POPULATION GENETIC STRUCTURE OF NORTH AMERICAN BROAD WHITEFISH, *COREGONUS NASUS* (PALLAS), WITH EMPHASIS ON THE MACKENZIE RIVER SYSTEM

by

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ABSTRACT

Broad whitefish, *Coregonus nasus*, is an important subsistence fish species in Arctic North America, yet virtually nothing is known regarding the genetic population structure of Nearctic populations of this species. In this thesis, microsatellite DNA variation was assayed among 1213 broad whitefish from 47 localities throughout North America, with emphasis on the Mackenzie River system, Northwest Territories. Specifically, I examined geographic variation in allele frequencies to assess how historical factors (Pleistocene glaciations) have shaped the current structuring of genetic variability and population differentiation. Microsatellite data was also used to resolve the relative contributions of broad whitefish populations to subsistence fisheries in the Mackenzie River system. Overall, broad whitefish exhibit relatively high intrapopulation microsatellite variation (average 12.29 alleles/locus, average $H_E = 0.58$) and there were declines in these measures of genetic diversity with distance from putative refugia suggesting historical factors, namely post-glacial dispersal, have influenced current microsatellite variation. Interpopulation divergence was low (overall $F_{ST} = 0.07$), but the main regions assayed in this study (Russia, Alaska, Mackenzie River and Travaillant Lake systems) are genetically differentiated. Strong isolation-by-distance among samples was resolved when including only those populations occupying former Beringia, but not when assaying those at the periphery of the range in the Mackenzie River system, suggesting that broad whitefish in the Mackenzie system have not occupied the region long enough since their invasion post-glacially to have approached equilibrium between gene flow and drift. Mixture analysis indicated that most fish from the lower Mackenzie River subsistence fishery originated from the Peel River, highlighting the importance of this tributary. Additionally the mixture analysis provides evidence for a putative riverine life history form in the Mackenzie River. My results indicate that glaciation and post-glacial colonization have been important in shaping the current genetic population structure of North American broad whitefish. They also illustrate the utility of microsatellite DNA to delineate population structure and patterns of genetic diversity in recently founded populations in addition to resolving contributions to fisheries. My data also support the hypothesis that there are several designatable units of conservation among broad whitefish populations and that management strategies should be implemented accordingly.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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DEDICATION

To “POPS”. Thank you for putting a fishing rod in my hands at the age of two. Also, to my parents for believing that I would not be in school forever. Sometimes I was not sure.

CHAPTER 1: GENERAL INRTODUCTION AND BACKGROUND INFORMMATION

Genetic Variation and the Genetic Structure of Populations

Quantifying the amount of genetic variation within natural populations is one of the fundamental goals in population and conservation genetics. Essentially, this field attempts to determine what causes genetic differentiation and what promotes variation between and within species, populations and individuals. Populations can be genetically subdivided into smaller units because of geographic, ecological or behavioural factors (Hedrick 2000), and it is now apparent that populations of most, if not all, species show some level of genetic structuring or some degree of genetic differentiation among geographical locations (Avise 1994; Balloux and Lugon-Moulin 2002). For instance, even within the European eel (*Anguilla anguilla*), a species once thought to be completely panmictic, recent investigations have shown that this species is genetically structured, therefore refuting the idea of random mating among all conspecifics (Wirth and Bernatchez 2001). Research emphasizing the study of population differentiation is informative as it can contribute to understanding how microevolutionary forces have acted throughout the history of a species and how these processes, within and between populations, generate and sustain such differentiation (Bohonak 1999).

The genetic diversity within and among populations is a direct consequence of mutation (Jarne and Lagoda 1996; Hallerman and Epifanio 2003) and the frequency of the different alleles that persist within a population will be dependent on dispersal of individuals and migration (gene flow) between populations (Hutchison and Templeton 1999; Balloux *et*

al. 2000), selection (Bohonak 1999; Hartl and Clarke 1989) and random genetic drift (Slatkin 1977; Frankham *et al.* 2002). The genetic structure of most natural populations at neutral loci, however, is most influenced by both random genetic drift and ongoing gene flow (Hutchison and Templeton 1999). Furthermore, the genetic structuring of fish populations is influenced by the roles of historical factors such as glaciation (Hewitt 1996; Angers and Bernatchez 1998; Bernatchez and Wilson 1998), contemporary factors such as current landscape features (Costello *et al.* 2003; Taylor *et al.* 2003) and environmental factors (e.g. MacLean *et al.* 1999).

Variation in allele frequencies at polymorphic loci are of particular interest to population geneticists because differences among populations or species will provide insight into how these evolutionary forces have acted throughout the history of a species. Data on genetic variation within and among populations are also important to fisheries researchers and managers because such data can provide information on population subdivision and relatedness in fish (Patton *et al.* 1997; Koskinen *et al.* 2001), post-glacial dispersal history (Bernatchez and Dodson 1991, 1994; Wilson and Hebert 1998; Turgeon and Bernatchez 2001a, 2001b), the frequency of hybridization among closely related species (Rubidge and Taylor 2005; Taylor and Costello 2006), the evolutionary history and evolutionary relationships of closely related species or populations (Bernatchez and Wilson 1998), and the evolutionary and adaptive potential of individuals, populations and species (Holderegger *et al*. 2006). Consequently, knowledge of how genetic variation is partitioned among populations may have important implications not only in evolutionary

biology and ecology, but also in conservation biology (Balloux and Lugon-Moulin 2002; Allendorf and Luikart 2007).

Dispersal markedly affects the population structure of the majority of animal species in nature (Bohonak 1999). Dispersal and subsequent gene flow play important roles in the partitioning of genetic variation between populations (Slatkin 1985); therefore, dispersal ability is consistently related to population structure (Bohonak 1999). Interpopulation dispersal is defined as the interpopulation movement of individuals between the natal area and the area where breeding first takes place (Fraser *et al.* 2004), and is a prerequisite of gene flow, but only results in gene flow when followed by successful establishment of the individual, and its descendents, in the new habitat (Michels *et al.* 2001). Gene flow is the movement of genes from one population to another (Slatkin 1985) via dispersal of individuals and the persistence of new genes or alleles in recipient populations influences the extent of genetic differentiation or subdivision among populations. Gene flow has homogenizing effects that lessen the degree of genetic differentiation between populations. Consequently, at equilibrium, and in the absence of any influence of selection, populations that are more closely situated to each other geographically should be more closely related genetically (Hutchison and Templeton 1999) due to increased potential for gene flow between them (Kimura 1953; Kimura and Weiss 1964).

Although migration and subsequent mating homogenizes genetic differentiation, gene flow may increase intrapopulation diversity through the potential addition of new alleles

into the recipient population (Slatkin 1985, 1987). Instead of constraining evolution through the homogenization of genetic variation, occasionally gene flow may spread new genes, or combinations of genes, throughout a species range (Slatkin 1985, 1987). Therefore, population geneticists and ecologists are often interested in understanding how dispersal, gene flow and population structure interrelate (Fraser *et al.* 2004). Understanding the extent of gene flow among and between populations is fundamentally important because gene flow may either constrain evolution by preventing adaptation to local conditions or promote evolution by spreading new alleles and combinations of alleles throughout a species' range (Slatkin 1985, 1987).

In addition to dispersal and subsequent gene flow, genetic drift also plays an important role in the partitioning of genetic variation and, consequently, influencing the genetic structuring of populations. In the absence of mutation, migration or selection, allele frequencies in finite populations may also change due to sampling error of gametes across generations (Hallerman 2003). Random genetic drift, the term applied to several nonselective processes driven by this sampling error, can result in allele frequency changes (Hartl and Clarke 1989) depending on several demographic and historical factors. Random genetic drift tends to lower genetic variation within populations and results in local differentiation among populations unless opposed by gene flow (Slatkin 1985, 1987). The effects of genetic drift are greater in small and fragmented populations, for example, those affected by founder events and bottlenecks associated with post-glacial colonization (Hewitt 1996), and can have major impacts on the evolution of such populations (Frankham *et al.* 2002). Drift leads to a loss in heterozygosity which, as a

long-term consequence, may result in the reduced capacity of a population to adapt to changing environmental conditions (Frankham *et al.* 1999, 2002). Drift, when acting alone, will ultimately cause the loss of all but one allele in finite populations (Slatkin 1987; Frankham *et al.* 2002). Ultimately, drift-induced changes can produce strong genetic differences among populations by altering allele frequencies and may play an important role in species divergence (Knowles and Richards 2005).

Microsatellite DNA

Genetic analyses of population structure have become commonplace since the advent of protein electrophoresis in the late 1960s (Bohonak 1999). More recently, microsatellite DNA markers have become one of the most powerful genetic assays for resolving population structure due to their exceptional variability and relative ease of scoring (Goldstein and Pollock 1997). With the development of the polymerase chain reaction (PCR), it was realized in the late 1980s that microsatellite DNA markers may be some of the most powerful Mendelian markers ever discovered (Jarne and Lagoda 1996; Goldstein and Pollock 1997). Microsatellite loci are a special class of tandemly-repeated DNA that are increasingly replacing or complementing other markers for numerous applications in evolutionary and conservation genetics (Angers and Bernatchez 1998). They are often highly polymorphic, appear to be largely immune to the action of selection, unless physically linked to a coding region of DNA, and may follow either the stepwise mutation model (SMM, Kimura and Crow 1964) or infinite alleles model (IAM, Ohta and Kimura 1973; Jarne and Lagoda 1996). The recent advent of microsatellite

DNA assays permits re-examination of various population-level issues, such as the magnitudes of genetic variation, from the perspective of a more highly mutable set of markers (DeWoody and Avise 2000). Specifically, within the last two decades, these codominant markers have proven useful in resolving finer scale population structure and studying closely related populations of fish (e.g., Stamford and Taylor 2005; Primmer *et al.* 2006), especially within short time frames (Hansen *et al.* 1999), assigning individual fish from unknown populations to populations of origin (Paetkau *et al.* 1995; Waser and Strobeck 1998; Primmer *et al.* 2000; Hansen *et al.* 2001), revealing dispersal patterns in fish populations (Castric and Bernatchez 2004; Fraser *et al.* 2004), and resolving phylogeographic patterns and postglacial dispersal and evolutionary histories (Angers and Bernatchez 1998; Hansen *et al.*1999, Koskinen *et al.* 2002, Costello *et al.* 2003; Tonteri *et al.* 2005; Sonstebo *et al*. 2007). Previously, the usefulness of these markers for addressing population structure and genetic differentiation has been demonstrated in members of the whitefish genus *Coregonus* (Pisces: Coregonidae), such as the lake whitefish *C. clupeaformis* (Bernatchez *et al.* 1999; Lu *et al.* 2001), the lake cisco *C. artedi* (Turgeon and Bernatchez 2001a), the European whitefish *C. lavaretus* (Hansen *et al.* 1999), and, on a limited scale, broad whitefish *C. nasus* (Patton *et al.* 1997).

Broad Whitefish in North America with Emphasis on the Mackenzie River System - Distribution, Life History and Cultural Importance

In North America, broad whitefish are distributed from the Perry River, Northwest Territories, west in numerous river systems in arctic Canada (such as the Coppermine, Mackenzie and Anderson rivers), and offshore in the Beaufort Sea, to the Kuskokwim

River, Bering Sea drainages in Alaska (McPhail and Lindsey 1970; Scott and Crossman 1998). They are also distributed throughout many of the larger river systems on the North Slope of Alaska such as the Colville and Sagavanirktok rivers (Scott and Crossman 1998; Morris 2003) and have been reported in the headwaters of the Yukon River as far upstream as Teslin Lake (Scott and Crossman 1998; McPhail 2007). Within the Mackenzie River system, depending on the life history stage, broad whitefish can be found in coastal lakes of the Tuktoyuktuk Peninsula, lakes of the Mackenzie Delta, the Mackenzie River proper to the village of Fort Good Hope, major tributaries of the lower Mackenzie River such as the Peel and Arctic Red rivers and some lakes such as Campbell, Andrew and Travaillant lakes (Reist and Chang-Kue 1997). Lindsey and McPhail (1986) have postulated that North American broad whitefish survived the last glacial maxima in the Beringian refuge (unglaciated portions of the Yukon River Valley and adjacent regions of Siberia), which is reasonable given their North American distribution (Scott and Crossman 1998).

Similar to many other coregonids, *C. nasus* exhibits diverse life history patterns. The current understanding of their life history, in the lower Mackenzie River system specifically, reveals a complex pattern that involves multiple life history types and the use of numerous aquatic habitats throughout the area (Reist and Chang-Kue 1997). In general, broad whitefish may display lacustrine, riverine, potamodromous (defined as making movements between freshwater and estuaries), and fully anadromous life histories (Reist and Bond 1988, Chang-Kue and Jessop 1997, Harris and Howland 2005). In the lower Mackenzie River system in particular, both lacustrine and semi-anadromous

life histories of broad whitefish are known to exist (Reist and Chang-Kue 1997), although it has only been recently that the purported lacustrine life history has been confirmed (Harris and Howland 2005). Additionally, a riverine (fluvial) form may inhabit this system, but concrete evidence of this is still lacking (but see Babaluk and Reist 1996). In Siberia, all three life histories (anadromous, lacustrine and riverine) have been known for quite some time (Berg 1962) possibly relating to a Eurasian centre of origin (see Politov *et al.* 2004). Broad whitefish are broadcast spawners (Scott and Crossman 1998) and typically mature at approximately seven to eight years of age (Bond 1982).

Anadromous broad whitefish utilize several areas within the Mackenzie River basin and its major tributaries for spawning (Chang-Kue and Jessop 1997; Reist and Chang-Kue 1997; Babaluk *et al.* 1997). These locations, identified using radio-telemetry, include Point Separation, several sites on the Mackenzie River mainstem, Ramparts Rapids near the town of Fort Good Hope and at least one upstream site in both the Peel and Arctic Red rivers (Chang-Kue and Jessop 1983, 1997; Reist and Chang-Kue 1997). In lacustrine populations there is less information available on possible spawning locations, and it has been only recently that such populations have been confirmed to be truly lake-locked (i.e., the Travaillant Lake system, Harris and Howland 2005). Harris and Howland (2005), with the use of radio-telemetry, identified three specific reaches of the Travaillant River upstream of Travaillant Lake as potential spawning locations for these lacustrine fish. Furthermore, these authors also found that spawning occurs at the outlet of Travaillant Lake; however, these are most likely lacustrine fish from Andrew Lake to the south. The capture of ripe or spent fish also indicates that spawning may also occur in

other lake systems in the Mackenzie River drainage, such as Campbell Lake (J. Reist, Fisheries and Oceans Canada, Central and Arctic Region, Winnipeg, unpublished data).

In the past extensive research has been conducted on broad whitefish from the Mackenzie River system to address questions concerning their biology (Treble and Reist 1997; Treble and Tallman 1997), morphology (Chudobiak *et al.* 2002), life history variation (Chudobiak 1995; Tallman *et al.* 2002), and specific habitat requirements (Chang-Kue and Jessop 1983, 1997; Harris and Howland 2005). Very little research, however, exists regarding genetic variation within and among these broad whitefish populations, within this system specifically, and within North America as well. Although a few genetic related broad whitefish studies have been conducted in North America (e.g., Patton *et al.* 1997; Reist 1986, 1997) this work was focused on variation in allozymes and there are now other techniques that allow for fine-scale assessments of molecular variation such as the use of microsatellite DNA. Although there is one microsatellite analysis of broad whitefish (Patton *et al.* 1997), this study was conducted on a small geographic scale with very little temporal consideration, and did not attempt to answer many other important questions regarding genetic variation, population structure and phylogeography of this species.

Anadromous broad whitefish are exploited during fall subsistence (Sparling 1988; Treble 1996) and commercial fisheries (Treble 1996) in the Mackenzie River, and it is currently not known what the relative contributions are of these various anadromous populations to the Mackenzie Delta fisheries. Several broad whitefish populations migrate through the

Mackenzie River Delta at any given time during the fall fishery (Reist and Chang-Kue 1997) and, consequently, potentially any of them are vulnerable to harvest. The subsistence fishery has, unlike the intermittent commercial fishery, been conducted since the first human habitation of the area (Treble and Reist 1997), and currently broad whitefish are being harvested within three different aboriginal land claim settlement areas within the region. Although physical tagging studies have been conducted to determine catch composition of this harvest with limited success (Babaluk *et al.* 1997), molecular techniques have never been used to assess contributions of populations to this mixed-stock fishery despite the power of their application in other fisheries (e.g., Beacham *et al.* 2005).

Recently, the Travaillant Lake system has also been the focus of numerous broad whitefish population, life history and habitat use studies. It was previously unknown whether the broad whitefish inhabiting Travaillant Lake system were in fact lacustrine or whether they were anadromous, and it was unknown what mixing, if any, there was between these two life history types. Comparisons of genetic variation (allozyme variation), vital rates, morphology and habitat use suggest that they are a distinct group of broad whitefish, different from their Mackenzie River counterparts (Reist 1986, 1997; Chudobiak 1995; Chudobiak *et al.* 2002; Tallman *et al.* 2002; Harris and Howland 2005). Comparatively, an examination of sulphur isotopes in the flesh of broad whitefish from Travaillant Lake suggested that they are visitors to the lake, which have assimilated a significant fraction of their sulphur from food sources outside of this lake (Hesslein *et al.* 1991), presenting the possibility that there may be several populations, or even life

history types, within this system. Given that genetic samples have been collected from the two spawning populations migrating up rivers from two separate lakes in the fall, and that summer samples have also been collected within the lakes over several consecutive years, I had the unique opportunity to address some of these questions regarding the life history and genetic variation persisting within this species in a lacustrine setting. These genetic data will also be used to corroborate the findings from the recent radio-telemetry studies (Chang-Kue and Jessop 1997; Harris and Howland 2005) and past morphological, allozyme, stable isotope and otolith microchemistry data (Reist 1986, 1997; Hesslein *et al.* 1991; Chudobiak 1995; Chudobiak *et al.* 2002; Tallman *et al.* 2002).

Thesis Objectives

In this thesis, broad whitefish are used as a model species to infer the historical and contemporary evolutionary processes that have influenced the spatial genetic variation within and between populations of Arctic fish species as assessed by variation at microsatellite loci. I used molecular data to assess the evolution and population genetics of broad whitefish throughout its North American range. The objectives of this thesis are two-fold. First, the thesis begins with a broad-scale phylogeographic study of the species and assesses how historical factors such as post-glacial recolonization patterns and historical reproductive isolation have influenced current genetic structuring. Second, I focus on microgeographic investigations within the Mackenzie River system to assess the address some unknowns regarding differentiation and contributions to this mixed-stock fishery. Specifically, microsatellite data was used to determine the number of discrete populations within this system, to evaluate the current going gene flow between

populations in this system and to resolve the contributions of anadromous populations to the mixed-stock subsistence fishery. In this study I use all sampling locations identified as putative populations to assess the North American phylogeographic structure, but only those from the Mackenzie River system for a fine scale genetic assessment of this species. Finally, in my fourth chapter, I tie all of the information together for a final synthesis and conclusions and then relate my findings to the conservation and management of this species. This is of particular importance as little is known regarding its population structure of broad whitefish, it is the coregonid species most valued in the local subsistence fisheries and has been the target of intermittent commercial fishery efforts in the Mackenzie River Delta (Chang-Kue and Jessop 1997).

CHAPTER 2: THE EFFECTS OF PLEISTOCENE GLACIATIONS AND POST-GLACIAL RECOLONIZATION ON PATTERNS OF GENETIC DIVERSITY

Introduction

In order to explain the geographical distribution, and the partitioning of genetic variation within North American broad whitefish, it is necessary to examine the phylogeography for this species and to try and determine possible recolonization routes following the last glaciation. As defined by Avise (2000), phylogeography is the field of study concerned with the principles and processes governing the geographic distributions of genealogical lineages, especially those within and among closely related species. Essentially, phylogeography, a rapidly expanding field of study, is the synthesis of phylogenetics and population genetics with biogeography and, since its introduction, has contributed to the reinvigoration of the role of history in evolutionary biology and population genetics (Avise *et al.* 1987).

Glaciation and Population Structure

The Pleistocene glacial epoch had major impacts on the ecology, genetic structure and biodiversity of North American species (Pielou 1991; Hewitt 1996) and the most recent, Wisconsinan, glaciation, in particular, had a profound impact on fish fauna in North America (Lindsey and McPhail 1987; Rempel and Smith 1997). The genetic and ecological upheavals caused by repeated glacial advances and retreats were especially pronounced among freshwater species due to their relatively restrictive dispersal abilities (Pielou 1991; Bernatchez and Wilson 1998). Fish were confined to areas of refuge

during glaciation and the sequence of deglacial events produced enormous volumes of water, which created temporary glacial lakes and spillways or reduced the salinity of coastal waters (Lindsey and McPhail 1987; Rempel and Smith 1997). Drainage divides were often inundated and watersheds temporarily connected by large meltwater lakes, providing opportunities for fish to disperse from the confines of refugia into newly deglaciated regions (Rempel and Smith 1997). Additionally, shifting ice fronts frequently altered habitat conditions drastically through the formation and failure of ice dams, drainage shifts, cascading overflows, and sudden emptying or flooding of ice-margin lakes (Pielou 1991). As a result of these large-scale disruptions, species diversities in formerly glaciated areas are far below those observed in neighbouring nonglaciated regions (McAllister *et al.* 1986). Conversely, the vast proglacial lakes formed from glacial meltwater provided tremendous dispersal opportunities for many aquatic species (Rempel and Smith 1997; Wilson and Hebert 1998).

Fish species were often confined to ice-free areas known as glacial refugia during times of ice advance. Studies of DNA polymorphisms have revealed that postglacial colonization events from such purported glacial refugia have markedly shaped the contemporary distribution of genetic variation in the wild (reviewed in Avise 2000). Several studies have provided evidence showing that postglacial dispersal from putative refugia has left a genetic signature on freshwater fish species in terms of a progressive decline in genetic diversity with increasing distance from the refuge (Bernatchez and Wilson 1998; Hewitt 2004), and elevated genetic diversity tends to occur in regions located in or close to putative glacial refugia (Hewitt 1996). When species recolonized

previously glaciated areas following deglaciation, dispersal often occurs in a stepping-stone-like pattern along coastal environments or via series of post-glacial lakes created as ice retreated (e.g., Rempel and Smith 1998; Wilson and Hebert 1998). The subsequent founder effects and bottlenecks associated with a stepping-stone pattern of post-glacial dispersal and subsequent range expansion that took place behind the receding glaciers are often thought to have driven the reduction in genetic diversity following Wisconsinan deglaciation (Hewitt 1996) that ended 10 to 12,000 years ago (Lindsey and McPhail 1986). Furthermore, populations following the margins of the ice sheets closely during post-glacial dispersal from putative refugia often had reduced population sizes where the effects of genetic drift may have been pronounced on these small founding populations (Avise 2000).

Beringia, one such glacial refuge which spans northeast Siberia, Alaska and northwest Canada, provides a natural laboratory for examining the effects of Pleistocene glaciations and their genetic consequences for northern species (Galbreath and Cook 2004). Broad whitefish provide an opportunity to examine the effects Pleistocene glaciations on sculpting the current patterns of genetic variation in an Arctic fish species because the current distribution of broad whitefish spans that of former Beringia, and this species likely survived solely within this refuge, dispersing following the Wisonsinan glaciation. In the case of North American broad whitefish, I predicted that the highest genetic diversity would be observed in populations occupying drainages covering areas of the former Beringian refuge (i.e. populations from Alaska) and genetic diversity would decrease in populations at the periphery of the range farther away from the refuge (i.e.,

populations in the Mackenzie River system including Travaillant Lake, see Stamford and Taylor 2004). If broad whitefish do show reduced variation in the Mackenzie River populations it could be because they are peripheral populations that represent the leading edge of dispersal from Beringia, that have been subject to serial founding events or bottlenecks associated with postglacial recolonization (see Costello *et al.* 2003). Furthermore, fish displaced by glaciers, such as those now inhabiting the Mackenzie River system should have lower genetic diversity than those that have survived solely in ice-free areas (Bernatchez and Wilson 1998).

Survival in North American Beringia and the Possibility of Two Post-Glacial Dispersal Routes

Within Beringia, it is possible that broad whitefish survived in two separate refugia; a north Beringian refuge, north of the Brooks Mountain Range and a south Beringian refuge, south of the Brooks Range. Genetic (mtDNA) data for Arctic grayling (*Thymallus arcticus*) has been used to provide evidence for two distinct Beringian refugia (Stamford and Taylor 2004). Such a scenario may also accurately depict broad whitefish survival in Beringia during past glaciations. Their current distribution suggests that they survived within Beringia, but whether there were multiple refugia within this region available for broad whitefish during Pleistocene glaciations is still unknown. Employing microsatellites, it is possible to determine if fish survived in separate refugia or sub-refugia (i.e., constitute separate glacial lineages) depicted by the presence of unique alleles, or differing allelic size ranges at the same locus (Lu *et al.* 2001).

Dispersal of broad whitefish from Beringia into Mackenzie River drainages may have ensued via two separate post glacial routes; dispersal solely through freshwater and dispersal through marine habitats (Figure 1). In the first scenario, meltwater from retreating glaciers during deglaciation events formed large proglacial lakes that could facilitate in the dispersal of freshwater fish (Pielou 1991; Bernatchez and Wilson 1998). Additionally, advancing ice sheets blocked and diverted numerous rivers such that some of these rivers reversed their flow into a different drainage (Lindsey and McPhail 1986). These proglacial lakes and drainage reversals connected separate drainages that are now currently distinct, and allowed for dispersal of freshwater fish between them (Lindsey and McPhail 1986; Pielou 1991). Two such lakes existed in Beringia during glacial maxima, Lake Old Crow and Lake Bonnet Plumme, and several examples of drainage reversal caused by blocking ice sheets exist (Peel and Porcupine River diversions, see Lindsey and McPhail 1986). Both of these deglaciation events created temporary connections between the Yukon and Mackenzie River systems (Lindsey and McPhail 1986; Pielou 1991); drainages that currently have no connections.

In contrast to a freshwater dispersal route from Beringia, broad whitefish may have also dispersed into the Mackenzie River system utilizing coastal habitats of the Beaufort Sea. Lindsey and McPhail (1986) discuss the potential for coastal migrations of other fish species along the North Slope of Alaska and postglacial dispersal via coastal migrations have also been suspected for lake trout ((*Salvelinus namaycush*, Wilson and Hebert 1998) and Arctic char (*S. alpinus*, Wilson *et al.* 1996). Broad whitefish are well established in

most major river systems flowing on the north slope of Alaska into the Beaufort Sea (Patton *et al*. 1997; Morris 2003) to the Mackenzie River and east to the Anderson and Coppermine rivers (Scott and Crossman 1998) and are well adapted to brackish water environments as evidenced by the anadromous life history observed in most populations. Broad whitefish therefore could have potentially dispersed along the Alaskan Arctic coast, following glacial margins closely, colonizing North Slope river systems in a stepping-stone like fashion until reaching the Mackenzie River system. Although phylogeographic assessments of broad whitefish have never be conducted, and their post glacial dispersal history is virtually unknown, they likely used one or several of the routes discussed above based on their current North American distribution. Several other fish species were also able to disperse post glacially from Beringia to the Mackenzie River system: Arctic grayling (Stamford and Taylor 2004), lake trout, Wilson and Hebert 1998) and lake chub (*Coueisus plumbeus*, Lindsey and McPhail 1986**)**.

Taking into consideration the current distribution and different life histories of broad whitefish in the Mackenzie River basin, the use of two separate dispersal routes from Beringia seems very plausible. I hypothesized that the genetic variation observed in populations of broad whitefish in North America, and specifically within populations inhabiting the Mackenzie River drainage, can be explained by their survival in the Bering refuge and by the possibility of two separate dispersal events. Conceivably, anadromous broad whitefish dispersed through coastal habitats explaining their possible life history and tolerance to saline environments and perhaps lacustrine broad whitefish inhabiting Travaillant Lake dispersed to the Mackenzie drainage solely through freshwater,

explaining the limited anadromy observed in these populations (Babaluk and Reist 1996; Harris and Howland 2005). If this is the case, I expected that populations inhabiting rivers on the north slope of Alaska (north of the Brooks Range) would be more similar genetically to anadromous Mackenzie River populations, and upper Yukon River populations (south of the Brooks Range) to be more similar to lacustrine populations in the Travaillant Lake system. My study could test the notion that broad whitefish populations may have survived in two separate Beringian refugia as has been shown for Arctic grayling (Taylor and Stamford 2004). If broad whitefish did survive in two separate Beringian refugia, I expected there to be major genetic discontinuities between populations located north and south of the Brooks Mountain range in Alaska (see Taylor and Stamford 2004).

Isolation-by-Distance

Geographic distance between populations can play an important role in the genetic structuring of populations. The term "isolation-by-distance" (IBD) was first used by Sewall Wright to describe patterns of population genetic variation that derive from spatially limited gene flow (Wright 1943) and is a commonly observed phenomenon in natural populations (Slatkin 1993). Isolation-by-distance analyses assess whether more distant population pairs are more genetically divergent from each other than are populations in closer proximity, can reveal the importance of specific barriers to gene flow, and may help separate the effects of population history from ongoing gene flow (Bohanak 2002).

