Assessing Genetic Diversity of Lake Trout (*Salvelinus namaycush*) Populations

in Saskatchewan

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ABSTRACT

Climate change may lead to declines in lake trout (*Salvelinus namaycush*) populations and change the structure of the ecosystem in which they live. The lake trout is a keystone species in ecosystems of northern temperate lakes and these declines may subsequently reduce the genetic diversity found in these salmonids. Populations that contain greater genetic variation may have an increased capacity to adapt to changes in the ecosystem. Therefore, an understanding of the genetic diversity found in lake trout populations is required for their effective conservation and management. As a result, this study aimed to examine the genetic diversity and phylogeography of lake trout populations in north central Canada.

The genetic diversity of lake trout from 19 lakes in Saskatchewan was examined using partial regions of the ND2 and ND5 mtDNA genes. A total of 607 tissue samples were analyzed using PCR-based single stranded conformation polymorphism (SSCP) and DNA sequencing. Although the ND5 gene fragment had minimal intraspecific variation, eleven sequence types were detected in the ND2 gene. Each sequence type differed in relative frequency between and among the lake trout populations sampled.

One particular southern lake trout population, Crean Lake, had markedly different genetic composition in comparison to other lakes in the region. In the 1950's and 1960's, Crean Lake was stocked with lake trout from neighbouring Wassegam Lake in an attempt to increase population numbers. The sequence types of Crean Lake trout and their relative frequencies were dramatically different to those in Wassegam Lake. This suggests that the stocked fish may have been unsuccessful in their establishment/reproduction within Crean Lake. Lake trout from this lake also contained the highest frequency (44%) of rare ND2 sequence type "B". Sequence type B was only detected in one other lake (La Ronge), at a very low frequency.

The mutational changes in the eleven ND2 mitochondrial DNA sequence types represented three different amino acid sequence types. Substitutions of Threonine and Isoleucine occurred, resulting in two polar amino acids with much different hydropathy indexes. This may affect the tertiary structure of the protein, possibly indicating functional differences. Functionally different proteins may be exhibiting characteristics that allow lake trout to flourish in their environment.

The fragments of both the ND2 and ND5 genes proved to be valuable for phylogenetic analyses within the Salmonidae. The genetic markers established in the present study provide the basis for future work on population genetics of lake trout. It would be advantageous to broaden the area of study in order to compare the genetic diversity found within the study area to other regions of Canada. This would determine whether the genetic diversity detected in this study is significantly greater than in other populations at a national scale. Management strategies should ultimately attempt to conserve the genetic diversity found within the lake trout populations of north central Saskatchewan.

Chapter 1: Introduction

1.0   The effects of climate change on fishes and aquatic ecosystems
Climate change is predicted to cause significant alterations to ecosystems, including changes in biogeochemical processes, food web structure, and primary and secondary production (Arnell *et al.* 1996; Cushing 1997). Specific responses of aquatic ecosystems to climate change may include warming of waters, reductions in ice cover, reduction in dissolved oxygen in deep waters, changes in the interaction between waters and their watersheds, a greater frequency of extreme events (*e.g.* flood and drought). Changes in growth, reproduction, and distribution of organisms including the poleward movement of climate zones for organisms are also expected (IPCC 2001; Wrona *et al.* 2006).

Climate change and its resultant affects to aquatic ecosystems is accelerating the decline of fish populations (IPCC 2001) and is of primary concern for fish conservation and management. Fishes are probably one of the most threatened of all vertebrate groups with regard to environmental change, with freshwater species ten times more likely to be threatened than marine and brackish water species (Froese & Torres 1999).   By effectively monitoring these populations, further effects can be identified and the mitigation of negative effects is possible.

1.0.1 Temperature
Changes in temperature and precipitation have considerable potential to impact freshwater fishes (Regier *et al.* 1990). Such effects are likely more prominent in small rivers and lakes compared to those in large lakes and rivers. Schindler *et al.* (1990) reported that some boreal lakes have seen an increase in temperature by 1-2°C from 1969-1988. These warmer conditions make aquatic habitats more suitable for flora and fauna that are adapted to warmer environments and subsequently less suitable for cold habitat flora and fauna (Schindler *et al.* 1990; Stefan *et al.* 2001). In fact, a 1°C increase in mean July air temperatures is predicted to decrease the length of habitable streams by salmonid fish by 8%; a 2°C increase causes a reduction of 14%, a 3°C increase causes a 21% decline (Keleher & Rahel 1996). This would cause the boundaries of southern species to move northward to cooler waters as surface temperatures increase to lethal ranges on occasion (McCauley & Beitinger 1992). The boundaries of individual warm-water species ranges were projected, in simulation studies, to move northward by as much as 400-500 km in Ontario, Canada (Minns & Moore 1995). However, dispersal is especially difficult for fishes because many inland lakes do not have surface water connections to adjacent waters, making it very difficult to keep up with the rates of change in freshwater habitats. Therefore, where temperatures are at the warmer limits of a species range and migration poleward or to higher elevations is not possible, coldwater and some coolwater species could become extirpated or go extinct.

Warmer water temperatures may also lead to higher metabolic rates in fish, which could increase their growth rate (IPCC 2001). However, if productivity of prey species does not increase along with the growth rate, reductions in growth would occur due to lack of an adequate food source (Arnell *et al.* 1996; Magnuson *et al.* 1997; Rouse *et al.* 1997).

An increase in water temperature reduces dissolved oxygen (Stefan *et al.* 1996) which is critical for a healthy aquatic ecosystem.

1.0.2 Ice Cover
Climate change has also decreased the duration of ice cover on inland lakes in the northern hemisphere (Schindler *et al.* 1990; Stefan & Fang 1997). The earlier melting of snow and ice in the spring months has resulted in an increase in solar radiation being absorbed by the lakes (Schindler *et al.* 1990). Some inland lakes have seen a dramatic change in deepwater oxygen concentrations and water temperatures, resulting in significant reductions in suitable habitat for fish such as lake trout (*Salvelinus namaycush*). A decrease in ice cover duration also effects biogeochemical cycling, gas exchange with the atmosphere, fish habitat availability (through changes in pH and dissolved oxygen), biodiversity, and seasonal succession (Arnell *et al.* 1996; Cushing 1997; Schindler *et al.* 1990). The uncoupling of trophic interactions has also been observed in aquatic ecosystems where herbivores are mismatched with the spring diatom bloom (Winder & Schindler 2004). This has the potential to have severe consequences for resource flow to upper trophic levels, including lake trout.

1.0.3 Eutrophication
Climate change will alter nutrient cycling in lakes in ways that would exacerbate existing water quality problems, such as eutrophication. Eutrophication of lakes results when nutrient inputs from catchments and recycling from bottom sediments is large. In warmer climates, the uptake of phosphorus by macrophytes from sediment may accelerate the eutrophication of lakes in the boreal zone (Kankaala *et al.* 2002). The result is excessive production of algae; blue-green algae in particular reduce water quality. Deep coldwater habitats may then become anoxic because of the increased decomposition rates that would occur when organic matter sinks to the bottom (Horne & Goldman 1994). Eutrophic conditions were more pronounced in small lakes during summer months when a decrease in oxygen beneath the thermocline was observed (Stefan & Fang 1993).

1.0.4 Acidification
The acidification of streams and the recovery of acidified lakes would be altered by climate change (Magnuson *et al.* 1997; Yan *et al.* 1996). Lakes would receive less buffering materials in dryer climates and more in wetter climates. Lakes high in the landscape that receive less groundwater in dry years would be more vulnerable to acidification (Webster *et al.* 1996). In experimental lakes where pH values were brought to as low as 5.8, irreversible stresses on aquatic ecosystems have been observed (Schindler *et al.* 1985). For example, Schindler *et al.* (1985) observed some important prey species for lake trout (*i.e. Mysis relicta* and *Pimephales promelas*) being extirpated from high acidity lakes, thereby resulting in the slow decline of lake trout populations. In addition, fish could not reproduce at pH values below 5.4, thereby rendering the lake fishless within a decade on the basis of the natural mortalities of most long-lived species (Schindler *et al.* 1985).

1.0.5 Dissolved Organic Carbon

Dissolved organic carbon (DOC) concentrations have decreased 15-25% in boreal lakes affected by climate change, drought and increased forest fires (Schindler *et al.* 1997). This decrease is of concern, since it may result in an increased penetration of UV and photo-synthetically active radiation (PAR), which are harmful to freshwater organisms (Bothwell *et al.* 1994; Williamson *et al.* 1996). In addition, thermoclines and euphotic zones may subsequently deepen, reducing the amount of available habitat to lake trout. DOC budgets of boreal lakes may also be affected by forest fires brought on by drought, which is expected to occur at a greater rate with climate change (Schindler *et al.* 1997). Deeper thermoclines and changes to the amounts of cold and warm thermal habitat available for fishes have resulted from an increased frequency in forest fires in the watersheds of boreal lakes. This was especially apparent when burned areas included the catchments of a lake, resulting in greater wind mixing of the water with the surrounding trees gone.

1.0.6 Invasive Species & Stocking

As climate change impacts the abundance of native and non-native species in aquatic ecosystems, stocking with introduced species will probably be used to maintain recreational fishing stability (IPCC 2001). However, stocking from captive-reared parents may result in the decrease in reproductive success of some fish by as much as 40% (Araki *et al.* 2007), making stocking a short-term solution.

Changes in water temperature will enable the spread of exotic and invasive species into more northern lakes. The presence of these species could impact trophic structure by an increased predation on important prey species (McDonald *et al.* 1996; Schindler DW 1990).

1.0.7 Keystone species

Although the previous discussion on the effects of climate change on lake ecosystems is not exhaustive, it becomes apparent that the additive effects of climate change are having direct impacts on inland lake ecosystems; entire ecosystems and their food webs are being altered. Trophic structures can be influenced by a multiple of factors, however, often the presence of a particular species that exerts a high influence on their associated ecosystem is so significant that they are capable of contributing directly to the diversity within the community. Such species are referred to as keystone species. Monitoring keystone species is of primary importance because they can play an important role in understanding the health of the environment.

1.1 *Salvelinus namaycush*: a keystone species of northern boreal lakes

The lake trout, *Salvelinus namaycush,* is the keystone species of northern temperate lakes in North America. Adult lake trout are the top carnivorous species in oligotrophic lakes, and their trophic interactions follow the typical pattern of multiple-trophic-level structuring of aquatic ecosystems (i.e. linkages among piscivores, zooplanktivores, zooplankton and phytoplankton). For example, lake trout predation directly controls certain benthic invertebrate populations and community dynamics (Goyke & Hershey 1992; Merrick *et al.* 1992). Lake trout have also been identified as integral parts of both

the epilimnetic and littoral food webs (France & Steedman 1996). *Salvelinus* are found in harsh environments; they require cold, deep, well-oxygenated water that stratifies thermally in the summer. Living at extreme habitats, along with other demographic characteristics such as slow growth and late maturity make *Salvelinus* more susceptible to extinction from habitat change and overexploitation. At their southernmost distribution, lake trout are at their physiological limits for survival as some of these requirements become limited. Having adapted to such precise ecological conditions, trout may be susceptible to even minor variations in temperature and food availability. The extent of impacts of global warming and subsequent environmental changes on lake trout will only be understood by examining the life history and biology of the fish.

1.1.1 Life History
The projected increase in temperature due to climate change has the potential to directly affect the timing of the lake trout spawn. When lake waters begin declining in temperature and day length shortens in the late summer or early fall, lake trout spawn is triggered along shorelines or on reefs (Hansen *et al.* 1995; Martin & Olver 1980). With climate change, the photoperiod and water temperature may no longer be in sync and confound the spawning triggers. This is critical since lake trout are iteroparous, and spawn only once a year or every other year.

Selection of spawning sites by adult lake trout may be influenced by a variety of factors. Those factors that could potentially be influenced by climate change include: water depth, proximity to deeper water, water temperature at spawning time, water quality in interstitial spaces and presence or absence of other species (Marsden *et al.* 1995b). The composition of the spawning reef is also an important factor for the survival and development of eggs. The size of cobble or gravel needs to be appropriate to secure the eggs and at the same time allow for water to circulate around the eggs, providing them with the required dissolved oxygen that they need to survive. This makes the trout eggs very sensitive to sedimentation and the resultant lack of oxygen (Marsden *et al.* 1995a; Sly & Schneider 1984). In addition, low levels of dissolved oxygen (2.6 to 4.5 mg/L), which can be caused by climate change, affect the larval development of trout, and can result in a delay in development rates (Garside 1959).

As eggs are broadcast over cobble and gravel substrates, they fall into the interstitial spaces where they may be incubated for up to five months over the winter and early spring (Marsden *et al.* 1995a). This incubation time varies considerably depending upon the latitudinal location and water temperature of the lakes, which along with genetic origin of the stock, are factors affecting the development and survival of eggs (Martin & Olver 1980). The temperature increase associated with climate change has the potential to adversely affect the development and survival of eggs.

Young of year (YOY) to 4 year old juveniles move to the cool, deep waters of summer stratified oligotrophic lakes in the southern part of their range (Carl *et al.* 1990; Martin & Olver 1980). These young trout are usually solitary, and tend to stay very close to the lake bottom, from <0.3 m from lake sediments (Davis *et al.* 1997). However, YOY have been documented as using inshore habitat with suboptimal conditions for growth to avoid

predation by larger fish (McDonald *et al.* 1992). Any decrease in the available habitat for YOY could stress lake trout populations in lakes where temperature and oxygen availability is adversely affected by climate change.

Adult lake trout are a top predator and as such do not experience high predation; however lake trout eggs and fry are vulnerable to predation by other fishes. These predators can include slimy sculpin (*Cottus cognatus*), burbot (*Lota lota*), round whitefish (*Prosopium cylindraceum*), lake whitefish (*Coregonus clupeaformis*), lake chub (*Couesius plumbeus*), longnose sucker (*Catostomous catostomus*), seven-spine stickleback and other lake trout (Jones *et al.* 1995). They are particularly vulnerable to predation by benthic predators (such as sculpins or burbot) as fry move out of the spawning substrate (Baird & Krueger 2000). Temperature increases may result in favorable conditions for lake trout predators and invasive species, resulting in an increase in predation on eggs and fry. It is predicted that biological responses to climate change may have an impact on these ecological interactions within lakes (DeStasio *et al.* 1996).

1.1.2 Habitat requirements
The habitat needs of lake trout vary with season and stage of their life cycle (Bjorn & Reiser 1991). Habitat selection and use by these fish is highly dependent on water temperature and oxygen, which are factors that could be potentially lethal at their extremes (Ryder & Edwards 1985). Other factors include subsurface light, and dissolved nutrients (Marshall 1996).

Adult lake trout inhabit the deeper parts of lakes where oxygen requirement can still be met. The movement of lake trout is related to variations in surface water temperatures, and as such reflects adaptive thermoregulatory behaviour. In summer, after thermal stratification, lake trout are reported to move below the thermocline (McPhail & Lindsey 1970). With temperature and oxygen being such an important factor for habitat in lake trout, climate change will directly affect the ability of lake trout to proliferate.

1.1.3 Foraging habits
Juvenile lake trout are omnivorous and display seasonality in their diet (Martin 1952) and this seasonality is triggered by the warming of surface-water temperatures in the summer. Juvenile lake trout move into deeper waters, shifting their diet from shallow-water invertebrates to deeper-water benthic organisms and fishes. In the fall, the diet again reverts to the shallow-water forage organisms as the juveniles move to shallower areas for feeding, and predator avoidance (Martin & Olver 1980). Adult lake trout are essentially piscivorous and, as such, demonstrate relatively rapid growth (Martin 1966); however, reduced prey densities in the littoral zone can prompt lake trout to switch to zooplankton and benthic invertebrates (Casselman & Grant 1998; Pazzia *et al.* 2002). The YOY are known to feed almost exclusively on zooplankton and aquatic insects (Martin & Olver 1980).

1.2 Measurement of genetic diversity as a conservation/management tool

1.2.1 Genetic Diversity

Genetic diversity is a measurement of the variation in the nucleotides, genes, chromosomes, whole genome, or phenotype of an organism. The greater the diversity of alleles a population contains the greater the potential for the evolution of new combinations of genes to occur (Jones *et al.* 1996; Taylor *et al.* 2001). Subsequently, populations that contain greater variation may experience an increased capacity for evolutionary adaptation to different environmental conditions. Genetic diversity therefore becomes very important in light of climate change.

A great amount of genetic diversity within a population of *Salvelinus* is a key factor in their survival when experiencing environmental changes (Amos & Balmford 2001). The presence of unique genetic mutations in individuals increases the genetic diversity within and between populations; therefore, it is important to identify and conserve this genetic diversity when implementing conservation measures (Ferguson 1989; Moritz 1994; Page *et al.* 2004; Rauch & Bar-Yam 2004; Taylor *et al.* 2001).

Genetic variation can be measured using a variety of markers. Molecular markers are small sections of a genome, which are chosen in the hope that they are representative of much larger stretches of DNA (Beebee & Rowe 2004). Each section is treated as a single 'locus', which may or may not be a functional gene. Most molecular markers that are used in population or conservation genetics are polymerase chain reaction (PCR) based. These markers allow for a minimum amount of tissue to be collected, making them particularly useful in cases where rare or endangered species are studied. Microsatellites and mitochondrial DNA are examples of markers that use PCR. For population genetic analysis, markers that can detect polymorphism is favorable because this will produce larger sample sizes of each allele for statistical treatments. For these and other studies that look at phylogenetics or phylogeography, mitochondrial DNA sequences are usually the marker of choice (*e.g.* (Bernatchez & Wilson 1998; Grewe & Hebert 1988; Grewe *et al.* 1993; Vitic & Strobeck 1996; Wilson & Hebert 1998).

1.2.2 Mitochondrial DNA

Mitochondrial DNA (mtDNA) is a circular, double stranded genome of eukaryotes. In fish, this extra-nuclear DNA ranges in size from 16.2 to 16.9 kbp. It encodes for 13 proteins, 22 tRNA and one each for the small and large subunits of rRNA. Proteins necessary for oxidative phosphorylation are found within the inner mitochondrial membrane. MtDNA encodes for seven proteins, which are subunits of the respiratory chain NADH dehydrogenase complex (ND1-ND6 and ND4L).

This region of DNA makes an excellent marker for population studies and it is possible to construct networks of genetic relationships within and among populations. This becomes a problem with allozymes, for example, where historical relationships between protein variants cannot usually be determined because there is much less variation in allozyme markers. Mitochondrial DNA is generally a more suitable marker because it does not undergo significant recombination, it is maternally inherited, has a high mutation rate and can be isolated and purified easily (Beebee & Rowe 2004). In addition, mtDNA is present at several copies per mitochondrion and there are several mitochondria per cell.

Therefore, there may be 100 to 10,000 mtDNA copies per cell. This makes mtDNA a very useful marker in situations where only very small amounts of tissue are available. Differences in mitochondrial DNA sequences can be displayed using single stranded conformation polymorphism analysis.

1.2.3 Single Stranded Conformation Polymorphism Analysis
Single stranded conformation polymorphism (SSCP) analysis is an effective tool for population level research since it can detect mutations between genes at a high capacity but also allows for a large number of samples to be analyzed in a relatively short amount of time (Noakes *et al.* 2003; Silva & Russo 2000; Sunnucks 2000). In other PCR-based analysis (*e.g.* restriction fragment length polymorphism (RFLP)), it can become difficult to detect mutations since only a small number of restriction enzymes scan a subset of variable sites, is time-consuming, labour-intensive and requires high-grade DNA for restriction digestion (Langefors *et al.* 2000). Although sequencing is the most accurate and sensitive method, sample preparation and analysis are time-consuming and expensive, therefore screening samples with SSCP analysis is time and cost effective.

SSCP is a process whereby amplicons of a specific marker are heated to separate the double-stranded DNA into single strands. The samples are then snap-cooled to prevent annealing; leaving single strands that fold over onto each other into different conformations based on their nucleotide sequences. These single-stranded amplicons are then loaded into a gel and subjected to electrophoresis. These amplicons then migrate through the gel as determined by its 3-dimentional shape. The conformation of the single-stranded DNA is determined by the sequence of the nucleotides, and a single base change is sufficient to change the structure. Therefore, SSCP analysis is capable of detecting slight changes in the sequences.

Numerous studies have used fragments of the mtDNA as a marker when studying fish populations (Apostolidis *et al.* 1996; Brunner *et al.* 2001; Nielsen *et al.* 1996; Oleinik *et al.* 2003), however, to date, the ND2 or ND5 region has not been explored in *Salvelinus namaycush*. These regions seem promising due to the variability demonstrated between salmonid species (Apostolidis *et al.* 1996) (ND5 variable in brown trout). By studying these regions of the mtDNA, the phylogeography of lake trout in Saskatchewan can be determined.

1.3 Phylogeography
Phylogeography can be described as the integration of the fields of population genetics and phylogenetic biology. It attempts to determine the role of different historical forces that helped shape the genetic structure of populations (Avise 2000). This genetic structure becomes important for the management of species by identifying the critical areas that are valued for conservation and monitoring.

1.3.1 Phylogeography of lake trout
Salvelinus namaycush distribution is reflective of influences of climate and topography changes due to many Pleistocene glaciations. Multiple glaciers poured into what are now the Canadian prairies, facilitating fish dispersal by postglacial drainage events (Wilson &

Hebert 1998). It is hypothesized that freshwater species were restricted in their dispersal, and therefore the genetic and ecological changes caused by repeated glacial advances and retreats were especially pronounced (Pielou 1991). Specifically, the glacial cycles from the Pleistocene events caused severe reductions in habitat availability, dramatically influencing the genetic structure of lake trout (Wilson & Hebert 1998). The geographic distribution of mitochondrial diversity suggests that lake trout dispersal was primarily dependent on the network of proglacial lakes that spanned much of Canada (Martin 1980; Wilson & Hebert 1998).

Three lineage groups co-occurred throughout central Canada, and extended northwest into Lake Athabasca and Great Slave Lake (Wilson & Hebert 1998). Areas formerly covered by the proglacial lakes Algonquin, Agassiz and McConnell created the greatest regional diversity in fish populations that occurred within or near these areas. Distinct lineages of fish found themselves in this melting pot resulting in high genetic diversity within these inland lakes (Wilson & Hebert 1998). The province of Saskatchewan falls into this area of high genetic diversity. To further substantiate this theory, all populations that occurred beyond the former proglacial lake margins contained only single haplotypes (Wilson & Hebert 1998).

This study looked at the genetic diversity of lake trout in a particular region (Saskatchewan) that had previously never been studied in detail. If the populations found in this province exhibited great genetic diversity, they may become very important for conservation at both the local and national scale.

1.4 Objectives
Through global warming, trout may be losing genetically unique, diverse populations due to the strain of a changing inhospitable environment. Many genetically unique populations may have been lost in the past and there is a need to identify and conserve the remaining genetic diversity.

The main objective of this research is to compare the genetic variation of lake trout populations in north central Canada. The following questions will be addressed:

1. Is there genetic variation among individuals within a lake trout population in the ND2 and ND5 regions of mitochondrial DNA?

2. Is there genetic variation among populations of lake trout i.e. differences in genetic diversity among lakes?

3. Are there genetically unique populations among lake trout within the study area?

4. Do stocking practices significantly influence the genetic diversity of lake trout in study area?

5. Do the southernmost populations of lake trout in Saskatchewan differ in diversity compared to northern populations?

The data from this study can be used as a baseline for the long-term monitoring of the effects of global warming on lake trout populations.

