MARKER ASSISTED SELECTION FOR ARCTIC CHAR (Salvelinus alpinus) BROODSTOCK DEVELOPMENT

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Marker Assisted Selection for Arctic Char (*Salvelinus alpinus*) Broodstock Development

by

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A thesis Submitted to the

School of Graduate Studies

in partial fulfilment of the

requirements for the degree of

Master of Science

Aquaculture Programme

Memorial University of Newfoundland

June 1999

St. John's Newfoundland

ABSTRACT

Arctic char is regarded as an excellent candidate for aquaculture. However, the size variability exhibited by this species hampers large scale production. An association study comparing individuals from a population with and without the trait in question, was carried out in combination with selective genotyping in order to determine if there was an association between marker loci and growth.

The Daniel's Harbour Hatchery Arctic char were found to have very low levels of genetic variation, with only 13 of 74 salmonid microsatellites showing variation. This result was not unexpected as the founding population consisted of two males and two females. Three microsatellite loci appeared to be associated with growth in two year classes of Arctic char. As these results were reproducible from year to year it is likely that this is a true association between marker and growth. This hypothesis can be tested by producing families from broodstock containing specific alleles at these loci and examining the association of specific alleles within family members. If the hypothesis is verified, it would be of great benefit to the Arctic char aquaculture industry.

ACKNOWLEDGMENTS

First and foremost I must thank my supervisor, Dr. Willie Davidson, for giving me the opportunity to work on this project and his encouragement along the way, as well as my supervisory committee, Dr. Joe Brown, for his comments and suggestions and Dr. Dave Innes for his comments and statistical advice.

Secondly, I must thank the organisations which supported this research: Gary Wilton and Daniel's Harbour Hatchery for setting up the crosses and collecting samples which were used in this study; ACERA and CCFI for providing funding; the Natural Sciences and Engineering Research Council for a strategic grant to Dr. Willie Davidson; and Memorial University for granting a Graduate Fellowship to the author.

Thanks to Hughie Broders and Sylvia Bartlett for their technical advice when I was first starting and didn't have a clue!! My lab mates have been T.L. Young, M.O. Woods, C.Walsh, H. Broders, D. Compton and T. Frost.

Next I would like to thank Michelle Hendrie, Sherene Faulkner, Krista Johansen and Myrtle Bateman, for listening to me rant and rave about the trials and tribulations of the gels. Robert P. Evans provided friendship, laughter, mini lecture series and rude

commentaries throughout, thanks R.P. I owe you the usual!! And to all the others who at some point were forced to listen to me cursing the gels and who are to numerous to name, thanks!

Finally I must thank my mother, Noreen A. Johansen, for her encouragement and support throughout my seemingly never-ending academic career; and my father, the late Dr. Peter H. Johansen whose Saturday morning fish feeding excursions started me on the path I have chosen to follow.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

1.1 INTRODUCTION

1.1.1 Background

Arctic char (*Salvelinus alpinus*) (Linnaeus 1758) has a northern circumpolar distribution and occurs in both anadromous and land locked forms (Leim and Scott 1966; Andersson *et al.* 1983; Hartley 1991; Jobling *et al.* 1993). Southwards, along the southern continental margins the anadromous form can be found up to the limit of sea ice in winter. Further south only non migratory stocks occur (Johnson 1980). Arctic char reaches its maximum density and individual size along the coast line of the Arctic Ocean (Johnson 1980).

Wide variation in the phenotypic characteristics of the char have been found and this contributes to the poor understanding of the taxonomy and relationships within the genus *Salvelinus* (Cavender 1980). Within *Salvelinus alpinus* this wide variation may be due to the geographic isolation of different populations from each other over several thousands of years (Jobling *et al.* 1993), or selection for resource polymorphisms (Smith and Skulason 1996). The occurrence of discrete intraspecific morphs which show differential niche use are referred to as resource polymorphisms and are common among fishes such as Arctic char which occur in lakes in recently glaciated regions (Smith and Skulason 1996). However, the exact systematics and evolution of the char is still the subject of much debate (Behnke 1980, 1984; Klemetsen and Grotnes 1980; Savvaitova 1980).

Examination of the cytochrome b gene of mitochondrial DNA (mtDNA) in Arctic char revealed four major genotypes (I, II, III, IV) (Bartlett *et al.* unpublished). It was found that char from the Northwest Territories, including Nauyuk Lake, possess genotype III. However, populations from Tree River exhibit genotype IV which suggests an Arctic refugium in the Tree River area. Newfoundland and Labrador populations exhibit genotypes III, I and II which suggest these areas were colonised by char from different refugia, most likely char which originated in Northwest Territories, Europe (genotype I) and southeastern North America (genotype II) (Bartlett *et al.* unpublished). However, a study which used restriction fragment length polymorphism (RFLP) to examine mtDNA found only two distinct lineage's which suggests separate northern and eastern glacial refugia (Wilson *et al.* 1996)

1.1.2 Aquaculture

Arctic char is regarded as an excellent candidate for aquaculture. Its cold water requirement and high stocking density make it ideally suited for growth in Canada and other northern countries. The farming of Arctic char first became widespread in the late 1970s and 1980s (Delabbio 1995). The cold water distribution of Arctic char has stimulated interest in the feasibility of farming this species in countries such as Norway, Scotland, Iceland, Ireland and Canada (Torrissen and Barnung 1991). It was originally thought that char could be cultured in both fresh and salt water; however, marine farming is still in the experimental stage (Delabbio 1995).

In the early 1980s, the only broodstock which was available in Canada originated from the Fraser River, Labrador (Figure 1a). Between 1981 and 1984 eggs were collected from the Fraser River under the direction of the Department of Fisheries and Oceans. The eggs were brought back to hatcheries, fertilised, reared and subsequently used for broodstock (Delabbio 1995). The eggs and milt were collected from four males and four females; however, the first generation survivors were from only two families made up of two males and two females (Olson 1992). A second stock which originated from Nauyuk Lake, Northwest Territories has since been made available for commercial operations. A third stock, from Tree River Northwest Territories is also currently being used as a source of Arctic char (Figure 1b).

There are several features which make the culture of Arctic char attractive: (i) they grow well at low water temperatures, demonstrating a better growth performance than rainbow trout or Atlantic salmon (DeMarch 1991; Delabbio 1995), (ii) they are an extremely hardy fish which have been found to grow well at high densities (DeMarch 1991; Brown *et al.* 1992; Delabbio 1995), (iii) it is a speciality item which secures a higher price than the other commercially grown salmonids (Delabbio 1995) and (iv) due to the small head and body shape the processing of char results in a fillet yield which is 7-8% higher than other salmonids (Delabbio 1995). Undesirable characteristics such as its lack of

synchrony of maturation, low fertilisation rates and size variability have hampered large scale production of this species (DeMarch 1991).

Studies on the growth rates of Arctic char have indicated that some individuals may never grow to harvest size and that these "runts" may be genetically programmed for slow growth (Papst and Hopky 1983; Jobling and Reinsnes 1986). To date, little effort has gone into documenting the genetic background of farmed Arctic char and the genetic basis for differences in growth rate within strains is not known (DeMarch 1991). Very few studies have looked at intra- and inter-strain genetic differences; however, it has been suggested these may be large (Jobling *et al.* 1993). If this is the case, there may be considerable potential for stock improvement through selective breeding (Jobling *et al.* 1993).

Figure 1a: Location of wild Arctic char stocks which are used as the source of commercialised stocks for culture: Fraser River char, Labrador.

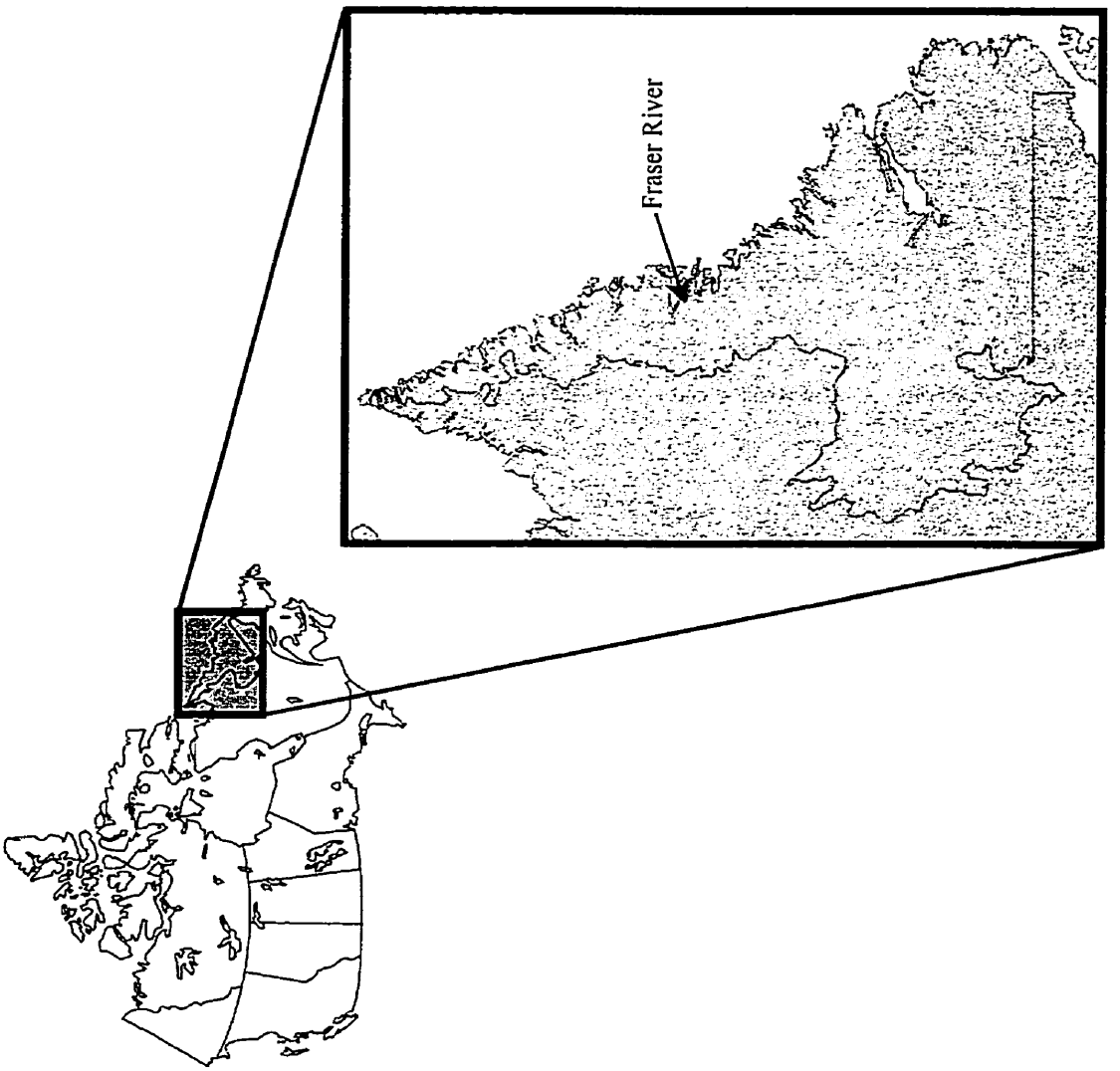
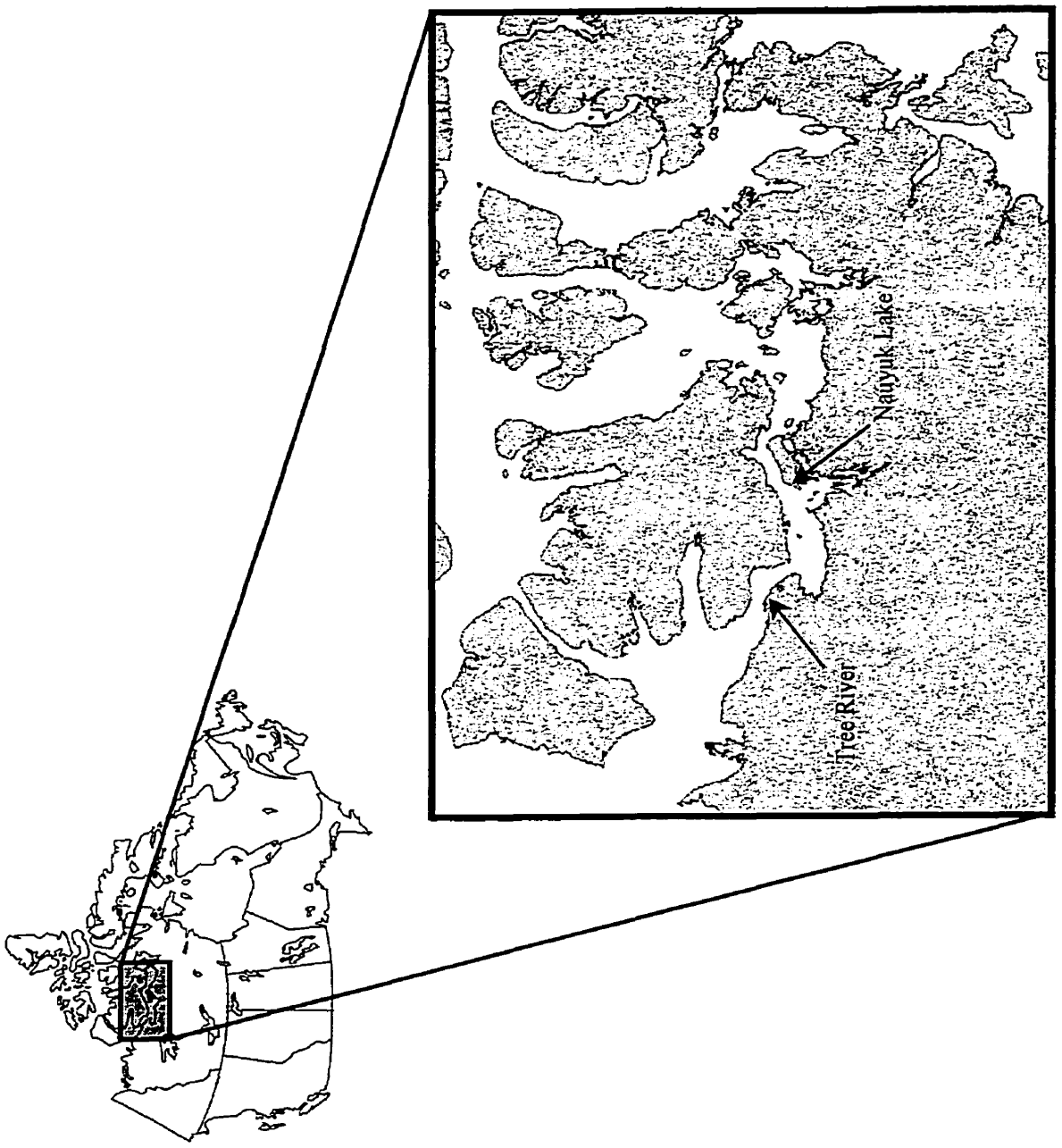