Populations occupying ice free areas (refugia), or those at the heart of their range are more likely to be older and exhibit patterns of regional migration–drift equilibrium than populations at the periphery of their range (e.g., Turgeon and Bernatchez 2001). This regional equilibrium refers to a condition of balance between loss of alleles due to drift and their replacement by gene flow between populations of a region (Hutchison and Templeton 1999). Under equilibrium conditions, gene flow offsets the effects of genetic drift and, thus, pairwise genetic distance estimates (e.g., F_{ST}) will increase with geographical distance (Slatkin 1993). In contrast, when genetic drift is more powerful than gene flow and populations are no longer in equilibrium, measures of F_{ST} should be more variable, resulting in a lack of relationship between genetic distance estimates and geographical distances (Hutchison and Templeton 1999). Therefore, genetic analyses incorporating isolation-by-distance can be useful for identifying populations that are not under regional equilibrium, and, thereby, stimulate further investigation of how population structure has been affected by isolation, gene flow and genetic drift. The expected patterns in regions not yet at equilibrium are influenced both by the time since colonization and the degree to which dispersal and subsequent gene flow is inhibited within the region (Hutchison and Templeton 1999; Crispo and Hendry 2005).

In this study, the relationship between increasing levels of geographic distances between populations and its relationship with measures of genetic distance was investigated. I predicted that as geographic distance increases between populations of broad whitefish, genetic distance measures will increase due to reduced levels of gene flow. Additionally,

since the relative strength of isolation-by-distance may differ between geographic regions depending on how far constituent populations are from drift-migration equilibrium (Hutchison and Templeton 1999), I examined the patterns of isolation-by-distance to provide another test of the influence of historical events on current population structure. I expected to observe that populations located farther away from Beringia (i.e., the Mackenzie River system) should have a less developed pattern of isolation-by-distance (e.g. Turgeon and Bernatchez 2001), owing to a more recent colonization.

Microsatellites vs. Mitochondrial DNA for Phylogeographic Resolution

Most phylogeographic studies of animals have relied on the analysis of mitochondrial DNA (mtDNA) sequence variation (reviewed in Avise 2000) and several examples exist for Arctic North American freshwater fish species (e.g., Wilson and Hebert 1998; Stamford and Taylor 2004) including coregonids specifically (e.g., Bernatchez and Dodson 1991, 1994). Mitochondrial DNA, often considered the classical marker most suitable for phylogeographic studies because of its haploid, non-recombining mode of inheritance, relatively rapid mutation rate, and because it is usually considered to be selectively neutral (Avise 2004). MtDNA, however, is not without its limitations. For example the usefulness of mtDNA in inferring population relationships may be limited when divergence times are quite recent (Avise *et al.* 1984; Brunner *et al.* 1998) as mtDNA may not evolve fast enough, in terms of mutation, to resolve evolutionary histories in recent times (Angers and Bernatchez 1998). Furthermore, phylogeographic studies utilizing mtDNA rely on a small number of linked genes, providing information

of a single gene tree, potentially leading to inferences that do not match organismal history (e.g., Pamilo and Nei 1988, Ballard and Whitlock 2004). Due to these, and other, limitations, the need to employ nuclear markers in phylogeographic studies has become apparent (Hare 2001)

Microsatellites are a nuclear marker that have been receiving increasing attention for their use in phylogeographic studies and for resolving fine-scale population structure (e.g., Angers and Bernatchez 1998; Koskinen *et al.* 2002). Microsatellites are bi-parentally inherited, short segments of DNA, that are tandomly repeated in the nuclear genome, in which the unit of repetition is usually one to five base pairs in length (Goldstein and Pollock 1997; Jarne and Lagoda 1996). These presumably selectively neutral markers have an exceptionally high rate of mutation, around the order of 10^{-5} to 10^{-2} per generation (Jarne and Lagoda 1996), which lead to extensive polymorphism between populations, and increase the probability that, even over short time frames, populations have diverged at these loci (Angers and Bernatchez 1998). Because of this high mutation rate, it is suggested that the information these markers provide may be hindered over time frames as recent as 30,000 years since divergence (Paetkau *et al.* 1997). In contrast, the utility of microsatellites in studies of phylogeography, or studies of fine-scale population structure in recently diverged populations, has been proven to be quite informative. Phylogeographic studies employing microsatellites have recently exemplified that these markers provide additional phylogeographic resolution across the natural range of widely distributed and highly structured species (see Angers and Bernatchez 1998, Koskinen *et al.* 2002; Stamford and Taylor 2004). These features, coupled with their relative ease of

application in the laboratory, have made microsatellites the preferred marker in the majority of recent genetic studies of population structure.

Understanding mutational processes responsible for microsatellite evolution is relevant to the development and choices of appropriate statistical analyses for studies employing these markers. There are currently two alternative mutational models that have been developed to explain genetic variation and both may be applied to microsatellite data. The stepwise mutation model (SMM, Ohta and Kimura 1973) contends that mutations only occur to adjacent states. That is, each mutation creates in new allele by the addition or deletion of a single microsatellite repeat with equal probability (Balloux and Lugon-Moulin 2002). Consequently, alleles more similar in base-pair length are expected to be more closely related and have diverged more recently than alleles of very different sizes (Primmer *et al.* 1998). Since polymerase slippage during replication is likely the main mode of microsatellite mutation (Levinson and Gutman 1987), in which there is an increase or a decreases of a microsatellite repeat unit (Jarne and Lagoda 1996), the SMM appears to be most suited to accommodate mutations at these markers, although microsatellites often violate the assumptions of this model (Primmer *et al.* 1998). Another problem regarding the application of the SMM to microsatellite data is that, under this model, an allele may be created that is already present in the population. This phenomenon known as size homoplasy may give false insight into the true relatedness of individuals and populations (see Estoup *et al.* 2002 for a review), due to the occurrence of the same alleles in separate populations that are identical in state but not identical by descent. Alternatively, the infinite allele model (IAM, Kimura and Crow 1964), states

that a mutation may involve any number of tandem repeats, and the newly created allele is a unique allele not previously encountered. Because of this assumption, size homoplasy is not a factor if this model is used, but given the "slippage" mode of mutation for microsatellites (Levinson and Gutman 1987), it is perhaps unrealistic to assume strict adherence to the IAM. Therefore, it is important to take into consideration both the mode of mutation for microsatellites and the assumption of each mutational model before deciding which may be more appropriate.

Objectives

In this study, microsatellites were used to assess how historical evolutionary processes have influenced the spatial genetic variation within and between populations of North American broad whitefish. I tested the hypothesis that glaciation and subsequent recolonization have had a significant impact on current levels of genetic variation in broad whitefish. The analysis of microsatellites offers the potential to gain further insight into, and possibly a more realistic view of, the evolutionary history of recently diverged populations (Angers and Bernatchez 1998). The fact that broad whitefish have likely recently dispersed from Beringia to occupy its current range within the last 10-12,000 years makes them an excellent candidate for microsatellite analysis. Furthermore, phylogeography and post glacial dispersal history of broad whitefish in North America has never been addressed.

Materials and Methods

Sample Collection and DNA Extraction

A total of 1476 samples were collected from 47 localities (Table 1, Figures 2 and 3). Samples were collected from the Pechora River, Russia, from several locations throughout Alaska and from the lower Mackenzie River System and its major tributaries, Northwest Territories, Canada. The lower Mackenzie River system, however, was sampled extensively in comparison to Russia and Alaska, comprising approximately 85% of all samples. Furthermore, although there are samples from 46 locations, each locality likely does not represent a distinct population, especially for samples collected throughout the Mackenzie River Delta through which several populations are known to migrate (Reist and Chang-Kue 1997). In this chapter only 14 of the 47 sampling locations were used because these were sampling locations assumed to be "true" populations and not areas of potentially mixed stocks as identified previously by radio-telemetry (Chang-Kue and Jessop 1997) or based on their geographic location. The majority of samples were collected via gill netting except for those from the Yukon River sampling location at the Rampart Rapids that were collected using a fish ladder. Owing to their remoteness, some of my localities could not be sampled extensively and consisted of samples of less than ten individuals. Samples consisted largely of fin clips or muscle tissue that was preserved in 95% ethanol. DNA was isolated from approximately 5 mg of tissue using the Qiagen DNA extraction kits.

Microsatellite Amplification and Scoring

I surveyed nuclear DNA variation by examining allele frequency variation at seven microsatellite loci. Microsatellite loci used in this study were screened and chosen for inclusion based on the clarity of resolution and degree of polymorphism. After screening microsatellite loci, seven were chosen for use in this study. In population genetics studies, increasing the number of loci from six will have substantial benefits (Pritchard *et al.* 2000; Koskinen *et al.* 2004), so seven loci seemed sufficient. Specific loci used in this study include Cocl-Lav4, Cocl-Lav6, Cocl-Lav8, Cocl-Lav10, Cocl-Lav18, Cocl-Lav27 (Rogers et al. 2004, Table 2). These primers were developed for lake whitefish, but were successfully cross-amplified in six additional taxa, including two other members of the genus *Coregonus*. An additional primer, Ots103 (Small *et al.* 1998, Table 2), was successfully amplified in broad whitefish, and also included for use in this study

Polymerase chain reaction (PCR) protocols were as described in Rogers *et al.* (2004) for the Cocl-Lav primers and Small *et al.* (1998) for Ots103, with slight modifications. Each PCR was performed in an 10-µL volume with 1-µL of genomic DNA, 5 µM of the reverse primer and 2 µM of the fluorescently-labelled forward primer, 10 mM dNTP, 1 µl reaction buffer (New England Biolabs) and 0.1 U of *Taq* polymerase (New England Biolabs).

For the Cocl-Lav primers, the following thermal profile was used: 1 x (95^{o}C for 3 min, locus-specific annealing temperature (Ta, Table 2) + 1^{o}C for 1 min, 72^{o}C for 1 min), 30 x

(95°C for 30 sec, Ta for 30 sec, 72°C for 1 min) and a final elongation step of 72°C for 5 min. For Ots103, the PCR protocol was as follows: 1 x (95 °C for 2 min, 58°C for 1 min, 72°C for 1 min), 8 x (94°C for 20 sec, 58°C 20 sec, 72°C for 20 sec), 32 x (94°C for 20 sec, 56°C 20 sec, 72°C for 20 sec) and a final elongation step of 72°C for 5 min.

PCR products were examined using fluorescently labeled primers and assayed on a Beckman-Coulter CEQ 8000 automated genotyper where alleles were scored by eye.

Genetic Analysis

Basic descriptive statistics of microsatellite variation, including number of alleles (N_a), expected heterozygosity (H_E) and observed heterozygosity (H_O) were calculated using TFPGA ver. 1.3 (Miller 1997). Allelic richness (A_r) was calculated using FSTAT ver. 2.9.3.2 (Goudet 2002). Since the number of alleles per locus (N_a) is highly dependant on the sample size, allelic richness was calculated to bypass this problem. Essentially, allelic richness is the number of alleles per locus, corrected for sample size using the rarefaction method (El Mousadik and Petit 1996), hence, allowing comparisons of this quantity between samples of different sizes (Goudet 2002).

I also tested for differences in allelic richness (A_r) and expected multilocus heterozygosity (H_E), between populations from Alaska (presumably populations occupying drainages covering areas of the former Beringian refuge) and populations from the Mackenzie River System (populations further away from potential refugia) using the

permutation approach in FSTAT. Specifically, since greatest genetic diversity tends to occur in regions located in, or close to, putative glacial refugia with a progressive decline at more distant localities (Costello *et al.* 2003, Stamford and Taylor 2004), I predicted that populations further away from putative refugia, in this case those from the Mackenzie River system, would show lower levels of genetic variation. Additionally, I organized populations by straight geographic distance from the putative Beringian refuge (at the confluence of the Koyukuk and Yukon rivers, see Stamford and Taylor 2004) and regressed this variable against several measures of genetic variation to further resolve the occurrence of trends in genetic variation and distance from refugia. If North American broad whitefish did survive the last glaciation in Beringia, I expect to observe a reduction in genetic diversity in populations located progressively farther from the North American centre of this refuge (i.e., negative correlation between measures of genetic diversity and geographical distance). Measures of genetic variation used were, once again, allelic richness and expected heterozygosity and the regression analysis was conducted using JMPin (version 3.2.1).

Tests for deviations from Hardy–Weinberg equilibrium of observed genotypes were performed using GENEPOP Ver. 3.4 (Raymond and Rousset 2003) for each locus-population combination using an exact test in which two-tailed *P*-values were estimated using a Markov chain method of Guo and Thompson (1992). Tests for genotypic linkage disequilibrium for all combinations of locus pairs within populations were also conducted using a Markov chain method in GENEPOP. Finally, employing GENEPOP, tests for population differentiation between all pairs of populations were performed over all loci

combined using log-likelihood (G)–based exact tests (Goudet *et al*. 1996) with default values. I adjusted the results from tests for conformation to Hardy–Weinberg proportions, linkage disequilibrium and population differentiation for multiple tests using the sequential Bonferroni procedure (Rice 1989) with an initial alpha level of 0.05.

Spatial genetic structure was also calculated at the population level by estimating F-statistics (F_{ST}) in order to measure the extent of genetic differentiation between populations. F_{ST}, a drift-based method of population subdivision calculated assuming that the loci assayed follow the infinite allele model (IAM, Kimura and Crow 1964), is one of the two most commonly reported statistics for the estimation of population structure (Balloux and Lugon-Moulin 2002). Although microsatellites are often assumed to mutate following the stepwise mutation model (SMM, Ohta and Kimura 1973) and a statistic, R_{ST} (Slatkin 1995), an F_{ST} analogue that assumes the SMM, has been developed specifically for SMM-based loci, adherence to this assumption can vary from locus-to-locus. F_{ST}, the drift-based method of population subdivision was considered to be most appropriate for several reasons. First, broad whitefish are likely Beringian in origin, and they likely dispersed from this refuge within the last 12,000-18,000 years (Lindsey and MacPhail 1986). Over such short time frames, particularly when population histories may have involved large changes in population sizes, demographic processes probably overwhelm any post-colonization mutation-based differentiation patterns (Taylor *et al.* 2001). In addition, simulation studies have demonstrated that F_{ST} typically outperforms R_{ST} in recently diverged populations under typical conditions of moderate or small sample sizes (i.e., $n < 50$) and when the number of loci scored is low (i.e., $n < 20$,

Gaggiotti *et al.* 1999). Additionally, if population structuring is weak, F_{ST} appears to perform better than R_{ST} (Gaggiotti *et al*. 1999; Balloux and Goudet 2002). Lastly, there is some evidence that microsatellites may not mutate perfectly under the SMM (Balloux and Lugon-Moulin 2002) and that this mutational model may be an oversimplification of the true mutational process (Ellgren 2000).

Consequently, I focussed on F_{ST}–based statistics, which appear to be more conservative with small sample sizes, few loci and recently diverged populations (Gaggiotti *et al.* 1999). Specifically, I used the classical estimator (θ) of Weir & Cockerham (1984). Pairwise F_{ST} (θ) values were calculated using FSTAT ver. 2.9.3.2 (Goudet 2002) and GENEPOP. Variances and robustness of *F*-statistics estimates were assessed by resampling procedures using jack-knife and bootstrap methods over loci to generate P values and 95% confidence intervals using FSTAT. F_{ST}, typically ranges from 0, which indicates no genetic differentiation, to 1, which indicates complete fixation of alternate alleles. Note that, sometimes using θ which relates to probability of identities, it is possible to obtain negative values for θ under certain situations (Balloux and Lugon-Moulin 2002).

Hierarchical analysis of molecular variance (AMOVA), based on allele frequency information (Excoffier *et al*. 1992), was conducted using ARLEQUIN version 2.0 (Schneider *et al*. 1997), to determine how the genetic variation is partitioned. AMOVA is similar to an analysis of variance (ANOVA) procedure in that it partitions estimates of population genetic variation. The percentage of the total genetic variation explained by

allele frequency variation within populations (Vc), among populations within groups (Vb), and by differences among groups (Va) was calculated under a variety of grouping hypotheses. For example, I tested for a major genetic division between Alaskan samples (those that presumably inhabit the area of the Beringian refuge) and Mackenzie River drainage samples (those not currently inhabiting formerly ice free areas) to determine if this represents a distinct grouping sufficient enough to explain the patterns of variation observed. Other groupings included separation of distinct watersheds and life-history type (i.e., lacustrine and anadromous). Finally, AMOVA was used to partition differentiation into components due to variation where samples have been collected over several consecutive years (e.g., the Travaillant Lake system) to check for temporal differences in population structuring.

I also attempted to identify population groups exhibiting major genetic discontinuities that could be revealed in the topology of a phenogram using genetic distances between population pairs. Genetic distances were estimated using the pairwise chord distance (D_{CE}) of Cavalli-Sforza and Edwards (1967) which has been shown to estimate tree topologies well in very closely related populations. I used PHYLIP version 3.5 (Felsenstein 1993) to calculate $D_{CE,}$ with the GENDIST module and used the corresponding genetic distance matrices to construct an unrooted neighbour-joining (N-J) phylogenetic tree to visualize genetic relationships among populations and sampling localities. Specifically, from these distance matrices, the neighbor-joining algorithm in the NEIGHBOR module was used to generate the trees, while CONSENSE was used to generate a consensus tree with bootstrap values from 1000 replicate datasets created in

SEQBOOT. The final tree was drawn in the DRAWTREE module. I chose to analyze genetic divergence between populations using D_{CE} because it is a drift-based estimate of genetic distance, it does not assume any models of molecular evolution and in the past it has performed well in simulations of microsatellite data (Takezaki and Nei 1996). Again, given the probable recent origin of broad whitefish in North America, drift, rather than mutation, has likely contributed the most to population differentiation in this species.

To further detect patterns of genetic differentiation between samples a factorial correspondence analysis (FCA), was conducted using Genetix 4.05.02 (Belkhir *et al.* 2004). FCA is a type of factor analysis that seeks to find the best linear combination of variables (in this case allele frequencies at different microsatellite loci) that best describe variation between individual observations (fish, Taylor *et al.* 2006). FCA graphically projects the individuals or populations on the factor space based on the similarity of their allelic states (Barluenga *et al.* 2006) and is best suited for categorical data, such as allele frequency counts (Taylor *et al.* 2006). FCA essentially determines the first K-orthogonal axes of an orthogonal number of axes that describe the most variance from a ‘‘cloud’’ of observations (Taylor *et al.* 2006). The main advantage of the FCA is that each individual can be represented using each allele as an independent variable, contrary to other multivariate analyses, that generally use a combined parameter as descriptor (Roques *et al.* 2001).

The isolation-by-distance (IBD) model predicts that genetic distance between populations will increase exponentially as the geographic distance between them increases, because of the limiting effect of geographic distance on rates of gene flow (Relethford 2004). This

pattern of IBD is clearly revealed by a direct relationship between estimates of genetic differentiation (e.g., F_{ST}) and geographical distances when populations are at equilibrium under genetic drift and migration (gene flow, Castric and Bernatchez 2003). To assess whether the association between genetic distance, in this case F_{ST}, and geographic distance is statistically significant, and to reveal insights into the stages of equilibrium in North American broad whitefish populations, a Mantel Test was employed in this study. This test assesses whether the pairwise genetic distance (F_{ST}) matrix is correlated with the pairwise geographic distance matrix (Bohanak 2002). Specifically, to test for isolation-by-distance, the Mantel test option in FSTAT was used to assess the significance of correlations between geographical distance (i.e. fluvial distance between sampling locations) and genetic distance (F_{ST}). Geographic distances between sampling locations within the study area was determined using the Geographic Information System (GIS) program, ArcView (version 3.14, ESRI). Isolation-by-distance was tested over all sampling locations, then separately for all locations in Alaska (those closer to putative refugia) and all locations in the Mackenzie River drainage (those further away from putative refugia). Differences in the isolation by distance patterns observed between these locations would suggest that broad whitefish populations from both areas may be at different stages of migration-drift-equilibrium, likely due to differences in timing regarding post-glacial colonization from the Beringian refuge. Specifically, I predicted that populations from Alaska will show a stronger pattern of isolation-by-distance because they currently occupy areas that were ice-free during the glaciation, providing more time to reach equilibrium. Following a more recent expansion and colonization

(e.g., populations in the Mackenzie River system) an IBD pattern is not expected, or it may be present only at short distances (Turgeon and Bernatchez 2001).

RESULTS

Intrapopulation Genetic Variation

A total of 648 individuals were genotyped from 13 sampling locations, representing year classes from 1998 to 2006, and, in general, microsatellite polymorphism in broad whitefish across all loci was variable across loci and populations. All seven microsatellite loci were polymorphic, with allele numbers ranging from 4 (Cocl-Lav27) to 18 (Cocl-Lav8), and H_E ranging from 0.47 (Cocl-Lav27) and 0.74 (Ots103, Table 3). Within sampling locations, the mean number of alleles (averaged across all loci) ranged from 3.71 in the Travaillant River south population, to 6.71 in the Peel River when all sampling locations were combined (Table 4). The overall mean allelic richness was 3.54 (based on a minimum sample size of nine diploid individuals), and varied from 2.60 in the Pechora River, Russia sample to 4.35 in the Yukon River, Alaska at the Rampart Rapids (Table 4). The low allelic diversity exhibited in the Pechora River sample was due the Cocl-Lav10 locus that was nearly monomorphic whereas at all other locations this locus was highly polymorphic. The mean gene diversity (H_E) over all sampling locations was 0.577, and ranged from 0.422 in the Pechora River, Russia, to 0.644 in the Yukon River, Alaska, at the Rampart Rapids (Table 4). The Pechora River population displayed extremely low levels of genetic variation in comparison to other sampling locations because it showed low polymorphism at three of the seven loci used in this study (Cocl-Lav14, Cocl-Lav10 and Ots103).

When sampling locations were grouped into those from areas that served as putative refugia (non-glaciated) during the last Wisconsinan glaciation (i.e., samples from Alaska) and those from previously glaciated areas at the periphery of the current range (i.e., the Mackenzie River system), significant differences, based on the permutation process, were observed between allelic richness and expected heterozygosity (all $P<0.05$). As predicted, samples from Alaska had significantly higher average allelic richness (3.96 compared to 3.45) and average expected heterozygosity (0.61 compared to 0.54) when compared to samples from the Mackenzie River system. In this study, as predicted, North American broad whitefish showed that genetic diversity declined with straight-line geographical distance from the lower Yukon River in Alaska (Figure 4). Among populations there was a significant negative correlation between geographical distance and allelic richness ($r = -0.734$, $P = 0.0028$). Furthermore, there was also a significant negative correlation between geographical distance and heterozygosity ($r = -0.772$, $P = 0.0012$).

Following Bonferroni corrections for simultaneous multiple tests (new alpha = 0.00052), conformation to Hardy-Weinberg equilibrium was not rejected in any of the 98 tests. Significant genotypic linkage disequilibrium was not detected in any of 924 tests following Bonferroni corrections.

Population Divergence and Genetic Structure

Log-likelihood (G)–based exact tests of population differentiation (e.g., Goudet *et al.* 1996) suggested that the two main regions included in my study (i.e., Mackenzie River system populations and Alaska/Russia populations) are significantly differentiated ($P < 0.0001$). Within Alaska, virtually all populations were differentiated from each other, with the exception of the two Yukon River samples and the sample from the Tanana River which was only significantly differentiated from the Travaillant Lake populations.

Overall, F_{ST} (θ) ranged from 0.034 (Cocl-Lav8) to 0.206 (Cocl-Lav6) and the overall level of population subdivision based on pairwise estimates was low to moderate (θ = 0.10, % 95 CI 0.059-0.138) among all populations. Among populations, pairwise θ values ranged from -0.00350 (between two populations sampled from the Yukon River) to 0.454 (between the Pechora River in Russia and a sample from the Travaillant Lake system, Table 4). Most differences in θ were substantial, and the majority of comparisons were statistically significant (83 out of 105 population only comparisons, $P<0.01$, Table 5). There were, however, several comparisons that were not significant and these were largely found in comparisons between populations within the same river or lake system (e.g., with the Yukon River and within the Travaillant Lake systems).

Cavalli-Sforza's chord distance was used to generate an unrooted tree through a neighbor-joining analysis (Figure 5). Overall, the topology of the resulting tree coincided well with physiogeographic region in that geographically proximate locations clustered

together. Populations or sampling locations from Alaska and Russia were well separated from Mackenzie River system populations (Figure 5), clearly illustrating the high degree of genetic differentiation between broad whitefish inhabiting the western and eastern regions within their North American distribution (Figure 5), although bootstrap support was generally low ranging from 20-97%. Within the Mackenzie River system samples from the Mackenzie River Delta, the Mackenzie River and major tributaries were well separated from samples collected in the Travaillant Lake system and the two Travaillant Lake populations clustered well with high bootstrap support (97%). Within Alaska samples collected from the Yukon River system in Alaska also clustered together, although bootstrap support was generally low. Furthermore, there was evidence of groupings based on whether samples were collected north or south of the Brooks Mountain Range in Alaska as has been documented for Arctic grayling (Stamford and Taylor 2004). All samples collected south of this mountain range (i.e., samples from the Yukon River drainage, Selawik River and Whitefish Lake) clustered separately from Teshekpuk Lake, which is north of the Brooks Range.

Several grouping hypotheses were tested for AMOVA analysis, largely based on the topologies of the NJ tree. In all grouping scenarios, analysis of molecular variance (AMOVA) revealed that the greatest amount of allele frequency variance occurs within populations (Table 6). When all North American samples were pooled and compared against samples from Russia, 30.6% of the variation in allele frequencies was attributable to this grouping (Table 6, $P < 0.001$). When Alaskan samples and Russian samples were combined and compared to all samples from the Mackenzie River system (to compare

samples currently occupying putative Beringian refuge to those currently inhabiting areas that were previously glaciated) only 3.8% of the variation in allele frequencies was attributable to this grouping (Table 6, $P < 0.001$), but when samples were grouped into Russian vs. Alaskan vs. Mackenzie River system, 13.8% variance was explained by this grouping ($P<0.001$). These groupings highlight the significance of the Pechora River sample in contributing to the variance among groups and hence the remarkable differentiation across continents. It should be noted however that there is only one Russian population and therefore variation among populations within regions (Vb) is only calculated for North American populations.

The genetic variation among all samples was also summarized by factorial correspondence analysis (FCA, Figure 6) which revealed a strong geographical pattern of genetic variation in accordance with the results of NJ tree, similarly suggesting the distinct genetic composition of Alaskan and Mackenzie River system groups of populations. In the FCA, three broad groupings of broad whitefish were resolved in addition to an outlier consisting of the Pechora River which was largely divergent from North American samples as indicated by FCA Axis 1 (Figure 6). Within North America, similar to the both the NJ tree, groupings were largely influenced by the geographic location of the collection site. One group (‘‘A’’) consisted of a clustering which included all broad whitefish collected from Alaska, with the exception of the Teshekpuk Lake population. A second group (‘‘B’’) consisted of all samples collected from the Mackenzie River system, excluding Travaillant Lake (i.e., Mackenzie River Delta, Mackenzie River proper, Peel and Arctic Red rivers, Figure 6). Interestingly, this Mackenzie River system

grouping also contained the Teshekpuk Lake population which is found on the North Slope of Alaska. The third group (‘‘C’’), a very tight clustering, consisted of all samples collected in the Travaillant Lakes system over several consecutive years (Figure 6). Grouping “A” was not as tightly clustered as was shown for groups “B” and “C” but this is likely due to the large geographical are sampled throughout Alaska, compared to that of the Mackenzie River and Travaillant Lake systems. Results of a principal components analysis were nearly identical in that there were the same three groupings mentioned above in addition to highly divergent position of the Pechora River (data not shown).

Isolation-by-Distance

Patterns of isolation-by-distance (IBD) were highly dependant on the geographic region surveyed. Throughout the entire study area, the extent of genetic differentiation between populations was strongly related to geographic distance and resolved a highly significant pattern of IBD ($r = 0.86$, $P < 0.0001$, Figure 7). As predicted, however, within this overall pattern there were some notable deviations in patterns of IBD between regions within the study area. For example, tests for IBD within the Mackenzie River system broad whitefish populations revealed a nonsignificant pattern of IBD ($r = 0.062$, $P = 0.79$, Figure 7). Alternatively, as predicted, when samples from Alaska and Russia (the Beringian refuge sample) were combined and tested for patterns of IBD, a highly significant relationship ($r = 0.95$; $P < 0.0001$) between the pairwise genetic distance (F_{ST}) and fluvial distance (km) was identified indicating equilibrium conditions between genetic drift and gene flow between these populations. These populations likely inhabit

areas that were ice-free during the Wisconsinan glaciation, therefore did not disperse to occupy their current locations post-glacially, and likely have had sufficient time to reach equilibrium.

Discussion

Genetic Differentiation Within and Between C. nasus *Populations*

My molecular data are the first large scale analysis of genetic diversity conducted specifically on North American broad whitefish using microsatellites (but see Patton *et al.* 1997, for a smaller scale study). In the current study, the number of alleles (4-18 across all loci and averaging 3.71 -6.71 across populations), allelic richness (2.60-4.35) and heterozygosity (0.47 - 0.74, with an average of 0.61 across all sites) estimates within *C. nasus* populations were comparable to reported values of previous studies of microsatellite analyses conducted on other salmonids (e.g., Angers *et al.* 1995; Taylor *et al.* 2003; Fraser *et al.* 2004; Whitley *et al*. 2004; Sonstebo *et al.* 2007) and on other coregonids (Turgeon and Bernatchez 2001a; Huuskonen *et al.* 2003; Rogers *et al.* 2004) specifically. Furthermore, the values reported in this study are roughly similar to the values reported in the only other microsatellite analysis conducted on North American broad whitefish (Patton *et al.* 1997). Unique alleles to specific populations were not common in this study. The Teshekpuk Lake population was the only one to show allele 146 at the Cocl-Lav18 locus which could be explained by isolation in a sub-refugium North of the Brooks Mountain range which is also suggest by the results of the NJ tree (Figure 5). The presence of unique alleles is often explained by isolation of populations in

distinct glacial refugia (Lu *et al.* 2001). Not surprisingly, the Pechora River population had several unique alleles at Cocl-Lav18 which suggests that Russian and North American populations have been isolated for quite some time. Additionally, there were many alleles that were unique to Alaskan populations that were not found in any of the Mackenzie River populations. This is more likely attributed to the loss of alleles due to founding events and subsequent drift in the small colonizing populations that dispersed post-glacially to the Mackenzie River system from Alaska, instead of survival in separate refugia.

