Developing Broodstock of Arctic charr (*Salvelinus alpinus* L.)

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Abstract

Most of the economically important traits in animal breeding programs are quantitative in nature. Detecting major genes and/or blocks of genes influencing these traits has been made possible by the availability of hypervariable DNA markers. In this study, phenotypic variations related to growth and body girth in the two domesticated strains of Arctic char (*Salvelinus alpinus* L.) at Icy Waters Ltd. (Whitehorse, Yukon, Canada) were examined and then quantitative trait loci for growth were identified using a genome wide scan approach. Twelve crosses involving the pure strains (Tree River and Yukon Gold™), the reciprocal hybrids, and the reciprocal backcrosses were set up with ten families per cross. After 18 months of rearing in the hatchery environment under identical culture conditions, it was observed that backcrosses with a 75% Tree River genome contribution ($(YG_fxTR_m)_fxTR_m$) grew fastest and possessed greatest variance. A total of 198 highly polymorphic microsatellite markers, from various salmonid species, covering 41 linkage groups on the current Arctic charr linkage map were tested for a genome scan. Sixty two highly polymorphic markers were chosen to perform a genome wide scan on a full-sib backcross family, namely 6-10, to detect genetic factors responsible for the variation of growth in Arctic charr. These markers cover 28 of the 46 linkage groups in the currently available, low-resolution genetic map of Arctic charr. Results from a transmission disequilibrium test (TDT) indicate a significant association ($0.001<p<0.05$) between growth parameters and several markers on the linkage group AC-25. While, the analysis of variance components demonstrate continuously decreasing effects on the either sides of a putative QTL location. QTL effects at these marker locations have also

been reported in Fraser River Arctic charr (Somorjai 2001) and in the rainbow trout (*Oncorhynchus mykiss*) (O'Malley *et al.* 2003). These results indicate the probable existence of one or more stable growth QTL in this region of the Arctic charr genome. A sex-specific (male) marker Sfo8LAV was also identified in Arctic charr from Icy Waters Ltd.

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AC	Arctic charr
bp	Allele size in base pair
DM	Differentially monomorphic
DP	Differentially polymorphic
H1	Hybrid 1; TR_fxYG_m
H2	Hybrid 2; YG_fxTR_m
Hybrid 1	Hybrid 1; TR_fxYG_m
Hybrid 2	Hybrid 2; YG_fxTR_m
KTL	Fulton's Condition factor
$K_{TL}R^2$	Regression value for K_{TL}
$logK_{TL}$	log of K_{TL}
logTL	log of TL
logWT	log of WT
LRM	Linear Regression Model
M	Monomorphic
N	Number
n	Number
NA	No amplicon
NL	Nauyuk Lake or Nauyuk Lake Arctic charr
NLf	Female Nauyuk Lake Arctic charr
NLm	Male Nauyuk Lake Arctic charr
P	Polymorphic
TDT	Transmission Disequilibrium Test
TL	Total length
TLR^2	Regression value for TL
TR	Tree River or Tree River Arctic charr
TRf	Female Tree River Arctic charr
TRm	Male Tree River Arctic charr
UA	Unsuitable amplicon
WT	Weight
WTR^2	Regression value for WT
YG	Yukon Gold™

Chapter 1. Introduction

1.1 Arctic Charr: biology, culture and issues

1.1.1 Biology of Arctic charr

Arctic charr (*Salvelinus alpinus* L.) is generally deemed to be a highly plastic salmonid fish species i.e. adapted to varied environmental conditions. It has a holarctic distribution with both landlocked and anadromous populations (Maitland 1995; Brunner *et. al.* 2001). A wide range of size variation per year class, varying growth rates, variable spawning time, extremely variable body colors, and tolerance to a wide range of temperatures are common biological features of the Arctic charr (Rogers and Davidson 2001). Although Arctic charr performs well in temperatures ranging from 0-22^{O}C, the optimum temperatures for the growth of Arctic charr under culture conditions are in the 14-17°C range (Glebe and Turner 1993; Sullivan *et al.* 2000; Larsson 2002). Furthermore, for different Arctic charr populations of the same year class, the differences in length and weight may vary up to 800% and 4000%, respectively (Johnson 1980; Baker and Ayles 1986). These basic biological attributes and a high market value are comparable to other salmonid species for commercial culture (Johnston 2002). Therefore, in recent years the Arctic charr has been viewed as a new potentially cultivable coldwater fish species among salmonid farmers in North America (Jobling *et. al.* 1993).

1.1.2 Arctic charr aquaculture

Although Arctic charr is regarded as an excellent candidate salmonid for aquaculture, unlike Atlantic salmon or rainbow trout, its farming is still in its infancy.

Due to the ocean dwelling part of its life cycle, it was believed that anadromous populations of Arctic charr could be raised both in the fresh and saline water; however, seawater acclimation was not very beneficial (Staurnes *et al.* 1994; Dumas *et. al.* 1995). Therefore, most Arctic charr culture is limited to freshwater. The optimal stocking density in sea-cages for Arctic charr ranges from 50-70Kg/m^3, which is significantly higher than for Atlantic salmon (25kg/m^3) (Jobling *et al.* 1993). Hence, Arctic charr can utilize the farming space better. Furthermore, the optimal growth temperature for Arctic charr is much lower than other salmonids, providing a great opportunity to exploit temperate niches where farming of Atlantic salmon or rainbow trout proved uneconomical (Jobling *et al.* 1993; Johnston 2002; The Charr network http://www.charrnet.org).

There are no published studies comparing the commercial attributes of the Norwegian, Icelandic and Canadian strains of Arctic charr under similar culture conditions. However, Johnston (2002) recorded that the Nauyuk Lake Arctic charr, a Canadian strains, grew to a larger market size (2-3Kg) in 3years before reaching sexual maturity (5+ years) and egg size was also bigger (4.0-5.1mm) than the most other commercial strains of Arctic charr in the world. However, the Tree River strain of Arctic charr was not included in these observations.

Among all Arctic charr, the native Canadian strains are recognized as having the best potential for development as an aquaculture strain (Lundrigan 2001; Johnston 2002). Therefore, in the early 1980s, the Department of Fisheries and Ocean (DFO), Canada, started to provide the Arctic charr seedlings for commercial production in Canada (Delabbio 1995; Johansen 1999). Like most other fish species, the broodstock for the

purpose of artificial propagation of Arctic charr was collected from a wild population, in this case from the Fraser River, Labrador (Appendix I) (Johansen 1999). Later, two other stocks were introduced into the Arctic charr breeding program (see section 1.3 for details). Thus far, Arctic charr breeding has been based on phenotypic selection (PS); the process of identifying the best individuals, families, or lines to breed for the next generation and the process has contributed significantly to these gains (Johnston 2002).

The ultimate goal of Arctic charr farming is a high return on investment for farmers. Apart from financial gains, another factor motivating the breeding of fast growing individuals is related to the maturation-induced changes in appearance and fillet quality. At maturity, salmonids cease feeding and proteins and lipids are mobilized from muscle and utilized in developing gonads, leading to deterioration in fillet quality and color (Aksnes *et al.* 1986). In North America a three to four year old farmed Arctic charr provides a good trade-off between commercial gains and consumer satisfaction (Eric Johnson pers. comm.).

Currently, Arctic charr is being cultured in more than twenty countries in the world (Food and Agricultural Organization of the United Nations; http://www.fao.org/fi/statist/FISOFT/FISHPLUS.asp; The Charr network http://www.charrnet.org; The Irish Char Conservation Group http://www.charr.org). The global production of cultured Arctic charr in the year 2000 was estimated to be 3000 metric tons and Canada contributed 720 metric tons to it (Rogers and Davidson 2001; Johnston 2002). Iceland is the number one Arctic charr producing country in the Europe and the world, producing more than 1000 metric tons in the year 2000. Based on the current trends, the extrapolated production of Arctic charr would reach a total of around

5600 metric tons by the year 2006, with a Canadian contribution of 2000 metric tons (The Charr network http://www.charrnet.org). The culture of Arctic charr is also growing in parts of Europe and China (Johnston 2002)

1.1.3 History of strains under culture in Canada

Like most aquaculture fish species, the currently utilized broodstock of Arctic charr was collected from the wild and has undergone only a few generations of domestication. The literature suggests that in Canada, the culture of Arctic charr started at the Rockwood Aquaculture Research Center in Manitoba in 1978. For the purpose of Arctic charr aquaculture, DFO retained juveniles of the unknown generation (F_n) resulting from the artificial propagation of wild adults collected over a ten year period from 1978 to 1988, from three different locations in Canada: Fraser River (Labrador); Nauyuk Lake (Nunavut); and the Tree River system (Nunavut) (Appendix I). It is not clear exactly how many females and males were used to propagate the Fraser River strain, which was collected once in both 1980 and 1981, and more than twice in 1984, but the exact number of collections made in 1984 is not known (Johnston 2002). Only seven females and seven males contributed as founders to the culture of the Nauyuk Lake population. The Tree River strain was started with fifteen females and nine males. The Nauyuk Lake population is a combination of resident and anadromous Arctic charr, while the Tree River and the Fraser River populations of Arctic charr are anadromous only (Lundrigan 2001). Later, the F_n generation individuals were supplied in the form of brooders to the Arctic charr farming industry in North America. The precise record of generation number or pedigree information on widely distributed Arctic charr broodstock is not known (Somorjai 2001). It is apparent therefore, that all the hatchery strains of Arctic charr in

Canada should be considered genetically different from one another (Rogers and Davidson 2001). This becomes vital in developing a selective breeding program for a species which was founded with a very small number of individuals and may be suffering from inbreeding depression through genetic bottlenecks.

1.1.4 Arctic charr production by Icy Waters Ltd.

Icy Waters Ltd. (1986) is one of the largest Arctic charr producers in North America. It is a private enterprise which sells Arctic charr eggs around the world and contributes up to 150 metric tons to the global Arctic charr production through their own grow out facilities. In 1996, Icy Waters Ltd acquired two stocks of Arctic charr from the Rockwood hatchery, Manitoba. The Tree River Arctic charr population is believed to be one of the largest growing Arctic charr in the world and individual fish may weigh up to 14 Kg in a life time (Moshenko *et al.* 1984). The Nauyuk Lake strain has more orange/red flanks when compared to the silvery Tree River strain (personal observations). This is one of the reasons that the Nauyuk Lake Arctic charr is sold under the trade name of Yukon Gold^{TM} by Icy Waters Ltd. The fecundity of Arctic charr from Tree River is similar to those from Nauyuk Lake but the egg size of Nauyuk Lake Arctic charr is slightly larger than those from Tree River at the beginning of the spawning season (Moshenko *et al.* 1984). Hybridizing the two lines produces an excellent, fast growing fish, with a pleasing body color and a good market value (Eric Johnson pers. comm.). For seed production, the two pure strains (from Tree River and Nauyuk Lake) and their reciprocal hybrids (H1; TR_{female} x YG_{male} and H2; YG_{female} x TR_{male}) are maintained at Icy Waters Ltd., Whitehorse, Yukon, Canada.

1.1.5 Issues in Arctic charr aquaculture in North America

Despite its great potential, past attempts at Arctic charr farming have not been very successful. Declining growth rates, decreasing food conversion ratios (FCR), increased number of deformed fish per generation, lack of a steady supply of quality fingerlings, and increased cost of production are matters of concern. Issues concerning the control of Arctic charr fisheries by the Aboriginal communities in Canada have further complicated the problem (Johnston 2002).

Arctic charr farming is also hindered by inherent species plasticity and lack of knowledge-based selective breeding programs. Studies intended to evaluate growth performance of Arctic charr suggested that some individuals could never attain a marketable size and may remain runts even after a prolonged growth period (Jobling *et al.* 1983). It is believed that the genetic makeup of these runts is responsible for slow growth (Papst and Hopky 1983 as cited in Johansen 1999). Several studies suggest that growth related traits have a high phenotypic and genetic correlation in salmonids, although the magnitude and directions of these effects depend on the culture environment and appear to be complex and difficult to predict (Nilsson 1992, 1994; Silverstein and Hershberger 1992; Heath *et al.* 1994).

Inappropriate aquaculture practices, such as breeding of closely related individuals, undocumented spawning, and small number of founding individuals, may inadvertently result in the loss of genetic variability and an increased probability of inbreeding depression. Because of the much higher fecundity in most fish species compared to terrestrial animals, even large-scale hatcheries may be operated with the use of a few breeders (Jackson *et al.* 2003; Evans *et al.* 2004). This frequently seems to result in

genetic degradation of hatchery stocks because of rapid accumulation of inbreeding and an unintended selection response in closed aquaculture populations (Eknath and Doyle 1990)

Inbreeding arises from the mating of related individuals. In populations under artificial selection, inbreeding can occur randomly from the use of a limited number of breeding individuals. The classical effect of inbreeding, the reduction of phenotypic performance, has been widely recognized for farm animals (Falconer 1989). An important attribute of fish physiology that has aided to the inbreeding depression is the high fecundity in fish. This has facilitated the mating of close family members to propagate domesticated lines in a short period of time of one to three generations. Therefore, studies estimating the inbreeding effects on fitness traits in fish have shown, in general, the detrimental effects of inbreeding such as reduced growth, viability and survival and increased number of abnormalities (Su *et al.* 1996; Rye and Mao 1998; Pante *et al.* 2001). Reduction in genetic diversity has also been shown to result in decreased fitness and survival in salmonids, cyprinids and cichlids (Falconer and Mackay 1996; Graham Mair pers. comm.). Additionally, an increased frequency of deformed individuals has also been found to be associated with the loss of heterozygosity and could be used as an indicator of the loss of genetic variation due to breeding practices (Allendorf and Ryman. 1987 and references cited there in).

One common practice that has been reasonably beneficial and has been put into practice by the hatchery operators around the world to maintain genetic diversity is the periodic hybridization of domesticated brooders with wild-caught adults, presumably unrelated. However, introducing a wild-caught fish may very well reduce the overall

selection response in a domesticated hatchery population (Kirpichnikov 1981; Tave 1993). In the case of Icy Waters Ltd. Arctic charr, this approach cannot be applied because the Aboriginal communities have exclusive control over the Tree River Arctic charr fisheries and thus have restricted the drawing of more fast growing adults from the wild for artificial breeding purposes (Eric Johnson pers. comm.; Johnston 2002).

Given the history of domestication of Arctic charr there are two issues faced by the Canadian Arctic charr aquaculture industry: (1) are the available levels of genetic variation in the broodstock enough to sustain the Arctic charr farming industry in the long run, and (2) what is the potential for the enhancement of Arctic charr aquaculture through a steady supply of quality seedlings in the 21st century?

The loss of genetic diversity under domestication is a common problem among cultured finfish. Arctic charr can be added to the list of species that suffers a major loss of genetic diversity due to their domestication. Due to a strong founder effect, the strains of Arctic charr currently being used in the aquaculture industry in Canada might be lacking the genetic variation that is required to carry out an effective selective breeding program (Lundrigan 2001). Founder effects and the gradual domestication of a wild population through artificial selection are known to reduce the genetic variation of hatchery-reared fish stocks (Dickson and MacCrimmon 1982; Cross and King 1983; Allendorf and Ryman 1987; Fleming and Gross 1992; Crozier 1994; Doyle *et al.*1995; Dowling *et al.* 1996). The loss of genetic variation results in a loss of potential genetic gain (Allendorf *et al.* 1987). Assortative mating, a process of mating unrelated individuals with a common characteristic, can result in an excess of homozygosity for associated genes or alleles (Beaumont and Hoare 2003). Cultured strains of Nauyuk Lake and Tree River Arctic

charr populations are no exception. At the Rockwood Aquaculture Research Center, Manitoba, these two strains have undergone several generations of genetic selection and the subsequent loss of genetic variation under artificial selection is evident (Lundrigan 2001).

In a comprehensive review compiled from 36 studies conducted on various plant and animal species, Reed and Frankham (2003) observed a highly significant correlation between measures of genetic diversity and population fitness, concluding that the loss of heterozygosity has a deleterious effect on population fitness. Jackson *et al.* (2003) reported a decrease of 26% in total allele numbers and a 36% reduction in heterozygosity in Atlantic halibut (*Hippoglossus hippoglossus*) from three Canadian hatcheries as compared to their wild counterparts, after one generation of domestication. Comparable observations were also made in two domesticated populations of abalone (*Haliotis* sp.) from South Africa and Australia (Evans *et al.* 2004). Reduction in genetic variation has been shown to be detrimental to commercially important traits such as growth rate and fitness in several fish species (Cross and King 1983; Koehn *et al.* 1988; Danzmann *et al.* 1989; Pante *et al.* 2001). These observations emphasize the necessity of close monitoring of breeding among fish and suggest that there is a need to apply innovative approaches to maintain genetic variation in Arctic charr broodstock, without introducing adults from the wild. Therefore, the viability of Arctic charr aquaculture primarily depends upon developing a superior broodstock so that the supply of quality seeds can be regularized and fingerlings can be raised economically (Rogers and Davidson 2001).

1.2 Aquaculture enhancement: A molecular genetic approach

Over the last half century, genetics of crossbreeding, hybridization, ploidy manipulation, and pure phenotypic selection, have done a commendable job in improving fish productivity (Dunham *et al.* 2001; Lutz 2001; Hussain *et al.* 2002). These genetic approaches, however, are slow and time consuming, and hence did not succeed in securing a status of a dependable farming industry for most cultivable fish species. Selection utilizing DNA tools has the potential to accelerate genetic gains in aquatic organisms. Use of novel genetic technologies has already proven to be economically efficient in livestock and plants in enhancing productivity.

The future of 21st century aquaculture in the world is dependent on genetically identifying or establishing high quality strains that are fast growing as well as able to survive better and possess traits that consumers are willing to pay a higher price for, such as better color, texture and taste. Improved profits will depend on the use of biotechnology to enhance growth rates, control reproductive cycles, improve feed composition and conversion, produce new vaccines, and develop a hardier disease resistant genetic stock (Beaumont and Hoare 2003). However, the majority of fish species used for farming have not been improved genetically for commercially important traits (Wright and Bentzen 1994).

In recent years, many molecular genetic techniques have been refined to a point where they are becoming practical for commercial aquaculture and are now being combined to improve production within a modern aquaculture industry (Sorgeloos 2001). Chiefly, two strategies of molecular mechanisms have been proposed to improve the efficacy of genetic improvements in cultured plant and animal species: the production of

introgressed transgenic lines and the use of marker-assisted breeding (Dekkers and Hospital 2002). Similar approaches have also been suggested to enhance aquaculture production through artificial fish breeding programs (Fjalestad *et al.* 2003).

Genetic introgression aims to introduce a 'target' gene(s), regulating a desired trait, from an inbred line (donor) into a productive line that lacks that particular gene (recipient). This can be achieved either via direct gene transfer (i.e. transgenic) or via hybridization of donor species with the recipient organism, followed by several generations of selective introgression of targeted foreign genes into the recipient strain (Macaranas 1986). In aquaculture, the production of transgenic fish can be a powerful means to improve the performance of many farmed fish (Fletcher and Davis 1991; Lutz 2001). For example, transgenic Atlantic salmon are made triploid to reduce the chance of them breeding if they escape (Entis 1997). Triploids, which have been created in a wide variety of fish, are believed to be reproductively sterile producing no or unviable germ cells (Allen *et al.* 1986). Therefore, sterile triploids have been suggested as a means to contain transgenes in transgenic stocks released to the natural environment, as if the method were "fool proof". In reality, a number of studies suggest that sterile triploids are "leaky", and some fertile gametes are sometimes produced (ISIS 2002, http://www.i-sis.org.uk/transfish.php). Then again, the effectiveness of hybrid introgression schemes, however, is conditional upon identifying individuals with the target gene(s) (Dekkers and Hospital 2002). Furthermore, there are numerous concerns regarding consumer acceptance of transgenically modified organisms (Edmonds Institute Manual 1998; Walter 1997; Sagoff 1998; Fletcher 1999).

On the other hand, during the last century, genetic improvements through selective molecular breeding programs have proved to be exceptionally successful in increasing livestock production steadily and are publicly well acknowledged (Lande and Thompson 1990; Gjedrem 1997; Rosegrant *et al.* 1999; Swick and Cremer 2001; Dekkers and Hospital 2002; Kutzer *et al.* 2003). Therefore, molecular genetics can be integrated with conventional methods of artificial selection through the application of marker assisted selection (MAS) to enhance aquaculture production (Fjalestad *et al.* 2003).

1.3 Marker Assisted Selection

1.3.1 Concept and requirements

Marker-assisted selection (MAS), which can also be described as marker-assisted breeding, is a process of identifying potential individuals that are enriched for desirable alleles that are associated with traits of interest. In other words, MAS is a process of incorporating the use of molecular markers linked to specific traits in genetic improvements programs (Fjalestad *et al.* 2003). Molecular marker analysis allows the identification of genome segments, so-called quantitative trait loci (QTL), contributing to the genetic variance of a quantitative trait and thus to select superior genotypes at these loci (Cannai *et al.* 2003). The ultimate goals of QTL analysis are to identify and locate the gene(s) underlying a quantitative trait (Liu 1998; Korstanje and Paigen 2002). However, for the practical application of MAS, one does not need to discover which genes are involved (Andersson 2001), because the information on the genetic markers flanking the gene of interest can be applied to make future selection decisions, since animals that inherit the marker will also inherit the useful effects of gene(s) associated

with it (Meuwissen and Goddard. 1996; Liu *et al.* 2003). Besides assisting in the breeding process and improving breeding predictions, MAS provides the capability of culturing a better quality animal in less time with greater survival and at lower cost than individuals selected conventionally (Weller 2001; Dekkers and Hospital 2002). Overall, along with genetic improvement, MAS brings gains in industrial efficiency, consistency in product quality and availability, and reduction in prices for consumers (Swick and Cremer 2001).

Successful execution of MAS requires information about available phenotypic and genetic variation within inbred lines or populations and a wide array of molecular markers, preferably mapped onto a high-density linkage map that can be linked to a putative QTL under measurement (Liu 1998). Not knowing the reliable estimates of QTL positions may seriously inflate the expected gain from MAS (Knapp 1998), especially in animals where the vast majority of production traits undergoing selection are typically polygenic (Lasley 1972; Ferguson and Danzmann 1998; Andersson 2001). Highly complex traits, such as growth and disease resistance, may not necessarily follow classical Mendelian inheritance (Liu 1998), and thus can be referred to as oligogenic (Risch 2002). Nevertheless, numerous genetic markers, each explaining a small part of the variation in a complex trait, can be determined by using well suited mapping populations and a marker set with a good genome coverage (Lander and Bottstein 1989; Risch 2002).

A number of genetic maps have been developed specifically to locate QTL in several fish species. The first such map was produced in zebrafish (Postlethwait *et al.* 1994; Shimoda *et al.* 1999), which is a non-aquacultural species. Among cultivable fish groups low-density maps have been developed for salmonids (Sakamoto *et al.* 2000;

Gharbi 2001 as cited in Woram 2001; Woram *et al.* 2004), for catfish (Liu *et al.* 2003; Poompuang and Na-Nakorn 2004), for tilapia (Kocher *et al.* 1998; Cnaani *et al.* 2003), for Japanese flounder (Sanchez *et al.* 2003), for Ayu (Sakamoto *et al.* 2003), for red sea bream (Sakamoto *et al.* 2003), for oysters (Yu and Guo 2003), and for shrimp (http://shrimpmap.tag.csiro.au). However, a genetic map is an accessory to precisely conduct molecular marker mediated selection in any given species (Liu *et al.* 2003).

1.3.2 Effectiveness of MAS for selection of economically important traits into existing breeding programs

Most economically important traits of farmed animals are quantitative and follow a continuous distribution caused by the action and interactions of many genes and the environment (Falconer and Mackay 1996; Liu 1998). In evaluating the possible contribution of MAS it is important to know in general how many QTL contribute to the trait of interest (Lynch and Walsh 1998). Therefore, most applications of MAS in selection programs are preceded by an analysis aimed at QTL detection, and only QTL that are shown to have a significant effect on phenotype are subsequently used for selection (Weller 2001). Selecting for favorable QTL effects based on marker data, therefore, has a great potential for improving quantitative traits (Schechert *et al.* 1999). If unbiased and true QTL estimates exist, even markers explaining a small part of the total genetic variance could increase the effectiveness of MAS substantially (Hospital *et al.* 1997; Soller and Medjugorac 1999). The same holds true for phenotypes with low heritabilities or phenotypes that are difficult to quantify (Moreau *et al.* 1998). An additional need to verify estimated QTL effects is possible epistatic interactions of the

QTL alleles with the genetic background of the strain one aims to improve (Soller *et al.* 1976; Meuwissen and Goddard 1996; Danzmann *et al.* 1999).

1.3.3 Potential limitations of MAS

There are two potential limitations of MAS. The first is the economics of MAS and the second is the efficiency. Although studies on the economics of MAS are scarce so far, based on a theoretical study, Xei and Xu (1998) calculated that MAS is only economical over phenotypic selection if the costs of the phenotypic data are higher than the marker data. At the present state of the art of molecular technology, in most practical cases this condition is only met if mapping costs are not included in the breeding program (Dreher *et al.* 2000), because the expected economic return of MAS compared with phenotypic selection decreases with the increasing cost of genotyping (Weller 2001). When the cost of genotyping is high, only a small proportion of the genome can be examined to identify a tightly linked QTL in a large population, which is a necessary condition to explain a large part of genetic variation (Moreau *et al.* 2000). A small population size would affect the precision of the QTL estimates, eventually compromising the efficiency of the MAS (Moreau *et al.* 1998).

In addition to finance, the issue of efficiency of MAS is associated with the uncertainties associated with the QTL estimations. A QTL can only be utilized by MAS if the marker and the trait loci are tightly linked i.e. the two are in complete disequilibrium (Lande and Thompson 1990). Computer simulations by Hospital *et al.* (1997) show that under long-term MAS program, while selecting for target QTL with large effects, the fixation of non-target unfavourable alleles at QTL with small effects

could be a potential problem. At the same time, another issue that may arise is the loss of favourable genes or QTL with small effects (Lande and Thompson 1990), which are difficult to map precisely (Liu 1998). One way of solving these problems is by reducing selection intensity, but this would lessen the efficiency of MAS, which is not desirable (Hospital and Chevalet 1993). However, the problem of the fixation of unfavourable alleles or the loss of small QTL may not be that consequential because the loss of small-effect QTL in the long term is small, compared with the gain of large-effect QTL in the short term (Hospital *et al.* 1997). Also, salmonids and some other aquacultural species have such a long generation intervals that a MAS-QTL mediated rapid selection response could actually be worth more from an economic point of view such as net present value, than a high selection (phenotypic) plateau that might not be acquired in several decades.

1.3.4 Selective breeding and potential contributions of MAS in aquaculture

Arguably among all the aquacultural finfish, tilapia are the most studied and carps are the most cultivated fish. Among salmonids the Atlantic salmon has been the most produced fish so far. The three fish, tilapia, carps and Atlantic salmon contributed at a rate of 18%, 32% and 22.4% annually between 1987 and 1997, respectively (Naylor *et al.* 2001). There are several other finfish and shellfish species that led to the growth of the aquaculture industry in last two decades (Food and Agricultural Organization of the United Nations; http://www.fao.org/fi/statist/FISOFT/FISHPLUS.asp). Credit for the successful aquaculture of these species goes to their high phenotypic plasticity, which gave an opportunity for geneticists to perform a wide variety of phenotypic selections.

Gjedrem (1997) reported a 20-35% genetic variation for growth rate in fish species compared to 7-10% in farmed terrestrial animals.

Given the huge species plasticity, until the mid 1990s, most genetic selection programs in aquaculture primarily focused on quantitative genetic analysis of body growth, body shape, disease resistance, and skin coloration of farmed fish (Dunham *et al.* 2001) and the use of molecular markers was limited to stock identifications (Ferguson 1994). Selective breeding programs for example, Atlantic salmon in Norway, tilapia in the Philippines, catfish in the southern USA and Thailand, and oysters in North America (Dunham, 1995; Gjedrem, 1997), represent a long-term genetic improvement strategy and are the best examples of selective breeding programs making full use of the genetic resources of aquatic species.

Although selection for body weight generally has been associated with increased performances, there are examples of long-term selections resulting in decreased fitness, lower survival, and reduced fecundity, possibly due to a genetic correlation between growth and fitness traits or inbreeding depression (Dunham *et al.* 2001 and references therein). In other words, the success of selective breeding is influenced by genetic properties of traits: a trait needs to exhibit additive genetic variation in subsequent generations (Dekkers and Hospital 2002) and, preferably, there should not be any strong disadvantageous genetic correlations with other traits of importance (Falconer and Mackay 1996).

Taking these concerns into account, molecular-genetics based knowledge is rapidly developing for aquatic species domestication. Furthermore, the stimulating results

produced from the application of MAS in breeding programs, in terms of detecting QTL and integrating the acquired knowledge, both in plants and animals (Soller and Medjugorac 1999; Andersson 2001; Pillen *et al.* 2003; Zheng *et al.* 2003), have also contributed to the cause. At the moment, MAS is not employed in any fish-breeding scheme (Sonesson 2003) and much effort is devoted to QTL mapping for growth, feed conversion efficiencies, disease resistance, fecundity, and spawning time (Dunham *et al.* 2001). In fish, several QTL studies have been published; in salmonids (Jackson *et al.* 1998; Johansen 1999; Robinson *et al.* 1999; Sakamoto *et al.* 1999; Martyniuk 2001, Ozaki *et al.* 2001; Somorjai 2001; Tao and Boulding 2003), in catfish (Liu *et al.* 2003), in tilapia (Cnaani *et al.* 2003), and in silver barb (Hussain *et al.* 2002).

In general, the application of MAS for the founding and maintenance of hatchery populations is straightforward. The first step is to define the goal of the hatchery project. For instance, a selective breeding program that aims at producing fast-growing fish with a high food conversion ratio would start from a base population containing a large amount of genetic variation governing required growth variations (Allendorf and Ryman. 1987). Knowing the amount of available genetic variation is a prerequisite to being able to respond to altered selection strategies (Hedrick and Miller 1992; Flint and Mott 2001). The next stage is the preparation of a suitable mapping population, followed by QTL estimation i.e. identification of marker-phenotype association (Weller 2001). Once effects of potential QTL are reliably estimated, two or more QTL can be combined into an inbred line, and progeny carrying QTL having positive effects may be selected for future breeding.

Although it is yet to be proven in aquaculture, undoubtedly together with 'traditional' selective breeding, MAS would be more beneficial for the fish breeding industry (Asins 2002; Koning 2003; Fjalestad *et al.* 2003). However, for MAS to be precisely effective, the unbiased marker-trait associations need to be estimated and verified across populations (Moreau *et al.* 1998) because only the reliable QTL effects should be included in the selection process (Montaldo and Meza-Herrera 1998).

