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**POPULATION GENETIC ANALYSES OF ARCTIC CHAR (SALVELINUS ALPINUS)
LIFE HISTORY TYPES IN NETTILLING LAKE & AMADJUAK RIVER
ECOSYSTEM: A TEST OF REPRODUCTIVE ISOLATION**

by

Chen Liu

A Thesis

Submitted to the Faculty of Graduate Studies
Through the Great Lakes Institute for Environmental Research
in Partial Fulfilment of the Requirements for
the Degree of Master of Science
at the University of Windsor

Windsor, Ontario, Canada

2021

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Population genetic analyses of Arctic char (*Salvelinus alpinus*) life history types in
Nettilling Lake & Amadjuak River ecosystem: a test of reproductive isolation

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May 28th, 2021

DECLARATION OF CO-AUTHORSHIP AND PREVIOUS PUBLICATION

I. Co-Authorship

I hereby declare that this thesis incorporates material that is result of joint research, as follows: This thesis incorporates the outcome of joint research undertaken in collaboration with Dr. Aaron Fisk and Dr. Sarah Larocque under the supervision of my supervisor Dr. Daniel D. Heath; this collaboration covers Chapter 2. In all cases, the key ideas, primary contributions, experimental designs, data analysis and interpretation, were performed by the author (myself), with additional input on data analysis, interpretation of data, and written discussion by co-authors.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis and have obtained written permission from each of the co-author(s) to include the above material(s) in my thesis.

I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

II. Previous Publication

This thesis includes material from one original paper that have been submitted for publication in peer reviewed journals, as follows:

Thesis Chapter	Publication Title	Publication Status
Chapter [2]	Liu C, Larocque S, Fisk A and Heath DD. "Population genetic analyses of migratory life history types of Nettilling Lake and Amadjuak River Arctic char"	Submitted to Molecular Ecology, "preparing for publishment"

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ABSTRACT

A great number of studies have identified strong genetic differences between sympatric anadromous and resident populations of Salmonidae. However, Arctic char (*Salvelinus alpinus*) migratory phenotypes in the Nettilling Lake and Amadjuak River ecosystem in Nunavut, Canada have not been genetically characterized, and it remains unclear if distinct genotypes and phenotypes associated with migratory life history differences are maintained through reproductive isolation, and they have been assumed to be sympatric populations, or co-occurring populations. Co-occurring Arctic char (n=225) were sampled from eleven sites along the Amadjuak River in 2014 and 2015. Twelve microsatellite loci were used to quantify genetic variation among the sampled fish. The genetic data showed two genetic clades (populations) of Arctic char living in the ecosystem. However, each genetic population contained both resident and anadromous individuals (migratory life histories). These results suggest that genotype should be considered when identifying populations of Arctic char for conservation and management purposes. Fish from the two different clades were captured at the same site, indicative of possible sympatry, increasing the complexity of effective management of this important fishery resource. We thus suggest using genetic methods to categorize individual fish to their respective genetic population, while further work should be done to explore morphological and physiological trait differences to simplify the management of the fish from the two cryptic populations.

DEDICATION

I dedicate this thesis to my parents for their continued support, patience, reliance, and love over the years.

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I would like to first acknowledge my supervisor, Dr. Daniel D. Heath, for giving me the chance to conduct research in his lab and for his unrelenting optimism and guidance over the years. His supervision, troubleshooting, and support is greatly appreciated by me; this thesis would not have been possible without him. I also want to acknowledge my committee members Dr. Aaron Fisk and Dr. Douglas Haffner from the Great Lakes Institute for Environmental Research, for their decisive optimism, advice, critical input, patience, which helped me improve in my critical thinking and research capabilities.

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LIST OF ABBREVIATIONS

bp: Base Pairs

ddH₂O: Double Distilled Water

dNTP: Deoxy-nucleotide Triphosphate

DNA: Deoxyribonucleic Acid

MgSO₄: Magnesium Sulfate

PCR: Polymerase Chain Reaction

Taq: *Thermus aquaticus*

Chapter One

General Introduction

Issues of overfishing

Marine and freshwater environments provide significant resources which support human beings' requirement for food. Historically, the fishing industry grew dramatically in early 1950s, and fish in general, including Salmonidae, became one of the most important food resources for people world-wide (Pinkerton, 1994). Fish and fisheries products provided approximately 15% of the total animal protein for global consumption (Allan et al., 2005), so fish resources contribute substantially to the global food supply (Allan et al., 2005). However, ocean and freshwater ecosystems are currently facing unprecedentedly high levels of exploitation (Crespo & Dunn, 2017; Pander & Geist, 2013). Generally, there are four different human disturbances that contribute to the degradation of aquatic biodiversity; pollution, low water quality, anthropogenic climate change and overfishing (Jackson et al., 2001). With the increasing demand for fish resources, overfishing is occurring frequently, and it leads to sequential depletion of fish stocks (Orensanz, 1998; Coll et al., 2008), especially highly desirable species such as Pacific salmonids (*Oncorhynchus* spp.) around the Pacific Northwest Coast regions. According to historical records, approximately 140 salmon populations had gone extinct by the middle of the 20th century, and ~620 populations of salmon have been identified as being at high risk of extinction (Healey, 2009). Similarly, Atlantic cod (*Gadus morhus*) in Atlantic Canada experienced dramatic population declines, in part due to overfishing (Myers, 1997). Indeed, the loss of biodiversity and population extinction due to overfishing is perhaps the primary human disturbance impacting fish stocks in the oceans and coastal ecosystems. Moreover, species are also experiencing population declines and extirpation

due to overfishing in the freshwater environment (Arthington, 2016). Additionally, overfishing can impact the entire ecosystem's food web, because it can change or simplify the links of the food web (Pauly, 2002). This is especially true in habitats with diverse prey and predators, because overfishing will affect many trophic linkages at once. This situation will lead to serious ecological problems, because the predators may not have the opportunity to feed on their preferred prey, as they may be sharply declining in the area (Scheffer et al., 2005). As a result, managing the ecosystem to ensure its sustainability and standing biodiversity must include a detailed understanding of the effects of exploitation (Murawski, 2000).

Inland water bodies are important freshwater environments for wild stocks of freshwater fish, although they may also contain migratory species, which move between marine and freshwater bodies (Allan et al., 2005). Since inland fisheries have unique characteristics, such as having multiple year classes and complex species communities (Allan et al., 2005), overfishing can lead to rapid undesirable changes in the target fish populations and the fish assemblage in general (Allan et al., 2005). Therefore, overfishing will not only impact the target fish species, but also rare fish species and weak subpopulations; such impacts would be irreversible and not addressed by target species management actions.

Molecular genetic technology has been widely used in conserving and managing fished species, specifically, to ensure that specific stocks have harvestable surplus and current fisheries do not compromise individual stock perpetuation (Carvalho, 1994). For example, Pacific salmon management has for many years focused on determining the escapement goal for sub-populations, defined by population genetic methods, that may be captured in a mixed-stock fishery (Ramstad et al., 2002; Zhivotovsky, 2015). Molecular genetic technology has made great contributions to identifying and characterizing cryptic sub-populations in complex ecosystems, and thereby

ensuring more effective management (Ramstad et al., 2002). Indeed, an overexploited population may have a loss of genetic diversity and reduced effective population size, metrics easily measured using molecular genetics; the New Zealand snapper (*Pagrus auratus*) is an excellent example (Hauser, 2002).

