variation is a fundamental concern in conservation biology. Consequently, to understand how much genetic diversity a population may have helps to understand the forces that influence it. Some empirical evidence based on comparisons of inter and intra-specific relationships between population size and genetic variation in the wild have been demonstrated in a wide range of species (Frankham 1996) including mammals and fish (Nevo et al. 1984; Bouzat et al. 1998; Heath et al. 2001). A positive relationship between population size and genetic variation is theoretically expected (Avice 1994;

Bouzat et al. 1998) and, therefore, a reduction in population size is generally accompanied by a decrease in heterozygosity (Waples 1989). In nature, it is often difficult to assess true population size, particularly for aquatic species such as fish. In such cases, it may be possible to measure geographic variables such as habitat area that may act as an indirect estimator of population size. The influences of habitat size on levels of genetic variation among some Salmonidae populations in the wild have been investigated (e.g. Angers et al. 1999; Castric et al. 2001; Heath et al. 2001), but variable results have been obtained. For instance, lake size in the brown trout (*Salmo trutta*; Jorde and Ryman 1996) and brook char (*Salvelinus fontinalis*; Angers et al. 1999 and Castric et al. 2001) were documented to have no relationship with the level of genetic variation observed in those studies, however, Heath et al. (2001) had found that fry rearing habitat area was related to the level of genetic variation in *Oncorhynchus mykiss*.

In my study, there were no estimates of population size, but variables that reflected habitat size, including lake surface area, lake perimeter, and stream/lake order. I found that my measures of habitat size, particularly lake perimeter and lake surface area, were not good predictors of genetic variation (Jorde and Ryman 1996; Angers et al. 1999; Castric et al. 2001). Although relationships between lake habitat size and average number of alleles, and expected heterozygosity and allelic richness were found to be statistically significant, they were very weak. The level of genetic variation observed in a population is largely influenced by the numbers of original founders, and over

time, its effective population size. Effective population size (N_e) is the number of individuals in a theoretically ideal population having the same magnitude of random genetic drift as the actual population (Hartl and Clark, 1989); therefore, as N_e increases, the effect of genetic drift decreases. It may be possible that regardless of how large the lake may be, other geographic factors are more likely responsible for influencing N_e such as those associated with reproduction including spawning or fry rearing habitat. Heath et al. (2001) had found that fry rearing habitat was related to the level of genetic variation observed in nature. Heath and his colleagues suggested that the mechanism behind the positive relationship between rearing habitat and genetic variation is that large rivers were capable of supporting larger populations which are characterised by high genetic variation and that this reduced loss of variation by drift. It is possible that the statistically significant, but weak relationship between lake surface area and perimeter with genetic variation in my study reflects the likelihood of a particular lake having its own spawning and/or fry rearing habitat(s) because some lakes may contain trout that are produced in adjacent, interconnected systems. Alternatively, larger systems are also more likely to have many streams associated with them. These streams may serve as specialized habitats required at various life stages (Schlosser 1995a, b) as documented in steelhead (Bisson et al. 1988; Bramblett et al. 2002) and lake/stream resident rainbow trout (Paul and Post 2001; James and Graynoth 2002) which may ultimately promote greater genetic variation via gene flow from neighbouring populations. The effects of lake surface area and perimeter do not, however, adequately explain

the level of genetic variation observed over the entire region because other variables such as post-glacial history, spawning and fry rearing habitat size and quality, and differences in effective population sizes may also be likely explanations.

Alternatively there may actually be a strong relationship between lake size and population size, but, the level of genetic variation observed may be the result of a historical founding event. Consequently, large post-founding population sizes may be able to limit the loss of genetic variation, but if the level of genetic variation was low to begin with then the pattern of increased genetic variation with lake size may not be resolvable. Likewise, in a situation where a small area is colonized by many individuals with high levels of genetic variation, the subsequent erosion of such variation via drift may be rapid if the population size declines to match that more closely tied to lake carrying capacity (Hartl and Clark 1989).

The level of genetic variation observed is also likely related to the level of connectivity between populations as depicted by stream/lake order. My results demonstrated that for all four measures of genetic variation, stream order revealed a stronger and significantly positive relationship compared to the other measures of habitat size. Shaw et al. (1991, 1994) found that the levels of observed polymorphism and heterozygosity were correlated with river order in Trinidadian guppies. The higher levels of genetic variation among lowland

populations with larger order streams may be explained by the amount of gene flow occurring through migration of individuals where lowland sites received continual genetic input from different upstream sources (unidirectional gene flow downstream) as well as greater gene flow by migration between different lowland rivers. The ultimate effect is a greater neighbourhood effective population size (Wright 1969; Jorde and Ryman 1996) in large stream order populations, which would reduce the effect of drift and bottlenecks compared to headwater populations.

The results of the assignment tests also support my general findings. There were more misassigned individuals documented within larger streams (as represented by stream order) as compared to smaller streams e.g. Columbia River system versus upper Fraser River lake chains, suggesting greater fish movement between populations joined by larger streams. Likewise, my genetic data suggested that contiguous populations have lower levels of population subdivision especially when they are interconnected by large river systems (Beacham et al. 2000; Hendry et al. 2002).

Jorde and Ryman (1996) demonstrated that lake size (from 300 to 1500 meters across) had no significant relationship with the amount of genetic variation (heterozygosity) observed in populations of brown trout (*Salmo trutta*). Their smallest lake studied not only showed the highest levels of genetic variation, but also showed the lowest effective population size. They explained this anomalous

result by suggesting that small population had high levels of heterozygosity due to the migration of fish from neighbouring populations which ultimately reflected a much larger effective population size (Jorde and Ryman 1996). Consequently, observed levels of genetic variation in nature appear to be profoundly influenced by the level of connectivity between populations. Jorde and Ryman (1996) also pointed out that there is a crude correspondence between the number of creeks flowing in and out of the lakes and the effective population size, i.e. small effective population sizes were estimated for those populations that had the fewest number of suitable breeding and/or nursing grounds (Jorde and Ryman 1996).

Geographic distance

Patterns of genetic differentiation and gene flow in various organisms are highly influenced by both macro and micro-geomorphic conditions experienced by the organisms and their dispersal abilities. Many species of salmonids such as *Oncorhynchus mykiss* undertake long migrations or dispersal between foraging areas in the ocean or lakes, and spawning grounds in rivers. Even though this species often exhibit very precise homing (e.g. Quinn 1993; Bagley and Gall 1998), dispersal (e.g. Schroeder et al. 2001) and successful reproduction of strays naturally occur at low levels (e.g. Reisenbichler et al. 1992; Nielson 1999) particularly among geographically proximate streams (e.g. Quinn and Fresh 1984).

The genetic structure of rainbow trout populations observed was largely influenced by local geomorphic features. The strong correlation between genetic distance and geographical distance suggests that population differentiation is largely governed by isolation-by-distance (IBD), at least within the upper Columbia River, Glatheli Lake chain and the upper Thompson River localities. Following the pooling of genetically similar localities, IBD was, however, only observed among upper Columbia River localities.

The lack of IBD within some drainages may be the result of small effective population size (N_e) and greatly reduced genetic variation via drift in tributary populations among the above barrier Columbia River localities (upper Sullivan, upper Murphy and Clearwater Creek). Also, above barrier localities demonstrated high levels of genetic differentiation between other sites regardless of their proximity and probably clouded the resolution of IBD (Hutchison and Templeton 1999). After removing the above barrier sample sites and controlling for elevation effects in my analyses, strong patterns of IBD were found. More generally, my results suggest that the patterns of genetic variation and differentiation in the Columbia River system are largely influenced by elevation, which is strongly associated with the presence of migration barriers. The AMOVA results also supported the influence of migration barriers in structuring genetic variation (Costello et al. 2003, Taylor et al. 2003).

Within each of the upper Fraser River lake chains, there were no localities that were as genetically differentiated from each other or as genetically depauperate as observed in the above barrier sample sites in the Columbia River system. Consequently, the lack of IBD in the upper Fraser system is unlikely to be explained by the presence of isolated populations. Rather, the apparent lack of IBD in the upper Fraser River drainage may be the result of ongoing gene flow, across relatively small spatial scales (e.g. tens of kilometres). The assignment tests and observed level of genetic differentiation between populations suggested that there were high levels of dispersal and gene flow between sample sites within each drainage (lake chain). Although IBD as measured by comparisons between multilocus F_{st} estimates and waterway distances were weak, there was a significant tendency for incorrectly classified individuals to be assigned to one of the nearest populations. Castric and Bernatchez (2004) found similar results and that assignment tests were a more sensitive test of IBD than F_{st} versus distance tests. Theoretical models have shown that few effective migrants are necessary to prevent strong differentiation between populations (Wright 1978, Slatkin 1985), which can compromise detection of IBD at small spatial scales.

My results investigating the relationship between fluvial distance and significance for population differentiation also supports the idea that geographically proximate localities are likely to exchange migrants and consequently be somewhat genetically similar. This is especially true for localities that are separated by less

than eight kilometres, such as many of the upper Fraser chain localities, and that have not had enough time to approach migration drift equilibrium. Another explanation may be that there are other migration barriers present which were not investigated in this study. Although many of the Columbia River localities were also separated by less than eight kilometres, significance of IBD in this area further suggests that these populations are in or closer to migration drift equilibrium than the upper Fraser River localities (Chapter 2).

Several authors (e.g. Slatkin 1993; Moran et al. 1995; Rousset 1997) have suggested that the apparent absence of IBD may be due to the scale at which IBD is investigated. Localities that are locally contiguous are likely to have gene flow occurring among them, resulting in no spatial pattern of population structure due to the homogenizing affect of gene flow. The detection of significant IBD is expected to result in reduced gene flow and high differentiation as populations become more physically separated. Hendry et al. (2002) reviewed studies of salmonid population structure and showed that studies at larger geographical scales tended to show higher levels of genetic differences among populations (F_{st}) than studies at smaller scales. Alternatively, the lack of statistical power to detect IBD may be due to the limited number of populations analysed. In a review of allozyme variation in phytophagous insects, Peterson and Denno (1998) demonstrated that with fewer than fifteen populations analyzed, one might conclude erroneously that IBD is lacking in a species in which gene flow does indeed decline with distance. This may hold true particularly among the upper

Fraser River lake chain populations where some lake chains only had four sample sites (e.g., Nutli Lake chain).

It is also likely that the failure to detect IBD is a result of a more historical effect. Given its more northerly location, the upper Fraser River was colonized postglacially after the upper Columbia River (McPhail and Lindsey 1986). Consequently, populations at the periphery of the range are expected to have lower levels of genetic variation than populations nearest to the original source, and also show reduced IBD owing to more recently founding. My results are consistent with relationships documented for bull trout (*Salvelinus confluentus*; Costello et al. 2003) within BC and the eastern collared lizard (*Crotaphytus collaris collaris*; Hutchison and Templeton, 1999). In both species, older populations had stronger patterns of IBD than more northerly areas that were established more recently.

Geographic distance alone was not the only variable influencing the genetic structure of populations. Although the patterns of genetic variation and differentiation in the Columbia River system was largely affected by elevation and presence of barriers, my results indicate that genetic structure in the different river drainages are affected by different suites of geomorphic features. For instance, within the upper Fraser River drainage, there was no IBD found after controlling the effects of elevation and the presence of nodes. Instead, the influence of nodes and elevation was found to play a significant role in shaping

the level of differentiation among populations, consequently masking or preventing any signs of IBD. Johnson and Black (1995) found significant IBD in regions where habitat was continuous and no IBD in regions that were highly fragmented. Both their and my results suggest, therefore, that when landscape features other than geographic distance influences population structure, IBD is not detected. My results suggest that each drainage is influenced differently by the studied geomorphic variables and that there is not just one variable that is responsible for the genetic structuring of all populations.

Connectivity

The genetic relationships of rainbow trout populations found in my study were those that reflected both current and historical hydrological connections as observed in other fishes (Meffe and Vrijenhoek 1988; Hansen and Mensberg 1998; Hurwood and Hughes 1998; Hebert et al. 2000; Costello et al. 2003). My hierarchical analysis of genetic variation demonstrates the importance of hydrological network in structuring genetic variation with drainages. In all regions, grouping of populations into major drainages explained a significant amount of structuring of genetic variation. Within the upper Fraser River and Thompson River drainages, however, further structuring of genetic variation into major tributaries explained more of the genetic structure than any other type of grouping. In addition, the N-J tree based on C-S chord distances and PCA based on allele frequencies also demonstrated structuring of populations based on major drainage systems. These results are similar to those in other salmonids

where a large portion of the genetic variation was explained by the differences between drainages (Reisenbichler and Phelps 1989; Reisenbichler et al. 1992; Nielsen et al. 1997; Hansen and Mensberg 1998; Knudsen et al. 2002).

The importance of connectivity to the structuring of genetic variation in rainbow trout was further supported by the canonical correspondence analysis. Although results were statistically non-significant, the forward selection process singled out drainage and environmental variables as the most likely ones to explain the observed genetic diversity. Results from my other analyses further supported these findings. In the environmental and drainage matrixes, the number of nodes, node UF2 and elevation were selected as predictors of the structuring of genetic diversity in the upper Fraser River drainage. In addition, there was a significant positive relationship between the number of nodes present between populations and the level of genetic differentiation within the upper Fraser River drainage even after controlling for the effects of geographic distance and elevation. Within the Thompson River drainage, the environmental variable, node TR2, was selected all thirteen times as a predictor for the structuring of genetic diversity. This node separated lakes of the LNTH drainage from those of the DEAD drainage. Results from assignment tests also supports my results suggesting no migration of individuals between the DEAD and LNTH drainages occurred beyond node TR2. In the Columbia River system, elevation and migration barriers were selected as predictors of the structuring of genetic diversity. These results are further supported by results from my Mantel tests

and AMOVA which demonstrated both that elevation and migration barriers play an important role in the genetic diversity of Columbia River populations.