1.4 Quantitative traits and QTL estimation in fish

Ubiquitous to all the breeding programs is their reliance on genetic variation and use of artificial selection to improve individuals for quantitative traits that are of interest for growers and the consumer. Hence, means of uncovering the potential genetic basis of quantitative variation and identification of genes which regulate quantitative variation could be profoundly important for artificial breeding (Liu 1998; Flint and Mott 2001; Shavorskaya 2004). Through the 1920s to 1980s, the use of statistical-genetic techniques (population means, variances, heritabilities, etc.) provided sufficient evidence to believe that there are several genes segregating in a population and that certain combinations are responsible for phenotypic variations in progeny (Liu 1998; Asins 2002). However, the molecular basis of such variation remained unclear until the 1980s (Weller 2001).

Marker-QTL studies rely on physical associations between markers and functional genes with effects on the trait of concern. The assumption is that in a sufficiently large population of recombining chromosomes, the QTL will be linked to different alleles and that a common QTL effect can be recognized at an observable locus (Flint and Mott 2001), which means that in a given population the marker allele and the QTL are in

linkage disequilibrium (LD) (Kocher *et al.* 1998; Liu *et al.* 1998). The required components for marker-QTL analysis are: a suitable resource population, molecular genetics, and statistical significance (Soller and Medjugorac 1999; Dekkers and Hospital 2002; Doerge 2002). In animals, generally, marker-QTL linkage studies are carried out within-family and between-families, and require markers that are polymorphic within the population or lines under study (Weller 2001). This approach also requires that the pedigree information is available (Estoup *et al.* 1998).

1.4.1 Molecular genetic approaches for QTL detection

The primary interest of animal breeding and selection in molecular genetics is in finding all the QTL influencing the performance of a trait and being able to distinguish among allele effects. There are two strategies available to locate a QTL: the genome-wide scan approach and the candidate genes approach (Andersson 2001; Pagnacco and Carta 2003).

A genome-wide scan approach using anonymous molecular markers is a process of identifying chromosomal regions influencing quantitative traits of commercial significance. This analysis assumes that a statistically significant association between the inheritance of a particular marker allele and a measured quantitative trait provides evidence that a QTL is linked to the marker in question (Malek *et al.* 2001). Provided an adequate proportion of a genome is covered (Liu 1998), genome-wide scans guarantee that a QTL with a given effect will be detected in a segregating population (Haley 1999). According to Ashwell *et al.* (2001), in an appropriate pedigree, by following the inheritance of 100 to 200 markers approximately evenly localized across the animal

genome, it is possible to trace all the major QTL influencing variation in a trait. In a given species, however, approximation of the optimum number of markers to carryout a genome-wide scan relies on the size of the genome, i.e. the number of linkage groups (or chromosomes) and the marker density on them (Lande and Thompson 1990). Usually, in a population with sufficient linkage disequilibrium (e.g. backcross population), choosing markers at an interval of 10-20cM is found to be appropriate to scan the entire genome in search of a QTL in an animal species (Lander and Botstein 1989; Piepho 2000; Asins 2002). For the first time, Paterson *et al.* (1988) used this approach in plants to determine a QTL controlling the difference between genetically divergent lines, whereas in livestock the first such study was conducted on pigs to identify genes controlling differences between the wild boar and commercial pigs (Andersson *et al.* 1994). Since then the genome-wide scan approach has been widely applied to identify QTL in several plant and animal species (Liu 1998). However, the genome-wide scan approach is relatively lengthy (Haley 1999), and without fine mapping (1 to 3cM) it is difficult to move from mapping a QTL to identifying the actual gene (Asins 2002; Pagnacco and Carta 2003). Furthermore, gene-hunting can be complicated by the lack of sufficient observable recombination between two closely linked markers flanking the actual gene (Kearsey and Pooni 1996; Lynch and Walsh 1998). More advanced mapping designs and increased population size, however, can solve the problem of fewer recombination events (Liu 1998; Asins 2002). This will inflate the cost of mapping though (Weller 2001). These problems partly explain why an alternative strategy, the candidate gene approach for mapping QTL, has been adopted by some animal geneticists more recently (Haley 1999; Linville *et al.* 2001).

A candidate gene approach is a second phase of association based QTL analysis (Haley 1999). This analysis is undertaken when a gene, which is assumed to affect trait performance, is chosen based on its physiological relevance to the trait (Montaldo and Meza-Herrera. 1998). There are two tactics to this approach: comparative and physiological (Haley 1999). The comparative approach explores the patterns of the genomic location of known genes in different species as candidates to detect similar QTL effects in the species under study (Pagnacco and Carta 2003). Johansson-Moller *et al.* (1996) found similar effects of a color coding gene, originally identified in the mouse, in a domesticated strain of pig. The results of Hirooka *et al.* (2002) confirmed this finding. The physiological candidate gene tactic, the second of the two, focuses on within species polymorphism in, or close to, genes directly contributing to the variation of the trait of interest (Montaldo and Meza-Herrera. 1998; Haley 1999). An association between the candidate gene polymorphism and the trait performance insinuates the presence of a causative mutation in that gene (Grobet *et al.* 1997; Pagnacco and Carta 2003). To date, in combination with the positional information (Haley 1999), the candidate gene approach has been very successful in identifying large effect-genes both in plants and animals (Flint and Mott 2001; Shavorskaya 2004). Hence, utilizing a candidate gene approach, selection can be based on the gene itself, rather than MAS. The gene based selection is known as gene-assisted selection (GAS) (Pagnacco and Carta 2003). However, we are still long away from applying GAS in aquacultural species, as the identification of conserved sequences in the region of genes of interest is not accomplished yet.

An alternative strategy to detect QTL in a domesticated population is to follow frequency changes at marker loci in selected lines (Keightley and Bulfield 1993). Under the assumption that artificial selection brings drastic changes to allele frequencies (Liu 1998) this approach, after several generations of unidirectional selection, estimates linked QTL effects by tracking changes to allele frequencies of anonymous markers and, the marker alleles showing large difference in allele frequencies between the high and the low (or no) selected lines would suggest the presence of QTL (Lebowitz *et al.* 1987 as cited in Rocha *et. al.* 1997). However, there are two major limitations of this approach: a) uncertainty whether loci with smaller differences in allelic frequencies contribute to quantitative trait variation or not and, b) prolonged selection time (Keightley *et al.* 1996). This method can be considered as a variation of the genome-wide scan approach because we are browsing the genome to detect a tight linkage between a marker and the QTL, by finding statistically significant differences between two ends of the selection (i.e. with and without selection). Apparently, this approach would be applicable to any farm species with or without availability of a dense linkage map (Keightley *et al.* 1996).

A similar approach is to test for differences in allele frequencies at a locus between the two extremes of the population (Lander and Bottstein 1989). This is known as selective genotyping where, from a large population equal numbers of individuals at each tail of the distribution are genotyped to determine if the genotype of individuals at the high end is significantly different from the genotype of individuals at the other end (Tanksley 1993). Assuming that two tails would be most varied (±1.96 standard deviations) for fixed QTL effects (Darvasi 1997); usually 40% of individuals (20% at each tail end) are sampled for this analysis (Darvasi and Soller 1992; Weller 2001), and

the remaining 60% of the population would represent the mean differences between the two genotypic classes (Liu 1998). To avoid any family effect, however, individuals should be sampled at the extremes within a well defined family (Muranty and Goffinet 1997). Another potential problem is that the savings from the selective genotyping could be nullified by the cost of maintaining a large number of individuals of one or more families (Weller 2001). In addition, searching for QTL for several correlated traits at the same time could compromise the reliability of QTL estimates (Ronin *et al.* 1998; Liu 1998) or one would end up sampling the most of the population (Tanksley 1993; Darvsai 1997; personal observations).

1.4.2 Experimental designs for QTL mapping

In general, obtaining a resource population involves selecting and hybridizing parental lines that differ in one or more quantitative traits and analyzing the segregating progeny in order to link the QTL to known DNA markers. According to Montaldo and Meza-Herrera (1998), in livestock, there are basically four designs possible for marker-QTL linkage analysis: 1) a half-sib sire design in which heterozygous males for the markers are mated with random females, 2) a grand-daughter design in which a sire and his sons are evaluated, 3) a backcross between the F1 and one of the original parent populations, and 4) mating of individuals from divergently selected lines or from populations with wide variations for traits under study. Methods 1, 2, and 4 are suitable for predicting QTL effects for within-population selection, while method 3 allows detection of marker-QTL associations in which QTL are already fixed in one breed. Other complicated designs are basically variations on these four designs (Weller 2001). Nevertheless, the purpose of such an exercise is to create a population-wide LD (Koning

et al. 2003). Because with LD, alleles at a marker locus and an associated trait locus are non-randomly associated across the population, allowing detection of a tightly linked marker-QTL association with a significant effect on the phenotype (Haley 1999).

In an ongoing fish breeding program, where individuals are under extreme selective pressure (Falconer 1983), the production and preservation of separate inbred lines facilitates in creating desirable LD (Sonesson and Meuwissen 2000). Two or more of these inbred populations that are representatives of the overall genetic diversity of the species can be combined into a single strain, and therefore genetic rehabilitation of a population can be engineered (Krueger *et al.* 1981; Ferguson 1994). Also, different inbred lines will be homozygous for many of the alleles of the loci under selection (Falconer and Mackay 1996; Beaumont and Hoare 2003), and QTL are expected to be fixed for alternative alleles in two populations (Haley 1999). Therefore, when two separate inbred lines are crossed, the 'F1 hybrid' is expected to be heterozygous (Weller 2001) and may express hybrid vigor (Krasznai 1987). Using a backcross system maximizes the likelihood of polymorphism while searching for QTL, because QTL tend to co-segregate with the associated molecular markers (Lynch and Walsh 1998; Sakamoto *et al.* 1999; O'Malley 2001; Perry 2001). The backcross families also facilitate the mapping of those markers that may not be informative in a single line (Liu *et al.* 1998). Hence, the backcross hybrid system offers a powerful system for rapid QTL detection (Slate *et al.* 2002) and MAS of economically important traits in fish breeding programs (Liu 1998).

1.4.3 Molecular markers for QTL mapping

Prior to the 1980s QTL analysis was limited to morphological (e.g. bean length and seed weight) and biochemical polymorphism (e.g. use of blood groups or allozymes) (Liu 1998). However, it was quite clear that neither of these two types of polymorphisms was sufficient to explain the total genetic variation for a given trait (Weller 2001) and the large quantity of naturally occurring polymorphism could only be detected by DNA-markers (Ferguson and Danzmann 1998). With the advent of DNA markers and PCR technology in the 1980s, came the ability to generate large numbers of polymorphic genetic markers in any given species (Liu 1998). Such genetic markers enhanced scientific capabilities to track the inheritance of a particular segment of the genome in a suitable pedigree (Haley 1999). Therefore, the utilization of DNA markers for the joint analysis of segregation of marker genotypes and phenotypic values of individuals or lines has simplified QTL detection (Liu 1998; Asins 2002).

Based on their transmission, DNA markers can be categorized into two types; mitochondrial and nuclear. Mitochondrial DNA (mtDNA) markers are maternally inherited and non-recombining which limits their ability to provide information on the male component of the genome (Ferguson and Danzmann 1998) and suitability for QTL mapping. The nuclear DNA markers, such as randomly amplified polymorphic DNA (RAPDs), restriction fragment length polymorphism (RFLPs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs; mini/ micro-satellites), and more recently, single nucleotide polymorphism (SNPs) allow the detection of variations or polymorphisms that exist among individuals in a domesticated population (Montaldo and Meza-Herrera 1998). RFLPs were the first DNA-markers to be employed in a

genome-wide scan for QTL in tomatoes (Paterson *et al.* 1988). Since then, using various DNA-markers, many more QTL studies have been carried out successfully both in plants and animals (Weller 2001). However, the choice of genetic marker used for QTL detection depends upon the model system used and the species under study (Park and Moran 1994).

Among all the nuclear markers developed in fish species so far, microsatellites are capable of detecting the greatest amount of genetic differentiation (Ferguson and Danzmann 1998). Microsatellites, which are prevalent throughout all genomes, are highly variable molecular markers (Tautz 1989). Furthermore, microsatellites are codominant DNA-markers and have high reproducibility (Cross *et al.* 1998). Utilizing these attributes of microsatellite markers, relatively dense genetic maps are being generated for most domesticated species (Weller 2001), allowing the complete genome to be examined for QTL with a major effect on the phenotype (Montaldo and Meza-Herrera 1998).

The recent surge in the application of SNPs to dissect the molecular basis of complex traits in humans (Trikka *et al.* 2002) has made them candidate markers for QTL mapping in other animals as well (Jungerius *et al.* 2002; Curtsinger 2003; Kutzer *et al.* 2003). According to Brookes (1999), SNPs are single base pair positions in genomic DNA at which different sequence alternatives (alleles) exist in normal individuals in some population(s), wherein the least frequent allele has an abundance of one percent or greater. Unlike microsatellites, which as are usually multiallelic, SNPs can be bi-, tri-, or tetra-allelic (Weller 2001). SNPs are numerous, more stable and potentially easier to score than microsatellites (Weiss 1998). SNPs can be found anywhere in the genome. SNPs in the coding regions of genes (cSNPs) or in regulatory regions are more likely to

cause functional differences than SNPs elsewhere in the genome (Collins *et al.* 1998). Similar to microsatellite analysis, SNP-linkage studies also assume that the marker allele and the QTL are in LD (Long and Langley 1999). Due to lower mutation rates, however, SNPs may provide a better platform for LD mapping than microsatellites (Weiss 1998). In Arctic charr, Tao and Boulding (2003) successfully identified a SNP associated with growth rates in juveniles. However, it will take some time to put SNPs into use because the DNA sequence is not available yet for any aquacultural species. Therefore, microsatellites remain the largest class of molecular markers available for QTL mapping in fish.

1.4.4 Statistical associations in QTL analyses

A QTL is essentially a statistical creation that identifies a particular region of the genome as containing a gene (or genes) that is associated with the trait being assayed (Weller 2001). In LD-based methods, a statistically positive association between neutral DNA polymorphisms and the variation in the trait performance is observable (Long and Langley 1999). A significant association between the trait and the markers may be evidence of a QTL near the marker (Liu 1998). Lander and Kruglyak (1995) examined three main causes of occurrence of a positive association in an association study: a) marker-allele carries causative mutation and thus is the actual cause of the variation in trait; b) marker-allele does not influence the trait but is in close LD with the cause, and c) is an artefact. To counter the number of artefects in QTL analysis, the use of stringent statistical significance thresholds (Lander and Kruglyak 1995; Lynch and Walsh 1998; Doerge 2002) and increasing the QTL-reliability by replicating studies (Liu 1998; Long and Langley 1999) have been proposed.

To date, there has been little consensus in the QTL mapping community on how data from QTL studies should be analyzed and what significance thresholds should be used to detect and report QTL (Van Tassell *et al.* 2000; Doerge 2002; Cnaani *et al.* 2003). Therefore, it is not surprising that results from these studies sometimes confirmed the same QTL and, in other cases, provided conflicting results (Ashwell *et al.* 2001). Nonetheless, most studies agree that to gain experimental power establishing an appropriate significance threshold is important before reporting a QTL (Lander and Kruglyak 1995; Lynch and Walsh 1998; Schrooten *et al.* 2000; Weller 2001; Doerge 2002). Churchill and Doerge (1994) proposed a permutation test to assess chromosome-wide statistical significance in QTL analysis. Similarly, a threshold for genome-wide significance can be obtained by correcting the chromosome-wide significance threshold for the number of chromosomes (or linkage groups) analyzed (Schrooten *et al.* 2000). For example; a permutation tests for a given trait yielded a chromosome-wise significance threshold of $p<0.05$, and assuming that 40 chromosomes were analyzed then a threshold of $p<0.00125$ represents genome-wide significance. However, in a QTL analysis using anonymous markers for which no chromosomal linkage information is available, i.e. marker location on a linkage map is not known, a nominal significance threshold of $p<0.05$ can be considered (Churchill and Doerge 1994; Robinson *et al.* 1999; Cnaani *et al.* 2003). Furthermore, according to the statistics of normal distribution 95% of the data points fall within ± 1.96 standard deviations (Cann 2003). Therefore, for a population possessing ± 1.96 or more standard deviations, using $\alpha=0.05$ appears to be statistically sound. In a QTL study in cattle, Grosz and MacNeil (2001) found that a 95% confidence interval (or $\alpha=0.05$) is significant enough to report a putative QTL, as the location of

detected QTL effects range from 28 to 83cM, which is substantially broader than those estimated for effective MAS by Lande and Thompson (1999) using the α=0.01 level of significance for detection of additive genetic variance at any QTL. Subsequently, these findings were confirmed in a comprehensive QTL analysis using 229 microsatellite markers, spanning 2.413 morgans mapped on to 29 bovine autosomes (MacNeil and Grosz 2002). In another study, Robinson *et al.* (1999), tested 222 unlinked marker loci at a significance threshold of $p<0.05$, to detect QTL influencing embryonic development in rainbow trout. To achieve required levels of stringency, however, applying adequate statistical corrections (e.g. Bonferroni, Tukey etc.), across multiple markers is advisable (Knott *et al.* 1998; Schrooten *et al.* 2000; Doerge 2002) and only effects that display statistical significance on two or more independent studies should be considered as confirmed (Lander and Kruglyyak 1995; Long and Langley 1999; Cnaani *et al.* 2003). The associations showing marginal significance or those that cannot be replicated or confirmed in subsequent studies, however, should be reported and be clearly identified for future references (Cheverud *et al.* 1996). Otherwise, many true effects will be missed out (Weller 2001).

In general, the basic hypothesis of a genome-wide scan approach is to detect one or more QTL on different chromosomes or linkage groups, one of which would be significant at the genome-wide level i.e. it exceeds the stringent confidence-interval limit required by a large number of simultaneous tests (Schrooten *et al.* 2000; Ashwell *et al.* 2001; Hirooka *et al.* 2002). In the single-QTL single-marker model, two basic statistical approaches are used to identify QTL (Liu 1998). These approaches are equivalent or similar under the assumption that if the genes and the markers are segregating in a

genetically defined population, then the linkage relationships among them may be discoverable by looking at the association between the trait variations and the marker segregation pattern (Liu 1998). In one approach, the phenotype of an offspring is regressed against the probability that it has inherited a QTL (actually, the QTL linked marker), which compares the trait variation between two allele classes as inherited from either sire or dam. Typically, this test is referred to as a transmission disequilibrium test (TDT) (Spielman *et al.* 1993). The second method is the linear regression model (LRM), which is the concept of variance of the inheritance of quantitative trait (Johansson and Rendel 1968). The TDT examines a one trait-one allele effect (Spielman *et al.* 1993) while the LRM (ANOVA) explains the amount of predictable variation at a marker location i.e. the effect-size of a QTL (Whittaker *et al.* 1995; Cann 2003). Having several closely-linked markers on a chromosome each explaining a significant portion of the phenotypic variation would be good evidence of a critical region (at the genome level) influencing the trait performance (Malek *et al.* 2001).

The basic concept of TDT is that marker alleles associated with high or low trait performance have a high probability of being transmitted to superior or inferior individuals. Typically, the TDT starts with a set of parents who differ in their phenotypic expression and are heterozygous for a marker under investigation (Liu 1998). Then a test, whether or not a marker-allele (let us assume 'M1') is associated with the superior phenotype, is carried out. If so, confirming the association by selecting other parents who are heterozygous for M1 is requisite. This way, the TDT approach can be generalized to an arbitrary number of alleles in a stock population (Spielman *et al.* 1993). However, the TDT is effective only if association (due to LD) is present (Liu 1998) and the differences

in effects are large enough to detect (Spielman *et al.* 1993). Additionally, due to the fact that TDT only uses a portion of the data, the statistical power of TDT is believed to be low (Liu 1998). Despite these limitations TDT is recommended for QTL analysis, because in the absence of any linkage the probability of detecting a false positive is low (0.05), if we select 0.05 as the significance threshold (Liu 1998).

Since the TDT is a linkage-based test and does not estimate the magnitude of allele effect, the LRM has been a model of choice for QTL estimation in both plants and animals over the last half a century (Liu 1998). The LRM uses a least squares method to find a linear relationship between a response variable (phenotype) and a possible predictor variable (marker) and depending upon the number of markers used in the model, it can be of two types: simple linear regression and the multiple linear regression models (Cann 2003). The simple linear model aims to find a linear relationship between a response variable (phenotype) and a possible predictor variable (marker). The multiple linear regression model aims to find a linear relationship between one or more phenotypes and several markers at the same time (Liu 1998). These models can also be modified to evaluate the environmental contributions to the overall trait variance (Liu 1998; Weller 2001). For more complex experimental designs, several other variations on these basic approaches have been proposed and used (Liu 1998).

The concept of variance is fundamental to the inheritance of quantitative trait. The total variance of a trait or a set of correlated traits has two main components (i) directly observable components of variance caused by, for example, the differences between group means and within groups and (ii) causal component derived from theoretical model (Johansson and Rendel 1968). The equation; $y=\mu+\beta x+\varepsilon$, represents the standardized

LRM applied to QTL analyses (Liu 1998), where y is the observed phenotypic value of the n^{th} individual in a mapping population (N=n), μ is the trait mean of the population, β is the effect of marker-allele 'x' as inherited from the male or female parent, and ε is the residual error of the model. The output of this model is an R^2 value (Liu 1998). This value tells us the amount of the variance of phenotype 'y' explained (or predicted) by the marker 'x', e.g. an R^2-value of 0.5 means that 0.5*100= 50% of the variance of phenotype y is due to the marker x. Suggesting up to 50% contribution of marker-locus to the total variance of the phenotype for a population under investigation (Cann 2003).

This model can also be applied to a situation where several alleles at a locus are found to be positively associated with the trait across multiple families (Simianer 1994; Meuwissen 2003). This would indicate genome-wide random QTL effects, as opposed to a locally fixed effect due to the marker-allele (Weller 2001). This is more likely to happen in backcross pedigrees whose parents are derived from a population carrying a large number of alleles at a locus (Weller 2001) and also the two parents may differ in state and in phase for QTL alleles (Simianer 1994; Asins 2002). The simple LRM, which assumes a single genotype per locus per individual i.e. two alleles per parent, can be modified to multiple genotype per locus per animal (Weller 2001). Fernando and Grossman (1989) devised a mixed variance-covariance model to estimate a population wide QTL effect (Meuwissen 2003). While combining data across families to estimate QTL effects, however, this approach assumes that the QTL location is the same for all the families and each family is considered to be heterozygous for two different QTL alleles (Weller 2001).

Taking a genome-wide scan approach, Slate *et al.* (2002) found evidence for the presence of several QTL for birth-weight (a fitness-related trait) in different linkage groups, even in a wild population of red deer (*Cervus elaphus*). This suggests a great suitability of genome-wide scan approach for QTL detection in fish stocks, which have undergone only a very few generations of domestication since their drawing from their natural habitat. Furthermore, being able to detect QTL in a natural population could be very useful in species that have a long first maturity time (e.g. salmonids) and hence, the time needed for making crosses among inbred lines can be reduced considerably (Liu 1998).

1.4.5 QTL mapping in fish, salmonids and Arctic charr

Although in fish several studies have confirmed the existence of significant genetic variation for quantitative traits of commercial importance (Kause *et al.* 2003), and have recognized the potential of MAS for their genetic improvement (Flint and Mott 2001), the application of QTL-mediated MAS in fish breeding is relatively insignificant compared to other agribusinesses in the world (Sonesson 2003). This is primarily due to the lack of reliable QTL estimates in fish species.

Thus far, very few QTL for production traits have been identified in fish (Sonesson 2003). Much effort is devoted to QTL mapping for growth, feed conversion efficiencies, disease resistance, fecundity, and spawning time (Dunham *et al.* 2001). QTL associated with the growth hormone gene have been reported in coho salmon (Forbes *et al.* 1994); brown trout (Gross and Nilsson 1995), chinook salmon (Park *et al.* 1995), Atlantic salmon (Gross and Nilsson 1999) and Arctic charr (Tao and Boulding 2003). Several

QTL studies have been published in rainbow trout for temperature tolerance (Jackson *et al.* 1998; Danzmann *et al.* 1999; Perry 2001), spawning time (Sakamoto *et al.* 1999; Fishback *et al.* 2000; O'Malley 2001); growth (Martyniuk 2001), disease resistance (Ozaki *et al.* 2001), and fitness traits (Somorjai 2001). Other notable QTL studies published in aquacultural fish species include: in tilapia for temperature and salinity tolerance (Streelman and Kocher 2002; Cnaani *et al.* 2003), in catfish for feed conversion efficiency and bacterial septicemia resistance (Liu 2003), in guppy for growth (Nakajima and Taniguchi 2002), in shrimps for viral resistance (http://shrimpmap.tag.csiro.au), in Atlantic salmon for infectious anemia resistance (Moen *et al.* 2003 as cited in Sonesson 2003) and in Arctic charr for growth rates and fitness traits (Johansen 1999; Somorjai 2001).

One important application of accumulating QTL information for many species is comparative mapping. In the future, genetic mapping may possibly be carried out by comparing genome maps among relatives in the same species or between different species (Kutzer *et al.* 2003). The purpose of comparative mapping is twofold: the transfer of mapping information across species and to achieve a better understanding of genomic evolution (Sankoff 1999). For example, co-linear genetic maps have been uncovered in plants; among maize, rice and sorghum, and animals; mouse, humans and other mammals (Liu 1998). While searching for birth-weight QTL in red deer, Slate *et al.* (2003) found a conserved marker order by comparing homologous linkage groups between cattle and deer. Using highly variable microsatellite markers from various salmonids and possibly from other fish species for mapping purposes might help in integrating QTL information across several fish species. Regions of genome flanking microsatellite in fish may have

evolved at a slower rate than those of terrestrial animals and therefore, the conservation of microsatellite loci across a broad range of species is evident among various teleost taxa (Rico *et al.* 1996; Dunham 2004). However, due to ecological variations and local adaptations, high polymorphism at a DNA marker in a species from one location does not mean that there will be high polymorphism in another population (Liu 1998). This might explain why several markers that are polymorphic in the Fraser River Arctic charr were found to be monomorphic in the Tree River or Nauyak Lake Arctic charr and *vice versa.* Slate *et al.* (2003) observed the same problem in a study conducted in cattle and deer, where due to lack of comparable density in syntenic regions desirable results could not be reached. Nonetheless, comparative mapping is a cost effective way of QTL mapping in several populations in a fish species and possibly, from one teleost to other.

Furthermore, it is also believed that in a broad breeding program a very few QTL/ marker associations are reliable because different mapping populations of a species share only a small set of common QTL (Kearsey and Farquhar. 1998; Lynch and Walsh 1998). This attribute is very important in composite QTL-mapping in salmonids because they seem to share life history characteristics and phenotypes observed across species and thus, it is likely that common genetic effects will be detected among them (Somorjai 2001). The availability of genome-wide microsatellite maps for an increasing number of animal species has facilitated QTL identification and eventually in dissecting the genetic architecture of variety of important quantitative traits in livestock (Andersson 2001; Flint and Mott 2001), and the same is due to be applicable in salmonids (Martyniuk 2001) and fish in general.

1.4.6 QTL mapping in Arctic charr from Icy Waters Ltd.

Arctic charr aquaculture has the advantage of learning from past mistakes made during the aquaculture of other salmonid species. Identification of QTL in the moderately inbred Arctic charr stocks maintained at Icy Waters Ltd. is facilitated by a consolidated linkage map of Arctic charr, which was developed through collaboration between groups at the University of Guelph and Simon Fraser University. More than 300 microsatellite markers have been placed on the genetic map of the Arctic charr, which is comprised of 46 linkage groups (Woram *et al.* 2004). Backcrosses between two strains with strong phenotypic divergence are appropriate for detecting and mapping of QTL in the Tree River and Nauyuk Lake populations of Arctic charr (Lynch and Walsh 1998; Weller 2001). Furthermore, backcrosses will contribute to polarized genetic variations (O'Malley 2001; Pillen *et al.* 2003). Such an approach should maximize the likelihood of detecting polymorphism with the markers used because the genotypes of the backcross parents are not expected to be completely homozygous at all the microsatellite loci (Hallerman and Beckmann 1988). The QTL of interest in Arctic charr include growth, length, condition factor (K_{TL}), feed conversion ratio (FCR), disease resistance, upper temperature tolerance (UTT), coloration, levels of omega3 fatty acids, and size at harvest. Considering, the small number of founders and few generations under selective pressure, this approach should be effective in detecting QTL of economic interest in Icy Waters Arctic charr.

1.5 Aim of the thesis.

The aim of this thesis is to evaluate the growth performance of various hybrid cross combinations and to search for QTL associated with growth in Arctic charr, which will enable us to design MAS strategies for the charr from Icy Waters Ltd.

Chapter 2. Material and Methods

2.1 Background information on Arctic charr crosses and families

Source material for this study was derived from the two domesticated strains of Arctic charr raised at Icy Waters Ltd. (as described in section 1.1.4). Icy Waters Arctic charr is comprised of the Tree River (TR) strain, the Nauyuk Lake strain (NL) and their reciprocal hybrids (Hybrid1: TR_{female} x YG_{male} and Hybrid2: YG_{female} x TR_{male}). At this facility, the Tree River strain was selected for growth and the Nauyuk Lake strain was selected for its appealing coloration. For the purpose of this study the Nauyuk Lake Arctic charr may also be referred to as Yukon Gold™ Arctic charr (i.e. NL or YG).

In May 2001, the entire 1996 Arctic charr broodstock (n=848: TR= 250, YG=210, Hybrid1=185 and Hybrid2=203) at Icy Waters Ltd. was physically tagged with passive integrated transponder (PIT) tags. Also, to measure genetic relatedness among them, all 848 broodfish were genotyped at four microsatellite loci (Ssa84DU, SalE38SFU, Sfo8LAV and Sfo23LAV). This experiment was important to propagate genetically identifiable families of unrelated individuals that will set-up the platform to track the genetic basis of phenotypic variations in the Arctic charr. Subsequently, in the fall of 2001, 123 genetically identifiable full-sib families were propagated from the 1996 broodstock. Dr. Colin McGowan carried out the preliminary genetic analysis of 848 broodfish at four microsatellite markers (Ssa85DU, SalE38SFU, Sfo8LAV and Sfo23LAV) and designed the following mating schema at the Icy Waters Ltd., Whitehorse, Yukon, Canada (Table 2.1).