Fraser River

Figure 1b: Locations of wild Arctic char stocks which are used as the source of commercialised stocks for culture: Nauyuk Lake char, Northwest Territories; Tree River char, Northwest Territories.


Nauyuk Lake
Tree River

1.1.3 Genetic makeup of salmonids

The nuclear genomes of bony fishes vary from 0.3-4 billion base pairs in size (Park and Moran 1994). In the past, studies tended to concentrate on mtDNA rather than nuclear DNA (nDNA). This is partly due to the ease of extraction and haploid nature of the mtDNA and partly because of the success with this form of DNA in other species (Park and Moran 1994).

There is some difficulty with studying nDNA with salmonids as approximately 25-100 million years ago the ancestor of all modern salmon went from diploid to tetraploid (Hartley and Davidson 1994; Lie *et al.* 1994; Park and Moran 1994) Most salmonid species have reverted to the diploid state and duplicate loci may be nonfunctional due to the accumulation of mutations. However, some duplicate genes are still present and may be expressed and functional (Ohno 1970).

1.1.4 Satellite DNA

The genomes of most eukaryotic organisms contain tandemly repeated blocks of DNA (O'Reilly and Wright 1995) which may make up to 66% of the nuclear DNA (Skinner 1977). These regions are termed satellite DNA because they orbit bulk DNA in buoyant density centrifugation. The satellite DNA consists of repeats which may be anywhere from two to thousands of base pairs (bp) in length (Franck *et al.* 1990; O'Reilly and Wright 1995). Satellite DNA has been further subdivided into maxisatellite, minisatellite

or microsatellite based on the size of the monomeric unit of the tandem array (Franck *et al.* 1990). Maxisatellite DNA ranges from hundreds to thousands of bp in length (Park and Moran 1994; O'Reilly and Wright 1995), minisatellites are shorter in repeat units, with repeat sequence of 10 to 100 bp in length and a total length of 0.1 to 7 kilobase pairs (Kb) (Estoup *et al.* 1993; Wright 1993; O'Reilly and Wright 1995), and microsatellites are characterised by repeat units which are 1 to 4 bp in length with an average length of 20-300 bp and an average distance between microsatellite of 50-100 Kb (Estoup *et al.* 1993; Queller *et al.* 1993; Slettan *et al.* 1993; Wright 1993; Park and Moran 1994; Wright and Bentzen 1994; Angers *et al.* 1995; O'Reilly and Wright 1995).These repetitive sequences exhibit polymorphism in the sizes of amplified fragments, a feature which is universal in eukaryote DNA regardless of length of the repeat unit (Weber and May 1989).

There is some debate over the function, or lack of function, of these repeat sequences. Some researchers have attributed functions such as maintenance and evolution of the genome to these noncoding regions whereas others have stated that these areas have no function (Weber and May 1989; Franck *et al.* 1990; Stallings *et al.* 1991). Pardue *et al.* (1987) reported that the repetitive sequences may play a role in genetic recombination; however, a study by Stallings *et al.* (1991) found no such correlation. It has also been shown that $(GT)_n$ sequences can enhance the transcriptional activity of genes in plasmid constructs (Hamada *et al.* 1984). Whatever their function, these areas are invaluable for

the construction of high resolution genetic maps and the localisation of disease genes (Richard and Sutherland 1994).

Minisatellites and microsatellites are both inherited in a Mendelian fashion and can therefore be used for pedigree reconstruction (Herbinger *et al.* 1995). Herbinger *et al.* (1995) found that 91% of rainbow trout offspring could be matched to one or two parental couples using only four or five microsatellite probes. There were no mutations at any of the loci used in the study by Herbinger *et al.* (1995). These extremely low mutation rates indicate that mutations are not a serious problem for those wishing to use these markers for genetic analysis.

1.1.5 Microsatellites

Microsatellites, which are common in all eukaryotic organisms, are also known by several other names: e.g., simple sequence repeats (SSRs), simple tandem repeats (STR) and simple sequence length polymorphisms (SSLPs) (Hughes and Queller 1993). The salmonid genome contains large numbers of these repeat units (Slettan *et al.* 1993; McConnell *et al.* 1997).

Microsatellites demonstrate a high degree of length polymorphism which, provided the sequences flanking the microsatellite are known, can be easily analysed using the

polymerase chain reaction (PCR) followed by electrophoresis on polyacrylamide or agarose gels (Beckman and Soller 1990; Hughes and Queller 1993; Slettan *et al.* 1993).

There are a number of reasons why there has been a search for genetic markers which exhibit Mendelian inheritance. If a relationship between single locus characters and production traits exist this would enhance breeding programmes. A microsatellite may be linked to a genetic locus affecting growth. If an allele is found to be positively correlated with growth it is probably linked to such a gene and therefore, by selecting this allele there is indirect selection for increased growth. Also, markers would help genetically discriminate between populations of fish species (Utter 1991). One of the main uses of microsatellites has been in genome mapping; however, in salmonids microsatellites have been more frequently used for the analysis of population structure (McConnell *et al.* 1995a).

Highly polymorphic microsatellite loci appear to be abundant in the genomes of teleost fish (Brooker *et al.* 1994; O'Reilly and Wright 1995; Olsen *et al.* 1996). The microsatellites isolated from fish have been found to be predominantly dinucleotide repeats, generally $(GT)_n$ or $(CT)_n$ (Estoup *et al.* 1993; Brooker *et al.* 1994; McConnell *et al.* 1995a). These structures are useful in gene mapping for a number of reasons. It is common for a microsatellite to display multiple alleles which can be easily resolved when run on polyacrylamide gels (Estoup *et al.* 1993; Queller *et al.* 1993; Pemberton *et al.*

1994; O'Reilly and Wright 1995). The polymorphisms are most likely generated by mutations as a result of slippage during DNA replication, unequal exchange during recombination, or gene conversions (Franck *et al.* 1990). These high levels of allelic variation are important for researchers studying species that show low overall levels of variation with conventional markers such as allozymes and mtDNA; populations that are inbred, recently derived or geographically proximate populations where genetic differentiation may be limited; and pedigree analysis (Wright and Bentzen 1994). There has been very little research into tandemly arrayed repetitive DNAs in lower vertebrates such as teleosts (Franck *et al.* 1990). Recent interest in the use of salmonids in aquaculture has led to an increase in the research on the genomes of these species (Goodier and Davidson 1998).

1.1.6 Use of microsatellites between species

One of the major constraints in using microsatellites is the need to isolate and characterise them by cloning and sequencing (Angers and Bernatchez 1996). One method to avoid this problem is to test microsatellites which were developed for other species in order to find conserved priming sites (Estoup *et al.* 1993; Wright and Bentzen 1994; Angers and Bernatchez 1996). As microsatellite loci and their flanking sequences are often conserved in related species this method frequently, but not always, works (Olsen *et al.* 1996). An increase in phylogenetic distance from the species the primers were designed for usually

results in a decrease in the ability to amplify and subsequently detect the microsatellite sequence (Angers *et al.* 1995).

Angers and Bernatchez (1996) found that primers developed for *Salvelinus fontinalis* could be used in other *Salvelinus* species. Their study found that Sfo-1, Sfo-3 and Sfo-20 were monomorphic for all *Salvelinus* sp., Sfo-11 is monomorphic but each species has a different size PCR product. Sfo-12 and Sfo-18 have some variation except in *Salvelinus alpinus* and *S. confluentis*, which were monomorphic at Sfo-12 and *S. malma*, which was monomorphic at Sfo-18. Sfo-8 and Sfo-23 were found to be highly variable in all *Salvelinus* species (Angers and Bernatchez 1996).