Testing for genetic differentiation among populations of aquatic species in freshwater and marine environments using molecular genetic markers has been a standard approach for decades (Kirk et al., 2011). Population genetic analyses can help determine if there is a genetic component to such variation, and that information can help describe the evolutionary or ecological factors driving the divergence (Schluter, 1996). Moreover, microsatellite markers are especially valuable due to their high level of polymorphism and simple and rapid genotyping protocols (Chistiakov, 2005). There are examples of using microsatellite markers in the analysis of genetic divergence among and within Arctic char in Labrador, Canada (Salisbury et al., 2018). There are also detailed analyses of genetic divergence between kokanee and sockeye salmon in Gates and Portage creeks in British Columbia, Canada (Moreira & Taylor, 2015), and genetic divergence among Pacific salmon populations and steelhead salmon populations in the Columbia River, US (Johnson et al., 2019). Those studies highlight the power of microsatellite markers to detect sympatric genetic divergence, and hence reproductive isolation. In an analysis of the persistent reproductive isolation of Chinook salmon (*Oncorhynchus tshawytscha*) in White Salmon River, Washington (Smith & Engle, 2011), microsatellite markers detected multiple allopatric populations. Additionally, microsatellite makers have been widely used in detecting micro-geographic population subdivision and allele frequency differences among diverse morphotypes within species (Englbrecht et al., 2002; Wilson et al., 2004; Lundrigan et al., 2005; Wilson et.al., 2019). Indeed, using microsatellite markers to address questions of genetic structure in contemporary and important fisheries, such as

Arctic char, is essential (Rogers, 2011). Moreover, Arctic char exhibit both resident and migrating (between fresh and salt water) life histories. However, there are not many previous studies that demonstrate genetic divergence between freshwater and sea-run Arctic char (*Salvelinus alpinus*) (but see Moore, 2016; Salisbury et al., 2018). Therefore, a quantitative measurement of the reproductive isolation between resident and migratory Arctic char would be useful in the management and conservation of this contemporary fished migratory species.

In conclusion, overfishing is a serious issue for the viability of marine and freshwater fish populations and species, and it is necessary to explore how to exploit species in the ocean and freshwater environments sustainably. Population genetics is a valuable tool in this regard. Therefore, this thesis uses genetic information and other population characteristics to contribute to better conservation of an exploited population of Arctic char in Canada's north.

Reproductive isolation

It is obviously important to protect fish species in general from overfishing; however, it is also necessary, but not easy, to identify the weak populations and groups within the species for special conservation efforts. Therefore, research on reproductive isolation among groups or populations of fish provides important data to identify populations that require specific management actions (Zhivotovsky et. al., 2019), especially with the potential for overfishing. Thus, it is necessary to define what reproductive isolation is.

Reproductive isolation results from natural barriers which prevent individuals of the same species from interbreeding (Mayr, 1963), and is also an essential condition for the evolution of local adaptation and speciation, ultimately. Spatial and temporal reproductive isolation will lead to a reduction in gene flow among subpopulations (Taylor, 1991), and serves to preserve isolated

gene pools (Dobzhansky, 1970). Therefore, measuring reproductive isolation using genetic divergence as a proxy is a reasonable method to identify different stocks, populations and species.

Habitat (ecological) isolation is one form of reproductive isolation and was first defined in Mayr's study of animal speciation and evolution (Mayr, 1963) where he explained how populations in different microhabitats may be reproductively isolated despite no obvious physical barriers. A classic example among salmonid species are sockeye salmon (*Oncorhynchus nerka*) which exhibit seasonal migratory behavior ocean and freshwater environments (Ramstad et al., 2002). However, some sockeye salmon stay in in the freshwater environment without migration ("kokanee"). Despite these two types of sockeye salmon possibly sharing spawning areas, high levels of genetic differentiation is observed (Ramstad et al., 2002). Ultimately, specialization of the kokanee salmon to the freshwater environment versus anadromous sockeye salmon specialized to migrate between ocean and fresh water promoted their reproductive isolation. Natural selection is commonly evoked in cases where populations exist in different ecological environments (Nosil et al., 2005), and sockeye salmon have sympatric (and parapatric) reproductive isolation that results in the evolution of genetically isolated populations (Schluter, 1996). Overall, ecological isolation can be an important evolutionary mechanism to generate genetic divergence, while habitat isolation in sympatric populations of salmonids is widespread (Nosil et al., 2005). Rainbow trout (*Oncorhynchus mykiss*) and steelhead trout are two different migratory life history types (resident and migratory, respectively), and are defined as the same species based on genetics, even though they have consistent morphological differences (likely due to parallel evolution; Docker & Heath, 2003).

Though reproductive isolation is common in subpopulations of salmonids, it is necessary to consider the effects of gene flow, genetic drift and natural selection to determine the expected

level of genetic divergence that results from reproductive isolation. Reproductive isolation is generally associated with the early stages of speciation (Palumbi, 1994) and it can result in adaptive (selection) and neutral (genetic drift) genetic divergence among populations. Indeed, a situation that involves genetic drift and natural selection can induce and enlarge both physiological and morphological differences between the populations which are reproductively isolated, even if they share the same ecosystem (Coyne, 1992). Therefore, it is ideal to use neutral and functional markers to test genetic differences among populations or reproductively isolated groups, because genetic data can be used as the base information on not only the level of reproductive isolation, but on the mechanism of genetic divergence (selection versus drift). Such information can be critical in the management and conservation of wild species and populations, including those which are exploited.

Local adaption

Local adaption is when individuals from one habitat and population perform better in their local environment than individuals from a non-local population and environment (Kawecki, 2004). Natural selection acts within the local environment to promote the evolution of traits adapted to local environmental conditions (Kawecki, 2004). Local adaption has been identified as common in fish species which have homing behavior (migratory behaviour), such as salmonids, where it is an important evolutionary process (Taylor, 1991; Hansen et al., 2002; Fraser et al., 2011). Many anadromous Pacific salmon species, which show homing behavior, are known to be well adapted to their natal freshwater environment when they move back to their spawning area (Fraser, et al., 2011). In general, Pacific salmon populations are reproductively isolated from conspecific populations (Waples, 1991), and are thus capable of evolving separately in response to local

conditions. Local adaptation can then lead to pre-zygotic reproductive barriers, such as habitat isolation that will serve to reinforce the reproductive isolation, even if straying occurs. Thus, natural selection in divergent habitats (i.e., local adaptation) can act as the evolutionary impetus that results in reproductive isolation (Schluter, 2009) which can, in turn, lead to ecological speciation (Nosil et al., 2012).