On a smaller geographic scale within drainages, the lack of structuring among lacustrine rainbow trout populations suggests that there is considerable gene flow among lakes. The lack of significance for population differentiation and low F_{st} values imply that gene flow is occurring among lakes in the upper Fraser River and among lakes in the upper Thompson River. These results are surprising considering that anadromous rainbow trout are known for their specific homing ability (Quinn 1993) and other salmonids such as brook char (Angers et al. 1995) and brown trout are known to have strong population structure (Estoup et al. 1998) even after excluding isolated populations (Bouza et al. 1999). Theoretical models have shown that only few effective migrants are necessary to prevent strong differentiation between populations (Wright 1978; Slatkin 1985). It, therefore, seems reasonable to conclude that the observed trout movements result in a large amount of gene flow hence a low degree of differentiation at a local scale (e.g. tens of kilometers) among interconnected habitats.

Overall my results suggest that localities within a drainage may act more as a metapopulation. Individuals disperse often when not limited by migration barriers and gene flow between localities is common. The result is effectively lower values of F_{st} and the homogenization of genetic variation between these localities (Nei et al. 1975). Consequently, within drainages, localities which are contiguous

are likely 'genetically linked' by random gene flow and it is the habitat component which manages the degree of connectivity between localities. Therefore, the definition of 'populations' may not necessarily be defined by individual lakes or streams, but more a function of habitat parameters which may encompass many lakes and streams.

Prior to pooling of sample sites, assignment results revealed low assignment success to correct population of origin. In particular, all lake and stream chains demonstrated very high levels of misassignment with the exception of above barrier populations (upper Sullivan Creek and Clearwater Creek). These results, corroborated by tests for population differentiation and low pairwise F_{st} suggests that many sample sites within each lake chain were in fact the same genetic population that utilized many different habitats. This situation has rarely been reported in salmonids and very little is known about dispersal and population structure among lacustrine rainbow trout.

One explanation for the high rate of dispersal and gene flow is that each lake may possess a particular habitat type which is required for a particular life history stage. Fish may have to migrate long distances to achieve specialized habitats required at various life stages (Schlosser 1995a, b) as documented in steelhead trout (Bisson et al. 1988; Bramblett et al. 2002) and lake/stream resident rainbow trout (Paul and Post 2001; James and Graynoth 2002). Such movement promotes sharing of spawning habitats resulting in increased gene flow.

Such inter-habitat dispersal is probably promoted by low geographic distances between lakes in my study areas. Previous studies have reported similar low population structure of *O. mykiss* within drainages (Reisenbichler and Phelps 1989; Reisenbichler et al. 1992; Nielsen et al. 1997; Hansen and Mensberg 1998; Knudsen et al. 2002). These results suggest that there is high gene flow between sites within the same drainage as compared to those between drainages in the absence of within drainage migration barriers.

The assignment tests revealed, however, relatively high assignment success of individuals into their correct population of origin following the pooling of sample sites. These results suggest that individuals are more likely to disperse to nearby habitats within the same drainage and less to neighbouring drainages. Although long distance dispersal has been documented in anadromous rainbow trout, I consider it highly unlikely that lacustrine rainbow trout disperse very long distances. Rather, it is more likely that the long distance gene flow into neighbouring drainages occurs through a series of smaller dispersal events such as that of a stepping stone model (Kimura and Weiss 1964). The detection of significant reduction in misassignment with reduced geographic distance between sites supports the stepping stone model of dispersal in lacustrine rainbow trout.

The level of connectivity is much higher among anadromous populations compared to stream or lake resident rainbow trout because fresh water populations are restricted via a lack of an oceanic dispersal phase (Gyllensten 1985). The relatively high levels of genetic variation coupled with the low level of differentiation supports the idea that anadromous populations are likely to have higher N_e than lake or stream resident populations of rainbow trout from the continual gene flow between neighbouring populations (Reisenbichler and Phelps 1989, see also for cutthroat trout Weinburg et al. 1998; see also Bouza et al. 1999 for brown trout, *Salmo trutta*). Results from assignment tests also supports the idea that greater dispersal occurs between anadromous populations and that movement occurs more frequent between geographically proximate populations than those that are further away (Castric and Bernatchez 2004; Rogers and Curry 2004).

Migration Barriers

My data suggest that above barrier sample sites had lower genetic variation and greater differentiation from other sites as compared to below barrier sample sites. These results are similar to those found in many species of fish including the Trinidadian guppy (Shaw et al. 1991), westslope cutthroat trout (Taylor et al. 2003), bull trout (Costello et al. 2003), brown trout (Marshall et al. 1992), and in other rainbow trout (Chilcote 1976; referenced in Zimmerman and Reeves 2000; Parkinson et al. 1984; Currens et al. 1990). The typically smaller effective

population sizes of headwater and some above barrier populations is believed to be the major reason for this pattern (Hartl and Clark 1989).

One cannot, however, dismiss the possibility that the higher level of genetic variation among below barrier populations may be the result of greater gene flow from the lack of isolation between neighbouring populations (Slatkin 1981, 1985; Slatkin 1987; Preziosi and Fairbairn 1992; Hughes et al. 1996; Laikre et al. 1998; Riginos and Nachman 2001). In addition, the downstream movement of fish over barriers which may result in an increase in genetic variation of below barrier populations (Shaw et al. 1991; Marshall et al. 1992; Shaw et al. 1994).

The unique characteristics of above barrier populations e.g. low genetic variation and high differentiation, suggests that 00466DEAD Lake and Khatada Lake are located above migration barriers even though such barriers on these systems have not been formally documented. The distinct clustering in CSE N-J tree and PCA analysis of DEAD lakes and Khatada Lake indicate that both are isolated from other populations within their drainage. Also, the low level of genetic variation (similar to those which were classified as above barrier within in Columbia River system) and the lack of misassigned individuals from other sources further suggest strong geographical isolation of these populations.

Due to the loss of genetic variation and near fixation of particular alleles, above barrier populations tend to cluster with other populations from other river

systems. The N-J dendrogram and PCA clustering of sample sites based on allele frequencies, generally demonstrated close relationships between populations from the same river system/region. Above barrier populations, however, were exceptions to the general pattern. For instance, Khatada Lake (belonging to the Skeena watershed) was found to cluster with sample sites from the Salmo River (Columbia River). The upper Sullivan Creek (belonging to the Columbia watershed) was found to cluster with the Thompson River sample sites. Also, although the upper Murphy Creek (belonging to the Columbia watershed), Clearwater Creek (belonging to the Columbia watershed), and 00466DEAD Lake (belonging to the Thompson watershed) did group with their appropriate drainage, there was no tight clustering as observed with other contiguous populations within a drainage e.g. upper Fraser River lake chains. The PCA analysis further suggested that Khatada Lake, Clearwater Creek, upper Sullivan Creek, and 00466DEAD Lake were genetically different from populations within the same drainage. Upper Murphy Creek, however, clustered closely with populations of the same drainage suggesting genetic similarity. Upon further examination of upper Murphy Creek, the level of genetic variation was found to be intermediate between those of above barrier populations and those of below barrier populations. Not only was the level of genetic variation intermediate between above and below barrier sites, but pairwise genetic distances between upper Murphy Creek and populations within the drainage were also intermediate. The results from the assignment tests also suggest dispersal of upper Murphy Creek individuals with nearby sample sites in contrast to results for the other

above barrier populations. Upper Murphy Creek may not necessarily be a strictly isolated population, but rather a headwater population that is capable of exchanging individuals with downstream sites.

Not all populations located above migration barriers demonstrated low genetic variation and high genetic differentiation. While a few lake chains such as those within the upper Fraser River drainage e.g. Blanchet Lake chain, are located above migration barriers, they are interconnected amongst members of the chain. Costello et al. (2003) found that while most above barrier bull trout populations show reduced genetic variation and were often monomorphic at several loci those isolated above barriers, but in larger watersheds, above barrier populations retained higher levels of genetic variation. Knudsen et al. (2002) found similar results where they looked at rainbow trout populations in the Kootenay River. Mainstream populations demonstrated the highest level of genetic variation whereas populations isolated above migration a barrier, but interconnected above the barrier, demonstrated intermediate levels of genetic variation, and isolated headwater populations showed the lowest levels of variation. Their observations demonstrate the nested nature of connectivity. Above barrier populations that are interconnected with other streams above the same barrier have potential for inter population gene flow among neighbouring populations resulting in a larger N_e . By contrast, single populations isolated above barriers have the lowest levels of genetic variation.

Contemporary factors can strongly influence patterns of genetic diversity initially set by founder events and postglacial dispersal. For example, the analysis of molecular variance points to the importance of migration barriers in structuring the genetic variation within and between watersheds in the rainbow trout. In all the regions, grouping of populations isolated above migration barriers against those below migration barriers explained a large component of the genetic variation in my dataset, comparable to that among major regions throughout BC. In addition, the greatest pairwise genetic distance within regions always occurred between populations isolated above migration barriers, and these distances were often greater than the average genetic distance between regions (c.f. Costello et al. 2003). The importance of barriers to structuring genetic variation among rainbow trout populations was further supported by the canonical correspondence analysis. In both spatial and environmental analyses, barriers were selected more often than all other variables as predictors of the structuring of genetic diversity within the regions (c.f. Costello et al. 2003). Migration barriers therefore appear to be important factors influencing observed patterns of genetic variation among populations over a large geographic scale.

Elevation and genetic variation

The general pattern of deglaciation in BC was from lowland areas northwards up major valleys and back into mountainous areas (McPhail and Lindsey 1986). Because founding individuals may represent only a fraction of its original population and higher elevation habitats are generally less accessible,

populations colonizing such areas are expected to exhibit lower levels of genetic variation as compared to their lowland ancestors.

In my study I found that populations at higher elevations had lower levels of genetic variation than sample sites at lower elevations. Higher elevated populations commonly displayed the same or fewer alleles as populations at lower elevations within the same drainage. This suggests that the higher elevated populations were most likely founded by their lowland ancestors and through founder effects and drift, have lost some genetic variation. Such trends have also been observed in the alpine snail (*Arianta arbustorum*, Arter 1990) as well as in Trinidadian guppies where lowland populations have significantly higher observed heterozygosity than upland populations which have less number of species and lower observed heterozygosity (Shaw et al. 1991).

Within the Thompson River drainage there was no significant relationship found between elevation and genetic variation. One reason for this is the elevation differences between sample sites were generally low. The range in elevation within the Thompson Lake chains was from 434 to 484 meters, whereas in the upper Fraser River lake chains and upper Columbia River sample sites it was 295 to 464 meters and 128-338 meters, respectively. As in the detection for IBD, there may not be enough elevation differences to detect significant trends.

Conclusion

In conclusion, the results from my study point to the importance of local hydrological features in shaping patterns of genetic variation and differentiation observed in *Oncorhynchus mykiss*. Particular geomorphic variables observed to have influenced patterns of genetic diversity include the presence of migration barriers, elevation, stream/lake order, presence of nodes and drainage pattern, and geographic distance between populations. The common thread which holds these variables together is the influence they all have on connectivity. Ultimately the level of genetic variation is governed by the level of inter-connectiveness and its interaction with population history. Populations that are less interconnected e.g. more isolated, are likely to become more influenced via drift than those that are interconnected and are exchanging individuals. The observed pattern of genetic differentiation between populations is, therefore, strongly influenced by the level and pattern of genetic variation within populations.

Figure 3.1 Drainage matrices for the upper Fraser, Thompson, and upper Columbia River regions illustrating the spatial arrangement of sample locations in the hydrographic matrix prior to pooling of sample sites (n=69). Population numbers correspond to those in Figure 2.1 and the names in Table 2.1. Nodes are numbered from (a) UF1-UF8 (upper Fraser River) (b) TR1-TR6 (Thompson River), and (c) CL-CL4 (upper Columbia River), except for barrier nodes that are named and underlined.