1.1.7 Genetic studies of aquaculture species

The majority of species used for aquaculture have not been improved genetically for commercially important traits (O'Connell and Wright 1997). As genetic identification and discrimination of aquaculture stocks is a fundamental requirement in any culture programme, increasing genetic information and the use of marker assisted selection would enhance breeding programmes and minimise inbreeding (Goodier and Davidson 1993; Ferguson 1994). At present the genetic variability in broodstock used in aquaculture is not routinely assessed. This lack of knowledge leads to the practice of not using enough broodstock which in turn results in the loss of genetic variability and an increased chance of inbreeding depression (Herbinger *et al.* 1995).

Genetic variation in salmonid populations has been widely studied using allozymes, mtDNA markers and nuclear ribosomal DNA markers. These methods have sometimes revealed genetic variation but often only detect low levels of genetic variation (McConnell *et al.* 1995b); however, the high levels of variability found in microsatellites mean they have the potential to be used as effective and efficient markers.

Due to the highly polymorphic nature of microsatellites they can be directly applied to the aquaculture industry for use in genetic mapping by linkage analysis, as well as population and pedigree studies (Franck *et al.* 1990; Slettan *et al.* 1993). With an increase in the number of repeats in a sequence the usefulness of a microsatellite as a variable genetic marker also increases. Salmon $(dG\text{-}dT)_n$ microsatellites usually have long stretches of repeats and generally occur 90 Kb apart. This will be very useful in constructing an Atlantic salmon marker map (Slettan *et al.* 1993) as well as maps of other salmonid species such as Arctic char. The large number of microsatellites and the high variability expressed by these regions means they are important tools for any problem which relies on Mendelian markers (Queller *et al.* 1993).

Research has begun on the use of microsatellites to assist fish farms in selective breeding of aquaculture species. Highly polymorphic microsatellites allow parents of superior

progeny to be identified in mixed family rearing environments therefore enabling selective breeding to occur on commercial fish farms (Wright and Bentzen 1994).

1.2 ASSOCIATION STUDIES AND QUANTITATIVE TRAIT LOCI

1.2.1 Background on association studies and quantitative trait loci

A quantitative trait is a character whose phenotypic variation is continuous and determined by segregation of multiple loci; the loci which control the quantitative trait are referred to as polygenes or quantitative trait loci (QTL) (Tanksley 1993; Casas-Carrillo *et al.* 1997). Characters of economic importance tend to be quantitative traits which are influenced by numerous loci throughout the genome. The individual loci often have small effects individually (Geldermann 1975; Lande and Thompson 1990). These QTL can be identified and mapped when they co-segregate with marker loci (Muranty *et al.* 1997). Identification of the genes which control quantitative variation could be of great value for genetics and breeding (Muranty and Goffinet 1997), and has occupied quantitative geneticists since early this century (Michelmore and Shaw 1988). These types of experiments have become more common and are driven by the potential for an increase in genetic gain in traits of economic importance (Henshall and Goddard 1999).

Phenotypic variation for quantitative traits is continuous and conditioned by allelic variation at several genetic loci each with relatively small effects (Tanksley 1993). It has been found that marker loci with no direct effect on the character of interest can be utilised in selection because of statistical and genetic association between alleles at the marker loci and the QTL (Lande and Thompson 1990). The QTL can be detected and mapped when they co-segregate with these marker loci (Muranty and Goffinet 1997).

Phenotypically neutral molecular markers such as microsatellites have made it easier to detect linkage between segregating markers and QTL. In addition, they have provided an unbiased way to estimate the phenotypic effect of each polygene without interference by the marker locus (Tanksley 1993).

Within the aquaculture industry selection programmes are not yet commonly used (Gjerdrem 1983). Growth rate is of economic importance for all species farmed in both agriculture and aquaculture. In farm animals a high genetic correlation has been reported between growth rate and food conversion; therefore, selection for high growth rate results in a rapid correlated improvement in food conversion (Gjerdrem 1983). Investing in selection programmes for traits such as rapid growth and disease resistance could greatly benefit the aquaculture industry.

1.2.2 Marker assisted selection

The first report of a QTL was in 1923 by Sax, who reported a QTL for rust and bunt resistance in wheat. Since then QTL have been reported in a number of commercially important species such as the tomato (Paterson *et al.* 1988), pigs (Andersson *et al.* 1994; Casas-Carrillo *et al.* 1997; Knott *et al.* 1998) and rainbow trout (Jackson *et al.* 1998; Dansmann *et al.* 1999; Robison *et al.* 1999; Sakamoto *et al.* 1999).

Marker assisted selection is a method which is used to integrate molecular genetics with artificial selection (Lande and Thompson 1990). One method which can be used to carry

out selection is to use association studies which compare unrelated individuals from a population with and without the trait in question (Lander and Schork 1994). An allele which occurs at a significantly higher frequency among the group with the trait is said to be associated with the trait (Lander and Schork 1994). The use of marker loci to detect QTL relies on the underlying assumption that the alleles at the marker loci and the QTL are in linkage disequilibrium (Tanksley 1993). Lander and Schork (1994) reported three reasons why positive association may occur in an association study:

1. The allele is the actual cause of the trait
2. The allele does not cause the trait but is in linkage disequilibrium with the cause
3. Result may be an artefact as a result of population admixture

To prevent artefacts from arising the study should be carried out within a relatively homogenous group and there should be some kind of internal control for allele frequency.

Young, genetically isolated populations tend to lend themselves to this type of study as the linkage disequilibrium extends over greater distance and the number of alleles associated with the trait is likely to be fewer (Lander and Schork 1994). It has been reported that a greater map distance between the marker loci and the QTL will cause a reduction in the efficiency of marker assisted selection; recombination reduces the linkage disequilibrium and thus decreases the effectiveness of selection of the marker loci (Lande and Thompson 1990). Over time, it may become necessary to re-evaluate the

association between marker loci and the QTL as recombination, random genetic drift and selection will have affected the association (Lande and Thompson 1990).

1.2.3 Selective Genotyping

Most traits which animal breeders select for show a continuous quantitative distribution (Muranty *et al.* 1997). Selective genotyping is a method which can be used in selection experiments to decrease the number of individuals being genotyped. It involves genotyping individuals from the high and low tails of the phenotypic range of the entire population (Lebowitz *et al.* 1987; Darvasi and Soller 1992; Tanksley 1993; Darvasi 1997) and makes use of the fact that most of the information for QTL effects is found in the top and bottom 20% of the quantitative trait distribution (Martinez *et al.* 1998; Ronin *et al.* 1998). This means that the number of individuals genotyped can be decreased significantly at the expense of a slight increase in the number of individuals phenotyped (Lebowitz *et al.* 1987; Lander and Botstein 1989; Darvasi and Soller 1992; Martinez *et al.* 1998). Correlative statistics can then be used to determine if the individuals with one genotype are significantly different from individuals of other genotypes with respect to the trait being measured (Tanksley 1993). If the allele frequency at one locus is found to differ between the two tails of the populations then it is assumed that there is a link between a QTL which controls the character of interest and the marker (Tanksley 1993). This method works best when studying one trait at a time because as the number of traits

being analysed increases, then selecting the extremes of each trait would ultimately result in the selection of most of the population (Tanksley 1993; Darvasi 1997).

Darvasi and Soller (1992) carried out a study to determine the efficiency of selective genotyping in marker-QTL linkage data studies with respect to a single trait and found that it is never more useful to genotype more than the upper and lower 25% of a population. The phenotype of an individual is the result of interaction between the genotype and the environment (Michelmore and Shaw 1988; Tanksley 1993), and the distribution of the phenotype most likely arises from the action of the variable environment and genes located at QTL (Muranty *et al.* 1997). Therefore, a QTL which is found to be important in one environment may not be as important in another environment (Tanksley 1993). If individuals are chosen at the extreme tails of the population then individuals from some families and none from other families would be chosen due to the family effect. Therefore, in order to apply selective genotyping the individuals should be chosen at the extremes within the family (Muranty *et al.* 1997).

1.2.4 Applicability of selective genotyping to Arctic char from Daniel's Harbour Hatchery

The fish used in this study are at most four generations removed from the original Fraser River stock which consisted of two families made up two males and two females. This domestic stock has been inbred ever since the original collection of eggs and milt in the

early 1980s and therefore it is highly likely that linkage disequilibrium extends over large areas of the genome, as this is most likely to occur in a young isolated population (Lander and Schork 1994). If a marker for a particular trait lies close enough to the trait causing locus on a chromosome then, in a population such as the Arctic char population used in this study, the correlation between the marker and the trait locus will not have been eroded by recombination (Lander and Schork 1994). Poompuang and Hallerman (1997) reported that QTL detection and marker assisted selection are not being practised on an aquaculture species. For these reasons it was decided that the Daniel's Harbour hatchery Arctic char fulfilled the requirements for an association study.

1.3 HYPOTHESES

This study looked at microsatellite loci as they relate to growth rates in Arctic char. The objectives of this study were to (1) test primers designed to amplify microsatellites in other salmonid species to see if they also amplify a homologous site in the Arctic char genome, (2) determine if there is a correlation between certain alleles with growth rate and (3) if such a correlation exists, to test if they can be used as markers for selecting broodstock.

Hypothesis 1:

H_0: The allelic distribution in the groups of small and large Arctic char is identical and therefore there is no association between the microsatellite locus and rapid growth.

H_A: The allelic distribution in the groups of small and large Arctic char is not identical and therefore there is an association between the microsatellite locus and rapid growth.

Hypothesis 2:

H_0: The genotypic distribution in the groups of small and large Arctic char is identical and therefore there is no association between the microsatellite locus and rapid growth.

H_A: The genotypic distribution in the groups of small and large Arctic char is not identical and therefore there is an association between the microsatellite locus and rapid growth.

CHAPTER 2

2.1 MATERIAL AND METHODS

2.1.1 Production of Arctic char families

The fish used in this study were descended from broodstock which originated from the Fraser River, Labrador. The fish were held at Daniel's Harbour Hatchery, Daniel's Harbour, Newfoundland, where 15 families were produced in the autumn of 1995 by crossing each of five males with three females. The eggs were incubated as individual families until first feeding. At this point equal numbers (n=50) from each family were pooled, this was done in triplicate. The fish were grown under identical conditions in the hatchery for nine months at which point they were transferred to the Animal Care Unit at Memorial University of Newfoundland where they were held in 0.21 m^3 tanks, with a water temperature of 10^{o}C and a light/dark regime of 12/12 hours. The fish were graded in April 1997 when the largest 5% had part of their tail removed and the bottom 5% were killed and stored frozen.

In the autumn of 1996, six different families were produced by crossing six males with six females. These families were pooled with equal numbers from each family (n=100) in triplicate and held at Daniel's Harbour Hatchery until October 1997. The fish were then transferred to the Animal Care Unit at Memorial University of Newfoundland where they were subjected to the same conditions as the previous families. Grading of these fish took place in February 1998, the smallest 5% were killed and stored frozen and the largest 5%

had part of their tail removed. Tissue samples were not available from the parents used from either year class.