Chapter 2: The genetic diversity of a remnant population of *Salvelinus namaycush* in Crean Lake, Saskatchewan (Canada)

2.0 Introduction

The lake trout, *Salvelinus namaycush*, is indigenous to North America (Scott & Crossman 1973). The range of this salmonid extends throughout Canada and into some states of north-eastern USA (Lee *et al.* 1980; Scott & Crossman 1973). This species is adapted to specific ecological conditions, that is, cold, deep, clear lakes that stratify thermally during summer (McPhail & Lindsey 1970) and that have high concentrations of dissolved oxygen (Marshall 1996; Ryder & Edwards 1985). The food webs of these types of lakes can vary in trophic levels depending on fish species richness and lake area (Vander Zanden *et al.* 1999). Some lakes may have as low as three trophic levels *i.e.* piscivores (lake trout)– primary consumers – primary producers, or contain up to 5 trophic levels: piscivores – forage fish (*e.g. Coregonus clupeaformis*) – Mysis/Diporia – primary consumers – primary producers (Carpenter *et al.* 1985; Mittelbach *et al.* 1995; Pazzia *et al.* 2002; Rasmussen *et al.* 1990). *S. namaycush* is a keystone species in northern temperate lakes because of its specialized niche as a top carnivore. Thus, removal of *S. namaycush* from these ecosystems by commercial and recreational fishing will dramatically change the composition and structure of the food webs by increasing small prey fish or by leading to a switch in the top predator (*e.g.* Carpenter *et al.* 1985). Overexploitation of fish species, due to recreational and commercial fishing, has lead to the depletion of populations (Post *et al.* 2002) and may lead to local extinctions in some inland lake systems. In addition, climate change may be adding to the fragility of the ecosystem (Carpenter *et al.* 1992; McDonald *et al.* 1996; Stefan *et al.* 2001). Extinction of species on a continental scale is the result of a series of local extinctions. Therefore, any local declines in lake trout populations should become a priority for conservation. These declines compromise biodiversity, shift trophic pathways (Carpenter *et al.* 1985), alter habitat for other species and affect genetic diversity (Guinand *et al.* 2003).

There are several lakes where there has been a major reduction in the effective population size of *S. namaycush* (Evans & Willox 1991; Golumbia 1988). Reduction in the effective population size of *S. namaycush* within a lake may lead to decreased genetic variability of that population and thus has a significant impact on reproductive fitness of individuals (Garant *et al.* 2005) and/or their ability to cope with any environmental changes that may occur in the future. Maintaining the genetic diversity within different populations of lake trout is essential for the conservation of this keystone species (Ferguson 1989; Moritz 1994; Page *et al.* 2004; Taylor *et al.* 2001). This requires knowledge of the magnitude of genetic diversity within and between populations of *S. namaycush*. However, few studies have addressed this (Vitic & Strobeck 1996; Wilson & Hebert 1998), and there has been no detailed population genetic study of *S. namaycush* in Saskatchewan.

The population of *S. namaycush* in Crean Lake, Saskatchewan (Canada) (Fig 2.1), has experienced a significant decline since the 1920's. Crean Lake was commercially fished from 1921-1927 (Rawson 1929). During this time, approximately 91,000 kg of mature *S. namaycush* was harvested. Upon establishment of the Prince Albert National Park (PANP) in 1928, commercial fishing was prohibited until 1943. Then between 1943-1944, an additional 5000 kg of *S. namaycush* were removed from the lake by commercial fishing (Andres & Corrigal 1965). In the early years of park establishment, recreational angling success was reported by creel census to be excellent (Solman 1948); however, there was a reduction in the number of trout caught between 1945 and 1951 (Cuerrier & Ward 1952). During this time, anglers reported catching only a total of 10 lake trout per year (Golumbia 1988). Attempts were then made to increase population numbers by transferring 1110 *S. namaycush* adults from Wassegam Lake to Crean Lake in the 1950's (Cuerrier 1952). An initial increase in population size was observed in Crean Lake immediately following the stocking event, this was measured by a corresponding increase in angling success; however, this was short-lived. In 1964, there was another release of *S. namaycush* (886 mature adults and 58,000 eggs) from Wassegam Lake into Crean Lake. This was followed by other stocking events from Wassegam Lake into Crean Lake in 1966 (424 adults) and 1968 (452 adults and 5400 yearlings). After these transfers, it was observed by Didiuk (1986) that two size-classes were present, smaller, young fish introduced from Wassegam (with clipped fins) and larger, older fish from the original Crean Lake population. There was no evidence of an increase in population recruitment following the introductions of the 1950s and 1960s, potentially leading to a population bottleneck (Corrigal 1973; Melville & Fitzsimmons 2004). Parks Canada closed Crean Lake to lake trout angling in 1989 to reduce fishing pressure (Parks Canada 1995).

The relatively low population size of *S. namaycush* in Crean Lake, despite the introductions of adult and/or immature individuals from Wassegam Lake, raises a number of fundamental biological questions. For instance, 1. Is there any evidence that *S. namaycush* from the introduced stock established successfully in Crean Lake, or are all the individuals present in Crean Lake descended from the original population in that lake? 2. If the brood stock introduced into Crean Lake from Wassegam Lake established successfully, then did they successfully reproduce and provide new individuals? 3. Are *S. namaycush* from Crean Lake genetically similar to those from Wassegam Lake? 4. What is the extent of genetic variation among individuals in Crean Lake with respect to those in Wassegam Lake? Thus, the aim of the present chapter was to compare the magnitude of genetic diversity of *S. namaycush* within Crean Lake with that of the *S. namaycush* population in Wassegam Lake, the source of introductions to Crean Lake. Also included for comparison were *S. namaycush* from Kingsmere Lake. This lake is located near Crean Lake, but unlike in Crean Lake, commercial fishing in Kingsmere Lake during the 1930's had only a limited impact on population size (Kooyman 1970).

2.1 Materials and Methods
2.1.1 Sample collection
S. namaycush were captured from three lakes in Prince Albert National Park; Crean Lake (54° 05', 106° 09' W), Kingsmere Lake (54° 06' N, 106° 27' W) and Wassegam Lake (54°17'N, 106°14'W) (Fig**.** 2.1) in the summer of 2005. Tissue samples taken from lake

trout in Crean Lake were obtained with the cooperation of PANP staff while they conducted research on the lake. Small-mesh gill nets were set along spawning reefs in the fall and in random plots throughout the lake. In contrast, traditional angling was used to collect fish from Kingsmere and Wassegam Lake (over 4 and 2 days, respectively). This provided random sampling from each lake and allowed the fish to be released after collection of the tissue samples. Angling was concentrated on two regions of Wassegam Lake; the southernmost tip and the area directly in front of the warden's cabin (Figure 2.2 for map of lake and sample locations). Collection sites were chosen based on anecdotal information on locations of spawning reefs and lake trout from Parks Canada wardens (M. Fitzsimmons, pers. comm) and due to wind direction/weather during sample time. Injury of the lake trout was reduced by angling in the spring, when they were found at shallower depths. In addition, opportunistic creel surveys were also used at Kingsmere Lake, which was not possible at Wassegam Lake due to its remote location.

A total of 172 *S. namaycush* were captured from Crean Lake (n=63), Kingsmere Lake (n=63) and Wassegam Lake (n=46) (Appendix B). A portion of the adipose fin was removed from each *S. namaycush* using sterile scissors. Sampling from the adipose fin was particularly important at Crean Lake because this reduced the mortality rate, which was <10%. Samples of adipose tissue were placed in vials containing 70% ethanol and stored at -20˚C until used for molecular analysis.   Animal care protocols were approved and permitted by the University of Saskatchewan Committee on Animal Care and Supply (#20050026).

2.1.2 DNA extraction & purification
Samples were removed from the vials containing ethanol using sterile forceps. A small piece of tissue was cut from the adipose fin (~0.25 mg fin tissue) with sterile scissors and transferred to a 1.5 ml microcentrifuge tube. The remaining tissue was stored at -70˚C. The scissors and forceps were thoroughly washed in distilled water and followed by a 70% ethanol rinse between samples. Genomic (g) DNA was extracted and purified from the adipose tissue using a DNeasy tissue kit (Qiagen; Cat. No. 69504).   This was conducted using manufacturer's protocol with the following modifications: only 10 µl of 15 µg/µl of proteinase K was used. Samples were maintained at 55˚C for 18 hours for complete digestion of tissues. Following digestion, each sample was centrifuged to remove debris and the supernatant was transferred to individual MinElute spin columns.

2.1.3 PCR
Two genes from mitochondrial DNA (mtDNA) were used as the targets to assess the genetic variation within and among populations of *S. namaycush*. These targets were a 322 bp region of the NADH dehydrogenase subunit 2 (ND2 gene) and a 332-bp region of the NADH dehydrogenase subunit 5 (ND5 gene). These regions were selected based on the interspecific variation reported for related salmonids, *S. fontinalis, S. alpinus, Salmo salar* and *Oncorhynchus mykiss* (Doiron *et al.* 2002).   These mtDNA genes were amplified by polymerase chain reaction (PCR). The two primers used to amplify ND5 were   ND5-F   (5'-TCTTGGTGCAAATCCAAGTAG-3')   and   ND5-R   (5'-ACACTTTTTGTGGTGTTGGA-3'). The primers used to amplify ND2 gene fragments were   NCND2-F1   (5'-AATTAAGCTTTCGGGCCCAT-3')   and   NCND2-R1   (5'-

TCAGAAGTGAACGGGCGCTA-3'). The primers were designed based on a comparison of the mtDNA sequences from four species of salmonid: *S. fontinalis* (Genebank Accession number AF154850), *S. alpinus* (AF154851), *Oncorhynchus mykiss* (NC001717), and *O. clarki henshawi* (NC006897). PCR was performed in 25 µl reaction volumes consisting of PCR buffer (Promega-M190G), DNase-free "UltraPure" water (Invitrogen Prod# 10977-015), 2 mM MgCl$_2$ (Promega-A351H), 200 µM of each dNTP (Promega-U1240), 100 pmoles of each primer (Alpha DNA), 1 U of *Taq* DNA polymerase (Promega-M1665) and 2 µl gDNA. Negative (i.e. no gDNA) controls were included in each PCR run. The conditions for PCR amplification of the ND2 gene fragment were: 1 cycle of 95°C for 5 min, followed by 30 cycles of 95°C for 30 s (denaturation), 56°C for 30 s (annealing), 74° for 30 s (extension), and a final extension of 74°C for 5 min. Amplification of the ND5 gene fragment used the same conditions, except that an annealing temperature of 50°C was used. Amplicons were detected on 1.5% agarose-TBE (EMD Biosciences; 89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3) gels, prepared using Invitrogen UltraPure™ Agarose (Prod. No. 15510-027) to which SYBR safe (Invitrogen S33102) DNA stain was added. A volume of 1 µl of TrackIt Cyan/Orange Loading Buffer (Invitrogen) was mixed with 5 µl of each amplicon and loaded in the gel. A 100 bp DNA ladder (O'GeneRuler, Fermentas) was included for comparison. Each gel was subjected to electrophoresis at 100 volts for 60-90 min. The bands on the gel were visualized and photographed with filter #2051SY, UV transilluminator at UV-C (254nm wavelength) and a Canon A80 digital camera, or with UVP BioDocIt Imaging System.

2.1.4 Single Strand Conformation Polymorphism (SSCP) analyses
Genetic variation within trout populations were evaluated with SSCP analysis of both target gene fragments. Samples were prepared for SSCP by mixing 1 µl of amplicon with 5 µl of Gel Tracking Dye (Elchrom's Loading Dye) and 4.0 µl of ultra pure water. PCR products were denatured at 95°C for 5 minutes, followed by snap cooling in an ice bath for 5 min. The amplicons were subjected to electrophoresis using an Elchrom SEA2000 Electrophoresis unit set to 7.4°C for 18 hours in GMA ™ for SSCP Wide-Mini gels (Elchrom). Gels were stained with 40 ml SYBRGold (Molecular Probes) in TAE buffer for 30 minutes and banding patterns were visualized with a digital camera with filter #2051SY, UV transilluminator at UV-C (254 nm wavelength), or in UVP BioDocIt Imaging System. On most gels, representative samples of each unique SSCP profile were included as controls. Gels were repeated to establish that no difference in relative migration of bands occurred on different days. The DNA sequence was determined for representative PCR amplicons that produced different SSCP patterns.

2.1.5 DNA sequencing
At least two representative samples of each SSCP profiles (i.e. banding patterns) detected were selected for DNA sequencing. Where possible, multiple samples from each of the three locations were included. Amplicons of representative samples were column purified using Qiagen MinElute PCR Purification Kits (Prod. No. 28004). These samples were subjected to automated DNA sequencing (U of S Biology and Plant Biotechnology Institute – Saskatoon) using forward primers (NCND2-F1, ND5-F), and in some cases,

the reverse primer was used for confirmation of the DNA sequence (NCND2-R1, ND5-R).

2.1.6 Data analyses
To confirm whether the fragment amplified by PCR was indeed a portion of the ND5 gene, sequences were manually aligned with ND5 regions from other salmonids (*e.g. Oncorhynchus clarkii henshawi*; GenBank accession number NC006897). This was also confirmed for the ND2 gene fragment sequences, by manually aligning with published ND2 regions from other salmonids (*e.g. S. alpinus*; accession number AF154851).

The ARLEQUIN software (Schneider *et al.* 2000) was used to calculate haplotype frequencies (= frequency of different sequence types), haplotypic diversities (*h*), nucleotide diversities (π) and F_{st} estimators between pairs of populations (θ;Weir & Cockerham 1984). Chi-square tests were used to compare the frequencies of sequence types among and between the three lakes. The program TCS (Clement *et al.* 2000) was used to produce a network tree depicting relationships between the different sequence types of the ND2 gene fragment. A similar network analysis was conducted to depict relationships between ND2 amino acid sequence types. Pairwise comparisons were made of the sequence differences between ND2 haplotypes (D) using the formula D=1-(M/L), where M is the number of alignment positions where the two haplotypes have a base in common, and L is the number of alignment positions over which the two haplotypes are compared. A phenogram was constructed from the matrix of pairwise sequence comparisons using the unweighted pair group method using arithmetic averages (UPGMA) (Sneath & Sokal 1978).

2.2 Results
2.2.1 ND5

Amplicons of the ND5 gene were obtained from 161 individual lake trout (63 from Crean Lake, 39 from Wassegam Lake and 59 from Kingsmere Lake). For each amplicon, only one band (~350 bp) was detected by agarose gel electrophoresis (Fig. 2.3). A total of 95 ND5 amplicons (36 Crean Lake, 27 Wassegam Lake and 32 Kingsmere Lake) were then subjected to SSCP analyses. The SSCP profile of each sample consisted of three bands and there was no evidence of any differences in banding pattern among samples (Fig. 2.4). DNA sequencing analyses were conducted on six samples selected at random from the three locations. A representative electropherogram of one sample (KG20) is shown in Fig. 2.5. A comparison of the partial ND5 sequences (332 bp) revealed no nucleotide differences among the six samples (Fig. 2.6).

2.2.2 ND2
Amplicons of the ND2 mitochondrial gene were obtained for 160 of the 172 (93%) lake trout gDNA samples. These included samples from Crean Lake (n=61), Kingsmere Lake (n=59), and Wassegam Lake (n=40). On agarose gels, each amplicon consisted of a single band of approximately 350 bp in length (Fig. 2.7).

Single-strand conformation polymorphism analyses of all 160 amplicons revealed significant variation in banding pattern among samples. Seven different SSCP profiles were detected among the 160 samples. Representative samples of each banding pattern are shown in Fig. 2.8. Table 2.3 shows the number of *S. namaycush* of the different ND2 SSCP profiles from each lake. Within Crean Lake and Wassegam Lake, four different SSCP profiles were detected while five different SSCP profiles were detected in *S. namaycush* from Kingsmere Lake. However, there was a significant difference in the SSCP profiles of *S. namaycush* among the three lakes. *S. namaycush* with SSCP profile 2 and 3 were only detected in Crean Lake whereas *S. namaycush* with SSCP profile 5, which were detected in relatively high frequency in Wassegam Lake and Kingsmere Lake, were not detected in Crean Lake.

Multiple samples of each SSCP profile type were sequenced to determine the number of sequence types present in each lake. No sequence differences were detected among samples with the same SSCP profile (data not shown). The partial ND2 sequences of a representative sample of each SSCP profile type were compared over an alignment length of 322 bp (Fig. 2.9). The results revealed that the different SSCP profile types (1-7) represented 6 different sequence types (A-F), with those samples of SSCP profile types 2 and 3 having the same DNA sequence (type B). These sequence types had between 1-6 nucleotide differences between them (Table 2.1). A pairwise comparison of the percent nucleotide difference between sequence types is shown in Table 2.3. The two most genetically distinct sequence types were "B" and "F". The phenogram (Fig. 2.10) derived from a UPGMA analysis of the percent nucleotide differences (Table 2.2) shows that the 6 sequence types can be separated into two major clusters; one containing sequence types A-C, the second containing sequence types D-F. This division of the sequence types into two distinct groups is also evident in the minimum spanning network tree (Fig. 2.11). Common to all three lakes were *S. namaycush* with sequence types A and C, while sequence types D and E, present in both Kingsmere Lake and Wassegam Lake, were absent in Crean Lake. Both Crean Lake and Kingsmere Lake contained unique sequence types; B and F respectively.

The greatest number of sequence types, and consequently the highest diversity was found in Kingsmere Lake. There were five different sequence types in Kingsmere Lake, four sequence types in Wassegam Lake, and three in Crean Lake (Table 2.3). There was a significant difference ($X^2_{10}=108.82$, p<0.001) in the frequency of the different sequence types among the three lakes, however, there was no significant difference ($X^2_4=5.83$, n.s.) in the frequency of different *S. namaycush* sequence types between Kingsmere Lake and Wassegam Lake. In contrast, the frequency of different sequence types of *S. namaycush* in Crean Lake was significantly different to those in both Kingsmere Lake ($X^2_5=87.5$, p<0.001) and Wassegam Lake ($X^2_4=60.2$, p<0.001). Sequence types A and B were the most common within Crean Lake, while the sequence type C was low in frequency. Of the six sequence types found among these three lakes, the B sequence type was unique to Crean Lake, with a frequency of 44%. The D sequence type, which was absent in Crean Lake, was prevalent in both Wassegam Lake and Kingsmere Lake with frequencies of 37% and 50% respectively. Sequence type F, which was only detected in Kingsmere Lake, was in low frequency (5%).

Since nucleotide differences were detected between ND2 samples, the corresponding amino acid sequences were also aligned to determine if these differences resulted in changes to the translated protein. The amino acid alignments of the six DNA sequence types (Fig. 2.12) revealed three different amino acid sequences. These differences were leucine (L) and phenylalanine (F) (position 6), and Isoleucine (I) and Threonine (T) (position 7) (Fig. 2.12). DNA sequence types A and B had identical amino acid sequences as did sequence types D, E and F. Sequence type C had a unique amino acid sequence. These diversions are also shown in the minimum spanning network (Fig. 2.13). The combined frequencies of corresponding sequence types found within each amino acid cluster were compared between lake trout from all three lakes (Table 2.4). The vast majority (98%) of Crean Lake trout were found within one cluster (amino acid cluster I). The remaining 2% were grouped into cluster II, leaving cluster III absent of Crean Lake trout. In contrast, *S. namaycush* from Wassegam Lake were more evenly distributed within all three amino acid clusters (I=30%, II=28%, III=42%). Kingsmere Lake trout were found at higher frequency in amino acid cluster III (63%), followed by cluster II and I, with 22% and 15% respectively.

2.3 Discussion

The aims of the study were to: 1) determine the extent of genetic diversity in a *S. namaycush* remnant population within Crean Lake (Saskatchewan) using two mitochondrial genes (ND2 and ND5) as the targets, and 2) compare the diversity within Crean Lake with those of Wassegam Lake and Kingsmere Lake. Wassegam Lake represents the source of several introductions of *S. namaycush* into Crean Lake between 1951-1968 (Didiuk 1986). The *S. namaycush* from Kingsmere Lake were included in the comparison because of the close proximity of this lake to Crean Lake (Fig. 2.1).

It is believed that the mitochondrial genomes of some fishes undergo a slow, constant rate of genetic change (Doiron *et al.* 2002; Rand 1993). This is particularly evident in some salmonids (Bernatchez & Danzmann 1993; Guiffra *et al.* 1994). The rate of nucleotide substitutions or mutations may be influenced by metabolism, through the influence of body size and environmental temperatures (Estabrook *et al.* 2007; Gillooly *et al.* 2005). The two mitochondrial genes used in the present study were selected because sequence comparisons of the complete mitochondrial genome of four salmonid species (*S. alpinus, S. fontinalis, Salmo salar* and *Oncorhynchus mykiss*) revealed the greatest magnitude of genetic difference (Doiron *et al.* 2002). It was expected that this would also be reflected in relative higher levels of intraspecific sequence difference for these genes.

The results of the present chapter revealed that sequence comparisons of the mtDNA ND5 gene failed to provide information on the genetic diversity of the three lake trout populations. Although no genetic variation was detected in ND5 gene for the samples tested, it may be useful to confirm this by subjecting more samples from surrounding lakes to SSCP and sequencing analysis (see Chapter 3). Nonetheless, the lack of intraspecific variation in ND5 gene may be of use in phylogenetic studies that examine the relationship of *S. namaycush* with other salmonids (see Chapter 4). The absence of genetic variation in the ND5 gene is in contrast to analyses of sequences of the ND2

gene, which were highly variable among *S. namaycush* individuals, both within and between populations. This genetic marker provided the opportunity to examine the magnitude of the genetic diversity of three different populations of lake trout.

Based on the small geographical area, the gentle topography (488-732 m ASL) and the possible historical ability of fish to disperse among the lakes, it was hypothesized that the extent of genetic diversity among *S. namaycush* individuals within a lake would be similar for all three lakes examined. The ND2 sequence types detected and their relative frequencies differed between and within the three lakes. For example, sequence type B was found exclusively in Crean Lake, and individuals with this sequence type were detected at high frequency (44%). High prevalence of this sequence type within Crean Lake may be the result of natural selection favouring *S. namaycush* with this sequence type. It may be possible in the future to determine if individuals of this sequence type are physiologically different from individuals of other sequence types. The haplotype diversity and nucleotide diversity in *S. namaycush* from Crean Lake were significantly lower than those in Wassegam Lake or Kingsmere Lake. The overexploitation of *S. namaycush* in Crean Lake may have lead to a decrease in genetic diversity and may have resulted in a bottleneck event (*e.g.* Guinand & Scribner 2003). In contrast, *S. namaycush* populations in Wassegam Lake and Kingsmere Lake have never experienced a population crash or a bottleneck due to extensive commercial fishing. This may be reflective of the inadequate habitat that is available to lake trout within Crean Lake compared to Wassegam Lake and Kingsmere Lake. Thus, *S. namaycush* may be more susceptible to population declines due to overexploitation in Crean Lake.

Some of the differences in nucleotide sequences of the ND2 are also reflected in changes in amino acid sequences. Such amino acid changes may potentially result in functional differences in the proteins. Leucine (L) and Phenylalanine (F) are two non-polar and neutral amino acids, while Threonine (T) and Isoleucine (I) are two polar amino acids with much different hydropathy index. Substitutions of the T and I may affect tertiary structure of the protein. Functional differences could be very significant because selection could be resulting due to favorable characteristics that these fish exhibit. Therefore, future research should examine the possibility of functional differences arising from these amino acid changes.