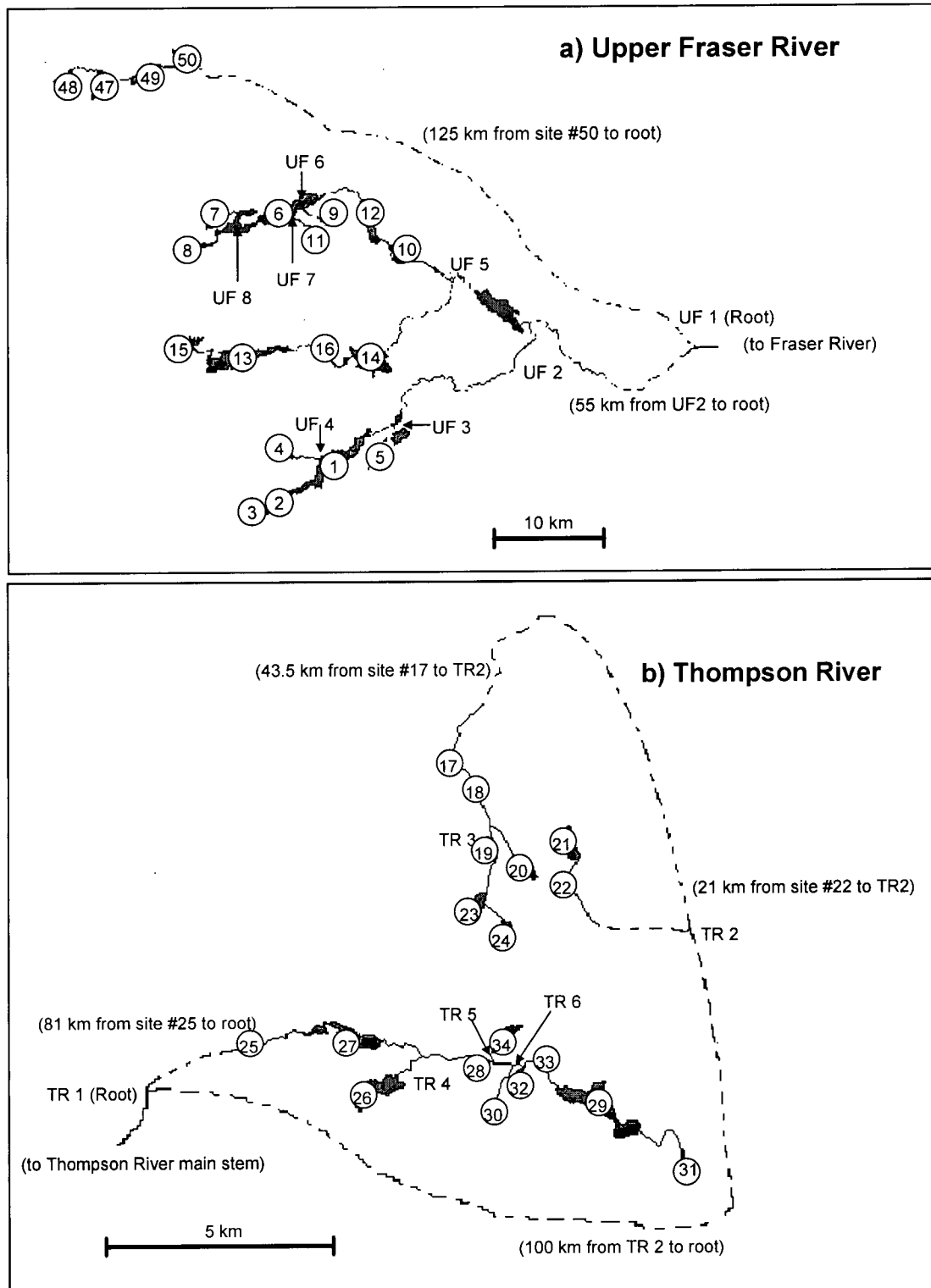


Figure 3.1 Continued

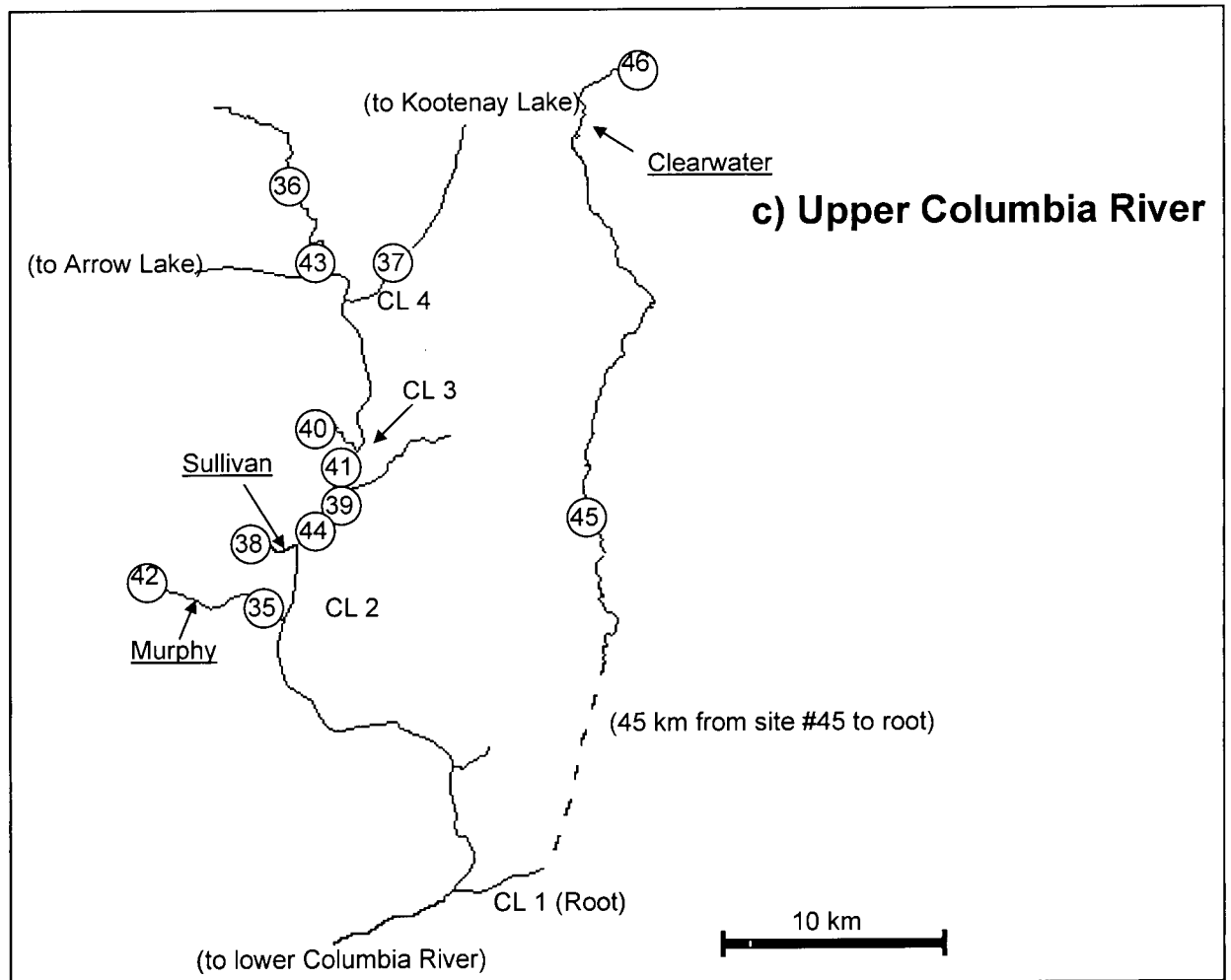


Figure 3.2 Drainage matrices for the upper Fraser, Thompson, and upper Columbia River regions illustrating the spatial arrangement of pooled sample locations (n=42) in the hydrographic matrix. Population numbers correspond to those in Figure 2.1 and the names in Table 2.1. Nodes are numbered from (a) UF1-UF4 (upper Fraser River) (b) TR1-TR2 (Thompson River), and (c) CL1-CL4 (upper Columbia River), except for barrier nodes that are named and underlined.

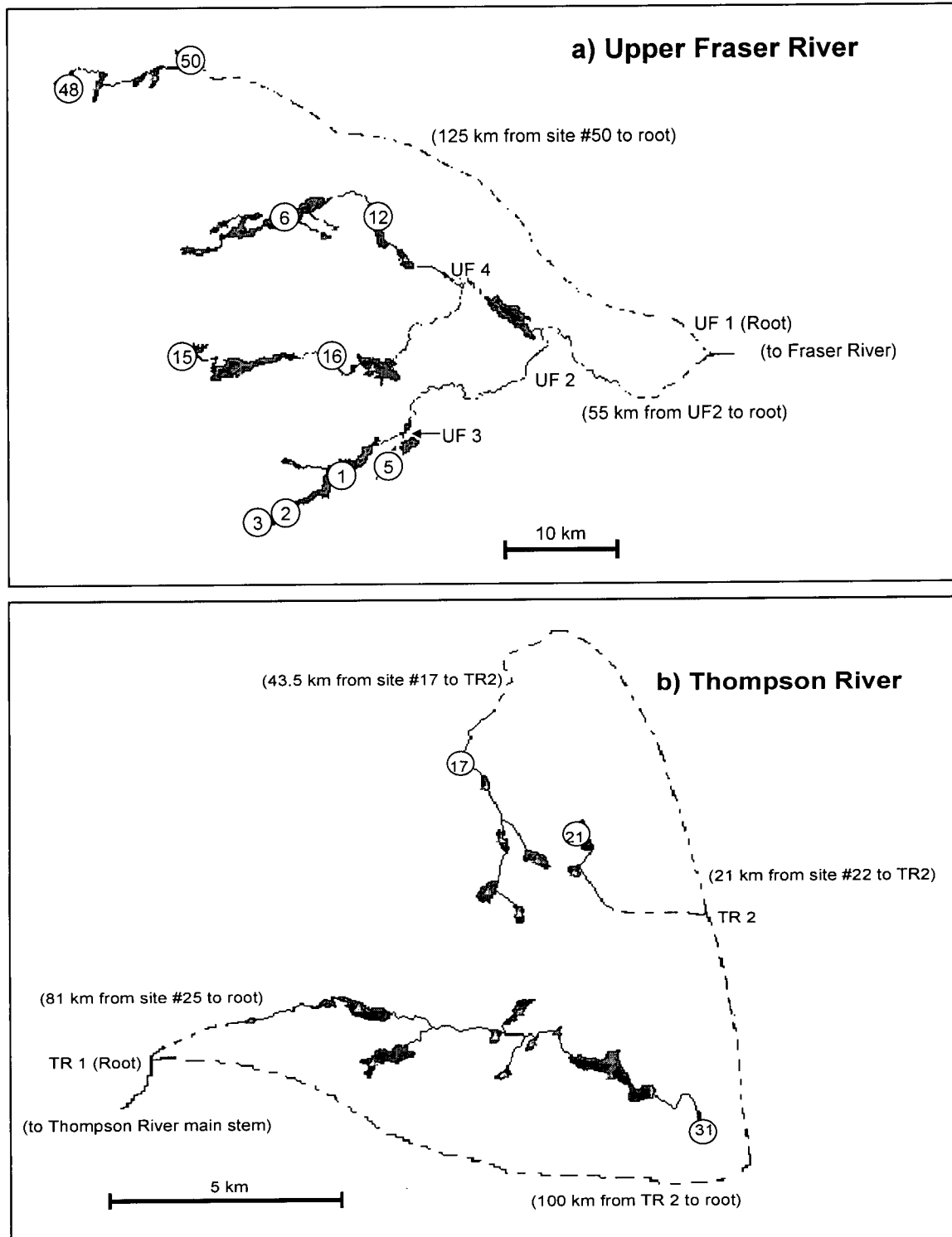


Figure 3.2 Continued

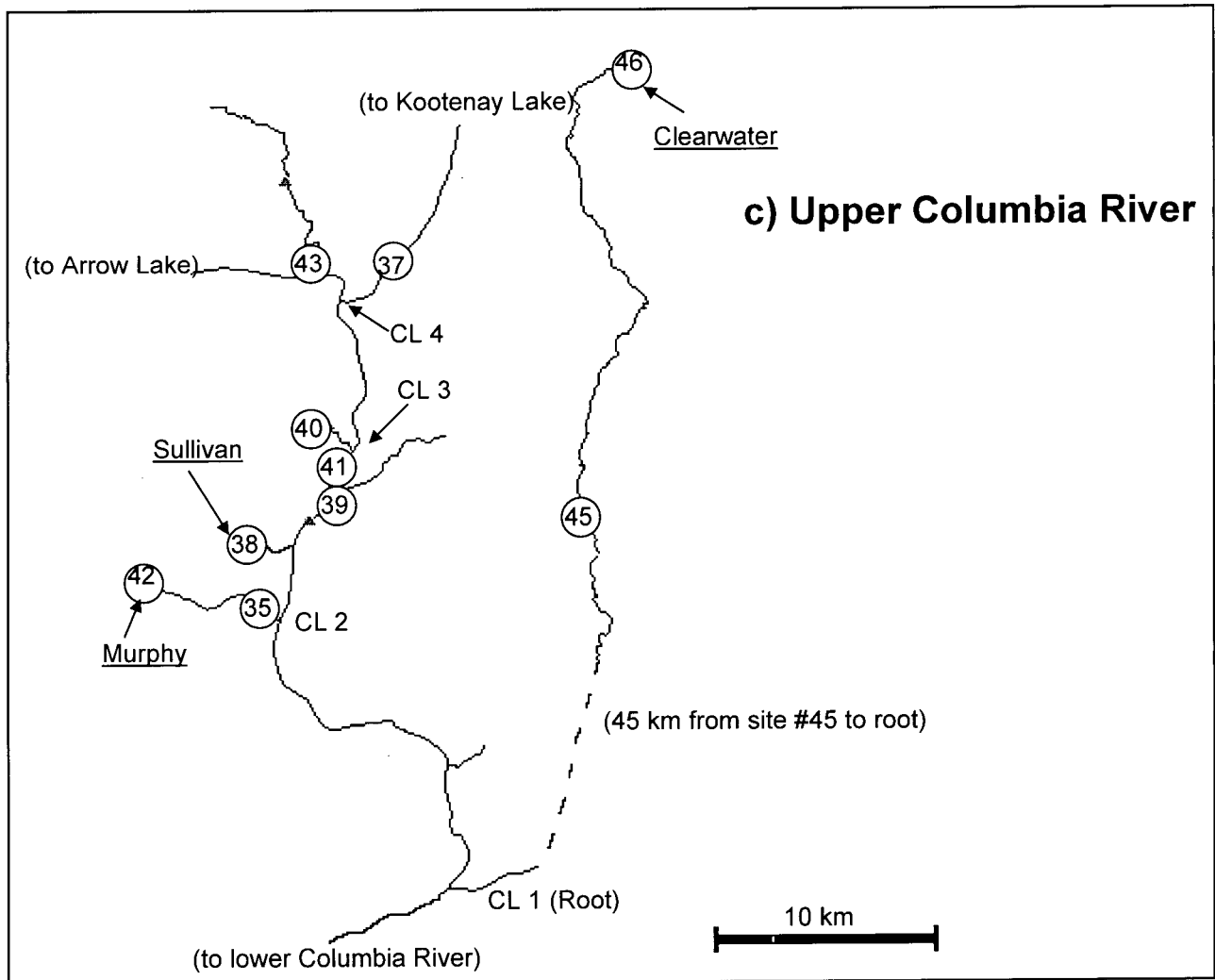


Figure 3.3 Results of principal components analysis of allele frequency variation among localities of *Oncorhynchus mykiss* assayed at ten microsatellite loci for the reduced sample set of 42 populations depicted as plots of mean component scores for each population along axes 1 and 2. Population sites are colour coded to represent the major drainage it belongs to. Groups of populations belonging to a major drainage are included within ellipses with the exception of coastal populations which were combined (green labelled). Note that for visual clarity, some populations from the upper Fraser, upper Columbia, and Thompson River drainages were not circled. Site # (Sample site): 1(Blanchet Lake), 2(Blanchet 2 Lake), 3(Blanchet 3 Lake), 4 (Tlutlias Lake), 5(Khatada Lake), 6(Fry Creek), 7(Nimpkish River), 8(Gold River), 9(Blackwater River), 10(Clearwater Creek), 11(Salmo River), 12(Kinbasket Reservoir), 13(Lardeau River), 14(Glatheli Lake), 15(Ghitzeli Lake), 16(Goodrich Lake), 17(Morgan Lake), 18(01179 LNTH Lake), 19(01189 LNTH Lake), 20(00466 DEAD Lake), 21(Murray Creek), 22(Kuyakuz Lake), 23(Coldwater River), 24(Clearwater River), 25(Fish Lake), 26(lower Murphy Creek), 27(upper Sullivan Creek), 28(China Creek), 29(lower Blueberry Creek), 30(lower Norns Creek), 31(upper Murphy Creek), 32(Norns Creek fan), 33(Kootenay River), 34(Copper Creek), 35(Mamin River), 36(Yakoun River), 37(Riley Creek), 38(Canyon Creek), 39(Moosevale Creek), 40(Eaulve Lake), 41(Twinkle Lake), and 42(Horseshoe Lake). Above barrier populations are underlined.