2.2 GENETIC ANALYSIS

2.2.1 DNA extraction

DNA was extracted using a modification of that developed by Taggart *et al.* (1992). Approximately 100 mg of frozen tail tissue was mixed with 375 mL of 0.2 M EDTA, 0.5% sacrosyl and 25 μL of 20 mg/ml pronase and incubated overnight in a 37°C water bath. Ten μL of 2 mg/mL DNAase free RNAase was added to each tube and incubated for one hour at 37°C. This was followed by three phenol/chloroform extractions. Four hundred μL of phenol (pH 8.0 equilibrated with 10 mM Tris/HCl) was added to each tube. The contents were mixed for 10 minutes, centrifuged for 10 minutes and the top layer was decanted into a new tube. Two hundred μL of phenol and 200 μL of chloroform:isoamyl alcohol (24:1 V:V) was added to each new tube, the contents mixed for 10 minutes, centrifuged for 10 minutes and the top layer decanted into a new tube. Four hundred μL of chloroform:isoamyl alcohol was added to each new tube, the contents mixed for ten minutes, centrifuged for ten minutes and the top layer decanted into a new tube. Two volumes (800 μL) of ice cold 95% ethanol was added to each new tube, mixed and placed at -20°C overnight. The tubes were centrifuged for 20 minutes and the ethanol was decanted. Sixty μL of 70% ice cold ethanol was added to each tube

followed by 5 minutes of centrifugation and the ethanol was decanted. The DNA was allowed to air dry overnight and then resuspended in 100 μL of TE.

2.2.2 Microsatellite analysis

Primers designed for amplification of microsatellite loci in other salmonid species were used in this study. It was hypothesised that many of the primers designed for other salmonid species would work with Arctic char because of the close phylogenetic relationship among these species (Estoup *et al.* 1993). Jackson (1995) reported that microsatellite primers developed for one salmonid easily amplify loci in other salmonid species with minimal or no adaptation of PCR conditions. However, this was not found to be the case in char; the published conditions for the primer pairs did not always result in amplification products. Initially eight individuals were set up with each primer pair to determine the correct annealing temperature. Each primer pair was set up with an annealing temperature of 48°C, if this did not result in an amplification product the annealing temperature was increased by two degrees. This process was repeated until the correct annealing temperature was determined or until 64°C was reached with no product. The forward primer from each pair was endlabelled using γ^{32}P ATP and polynucleotide kinase. A total volume of 12.5 μL was used in PCR. This volume was made up of approximately 50 ng of DNA, 3.2 pmoles of radioactive forward primer, 7.4 pmoles of unlabelled forward primer, 10 pmoles of reverse primer, 1 unit of TFL polymerase (Promega), 10X TFL buffer (final concentration of 20 mM Tris acetate (pH

9.0), 10 mM potassium chloride, 75 mM potassium acetate, 0.05% Tween-20), 1.5 mM of $MgSO_4$, 10 mM of each dNTP (dATP, dCTP, dGTP, dTTP) (Pharmacia). The reaction cocktail was placed in thin walled tubes and PCR was carried out in a Gene Amp® PCR system 9600 thermal cycler (Perkin-Elmer). The following PCR conditions were used: initial denaturation for 3 min at 95 °C followed by 35 cycles of 45 s at 94°C, 45 s at the primer specific annealing temperature and 30 s at 72°C. The completed reactions were held at 4°C. The resulting products were separated using electrophoresis on a 6% polyacrylamide sequencing gel containing 7 M urea with 1X TBE buffer. A constant power of 42 watts was applied for 2.5 hours. The gels were dried and visualised using autoradiography with an exposure time of 3-4 days. If a primer resulted in a monomorphic product then that primer was not included in any further analysis. If a polymorphic product resulted, then both year classes (1995 and 1996) were screened using this primer. Alleles were numbered arbitrarily.

2.3 STATISTICAL ANALYSIS

2.3.1 Initial data check

The relative frequencies of different alleles and genotypes at each polymorphic locus were calculated. Data sheets which could be used in GENEPOP (v3.1a; Raymond and Rousset 1995) were constructed to calculate genic (allelic) differentiation and genotypic differentiation.

Two hypotheses were tested using GENEPOP. The first hypothesis dealt with the allelic distribution of alleles and was tested using the Fisher exact test. The second hypothesis dealt with the distribution of genotypes and was tested using a log-likelihood (G) based test. These tests were used to determine if there was a significant difference between large and small fish at a particular locus. If a significant difference was detected at a given locus and if this result was reproducible (i.e., significant in both year classes), then it was hypothesised the locus is associated with growth.

The critical value (α) for statistical significance in this study was set at 0.05.

2.3.2 Analysis of hypothesis 1

Fisher exact tests were used to examine the allelic distribution of alleles between the small fish and the large fish. GENEPOP was used to compute an unbiased estimate of the P-value (Raymond and Rousset 1995). If the frequencies of alleles between groups were statistically significant ($p<0.05$) in both the 1995 and 1996 year class of fish, then it was hypothesised that there is an association with growth at that particular locus in the Fraser River strain of Arctic char originating from Daniel's Harbour Hatchery.

2.3.3 Analysis of hypothesis 2

An unbiased estimate of the P-value of a log-likelihood (G) based test (Goudet *et al.* 1996) was performed to examine the genotypic distribution between small fish and large fish. If the genotypic distributions between groups were statistically significant ($p<0.05$) in both the 1995 and 1996 year class of fish, and if the Fisher exact test was also statistically significant at the same locus then this locus was considered to be associated with growth in the Fraser River strain of Arctic char originating from Daniel's Harbour Hatchery.

2.3.4 Calculation of genetic distance

A programme was written in Fortran by Dr. D. Innes to calculate the genetic distance between each pair of individuals. The alleles of each individual were compared at each variable locus to determine how similar the genotypes were. Each individual could have 1 match if they had one allele in common, 2 matches if the genotypes at that locus was identical or 0 matches if they were completely different at that particular locus. Individuals which did not amplify at a specific locus were originally coded as 0 and were not compared to other individuals at that locus. The genetic distance was then calculated using the following formula:

M1 = # matches/ # of comparisons X (2 x # loci)

Distance = SQRT(1 - M1)

A matrix of genetic distances was created with identical individuals being coded as 0 and 1 representing completely dissimilar individuals. This matrix was then converted to two dimensions through nonmetric multidimensional scaling using NTSYS-pc (Applied Biostatistics Inc., Exeter Software) so that a two dimensional scatter plot of the genetic distances could be created. Nonmetric multidimensional scaling is a method similar to principal components analysis in that it represent the relationships among a set of points in a low dimensional space. The two dimensions represent the information which is contained in the multi-dimensional matrix. This was created to determine if the fish which had similar genotypes were also in the same size class. Analyses were also carried out using only those variable loci that did not show any association with growth and again with only the variable loci which were correlated with growth.

CHAPTER 3

3.1 RESULTS

3.1.1 Weight distributions of fish

At the time of grading the 1995 year class fish ranged in weight from less than 2 g to 34 g with a mean of 12.9 g (Figure 2). The 1996 year class fish ranged in weight from 0.2 g to 20 g with a mean of 6.15 g.(Figure 3). There was a ten- fold difference in weight between the smallest and largest fish in both year classes. Samples were taken from the 5% smallest and largest in both year classes for DNA analysis. In 1995, all fish under 6 g and over 20 g were sampled. This resulted in 22 small fish and 23 large fish. In 1996, all fish under 3 g and over 11 g were sampled. This resulted in 62 small fish and 60 large fish.

3.1.2 Analysis of microsatellite primers

The primer pairs used in this study were not developed for Arctic char; therefore, it was unknown if they would result in an amplification product. Seventy-four primer pairs were tested, 56 resulted in an amplification product and 18 did not amplify. Of the 56 which were successful, 41 produced monomorphic products and 15 produced polymorphic products. Twelve of the fifteen were analysed as two were not reproducible from year to year and one was a duplicated locus (Table 1). The number of alleles at the polymorphic loci ranged from 2 to 7 (Table 2). The sizes of alleles at each locus were determined by comparing the alleles to a sequence of known size (Table 3).

Table 1: Microsatellite primers tested on Arctic char DNA. Primers producing reproducible, scorable products are in boldface and those showing variation are identified by *, duplicated loci identified by λ

Primer	Reference or genbank accession number
Ssa 202	U43695
Ssa 171	U43693
Ssa 86	AF019191
Ssa 289	**McConnell *et al.* 1995a**
Ssa 85	U43692
Ssa 120	U58896
Ssa 76	**AF019188**
Ssa 4	**McConnell *et al.* 1995a**
Ssa 14	**McConnell *et al.*1995a**
Ssa 119	**U58892**
Ssa 197	**U43694**
Sfo 23*	**U50304**
Sfo 8	**U50305**
Sfo 11	**U50306**
Sfo 18*	**U50303**
Sfo 12	**U50302**
μF43*	**Sanchez *et al.* 1996**
μ20.19	**U37494**
μD30	U37493
μ5.27*	**U37491**
μ1.22*	**AF020848**
μ1.14	**AF020847**
Mst 79	AB001063
Mst 73	**AB001056**
Mst 60	AB001057
Mst 15	AB001058
Mst 3	AB001060
Mst 85	AB001059
Mst 591	AB001064
Mst 543	AB001062
OmyFGT 29TUF	**Pers. com. Takashi Sakamoto**
OmyRGT47TUF	**Pers. com. Takashi Sakamoto**
OmyRGT51TUF	**Pers. com. Takashi Sakamoto**
OmyRGT14TUF	**Pers. com. Takashi Sakamoto**
OmyPuPuPyDU	**Morris *et al.* 1996**
OmyRgt1TUF	**Pers. com. Takashi Sakamoto**

Table 1 continued

OmyRGt36TUF	**Pers. com. Takashi Sakamoto**
Omy 287*	**O'Connell *et al.* 1997**
Omy 77*	**Morris *et al.* 1996**
Omy 325	**O'Connell *et al.* 1997**
Ssosl 85	**Slettan *et al.* 1993**
Ssosl 311	**Slettan *et al.*1993**
Ssosl 417	**Slettan *et al.* 1993**
Ssosl 25	**Slettan *et al.* 1993**
Ssosl 436	**Z49118**
Ssosl 456*	**Z69645**
Sslee L53	**U86704**
Sslee N17	**U86705**
Sslee R15	**U86708**
Sslee P96	**U86707**
Sslee I84*λ	**U86703**
Sslee T47*	**U86709**
Ogo 1	**AF007827**
Ogo 2	**AF009794**
Ogo 4	**AF009796**
Ogo 5	**AF009797**
Ogo 6	**AF009798**
Ogo 7	**AF009799**
One 18*	**U56718**
One 10	**U56710**
One 5	**U56704**
One 8	U56708
One 15	U56715
One 12	U56712
One 7	**U56707**
One 1	**U56699**
One 14	U56714
One 6*	**U56706**
One 3	U56701
Ots4BML	**Pers. com. Takashi Sakamoto**
ApoAI 66 and 67	**Drover 1996**
ApoAI 66 and 65	**Drover 1996**
Cocl 3	**Pers. com. L. Bernatchez**
Cocl 1*	**Pers. com. L. Bernatchez**

Table 2: Number of alleles and PCR amplification conditions for the thirteen polymorphic microsatellite loci in Daniel's Harbour Hatchery Arctic char. Duplicated loci indicated by τ.

Locus	Number of Alleles	Annealing temperature (°C)	Number of cycles
Sfo 23	4	52	35
Sfo 18	2	52	35
μF43	4	55	35
μ5.27	3	52	35
μ1.22	6	50	35
Omy 287	2	52	35
Omy 77	4	50	35
Ssosl 456	7	50	35
Sslee I84/1 τ**	3	60	35
Sslee I84/2 τ**	3	60	35
Sslee T47	2	60	35
One 18	6	55	35
One 6	4	50	35
Cocl 1	2	50	35

**5 alleles in total amplified at this duplicate loci however, one allele amplified at both loci

Table 3: Size (in bp) for alleles at 13 variable microsatellite loci for Daniel's Harbour Hatchery Arctic char.