2.1.1 Mating design

During the 2001 spawning, 107 females and 90 males from the 1996 broodstock were artificially bred to produce two pure crosses, two reciprocal hybrid crosses, and eight backcrosses. The parent fish (n=197) consisted of 26 TR males, 34 TR females, 36 YG males, 32 YG females, 13 hybrid1 males, 15 hybrid2 males, 21 hybrid1 females, and 20 hybrid2 females. Each of the twelve propagated lines was composed of ten full-sib families of unrelated male and female parents. A total of 123 full-sib families from four hatchery-reared groups of Arctic charr were propagated at Icy Waters Ltd., Whitehorse. One of these families was used to identify putative growth QTL in Icy Waters Ltd. Arctic charr. A description of the families propagated from the four groups of Arctic charr is given in Table 2.1.

2.1.2 Incubation and Rearing

The sacfry from the 123 unique families were incubated separately in 123 Heath Tray incubators. After 85% of yolksac absorption, equal number of alevins from each family, representing a specific cross, were pooled and transferred into 12 newly purchased circular tanks (fiberglass) for rearing under identical conditions in an indoor hatchery. The ambient conditions such as water temperature and dissolved oxygen concentration, and daily feeding rates were kept consistent across the twelve lines throughout the rearing to eliminate any biases in stock performance. Alevins were monitored and maintained in the hatchery for the duration of the experiment.

Table 2.1. Families produced from four groups of Arctic charr at Icy Waters Ltd. in the fall of 2001.

FAMILY	FEMALE	MALE
Yukon Gold™	YG	YG
Tree River	TR	TR
TRf x YGm (Hybrid1)	TR	YG
YGf x TRm (Hybrid2)	YG	TR
Backcross (Hybrid2 x YGm)	YGf x TRm (hybrid2)	YG
Backcross (YGf x Hybrid2)	YG	YGf x TRm (Hybrid2)
Backcross (Hybrid1 x YGm)	TRf x YGm (Hybrid1)	YG
Backcross (YGf x Hybrid1)	YG	TRf x YGm (Hybrid1)
Backcross (Hybrid2 x TRm)	YGf x TRm (Hybrid2)	TR
Backcross (TRf x Hybrid2)	TR	YGf x TRm (Hybrid2)
Backcross (Hybrid1 x TRm)	TRf x YGm (Hybrid1)	TR
Backcross (TRf x Hybrid1)	TR	TRf x YGm (Hybrid1)

YGm; male from the Nauyuk Lake strain, YGf; female from the Nauyuk Lake strain, TRm; male from the Tree River strain, TRf; female from the Tree River strain.

2.2 Genetic Profiling of the 1996 broodstock

In May 2001, at the time of PIT tagging, samples of fin-tissue were collected from 848 broodfish. Collected tissues were stored in 95% ethanol at room temperature. DNA was extracted from fin tissue using the PUREGENE® kit (Gentra system, Minneapolis, MN. USA).

After testing several microsatellite markers (Appendix II), four additional polymorphic microsatellite markers (One18ASC, Sal5UG, SalP61SFU, and SalD39SFU) were used to complete the genetic profiling of the 1996 Arctic charr broodstock at eight microsatellite markers (Appendix III). The genetic profiling of the entire broodstock was important to consolidate the relatedness matrix, which will be a crucial element for the marker-assisted artificial breeding of these Arctic charr in the future.

Genotyping at loci Ssa85DU, SalE38SFU, Sfo8LAV, and Sfo23LAV was done using a radioactive technique, whereas, genotyping at loci One18ASC, Sal5UG, SalP61SFU, and SalD39SFU utilized a semiautomated fluorescent technique. Furthermore, to be consistent with the genotyping on progeny, all the parents (n=197) that were used to generate the 12 different lines were re-genotyped using the fluorescent technique at all eight microsatellite loci (Appendix III). The radioactive and fluorescent fingerprinting techniques are as described in section 2.6.2 of this chapter.

2.3 Growth performance of twelve lines

In July 2002, after eleven months of indoor rearing, the total-length (TL; cm, nearest 1mm) and the wet-weight (WT; g, nearest 0.1g) of 250 juvenile fish from each of

the 12 lines were recorded. In addition, at the time of adjusting for weekly feeding rates, hatchery staff also measured the batch weight from each of the 12 crosses on a weekly basis. All measurements were taken on randomly sampled juvenile fish.

2.4 Strategy for genome coverage in Arctic charr

It was hypothesized that one microsatellite marker per linkage group would be tested for initial genome scanning and, if a marker was found associated with a growth parameter in the most variable family, other neighboring markers from the same linkage group would also be tested to detect similar associations i.e. QTL effects, enabling the detection of region(s) of the genome on one or more linkage groups significantly responsible for superior growth performance in Arctic charr.

Due to the low resolution of the Arctic charr linkage map initially available (Woram 2001), however, other anonymous microsatellite markers cloned from various salmonids species were also included in this study to achieve greater genome coverage.

2.5 Marker suitability in Icy Waters Arctic charr

Using the radioactive genotyping technique, 198 microsatellite markers from various salmonid species were tested on twelve randomly chosen brooders (six NL and six TR) for their suitability in this study (Appendix II). Out of 198 microsatellite loci, only 75 markers were informative in the Icy Waters' populations, whereas, 123 markers were unsuitable for this study. Among non-informative markers, a marker locus producing one allele across the two strains or an allele size larger than 400bp was considered undesirable (n=54) for this work, whereas, the rest of the 69 loci produced either no amplicons or nonuseable amplicons. One hundred and eighteen microsatellites

of the total tested markers (n=198) have not been mapped on to the Arctic charr linkage map yet.

Although adequate polymorphisms at the 75 markers were observed, only 62 polymorphic markers (Appendix VI) were given priority to perform genome wide scans in the Icy Waters Arctic charr population. These markers were chosen based on polymorphism results obtained in this study and information from other QTL studies in Arctic charr (Somorjai 2001; Johansen 1999). The remaining thirteen markers were left for future analysis.

Forty-five of the 62 selected markers cover 28 linkage groups (AC-1, AC-3, AC-4, AC-6, AC-7, AC-8, AC-9, AC-10, AC-11, AC-12, AC-13, AC-14, AC-15, AC-16, AC-18, AC-20, AC-22, AC-23, AC-24, AC-25, AC-26, AC-27, AC-29, AC-30, AC-31, AC-33, AC-36, AC-38) on the consolidated Arctic charr map (Woram *et al.* 2004). The remaining 17 markers remain unassigned to any linkage group on the Arctic charr linkage map. To test if a polymorphic marker is also informative in a particular full-sib family, the two parents and two of their randomly selected progeny were screened for the 62 informative microsatellite loci prior to family-wide screening.

2.6 QTL analysis in Icy Waters Arctic charr

2.6.1 Phenotyping of the four most variable backcrosses

In February 2003, based on the phenotypic information obtained in July 2002, four backcrosses were selected for further genetic analysis in Icy Waters Arctic charr. The measurements of total-length (cm, nearest 1mm) and wet-weight (g, nearest 0.1g) were gathered for 500 randomly sampled individuals from each of four backcrosses: (tank1:

$(YG_fxTR_m)_fxYG_m$, tank3: $YG_fx(TR_fxYG_m)_m$, tank7: $TR_fx(TRf_xYG_m)_m$ and tank10: $(YG_fxTR_m)_fxTR_m$). Fulton's condition factor (K_{TL}) was used as an indicator of plumpness of a fish. It is a measure of the relationship between body weight and total length. [$K_{TL}=(W/TL^3)*100$] (Murphy and Willis 1996). The crosses $(YG_fxTR_m)_fxYG_m$ and $YG_fx(TR_fxYG_m)_m$ were the fastest growing among the four Nauyuk Lake backcrosses and the crosses $TR_fx(TRf_xYG_m)_m$ and $(YG_fxTR_m)_fxTR_m$ were the fastest growing among the four Tree River backcrosses propagated in the fall of 2001 (Table 2.1). At the time of phenotypic measurements, fin tissue from the randomly selected fish was also collected for genetic analysis. Fin tissue from all 2000 juvenile fish were preserved and stored as described in the section 2.2 of this chapter.

2.6.2 Genotyping of Tree River backcross

2.6.2.1 DNA extraction

For the purpose of identification of growth QTL in Icy Waters Arctic charr, based on the phenotypic information gathered in February 2003, the Tree River backcross $(YG_fxTR_m)xTR_m$) was selected for detecting growth QTL in Icy Waters Arctic charr. This backcross possessed the greatest amount of variance for growth parameters under consideration among all four sampled backcrosses, and thus had the greatest potential for detecting segregating QTL. The DNA from all the 500 fish sampled from this backcross, was extracted from fin tissue using the PUREGENE® kit (Gentra system, Minneapolis, MN. USA). Concentrations of purified DNA were determined and approximately 50 ng/μL of sample DNA were used for the polymerase chain reaction (PCR).

2.6.2.2 Radioactive genotyping

PCR was performed in a 10-20 μL volume containing 1X PCR buffer with 1.5mM $MgCl_2$, 1U Taq DNA polymerase (Amersham Biosciences Corp. NJ, USA), 0.05mM each dNTP, 0.1mM each primer and 50ng of genomic DNA. For each marker, the forward primer was end-labeled with [γ^{32}P]ATP (T4 polynucleotide Kinase kit, GIBCOBRL®, USA). The following PCR program, with locus specific modifications, was used: an initial denaturation cycle of 5 min at 95^{O}C, followed by 35 cycles of 45 sec at locus specific annealing temperature, 45 sec at 72^{O}C, 45 sec at 95^{O}C and a final extension time of 1 min at 72^{O}C, in a Biometra thermocycler. PCR products (or amplicons) were size fractionated using electrophoresis through an 8% highly denaturing polyacrylamide-formamide gel (Litt et al. 1993). Gels were dried and exposed to Kodak X-Omat Blue film for autoradiography. Genotypes were manually scored from the autoradiographs.

2.6.2.3 Fluorescent genotyping

PCR was carried out using the same method as described in the previous section (*2.6.2.ii*). For semiautomated fluorescent genotyping, fluorochromes of different colors (FAM, blue; HEX, green; TAM or TET, yellow) replaced the radioactive labeling. Gels containing fluorescently labeled amplicons were visualized using an ABI PrismTM 377-96 collection software (ABI, Foster City, California, USA). For analysis, 0.5-1.2 μL from as many as three different PCR reactions were pooled, and the mixture was added to 1.53 μL formamide-loading buffer and 0.17 μL GeneScan 400HD standard ladder (Applied Biosystems). After denaturation at 94^{O}C for 5 min, the solution was loaded on an ABI-377 DNA sequencer (8% polyacrylamide-formamide gel or 5% longranger from

GIBCOBRL®, USA). The DNA fragments were separated by electrophoresis and allele sizes were determined using Genescan® software (version 2.1), which uses a logarithmic scale to compare unknown band sizes to known base pair sizes. As an internal standard, Genescan® 400HD [ROX] (Applied Biosystems, California, USA) was used. Fragment size data for the upper and lower size range for each allele were determined. This information was used to determine the electrophoresis run time (2-8hours) and to bin sizes for all alleles (the nearest whole base pair number). One advantage of fluorescent genotyping is that multiple loci could be co-amplified in the same PCR tube (multiplex PCR). Ssa85DU(FAM) and SalE38SFU(TAM), and SSOSL456(HEX) and One8ASC(FAM) could be multiplexed successfully, while the other loci were amplified individually (for details see Appendix VI).

2.6.3 Parentage assignment

PROBMAX 1.2 (Danzmann 1997) was used to sort juvenile fish into 10 unique full-sib families per cross. When the parental mating combinations and genotypes of the parents and the progeny are known, this program calculates the maximum probability of progeny assignments to a mixture of parents. To achieve a precise assignment to one unique parent pair, all 500 fish sampled from tank 10 (Backcross $(YG_f x TR_m)_f x TR_m$) were genotyped at eight loci (Appendix III).

2.6.4 Statistical analysis

The ANOVA, Mann-Whitney U rank test, correlation tests, descriptive statistics, and frequency distribution were carried out using SPSS® 10.0 software, SPSS Inc.

Chicago, whereas the regression analysis was performed using SAS (version 8.0) software from the SAS Institute, Cary, North Carolina, USA.

2.6.5 Basic statistics and correlation tests

Using ANOVA, pairwise comparisons for growth data between all 10 full-sib families of cross 10 (backcross $(YG_fxTR_m)_fxTR_m$) were made. Within each family, the growth performances of male and female juvenile fish were also compared to assess any sex-influenced effects. Kolmogorov-Smirnov tests were performed to test for the normality of the weight, length, and condition factor data. Descriptive statistics were performed to assess the amount of phenotypic variation present in 10 full-sib families of cross 10. Significant differences in growth were determined using a 95% confidence interval and under the assumption of unequal variances.

To determine a correlation among three growth parameters a bivariate correlation analysis using the Pearson Product-Moment Correlation was performed on the transformed data (logtl, logwt, and logcf) and using the Kendall's tau-b correlation on the raw data for the three growth parameters (TL, WT, K_{TL}) for all six sizable full-sib backcross families (2-10, 3-10, 4-10, 5-10, 6-10, and 9-10). The Kendall's tau-b correlation estimation is a nonparametric test and measures an association between rank orders.

2.6.6 Statistical tests and thresholds for QTL detection

A sizable backcross family ($(YG_fxTR_m)_fxTR_m$), namely 6-10, derived from the Tree River and the Nauyuk Lake strains of Arctic charr was chosen for QTL analysis in the Icy Waters Arctic charr. Among six sizable families (35 or more sibs per family), family 6-10

(n=36) possesses the largest phenotypic variances for the three growth parameters under study.

Using the nonparametric Mann-Whitney U rank test, a statistical association ($p<0.05$) between a marker and a trait was examined by comparing the phenotypic means of two groups of individuals receiving different alleles from a heterozygous parent (female or male). If both parents were heterozygous for the same alleles, homozygous progeny were compared for their phenotypic means based upon their genotypes rather than allelic inheritance. The probability values were adjusted ($p<0.00081$) using the Bonferonni correction for multiple tests for 62 markers. An association that failed to pass this correction but for which α was 0.05 or less (i.e. $p<0.05$) during the initial analysis, was considered marginally significant.

To test for the proportion of phenotypic variation explained by a particular locus i.e. size of the QTL effect, an analysis of regressing phenotypes on to a molecular marker locus was carried out. To be conservative, before performing the regression analysis, the data were transformed by taking the natural log of the phenotypic values. The following generalized linear regression model was used to estimate the QTL effect;

$$y_{ij} = \mu + \beta j + \varepsilon_{ij},$$

where y_{ij} is the observed phenotypic value of the i^{th} individual in a mapping population ($N=1\text{-}n_i$) at $_j$th locus, μ is the trait mean of the group, β is the effect of marker-allele 'j' (j=1,2,12) as inherited from the male or female parent, and ε_{ij} is the residual error of the model. The R^2 value obtained from this model predicted the amount of the

variance of phenotype 'y' explained by the marker. A computer code was written to carry out the regression analysis for multiple markers simultaneously for all three normalized parameters total length (loglt), body weight (logwt) and condition factor (logcf) (Appendix IX).

Table 2.2. Revised designations for BHMS loci (clones) used in this study, as per SALMAP declaration.

Previous Designation	New Designation	Genbank accession number of the clone
BHMS121	Ssa132NVH	AF256769
BHMS130	Ssa12NVH	AF256663
BHMS142	Ssa135NVH	AF256772
BHMS206	Ssa30NVH	AF256680
BHMS217	Ssa152NVH	AF256786
BHMS330	Ssa105NVH	AF256748
BHMS356	Ssa109NVH	AF256751
BHMS409	Ssa180NVH	AF256811
BHMS411	Ssa121NVH	AF256761
BHMS429	Ssa71NVH	AF256719
BHMS431	Ssa72NVH	AF256720
BHMS490	Ssa76NVH	AF256724
BHMS540.1	Ssa198NVH	AF257059
BHMS546	Ssa200NVH	AF256829
BHMS7.030	Ssa209NVH	AF256838
BHMS7.033	Ssa5NVH	AF256658

Table 2.3. Sources of microsatellite primers used in this study.

Abbreviation	Common Name	Scientific Name
As	Atlantic salmon	*Salmo salar*
BFRO	Marble trout	*Salmo marmoratus*
BHMS	Atlantic salmon	*Salmo salar*
Cocl	Lake Whitefish	*Coregonus clupeaformis*
MBO	Arctic charr	*Salvelinus alpinus*
MST	Brown trout	*Salmo trutta*
Ocl	Cutthroat trout	*Oncorhynchus clarki*
Ogo	Pink salmon	*Oncorhynchus gorbuscha*
OMM	Rainbow trout	*Oncorhynchus mykiss*
Omy	Rainbow trout	*Oncorhynchus mykiss*
One	Sockeye salmon	*Oncorhynchus nerka*
Ots	Chinnok salmon	*Oncorhynchus tshawytscha*
Sal	Arctic charr	*Salvelinus alpinus*
Sap	Arctic charr	*Salvelinus alpinus*
Sco	Bull trout	*Salvelinus confluentus*
Sfo	Brook charr	*Salvelinus fontinalis*
Sma	Marble trout	*Salmo marmoratus*
Sox9-ms	microsate within SOX9 gene cloned from *S. alpinus*	
Ssa	Atlantic salmon	*Salmo salar*
SSLEE	Atlantic salmon	*Salmo salar*
SSOSL	Atlantic salmon	*Salmo salar*
Str	Brown trout	*Salmo trutta*

Table 2.4. Institute and country from where microsatellite primers used in this study were originated.

Abbreviation	Institute's Name
ASC	Alaska Science Center, USA
BRFO	University of Ljubljana, Slovenia
DIAS	Danish Institute of Agricultural Sciences, Denmark
DU	Dalhousie University, Canada
INRA	Institut Natioal de la Recherche Agronomique, France
LAV	University of Laval, Canada
LEE	National Fish Health Research Laboratory, USA
MBO	NRC Institute for Marine Biosciences, Canada
NUIG	National University of Ireland, Galway, Ireland
NVH	Norwegian College of Veterinary Medicine, Norway
NWFSC	North-West Fisheries Scienec Center, USA
SFU	Simon Fraser University, Canada
TUF	Tokyo University of Fisheries, Japan
UBC	University of British Columbia, Canada
UG	University of Guelph, Canada
UW	University of Washington, USA

Chapter 3. Results

3.1 Molecular tagging of 1996 Broodstock

All 1996 Arctic charr broodstock fish (n=848) from the four groups (Tree River, Nauyuk Lake, and two reciprocal hybrids) were genotyped to test for genetic variation at eight microsatellite markers (Ssa85DU, SalE38SFU, Sfo8LAV, Sfo23LAV, One18ASC, Sal5UG, SalP61SFU, and SalD39SFU). At the eight loci, a total of 69 alleles were detected across the four groups (Table 3.1).

The loci and genotyped brood fish showed a wide variation for the genetic variability in the two strains. Inter-locus allele size differences and the number of alleles per locus (i.e. allele diversity) varied. The locus Sfo23LAV possesses the greatest size range (176-300bp) of all alleles across all 848 samples. In contrast, locus One18ASC has the smallest size range (180-220bp) of all the loci tested. In general, hybrid fish revealed the greatest range of allele sizes and the greatest number of alleles per locus.

Overall, allelic diversity (A) in Icy Waters Arctic charr stocks is 8.6 per locus (Table 3.1). In ascending order, the average number of alleles overall in the four groups examined were: Hybrid1, Hybrid2, Tree River and Nauyuk Lake. The Tree River strain had a greater allelic variation (A=6.9) than the Nauyuk Lake strain (A=5.1). The larger allelic diversity in the two hybrid groups, Hybrid1 (A=8.1) and Hybrid2 (A=7.9), indicated a combined genetic diversity of the two original Tree River and Nauyuk Lake populations in them. At loci SalD39SFU, SalE38SFU, Sfo8LAV, and Sfo23LAV, the two pure strains (TR and YG) seem to carry 3-4 private alleles. Allele 187 at locus

Ssa85DU is exceptionally frequent in the Nauyuk Lake strain (0.98). Similarly, allele 220 at locus One18ASC is very frequent in the pure Tree River (0.86) strain (Figure 3.1)

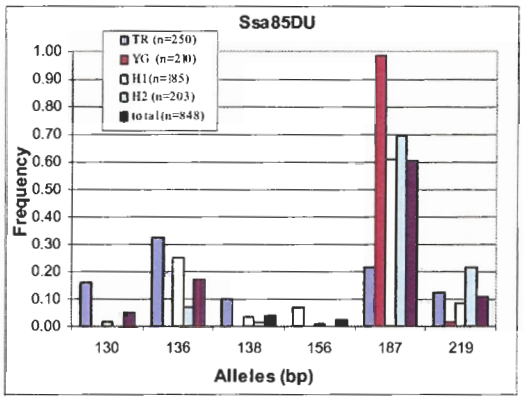
All of the 197 parents that were involved in generating the 12 different crosses were re-genotyped using the fluorescence-based automated genotyping technique at all eight loci (Appendix III). Using automated genotyping, the number of alleles across the four groups increased from 69 (autoradiography) to 81 and, thus the number of alleles per locus rose from 8.6 to 10.1 per locus. The allele 180 at the One18ASC locus is absent from the 197 parents used to generate the 12 family lines.

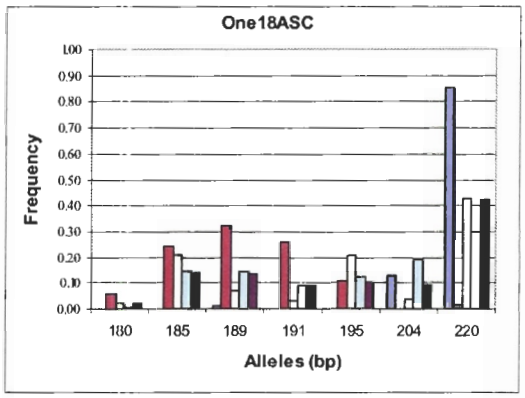
The fluorescence-based automated genotyping is a highly sensitive technique, which offers greater consistency when scoring electrophoresis gels. Ability to multiplex two or more loci in the same PCR tube and run the amplicons through the single gel lane is the key to this high-throughput technique (Chamberlain *et al.* 1988). Gels can be analyzed immediately after the electrophoresis is completed and genotypes can be obtained in much shorter time than with the radioactive technique (Olsen *et al.* 1996). However, scoring two alleles differing by only two base pairs can be difficult and may lead to genotyping errors. This problem can be minimized by comparing with other alleles in the population or by selecting microsatellites with tri- or tetra-nucleotide repeats. Nonetheless, the fluorescence-based automated genotyping technique offers several advantage over conventional and hazardous radioactive technique (Oda *et al.* 1997; Cawkwell1 and Quirke 2000).

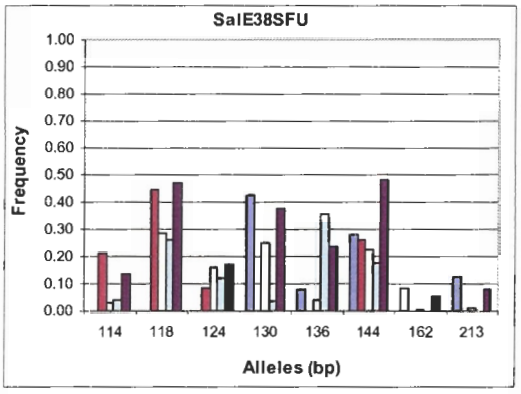
Table 3.1. Allelic diversity of the four Arctic charr broodstock groups at Icy Waters Ltd., using eight microsatellites (for details see Appendix IV).

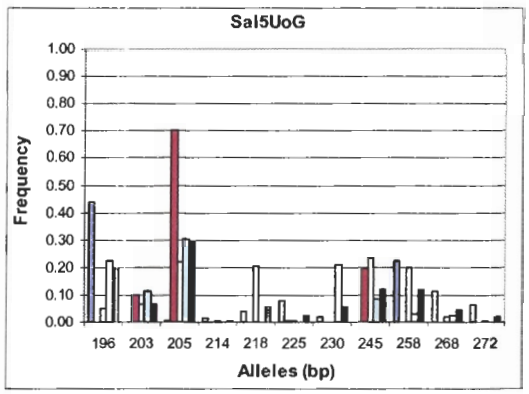
Marker	Allele size range (bp)	All brooders	Tree River	Yukon Gold	Hybrid1	Hybrid2
		Allele No.	Allele No.	Allele No.	Allele No.	Allele No.
SalE38SFU	114-213	8	6	4	8	8
Sfo8Lav	250-308	7	4	5	7	7
Ssa85DU	130-223	6	6	3	6	5
Sfo23Lav	176-300	9	4	5	7	9
One18ASC	180-220	7	6	6	7	7
SalP61SFU	139-193	10	9	7	9	9
SalD39SFU	194-290	11	10	5	11	9
Sal5UG	196-272	11	10	6	10	9
Total		69	55	41	65	63
Mean allele per locus		8.6	6.9	5.1	8.1	7.9

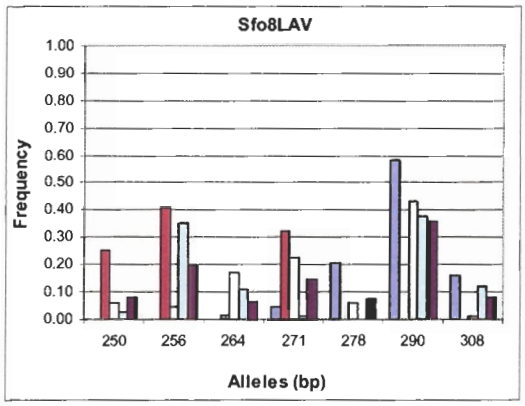
Figure 3.1. Allele frequencies for the eight microsatellites tested on the four groups. The number of samples tested per group: Tree River (TR; in blue)= 250, Yukon Gold (YG; in red)= 210, Hybrid1 (H1; in yellow)= 185, Hybrid2 (H2; in light blue)= 203, All 1996 broodstock (All; in brown)= 848.

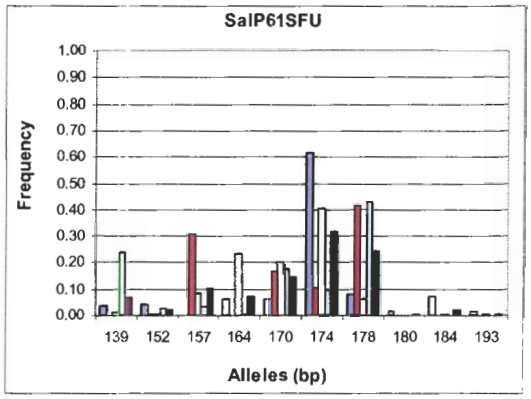

Ssa85DU
TR (n=250)
YG (n=240)
H1(n=385)
H2 (n=203)
total(n=848)
Frequency
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
130 136 138 156 187 219
Alleles (bp)

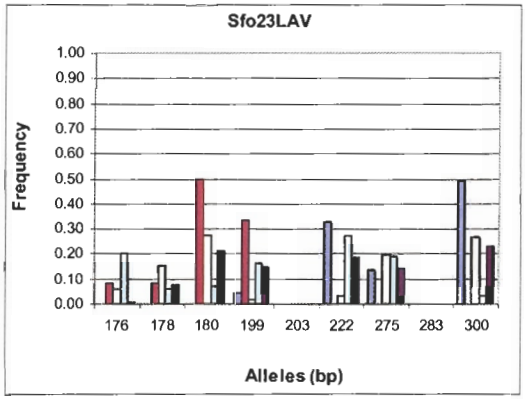

One18ASC
Frequency
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
180 185 189 191 195 204 220
Alleles (bp)

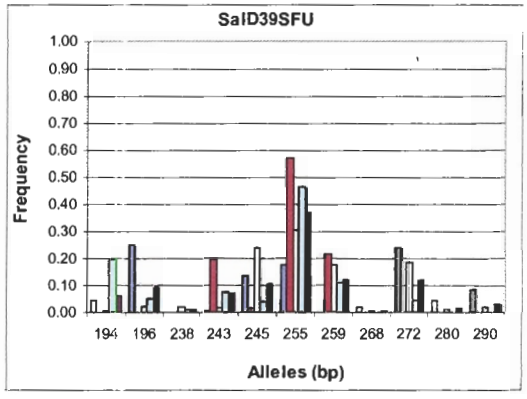

SalE38SFU
Frequency
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
114 118 124 130 136 144 162 213
Alleles (bp)


Sal5UoG
Frequency
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
196 203 205 214 218 225 230 245 258 268 272
Alleles (bp)


Sfo8LAV
Frequency
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
250 256 264 271 278 290 308
Alleles (bp)


SalP61SFU
Frequency
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
139 152 157 164 170 174 178 180 184 193
Alleles (bp)


Sfo23LAV
Frequency
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
176 178 180 199 203 222 275 283 300
Alleles (bp)


SalD39SFU
Frequency
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
194 196 238 243 245 255 259 268 272 280 290
Alleles (bp)

3.2 Male specific markers

Following fluorescent genotyping, a male-specific allele at the Sfo8Lav locus was observed in each of the two Arctic charr strains. At this locus marker-alleles Sfo8LAV-271 and Sfo8LAV-308 were exclusively found in males originating from the Nauyuk Lake and the Tree River populations, respectively. All of the 39 Nauyuk Lake males and 20 Hybrid1 males (TR_fXYG_m) possessed a 271 allele at this locus. Similarly, all 40 Tree River males and 20 Hybrid2 males (YG_fXTR_m) possess a 308 allele. None of the Nauyuk Lake, Tree River, Hybrid1, or Hybrid2 females carries either the 271 or the 308 allele at the Sfo8LAV locus. The only exception to this observation was one of the Hybrid1 male (PIT tag # 497249), which does not have a 271 allele, but possesses a 308 allele. For this fish, the genotypes at the other seven loci also suggest that it is an incorrectly identified individual and it is now believed to be a male fish from Hybrid2 (YG_fXTR_m) not Hybrid1 (TR_fXYG_m). This finding was confirmed by testing 25 males and 25 females from each of the four groups. Therefore, the Sfo8Lav marker is believed to be a male-specific marker and could prove to be invaluable during sex-reversal related genetic manipulations in Arctic charr at Icy Waters Ltd.

Additionally, all male fish carrying either allele 271 or 308 were heterozygous at the Sfo8LAV locus. This suggested a possible location of Sfo8LAV on the male-sex chromosome in Arctic charr. Results from the linkage analysis on the three Tree River backcross families (3-10, 4-10, and 6-10) indicated that the Sfo8LAV marker (allele 308) has zero percent recombination with the Omy6DIAS locus (allele 229) (Appendix X). The marker Omy6DIAS is 14cM from the phenotypically mapped 'SEX'-locus on the currently available Arctic charr linkage map (AC-4; Woram *et al.* 2004). The linkage

group AC-4 is believed to be a sex-specific linkage group in Arctic charr. Due to a lack of polymorphism in the two mapping families used by Woram *et al.* (2004), the marker Sfo8LAV could not be mapped to any linkage group (Dr. McGowan pers. comm.). Also, the two mapping families used to generate the current Arctic charr linkage map were created as Fraser River backcrosses (hybrid (Fraser x Nauyuk) x Fraser) (Woram *et al.* 2004), while the families used in this study were propagated as the Tree River backcross families ($(YG_fxTR_m)_fxTR_m$).