Figure 3.3 Continued

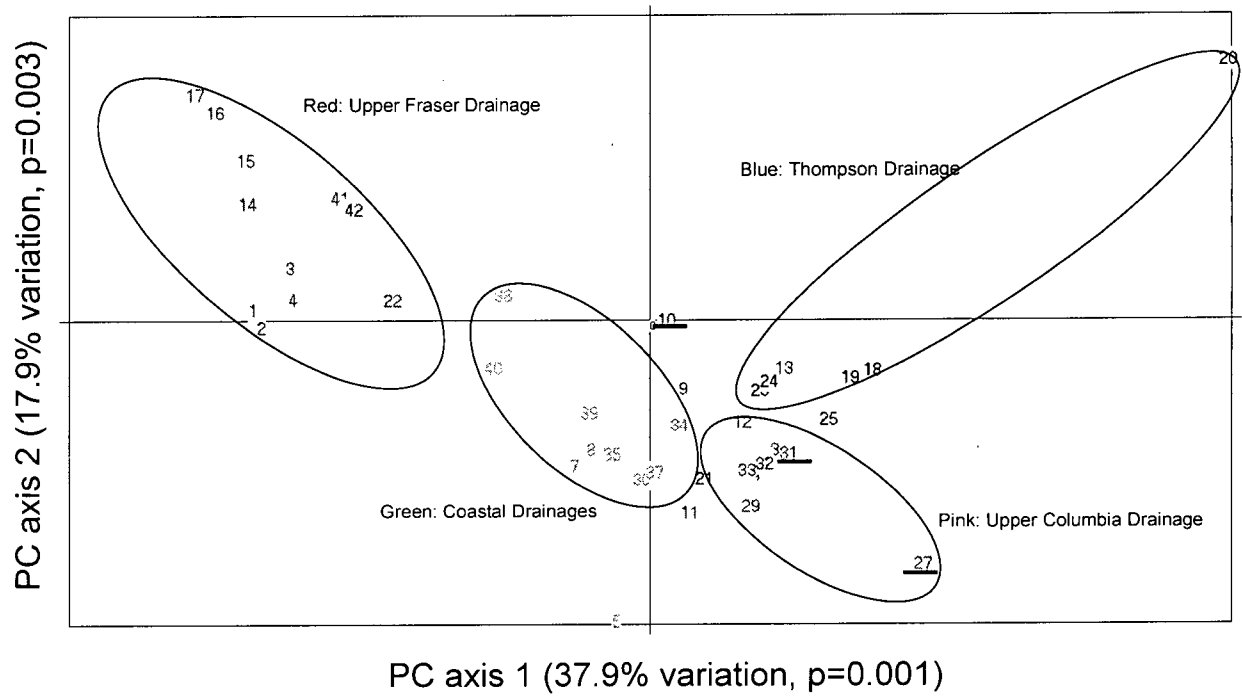


Figure 3.4 Neighbour-joining tree of relationships among populations of rainbow trout (*Oncorhynchus mykiss*) from British Columbia from the reduced data set of 42 populations. Clustering was based on Cavalli-Sforza and Edwards' (1967) chord distances (CSE) derived from allelic variation at ten microsatellite loci. Numbers at branch points represent bootstrap percentages from 1000 replicates (only those values $\geq 50\%$ are shown).

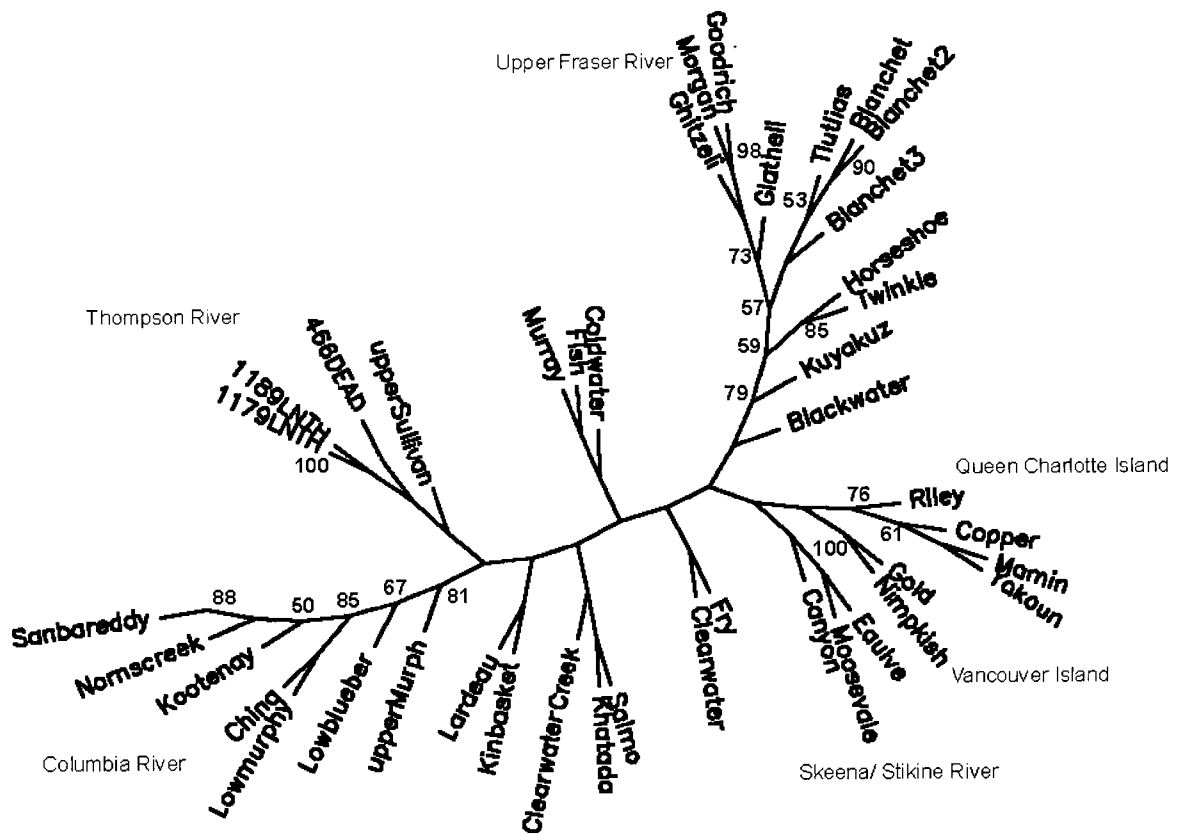


Figure 3.5 Isolation by distance analyses (IBD) for *Oncorhynchus mykiss* following pooling of sample sites throughout BC assayed at ten microsatellite loci. Pairwise F_{st} (θ) distances (y-axis) are plotted against pair-wise geographic distances (x-axis).

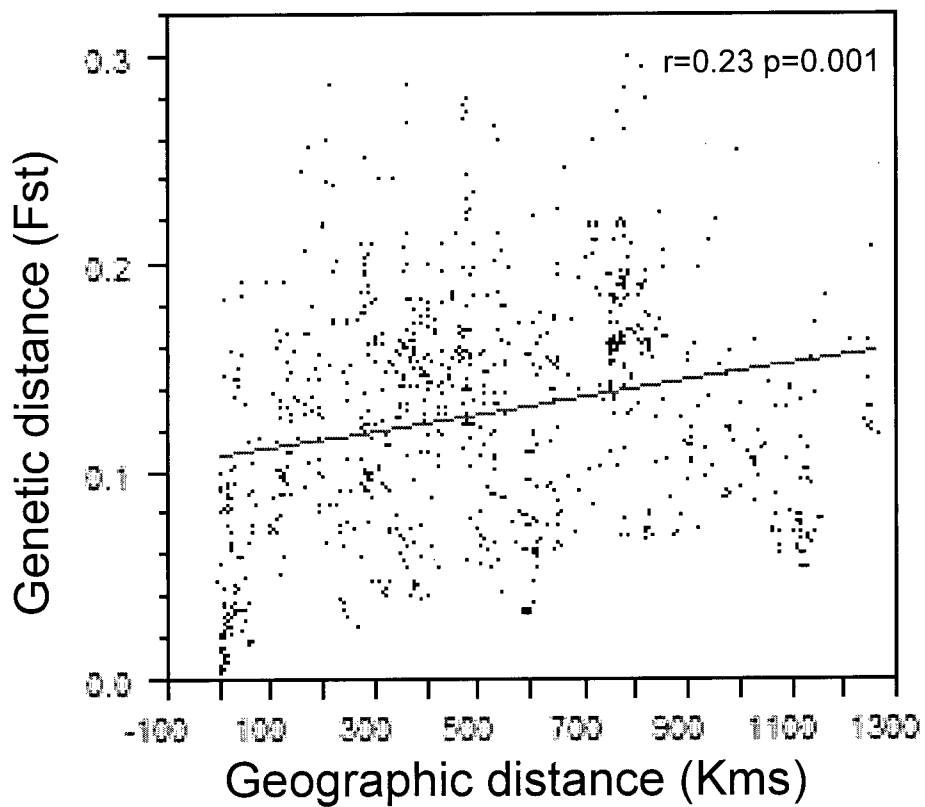


Table 3.1 Pooling strategy for populations within population chains of rainbow trout (*Oncorhynchus mykiss*). Pooling of sample sites were determined following tests for significance for genetic differentiation, Hardy-Weinburg equilibrium, and linkage disequilibrium. Population chain names are in italics and names of pooled populations, and new sample sizes are shown.

Sample site	Pooled with Sample site	Sample size	Drainage
<i>Blanchet Lake chain</i>			
Blanchet Lake	Grizzly Lake	100	Upper Fraser River
Blanchet 2 Lake		50	Upper Fraser River
Blanchet 3 Lake		50	Upper Fraser River
Tlutlias Lake		50	Upper Fraser River
<i>Glattheli Lake chain</i>			
Glattheli Lake	Unnamed 1 Lake, Michel Lake, Unnamed 2 Lake, Unnamed 3 Lake	160	Upper Fraser River
Theleteban Lake	Ghitzeli Lake	64	Upper Fraser River
<i>Fenton Lake chain</i>			
Goodrich Lake	Fenton Lake	64	
Morgan Lake	Nutli Lake	64	Upper Fraser River
<i>Skinny Lake chain</i>			
Twinkle Lake	Skinny Lake, Needle Lake	150	Upper Fraser River
Horseshoe Lake		32	Upper Fraser River
<i>Lower North Thompson chain</i>			
01157LNTH Lake	01166LNTH Lake, 01179LNTH Lake, 01193LNTH Lake, 01184LNTH Lake	139	Thompson River
01176LNTH Lake	01189LNTH Lake, 01201LNTH Lake	96	Thompson River
<i>Deadman chain</i>			
00466DEAD Lake	00422DEAD Lake, 00357DEAD Lake, 00409DEAD Lake, 00447DEAD Lake, 00416DEAD Lake, 00376DEAD Lake, 00439DEAD Lake, 00410DEAD Lake, 00369DEAD Lake	320	Thompson River
<i>Columbia River chain</i>			
Lower Norns Creek	Sand Bar Eddy	90	Upper Columbia River
Kootenay River	Columbia River at Genelle	214	Upper Columbia River
Lower Murphy Creek		50	Upper Columbia River
Upper Sullivan Creek		38	Upper Columbia River
China Creek		49	Upper Columbia River
Lower Blueberry Creek		49	Upper Columbia River
Upper Murphy Creek		50	Upper Columbia River
Norns Creek Fan		175	Upper Columbia River
Salmo River		60	Upper Columbia River
Clearwater Creek		27	Upper Columbia River

Table 3.2 Summary of allelic variation at ten microsatellite loci among pooled sample localities of rainbow trout (*Oncorhynchus mykiss*). Pooled sample sites are grouped into major watersheds in italics (*Upper Fraser River, Thompson River, Upper Columbia River, South Coast British Columbia, North Coast British Columbia, Stikine River, and Skeena River*). Number of alleles per locus (A), expected heterozygosity (H_e), observed heterozygosity (H_o), allelic richness (A_r), and the number of genotyped individuals (N) are given for each loci per population. Significant departures from HWE are denoted by "*" (using Bonferroni corrections for 42 populations; $\alpha = 0.05/42=0.0012$).