Locus	Allele	Size (bp)
Omy 287	5	130
	7	129
μ5.27	3	110
	5	108
	7	106
One 6	5	328
	8	324
μ1.22	4	162
	6	159
	10	152
Cocl 1	3	219
	5	215
μF43	1	107
	3	105
	5	102
	7	101
One 18	2	217
	8	197
	10	194
	12	189
	18	180
Sslee I84/1	7	219
	12	200
	3**	229
Sslee I84/2	2	237
	9	215
Sslee T47	3	170
	5	169
Sfo 18	1	162
	2	159
Omy 77	3	181
	7	170
	9	167

Table 3 continued

Ssosl 456	5	260
	6	250
	7	240
	9	222
	13	210
	21	157
	33	142
Sfo 23	1	244
	3	241
	7	217
	9	213

** Allele 3 at Sslee I84 amplifies at both Sslee I84/1 and Sslee I84/2

Figure 2 Weight distribution of Daniel's Harbour Hatchery Arctic char 1995 year class; mean of weight distribution is 12.9 grams with the top 5% being fish over 20 grams and the bottom 5% being fish under 6 grams

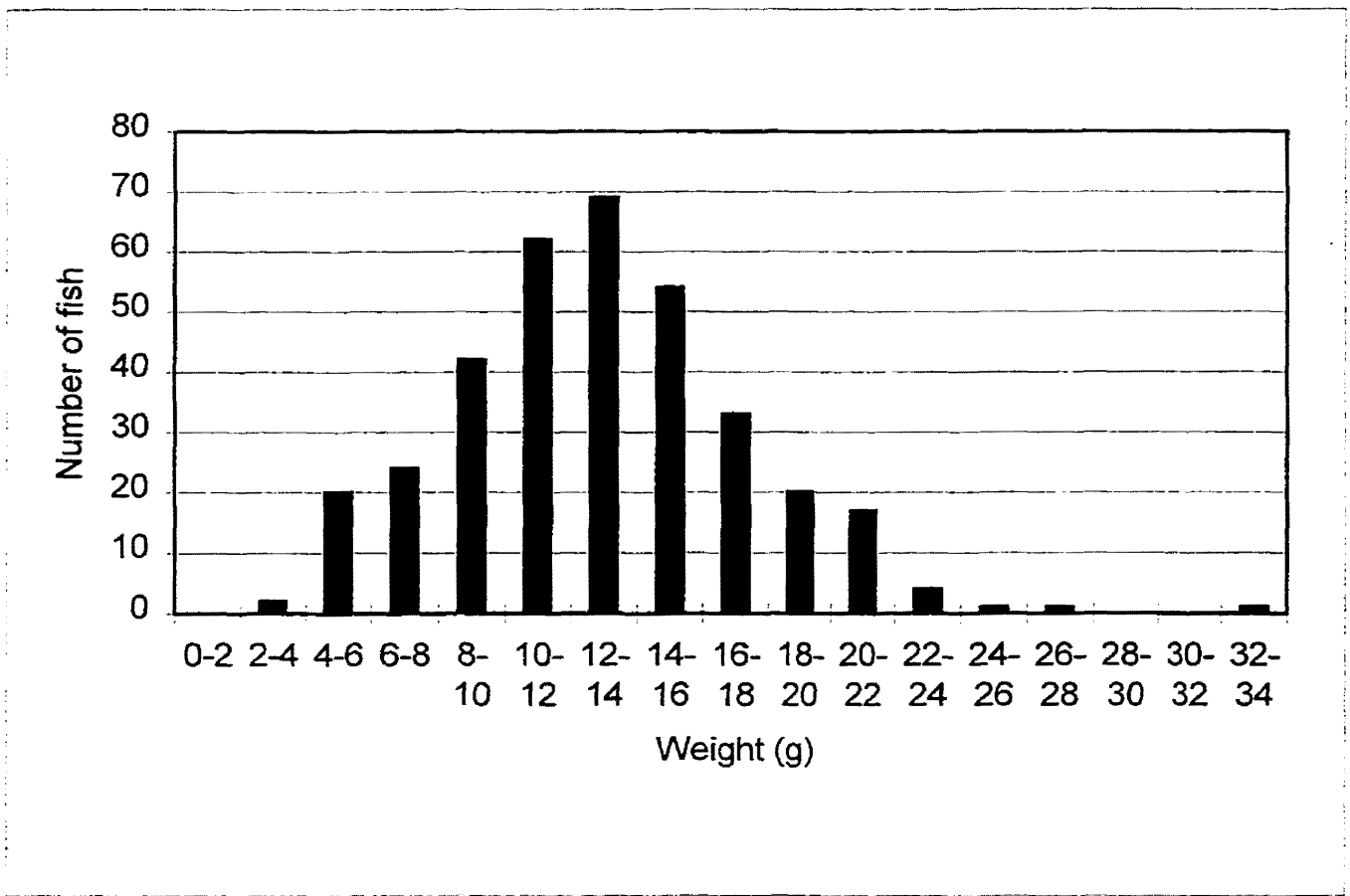
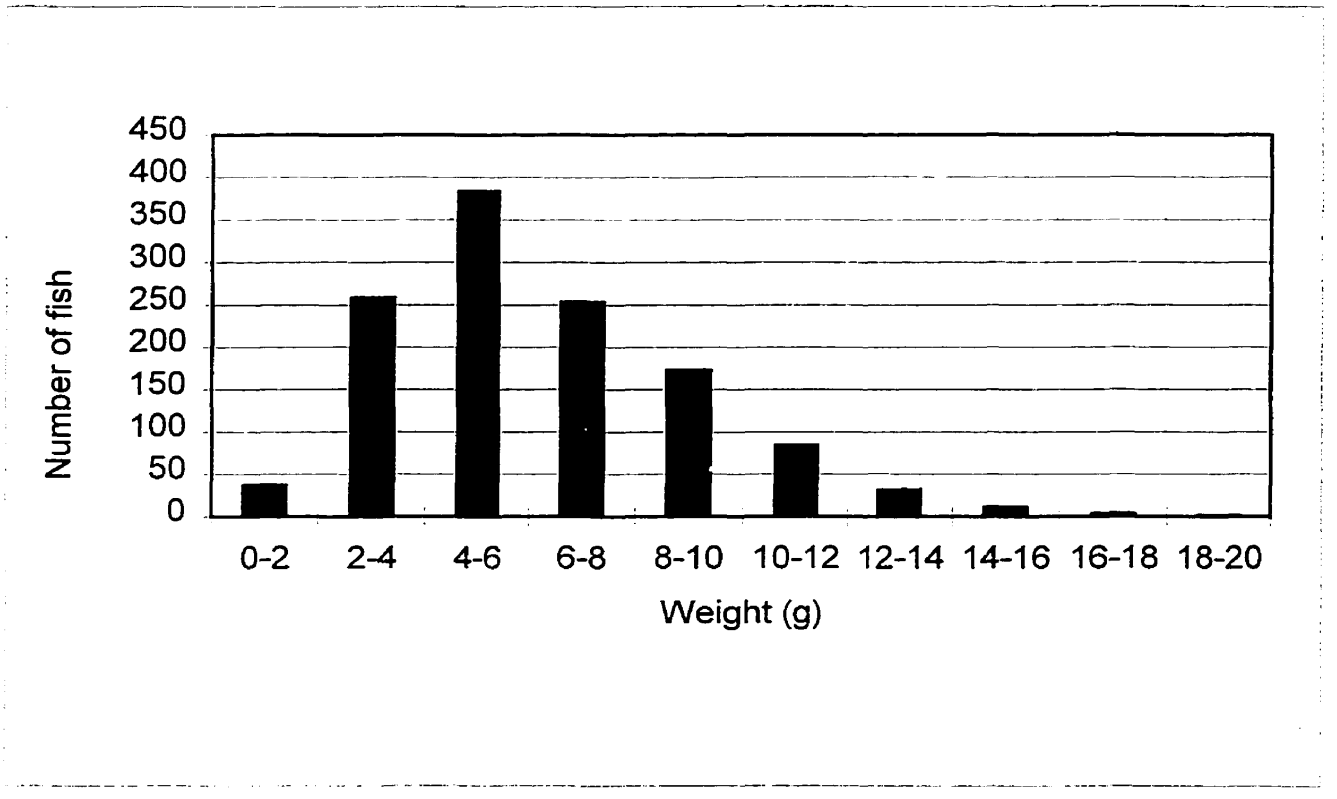

80
70
60
50
40
30
20
10
0
Number of fish
0-2
2-4
4-6
6-8
8-10
10-12
12-14
14-16
16-18
18-20
20-22
22-24
24-26
26-28
28-30
30-32
32-34
Weight (g)

Figure 3 Weight distribution of Daniel's Harbour Hatchery Arctic char 1996 year class; mean of weight distribution is 6.15 grams with the top 5% being fish over 11 grams and the bottom 5% being fish under 3 grams.


450
400
350
300
250
200
150
100
50
0
Number of fish
0-2
2-4
4-6
6-8
8-10
10-12
12-14
14-16
16-18
18-20
Weight (g)

3.1.3 Allelic frequencies of Daniel's Harbour Hatchery Arctic char

Fisher exact tests (probability test) showed there was a significant difference in allele frequency between small and large Arctic char in 3 of the polymorphic loci in the 1995 year class and 6 of the polymorphic loci in the 1996 year class (Table 4).

3.1.4 Genotypic frequencies of Daniel's Harbour Hatchery Arctic char

Log likelihood tests (G-test) showed there was a significant difference between the genotypic frequencies in small and large Arctic char in 3 of the polymorphic loci in the 1995 year class and 8 of the polymorphic loci in the 1996 year class (Table 5). Three loci were not considered in these statistical tests; Sslee I84 exhibited disomic inheritance making statistical testing difficult, whereas Ogo 4 and One 5 did not result in reproducible amplification products in both year classes and were therefore excluded from any further analysis.

In order for a locus to be considered associated with growth in Arctic char both the G-test and the probability test had to result in a significant P-value in both year classes of fish. Using these criteria three loci were associated with growth in the Daniel's Harbour Hatchery Arctic char.

The differences in allelic and genotypic frequencies differed from year to year due to the different parents used in the crosses (Tables 6,7,8,9). Using primer Ssosl 456 alleles 5

(260 bp) and 13 (210 bp) appeared only in the small fish in the 1995 year class whereas in the 1996 year class allele 13 appeared in both populations of fish and allele 5 was not present at all. The differences in allele and genotypic frequencies with primer Sfo 23 were consistent from year to year, with allele 1 (244 bp) occurring more frequently in the small fish and allele 7 (217 bp) much more frequently in the large. Fish which were homozygous for allele 7 occurred in the large fish at a much higher frequency than in the small group. Primer μ5.27 also showed consistent differences from year to year. Allele 3 (110 bp) occurs in the small fish but never in the large fish in both year classes.