Overall differences in genetic variation were observed between *C. nasus* populations located in or close to regions that may have served as putative refugia (i.e., Alaskan samples) and those at the periphery of their current North American range (Mackenzie River system samples). One trend that has become apparent in north temperate fishes of North America is that there is a pattern of declining genetic diversity further from putative refugia with populations at the periphery of a species' range showing the least amount of variation (Bernatchez and Wilson 1998; Turgeon and Bernatchez 2001; Castric and Bernatchez 2003; Stamford and Taylor 2004). In this study, a historical signature of the effects of postglacial colonization was evident in that samples from Alaska had significantly higher average allelic richness and average expected heterozygosity when compared to samples from the Mackenzie River system at the periphery of their range.

I also showed that genetic diversity (A_r and H_E) declined significantly with straight-line geographical distance from the lower Yukon River in Alaska (Figure 4). A decrease in

genetic variation with distance from putative refugia has been shown in many fish species previously isolated in refugia during Pleistocene glaciations in North America (Castric and Bernatchez 2003, Costello *et al.* 2003, Stamford and Taylor 2004, Adams *et al.* 2006). Founder events and bottlenecks associated with post-glacial recolonization from Beringia have likely played the most important role in shaping the patterns and levels of extant variation and it is quite likely that such colonization processes are still playing an important role in shaping spatial patterns of genetic variation in North American broad whitefish (Hewitt 1996). Through chance founding events following glaciations, populations surviving on the periphery of the colonization front would likely be composed of smaller, more isolated populations with reduced genetic variability (i.e., fewer alleles and lower heterozygosities) compared to source populations that survived in glacial refugia, and this low variability is quite common in newly founded or bottlenecked populations (Nei *et al.* 1975, e.g., Costello *et al.* 2003, Castric and Bernatchez 2003; England *et al.* 2003). This phenomenon may be particularly pronounced in Mackenzie River drainage broad whitefish because this area, except for the Anderson River and Coppermine Rivers, represents the eastern margin of its North American range (Scott and Crossman 1998) where pioneer-type range expansion may have contributed to low molecular variation presently observed (Ibrahim *et al.* 1996). As new founding populations from Beringia encountered the novel environments of the Mackenzie River system, founders would have dispersed to such areas, likely with lower levels of diversity in comparison to the contributing populations (e.g., Sage and Wolff 1986). Furthermore, these founding populations may have been quite small and the effects of drift would have been more pronounced also leading to a decrease in genetic

diversity of these populations. In this study, the decline in genetic diversity associated with increasing distance from Beringia may reflect a stepping-stone like post-glacial dispersal pattern from this refuge that would produce subsequent founder effects or bottlenecks (see Brunner *et al.* 2001). Such a model of range expansion produces a serial loss of alleles from the oldest to the youngest populations (Nei *et al.* 1975; Sage and Wolff 1986), in this case populations from Alaska to those at the eastern margin of the North American distribution (e.g., Mackenzie River system) respectively.

The NJ tree revealed four population clusters that are in general agreement with the geographic distribution of the populations: Russia, Alaska, the Mackenzie River system and the Travaillant Lake system. A substantial genetic discontinuity between North American populations and the Pechora River population from Russia was indicated by the NJ tree, the significant genic differentiation between the Pechora River population and all other populations, a high degree of population subdivision measured by AMOVA and by large F_{ST} values between the Pechora River samples and all other populations. This is not surprising because populations occupying the different continents are clearly geographically isolated, and likely have been since the end of the last glaciation (i.e., a minimum of 18,000 years (Lindsey and McPhail 1986), resulting in restricted gene flow. King *et al.* (2001), showed a similar genetic discontinuity in Atlantic salmon populations inhabiting Europe and North America and other authors (Politov *et al.* 2004) have found *Coregonus* spp. on opposite sides of the Bering Strait to be highly divergent. In contrast, Bernatchez and Dodson (1994) using mtDNA, showed the lake whitefish from Beringia clustered more closely to those from Eurasia (*C. laveratus*), than their North American

counterparts that survived Pleistocene glaciations in other distinct North American refugia (e.g., Mississippian, Atlantic and Acadian). Their results indicated that Beringia is a zone of secondary contact between Nearctic lake whitefish and those of a Eurasian origin. This was also illustrated by Brunner et al. (2001) and Stamford and Taylor (2004) who showed that Arctic char (*Salvelinus alpinus*) and Arctic grayling, respectively, inhabiting western Alaska have a greater genetic affinity to populations from Siberia when compared to other North American populations. Lastly, Galbreath and Cook (2004) found no evidence of differentiation Siberian and western North American populations of the tundra vole (*Microtus oeconomus*), which is interesting given that in this species dispersal is reliant on terrestrial environments.

Similar comparisons are not possible for North American broad whitefish (i.e., do they have a higher genetic affinity with Eurasian populations or other North American populations) since they likely survived solely within the Beringian refuge. Broad whitefish from North America and Eurasia are likely differentiated, even if populations were once continuous when the Bering land bridge persisted, by the opening of the Bering Sea which unquestionably promoted divergence between American and Eurasian continents (Brunner *et al.* 2001). Pechora River populations are highly divergent from all North American populations and do have unique alleles which may be a reflection of isolation on different continents, which has allowed enough time for other evolutionary forces (e.g., mutation and drift) to drive differentiation. The Bering Strait is likely still the most influential factor driving differentiation between the two continents, which has likely separated populations on each continent for 10,000 years (Lindsey and McPhail

1986). Although broad whitefish are anadromous (although semi-anadromous may be a better description), differentiation across continents is likely still persisting because broad whitefish can not tolerate full-strength sea water (Reist and Chang-Kue 1997) and therefore are confined to coastal environments, thus limiting gene flow of this species across the Bering Strait.

Within North America, populations from Alaska were almost always significantly differentiated from Mackenzie River system populations. This genetic discontinuity is also clearly illustrated by the separate clustering of theses groups in the neighbour-joining tree (Figure 5) and the FCA (Figure 6). Much of the current genetic structuring of North American broad whitefish can again be attributed to Pleistocene glaciations and subsequent recolonization (discussed in next section) from putative refugia, but currently it is the limited gene flow between these regions that is likely the main force promoting the genetic differentiation between Alaskan and Mackenzie River populations. There is however, the potential for some populations along the North Slope of Alaska to exchange genetic material with Mackenzie River populations, via coastal migrations, since several broad whitefish populations clearly exhibit an anadromous life history (Reist and Chang-Kue 1997). Since anadromous fish would be tolerant to higher levels of salinity, this may allow for the potential movement of individuals between the Mackenzie River system and proximate systems in Alaska (i.e., Sagnavirnoktok River System), or visa versa. If this was the case and on occasion there was a small degree of gene flow between Mackenzie River system broad whitefish and broad whitefish from the North Slope, populations on the North Slope of Alaska should be less genetically differentiated from Mackenzie

system populations compared to those from the Yukon River for example. This trend was not evident in this study. Future tagging studies (e.g., Floy, telemetry or acoustic) may provide more insight into the occurrence of broad whitefish coastal migrations between Alaskan and Mackenzie System populations. Additional microsatellite analyses utilizing more loci conducted on more sampling locations may also provide finer resolution into migrations or straying between systems.

There was a lack of genetic differentiation between anadromous populations in the Mackenzie River system (discussed in detail in chapter 3), yet other populations in this study (i.e., most populations from Alaska or Alaskan populations compared to Mackenzie River populations), were clearly differentiated. The Alaskan populations are separated much further geographically, resulting in reduced gene flow which would promote divergence. Furthermore these populations occupy putative refugial areas, in which sufficient evolutionary time has past to cause the present divergence. Accurate distinction between these alternatives may benefit from denser sampling across the natural range of *C. nasus,* including more microsatellite loci. Less intraspecific divergence should be expected in populations currently occupying previously glaciated areas (Bernatchez *et al.* 1998) especially when numerous colonists were involved in the founding process (Castric and Bernatchez 2003).

Isolation-by-Distance

Range-wide, a strongly significant relationship existed between genetic distance (F_{ST}) and geographical distance, indicating that there is a strong pattern of isolation–by-distance (IBD) within this study area (Figure 7). There were, however, some notable departures in patterns of IBD between regions within the study area, and the absence of IBD within some geographic scales (e.g., within the Mackenzie River system) allows me to reject the hypothesis of global migration-drift equilibrium (Hutchison and Templeton 1999). This may in part be due to the large spatial scale of this study, but is most likely due to the glacial history of the two areas compared. The pattern of IBD was still strongly significant when removing all populations from the Mackenzie River system indicating that there is regional equilibrium between gene flow and drift (Hutchison and Templeton 1999) within samples from Alaska and Russia. This trend is consistent with populations in the heart of their range, or those in areas that previously served as glacial refugia. Since Alaskan populations survived in or close to the heart of the Beringian refuge, and are at the heart of the North American range, they have been established longer regionally allowing enough time for migration-drift equilibrium to be established (Crispo and Hendry 2004) resulting in a signature of IBD as the effects of gene flow are more pronounced than that of genetic drift.

The lack of a correlation between F_{ST} and geographic distance in the Mackenzie River system indicates that broad whitefish have not occupied the region long enough since their invasion after the Wisconsinan glaciation to have approached any pattern

resembling IBD or equilibrium (Figure 7). This is consistent with a more recent expansion and subsequent colonization following the last glaciation in Mackenzie River broad whitefish, suggesting that these populations have not had sufficient time to reach equilibrium between gene flow and drift. Given its more easterly location, and assuming a stepping stone like pattern of dispersal along the Arctic coast from Beringia, the Mackenzie system would have been colonized later in deglaciation than areas in Alaska. Areas that were previously glaciated are considered to be "younger" and likely have not yet attained a state of equilibrium (Turgeon and Bernatchez 2001a). Other studies have shown that stronger patterns of IBD occur in areas where populations have been established longer in comparison to those at the periphery of the range (Hutchison and Templeton 1999; Castric and Bernatchez 2003; Costello *et al.* 2003; Taylor *et al.* 2003). Therefore, in this study, the recent colonization of the Mackenzie system likely explains the absence of any significant IBD patterns (reviewed in Crispo and Hendry 2004). Furthermore, within the Mackenzie system, the Travaillant Lake populations are highly genetically differentiated from all other Mackenzie River samples, and previous radio-telemetry data (Harris and Howland 2005) suggests that Travaillant Lake fish do not migrate out of the system, therefore rarely encountering their anadromous counterparts. The loss of anadromy in Travaillant Lake fish in this system that was colonized following deglaciation has led to increased fragmentation which may now be blurring patterns of IBD (see Castric and Bernatchez 2003 for a brook trout example). As genetic exchange is currently limited or absent between these two groups, populations drift or diverge independently from each other, thus disrupting the pattern of IBD observed in other North American populations (i.e., those from Alaska). Small isolated populations, such

as those in the Travaillant Lake system, should not conform to the IBD model, because they are unlikely to be equilibrium between genetic drift and gene flow (Johnson *et al.* 2003). Additionally since an IBD pattern is not evident in the Mackenzie River system, this may be indicative of historical patterns of random mating and on going gene flow among broad whitefish from different populations within the area that have recently recolonized. This is consistent with the lack genetic differentiation, low F_{ST} values and lack of variation explained among groups with AMOVA in the anadromous Mackenzie River broad whitefish. I contend that my data support 'nascent' population structure in Mackenzie River broad whitefish, consistent with its recent postglacial recolonization to the area in which there is still a high degree of gene flow (historical and possibly contemporary) limiting population structure and affecting equilibrium patterns of IBD. The nascent population structure in the Mackenzie River system was also evidence by significantly lower H_E, and A_r compared to that in Alaska. This combined with the highly fragmented Travaillant Lake populations is likely preventing patterns of IBD.

Historical Impacts on Microsatellite Variation - Zoogeographic Inferences

Although the utility of using microsatellites to assess phylogeography and post-glacial dispersal history has only recently come into light, there are now numerous studies that have shown these markers can be quite useful for such analyses (e.g., Angers and Bernatchez 1998; Hansen *et al.* 1999; Koskinen *et al.* 2002; Costello *et al.* 2003; Tonteri *et al.* 2005; Sonstebo *et al*. 2007). Even given the limited population structure within the anadromous populations of the Mackenzie River system, the microsatellite markers used

in this study have provided some relative insight into the phylogeography and post-glacial dispersal history of broad whitefish in North America. Given the current North American distribution of broad whitefish (Scott and Crossman 1998), the population structure resolved in this study and the geological history of the area, it is most likely that broad whitefish from North America survived in one refugium, the Beringian, and dispersed from there subsequent to the retreat of the glaciers approximately 12,000 – 10,000 years ago (Dyke and Prest 1987). Evidence for the single North American refugial origin is suggested by the current broad whitefish distribution, and by the clustering of all North American samples, low interpopulation divergence within North American samples (e.g., low F_{ST} values and lack of genetic differentiation), and, for the most part, the lack of unique alleles within North America populations. If extreme differentiation was noticed, especially over short geographic distances, this may have indicated survival in different ice-age refugia (Bernatchez and Wilson 1998; Koskinen *et al.* 2002), which was not observed in this study. Additionally, refugial areas can be identified either by a centre of increased genetic diversity (Cann *et al*. 1987) which was the case in this study in which samples from Alaska, those occupying past refugial areas, clearly had higher levels of genetic diversity, which declined eastward towards the Mackenzie River system (Figure 4).

Russian and North American populations were at one time likely continuous due to the existence Bering Land Bridge and subsequent freshwater habitats during glacial maxima (Lindsey and McPhail 1986). Therefore, current reproductive isolation has existed between these groups on opposite sides of the Bering Strait for a minimum of 12,000-

15,000 years, subsequent to the close of the land bridge (Lindsey and McPhail 1986; Pielou 1991), which probably explains the high levels of differentiation. Broad whitefish, however, likely originated in Eurasia, as evidenced by the results of this study (e.g., decline in genetic diversity as you move eastward and NJ analysis) and their three well established life histories and larger geographic distribution that persists within the Eurasian populations (Berg 1962). Politov *et al.* (2004) have also suggested that Siberia is the centre of origin for other coregonids with nearly identical distributions to broad whitefish, and that an eastward colonization route to Beringian Alaska would have been available subsequent to the opening of the Bering Land Bridge that connected the two continents during glacial maxima several times within the last 300,000 years (Pielou 1991, Lindsey and McPhail 1986). Broad whitefish may have also crossed part of the Bering Strait before the emergence of the land bridge since this species is tolerant to migrations in sea water as evidenced by an anadromous life history (Reist and Chang-Kue 1997). Even salt intolerant species had the potential to disperse this way when salinity decreased following massive freshwater discharges into marine environments from melting glaciers (Pielou 1991; Wilson and Hebert 1998).

Dispersal between Eurasia and Alaskan Beringia is supported by faunal resemblance index (FRI) analysis which shows that of 30 freshwater fish species, 23 are common to both sides of the present day Bering Sea (Lindsey and McPhail 1986). Additionally morphological evidence illustrates that some species occupying ranges on opposite sides of the Bering Strait may be more similar to each other morphologically even though they may show distinctive geographic variants elsewhere (see Lindsey and McPhail 1986 and

references therein for examples). Furthermore, as discussed previously, there is a wealth of genetic information that provides evidence of the close affinities, between Beringian populations of fishes those on the Eurasian side of the Bering Strait (Bernatchez and Dodson 1994; Brunner *et al*. 2001; Stamford and Taylor 2004) suggesting that there was historical gene flow between these groups of fishes.

In North America, as discussed earlier, it is possible that broad whitefish survived in two separate Beringian refugia; a north Beringian refuge, north of the Brooks Mountain Range and a south Beringian refuge, south of the Brooks Range. Genetic data, based on mtDNA, for Arctic grayling has been used to provide evidence of the potential for two distinct Beringian refugia (Stamford and Taylor 2004). These authors document two mtDNA haplotype groups that roughly coincide with a division north and south of the Brooks Range which is consistent with the idea that Arctic grayling survived glaciation in two isolated regions in Beringia (i.e. North and South Beringia). Lake whitefish have also shown meristic distinctions depending on which side of the Brooks Range the populations occur (Lindsey and MacPhail 1986). These differences may also be a result of two dispersal from Eurasia to North American Beringia; a northern and southern route, on opposite coasts of the Bering land bridge (Politov *et al.* 2004). My data do not conclusively show this, but there is some evidence suggesting the possibility of two separate refugia with Beringia. For example as shown in the NJ tree, Teshekpuk Lake clusters with the Pechora River, separate from other Alaskan populations (Figure 5), providing some evidence for the persistence in two Beringian refugia. Additionally, within Alaska, one unique allele was found in Teshekpuk Lake, but more generally there

was a lack of unique genetic variation on opposite sides of this mountain range. Furthermore, this unique allele found in the Teshekpuk Lake population may have persisted from founding populations, or may be a result of mutation. Although the scenario of the use of two distinct refugia may also accurately depict broad whitefish survival in Beringia during glacial times it was not distinctly apparent from my data. More thorough sampling should be conducted from populations north and south of the Brooks Range in order to fully resolve this issue.

Within North America, phylogenetic groupings of all populations coincided with their geographic origin. My results (e.g., NJ tree, Figure 5) suggest that Alaskan (Beringian) populations are ancestral to those populations from the Mackenzie River System. Within the Mackenzie system, it appears that populations of the Mackenzie River proper and major tributaries are ancestral to Travaillant Lake populations. Taken all together, my data suggest an eastward colonization from Eurasia, to Beringia, to the Mackenzie River system and then finally into Travaillant Lake. This is further indicated by the cline in genetic diversity moving eastward from the heart of the Beringian refuge suggesting that those in the Mackenzie River system are the youngest populations (Figure 4), and by the significant differences in H_E and A_r observed between the two areas. Furthermore, genetic diversity (H_E and A_r) in the Travaillant Lake system is significantly lower than that of the other Mackenzie system populations (see Chapter 3) suggesting that this was the most recent area colonized. The recent invasion into the Mackenzie System may also be indicated by their limited upstream distribution in the Mackenzie River, whereas they occur up to the headwaters of the Yukon River (Scott and Crossman 1998). Additionally,

allelic size distributions at some loci (e.g., Cocl-Lav27, Figure 8), clearly show trends in allele frequencies moving from the Pechora River to the Travaillant Lake system.

The question then becomes, how did broad whitefish recolonize the Mackenzie River system from Alaskan Beringia? Although three hypotheses were put forward regarding this post-glacial dispersal system (dispersal solely through freshwater, stepping-stone like dispersal through marine environments, or both), data in my study suggest recolonization of the Mackenzie River system from Beringia using marine environments. The neighbour-joining tree and the FCA both provide evidence that Yukon River populations are not more closely related to Mackenzie River populations in comparison to those from the North Slope of Alaska. For example, Teshekpuk Lake on the North Slope of Alaska, in the FCA, clusters more closely with Mackenzie River broad whitefish than to other Alaskan populations. This is consistent with the hypothesis of a marine dispersal from Beringia, in which broad whitefish colonized newly available habitat along the North Slope of Alaska in a stepping-stone-like fashion until eventually reaching the Mackenzie River system. Anadromous broad whitefish from the Mackenzie River system then eventually colonized the Travaillant Lake system where the only lacustrine population in this system has been confirmed (Harris and Howland 2005). If populations dispersed solely through freshwater, through any of the Yukon-Mackenzie headwater connections (see Lindsey and McPhail 1986) instead of along the Arctic coast, I predicted that populations from the Yukon River should be more closely related to populations in the Mackenzie system fish, and that they would be more closely related to the lacustrine Travaillant Lake populations (due to dispersal solely through freshwater). If Travaillant

Lake populations are intolerant of saline environments, although studies are needed to confirm this, then post-glacial recolonization to this system solely through freshwater seems plausible. Additionally, if both postglacial routes were used by broad whitefish, I would have expected to see large genetic discontinuities within the Mackenzie River system, similar to regions of secondary contact between different refugial races (e.g., Lu *et al.* 2001; Turgeon and Bernatchez 2001). These scenarios were clearly not the case based on my microsatellite analysis (Figures 5 and 6, Table 5).

My data argue that broad whitefish dispersed in a stepping-stone like fashion along the north slope of Alaska via the Arctic Ocean as the glaciers retreated, establishing in river systems along the way eventually and reaching the Mackenzie River system (Figure 1). Broad whitefish would have almost certainly followed glacial margins closely dispersing along the coast until reaching the Mackenzie roughly 14,000 ya (Lindsey and McPhail 1986). A stepping stone-like dispersal from Beringia eastward in marine waters of the Arctic coast has also been suggested for lake trout (Wilson and Hebert 1998), Arctic char (Wilson *et al.* 1996), Arctic grayling from a Northern Beringian refuge (Stamford and Taylor 2004) and likely applies to a number of coregonids species based on current distributions (e.g., Politov *et al.* 2004). For example, Arctic cisco (*C. autumnalis*) from the Coleville River system (near Teshekpuk Lake) on the North Slope of Alaska are genetically indistinguishable from Mackenzie River system populations (J.L. Nielsen, Alaska Science Center, US Geological Survey, Anchorage, AK, personal communication). The Coleville River is one of the first major river systems to the west of the Mackenzie River system on the North Slope of Alaska and my data, along with the

Arctic cisco data, argue that these populations have not been isolated in separate river systems for long periods of time or that gene flow between these systems is still quite high. Furthermore, coastal dispersal, rather than inland, seems extremely plausible for anadromous species of fish such as broad whitefish that are tolerant to saline environments.

The contribution of the Beringian refuge fish to the colonization of the Mackenzie River system apparently varies considerably among various fish species. For example, lake whitefish that survived in Beringia did not disperse to the Mackenzie system, and it was determined that this area was almost exclusively recolonized with Mississippian refuge lake whitefish (Bernatchez and Dodson 1991). Margaret Lake, however as discussed by Lindsey and McPhail (1986), once created a headwater connection between the Porcupine River (Yukon River drainage) and Peel River (Mackenzie River drainage) and this lake contains relict Beringian lake whitefish. Although it is currently not connected to any tributaries of the Mackenzie River, it is not inconceivable that Beringian lake whitefish could have colonized the Mackenzie River via this connection, but more likely this lake was inhabited by these fish after the Peel River resumed flow to the Beaufort Sea roughly 12,500 ya (Lindsey and McPhail 1986). By contrast, other species have been able to successfully disperse extensively from Beringia. Arctic grayling for example, have dispersed far south and east from Beringia following the retreat of the glaciers (Stamford and Taylor 2004). The majority of the current geographic range of lake trout in North America, approximately two-thirds, is thought to result from recolonization from the Beringian refuge (Wilson and Hebert 1998). The current distribution of broad

whitefish argues for a Beringian refuge exclusively, meaning there has been limited dispersal from this refuge, in comparison to other species such as lake trout. This may be due to a later colonization of Beringia from Eurasian populations, and hence a later dispersal opportunity form Beringia to the east. Additionally, a waterfall on the Mackenzie River that existed from approximately 11 500 to 6100 ya (Lindsey and McPhail 1986) or specific thermal preferences (McPhail and Lindsey 1970) may have prevented extensive upstream dispersal in the Mackenzie River system.

Conclusion

Molecular data included in this chapter of my thesis provide the first comprehensive study to evaluate how historical processes have influenced genetic variation within broad whitefish, but more specifically within North American populations of this species. My results provided strong evidence that North American broad whitefish represent a species of a single glacial lineage surviving solely within Beringia. Subsequent to Pleistocene glacial events, it appears that broad whitefish recolonized newly available habitat in an eastward direction from Beringia, via marine environments, until eventually reaching the current periphery of their range in the Mackenzie River system. This was suggested by several lines of evidence including allele frequency distributions, a decline in genetic diversity with increasing distance from Beringia, isolation-by-distance analysis, and results of the NJ tree and FCA. Furthermore, the results in this chapter also illustrate how postglacial range expansion has influenced genetic diversity within and among populations of this species, especially those at the periphery of their range. Founding events, and subsequent drift, associated with a stepping-stone like pattern of dispersal

from glacial refugia, have contributed the most to the extant genetic variation observed in the current study. Although populations at the heart of their range appear to be in migration-drift equilibrium, those at the periphery of the range are far from equilibrium between genetic drift and gene flow, which is consistent with populations in a newly inhabited area. In this chapter, my analysis of microsatellite markers did indeed provide new insights into issues related to the genetic population structure of North American broad whitefish, certainly in terms of historical impacts on genetic variation, however studying fine-scale genetic population structure in this species will provide further insights the evolutionary forces that are continuing to shape and maintain this genetic variation. I do this in chapter three of my thesis where I examine, on a finer scale, the genetic population structure of this species at the periphery of their range in the Mackenzie River system, Northwest Territories.

Table 1. Broad whitefish sampling locations and sample sizes (N).

REGION/SAMPLING LOCATION	LATITUDE	LONGITUDE	N
RUSSIA			
Pechora River	66°53'	53°47'	36
ALASKA			
Yukon River (1999)	65°34'	151°06'	30
Yukon River (2004)	65°11'	151°58'	50
Tanana River	64°45'	149°56'	12
Selawik River	66°57'	160°21'	30
Whitefish Lake	61°24'	160°01'	30
Teshekpuk Lake	70°45'	153°57'	32
MACKENZIE RIVER SYSTEM			
Peel River			
Scrapper Hill	67°15	134°53	99
F. Koe Camp	67°27'	134°52	30
Road River	66°52'	135°00'	60
Arctic Red River	66°58'	133°16'	35
Mackenzie River Delta			
East Channel - Inuvik (2003)	68°20'	133°24'	33
East Channel - Inuvik (2004)	68°20'	133°24'	11
East Channel - W. Simon Camp	68°14'	133°49'	17
West Channel - Destructon City (2004)	67°44'	135°22'	20
West Channel - Destructon City (2005)	67°44'	135°22'	19
West Channel - Rat River (2004)	67°45'	135°07'	25
West Channel - Rat River (2005)	67°45'	135°07'	32
West Channel - E. Lennie Samples	68°28'	135°34'	5
C. Allen Camp	68°40'	134°20'	30
B. Day Camp	68°18'	134°12'	30
Napoiak Channel	68°35'	134°53'	11
J.Firth Samples	68°13'	134°31'	30
J. Maring Samples	68°06'	134°30'	30
E. Lennie Camp	68°29'	134°33'	30
Bassook	67°44'	134°38'	27
Cuttoff	67°38'	134°38'	28
Point Seperation	67°35'	134°04'	23
Campbell Lake	68°12'	133°27'	29
Tuktoyuktuk Harbour	69°22'	133°38'	95
Shingle Point	69°00'	137°024'	5

Table 1. Continued.

REGION/SAMPLING LOCATION	LATITUDE	LONGITUDE	N
Mackenzie River Proper			
Mouth of Arctic Red River	67°26'	133°44'	12
Tree River	67°26'	132°31'	16
Mouth of Pierre Creek	67°19'	133°20'	9
Fort Goodhope	66°39'	129°25'	34
TRAVAILLANT LAKE SYSTEM			
Travaillant Lake (2003)	67°40'	131°53'	21
Travaillant Lake (2004)	67°40'	131°53'	20
Travaillant Lake (2005)	67°40'	131°53'	32
Travaillant River South (2003)	67°36'	131°51'	30
Travaillant River South (2004)	67°36'	131°51'	20
Travaillant River South (2005)	67°36'	131°51'	19
Travaillant River North (2004)	67°45'	131°51'	35
Travaillant River North (2005)	67°45'	131°51'	30
Andrew Lake	67°35'	131°53'	5

Table 2. Seven microsatellite loci used in this study, including references, annealing temperature used during polymerase chain reactions (Ta), and the size range of alleles in base pairs.

Locus	Source Species	Reference	Ta (^{o}C)	Size Range (bp)
Cocl-Lav4	*Coregonus clupeaformis*	Rogers *et al.* 2004	57	129-159
Cocl-Lav6	*Coregonus clupeaformis*	Rogers *et al.* 2004	60	125-151
Cocl-Lav8	*Coregonus clupeaformis*	Rogers *et al.* 2004	55	212-252
Cocl-Lav10	*Coregonus clupeaformis*	Rogers *et al.* 2004	57	249-271
Cocl-Lav18	*Coregonus clupeaformis*	Rogers *et al.* 2004	57	136-174
Cocl-Lav27	*Coregonus clupeaformis*	Rogers *et al.* 2004	55	176-206
Ots 103	*Oncorhynchus tshawytscha*	Small *et al.* 1998	58	75-111

Table 3. Individual and average values for the expected and observed heterozygosities (H_E and H_O) and the number of alleles per locus (N_A) for each locus.