Upper Fraser River

Blanchet Lake

	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	3.00	2.00	2.00	4.00	2.00	2.00	1.00	2.00	1.00	10.00	2.90
He	0.63	0.42	0.10	0.48	0.19	0.50	0.00	0.50	0.00	0.77	0.36
Ho	0.56	0.43	0.10	0.46	0.18	0.60	0.00	0.45	0.00	0.77	0.36
Ar	3.00	2.00	1.70	3.62	1.93	2.00	1.00	2.00	1.00	6.24	2.45
N	99.00	99.00	100.00	93.00	97.00	97.00	97.00	98.00	100.00	96.00	97.60

Blanchet 2 Lake

A	3.00	2.00	2.00	4.00	2.00	2.00	1.00	2.00	1.00	7.00	2.60
He	0.61	0.41	0.16	0.51	0.26	0.35	0.00	0.49	0.00	0.72	0.35
Ho	0.58	0.45	0.10	0.57	0.30	0.32	0.00	0.44	0.00	0.76	0.35
Ar	2.98	2.00	1.90	3.16	1.98	2.00	1.00	2.00	1.00	5.36	2.34
N	50.00	49.00	50.00	47.00	50.00	47.00	50.00	50.00	50.00	50.00	49.30

Blanchet 3 Lake

A	3.00	3.00	2.00	3.00	1.00	2.00	1.00	2.00	4.00	5.00	2.60
He	0.41	0.23	0.06	0.47	0.00	0.10	0.00	0.02	0.38	0.45	0.21
Ho	0.41	0.14	0.02	0.47	0.00	0.10	0.00	0.02	0.41	0.44	0.20
Ar	2.64	2.19	1.54	2.54	1.00	1.73	1.00	1.22	3.40	3.70	2.10
N	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00	50.00	49.10

Tlulias Lake

A	3.00	2.00	2.00	4.00	2.00	2.00	1.00	2.00	1.00	6.00	2.50
He	0.41	0.24	0.48	0.62	0.16	0.18	0.00	0.34	0.00	0.67	0.31
Ho	0.34	0.28	0.58	0.71	0.14	0.16	0.00	0.31	0.00	0.59	0.31
Ar	2.92	1.98	2.00	3.21	1.90	1.93	1.00	2.00	1.00	4.34	2.23
N	50.00	50.00	50.00	49.00	50.00	50.00	47.00	48.00	50.00	49.00	49.30

Blackwater River

A	9.00	5.00	3.00	5.00	3.00	8.00	3.00	2.00	9.00	12.00	5.90
He	0.77	0.58	0.14	0.59	0.30	0.81	0.51	0.50	0.78	0.89	0.59
Ho	0.66	0.54	0.14	0.41	0.27	0.91	0.64	0.51	0.73	0.80	0.56
Ar	5.77	3.49	2.18	4.29	2.63	6.66	2.23	2.00	6.21	9.47	4.49
N	47.00	48.00	49.00	46.00	48.00	47.00	47.00	47.00	45.00	46.00	47.00

Glatheili Lake

A	7.00	4.00	1.00	4.00	2.00	7.00	1.00	2.00	3.00	10.00	4.10
He	0.65	0.34	0.00	0.54	0.01	0.70*	0.00	0.49	0.07	0.79*	0.36
Ho	0.62	0.36	0.00	0.45	0.01	0.59	0.00	0.47	0.07	0.62	0.32
Ar	4.03	2.90	1.00	2.67	1.14	5.59	1.00	2.00	1.61	6.65	2.86
N	154.00	154.00	157.00	132.00	150.00	147.00	159.00	158.00	152.00	130.00	149.30

Upper Fraser River

Ghitze Lake

	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	6.00	4.00	1.00	5.00	1.00	6.00	1.00	2.00	2.00	11.00	3.90
He	0.62	0.57	0.00	0.62	0.00	0.63	0.00	0.22	0.02	0.75*	0.34
Ho	0.59	0.56	0.00	0.69	0.00	0.60	0.00	0.19	0.02	0.40	0.31
Ar	4.29	3.72	1.00	3.71	1.00	4.23	1.00	1.96	1.17	6.85	2.89
N	63.00	63.00	64.00	54.00	64.00	62.00	63.00	63.00	64.00	57.00	61.70

Goodrich Lake

A	2.00	2.00	2.00	3.00	2.00	4.00	1.00	1.00	1.00	10.00	2.80
He	0.29	0.02	0.13	0.29	0.19	0.52*	0.00	0.00	0.00	0.84	0.23
Ho	0.29	0.02	0.15	0.31	0.15	0.05	0.00	0.00	0.00	0.72	0.17
Ar	1.991	1.172	1.839	2.177	1.932	2.355	1	1	1	7.018	2.15
N	63.00	64.00	62.00	58.00	62.00	62.00	64.00	64.00	63.00	61.00	62.30

Morgan Lake

A	3.00	3.00	2.00	2.00	2.00	3.00	1.00	1.00	1.00	9.00	2.70
He	0.28	0.08	0.20	0.20	0.06	0.09	0.00	0.00	0.00	0.83	0.17
Ho	0.32	0.08	0.19	0.19	0.03	0.10	0.00	0.00	0.00	0.76	0.17
Ar	2.474	1.76	1.945	1.942	1.541	1.807	1	1	1	7.044	2.15
N	62.00	63.00	62.00	63.00	63.00	62.00	63.00	63.00	62.00	62.00	62.50

Kuyakuz Lake

A	5.00	4.00	3.00	5.00	1.00	7.00	1.00	2.00	5.00	9.00	4.20
He	0.74	0.37	0.64	0.65	0.00	0.72	0.00	0.06	0.55	0.82	0.46
Ho	0.80	0.39	0.58	0.62	0.00	0.82	0.00	0.06	0.48	0.78	0.45
Ar	4.21	3.03	3.00	4.18	1.00	5.33	1.00	1.55	3.84	6.81	3.39
N	50.00	49.00	48.00	50.00	50.00	50.00	48.00	48.00	48.00	49.00	49.00

Fish Lake

A	4.00	2.00	2.00	3.00	2.00	2.00	2.00	1.00	4.00	7.00	2.90
He	0.49	0.15	0.04	0.58	0.26	0.42	0.04	0.00	0.56	0.72	0.33
Ho	0.43	0.16	0.04	0.47	0.31	0.37	0.04	0.00	0.60	0.69	0.31
Ar	3.27	1.88	1.42	2.91	1.98	2.00	1.40	1.00	3.18	5.56	2.46
N	49.00	49.00	47.00	49.00	49.00	49.00	49.00	49.00	47.00	48.00	48.50

Horseshoe Lake

A	5.00	4.00	3.00	4.00	1.00	7.00	1.00	2.00	3.00	7.00	3.70
He	0.71	0.66	0.40	0.47	0.00	0.68	0.00	0.22	0.58	0.81	0.45
Ho	0.78	0.72	0.39	0.50	0.00	0.61	0.00	0.19	0.63	0.69	0.45
Ar	4.54	3.82	2.74	3.66	1.00	5.67	1.00	1.97	2.96	6.24	3.36
N	32.00	32.00	31.00	30.00	32.00	31.00	32.00	32.00	32.00	29.00	31.30

Upper Fraser River

Twinkle Lake

	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	5.00	3.00	3.00	4.00	1.00	6.00	1.00	2.00	3.00	10.00	3.80
He	0.64	0.62	0.25*	0.49	0.00	0.62	0.00	0.13	0.57	0.62	0.39
Ho	0.52	0.59	0.13	0.50	0.00	0.48	0.00	0.12	0.55	0.60	0.35
Ar	4.32	2.99	2.20	3.04	1.00	3.86	1.00	1.80	2.84	5.08	2.81
N	143.00	143.00	124.00	140.00	146.00	142.00	148.00	148.00	141.00	143.00	141.80

Thompson River

01157LNTH Lake

A	8.00	2.00	5.00	4.00	2.00	7.00	3.00	2.00	9.00	5.00	4.70
He	0.81	0.09	0.54	0.37	0.21*	0.70	0.51	0.09	0.61	0.51	0.44
Ho	0.75	0.08	0.55	0.26	0.14	0.73	0.58	0.09	0.57	0.47	0.42
Ar	6.25	1.67	2.86	2.34	1.95	4.68	2.40	1.67	3.87	3.43	3.11
N	138.00	139.00	113.00	121.00	135.00	133.00	137.00	137.00	127.00	135.00	131.50

01176LNTH Lake

A	5.00	2.00	5.00	2.00	2.00	6.00	2.00	2.00	6.00	4.00	3.60
He	0.60	0.13	0.70	0.46	0.16	0.57	0.47	0.11	0.65	0.60	0.45
Ho	0.51	0.13	0.68	0.44	0.16	0.55	0.34	0.12	0.66	0.51	0.41
Ar	3.66	1.81	4.06	2.00	1.89	3.35	2.00	1.76	3.90	3.10	2.75
N	83.00	89.00	81.00	91.00	89.00	89.00	92.00	93.00	83.00	86.00	87.60

00466DEAD Lake

A	3.00	1.00	2.00	1.00	2.00	3.00	1.00	2.00	1.00	5.00	2.10
He	0.5*	0.00	0.31	0.00	0.17	0.04	0.00	0.003	0.00	0.60	0.16
Ho	0.45	0.00	0.29	0.00	0.16	0.04	0.00	0.003	0.00	0.57	0.15
Ar	2.991	1	2	1	2	2.92	1	1.902	1	4.91	2.07
N	316.00	316.00	289.00	286.00	311.00	311.00	317.00	317.00	308.00	314.00	308.50

Murray Creek

A	4.00	3.00	1.00	2.00	2.00	4.00	2.00	2.00	3.00	5.00	2.80
He	0.62	0.27	0.00	0.50	0.21	0.59*	0.33	0.22	0.49	0.57	0.38
Ho	0.68	0.29	0.00	0.42	0.19	0.14	0.24	0.25	0.56	0.66	0.34
Ar	3.32	2.30	1.00	2.00	1.97	3.30	2.00	1.97	2.96	4.17	2.50
N	34.00	35.00	32.00	36.00	37.00	29.00	33.00	32.00	34.00	35.00	33.70

Coldwater River

A	6.00	4.00	3.00	4.00	2.00	8.00	4.00	2.00	8.00	11.00	5.20
He	0.72	0.36	0.31	0.67	0.38	0.85	0.30	0.49	0.58	0.78	0.54
Ho	0.79	0.26	0.34	0.66	0.34	0.80	0.29	0.49	0.54	0.76	0.53
Ar	4.62	2.99	2.52	3.31	2.00	7.25	3.23	2.00	5.90	7.34	4.12
N	34.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	34.00	34.80

*Thompson River***Clearwater River**

	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	8.00	4.00	3.00	2.00	3.00	8.00	4.00	2.00	8.00	10.00	5.20
He	0.80	0.55	0.26	0.02	0.34	0.69	0.48	0.34	0.66	0.75	0.49
Ho	0.70	0.44	0.19	0.02	0.31	0.67	0.43	0.31	0.59	0.62	0.43
Ar	6.20	2.89	2.66	1.21	2.20	5.65	3.09	2.00	4.65	6.58	3.71
N	54.00	54.00	54.00	53.00	54.00	54.00	54.00	54.00	54.00	53.00	53.80

*Upper Columbia River***Fry Creek**

A	6.00	8.00	1.00	2.00	2.00	4.00	2.00	2.00	3.00	5.00	3.50
He	0.76	0.62	0.00	0.37	0.40	0.72	0.44	0.24	0.44	0.68	0.47
Ho	0.67	0.45	0.00	0.34	0.42	0.69	0.50	0.24	0.53	0.67	0.45
Ar	5.25	5.36	1.00	2.00	2.00	3.98	2.00	1.98	2.81	4.73	3.11
N	43.00	42.00	43.00	41.00	43.00	42.00	42.00	42.00	40.00	42.00	42.00

Lower Murphy Creek

A	13.00	10.00	3.00	4.00	4.00	13.00	3.00	2.00	12.00	16.00	8.00
He	0.77	0.77	0.44	0.55	0.68	0.88	0.59	0.41	0.87	0.90	0.68
Ho	0.74	0.70	0.33	0.67	0.60	0.78	0.56	0.42	0.80	0.94	0.65
Ar	7.97	6.74	2.40	3.13	3.90	8.94	2.93	2.00	8.79	10.55	5.73
N	50.00	50.00	49.00	49.00	50.00	50.00	50.00	50.00	49.00	50.00	49.70

Lower Norns Creek

A	12.00	10.00	4.00	7.00	4.00	15.00	7.00	2.00	16.00	17.00	9.40
He	0.76	0.52	0.47	0.59	0.52	0.79*	0.60	0.50	0.80	0.84	0.64
Ho	0.76	0.47	0.48	0.56	0.49	0.61	0.44	0.41	0.76	0.83	0.58
Ar	6.81	4.98	2.29	4.12	3.00	7.48	3.98	2.00	7.64	8.47	5.08
N	89.00	88.00	75.00	84.00	90.00	88.00	90.00	90.00	87.00	89.00	87.00

Upper Sullivan Creek

A	2.00	2.00	2.00	2.00	1.00	3.00	2.00	1.00	3.00	2.00	2.00
He	0.46	0.47	0.43	0.50	0.00	0.59	0.10	0.00	0.52	0.40	0.35
Ho	0.45	0.53	0.42	0.53	0.00	0.76	0.05	0.00	0.58	0.55	0.39
Ar	2.00	2.00	2.00	2.00	1.00	3.00	1.75	1.00	2.98	2.00	1.97
N	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00