Migration

Long distance migration is a life history trait common in trout (Jonsson, 2011), salmon (McCormick, 1998) and char (Gulseth, 2001) and includes both migration within the freshwater environment or between saltwater and freshwater habitats (anadromous). For example, adult anadromous sockeye salmon migrate to the northern Pacific Ocean (Wood, 1996), while kokanee are the freshwater form that migrate from their nursery lake to share stream and beach spawning sites with returning sockeye salmon (Vernon, 1957). Though sockeye and kokanee salmon usually share spawning sites with similar spawning timing, they are not morphologically similar, and they are at least partially reproductively isolated (Wood, 1996). Indeed, anadromous species are ecologically important as they can transfer nutrients across different ecosystems (Vanni, 2002). Moreover, anadromous and non-anadromous species are both important examples for exploring how animals adapt to very different environments, such as freshwater and marine ecosystems. Migration in salmonids is evolutionarily and ecologically important but makes conservation and management complex.

Genetic markers, such as microsatellites, are commonly used to determine if natural populations are genetically differentiated from each other (Abdul-Munier, 2014). Microsatellite DNA markers allow genotyping of individual organisms (Rubtsova et al., 2016), and the variation

in allele size among individuals can be used to assign individuals to different “populations” using clustering software (Porras-Hurtado et al., 2013). Such genetic technologies currently are used in fisheries management to identify fish stocks (Vähä et al., 2017), cryptic populations (Finnegan et al., 2013), and potentially locally adapted stocks (Perrier et al., 2013).

Thesis objectives

The main objective of this thesis is to determine if there is reproductive isolation among the different migratory life history types of Arctic char in the Nettilling Lake ecosystem using genetic markers. This work will determine whether the genetic divergence is a result of genetic drift or is consistent with natural selection. The specific goals to accomplish this objective was to genotype Arctic char of known and inferred migratory life history and to test for reproductive isolation among the life histories using microsatellite markers (genetic drift).

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Chapter Two

Population genetic analyses of migratory life history types of Nettilling Lake and Amadjuak

River Arctic char

Introduction

One critical life history trait variant in fishes is migration, and for salmonids, this is often between fresh and salt water (McCormick, 1998; Gulseth, 2001; Jonsson, 2011). However, the proportion of migratory individuals in a population may not be 100%, with some proportion of the population being resident (McCormick, 1998) while some populations of salmonid fishes are entirely freshwater resident (Lin et al., 2008; McGlaufin et al., 2011). There are well-documented examples of separate resident and migratory populations living part of their life in a common freshwater ecosystem (Vernon, 1957; Lemopoulos et al., 2018). Some of these co-existing populations have been shown to be genetically differentiated, despite the opportunity for interbreeding and gene flow (Quinn, Stewart & Boatright, 2006; Van Doornik, Berejikian & Campbell, 2013; Adams, Cote & Hutching, 2014). In fact, some level of genetic isolation between migratory and resident populations of salmonid fishes is common (Verspoor & Cole, 1989; Scribner et al., 2012). One hypothesis for how this evolves involves local adaptation that drives the evolution of traits adapted to the specific environmental conditions, freshwater resident or saltwater migratory (Kawecki & Ebert, 2004). Therefore, local adaptation may contribute to genetic isolation among populations of fish, as species respond to different microhabitat characteristics (Schluter, 2000; Dionne et al., 2008). For example, sympatric migratory/resident life histories are commonly observed in trout (Wood, 1995), char (Fraser, Lippe & Bernatchesz, 2004), and salmon (Adams, Cote & Hutchings, 2014). Sockeye salmon (*Oncorhynchus nerka*) have two ecotypes with divergent migratory life histories with freshwater resident individuals termed “kokanee” (Nichols, Kozfkay & Narum, 2016). Such mixed life history stocks are difficult to manage for sport, commercial or indigenous subsistence fisheries, because it is unknown if the fish are a single population or if they are reproductively isolated, but co-existing migrant and

resident populations. A lack of knowledge of the underlying genetic population structure can lead to poor management decisions which will not effectively protect the population(s) or their life history diversity.

Generally, Arctic char are distributed across the Canadian Arctic and some islands of the Arctic Archipelago, especially river systems in Nunavut, Canada (De March, 1991; Evans et al., 2015). Arctic char can also be found in many lakes and rivers across Canada's North. Arctic char is a key traditional food source for Arctic region residents (Lundrigan et al, 2005), as well as a significant commercial fishery, with approximately 50,000 kgs landed in 2012 alone, at an estimated average of \$24 per kilogram or a market value of ~\$1,160,600 in the Cambridge Bay region (Fisheries and Oceans Canada, 2014). Therefore, Arctic char represent an important addition to local incomes, a critical factor in the Canadian Arctic regions. Substantial genetic structure found in Iceland showed that Arctic char from different sampling sites have very low genetic connectivity (Kapralova, 2011). Thus, the effective management of the Canadian Arctic char fisheries may not be straightforward.

As one of the most northerly fish species, the Arctic char (*Salvelinus alpinus*) has played an important role in the history of Northern Canada (Dempson & Green, 1985; Johnson, 1989), where they have been a key nutrient source for the Canadian Arctic region (Myers et al., 2005; Zeller et al., 2011). Anadromous Arctic char is the target of commercial and Indigenous fisheries, and large-scale gillnet fishing efforts are common in river mouths during the summer (Kristofferson et al., 1984). Canada's Wild Pacific Salmon Policy (WSP) stipulates the need to maintain genetic diversity among populations of harvested salmonids (Fisheries and Oceans Canada, 2000; Krkosek, 2010; Price et al., 2017), making it critical to know the population structure of the harvested northern Arctic char stocks. Moreover, although Arctic char have biological and ecological characteristics similar to many salmon species, we still need to better understand their genetic diversity and sub-species taxonomy (Reist, Power & Dempson, 2013). The situation is complicated by the fact that Nunavut's Arctic char fisheries management policy has not been revised since the 1980s (Roux et al., 2011), and there is no relevant genetic diversity

information about Arctic char in the southern Baffin Island region. Therefore, the Arctic char fishery on Baffin Island might be impacting genetic stocks or populations in ways we do not know. Both genetic and environmental conditions can contribute to diversity in salmon life histories (Heath et al., 2006; Heath et al., 2008), but genetic effects can be effectively managed for.

Here we test whether there are reproductively isolated populations of Arctic char in the Nettilling Lake and Amadjuak River ecosystem. This system has both freshwater (resident) and anadromous Arctic char co-existing, and we hypothesize that they are genetically divergent due to some level of reproductive isolation. We use microsatellite DNA markers to genotype Arctic char from Nettilling Lake and Amadjuak River and test for evidence of genetic structure in the population. We hypothesize that two genetic clusters will emerge, and they will represent the resident and migrant forms. The use of microsatellite makers to test for genetic structure in salmon, trout and char is widespread, and represents a powerful analytical approach (Arden et al., 1999; DeWoody & Avise, 2000; Rexroad III, et al., 2002; Palti, et al., 2002; Dehaan & Ardren, 2005; Melnik.et.al., 2020). This investigation of the Arctic char in the Nettilling Lake and Amadjuak River ecosystem will inform fisheries managers and build on the work of others who have used population genetic analysis to assess migratory and resident life histories in salmonids (Ozerov et al., 2010; Nilsen et al., 2008).