Locus	H_E	H_O	N_A
Cocl-Lav4	0.65	0.58	11
Cocl-Lav6	0.57	0.49	12
Cocl-Lav8	0.66	0.61	18
Cocl-Lav10	0.60	0.53	9
Cocl-Lav18	0.55	0.52	17
Cocl-Lav27	0.47	0.41	4
Ots 103	0.74	0.67	11
Average	0.61	0.54	11.71

Table 4. Summary of allelic variation at seven microsatellite loci for all broad whitefish sampling locations. Number of alleles per locus (A), expected heterozygosity (H_E), observed heterozygosity (H_O), allelic richness (A_R) and number of genotyped individuals are listed. Sample locations italicized refer to those assayed in chapter 2. Those values of H_E and H_O that were significantly different from tests of Hardy-Weinberg Equilibrium are underlined.

Peel River - Scrapper Hill	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	5.00	4.00	6.00	8.00	8.00	3.00	8.00	6.00
H_E	0.68	0.51	0.60	0.64	0.48	0.49	0.66	0.58
H_O	0.60	0.49	0.62	0.60	0.45	0.50	0.56	0.54
A_R	4.35	3.20	3.50	4.43	3.77	2.11	4.93	3.75
N	85.00	88.00	87.00	84.00	86.00	86.00	88.00	86.29

Peel River - Road River	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	6.00	5.00	5.00	7.00	5.00	2.00	7.00	5.29
H_E	0.64	0.55	0.61	0.61	0.52	0.47	0.59	0.57
H_O	0.60	0.53	0.55	0.55	0.46	0.41	0.63	0.53
A_R	3.92	3.62	3.95	4.34	3.38	2.00	4.49	3.67
N	58.00	58.00	58.00	55.00	56.00	56.00	56.00	56.71

Peel River - F. Koe Camp	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	5.00	4.00	4.00	5.00	4.00	2.00	5.00	4.14
H_E	0.59	0.61	0.64	0.53	0.51	0.44	0.72	0.58
H_O	0.59	0.66	0.56	0.65	0.46	0.33	0.57	0.55
A_R	3.77	3.79	3.82	3.50	3.23	2.00	4.23	3.48
N	29.00	25.00	26.00	26.00	28.00	27.00	28.00	27.00

All Peel River Samples	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	6.00	5.00	6.00	9.00	9.00	3.00	9.00	6.71
H_E	0.65	0.54	0.61	0.61	0.50	0.47	0.65	0.58
H_O	0.60	0.53	0.59	0.59	0.46	0.44	0.58	0.54
A_R	4.19	3.45	3.76	4.26	3.58	2.05	4.69	3.71
N	172.00	175.00	170.00	164.00	170.00	169.00	172.00	170.29

Arctic Red River	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	6.00	5.00	4.00	10.00	3.00	2.00	6.00	5.14
H_E	0.52	0.46	0.57	0.70	0.51	0.46	0.63	0.55
H_O	0.56	0.58	0.63	0.70	0.69	0.50	0.71	0.62
A_R	3.60	3.02	3.03	5.78	2.82	2.00	4.54	3.54
N	34.00	33.00	30.00	33.00	32.00	32.00	31.00	32.14

Point Separation	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	5.00	3.00	4.00	8.00	3.00	2.00	6.00	4.43
H_E	0.59	0.48	0.53	0.74	0.54	0.32	0.77	0.57
H_O	0.39	0.48	0.48	0.45	0.55	0.30	0.74	0.48
A_R	3.81	2.95	3.56	5.41	2.97	1.99	5.05	3.68
N	23.00	23.00	23.00	22.00	22.00	23.00	23.00	22.71

Table 4. Continued.

Mackenzie River - FGH	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	5.00	6.00	4.00	7.00	4.00	2.00	8.00	5.14
H_E	0.60	0.53	0.54	0.59	0.48	0.38	0.78	0.56
H_O	0.65	0.56	0.65	0.56	0.44	0.26	0.85	0.57
A_R	3.85	3.98	3.22	3.72	3.18	2.00	5.61	3.65
N	34.00	34.00	34.00	34.00	34.00	34.00	34.00	34.00

Tuktoyuktuk Harbour	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	5.00	5.00	6.00	8.00	4.00	2.00	6.00	5.14
H_E	0.55	0.40	0.46	0.58	0.48	0.44	0.34	0.46
H_O	0.64	0.38	0.32	0.80	0.62	0.51	0.25	0.50
A_R	3.26	2.95	3.01	3.23	2.92	2.00	3.32	2.95
N	89.00	91.00	87.00	84.00	91.00	92.00	91.00	89.29

Mackenzie Delta - C. Allen	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	4.00	4.00	5.00	7.00	5.00	2.00	7.00	4.86
H_E	0.53	0.44	0.64	0.68	0.47	0.39	0.73	0.55
H_O	0.67	0.34	0.76	0.55	0.50	0.38	0.77	0.57
A_R	3.55	3.20	3.90	5.16	3.66	2.00	5.22	3.81
N	30.00	29.00	29.00	29.00	30.00	29.00	30.00	29.43

Mackenzie Delta - B. Day	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	6.00	5.00	5.00	7.00	4.00	2.00	7.00	5.14
H_E	0.70	0.52	0.57	0.70	0.39	0.46	0.71	0.58
H_O	0.63	0.57	0.53	0.73	0.43	0.23	0.73	0.55
A_R	4.67	3.72	3.72	3.60	3.10	2.00	5.00	3.69
N	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00

Mackenzie River - Mouth of ARR	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	3.00	3.00	3.00	3.00	3.00	2.00	4.00	3.00
H_E	0.43	0.35	0.59	0.54	0.48	0.48	0.60	0.50
H_O	0.50	0.40	0.30	0.56	0.50	0.50	0.30	0.44
A_R	3.00	2.90	3.00	3.00	3.00	2.00	3.80	2.96
N	10.00	10.00	10.00	9.00	10.00	10.00	10.00	9.86

Mackenzie River - Tree River	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	4.00	3.00	4.00	8.00	3.00	2.00	6.00	4.29
H_E	0.63	0.42	0.50	0.75	0.41	0.48	0.61	0.54
H_O	0.81	0.50	0.44	0.69	0.44	0.50	0.69	0.58
A_R	3.90	2.92	3.13	6.50	2.81	2.00	4.76	3.72
N	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00

Table 4. Continued.

Mackenzie Delta - J. Firth	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	6.00	4.00	3.00	7.00	4.00	2.00	8.00	4.86
H_E	0.57	0.31	0.53	0.60	0.60	0.33	0.71	0.52
H_O	0.70	0.25	0.60	0.60	0.55	0.40	0.80	0.56
A_R	4.19	2.86	2.70	4.64	3.44	2.00	5.99	3.69
N	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00

Mackenzie Delta - J. Maring	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	5.00	4.00	5.00	6.00	3.00	2.00	7.00	4.57
H_E	0.66	0.53	0.56	0.56	0.51	0.24	0.68	0.53
H_O	0.73	0.57	0.77	0.63	0.63	0.20	0.67	0.60
A_R	3.99	3.76	3.33	3.67	2.95	1.95	5.25	3.56
N	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00

Mackenzie Delta - Bassook	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	4.00	3.00	4.00	6.00	3.00	2.00	4.00	3.71
H_E	0.65	0.31	0.57	0.64	0.54	0.34	0.59	0.52
H_O	0.81	0.36	0.50	0.75	0.69	0.42	0.46	0.57
A_R	3.69	2.34	3.07	3.07	2.97	2.00	3.32	2.92
N	26.00	25.00	22.00	24.00	26.00	26.00	26.00	25.00

Mackenzie Delta - Cuttoff	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	5.00	4.00	6.00	5.00	4.00	3.00	5.00	4.57
H_E	0.52	0.57	0.67	0.62	0.48	0.53	0.61	0.57
H_O	0.56	0.54	0.64	0.54	0.43	0.61	0.67	0.57
A_R	3.85	3.61	4.48	3.88	3.18	2.54	4.15	3.67
N	27.00	28.00	28.00	26.00	28.00	28.00	27.00	27.43

Mackenzie Delta - E. Lennie	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	4.00	4.00	6.00	7.00	5.00	2.00	3.00	4.43
H_E	0.61	0.25	0.43	0.65	0.51	0.48	0.48	0.49
H_O	0.92	0.19	0.14	0.73	0.68	0.67	0.41	0.53
A_R	3.41	2.75	4.09	4.54	3.41	2.00	2.78	3.28
N	26.00	26.00	28.00	22.00	28.00	27.00	29.00	26.57

Mackenzie Delta - Napoiak	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	4.00	5.00	4.00	5.00	3.00	2.00	5.00	4.00
H_E	0.58	0.61	0.65	0.68	0.47	0.48	0.62	0.58
H_O	0.55	0.64	0.40	0.64	0.60	0.36	0.60	0.54
A_R	3.97	4.61	3.90	4.61	2.90	2.00	4.80	3.83
N	11.00	11.00	10.00	11.00	10.00	11.00	10.00	10.57

Mackenzie Delta - WstDC04	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	5.00	3.00	4.00	6.00	4.00	2.00	6.00	4.29
H_E	0.64	0.50	0.58	0.68	0.53	0.38	0.62	0.56
H_O	0.60	0.55	0.45	0.50	0.35	0.20	0.40	0.44
A_R	4.14	2.96	3.15	4.70	3.37	2.00	4.92	3.61
N	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00

Table 4. Continued.

Mackenzie Delta - WstDC05	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	4.00	4.00	4.00	5.00	3.00	2.00	6.00	4.00
H_E	0.52	0.54	0.62	0.44	0.53	0.50	0.73	0.56
H_O	0.53	0.42	0.58	0.35	0.61	0.50	0.68	0.52
A_R	3.41	3.34	3.41	3.58	2.95	2.00	4.90	3.37
N	19.00	19.00	19.00	17.00	18.00	18.00	19.00	18.43

Mackenzie Delta - WstBW04	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	5.00	4.00	4.00	6.00	4.00	2.00	7.00	4.57
H_E	0.56	0.35	0.49	0.59	0.58	0.43	0.73	0.53
H_O	0.64	0.32	0.48	0.46	0.60	0.52	0.80	0.55
A_R	3.47	3.11	3.34	4.22	3.34	2.00	5.73	3.60
N	25.00	25.00	25.00	24.00	25.00	25.00	25.00	24.86

Mackenzie Delta - WstBW05	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	5.00	3.00	4.00	6.00	3.00	2.00	7.00	4.29
H_E	0.55	0.48	0.53	0.60	0.42	0.51	0.76	0.55
H_O	0.66	0.44	0.56	0.68	0.32	0.40	0.61	0.52
A_R	3.66	2.93	3.27	3.95	2.32	2.00	5.62	3.39
N	32.00	32.00	32.00	28.00	31.00	30.00	31.00	30.86

Mackenzie Delta - EstChInvk03	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	5.00	4.00	6.00	7.00	3.00	4.00	7.00	5.14
H_E	0.65	0.53	0.63	0.59	0.41	0.50	0.65	0.57
H_O	0.77	0.57	0.57	0.50	0.40	0.60	0.61	0.58
A_R	3.91	3.60	4.10	3.93	2.87	2.60	4.88	3.70
N	31.00	28.00	28.00	30.00	30.00	30.00	31.00	29.71

Mackenzie Delta - EstChInvk04	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	4.00	3.00	2.00	3.00	3.00	2.00	4.00	3.00
H_E	0.52	0.44	0.51	0.54	0.43	0.52	0.57	0.50
H_O	0.45	0.36	0.45	0.22	0.50	0.90	0.36	0.47
A_R	3.81	2.82	2.00	3.00	3.00	2.00	3.64	2.89
N	11.00	11.00	11.00	9.00	10.00	11.00	11.00	10.57

Mackenzie Delta - EstChWS	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	4.00	4.00	5.00	6.00	3.00	2.00	4.00	4.00
H_E	0.56	0.30	0.56	0.73	0.44	0.31	0.72	0.52
H_O	0.67	0.33	0.56	0.53	0.35	0.38	0.75	0.51
A_R	3.58	3.15	3.69	4.84	2.95	2.00	3.93	3.45
N	15.00	15.00	16.00	17.00	17.00	16.00	16.00	16.00

Campbell Lake	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	5.00	7.00	5.00	4.00	5.00	2.00	5.00	4.71
H_E	0.67	0.55	0.59	0.61	0.49	0.48	0.70	0.58
H_O	0.56	0.57	0.31	0.56	0.54	0.68	0.77	0.57
A_R	4.05	4.15	3.42	3.44	3.54	2.00	4.45	3.58
N	25.00	28.00	26.00	25.00	28.00	28.00	26.00	26.57

Table 4. Continued.

Travaillant Lake 2003	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	3.00	3.00	4.00	5.00	3.00	2.00	5.00	3.57
H_E	0.58	0.26	0.49	0.71	0.50	0.40	0.66	0.51
H_O	0.57	0.24	0.45	0.86	0.40	0.33	0.55	0.49
A_R	2.99	2.59	3.60	4.53	2.70	2.00	4.33	3.25
N	21.00	21.00	20.00	21.00	20.00	21.00	20.00	20.57

Travaillant Lake 2004	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	4.00	3.00	5.00	7.00	4.00	2.00	5.00	4.29
H_E	0.59	0.19	0.49	0.65	0.34	0.30	0.66	0.46
H_O	0.55	0.20	0.55	0.60	0.30	0.35	0.55	0.44
A_R	3.80	2.29	4.10	4.72	2.88	1.99	3.88	3.38
N	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00

Travaillant Lake 2005	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	3.00	3.00	5.00	4.00	3.00	2.00	6.00	3.71
H_E	0.65	0.12	0.61	0.53	0.38	0.22	0.62	0.45
H_O	0.63	0.09	0.67	0.61	0.46	0.25	0.72	0.49
A_R	2.99	1.92	3.91	2.79	2.54	1.94	3.90	2.86
N	32.00	32.00	30.00	31.00	28.00	32.00	29.00	30.57

Travaillant River South 2003	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	3.00	3.00	4.00	5.00	3.00	3.00	5.00	3.71
H_E	0.64	0.10	0.58	0.58	0.47	0.19	0.54	0.44
H_O	0.67	0.10	0.70	0.60	0.37	0.20	0.57	0.46
A_R	2.98	1.81	3.44	3.66	2.89	2.14	3.63	2.94
N	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00

Travaillant River South 2004	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	3.00	3.00	4.00	4.00	3.00	2.00	6.00	3.57
H_E	0.51	0.14	0.63	0.54	0.48	0.27	0.67	0.46
H_O	0.45	0.15	0.65	0.45	0.50	0.32	0.55	0.44
A_R	2.70	2.15	3.80	3.29	2.45	1.99	4.45	2.98
N	20.00	20.00	20.00	20.00	20.00	19.00	20.00	19.86

Travaillant River South 2005	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	3.00	4.00	5.00	5.00	4.00	2.00	4.00	3.86
H_E	0.52	0.20	0.52	0.55	0.50	0.23	0.53	0.44
H_O	0.47	0.11	0.47	0.50	0.44	0.26	0.68	0.42
A_R	2.97	2.68	3.68	3.50	3.68	1.97	3.66	3.16
N	19.00	19.00	19.00	19.00	18.00	19.00	19.00	18.86

Travaillant River North 2004	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	3.00	4.00	6.00	6.00	3.00	2.00	5.00	4.14
H_E	0.59	0.14	0.63	0.56	0.45	0.41	0.68	0.49
H_O	0.46	0.11	0.69	0.60	0.49	0.34	0.71	0.49
A_R	2.96	2.11	3.90	3.71	2.78	2.00	3.89	3.05
N	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00

Table 4. Continued.

Travaillant River North 2005	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	4.00	3.00	4.00	4.00	3.00	2.00	5.00	3.57
H_E	0.65	0.10	0.61	0.57	0.46	0.31	0.60	0.47
H_O	0.77	0.10	0.57	0.68	0.47	0.38	0.57	0.50
A_R	3.63	1.81	3.63	3.09	2.77	1.99	3.48	2.91
N	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00

All Travaillant System Samples	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	4.00	6.00	7.00	11.00	5.00	3.00	8.00	6.29
H_E	0.61	0.15	0.59	0.58	0.45	0.29	0.62	0.47
H_O	0.58	0.14	0.61	0.61	0.43	0.30	0.63	0.47
A_R	3.25	2.09	3.76	3.73	2.82	2.02	3.86	3.07
N	212.00	212.00	209.00	208.00	206.00	210.00	207.00	209.14

Yukon River	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	4.00	5.00	4.00	9.00	8.00	2.00	9.00	5.86
H_E	0.67	0.66	0.58	0.74	0.60	0.50	0.81	0.65
H_O	0.65	0.69	0.51	0.63	0.61	0.47	0.88	0.64
A_R	3.76	4.25	2.95	5.21	4.36	2.00	5.89	4.06
N	49.00	49.00	49.00	49.00	49.00	49.00	49.00	49.00

Yukon River - Ramparts	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	5.00	6.00	3.00	10.00	8.00	2.00	7.00	5.86
H_E	0.66	0.62	0.54	0.79	0.73	0.50	0.80	0.66
H_O	0.76	0.62	0.36	0.66	0.66	0.61	0.66	0.62
A_R	3.95	4.75	2.54	5.83	5.90	2.00	5.50	4.35
N	29.00	29.00	28.00	29.00	29.00	28.00	29.00	28.71

Whitefish Lake	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	7.00	5.00	3.00	7.00	5.00	2.00	8.00	5.29
H_E	0.64	0.76	0.34	0.56	0.64	0.48	0.82	0.61
H_O	0.60	0.80	0.37	0.63	0.67	0.43	0.83	0.62
A_R	4.17	4.48	2.49	4.39	3.79	2.00	6.34	3.95
N	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00

Teshekpuk Lake	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	5.00	5.00	4.00	7.00	5.00	2.00	5.00	4.71
H_E	0.56	0.61	0.52	0.57	0.56	0.49	0.65	0.57
H_O	0.69	0.63	0.63	0.60	0.63	0.37	0.66	0.60
A_R	3.05	3.87	2.97	4.54	3.46	2.00	4.18	3.44
N	32.00	32.00	32.00	32.00	32.00	30.00	32.00	31.71

Tanana River	Lav4	Lav6	Lav10	Lav8	Lav18	Lav27	Ots103	Average
A	3.00	4.00	4.00	5.00	5.00	2.00	6.00	4.14
H_E	0.60	0.63	0.63	0.69	0.71	0.47	0.78	0.65
H_O	0.89	0.67	0.56	0.44	0.56	0.22	0.75	0.58
A_R	3.00	4.00	4.00	5.00	5.00	2.00	5.45	4.06
N	9.00	9.00	9.00	9.00	9.00	9.00	12.00	9.43

Table 4. Continued.

Selawik River	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	4.00	7.00	4.00	6.00	5.00	2.00	6.00	4.86
H_E	0.65	0.73	0.58	0.75	0.42	0.44	0.80	0.62
H_O	0.47	0.67	0.60	0.67	0.50	0.43	0.73	0.58
A_R	3.48	4.94	3.07	4.82	3.54	2.00	5.32	3.88
N	30.00	30.00	30.00	27.00	30.00	30.00	30.00	29.57

Pechora River	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	3.00	4.00	2.00	12.00	3.00	2.00	3.00	4.14
H_E	0.38	0.16	0.03	0.84	0.63	0.33	0.51	0.41
H_O	0.33	0.33	0.08	0.92	0.42	0.58	0.58	0.46
A_R	2.46	2.19	1.30	7.08	2.99	1.99	2.25	2.89
N	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00

Mackenzie System Only	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	11.00	11.00	9.00	21.00	15.00	5.00	11.00	11.86
H_E	0.61	0.42	0.60	0.62	0.48	0.42	0.66	0.54
H_O	0.62	0.40	0.54	0.61	0.49	0.42	0.60	0.53
A_R	8.96	8.92	8.98	16.00	11.00	4.97	9.00	9.69
N	990.00	993.00	975.00	955.00	984.00	986.00	987.00	981.43

All Locations	**Lav4**	**Lav6**	**Lav10**	**Lav8**	**Lav18**	**Lav27**	**Ots103**	**Average**
A	11.00	12.00	9.00	21.00	17.00	5.00	11.00	12.29
H_E	0.63	0.50	0.60	0.65	0.51	0.45	0.70	0.58
H_O	0.62	0.44	0.52	0.62	0.51	0.42	0.62	0.54
A_R	10.90	11.93	8.99	21.00	16.94	4.97	10.97	12.24
N	1208.00	1213.00	1188.00	1170.00	1204.00	1203.00	1210.00	1199.43

Table 5. Above diagonal: log-likelihood exact tests for genetic differentiation among pairs of populations. Non-significant (NS) and significant (*) at the 5% nominal level following Bonferroni corrections. Below diagonal: pairwise F_{ST} (θ) comparisons among all pairs of populations. Underlined values represent comparisons that are not significant (P = 0.05) based on the permutation process. 1 = Peel River, 2 = Arctic Red River, 3 = Point Separation, 4 = Mackenzie River at Fort Good Hope, 5 = Campbell Lake, 6 = Travaillant Lake South, 7 = Travaillant Lake North, 8 = Yukon River, 9 = Yukon River at Ramparts, 10 = Whitefish Lake, 11 =Teshekpuk Lake, 12 = Tanana River, 13 = Selawik River and 14 = Pechora River.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	-	NS	NS	NS	NS	*	*	*	*	*	*	NS	*	*
2	0.0090	-	NS	NS	NS	*	*	*	*	*	*	NS	*	*
3	0.0187	0.0365	-	NS	NS	*	*	NS	*	*	*	NS	*	*
4	0.0052	0.0200	0.0002	-	NS	*	*	*	*	*	*	NS	*	*
5	0.0024	0.0058	0.0255	0.0112	-	*	*	*	*	*	*	NS	*	*
6	0.0418	0.0430	0.0487	0.0339	0.0602	-	NS	*	*	*	*	**	**	*
7	0.0376	0.0306	0.0490	0.0302	0.0393	0.0051	-	*	*	*	*	**	*	*
8	0.0285	0.0354	0.0344	0.0333	0.0185	0.1063	0.0767	-	NS	*	*	NS	NS	*
9	0.0524	0.0521	0.0502	0.0548	0.0375	0.1238	0.0875	-0.0035	-	*	*	NS	*	*
10	0.0687	0.0527	0.0978	0.0783	0.0429	0.1633	0.1334	0.0453	0.0615	-	*	NS	*	*
11	0.0327	0.0252	0.0725	0.0622	0.0152	0.0994	0.0820	0.0405	0.0515	0.0344	-	NS	*	*
12	0.0246	0.0454	0.0468	0.0508	0.0090	0.1365	0.1026	-0.0055	-0.0052	0.0457	0.0097	NS	NS	*
13	0.0507	0.0625	0.0840	0.0734	0.0363	0.1603	0.1215	0.0117	0.0209	0.0634	0.0464	-0.0063	-	*
14	0.3479	0.3731	0.4137	0.3876	0.3473	0.4542	0.4058	0.2939	0.2877	0.3136	0.3404	0.3319	0.3194	-

Table 6. Results of the analysis of molecular variance (AMOVA), showing the grouping hypotheses tested in this study. Variation among groups (Va), variation among populations within groups (Vb) and variation within populations (Vc).

Grouping Hypothesis	Va	Vb	Vc
1. North America vs. Russia	30.6 ***	3.3 ***	66.1 ***
2. Mackenzie System (Including Travaillant Lake) vs. Alaska	3.8 ***	2.7 ***	93.4 ***
3. Mackenzie System (Including Travaillant Lake) vs. Alaska vs. Russia	13.4 ***	3.2 ***	83.4 ***
4. Mackenzie System vs. Travaillant System vs. Alaska vs. Russia	9.8 ***	1.8 ***	88.6 ***
5. Mackenzie System vs. Travaillant Lake System	3.1 ***	0.9 ***	96.0 ***
6. Travaillant Lake System - 2003 vs.2004 vs. 2005	0.4	0.4	99.2 *

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

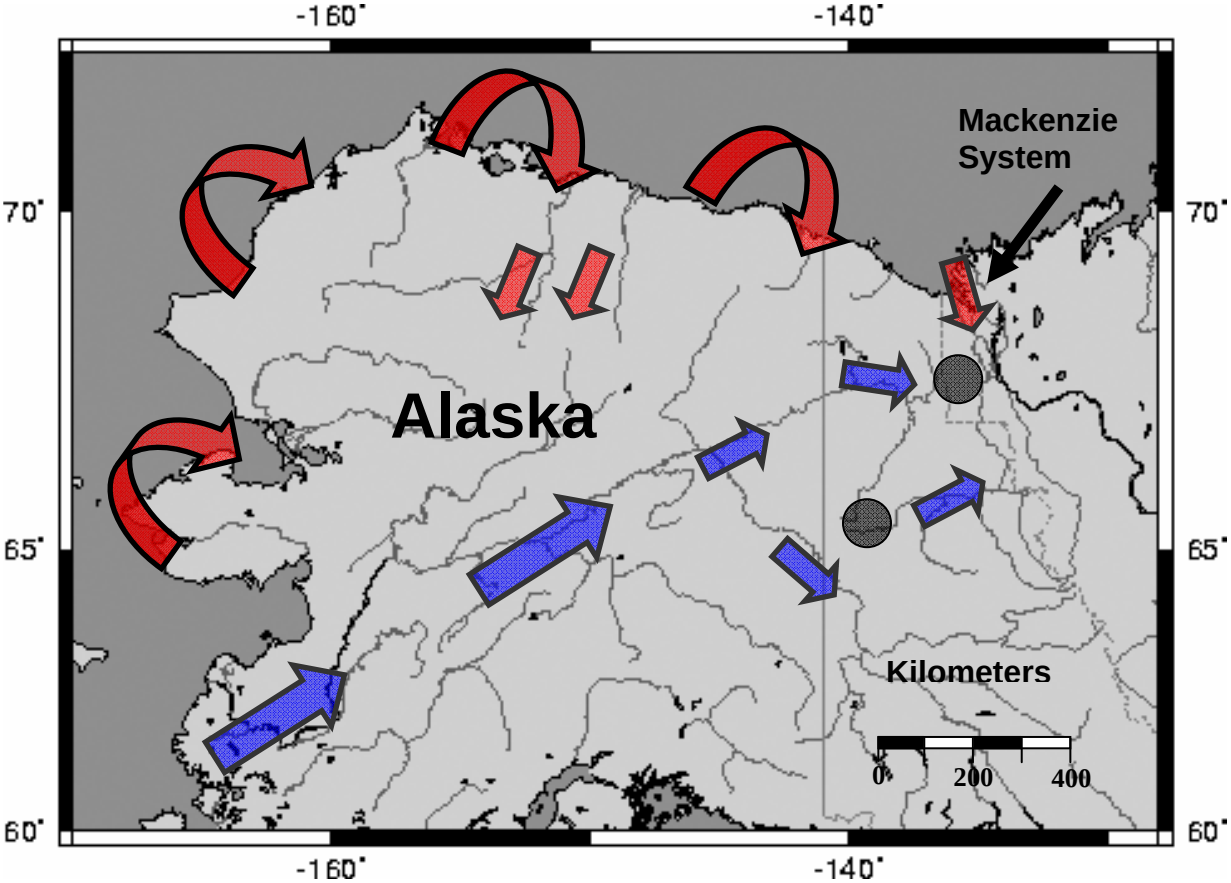

Figure 1. Map showing the two potential dispersal routes from Beringian North America to the Mackenzie River system. Red arrows show dispersal via marine environments and blue arrows represent a solely freshwater dispersal. The black circles indicate areas where there were, at one time, connections between the Yukon and Mackenzie river systems.