China Creek

A	11.00	7.00	3.00	5.00	4.00	13.00	6.00	2.00	16.00	12.00	7.90
He	0.75	0.70	0.38	0.47	0.51	0.80	0.60	0.50	0.84	0.87	0.64
Ho	0.71	0.60	0.25	0.44	0.35	0.82	0.60	0.43	0.83	0.86	0.59
Ar	6.82	5.46	2.23	2.69	3.36	7.72	3.94	2.00	9.04	9.13	5.24
N	48.00	48.00	48.00	48.00	49.00	49.00	47.00	47.00	48.00	49.00	48.10

*Upper Columbia River***Lower Blueberry Creek**

	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	12.00	10.00	3.00	5.00	3.00	9.00	5.00	2.00	15.00	12.00	7.60
He	0.73	0.65	0.26	0.56	0.55	0.85	0.58	0.42	0.87	0.84	0.63
Ho	0.67	0.61	0.26	0.54	0.52	0.83	0.48	0.39	0.77	0.80	0.59
Ar	6.87	5.95	2.22	3.55	2.95	7.38	3.35	2.00	9.24	8.15	5.16
N	49.00	49.00	46.00	46.00	44.00	47.00	48.00	49.00	47.00	49.00	47.40

Upper Murphy Creek

A	8.00	7.00	4.00	4.00	2.00	8.00	3.00	2.00	5.00	9.00	5.20
He	0.44	0.66	0.42	0.52	0.49	0.80	0.50	0.50	0.33	0.71	0.54
Ho	0.40	0.80	0.41	0.48	0.46	0.61	0.54	0.33	0.38	0.66	0.51
Ar	4.03	4.74	3.40	2.44	2.00	6.01	2.65	2.00	3.33	5.92	3.65
N	50.00	50.00	39.00	50.00	46.00	49.00	48.00	48.00	42.00	50.00	47.20

Norns Creek Fan

A	13.00	10.00	5.00	7.00	5.00	14.00	8.00	2.00	17.00	18.00	9.90
He	0.72	0.58	0.43	0.53*	0.47	0.80	0.62*	0.50	0.77	0.84	0.63
Ho	0.71	0.51	0.42	0.45	0.42	0.78	0.51	0.45	0.81	0.77	0.58
Ar	6.32	4.90	2.27	3.66	2.87	7.10	4.00	2.00	7.54	7.89	4.86
N	174.00	174.00	161.00	161.00	174.00	171.00	168.00	170.00	169.00	170.00	169.20

Kootenay River

A	13.00	13.00	5.00	7.00	4.00	18.00	7.00	2.00	21.00	20.00	11.00
He	0.75	0.60	0.42	0.53	0.53	0.80*	0.60*	0.49	0.80*	0.84	0.64
Ho	0.71	0.58	0.39	0.48	0.49	0.70	0.50	0.46	0.70	0.83	0.58
Ar	6.48	4.95	2.37	3.87	2.89	7.75	3.83	2.00	7.45	8.08	4.97
N	213.00	214.00	171.00	195.00	208.00	204.00	209.00	213.00	202.00	208.00	203.70

Clearwater Creek

A	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	3.00	1.30
He	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.05
Ho	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.06
Ar	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	2.41	1.24
N	27.00	27.00	27.00	19.00	27.00	27.00	27.00	27.00	27.00	19.00	25.40

Salmo River

A	11.00	9.00	2.00	5.00	4.00	13.00	5.00	2.00	12.00	15.00	7.80
He	0.84	0.78*	0.02	0.56	0.42	0.87*	0.62	0.37	0.79	0.84	0.61
Ho	0.93	0.78	0.02	0.47	0.32	0.77	0.63	0.39	0.68	0.70	0.57
Ar	7.30	6.23	1.20	3.51	3.11	8.50	3.68	2.00	6.35	7.92	4.98
N	59.00	60.00	56.00	47.00	60.00	60.00	59.00	59.00	60.00	57.00	57.70

*Upper Columbia River***Kinbasket Reservoir**

	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	9.00	3.00	2.00	3.00	2.00	5.00	3.00	2.00	4.00	9.00	4.20
He	0.76	0.45	0.40	0.62	0.48	0.67	0.14	0.50	0.69	0.80	0.55
Ho	0.86	0.43	0.18	0.50	0.36	0.57	0.14	0.64	0.69	0.71	0.51
Ar	7.92	2.99	2.00	3.00	2.00	4.78	2.57	2.00	4.00	8.06	3.93
N	14.00	14.00	11.00	14.00	14.00	14.00	14.00	14.00	13.00	14.00	13.60

Lardeau River

A	5.00	2.00	2.00	4.00	2.00	7.00	2.00	2.00	2.00	3.00	3.10
He	0.61	0.29	0.02	0.48	0.14	0.80	0.47	0.29	0.23	0.51	0.39
Ho	0.75	0.35	0.03	0.43	0.15	0.80	0.68	0.35	0.22	0.55	0.43
Ar	4.01	1.99	1.28	3.61	1.87	5.84	2.00	1.99	1.98	2.28	2.68
N	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	37.00	40.00	39.70

*South Coast BC***Nimpkish River**

A	7.00	8.00	1.00	4.00	5.00	7.00	6.00	2.00	6.00	9.00	5.50
He	0.75	0.79	0.00	0.53	0.57	0.73	0.68	0.48	0.74	0.86	0.61
Ho	0.65	0.72	0.00	0.66	0.61	0.73	0.53	0.41	0.74	0.90	0.59
Ar	5.61	6.13	1.00	3.25	4.39	5.16	4.85	2.00	5.28	7.49	4.52
N	34.00	32.00	33.00	35.00	33.00	33.00	34.00	34.00	31.00	30.00	32.90

Gold River

A	6.00	9.00	1.00	4.00	5.00	11.00	6.00	2.00	9.00	10.00	6.30
He	0.53	0.79*	0.00	0.58	0.60	0.81	0.72	0.50	0.76	0.88	0.62
Ho	0.63	0.55	0.00	0.56	0.54	0.83	0.59	0.45	0.59	0.78	0.55
Ar	5.10	6.62	1.00	3.30	4.64	7.39	5.36	2.00	6.47	8.58	5.04
N	35.00	33.00	35.00	34.00	35.00	35.00	32.00	33.00	27.00	32.00	33.10

*North Coast BC***Cooper Creek**

A	5.00	7.00	2.00	6.00	4.00	10.00	7.00	2.00	6.00	11.00	6.00
He	0.29	0.74	0.12	0.61	0.60	0.77	0.76	0.41	0.57	0.83	0.57
Ho	0.24	0.81	0.13	0.71	0.65	0.70	0.86	0.48	0.50	0.85	0.59
Ar	3.53	6.06	1.91	4.53	3.52	7.76	5.98	2.00	5.07	8.77	4.91
N	21.00	21.00	16.00	21.00	20.00	20.00	21.00	21.00	20.00	20.00	20.10

Mamin River

A	2.00	9.00	2.00	3.00	3.00	8.00	5.00	2.00	5.00	12.00	5.10
He	0.27	0.76	0.06	0.60	0.57	0.77	0.59*	0.50	0.72	0.88	0.57
Ho	0.26	0.90	0.06	0.48	0.61	0.80	0.30	0.58	0.77	0.87	0.56
Ar	1.99	7.03	1.59	3.00	2.96	6.41	4.52	2.00	4.54	9.52	4.36
N	31.00	31.00	31.00	31.00	31.00	30.00	30.00	31.00	31.00	31.00	30.80

North Coast BC

Yakoun River

	Onue8	Ssa85	Ots103	Ots3	Ssa456	Omy77	Oneu14	Ssa197	Ots100	Okia3	Average
A	3.00	7.00	2.00	3.00	3.00	9.00	4.00	2.00	4.00	12.00	4.90
He	0.18	0.70	0.06	0.61	0.49	0.83	0.69	0.50	0.50	0.86	0.54
Ho	0.10	0.55	0.07	0.63	0.35	0.65	0.50	0.35	0.58	1.00	0.48
Ar	2.47	5.45	1.73	3.00	2.55	7.75	3.97	2.00	3.90	9.46	4.23
N	20.00	20.00	15.00	19.00	20.00	20.00	20.00	20.00	19.00	19.00	19.20

Riley Creek

A	1.00	5.00	1.00	5.00	3.00	9.00	5.00	2.00	6.00	10.00	4.70
He	0.00	0.73	0.00	0.68	0.51	0.80	0.72	0.48	0.63	0.85	0.54
Ho	0.00	0.73	0.00	0.77	0.42	0.63	0.75	0.53	0.62	0.83	0.53
Ar	1.00	4.57	1.00	3.97	2.46	7.23	4.57	2.00	4.89	8.11	3.98
N	30.00	30.00	29.00	30.00	24.00	24.00	28.00	30.00	29.00	29.00	28.30

Stikine River

Ealue Lake

A	4.00	5.00	1.00	2.00	5.00	4.00	1.00	2.00	5.00	4.00	3.30
He	0.69	0.69	0.00	0.07	0.59	0.63	0.00	0.46	0.66	0.60	0.44
Ho	0.67	0.59	0.00	0.00	0.57	0.64	0.00	0.50	0.61	0.52	0.41
Ar	3.93	4.34	1.00	1.67	4.18	3.75	1.00	2.00	4.73	3.69	3.03
N	27.00	27.00	31.00	26.00	30.00	22.00	26.00	30.00	23.00	25.00	26.70

Skeena River

Khtada Lake

A	2.00	4.00	2.00	1.00	1.00	3.00	2.00	2.00	3.00	1.00	2.10
He	0.02	0.63	0.24	0.00	0.00	0.24	0.22	0.49	0.11	0.00	0.20
Ho	0.02	0.75	0.27	0.00	0.00	0.26	0.19	0.51	0.12	0.00	0.21
Ar	1.26	3.61	1.98	1.00	1.00	2.67	1.97	2.00	2.13	1.00	1.86
N	43.00	40.00	33.00	29.00	34.00	34.00	32.00	35.00	25.00	44.00	34.90

Canyon Creek

A	1.00	3.00	2.00	2.00	2.00	3.00	2.00	2.00	2.00	4.00	2.30
He	0.00	0.56	0.03	0.06	0.22	0.50	0.34	0.22	0.06	0.13	0.21
Ho	0.00	0.55	0.03	0.06	0.13	0.38	0.38	0.25	0.06	0.13	0.20
Ar	1.00	2.84	1.34	1.57	1.97	2.34	2.00	1.97	1.57	2.34	1.90
N	32.00	31.00	32.00	32.00	32.00	32.00	32.00	32.00	32.00	30.00	31.70

Moosevale Creek

A	5.00	6.00	3.00	4.00	5.00	6.00	4.00	2.00	8.00	10.00	5.30
He	0.44	0.68	0.39	0.67	0.47	0.52*	0.49	0.41	0.69	0.82	0.56
Ho	0.45	0.59	0.39	0.63	0.42	0.27	0.50	0.52	0.74	0.68	0.52
Ar	3.83	5.42	2.35	3.88	4.15	4.65	2.69	2.00	6.58	7.28	4.28
N	31.00	32.00	31.00	32.00	31.00	30.00	32.00	31.00	31.00	31.00	31.20

Table 3.3 Hierarchical analysis of the regional and subregional distribution of genetic diversity in 42 rainbow trout (*Oncorhynchus mykiss*) populations included in this study under various hypotheses. Calculated using ARLEQUIN ver 2.0, V_{bg} represents the percentage of variation existing between groups, V_{ap} , the amount existing among populations within groups, and V_{wp} is the percentage of variation existing within populations. The stated P value refers to the probability that the observed value for V_{bg} is equalled or exceeded by chance determined from 1000 permutations. Probability values for all observed values of V_{ap} and V_{wp} were <0.0001.

Comparison	Vbg	Vap	Vwp	P
Lakes vs. Rivers (coast populations)	9.0	19.1	71.9	0.0920
Lakes vs. Rivers (interior populations)	10.3	31.3	58.4	0.0009
Lakes vs. Rivers (among all populations)	8.6	33.8	59.6	0.0020
Above barriers vs. below barriers (collectively)	3.3	7.1	89.5	0.0090
Above barriers vs. below individual barriers (within streams)	17.8	1.8	80.4	0.0050
Life history (lake resident, stream resident, anadromous)	8.3	31.7	60.0	<0.0001
Regions (Coast, U. Fraser, U. Columbia, Thompson)	17.7	22.9	59.4	<0.0001

Table 3.4 Hierarchical analysis of the subregional distribution of genetic diversity of rainbow trout (*Oncorhynchus mykiss*) populations in the Columbia (upper Columbia River, mid-Columbia River, Salmo River, Duncan River), Thompson (Clearwater River, upper Thompson River, Bonaparte River, Nicola River, mid-Thompson River), upper Fraser (Nechako River, Blackwater River, Chilcotin River) and coastal British Columbia (Queen Charlotte Islands, Vancouver Island, Skeena River, Stikine River) by major tributary. Calculated using ARLEQUIN ver 2.0, V_{bg} represents the percentage of variation existing between groups, V_{ap} , the amount existing among populations within groups, and V_{wp} is the percentage of variation existing within populations. The stated P value refers to the probability that the observed value for V_{bg} is equalled or exceeded by chance determined from 1000 permutations. Probability values for all observed values of V_{ap} and V_{wp} were <0.0001.