The data in this study can be used to a limited extent to test whether fish from Wassegam Lake were successful in establishing in Crean Lake. Although there were two sequence types common to both Wassegam Lake and Crean Lake, they differed in relative proportions. Furthermore, sequence type D, which was very common in Wassegam Lake, was not detected in Crean Lake. From this, it may be deduced that establishment and/or reproductive success of some individuals (i.e. those of sequence type D) introduced into Crean Lake from Wassegam Lake was extremely low. In addition, it is likely that *S. namaycush* individuals of sequence type B may have been one of the original inhabitants of the Crean Lake trout population because this sequence type was not found within Wassegam Lake. Furthermore, since the frequency of sequence type B in Crean Lake was high, it could be assumed that the native population was successfully reproducing.   Future studies may look at introducing lake trout from Crean Lake with

known B sequence types into Wassegam Lake, or introducing the unique sequence type D lake trout from Kingsmere into Crean Lake where it is not found. By using these known markers, successful reproduction and recruitment could be monitored over time. This would further determine whether these unique sequence types reflect functional differences within the lake trout.

One of the assumptions made in this study is that the frequency of sequence types within the lakes has remained stable over time, because no genetic baseline is available for comparison. For example, sequence type D may have been found at a low frequency in the Wassegam Lake trout population during the stocking events, resulting in low rate of transfer. This study has established a baseline from which future studies can monitor frequencies of sequence types at a temporal scale. Continued genetic monitoring is recommended for lake trout from these three lakes.

The three localities examined in the present study are located in the same geographical area (Fig. 2.1) and represent the southern distribution limit of *S. namaycush* in Saskatchewan (Atton & Merkowsky 1983). However, there were significant differences in the genetic diversity of *S. namaycush* in Crean Lake compared to the other two lakes. In contrast, there were similarities in the genetic composition of *S. namaycush* from Wassegam Lake and Kingsmere Lake. The physical characteristics of the lakes may be important factors influencing the genetic diversity of fish populations. The size of the lakes varied between 5208 ha for Kingsmere Lake (47 m. maximum depth), and 1119 ha for Wassegam Lake (max. depth 38.2 m). In comparison, Crean Lake was the largest lake at 11,525 ha in size, but with the shallowest maximum depth at 27 m. Crean Lakes shallow depth creates a less than ideal habitat for lake trout. Its summer thermocline can range from 10-20 m in depth, but from mid-August to fall turnover, oxygen depletion is significant, and the water temperature increases to create a stressful environment for the trout (Fitzsimmons 2007; Melville & Fitzsimmons 2004). Although Wassegam Lake is not an 'ideal' lake trout lake (where oxygen levels are above 6 mg/l and temperatures below 10˚C (Marshall 1996; McLean *et al.* 1990)), this lake also experiences a depletion of oxygen in the late summer, however it is not as significant as in Crean Lake (Fitzsimmons 2007). Wassegam Lake's summer thermocline is found at a depth of 10-15 m. Kingsmere Lake has a strong thermocline that persists (Fitzsimmons *et al.* 1988) at a depth of approximately 35 m (unpublished data).

In conclusion, based on the results of this study, it may be more prudent to develop lake-specific breeding programs, using stock currently found within lakes as opposed to past attempts of stocking with neighbouring lake fish. This is particularly important for lake trout within Crean Lake. Based on the genetic marker used, this population has a significantly different gene pool than those *S. namaycush* from other lakes. This would ensure the conservation of local genetic forms and sustain intraspecific diversity, favouring fish that are adapted to the specific conditions of the lake (Utter 2004). Management strategies should also attempt to conserve the relatively high genetic diversity seen in lake trout within Kingsmere Lake and Wassegam Lake.
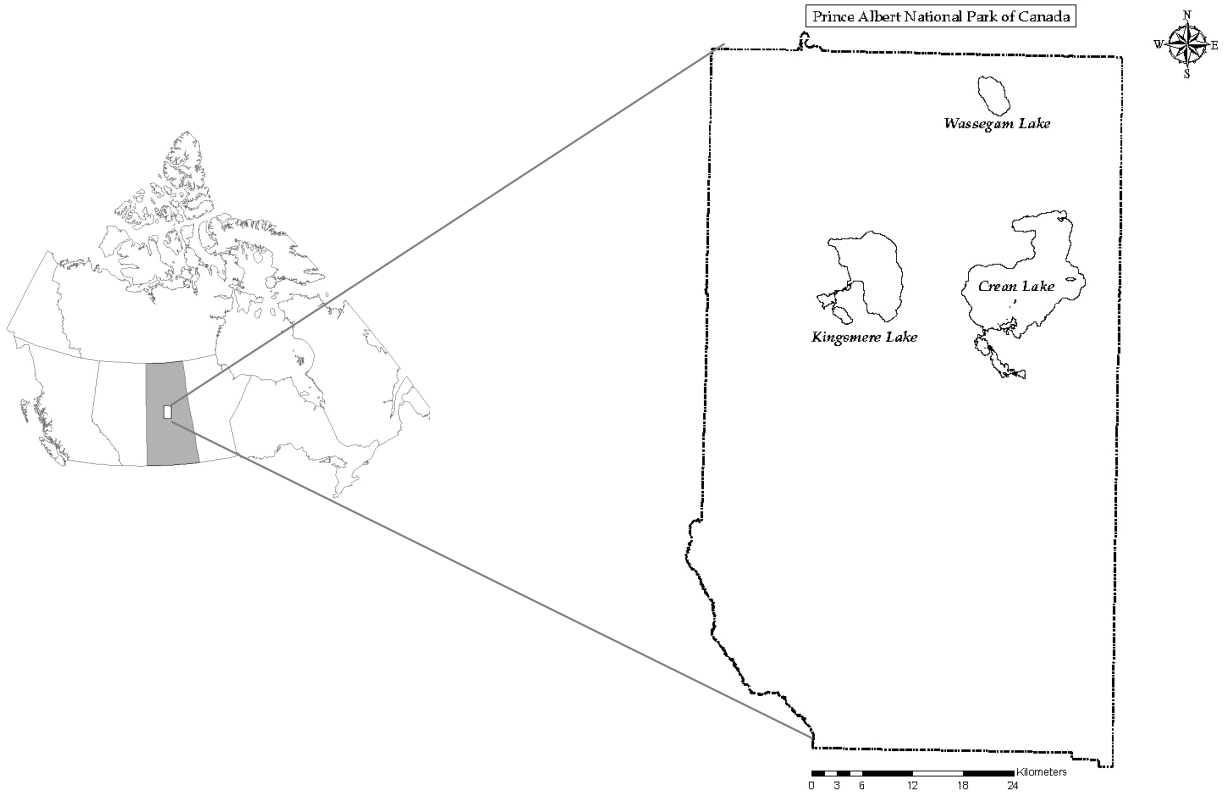
Figure 2.1 The three lakes in Prince Albert National Park (Saskatchewan, Canada) from where tissue samples of *S. namaycush* were obtained.
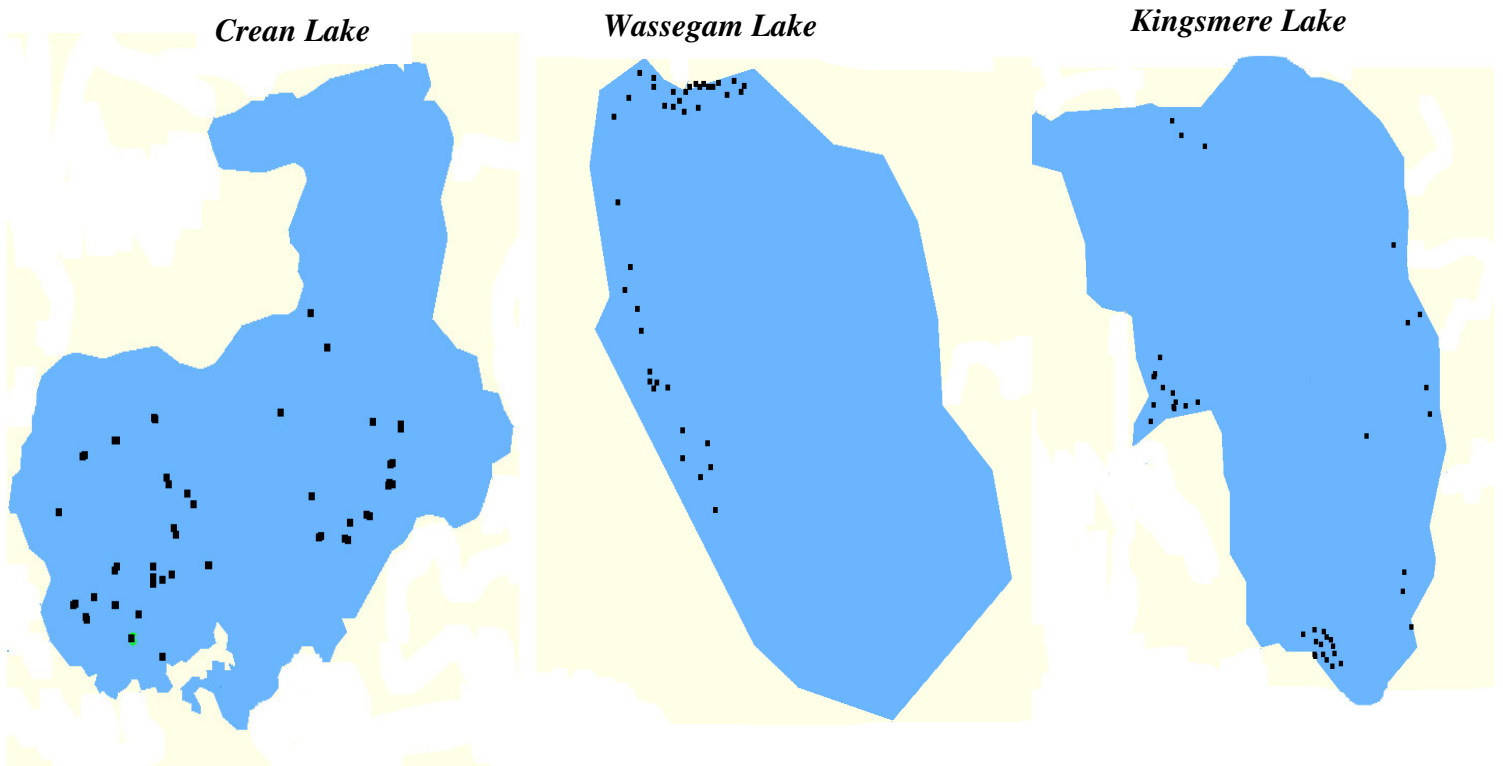
Figure 2.2 Sampling locations of *S. namaycush* **collected from Crean Lake, Wassegam Lake and Kingsmere Lake (Appendix B). Note: Not all points shown on Crean Lake represent a collected sample. (Lakes not shown to scale)**
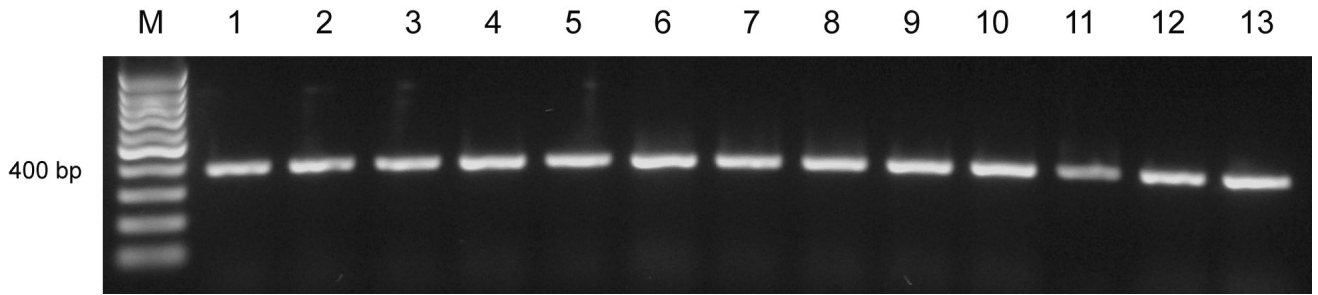
Figure 2.3 Agarose gel depicting the mtDNA ND5 amplicons of 13 representative *S. namaycush* samples (lanes 1-13) from Crean Lake. M=DNA size marker.
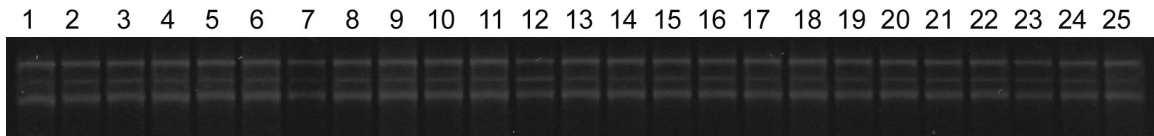
Figure 2.4 SSCP profiles of the ND5 mtDNA for 25 representative *S. namaycush* samples (lanes 1-25) collected from Crean Lake.
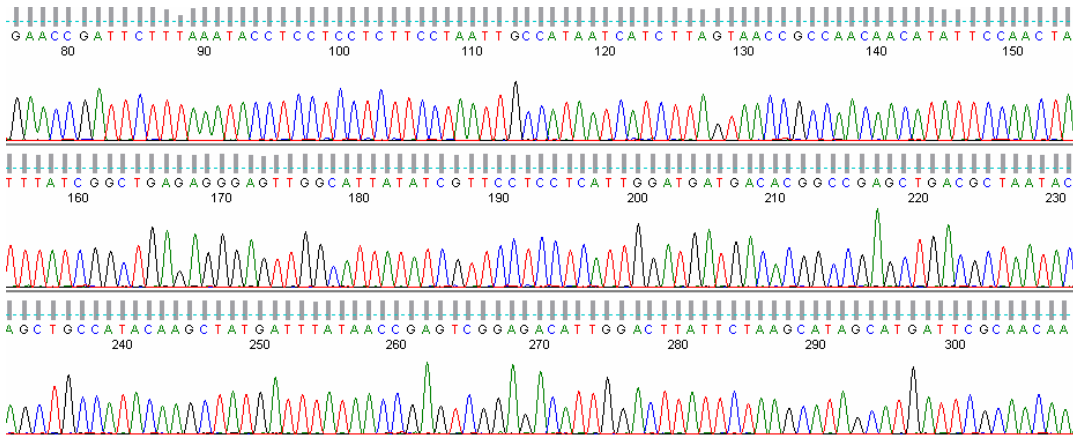
Figure 2.5 A representative electropherogram displaying the nucleotide sequence of part of the ND5 gene fragment for specimen KG20.

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                10        20        30        40        50        60        70
       .........+.........+.........+.........+.........+.........+.........+
CR29   CCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAAT
CR30   CCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAAT
KG20   CCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAAT
KG21   CCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAAT
WS24   CCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAAT
WS25   CCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAAT
       **********************************************************************
                80        90       100       110       120       130       140
       .........+.........+.........+.........+.........+.........+.........+
CR29   ATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAACA
CR30   ATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAACA
KG20   ATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAACA
KG21   ATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAACA
WS24   ATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAACA
WS25   ATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAACA
       **********************************************************************
               150       160       170       180       190       200       210
       .........+.........+.........+.........+.........+.........+.........+
CR29   TATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACGG
CR30   TATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACGG
KG20   TATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACGG
KG21   TATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACGG
WS24   TATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACGG
WS25   TATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACGG
       **********************************************************************
               220       230       240       250       260       270       280
       .........+.........+.........+.........+.........+.........+.........+
CR29   CCGAGCTGACGCTAATACAGCTGCCATACAAGCTGTGATTTATAACCGAGTCGGAGACATTGGACTTATT
CR30   CCGAGCTGACGCTAATACAGCTGCCATACAAGCTGTGATTTATAACCGAGTCGGAGACATTGGACTTATT
KG20   CCGAGCTGACGCTAATACAGCTGCCATACAAGCTGTGATTTATAACCGAGTCGGAGACATTGGACTTATT
KG21   CCGAGCTGACGCTAATACAGCTGCCATACAAGCTGTGATTTATAACCGAGTCGGAGACATTGGACTTATT
WS24   CCGAGCTGACGCTAATACAGCTGCCATACAAGCTGTGATTTATAACCGAGTCGGAGACATTGGACTTATT
WS25   CCGAGCTGACGCTAATACAGCTGCCATACAAGCTGTGATTTATAACCGAGTCGGAGACATTGGACTTATT
       **********************************************************************
               290       300       310       320       330
       .........+.........+.........+.........+.........+..
CR29   CTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAAA
CR30   CTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAAA
KG20   CTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAAA
KG21   CTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAAA
WS24   CTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAAA
WS25   CTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAAA
       ***************************************************
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Figure 2.6 Alignment of the partial mitochondrial ND5 sequences from six *S. namaycush* individuals collected from either Crean Lake (CR29 & CR30), Kingsmere Lake (KG20 & KG 21) or Wassegam Lake (WS24 & WS25). *'s denotes bases that are identical among all sequences.

22
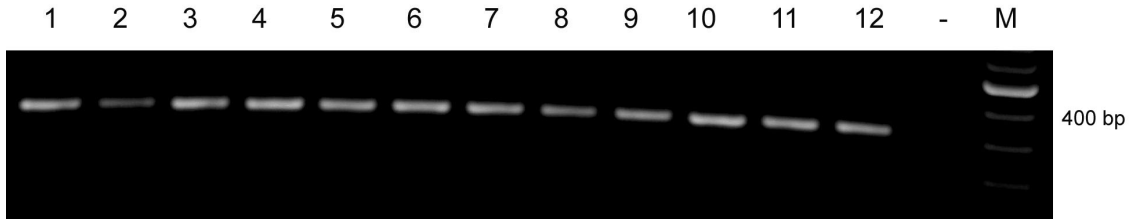
Figure 2.7 Agarose gel depicting the mtDNA ND2 amplicons of 12 representative *S. namaycush* samples (lanes 1-12) from Crean Lake. M=DNA size marker.
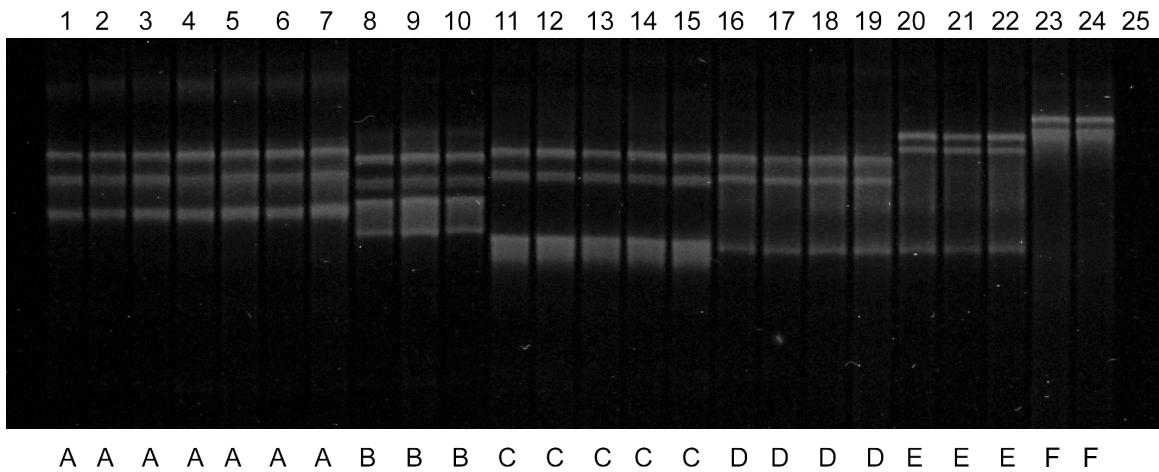
Figure 2.8 SSCP profiles (A-F) of the ND2 mtDNA for representative *S. namaycush* samples collected from Crean Lake (lane 2, 3, 8, 9, 10, 11), Kingsmere Lake (lanes 4, 5, 12, 13, 16, 17, 20, 21, 23, 24) and Wassegam Lake (lanes 1, 6, 7, 14, 15, 18, 19, 22). Lane 25 empty.

```
                 10        20        30        40        50        60        70
        .........+.........+.........+.........+.........+.........+.........+
CR12_A  ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
CR11_B  ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
WS03_C  ATGAACCCCTACGTACTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
WS30_D  ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
WS42_E  ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
KG10_F  ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
        ************** *** **************************************************

                 80        90       100       110       120       130       140
        .........+.........+.........+.........+.........+.........+.........+
CR12_A  CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
CR11_B  CACATTGACTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
WS03_C  CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
WS30_D  CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
WS42_E  CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
KG10_F  CACATTGGCTACTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
        ******* ** *********************************************************

                150       160       170       180       190       200       210
        .........+.........+.........+.........+.........+.........+.........+
CR12_A  ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTAACGCAAGCGACCGCCGCAGCAATGATC
CR11_B  ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTAACGCAAGCGACCGCCGCAGCAATGATC
WS03_C  ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTAACGCAAGCGACCGCCGCAGCAATGATC
WS30_D  ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTAACACAAGCGACCGCCGCAGCAATGATC
WS42_E  ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTAACACAAGCGACCGCCGCAGCAATGATC
KG10_F  ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTAACACAAGCGACCGCCGCAGCAATGATC
        ********************************************* *********************

                220       230       240       250       260       270       280
        .........+.........+.........+.........+.........+.........+.........+
CR12_A  CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
CR11_B  CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
WS03_C  CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
WS30_D  CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
WS42_E  CTGTTTGCTAGCACTACCAATGCCTGACTAATAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
KG10_F  CTGTTTGCTAGCACTACCAATGCCTGACTAATAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
        ****************************** ************************************

                290       300       310       320
        .........+.........+.........+.........+..
CR12_A  CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
CR11_B  CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
WS03_C  CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
WS30_D  CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
WS42_E  CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
KG10_F  CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
        ******************************************
```

Figure 2.9 Alignment of the partial mitochondrial ND2 sequences from *S. namaycush* individuals collected from Crean Lake (CR12-A & CR11-B), Kingsmere Lake (KG10-F) and Wassegam Lake (WS3-C, WS30-D & WS42-E).

Sequence type	Position of nucleotide in alignment sequence					
	16	20	78	81	186	241
A	C	T	G	T	G	G
B	C	T	A	T	G	G
C	C	C	G	T	G	G
D	T	C	G	T	A	G
E	T	C	G	T	A	A
F	T	C	G	A	A	A

Table 2.1 Location of the mutational changes in nucleotide sequence of the ND2 mitochondrial gene among the six sequence types of *S. namaycush* (A-F) from the three lakes.