3.3 Marker suitability and genome coverage in Icy Waters Arctic charr

One hundred and ninety eight microsatellite markers from various salmonid species were tested for their suitability in this study (Appendix II). Among the 198 markers, eighty have been mapped on to 39 various linkage groups on the current genetic map covering 85% of the Arctic charr genomic map leaving the remaining 118 unassigned (Woram *et al.* 2004). Out of the 198 markers, only 75 markers were informative in the Icy Waters Arctic charr populations. Among unsuitable markers, 54 were undesirable and other 69 loci either did not amplify or produced unusable amplicons. The 75 informative microsatellite markers covered 39 of the 46 linkage groups of the current Arctic charr genetic map (Woram *et al.* 2004). Among the seven linkage groups that were not represented in this study, AC-40 and AC-43 each only have one microsatellite whereas, linkage groups AC-39, AC-41, AC-42, AC-44, and AC-46 have no microsatellites mapped on them and were characterized by AFLP markers in mapping families. Overall, 39 of the possible 41 linkage groups were screened in this study and thus, only linkage groups AC-40 and AC-43 remain unanalyzed in this study.

Table 3.2. Summary of informative microsatellite markers (n=75) in Icy Waters Arctic charr (for details see Appendix II).

Polymorphism	Nauyuk Lake (N=6)	Tree River (N=6)
No. of Monomorphic loci	20	13*
No. of Polymorphic loci	55	62*

*35 loci carried non overlapping alleles.

Among informative microsatellite markers (Table 3.2), 35 loci have non-overlapping alleles, whereas, 40 markers are sharing one or more alleles between the two strains (TR and YG) at Icy Waters Ltd. Among microsatellites with non-overlapping alleles, seven of the markers are monomorphic in the respective strains. Overall, the number of monomorphic microsatellite markers in the Nauyuk Lake and the Tree River Arctic charr from Icy Waters Ltd. are twenty and thirteen, respectively, suggesting either a heavier selection pressure under domestication or a larger founder effect in the Nauyuk Lake Arctic charr than the Tree River Arctic charr. Considering that the domestication of the Nauyuk Lake Arctic charr started with fewer individuals than the Tree River strain, the latter reason seems more likely. However, the number of founders contributing to the existing 1996 broodstock at Icy Waters Ltd. is not available.

3.4 Growth performance assessment of twelve crosses

After eleven months of rearing, in July 2002, length and weight data were collected for the twelve lines of juvenile fish (Table 2.1). Table 3.3 summarizes the data on twelve crosses as sampled in July 2002. No bimodal distributions were observed and those groups that were not distributed normally were very close to being normal. Probability estimates for pairwise comparisons between the twelve lines are shown in Appendix V. For the purebred lines, Tree River Arctic charr appeared to grow faster than the Nauyuk Lake Arctic charr. Both hybrid lines grew at similar rates and were equivalent to the Tree River line. Three of the four backcross lines generated from hybrids backcrossed with Yukon Gold fish grew at similar rates to the pure Yukon Gold line, while the backcross $YG_f x(TR_f x YG_m)_m$ grew at a rate similar to the Tree River strain. One time data collected

Table 3.3. Summary of length (cm) and weight (g) data for twelve lines of juvenile Arctic char (July 2002)

Cross	Tank	Mean Length	SD	variance	distribution	Mean Weight	SD	variance	distribution
TRf x TRm	2	5.6	0.41	0.17	normal	1.29	0.29	0.084	normal
YGf x YGm	6	5.1	0.27	0.073	normal	1.02	0.16	0.026	normal
YGf x TRm	11	5.8	0.38	0.14	normal	1.35	0.28	0.078	normal
TRf x YGm	5	5.6	0.44	0.19	normal	1.34	0.3	0.09	normal
(YGf x TRm)f x YGm	1	5.2	0.34	0.12	not normal	1.09	0.22	0.048	not normal
(TRf x YGm)f x YGm	4	5.3	0.41	0.17	normal	1.11	0.26	0.068	normal
YGf x (TRf x YGm)m	3	5.5	0.47	0.22	normal	1.2	0.32	0.1	normal
YGf x (YGf x TRm)m	9	5.1	0.4	0.16	normal	0.98	0.23	0.053	not normal
(YGf x TRm)f x TRm	10	5.9	0.5	0.25	not normal	1.5	0.4	0.16	normal
(TRf x YGm)f x TRm	12	6	0.42	0.18	not normal	1.53	0.35	0.12	not normal
TRf x (TRf x YGm)m	7	6.1	0.53	0.28	not normal	1.75	0.5	0.25	normal
TRf x (YGf x TRm)m	8	5.6	0.5	0.25	normal	1.28	0.36	0.13	normal

in July 2002 showed a strong agreement with the weekly measurements taken at the hatchery over a period of 35 weeks from the first feeding (Figure 3.2, 3.3, 3.4, and 3.5).

Four backcross lines generated from hybrids backcrossed with Tree River fish, exhibited the best growth. The Tree River backcrosses with 75% male ($(YG_fxTR_m)_fxTR_m$) or 75% female ($TR_fx(TR_fxYG_m)_m$) contribution grew even faster than the other two Tree River backcrosses ($TR_fx(YG_fxTR_m)_m$ and $(TR_fxYG_m)_fxTR_m$).

Among the four Nauyuk Lake Arctic charr backcrosses, the backcrosses with 75% female ($YG_fx(YG_fxTR_m)_m$) contribution grew slowest. Although in general the Yukon Gold backcrosses were out-grown by the Tree River backcrosses, the Yukon Gold backcross families may be valuable in detecting QTL responsible for the attractive color and body shape in the Arctic charr at Icy Waters Ltd.

3.5 Growth performance of the four most informative backcrosses

In February 2003, four backcrosses were selected for the detection of QTL in Icy Waters Arctic charr populations. Two of the four sampled lines represent the Nauyuk Lake backcross ($(YG_fxTR_m)_fxYG_m$ and $YG_fx(TR_fxYG_m)_m$), while the other two were the Tree River backcrosses ($TR_fx(TR_fxYG_m)_m$ and $(YG_fxTR_m)_fxTR_m$). These lines were the fastest growing among eight backcrosses at that time and were expected to provide the most information while searching for growth QTL in Icy Waters Ltd. Arctic charr (Table 3.4.1).

Figure 3.2. Average batch weight of juvenile fish for the two pure (cross 2; TR_fxTR_m in gray, and cross 6; YG_fxYG_m in blue) and the two reciprocal hybrid (cross 5; TR_fxYG_m in green, and cross 11; YG_fxTR_m in yellow) crosses over 32 weeks of hatchery rearing between February 21, 2002 to October 22, 2002.

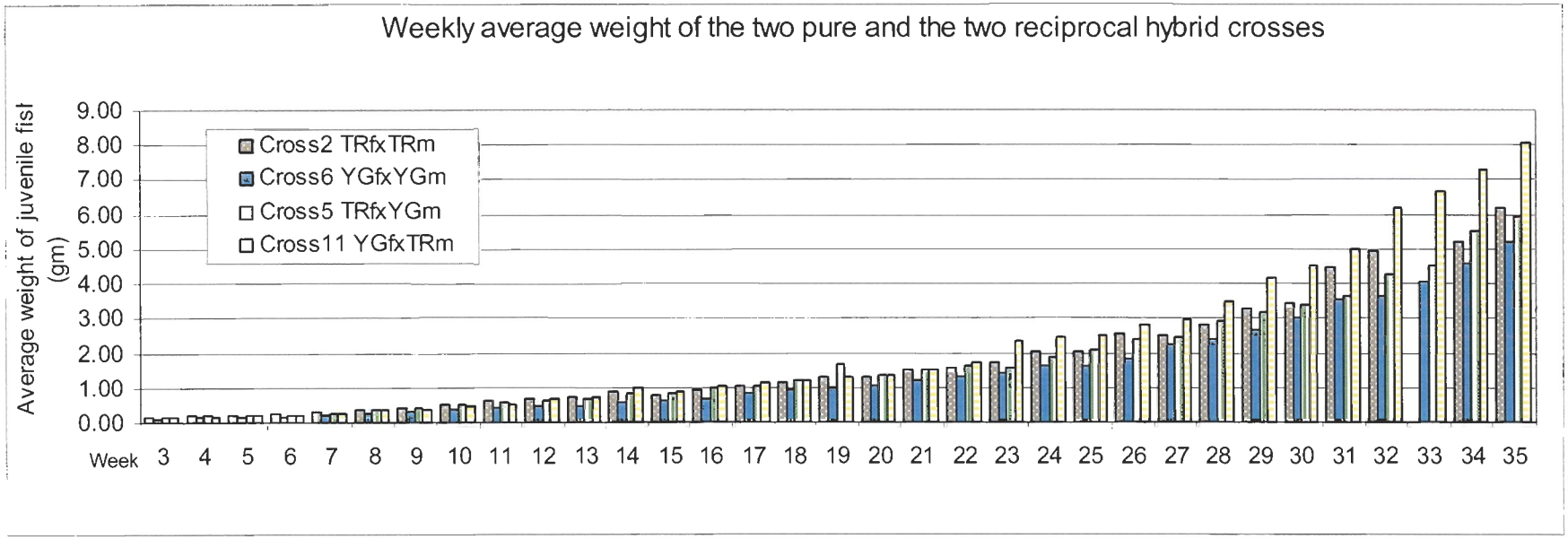

Weekly average weight of the two pure and the two reciprocal hybrid crosses
Average weight of juvenile fist (gm)
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Cross2 TRfxTRm
Cross6 YGfxYGm
Cross5 TRfxYGm
Cross11 YGfxTRm
Week 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35

Figure 3.3. Average batch weight of juvenile fish for the pure Nauyuk Lake (NL) cross (cross 6; $YG_f x YG_m$ in blue) and the four Nauyuk Lake backcrosses (cross 1; $YG_f x TR_m)_f x YG_m$ in brown, cross 4; $(TR_f x YG_m)_f x YG_m$ in dark blue, cross 3; $YG_f x (TR_f x YG_m)_m$ in pink, and cross 9; $YG_f x (YG_f x TR_m)_m$ in yellow) over 32 weeks of hatchery rearing between February 21, 2002 to October 22, 2002.

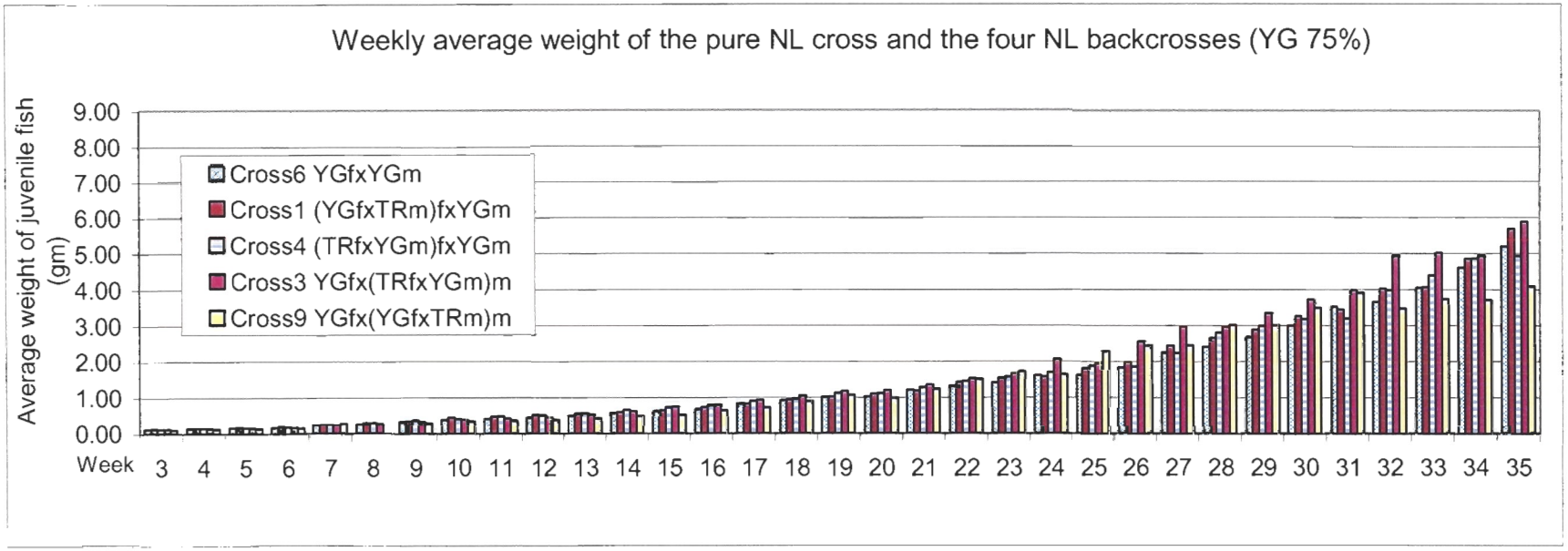

Weekly average weight of the pure NL cross and the four NL backcrosses (YG 75%)
Average weight of juvenile fish (gm)
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Cross6 YGfxYGm
Cross1 (YGfxTRm)fxYGm
Cross4 (TRfxYGm)fxYGm
Cross3 YGfx(TRfxYGm)m
Cross9 YGfx(YGfxTRm)m
Week
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35

Figure 3.4. Average batch weight of juvenile fish for the pure Tree River (TR) cross (cross 2; $TR_f x TR_m$ in light blue) and the four Tree River backcrosses (cross 10; $YG_f x TR_m)_f x TR_m$ in dark blue, cross 12; $(TR_f x YG_m)_f x TR_m$ in pink, cross 7; $TR_f x (TR_f x YG_m)_m$ in red, and cross 8; $TR_f x (YG_f x TR_m)_m$ in green) over 32 weeks of hatchery rearing between February 21, 2002 to October 22, 2002.

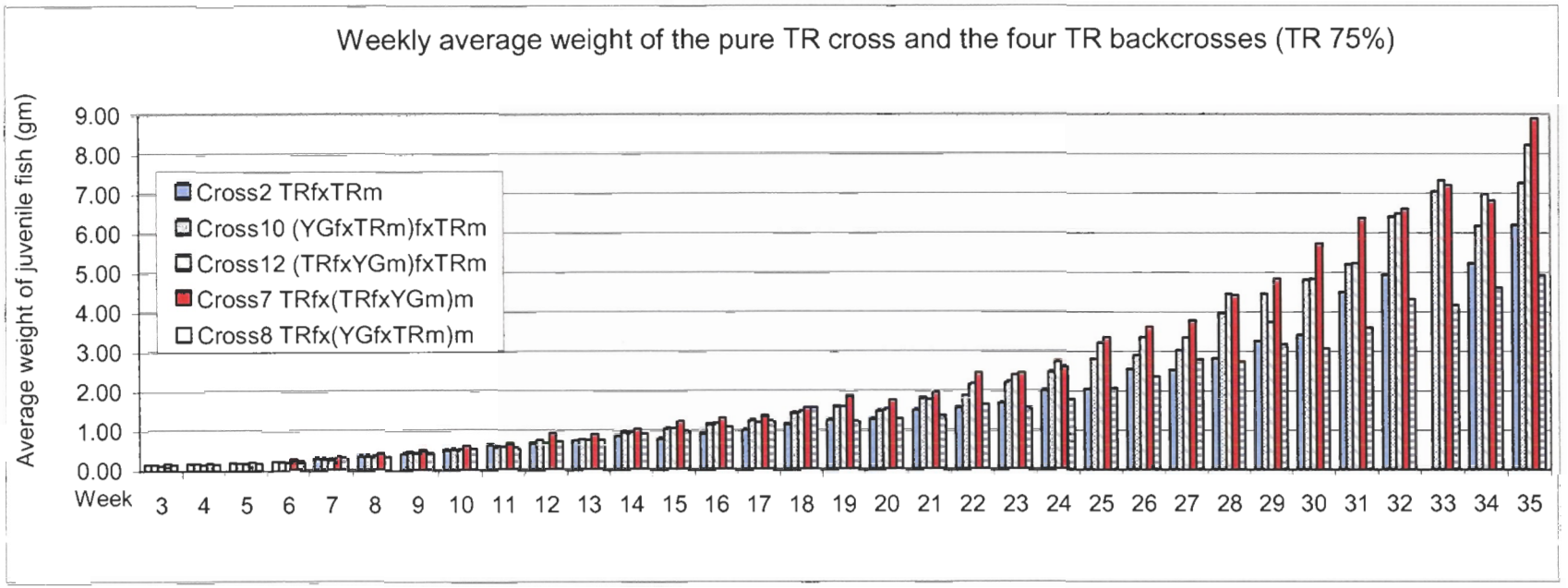

Weekly average weight of the pure TR cross and the four TR backcrosses (TR 75%)
Average weight of juvenile fish (gm)
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Cross2 TRfxTRm
Cross10 (YGfxTRm)fxTRm
Cross12 (TRfxYGm)fxTRm
Cross7 TRfx(TRfxYGm)m
Cross8 TRfx(YGfxTRm)m
Week
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35

Figure 3.5. Average batch weight of juvenile fish for the four most variable backcrosses (cross 1; $(YG_fxTR_m)_fxYG_m$ in brown, cross 3; $YG_fx(TR_fxYG_m)_m$ in pink, cross 7; $TR_fx(TR_fxYG_m)_m$ in red, cross 10; $(YG_fxTR_m)_fxTR_m$ in blue) over 32 weeks of hatchery rearing between February 21, 2002 to October 22, 2002.

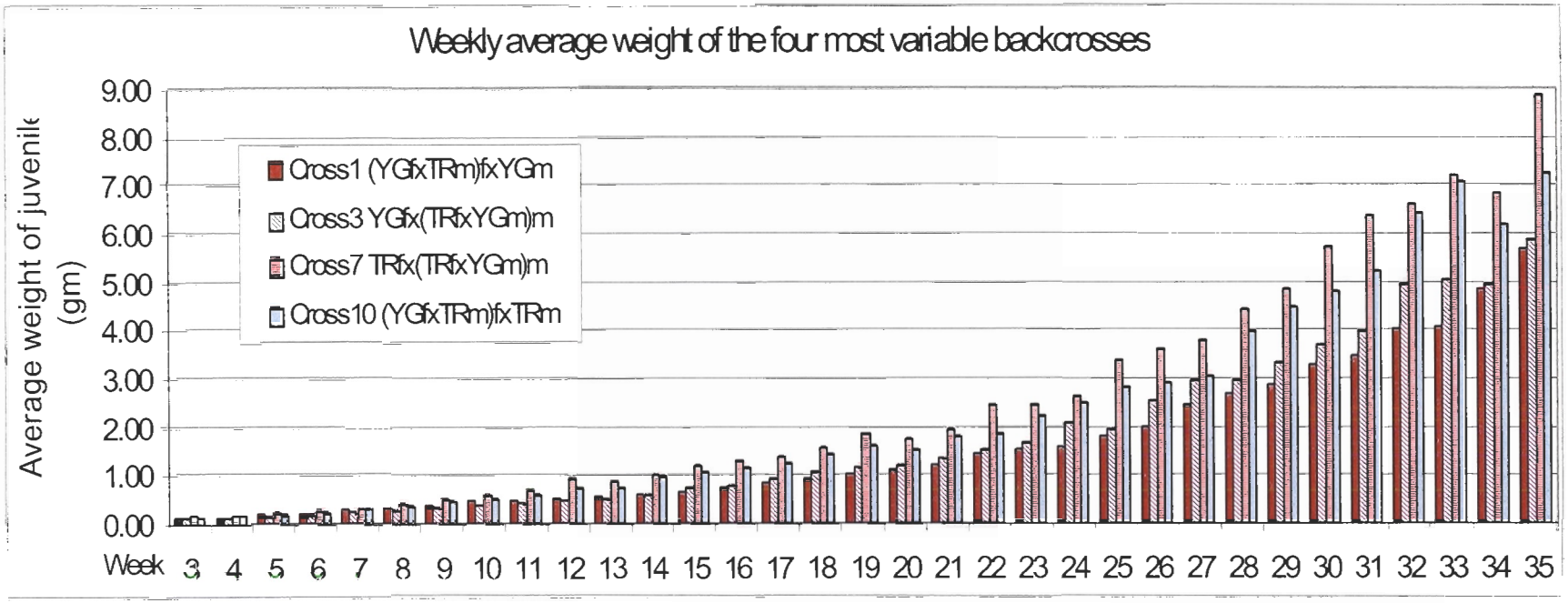

Weekly average weight of the four most variable backcrosses
Average weight of juvenile (gm)
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Cross1 (YGfxTRm)fxYGm
Cross3 YGfx(TRfxYGm)m
Cross7 TRfx(TRfxYGm)m
Cross10 (YGfxTRm)fxTRm
Week 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35

Table 3.4.1. Summary of three growth parameters and test of normality in the four backcrosses: tank1; $(YG_fxTR_m)_fxYG_m$, tank3; $YG_fx(TR_fxYG_m)_m$, tank7; $TR_f x(TR_fxYG_m)_m$, and tank10; $(YG_fxTR_m)_fxTR_m$(February 2003).

Cross	Tank	Length (cm)				Weight (g)				K_{TL}			
		Mean	SD	VAR	Distribution*	Mean	SD	VAR	Distribution*	Mean	SD	VAR	Distribution*
$(YG_fxTR_m)_fxYG_m$	1	10.71	1.31	1.72	not normal	11.3	4.55	20.70	normal	0.87	0.06	0.004	normal
$YG_fx(TR_fxYG_m)_m$	3	11.83	1.38	1.91	normal	16.4	6.18	38.20	normal	0.94	0.07	0.003	normal
$TR_fx(TR_fxYG_m)_m$	7	13.38	1.8	3.23	normal	22.65	9.81	96.32	normal	0.90	0.06	0.004	normal
$(YG_fxTR_m)_fxTR_m$	10	12.56	2.09	4.38	normal	19.26	10.63	113.07	normal	0.88	0.07	0.006	normal

*Kolmogorov-Smirnov test of normality was used ($p<0.5$).

Table 3.4.2. Summary of pairwise comparisons for three growth parameters in the four backcrosses: tank1; $(YG_fxTR_m)_fxYG_m$, tank3; $YG_fx(TR_fxYG_m)_m$, tank7; $TR_fx(TR_fxYG_m)_m$, and tank10; $(YG_f\ xTR_m)$ (February 2003).

		$(YG_fxTR_m)_fxYG_m$	$YG_f\ x(TR_fxYG_m)_m$	$TR_fx(TR_fxYG_m)_m$	$(YG_fxTR_m)_fxTR_m$	
Cross	Tank	1	3	7	10	
$(YG_fxTR_m)_fxYG_m$	1		xxx	xxx	xxx	Weight (WT)
$YG_f\ x(TR_fxYG_m)_m$	3	xxx		xxx	xxx	
$TR_fx(TR_fxYG_m)_m$	7	xxx	xxx		xxx	
$(YG_fxTR_m)_fxTR_m$	10	xxx	xxx	xxx		
		Length (TL)				
$(YG_fxTR_m)_fxYG_m$	1		***	***	***	K_{TL}
$YG_f\ x(TR_fxYG_m)_m$	3			***	ns	
$TR_fx(TR_fxYG_m)_m$	7				***•	

xxx; highly significant for TL& WT ($P<0.001$), ***; highly significant differences for K_{TL} ($p<0.001$), ns: not significant

The four backcrosses were significantly different from one another for total length and body weight ($p<0.001$; Table 3.4.2). Among these four crosses, the Tree River backcross (tank 10) had the largest variance for both total length and body weight and hence, families derived from this backcross were considered to have the greatest potential for detecting the genetic basis of growth in Arctic charr. Therefore, the Tree River backcross ($YG_fxTR_m)_fxTR_m$) was selected for growth QTL analysis.

3.6 Parentage assignment in the Tree River backcross: $(YG_fxTR_m)_fxTR_m$

All 500 fish from cross 10 ((YGfxTRm)$_f$xTRm) were genotyped for eight polymorphic loci (Appendix II) and sorted into ten full-sib families using PROBMAX 1.2. The PROBMAX is a software that calculates the maximum probability of progeny assignments to a mixture of possible contributing parents, when the genotypes of the parents and progeny (at the same loci), and the potential parental mating combinations are known. Only 321 juvenile fish could be assigned to ten unique full-sib families belonging to cross 10. Each family was comprised of 12 to 47 full-siblings (Table 3.5). One hundred and seventy nine (179) fish from 'tank 10' could not be assigned to a unique family and therefore were excluded from further analysis. Out of ten, only six families (2-10, 3-10, 4-10, 5-10, 6-10 and 9-10) were sufficiently large ($N>35$) to carry out QTL mapping analysis.

Table 3.5. Description of ten full-sib families from $((YG_fxTR_m)_fxTR_m$ backcross (tank 10).

Family	Female (YG_fxTR_m) PIT tag #	Male (TR_m) PIT tag #	No.of juvenile fish
1-10	497268	503443	16
2-10	497271	510037	47
3-10	497908	506556	35
4-10	497149	501520	36
5-10	503067	500055	46
6-10	497479	501764	36
7-10	503970	510460	33
8-10	502424	504560	20
9-10	511074	502067	40
10-10	500517	504242	12

3.7 Growth patterns of ten full-sib Tree River backcross families ($YG_fxTR_m)_fxTR_m$)

After sorting 321 fish into 10 unique full-sib families (Table 3.5), fish from each of the 10 families were sorted into male and female groups based on the presence or absence of the 308 allele at the Sfo8LAV locus. It was apparent that males and females had been randomly sampled in equal proportion. There were no differences ($p<0.05$) in early growth rates (all three parameters) between males and females (Appendix XI). These findings ruled out any possibility of a sex-associated effects on the early growth of the juvenile fish and therefore the entire family can be treated uniformly. Table 3.6 summarizes the mean phenotypic values for the three growth parameters and tests for normality of the data for all ten families.

Variation in the growth rates and the number of progeny in each family indicates the prevalence of family effects between families. For the purpose of genome wide scans to test for linkage between genetic markers and quantitative traits, family 6-10, the most variable family was selected. Among sizable families, family 6-10 (N=36) possessed the greatest phenotypic variance for the three growth parameters which was very important in analyzing the mechanisms underlying the phenotypic variation caused by genetic or environmental factors or their interaction. Since all families were raised under identical culture conditions, the effect of environment on the phenotypic variation was assumed insignificant.

Table 3.6. Summary of three growth parameters and test of normality in ten full-sib families of the Tree River backcross ($YG_fxTR_m)_fxTR_m$ (February 2003).

		Length (cm)			Weight (g)			K_{TL}		
Family	N	Mean	STDEV	*Sig. (p<0.5)	Mean	STDEV	*Sig. (p<0.5)	Mean	STDEV	*Sig. (p<0.5)
1-10	16	12.3	2.01	0.200	17.45	10.47	0.200	0.85	0.08	0.200
2-10	47	12.2	1.83	0.031	17.02	8.74	0.000	0.87	0.06	0.082
3-10	35	13.2	1.90	0.068	21.93	10.05	0.054	0.90	0.06	0.027
4-10	36	12.5	1.77	0.149	19.26	9.26	0.001	0.91	0.08	0.200
5-10	46	12.4	1.73	0.200	16.89	8.01	0.078	0.83	0.10	0.004
6-10	36	14.7	2.10	0.200	31.29	13.70	0.200	0.91	0.05	0.200
7-10	33	14.1	1.84	0.128	27.10	10.29	0.012	0.92	0.05	0.200
8-10	20	12.7	1.87	0.200	18.85	8.71	0.200	0.86	0.06	0.200
9-10	40	10.2	1.29	0.200	9.64	3.79	0.200	0.85	0.08	0.200
10-10	12	13.6	1.87	0.186	23.89	11.34	0.117	0.90	0.08	0.179

*Kolmogorov-Smirnov test of normality was used.

Table 3.7. Correlations among three growth parameters in six backcross families of Arctic charr calculated using the Kendall Tau-b Correlation Coefficients (above diagonal) and the Pearson Product Moment (below diagonal: after normalizing the data by taking the natural log of it). Where, TL; total length, WT; body weight, and K_{TL}; Fulton's condition factor. Values in bold indicate no correlation at $p>0.05$*.

Family	Trait	TL	WT	KTL
2-10 (N=47)	TL		0.94	0.36
	WT	0.99		0.43
	K_{TL}	0.53	0.63	
3-10 (N=35)	TL		0.95	**0.22**
	WT	0.99		**0.28**
	K_{TL}	**0.31**	0.43	
4-10 (N=36)	TL		0.89	0.38
	WT	0.99		0.50
	K_{TL}	0.54	0.67	
5-10 (N=46)	TL		0.85	**0.19**
	WT	0.94		0.35
	K_{TL}	**0.03**	0.36	
6-10 (N=36)	TL		0.94	0.43
	WT	0.99		0.50
	K_{TL}	0.46	0.56	
9-10 (N=40)	TL		0.90	0.32
	WT	0.98		0.43
	K_{TL}	0.54	0.68	

*Null rejected at $p<0.017$(Bonferonni 0.05/3).

3.8 Correlation among Growth traits in six full-sib Tree River backcross families

Correlations among all three growth parameters varied in both magnitude and pattern across families (Table 3.7). Total length and body weight were highly correlated in all families ($r>0.84$ and, $r>0.94$ for transformed data). TL and K_{TL} were weakly correlated in four families and were not correlated ($p>0.017$) in two families for both tests. WT and K_{TL} showed a moderate to weak correlation. Furthermore, the correlation between WT and K_{TL} greatly varies across families for both tests ($r=0.28$-0.50 and, $r=0.36$-0.67 for transformed data). In family 6-10, correlations between the TL and WT, TL and K_{TL} and, WT and K_{TL} were very similar across the two tests.

3.9 QTL Mapping: Genome wide scans in family 6-10.

Out of the 62 informative markers tested in family 6-10, 49 microsatellite markers were heterozygous for either of the two parents and 13 markers were uninformative (Appendix VI). Thirty two of the 49 informative markers were assigned to 27 linkage groups of the Arctic charr linkage map. The remaining seventeen informative markers were unassigned. Moreover, five microsatellite loci (MST85, Omy38DU, SapI26SFU, Ssa208, and Ssa20.19NUIG), which were each believed to be single loci, were found to be duplicated in Icy Waters Arctic charr. Among markers informative in family 6-10, however, the microsatellite loci (Ogo4UW, SalD100SFU, SalF41SFU, BHMS206, BHMS490, and SSOSL32) which were mapped as duplicate loci by Woram *et al.* (2004), produced only one locus in these populations. This supports the pseudo-tetraploid nature of Arctic charr. Furthermore, successful amplification of primers originally isolated from

other salmonids confirmed the high conservation of microsatellite flanking regions across several salmonid fish species (for details see Appendix II).