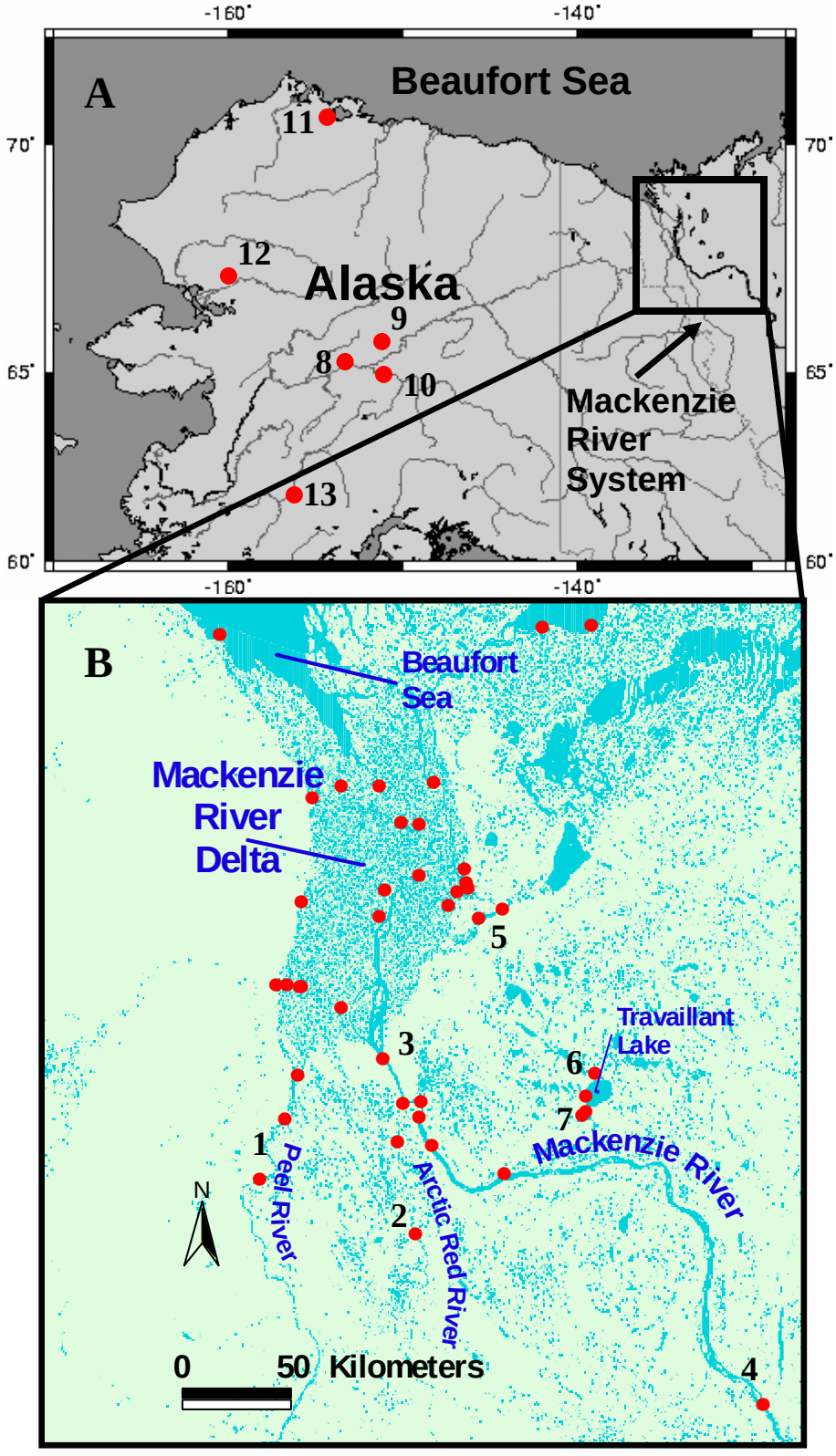

Figure 2. Map showing sampling locations for throughout Alaska (A) and the Mackenzie River system (B). Numbers correspond to populations outlined in Table 5.

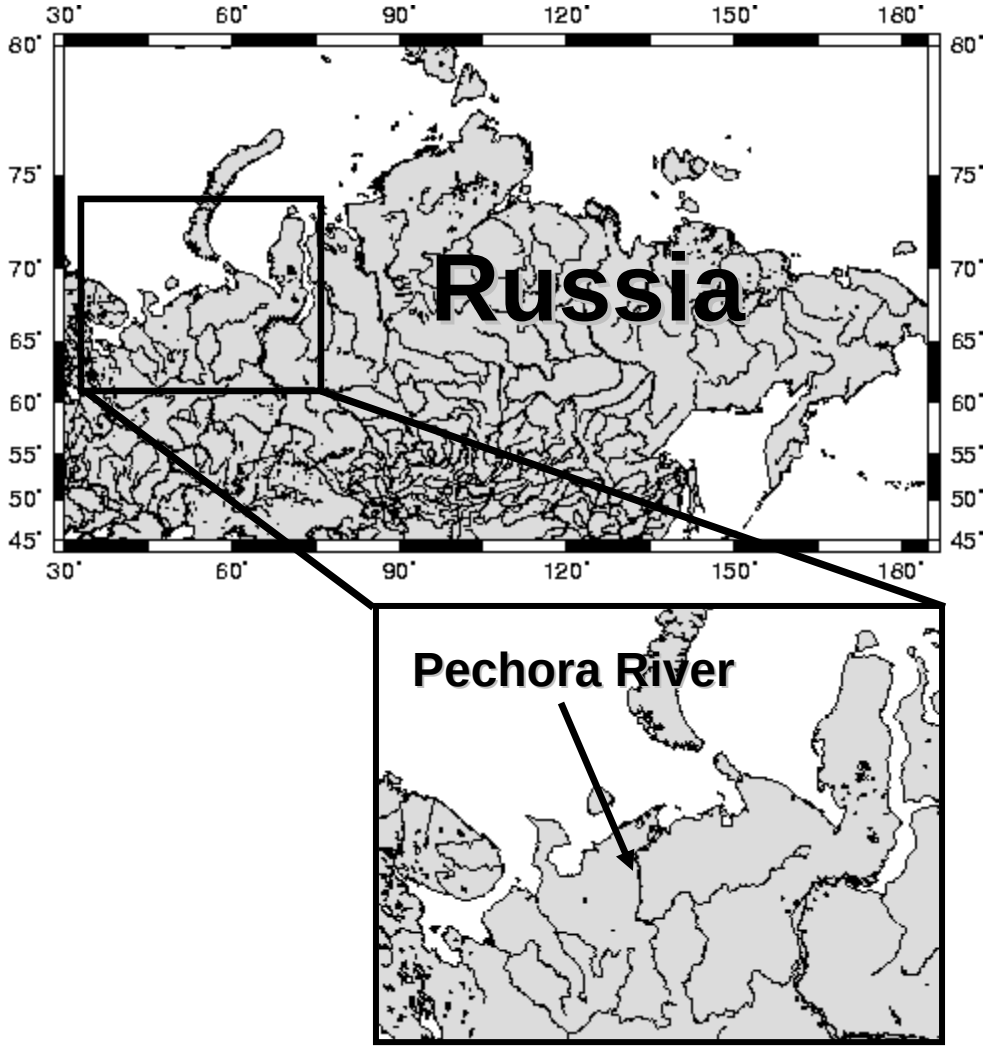

Figure 3. Map showing the Pechora River, Russia sampling location.

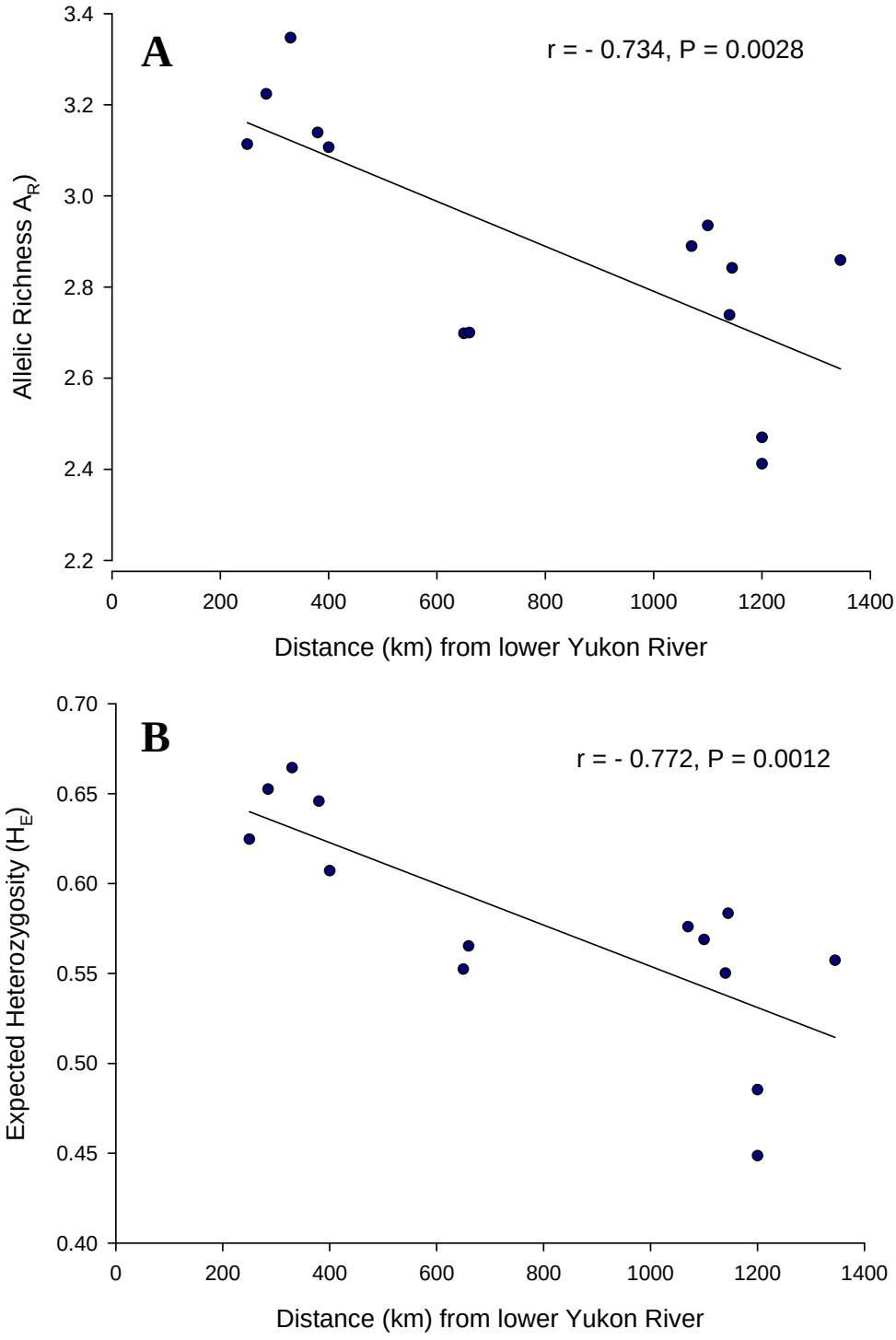

Figure 4. Change in microsatellite genetic diversity within broad whitefish populations vs. their geographical distance from the lower Yukon River, at the confluence with the Koyukuk River. (A) Allelic richness (A_R) vs. geographical distance (km); (B) Expected heterozygosity (H_E) vs. geographical distance (km).

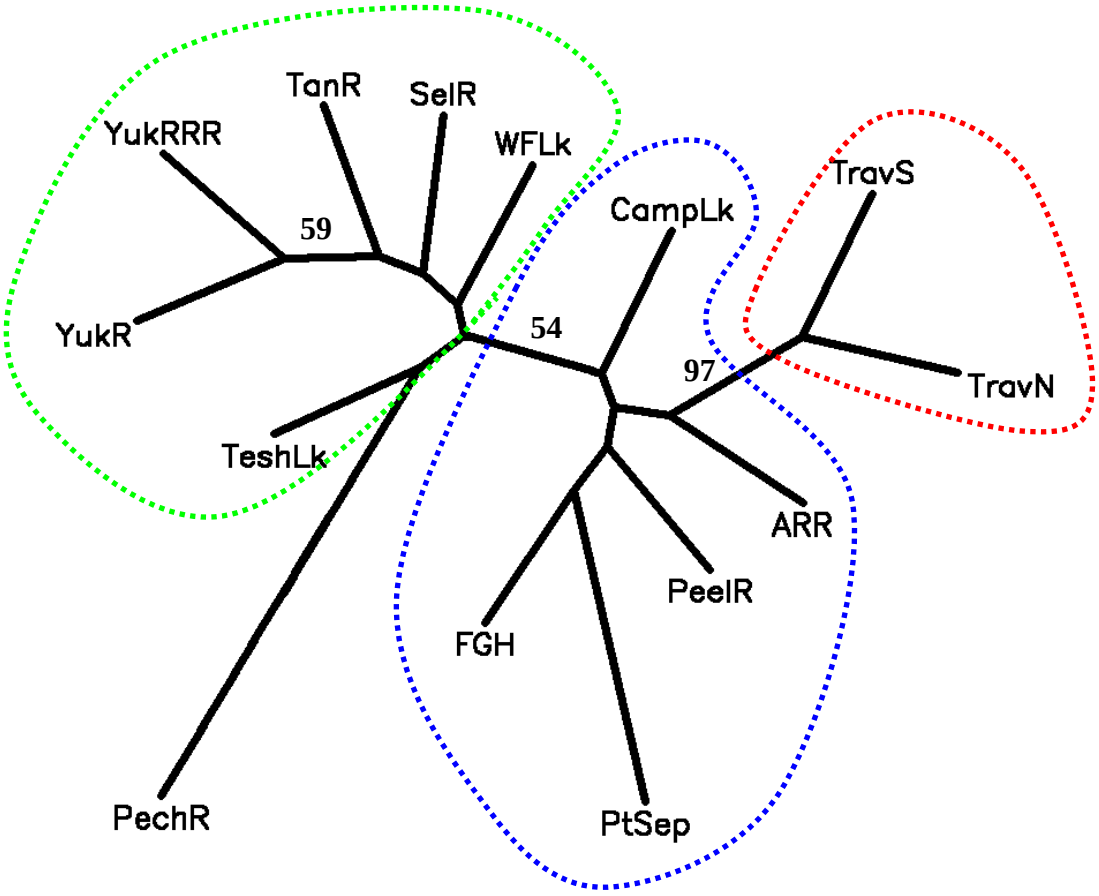

Figure 5. Neighbour-joining tree based on Cavalli-Sforza and Edwards (1967) chord distances for broad whitefish population surveyed in this study. Bootstrap values greater than 50% are shown. Dotted lines show geographic region. PeelR = Peel River, ARR = Arctic Red River, PtSep = Point Separation, FGH= Mackenzie River at Fort Goodhope, CampLk = Campbell Lake, TravS = Travaillant Lake South, TravN = Travaillant Lake North, YukR = Yukon River, YukRRR = Yukon River at Ramparts, WFLk = Whitefish Lake, TeshLk =Teshekpuk Lake, TanR = Tanana River, SelR = Selawik River and PechR = Pechora River.

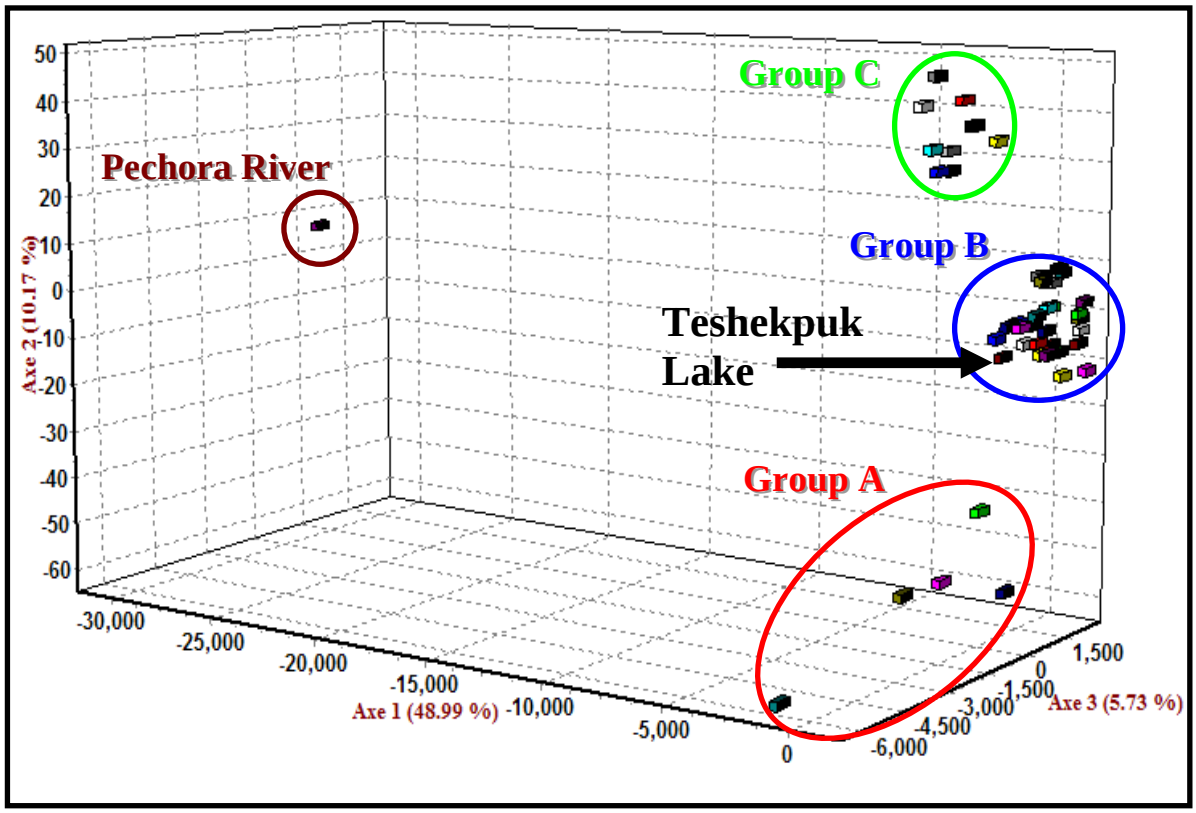

Figure 6. Results of the factorial correspondence analysis (FCA). All samples from Alaska (Group A), all samples from the Mackenzie River system excluding those from Travaillant Lake (Group B) and all samples solely from the Travaillant Lake system (Group C).

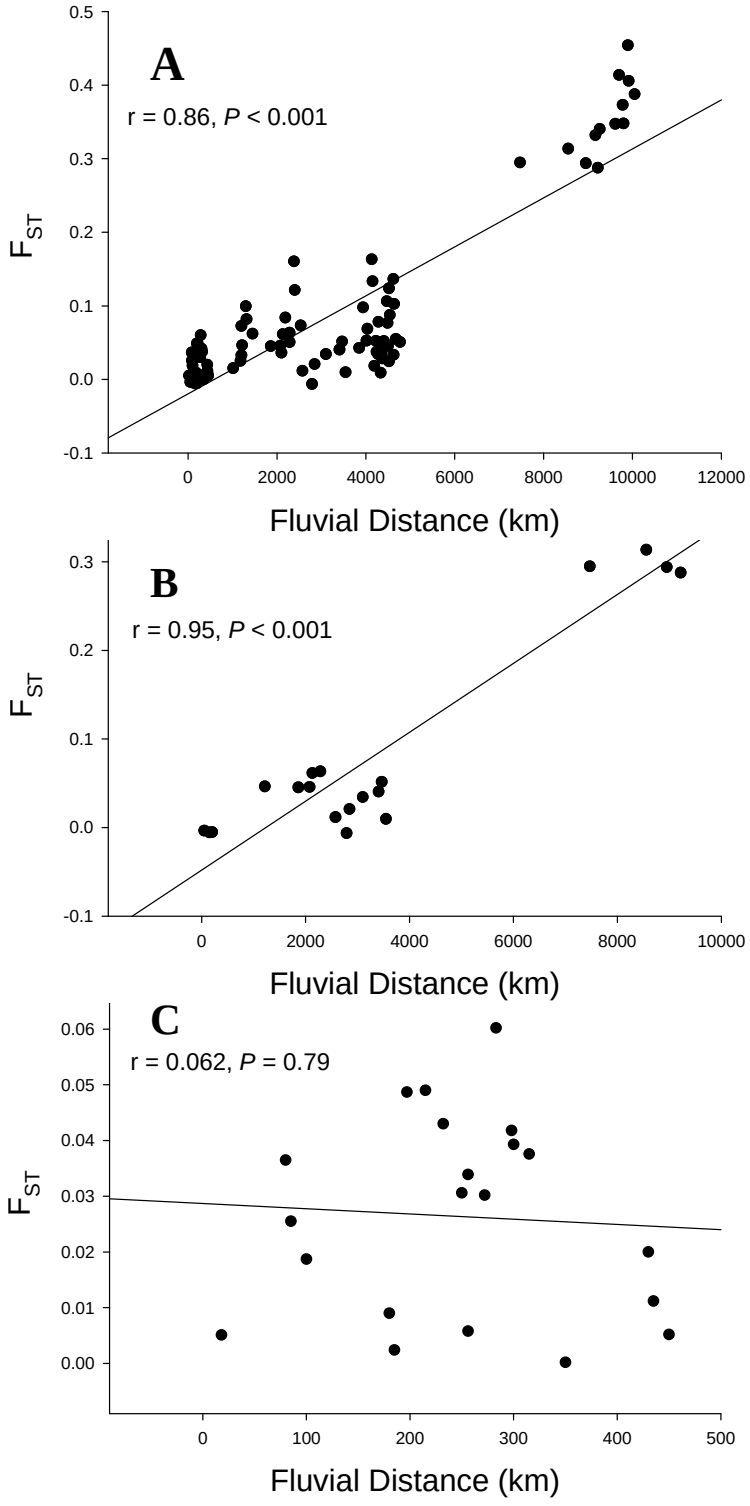

Figure 7. Isolation-by-distance patterns over all populations (A), only those from former Beringia (B) and those from the newly invaded Mackenzie River system (C). F_{ST} as defined by Weir and Cockerham (1984).

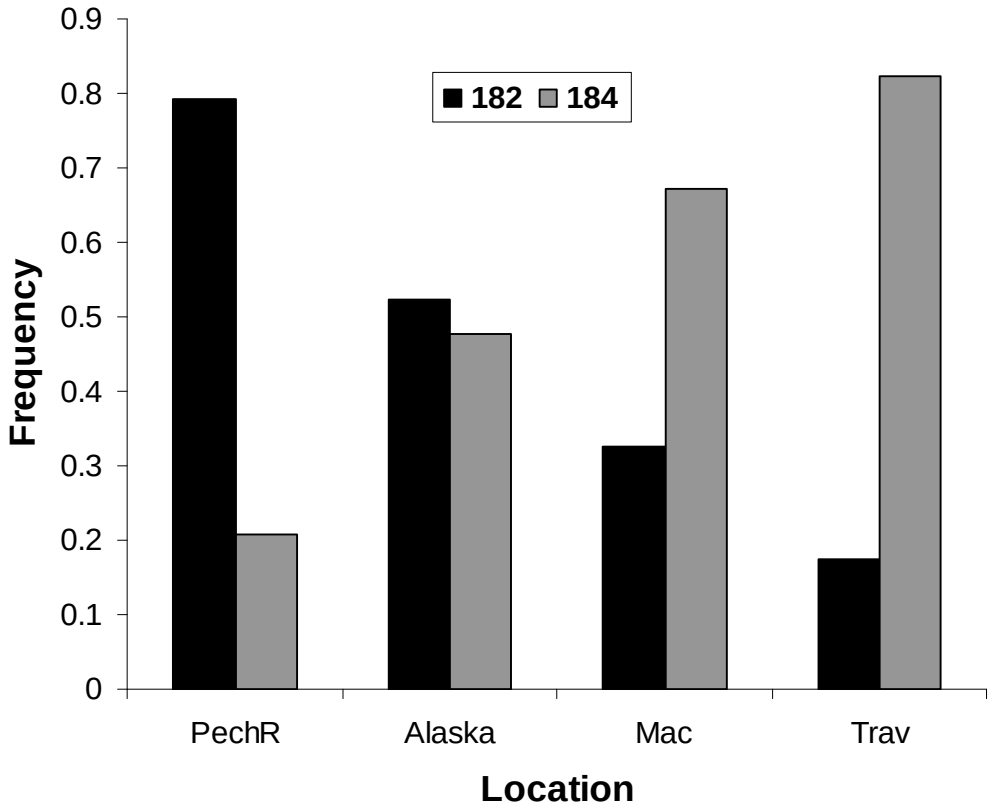

Figure 8. Allele frequencies at the Cocl-Lav 27 locus showing a progressive decline in allele 182 and an increase in allele 184 as you move from east to west (Pechora River – Travaillant Lake. PechR = Pechora River, Alaska = all Alaskan samples combined, Mac = all anadromous populations of the Mackenzie River system and Trav = Travaillant Lake.

CHAPTER 3: POPULATION STRUCTURE OF BROAD WHITEFISH AND MOLECULAR ASSESSMENT OF THE MIXED-STOCK FISHERY IN THE MACKENZIE RIVER SYSTEM

Introduction

Although a large body of research has focused on documenting variation in morphology, life history and in habitat preferences in broad whitefish of the Mackenzie River system (Chang-Kue and Jessop 1983, 1997; Chudobiak 1995; Treble and Reist 1997; Treble and Tallman 1997; Chudobiak *et al.* 2002; Tallman *et al.* 2002), little is known regarding genetic relationships and population structure (but see Reist 1986 for an allozymes example). Molecular assessments, specifically those employing microsatellite markers, of population structure can provide historical and contemporary evolutionary information on populations and species, information often required for effective management and conservation. The usefulness of microsatellites for addressing fine-scale population structure has been demonstrated numerous times in coregonids (e.g., Hansen *et al*. 1999; Lu *et al.* 2001; Huuskonun *et al.* 2004). In the only use of microsatellites in broad whitefish thus far, however, limited gene flow was reported for two populations inhabiting nearby rivers on the North Slope of Alaska, suggesting they are semi-isolated populations (Patton *et al.* 1997). Currently it is not known how much gene flow occurs between Mackenzie River broad whitefish populations and how many genetically distinct populations exist in this system.

Broad whitefish support a major subsistence fishery in the Mackenzie River Delta. Several populations of anadromous broad whitefish are known to be harvested in autumn

subsistence fisheries during upstream migrations to spawning locations, yet the relative contributions to the fishery of the various anadromous populations are unknown. Since several broad whitefish populations are migrating through the Mackenzie River Delta at any given time during the autumn fishery (Reist and Chang-Kue 1997), potentially all of them are vulnerable to harvest. This chapter uses microsatellite data to estimate the number of genetically distinct populations in the Mackenzie River system, and to identify which populations contribute to subsistence fisheries, and to what degree. Although physical tagging studies have tried to determine catch composition of this harvest with limited success (Babaluk *et al.* 1997), molecular techniques have never been used to assess contributions of populations to this mixed-stock fishery.

Assignment Tests, Mixed-Stock Analysis and Broad Whitefish

Assignment tests are statistical methods that use genetic information, such as multi-locus genotypes, to determine which populations certain individuals, or groups of individuals, are members of and in recent years they have been used to study a wide range of evolutionary and ecological processes (Manel *et al.* 2005). Assignment tests are essentially based on calculations of the relative likelihoods that given multilocus genotypes originate in a number of possible populations given the allele frequencies of the populations considered (Hansen *et al.* 2001). The original formulation developed by Paetkau *et al.* (1995), as well as all subsequent methods, employ the same basic methodology and criterion for assignment; expected genotypic probabilities are computed from samples from each potential source population, and genotypes of individuals in

question are assigned to the population in which that genotype is most likely to occur (Waser and Strobeck 1998). Due to numerous features, such as their high levels of polymorphism, microsatellites have proven to be particularly suited to studies of individual identification (Estoup and Angers 1998; Cornuet *et al.* 1999). Assignment methods have been used often for identification of migrants between populations (e.g. Fraser and Bernatchez 2004; Taylor and Costello 2006), detection of poaching (e.g. Primmer *et al.* 2000) and distinction between individuals of native and stocked origin (e.g. Primmer *et al.* 1999).

It may also be of interest to conduct an analysis of population divergence assuming no particular structure *a priori* in order to elucidate the most likely number of populations in a given system. A novel, Bayesian model-based clustering algorithm (implemented under the STRUCTURE software) has been developed to assess the level of population subdivision within a set of collections without any *a priori* designation of populations (Pritchard *et al.* 2000). This analysis uses a likelihood approach to find the most likely number (K) of populations that are most consistent with the observed allele frequency data. Individuals in the sample are probabilistically assigned to populations or jointly to two or more populations if their genotypes indicate that they are admixed (e.g., Stamford and Taylor 2005). The analysis estimates the minimum number of populations in the total dataset that minimizes departures from Hardy-Weinburg equilibrium and linkage equilibrium; deviations from such equilibria are typically found when mixtures of genetically divergent populations are analyzed as a composite. Within the present context, this will be an especially important approach to questions of the number of

genetic populations (as opposed to sampling localities) within the Travaillant Lake and Mackenzie River systems.

Sometimes, it may be of more interest to determine the overall composition of a mixture of individuals from different populations or an admixture (which results from interbreeding among populations) instead of conducting assignments of individual fish (Manel *et al.* 2005). The question of interest, in such cases, would not be the origin of individual fish, but rather the stock composition of a fishery and how it changes in space and time (Manel *et al.* 2005). As an alternative to assignment test-based analyses, genetic mixture analyses have been developed specifically for estimating the proportional contribution of contributing populations in areas of mixed exploitation. Estimating the proportion of fish that different spawning (baseline) populations contribute to commercial, recreational and subsistence fisheries is crucial for the management and conservation of exploited and harvested fish species. Mixed-stock analysis (MSA), a modelling procedure that estimates the relative contributions of such donor stocks to an admixture of fish that are being harvested (Utter and Ryman 1993) has been used for extensively to assist fisheries managers. Originally, MSA was most often employed in Pacific salmon (*Oncorhynchus* spp) fisheries and focussed on traits such as scale pattern analysis (e.g., Cook and Guthrie 1987), parasites (e.g., Margolis 1963) and physical tagging (e.g., Gilbert 1924) for stock identification and elucidating stock contributions. More recently, however, genetic stock identification (GSI) has been used most frequently to estimate contributions to mixed-stock fisheries (reviewed in Utter and Ryman 1993). Allozymes were the first molecular marker used in MSA studies employing GSI (e.g.,

Utter *et al.* 1974), and still remain important in such studies, but more modern tools such as mtDNA, minisatellites and microsatellites are rapidly becoming more popular (Shaklee and Currens 2003). The use of microsatellite DNA has been recommended for MSA and GSI by several authors, yet relatively few studies exist employing these nuclear markers (but see Beacham *et al.* 2005).