	A	B	C	D	E	F
A	-					
B	0.31	-				
C	0.31	0.62	-			
D	0.93	1.24	0.62	-		
E	1.24	1.55	0.93	0.31	-	
F	1.55	1.86	1.24	0.62	0.31	-

Table 2.2 Pair-wise comparison of the genetic differences (%) in DNA sequence among ND2 sequence types.
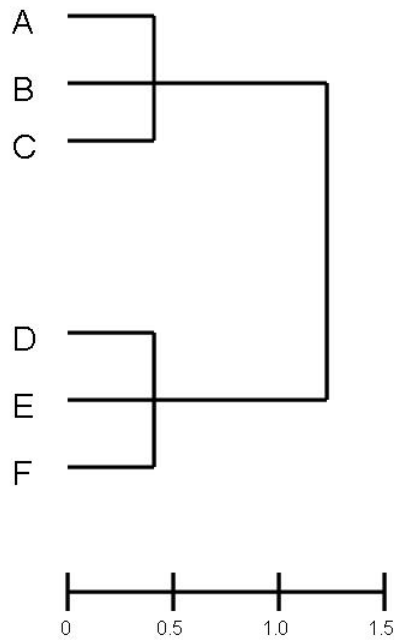
Figure 2.10 Phenogram depicting the genetic differences (%) among the six ND2 sequence types (A-F) of *S. namaycush* collected from Crean, Wassegam and Kingsmere Lakes.
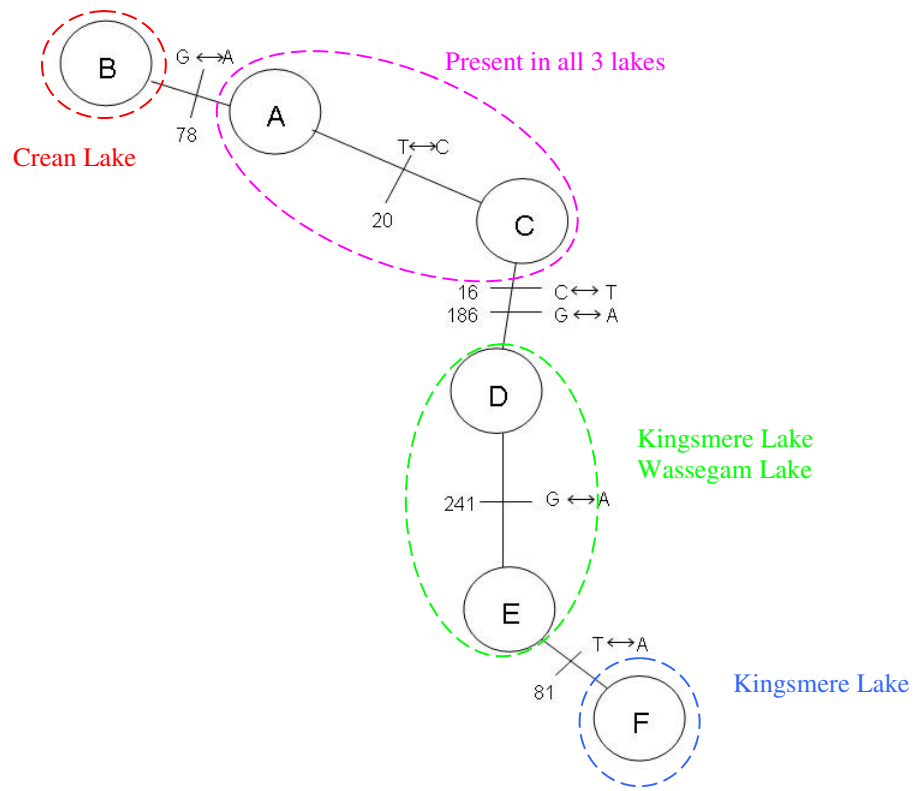
Figure 2.11 Minimum spanning tree depicting the positions of substitutional mutations relative to the six S. *namaycush* ND2 sequence types (A-F) and the lakes in which they were detected.

SSCP Profile type	Sequence type	Lake:		
		Crean no. (%)	Wassegam no. (%)	Kingsmere no. (%)
1	A	33 (54)	12 (30)	9 (15)
2+3	B	27 (44)	0 (0)	0 (0)
4	C	1 (2)	11 (28)	13 (22)
5	D	0 (0)	15 (37)	29 (50)
6	E	0 (0)	2 (5)	5 (8)
7	F	0 (0)	0 (0)	3 (5)
Haplotype Diversity (h)		0.5197±0.02320	0.7090±0.0282	0.6885±0.0456
Nucleotide Diversity (π)		0.00166±0.00152	0.00475±0.00324	0.00482±0.00324

Table 2.3 The number of different SSCP types (1-7) and sequence types (A-F) of Mt ND2 detected in Crean lake (n= 70), Wassegam Lake (n =40) and Kingsmere Lake (n = 59). Also included is the haplotype diversity and nucleotide diversity for each lake.

```
A    MNPYVLIILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
B    MNPYVLIILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
C    MNPYVLTILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
D    MNPYVFTILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
E    MNPYVFTILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
F    MNPYVFTILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE

A    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
B    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
C    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
D    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
E    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
F    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
```

Figure 2.12 Alignment of the amino acid sequences of the ND2 fragment for the six nucleotide sequence types (A-F) of *S. namaycush*. Highlighted positions indicate differences in amino acid differences.
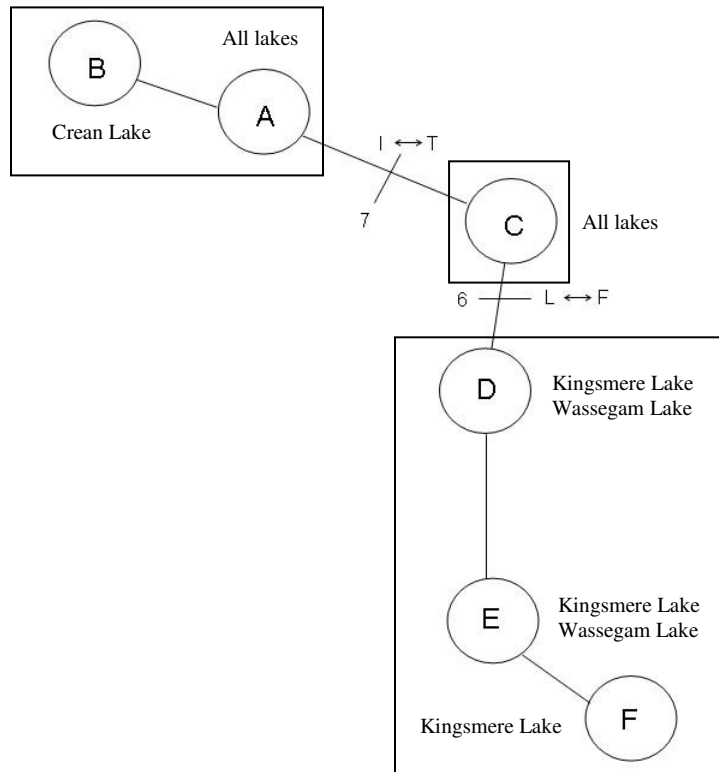
Figure 2.13 Minimum spanning network as shown in Fig. 11 but depicting the clusters of *S. namaycush* nucleotide sequence types (A-F) based on differences in their amino acid sequences of the ND2 gene fragment.

Amino acid clusters	Corresponding Sequence types	Crean Lake	Wassegam Lake	Kingsmere Lake
I	*A, B*	98%	30%	15%
II	*C*	2%	28%	22%
III	*D, E, F*	0%	42%	63%

Table 2.4 The frequency of sequence types (ND2) detected within each amino acid cluster from *S. namaycush* from each lake.

Chapter 3: Phylogeographic comparisons of lake trout populations in north-central

Saskatchewan

3.0 Introduction

Understanding the genetic diversity of *S. namaycush* within Saskatchewan has implications for the conservation and management of this species. Furthermore, by overlaying the genetic diversity with the geographical areas it is found, it is possible to examine the phylogeography of this important keystone species. Phylogeography is the geographical distribution of genealogical lineages (Avise 2000). In chapter 2 of this thesis, I examined the magnitude of genetic diversity of *S. namaycush* within three lakes in Saskatchewan that were in relatively close proximity (approx. 7 km) to each other and which represented populations of lake trout near the southern distributional limit for the species. Given the genetic diversity detected in the ND2 mtDNA gene of *S. namaycush* from these three southern Saskatchewan lakes, it may be possible to conduct a phylogeographic analysis on lake trout from different regions of the province using the combined SSCP and DNA sequencing methodology used in Chapter 2. Such an approach has not been used previously to examine the genetic diversity of *S. namaycush* in Saskatchewan, or in North America. There have been, however, a small number of phylogeographic studies conducted on *S. namaycush* (Vitic and Strobeck 1996; Wilson and Hebert 1998).

Wilson and Hebert (1998) examined the phylogeography of lake trout in North America on a broad scale. They used restricted fragment length polymorphism (RFLP) analyses of the whole mtDNA genome to assess the complex postglacial history of lake trout. Nine restriction enzymes were ultimately used in the analyses to compare the RFLP profiles of 1416 lake trout from 93 populations across the range in North America. Included in the study by Wilson and Herbert (1998) were *S. namaycush* from 8 lakes in Saskatchewan: Reindeer, Little Bear, La Ronge, Nemeiben, Kingsmere, La Plonge, Pierce, Milliken and Lake Athabasca. Their results revealed three major lineages of *S. namaycush* throughout the range of this species, and it appeared that various glacial patterns (i.e. Beringia, Nahanni, Montana and Mississippi refuges) resulted in an increased genetic diversity within the central region of Canada (Wilson & Hebert 1998). Although this study determined that secondary contact among refugial groups was extensive throughout central Canada, the genetic diversity of lake trout within the region was only determined on a broad scale. Only 1-5 haplotypes were detected within *S. namaycush* from Saskatchewan lakes. By using RFLP data, a detailed examination of the diversity found within the region was not possible. RFLP screened large areas of mtDNA and specific restriction enzymes cut only at specific sites, variation in the nucleotide sequence was determined when the length of the cut segments varied, which left much of the mtDNA variation undetectable.

The genetic diversity of lake trout distributed in central Canada was also examined by Vitic and Strobeck (1996). They used amplified restriction fragment length

polymorphism (ARFLP) analysis in combination with DNA sequencing of two genes in the mtDNA. The two genes used by Vitic and Strobeck (1996) were the ATPase subunit 6 and the cytochrome oxidase C subunit III genes. Their study included ARFLP analyses of 174 *S. namaycush* from 10 different populations. However, only a single lake from Saskatchewan, Whiteswan Lake (54˚5'N, 105˚10'W), was included in the study (Vitic & Strobeck 1996). This was because they were examining the impact of stocking on genetic diversity and this lake had been used as a stocking source for Cold Lake, Alberta. Vitic and Strobeck (1996) found that the ARFLP analyses revealed only limited polymorphism among populations, as a consequence of the limitations of the approach taken. In contrast, sequencing of the genes from individual fish revealed greater than four times the number of haplotypes than did the ARFLP analyses. Although the study by Vitic and Strobeck (1996) provided a more detailed look into the variation present in the region by the detection of a greater number of types through sequencing, it revealed little in terms of the genetic diversity and phylogeographic relationships of *S. namaycush* in Saskatchewan as only a single population from the province was included in the analyses. This highlighted the need for a more detailed study in this region. Hence, the aim of this chapter was to compare the relative genetic diversity of *S. namaycush* in several lakes situated in both the northern and southern range of lake trout in Saskatchewan.

The results in Chapter 2 for three lakes, Crean Lake, Wassegam Lake and Kingsmere Lake, revealed no genetic variation in the partial ND5 mtDNA gene within or among populations. In contrast, six haplotypes of the partial ND2 mtDNA gene were detected within and among populations of *S. namaycush*. Of particular interest was the detection of a unique sequence type (haplotype 'B') found at high frequency within Crean Lake. These findings raise numerous questions on the genetic diversity and phylogeography of lake trout from different regions of Saskatchewan.

Within this chapter, I will examine the following questions:
1. Is the partial ND5 mtDNA gene (as used in Chapter 2) invariant in lake trout throughout all populations in Saskatchewan? If there is little or no sequence variation for the ND5, then this gene region may be of use to examine phylogenetic (i.e. evolutionary) relationships of *S. namaycush* (see chapter 4).
2. With respect to the ND2 mtDNA gene, how many different sequence types are there within each lake? Do some lakes contain the same sequence types and are they in equal relative frequency? Are there any lakes, apart from Crean Lake, that contain the "B" haplotype? Are there any differences in the haplotypes present in lakes?

3.1 Materials and Methods
3.1.1 Sample Collection
A total of 607 *S. namaycush* were captured from 19 lakes in Saskatchewan, Canada (Table 3.1) during the summer of 2005. The methods used to collect tissue from each lake (*e.g.* netting, creel census), and the names of the outfitters and commercial fishers that participated are provided in Appendix A. In brief, tissue sampling kits were sent to volunteer participants in outfitting resorts in the most remote lakes to ensure the same protocols were used. A copy of the tissue sampling instructions used is provided in Appendix B. A portion of the adipose fin was removed from each *S. namaycush* using

scissors. Sampled fish were able to be released after sampling, ensuring a low mortality rate. Samples of adipose tissue were placed in vials containing 70% ethanol until received at the laboratory and stored at -20˚C until used for molecular analysis.

3.1.2 *Molecular methods and DNA analyses*

The methods used to extract and purify gDNA from samples, and the conditions used to conduct PCR, SSCP and DNA sequencing have been described previously in detail (see Chapter 2). For the analyses of the ND2 sequence data, the program TCS (Clement *et al.* 2000) was used to produce a network tree depicting relationships between the different sequence types of the ND2 gene fragment. A similar network analysis was conducted to depict relationships between ND2 amino acid sequence types. As in chapter 2, the ARLEQUIN software (Schneider *et al.* 2000) was used to calculate haplotype frequencies, haplotypic diversities (*h*) (the degree to which a haplotype has become distributed), nucleotide diversities (π) (estimated by averaging the estimated numbers of nucleotide changes over all the pairs in the sample) and F_{st} estimators (used to analyze the pair wise differences between genetic diversity of *S. namaycush* from the different lakes) (θ; Weir & Cockerham 1984). Populations were defined based on the lakes from where *S. namaycush* were collected. Other methods of data analyses follow those used in Chapter 2.

Chi-square tests were used to compare the frequencies of the major sequence types among lakes from different geographical locations. For example, the relative frequencies of the A, C and D haplotypes in Kingsmere Lake and Wassegam Lake in the Prince Albert National Park were compared to those in Little Bear and East Trout Lake. This comparison was conducted in order to determine whether lakes within the National Park were significantly different than those in lakes within the same geographical location. A second comparison was conducted between lakes in northern and southern Saskatchewan. Northern lakes were defined as those found in the boreal shield, while southern lakes were not.

3.2 Results
3.2.1 ND5

Amplicons of the ND5 gene were obtained from 583 individual lake trout. For each amplicon, only one band (~350 bp) was detected by agarose gel electrophoresis (data not shown). A total of 191 ND5 amplicons were then subjected to SSCP analyses (Table 3.2). The SSCP profiles of 190 amplicons were identical, whereas sample RD4 (from Reindeer Lake) had a unique banding pattern (Fig. 3.2). DNA sequencing analyses were conducted on seven samples, six with the common SSCP profile (two each from Crean Lake, Kingsmere Lake and Wassegam Lake) and sample RD4 (Appendix C). A comparison of the partial ND5 sequences (332 bp) revealed one nucleotide difference (at position 246; Fig. 3.3) among the seven samples. This purine transitional change in sample RD4 did not change the amino acid sequence (Fig. 3.4).

3.2.2 ND2

Amplicons of the ND2 mt gene were obtained for 576 of the 607 (95%) lake trout gDNA samples subjected to PCR analyses. On agarose gels, each amplicon consisted of a single band of approximately 350 bp in length (Fig. 3.5).

SSCP analyses of all 576 amplicons revealed significant variation in banding pattern among samples. Fourteen (14) different SSCP profiles were detected among the 576 samples. Representative samples of most of the different banding pattern types are shown in Fig. 3.6. The relative frequency of each SSCP profile type within the different lakes is shown in Table 3.3. Some lakes (*e.g.* Nettle) contained a single SSCP profile type, however these were lakes that had a relatively small sample size. Some lakes contained *S. namaycush* with two different SSCP profiles, whereas *S. namaycush* from Lac La Ronge belonged to one of 8 different SSCP profile types. Of the 14 SSCP profile types, four (1, 3, 5 and 7) were the most frequent (Table 3.3), with SSCP profile 3 occurring in Crean Lake and Lac La Ronge. Most of the other 10 SSCP profile types were detected in a single *S. namaycush* (Table 3.3).

Multiple samples, when possible, of each SSCP profile type were sequenced to determine the number of sequence types present in each lake. No sequence differences were detected among samples with the same SSCP profile (Appendix D). The partial ND2 sequences of a representative sample of each SSCP profile type were compared over an alignment length of 322 bp (Fig. 3.7). The results revealed that the different SSCP profile types (1-14) represented 11 different sequence types (A-K). Those samples of SSCP profile types 1 and 8 had the same DNA sequence as type A. Similarly, profile types 2 and 3 had sequence type B and SSCP profile 4 and 9 had the same DNA sequence as sequence type C (Appendix E). A comparison of the sequences of the 11 sequence types revealed variation at 12 alignment positions, consisting of six purine transitions, two pyrmidine transitions and four transversional changes (Table 3.4 and Figure 3.7) There were 1-8 nucleotide differences among the 11 sequence haplotypes (Table 3.4 and Figure 3.7). A pairwise comparison of the percent nucleotide difference between sequence types is shown in Table 3.5. The magnitude of the differences between sequences ranged from 0.3-2.8%. The two most genetically distinct sequence types were types "H" and "F".

A phenogram derived from a UPGMA analysis of the percent nucleotide differences (Table 3.5) is shown in Fig. 3.8. The 11 sequence types can be separated into 3 major clusters; one containing sequence types A, B, H, I, J and G the second containing sequence types C and K, and the third with sequence types D, E, and F.

Of the eleven sequence types found among these 19 lakes, the F sequence type remained unique to Kingsmere Lake, with a frequency of 5% (Table 3.6). Sequence type B was only found in two lakes (Crean Lake and Lac La Ronge), at very different frequencies (44% and 8% respectively) (Table 3.6). The highest frequency of a single sequence type within a lake occurred in Little Bear Lake, where sequence type A was found at a frequency of 91%. Its neighbouring lake, East Trout, had no sequence type A in the lake trout sampled. This was surprising since they were very close geographically to each other. The second highest sequence type frequency occurred in Hatchet Lake where 79%

of the lake trout population contained the C sequence type. The sequence types G, H, I, J and K were found at the lowest frequencies (1-3%) (Table 3.6).

The greatest numbers of sequence types were found in trout from La Ronge and Reindeer Lakes (Table 3.6). There were six different sequence types in both La Ronge and Reindeer Lake, five sequence types in Kingsmere Lake, four in Wassegam, Oliver and Wollaston Lakes, while Crean, Nemeiben, Hatchet and George Lakes all contained three sequence types. Lakes with the lowest number of sequence types were Little Bear (2), East Trout (2), Orr (2), and Tazin (2); the remaining lakes were found to have only one sequence type (Table 3.6).

Although the range of values for haplotype diversity (*h*) was relatively similar (0.3846-0.7090), Little Bear lake trout were markedly different at 0.1705 +/- 0.0822 (Table 3.7). The similarity between diversities may be due to the fact that there are on average only 2-3 haplotypes present per lake. In addition, the smaller sample sizes may have influenced these numbers since it decreases the likelihood of detecting other, less frequent sequence types.

The Fst values in Table 8 show that most lakes have sequence type frequencies that are significantly different to each other. Crean, Kingsmere and Little Bear Lakes had lake trout populations that had significantly different sequence type frequencies to every other lake trout population in the study (Table 3.8). Nemeiben Lake trout were not significantly different to any other lake except for the three aforementioned. The low sample size and the connectivity of this lake to Lac La Ronge may have been a factor.

Amino acid sequences were aligned to determine if the additional ND2 sequence types detected in this chapter resulted in more changes to the translated protein. The amino acid alignments of the eleven DNA sequence types (Fig. 3.10) revealed three different amino acid sequences. DNA sequence types A, B, G, H, I, and J had identical amino acid sequences as did sequence types C and K. The third group contained sequence types D, E and F. These diversions are shown in the minimum spanning network (Fig. 3.11). The three amino acid clusters reinforce the observation that there are three main sequence types (A, C, and D) within lake trout populations in this region, as each is found in its respective cluster (Figure 3.11). Amino acid cluster I contains main sequence type A, combined with sequence types B, G, I, H, and J. The second amino acid cluster (II) includes sequence types C and K, while cluster III is formed by sequence type D, E and F. These clusters follow the same pattern as the minimum spanning tree (Figure 3.9).

The combined frequencies of corresponding sequence types found within each amino acid cluster were compared between lake trout from all 19 lakes (Table 3.9). Amino acid cluster I contained the most sequence types with six (A, B, G, H, I, J), cluster II had two (C and K), and cluster III, three (D, E, F). The vast majority (98%) of Crean Lake and (91%) of Little Bear Lake trout were found within one cluster (amino acid cluster I) (Table 3.9). Hatchet Lake, Tazin Lake and East Trout Lake trout demonstrated a high frequency of amino acids from cluster II (76%, 70% and 68% respectively). Cluster III was notably more frequent in Kingsmere Lake trout at a frequency of 63%. Most lakes

contained all three amino acid clusters. However, exceptions included Crean Lake, Little Bear Lake, Orr Lake and Tazin Lake where cluster III was absent, while East Trout Lake was the only lake that did not contain any lake trout with amino acids from cluster I.

3.3 Discussion

The aims of this chapter were to use SSCP and DNA sequencing to determine: 1) if the sequence of the partial ND5 mtDNA gene was invariant among *S. namaycush* from a number of lakes within Saskatchewan, 2) if there were any differences in the ND2 haplotypes present in lakes, 3) how many different sequence types of the ND2 mtDNA gene there were within lakes in the province, 4) if some lakes contained the same ND2 sequence types and if they occurred in the same relative frequency, and finally 5) if the "B" ND2 sequence type occurred in any other lake besides Crean Lake.

The results revealed that the sequence of the ND5 mtDNA gene of *S. namaycush* samples from a larger number of lakes within the province was invariant except for a single lake trout sample from Reindeer Lake. This *S. namaycush* individual had a single mutation at position 246 within the 332 bp fragment of the ND5 gene. This single mutational change in DNA sequence did not correspond to a change in the amino acid sequence for the ND5 gene. The frequency of this rare sequence type (II) within Reindeer Lake was 1%. Small sample size may contribute to a failure to detect low levels of genetic variation within other lakes as the lake trout from Reindeer Lake were the most numerous sampled (n=93). Nonetheless, given that only a single individual of type II was detected in Reindeer Lake, it is probable that if this variant does occur in other lakes, it will be found at very low frequency. Although there was little sequence variation detected in this region of the ND5 gene among *S. namaycush* in 19 lakes in Saskatchewan, this gene region may be of use to examine the phylogenetic relationships of *S. namaycush* to other salmonids (see Chapter 4).

The ND2 fragment of mtDNA gene proved to be significantly more valuable to examine the genetic diversity within and among populations of *S. namaycush*. Six ND2 sequence types (A-F) had been detected previously from lake trout in three lakes (Chapter 2), however the number of sequence variants increased to 11 (A-K) based on analyses of lake trout from 19 lakes (this chapter).

A total of 14 SSCP profiles (1-14) of the ND2 mtDNA were detected among the 576 *S. namaycush* examined. Subsequent sequence analysis of 41 representative samples confirmed the utility of SSCP to accurately detect mutations even among samples with sequence sequences that differed by a single nucleotide. Thus, the initial screening of gDNA samples by SSCP substantially reduced the time and monetary constraints that often burden genetic research (Silva & Russo 2000; Sunnucks *et al.* 2000). Although 14 ND2 SSCP profiles were detected, subsequent sequence analyses revealed that some samples with a different SSCP profile had the same sequence. The sequence analyses therefore revealed 11 different sequence types (A-K) for *S. namaycush*. This finding of fewer sequence types to SSCP profiles for a given set of gDNA samples was not unexpected, as it has been shown previously that single-stranded DNA can take on more

than one conformational type during rapid cooling (Sunnucks *et al.* 2000). The optimum length that DNA strands can be for SSCP analyses is approximately 200-400 bp (Gasser & Chilton 2001). The ND2 fragment used in these analyses were a total length of 322 bp, and could result in an increased possibility that the single stranded DNA may have folded onto itself in different conformations. Alternately, the different conformations may have been due to the multiple cells found in each of the samples, as multiple cells result in multiple DNA strands, increasing the chance of different conformations forming.