3.10 QTL Mapping: TDT and LRM analyses

Eighteen allele-trait association effects ($p<0.05$) from both the female and male for the three growth parameters (four for TL, six for WT, and eight for K_{TL}) were detected at thirteen markers in the Icy Waters Arctic charr family 6-10 (Table 3.8). Furthermore, a marginal allelic variation ($p<0.053$) was detected at Sal5UG for K_{TL}. None of the 49 informative markers cleared the experiment-wide significance threshold of $p<0.001$ (Bonferonni 0.05/49). Thus, all reported associations were considered marginal ($p<0.05$). Two of the four informative markers on AC-25 passed a linkage group-wide threshold ($p<0.0125$; 0.05/4).

The proportion of phenotypic variation explained by the regression model ranged from 9.9–26.3% for significant or marginally significant associations (Table 3.8). Further, TL and WT showed nearly identical results at six loci both for the TDT and LRM analyses, which was not surprising given their high correlation ($r>0.94$) in family 6-10 (Table 3.7, Table 3.8).

Allelic variation at BHMS490 (AC-4 & AC-25) from the male parent was significantly associated with TL and WT. Another, two informative markers on AC-4, OMM1228 and Omy6DIAS, did not show any notable association for either of the growth parameters. However, the two markers flanking BHMS490 on AC-25, BHMS121 and OmyRGT39TUF, showed significant association ($p<0.05$). Linkage analysis between

Table 3.8. Putative QTL for total length (LT), body weight (WT), and Fulton's condition factor (K_{TL}) in family 6-10 of Icy Waters Arctic charr. Values in **bold** indicate significant allele effects at p<0.05, while values in ***bold-italics*** indicate marginal effects at 0.06>p>0.05 for the transmission disequilibrium test (TDT). R^2 is the proportion of phenotypic variance explained by the linear regression model, and represents the QTL effect. The underlined R^2 values are additional notable variations (for details see Appendix VII & VIII).

Linkage Group	Locus	Parental Sex	Allele (freq.)	TL (cm)		WT (gm)		K_{TL}	
				TDT-Stat. (p<0.05)*	R^2 %	TDT-Stat. (p<0.05)*	R^2 %	TDT-Stat. (p<0.05)*	R^2 %
AC-1	Ssa77NUIG	f	162(15) - 166(21)	0.785	2.0	0.585	9.0	**0.031**	15.2
		m	Homozygous(166)	-		-		-	
AC-4 & AC-25	BHMS490	f	111(22) - 115(14)	0.188	6.3	0.123	8.0	**0.008**	15.1
		m	109(19) - 111(17)	**0.002**	25.5	**0.002**	26.3	**0.016**	11.9
AC-6	One8ASC	genotypic	148/148(10)-158/158(7)	0.406	2.0	0.380	2.7	0.351	15.7
			148/148(10)-148/158(19)	0.396		0.371		**0.045**	
			158/158(7)-148/158(19)	1.000		0.862		0.505	
AC-8 & AC-6	BHMS206	f	174(24) - 180(12)	***0.056***	10.2	**0.048**	10.5	0.207	4.1
		m	Homozygous(209)	-		-		-	
AC-13	Ssa85DU	f	Homozygous(187)	-		-		-	
		m	187(18) - 223(18)	0.658	0.0	0.681	0.1	0.070	10.0
AC-15	BHMS356	f	189(17) - 202(19)	0.194	3.9	0.178	4.7	**0.043**	8.0
		m	Homozygous(202)	-		-		-	
AC-20	OmyRGT4TUF	f	140(18) - 142(18)	0.060	12.3	***0.052***	13.2	**0.044**	8.1
		m	132(22) - 140(14)	0.194	5.6	0.200	5.0	0.782	0.1
AC-25	OmyRGT39TUF	f	106(15) - 118(21)	0.072	9.9	**0.047**	11.6	**0.009**	14.5
		m	116(18) - 118(18)	**0.010**	19.2	**0.007**	20.3	**0.016**	12.1
AC-25	BHMS121	f	123(20) - 131(16)	0.052	12.4	**0.047**	11.3	0.346	0.7
		m	131(23) - 135(13)	**0.041**	14.6	***0.052***	11.6	0.779	1.4
AC-36	Sal5UG	f	230(20) - 245(16)	0.679	0.0	0.567	0.3	***0.053***	9.9
		m	196(21) - 258(15)	0.700	0.1	0.797	0.0	0.573	1.5
unassigned	OMM1037	f	124(18) - 128(18)	**0.019**	14.7	**0.019**	13.6	0.309	1.0
		m	Homozygous(124)	-		-		-	
unassigned	Ssa171	f	96(18) - 99(18)	***0.055***	12.5	**0.041**	14.0	**0.022**	12.1
		m	Homozygous(99)	-		-		-	
unassigned	Ssa208b	f	310(17) - 340(19)	0.064	8.1	***0.053***	8.4	0.089	3.5
		m	280(19) - 300(17)	0.428	0.5	0.254	1.7	**0.003**	24.4

*Experiment-wide null rejected at p<0.001(Bonferonni 0.05/49).

Table 3.9. Putative growth QTL (TL; total length, WT; body weight, and (K_{TL}; condition factor) on the Arctic charr linkage group AC-25, in the Icy Waters Arctic charr family 6-10 as inherited from the female and male parents. Values in **bold** indicate significant allele effects at P<0.05, while values in ***bold-italics*** indicate marginal effects at 0.06>p>0.05 for the transmission disequilibrium test (TDT). R^2 is the proportion of phenotypic variance explained by the linear regression model, and represents the QTL effect. For details see Appendix VII & VIII.

Locus	Parental Sex	Allele (freq.)	TL (cm)		WT (gm)		K_{TL}	
			TDT-Stat. (p<0.05)*	R^2 %	TDT-Stat. (p<0.05)*	R^2 %	TDT-Stat. (p<0.05)*	R^2 %
SalD39SFU	f	255(13) - 259(23)	ns	0.2	ns	0.5	ns	3.1
	m	272(24) - 290(12)	ns	7.6	ns	5.6	ns	2.3
BHMS121	f	123(20) - 131(16)	***0.052***	12.4	**0.047**	11.3	ns	0.7
	m	131(23) - 135(13)	**0.041**	14.6	***0.052***	11.6	ns	1.4
BHMS490	f	111(22) - 115(14)	ns	6.3	ns	8.0	**0.008**	15.1
	m	109(19) - 111(17)	**0.002**	25.5	**0.002**	26.3	**0.016**	11.9
OmyRGT39TUF	f	106(15) - 118(21)	ns	9.9	**0.047**	11.6	**0.009**	14.5
	m	116(18) - 118(18)	**0.010**	19.2	**0.007**	20.3	**0.016**	12.1

*Chromosome-wide null rejected at p<0.0125(Bonferonni 0.05/4). 1; OMM1184, one of the five tested markers was homozygous for both the parents

BHMS490 and OmyRGT39TUF detected a tight linkage (unpublished results). For allelic variation derived from the female parent, a significant association was detected at OMM1037 (unassigned) with WT. Furthermore, marginal maternal effects on WT were also detected at BHMS206 (AC-6 & AC-8), OmyRGT4TUF (AC-20), Ssa171 (unassigned), and Ssa208b (unassigned). The male parent was homozygous for the loci OMM1037, BHMS206, and Ssa171. Alleles derived from the male, however, did not yield any significant association at OmyRGT4TUF and Ssa208b.

The existence of significant or marginally significant associations with TL and WT and markers on AC-25 provides suggestive evidence for a LT-/ WT-QTL on this linkage group (Table 3.9). These effects were detected in alleles derived from the male parent. The variance for loci exhibiting significant association ($p<0.001$) for LT or WT ranged from 19.2-26.3%. The variance at the locus BHMS121 showed a marginal significance and contributed 14.6% and 11.6% to the variation in TL and WT, respectively. Overall, the genomic region on AC-25 spanning BHMS121 and OmyRGT39TUF loci contributed 14.6-25.5% and 11.6-26.3% to the variance of TL and WT, respectively. A total of five microsatellite markers were tested for an association on this linkage group, one of which, OMM1184, was homozygous for both the parents. The marker SalD39SFU did not show any association with any of the three growth parameters.

An unassigned marker, Ssa208b (paternal effect only) was associated with variation in K_{TL} (Table 3.8). The marker contributed the most (24.4%) to the total variation in K_{TL}. In addition, allelic variations at the loci BHMS490 and OmyRGT39TUF showed very similar associations with K_{TL} at AC-25. Furthermore, both BHMS490 and OmyRGT39TUF, contributed ~12% (paternal effect) and ~15% (maternal effect) to the

variation in K_{TL}. Unlike for TL/WT, however, the contribution of the locus BHMS121 from the same linkage group is negligible to the variation in K_{TL}. This supported the evidence of growth-QTL closely linked to BHMS490.

The existence of marginal associations (maternal effect) with K_{TL} at markers Ssa77NUIG (AC-1), Sal5UG (AC-36), and Ssa171 (unassigned) provided suggestive evidence for QTL in those locations (Table 3.8). In addition, two other marginal associations with K_{TL} at the locus Ssa85DU (AC-13; paternal effect) and One8ASC (AC-6; genotypic) were also observed. Although genotypic variation was marginal at the locus One8ASC, the model explains 15.7% of the variance which is similar to, or more than, any other marker with the exception of marker Ssa208b. This suggested the existence of a major QTL at this location. However, when comparing genotypic classes a/a and b/b alone, the genotypic variation did not show any significant differences between the two classes. Since both the parents were double-heterozygotic for the same alleles, however, effects of allelic variation (paternal or maternal) remain to be determined. Overall, the variance for loci exhibiting marginal associations with K_{TL} ranged from ~10-24%: ~10-15% (maternal) and ~10-24% (paternal).

A higher number of linkage groups associated ($p<0.05$) with QTL effects for K_{TL} (eight) than for TL/WT (six) and weak correlations between K_{TL} and TL ($r<0.53$), and K_{TL} and WT ($r<0.68$) supported the presence of QTL for K_{TL} and TL/WT in different chromosomal regions. In addition, genes responsible for the shape (girth) of fish might be more widespread than for the length or weight of the fish. Furthermore, genes responsible for the length and the weight in fish might be co-localized and could have evolved under similar selective pressure in the Tree River Arctic charr. Similarly, genes governing fish

girth could have been selected independently in the Nauyuk Lake Arctic charr at Icy Waters Ltd.

Chapter 4. Discussion

The aim of this study was to evaluate the growth performance of various hybrid cross combinations and to search for QTL associated with growth in Arctic charr. To find the favorable crosses for production of Arctic charr in the fish farming industry, the growth performance of juvenile fish from twelve crosses was evaluated. Crossing experiments also provided information on the amount of variation available for genetic selection. To apply MAS for the development of Arctic charr broodstock at in Canada, it was essential to identify molecular markers associated with growth and then to estimate the QTL effect. An analysis of 62 microsatellite markers was carried out to detect QTL. Utilizing the knowledge obtained from this study will make it possible to design and implement a MAS strategy for the integration of commercially important QTL in the Arctic charr breeding program at Icy Waters Ltd.

4.1 Growth performance in Arctic charr

4.1.1 Growth evaluation of Arctic charr at Icy Waters Ltd.

This study presents the most comprehensive growth trial ever performed on the TR Arctic charr and for the first time allows the comparison with other domesticated Arctic charr populations around the world. The eleven months old juvenile Arctic charr from the twelve crosses showed significant differences for growth (Fig. 3.2; Appendix V). The TR Arctic charr, which grew at a significantly faster rate than the NL Arctic charr, proved to be the fastest growing domesticated strain of Arctic charr in the world. Thus far, according to Johnston (2002), the NL Arctic charr was known to be the fastest growing domesticated strains of Arctic charr in the world. The data collected on the 1996

broodstock also showed significant differences in growth between the two strains (Appendix XII). Furthermore, these results are consistent with the growth data collected on the wild counterparts of these two Arctic charr populations (Moshenko *et al.* 1984).

The two reciprocal hybrid crosses performed better than the pure NL cross but did not show any crossbreeding advantage for growth over the pure TR cross for growth. In general, intraspecific hybrids between two inbred but divergent strains are expected to show heterosis and may express intermediate or better growth than the parent displaying the best growth rate (Alm 1955, as cited by Refstie and Gjerdrem 1975; Krasznai 1987; Tave 1993; Weller 2001). Aside from this study, no study comparing different domesticated strains of a salmonid fish species has been published. However, based on interspecific hybridization trials, Refstie and Gjerdrem (1975), reported that all salmonid hybrids involving Arctic charr were heavier than the better pure bred specimen at eleven months and all other hybrids were lighter than the best pure bred fish. From a hybridization trial between brook charr (*Salvelinus fontinalis*) and Arctic charr, Dumas *et al.* (1995) reported that after first feeding the two reciprocal hybrids grew at approximately the same rate, intermediate to the parental species. Both the hybrids grew faster than Arctic charr but slower than brook charr, suggesting little or no heterosis effects in F_1 hybrids. In another hybridization experiment on Atlantic salmon and brown trout (Genus *Salmo*), McGowan and Davidson (1992) observed that only one of the two hybrids (Atlantic $\text{salmon}_{\text{female}}$ X $\text{brown trout}_{\text{male}}$) grew faster than the two pure crosses, whereas the growth of the reciprocal hybrid ($\text{brown trout}_{\text{female}}$XAtlantic $\text{salmon}_{\text{male}}$) was significantly lower than the pure parents. In hybridization experiments carried out in catfish (Genus *Ictalurid*), it was observed that only one of the two reciprocal hybrids

performed significantly better than the slower growing parent strain (Smitherman *et al.* 1983; Argue *et al.* 2003). Similar results were also observed in a comprehensive hybridization experiment on the common carp (Genus *Cyprinus*) by crossbreeding twelve different genotypes, including backcross hybrids (Bakos and Gorda 1995). These crossing experiments support the results obtained in this study, suggesting that crossing different genotypes of Arctic charr can result in useful heterosis, but not for all crosses.

The four backcrosses generated by the mating of two hybrids and the NL Arctic charr showed little to no absolute growth differences compared with the pure NL cross (Fig. 3.3 ; Appendix V). Moreover, the backcross $YG_fx(YG_fxTR_m)_m$ (i.e. 75% NL female) grew slowest. This might be due to the accumulation of genes fixed for reduced growth rates in the NL population. Conversely, the four backcrosses generated by the mating of two hybrids and the TR Arctic charr exhibited the best growth and possessed the largest growth variances (Fig. 3.4; Appendix V). The backcross $TR_fx(TR_fxYG_m)_m$ (i.e. 75% TR female) grew fastest but the backcross $(YG_fxTR_m)_fxTR_m$ (i.e. 75% TR male) carried the largest variance for weight and length.

Physiologically, these differences in growth could be associated with an early or a late start of first feeding in these Arctic charr. In Atlantic salmon, McCarthy *et al.* (2003) reported significant effects of timing of first feeding on the growth. It was demonstrated that under identical hatchery conditions, early first feeding parr grew faster than late first feeding parr from the same family. In this study, it is possible to consider such a phenomenon. Weekly batch weight data suggest that at the time of first feeding (week 3) juvenile hybrids from all four TR backcrosses were heavier (avg. 0.16g) than the four NL backcrosses (avg. 0.13g) (Appendix XIV). The heavier backcross hybrids (at week 3)

continued to grow faster (avg. 7.3g *vs.* 5.1g) until week 35. The same holds true for the pure TR (0.16g) and NL (0.12g) crosses. The two reciprocal hybrids weighed the same (0.15g) at the start (week 3); however, after the mortality related reduction in stocking density during week 15, the hybrid2 (YG_fxTR_m) started growing more rapidly. Without concrete knowledge on exactly when the first feeding started for each cross and correlation between first feeding and growth in Arctic charr, this hypothesis remains unclear. Regardless, it appears to be a genetic component involved in the faster growth of juvenile Arctic charr.

In the genetic sense, crossbreeding between different selection lines may improve a farm animal by heterosis i.e. non-additive genetic effects. The process of epistasis, positive or negative interactions among alleles at different loci (Wright 1977, as cited by Hedgecock *et al.* 1996) may explain a significant source of this heterosis. However, due to segregation and recombination of gametes from crossbred parents, epistatic advantages present in the F_1 generation may be compromised in subsequent introgression efforts (e.g. F_2, F_n or backcross) (Kirpichnikov 1981; Rieseberg *et al.* 2000). The formation of homozygotes at certain loci can result in the observed losses in epistatic superiority (i.e. heterosis) in subsequent generations (Lutz 2001). One possibility of reduced growth in the NL backcrosses could be attributed to epistatic recombination loss, whereas epistatic gains might have contributed to the enhanced growth in the four TR backcross hybrids. It should also be realized that the TR strain at Icy Waters Ltd. represents the fastest growing known Arctic charr in the world (Moshenko *et al.* 1984), and the genes responsible for fast growth might be fixed in the TR Arctic charr genome. On the other hand, backcrossing F_1 hybrids onto NL would be diluting the genetic contribution of the TR

genome. Therefore, individuals could lose certain desirable genes and/or combinations of genes of interest, which are believed to be more active in TR. Nonetheless, the new additive genetic variance introduced from hybridization may be explored by fish breeders in selection programs (Lutz 2001). Selecting top performing hybrids and their mating with the original parents to produce backcross hybrids could be an excellent alternative for genome homogenization and selection for the best traits from the original parents (Dalton 1985, as cited by Argue *et al.* 2003). Taking advantage of this approach, Argue *et al.* (2003) have successfully demonstrated how a female F_1 hybrid (channel-blue catfish) upon breeding with a male channel catfish can be used to increase dress-out and fillet percentage. It was also reported that a backcross hybrid produced from the mating of a female channel catfish with a male F_1 hybrid did not produce these desirable gains.

For the selection of desired traits, the additive genetic variance obtained by crossbreeding different strains provides an opportunity to detect genetic factors contributing to the cause i.e. large genetic variance is critical in determining QTL effects in species under study (Weller 2001; Asins 2002). Backcross progeny derived from two genetically isolated and phenotypically divergent strains are expected to show either positive (QTL with negative effect) or negative (QTL with positive effect) skewness (Weller 2001). However, the backcrosses studied here did not show any significant skewness and appeared to be normally distributed for their growth (Table 2.1, Table 3.3). These results are rather surprising and may be explained by the early age at which the fish were sampled in February 2003 (Figure 4.1). In fish, growth can be represented by a sigmoid curve (Figure 4.2) (Hopkins 1992). Moreover, similar growth trajectories were observed by Glebe and Turner (1993) in a growth trial on Arctic charr from two different

hatcheries populations. The phenotype of an individual, including its size, shape, and metabolic rate changes with age (Wu *et al.* 2002).

Recently a widely accepted view of the genetic basis of growth proposed that a given set of genes affecting growth is progressively modified. Hence, growth at different ages should be treated as different traits (Wu *et al.* 2002a). The genetic basis of an age-dependent trait analysis is also important from an evolutionary and developmental point of view, which can help in predicting changes to phenotypes within particular environmental contexts (Rice 1997). Several QTL analyses have shown that growth variations may result from the activation or repression of genes responsible for changes in growth (Pletcher *et al.* 1998; Vaughn *et al.* 1999; Promislow *et al.* 2001; Wu *et al.* 2002b). The phenotypic data collected on the same eight backcrosses in January 2004 show much greater variances in weight than the data collected in February 2003 (McGowan 2004). These findings clearly suggest that there are age-dependent factors acting on growth in these hybrid Arctic charr. Hence, there is a potential of finding more QTL effects in a population of mature Arctic charr.

Figure. 4.1. Batch weight over time for the four most informative backcrosses (Cross 1: $(YG_fxTR_m)_fxYG_m$; cross 3: $YG_fx(TR_fxYG_m)_m$; cross 7: $TR_fx(TR_fxYG_m)_m$; and cross 10: $(YG_fxTR_m)_fxTR_m$) over 32 weeks of rearing in hatchery at Icy Waters Ltd., Whitehorse, Yukon, Canada.

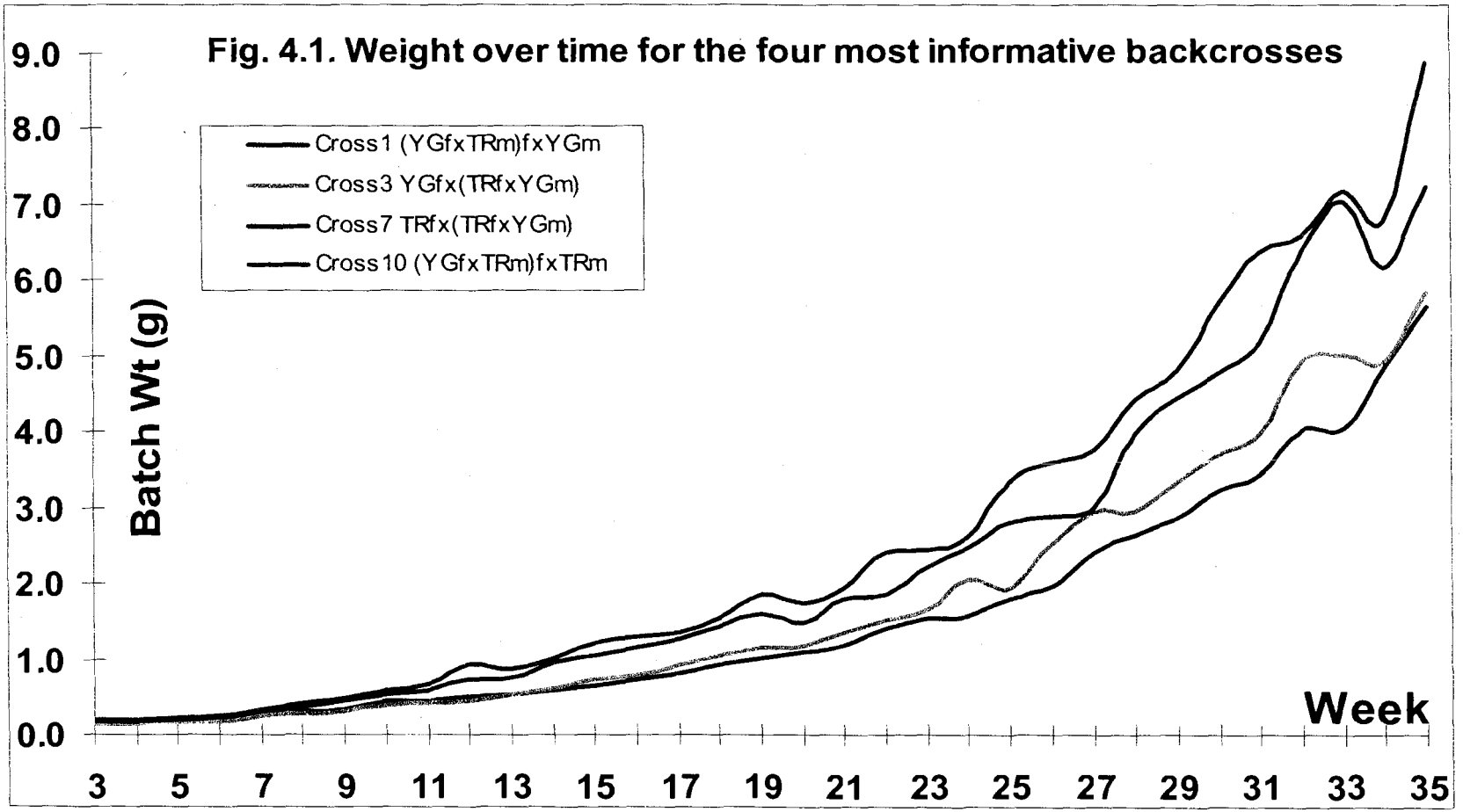
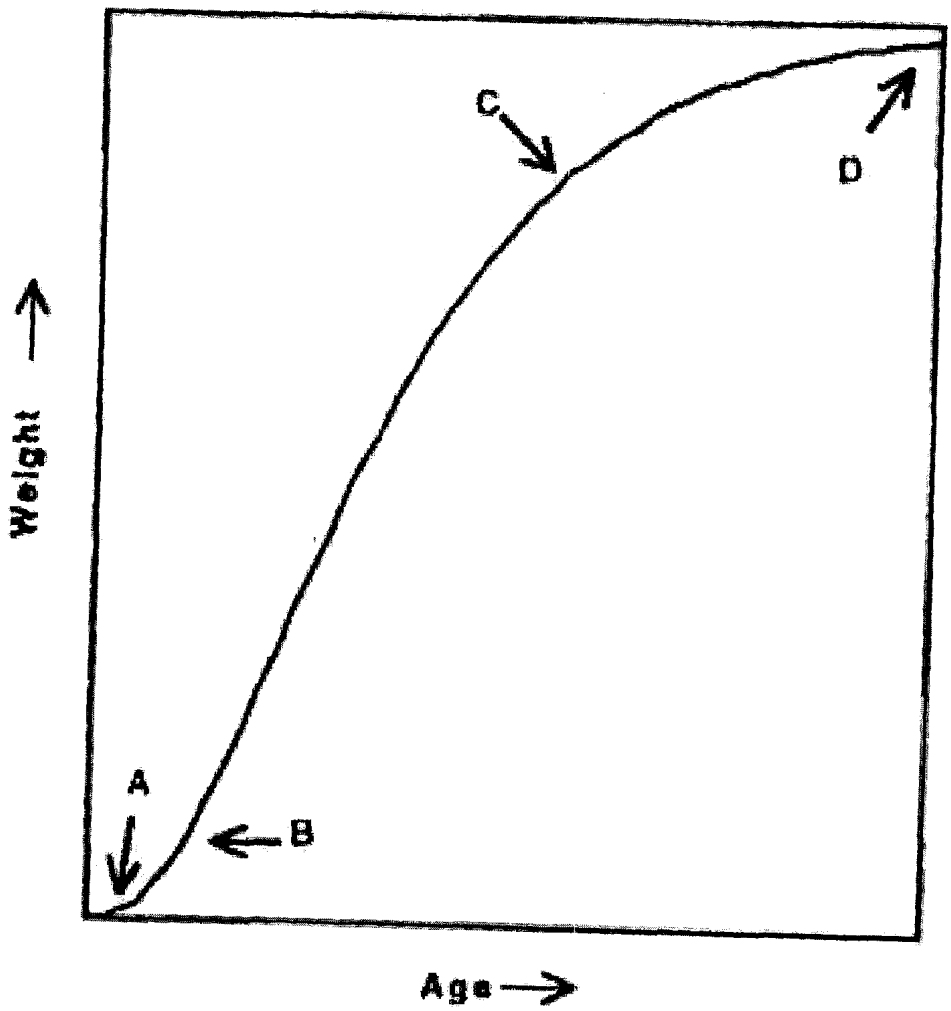


Fig. 4.1. Weight over time for the four most informative backcrosses
Cross1 (YGfxTRm)fxYGm
Cross3 YGfx(TRfxYGm)
Cross7 TRfx(TRfxYGm)
Cross10 (YGfxTRm)fxTRm
Batch Wt (g)
Week
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35

Figure. 4.2. A sigmoid growth curve in fish showing an exponential segment A→C, a relative linear segment B→C, the stabilizing segment C→D, and sigmoid section A→D. Source: Hopkins 1992.


C
D
Weight
A
B
Age

One of the aims of hybridizing the TR and YG Arctic charr at Icy Waters Ltd. was to obtain a fast growing fish with a pleasing body color, which will have a higher market value than the either of the two pure line fish. The observation of the two reciprocal hybrid broodstock (a part of 1996 broodstock), suggests that hybridizing the two pure strains offers a good mechanism for combining the two attributes without compromising the growth rates. Growth data at 11 months did not indicate any reduction in growth due to hybridization. Producing a backcross hybrid by mating F_1 hybrids with the TR Arctic charr offers another option of producing faster growing fish; however, the introgression of genes responsible for the body coloration in these backcross hybrids remain to be observed.

4.1.2 Growth patterns in the ten Tree River backcross families ($YG_fxTR_m)_fxTR_m$) and selecting the best resource family for QTL analysis

One of the two TR backcrosses (($YG_fxTR_m)_fxTR_m$), sampled in February 2003, carried the largest growth variances (TL; 2.1SD and WT; 10.6SD). The detected levels of phenotypic variances were considered large enough to detect QTL effects for growth in these strains of Arctic charr and hence, families derived from this backcross were considered to have the greatest potential to provide substantial information on the genetic basis of growth in Arctic charr.

With the exception of family 10-10, in each family females and males were sampled nearly in equal proportion. The significantly higher female to male ratio (9 *vs.* 3) in family 10-10 might be due to sampling error or could be due to reduced fitness of male

hybrids in this family. However, in the absence of mortality data or without performing any genetic analysis in this family, it is difficult to draw a conclusion on female dominance. There were no signs of a sex-associated affect on the early growth of the juvenile fish (Appendix XI) and therefore the entire family was treated uniformly to test for QTL analysis without discriminating between the two sexes (Liu 1998). However, it has been observed that adult females grow faster than males in the 1996 broodstock of the two pure Arctic charr strains and their reciprocal hybrids (Eric Johnson pers. comm.).

The observed differences in growth among the ten full-sib families from cross 10 could be attributed to family effects. The source of these phenotypic differences could be biological; differences in fecundities or egg sizes. Without the fecundity data on the individual brooder or egg size measurements, it is difficult to infer a possible cause of inter-family differences. Regardless of the cause, however, it is important to realize that without establishing proper relationships among backcross hybrids and their parents, the estimations of QTL effects might be inflated and thus could be misleading (Weller 2001; Slate *et al.* 2002). Furthermore, crosses between two divergent lines capture allele combinations that have segregated into progeny that display varying phenotypic performances.

In fish, the larger phenotypic variation of quantitative traits is due to genetic differences between individuals, rather than associated heritabilities. In fact, higher levels of phenotypic variations are found to be coupled with lower heritabilities, suggesting greater susceptibility to environmental factors (Gjederm 1983). In other words, in fish the contribution of environment-driven variance may be larger than the genetics-driven

inherited variance to the over all variance of a progeny. In an experiment designed to assess the impact of environmental tank effects versus genetic family effect during early growth performance of Atlantic salmon, Herbinger *et al.* (1999) observed significant differences in growth due to environmental differences among tanks rather than genetic differences among families grown in a single tank. Therefore, to eliminate environment related variations, it was crucial to raise all the families under identical culture conditions, i.e. in a single tank for the duration of the trials. Since all families were raised under identical culture conditions, no environment-associated phenotypic variation was considered. Furthermore, raising a large number of full-sib families in separate tanks would have been impractical due to considerations of space and expense.