Materials & Methods

Study area

Nettling Lake is a cold freshwater lake located on the southern end of Baffin Island, Nunavut, Canada (Figure 1). The lake has an area of approximately 5,500 km², a maximum length of 123 km and maximum depth of 132 m. This lake is frozen for most of the year, ice usually starts to break up in early July, mainly at the mouths of the rivers connecting the lakes, with surface

temperatures higher than 7°C in August, and the ice will reform between late September and early October (Oliver, 1964). Nettilling Lake is connected to the saltwater Foxe Basin by the Koukdjuak River and is fed by Amadjunk Lake (Figure 1). Amadjunk Lake is the second largest lake on Baffin Island after Nettilling Lake. There are only a few native vertebrate species found in Nettilling Lake, and they include ringed seals (*Phoca hispida*), Arctic char, ninespine sticklebacks (*Pugitius pungitus*) and threespine sticklebacks (*Gasterosteus aculeatus*) (Kristofferson et al., 1991). In particular, the unique diversity of stickleback in Nettilling Lake provides an unusual prey base for the Arctic char, quite different from that available in other lakes in Nunavut. These prey fish may contribute to the high growth rates of both the anadromous and non-anadromous life histories of Arctic char in this ecosystem (Kristofferson et al., 1991).

Study species

The target species in this study include two different life histories of Arctic char: anadromous (migratory) and non-anadromous (resident) Arctic char (*Salvelinus alpinus*). Arctic char were collected for this study in August of 2014 and 2015, and acoustic telemetry-based movement data were recorded for some of the sampled fish as part of another project.

Anadromous Arctic char move to the ocean when they reach four to five years old and can reach a size of 150 to 250 cm. In the freshwater environment, juvenile Arctic char feed on freshwater shrimp and insect larvae, adult Arctic char feed on small fish, insect larvae and snails. In salt water, Arctic char feed on invertebrates and marine fishes.

Sampling

Fin clips were collected from 225 individual fish in the riverine environment of Nettilling Lake (Amadjuak River; Figure 1) and were preserved in high salt buffer (25 mM sodium citrate, 10 mM EDTA, and 70 g ammonium sulfate) for later DNA extraction. In 2014, 40 Arctic char were captured and sampled by gillnet in small streams of the Amadjuak River and Koudjuak River. In 2015, 185 Arctic char were captured by gillnet and angling in the Amadjuak River; of those, 119 fish were terminally sampled, while 66 fish were released after sampling fin tissue for acoustic telemetry. Fin tissue samples were preserved in the high salt buffer and held at -20°C for later DNA extraction.

DNA extraction

Total genomic DNA was isolated from fish fin clip tissues using a commercially available proteinase K/Wizard Genomic DNA purification kit (Promega Corporation, Canada). Each fin clip was cut to approximately 20 mg using sterilized forceps and razor blades, the tissue was rinsed in 70% ethanol (to remove the high-salt buffer) and placed into 1.5mL tubes containing 600uL of Nuclei Lysis Solution and 2uL of proteinase K. The solution was incubated at room temperature with gentle shaking overnight. Subsequently, 200uL of chilled Protein Precipitation Solution was added. After centrifugation, the supernatant was transferred to fresh tubes and 600uL of room temperature isopropanol was added to precipitate the DNA. After a second centrifugation, the supernatant was removed and 600uL of 70% ethanol was added and the pellet was washed by gentle rocking in the ethanol. The washed pellet was air-dried and reconstituted with 100uL of ddH₂O. All DNA samples were run on 2% agarose gels to assess DNA quality, and if judged to be of acceptable quality, stored at -20°C for later PCR amplification. Samples that failed were re-extracted.

Microsatellite Genotyping

We selected 12 microsatellite loci from the literature for genotyping the Arctic char for this project and optimized specific PCR conditions for each of those primer sets (Table 1). All 225 DNA samples were genotyped at the 12 microsatellite loci by PCR amplification using dye-labelled primers for fragment analysis on a Li-Cor 4300 DNA Analyzer (Li-COR Biosciences, USA). The thermocycling regime consisted of initial denaturation at 95°C for 2 minutes, followed by 30 cycles of denaturing at 95°C then 30 cycles at various annealing temperatures (Table 1) and a 1-minute extension at 72°C, all followed by a final extension step at 72°C for 1 minute. Allele sizes were determined for each fish at each locus using Gene Imager software (version 4.05) after running PCR products on the Li-Cor fragment analyzer (Model 4300).

Population Genetic Analysis

This study was intended to determine genetic structure of 225 individual fish samples without pre-defined groups, and therefore STRUCTURE (Pritchard lab, Stanford University; Novembre, 2016) was used to identify and assign individual fish to the most likely number of genetic clusters (Parras-Hurtado et al., 2013). We evaluated the most likely number of clusters (K) using STRUCTURE Harvester (Earl & vonHoldt, 2012; STRUCTURE Harvester: web version 0.6.94). STRUCTURE generates Q values (probability of assignment) for each sample (genotype), Q varies from 0.0 to 1.0 for each genetic cluster. Generally, for samples with $K > 1$, Q values in STRUCTURE can be used to assign individual samples to genetic subgroups (clusters) (Salisbury et al., 2018). Hardy–Weinberg equilibrium (HWE) was tested in the STRUCTURE identified Arctic char subpopulations using GenePop (genepop.curtin.edu.au, version 4.7) (Raymond &

Rousset, 1995). Tests for HWE employed the Markov-chain method proposed by Guo and Thompson (1992), with the following chain parameters: 1000 dememorizations, 100 batches and 1000 iterations.

Our fish samples were captured at different sites in the freshwater habitat; however, based on the morphological and acoustic telemetry information for the sampled Arctic char, the 225 samples included both migratory and resident Arctic char. In our population genetics analysis of Arctic char, we explored genetic structure using the clustering method in STRUCTURE version 2.3.4 (Hubisz et al., 2009). We determined the most likely number of genetic clusters as the most probable value of K, where K is the number of genetically distinct populations, and we evaluated K values from 1 to 5. We allowed admixture in the analysis with possible correlation of the allele frequencies (Wilson et al., 2004; Falush et al., 2003). Markov Chain Monte Carlo (MCMC) estimation was used with a burn-in period equal to 100,000 iterations, and a chain length from 250,000 to 750,000, to perform multiple runs to get robust estimates of population genetic structure. Once STRUCTURE identified clusters, we used GenePop (genepop.curtin.edu.au, version 4.7) (Raymond & Rousset, 1995) to quantify cluster genetic divergence as F_{ST} .

We further refined our population assignment by scoring individual fish based on a threshold Q value: genotypes with Q values above 0.80 were identified as “pure” while Q values between 0.50 and 0.80 are putative hybrids. The genotype data from all cluster assigned fish were analyzed in PAST (Paleontological statistics software package, version 3, University of Oslo, Norway; Hammer, 2001) to generate a principal coordinate analysis to define the 95% confidence ellipse to visualize the relationship of individual fish samples in different clusters using the Q values (admixture values) estimated by STRUCTURE.