Three sequence types (A, C and D) were detected in high frequency (37%, 34% and 19%) within the analyzed samples. Statistical analyses (*e.g.* neighbor-joining and parsimony network analyses) also showed that each of these sequence types belonged to a separate cluster that contained other sequence types (data not shown, see Ch. 4). It may be hypothesized that these three clusters may represent remnants of the three main lineages detected within lake trout mtDNA in Wilson and Hebert (1998) from refugial dispersal patterns *i.e.* the Beringia, Nahanni and Mississippi that passed within the region studied.

The relative frequencies of the different ND2 fragment sequence types were not consistent throughout the lakes sampled. For example, the frequency of sequence types within East Trout and Little Bear Lake trout were markedly different despite the close proximity of these lakes (~30 km). A majority (91%) of lake trout in Little Bear Lake trout were sequence type A whereas none of the *S. namaycush* in East Trout Lake were sequence type A. There are numerous reasons why this may be, for example, a historical population bottleneck in one or both of these lakes (*e.g.*, Ramakrishnan *et al.* 2005) or different physical lake parameters (*e.g.* surface area) (*e.g.*, Cena *et al.* 2006) may have influenced the genetic diversity. A closer examination of the lake characteristics and history may be required to determine the cause of this significant difference in the population genetics. This is particularly important since these lakes are at the southern distribution of lake trout in the province, and *S. namaycush* in these lakes may be more vulnerable to selection pressures associated with global warming and climate change. It is also of interest to note that the *S. namaycush* from lakes at or near the southern distributional limit of this species in the province (i.e. Crean, Kingsmere and Little Bear Lakes) had significantly different sequence type frequencies to every other lake trout population in the study.

There were, however, significant differences in the relative frequencies of different ND2 sequence types among lake trout from different lakes. It was shown in Chapter 2 that sequence type B occurred in high frequency in lake trout within Crean Lake (44%) but was not detected for individuals within another two lakes (Wassegam Lake and Kingsmere Lake) in Prince Albert National Park. The results of this chapter, where lake trout were compared from a larger number of lakes, showed that sequence type B was detected in only one lake, Lac La Ronge, but only at a very low frequency (8%). The possible historical connectivity of these two lakes may have allowed for the dispersal of lake trout between the two lakes, hence resulting in the distribution of the sequence type. However, it remains unclear why the frequencies are significantly different. Further research into the biological significance of this sequence type is needed to more fully

understand the consequences of the elevated frequency of sequence type B in Crean Lake trout.

Several sequence types were detected in low frequency but only within a single lake. For instance, sequence type F was unique to Kingsmere Lake trout, with a frequency of 5%. Five other sequence types (G, H, I, J and K) were found only in one lake and they were only detected in a single individual *S. namaycush*. While H, I and J were all detected in Reindeer Lake trout, this may be indicative of the influence of lake size on genetic diversity (Cena *et al.* 2006; Heath *et al.* 2001; Yamamoto *et al.* 2004) and the larger sample size (n=98) enabling the detection of the less abundant types. Genetic diversity is theoretically a function of population size (Avise 1994), and because habitat size can be indicative of population size (Heath *et al.* 2001) genetic diversity is often hypothesized to correlate to lake size. This theory is held true in this study, with Reindeer (n=93) and La Ronge (n=79) lakes having the most variation in the ND2 fragment of the mtDNA gene. Although Wollaston Lake is also a large lake, fewer samples were collected from this lake (n=29), substantially reducing the ability of detecting more diversity. Nonetheless, all of these six ND2 sequence variants (F, G, H, I, J and K) differed by 1 or 2 mutations with respect to other sequence types present within a lake. This suggests that these sequence variants may have originated from single mutational changes in one genotype that occurred within the population of *S. namaycush* following isolation of the lake from neighbouring lakes. Although it is hypothesized that the deglaciation of Saskatchewan occurred from about 17000-10000 years ago (Christiansen 1979), there appears to be no information available as to how long the different lakes may have been isolated from one another. Furthermore, the significant differences detected in genetic diversity of *S. namaycush* among lakes, even within close proximity, may reflect different selection pressures and/or genetic drift since the isolation of the lakes.

It was demonstrated in Chapter 2, that some of the mutational changes in DNA sequence were associated with changes in amino acid sequence. It was shown that the 11 sequence types represented three different amino acid sequence types. However, the detection of an increased number of sequence types in this chapter did not result in an increased number of different amino acid sequence types. Thus, within Saskatchewan only three ND2 amino acid sequence types (I, II and III) were detected. Each of these represented the three main clusters of DNA sequence types. This strengthens the need to identify whether the clusters represent functional differences in the proteins. Functional differences could be very significant because selection could be resulting due to favorable characteristics that these fish exhibit. The majority of lake trout in Crean Lake and Little Bear Lake (98% and 91% respectively) belong to the amino acid sequence type I. Thus, lake trout from these two lakes are of particular interest due to their unique sequence type frequencies and because of their geographical location. If lake trout of amino acid sequence type I have functionally different proteins, these favorable characteristics exhibited by the fish may be the reason why these fish flourish within these lakes that are located at their southern geographical limit. Therefore, future research should examine the possibility of functional differences arising from these amino acid changes.

A hypothesis that was examined was whether there was a difference in genetic diversity between lake trout in the north versus those in the southern region of the province. The results showed that there was no significant relationship between the latitude of the lakes studied and the genetic diversity of *S. namaycush* contained within them. However, some caution is advised because the sample sizes in some lakes (*e.g.* Nemeiben and Hatchet) were low (i.e. fewer than 20 individuals). Nonetheless, further research is warranted to explore this hypothesis among the lakes studied. At the northern boundary of the province, there were only two sequence types detected. More detailed sampling should be attempted in this region, as northern lakes are known to have reduced carrying capacity, thereby theoretically having lower genetic diversity (Rigler 1977). Also, it would be of advantage to examine the genetic diversity of *S. namaycush* within Lake Athabasca, the largest lake in the province. This would provide the opportunity to examine the hypothesis that larger lakes tend to contain the greater genetic diversity than smaller lakes.
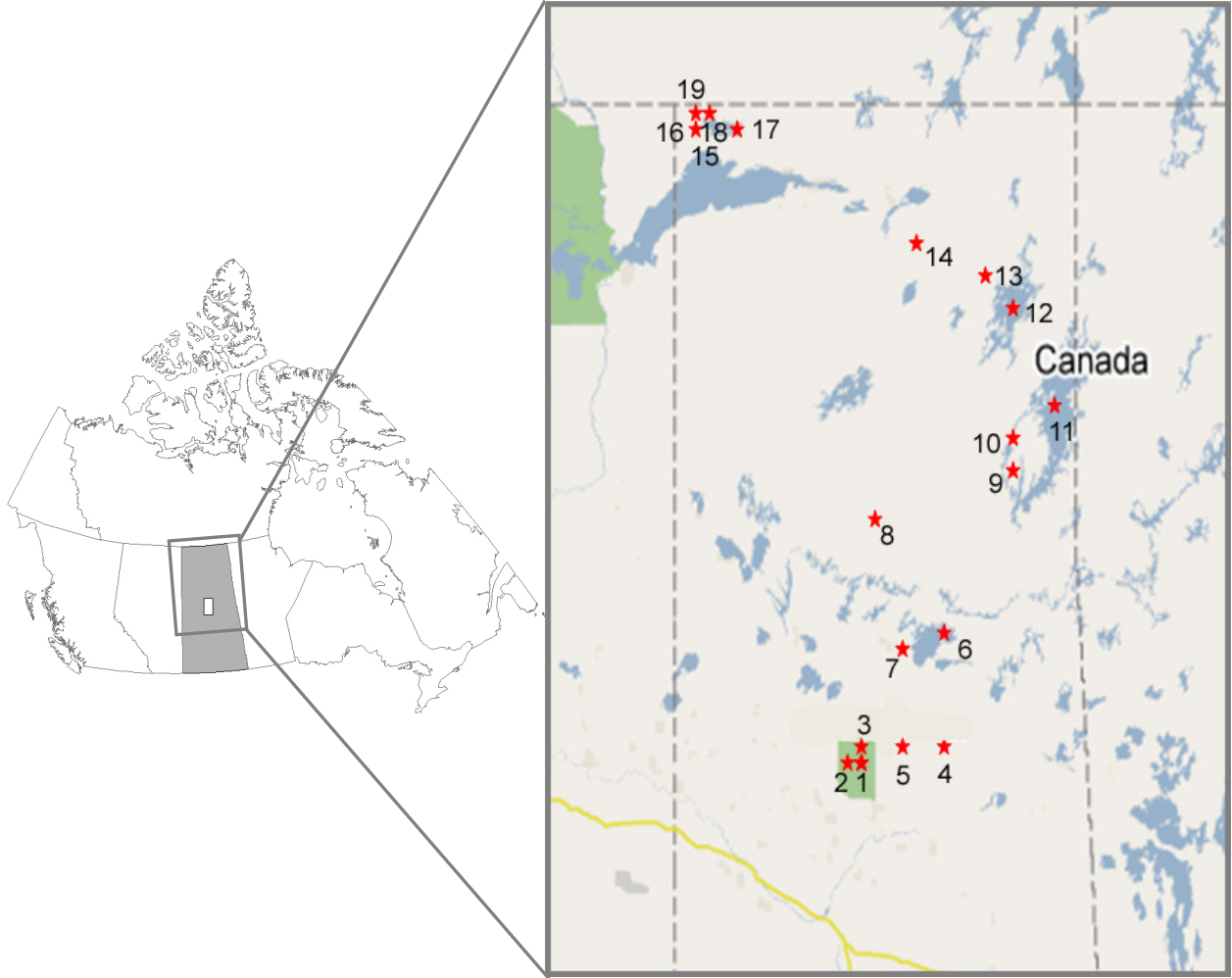
Figure 3.1 The 19 lakes in Saskatchewan, Canada from where tissue samples of *S. namaycush* were obtained. Numbers are the lakes as indicated in Table 1.

Table 3.1 The number of *S. namaycush* tissue samples collected from 19 lakes in Saskatchewan. *N*, sample size of fish from each population.

#	Location	Population	Code	*N*	Geographical position	
1	South	Crean	CR	63	54° 5' North	106° 9' West
2	South	Kingsmere	KG	63	54° 6' North	106° 27' West
3	South	Wassegam	WS	46	54° 17' North	106° 14' West
4	South	Little Bear	LB	33	54° 20' North	104° 35' West
5	South	East Trout	ET	27	54° 22' North	105° 5' West
6	North	La Ronge	LR	84	55˚ 10'North	105˚00' West
7	North	Nemeiben	NB	10	55° 20' North	105° 20' West
8	North	George	GL	35	56˚ 12' North	106˚ 19' West
9	North	Ghana	GH	1	56° 40' North	103° 28' West
10	North	Oliver	OL	49	56° 48' North	103° 29' West
11	North	Reindeer	RD	98	57° 15' North	102° 15' West
12	North	Wollaston	WL	29	58° 15' North	103° 15' West
13	North	Hatchet	HT	15	58° 38' North	103° 40' West
14	North	Orr	OR	23	58° 57' North	104° 47' West
15	North	Thluicho	THL	4	59° 43' North	109° 16' West
16	North	Tsalwor	TSA	1	59˚ 45' North	109˚ 22' West
17	North	Clinkskill	CLK	3	59˚ 48' North	108˚ 44' West
18	North	Tazin	TZ	21	59° 48' North	109° 5' West
19	North	Nettell	NTL	2	59˚55' North	109˚ 29' West
			Total	**607**		

Table 3.2 A total of 190 *S. namaycush* tissue samples were screened by SSCP for the ND5 region of mtDNA. All samples produced the same haplotype (I), with the exception of one (1) from Reindeer Lake (II).

	Lake	SSCP type	
		I	**II**
1	Crean	63	
2	Kingsmere	59	
3	Little Bear	2	
4	East Trout	2	
5	Wassegam	39	
6	La Ronge	9	
7	Oliver	1	
8	Wollaston	2	
9	Hatchet	2	
10	Reindeer	5	1
11	George	2	
12	Orr	2	
13	Tazin	2	
	Total	**190**	**1**
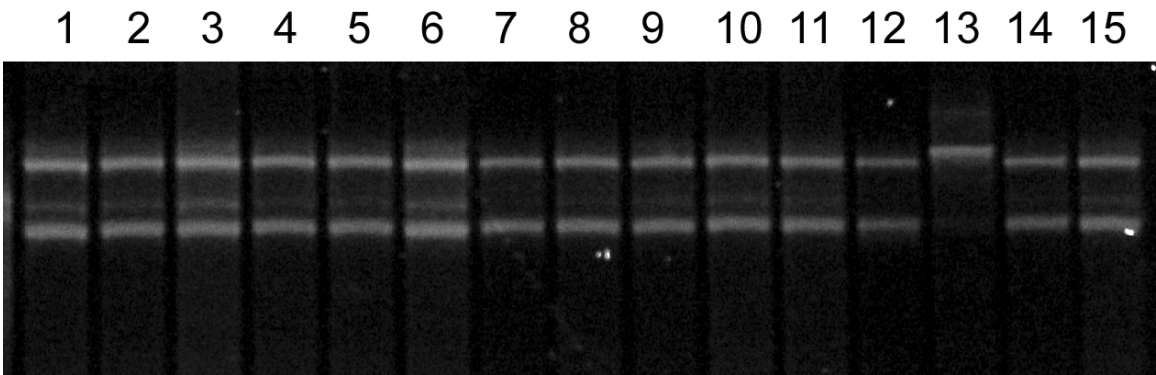
Figure 3.1 SSCP profiles of the ND5 mtDNA for 15 representative *S. namaycush* **samples collected from Lac La Ronge (lanes 1-6), Kingsmere Lake (lanes 7-11) and Reindeer Lake (12-15).   The sequence of the sample in Lane 13 differs from those in the other lanes (see Fig 3).**

```
                 10        20        30        40        50        60        70
        .........+.........+.........+.........+.........+.........+.........+
I       CCCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAA
II      CCCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAA
        ********************************************************************
                 80        90       100       110       120       130       140
        .........+.........+.........+.........+.........+.........+.........+
I       TATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAAC
II      TATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAAC
        ********************************************************************
                150       160       170       180       190       200       210
        .........+.........+.........+.........+.........+.........+.........+
I       ATATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACG
II      ATATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACG
        ********************************************************************
                220       230       240       250       260       270       280
        .........+.........+.........+.........+.........+.........+.........+
I       GCCGAGCTGACGCTAATACAGCTGCCATACAAGCTGTGATTTATAACCGAGTCGGAGACATTGGACTTAT
II      GCCGAGCTGACGCTAATACAGCTGCCATACAAGCTATGATTTATAACCGAGTCGGAGACATTGGACTTAT
        *********************************** ********************************
                290       300       310       320       330
        .........+.........+.........+.........+.........+..
I       TCTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAA
II      TCTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAA
        ***************************************************
```

Figure 3.2. Representative alignment of the partial mitochondrial ND5 sequences from _S. namaycush_ individuals collected from Crean Lake (CR29-I) and Reindeer Lake (RD4-II). *'s denotes bases that are identical among both sequences.

```
II  PPLPSTPDPFSNLHPDTCMPTPITDSLNTSSSSLPSSPPTTYSNYLSAERELAL
I   PPLPSTPDPFSNLHPDTCMPTPITDSLNTSSSSLPSSPPTTYSNYLSAERELAL


II  YRSSSLDDDTAELTLIQLPYKLFITESETLDLFAHDSQQTLTPEKFN
I   YRSSSLDDDTAELTLIQLPYKLFITESETLDLFAHDSQQTLTPEKFN
```
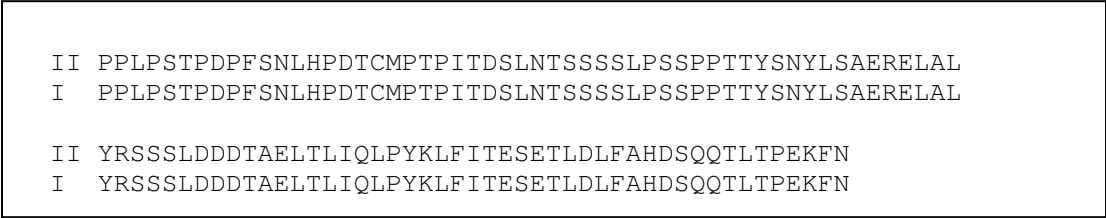
Figure 3.3. Alignment of the amino acid sequences of the ND5 fragment for the two nucleotide sequence types (I-II) of _S. namaycush_. No differences in protein sequence was detected.
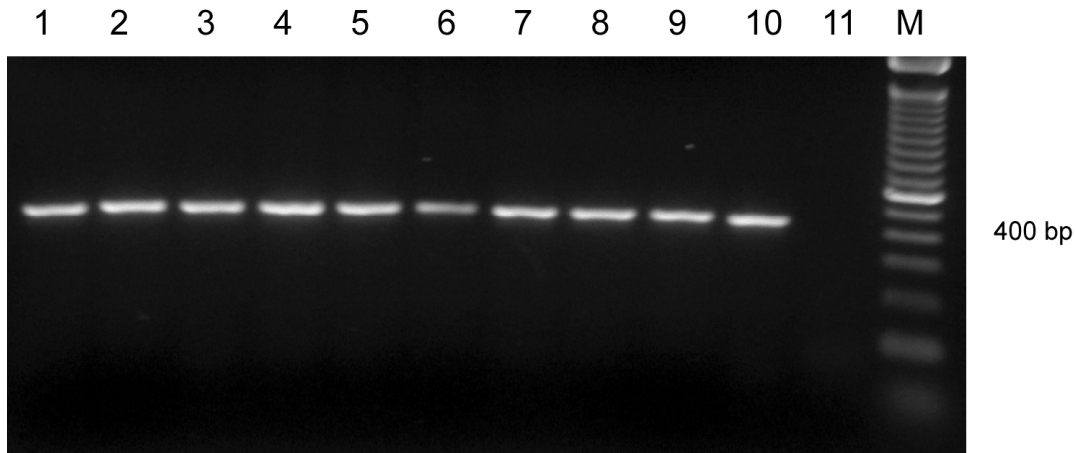
Figure 3.5 Agarose gel depicting the mtDNA ND2 amplicons of 10 representative *S. namaycush* samples (lanes 1-10) from East Trout Lake. M=DNA size marker, lane 11= negative (i.e. no gDNA) control.
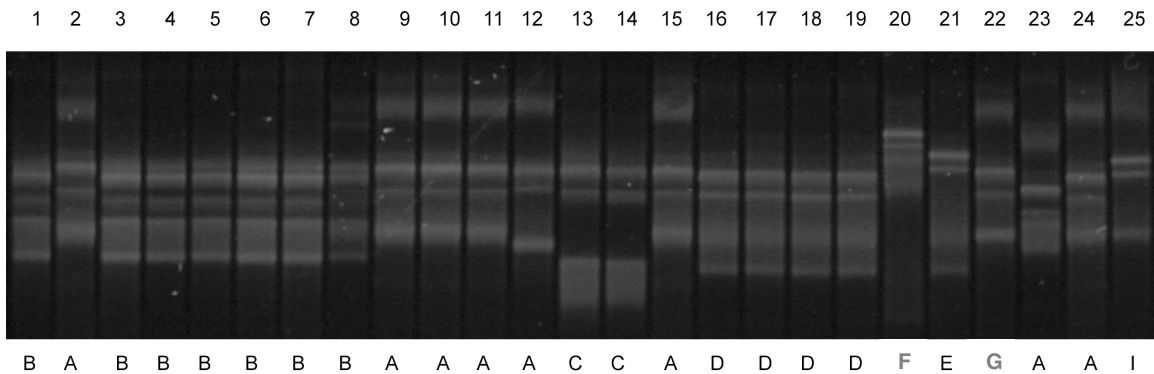
Figure 3.6 SSCP profiles (A-G, I) of the ND2 mtDNA for representative *S. namaycush* samples collected from Hatchet Lake (lane 16), Kingsmere Lake (lane 20), Reindeer Lake (lanes 11-12, 25), Oliver Lake (lanes 23-24) , Crean Lake (lanes 1-5, 7-9, 13, 15), and Lac La Ronge (lane 6, 10, 14, 17-19, 21-22).

Table 3.3. The number of each ND2 mtDNA SSCP type (1-14) of *S. namaycush* detected in each lake.