For the purpose of genome-wide scans to test for association between genetic markers and quantitative traits, it was important to select a highly variable full-sib family. Among six sizable families, family 6-10 (N=36) possessed the greatest phenotypic variance for the three growth parameters, which was very important for analyzing the mechanisms underlying the phenotypic variation caused by genetic factors (Table 3.6). Therefore, family 6-10 was selected for the analysis of growth QTL in these Arctic charr.

4.1.3 Correlation among three growth parameters

Recently QTL analysis is being used as a complementary mechanism to test the relationship between physiological processes or traits (Thumma *et al.* 2001and references therein). Correlations between the related traits is due either to a tight linkage between QTL or to a single QTL that affects multiple traits (Yin *et al.* 2003). Thus, to test whether or not QTL for LT, WT and K_{TL} are on the same chromosomes and share QTL-effects, it

was important to determine the phenotypic correlation between the parameters. Although, TL and WT were highly correlated across six families from cross 10 ($r>0.85$-0.99) and QTL analysis for either TL or WT alone would have been sufficient, a precaution was taken by performing QTL analysis for both TL and WT separately. Furthermore, results from the QTL analyses on the TL and WT showed that if QTL analysis had been performed on either TL or WT alone, some marginal effects would have been missed (Table 3.8). After 13 months of hatchery rearing, significantly high phenotypic correlations were also observed in five full/half-sib Arctic charr families produced by hybridization of domesticated strains of Arctic charr originated from the Fraser River and NL (Somorjai 2001).

Weak correlations ($r<0.68$; $p>0.017$) between TL/WT and K_{TL} in family 6-10 were consistent with other families from cross 10. Furthermore, the number of associations between K_{TL} and marker alleles on six linkage groups and with two unassigned markers (Table 3.8) indicate that K_{TL} and TL or WT might be two unrelated phenotypes. These results are consistent with the observations made by Somorjai (2001) among hybrid families produced from the Fraser River and NL after 13 months of hatchery rearing.

Very similar results obtained on the phenotypic correlation from the parametric and nonparametric tests suggest little or no advantage of comparing allele or genotypic groups by using two types of statistical methods; i.e. parametric and nonparametric tests. Hence, it was decided that using only a nonparametric Mann-Whitney U rank test would be sufficient to perform TDT in the selected family. However, to perform linear

regression analysis it was important to transform the data as the ANOVA is a parametric test and assumes a uniform variance between the groups (Weller 2001; Cann 2003).

4.2 Parentage assignment in the Tree River backcross: $(YG_fxTR_m)_fxTR_m$

The use of molecular markers for parentage assignment in the TR backcross eliminated the need for costly multi-tank rearing of the ten full-sib families. This also helped to avoid confounding environmental effects common to full-sibs. Using eight polymorphic microsatellites, only 64% of the fish (n=321/500) from the cross 10 could be assigned to a unique full-sib family (Table 3.5). The remaining 36% were excluded from further analysis. The proportion of assignment was relatively low compared to an 82% assignment of the 80 offspring from fourteen parental combinations for an Atlantic salmon study conducted for another hatchery population from Canada using the same analytical tools (Dr. McGowan pers. comm.). In a parentage analysis applied on Kurma prawn (*Penaeus japonicus*), Jerry *et al.* (2003) reported that using eight microsatellite markers (average allele per locus; A=13.2), only 80% of the 288 adult shrimps could be assigned to a unique family. The allocation success was lower (47%) while testing nauplii for six loci (A=10.2) in the same breeding experiment. A much higher (95%) correct parentage assignment was reported by Letcher and King (2001) in a study conducted on hatchery released wild Atlantic salmon populations from the Connecticut River, using fourteen microsatellite markers (A=12.0). Using computer simulations, Letcher and King (2001) also demonstrated that in a large population where the mating structure of parents is known, a correct parentage assignment up to 99% could be obtained using eight loci

with eleven alleles per locus. A study published by O'Reilly *et al.* (1998) supported these results.

In this study, the lower assignment rate could have arisen due to possible genotyping errors or the mixing of individuals from other crosses. Here, the best results were obtained when using Ssa85DU, Sfo8LAV, One18ASC, Sal5UG, and SalD39SFU. Although the number of alleles for SalE38SFU (A=11), Sfo23LAV (A=12), and SalP61SFU (A=10) was greater, the discriminatory power was lower than for the other five loci used for parentage assignment in this study. This was probably due to difficulties in genotyping at these dinucleotide (repeats of two) loci. For future work, these loci should be replaced by other easier to score loci, such as loci with no stutter bands or tri-/tetranucleotide (repeats of three or four) loci. The issue of errors in genotyping has been addressed by O'Reilly *et al.* (1998). Besides typing error, the assignment accuracy is also affected by the number of loci and the average number of alleles. In general, assignment accuracy is greatest when using a modest number of loci (8-10) with a modest number of alleles per locus (A=6-8) (Bernatchez and Duchesne 2000; Banks *et al.* 2003).

The size of a family under investigation is also critical in determining reliable QTL estimates and thus the efficiency of future MAS (Moreau *et al.* 1998, 2000). A variable number of fish per family observed in this study requires some attention. Only six out of ten families were sufficiently large (N>35; 10% of 321) to carry out QTL mapping analysis. In a simulation study, Gjerde (2003) reported that only half of the pooled families had ten percent or more fish, while the number of fish per family can be

increased by pooling more fish per mating combination. However, increasing the sample size will result in an increase in the cost of genotyping (Weller 2001), which is undesirable.

4.3 Genetic analysis of 1996 Broodstock

The genetic analysis of the 1996 Arctic charr broodstock at Icy Waters Ltd. is crucial for two main reasons: a) individual tagging for broodstock management and b) to identify genetic factors underlying the fixed phenotypic differences between the two domesticated strains.

4.3.1 Molecular tagging and broodstock management

The molecular tagging of the entire broodstock allows identification of individual fish (Estoup *et al.* 1998), tracking genetic relatedness (Sonesson and Meuwissen 2000), identification of broodstock parents of juveniles (Norris *et al.* 2000), identification of sibs and half-sibs in a mixed-parentage spawning (Letcher and King 2001), establishment of pedigree lines centered around desired expression of certain traits (Kumar and Garrick 2001), identification of genotypic sex of an individual (Schutz and Harrell 1998), identification of QTL and its use in MAS of economically important traits (Ferguson and Danzmann 1998), identification of source population of hatchery stock, (Davidson *et al.* 1989), and the determination of vertical and horizontal lineage should true bio-secure measures ever be implemented at a breeding center (Ferguson 1994; Dunham 2004).

In the present study, molecular and physical tagging of all 1996 broodstock was carried out. A wide genetic variation in the two strains was observed. Allelic diversity,

which may be a more sensitive measure to test genetic variation in short founder populations (Norris *et al.* 1999), was calculated for the four groups. It has been demonstrated that allelic loss may occur faster than the loss of genetic heterozygosity because low frequency alleles contribute little to overall heterozygosity (Allendorf and Ryman 1987; Tessier *et al.* 1997; Norris *et al.* 1999). Therefore, allelic diversity provides a good measure of genetic variability in these Arctic charr.

The NL strains of Arctic charr were produced from fewer wild founders than the TR strain and they had the fewest average number of alleles over six of the eight loci used for molecular tagging (Table 3.1, Figure 3.1, refer to section 1.1.3 for strain history). Furthermore, the fewer alleles observed in the NL strain compared to the TR strain (178 *vs.* 210) at 75 polymorphic microsatellite (Table 3.2) support the hypothesis of reduced levels of genetic variability available in the NL stocks. It is also possible, however, that fish from NL naturally have less genetic variation than fish from TR.

The high number of loci with non-overlapping alleles (n=35; Table 3.2), the number of population-specific alleles, and the presence of different male specific alleles at Sfo8LAV (308 for TR and 271 for NL) suggest that the TR and NL are genetically different strains, and geographic isolation has limited gene flow between them. These results are consistent with population structure studies conducted on Arctic charr from Canadian waters (Lundrigan 2001), and from North America and Europe (Bernatchez *et al.* 1998, 2002; Brunner *et al.* 1998, 2001). Although this study does not compare the loss of genetic diversity due to domestication, comparable genetic data presented by Lundrigan (2001) clearly demonstrated a reduced amount of genetic variation in

aquaculture stocks compared to their wild counterparts of the TR or NL Arctic charr populations in terms of allelic diversity at three markers: Sfo8LAV, Sfo23LAV, and MST85.

The two Arctic charr strains at Icy Waters Ltd. were founded by only a few individuals. Strong founder effects and artificial selection under domestication are known to reduce the genetic variation of hatchery-reared fish stocks (Dickson and MacCrimmon 1982; Cross and King 1983; Crozier 1994; Doyle *et al.*1995; Dowling *et al.* 1996). The loss of genetic variation results in a loss of potential genetic gain (Allendorf *et al.* 1987). In a genetic improvement program, knowing the amount of available genetic variability is imperative and forms the basis of MAS in Arctic charr. In addition, measurement of genetic divergence between the hybridizing strains is also important because intraspecific genetic variation varies as a function of time as strains develop adaptations to local environments and selection pressures (Na-Nakorn *et al.* 1999). Thus, the aim of genotyping the entire 1996 broodstock of Arctic charr at Icy Waters Ltd. was twofold: a) to determine the amount of genetic variability available for selection and b) to measure the genetic divergence between the two reproductively isolated populations.

Another advantage of genotyping the entire 1996 broodstock is to generate baseline data, which are crucial in identifying sibs and avoiding inbreeding while establishing pedigrees for future breeding (Taniguchi *et al.* 1999; Norris *et al.* 2000; Bentsen and Olesen 2002). Therefore, it is important to maintain the genetic variability in the broodstock. This should be assessed at regular intervals and individuals carrying rare alleles could provide a good measure of the amount of the amount of genetic variation

present in broodstock at any given time (Crozier 1994; Woods *et al.* 1996). Without proper management, the genetic diversity of domesticated stocks can be lost in only a few generations (Cross and King 1983; Waples 1991; Cross *et al.* 1998; Evans *et al.* 2004). Reduction in genetic variation has been shown to be detrimental to commercially important characteristics such as growth rates (Koehn *et al.* 1998), fitness (Danzmann *et al.* 1989), and disease resistance (Palti *et al.* 1999) in fish.

4.3.2 Male-specific microsatellite marker-allele

In the present study, two male-specific marker-alleles at a microsatellite (Sfo8LAV) were observed in the two Arctic charr strains. At this locus NL males (NL_m) carried Sfo8LAV-271, whereas, TR males (TR_m) had the Sfo8LAV-308 allele. All the brood males examined were heterozygous at this locus. Furthermore, the localization of Sfo8LAV on the linkage group AC-4 (Woram *et al.* 2004) is evidence of the sex specificity of this marker (Appendix X). This is the first observation of a fixed association between a microsatellite marker-allele and male sex in a salmonid species (Dr. Davidson pers. comm.; Devlin and Nagahama 2002). These findings have both aquacultural and evolutionary implications for Arctic charr.

In fishes, genetic sex can be permanently reversed by exposure to androgens or estrogens during early juvenile development. Depending upon goals, monosex populations can be produced by crossing experimentally produced all male or all female populations with normal male or females. However, one common problem of such manipulations is lack of consistency i.e. a 100% male or female population is difficult to obtain (Mair *et al.* 1997b; Bongers *et al.* 1999). In addition, growing individuals of the

undesirable sex unnecessary inflates the cost of operation at a breeding facility (Hunter *et al.* 1983).

Sexual dimorphism, where one sex grows faster than the other, is one of the primary objectives for the production of monosex populations in aquaculture (Shelton 1986; Mair *et al.* 1997a). The manipulation of sex to produce genetically male tilapia (GMT) has been extremely successful (96.5% male) (Mair *et al.* 1997b). Among salmonids, sexual manipulation to produce all female populations started in rainbow trout (Mair *et al.* 1997a). It has been observed that in Arctic charr adult females grow faster than males (Eric Johnson pers. comm.). Identifying the genetic sex of a sex-reversed individual early during juvenile development will not only be economical but will also increase the success rates in sex reversal processes. Removing genetic males from an androgen treated population of juvenile Arctic charr would leave only sex-reversed females (males with XX genotype) behind (Schutz and Harrell 1999). Hybridizing sex-reversed female with normal Arctic charr female will give rise to a 100% female progeny (Bonger *et al.* 1999; Schutz and Harrell 1999). Additionally, by producing an all female population, sexual maturity can be delayed in protandrous Arctic charr (Moshenko *et al.* 1984). This also means that harvest size can be reached before the onset of sexual maturity (O'Malley *et al.* 2003). In salmonids, during the course of gonadal development, the pigment starts relocalizing from muscles (filet) to ova in females (Youngson *et al.* 1997) and to the dermis in males. Therefore, harvesting fish prior to the onset of sexual maturity is critical in terms of retaining pigment in the tissue. Trials of hormonally

induced sex reversal in Arctic charr have been achieved at the Icy Waters Ltd. (Dr. McGowan pers. comm.).

Besides identification of sex, the marker-alleles Sfo8LAV-271 and Sfo8LAV-308 were also useful in the identification of the lineage of an anonymous hybrid fish at Icy Waters Ltd. For example, the fish with PIT tag # 497249), which was believed to be Hybrid1 male (YG_fXTR_m) was in fact a hybrid 2 male (TR_fXYG_m) based on its genotype at the Sfo8LAV locus.

For evolutionary purposes, these findings provide a useful tool for examining sex linkage in Arctic charr and salmonids in general. Following the inheritance of sex specific microsatellite markers in heterogametic males and the examination of genomic regions flanking these microsatellites can provide important insights into the evolutionary processes that are acting on the sex-chromosome structure of Arctic charr, and ultimately, can yield information on the conservation of the sex-determination process among salmonids and fish in general (Phillips and Rab 2001; Devlin and Nagahama 2002; Harvey *et al.* 2002).

4.3.3 Introgressive hybridization between two divergent populations

Knowing whether two populations are in karyotypic complement has significant bearing on long-term hybrid introgression programs (Goel 2000). Karyotypic information addresses the issue of reproductive competence and thus the hybrid fertility/sterility. Despite the fact that Atlantic salmon and brown trout having significantly different karyotypes (Phillips and Rab 2001), the F_1 hybrids between the two species are viable

and may be fertile (Refstie and Gjerdrem 1975; McGowan and Davidson 1992); however, their backcross hybrids were viable but completely sterile (Johnson and Wright 1986; Galbreath and Thorgaard 1995). It was hypothesized that the disparate parental karyotypes of the F_1 hybrids (brown trout 2n=80, Atlantic salmon 2n=54-60) most likely caused disruption of meiosis (Johnson and Wright 1986). Similar results have also been noticed in other fish species (Johnson and Wright 1986 and references therein). Therefore, knowledge of the reproductive competence of backcross hybrid Arctic charr is critical for the future of hybrid introgression. The F_1 hybrids produced by the mating of TR and NL Arctic charr are fertile.

Stock-specific karyotypic differences in the size, number and chromosome positions of the nucleolar organizer regions (NOR) exist in Arctic charr (Phillips *et al.* 1988). In addition to Arctic charr, stock-specific variations in NOR size have also been observed in other species of the genus *Salvelinus*. In a study of six populations of lake trout (*Salvelinus namaycush)* from the Great Lakes, even though chromosome arm number (NF) was the same for all six populations, significant NOR polymorphism was detected (Phillips *et al.* 1989). Similar chromosomal variations also exist in other salmonids (Phillips and Rab 2001). Variations in the size of NOR at homologous chromosomes, which probably occurs as a result of unequal crossing-over affects the frequency of recombination in this region (Reed and Phillips 1997), may account for inter-individual differences in genome size (Lockwood and Derr 1992) and hence, may affect the reproductive success of fish. Therefore, it is important to know whether or not the backcross hybrids produced in this study are fertile. Fertile backcross hybrids will be

an asset for performing a series of introgression experiments in these Arctic charr. On the other hand, infertile backcross hybrids will leave us with an opportunity to grow TR backcross hybrids without worrying about the dangers of interbreeding between farmed fish and wild stocks (Waples 1991; Fleming and Gross 1992).

4.4 QTL analysis in Icy Waters Arctic charr

To search for growth-QTL in Arctic charr, a genome wide scan involving markers on 27 linkage groups was performed. To increase the coverage, 17 unassigned markers were also tested for their association with growth parameters. Thirteen of the of the 62 microsatellite tested, which were found to be informative in two pure populations, were uninformative in family 6-10. Five microsatellite loci (MST85, Omy38DU, SapI26SFU, Ssa208, and Ssa20.19NUIG), which were found to be duplicated in Icy Waters Arctic charr, further increased coverage of the Arctic charr genome. However, the linkage groups of unassigned and duplicated loci remain to be determined. Overall, a significant proportion of the Arctic charr genome was covered, meeting a basic criterion for the application of genome-wide scans for QTL analysis (Liu 1998; Ashwell *et al.* 2001; Hirooka *et al.* 2002). Eighteen significant allele-trait associations ($p<0.05$) at eight linkage groups (AC-1, AC-6, AC-8, AC-13, AC-15, AC-20, AC-25, and AC-36) were detected under the single-QTL single-marker model of QTL analysis (Table 3.8). Additional QTL may be linked to three unassigned markers: OMM1037, Ssa171, and Ssa208b.

In this study, no significant association was detected on AC-4, which was reported to be carrying several growth-QTL in Arctic charr (Woram pers. comm., as cited by

Somorjai 2001). Similarly, this study did not detect an association between growth and AS1.22 (unassigned), Omy77DU (AC-12), Sfo23LAV (unassigned), SSOSL456 (AC-29), or μ5.27NUIG (unassigned), which were reported to be associated with growth in previous analyses (Johansen *et al.* 1998; Johansen 1999). Somorjai (2001) also did not detect any association at SSOSL456. The broodstock used to produce families in the previous two studies was, however, derived from the Fraser River population (Johansen 1999; Somorjai 2001). These findings suggest that there is no major QTL at these locations affecting growth in Arctic charr. Further analyses of other variable families, however, are required to confirm these results and to determine the usefulness of these loci in future QTL analyses for growth related traits.

4.4.1 QTL for TL/WT and K_{TL}

Marginally significant associations between LT/WT and marker alleles on three linkage groups (AC-6/-8, AC-20, and AC-25) were detected in Arctic charr from Icy Waters Ltd. In addition, a maternal allele-effect is also evident at OMM1037. QTL for WT/TL have also been reported by Somorjai (2001) at these locations. These findings support the existence of growth-QTL on their respective linkage groups, rendering them as candidate locations to look for major QTL affecting growth in Arctic charr (Liu 1989; Long and Langley 1999; Robinson *et al.* 2001; O'Malley *et al.* 2003).

A marginal effect at BHMS206 is consistent with the findings of Somorjai (2001). On the Arctic charr linkage map, BHMS206 was reported as a duplicated locus and was mapped onto two linkage groups: BHMS206(ii) on AC-6 and BHMS206(i) on AC-8 (Woram *et al.* 2003). From the results obtained here, however, it is not clear which

linkage group the effect is associated with. Unfortunately, another marker BHMS330 from the linkage group AC-8 was uninformative in family 6-10, whereas, One8ASC (AC-6) did not show any TL-/WT-QTL effect in this study. Another association ($p<0.052$) at OmyRGT4TUF (AC-20) appears to be critical, as this locus contributes 12-13% to the total phenotypic variance for the growth, which is comparable with other markers showing significant associations with LT/WT. A highly significant QTL effect at OmyRGT4TUF ($R^2>20\%$) has also been reported in Fraser River Arctic charr by Somorjai (2001). However, results at SalD100SFU (AC-20) did not corroborate the findings of Somorjai (2001). At SalD100SFU, the two parents in family 6-10 were heterozygous for the same alleles, and the genotypic ratio of the progeny did not follow a typical Mendelian inheritance. On the linkage group AC-25, of the five markers tested, three associations between TL/WT and marker-alleles (BHMS121-131, BHMS490-109, and OmyRGT38TUF-118) were observed (Table 3.9; Figure 4.3). Furthermore, these markers contributed up to 26.3%, a maximum for any locus studied, of the total phenotypic variance of LT/WT (Table 3.9; Figure 4.4). The significant associations at these locations have also been reported in Fraser River Arctic charr (Somorjai 2001). In contrast, a significant association at SalD39SFU remained undetected in family 6-10 from this study. Recent reviews on QTL estimation revealed that different mapping populations generally share only small sets of common alleles (Kearsey and Farquhar 1998; Lynch and Walsh 1998) and thus very few QTL-marker associations are expected to be valid in a whole gene pool of a species or in an extensive breeding program comprising genetically diverge populations. Hence, these observations strongly advocate

for a species-specific major growth QTL in these regions (AC-6/-8, AC-20, and AC-25) of the Arctic charr genome (Malek *et al.* 2001).

Thirteen marker-K_{TL} associations ($p<0.05$) were detected in this study (Table 3.8). Furthermore, a marginal allelic variation ($p<0.053$) was detected at Sal5UG. These K_{TL}-QTL effects span seven linkage groups on the current Arctic charr linkage map. A significant association was also observed with an unassigned marker Ssa208b (paternal allele) (Table 3.8). In addition, at Ssa208b the effect-size is maximum (24.4%). Maternal allele-effects are larger than paternal allele-effects (15% *vs.* 12%) for BHMS490 and OmyRGT39TUF (AC-25). Unlike TL/WT, however, the contribution of the locus BHMS121 from the same linkage group is negligible to the variation of K_{TL}. QTL effects at these locations have not been reported previously. The existence of a K_{TL}-QTL effect (maternal allele) at Ssa77NUIG and Ssa85DU are consistent with the findings of Somorjai (2001). Although the association at One8ASC (AC-6; genotypic) is marginal, the amount of variance (15.7%) explained at this location is second only to Ssa208b. These findings suggest the existence of major QTL in the vicinity of One8ASC and Ssa208b. A marginal association at OmyRGT4TUF (AC-20) appears to be a statistical artefact, as the R^2 at this location is very low. Furthermore, no K_{TL}-QTL has been reported previously at the linkage group AC-20 (Somorjai 2001). Overall, the variance for loci exhibiting marginal associations with K_{TL} was ~10-15% for maternal allele contribution and ~10-24% for paternal allele contribution.

A higher number of linkage groups associated with QTL effects for K_{TL} (eight) than for TL/WT (six) and weak correlations between K_{TL} and TL ($r<0.53$), and K_{TL} and

WT ($r<0.68$) support the presence of QTL for K_{TL} and TL/WT in different chromosomal regions. In addition, genes responsible for the K_{TL} of fish might be more widespread than for the TL or WT of fish. Furthermore, genes responsible for the TL and WT in fish might be co-localized and could have evolved under similar selection pressures in the TR Arctic charr. Similarly, genes governing K_{TL} would have been selected independently in the NL Arctic charr at Icy Waters Ltd. In other words, according to the oligogenic model reviewed by Tanksley (1993), LT/WT appear to be a continuous variation as a result of few loci with very large effects (Risch 2002), whereas, the K_{TL} might follow Fisher's infinitesimal model, where quantitative traits are controlled by a very large number of loci, each with a small phenotypic effect (Lander and Bottstein 1989; Risch 2002). Regardless, testing the number of QTL effects and the magnitude of these effects on quantitative traits facilitates an understanding of the underlying genetics of these traits (Falconer and Mackay 1996) and provides an opportunity to exploit the knowledge for the MAS of economically important quantitative traits (Knapp 1998; Andersson 2001; Liu *et al.* 2003).

Figure 4.3. In family 6-10, probability estimates (TDT) showing significant QTL-effects for total length (TL), body weight (WT), and Fulton's condition factor (K_{TL}) for three microsatellite markers (in bold) mapped on the Arctic charr linkage group AC-25 (modified from Woram *et al.* 2004). Map distances calculated for family 6-10 are given in Appendix XIII. A putative growth-QTL is shown in red. Chromosome-wide null rejected at $p<0.0125$ (Bonferonni 0.05/4).

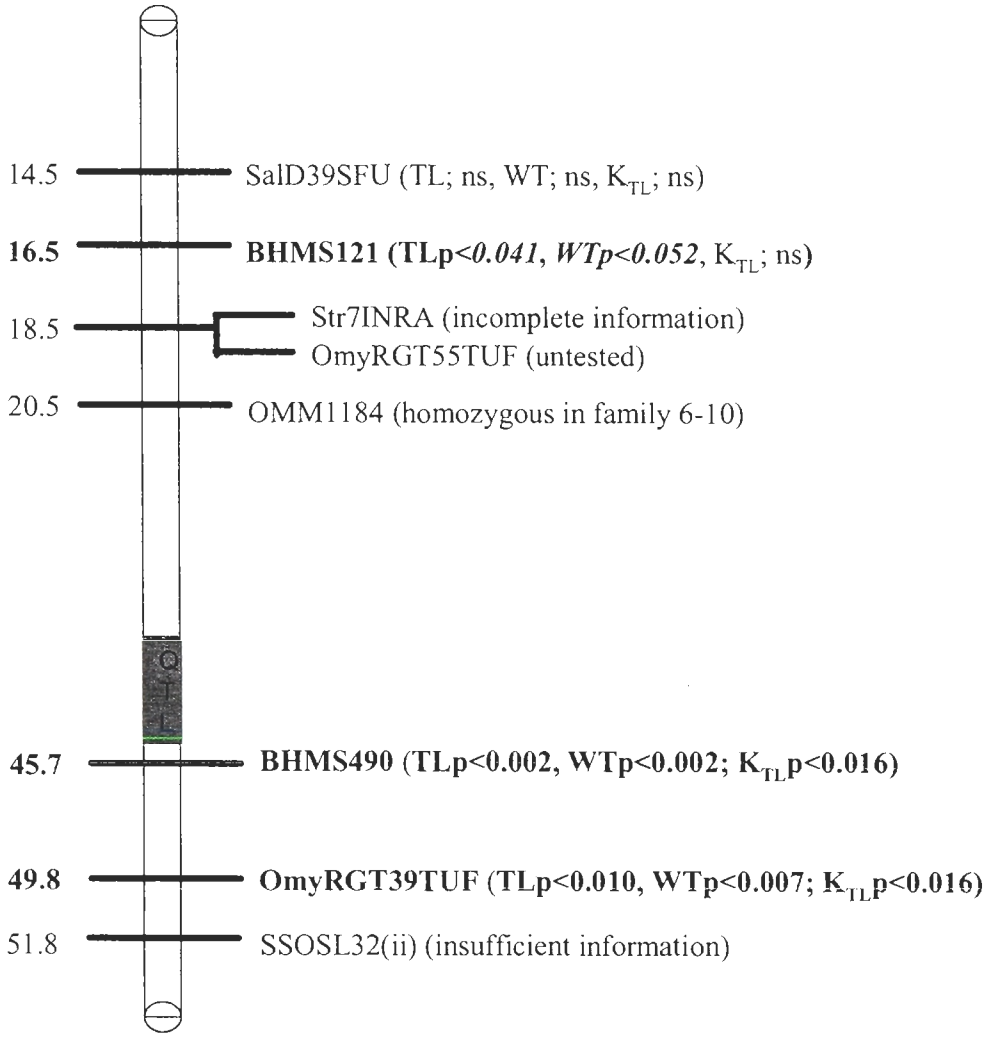
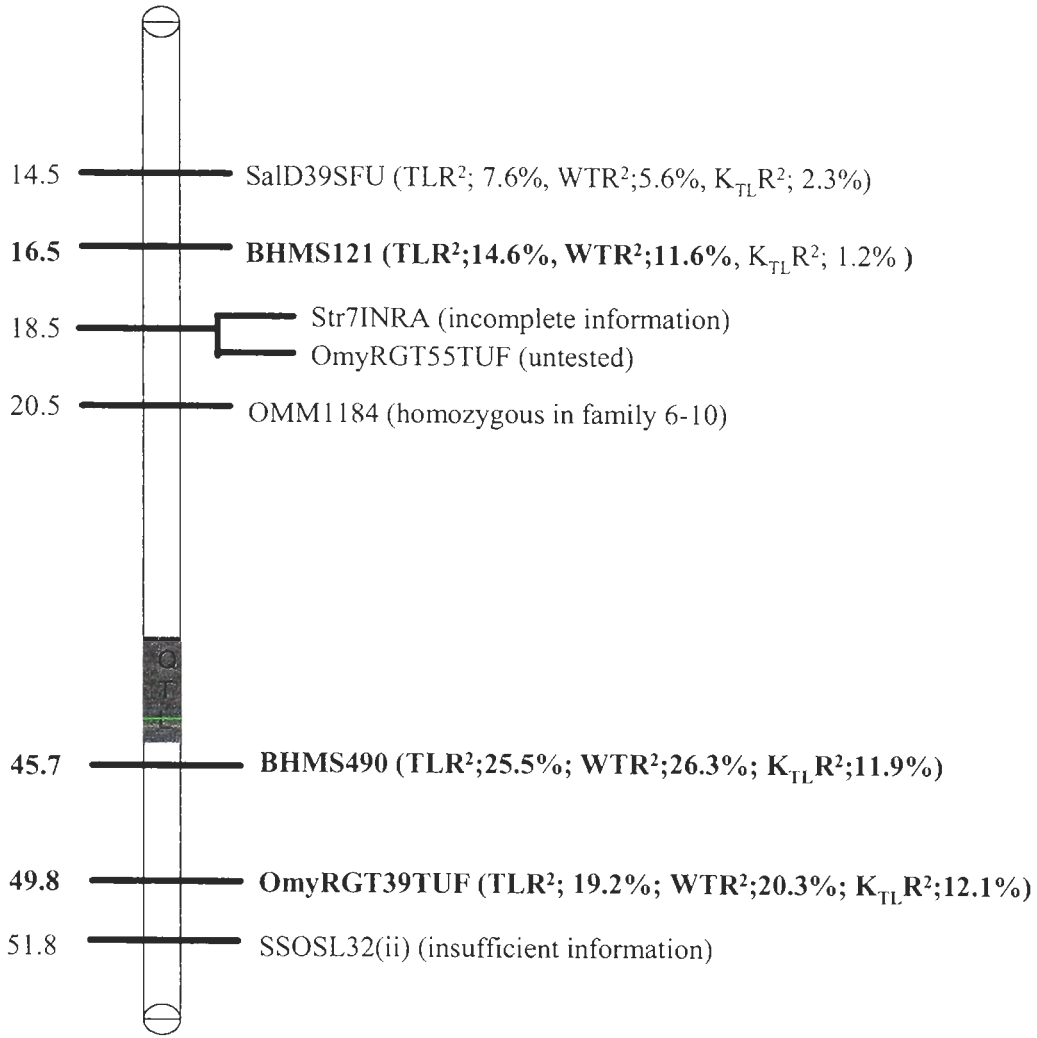

14.5
SalD39SFU (TL; ns, WT; ns, K_{TL}; ns)
16.5
BHMS121 (TLp<0.041, WTp<0.052, K_{TL}; ns)
18.5
Str7INRA (incomplete information)
OmyRGT55TUF (untested)
20.5
OMM1184 (homozygous in family 6-10)
45.7
BHMS490 (TLp<0.002, WTp<0.002; K_{TL}p<0.016)
49.8
OmyRGT39TUF (TLp<0.010, WTp<0.007; K_{TL}p<0.016)
51.8
SSOSL32(ii) (insufficient information)

Figure 4.4. In family 6-10, the amount of phenotypic variation explained by the LRM for total length (TLR^2), body weight (WTR^2), and Fulton's condition factor ($K_{TL}R^2$) as contributed by the linkage group AC-25, (modified from Woram *et al.* 2004). Map distances calculated for family 6-10 are given in Appendix XIII. A putative growth-QTL is shown in red.