Genetic stock identification used in MSA essentially tries to resolve the source proportions contributed by separately breeding populations that differ genetically (Pella and Maduda 2001). Thus, an important assumption for this type of analysis is that distinct stocks exhibit detectable divergence and that this divergence is large enough for reliable stock contribution estimates (Shaklee and Currens 2003). For MSA and GSI, allele frequencies are first determined in all populations potentially contributing to the mixed fishery (Potvin and Bernatchez 2001). That is "learning" or "baseline" sample genetic data are collected from each major spawning population (Tallman and Reist 1997). Second, the most likely proportions of each population in the mixed fishery (from samples collected where stocks are mixing) are then determined based on the maximum likelihood of allelic composition of the mixture, given allelic frequencies in baseline populations (Potvin and Bernatchez 2001). Bayesian methods, in addition to maximum likelihood methods, are also available for estimating the proportions of contributing populations to a mixed stock fishery (e.g., Millar 1987; Pella and Masuda 2001) and although these methods differ computationally and philosophically, both are built upon estimates of the probability of a fish in the baseline populations having the genotype of a fish observed in the fishery (Kalinowski 2003). One such system where the application of

MSA by GSI techniques might prove to be extremely informative is the lower Mackenzie River system, Northwest Territories, Canada, where broad whitefish are harvested extensively in subsistence fisheries and periodically in commercial fisheries (Treble and Reist 1997). For example, understanding which populations contribute most to the lower Mackenzie River fishery might help to prioritize populations and possibly their habitats for protection.

The migratory behaviour of the anadromous broad whitefish presents many opportunities for their exploitation and a wide range of fisheries have developed, operating in the Beaufort Sea, the Mackenzie River Delta and the mainstem Mackenzie River. All these areas and their tributaries encompass three land claim settlement areas of First Nations (Inuvialuit, Gwich'in and Sahtu). Prior to spawning, maturing anadromous broad whitefish spend the summer feeding in the outer Mackenzie Delta and nearshore estuarine environments of the Beaufort Sea and in late July and early August they move towards pre-spawning aggregation sites in the inner Mackenzie River Delta at major eddies (e.g., the large eddy downstream from Horseshoe Bend in the Middle Channel, Chang-Kue and Jessop 1983, 1997; Reist and Chang-Kue 1997) on their way to what are presumed to be their natal spawning areas. Prespawning individuals may aggregate in these areas for weeks before proceeding with their upstream migration to spawning areas on the mainstem Mackenzie River or Arctic Red and Peel rivers (Reist and Chang-Kue 1997). The concentration of major assemblages of fish into these local habitats at specific times of the year, make these fish extremely susceptible to exploitation. As such, subsistence fisheries for broad whitefish in the Mackenzie River system have been

operating in the region since the first human habitation (Treble and Reist 1997). In addition, because broad whitefish are iteroparous, meaning they do not die after spawning, they can potentially be harvested during post-spawning migrations from natal spawning areas to overwintering areas of the outer Mackenzie Delta.

Although several radio-telemetry studies have been conducted in order to determine the potential number of spawning populations in this system and to identify specific areas where spawning takes place (see Chang-Kue and Jessop 1997 for a summary), little has been done in the way of determining the contributions of such spawning populations to subsistence fisheries in the Mackenzie River system. Physical tagging (T-bar anchor (Floy) tags) studies been conducted, but with little success in terms of estimating stock contributions (Babaluk *et al.* 1997). In 1992, 113 fish were tagged at Aklavik on the West Channel of the Mackenzie Delta, and in 1993, 1225 fish were tagged at a major congregating area, Horseshoe Bend on the Main Channel of the Mackenzie Delta (Babaluk *et al.* 1997). Of the broad whitefish that were tagged, only 23 were recaptured. This study did provide information regarding long distance spawning migrations that span several land claim boundaries; however, the contributions of individual broad whitefish populations to subsistence fisheries are still unknown and have not been addressed since then. In response to this information gap, I used Bayesian mixture analysis methods to determine which populations are contributing to subsistence fisheries, and to what degree, at several locations throughout the Mackenzie River Delta (including the West, East and Main Channels) and mainstem Mackenzie River proper. In order to do this, learning, or baseline samples were collected from spawning locations

outlined previously (Point Separation, Peel River, Arctic Red River and upper mainstem Fort Good Hope populations, Tallman 1997) and fishery samples were collected by local harvesters during the fall subsistence fishery.

The Travaillant Lake system, known to contain the only confirmed population of lacustrine broad whitefish in the Mackenzie River system, has recently been the focus of numerous population, life history and habitat use studies. Studies of genetic variation (allozyme variation), vital rates, and morphology suggest that broad whitefish in this system are distinct from their Mackenzie River counterparts (Reist 1986, 1997; Chudobiak 1995; Chudobiak *et al.* 2002; Tallman *et al.* 2002), but it has been only recently that that their lacustrine life history was confirmed (Harris and Howland 2005). Comparatively, an examination of sulphur isotopes in the flesh of broad whitefish from Travaillant Lake suggested that they are not lake-resident, but rather occasional visitors that have assimilated a significant fraction of their sulphur from food sources outside of Travaillant Lake (Hesslein *et al.* 1991). These data suggest that there may be several populations, or even life histories, within this system. Although subsistence fishing has not been active in this lake for several years, during past fisheries it was unknown how many populations were being harvested and to what extent. With the proposed Mackenzie Valley pipeline planned to pass within 12 km of the lake, the potential for the development of future fisheries will increase. In this chapter, samples were collected from the two spawning populations in Travaillant Lake (learning or baseline samples), and samples have also been collected within the lake itself (likely mixed stock samples) over several consecutive years. That provided me with a unique opportunity to address

some of these questions regarding the life history and genetic variation persisting within this species in a lacustrine setting. Additionally, the use of assignment methods and mixture stock analysis will allow me to determine contributions of the spawning populations to fish being harvested from Travaillant Lake.

Genetics of Anadromous vs. Lacustrine Population of Fishes

Several studies examining a variety of molecular markers have shown that lacustrine fish are usually much less genetically diverse in comparison to their marine or anadromous counterparts (Ward *et al.* 1994; Gyllensten 1985, DeWoody and Avise 2000). Empirical evidence showing this trend is also becoming more commonplace for species of north temperate fishes (e.g., Castric and Bernatchez 2003; Tonteri *et al.* 2007). The larger effective population sizes of marine and anadromous fishes are often thought to be responsible for such differences (DeWoody and Avise 2000), but investigations of geographic population structure and phylogeography are now shedding light on other processes such as post-glacial dispersal history and the role founding events and subsequent drift that may have influenced, or are continuing to impact, current levels of diversity (e.g., Tonteri *et al.* 2007). Additionally, lacustrine populations are often shown to be genetically differentiated from nearby anadromous populations (King *et al.* 2001; Tonteri *et al*. 2007), which may be a result of historical isolation in separate refugia (Bernatchez *et al.* 1998), or contemporary isolation given current hydrological features, where drift and mutation would be acting independently on these isolated populations in the absence of gene flow. Additionally, if a small number of founders are contributing to

these lacustrine populations, the influence of drift would be stronger on such populations, further promoting divergence (Tonteri *et al.* 2007). Furthermore, if anadromous and lacustrine populations are highly differentiated, this could be the result of differential ecological selection acting to maintain local adaptation in these different aquatic habitats (Tessier *et al*. 1997). In the Mackenzie River system it is of interest to determine if lacustrine Travaillant Lake populations do show lower levels of genetic diversity and if they are highly differentiated from anadromous populations. If they do show lower levels of diversity and are highly differentiated from anadromous populations, determining the causes for reduced diversity and high differentiation will provide insight into the evolutionary history of this species and perhaps be useful for managing the conservation of broad whitefish diversity in the Mackenzie River system.

Objectives

In this chapter polymorphic microsatellite loci were used to determine if gene flow still plays an important role in the organization, pattern and levels of genetic variation in broad whitefish by addressing the affects of geography and location of spawning on the levels of genetic variation in populations. Broad whitefish segregate into several spawning populations (lacustrine and anadromous; Chang-Kue and Jessop 1983, 1997, Reist and Chang-Kue 1997; Harris and Howland 2005) and if gene flow is limited between these putative reproductively isolated populations, I expect a high degree of genetic subdivision between these populations to be apparent. In view of such spawning migrations, for broad whitefish populations to be genetically structured and differentiated, fidelity of homing to natal spawning areas is necessary. Alternatively, if

gene flow between these populations is high, I expect the allelic and genotypic frequencies to be quite similar, and population subdivision to be reduced.

In addition, I used assignment methods to determine the probability that individual fish would be assigned to the population in which it was sampled, providing insights into the movement of individuals, and subsequent gene flow, between populations. Lastly, I used assignment methods and mixed-stock analysis to determine how many populations exist in this system and what the contributions are of these various populations to areas of subsistence harvest where several populations are likely being exploited. In this chapter I use all samples collected from the Mackenzie River system including those that are considered to be "true" populations and those collected in areas where several populations might be contributing to the sample. Using these techniques, I: demonstrate the genetic uniqueness of the Travaillant Lake system, highlight the lack of divergence among Mackenzie River populations, provide evidence for the existence of a riverine life history form which has never been confirmed, and show that there are unequal contributions to the subsistence harvest of broad whitefish in the Mackenzie River Delta and the Mackenzie River proper.

Materials and Methods

Sample Collection and DNA Extraction

See Chapter 2 Materials and Methods

Microsatellite Amplification and Scoring

See Chapter 2 Materials and Methods

Genetic Analysis

Basic descriptive statistics of microsatellite variation, including number of alleles (N_a), expected heterozygosity (H_E) and observed heterozygosity (H_O) were calculated using TFPGA ver. 1.3 (Miller 2000) and allelic richness (A_r) was calculated using FSTAT ver. 2.9.3.2 (Goudet 2002). I also tested for differences in allelic richness (A_r) and expected multilocus heterozygosity (H_E), between populations from Mackenzie River system and Travaillant Lake using the permutation approach in FSTAT. Because lower genetic diversity tends to occur in more recently inhabited regions, often due to smaller population sizes and founding events associated with range expansion, especially at the periphery of a range, I predicted that populations from Travaillant Lake would show lower levels of genetic variation. Furthermore, populations that exhibit freshwater life histories (e.g., Travaillant Lake populations) are often less genetically diverse than anadromous (Mackenzie River system populations) or marine populations of the same species (e.g., DeWoody and Avise 2000).

Tests for deviations from Hardy–Weinberg equilibrium of observed genotypes were performed using GENEPOP Ver. 3.4 (Raymond and Rousset 2003) for each locus-population combination using an exact test in which two-tailed *P*-values were estimated using a Markov chain method of Guo and Thompson (1992). Tests for genotypic linkage disequilibrium for all combinations of locus pairs within populations were conducted using a Markov chain method in GENEPOP. Finally, employing GENEPOP, tests for population differentiation between all pairs of populations were performed over all loci combined using log-likelihood (G)–based exact tests (Goudet *et al*. 1996) with default values. I adjusted the results from tests for conformation to Hardy–Weinberg proportions, linkage disequilibrium and population differentiation for multiple tests using the sequential Bonferroni procedure (Rice 1989) with an initial alpha level of 0.05. The statistical software program JMP was used for all other standard statistical tests.

Spatial genetic structure was also calculated at the population level by estimating F-statistics (F_{ST}) in order to measure the extent of genetic differentiation between populations. $F_{ST,}$ a drift-based method of population subdivision calculated assuming that the loci assayed follow the infinite allele model (IAM; Kimua and Crow 1964), was chosen instead of R_{ST} (Slatkin 1995), an F_{ST} analogue that assumes the SMM, has been developed specifically for SMM-based loci, adherence to this assumption can vary from locus-to-locus. F_{ST}, the drift-based method of population subdivision was considered to be most appropriate for the reasons outlined in Chapter 2 methods. Specifically, I used the estimator (θ) of Weir & Cockerham (1984). Pairwise F_{ST} (θ) values were calculated FSTAT ver. 2.9.3.2 (Goudet 2002) and GENEPOP. Variances and robustness of F_{ST}

estimates were assessed by resampling procedures using jack-knife and bootstrap methods over loci to generate *P*-values and 95% confidence intervals using FSTAT. F_{ST}, typically ranges from 0, which indicates no genetic differentiation, to 1, which indicates fixation of alternative alleles.

Genetic distances were estimated using the pairwise chord distance (D_{CE}) of Cavilla-Svorza and Edwards (1967) which has been shown to estimate tree topologies well in very closely related populations. PHYLIP version 3.5 (Felsenstein 1993) was used to calculate $D_{CE,}$ with the GENDIST module and used the corresponding genetic distance matrices to construct an unrooted neighbour-joining (N-J) phylogenetic tree to visualize genetic relationships among populations and sampling localities. Specifically, from these distance matrices, the neighbor-joining algorithm in the NEIGHBOR module was used to generate the trees, while CONSENSE was used to generate a consensus tree with bootstrap values from 1000 replicate datasets created in SEQBOOT. The final tree was drawn in the DRAWTREE module. I chose to analyse genetic divergence between populations using D_{CE} because it is drift based estimate of genetic distance, it does not assume any models of molecular evolution and in the past it has performed well in simulations of microsatellite data (Takezaki and Nei 1996). Again, given the probable recent origin of broad whitefish in North America, drift, rather than mutation has likely contributed the most to population differentiation in this species.

To further detect patterns of genetic differentiation between samples a factorial correspondence analysis (FCA), which is a type of factor analysis that seeks to find the

best linear combination of variables (in this case allele frequencies at different microsatellite loci) that best describe variation between individual observations (e.g. Taylor *et al.* 2006), of the microsatellite genotype data was conducted with the program Genetix 4.05.02 (Belkhir *et al*. 2004).

To estimate the most likely number of populations in the Mackenzie River and Travaillant Lake systems, I conducted an analysis of population divergence assuming no particular structure *a priori*. A novel, Bayesian model-based clustering algorithm (implemented under the STRUCTURE software), using a Markov chain Monte Carlo (MCMC) sampling method, has been developed to assess the level of population subdivision within a set of collections without any a priori designation of populations (Pritchard *et al.* 2000). This analysis uses a likelihood approach to find the most likely number of *K* populations (i.e., the *K* value with the highest log-likelihood score) that are most consistent with the observed microsatellite allele frequency data. Individuals in the sample are probabilistically assigned to populations or jointly to two or more populations if their genotypes indicate that they are admixed (e.g., Stamford and Taylor 2005). The analysis estimates the minimum number of populations in the total dataset that minimizes departures from Hardy-Weinburg equilibrium and linkage equilibrium; deviations from such equilibria are typically found when mixtures of genetically divergent populations are analyzed as a composite. This analysis is especially important when addressing questions regarding the most likely number of genetic populations (as opposed to sampling localities) within the Travaillant Lake and Mackenzie River systems, especially

the Mackenzie River Delta where it is unknown how many distinct populations are being harvested during the subsistence fishery.

The STRUCTURE analysis was conducted using a burn-in period of 10 000 replications with subsequent analysis continuing for a further 10 000 MCMC replications. This number of replications was suggested to be sufficient based on simulation studies conducted by Evanno *et al*. (2005). For each value of *K* (from 1 to the most likely number of populations plus two in our study, suggested by Evanno *et al.* 2005) that was assessed, I conducted 20 independent iterations to check for variability of obtained log-likelihood values (Pritchard *et al*. 2000). Two models were run for comparison. First, allele frequencies were selected to be correlated among localities based on previous tests of population differentiation suggesting that Mackenzie River system broad whitefish populations are not highly differentiated and may be closely related. This option is used when it is suspected that some of the putative populations share allele frequencies, and may allow for the accurate assignment of individuals in very closely related populations (Pritchard *et al.* 2000), which is likely the case in my study system. Default values implemented in STRUCTURE were selected for all other model parameters. Upon generation of the log probability given the data ($Pr(X \mid K)$ (equation 12 in Pritchard *et al.* (2000)), where applicable, the posterior probability of *K* was calculated as suggested in the STRUCTURE manual.

I also used maximum likelihood-based assignment procedures to determine the likelihood of each individual's genotype being found in the locality from which it was sampled.

Assignment methods attempt to correctly classify individual fish to their most likely population of origin based on their composite microsatellite genotypes given the allele frequencies of the populations considered (Hansen *et al*. 2001). Rannala and Mountain's (1997) Bayesian individual assignment method, followed by the permutation procedure (10 000 simulated individuals) of Paetkau *et al*. (2004) implemented in GENECLASS2 (Piry *et al.* 2004) was used to estimate the likelihood that a fish originated from the location from which it was sampled. It has been noted that previous Monte Carlo resampling procedures (e.g., Rannala and Mountain 1997, Cornuet *et al.* 1999) may introduce a bias that leads to over-rejection of resident individuals. Because of this I employed the resampling method of Paetkau *et al.* (2004) which generates population samples as the same size of the reference population (Piry *et al*. 2004). This method takes into account the sample size of the reference or baseline population, and therefore, it better reflects the sampling variance associated with the analyzed dataset compared to earlier resampling methods (Piry *et al.* 2004). See Piry *et al.* (2004) for a review of this resampling procedure. To avoid unduly biasing the sample, I used a low significance level of 0.05 for excluding individuals and incorporating the 'jackknife' or 'leave one out' option to reduce assignment bias (Efron 1983). I also used GENECLASS2 to evaluate the effects of individual loci on classification accuracy by leaving each locus out of the analysis sequentially and utilizing the remaining 6 for the assignment tests. An individual fish whose sampling location differed from the assigned location in which the genotype was most likely to originate from was referred to as being miss-assigned.

I used GMA (Genetic Mixture Analysis, Kalinowsky 2003) to determine the relative contributions of populations that contribute fish to those caught during subsistence fisheries in the lower Mackenzie River system, including Travaillant Lake. This is why it was extremely important to collect samples from fish harvested during the Mackenzie River Delta subsistence fishery. I included simulation analyses as well as a method to assess the robustness of mixture estimates. Replicate mixtures and baselines are simulated from the actual baseline by drawing individuals randomly from the baseline file (e.g. Taylor and Costello 2006). I simulated mixtures of 1000 individuals (i.e., multilocus genotypes) by random sampling with replacement and estimated mixture proportions for 10000 replicate analyses. The variability about the mixture proportions across all replicates gives an indication of how well baseline data can estimate mixture proportions (Kalinowski 2003).

Results

Intrapopulation Genetic Variation

Across 1013 individuals that were genotyped from 36 locations throughout the Mackenzie River system, all seven microsatellite loci were polymorphic with the number of alleles detected ranging from 5 (Cocl-Lav27) to 21 (Cocl-Lav8, Table 7) and H_E ranging from 0.41 (Cocl-Lav27) and 0.70 (Ots103, Table 7). Within sampling locations, the mean number of alleles (averaged across all loci) ranged from 3.00 in the East Channel of Mackenzie Delta sampled in 2004, to 6.71 in the Peel River when all sampling locations were combined (Table 4). The overall mean allele richness was 3.4 (based on a minimum sample size of nine diploid individuals), and varied from 2.90 in an

East Channel of the Mackenzie Delta sample collected in 2004 to 3.93 in a sample from the Napoiak Channel in the Mackenzie River Delta (Table 4). The mean gene diversity (H_E) over all sampling locations was 0.543, and ranged from 0.434 in a Travaillant River South sample collected in 2003, to 0.584 in the Napoiak Channel sample (Table 4). Based on the FSTAT permutation process, the Travaillant Lake system had significantly lower He (0.467 compared to 0.544) and allelic richness (2.466 compared to 2.722) compared to the Mackenzie River system ($P<0.001$).

Following Bonferroni corrections for simultaneous multiple tests conformation to Hardy-Weinberg equilibrium was rejected in only three tests, two of which involved deficits in heterozygotes. Two of the significant departures from HW equilibrium occurred in a sample collected from Tuktoyuktuk Harbour, and the other two significant departures were from samples collected from one sampling location in the Mackenzie River Delta. Significant genotypic linkage disequilibrium was detected in only one of 924 tests. The significant result involved the sample from the Tuktoyuktuk Harbour and the loci Cocl-Lav8 and Cocl-Lav10.

Population Divergence and Genetic Structure

Log-likelihood (*G*)–based exact tests of population differentiation (e.g., Goudet et al. 1996) suggest that the two main regions included in my study (i.e., Mackenzie River system and Travaillant Lake system) are significantly differentiated (Table 8, $P < 0.01$). None of the anadromous Mackenzie River populations were differentiated from each

other, but all were differentiated from both Travaillant Lake system populations ($P <$ 0.01). The two Travaillant Lake populations were not differentiated from each other ($P <$ 0.01).

F_{ST} (θ) ranged from 0.001 (Cocl-Lav4) to 0.072 (Cocl-Lav6) and the overall level of population subdivision based on average pairwise estimates was low (θ = 0.026, 95 % CI 0.01-0.045) among all populations. Among populations, pairwise F_{ST} values ranged from 0.0002 (between Point Separation and the mainstem Mackenzie River at Fort Good Hope) to 0.0602 (between the Travaillant River South and Campbell Lake, Table 8). Differences in θ were not substantial, and the comparisons were statistically significant in only 10 out of the 21 comparisons ($P<0.01$, Table 8). Samples from the Travaillant Lake system were, however, always significantly different than samples from the anadromous Mackenzie River populations.

Relatively clear genetic relationships were observed among the broad whitefish populations. The most striking feature of the NJ tree based of Cavilla-Sforza's chord distance was the major genetic discontinuity between Mackenzie System populations and those sampled from Travaillant Lake with 100% bootstrap support (Figure 9). When all sampling locations were included (i.e., those from mixed-stock fisheries) the major population clusters of Travaillant Lake and all other Mackenzie System samples were also clearly shown.

The genetic variation among all samples, as summarized by factorial correspondence analysis (FCA, Figure 10) also revealed a strong geographical pattern of genetic variation. Similar to the results of NJ dendogram, two distinct groupings were resolved showing the distinct genetic composition of Travaillant Lake and Mackenzie River system groups of populations. In this analysis, when all sample sites were compared in both systems, there was no overlap between any samples, and no extreme intermediates between the two. There were, however, two outliers in the Mackenzie River group of samples, and they may potentially be explained by the presence of a riverine life history form (discussed later). Results of a principal components analysis were nearly identical in that there were the two distinct groupings of Travaillant Lake samples and all other Mackenzie system samples (data not shown).

Assignment Tests and Mixed-Stock Analysis

The STRUCTURE results revealed that that the most likely number of distinct genetic groups in the Mackenzie River system is two (mean –ln likelihood over the 20 runs = –14531.3, Table 9). This is in concordance with the NJ tree and also the results of the FCA which all showing that there are at least two distinct clusters in this system. When Mackenzie River and Travaillant Lake samples were compared separately, results showed that in both cases, the most likely number of populations within each system was one (K = 1 with the mean –ln likelihood over the 20 runs = –11619.4 and -2600.0 respectively, Table 9). Similarly, when both the Peel River and the Arctic Red River were analyzed by themselves, in both cases the most likely number of populations in these rivers was one (K = 1 with the mean –ln likelihood over the 20 runs = –2665.2 and -

3612.2 respectively, Table 9). In all cases, the posterior probability of the most likely *K* value indicated by the log probabilities was 1.0 and any *K* value greater than or less than most likely value had a posterior probability of 0.0 (Table 9).

The "learning" samples of broad whitefish (i.e., fish collected from localities known to contain spawning or potential source populations contributing to mixed-stock subsistence fisheries in this system) consisted of 401 fish from six localities (two in the Travaillant Lake system of lacustrine broad whitefish populations and four in the Mackenzie system of anadromous broad whitefish). Self-assignment of these fish resulted in low overall assignment success; only 51.6% of these learning sample fish were correctly assigned to their collection localities. The success of these tests, however, varied greatly among source populations. For instance, assignment success was as high as 80.0% in the Travaillant Lake North sample collected in 2004, but as low as 17.1% in the Arctic Red River sample (Table 10). In most cases, low assignment success was attributable to miss-assignment to the Peel River sample. When the all Mackenzie samples were combined and compared to all combined Travaillant Lake samples, and assignment success was tested, results were still variable. High assignment success was revealed for the Mackenzie River populations in that 95.3% percent of the time they were assigned to the Mackenzie system instead of the Travaillant Lake system. Alternatively, however, Travaillant Lake samples were correctly self-assigned only 51.9% of the time.

The mixture analysis revealed that all baseline or source populations contribute to the Mackenzie Delta and Mackenzie River subsistence fishery and that there is variation in

the contributions to the fishery depending on where fish are being exploited. Overall, when all fishery samples from the Mackenzie Delta and Mackenzie River were combined, the Peel River was estimated to contribute the most (54 ± 9% S.D.), followed by the Arctic Red River (25 ± 7), the mainstem Mackenzie River population at Fort Good Hope (16 ± 7), and finally Point Separation (5 ± 4) (Table 11, Figure 11). There was, however, considerable variation in contributions to fisheries depending on harvest locations although the Peel River typically contributed the most in all cases (Table 11). For instance, the Peel River still contributed the most to fisheries in the east channel, west channel and main channels of the Mackenzie Delta and the mainstem Mackenzie River when sampling locations in each region were combined at a contribution of 58% (9), 57% (9), 52% (10) and 71% (8) respectively. It was estimated that the Arctic Red River contributes the most to the Beaufort Sea fishery (67% (8)) and in most the majority of cases, the Fort Good Hope source population contributed the least to fisheries (Table 11).

In the Travaillant Lake system, when all years were combined, the population in the Travaillant River upstream of the lake contributed the most (69% (9)) to samples harvested from Travaillant Lake (Table 11). When temporal analyses were conducted on fisheries contributions there was considerable variation in this system. In 2004, the majority of fish harvested in Travaillant Lake were estimated to have originated from the Travaillant River population downstream from Travaillant Lake (69% (7)), whereas in 2005 virtually all harvested fish ((97% (3)), were Travaillant River fish, from the upstream spawning location north of the lake (Table 11). Additionally, fish were captured in Andrew Lake in 2005, and subsequently it was estimated that 75% (10) of the catch

was contributed by the south spawning location, although sample size was quite low (n = 5).

Discussion

Genetic Differentiation Within and Between C. nasus *Populations*

In the current study, genotypes met Hardy–Weinberg equilibrium expectations in the majority of comparisons and a limited amount of linkage disequilibrium was observed throughout the study area. The only case of disequilibrium was observed in the Tuktoyukyuk Harbour sample and could indicate population subdivision (or mixing of distinct populations or generations) within my samples, but physical linkage of loci appears unlikely. These samples were collected in the nearshore marine environment of the Tuktoyuktuk Harbour in the Beaufort Sea, where there may be several distinct populations occurring (Reist 1997; Reist and Chang-Kue 1997). For example, the Anderson River system, 50 km east of the Mackenzie River system, also contains populations of broad whitefish (Scott and Crossman 1998), that may likely contribute to those fish captured in the Tuktoyuktuk Harbour.

This study represents the first detailed examination of population structure in Mackenzie River broad whitefish and I detected significant genetic divergence among the two main sampling regions in this system (i.e., the Mackenzie River system and the Travaillant Lake system) and was able to resolve contributions of spawning populations to subsistence fisheries in the area. As predicted, marked differences in genetic diversity was observed between freshwater (lacustrine) and anadromous populations of *C. nasus*; a

trend that has been previously documented (see Ward *et al.* 1994, DeWoody and Avise 2000). In this study, the mean number of alleles and expected heterozygosity averaged 4.5 and 0.54 in anadromous fish respectively, compared to 4.1 and 0.47 in lacustrine fish from the Travaillant Lake system, although due to the lack of replication of freshwater populations, further lacustrine samples are needed to further test this quantitatively. Castric *et al.* (2001) and Castric and Bernatchez (2003) also reported similar differences between land-locked and anadromous char. More recently, Tonteri *et al.* (2007) showed that anadromous populations of European Atlantic salmon, *Salmo salar*, always had higher levels of genetic diversity at microsatellite loci than their freshwater counterparts.

Greater genetic variation in anadromous fish can likely be explained by larger evolutionarily effective population sizes of marine and anadromous fish species (Ward *et al.* 1994), on average, which in turn may be related to the larger and more continuous nature of the marine environment (DeWoody and Avise 2000). Populations of freshwater fishes are often limited to particular drainages over short to moderate evolutionary time, and, therefore, should be smaller in size compared to marine or anadromous populations which are open to potential genetic exchange with conspecifics over much larger areas (Dewoody and Avise 2000). Additionally, historical factors such as Pleistocene glaciations have likely contributed to the observed differences in genetic variation in that demographic events, such as bottlenecks or founder events associated with post-glacial dispersal from Beringia, would likely affect a more recently occupied freshwater habitat in comparison to that in the marine environment (see Hewitt 1996; Bernatchez and Wilson 1998). For instance, populations at the periphery of their range, those that

colonized later after deglaciation such as those in the Travaillant Lake system, typically show reduced genetic diversity, associated with chance founder events and bottlenecks (Ibrahim *et al.* 1996). The lower genetic diversity observed in the Travaillant Lake system may be the result of a small number of founding individuals where subsequent drift has acted to lower genetic diversity in these populations that have been isolated for time from anadromous Mackenzie River populations (e.g., Tonteri *et al.* 2007). This is assuming that broad whitefish dispersed in a stepping-stone like fashion from Beringia east to the Mackenzie River system and then finally into the Travaillant Lake system (Chapter 2) which appears likely given the results of this study. This trend, reduced genetic diversity in populations at the periphery of the range, has been observed in many north temperate fish species (e.g., Taylor *et al.* 2003; Costello et al. 2003), which is not surprising given the glacial history of the area which has provided many opportunities for post-glacial dispersal into previously unoccupied habitats (Bernatchez and Wilson 1998).