Results

DNA extraction and genotyping

We were able to extract high quality DNA from fin clip tissue samples for all 225 fish samples, and all fish were successfully genotyped at the 12 microsatellite loci (Supplementary Table S1).

Population genetic results:

We determined the most probable cluster number using STRUCTURE Harvester, which indicated that the most probable value of K was 2.0, based on the maximum value of K (Supplementary Figure S2). The global F_{ST} value between those two clusters was 0.06, while we used the individuals which have the Q value higher than 0.80; the locus specific F_{ST} values showed considerable variation among the 12 marker loci (Supplementary Table S1). We also found considerable variation among the locus-specific estimates for allele numbers (A), observed heterozygosity (H_o), expected heterozygosity (H_e) and allelic richness (A_R) values (Supplementary Table S1). Genomic admixture analysis using the Bayesian STRUCTURE results indicated hybridization between the two genetic clusters; there are 30 individuals ($0.50 < Q < 0.80$) identified as “hybrids” among the 225 individuals fish samples, 195 individuals were pure types (Figure 2). Note that our threshold for identifying “pure” cluster membership is very conservative: the threshold Q values for pure genotypes was $Q > 0.80$ (hybrid genotypes were $0.50 < Q < 0.80$). The F_{ST} values calculated and shown in Supplementary Table S1 only included the pure assigned fish samples, and included 103 individuals in “pure” cluster 1, and 92 individuals in “pure” cluster 2 ($P < 0.001$ for all loci; Supplementary Table S1). The principal coordinate analysis (PCoA) plot of the first two PCs highlights the genetic overlap between the two genetic clusters (“hybrid”

genotypes are included in this analysis), indicating on-going gene flow (Figure 3). Interestingly, the genetic differentiation between the two clusters is very high at locus One11ASC ($F_{ST} = 0.650$) (Supplementary Table S1), which may reflect linkage disequilibrium with a locus under selection (Meuwissen et al., 2001; Zenger et al., 2019).

Discussion

We found two partially reproductively isolated populations of Arctic char in the Nettilling Lake and Amadjuak River system. The two populations (genetic clusters) diverged with an F_{ST} value of ~ 0.06 which, while not very high, is similar to isolated locally adapted populations of Pacific salmonids on the west coast of Canada (Waples et al., 2008). However, the two Arctic char populations are not like the sockeye-kokanee systems, which have the distinctive migratory life histories (Moreira & Taylor, 2015), but is more similar to rainbow-steelhead systems where reproductively isolated populations consist of a mixture of migratory and resident fish (Docker & Heath, 2003). While there are two distinct migratory life histories in the Arctic char in this ecosystem, they surprisingly did not correspond to the two well-supported genetic populations. In a similar situation, populations of rainbow trout in California exhibit “partial migration”, where some fish are resident and some migrate (Kelson et al., 2019). The migratory fish in these mixed populations were generally female fish and did not always show the correct “migratory genotype” (Kelson et al., 2019). Thus, genetic sex may be correlated with migratory life history in salmonids, and the migratory and resident Arctic char may be reflecting a combination of their genotypes and their environment conditions. In another example, chromosomal inversions led to the sympatric fluvial and ad-fluvial (non-anadromous) ecotype rainbow trout genetic differences in a southeastern Alaska watershed, which contributed disproportionately to the intraspecific diversity

of local rainbow trout populations (Arostegui et al., 2019). Moreover, Puget Sound Chinook salmon (*Oncorhynchus tshawtscha*) also showed differences in migration patterns (Kagley et al., 2017), where some tagged fish remained in the area of Puget Sound, but some tagged were “transients” and migrated out of Puget Sound, which is analogous to our Arctic char situation, although both Chinook salmon life histories were anadromous. Since the Arctic char sampled in this project exhibit two genetic populations, that are not related to migratory life history, there must be two spawning populations with at least partial reproductive isolation. It is likely that the two main spawning areas in the ecosystem are Nettilling Lake (beach spawners), and Amadjuak River (river spawners), although our data do not allow us to test this hypothesis. Our data shows the genetic populations are not 100% reproductively isolated (some fish were identified as possible hybrids), and we postulate that the migratory behaviour of Arctic char may be sex-linked, although unknown genetic or environmental factors may also contribute (Kelson et al., 2019). Although the Arctic char migratory life histories in the Nettilling Lake and Amadjuak River ecosystem are not reproductively isolated, that diversity should be conserved to sustain intraspecific diversity in Arctic char.

Many factors can lead to the breakdown of reproductive isolation in fish populations; environmental changes that erode reproductive barriers or cues, coupled with weak reproductive isolation will result in hybridization, although this would not necessarily lead to complete loss of reproductive isolation. Our genetic data indicates gene flow between the two genetic populations of Arctic char, with ~13.4% intermediate genotypes (or hybrids). Human disturbance can be associated with the loss of reproductive isolation in fish (Heath et al., 2009), and it is also one of the factors of hybridization between wild and domestic fish, such as Atlantic salmon (Wringe et al., 2018). The timing of migration and breeding (temporal isolation) (Quinn et al., 2007), and

ecological isolation (habitat) can contribute to reproductive isolation in fish species (Miller et al., 2011). However, hybridization can be a consequence of fish “straying” (fish migrate to non-original habitats and reproduce with the fish from other population), and the straying problem of Pacific salmon has been well documented (e.g., Bett et al., 2017). In another example, the Klukshu River sockeye salmon showed hybridization between two sympatric genetic populations due to high levels of straying (Fillatre et al., 2003). Although the two genetic populations of Arctic char in this study show evidence for hybridization, we do not have data on what may be leading to the breakdown of reproductive isolation; however, we speculate that climate change and human activity (perhaps fishing pressures) in the region may be contributing to it over time.

Our results contribute to the conservation and management of the Arctic char in the Nettilling Lake and Amadjuak River ecosystem because they show the presence of two, previously unknown, cryptic populations of Arctic char. Unfortunately, the lack of consistent life history trait differences complicates the management of this stock as only genetic data can identify which population an individual fish belongs to. However, gene transcription data (data not shown) hints that there may be functional differences between the genetic populations that would help in identifying and managing these two groups. Finally, our genetic analyses show that the cryptic populations appear to be hybridizing, indicating that the Arctic char diversity in the Nettilling Lake and Amadjuak River system may be in the process of being lost. These fish are a valuable resource that may be managed poorly due to a lack of understanding of the complex stock structure present in the system. This population should be monitored for the progress of the hybridization or loss of reproduction isolation over the time. Thus, we propose that it is critical to find out where these fish spawn and how they are reproductively isolated to allow effective and long-term management of this important local fishery and for conserving biodiversity in this Arctic ecosystem.