14.5
SalD39SFU (TLR²; 7.6%, WTR²;5.6%, K_TLR²; 2.3%)
16.5
BHMS121 (TLR²;14.6%, WTR²;11.6%, K_TLR²; 1.2%)
18.5
Str7INRA (incomplete information)
OmyRGT55TUF (untested)
20.5
OMM1184 (homozygous in family 6-10)
45.7
BHMS490 (TLR²;25.5%; WTR²;26.3%; K_TLR²;11.9%)
49.8
OmyRGT39TUF (TLR²; 19.2%; WTR²;20.3%; K_TLR²;12.1%)
51.8
SSOSL32(ii) (insufficient information)

4.4.2 Chromosome-wide QTL-effects for growth on AC-25

Examination of the coefficient of variance (R^2) for each locus on AC-25 indicates decreasing QTL effects on either side of BHMS490. This observation is supported by the probability estimates obtained by TDT ($p<0.0125$). SalD39SFU, which is furthest away from BHMS490, did not show any significant effect between the two allele groups. Also, the effect-size at SalD39SFU was nearly half that seen at BHMS121 (Figure 4.3 & 4.4)Despite being 29.2cM away from BHMS490, BHMS121 contributed ~11-15% to the total variance for LT/WT at this linkage group. OmyRGT39TUF, which is only 4.1cM away from BHMS490 explained 20% of the variance. Thus, logically a putative growth-QTL is expected to be located between BHMS121 and BHMS490, but closer to BHMS490. Evidently as the map distance increases, the covariance of the trait values becomes less dependent on the value of the coefficient of variance at the map location and so the value of QTL variance component will decrease with distance away from the actual QTL (Andersson *et al.* 1994; Knott *et al.* 1998; Piepho 2000). Several studies in plants and animals have proved that QTL resolution is most accurate within 10cM from the actual QTL (Lande and Thompson 1990; Piepho 2000). In addition, to identify the actual gene underlying the QTL-effects, mapping to within 1 to 3cM is required (Asins 2002; Pagnacco and Carta 2003). Therefore, to get precise estimates of growth-QTL on this linkage group, fine mapping of the region spanning BHMS121 and OmyRGT39TUF is required, which will be possible after increasing the marker density on this linkage group in Arctic charr.

In an extensive analysis at 201 microsatellites on two year old rainbow trout, O'Malley *et al.* (2003) reported a marginal growth-QTL (male inheritance) associated

with SalD39SFU. Two of the three markers (SalD39SFU and Str7/INRA) mapped on the linkage group S-male in rainbow trout have also been mapped onto AC-25 in Arctic charr (Woram *et al.* 2004). This provides marginal evidence that linkage groups S-male in rainbow trout and AC-25 in Arctic charr are ancestral homologues containing detectable QTL for the same trait (growth). These findings, provide the first evidence for the detection of a QTL affecting growth in salmonids. Evidence of the existence of homologous linkage groups containing two upper temperature tolerance QTL (UTT-QTL) in rainbow trout and Arctic charr have been provided (Somorjai *et al.* 2003). These results also signify the importance of conservation of microsatellite loci among fish species over the past 470 million years (Rico *et al.* 1996).

4.4.3 Comparative mapping approach for QTL detection

Comparative mapping has proven to be a very effective approach to identify QTL in both experimental and commercial populations. However, high-density genome maps with comparable polymorphic markers are essential for such projects (Kutzer *et al.* 2003; Liu *et al.* 2003). Kappes (1999) described the process of identifying QTL for reproductive traits in sheep, pigs, and cattle. In an excellent example of the utility of comparative genomics, Johansson-Moller *et al.* (1996) demonstrated how loci controlling color and patterning in the mouse have similar effects in livestock.

In fish, Sakamoto *et al.* (1999) hypothesized that provided a QTL is functionally conserved, the heterologous primers, which amplify a locus associated with a QTL, may also identify similar QTL-effects in species of common origin. Sakamoto *et al.* (1999) identified One14ASC and Ssa85DU associated with UTT in rainbow trout. Both

One14ASC and Ssa85DU are heterologous to rainbow trout. In this study, twelve out of thirteen marker-QTL associations were detected using heterologous primer sets, being cloned from Atlantic salmon, rainbow trout, and sockeye salmon. The findings of Sakamoto *et al.* (1999) and results obtained in this study, advocate for the hypothesis of functional conservation of major QTL regions in the genomes of salmonids. Taking advantage of this phenomenon, a comparative mapping project for UTT-QTL in Atlantic salmon is in progress (Dr. Davidson pers. comm.). Therefore, salmonids, which are believed to be derived from a common tetraploid ancestor ~25-100 million years ago (Allendorf and Thorgaard 1984), offer a good model for comparative genomic studies following a duplication event. Comparing linkage maps of salmonids has elucidated both molecular marker-based homology and significant divergence between species (Somorjai 2001; Woram *et al.* 2001; O'Malley *et al.* 2003; Somorjai *et al.* 2003; Woram *et al.* 2003). Broad homologies among chromosomes of different species can be determined by fluorescent in-situ hybridization (FISH) (Phillips and Ihssen 1985). Thus, through in-situ hybridization of mapped markers, syntenic regions can be assigned to specific chromosome pairs (Phillips and Rab 2001). In this case, probes specific to the conserved flanking regions of SalD39SFU and Str7/INRA can be labeled with a fluorescent tag and hybridized to metaphase chromosome spreads of various salmonids species (Dr. Noakes pers. comm.), thus establishing the comparative relationship between the genes located on the respective chromosomes.

4.5 MAS in Icy Waters Arctic charr

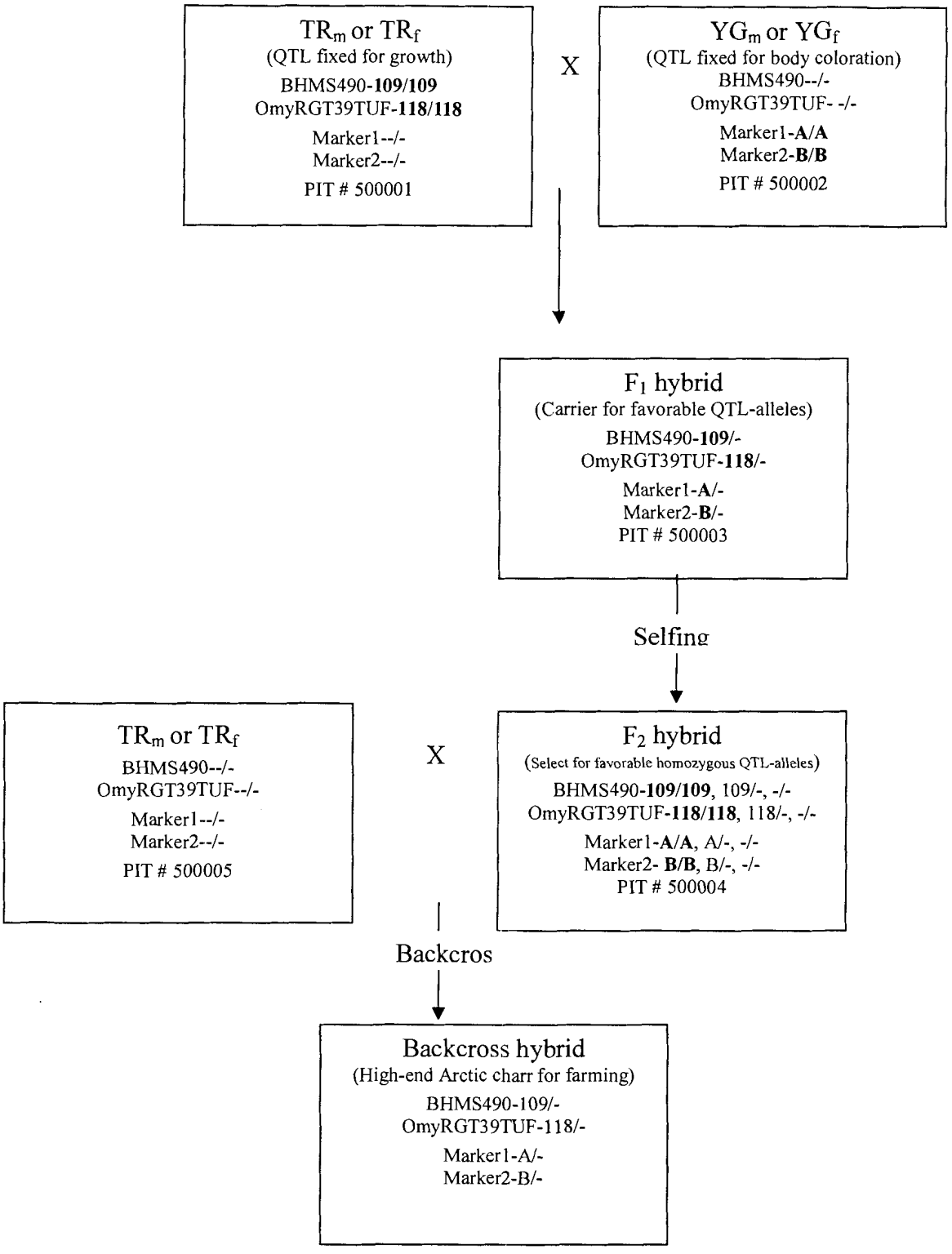
There are two possible approaches for the application of molecular markers in Icy Waters Arctic charr. Genotyping of 1996 Arctic charr broodstock at eight DNA markers

provided a unique fingerprint of 848 broodfish at Icy Waters Ltd. By combining genotypic information with the unique PIT tag numbers, genetic relationships between individual fish were established by Dr. Colin McGowan, and this can assist in avoiding mating of closely related individuals. More importantly, this molecular information offers a great potential for an accelerated improvement of commercially important traits in Arctic charr via MAS. Once the linkage phase of marker and QTL-alleles are determined, spawners carrying the maximum numbers of high-performance QTL-associated alleles can be selected for future breeding (Hallerman and Beckmann 1988). Breeding efficiencies can be improved by combining the QTL building and phenotypic selection. QTL building aims to establish lines that combine favorable alleles (linked to genes) from different lines. To be effective and superior over phenotypic selection, selection based on QTL information must be combined with selection on phenotype (Hospital *et al.* 1997; Dekkers and Hospital 2002). Otherwise, epistatic effects, gene-environment interactions, genetic recombination between marker-allele and the QTL, and incomplete information on the role of genes involved with a trait-QTL may seriously affect the results of MAS (Martyniuk 2001; Dekkers and Hospital 2002). Additionally, due to repulsive epistatic interactions with other genes, the performance of QTL-alleles may be altered in different genomic backgrounds (Danzmann *et al.* 1999). Therefore, marker-QTL associations should also be re-evaluated every few generations in species that demonstrate high rates of recombination (Lande and Thompson 1990).

The primary goal of the hatchery operations at Icy Waters Ltd., Whitehorse, is to produce fast growing Arctic charr with a pleasing body color. Based on the information

obtained from this study, a schema for an efficient application of MAS in two moderately inbred selection lines of Arctic charr is proposed (Figure 4.5).

Figure 4.5. A proposed MAS scheme for hybrid introgression of QTL (marker-alleles) responsible for growth and coloration in the Arctic charr at Icy Waters Ltd. Marker-genotypes in bold are preferentially selected for. BHMS490-109; favorable allele 109 at locus BHMS490, and OmyRGT38TUF-118; favorable allele 118 at locus OmyRGT39TUF.


TRm or TRf
(QTL fixed for growth)
BHMS490-109/109
OmyRGT39TUF-118/118
Marker1--/-
Marker2--/-
PIT # 500001
X
YGm or YGf
(QTL fixed for body coloration)
BHMS490--/-
OmyRGT39TUF- -/-
Marker1-A/A
Marker2-B/B
PIT # 500002
F1 hybrid
(Carrier for favorable QTL-alleles)
BHMS490-109/-
OmyRGT39TUF-118/-
Marker1-A/-
Marker2-B/-
PIT # 500003
Selfing
TRm or TRf
BHMS490--/-
OmyRGT39TUF--/-
Marker1--/-
Marker2--/-
PIT # 500005
X
F2 hybrid
(Select for favorable homozygous QTL-alleles)
BHMS490-109/109, 109/-, -/-
OmyRGT39TUF-118/118, 118/-, -/-
Marker1-A/A, A/-, -/-
Marker2- B/B, B/-, -/-
PIT # 500004
Backcros
Backcross hybrid
(High-end Arctic charr for farming)
BHMS490-109/-
OmyRGT39TUF-118/-
Marker1-A/-
Marker2-B/-

4.6 Summary

Significant measurable differences for growth and DNA polymorphism exist between the two Arctic charr strains reared at Icy Waters Ltd. Hybrid juveniles with 50% or more Tree River genome contribution grow significantly faster than their counterparts from Nauyuk Lake. Considering the significant founder effects in these Arctic charr, adequate but moderate levels of genetic variation exist in the two domesticated strains of Arctic charr at Icy Waters Ltd., and private and non-overlapping alleles can be observed at several loci in the two strains. Two male specific marker-alleles at Sfo8LAV will be invaluable to monitor sex-reversal experiments when producing mono-sex populations of Arctic charr at Icy Waters Ltd.

The genome-wide scan is a powerful approach for identifying QTL of economic importance and for investigating the genetic basis of complex traits in fish populations exhibiting noticeable phenotypic and genetic differences. In Arctic charr, genetic factors for length and weight appear to be clustered together. However, genes regulating body girth appear to be distributed across several chromosomes. The discovery of a reliable growth-QTL on AC-25 across isolated populations of Arctic charr (Fraser River and Tree River), and the possible existence of a homologous QTL in rainbow trout, highlights the need for a comparative functional genetic analysis at conserved QTL loci in salmonids. Furthermore, amplification of QTL-associated microsatellite using heterologous primer sets provides further evidence for the common ancestry of salmonid species, which has evolutionary implications.

Given the breadth of phenotypic and genetic variation present in the two domesticated strains of Arctic charr at Icy Waters Ltd., for the first time the usefulness of

MAS for an accelerated improvement of growth and coloration in Arctic charr can be determined within a reasonable timeframe. Hence, the future of MAS for growth and other desirable traits appears to be promising for the advancement of Arctic charr aquaculture in Canada.

References

Aksnes A., B. Gjerdeand, S.O. Roald. 1986. Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. Aquaculture 53: 7–20.

Allen S.K., R.G. Thiery and N.T. Hagstrom 1986. Cytological evaluation of the likelihood that triploid grass carp will reproduce. Trans. Amer. Fish. Soc. 115: 841-848.

Allendorf F. and G. Thorgaard. 1984. Polyploidy and the evolution of salmonid fishes. Pp. 1–53 in B. J. Turner, ed. The evolutionary genetics of fishes. Plenum Press, New York.

Allendorf F. and N. Ryman. 1987. Genetics management of hatchery stocks. In Ryman & Utter (Ed). Population Genetics & Fishery Management, pp.141-160.

Allendorf F., N. Ryman and F. Utter. 1987. Genetics and fisheries management: past, present and future. In Ryman & Utter (Ed). Population Genetics & Fishery Management, pp.1-20.

Andersson L. 2001. Genetic dissection of phenotypic diversity in farm animals. Nature Reviews Genetics 2: 130-138.

Andersson L., C. S. Haley, H. Ellegren, S. A. Knott, M. Johansson, K. Andersson, L. Andersson-Eklund, I.Edfors-Lilja, M. Fredholm, I. Hansson, J. Hakansson and K. Lundstrom. 1994 Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science 263: 1771-1774.

Angers B., L. Bernatchez, A. Angers and L. Desgroseillers. 1995. Specific microsatellite loci of brook charr (*Salvelinus foninalus* (Mitchill)) reveals strong population subdivision on microgeographic scale. Journal of Fish Biology 47 (Suppl. A): 177-185.

Ashwell M.S., C.P. Van Tassell and T.S. Sonstegard. 2001. A genome scan to identify quantitative trait loci affecting economically important traits in a US Holstein population. J. Dairy Sci. 84: 2535-2542.

Asíns M. J. 2002. Present and future of quantitative trait locus analysis in plant breeding. Plant Breeding 121(4): 281-291.

Azuma T., K. Takeda, T. Doi, K. Muto, M. Akutsu, M. Sawada and S. Adachi. 2004. The influence of temperature on sex determination in sockeye salmon *Oncorhynchus nerka*. Aquaculture 234: 461-473.

Baker R. F. and G.B. Ayles. 1986. Effects of temperature, size and rations on the growth of strains of Arctic charr in intensive aquaculture. Aquaculture 57: 360-366.

Bakke T.A. and P.D. Harris. 1998. Diseases and parasites in wild Atlantic salmon (*Salmo salar*) populations. CJFAS 55(Suppl. 1): 247–266.

Bakos J. and S. Gorda. 1995. Genetic improvement of common carp strains using intraspecific hybridization. Aquaculture 129: 183-186.Argue B.J., L. Zhanjiang, R.A. Dunham. 2003. Dress-out and fillet yields of channel catfish, *Ictalurus punctatus*, blue catfish, *Ictalurus furcatus* and their F1, F2 and backcross hybrids. Aquaculture 228: 81-90.

Banks M.A., W. Eichert and J.B. Olsen. 2003. Which genetic loci have greater population assignment power? Bioinformatics 19(11): 1436-1438.

Baroiller A. J.-F., Y. Guiguenb and A. Fostierb. 1999. Endocrine and environmental aspects of sex differentiation in fish. Cellular and Molecular Life Sciences 55: 910-931.

Bartfai R., S. Egedi, G.H. Yue, B. Kovacs, B. Urbanyi, G. Tamas, L. Horvath and L. Orban. 2003. Genetic analysis of two common carp broodstocks by RAPD and microsatellite markers. Aquaculture 219: 157-167.

Beaumont A.R. and K Hoare. 2003. Biotechnology and genetics in fisheries and aquaculture. Blackwell Science Ltd. Oxford. UK.

Bernatchez L. and P. Duchesne. 2000. Individual based genotype analysis in studies of parentage and population assignment: how many loci, how many alleles? CJFAS 57: 1-12.

Bernatchez L., J.B. Dempson and S. Martin. 1998. Microsatellite gene diversity analysis in anadromous arctic char, *Salvelinus alpinus*, from Labrador, Canada. CJFAS 55: 1264-1272.

Bernatchez L., J.G. Rhydderch and F.W. Kircheis. 2002. Microsatellite gene diversity analysis in landlocked Arctic charfrom Maine. Trans. Am. Fish. Soc. 131: 1106-1118.

Bongers A.B., B. Zandieh-Doulabi, C.J.J. Richter and J. Komen. 1999. Viable androgenetic YY genotypes of common carp (*Cyprinus carpio* L). J. Heredity 90: 195-198.

Brunner P.C., M.R. Douglas M.R. and L. Bernatchez. 1998. microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Arctic charr *Salvelinus alpinus* (Teleostei: Salmonidae) from central Alpine Lakes. Mol. Ecol. 7: 209-223.

Brunner P.C., M.R. Douglas, A. Osinov, C.C. Wilson and L. Bernatchez. 2001. Holarctic phylogeography of Arctic charr (*Salvelinus alpinus* L.). Evolution 55: 573-586.

Cann A.J. 2003. Maths from scratch for biologists. John Willey & Sons Ltd. Chichester, England.

Carvalho G. R. and L. Hauser. 1998. Advances in the molecular analysis of fish population structure. Int. J. Zool. 65 (Suppl.): 21-33.

Carvalho G.R. 1993. Evolutionary aspects of fish distribution: genetic variability and adaptation. J. Fish Bio. 43 (A): 53-73.

Cawkwell1 L. and P. Quirke. 2000. Direct multiplex amplification of DNA from a formalin fixed, paraffin wax embedded tissue section. Clin Pathol: Mol Pathol. 53:51-52.

Chamberlain J.S., R.A. Gibbs, J.E. Ranier, P.N. Nguyen and C.T. Caskey. 1988. Deletion screening of the duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic Acids Res, 16(23)" 11141-11156.

Cheverud J.M., E.J. Routman, F.A.M. Durate, B. van Swinderen, K. Colthran and C. Parel. 1996. Quantitative trait loci in murine growth. Genetics 142: 1305-1319.

Churchill G.A. and R.W. Doerge. 1994. Empirical threshold vales for quantitative trait mapping. Genetics 138:963-971.

Cnaani A., E.A. Hallerman, M. Ron, J.I. Weller, M. Indelman, Y. Kashi, G.A.E. Gall and G. Hulata. 2003. Detection of a chromosomal region with two quantitative trait loci, affecting cold tolerance and fish size, in an F2 tilapia hybrid, Aquaculture 223: 117-128.

Collins F.S., L.D. Brooks and A. Chakravarti. 1998. A DNA polymorphism discovery resource for research on human genetic variation. Genome Res. 8: 1229–1231.

Cross T. and J. King. 1983. Genetic effects of hatchery rearing in Atlantic salmon. Aquaculture 33:33-40.

Cross T., P. Galvin and E. Dillane. 1998. The use of molecular markers in Aquaculture Genetics. (Position paper presented at WGAGFM/ICES meeting at Cork, August 1998). CM 1998/K: 6 Symposium K.

Crozier W.W. 1994 Maintenance of genetic variation in hatchery stocks of Atlantic salmon, *Salmo salar* L.: Experiences from the River Bush, Northern Ireland. Aqua. Fish. Manag. 25:383-392.

Curtsinger J.W. 2003. Peeking under QTL peaks. Nature Genetics 34(4): 358-359.

Danzmann R.G. 1997. PROBMAX: a computer program for assigning unknown parentage in pedigree analysis from known genotypic pool of parents and progeny. J. Heredity 88: 333-337.

Danzmann R.G., M.M. Ferguson and F.W. Allendorf. 1989. Genetic variability and components of fitness in hatchery strains of rainbow trout. Journal of Fish Biology 35(A), pp. 313–319.

Danzmann R.G., T.R. Jackson and M.M. Ferguson. 1999. Epistasis in allelic expression at upper temperature tolerance QTL in rainbow trout. Aquaculture 173: 45-58.

Darvasi A. 1997. The effect of Selective genotyping on QTL mapping accuracy. Mammalian genome 8: 67-68.

Darvasi A. and M. Soller. 1992. Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. Theor. Appl. Genet. 85:353-359.

Davidson W.S., T.P. Birt and J.M. Green. 1989. A review of genetic variation in Atlantic salmon, *Salmo salar* L. and its importance for stock identification, enhancement programmes and aquaculture. J. Fish Biol. 34: 547-560.

Dekkers J.C.M. and F. Hospital. 2002. The use of molecular genetics in the improvement of agricultural populations. Nature Reviews Genetics. 3:22-32.

Devlin R.H. and Y. Nagahama. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological and environmental influences. Aquaculture 208: 191-364.

Dickson T.A. and H.R. MacCrimmon. 1982. Influence of Hatchery Experience on Growth and Behavior of Juvenile Atlantic Salmon within Allopatric and Sympatric Stream Populations. CJFAS. 39:1453-1458.

Doerge R. W. 2002. Mapping and analysis of quantitative trait loci in experimental populations. Nature Reviews Genetics 3:43-52.

Dowling T.E., G.R. Smith and W.M Brown. 1989. Reproductive Isolation and Introgression between *Notropis cornutus* and *Notropis chrysocephalus* (family cyprinidae): comparison of morphology, allozyme and Mitochondrial DNA. Evolution 43(3): 620-634.

Dowling T.E., W.L. Minckley, P.C. Marsh and E.S. Goldstein. 1996. Mitochondrial DNA variability in the endangered razorback sucker (*Xyrauchen texanus*): Analysis of hatchery stocks and implications for captive propagation. Conserv.Biol. 10:120-127.

Doyle R.W. and A. Talbot. 1986. Effective population size and selection in variable aquaculture stocks. Aquaculture 57: 27–35.

Doyle R.W., C. Herbinger, C.T. Taggart and S. Lochmann. 1995. Use of DNA microsatellite polymorphism to analyze genetic correlations between hatchery and natural fitness. Am.Fish.Soc.Symposium. 15:205-211.

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Dreher K., M. Morris, M. Khairallah, J-M. Ribaut, S. Pandey and G. Srinivasan. 2000. Is marker-assisted selection cost-effective compared to conventional plant breeding methods? The case of quality protein maize. The Fourth Annual Conference of the International Consortium on Agricultural Biotechnology Research (ICABR), "The Economics of Agricultural Biotechnology, Ravello, Italy.

Dumas S., C. Audet, J.M. Blanc and J. De la Nouee 1995a. Seawater acclimation of diploid and triploid brook charr (*Salvelinus fontinalis*), diploid Arctic charr (*Salvelinus alpinus*) and their diploid and triploid hybrids. Journal of Fish Biology 46(2):302-316.

Dumas S., J.M. Blanc, C. Audet and J. De la Nouee, 1995b. Variation in yolk absorption and early growth of brook charr (*Salvelinus fontinalis* (Mitchill)), Arctic charr (*Salvelinus alpinus* (L.) and their hybrids. Aquaculture research 26(Suppl.10): 759-764.

Dunham R.A. 1995. The contribution of genetically improved aquatic organisms to global food security. Thematic paper presented at the Japan/FAO International Conference on Sustainable Contribution of Fisheries to Food Security, 4 - 9 December 1995, Kyoto, Japan.

Dunham R.A. 2004. Aquaculture and fisheries biotechnology: genetic approaches. CABI Publishing, Cambridge, MA. USA.

Dunham R.A., K. Majumdar, E. Hallerman, D. Bartley, G. Mair, G. Hulata, Z. Liu, N. Pongthana, J. Bakos, D. Penman, M. Gupta, P. Rothlisberg and G. Hoerstgen-Schwark. 2001. Review of the status of aquaculture genetics. In R.P. Subasinghe, P. Bueno, M.J. Phillips, C. Hough, S.E. McGladdery & J.R. Arthur, eds. Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20-25 February 2000. pp. 137-166. NACA, Bangkok and FAO, Rome.

Edmond Institute Manual. 1998. Scientists' Working Group on Biosafety. Manual for assessing ecological and human health effects of genetically engineered organisms. http://www.Edmonds-Institute.org.

Eknath A.E. and R.W. Doyle. 1990. Effective population size and rate of inbreeding in aquaculture of Indian major carps. *Aquaculture* 85. 293-305.

Entis E. 1997. AquAvantage Salmon: Issues in the Introduction of Transgenic Foods. In Transgenic Animals & Food Production. Royal Swedish Academy of Agriculture & Forestry: Stockholm.

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Estoup A., K. Gharbi, M. SanCristobal, C. Chevalet, P. Haffray and R. Guyomard. 1998. Parentage assignment using microsatellites in turbot (*Scophthalmus maximus*) and rainbow trout (Oncorhynchus mykiss) hatchery populations. CJFAS 55: 715-723.

Evans B., J. Bartlett, N. Sweijd, P. Cook and N. G. Elliott.2004. Loss of genetic variation at microsatellite loci in hatchery produced abalone in Australia (Haliotis rubra) and South Africa (Haliotis midae). 2004. Aquaculture 233:109-127.

Falconer D. S. 1983. Problems on quantitative genetics. Longman publication, London; New York.

Falconer D.S. and T.F.C. Mackay. 1996. Introduction to quantitative genetics, 4th edition. Longman Science and Technology, Toronto, Canada.

Falconer, D.S., 1989. Introduction to quantitative genetics. 3rd edn. Longman Scientific and Technical, England.

Ferguson M.M. 1994. The role of molecular genetic markers in the management of cultured fishes. In Carvalho & Pitcher (Ed) Reviews in Fish Biology and Fisheries. Vol. 4(3), pp. 351-373.

Ferguson M.M. and R. G. Danzmann. 1998. Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? CJFAS 55: 1553-1563.

Ferguson M.M., P. Ihssen and J.D. Hynes. 1991. Are cultured stocks of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) genetically similar to their source populations. CJFAS 48 (supplement 1): 118-123.

Fishback, A.G., R.G. Danzmann and M.M. Ferguson. 2000. Microsatellite allelic heterogeneity among hatchery rainbow trout maturing in different seasons. Journal of Fish Biology 57 (6):1367-1380.

Fjalestad K.T., T. Moen and L. Gomez-Raya. 2003. Prospects for genetic technology in salmon breeding programmes. Aquaculture Research 34 (5): 397-406.

Fleming I.A. and M.R. Gross. 1992. Reproductive behavior of hatchery and wild coho salmon (*Oncorhynchus kisutch*): Does it differ? Aquaculture 103:101-121.

Fleming I.A. and S. Einum. 1997. Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication, ICES Journal of Marine Science, 54: 1051-1063.

Fletcher G.L., M.A. Shears, S.V. Goddard, R. Alderson, E.A. Chin-Dixon and C.L. Hew. 1999. Transgenic fish for sustainable aquaculture. In Sustainable aquaculture: Food for the future? Eds. Svennevig N, Reinertsen H and New M, 193-201. Rotterdam: AA Balkema.

Flint J. and R. Mott. 2001. Finding the molecular basis of quantitative traits: success and pitfalls. Nature Review Genetics. 2:437-445.

Forbes S.H., K.L. Knudsen, T.W. North and F.W. Allendorf. 1994. One of Two Growth Hormone Genes in Coho Salmon is Sex-Linked. Proceedings of the National Academy of Sciences, Vol 91, 1628-1631.

Galbreatha P.F. and G. H. Thorgaard. 1995. Sexual maturation and fertility of diploid and triploid Atlantic salmon X brown trout hybrids. Aquaculture 137: 299-311.

Gall G. A.E. and Y. Bakar. 2002. Application of mixed-model techniques to fish breed improvement: analysis of breeding-value selection to increase 98-day body weight in tilapia. Aquaculture 212: 93– 113.

Gallardoa J.A., X. Garcia, J.P. Lhorenteb and R. Neiraa. 2004. Inbreeding and inbreeding depression of female reproductive traits in two populations of Coho salmon selected using BLUP predictors of breeding values. Aquaculture 234: 111– 122.