Of special interest, was the concordance between the STRUCTURE results, the unrooted NJ tree and the factorial correspondence analysis all suggesting that lacustrine Travaillant Lake populations are highly differentiated from anadromous Mackenzie River populations. This is likely a result of the lake-locked lacustrine life history of Travaillant Lake fish which has likely isolated them from their Mackenzie River counterparts for some time. Virtually all analyses showed that Travaillant Lake fish form a genetic cluster that is highly divergent from the cluster comprised of anadromous Mackenzie River populations. Several studies have also found significant differences between these two groups of fish. For example, Tallman *et al.* (2002) found that anadromous broad

whitefish had greater reproductive investment (fecundity) and a greater age-at-maturity in comparison to lacustrine populations, although growth appeared to be similar among populations. Additionally, Chudobiak *et al.* (2002) found that anadromous populations from the Arctic Red River and lacustrine populations from the Travaillant Lake differed morphologically and Reist (1986, discussed in Reist 1997) using allozyme loci provided the first insights that lacustrine Travaillant Lake fish and anadromous Mackenzie River fish are genetically differentiated. Furthermore, although broad whitefish can migrate upwards of 800 km, (Chang-Kue and Jessop 1997; Babaluk *et al.* 1997), no previous or ongoing radio-telemetry studies (Chang Kue and Jessop 1997; Melanie Toyne, Fisheries and Oceans Canada, Winnipeg, MB, personal communication) have documented the movement of Mackenzie River broad whitefish into the Travaillant Lake system. Furthermore, even though classified as part of the Mackenzie River drainage, previous radio-telemetry studies have shown that Travaillant Lake broad whitefish do not migrate to the mainstem Mackenzie River (Harris and Howland 2005). The genetic effects of such isolation, and subsequent lack of gene flow, are reflected by the FCA, NJ trees and differential allelic frequencies among populations from Travaillant Lake and those from the Mackenzie River and tributaries.

Other studies have shown significant differentiation between anadromous and lacustrine populations of fishes. Bernatchez *et al.* (1998) found similar results in land-locked Arctic char (*S. alpinus*) that were clearly differentiated from their anadromous counterparts and King *et al.* (2001) showed that anadromous and land locked Atlantic salmon (*S. salar*) from Maine were also highly divergent from each other. In addition to contemporary life

history differences, genetic differentiation could also be explained by historical factors, such as timing of post-glacial colonization and refugial origin. For example, it is hypothesized that distinct post-glacial origins for anadromous and lacustrine populations of Arctic char, may explain why these populations highly differentiated (Bernatchez *et al.* 1998). Furthermore, secondary contact of distinct glacial races is not uncommon when examining the evolutionary history of north temperate fishes (e.g., Wilson and Hebert 1998, Lu *et al.* 2001, Turgeon and Bernatchez 2001). In cases like this, where survival in separated refugia may account for high degrees of divergence, populations surviving in isolation have likely drifted independently from each other in separate refugia and when there is secondary contact among such groups, the result is one of significant divergence. Given the geological history of the area, the current North American distribution, and lack of unique allele between anadromous and lacustrine broad whitefish, separate glacial refugia are unlikely for these fish. Secondary contact between lake whitefish, however, has likely occurred in the Mackenzie Rivers system (Bernatchez and Dodson 1991). These fish survived in five glacial refugia (Bernatchez and Dodson 1991), making secondary contact much more likely in comparison to broad whitefish that probably survived solely within Beringia (Chapter 2).

Although gene flow has likely been restricted between Travaillant Lake and Mackenzie River populations, and other evolutionary forces are likely acting independently to promote divergence, this divergence may be relatively recent given the low (but significant) F_{ST} values observed (Table 8) Further evidence supporting the more recent origin of these lacustrine fish is provided by the lack of unique alleles between

Travaillant Lake fish and those from the anadromous populations suggesting not enough time has past since broad whitefish colonized Travaillant to allow for the accumulation of new alleles driven by mutation. Lu *et al.* (2001), showed in lake whitefish of the St. John River system that, within a similar time frame, mutations had also not accumulated in many populations to drive differentiation The paucity of unique genetic variation (as measured by unique alleles) in the lacustrine broad whitefish from Travaillant Lake suggests that Travaillant Lake was founded from a small number of anadromous source populations after recolonization of the Mackenzie River system (Chapter 2). That is non-anadromous populations of broad whitefish were derived from anadromous populations in postglacial times. This phenomenon has been suggested for several other salmonid species (see review by Behnke 1972; King *et al.* 2001).

Surprisingly in this study, there was a lack of inter-population genetic differentiation among anadromous populations in the Mackenzie River system (i.e., excluding the Travaillant Lake system) despite previous work (molecular and tagging studies) that suggests that gene flow is limited between these groups (Reist 1997, Babaluk *et al*. 1997, Chang-Kue and Jessop 1997, Reist and Chang-Kue 1997). This is further evidenced by the low F_{ST} values calculated between all Mackenzie River system populations (Table 8), however, a few of the pairwise F_{ST} comparisons were significant in this system (Table 8). In anadromous fish populations, low F_{ST} values (less than 0.1) are often common which may be a result of the greater dispersal potential compared to land-locked or freswhwater resident populations (Ward 1994, Waples 1998, Bernatchez *et al*. 1998, Castric and Bernatchez 2003). In the only other broad whitefish study incorporating microsatellites

(Patton *et al.* 1997) extremely low values of F_{ST} between river systems (Sagnavirnoktok and Coleville rivers, Alaska) were also reported, but they found genetic differentiation between them was significant. Comparisons of microsatellite DNA variation between Arctic cisco (*Coregonus autumnalis*) populations from the Coleville River and from the Mackenzie River system also revealed a significant lack of differentiation despite the large geographic area between the two systems (J.L. Nielsen, Alaska Science Center, US Geological Survey, Anchorage, AK, personal communication). Numerous studies, on the other hand, (e.g., Bernatchez *et al.* 1998, Koskinen *et al.* 2001, Bernatchez *et al*. 2002, Castric and Bernatchez 2003, Costello *et al.* 2003, Whiteley *et al.* 2004, Stamford and Taylor 2005, Neville *et al.* 2006,) have shown differentiation among fish populations in rivers separated by very short distances, but lack of differentiation in marine (Hutchings *et al.* 2007) and some anadromous fish populations (MacLean *et al.* 1999) is not uncommon. Several hypotheses may explain the lack of differentiation and the low F_{ST} values in the Mackenzie River system.

First, homing behaviour may be imprecise resulting some straying between populations and limited reproductive isolation. Natal homing has evolved in several groups of fishes, typified by salmonids, and is a precursor to fine scale genetic structuring in these fish (see Hendry *et al.* 2004 for a review; Rich *et al.* 2006) or formation of populations (Stewart *et al.* 2003) because of the clustering of related individuals on spawning grounds (Neville *et al.* 2006). Because of this natal philopatry in anadromous fish, populations with this life history are typically show genetic differentiation and subdivided population structure between freshwater spawning tributaries (MacLean *et al.* 1999, Hendry *et al.* 2004). In

some cases, however, selection may favour straying if population sizes or spawning habitat quality are typically variable (Hendry *et al.* 2004, Esteve 2005), or to reduce inbreeding in small populations (Hendry *et al.* 2004). In such cases, population structure is less developed. For instance, Neville *et al.* (2006) showed that a lack of fine-scale homing or movement at some stage subsequent to homing in chinook salmon (*O. tshawytscha*) resulted in a lack of genetic structuring in males spawning in an Idaho river system. In another anadromous species, the eulachon (*Thaleichthys pacificus*), Maclean *et al.* (1999) also found little genetic differentiation among populations from distinct freshwater locations throughout their range, which may also be a result of a high degree of straying. For example, straying rates can be as high as 41.6% the semelparous chinook salmon (Pascual *et al.* 1995, c.f. Hendry and Stearns 2004). Comparing a life-history more similar to that of the broad whitefish, in the iteroparous coastal cutthroat trout (*O. clarkii clarkii*) straying rates can be as high as 35% (Michael 1989, c.f. Hendry and Stearns 2004). Broad whitefish may have an increased chance of straying because all populations are mixed when feeding in the nearshore environments prior to returning to spawn and during upstream spawning migrations (Reist and Chang-Kue 1997, Chang-Kue and Jessop 1997). Near shore feeding grounds of the Beaufort Sea may be upwards of 1200km away from their home river, such that broad whitefish can potentially stray to virtually any spawning river or spawning ground within its range, although rivers closest to their home river are likely the most probable river in which they stray (Castric and Bernatchez 2004). Furthermore, if broad whitefish do not spend enough time in their freshwater natal streams to imprint since young of the year are carried to nearshore marine environments and eventually to coastal lakes in the Tuktoyuktuk Peninsula during

the spring freshet (Reist and Chang-Kue 1997), imprinting may be to this nearshore environment or these coastal lakes rather than to the spawning locality *per se*. Similarily, pink salmon (*O. gorbuscha*) have a tendency to stray more than other species of Pacific salmon, possibly due to their short freshwater residency, and therefore reduced opportunity for imprinting (Hendry *et al.* 2004). Although there is a wealth of knowledge on the straying rates of salmonids (see Hendry *et al.* 2004 for a review), empirical and experimental data on the degree of straying of coregonids is lacking. In my study, results based on population assignment (discussed in the next chapter) provide evidence that there is some straying among populations based on the relatively high percentages of fish that were miss-assigned from their population of origin. Evidence of straying was also found in earlier physical tagging studies where Babaluk *et al.* (1997) found that a broad whitefish tagged in the west channel of the Mackenzie Delta was recaptured the following year migrating up the east channel of the Delta. Although straying may be the main cause for a lack of differentiation in this system, there are very few cases of heterozygote deficiency, indicative of the Wahlund effect suggesting a mixture of populations. Further work is needed to determine if straying is the main cause for the lack of differentiation in this system.

Second, given the broadcast spawning nature of this species (Scott and Crossman 1998) and ability to migrate over large distances (Chang-Kue and Jessop 1997, Babaluk *et al.* 1997), limited differentiation and low F_{ST} values may not be surprising. Previous studies have shown that broadcast spawning behaviour coupled with high levels of gene flow has been indicative of low levels of population differentiation and genetic sub-structuring.

For example, in the widely distributed, broadcast spawning, Atlantic cod (*Gadus morhua*), genetic differentiation was undetected using microsatellite DNA over large spatial scales ranging 600-800km (Hutchings *et al.* 2007). In their study, however, it was shown that these populations differ genetically in quantitative traits related to their response to environmental factors such as temperature. A similar situation may exist for Mackenzie River system broad whitefish, but future studies are needed to shed light in this area. Most examples highlighting a lack of genetic differentiation among populations are taken from strictly marine species in the marine environment where gene flow is likely quite extensive due to the lack of physical obstructions to long-distance dispersal (Hilbish 1996; Avise 2000). While Mackenzie River system broad whitefish are largely anadromous, the spawning nature (broadcast) of these fish has likely also played an important role in the lack of differentiation observed.

Finally, the genetic effects of historical factors may still be leaving a pronounced signature of past evolutionary processes. For example, a lack of differentiation in the Mackenzie River system may be evidence of a recently colonized area in which there was extensive historical gene flow between founding populations (i.e., lack of reproductive isolation or one genetically homogenous founding population), or one that has recently been colonized by a single founding panmictic population and not enough evolutionary time has passed to promote divergence. If broad whitefish were isolated in a single refugium during the last Pleistocene glaciation, then they have had approximately 14,000 years to diverge into discrete populations (Lindsay and McPhail 1986; Pielou 1991). This time may be even less for Mackenzie River broad whitefish that are near the

periphery of their range and that appear to have large population sizes. This evolutionarily short period, although allowing enough time for other North American fish species to diverge into populations (Lu *et al.* 2001), may not have been long enough for distinct broad whitefish populations to form, at least when assessed with the loci used in this study in this system. Estoup and Angers (1998) suggest 2000 generations since population founding is needed to promote significant differentiation, which is clearly not the case in this study since broad whitefish are mature at approximately 7 years of age (Bond 1982) although this will also be dependant on the effective population size.

Assignment Tests and Mixture Analysis

The low level of differentiation between these populations is further evidenced by the results of the assignment tests. In my study, there was low overall self-assignment success (51.6%), which is highly variable depending on the population. Assignment success was as high as 80% in the Travaillant Lake North sample collected in 2004, but as low as 17.1% in the Arctic Red River sample. Several explanations may account for why self-assignment in this study was, on average, quite, low. First, miss-assigned fish can be immigrants that are assigned to the river in which they immigrated instead of the river in which they were sampled (Waser and Strobeck 1998; Castric and Bernatchez 2004). Second, some fish, by chance, may be assigned to another population when the likelihood functions of two rivers overlap (Waser and Strobeck 1998) and third, miss-assigned individuals may originate from populations that were not sampled in this study (Castric and Bernatchez 2004), although this is unlikely given the current knowledge on

broad whitefish spawning locations (see Tallman and Reist 1997 and references therein for a review).

Typically the accuracy of assignment tests are highly influenced by three factors: the number of loci employed the level of variability of these loci and differentiation between populations (e.g., F_{ST}, Cornuet *et al.* 1999; Berry *et al*. 2004). My assignment tests indicate that there is relatively weak divergence between some populations and this would correspond to the hypothesis that there is a high degree of straying or interpopulation dispersal in this system as was discussed previously. A high degree of straying is also evidenced by the low inter-population differentiation (F_{ST}), lack of genic differentiation observed in this study and the STRUCTURE results. Migration between populations can effectively be studied using assignment tests (Berry *et al.* 2004). Such studies have revealed that miss-assignment should be biased toward geographically proximate locations (i.e., miss-assignment should be more common among populations which are closer geographically, Castric and Bernatchez 2004). In terms of my study, this expectation was clearly not the case as the majority of miss-assigned anadromous fish in this system were assigned to the Peel River regardless of the sample origin.

The low to moderate F_{ST} values observed in my study and the moderate number of loci (7) used likely hindered the assignment success. F_{ST} values of 0.05-0.1 are typically reported for reasonable assignment success (Cornuet *et al.* 1999, Manel *et al.* 2002) depending on the number of loci, the mutation model and the assignment method used (Hauser *et al.* 2006). For example, Cornuet *et al.* (1999), using simulated data, achieved

100% assignment success with an F_{ST} value near 0.1, although they employed more loci (10 vs. 7) and higher H_E (0.60 vs. 0.53) compared to the current study. Hauser *et al.* (2006) studying the origin of hatchery verses wild steelhead trout had over 90% assignment success using eight microsatellite loci despite relatively low differentiation (F_{ST} = 0.02). On the other hand, Taylor and Costello (2006) employed 7 microsatellite loci and reported low overall assignment success (averaged 53.4% correct assignment among populations), such as has been reported in this study, and they attribute this in part to the low number of loci used since they had high values of population differentiation (e.g., F_{ST} = 0.60 between the Chehalis and Squamish Rivers). It is quite apparent that my study would have benefited from employing more polymorphic loci, which may have in turn increased multilocus differentiation between populations, both of which are correlated to increased assignment success (Cornuet *et al.* 1999; Berry *et al.* 2004). When differentiation between populations is quite low, such as the case in this study, it has been shown that the performances of assignment methods always improve with larger population samples and larger numbers of loci (Cornuet *et al.* 1999). Since the sample sizes of the reference populations in this study are sufficient for accurate assignment (Cornuet *et al.* 1999), increasing the number variable loci within populations would be extremely beneficial for increasing correct assignment of individual fish in this system.

Although regarded as an important step towards the effective management of broad whitefish in the Mackenzie River system (Tallman and Reist 1997), contributions to fisheries (mixed-stock assessment) have never been resolved. In my study, I was able to take advantage of a large subsistence fishery sample from very diverse locations

throughout the system to address questions regarding which populations contribute to the fishery and to what degree. In this study, the mixture analysis highlighted two clear results and provides evidence of the existence of a riverine life-history form of broad whitefish in this system.

First, my data indicate that all populations outlined previously as the major spawning stocks (Tallman 1997) do contribute fish to this fishery. Second, although all populations contribute to the fishery, the catch appears to be dominated by fish from the Peel River. Overall, the Peel River contributed the most to the subsistence fishery in this system, and when mixture analysis was conducted on specific regions (e.g., east channel verses west channel) the Peel River was also important to the fishery. This system is the first major river system broad whitefish encounter during upstream spawning migrations, so it is perhaps not surprising that the Peel River would contribute the most. In a study in which 113 broad whitefish were physically tagged near the town of Aklavik, four fish were recaptured: one in the Aklavik channel downstream of the townsite, two in the Peel River and one in the Arctic Red River (Babaluk *et al.* 1997). The results of my study are consistent with the tagging study, although sample sizes were quite limited in the latter. The following year, 1225 broad whitefish were tagged at Horseshoe Bend and although the majority of these fish were recaptured at the tagging location, fish were also recaptured in the Peel River, the Arctic Red River and at the spawning location near Fort Good Hope also indicating that more than one population contributes to the fishery.

Radiotelemetry studies have suggested that Point Separation is an important spawning location for broad whitefish in this system (Chang-Kue and Jessop 1997); however, this was not apparent in the current genetic study. First the timing of sampling may help explain this. The "learning" samples collected at Point Separation may have been collected before the Point Separation population reached these spawning grounds, and therefore my learning sample may contain other populations migrating through this section of river on their way to spawning locations upstream on the Peel, Arctic Red and Mackenzie rivers. Radio-telemetry data has shown that spawning at Point Separation takes place in late October and early November (Chang-Kue and Jessop 1997). The Point Separation sample was collected at the end of September, allowing for the possibility that other populations were potentially being harvested. It is, however, extremely difficult to collect samples in the main channel of the Mackenzie River, such as Point Separation, at the end of October or early November because of the dangers associated with sampling during freeze-up at that time of year (Sonny Blake, Gwich'in Beneficiary, personal communication.). Second, the authors of past radio-telemetry studies (i.e., Chang-Kue and Jessop 1997) also hypothesized that extensive gill netting in the area at the time of their radio telemetry studies may have caused some broad whitefish to artificially spawn at Point Separation, giving the false impression that this area is an important spawning location (R. Tallman, Fisheries and Oceans Canada, Winnipeg, MB, personal communication). As such, my results may not be that surprising. In fact, these authors have indicated that they never really considered Point Separation as having the same characteristics as the Peel or the Arctic Red rivers in terms of features important for

broad whitefish spawning (R. Tallman, Fisheries and Oceans Canada, Winnipeg, MB, personal communication).

A large majority of broad whitefish captured upstream in the Mackenzie River (e.g., at Tree River) were estimated to have been composed of fish from the Peel River. The expectation was that most of these fish would be assigned to the next downstream spawning population at Fort Good Hope, but this was not the case. Even though 24% of these fish were apparently derived from the Fort Good Hope population, 71% of these fish appear to be of Peel River origin. This may potentially be explained by a river-resident life history form of broad whitefish in the Mackenzie River that has yet to be confirmed. It is unlikely that broad whitefish would migrate that far upstream of the Peel River, and then migrate back to that system to spawn given the energetic requirements of migration. One would assume that, if anadromous, the fish captured well upstream of the Peel River are likely individuals of the Fort Good Hope spawning population, yet only 24% of these fish were attributed to that location. Instead, I propose that there may be a potential riverine population in the Mackenzie River system that uses the Peel River to spawn. There is no confirmation of the existence of this life history in the area, but given the size and complexity of this system, the existence of riverine form would not be surprising. Babaluk and Reist (1996), assessing otolith strontium distribution in broad whitefish otoliths throughout the Mackenzie River system, found that some fish inhabiting the Peel River appeared to be of a completely freshwater form. It is unknown if those fish were Peel River residents or a Mackenzie River resident form that migrates to the Peel River during spawning season. Regardless, my GMA results corroborate the

suggestion by Babaluk and Reist (1996) of the existence of a freshwater-resident form and suggest that there is a higher level of life history complexity in this system than previously appreciated. A riverine form of this species exists in Siberia (Berg 1962), and given its distribution in the Yukon River system (Scott and Crossman 1998) a riverine form likely exists in Alaska as well. Future radio-telemetry or physical tagging studies, or perhaps otolith microchemistry investigations, will be able to provide some insight into this idea of a riverine life history form in the Mackenzie River system.

Results of the mixture analysis in the Travaillant Lake system are consistent with previous radio-telemetry data (Harris and Howland 2005), although a great deal of temporal variation was observed. Consistent with this previous radio-telemetry study, the spawning population upstream of Travaillant Lake contributed the most to this lake fishery. In contrast, although sample sizes were low, the mixture analysis indicated that the majority of Travaillant Lake samples collected in 2004 were derived from the southern spawning population in the Travaillant River downstream of Travaillant Lake, an area thought to be a spawning location for Andrew Lake fish to the south. In 2005 the northern spawning population in the Travaillant River upstream of the lake made up the majority of the Travaillant Lake fishery. The results of 2005 GMA analysis are congruent with previous radio-telemetry data that showed the majority of Travaillant Lake broad whitefish migrated to the Travaillant River upstream of the lake to spawn (Harris and Howland 2005). Because there are several spawning populations in this system that are separated by no more than 30 km (Harris and Howland 2005), this may allow for a high degree of straying between them, which could account for the temporal variation

observed with the mixture analysis. Furthermore, as has been suggested for anadromous populations of broad whitefish (Bond and Erickson 1985; Tallman *et al.* 2002), broad whitefish in this system may not spawn annually and perhaps use different areas to spawn depending on the year. Continuing radio-telemetry studies in the area will perhaps be able to provide more detailed information on habitat use in this system and frequency of spawning.

Conclusions

This chapter provides the first comprehensive study of the fine-scale genetic population structure of broad whitefish in the Mackenzie River system, and is the largest study to date focussing on North American broad whitefish. Virtually all analyses conducted in this study have provided evidence indicating that populations of lacustrine broad whitefish from the Travaillant Lake system are genetically divergent from anadromous populations in the Mackenzie River system. This is undoubtedly the result of the lacustrine life history of Travaillant Lake fish which has likely isolated them from their Mackenzie River counterparts for some time, resulting in restricted gene flow between these groups of fish that in turn is promoting divergence. This divergence, however, may be relatively recent given the low (but significant) F_{ST} values observed. Surprisingly, populations from the Mackenzie River system were not genetically differentiated which is likely due to possible straying among spawning populations, the broadcast spawning nature of this species coupled with extensive migrations and possibly because a strong signal of historical gene flow still persists given the recent invasion to this system. Lastly, the genetic mixture analysis highlights two important findings. First, the Peel River system contributes the most to the mixed-stock fishery for broad whitefish in the

Mackenzie River system which will likely have several management and conservation implications (discussed in Chapter 4). Second, the GMA results have provided evidence that there may be the existence of a riverine life history form of broad whitefish in the Mackenzie River system, although future radio-telemetry and otolith micro-chemistry studies would be useful to further confirm this.

Table 7. Individual and average values for the expected and observed heterozygosities (H_E and H_O) and the number of alleles per locus (N_A) for each locus (Mackenzie River system broad whitefish only).

Locus	H_E	H_O	N_A
Cocl-Lav4	0.61	0.62	11
Cocl-Lav6	0.41	0.40	11
Cocl-Lav8	0.62	0.61	21
Cocl-Lav10	0.60	0.54	9
Cocl-Lav18	0.48	0.49	15
Cocl-Lav27	0.42	0.42	5
Ots 103	0.66	0.60	11
Average	0.54	0.53	11.86

Table 8. Above diagonal: genetic differentiation among pairs of populations. Non-significant (NS) and significant (**, $P < 0.05$). Below diagonal: pairwise F_{ST} (θ) comparisons among all pairs of populations. Underlined values represent comparisons that are not significant ($P = 0.05$) based on the permutation process. 1 = Peel River, 2 = Arctic Red River, 3 = Point Separation, 4 = Mackenzie River at Fort Good Hope, 5 = Campbell Lake, 6 = Travaillant Lake South and 7 = Travaillant Lake North.

Population	1	2	3	4	5	6	7
1	-	NS	NS	NS	NS	**	**
2	0.0092	-	NS	NS	NS	**	**
3	0.0187	0.0365	-	NS	NS	**	**
4	0.0052	0.02	0.0002	-	NS	**	**
5	0.0024	0.0058	0.0255	0.0112	-	**	**
6	0.0418	0.043	0.0487	0.0339	0.0602	-	NS
7	0.0376	0.0306	0.049	0.0302	0.0393	0.0051	-

Table 9. Mean likelihood scores, their standard deviations and posterior probabilities from 20 iterations of the structure program for each hypothesized number of populations (*K*) of broad whitefish inferred from variation at seven microsatellite loci. Bold values are the most likely population structure.

Samples Included	K	log-likelihood	SD	Posterior Probability of *K*
Entire Mackenzie River System	1	-14571.9	0.20	0.0
	2	**-14531.3**	**47.79**	**1.0**
	3	-14611.5	123.83	0.0
Mackenzie River System Without Travaillant Lake	**1**	**-11619.4**	**0.32**	**1.0**
	2	-11663.3	41.56	0.0
	3	-11975.1	103.39	0.0
Travaillant Lake	**1**	**-2600.0**	**1.02**	**1.0**
	2	-2615.6	9.89	0.0
	3	-2727.1	83.54	0.0
Peel River	**1**	**-2665.2**	**0.65**	**1.0**
	2	-2681.4	7.53	0.0
	3	-2712.0	32.60	0.0
Arctic Red River	**1**	**-3612.2**	**0.18**	**1.0**
	2	-3631.8	9.11	0.0
	3	-3688.3	38.95	0.0

Table 10. Results from the self-assignment tests: only anadromous populations (A), comparisons between the Mackenzie and Travaillant systems (B), Travaillant Lake sytem over all years (C), and the Travaillant Lake system in 2004 (D) and 2005 (E). PEEL = Peel River, ARR = Arctic Red River, PT SEP = Mackenzie River at Point Separation, FGH = Mackenzie River at Fort Goodhope and TravNorth and TravSouth = the Travaillant River north and South of Travaillant Lake respectively.

A

Sampled in	Excluded from all rivers	Assigned to (%)			
		1	2	3	4
1. PEEL	2.9	**69.1**	1.7	13.7	12.6
2. ARR	0.0	62.9	**17.1**	17.1	2.9
3. PTSEP	4.3	47.8	4.3	**39.1**	4.3
4. FGH	2.9	50.0	0.0	5.9	**41.2**

B

Sampled in	Excluded from all rivers	Assigned to (%)	
		1	2
1. Mac. R. System	2.6	**95.3**	1.1
2. Trav Lk. System	2.2	48.1	**51.9**

C

Sampled in	Excluded from all rivers	Assigned to (%)	
		1	2
1. TravNorth	3.1	**76.9**	20.0
2. TravSouth	4.3	37.7	**58.0**

D

Sampled in	Excluded from all rivers	Assigned to (%)	
		1	2
1. TravNorth (2004)	5.7	**80.0**	14.3
2. TravSouth (2004)	15.0	55.0	**30.0**

Table 10. Continued

E

Sampled in	Excluded from all rivers	Assigned to (%)	
		1	2
1. TravNorth (2005)	0.0	**73.3**	26.7
2. TravSouth (2005)	10.5	47.4	**31.6**

Table 11. Results of the genetic mixture analysis showing the estimated percent contributions of source populations of broad whitefish to fish captured during summer and fall subsistence fisheries from various geographic locations from the lower Mackenzie River and Mackenzie River Delta (A) and from Travaillant Lake during a concurrent populations assessment (B). The values represent the mean estimated percent contributions (±1 standard deviation) from 5000 simulated mixtures from the baseline data (N = 1000 fish each). Peel = Peel River, ARR = Arctic Red River, PtSep = Mackenzie River at Point Separation, FGH = Mackenzie River at the town of Fort Good Hope, TravSouth = Travaillant River South downstream of Travaillant Lake, TravNorth = Travaillant River South upstream of Travaillant Lake.