Tables & Figures

Locus	Primer sequence 5'-3'	T _A (°C)	Size range (bp)	Citation
BHMS206	CCAAATAACTGACAAGTGAG CAGAGGTTGATAATGGGG	54	186-252	Kapralova et al. (2011)
OtsG253b	GAGAAGGCCGAGCAGGTGTCT AATTGGGTCATTAAGGCTCTGTGG	52	100-130	Williamson et al. (2002)
One11ASC	GTTTGGATGACTCAGATGGGACT TCTATCCTTTCCTGTCAACTTCCA	54	139-157	Kapralova et al. (2011)
Omi127	GGGAACATCCCACACCTTA CAGGGCTACAGGTAAGTGG	54	147-157	Kapralova et al. (2011)
Omi187	AATAGCCCTGCTGTGCTGTT GAACTCTGATTCCGCGTCTC	50	152-180	Kapralova et al. (2011)
Sco19	CCTGAAATTAGTTAAACAGC CCAAACTACCCAATAATC	50	210-262	Taylor et al. (2001)
Sco19SFU	CTTGAAATTAGTTAAACAGC CCAAACTACCCAATAATC	50	207-263	Kapralova et al. (2011)
Bx079862	TGTGAGAAGAACACGAGAGTTGG GAATGAGGTGTTAGAACGACTGC	50	148-186	Kapralova et al. (2011)
SalD41SFU	ATCCGCTATGAACCACAGG ACTGCTCCGGCAACTACAG	60	222-250	McGowan et al. (2004)
SalD56SFU	TGCAGTTCACAATATATCCC AAGGGCACACTCAGATTTTG	60	272-292	McGowan et al. (2004)
SalD38SFU	CGCCTTGTCATACATTACACC ACGCTACAGAAACAGGAGAAAG	60	135-231	McGowan et al. (2004)
SalD23SFU	TCTGAATGCAGCCCCACAG TTCAAGCCAAGGACACATGG	60	241-261	McGowan et al. (2004)

Table 1 List of the microsatellite markers and primer sequences with their annealing temperature (T_A) and amplicon size ranges (bp) used for genotyping Arctic char for population genetics analyses.

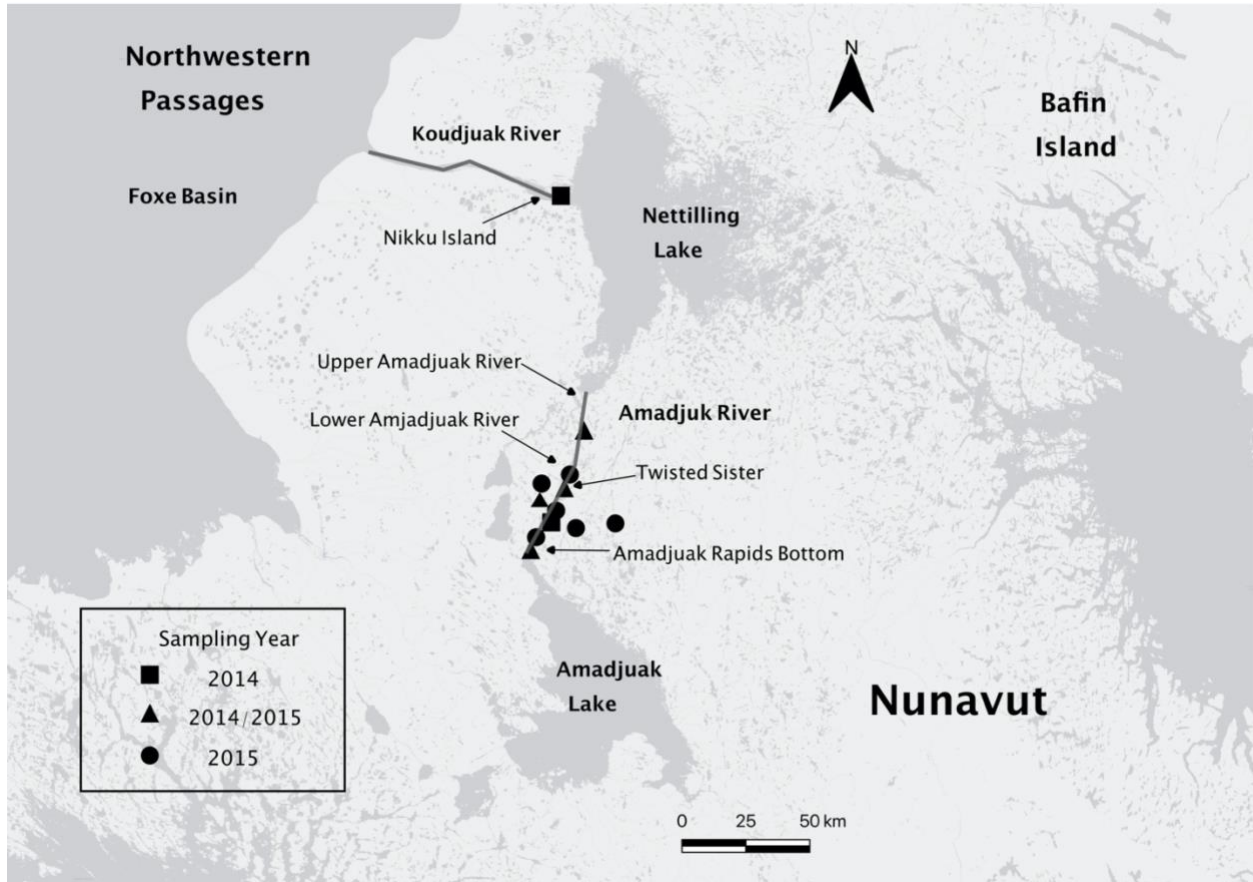


Figure 1 Sampling locations for Arctic char (*Salvelinus alpinus*) in Nettilling Lake and Amadjuak River. Fish samples were collected at freshwater locations in Nettilling Lake & Amadjuak River ecosystem. In 2014, fish samples were collected from the upper Amadjuak River (near the mouth of Amadjuak River), Twisted Sister, Lower Amadjuak Rapids and Nikku Island. In 2015, fish samples were collected in capture sites of Upper Amadjuak River (near the mouth of Amadjuak River), Lower Amadjuak Rapids (sites between Twisted Sister and Amadjuak Rapids Bottom), Twisted Sister and Amadjuak Rapids bottom.

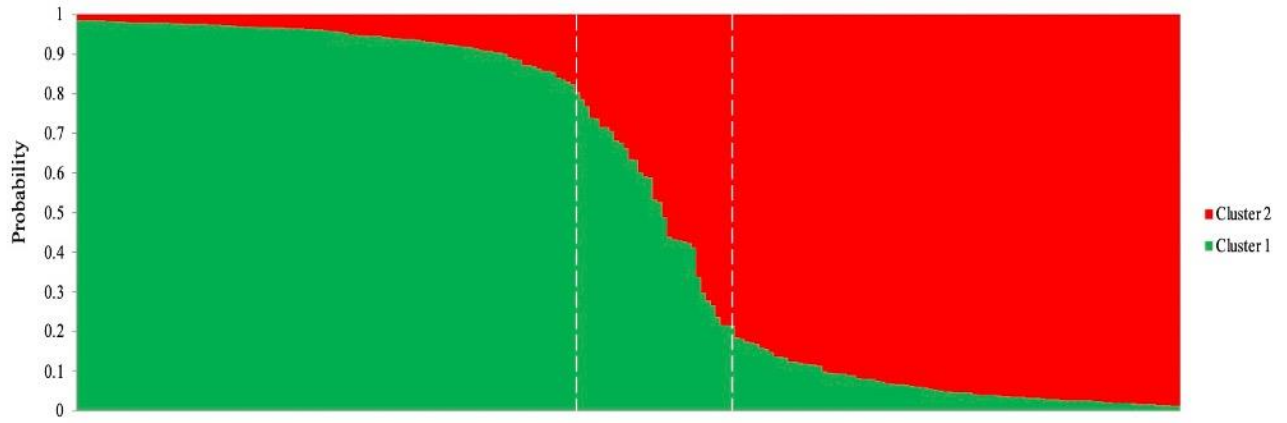


Figure 2 Bayesian admixture proportions (Q) of each Arctic char estimated in STRUCTURE, with K=2. Individual fish sampled are on the X axis, and the Y axis is the Q value from STRUCTURE (Q = probability of cluster membership). Each vertical line represents an individual genomic DNA sample. The green colour represents cluster (population) one, and the red colour represents cluster (population) two, the vertical dashed lines show the demarcation of “pure” versus “hybrid” genotypes (Pure genotypes have Q values >0.080).