Table 4: P-values for probability test for allelic frequency differences between large and small Arctic char at 12 polymorphic loci (1995 and 1996 year classes)

Primer	P-value (1995)	P-value (1996)
Omy 287	0.735	0.0952
One 6	0.920	0.2849
Omy 77	0.5004	0.0161*
Cocl 1	0.8054	0.0923
One 18	0.3232	0.1079
μ5.27	0.0021*	<0.00001*
μ1.22	0.0999	0.00118*
Sslee T47	0.5592	0.8748
Sfo 18	0.0839	0.509
μF43	0.6132	<0.00001*
Ssosl 456	0.0083*	<0.00001*
Sfo 23	0.0349*	<0.00001*

*= significant at α=0.05

Table 5: P-Values for log likelihood test (G-test) for genotypic frequency differences between large and small Arctic char at 12 polymorphic loci (1995 and 1996 year classes)

Primer	P-value (1995)	P-value (1996)
Omy 287	0.6266	0.0396*
One 6	0.9406	0.3095
Omy 77	0.5548	0.0139*
cocl 1	0.7863	0.0150*
One 18	0.2491	0.1931
μ5.27	0.0366*	<0.00001*
μ1.22	0.0562	0.0008*
Sslee T47	0.5219	0.8685
Sfo 18	0.1072	0.3317
μF43	0.5704	0.0029*
Ssosl 456	0.0459*	<0.00001*
Sfo 23	0.0086*	<0.00001*

*=significant at α=0.05

Table 6: Allelic frequencies for primers Sfo 23, Ssosl 456 and μ5.27 for the small and large groups of the 1995 and 1996 year classes of Arctic char.

Primer (allele)		Allelic Frequency 1995		Allelic Frequency 1996	
		small	large	small	large
Sfo 23	(1)	0.26	0.0	0.28	0.06
	(3)	0.07	0.0	0.07	0.04
	(7)	0.52	0.86	0.39	0.9
	(9)	0.14	0.14	0.26	0.0
Ssosl 456	(5)	0.125	0.0	0.027	0.0
	(6)	0.0	0.0	0.009	0.375
	(7)	0.063	0.077	0.173	0.0
	(9)	0.0	0.0	0.055	0.333
	(13)	0.0	0.269	0.282	0.021
	(21)	0.656	0.538	0.436	0.27
	(33)	0.156	0.115	0.018	0.0
μ5.27	(3)	0.22	0.0	0.16	0.0
	(5)	0.59	0.5	0.11	0.35
	(7)	0.18	0.5	0.73	0.65

Table 7: Genotypic frequencies for primers Sfo 23, Ssosl 456 and μ5.27 for the small and large groups of the 1995 and 1996 year classes of Arctic char.

Primer	Genotype	Frequency small 1995	Frequency large 1995	Frequency small 1996	Frequency large 1996
Sfo 23	1,1	0.05	0	0.09	0
	1,3	0.05	0	0	0
	1,7	0.33	0	0.28	0.12
	7,7	0.19	0.71	0.14	0.81
	7,9	0.24	0.29	0.09	0
	3,7	0.1	0	0.14	0.08
	1,9	0.05	0	0.12	0
	9,9	0	0	0.16	0
Ssosl 456	5,5	0.063	0	0	0
	5,7	0.125	0	0.036	0
	6,6	0	0	0	0.042
	6,9	0	0	0	0.438
	7,7	0	0	0.11	0
	7,13	0	0.153	0.091	0
	9,9	0	0	0.036	0.104
	9,13	0	0	0.036	0.021
	13,13	0	0.153	0.145	0
	5,21	0	0	0.018	0
	6,21	0	0	0.018	0.229
	13,21	0	0.077	0.145	0.021
	21,21	0.625	0.462	0.345	0.146
	21,33	0.125	0.077	0.018	0
	33,33	0.0625	0.077	0	0
μ5.27	3,3	0.22	0	0.16	0
	5,5	0.59	0.46	0.04	0.22
	5,7	0	0.07	0.12	0.26
	7,7	0.18	0.46	0.65	0.52

Table 8: Allelic frequencies for nine polymorphic microsatellite loci for the small and large groups of the 1995 and 1996 year classes of Arctic char.

Primer (allele)		Allelic Frequency 1995		Allelic Frequency 1996	
		small	large	small	large
One 18	(2)	0.12	0.06	0.08	0.22
	(8)	0.31	0.4	0.08	0.19
	(10)	0.07	0.13	0.26	0.21
	(12)	0.36	0.2	0.37	0.24
	(19)	0.14	0.2	0.14	0.14
	(21)	0	0.033	0	0
Omy 77	(3)	0.5	0.33	0.44	0.43
	(7)	0.35	0.44	0.52	0.39
	(9)	0.15	0.22	0.04	0.17
	(12)	0	0	0	0.01
Omy 287	(5)	0.53	0.5	0.39	0.52
	(7)	0.47	0.5	0.61	0.48
One 6	(5)	0.37	0.43	0.4	0.32
	(8)	0.37	0.3	0.6	0.68
	(10)	0.08	0.07	0	0
	(12)	0.18	0.2	0	0
Cocl 1	(3)	0.57	0.6	0.43	0.3
	(5)	0.43	0.39	0.57	0.71
SsleeT47	(3)	0.18	0.13	0.22	0.22
	(5)	0.82	0.87	0.78	0.78
µ1.22	(4)	0	0	0.13	0.3
	(6)	0.5	0.55	0.08	0.14
	(10)	0.33	0.42	0.08	0.14
	(13)	0.14	0.02	0	0
	(15)	0.05	0	0	0
	(16)	0.02	0	0	0
Sfo 18	(1)	0.29	0.5	0.4	0.46
	(2)	0.71	0.5	0.6	0.54
µF43	(1)	0.75	0.69	0.45	0.46
	(3)	0	0	0.13	0
	(5)	0	0	0.06	0.19
	(7)	0.25	0.31	0.37	0.35

Table 9: Genotypic frequencies for nine polymorphic microsatellite loci for the small and large groups of the 1995 and 1996 year classes of Arctic char.

Primer	Genotype	Frequency small 1995	Frequency large 1995	Frequency small 1996	Frequency large 1996
One 18	2,2	0	0	0.09	0.08
	2,8	0.14	0.05	0.02	0.11
	8,8	0	0	0	0.28
	2,10	0	0	0.06	0.17
	8,10	0.09	0.13	0	0.06
	10,10	0	0	0.16	0
	2,10	0	0	0.02	0
	8,12	0.24	0.33	0.09	0.11
	10,12	0.05	0	0.13	0.19
	12,12	0.14	0	0.22	0.06
	2,12	0.05	0	0.02	0
	8,19	0.14	0.27	0.04	0.06
	12,19	0.09	0	0.04	0.06
	19,19	0	0	0.09	0.08
	10,19	0	0.07	0	0
	10,21	0	0.07	0	0
	2,19	0.05	0.07	0	0
Omy 77	3,3	0.31	0.11	0.24	0.07
	3,7	0.23	0.28	0.33	0.44
	7,7	0.23	0.28	0.36	0.16
	3,9	0.15	0.17	0.07	0.27
	7,9	0	0.05	0	0.02
	9,9	0.07	0.11	0	0.02
	3,12	0	0	0	0.02
Omy 287	5,7	0.75	0.76	0.65	0.66
	5,5	0.15	0.12	0.065	0.2
	7,7	0.1	0.12	0.283	0.15
One 6	5,5	0.11	0.2	0.21	0.096
	5,8	0.26	0.13	0.38	0.45
	5,10	0.16	0.13	0	0
	5,12	0.1	0.2	0	0
	8,8	0.21	0.2	0.4	0.45
	8,12	0.05	0.06	0	0
	12,12	0.1	0.06	0	0
Cocl1	3,3	0.21	0.37	0.026	0
	3,5	0.71	0.47	0.82	0.59
	5,5	0.07	0.16	0.16	0.41

Table 9 continued

SsleeT 47	3,3	0	0	0.03	0
	3,5	0.36	0.26	0.38	0.44
	5,5	0.64	0.74	0.59	0.56
μI.22	4,4	0	0	0.07	0.04
	4,6	0	0	0	0.14
	4,10	0	0	0.12	0.37
	6,6	0.14	0.14	0	0
	6,10	0.45	0.76	0.16	0.14
	6,13	0.18	0.05	0	0
	6,16	0.05	0	0	0
	10,10	0.05	0.05	0.66	0.32
	10,15	0.09	0	0	0
	13,14	0.05	0	0	0
Sfo 18	1,1	0.05	0.35	0.1	0
	1,2	0.48	0.29	0.6	0.91
	2,2	0.48	0.35	0.3	0.09
μF43	1,1	0.55	0.39	0.38	0.43
	1,7	0.4	0.61	0.14	0.07
	3,3	0	0	0.13	0
	5,5	0	0	0.05	0.14
	5,7	0	0	0.02	0.09
	7,7	0.05	0	0.29	0.27

3.1.5 Measurement of genetic distance

Evidence for genetic differences between small and large populations in both the 1995 and 1996 year classes was found when genetic distances for each individual at the 12 polymorphic loci were calculated. These differences were even more apparent when the genetic distances for the 1996 year class alone were compared (Figure 4). As there was a distinct clustering based on size in both year classes of fish, these results support the hypothesis that there is a genetic difference between small and large populations of the Daniel's Harbour Hatchery Arctic char.

The calculations were carried out using only the nine microsatellite loci which were not found to be statistically significant. This analysis was performed to determine if the three loci (Sfo 23, Ssosl 456 and μ5.27) which appear to be associated with growth were a contributing factor to any clustering of genotypes. If these three loci were contributing to the difference in genotypes then once they were removed from the calculations, the small and large groups of fish should be randomly distributed.

When the three microsatellite loci (Sfo 23, Ssosl 456 and μ5.27) which appear to be associated with growth were removed from the calculation of genetic distances there was a more random distribution of genotypes (Figure 5). When the genetic distances were calculated for the three microsatellite loci which appear to be associated with growth, there was again a clustering based on size (Figure 6). This indicates that these three loci

have a strong influence on the differences in genotypes in the small and large groups of fish, and are contributing to the differences in growth rate.

Figure 4: Scatter plot showing genetic distance calculated using 12 polymorphic microsatellite loci, between small and large groups of Arctic char, 1996 year class. For explanation of dimension 1 and 2 refer to methods section 2.3.4