A

	Peel	ARR	PtSep	FGH
All Mackenzie Samples	**54** (9)	**25** (7)	**5** (4)	**16** (7)
Beaufort Sea *	**27** (8)	**67** (8)	**2** (2)	**4** (4)
East Channel **	**57** (9)	**19** (7)	**14** (6)	**9** (6)
West Channel **	**58** (10)	**6** (5)	**7** (5)	**28** (9)
Middle Channel **	**52** (10)	**18** (7)	**3** (3)	**26** (8)
Mouth of Peel **	**50** (9)	**29** (8)	**18** (6)	**2** (3)
Mackenzie River ***	**71** (8)	**3** (3)	**2** (2)	**24** (8)

B

	TravSouth	Trav North
Travaillant Lake (2004 and 2005)	**31** (9)	**69** (9)
Travaillant Lake (2004)	**69** (7)	**31** (7)
Travaillant Lake (2005)	**3** (3)	**97** (3)
Andrew Lake	**75** (10)	**25** (10)

* includes samples from several locations in the Tuktoyuktuk Harbour
** indicates the different channels of the Mackenzie River Delta
*** includes all Mackenzie River sampling sites upstream of the Arctic Red River

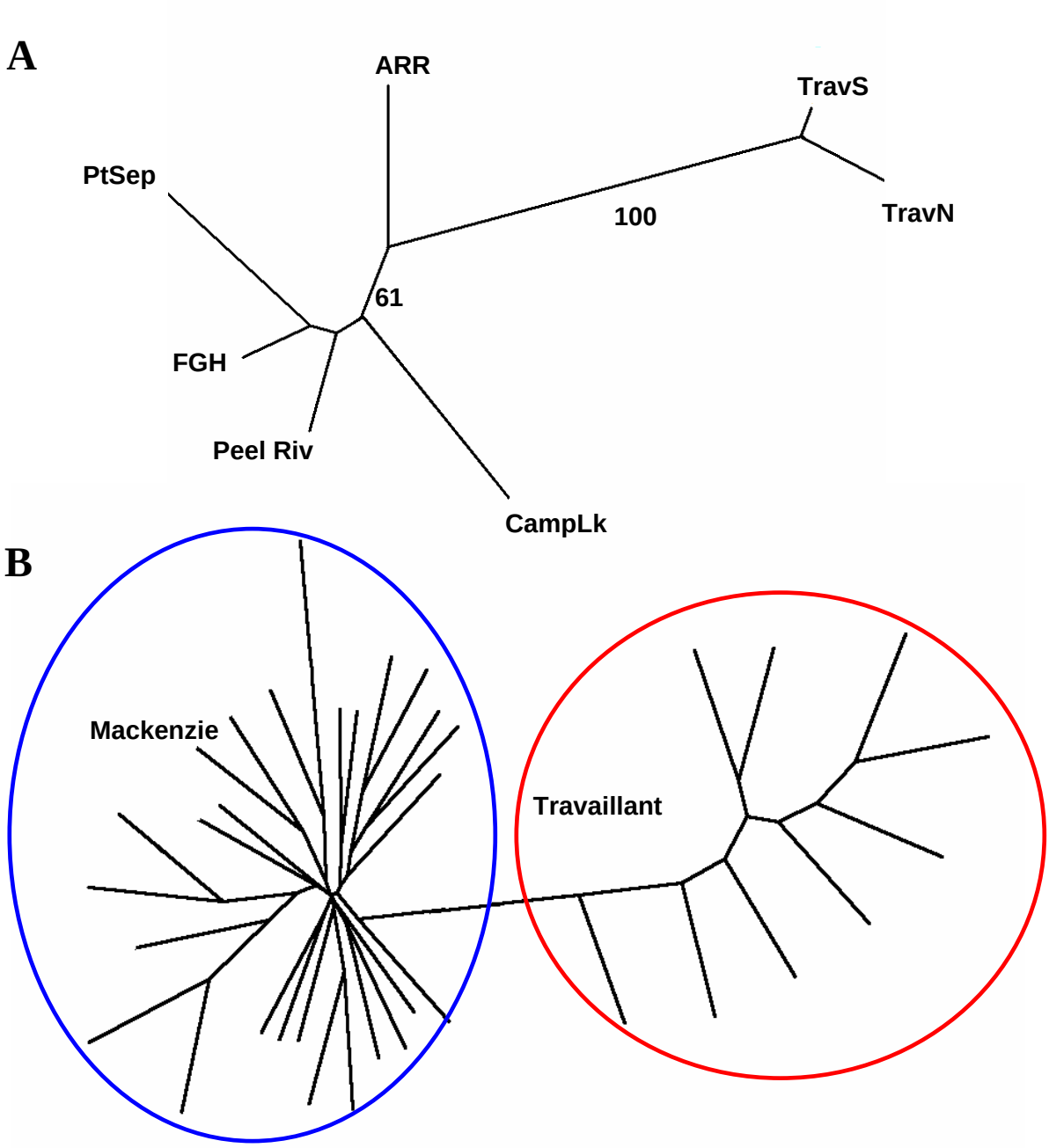

Figure 9. **.** Neighbour-joining tree based on Cavalli-Sforza and Edwards (1967) chord distances for broad whitefish population surveyed in this study. Only *a priori* designated populations based on previous literature (A) and all sample sites in the Mackenzie River system (B). Bootstrap values greater than 50% are shown. Dotted lines show geographic region. PeelR = Peel River, ARR = Arctic Red River, PtSep = Point Separation, FGH= Mackenzie River at Fort Goodhope, CampLk = Campbell Lake, TravS = Travaillant Lake South and TravN = Travaillant Lake North.

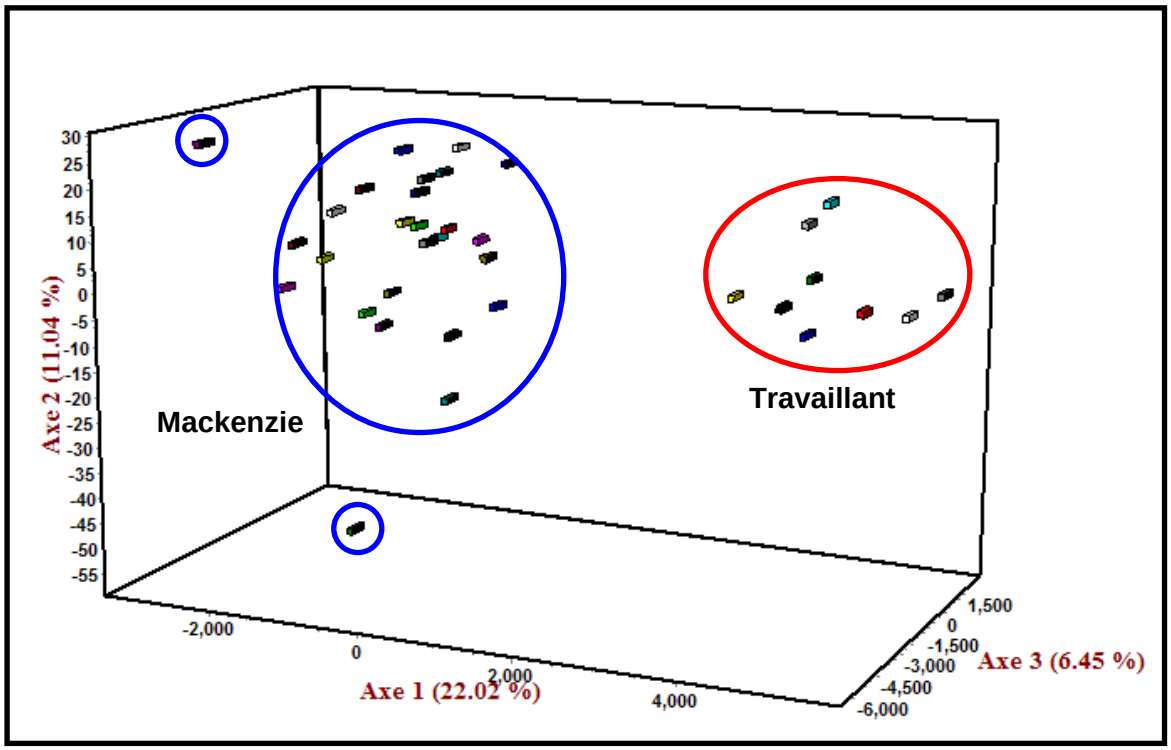

Figure 10. Results of the factorial correspondence analysis (FCA), showing differentiation between Travaillant Lake system and Mackenzie River system broad whitefish.

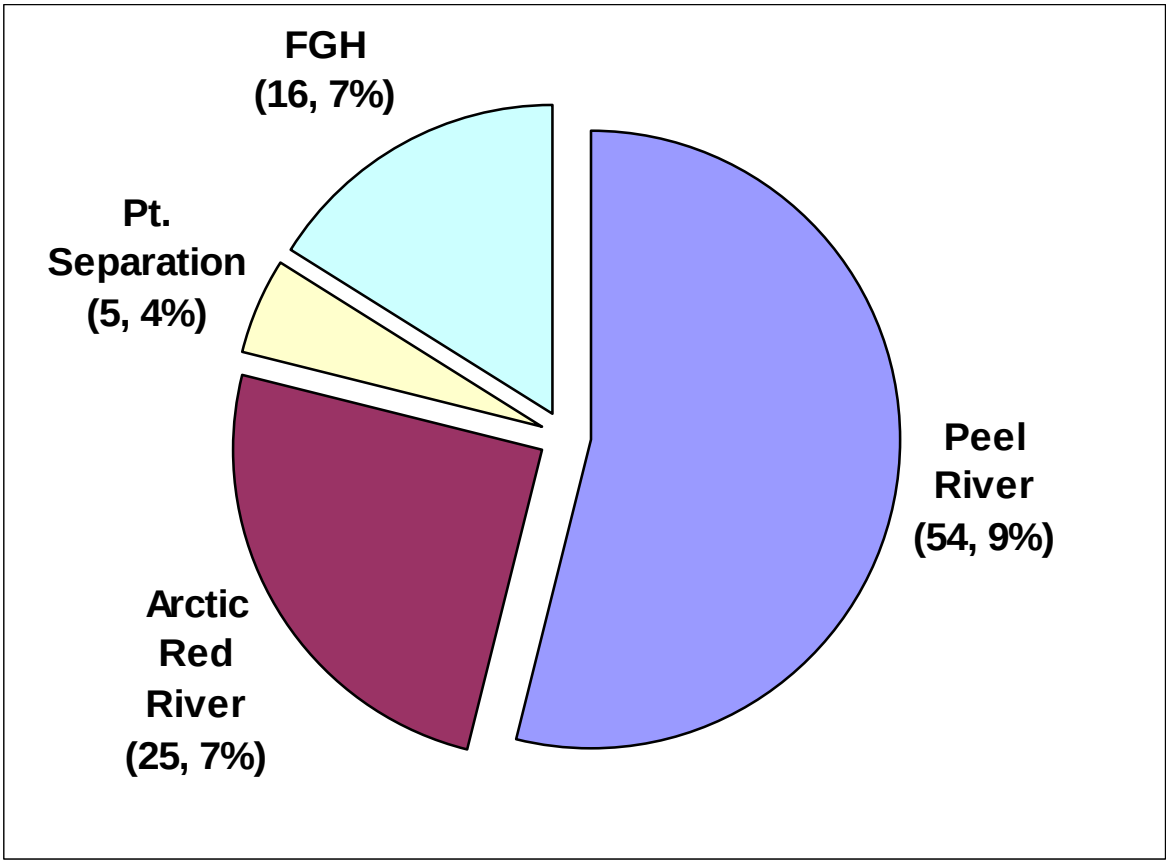

Figure 11. Results of the genetic mixture analysis showing the estimated percent contributions of source populations of all broad whitefish to fish captured during summer and fall subsistence fisheries from the lower Mackenzie River in this study. The values represent the mean estimated percent contributions (±1 standard deviation) from 5000 simulated mixtures from the baseline data (N = 1000 fish each) and represent variability about the empirical estimated percentages of 55.4%, 25.1%, 3.8%, and 15.8% for the Peel River , Arctic Red River, Mackenzie River at Point Separation (Pt Separation) and Mackenzie River at Fort Good (FGH) respectively.

CHAPTER 4: CONSERVATION IMPLICATIONS AND FINAL CONCLUSIONS

Conservation Implications

In the Arctic, the resolution of taxonomic relationships among many north temperate species is quite poor and, in many case, the knowledge of variation within such species is also not known (Tallman and Reist 1997). With respect to coregonids, and especially broad whitefish, the management problems pertinent to these species of the lower Mackenzie River and surrounding water bodies are, in many cases, a complex array of unresolved taxonomic issues, poorly known life history accounts and a significant lack of accurate distributional information (Reist and Bond 1988). This has underlying ecological and utilitarian importance in that coregonids play a principal role in food chains of the tundra zone (Politov e*t al.* 2000) in addition to being of great importance culturally and in subsistence (Freeman 1997) and commercial fisheries (Treble and Reist 1997) in North America and Eurasia. In addition, broad whitefish exhibit a complex and diverse range of life histories (Reist and Chang-Kue 1997; Harris and Howland 2005, this study) which may complicate the development of a general set of management guidelines (e.g., Primmer *et al.* 1999). The results of my study highlight several important implications for the management and conservation of such a culturally, commercially and ecologically important fish species.

One application of genetic data is the development of appropriate conservation guidelines or management plans aimed at defining or recognizing so-called evolutionarily significant units (ESU; Waples 1991; reviewed in Fraser and Bernatchez 2001) and or management units (MU; Moritz 1994). Although revised several times since it was first

proposed by Ryder (1986), an ESU can be defined as a population (or set of populations) that is 'reciprocally monophyletic for mtDNA alleles' and 'shows significant divergence of allele frequencies at nuclear loci' (Moritz 1994) and 'represents an important component in the evolutionary legacy of the species' (Waples 1991). Evolutionary significant units are deep historical lineages that represent long-term divergences (i.e., historically isolated for quite some time), or more recently they have been defined as a lineage demonstrating highly restricted gene flow from other such lineages within the higher organizational level (lineage) of the species (Fraser and Bernatchez 2001). Management units, on the other hand, are defined as populations connected by little or no contemporary gene flow, but not separated historically for very long periods of time (Waples 1991; Moritz 1994). The focus of the MU is on contemporary population structuring and short-term monitoring rather than historical factors (Fraser and Bernatchez 2001). Although a rigid, universal definition of such units across all species may not be possible (Fraser and Bernatchez 2001), both levels of genetic divergence should aim to preserve the genetic bioheritage and potential of a species (Bowen 1999).

In Canada, ESUs, however, are not recognized from a legislative point of view. Rather, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has provided a more practical concept of a unit of conservation below the species level, thus accurately conserving species biodiversity, with a more structured approach for identifying such units (herein referred to as designatable units, DUs, COSEWIC 2005). As such, DUs provide the concept that is recognized by the law (i.e., Species at Risk Act (SARA)). Although this unit of conservation takes into consideration some of the

qualifications described above for discerning previously defined units of conservation (e.g., similar to an ESU the unit is important to the evolutionary legacy of the species as a whole) the concept of identifying DUs also includes data on morphology, physiology, and ecology including life history variation, range disjunctions among populations and habitat (COSEWIC 2005). Simply conveyed, a species, subspecies or taxonomic unit below that of a species or subspecies that is being considered for designatable conservation status (i.e., is a putative designatable unit (PDU), Taylor 2006)) warrants classification as a DU if it fulfills at least one of the following criteria as described by Taylor (2006): (1) it is a distinct taxonomic entity or qualifies as an distinct biological species, (2) represents a major phylogentic lineage or grouping within the species, (3) has a distinctive and rare trait or traits (behaviour, life history, physiology, morphology) that represents local adaptation, (4) occupies a range that is significantly disjunct from other populations of the species and (5) inhabits a different aquatic ecoregion (Taylor 2006). Taking these criteria into consideration it appears that several broad whitefish populations resolved in the sampling scheme of this study undoubtedly fit the classification of a DU for several reasons discussed in subsequent sections. Similar to other concepts, DUs intend to prevent wildlife species from becoming extinct or extirpated and while aiming to conserve biodiversity for taxonomic units at and below the species level (COSEWIC 2005).

The results of the current study have several implications for conservation and management of North American, but more specifically, Mackenzie River system broad whitefish. Since the NJ and FCA analyses divide the populations into four geographical

groups (Pechora River, samples from Alaska, anadromous Mackenzie River samples and lacustrine Travaillant Lake samples in the Mackenzie system) this implies that those groups that exist in Canada should be managed as separate units as described above (i.e., separate DUs). In this study, the sampling scheme was limited in several areas, for example, samples from the western Bering Sea area consisted of a single sample from the Pechoran River. In addition, the Alaskan samples included several locations, but they were highly isolated geographically from all other localities. In light of that, a more sensitive analysis (e.g., more sampling locations encompassing both macro- and micro-scale comparisons) would be warranted before specific recognition of major lineages (DUs or ESUs) across such a broad region. For that reason, conservation inferences deduced from this study focus on the Mackenzie River system.

Within the Mackenzie River system, population structure was investigated on a much finer geographic scale and several points regarding the management of broad whitefish in this system are highlighted. First is the clear genetic distinction observed between anadromous and lacustrine broad whitefish which indicates that more than one management unit occurs in this system. Virtually all analyses (dendograms, FCA, genic differentiation, etc.) show concordance in this divergence and indicate that each could be considered as a single management unit relative to each other. Although all Mackenzie River system broad whitefish appear to belong to a single evolutionary lineage (i.e., all from the same glacial lineage), sufficient divergence between anadromous and lacustrine populations was demonstrated, therefore, the hypothesis that all populations in this system should be considered as genetically equivalent for conservation can be rejected.

My data argue that there are two distinct units of conservation (DUs) that should be considered important in terms of management in this system: (1) anadromous populations of the Mackenzie River and its tributaries and (2) lacustrine populations from the Travaillant Lake system.

It is, however, evident that these populations of broad whitefish within the Mackenzie River system have not been isolated from each other for a long time as indicated by the lack of novel mutations in the Travaillant Lake populations (see Fraser and Bernatchez 2001), but within each, unique local adaptations may have evolved, which would warrant their separate conservation status. For example, Travaillant Lake populations, are clearly adapted to live solely within freshwater, although salinity tolerance differences between anadromous and lacustrine populations of broad whitefish have yet to be tested. In inconnu, *Stenodus leucichthys*, another Arctic coregonid, Howland *et al.* (2001), showed experimentally that there are marked differences in salinity tolerance between anadromous populations and those that are solely freshwater, with the latter showing diminished salinity tolerance. Similar analyses conducted on anadromous and lacustrine broad whitefish would be beneficial in understanding the existence of locally adapted traits, if any have evolved within these groups. For example, the Travaillant Lake system consists of roughly a series of approximately a dozen lakes, although to the author's knowledge, only two of these are inhabited by broad whitefish even though no physical barriers to migration exist. Broad whitefish may, however, have specific habitat requirements that are not provided by these apparently unoccupied lakes.

Several other lakes in the Travaillant Lake system do contain populations of the closely related, lake whitefish, which can surely be attributed to the fact that they are of a different glacial refugial origin (see Dodson and Bernatchez 1991). Lake whitefish in this area Mississippian in origin (Dodson and Bernatchez 1991), in contrast to Beringian broad whitefish, and may have had access to the area through extensive glacial lake connections, well before broad whitefish inhabited the area, which would explain their larger distribution. Broad whitefish populations are at the utmost periphery of their range in the Mackenzie River system, and if post-glacial colonization is still occurring in this system, locally adapted traits such as those associated with a solely freshwater lifestyle, will be extremely important in the habitation of these novel freshwater environments. Adaptation to local environments however will also essentially depend on the relative rates of gene flow, selection and genetic drift (Castric and Bernatchez 2004). Additionally, conservation of Travaillant Lake fish may be particularly important to because peripheral populations are often the most genetically divergent they may harbour distinct traits important for adaptation to changing environments or local or new conditions (Taylor *et al.* 2003). Given the above information, the Travaillant Lake system therefore represents an important unit of conservation in the Mackenzie River system. Finally, the results of my study emphasize the importance of considering life history variation when developing conservation strategies. Travaillant Lake fish are clearly reproductively isolated from anadromous Mackenzie populations, and since this limits the possibility for new genetic diversity via gene flow, it is expected that these lacustrine populations may be more vulnerable to extinction following a population crash (Tonteri

et al. 2007). As such, high conservation status should be given to populations that exhibit this life history, at least in the Mackenzie River system.

On the other hand, my analyses suggest that all anadromous populations in the Mackenzie River system should be managed as one unit. Lack of genic differentiation and low F_{ST} values between anadromous populations and the tight clustering within the NJ tree and FCA all indicate these broad whitefish clearly consist of a single unit of conservation. Ideally, all populations should be treated as independent management units, maintaining maximum genetic and phenotypic diversity, thereby preserving all local adaptations, however, this is not always possible given limited management resources in many situations. Consequently, in the absence of any other data (e.g., morphological, ecological, physiological, etc.) anadromous populations of the Mackenzie River system appear to represent a single DU and although individual population management units represent much shallower divergences than do DUs, the anadromous populations of broad whitefish in this system may only comprise one management unit as well. If all anadromous populations are taken to be equal with respect to conservation, then priority for protection, as recommended by Bernatchez (2005) should be given to those that are particularly threatened by human impact. This may mean protecting those populations most harvested during subsistence fisheries (e.g., the Peel River), those that may contain unique life history variants (potential riverine stocks of the Peel River; see Chapter 3) or those most likely to be impacted by oil and gas exploration and development (e.g., populations in closest proximity to construction of the potential Mackenzie Valley pipeline). Additionally, similar to the lacustrine populations of Travaillant Lake,

anadromous or riverine populations in this system also represent fish near the eastern periphery of their range, and therefore may also be important to conservation because they may exhibit unique adaptations to marginal environments and because there is increasing evidence that species may collapse to the periphery of their ranges, not the geographic centre (Lesica and Allendorf 1995; Channell and Lomolino 2000). My data clearly indicate that population structure is only weakly developed in anadromous Mackenzie River broad whitefish, possibly due to the recent colonization of this system . The apparently high levels of gene flow, as indicated by low divergence and poor assignment success, in any particular year suggests that, regardless of the level of genetic structure, habitat preservation of current and recent spawning areas must still be a high priority (e.g., Maclean *et al.* 1999).

Major mixed-stock broad whitefish subsistence fisheries exist in the Beaufort Sea, the Mackenzie River Delta and the Mackenzie River covering three different aboriginal land claim settlement areas (Inuvialuit, Gwich'in, Sahtu). The results of mixture analysis in this study bring up several important management considerations. First, even though all populations contribute to subsistence fisheries of the Mackenzie Delta and Mackenzie River, my data indicate that the fishery is dominated, for the most part by Peel River fish. In the absence of any population estimate data, the results of my study suggest that it may be very important to define and protect critical habitats, such as those used for spawning, in the Peel River system. Second, since the results of my thesis showed that contributions to the subsistence fishery are variable depending on the harvest location within this system (i.e., east, west, or main channel), different management regimes may be useful,

depending on the fishing area. Because this subsistence fishery spans three land claims, coordination among the co-management boards within these regions is a necessity for effective management of the species. Third, the results of the mixed-stock analysis have provided evidence that there may be a riverine life-history form of broad whitefish present in this system, something that has never been confirmed in this area. Conservation of such populations may be important as they could represent a distinct evolutionary lineage, separate from lacustrine and anadromous populations of the system, or they may have evolved locally adapted traits important for a riverine life history. Ideally future radio-telemetry or otolith microchemistry studies can provide more insight into the possibility of this in the Mackenzie River system, which will help guide specific management decisions

The results of this study will also be important for the management of Mackenzie River system broad whitefish in an area that has been the focus of increased exploration and development of both renewable and non-renewable resources. Such an example is the Mackenzie Valley pipeline that is proposed to carry natural gas from the Mackenzie River Delta to the Alberta-Northwest Territory border, passing directly through or within the vicinity of several areas of fisheries importance, including the Travaillant Lake system. Pipeline construction can result in complicated and often long term effects on aquatic environments, particularly on fish (Stein *et al.* 1973) and consequently there is local concern that the construction of the Mackenzie Valley pipeline will cause irreversible negative impacts on fish and water quality in local lakes and other important tributary systems of the Mackenzie River by potentially adding contaminants, increasing

sedimentation and erosion, and increasing access to these otherwise inaccessible areas (Harris and Howland 2005). Due to these impacts, there was a need to classify population structure and describe the current genetic diversity of important fish species such as broad whitefish in this system. The genetic information collected in my thesis will be essential for the management of fisheries in the face of potential industrial impacts and will allow for the post-development monitoring of potential changes in fish and fish behaviour. Additionally, more sensitive analyses (e.g. larger samples, more variable loci, more sampling locations) may produce more powerful tests of genetic differentiation among populations in local geographical areas, which may increase precision and effectiveness of any conservation strategy. Finally, it would be extremely important to assess the genetic population structure of broad whitefish on similar geographical scales in other regions of North America, in order to determine if the low levels of genetic differentiation demonstrated within Mackenzie River broad whitefish is a common feature across the natural range of this culturally important coregonid species.

Final Conclusions

This thesis provides the first comprehensive study on the genetics diversity of broad whitefish in North America, assessing the population structure of this species at two different scales: a large scale including populations from throughout North America (Chapter 2), and a much finer scale focussing on populations found solely within the Mackenzie River system (Chapter 3). Furthermore, this thesis clearly exemplified that microsatellite DNA markers can be extremely informative in resolving evolutionary relationships and in determining how evolutionary forces have acted to shape the current portioning of genetic variation within and among populations of broad whitefish.

In the second chapter of my thesis, I evaluated how historical processes have influenced genetic variation in broad whitefish populations throughout their North American range and have proposed how evolutionary forces, namely migration and drift, have been responsible for, and are still continuing to shape patterns of genetic diversity in this species. My data contribute to understanding the complex nature of glacial refugia in Arctic regions of North America and how the evolutionary processes associated with such events have impacted a single species. The genealogical evidence provided in this chapter suggest that broad whitefish from North America likely survived Pleistocene glaciations in one refugium (Beringia) and therefore comprise a single glacial race as evidenced by the overall lack of unique alleles and low estimates of population divergence (F_{ST}) in virtually all of the North American populations. Dispersal, post-glacially to the Mackenzie River system from this refugium, appears to have ensued in an eastward direction via coastal marine environments in a stepping-stone like pattern of dispersal instead of through freshwater via Porcupine-Peel River connections. A progressive decline in genetic diversity with distance from putative refugia was observed in this study which is undoubtedly the result of postglacial range expansion (Bernatchez and Wilson 1998; Stamford and Taylor 2004) where founder events and loss of diversity due to drift would be pronounced. Populations at the periphery of the range (i.e., those from the Mackenzie River and Travaillant Lake system) showed the lowest levels of genetic diversity which is consistent with theoretical and empirical expectations (Bernatchez and Wilson 1998; Costello *et al.* 2003; Taylor *et al.* 2003; Stamford and Taylor 2004).

Isolation-by-distance patterns observed in this study were highly variable depending on the geographic region of the populations assayed. Those in inhabiting area covered by the putative Beringian refuge are in equilibrium and gene flow while those from the Mackenzie River system are still far from migration-drift equilibrium. These populations are the periphery of the range and therefore it is quite plausible that sufficient evolutionary time has not passed since postglacial colonization for IBD patterns to develop. In the Mackenzie River it is likely that the historical influence of gene flow as a result of a very recent homogenous founding population(s) is still much stronger than the influence of drift (Hutchison and Templeton 1999). Finally most results have shown that there are major genetic discontinuities between the main geographic areas sampled in this study. That is, populations from Russia, populations from Alaska and those from the Mackenzie River system were all genetically divergent from each other as evidenced by the allele frequency distributions, genic differentiation, AMOVA and results of the NJ tree and FCA. Although there was divergence among these regions, and it is clear that there is little contemporary gene flow Alaska and the Mackenzie River system populations, more thorough sampling especially throughout Alaska will be beneficial in further resolving genetic signatures of both long-term and short-term isolation in refugia and postglacial range expansion.

In chapter three, I assessed fine-scale population structuring of broad whitefish solely within the Mackenzie River system, and I provided the first account of the genetic population structure of this species in this system using microsatellite markers. First, my

results provided evidence indicating that populations of lacustrine and anadromous populations inhabiting this system are clearly divergent which is consistent with previous morphological, life history, otolith microchemistry and allozymes data (Hesslein *et al.* 1997; Reist 1997; Chudobiak *et al.* 2002; Tallman *et al.* 2002). This is the result of restricted gene flow owing to the differences in life history between these populations and therefore my study highlights the importance of life history traits (anadromy vs. non-anadromy) in the genetic structuring of these populations. The genetic divergence between these populations, however, is likely quite recent given the low (but significant) F_{ST} values observed. Populations from the Travaillant lake system also showed reduced levels of genetic diversity compared to Mackenzie River populations, which can also be attributed to a recent colonization in which founder events associated with dispersal into new habitats are pronounced. Surprisingly, in this study, and in contrast with previous research (Reist 1997), Mackenzie River system populations were not genetically differentiated. Straying among populations and possibly because a strong signal of historical gene flow still persists given the recent invasion to this system, likely explains this lack of differentiation. Finally, in this chapter, my genetic mixture analyses highlight the importance of the Peel River in contributing to the Mackenzie River mixed-stock fishery and have provided preliminary evidence that there may be the existence of a riverine life history form of broad whitefish in this system.

Lastly, this thesis highlights how genetic data, specifically those generated from microsatellite markers, may be relevant when considering the management and conservation of a species. For example, my data provides an objective way to discern

units of management within the Mackenzie River system. According to the criteria of several authors (e.g., Waples 1991; Moritz 1994) within the Mackenzie River system, populations from Travaillant Lake and those from the Mackenzie River system comprise separate evolutionarily significant units (ESUs) and therefore could be managed separately for conservation motivation (DUs). In the Mackenzie River system, although several populations would then be included in one in one management unit (e.g., Peel and Arctic Red Rivers), priority for conservation could then be given to those populations with, for example, unique life history variants, those that contribute the most to local fisheries or those most likely to be impacted by future development. The genetic data resolved in this study would then suggest high conservation status be given to the Peel River population which contributes the most fish to the subsistence broad whitefish fishery of the lower Mackenzie River system or perhaps the potential riverine life history form of the Mackenzie River proper. Further studies, however are needed to further confirm the latter.

Overall this thesis has contributed to an increased understanding of the forces shaping the genetic populations structure of a high latitude species. My data also contribute to a better understanding of Beringia, and how isolation in, and post-glacial dispersal from, have impacted the genetic diversity in an Arctic species that survived solely within this refugium. This study therefore highlights the importance of considering biogeographic perspectives is essential for understanding how patterns of genetic variation within and among population has been shaped, throughout the history of a species. My study illustrates the complex nature of population structure in an Arctic coregonid that is

probably characteristic of most species that have dispersed from glacial refugia, especially for populations at the periphery of their range where range expansion is still continuing. Taken together, this thesis has shown how examination of population structure at different spatial scales is necessary for a comprehensive understanding of population structure and its implications for conservation.

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