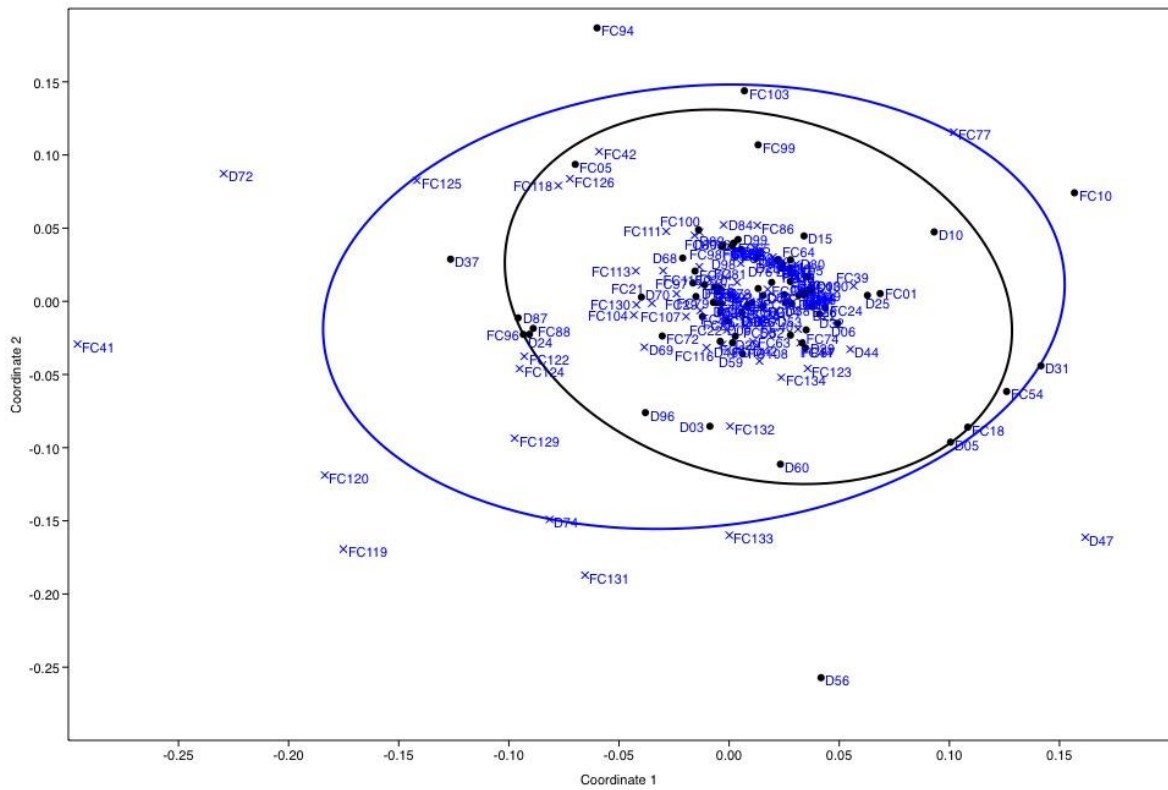


Figure 3 Scatterplot (with 95% confidence ellipses) for the first two principal coordinate axes (from the PCoA) of microsatellite genetic variation among 225 individuals of Arctic char in Nettilling Lake and Amadjuak River ecosystem. The black ellipse is 95% confidence of cluster 1 versus cluster 2 (blue ellipse), each black dot represents individual fish sample in cluster 1, and each blue “X” represents individual fish sample in cluster 2.

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Chapter Three

General Conclusion

Climate change and overfishing are increasingly disrupting the balance of cold-adapted fish, such as Arctic char (Gilbert et al., 2020). Thus, any data that add to our understanding of population structure, habitat use and diversity in those highly impacted fishes is valuable for their effective management and conservation. Population genetic methods, such as those used here, allow genetic analyses of individual fish to assess reproductive isolation, and the identification of possible cryptic populations in species with complex life histories. Our results provide information on the nature of freshwater and marine resources in a resource-scarce region, and thus help to resolve fisheries issues, and ultimately maintain genetic diversity of the vulnerable fish populations in the Nettilling Lake system.

The research presented in this thesis is the first monitoring of the genetic structure of Arctic char in the Nettilling Lake and Amadjuak River ecosystem. The two identified genetic populations are clearly reproductively isolated, but curiously consist of both resident and anadromous individuals. This result conflicts with past practice of managing the stock based on morphological differences or migratory behaviours. The outcome of the present genetic analyses indicates cryptic, but robust, genetic structure of Arctic char in the ecosystem, thus making it possible to now manage to maintain the genetic diversity of the individuals that rely on the Nettilling Lake and Amadjuak River freshwater ecosystem.

The two cryptic populations are genetically distinct (global $F_{ST} = 0.06$, Supplementary Table S1) and thus harbor different genetic diversity and should be managed separately, especially in such a species-poor ecosystem. Sockeye salmon and kokanee are sympatric, but are reproductively isolated because they select different spawning sites, spawning on the shore or the stream (Wood

et al., 2008). However, “straying” (Brenner et al., 2012) and environmental stressors or changes (Sandø et al., 2020) may lead to hybridization among sympatric fish populations. Moreover, if the alleles that control spawning behaviour (Veale and Russello, 2017) are lost, ecotypes such as sockeye salmon and kokanee could also be lost, and biodiversity would decrease.

As we found evidence for genetic intermediate individuals, indicating that hybridization between the two cryptic populations is likely, perhaps some of the Arctic char from the two clusters are now spawning in the same sites in the Nettilling Lake and Amadjuak River ecosystem. If some prey fish develop novel habitat use patterns due to environmental change and stress, it might lead to changes in the trophic structure of entire simple ecosystems, preferentially impacting non-migratory predator fish (Brönmark et al., 2008). This would be expected to be pronounced in salmonids as they are known to search for richer food resources (McDowall, 2001) for smolting or spawning. Thus, we predict that low resources in a freshwater ecosystem may force some Arctic char to move to other habitats and waterbodies to find food.