Lake	1	8	2	3	4	9	5	6	7	10	11	12	13	14	
					SSCP Profile										
Crean	33	0	26	1	1	0	0	0	0	0	0	0	0	0	61
Kingsmere	9	0	0	0	13	0	29	5	3	0	0	0	0	0	59
Little Bear	30	0	0	0	3	0	0	0	0	0	0	0	0	0	33
East Trout	0	0	0	0	17	0	8	0	0	0	0	0	0	0	25
Wassegam	12	0	0	0	11	0	15	2	0	0	0	0	0	0	40
La Ronge	17	1	6	0	41	2	8	3	0	1	0	0	0	0	79
Nemeiben	2	1	0	0	5	0	2	0	0	0	0	0	0	0	10
Oliver Lake	15	2	0	0	11	0	16	3	0	0	0	0	0	0	47
Wollaston	12	0	0	0	10	0	6	0	0	0	0	0	1	0	29
Hatchet	2	0	0	0	11	0	1	0	0	0	0	0	0	0	14
Reindeer	44	0	0	0	32	0	14	0	0	0	1	1	0	1	93
George	18	0	0	0	5	0	11	0	0	0	0	0	0	0	34
Orr	7	0	0	0	14	0	0	0	0	0	0	0	0	0	21
Tazin	6	0	0	0	14	0	0	0	0	0	0	0	0	0	20
Ghana	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Nettle	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Tsalcior	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Clinksvill	0	0	0	0	3	0	0	0	0	0	0	0	0	0	3
Thluicho	0	0	0	0	4	0	0	0	0	0	0	0	0	0	4
Total	**211**	**4**	**32**	**1**	**195**	**2**	**110**	**13**	**3**	**1**	**1**	**1**	**1**	**1**	**576**

```
                10        20        30        40        50        60        70        80
        .........+.........+.........+.........+.........+.........+.........+.........+
A       ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCTCACATTGGCT
B       ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCTCACATTGACT
C       ATGAACCCCTACGTACTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCTCACATTGGCT
D       ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCTCACATTGGCT
E       ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCTCACATTGGCT
F       ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCTCACATTGGCT
G       ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCTCACATTGGCT
H       ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCTCACATTGGCT
I       ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCTCACATTGGCT
J       ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCTCACATTGGCT
K       ATGAACCCCTACGTACTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCTCACATTGGCT
        ************** *** *********************************************************** **
                90       100       110       120       130       140       150       160
        .........+.........+.........+.........+.........+.........+.........+.........+
A       TCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTG
B       TCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTG
C       TCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTG
D       TCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTG
E       TCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTG
F       ACTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTG
G       TCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTG
H       TCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTG
I       TCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTG
J       TCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTG
K       TCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTG
         **************************************************************************** *
               170       180       190       200       210       220       230       240
        .........+.........+.........+.........+.........+.........+.........+.........+
A       AAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTACCAATGCCTGACTA
B       AAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTACCAATGCCTGACTA
C       AAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTACCAATGCCTGACTA
D       AAGCTACTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTACCAATGCCTGACTA
E       AAGCTACTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTACCAATGCCTGACTA
F       AAGCTACTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTACCAATGCCTGACTA
G       AAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTACCAATGCCTGACTA
H       AAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAACAATGATCCTGTTTGCTAGCACTACCAATGCCTGACTA
I       AAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTACCAATGCCTGACTA
J       AAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTACCAATGCCTGACTA
K       AAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTATTTGCTAGCACTACCAATGCCTGACTA
        *************************** ************** ********* ***********************
               250       260       270       280       290       300       310       320
        .........+.........+.........+.........+.........+.........+.........+.........+..
A       GTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
B       GTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
C       GTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
D       GTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
E       ATAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
F       ATAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
G       GTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTAAAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
H       GTAGGAAAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
I       ATAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
J       GTAGGAAAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTACAACACAATACTGGCCCTTGCACTTAAACTTGGGC
K       GTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
        ***** ******************************************** **** ***************************
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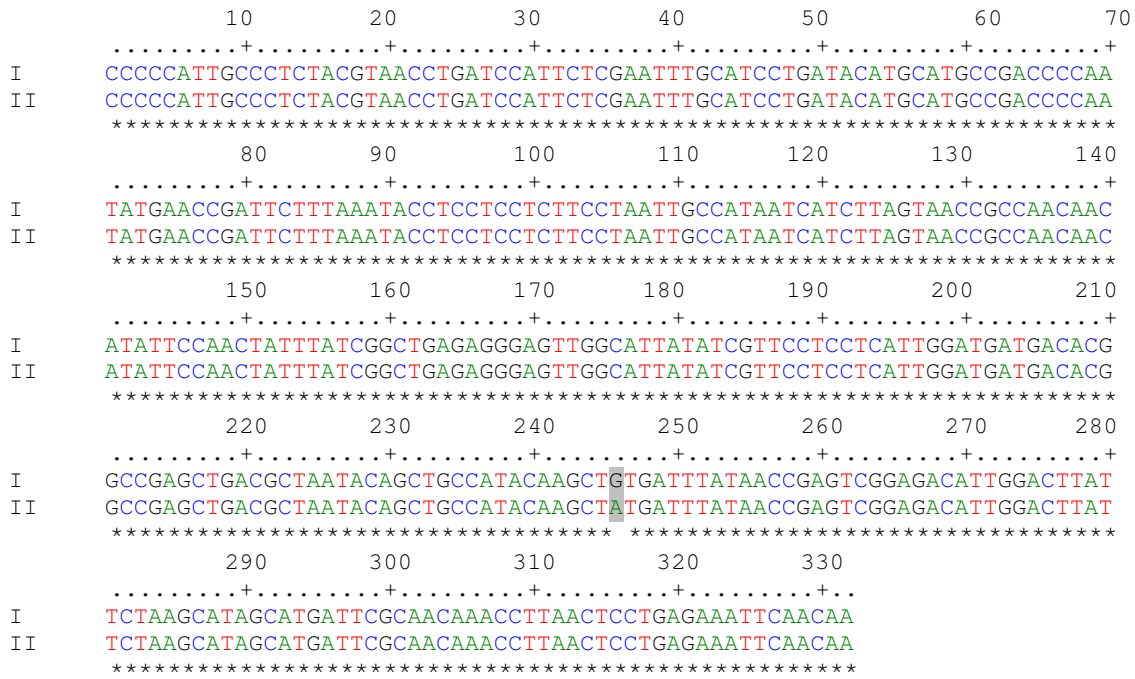
Figure 3.7 Alignment of the partial mitochondrial ND2 sequences from *S. namaycush* individuals collected from Crean Lake (CR12-A , CR11-B), Kingsmere Lake (KG10-F), Reindeer Lake (RD83-C, RD6-H, RD20-I, RD64-K), Hatchet Lake (HL14-D); La Ronge (LA2-E, LA9-G) and Wollaston Lake (WL12-J).

Table 3.4 Location of the mutational changes in nucleotide sequence of the ND2 mitochondrial gene among the 11 sequence types of *S. namaycush* (A-K) from the 19 lakes.

Sequence Type	Position of nucleotide in alignment sequence											
	16	20	78	81	186	202	213	241	286	287	292	304
A	C	T	G	T	G	G	G	G	A	C	G	C
B	C	T	A	T	G	G	G	G	A	C	G	C
C	C	C	G	T	G	G	G	G	A	C	G	C
D	T	C	G	T	A	G	G	G	A	A	G	C
E	T	C	G	T	A	G	G	A	C	A	G	C
F	T	C	G	A	A	G	G	A	C	A	G	C
G	C	T	G	T	G	G	G	G	A	A	G	A
H	C	T	G	T	G	A	G	G	A	C	G	C
I	C	T	G	T	G	G	G	A	A	C	G	C
J	C	T	G	T	G	G	G	G	A	C	A	C
K	C	C	G	T	G	G	A	G	A	C	G	C

Table 3.5 Pairwise comparison of the percent difference in sequences among haplotypes.

	A	B	C	D	E	F	G	H	I	J	K
A	-										
B	0.3	-									
C	0.3	0.6	-								
D	1.2	1.6	0.9	-							
E	1.9	2.2	1.6	0.6	-						
F	2.2	2.5	1.9	0.9	0.3	-					
G	0.6	0.9	0.9	1.2	1.9	2.2	-				
H	0.3	0.6	0.6	1.6	2.2	2.8	0.9	-			
I	0.3	0.6	0.6	1.6	1.6	1.9	0.9	0.6	-		
J	0.3	0.6	0.6	1.6	2.2	2.5	0.9	0.6	0.6	-	
K	0.6	0.9	0.3	1.2	1.9	2.2	1.2	0.9	0.9	0.9	-

Table 3.6. The number and frequency of each ND2 mtDNA sequence type (A-K) of
S. namaycush **within each lake.**

Lakes	A	B	C	D	E	F	G	H	I	J	K	*n*
				Sequence Types No. (%)								
Crean	33 (54)	27 (44)	1 (2)	0	0	0	0	0	0	0	0	61
Kingsmere	9 (15)	0	13 (22)	29 (50)	5 (8)	3 (5)	0	0	0	0	0	59
Little Bear	30 (91)	0	3 (9)	0	0	0	0	0	0	0	0	33
East Trout	0	0	17 (68)	8 (32)	0	0	0	0	0	0	0	25
Wassegam	12 (30)	0	11 (28)	15 (37)	2 (5)	0	0	0	0	0	0	40
La Ronge	18 (23)	6 (8)	43 (54)	8 (10)	3 (4)	0	1 (1)	0	0	0	0	79
Nemeiben	3 (30)	0	5 (50)	2 (20)	0	0	0	0	0	0	0	10
Oliver Lake	17 (36)	0	11 (23)	16 (35)	3 (6)	0	0	0	0	0	0	47
Wollaston	12 (41)	0	10 (35)	6 (21)	0	0	0	0	0	1 (3)	0	29
Hatchet	2 (14)	0	11 (79)	1 (7)	0	0	0	0	0	0	0	14
Reindeer	44 (47)	0	32 (35)	14 (15)	0	0	0	1 (1)	1(1)	0	1 (1)	93
George	18 (53)	0	5 (15)	11 (32)	0	0	0	0	0	0	0	34
Orr	7 (33)	0	14 (67)	0	0	0	0	0	0	0	0	21
Tazin	6 (30)	0	14 (70)	0	0	0	0	0	0	0	0	20
Ghana	1 (100)	0	0	0	0	0	0	0	0	0	0	1
Nettle	2 (100)	0	0	0	0	0	0	0	0	0	0	2
Tsalcior	1 (100)	0	0	0	0	0	0	0	0	0	0	1
Clinksvill	0	0	3 (100)	0	0	0	0	0	0	0	0	3
Thluicho	0	0	4 (100)	0	0	0	0	0	0	0	0	4
Total	**215**	**33**	**197**	**110**	**13**	**3**	**1**	**1**	**1**	**1**	**1**	**576**

Table 3.7 Intrapopulation analyses of lake trout haplotype diversity (h) and the nucleotide diversity (π) from 14 lakes in Saskatchewan

| Lake	Haplotype diversity (*h*)	Nucleotide diversity (π)
Crean	0.5197 ± 0.0230	0.001660 ± 0.001552
Wassegam	0.7090 ± 0.0282	0.004754 ± 0.003235
Kingsmere	0.6885 ± 0.0456	0.004817 ± 0.003240
Little Bear	0.1705 ± 0.0822	0.000529 ± 0.000805
East Trout	0.4533 ± 0.0717	0.002816 ± 0.002253
La Ronge	0.6423 ± 0.0460	0.003619 ± 0.002613
Nemeiben	0.6889 ± 0.1038	0.003658 ± 0.002891
Reindeer	0.6417 ± 0.0276	0.003442 ± 0.002516
Wollaston	0.6897 ± 0.0419	0.004131 ± 0.002942
Oliver	0.7095 ± 0.0271	0.004901 ± 0.003297
Hatchet	0.3846 ± 0.1494	0.001706 ± 0.001672
George	0.6114 ± 0.0522	0.004395 ± 0.003064
Orr	0.4667 ± 0.0751	0.001449 ± 0.001472
Tazin	0.4421 ± 0.0875	0.001373 ± 0.001427
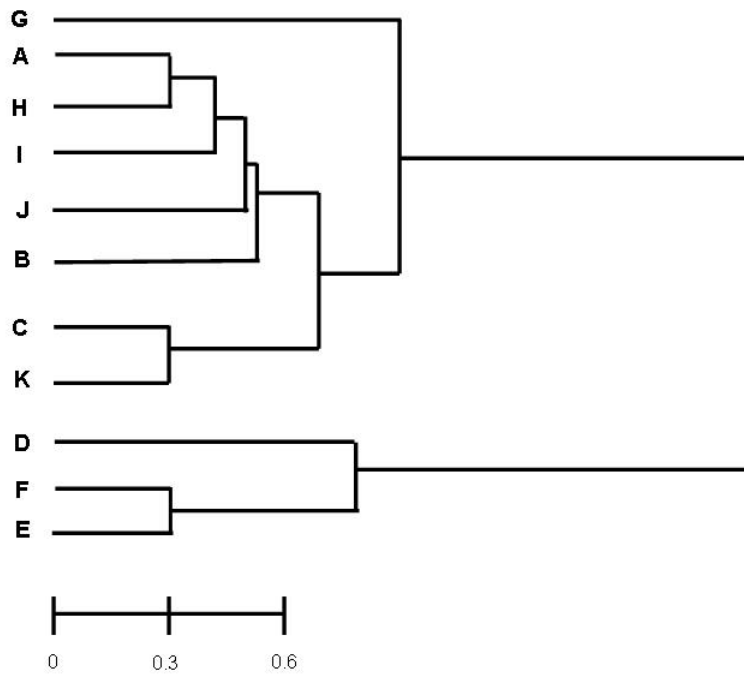
Figure 3.8 Phenogram depicting the genetic differences (%) among the 11 ND2 sequence types (A-K) of *S. namaycush* collected from 19 lakes in Saskatchewan.
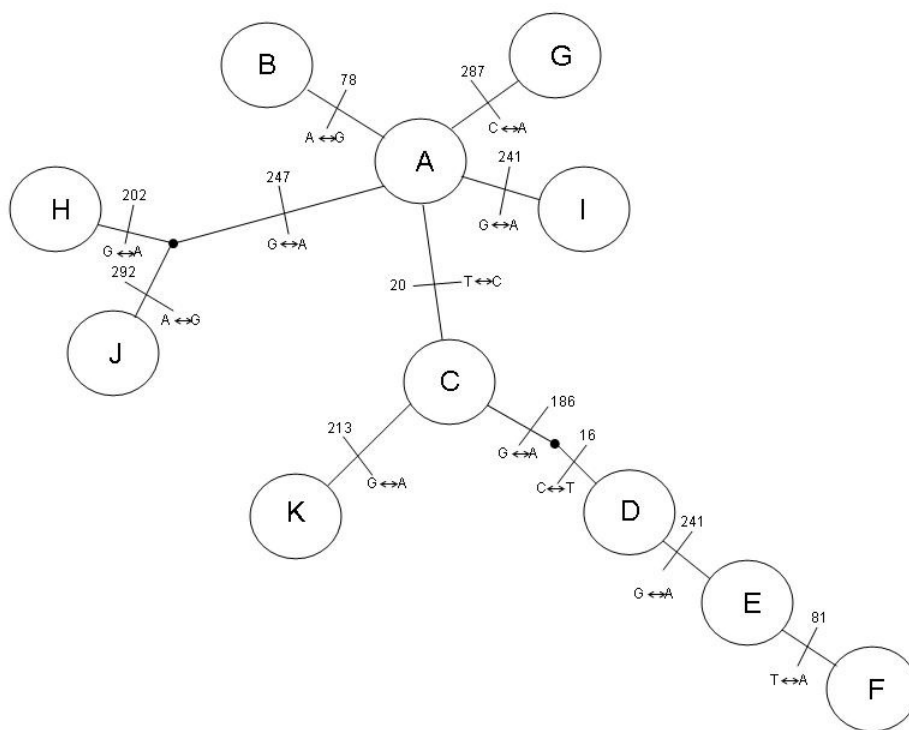
Figure 3.9 - Minimum spanning tree depicting the positions of substitutional mutations relative to the 11 S. *namaycush* ND2 sequence types (A-K).

```
A    MNPYVLIILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
B    MNPYVLIILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
G    MNPYVLIILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
H    MNPYVLIILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
I    MNPYVLIILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
J    MNPYVLIILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
----------------------------------------------------------------
C    MNPYVLTILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
K    MNPYVLTILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
----------------------------------------------------------------
D    MNPYVFTILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
E    MNPYVFTILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE
F    MNPYVFTILLSSLGLGTVLTFASSHWLLAWMGLEINTLAIIPIMAQQHHPRAIE