Comparison	V_{bg}	V_{ap}	V_{wp}	P
Coastal	0.3	23.9	75.8	0.5083
Upper Fraser	26.4	21.6	52.0	<0.0001
Thompson	50.0	1.4	48.6	0.0049
Upper Columbia	2.1	9.5	88.4	0.1750
Upper Columbia without barrier populations	4.4	3.7	91.8	0.0528

Table 3.5 The relationship between habitat size and genetic variation in rainbow trout (*Oncorhynchus mykiss*). Genetic variation variables include average number of alleles, expected heterozygosity, observed heterozygosity, and allelic richness. Habitat size variables include lake surface area in hectares (ha), lake perimeter in kilometers (km), and stream/lake order as described in Arcview.

	Average number of alleles		Expected Heterozygosity		Observed Heterozygosity		Allelic richness	
	r	p-value	r	p-value	r	p-value	r	p-value
Area (ha)	0.28	0.0700	0.30	0.0400	0.28	0.0600	0.30	0.0500
Perimeter (km)	0.30	0.0500	0.32	0.0400	0.30	0.0600	0.32	0.0400
Area (ha) log transformed	0.40	0.0080	0.24	0.1200	0.22	0.0400	0.36	0.1100
Perimeter (km) log transformed	0.37	0.0200	0.22	0.1600	0.39	0.1800	0.14	0.1800
Stream/ lake order	0.48	<0.0001	0.40	0.0008	0.37	0.0020	0.44	0.0002

Table 3.6 The relationship between observed level of genetic variation and elevation in rainbow trout (*Oncorhynchus mykiss*). The effects of elevation was conducted within each lake/stream chain of the three major watersheds (upper Fraser River, upper Columbia River, and Thompson River).

	Allelic richness		Average number of alleles per loci		Expected heterozygosity	
	r	p-value	r	p-value	r	p-value
All populations	-0.67	<0.0001	-0.71	<0.0001	-0.66	<0.0001
upper Fraser River lake chains	-0.65	0.002	-0.84	<0.0001	-0.57	0.01
upper Columbia River chains	-0.81	0.002	-0.81	0.001	-0.73	0.01
Thompson River lake chains	-0.45	0.06	-0.45	0.07	-0.4	0.10

Table 3.7 Assignment tests of population chains in each drainage (upper Fraser River, Thompson River, and upper Columbia River) based on ten microsatellite loci in rainbow trout (*Oncorhynchus mykiss*) prior to pooling of sample localities. Values are the proportion of individuals 'assigned' to each sample site and numbers in bold are the proportion of fish classified into their sampling lake. Excluded rainbow trout (in %) had multilocus genotypes with a probability of belonging to any locality where they were collected of lower than 5%, or when individuals were missing genotypic data from more than 3 microsatellite loci.

Sampled in	Excluded from all samples	Classified in																			
		Blanchet Lake	Blanchet 2 Lake	Blanchet 3 Lake	Tlulias Lake	Grizzly Lake	Nutli Lake	Fenton Lake	Goodrich Lake	Morgan Lake	Theleteban Lake	Glattheli Lake	Michel Lake	Unnamed 301 Lake	Unnamed 401 lake	Unnamed 451 Lake	Ghitzeli Lake	Horseshoe Lake	Skinny Lake	Needle Lake	Twinkle Lake
Blanchet Lake	0.04	0.18	0.20	0.00	0.02	0.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Blanchet 2 Lake	0.02	0.22	0.62	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Blanchet 3 Lake	0.14	0.00	0.00	0.84	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tlulias Lake	0.04	0.06	0.02	0.00	0.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Grizzly Lake	0.02	0.42	0.16	0.00	0.02	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nutli Lake	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.00	0.09	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
Fenton Lake	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.88	0.03	0.00	0.03	0.00	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Goodrich Lake	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.03	0.50	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
Morgan Lake	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.16	0.61	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
Theleteban Lake	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.72	0.03	0.03	0.13	0.00	0.00	0.06	0.00	0.00	0.00	0.00
Glattheli Lake	0.01	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.00	0.00	0.09	0.21	0.16	0.19	0.19	0.03	0.06	0.00	0.00	0.00	0.00
Michel Lake	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.03	0.19	0.12	0.31	0.28	0.03	0.00	0.00	0.00	0.00	0.00
Unnamed 301 Lake	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.03	0.25	0.22	0.22	0.13	0.09	0.00	0.00	0.00	0.00	0.00
Unnamed 401 lake	0.02	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.03	0.00	0.03	0.13	0.19	0.19	0.38	0.00	0.00	0.00	0.00	0.00	0.00
Unnamed 451 Lake	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.03	0.75	0.13	0.00	0.00	0.00	0.00
Ghitzeli Lake	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.03	0.06	0.03	0.00	0.00	0.00	0.19	0.53	0.00	0.00	0.00	0.00
Horseshoe Lake	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94	0.03	0.00	0.03
Skinny Lake	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.30	0.32
Needle Lake	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.43	0.37
Twinkle Lake	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.35	0.44

Sampled in	Classified in												
	Excluded from all samples	Lower Murphy Creek	Lower Norms Creek	Kootenay River	Upper Sullivan Creek	China Creek	Lower Blueberry Creek	Sand Bar Eddy	Upper Murphy Creek	Norms Creek Fan	Genelle	Salmo River	Clearwater Creek
Lower Murphy Creek	0.11	0.63	0.05	0.00	0.00	0.07	0.02	0.02	0.02	0.09	0.00	0.00	0.00
Lower Norms Creek	0.06	0.04	0.26	0.10	0.00	0.14	0.02	0.14	0.00	0.14	0.10	0.00	0.00
Kootenay River	0.12	0.02	0.15	0.24	0.00	0.14	0.03	0.08	0.00	0.14	0.08	0.00	0.00
Upper Sullivan Creek	0.03	0.00	0.00	0.00	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
China Creek	0.09	0.11	0.09	0.24	0.00	0.15	0.11	0.07	0.00	0.06	0.07	0.00	0.00
Lower Blueberry Creek	0.08	0.04	0.08	0.08	0.00	0.08	0.49	0.02	0.02	0.06	0.08	0.00	0.00
Sand Bar Eddy	0.09	0.02	0.11	0.09	0.00	0.04	0.11	0.21	0.00	0.21	0.13	0.00	0.00
Upper Murphy Creek	0.06	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.89	0.02	0.00	0.00	0.00
Norms Creek Fan	0.07	0.03	0.11	0.11	0.00	0.06	0.05	0.12	0.01	0.28	0.16	0.01	0.00
Genelle	0.06	0.03	0.11	0.17	0.00	0.09	0.08	0.06	0.00	0.17	0.21	0.01	0.00
Salmo River	0.18	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.78	0.00
Clearwater Creek	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Sampled in	Classified in												
	Excluded from all samples	00376 DEAD Lake	00422 DEAD Lake	00357 DEAD Lake	00409 DEAD Lake	00439 DEAD Lake	00447 DEAD Lake	00466 DEAD Lake	00416 DEAD Lake	00410 DEAD Lake	00369 DEAD Lake		
00376 DEAD Lake	0.00	0.29	0.03	0.10	0.10	0.03	0.06	0.06	0.16	0.06	0.10		
00422 DEAD Lake	0.03	0.09	0.03	0.06	0.25	0.03	0.03	0.25	0.00	0.03	0.19		
00357 DEAD Lake	0.00	0.22	0.00	0.09	0.06	0.00	0.00	0.19	0.19	0.06	0.19		
00409 DEAD Lake	0.00	0.19	0.19	0.00	0.09	0.06	0.06	0.22	0.03	0.00	0.16		
00439 DEAD Lake	0.03	0.06	0.03	0.03	0.03	0.26	0.03	0.39	0.03	0.03	0.06		
00447 DEAD Lake	0.00	0.19	0.13	0.03	0.13	0.03	0.00	0.00	0.09	0.06	0.16		
00466 DEAD Lake	0.06	0.03	0.00	0.00	0.06	0.00	0.00	0.81	0.00	0.03	0.00		
00416 DEAD Lake	0.00	0.09	0.09	0.16	0.13	0.13	0.03	0.13	0.06	0.03	0.16		
00410 DEAD Lake	0.06	0.06	0.03	0.00	0.06	0.06	0.00	0.23	0.13	0.13	0.19		
00369 DEAD Lake	0.03	0.25	0.00	0.09	0.00	0.06	0.00	0.34	0.03	0.00	0.19		

Sampled in	Classified in									
	Excluded from all samples	01157 LNTN Lake	01166 LNTN Lake	01179 LNTN Lake	01184 LNTN Lake	01176 LNTN Lake	01189 LNTN Lake	01193 LNTN Lake	01201 LNTN Lake	
01157 LNTN Lake	0.14	0.10	0.29	0.19	0.14	0.05	0.00	0.05	0.05	
01166 LNTN Lake	0.00	0.32	0.09	0.09	0.09	0.05	0.09	0.14	0.14	
01179 LNTN Lake	0.00	0.09	0.06	0.06	0.38	0.03	0.00	0.38	0.00	
01184 LNTN Lake	0.06	0.09	0.03	0.19	0.28	0.06	0.00	0.22	0.06	
01176 LNTN Lake	0.06	0.03	0.03	0.00	0.06	0.22	0.38	0.06	0.16	
01189 LNTN Lake	0.09	0.03	0.03	0.00	0.03	0.41	0.19	0.03	0.19	
01193 LNTN Lake	0.03	0.13	0.09	0.19	0.34	0.06	0.00	0.16	0.00	
01201 LNTN Lake	0.09	0.06	0.06	0.00	0.06	0.06	0.22	0.03	0.41	

Table 3.8 Assignment tests for rainbow trout (*Oncorhynchus mykiss*) from population chains following the pooling of sample sites in each drainage (upper Fraser River, Thompson River, and upper Columbia River) based on ten microsatellite loci. Values are the proportion of individuals 'assigned' to each sample site and numbers in bold are the proportion of fish classified into their sampling lake. Excluded rainbow trout (in %) had multilocus genotypes indicating a probability of belonging to any locality of lower than 5% or when individuals were missing genotypic data from more than 3 microsatellite loci.

Sampled in	Excluded from all samples	Classified in									
		Blanchet Lake	Blanchet2 Lake	Blanchet3 Lake	Tluttias Lake	Glatheili Lake	Twinkle Lake	Horseshoe Lake	Theleteban Lake	Goodrich Lake	Morgan Lake
Blanchet Lake	0.01	0.76	0.20	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00
Blanchet2 Lake	0.00	0.38	0.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Blanchet3 Lake	0.02	0.02	0.00	0.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tluttias Lake	0.00	0.06	0.02	0.00	0.92	0.00	0.00	0.00	0.00	0.00	0.00
Glatheili Lake	0.07	0.00	0.00	0.00	0.01	0.90	0.00	0.00	0.00	0.02	0.01
Twinkle Lake	0.04	0.00	0.00	0.00	0.00	0.00	0.95	0.01	0.01	0.00	0.00
Horseshoe Lake	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Theleteban Lake	0.00	0.00	0.00	0.00	0.00	0.16	0.02	0.05	0.66	0.02	0.11
Goodrich Lake	0.02	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.64	0.30
Morgan Lake	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.06	0.89

Sampled in	Classified in			
	Excluded from all samples	1157LNTH Lake	1176LNTH Lake	00466DEAD Lake
1157LNTH Lake	0.03	0.83	0.14	0.00
1176LNTH Lake	0.07	0.15	0.78	0.00
00466DEAD Lake	0.00	0.00	0.00	1.00

Sampled in	Excluded from all samples	Classified in								
		Clearwater Creek	Salmo River	lower Murphy Creek	upper Sullivan Creek	China Creek	lower Blueberry Creek	San Bar Eddy	upper Murphy Creek	Norns Creek Fan
Clearwater Creek	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Salmo River	0.02	0.00	0.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00
lower Murphy Creek	0.00	0.00	0.00	0.72	0.00	0.08	0.02	0.08	0.02	0.08
upper Sullivan Creek	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
China Creek	0.00	0.00	0.00	0.12	0.00	0.22	0.12	0.10	0.00	0.12
lower Blueberry Creek	0.04	0.00	0.00	0.04	0.00	0.09	0.59	0.06	0.02	0.09
San Bar Eddy	0.02	0.00	0.02	0.00	0.00	0.09	0.10	0.40	0.00	0.22
upper Murphy Creek	0.02	0.00	0.00	0.02	0.00	0.02	0.00	0.00	0.92	0.02
Norns Creek Fan	0.01	0.00	0.01	0.05	0.00	0.07	0.06	0.15	0.01	0.39
Kootenay River	0.03	0.00	0.00	0.03	0.00	0.14	0.09	0.14	0.00	0.25

Table 3.8 Continued

Sampled in	Classified in						
	Excluded from all samples	Copper Creek	Mamin River	Yakoun River	Riley Creek	Nimpkish River	Gold River
Copper Creek	0.00	0.62	0.19	0.10	0.10	0.00	0.00
Mamin River	0.03	0.06	0.68	0.13	0.06	0.00	0.03
Yakoun River	0.00	0.20	0.20	0.40	0.20	0.00	0.00
Riley Creek	0.03	0.03	0.03	0.10	0.80	0.00	0.00
Nimpkish River	0.09	0.00	0.00	0.00	0.00	0.77	0.14
Gold River	0.06	0.00	0.00	0.00	0.06	0.23	0.66

Table 3.9 Influence of geographic distance on misassignment before pooling of sample sites in rainbow trout (*Oncorhynchus mykiss*).

	r	p-value
Upper Fraser River lake chains	-0.33	0.005
Columbia River chains	-0.48	<0.0001
Thompson River lake chains	-0.20	0.01
Coastal populations	-0.64	0.003
All populations	-0.10	0.02
All populations without coastal populations	-0.33	<0.0001

Table 3.10 Influence of geographic distance on misassignment following pooling of sample sites in rainbow trout (*Oncorhynchus mykiss*).

	r	p-value
Upper Fraser River lake chains	-0.20	0.06
Columbia River chains	-0.46	<0.0001
Coastal populations	-0.64	0.003
All populations	-0.04	0.51
All populations without coastal populations	-0.32	<0.0001

Table 3.11 Correlation between genetic differentiation (Gen) and elevation (Elev), and fluvial distance (Geo), along with results between genetic differentiation and elevation, fluvial distance, and presence of nodes (Nodes) controlling (contr) the effect of Geo, Nodes, and Elev, respectively among population chain localities in rainbow trout (*Oncorhynchus mykiss*) prior to pooling of localities.