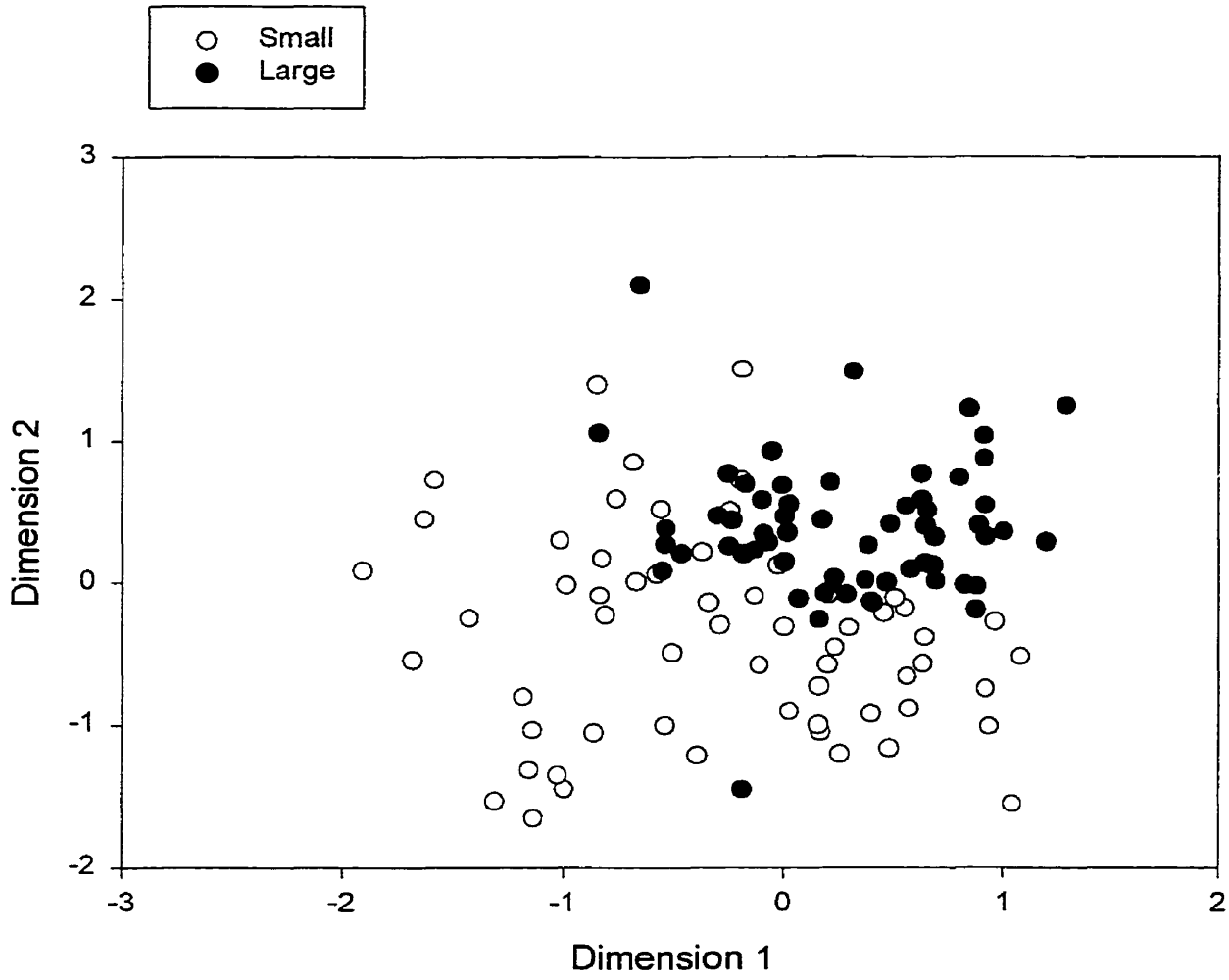

Small
Large
3
2
1
0
-1
-2
-3
-2
-1
0
1
2
Dimension 2
Dimension 1

Figure 5: Scatter plot showing genetic distance calculated using 9 polymorphic microsatellite loci, between small and large groups of Arctic char, 1996 year class. These 9 loci do not show any association with growth.

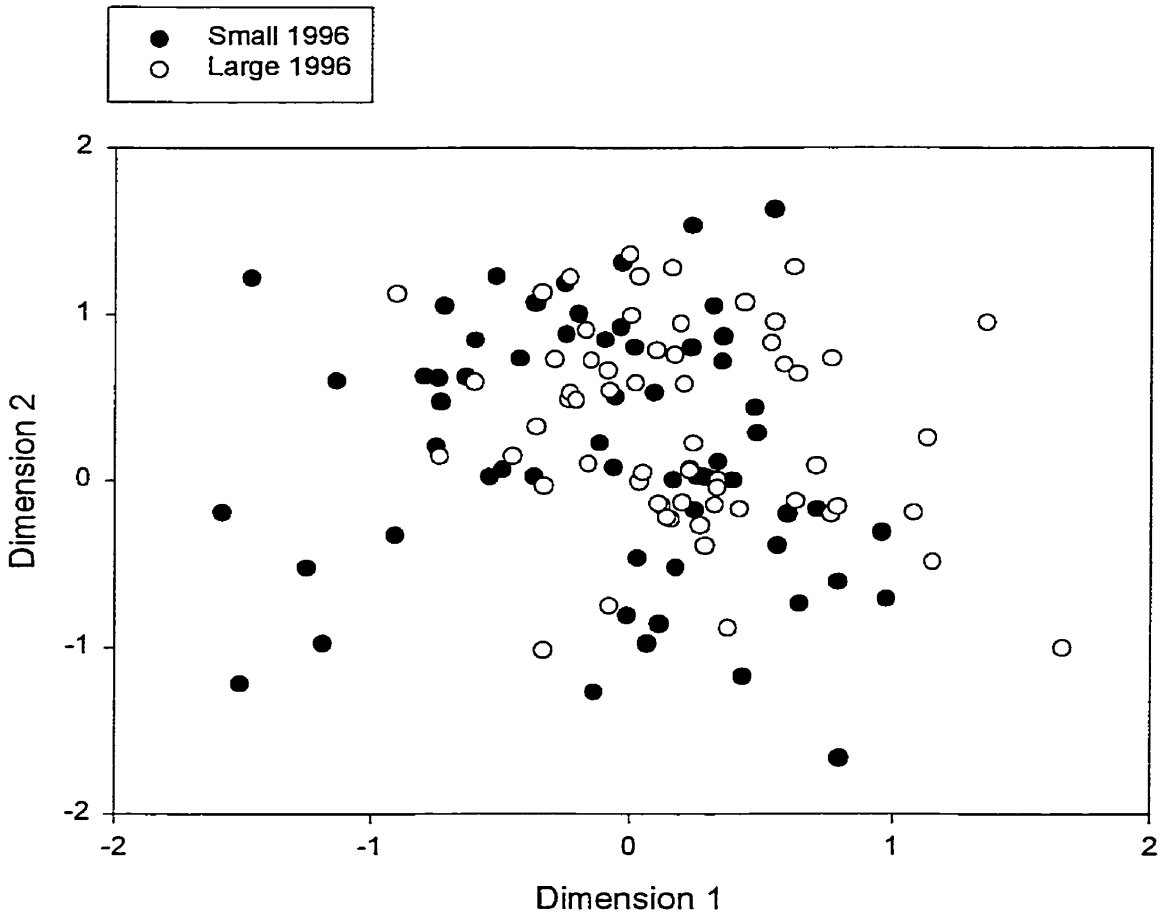
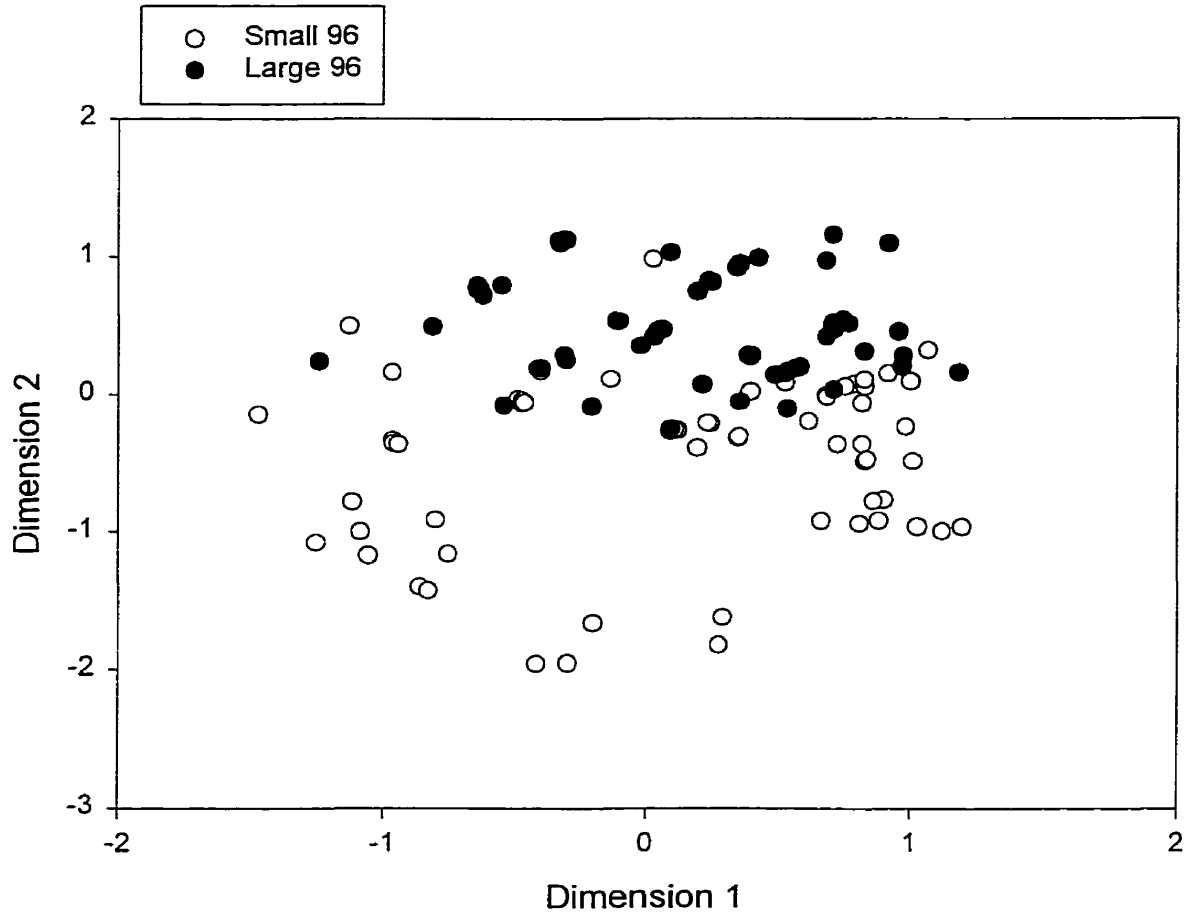

Small 1996
Large 1996
2
1
0
-1
-2
Dimension 2
-2
-1
0
1
2
Dimension 1

Figure 6: Scatter plot showing genetic distance calculated using 3 polymorphic microsatellite loci, between small and large groups of Arctic char, 1996 year class. These 3 loci show an association with growth.


Small 96
Large 96
Dimension 2
Dimension 1
2
1
0
-1
-2
-3
-2
-1
0
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2

CHAPTER 4

4.1 DISCUSSION

Arctic char has great potential as an aquaculture species and the growing conditions required indicate it is well suited for growth in Canada. However, the wide variation in growth rate found in the Fraser River strain has inhibited the commercialisation of this species. Jobling and Reinsnes (1986) found that even when runts of the Hammerfest strain were reared in isolation the growth rates were still much lower than the normal individuals; which in turn led to the hypothesis that these runts are genetically programmed for slow growth. Marker loci which co-segregate with QTL can be used to select the strains with commercially important traits such as fast growth. By genotyping only those individuals at the high and low tails of the phenotypic distribution the number of individuals genotyped is decreased (Lebowitz *et al.* 1987; Lander and Botstein 1989; Darvasi and Soller 1992; Martinez *et al.* 1998). Statistics can then be used to determine if there is an association between a particular marker and the trait in question (Tanksley 1993). By determining if there is a correlation between certain marker loci and growth rate and increasing the knowledge of the genetic makeup of different strains, those strains that exhibit commercially important traits may be selected and the culture of Arctic char could be greatly enhanced.

Delabbio (1995) reported that the Arctic char used for culture in Canada were, at most, three generations removed from the wild. Therefore the Arctic char used in this study are

at most four generations removed from the originating two families. This in turn has led to inbreeding and most likely the loss of alleles compared to the original wild population. As this is a young, isolated population it is probable that it is in linkage disequilibrium over large segments of the genome (Houwen *et al.* 1994). If a marker for growth lies close to a locus which affects growth then this correlation should still be evident.