A    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
B    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
G    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATKTAMLALALKLG
H    ATTKYFLTQATAATMILFASTTNAWLVGKWEIHQLSHPLATTTAMLALALKLG
I    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
J    ATTKYFLTQATAAAMILFASTTNAWLVGKWEIHQLSHPLATTTTMLALALKLG
----------------------------------------------------------------
C    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
K    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
----------------------------------------------------------------
D    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
E    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
F    ATTKYFLTQATAAAMILFASTTNAWLVGEWEIHQLSHPLATTTAMLALALKLG
```

Figure 3.10 Alignment of the amino acid sequences of the ND2 fragment for the 11 nucleotide sequence types (A-K) of *S. namaycush*. Highlighted positions indicate differences in amino acids. Dashed line separates the 3 groups described in Figure 3.11 and Table 3.9.
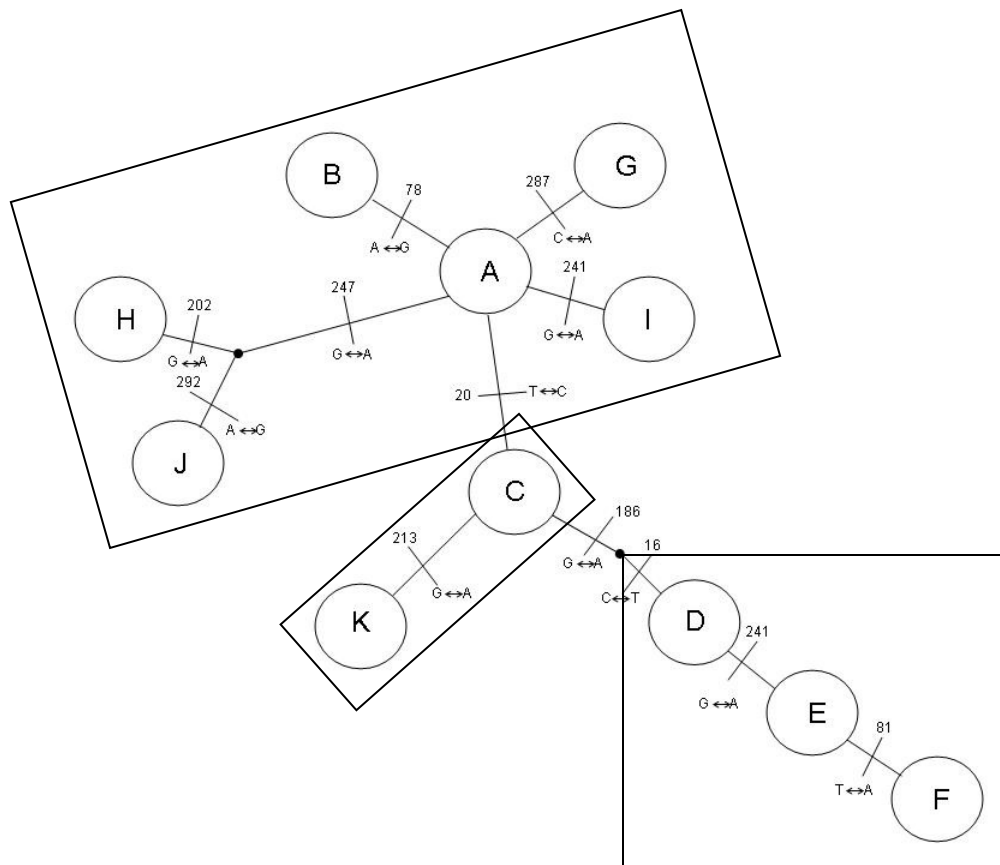
Figure 3.11 Minimum spanning network as shown in Fig. 9 but depicting the clusters of *S. namaycush* nucleotide sequence types (A-K) **based on differences in their amino acid sequences of the ND2 gene fragment.**

Table 3.8 Pair wise differences between genetic diversity of S. namaycush from different lakes were analyzed using Fst. values (distance method). 1-Crean, 2-Wassegam, 3-Kingsmere, 4-Little Bear, 5-East Trout, 6-LaRonge, 7-Nemeiben, 8-Oliver, 9-Hatchet, 10-George, 11-Orr, 12-Tazin, 13-Reindeer and 14-Wollaston. Significantly different diversities are highlighted.

	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.52090												
3	0.61853	0.04988											
4	0.32003	0.43599	0.56099										
5	0.67815	0.05311	0.12469	0.67605									
6	0.40521	0.10152	0.27376	0.30178	0.11520								
7	0.53353	0.00827	0.17564	0.52075	0.04866	-0.04542							
8	0.47546	-0.02043	0.07182	0.37670	0.07405	0.08770	-0.00027						
9	0.62348	0.14343	0.29714	0.67563	0.11395	0.00131	-0.01430	0.13497					
10	0.41146	0.02347	0.17224	0.29111	0.16573	0.07089	-0.00378	0.00459	0.15669				
11	0.53783	0.21601	0.38564	0.52979	0.28736	0.01503	0.04232	0.18883	0.03249	0.16376			
12	0.55784	0.21402	0.38106	0.56999	0.27289	0.01383	0.04113	0.18896	0.01101	0.17184	-0.04862		
13	0.34710	0.12004	0.30894	0.18738	0.20525	0.02218	-0.01687	0.09274	0.08296	0.03067	0.04128	0.05102	
14	0.41139	0.05175	0.22358	0.28041	0.14127	0.00640	-0.05078	0.03288	0.06482	-0.00570	0.05949	0.06630	-0.01367

Table 3.9   The frequency of amino acid ND2 sequence types within each lake.

Amino acid clusters *Corresponding Sequence Types*	**I** *A,B,G,H,I,J*	**II** *C, K*	**III** *D,E,F*	*n*
Crean Lake	98%	2%	-	61
Kingsmere Lake	15%	22%	63%	59
Little Bear Lake	91%	9%	-	33
East Trout Lake	-	68%	32%	25
Wassegam Lake	30%	28%	42%	40
Lac La Ronge	32%	54%	14%	79
Nemeiben Lake	30%	50%	20%	10
Oliver Lake	36%	24%	40%	47
Wollaston Lake	45%	34%	21%	29
Hatchet Lake	15%	76%	7%	14
Reindeer Lake	50%	35%	15%	93
George Lake	53%	15%	32%	34
Orr Lake	33%	67%	-	21
Tazin Lake	30%	70%	-	20
Ghana Lake	100%	-	-	1
Nettle Lake	100%	-	-	2
Tsalcior Lake	100%	-	-	1
Clinksvill Lake	-	100%	-	3
Thluicho Lake	-	100%	-	4

Chapter 4: Phylogenetic relationships of *Salvelinus namaycush* based on analyses of

the mitochondrial DNA sequences

4.1 Introduction

In the previous two chapters, the genetic diversity of *S. namaycush* within and among different lakes in Saskatchewan was examined using partial regions of two mitochondrial DNA genes, the ND2 and ND5. The aim of this chapter was to gain insight into the evolutionary relationships of *S. namaycush* within the Family Salmonidae (Order Salmoniformes) using the sequence data obtained in chapters 2 and 3.

The lake trout, *S. namaycush* is one of at least 11 recognized species within the genus *Salvelinus*, however the status of some species within the genus and the systematic relationships of species have a long history of uncertainty (Behnke 1980). The genus *Salvelinus*, together with four other genera (*Brachymystax, Hucho, Oncorhynchus* and *Salmo*), belong to the subfamily Salmoninae (Scott & Crossman 1973). The Salmoninae (i.e. char, trout, and salmon), Coregoninae (i.e. whitefish and ciscoes) and Thymallinae (i.e. grayling) are the three subfamilies that constitute the family Salmonidae (i.e. the salmonids). However, the phylogenetic relationships of salmonids have been the subject of considerable debate (Behnke 1980; Crespi & Fulton 2004; Ishiguro *et al.* 2003; McKay *et al.* 1996). For instance, questions remain concerning the relationships of genera within the Salmonidae and species-level relationships within the genera *Oncorhynchus* and *Salvelinus* (Crespi & Fulton 2004).

In the most comprehensive study of the phylogeny of the Salmonidae, Crespi and Fulton (2004) combined both nuclear DNA and mitochondrial DNA (from 16 genes) data to infer phylogenies for 30 species of salmonids. However, in this and in other phylogenetic studies, few have included *S. namaycush* and/or the data on the ND2 gene and/or the ND5 gene (Crespi & Fulton 2004; Ishiguro *et al.* 2003; McKay *et al.* 1996). There has been no study that has determined the relative value of the ND2 gene and/or ND5 gene to examine the evolutionary relationships of salmonids. Furthermore, there are no previously published sequences of the ND2 and ND5 genes for *S. namaycush*.

The objective of this chapter was to examine the phylogenetic relationships of *S. namaycush* within the Salmonidae using sequences of the ND2 and ND5 mt DNA genes**.** This is now possible given that sequences of the ND2 and ND5 genes for *S. namaycush* have been established (chapters 2 and 3)**,** and that there are sequence data available for several other species of salmonids, including other species of *Salvelinus*.

4.2 Materials and Methods
The ND2 and ND5 sequence data obtained in chapters 2 and 3 for *S. namaycush* were aligned manually with previously published mtDNA sequence data for several other species of salmonid (Table 4.1). Phylogenetic analyses were carried out using a neighbor-

joining (NJ) and maximum parsimony (MP) methods in PAUP v4.0b2 (Swofford 2001). Trees were inferred for the ND2 and ND5 in separate analyses. For the MP analyses, heuristic searches were carried out with random addition of sequences (n=1000), tree-bisection-reconstruction (TBR) branch swapping, the MulTrees option in effect and saving all equally parsimonious trees. All characters were equally weighted and unordered. The tree length (L), consistency index excluding uninformative characters (CI) and retention index (RI) were recorded for each MP analysis. The northern pike, *Esox lucius*, was used as the outgroup for the MP analyses as this species belongs to the order Esociformes, which was shown by Ishiguro *et al.* (2003) to be a sister group to the order Salmoniformes. The relative support for clades in NJ and MP analyses was determined using 1,000 bootstrap replicates.

4.3 Results
The phylogenetic relationships of *S. namaycush* to other salmonids was examined using NJ and MP analyses of sequence data for partial regions of the ND5 and ND2 mtDNA genes. Each gene was treated separately as sequence data for both genes were not available for all salmonids used in this study.

4.3.1 ND5
The MP analysis of the 322 alignment positions of ND5 mtDNA gene produced the two most equally parsimonious trees with a length of 219, a consistency index (excluding 247 uninformative characters) of 0.58 and a retention index of 0.54. The topology of the strict consensus tree (not shown) was very similar to the tree produced by the NJ analysis (Fig. 4.1). The results showed that *S. namaycush* had a sister-taxa relationship with *S. alpinus* for which there was strong statistical support in the NJ analysis. There was no support for such a relationship in the MP analyses. In the NJ analyses there was very strong support (93%) for the three species of *Salvelinus* (*S. namaycush, S. alpinus* and *S. fontinalis*) representing a clade. There was however, only limited support (54%) for such a clade in the MP analyses. In both the NJ and MP analyses, there was very strong statistical support (90-93%) for a clade containing the six species of *Oncorhynchus*. Within the *Oncorhynchus* clade, *O. keta* and *O. gorbuscha* formed one clade (bootstrap values of 87-97%), while the other 4 species (*O. mykiss*, O. *clarki henshawi, O. clarki* and *O. tshawytscha*) formed a second clade (bootstrap values of 79-89%). Within this second clade, there was total statistical support (bootstrap values of 100%) for a sister-taxa relationship between *O. clarki henshawi* and *O. clarki*. There was also very strong support for a clade containing three genera, *Salvelinus, Oncorhynchus* and *Salmo*. The phylogenetic position of *Salmo salar* with respect to the other two genera was not resolved. There was also very strong support for a sister-taxa relationship between *Coregonus lavaretus* and *Osmerus morax* (Fig. 4.1).

4.3.2 ND2

The MP analysis of the 322 alignment positions of ND2 mtDNA gene produced five most equally parsimonious trees with a length of 228, a consistency index (excluding 246 uninformative characters) of 0.65 and a retention index of 0.70. As in the analysis of the

ND5 sequence data, the topology of the strict consensus tree for the ND2 data (not shown) was very similar to the tree produced by the NJ analysis for this gene (Fig. 4.2). The results showed that 11 *S. namaycush* ND2 haplotypes represented a clade with strong statistical support (bootstrap values of 96% and 78% for the NJ and MP analyses, respectively). There was 100% statistical support in both the NJ and MP analyses for a clade containing the three species of *Salvelinus* (*S. namaycush, S. alpinus* and *S. fontinalis*). Within this clade there was only weak support (54-67%) for a sister-taxa relationship between *S. namaycush* and *S. fontinalis*. The three species of *Oncorhynchus* (*O. mykiss*, *O. clarki henshawi* and *O. tshawytscha*) included in the analyses formed a clade with very strong statistical support (bootstrap values of 90-97%). In the NJ tree, *Salmo salar* was placed on a branch external to the genus *Salvelinus*, however there was no statistical support for such a relationship between the two genera. As in the ND5 tree (Fig. 1), there was very strong support (bootstrap values of 90-96%) for a clade containing species from three genera, *Salvelinus*, *Salmo* and *Oncorhynchus*, to the exclusion of *C. lavaretus* (Fig. 4.2).

4.4 Discussion

The aim of this chapter was to examine the phylogenetic relationships of *S. namaycush* within the Salmonidae using sequences of the ND2 and ND5 mtDNA genes. The use of genetic markers from multiple genes is important to gain insight into the evolutionary history of *S. namaycush* and other salmonids because these relationships are not well defined. It also becomes important in order to compare the adaptations of salmonids, *e.g.* the evolution of semelparity (Crespi & Teo 2002), for comparative genomics, and for the evaluation of conservation priorities (Crandall *et al.* 2000).

There were differences between analyses in the relative support for a clade comprising the three species of *Salvelinus*, with total statistical support for the relationship using the ND2 gene, but only weak support in the ND5 tree. On the other hand, there was strong statistical support for an *Oncorhynchus* clade in the separate analyses of the ND2 and ND5 genes.

Within the *Salvelinus* clade, *S. namaycush* and *S. fontinalis* were more closely related to each other than to *S. alpinus* based on analyses of the ND2 gene. There was, however, only limited support for a sister-taxa relationship between *S. namaycush* and *S. fontinalis.* In contrast, analyses of the ND5 gene revealed strong support for a sister-taxa relationship between *S. namaycush* and *S. alpinus*. In the study by Crespi and Fulton (2004), analyses of the NADH dehydrogenase subunit 3 (ND3) of the mtDNA gene revealed that there was also strong support for a clade comprising *S. namaycush, S. alpinus* and a third species *S. malma* to the exclusion of *S. fontinalis*.

Within the *Oncorhynchus* clade, there was total statistical support for the sister-taxa relationship between the *O. clarki henshawi* and *O. clarki* in the ND5 tree. This was expected as *O. clarki henshawi* (Lahontan cutthroat trout) is a genetically distinct subspecies of *O. clarki* (cutthroat trout) (Machtinger 2007). The relative positions of the *Oncorhynchus* species to each other in the ND2 and ND5 trees were similar to those in trees derived from separate analyses of the ND3 gene and nuclear growth hormone type-2

gene (GH2) (McKay *et al.* 1996). For example, in the study by McKay *et al.* (1996) an *O. mykiss* and *O. clarki* clade was formed, which strengthens the results of the ND2 gene analyses where *O. mykiss* and *O. clarki henshawi* formed a clade. In addition, there was a sister-taxa relationship between *O. keta* and *O. gorbuscha* in the present study (i.e. in the ND5 tree) and in the study of McKay *et al.* (1996).

From the analysis of the partial ND5 and ND2 gene sequences, the genus *Oncorhynchus* represented a sister taxon to lake trout and other species of the genus *Salvelinus*. The two genera are closely related based on life histories, morphological traits, molecular clock evidence, vitellogenin gene and microsatellite gene evidence (see Crespi & Fulton (2004) for review). This sister-taxa relationship is in agreement with the findings of Crespi & Fulton (2004) who conducted phylogenetic analyses using three other genes, a nuclear growth hormone (GH1C), the vitellogenin gene (VIT), and the ND3 of mtDNA. Both genera are believed to have radiated in parallel on a large scale (Angers & Bernatchez 1997), such that the each genus has given rise to exclusively freshwater species, diadromous populations, and exclusively Asian species (Stearley & Smith 1993). However, it is still unclear as to whether the origins of these groups are marine or freshwater (McDowall 2002).

Although there was the strong support for a close relationship between the *Oncorhynchus* and *Salvelinus* based on analyses of the ND2 and ND5 genes analyses, these two mtDNA genes did not always resolve the relationships of all genera within the Salmonidae. The relative position of *Salmo salar* differed between the ND2 and the ND5 trees, forming a sister-taxon relationship with *Salvelinus* in the ND2 tree, while the ND5 grouped *Sa. salar* with *Oncorhynchus*, and in both cases there was no statistical support for its position. There is ongoing debate as to whether *Salmo* and *Oncorhynchus* form a sister-taxon relationship (Angers & Bernatchez 1997; Crespi & Fulton 2004; McKay *et al.* 1996; Oakley & Phillips 1999). In a previous phylogenetic study, *Sa. salar* and the genus *Oncorhynchus* were shown to be closely related using sequence data of the ND3 mtDNA gene and a portion of the nuclear growth hormone type-2 gene (McKay *et al.* 1996). The sister group relationship between *Oncorhynchus* and *Salmo* has long been hypothesized because *Oncorhynchus* is believed to have arisen from a single ancestral species derived from the *Salmo* evolutionary line (McKay *et al.* 1996). The similarities in morphological characters caused *O. mykiss* and *O. clarkii* to be retained in the genus *Salmo*. However, increasing resolution of systematic analysis suggested a closer relationship to other salmonids, leading to the eventual placement of *O. mykiss* and *O. clarkii* in *Oncorhynchus* (McKay *et al.* 1996). As indicated previously, the precise relationship of *Sa. salar* to the genera *Oncorhynchus* and *Salvelinus* was not resolved using the ND2 and ND5 genes. Nonetheless in both analyses, there was very strong statistical support for the inclusion of *Sa. salar* in a clade with species of *Oncorhynchus* and *Salvelinus,* to the exclusion of *Coregonus lavaretus* and *Osmerus morax.*

In conclusion, the ND2 and ND5 mtDNA genes each provide valuable genetic markers to infer the relationships of species within the Salmonidae at various taxonomic studies. Further work in ND2 and ND5 analyses of Salmonidae phylogeny should include more species, such as the *S. malma* or other species from the genus *Salmo*, as well as including

sequence data for other mtDNA genes in the analyses. This may provide further resolution to the relationships found within this economically and ecologically important family of fishes.
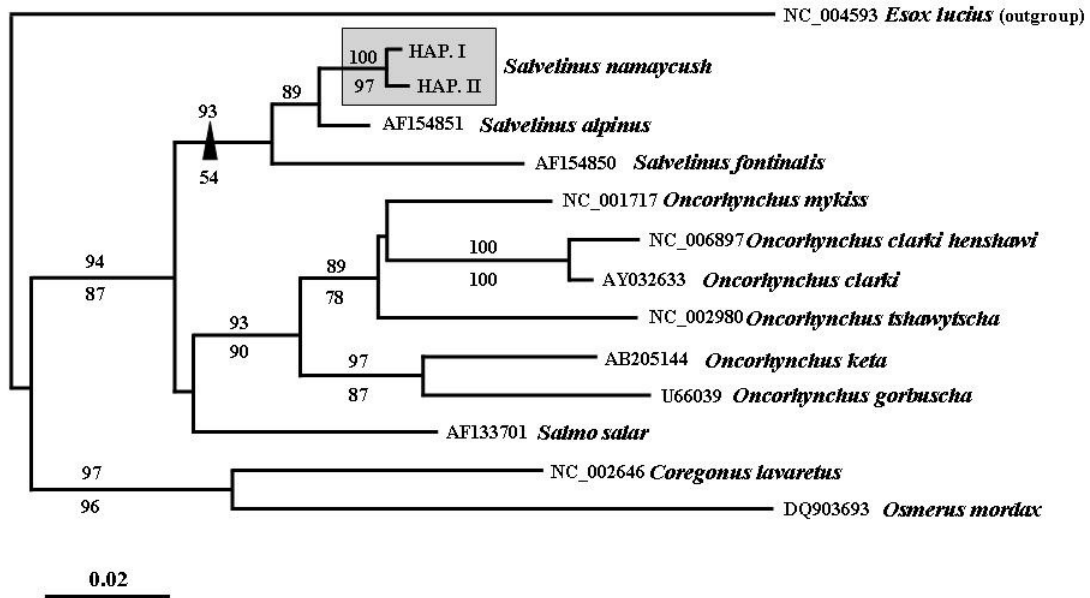
Figure 4.1 – Phylogenetic relationships of *Salvelinus namaycush* to other salmonids based on NJ-analysis of part of the ND5 MtDNA gene. Bootstrap values (over 50%) for NJ (above) and MP (below) are shown. Shaded area shows the *Salvelinus namaycush* clade.
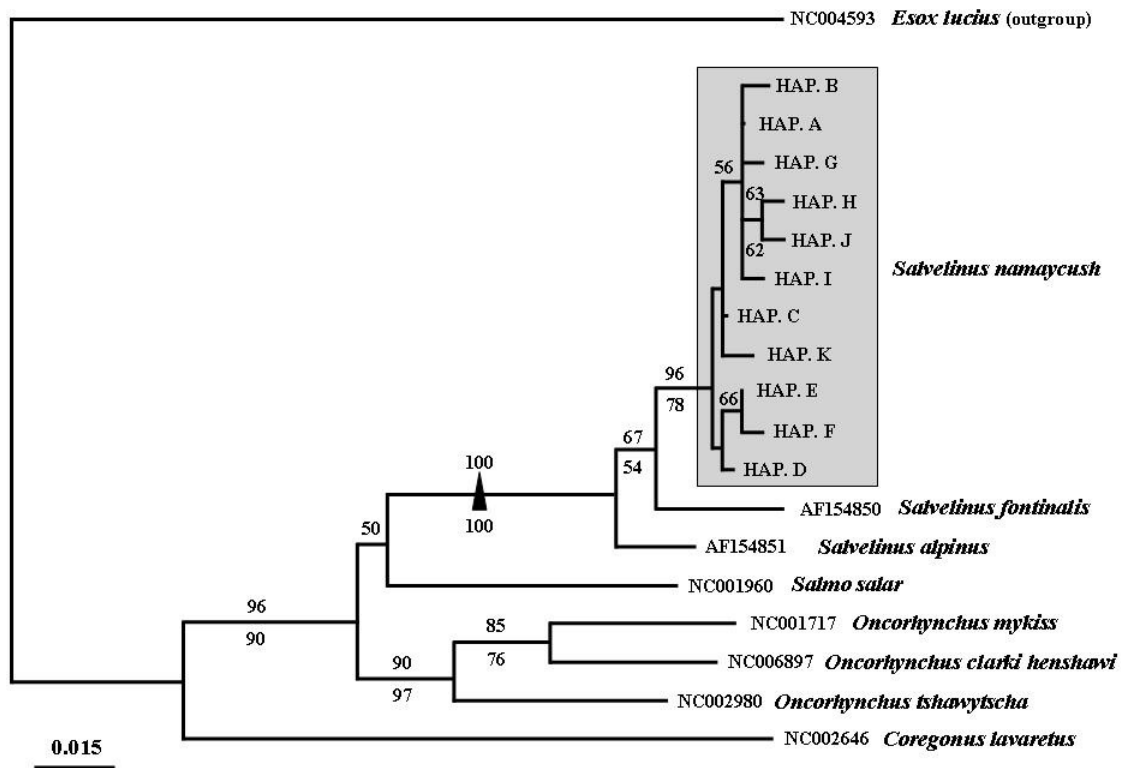
Figure 4.2 Phylogenetic relationships of *Salvelinus namaycush* to other salmonids based on NJ analysis of part of the ND2 MtDNA gene. Bootstrap values (over 50%) for NJ (above) and MP (below) are shown. Shaded area shows the *Salvelinus namaycush* clade.

Table 4.1   The GenBank accession numbers for species included in the phylogenetic analyses of the ND5 and ND2 mtDNA genes

Species	GenBank Accession no:	
	ND5 gene	ND2 gene
Salvelinus fontinalis	AF154850	AF154850
Salvelinus alpinus	AF154851	AF154851
Oncorhynchus mykiss	NC001717	NC001717
Oncorhynchus clarki henshawi	NC006897	N/A
Oncorhynchus clarki	AY032633	N/A
Oncorhynchus tshawytscha	NC002980	NC002980
Coregonus lavaterus	NC002646	NC002646
Osmerus mordax	DQ643256	N/A
Oncorhynchus gorbuscha	U66039	U66039
Oncorhynchus keta	AB205144	N/A
Salmo salar	NC001960	NC001960
Esox lucius (outgroup)	NC004593	NC004593

Chapter 5: General Discussion

Genetic diversity within a population serves as a basis for evolutionary change. A decrease in genetic variability over time may negatively impact a population's viability (Lande & Shannon 1996; Wang *et al.* 2002). Genetic variation can play a critical role in the long-term viability of a population by affecting its ability to persist and adapt to stochastic environments, such as those predicted to occur in light of climate change. This will be particularly important for species, such as the lake trout (*Salvelinus namaycush*), at the extremes of their distributional range, where the precise ecological conditions that they require are not being fully met, making them susceptible to even minor changes in temperature and food availability. Understanding the population genetics in *S. namaycush* is important because of the role of genetic variation as a source for potential adaptation to changes in strenuous environments.

The main objective of this research project was to examine the magnitude of genetic variation within and among populations of lake trout in north central Canada. In particular, to compare the populations of *S. namaycush* that occur in the southern-most range of the species, which includes Crean Lake, Kingsmere Lake, East Trout and Little Bear Lakes. Two mitochondrial genes, the NADH dehydrogenase subunit 2 (ND2) and the NADH dehydrogenase subunit 5 (ND5) were selected as the target to compare the genetic diversity in populations of lake trout. These two genes were chosen based on the highest levels of interspecific sequence differences between four related salmonid species *(S. fontinalis, S. alpinus, Salmo salar* and *Oncorhynchus mykiss)* reported in Dorion *et al.* (2002). It was predicted, based on the data of Dorion *et al.* (2002), that the ND5 gene would be more variable in sequence for *S. namaycush* than an equivalent-sized portion of the ND2 gene. However, my results clearly showed this not to be the case. There was almost no intraspecific variation for the 332 bp of the *S. namaycush* ND5 gene used. Only one lake trout differed in sequence compared to the other 190 individuals examined, and this difference represented only a single mutational change. This made this gene fragment unsuitable as a marker to study the population genetics of lake trout. This is in contrast to the 322 bp of the ND2 gene where 11 different sequence genotypes were detected and where mutational changes were found at 12 nucleotide positions. In addition, some of these mutational changes in the ND2 gene resulted in changes in amino acid sequence, the implications of which are discussed later on in this chapter.

One of the important objectives of my research project was to compare the genetic diversity of *S. namaycush* populations among lakes, and to determine if any lakes contained unique genotypes. In addition, the sequence data were analyzed to determine if genetic diversity was related to the latitudinal position of lakes. The relative frequency of this diversity differed markedly between the lakes, with the lakes in the southern distribution of lake trout (Crean, Wassegam, East Trout and Little Bear Lakes) having greater variation between them than lakes at different latitudes. The significant differences detected in genetic diversity of *S. namaycush* among lakes, even within close proximity, may have resulted from a combination of different factors, including ecological factors, environmental stochastic events, mating systems, and various genetic

processes (*i.e.* gene flow, population bottlenecks, and genetic drift) (Roff & DeRose 2001). Where populations are relatively small and isolated, long-term viability and the ability to adapt to stochastic environmental events may be compromised since genetic variation decreases as the likelihood of inbreeding events increase (Frankham 1995b). The monitoring of genetic diversity to determine if there are any temporal changes to frequency is also recommended. For example, some lakes could be monitored every 5 years to determine if there are changes in frequencies of sequence types and possibly establish if there is selection acting on a specific sequence type(s). This approach would also be useful to detect new sequence types and/or sequence types that occur at a very low frequency. This will be useful for monitoring the genetic diversity of *S. namaycush* populations in the southern lakes (Kingsmere, Crean, Wassegam, Little Bear and East Trout), where the impacts of environmental changes due to global warming may become more important on the survival of these populations.

Another important question examined in this thesis was whether the practice of stocking affected the genetic diversity of lake trout in the study area. Stocking has often been used as a management tool in an attempt to supplement declining fish populations. Stocking can affect genetic diversity by either artificially increasing the genetic variation in populations that are experiencing inbreeding depression, or it can result in an outbreeding depression (Frankham 1995a). An outbreeding depression can cause a loss of local adaptation due to the mixing of genomes that evolved independently in different environments, resulting in a less adaptable and persistent population in light of environmental change (Lande & Shannon 1996). To examine, the question of the impact of stocking on the genetic diversity of pre-existing population, a study was conducted to compare the genetic diversity of *S. namaycush* from Crean Lake and Wassegam Lake. These two lakes were used because of the well documented stocking events that occurred within Crean Lake with fish from Wassegam Lake. The results of the present study showed that the genetic composition of lake trout population in Crean Lake was dramatically different to that in Wassegam Lake. This finding raises the possibility that the previous stocking attempts of fish into Crean Lake from Wassegam Lake may have been of little or no success. Therefore, even though stocking may be used as an attempt to enhance fish populations, the results of this study suggest that lake trout of a specific genotype(s) from donor lakes may not be able to reproduce successfully in the introduced lake. Given the questionable stocking success identified in Crean Lake, and with the threat of outbreeding depression, it is recommended that lake-specific breeding programs be initiated in order to conserve the locally-adapted genetics found within the lake trout, especially in the lakes at their southern distribution limit. In addition, the difference in sequence type frequencies and the identification of unique sequence types within some *S. namaycush* lakes, including Crean Lake, require the examination of the functionality of the corresponding amino acid sequences. Functional differences could be resulting in the selection of specific characteristics which result in those fish exhibiting favorable traits.

Genetic markers have not only been used to compare the genetic variation within and among populations, but they have also been used to infer the phylogeography (*i.e.* the geographical distribution of genealogical lineages) of species (Avise 2000). For instance, Wilson and Hebert (1998) identified high genetic diversity in lake trout in the central

region of Canada due to overlapping glacial refuges from the Pleistocene era**. Different lineages were identified, and most co-occurred throughout the region. However, even in light of this finding, no detailed studies had been conducted on populations of lake trout in Saskatchewan. In addition, the ND2 mtDNA gene has not been utilized in any population genetics study of *S. namaycush*, although the sequences of this gene differ among species of other salmonids (Doiron *et al.* 2002). Analyses of the ND2 gene for *S. namaycush* resulted in the detection of 11 different sequence types in the sampled lakes, however it remains to be determined whether this genetic diversity is significantly larger than in other geographical regions where lake trout occur. What is needed is a more comprehensive study of the genetic diversity of *S. namaycush* from lakes in regions across Canada using the ND2 and ND5 genes. This would provide more insight into the relative significance of the genetic diversity detected in *S. namaycush* populations within Saskatchewan. Studies could also expand to include co-dominant markers, such as nuclear DNA (microsatellites), in order to further understand the genetic composition of lake trout populations in Saskatchewan. Also, differences at a latitudinal scale may also be identified using the approach adopted in the present study. If the unique sequence types such as the ND2 genotypes B & F remain exclusive to this geographical region, then they may be important with respect to the genetic diversity of the species at a national level.

There have been previous molecular studies (Behnke 1980; Crespi & Fulton 2004; Ishiguro *et al.* 2003; McKay *et al.* 1996) on the phylogeny (*i.e.* evolutionary relationships) of the salmonids that have included *S. namaycush*. There has however, been no previous attempt to determine the relative utility of the ND5 and/or the ND2 mtDNA genes for this purpose. Hence, one of the aims of my research project was to determine if these two target genes could also be used to infer the phylogenetic relationships of *S. namaycush* to other salmonid species. In Chapter 4, it was shown that both the ND2 and ND5 genes could be used to infer the evolutionary relationships among members of the family Salmonidae. Furthermore, some of the relationships depicted in trees generated from the analyses of the ND2 and ND5 sequence data (*e.g.* between genera and within a genus) were consistent with the findings of other studies (*e.g.* Crespi & Fulton 2004: McKay *et al.* 1996). Thus, future studies could expand to examine, in greater detail, the phylogenetic relationships of all species within the family Salmonidae using genetic markers in these two mtDNA genes.

In conclusion, genetic diversity has been identified as critical in the long-term viability and persistence of populations and as such, the maintenance of genetic variation within and among conspecific populations should be considered a primary goal of conserving salmonid fishes (Wang *et al.* 2002). Molecular ecology is becoming a powerful tool in the management and conservation of fish populations around the world. One of the novel aspects of my research project with respect to examining genetic variation in salmonids was the use of SSCP to genetically screen multiple samples prior to DNA sequencing. This approach of pre-screening proved to be very valuable by reducing the time taken and costs incurred for the project. Furthermore, for the genetic analyses conducted, only a small portion of the adipose fin was removed from each *S. namaycush* used in this study. Thus, *S. namaycush* could be returned back relatively quickly into the lake from where it

was captured with minimal impact on its health. This is particularly important for individuals from Crean Lake, where population size is very low and of major concern to park managers. The methodology used for obtaining material and to conduct genetic analyses employed in the current study could be used very effectively to identify the genetic diversity among and within populations of other fish species such as the endangered lake sturgeon (*Acipenser fulvescens*) (COSEWIC 2007). Thus, it could be used to monitor changes in the genetic makeup of populations over time and to monitor the long-term recovery of the depleted populations (Peterson *et al.* 2007). It would also be interesting to use the SSCP-DNA sequencing approach to determine if the differences in genetic diversity of *S. namaycush* in Crean Lake compared to those in Wassegam and Kingsmere lakes are similar for other fish species that inhabit the lakes. The data collected during this study provides a strong foundation to monitor the long-term effects of global warming on the population dynamics and population genetics of *S. namaycush*.

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APPENDIX A
Methods for collecting lake trout tissue samples by lake.

#	Lake	Collectors	Method
1	Crean	1) Prince Albert National Park staff (PANP) Tina Giroux	- Nets set along spawning reefs and in random plots throughout the lake
2	Kingsmere	1) University of Saskatchewan Chivers lab	- angling
		2) PANP	- opportunistic creel survey
3	Wassegam	1) T. Giroux and H. Giroux	- angling
4	Little Bear	1) Little Bear Lake Resort	- angling
		2) Little Bear Lake Campground	- angling
5	East Trout	1) Eagle Bay Resort	- angling
6	Lac La Ronge	1) Pickerel Bay Resort	- angling
		2) Saskatchewan Environment	- netting at Hunters Bay
7	Nemeiben	1) Nemeiben Lake campground	- creel survey
		2) T. Giroux	- angling
8	George	1) George Lake Lodge	- angling
9	Ghana	1) Oliver Lake Lodge	- angling
10	Oliver	1) Oliver Lake Lodge	- angling
11	Reindeer	1) Commercial fishers - T. Giroux and N. Carriere at fish plant in Kinisoo, MB	- netting
		2) Nordic Lodge	- angling
12	Wollaston	1) Wollaston Lake Lodge	- angling
		2) T. Giroux	- angling
		3) Sustenance nets by local community members	- netting
13	Hatchet	1) Hatchet Lake Lodge	- angling
14	Orr	1) Hawkrock Wilderness	- angling
15	Thluicho	1) Tazin Lake Lodge	- angling
16	Tsalwor	1) Tazin Lake Lodge	- angling
17	Tazin	1) Tazin Lake Lodge	- angling
18	Clinskill	1) Tazin Lake Lodge	- angling
19	Nettell	1) Tazin Lake Lodge	- angling

APPENDIX B
Example of the lake trout sample collection letter sent to outfitters for voluntary tissue collection.

Contact information for Tina Giroux:

Phone: (306) 663-4532 [summer – work]
      (306) 960-1735 [cell]
      (306) 982-4932 [summer – home]
      (306) 966-4455 [leave message]
      (306) 982-2099 [fax]

Email: tina.giroux@usask.ca

IMPORTANT NOTICE

➢ **Ethanol (ETOH) is a toxic substance that is poisonous if consumed.**
➢ **ETOH is combustible and must be kept away from heat / flame.**

Tissue Sample Kit includes:

- **Ethanol (ETOH) filled containers (x 40)**
- **2 permanent markers**
- **2 pencils**
- **1 pair scissors (please return at the end of study!)**
- **Instruction sheet**
- **1 Notebook**
- **Copy of Special Collection Permit (Saskatchewan Environment)**

*NOTE: Special Collection Permit **DOES NOT** allow outfitters/anglers to retain any fish beyond the regular license limits, but only allows for tissue sample collection*

LAKE TROUT TISSUE SAMPLE COLLECTION

1. Only **LAKE TROUT** (*Salvelinus namaycush*) tissue is to be collected.

2. Only **ONE** sample is to be collected **per fish**.

3. Tissue can be taken from any part of the trout, but the **adipose fin** is preferred (refer to diagram). Fish can be released after fin is clipped, ensure that only the fin is clipped, and not the fleshy tissue beneath it.
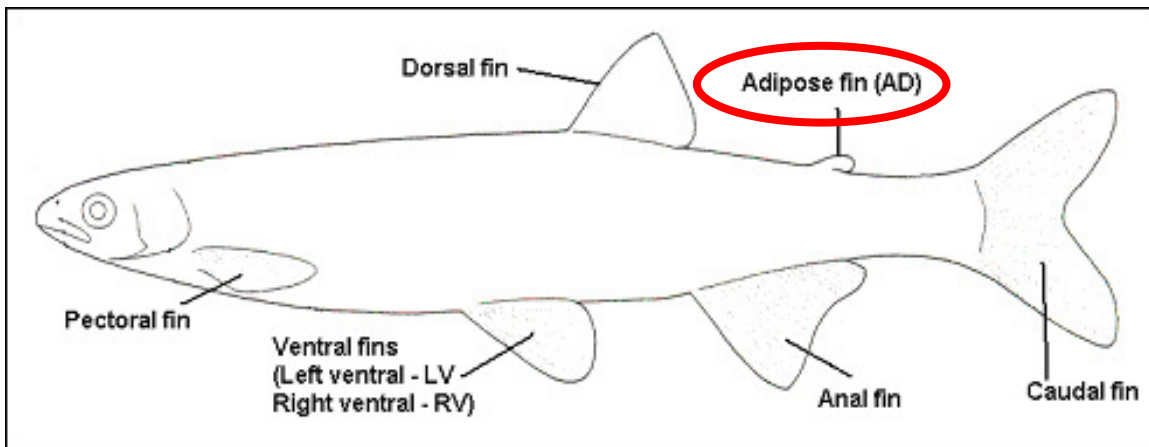
Adipose fin and other tissues should be cut with a pair of scissors (supplied), however a clean knife can also be used. This sample should be **small** enough to fit in the sample container provided in the kit. To avoid contamination of samples, knife/scissors should be **washed** off with lake water between each fish.

4. Only **ONE** tissue sample should be included per **CONTAINER** / **BAG**.

5. To avoid spillage of the ethanol, handle container carefully and ensure cap is closed **tightly**.

6. **Label** each Whirl-pak bag with **permanent marker**:
   - SAMPLE NUMBER
   - LAKE NAME
   - AREA OF LAKE where caught (on large lakes only)

7. Place container in bag **immediately** after sample is collected and close bag.

8. Place in **Ziploc bag** provided

9. Record in **notebook (pencil)**:
   - SAMPLE NUMBER
   - LAKE NAME
   - AREA OF LAKE
   - OPTIONAL MEASUREMENTS (length, weight, comments, etc.)

These samples can remain in the ethanol mixture for several months. There is no need to refrigerate the samples.

If samples will be kept **frozen, they should be stored in the Whirl-pak bags. Please label the bag with 1) Lake Name, 2) date and 3) area of lake with permanent marker. These samples should only be sent frozen if you are confident that they will be returned in a timely manner, and **remain** frozen. If not, please send them in ethanol vials.

Samples can be returned by the following methods:

- Via scheduled **flight** on TransWest Airline to Prince Albert or La Ronge, please contact Tina Giroux before sending to ensure timely pickup. Send C.O.D.

- For **pick-up** in La Ronge, Prince Albert or Saskatoon, contact Tina at the coordinates found on Page 1.

- Tissues can be left at the following **drop-off** locations:

    ✓ **Henry's Taxidermy & Fur Tanning**
       Located ½ miles North of Christopher Lake turnoff
       Phone: (306) 982-4932
       **extra supplies can also be picked up at this location if needed

    ✓ **Prince Albert National Park**
       Warden Station
       Phone: (306) 663-4532

    • **Waterbase Inn**
       303 La Ronge Ave
       La Ronge, SK.
       Phone: (306) 425-5550

    ✓ **Prince Albert Inn**
       Front Desk
       3680 2nd Ave West
       Prince Albert, SK.
       Phone: (306) 922-5000

OPTIONAL DATA COLLECTION

Additional information may be collected if desired. This information will help in determining the status of the lake trout population within your sampled lakes. If you provide this additional data, a brief summary of the analysis will be forwarded to you if desired.

Length/Weight: If you wish to include measurements for length/weight of each fish sampled, please follow these instructions.

Fork Length

Total Length
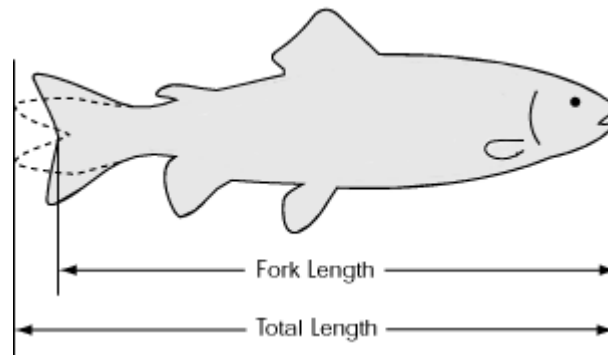
Fork length: Fork length is a measure from the tip of the mouth with the jaws closed to the central part of the tail fin. Record this measurement in notebook provided, along with the weight and the sample ID #.

There also exists the possibility to age lake trout through scales. If your camp would like to participate in this type of data collection, please inform Tina and further instructions will be forwarded to you. Length-weight and age distribution is useful in determining the health of your trout population.

Please keep in mind that the more samples/measurements you provide the more accurate the analysis will be. There is an endless supply of sample containers so feel free to request more if you run out!

- Tina Giroux

APPENDIX C
Alignment of the partial mitochondrial ND5 sequences from seven *S. namaycush* individuals collected from either Crean Lake (CR29-I & CR30-I), Kingsmere Lake (KG20-I & KG 21-I), Wassegam Lake (WS24-I & WS25-I) or Reindeer Lake (RD4-II). *'s denotes bases that are identical among all sequences.