Population Chain	Gen vs Elev		Gen vs Geo		Gen vs Elev contr Geo		Gen vs Elev contr Nodes	
	Mantel r value	p-value	Mantel r value	p-value	Mantel r value	p-value	Mantel r value	p-value
Columbia Chain	0.624	0.034	0.236	0.144	0.596	0.032	0.450	0.040
Columbia Chain without barrier populations	0.048	0.387	0.405	0.030	0.185	0.161	0.920	0.003
Deadman Chain	0.755	0.010	0.663	0.002	0.653	0.038	0.790	0.003
LNTH Chain	0.376	0.041	0.607	0.007	0.363	0.048	0.450	0.024
Blanchet Chain	-0.440	0.125	0.271	0.281	-0.433	0.204	-0.440	0.190
Nutli Chain	-0.247	0.438	-0.300	0.315	-0.004	0.461	N/A	N/A
Glattheli Chain	0.610	0.008	0.503	0.038	0.498	0.040	0.600	0.020
Horseshoe Chain	-0.217	0.598	0.438	0.296	-0.736	0.090	N/A	N/A

Population Chain	Gen vs Geo contr Elev		Gen vs Geo contr Nodes		Gen vs Nodes contr Elev		Gen vs Nodes contr Geo	
	Mantel r value	p-value	Mantel r value	p-value	Mantel r value	p-value	Mantel r value	p-value
Columbia Chain	0.052	0.410	0.71	0.02	0.01	0.45	-0.13	0.33
Columbia Chain without barrier populations	0.436	0.017	0.67	0.08	-0.007	0.45	0.29	0.05
Deadman Chain	0.502	0.024	0.7	0.001	0.35	0.02	-0.33	0.08
LNTH Chain	0.601	0.021	0.59	0.015	0.43	0.013	0.32	0.05
Blanchet Chain	0.258	0.295	0.19	0.35	0.21	0.34	-0.11	0.35
Nutli Chain	-0.177	0.678	N/A	N/A	N/A	N/A	N/A	N/A
Glattheli Chain	0.324	0.136	0.53	0.03	0.08	0.44	-0.26	0.23
Horseshoe Chain	0.782	0.123	N/A	N/A	N/A	N/A	N/A	N/A

Table 3.12 Correlation between genetic differentiation (Gen), elevation (Elev), fluvial distance (Geo), and presence of nodes (Nodes) controlling (contr) the effect of Geo, Nodes, and Elev, respectively among population chain localities in rainbow trout (*Oncorhynchus mykiss*) following pooling of localities.

	Gen vs Geo cont Elev		Gen vs Nodes cont Elev		Gen vs Elev cont Geo		Gen vs Nodes cont Geo		Gen vs Elev cont Nodes		Gen vs Geo cont Nodes	
	Mantel r value	p-value	Mantel r value	p-value	Mantel r value	p-value	Mantel r value	p-value	Mantel r value	p-value	Mantel r value	p-value
Fraser River chains	0.200	0.100	0.370	0.003	0.290	0.004	0.370	0.002	0.225	0.013	0.096	0.240
Columbia River chains	0.650	0.020	0.030	0.450	0.350	0.046	-0.130	0.270	0.480	0.030	0.710	0.020
Columbia River chains without barrier populations	0.876	0.002	0.380	0.036	-0.030	0.560	-0.007	0.480	0.870	0.060	0.920	0.007

Table 3.13 Forward selection results for each genetic diversity measure in rainbow trout (*Oncorhynchus mykiss*) for pure drainage and environmental categories prior to pooling of sample sites (n=69). The abbreviations under the Pure drainage column (UF, upper Fraser River; TR, Thompson River, and CL, Columbia River) represents specific nodes labelled in Figure 3.4, and sample site names represents specific barriers to above barrier populations. Environmental variables are represented by number of species (Spp), longitude (Alb Y), latitude (Alb X), elevation (Elev), lake surface area (Area), number of nodes, presence of migration barriers, and perimeter. Under the Pure drainage or Pure environmental heading, variables were selected on the basis of the proportion of variance that they explained and its significance (based on 1000 Monte Carlo permutations with cut-off point for selection of $p = 0.10$).

Measure	Pure drainage	Pure environmental
Upper Fraser River		
Allelic richness	UF2, UF6	Spp, Alb X, Nodes, Area
Alleles	UF2, UF6	Spp, Alb X, Nodes, Elev
Heterozygosity	UF2, UF6	Spp, Alb Y, Nodes, Area
Okia3	UF2, UF3	-
Omy77	UF7, UF8	-
Onue8	UF2, UF4	Spp, Alb Y, Elev, Nodes
Oneu14	na	na
Ots3	UF2, UF4, UF, UF6	Alb Y, Nodes
Ots100	UF2	Alb Y, Alb X, Elev
Ots103	UF4, UF5	Alb Y, Nodes
Ssa85	UF2, UF4	Alb Y, Perimeter
Ssa197	UF3, UF4, UF6	-
Ssa456	UF3	Spp, Alb X, Alb Y, Elev
Thompson River		
Allelic richness	TR2	Alb Y
Alleles	TR2	Alb Y, Elev
Heterozygosity	TR2, TR5	Alb Y, Elev
Okia3	TR2, TR3, TR4	Alb X, Alb Y
Omy77	TR2, TR3	Alb Y, Elev
Onue8	TR2, TR3	Alb Y
Oneu14	TR2	Alb Y
Ots3	TR2	Alb Y
Ots100	TR2, TR3	Alb Y
Ots103	TR2	Alb Y
Ssa85	TR2, TR3	Alb Y
Ssa197	TR2	Alb Y, Elev
Ssa456	TR2	Alb X, Alb Y
Upper Columbia River		
Allelic richness	Upper Murphy	Barriers
Alleles	-	Barriers
Heterozygosity	Upper Murphy	-
Okia3	-	Elev, Alb X
Omy77	-	Alb Y, Elev, Barriers
Onue8	-	Alb X, Barriers
Oneu14	-	-
Ots3	-	-
Ots100	CL2, Clearwater Creek	Alb X, Barriers
Ots103	CL2	Alb Y, Alb X, Barriers
Ssa85	Clearwater Creek	Barriers, Elev
Ssa197	Upper Sullivan	Alb X, Alb Y
Ssa456	-	Alb Y

Table 3.14 Forward selection results for each genetic diversity measure in rainbow trout (*Oncorhynchus mykiss*) for pure drainage and environmental categories following pooling of sample sites (n=42). The abbreviations under the Pure drainage column (UF, upper Fraser River; TR, Thompson River, and CL, Columbia River) represents specific nodes labelled in Figure 3.4, and sample site names represents specific barriers to above barrier populations. Environmental variables are represented by number of species (Spp), longitude (Alb Y), latitude (Alb X), elevation (Elev), lake surface area (Area), number of nodes, presence of migration barriers, and stream order. Under the Pure drainage or Pure environmental heading, variables were selected on the basis of the proportion of variance that they explained and its significance (based on 1000 Monte Carlo permutations with cut-off point for selection of $p = 0.10$).

Measure	Pure drainage	Pure environmental
Upper Fraser River		
Allelic richness	UF3	Elev, Alb Y, Area
Alleles	UF3	Elev, Alb Y, Stream order
Heterozygosity	UF2	Nodes, Alb Y
Okia3	UF2	Nodes
Omy77	UF2	Spp
Onue8	UF2	Nodes
Oneu14	na	-
Ots3	UF4	Area
Ots100	UF2	Alb Y, Nodes
Ots103	-	Spp
Ssa85	-	-
Ssa197	UF3	Elev
Ssa456	UF3	Alb X, Spp, Alb Y
Upper Columbia River		
Allelic richness	Upper Murphy	Barriers, Alb Y
Alleles	Upper Murphy	Barriers
Heterozygosity	-	-
Okia3	-	Elev, Alb X
Omy77	-	Elev, Alb Y
Onue8	-	Alb X, Barriers
Oneu14	CL3	Alb X
Ots3	Upper Sullivan	-
Ots100	Clearwater Creek	Alb X, Barriers
Ots103	-	Alb X
Ssa85	-	Barriers
Ssa197	Upper Sullivan	Alb Y
Ssa456	-	Alb Y, Elev

CHAPTER 4: Overview and conclusions

Mitochondrial DNA has proven to be useful for genealogical and evolutionary studies of animal populations, while microsatellite sequences are the most revealing DNA markers available so far for inferring population structure and dynamics. Together these markers are complementary in the sense that they reveal different aspects of a complex story at different depths of population history (Zhang and Hewitt 2003). Previous studies have demonstrated that although most valuable in describing the evolution of genetic diversity over short time scales, microsatellites have also been useful in describing historical events such as postglacial colonization which is typically studied using mtDNA (Nielsen et al 1997; Koskinen et al. 2001, 2002). I begin this final chapter by discussing some of the issues involved in inferences based on microsatellite DNA.

Selection, mutation, sampling effects, and life history differences

Gene flow, genetic drift, natural selection and mutation are major forces that ultimately shape genetic diversity. Natural selection is unlikely to have influenced my results since microsatellites are presumed to be selectively neutral (Jarne and Lagoda 1996). This is further supported by the coherence to Hardy Weinberg Equilibrium across all ten loci. I cannot, however, rule out the possibility that some markers could be linked to adaptive loci.

Mutation is more likely to have influenced patterns of observed genetic diversity. Microsatellite DNA are characterized as having high mutation rates (Jarne and Lagoda 1996) consequently resulting in high allelic polymorphism. Mutation may result in over estimation of gene flow between lacustrine localities especially if homoplasy is frequent (Jarne and Lagoda 1996).

The level of genetic differences between populations may be a result of sampling artifacts. For many sample sites I was limited in the range of age classes collected. Particularly populations within the Queen Charlotte Islands, the sampling season did not coincide with adult run timing and consequently, I was limited to sampling subadults (i.e. smolts). Among lacustrine populations, many lakes had fish of similar age classes and as a result few lakes were represented by individuals of more than two age classes. The possibility of sampling related individuals violates the assumption of random sampling and may exaggerate levels of genetic differentiation.

There were noticeable differences between lake-resident and anadromous or stream-resident populations of rainbow trout in terms of genetic variation and differentiation. One cannot dismiss the possibility that observed differences may be the result of different life history characteristics rather than just pure physical environment. For instance, the stream resident behaviour of fluvial populations is likely to promote straying between populations not because they are any more physically interconnected, but because the flowing nature of streams may

promote movement in fishes and thus may lead to greater dispersal. Although speculative, the direct comparisons of genetic information between populations with different life history trajectories may not necessarily be completely accurate.

Funnel of structure

It was evident from my results that river drainage played an important role in the genetic structuring of *Oncorhynchus mykiss* populations over a large geographic scale. Analysis of molecular variance, grouping of populations based on the Neighbour-Joining generated tree and clustering of populations using principal component analysis all point to the influence of drainage pattern in shaping genetic diversity. This is not surprising since the genetic structure of fish populations should reflect the restrictive nature of freshwater populations. Within large drainages, populations within these drainages were also clustered into smaller groups based river assemblages. For example, over a large scale the upper Fraser River population grouped together to form a major cluster separate from other major regions. Within the upper Fraser River group, however, there were further groupings into smaller drainages.

Within drainages, I found individual populations with varying degrees of genetic differentiation and variation. Among small drainages where relatively short distances separate populations, sample sites are generally observed to be genetically similar due to dispersal and gene flow. Likewise, when there were migration barriers to gene flow present, isolated populations were more

genetically distinct. Ultimately, the degree of connectiveness shaped patterns of genetic diversity and divergence by influencing effective population size. The more connected populations are, the more likely it is that individuals from nearby populations contribute genetic material to the pool of diversity. By contrast, environmental variables that help promote isolation (habitat size, barriers, distance, and elevation) are those that are likely to reduce effective neighborhood size and consequently result in the reduction of genetic variation.

Against this template of contemporary factors that influence genetic variation and differentiation (Chapter 3) lies the important influence of population history. In chapter 2, I showed that rainbow trout were structured into two major lineages defined by microsatellite DNA variation: a coastal lineage and an interior (upper Fraser and Columbia rivers) lineage. I also showed that genetic traces of postglacial colonization from an inland refuge could be resolved in the microsatellite data. Consequently, at the largest spatial (across BC) and temporal scale (Pleistocene glaciations), my results demonstrate the importance of considering the role of history in explaining patterns of genetic variation and differentiation in contemporary populations. In general then, my thesis provides a contribution towards 'waterscape' ecology in understanding the mechanisms that govern genetic diversity within species.

Geography and conservation

From a conservation viewpoint, results from my study indicate that genetic diversity in rainbow trout is nested: major differences exist among regions, among drainages within regions, and differences within drainages are variable and often depend on the localized geographic matrix of topography across which populations are located.

Biodiversity managers must appreciate this hierarchical nature of genetic diversity within rainbow trout to properly plan for its conservation. At the most local scale, my results highlight the often highly interconnected nature of apparently discrete habitats (i.e. adjacent lakes interconnected via streams) and that a single genetic population of fish may use multiple habitats. Clearly, fisheries habitat managers must account for such 'dispersal corridors' in much the same way as wildlife managers do for terrestrial vertebrates (Harrison 1992; Rosenberg et al. 1997).

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