Microsatellite linkage maps have improved the ability to dissect quantitative traits (Andersson *et al.* 1994). The maps can be used to trace the segregation of individual chromosome segments within pedigrees and therefore map QTL (Andersson *et al.* 1994). Evidence has been found for a QTL for growth in pigs on chromosome 4 and on chromosome 13 (Andersson *et al.* 1994). As well, there is a suggestion of linkage with average daily gain (ADG) in pigs on chromosome 3 (Casas-Carrillo *et al.* 1997).

The current study found that 20% of the 74 salmonid microsatellite primers tested were polymorphic, 55% were monomorphic, and 24% resulted in no amplification product. These results were expected as other studies (Presa and Guyomard 1996; Scribner *et al.* 1996) have found that the majority of microsatellite loci tend to be conserved across closely related genera and species and amplify homologous regions of the genome. Therefore, it was hypothesised that the majority of salmonid primers used would result in an amplification product. The primers used in this study were designed for Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*),

rainbow trout (*Oncorhynchus mykiss*), pink salmon (*Oncorhynchus gorbuscha*), sockeye salmon (*Oncorhynchus nerka*) and whitefish (*Coregonus* sp.). The primers developed for the genus *Salmo* resulted in a 66% success rate in terms of amplification, *Oncorhynchus* resulted in an 82% success rate and *Salvelinus and Coregonus* both resulted in a 100% success rate. Only one of these species (*Salvelinus fontinalis*) is within the same genus as Arctic char and the remainder of the species are quite distant in phylogenetic terms. Thus, it is possible that the loci which did not amplify are no longer, or never were present in the Arctic char genome. Alternatively, the loci may be present but the primer sequences may have diverged to such an extent that one or other of the primers at a particular locus did not anneal.

Presa and Guyomard (1996) and McConnell *et al.* (1995a) found that the level of polymorphism at microsatellite loci tended to be conserved across species. Scribner *et al.* (1996) surveyed 22 microsatellite loci in ten salmonid species using primers developed for sockeye salmon and found that the majority of these loci were polymorphic. Angers and Bernatchez (1996) surveyed six *Salvelinus* species using eight microsatellite primer pairs developed for brook char and found that five of the primer pairs amplified a homologous microsatellite locus in each of the species. In Arctic char the microsatellite loci were monomorphic in two of the five primer pairs which successfully amplified. Similar results were not found in this study as 55% of the microsatellite loci were monomorphic in Arctic char. The majority of these loci were polymorphic in the species

for which the primers were designed. It has been found that microsatellite regions tend to evolve rapidly which results in differences in base sequences in the repeats between closely related species (Phillips and Pleyte 1991). This strain of Arctic char may have little genetic variation partly because the founding population consisted of two males and two females which means there would not have been much genetic variation to start with. It is also possible that at some point in their evolutionary past a bottleneck occurred in the originating Labrador population which would have resulted in a reduction in the number of alleles present.

The overall genetic variation found in the Daniel's Harbour Hatchery Arctic char was quite low. There is a tendency for salmonids to evolve ecologically specialised populations which have a degree of reproductive isolation which in turn results in a large fraction of the total genetic variation being distributed between local populations (Andersson *et al.* 1983; Smith and Skulason 1996). This means salmonids are inclined to lose genetic variation due to the extinction of genetically unique populations (Andersson *et al.* 1983). The lack of genetic variation found in this study is supported by results from other studies which examined protein loci. Andersson *et al.* (1983) examined 37 protein loci in Arctic char and found only four exhibited genetic variation. Brunner *et al.* (1998) detected low polymorphism using PCR-RFLP analysis of mtDNA in wild populations of Arctic char, and that the cytochrome b / D loop segment was monomorphic for all

enzymes. In addition, the ND5/6 region was polymorphic for Ava I only. However, the same study looked at 16 microsatellite loci and found that only four were monomorphic, the rest being polymorphic. Bernatchez *et al.* (1998) analysed six loci in 257 Arctic char which represented 7 different locations in Newfoundland and Labrador. The number of alleles reported varied from 9 to 48. These contradictory results may be due to the different populations studied. The fish used in my study are descendants of the original Fraser River broodstock which were collected at the Fraser River Labrador in 1984 and were made up of offspring from two families. There may have been very little genetic variation present in the original wild population or the subsequent inbreeding which occurred may have resulted in a loss of genetic variation.

Marker loci with no direct effect on the trait of interest have been found to be of use in selection experiments if there is a statistical association between a particular allele at the marker loci and the QTL (Lande and Thompson 1990). Loci which were statistically significant in only one year class and not the other were assumed to be artefacts as the results were not consistent from year to year. Through chance the alleles could have segregated in such a way that one group (i.e., small or large) has a slightly different allele frequency than the other. Also, random selection of the parents meant that not every allele was represented. It is also possible the difference in the number of loci found to be associated with growth between the 1995 and 1996 fish may be due to statistical power. The 1995 experiment included 45 fish, while the 1996 experiment included 122 fish. The

larger the sample size is the more accurate the statistical analysis is. Therefore, if the number of fish used in the 1995 experiment had been larger there may not have been this difference between the number of loci associated with growth. Three microsatellite loci appear to be associated with growth in the Daniel's Harbour Hatchery Arctic char. Sfo 23, Ssosl 456 and μ5.27 all appear to be associated with growth in the Daniel's Harbour Hatchery Arctic char. The major allele which appears to be associated with growth at each loci is not necessarily the same from year to year. At Ssosl 456 allele 13 (210 bp) occurs only in the small fish in the 1995 year class; however, in the 1996 year class this allele occurs in both small and large fish. Alleles 6 (250) and 9 (222) occur much more frequently in the large fish in the 1996 year class but are not present at all in the 1995 year class. This discrepancy may have occurred because different parents were used to make the crosses in 1995 and 1996 which means it is possible that there are alleles present in some of the fish that were not present in the fish used in the other year. Both Sfo 23 and μ5.27 were consistent for which alleles appear to be associated with growth. At Sfo 23 allele 7 (217 bp) occurred at a much higher frequency in the 1995 and 1996 year class of large fish (86% and 90%, respectively) whereas at μ5.27 allele 3 (110 bp) occurred only in the small fish in both year classes.

The measurement of genetic distance further supports a genetic difference between the small and large fish. There is a distinct clustering based on genotypes which separates the small fish from the large fish (Figure 4). When the three statistically significant

microsatellite loci (Sfo 23, Ssosl 456 and μ5.27) were removed from the calculation of genetic distance there was a more random distribution based on genotypes (Figure 5). Also, when genetic distances were calculated using Sfo 23, Ssosl 456 and μ5.27 there was a clustering of individuals by size class (Figure 6). This indicates that these three loci are a major contributing factor to the genetic differences of the small and large groups of fish. It was thought that when the 3 statistically significant loci were analysed the genetic distances would be such that there would be two very distinct clusters based on size. However, that does not appear to be the case. There is clustering based on size but there is also still some overlap between the two size groups. The large fish tend to be in a tighter cluster whereas the small fish tend to be more spread out. This may be because there is less difference in the genotypes in the large fish. At all three loci there were fewer genotypes displayed by the large fish which would result in a smaller genetic distance between the large individuals. However, as the offspring were pooled after first feeding it cannot be said with certainty that this genetic difference between the small and large fish is due to the three loci being associated with growth or if it is a difference between families. The parental genotypes could not be determined which means it is not possible to assign the offspring to a particular family. Selective genotyping works best when the individuals chosen to be genotyped are selected from the extremes within each family so as to ensure that individuals from all families are selected. It is possible that the large fish tend to come from one or two parental pairings as do the small fish and therefore the clustering observed would be due to familial differences rather than differences due to an

association between a microsatellite locus and a QTL. However, it can also be argued that if these differences are due to a familial difference rather than an association with a QTL, it is likely that the alleles which appear to be associated with growth would occur only in combination with one or two other alleles and would not occur in the opposite size population. This is not supported by the data. Allele 7 (217 bp) at Sfo 23 is homozygous in the majority of the large fish; however, it also occurs as homozygous in the small fish and also with allele 9 (213 bp), 3 (241 bp) and 1 (244 bp) in both the small and large fish. If the difference was due to the different size fish being offspring from different families then allele 7 (217 bp) would not occur in the small fish and it would not occur in combination with the other alleles in both size groups of fish. This pattern occurs at Ssosl 456 as well. μ5.27 does not show this pattern to such an extent. Alleles 5 (108 bp) and 7 (106 bp) occur in both size groups of fish; however, allele 3 (110 bp) occurs only in the small fish and never with the large. As this occurs in both year classes it is unlikely to be due to family groups as different parents were used in the crosses. It does not seem likely that the crosses in both years resulted in fish from one family being homozygous for allele 3 and by chance these fish were also the small fish. Also, if the differences were simply a familial difference then one would not expect the same loci to be significantly associated with growth in the separate year classes. In addition, it was shown that when genetic distances are calculated there is separation of individuals into two groups based on size. However, there is overlap in genetic distance between the two size groups. If the two size groups came from entirely different families then they would be extremely

dissimilar genetically and there would be two very distinct groups when genetic distances were calculated. However, as there is some overlap between the groups this indicates that there are some similarities between the genotypes which may indicate that the size differences are not simply due to the selection of one particular family when selective genotyping was carried out. Rather, these fish are representative of a number of families and the association between the microsatellite loci and growth is a true association. It is also possible that this overlap in genetic distances between the size groups of fish occurs because the loci cancel each other out; as the genetic distance for each locus is calculated the difference is in the positive direction at one locus and the negative direction at another locus.

4.2 FUTURE STUDIES

In order to determine with certainty whether these microsatellite loci are really associated with growth, crosses need to be set up using parents with known genotypes. Individuals heterozygous for allele 7 (217 bp) at Sfo 23 and heterozygous for alleles 6 (250 bp), 9 (222 bp) or 21 (157 bp) at Ssosl 456 should be used to determine if the offspring which are homozygous for allele 7 at Sfo 23 and heterozygous for alleles 6 and 9 or 21 at Ssosl 456 are also the offspring which exhibit rapid growth. Other crosses should be set up using individuals heterozygous for allele 3 (110 bp) at μ5.27 to determine if the offspring that inherit allele 3 are smaller then their siblings. The parental genotypes should be known and the families kept distinct so it is possible to assign offspring to particular

parents and therefore rule out familial differences as being the cause of the difference in growth rate. Also, the offspring from each family should be kept in separate tanks and separation into small and large groups carried out on each family so it is known that offspring from each family are being used for analyses. If a similar pattern of association is found within each family then it will be possible to say with more certainty that these microsatellite loci are associated with rapid growth in the Daniel's Harbour Hatchery Arctic char. In order to eliminate the chance of tank effects on the experiment the families should be divided in three at first feeding so each family is set up in triplicate.

4.3 SUMMARY AND CONCLUSION

The results described in this thesis point to an association between certain microsatellite loci and rapid growth in the Fraser River strain of Arctic char. Further testing of this hypothesis is vital to the Arctic char aquaculture industry. Only by eliminating the extreme size variation exhibited by this species will the industry become economically viable and the potential exhibited by this species realised.

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APPENDIX I

An example of polymerase chain reaction products for a polymorphic locus (Sfo 23) in several random individuals resolved using denaturing polyacrylamide gel electrophoresis. Alleles are randomly numbered with the smallest number corresponding to the largest size allele. Allele sizes (Table 3) were determined by comparing alleles to known sequences. Allele numbers are shown.

Locus Sfo 23