```
                10        20        30        40        50        60        70
        .........+.........+.........+.........+.........+.........+.........+
CR29-I  CCCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAA
CR30-I  CCCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAA
KG20-I  CCCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAA
KG21-I  CCCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAA
WS24-I  CCCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAA
WS25-I  CCCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAA
RD4-II  CCCCCATTGCCCTCTACGTAACCTGATCCATTCTCGAATTTGCATCCTGATACATGCATGCCGACCCCAA
        **********************************************************************
                80        90       100       110       120       130       140
        .........+.........+.........+.........+.........+.........+.........+
CR29-I  TATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAAC
CR30-I  TATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAAC
KG20-I  TATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAAC
KG21-I  TATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAAC
WS24-I  TATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAAC
WS25-I  TATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAAC
RD4-II  TATGAACCGATTCTTTAAATACCTCCTCCTCTTCCTAATTGCCATAATCATCTTAGTAACCGCCAACAAC
        **********************************************************************
               150       160       170       180       190       200       210
        .........+.........+.........+.........+.........+.........+.........+
CR29-I  ATATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACG
CR30-I  ATATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACG
KG20-I  ATATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACG
KG21-I  ATATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACG
WS24-I  ATATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACG
WS25-I  ATATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACG
RD4-II  ATATTCCAACTATTTATCGGCTGAGAGGGAGTTGGCATTATATCGTTCCTCCTCATTGGATGATGACACG
        **********************************************************************
               220       230       240       250       260       270       280
        .........+.........+.........+.........+.........+.........+.........+
CR29-I  GCCGAGCTGACGCTAATACAGCTGCCATACAAGCTGTGATTTATAACCGAGTCGGAGACATTGGACTTAT
CR30-I  GCCGAGCTGACGCTAATACAGCTGCCATACAAGCTGTGATTTATAACCGAGTCGGAGACATTGGACTTAT
KG20-I  GCCGAGCTGACGCTAATACAGCTGCCATACAAGCTGTGATTTATAACCGAGTCGGAGACATTGGACTTAT
KG21-I  GCCGAGCTGACGCTAATACAGCTGCCATACAAGCTGTGATTTATAACCGAGTCGGAGACATTGGACTTAT
WS24-I  GCCGAGCTGACGCTAATACAGCTGCCATACAAGCTGTGATTTATAACCGAGTCGGAGACATTGGACTTAT
WS25-I  GCCGAGCTGACGCTAATACAGCTGCCATACAAGCTGTGATTTATAACCGAGTCGGAGACATTGGACTTAT
RD4-II  GCCGAGCTGACGCTAATACAGCTGCCATACAAGCTATGATTTATAACCGAGTCGGAGACATTGGACTTAT
        ***********************************  *********************************
               290       300       310       320       330
        .........+.........+.........+.........+.........+..
CR29-I  TCTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAA
CR30-I  TCTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAA
KG20-I  TCTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAA
KG21-I  TCTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAA
WS24-I  TCTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAA
WS25-I  TCTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAA
RD4-II  TCTAAGCATAGCATGATTCGCAACAAACCTTAACTCCTGAGAAATTCAACAA
        ****************************************************
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84

APPENDIX D

Alignment of all partial mitochondrial ND2 sequences from 38 _S. namaycush_ individuals from Crean (CR), La Ronge (LA), Wassegam (WS), Reindeer (RD), Wollaston (WL), Oliver (OL), Hatchet (HL), and Nemeiben (NB) Lakes.

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CR48-A    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
WS36-A    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
RD31-A    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
LA49-A    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
WS17-A    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
LA57-A    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
CR12-A    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
OL26-A    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGTCTAGGTTTAG
OL39-A    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
CR47-A    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
NB3 -A    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
WS34-A    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
RD26-A    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
CR1 -B    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
CR54-B    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
LA17-B    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
CR37-B    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
CR32-B    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
CR11-B    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
Rd83-C    ATGAACCCCTACGTACTTACCATCTTACTTTCTAGCCTAGGTTTAG
WS3 -C    ATGAACCCCTACGTACTTACCATCTTACTTTCTAGCCTAGGTTTAG
LA7 -C    ATGAACCCCTACGTACTTACCATCTTACTTTCTAGCCTAGGTTTAG
CR44-C    ATGAACCCCTACGTACTTACCATCTTACTTTCTAGCCTAGGTTTAG
LA41-C    ATGAACCCCTACGTACTTACCATCTTACTTTCTAGCCTAGGTTTAG
LA58-C    ATGAACCCCTACGTACTTACCATCTTACTTTCTAGCCTAGGTTTAG
HL14-D    ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAG
LA71-D    ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAG
WS30-D    ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAG
LA4 -D    ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAG
LA6 -D    ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAG
LA2 -E    ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAG
WS42-E    ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAG
KG10-F    ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAG
LA9- G    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
RD6- H    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
RD20-I    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
WL12-J    ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAG
RD64-K    ATGAACCCCTACGTACTTACCATCTTACTTTCTAGCCTAGGTTTAG
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CR48-A    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
WS36-A    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
RD31-A    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
LA49-A    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
WS17-A    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
LA57-A    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
CR12-A    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
OL26-A    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
OL39-A    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
CR47-A    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
NB3 -A    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
WS34-A    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
RD26-A    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
CR1 -B    GCACAGTCCTCACCTTCGCCAGCTCACATTGACTTCTCGCATGAATAGGTCTAGAAATCA
CR54-B    GCACAGTCCTCACCTTCGCCAGCTCACATTGACTTCTCGCATGAATAGGTCTAGAAATCA
LA17-B    GCACAGTCCTCACCTTCGCCAGCTCACATTGACTTCTCGCATGAATAGGTCTAGAAATCA
CR37-B    GCACAGTCCTCACCTTCGCCAGCTCACATTGACTTCTCGCATGAATAGGTCTAGAAATCA
CR32-B    GCACAGTCCTCACCTTCGCCAGCTCACATTGACTTCTCGCATGAATAGGTCTAGAAATCA
CR11-B    GCACAGTCCTCACCTTCGCCAGCTCACATTGACTTCTCGCATGAATAGGTCTAGAAATCA
RD83-C    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
WS3 -C    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
LA7 -C    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
CR44-C    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
LA41-C    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
LA58-C    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
HL14-D    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
LA71-D    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
WS30-D    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
LA4 -D    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
LA6 -D    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
LA2 -E    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
WS42-E    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
KG10-F    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTACTCGCATGAATAGGTCTAGAAATCA
LA9- G    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
RD6- H    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
RD20-I    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
WL12-J    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
RD64-K    GCACAGTCCTCACCTTCGCCAGCTCACATTGGCTTCTCGCATGAATAGGTCTAGAAATCA
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CR48-A    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
WS36-A    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
RD31-A    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
LA49-A    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
WS17-A    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
LA57-A    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
CR12-A    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
OL26-A    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
OL39-A    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
CR47-A    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
NB3 -A    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
WS34-A    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
RD26-A    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
CR1 -B    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
CR54-B    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
LA17-B    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
CR37-B    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
CR32-B    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
CR11-B    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
RD83-C    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
WS3 -C    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
LA7 -C    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
CR44-C    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
LA41-C    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
LA58-C    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
HL14-D    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
LA71-D    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
WS30-D    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
LA4 -D    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
LA6 -D    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
LA2 -E    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
WS42-E    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
KG10-F    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
LA9 -G    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
RD6 -H    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
RD20-I    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
WL12-J    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
RD64-K    ATACACTCGCTATTATTCCGATCATGGCACAACAACATCACCCTCGAGCAATTGAAGCTA
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CR48-A  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
WS36-A  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
RD31-A  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
LA49-A  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
WS17-A  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
LA57-A  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
CR12-A  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
OL26-A  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
OL39-A  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
CR47-A  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
NB3 -A  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
WS34-A  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
RD26-A  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
CR1 -B  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
CR54-B  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
LA17-B  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
CR37-B  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
CR32-B  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
CR11-B  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
RD83-C  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
WS3 -C  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
LA7 -C  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
CR44-C  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
LA41-C  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
LA58-C  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
HL14-D  CTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
LA71-D  CTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
WS30-D  CTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
LA4 -D  CTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
LA6 -D  CTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
LA2 -E  CTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
WS42-E  CTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
KG10-F  CTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
LA9 -G  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
RD6 -H  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAACAATGATCCTGTTTGCTAGCACTA
RD20-I  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
WL12-J  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTGTTTGCTAGCACTA
RD64-K  CTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATCCTATTTGCTAGCACTA
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CR48-A   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
WS36-A   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
RD31-A   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
LA49-A   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
WS17-A   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
LA57-A   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
CR12-A   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
OL26-A   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
OL39-A   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
CR47-A   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
NB3 -A   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
WS34-A   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
RD26-A   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
CR1 -B   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
CR54-B   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
LA17-B   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
CR37-B   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
CR32-B   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
CR11-B   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
RD83-C   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
WS3 -C   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
LA7 -C   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
CR44-C   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
LA41-C   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
LA58-C   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
HL14-D   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
LA71-D   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
WS30-D   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
LA4 -D   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
LA6 -D   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
LA2 -E   CCAATGCCTGACTAATAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
WS42-E   CCAATGCCTGACTAATAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
KG10-F   CCAATGCCTGACTAATAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
LA9 -G   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
RD6 -H   CCAATGCCTGACTAGTAGGAAAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
RD20-I   CCAATGCCTGACTAATAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
WL12-J   CCAATGCCTGACTAGTAGGAAAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
RD64-K   CCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGGCGACTA
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CR48-A    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
WS36-A    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
RD31-A    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
LA49-A    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
WS17-A    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
LA57-A    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
CR12-A    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
OL26-A    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
OL39-A    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
CR47-A    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
NB3 -A    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
WS34-A    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
RD26-A    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
CR1 -B    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
CR54-B    CAACAGCAATACTG
LA17-B    CAACAGCAATACTGGCCCT
CR37-B    CAACAGCAATACTG
CR32-B    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
CR11-B    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
RD83-C    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
WS3 -C    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
LA7 -C    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
CR44-C    CAACA
LA41-C    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
LA58-C    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
HL14-D    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
LA71-D    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
WS30-D    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
LA4 -D    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
LA6 -D    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
LA2 -E    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
WS42-E    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
KG10-F    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
LA9 -G    AAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
RD6 -H    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
RD20-I    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
WL12-J    CAACAACAATACTGGCCCTTGCACTTAAACTTGGGC
RD64-K    CAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
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90

APPENDIX E
Alignment of 14 SSCP profile types from Crean (CR), La Ronge (LA), Reindeer (RD), Wollaston (WL), Hatchet (HL) and Kingsmere (KG) lakes. *'s denote the corresponding 11 different sequence types.

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            10        20        30        40        50        60        70
         .........+.........+.........+.........+.........+.........+.........+
CR12-A   ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
LA57-A*  ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
CR11-B   ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
CR32-B*  ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
RD83-C   ATGAACCCCTACGTACTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
LA41-C*  ATGAACCCCTACGTACTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
HL14-D   ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
LA2 -E   ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
KG10-F   ATGAACCCCTACGTATTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
LA9 -G   ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
RD6 -H   ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
RD20-I   ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
WL12-J   ATGAACCCCTACGTACTTATCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
RD64-K   ATGAACCCCTACGTACTTACCATCTTACTTTCTAGCCTAGGTTTAGGCACAGTCCTCACCTTCGCCAGCT
         ************** *** *************************************************
                80        90       100       110       120       130      140
         .........+.........+.........+.........+.........+.........+.........+
CR12-A   CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
LA57-A*  CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
CR11-B   CACATTGACTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
CR32-B*  CACATTGACTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
RD83-C   CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
LA41-C*  CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
HL14-D   CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
LA2- E   CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
KG10-F   CACATTGGCTACTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
LA9- G   CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
RD6- H   CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
RD20-I   CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
WL12-J   CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
RD64-K   CACATTGGCTTCTCGCATGAATAGGTCTAGAAATCAATACACTCGCTATTATTCCGATCATGGCACAACA
         ******* ** *******************************************************
                150       160       170       180       190       200      210
         .........+.........+.........+.........+.........+.........+.........+
CR12-A   ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATC
LA57-A*  ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATC
CR11-B   ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATC
CR32-B*  ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATC
RD83-C   ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATC
LA41-C*  ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATC
HL14-D   ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATC
LA2- E   ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATC
KG10-F   ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACACAAGCGACCGCCGCAGCAATGATC
LA9- G   ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATC
RD6- H   ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAACAATGATC
RD20-I   ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATC
WL12-J   ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATC
RD64-K   ACATCACCCTCGAGCAATTGAAGCTACTACCAAATATTTTTTAACGCAAGCGACCGCCGCAGCAATGATC
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                220       230       240       250       260       270       280
       .........+.........+.........+.........+.........+.........+.........+
CR12-A    CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
LA57-A*   CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAAAATGAAAATTCACCAGCTATCCCACCCCCTGG
CR11-B    CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
CR32-B*   CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
RD83-C    CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
LA41-C*   CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
HL14-D    CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
LA2- E    CTGTTTGCTAGCACTACCAATGCCTGACTAATAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
KG10-F    CTGTTTGCTAGCACTACCAATGCCTGACTAATAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
LA9- G    CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
RD6- H    CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAAAATGAGAAATTCACCAGCTATCCCACCCCCTGG
RD20-I    CTGTTTGCTAGCACTACCAATGCCTGACTAATAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
WL12-J    CTGTTTGCTAGCACTACCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
RD64-K    CTATTTGCTAGCACTACCAATGCCTGACTAGTAGGAGAATGAGAAATTCACCAGCTATCCCACCCCCTGG
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                290       300       310       320
       .........+.........+.........+.........+..
CR12-A    CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
LA57-A*   CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
CR11-B    CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
CR32-B*   CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
RD83-C    CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
LA41-C*   CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
HL14-D    CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
LA2- E    CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
KG10-F    CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
LA9- G    CGACTAAAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
RD6- H    CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
RD20-I    CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
WL12-J    CGACTACAACACAATACTGGCCCTTGCACTTAAACTTGGGC
RD64-K    CGACTACAACAGCAATACTGGCCCTTGCACTTAAACTTGGGC
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