The management of the wild Arctic char in Nettilling and Amadjuak ecosystem is important for their long-term sustainable harvest. Telemetry data showed diverse movement patterns of Arctic char in the ecosystem; therefore, habitat connectivity should be high. There are many examples of using adult fish translocation across barriers for conservation (Geist et al., 2016; Lusardi & Moyle, 2017), and while there are no artificial barriers in the Nettilling and Amadjuak ecosystem, such methods might be effective in the aggressive management of Arctic char diversity to promote habitat connectivity and long term conservation.

Future directions

The main goal of this thesis was to assess the population structure of Arctic char in the Nettilling and Amadjuak ecosystem using genetic methods. The main hypothesis of this work was that migratory life histories would show reproductive isolation, similar to that seen in migratory sockeye and the sympatric kokanee. However, that is not what we found. For future studies I suggest:

- 1) genetically characterize spawning fish to test if separate spawning sites reflect the two genetic clusters.
- 2) test for morphological and gene transcription differences between the two genetic clusters, indicative of possible local adaptation effects
- 3) test for environmental effects, such as climate changes and human activity, that may be driving fish to either migrate or stay in the freshwater environment.
- 4) collect new fish samples and use historical samples with the new samples to test for temporal trends in the level of hybridization between the two genetic populations.

Moreover, it is important to test for trophic specializations occurring between the two populations, as some form of phenotypic divergence would also be expected if that were the case (Hooker et al., 2016). This could be done in the Arctic char populations in Nettilling and Amadjuak ecosystem by collecting prey items in the stomachs of the cryptic populations to test whether trophic ecological variables are contributing to the reproductive isolation of the two populations. Thus, I suggest combining genetic, morphological and ecological information to assess the ecomorphological diversity of Arctic char in the Nettilling and Amadjuak ecosystem.

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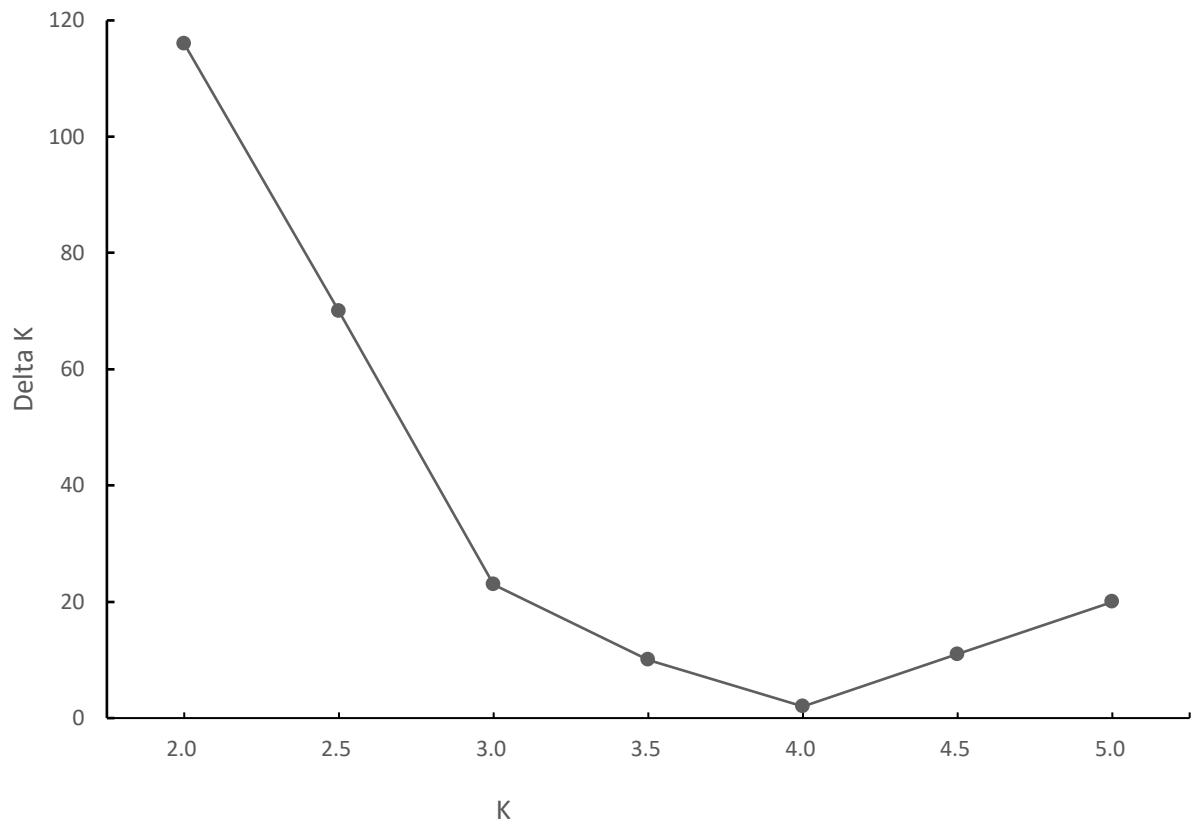
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Supplementary Material

Locus	Cluster	A	H _o	H _e	A _R	F _{st}	HWE (P)
SalD23SFU	1	15	0.26	0.81	13.74	0.004	<0.001
	2	11	0.14	0.8	9.94		
SalD38SFU	1	45	0.54	0.92	36.5	0.007	<0.001
	2	45	0.42	0.91	34.76		
SalD56SFU	1	18	0.21	0.9	17.06	0.002	<0.001
	2	19	0.06	0.88	16.62		
SalD41SFU	1	13	0.25	0.81	11.09	0.012	<0.001
	2	13	0.3	0.75	11.48		
Bx079862	1	12	0.28	0.82	10.8	0.085	<0.001
	2	17	0.42	0.85	14.69		
Sc019SFU	1	15	0.67	0.91	14.15	0.017	<0.001
	2	18	0.77	0.9	16.71		
Sco19	1	15	0.67	0.9	13.71	0.022	<0.001
	2	17	0.74	0.91	15.96		
Omi187	1	12	0.4	0.77	10.62	0.02	<0.001
	2	16	0.26	0.8	14.12		
Omi127	1	6	0.43	0.57	5.18	0.021	<0.001
	2	6	0.45	0.71	5.97		
One11ASC	1	7	0.3	0.73	6.78	0.418	<0.001
	2	7	0.35	0.78	6.99		
OtsG253b	1	19	0.78	0.92	18.54	0.006	<0.001
	2	23	0.72	0.9	20.71		
BHMS206	1	24	0.76	0.9	21.06	0.01	<0.001
	2	34	0.66	0.91	28.95		

Supplementary Table S1 Table of microsatellite makers characteristics of 12 loci of two clusters, and the data contained all 225 fish samples genetic data. A, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; A_R, allelic richness; F_{ST}, F-statistics; Global F_{ST} = 0.06; HWE (P), P-value corresponding to the null hypothesis that allele frequencies among the populations follow Hardy-Weinberg expectations.



Supplementary Figure S2: Figure showing the relationship between K value and ΔK statistics.

The most likely K value is where ΔK reaches a maximum, thus the most likely cluster number is at K=2.0, where ΔK reached the peak value.

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