Garibaldi L. 1996. List of animal species used in aquaculture. FAO, Fisheries Circular No. 914. FAO, Rome.

George A. H., E. M. Donaldson, J. Stoss and I. Baker. 1983. Production of monosex female groups of chinook salmon (Oncorhynchus tshawytscha) by the fertilization of normal ova with sperm from sex-reversed females. Aquaculture 33: 355-364.

Georges M., D. Nielsen, M. Mackinnom, A. Mishra, R. Okimoto, A. Pasquino, L. Sargeant, A. Sorensen, M. Steele, X. Zhao, J. Womack and I Hoeschele. 1995. Mapping quantitative trait loci controlling production in dairy cattle by exploring progeny testing. Genetics 139: 907-920.

Gislason D., M.M. Ferguson, S. Skulason and S.S. Snorrason. 1999. Rapid and coupled phenotypic and genetic divergence in Icelandic Arctic charr (*Salvelinus alpinus.*). CJFAS 56: 2229-2234.

Gjedrem T. 1983. Genetic variation in quantitative traits and selective breeding in fish and shellfish. Aquaculture 33: 51-72.

Gjedrem T. 1997. Selective breeding to improve aquaculture production. World Aquaculture 28: 33- 45.

Gjerde B. 2003. Genetic markers for parental assignment in fish breeding programs. Presentation at Genetics in aquaculture VIII: International Symposium Genetics in Aquaculture. 9 to 15 November 2003, Puerto Varas, Chile.

Glebe B.D. and T. Turner. 1993. Alternate commercial rearing atrategies for Arctic charr (*Salvelinus alpinus*). Bulletin of Aquaculture Association. Canada 93: 2-9.

Goel A.K. 2000. Genetic characterization of Clariid catfish (*C. macrocephalus*) and its hybrid (♀*Clarias macrocephalus* X ♂*Clarias gariepinus*) in Thailand. Masters Thesis. Asian Institute of Technology, Bangkok, Thailand.

Gregg R.E., Jr O.E. Rhodes and G. Armstrong. 2001. A genetic analysis of the London strain of rainbow trout. Animal Genetics 32(4) 210 – 214.

Grobet L., L.J.R. Martin, D. Poncelet, D. Pirottin, B. Brouwers, J. Riquet, A. Schoeberlein, S. Dunner, F. Menissier, J. Massabanda, R. Fries, R. Hanset. and M. Georges. 1997. A deletion in the bovine myostatingene causes the double muscled phenotype in cattle. Nature Genetics 17: 71-74.

Gross R. and J. Nilsson. 1995. Application of heteroduplex analysis for detecting variation within the growth hormone 2 gene in *Salmo trutta* L. (brown trout). Heredity 74: 286–295.

Gross R. and J. Nilsson. 1999. Restriction fragment length polymorphism at the growth hormone 1 gene in Atlantic salmon (*Salmo salar* L.) and its association with weight among the offspring of a hatchery stock. Aquaculture 173 1999 73–80.

Grosz M.D. and M.D. MacNeil. 2001. Putativeqtt locus affecting birth weight on bovine chromosome 2. J. Anim. Sci. 79: 68-72.

Guo S.W. and E.A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48: 361-372.

Haley C. 1999. Advances in quantitative trait locus mapping. Dekkers et al.)Eds.) Proc. of the Conf. "From Jay L. Lush to genomics: visions for animal breeding and genetics.

Hallerman E.M. and J.S. Beckmann. 1988. DNA-level polymorphism as a tool in fisheries science. CJFAS 45: 1075- 1087.

Harvey S.C., J. Masabanda, L.A.P. Carrasco, N.R. Bromage, D.J. Penmann and D.K. Griffin. 2002. Molecular-cytogenetic analysis reveals sequence differences between the sex chromosomes of *Oreochromis niloticus*: evidence for an early stage of sex-chromosome differentiation. Cytogenet. Genome Res. 79: 76-80.

Heath D.D., R.H. Devlin, J.W. Heath and G.K. Iwama. 1994. Genetic, environmental and interaction effects on the incidence of jacking in chinook salmon (*Oncorhynchus tshawytscha*). Heredity 72: 146-154.

Hedgecock D., D.J. McGoldrick, D.T. Manahan, J. Vavra, N. Appelmans and B.L. Bayne. 1996. Quantitative and molecular genetic analysis of heterosis in bivalves mollusks. Journal of Experimental Marine Biology and Ecology 203: 49-59.

Hedrick P.W. and P.S. Miller. 1992. Conservation genetics: techniques and fundamentals. Ecol. Appl. 2: 30–46.

Herbinger C.M., P.T. O'Reilly, R.W. Doyle, J.M. Wright and F.O'Flynn. 1999. Early growth performance of Atlantic salmon full-sib families reared in single family tanks versus in mixed family tanks. Aquaculture 173: 105-116.

Hirooka H., D.J.de Koning, J.A.M. van Arendonk, B. Harlizius, P.N.de Groot and H. Bovenhuis. 2002. Genome scan reveals new coat color loci in exotic pig cross. J. of Heredity 93(1): 1-8.

Hopkins K.D. 1992. Reporting fish growth: a review of the basics. Journal of the World Aquaculture Society 23(3): 173-179.

Hospital F. and C. Chevalet. 1993. Effects of population size and linkage on optimal selection intensity. Theor Appl Genet 86: 775–780.

Hospital F., L. Moreau, F. Lacoudre, A. Charcosset and A. Gallais. 1997. More on the efficiency of marker-assisted selection. Theor. Appl. Genet. 95:1181–1189.

Hussain M.G., M.S. Islam, M.A. Hossain, M.I. Wahid, A.H.M. Kohinoor, M.M. Dey and M.A. Mazid. 2002. Stock improvement of silver barb (*Barbodes gonionotus* Bleeker) through several generations of genetic selection, Aquaculture 204 (3-4): 469-480.

Jackson T.R., M.M. Ferguson, R.D. Danzmann, A.G. Fishback, P.E. Ihssen, M. O'Connell and T. Crease. 1998. Identification of two QTL influencing upper temperature tolearance in three rainbow trout (*Oncorhynchus mykiss*) half-sib families. Heredity 80: 143-151.

Jackson, T. R., D. J. Martin-Robichaud and M. E. Reith. 2003. Application of DNA markers to the management of Atlantic halibut (*Hippoglossus hippoglossus*) broodstock. Aquaculture 220:245-259.

Jenkins W.E. and T.I.J. Smith. 1990. Use of PIT tags to individually identified stripped bass and red drum brood stocks. American Fish. Society Symposium 7: 341-345.

Jerry D.R., N.P. Preston, P.J. Crocos, S. Keys, J.R.S. Meadows and Y. Li. 2003. DNA parentage analysis applied to the kurma shrimp *Penaeus japonicus*. Presentation at Genetics in aquaculture VIII: International Symposium Genetics in Aquaculture. 9 to 15 November 2003, Puerto Varas, Chile.

Jobling M., E.H. Jorgensen, A.M. Arnsen and E. Ringo. 1993. Feeding, growth and environmental requirements of Arctic Char: A review of aquaculture potential. Aquaculture International, 1, 20-46.

Johansen A.D. 1999. Marker assisted selection for Arctic charr (*Salvelinus alpinus* L.) broodstock development. Master's thesis, Memorial Univ. of Newfoundland, Nfld., Canada.

Johansen A.D., G. Wilton and W.S. Davidson. 1998. Marker assisted selection for Arctic charr (*Salvelinus alpinus*) broodstock development. Bull. Aquacul Assoc. Canada 2: 30-31.

Johansson I. and J. Rendel. 1968. Genetics and animal breeding. W.H. Freeman and Company. San Francisco, USA.

Johansson-Moller M., R. Chandhary, E. Hellman, B. Hoyheim, B. Chowdhary and L. Andersson. 1996. Pigs with the dominant white coat color phenotype carry a duplication of the KIT gene encoding the mast/stem cell growth factor receptor. Mammalian Genome, 7:822–830.

Johnson K.R. and J.E. Wright. 1986. Female brown troutX Atlantic salmon hybrids produce gynogens and triploids when backcrossed to male Atlantic salmon. Aquaculture 57: 345-358.

Johnson L. 1980. The Arctic charr, *Salvelinus alpinus*. In Charrs, Salmonid fishes of the genus Salvelinus, Ed E.K. Balon. W. Junk. Publishers, The Hague.

Johnston G. 2002. Arctic charr aquaculture. Fishing News Books, Blackwell Publishing, Oxford, UK.

Jungerius B., A. Rattink, B. Harlizius, B. Oost, M. Pas and M. Groenen. 2002. Development and typing of single nucleotide polymorphism (SNP) markers in a QTL region for fatness traits on porcine chromosome 2. Poster presentation at the Plant, Animal & Microbe Genomes X Conference, San Diego, CA, USA.

Kappes S.M. 1999. Utilization of gene mapping information in livestock animals. Theriogenology 51: 135-147.

Kause A., O. Ritola, T. Paananeu, U. Eskelinen and E. Mantysaari. 2003. Big and beautiful? Quantitative genetic parameters for appearance of large rainbow trout. Journal of Fish Biology 62, 610–622.

Kearsey M.J. and G.L. Farquhar. 1998. QTL analysis in plants; where are we now? Heredity 80, 137142.

Kearsey M.J. and H.S. Pooni. 1996. The Genetical Analysis of Quantitative Traits. Chapman and Hall, London.

Keightley P.D. and G Bulfield. 1993. Detection of quantitative trait loci from fequency changes at marker loci under selection. Genetic Resources, 62: 195-203.

Keightley P.D., T. Hardge, L. May and G Bulfield. 1996. A genetic map of quantitative trait loci for body weight in the mouse. Genetics 142: 227-235.

Kirpichnikov V.S. 1981. Genetic bases of fish selection. Springer-Verlag Berlin Heidelberg.

Knapp S.J. 1998. Marker-Assisted Selection as a Strategy for Increasing the Probability of Selecting Superior Genotypes. CROP Science. 38, 1164-1175.

Knott S. A., L. Marklund, C. S. Haley, K. Andersson, W. Davies, H. Ellegren, M. Fredholm, I. Hansson, B. Hoyheim, K. Lunkstrom, M. Moller and L. Andersson. 1998. Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and larger white pigs. Genetics 149:1069–1080.

Kocher T.D., W.J. Lee, H. Sobolewska, D. Penman and B. McAndrew. 1998. A genetic linkage map of a cichlid fish, the tilapia (*Oreochromis niloticus*). Genetics 148, 1225-1232.

Koehn R.K., W.J. Diehl and T.M. Scott. 1988. The different contribution by individual enzymes of glycolysis and protein catabolism to the relationship between heterozygosity and growth rate in the coot clam *Milinia lateralis*. *Genetics* 118, pp. 121–130

Koning D.J., J.C.M. Dekkers and C.S. Haley. 2003. Designs for QTL detection in livestock and their implications for MAS. Paper presented at the workshop entitled "Marker assisted selection: A fast track to increase genetic gain in plant and animal breeding?", held during a conference on "Molecular marker assisted selection as a potential tool for genetic improvement of crops, forest trees, livestock and fish in developing countries". 17 November to 12 December 2003, Turin, Italy.

Korstanje R. and B. Paigen. 2002. From QTL to gene: the harvest begins. Nature genetics; 31: 235-236.

Krasznai Z.L. 1987. Interspecific hybridization of warm water finfish. Proceedings of World Symp. On Selection, Hybridization and Genetic Engineering in Aquaculture Bordeaux 27-30 May, 1986. Vol. II. Berlin 1987.

Krueger C.C., A.J. Gharrett, T.R. Dehring and F.W. Allendorf. 1981. Genetic aspects of fisheries rehabilitation programs. CJFAS 38: 1877-1881.

Kumar S. and D.J. Garrick. 2001. Genetic response to within-family selection using molecular markers in some radiate pine breeding schemes. Can. J. For. Res., 31: 779-785.

Kutzer T., T. Leeb and B. Brenig. 2003. Current state of development of genome analysis in livestock. Current Genomics 4: 487-525.

Lande R. and R. Thompson. 1990. Efficiency of marker assisted selection in the improvement of quantitative traits. Genetics 124: 743- 456.

Lander E.S. and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185- 199 and 136: 705.

Lander E.S. and L. Kruglyak. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nature Genetics 11: 241-247.

Larsson S. 2002. Thermal performance of Arctic charr: intraspecific variation and competitive ability. Doctoral Thesis, Swedish Univ. of Agricultural Science, Umea, Sweden. ISSN 1401-6230, ISBN 91-576-6335-1.

Lasley J. F. 1972. Genetics of livestock improvement, 2nd edition. Prentice-Hall, Inc. Englewood Cliffs, N.J.

Letcher B.H. and T.L. King. 2001. Parentage and granparent assignment with known and unknown matings: application to Connecticut River Atlantic salmon restoration. CJFAS 58: 1812-1821.

Linville R.C., D. Pomp, R. K. Johnson and M. F. Rothschild. 2001. Candidate gene analysis for loci affecting litter size and ovulation rate in swine. J. Anim. Sci. 79:60–67.

Litt M., X. Hauge and V. Sharma. 1993. Shadow bands seen when typing polymorphic dinucleotide repeats: some causes and cures. Biotechniques 15: 280-284.

Liu B. H. 1998. Statistical genomics: Linkage, mapping and QTL analysis. CRC press LLC, Boca Raton. Florida, USA.

Liu P., J. Zhu, X. Lou and Y. Lu. 2003c. A method for marker-assisted selection based on QTLs with epistatic effects. Genetica 119: 75–86.

Liu Z. 2003. A review of catfish genomics: progress and perspectives. Comparative and Functional Genomics 4: 259–265.

Liu Z., A. Karsi, P. Li, D. Cao and R.A. Dunham. 2003. An AFLP-Based Genetic Linkage Map of Channel Catfish (*Ictalurus punctatus*) Constructed by Using an Interspecific Hybrid Resource Family. Genetics 165: 687-694.

Liu Z., A. Nichols, P. Li and R.A. Dunham. 1998. Inheritance and usefulness of AFLP markers in Channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*) and their F1, F2 and backcross hybrids. Molecular. Gen. Genet. 258: 260-268.

Lockwood S.F. and J.N Derr. 1992. Intra- and interspecific genome size variation in the salmonidae. Cytogenetics and Cell Genetics 59: 303-306.

Long A.D. and C.H. Langley. 1999. The power of association studies to detect the contribution of candidate genetic loci to variation in complex traits. Genome Research, 9: 720-731.

Lund T., T. Gjedrem, H. B. Bentsen, D. M. Eide, H. J. S. Larsen and K. H. Roed. 1995. Genetic variation in immune parameters and associations to survival in Atlantic salmon. Jn. of Fish Bio. 46: 748-758.

Lundrigan T.A. 2001. Genetic variation in cultured and natural populations of Arctic charr (*Salvelinus alpinus*) from North America. Master's Thesis. Univ. of Guelph, Ontario, Canada.

Lutz C.G. 2001. Practical genetics for aquaculture. A Blackwell Science Company. Iowa.

Lynch M. and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer Associates, Inc. Sunderland, M.A.

Macaranas J.M., N. Taniguchi, M.J.R. Pante, J.B. Capili and R.S.V. Pullin. 1986. Electrophoretic evidence for extensive hybrid gene introgression into commercial *Oreochromis niloticus* (L.) stock in the Philippines. Aquaculture and Fisheries management, 17: 249-258.

MacNeil M.D. and M.D. Grosz. 2002. Genome-wide scans for QTL affecting carcass traits in Hereford X composite double backcross populations. J. Anim. Sci. 80: 2316-2324.

Mair G.C. 2001. Monosex tilapia: factors, effects and methods for male-only production. Global Aquaculture Advocate 4(4): 79-81.

Mair G.C., J.S. Abucay, D.O.F. Skibinski, T.A. Abella and J.A. Beardmore. 1997b. Genetic manipulation of sex ratio for the large scale production of all-male tilapia *Oreochromis niloticus* L. CJFAS 54(2): 396-404.

Mair G.C., L.R. Dahilig, E.J. Morales, J.A. Beardmore and D.O.F. Skibinski. 1997. Application of genetic techniques for the production of monosex male tilapia in aquaculture: Early experiences from the Philippines. Proceedings of the Fourth Central America Symposium on Aquaculture, Tegucigalpa, Honduras, April 22-24, 1997a. 225-227.

Maitland P.S. 1995. World status and conservation of the Arctic charr *Salvelinus alpinus* (L.). Nordic journal of freshwater research 71, 113-127.

Malek M., J.C. Dekkers, H.K. Lee, T.J. Baas and MF Rothschild. 2001. A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. I. Growth and body composition. Mammalian genome. 12(8): 630-636.

Martyniuk C.J. 2001. A microsatellite DNA analysis examining the relationship between growth and maturation rate genes in rainbow trout. Masters Thesis. University of Guelph, Ontario.

McCarthy I.D., J.A. Sanchez and G. Blanco. 2003. Allozyme heterozygosity, date of first feeding and life history strategy in Atlantic salmon. J. of Fish Biology 62: 341-357.

McGowan C. 2004. Enhancing the diversity of Arctic charr (*Salvelinus alpinus*) broodstock. A final report prepared for Icy Waters Ltd. describing the genetic analysis carried out by the Davidson lad at Simon Fraser University (project # 01-01-BSD). April 2004.

McGowan C. and W.S. Davidson. 1992. Artificial fertilization of Newfoundland brown trout and Atlantic salmon: hatchability, survival and growth to first feeding. Aquaculture 106: 117-125.

Metusalach M.S., J.A. Brown and F. Shahidi. 1999. Effects of Stocking Density on Composition and Performance of Reared Arctic Charr (*Salvelinus alpinus*). Journal of Aquatic Food Product Technology 8(2):39 – 57.

Meuwissen T. 2003. Genomic selection: The future of marker assisted selection and animal breeding. Paper presented at the workshop entitled "Marker assisted selection: A fast track to increase genetic gain in plant and animal breeding?", held during a conference on "Molecular marker assisted selection as a potential tool for genetic improvement of crops, forest trees, livestock and fish in developing countries". 17 November to 12 December 2003, Turin, Italy.

Meuwissen T.H.E. and M.E. Goddard. 1996. The use of marker haplotypes in animal breeding schemes. Genet. Sel. Evol. 28:161-176.

Montaldo H.H. and C.A. Meza-Herrera. 1998. Use of molecular markers and major genes in the genetic improvement of livestock. Electronic Journal of Biotechnology 1(2): 83-89.

Moreau L., A. Charcosset, F. Hospital and A. Gallais. 1998. Marker-assisted selection efficiency in populations of finite size. Genetics 148; 1353-1365.

Moreaua L., S. Lemarieb, A. Charcosseta and A. Gallaisa. 2000. Economic efficiency of one cycle of marker-assisted selection. Crop Science 40:329-337.

Moshenko R.W., R.E. Peet, W. Dahlke and D.H. Dowler. 1984. The Arctic charr sport fishery at Tree River, Northwest Territories, Canada, 1964-78, p.349-364. In L. Johnson and Burns B.L. (Eds.). Biology of the Arctic charr, proceedings of the international symposium on Arctic Char, Winnipeg, Manitoba, May 1981. University of Manitoba Press, Winnipeg, Canada.

Muranty H. and B. Goffinet. 1997. Selective genotyping for location and estimation of the effect of a quantitative trait locus. Biometrics 53: 629-643.

Murphy B.R. and D.W. Willis. 1996. Fisheries Techniques, Second Edition. American Fisheries Society, Bethesda, Maryland.

Nakajima M. and N. Taniguchi. 2002. Genetic control of growth in the guppy (*Poecilia reticulata*). Aquaculture 204: 393-405.

Na-Nakorn N., N. Taniguchi, S. Seki and W. Kamonart. 1999. Isolation and Characterization of microsatellite Loci of *Clarias macrocephalus* and their application to Genetic Diversity Study. Fisheries Science. Vol. 65(4), pp. 520-526.

Naylor R.L., R.J. Goldburg, J.H. Primavera, N. Kautskybk, M.C. Beveridge, J. Clay, C. Folke, J. LubchencoI, H. Mooney and M. Troellbk. 2001. Effect of aquaculture on world fish supplies. Nature 405: 1017-1024.

Nilsson J. 1992. Genetic parameters of growth and sexual maturity in Arctic charr (*Salvelinus alpinus*). Aquaculture 106: 9-19.

Nilsson J. 1994. Genetics and growth of juvenile Arctic charr Trans. Am. Fish. Soc. 123, 430-434.

Norris A. T., D. G. Bradley and E. P. Cunningham . 1999. Microsatellite genetic variation between and within farmed and wild Atlantic salmon (*Salmo salar*) populations. Aquaculture 180: 247-264.

Norris A. T., D. G. Bradley and E. P. Cunningham . 2000. Parentage and relatedness determination in farmed Atlantic salmon (*Salmo salar*) using microsatellite markers. Aquaculture 180: 73-83.

O'Malley K.G. 2001. Quantitative trait loci for spawning time and body weight in rainbow trout: testing for conserved effects across ancestral homeologues. Masters Thesis. University of Guelph, Ontario.

O'Malley K.G., T. Sakamoto, R.G. Danzmann and M.M. Ferguson. 2003. Quantitative trait loci for spawing date and body weight in rainbow trout: testing for conserved effects across ancestrally duplicated chromosomes. Journal of Heredity 94(4): 273-284.

Oda S., E. Oki, Y. Maeharal and K. Sugimachi. 1997. Precise assessment of microsatellite instability using high resolution fluorescent microsatellite analysis. Nucleic Acids Research, 25(17): 3415–3420.

Olsen J.B., J.K. Wenburg and P. Bentzen. 1996. Semiautomated multilocusgenotyping of pacific salmon (*Oncorhynchus* spp.) using microsatellites. Mol. Mar. Biol. Biotch 5: 259-272.

Olsen J.B., P. Bentzen and J.E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. Mol. Ecol. 7:1087-1088.

O'Reilly P.T., C. Herbringer and J.M. Wright. 1998. Analysis of parentage determination in Atlantic Salmon (*Salmo salar*) using microsatellites. Animal genetics 29: 363-370.

O'Reilly P.T., L.C. Hamilton, S.K. McConnell and J.M. Wright. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Canadian Journal of Fisheries and Aquatic Science 53: 2292-2298.

Ozaki A., T. Sakamoto, S. Khoo, K. Nakamura, M. R. M. Coimbra, T. Akutsu and N. Okamoto. 2001. Quantitative trait loci (QTLs) associated with resistance/susceptibility to infectious pancreatic necrosis virus (IPNV) in rainbow trout (*Oncorhynchus mykiss*). Molecular Genetics and Genomics 265: 23 – 31.

Pagnacco G. and A. Carta. 2003. Animal breeding from infinitesimal model to MAS: the case of backcross design in dairy sheep (Sarda x Lacaune) and its possible impact on selection. Paper presented at the workshop entitled "Marker assisted selection: A fast track to increase genetic gain in plant and animal breeding?", held during a conference on "Molecular marker assisted selection as a potential tool for genetic improvement of crops, forest trees, livestock and fish in developing countries". 17 November to 12 December 2003, Turin, Italy.

Palti Y., JE. Parsons and GH. Thorgard. 1999. Identification of candidate DNA markers associated with IHN virus resistance in backcross of rainbow (*O. mykiss*) and cutthroat trout (*O. clarki*). Aquaculture 173: 81-94.

Pante M.J.R., B. Gjerde and I. McMillan. Effect of inbreeding on body weight at harvest in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 192, 201–211

Park L.K. and P. Moran. 1994. Developments in molecular genetic techniques in fisheries. In Carvalho G. R. and Pitcher T. J. (Ed) 1994. Reviews in Fish Biology and Fisheries. Vol. 4 (3), pp. 272-299.

Park L.K., P. Moran and D.A. Dightman. 1995. A polymorphism in intron D of the chinook salmon growth hormone 2 gene. Anim. Genet. 26: 285.

Paterson A.H., E.S. Lander, J.D. Hewitt, S Peterson, S. Lincoln and S.D. Tanksley. 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphism. Nature 335:721-726.

Perry G.M.L. 2001. The genetic architecture of thermal tolerance and correlated growth-related traits in rainbow trout, *Oncorhynchus mykiss*. Masters Thesis. University of Guelph, Ontario.

Phillips R. and P. Rab. 2001. Chromosome evolution in the salmonidae (Pisces): an update. Biol. Rev. 79: 1-25.

Phillips R.B. and P.E. Ihssen. 1985. Chromosome banding in salmonid fish: nucleolar organizer regions in *Salmo* and *Salvelinus*. Can J Genet Cytol 27: 433-440.

Phillips R.B., K.A. Pleyte and S.E. Hartley. 1988. Stock-specific differences in the number and chromosome positions of the nucleolar organizer regions in Arctic char (*Salvelinus alpinus*). Cytogenet Cell Genet 48: 9-12.

Phillips R.B., K.D. Zajicek and P.E. Ihssen. 1989. Population differences in chromosome-banding polymorphisms in lake trout. Trans. Am. Fish Soc. 118: 64-73.

Piepho H. 2000. Optimal marker density for interval mapping in a backcross population. Heredity 84: 437-440.

Pillen K., A. Zacharias and J. Leon. 2003. Advanced backcross QTL analysis in barley. Theor. Appl. Genet., 107:340-352.

Pletcher S.D., D. Houle and J. W. Curtsinger. 1998. Age-specific properties of spontaneous mutations affecting mortality in *Drosophila melanogaster*. Genetics 148: 287-304.

Poompuang S. and U. Na-Nakorn. 2004. A preliminary genetic map of walking catfish (*Clarias macrocephalus*). Aquaculture 232:195-203.

Postlethwait, J., S. Johnson, C.N. Midson, W.S. Talbot, M. Gates, E.W. Ballinger, D. Africa, R. Andrews, T. Carl, J.S. Eisen, S. Horne, C.B. Kimmel, M. Hutchinson, M. Johnson and A. Rodriguez. 1994. A genetic map for the zebrafish. Science 264:699-703.

Prentice E.F., T.A. Flagg and C.S. McCutcheon. 1990. Feasibility of using implantable passive integrated transponder (PIT) tags in salmonids. American Fish. Society Symposium 7: 317-322.

Presa P. and R. Guyomard. 1996. Conservation of microsatellites in three species of salmonids. J. Fish Biol. 49:1326-1329.

Promislow D. E. L., C. F. Jung and M. L. Arnold. 2001. Age-specific fitness components in hybrid females of *Drosophila pseudoobscura* and *D. persimilis*. J. Heredity 92(1): 30-37.

Quillet E., G. Aubard and I. Queau. 2002. Mutation in a sex-determining gene in rainbow trout: detection and genetic analysis. Journal of Heredity 93(2): 91-99.

Raymond M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248-249.

Reed K.M. and R.B. Phillips. 1997. Polymorphism of the NOR on the putative sex chromosome of Arctic charr(*Salvelinus alpinus*) is not sex related. Chromosome Research 5: 221-227.

Reed, D. H. and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conservation Biology 17:230-237.

Refstie T. and T. Gjerdrem. 1975. Hybrids between salmonidae species. Hatchability and growth rate in the freshwater period. Aquaculture 6: 333-342.

Rice S.H. 1997. The analysis of ontogenetic trajectories: when a change in size or shape is not heterochrony. Proceedings of the National Academy of Sciences, 94: 907-912.

Rico C., I. Rico and G. Hewitt. 1996. 470 million years of conservation of microsatellite loci among fish species. Proc. R. Soc. Lond. B, 263: 549-557.

Rieseberg L. H., S. J. E Baird and K. A. Gardner. 2000. Hybridization, introgression and linkage evolution. Plant Molecular Biology 42: 205-224.

Risch N.J. 2002. Searching for genetic determinants in the new millennium. Nature 405: 847-856.

Robinson B.D., P.A. Wheller and G.H. Thorgard. 1999. Variation in development rate among clonal lines of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 173: 131-141.

Rocha J.L., J.O. Sanders, D.M. Cherbonnier, T. J. Lawlor and J. F. Taylor. 1997. Trait-Based Analysis in Dairy Cattle Using Blood Group Polymorphisms. J. Dairy Sci 81:1681–1689.

Rogers B. and Davidson W.S. 2001. Arctic charr aquaculture review. Report to PEI aquaculture alliance and aquaculture association of Nova Scotia. Office of Commissioner for Aquaculture development.

Ronin Y.I., A. Korol and I.J. Weller. 1998. Selective genotyping to detect quantitative trait loci affecting multiple traits: interval mapping analysis. Theor. Appl. Genet., 97:1169-1178.

Rosegrant M., H. Steinfeld, S. Ehui and C. Courbois. 1999. Livestock to 2020: The Next Food Revolution. Delgado C.; IFPRI, FAO and ILRI. Washington, D.C., ISBN 0-89629-632-6. (http://64.95.130.128/ilri/InfoServ/Webpub/Fulldocs/Lvst2020/Lvst2020.pdf)

Rye M. and I.L. Mao. 1998. Nonadditive genetic effects and inbreeding depression for body weight at harvest in Atlantic salmon *Salmo salar* L. . Livest. Prod. Sci. 57, 15–22.

Sagoff M. 1998. Patented Genes:An Ethical Appraisal. Issues in Science & Technology.

Sakamoto T., N. Okamoto, M. Okochi, M. Inami and H. Fukuda. 2003. Development of microsatellite markers from Ayu (*Plecoglossus altivelis*) and read sea bream (*Pagrus major*) for genome mapping. Presentation at Genetics in aquaculture VIII: International Symposium Genetics in Aquaculture. 9 to 15 November 2003, Puerto Varas, Chile.

Sakamoto T., R.D. Danzmann, K. Gharbi, P. Howard, A Ozaki, S.K. Khoo, R.A. Woram, N. Okamoto, M.M. Ferguson, L.E. Holm, R. Guymound, and B. Hoyheim. 2000. A microsatellite linkage map of rainbow trout (*Oncorhynchus mykiss*) characterized by large sex-specific differences in recombination rates. Genetics 155: 1331-1345.

Sakamoto T., R.G. Danzmann, N. Okamoto, M.M. Ferguson and P.E. Ihssen. 1999. Linkage analysis of quantitative trait loci associated with spawning time in rainbow trout (*Oncorhynchus mykiss*), Aquaculture 173(1-4): 33-43.

Sanchez C.C., K. Fuji, T. Sakamoto, O. Hasegawa, I. Nakayama, T. Kobayashi, K. Morishima, H. Okamoto and N. Okamoto. 2003. Detailed genetic map of the Japanese flounder. Presentation at Genetics in aquaculture VIII: International Symposium Genetics in Aquaculture. 9 to 15 November 2003, Puerto Varas, Chile.

Sanchez J.A., C. Clabby, D. Ramos, G. Blanco, F. Flavin, E. Vazquez and R. Powell. 1996. Protein and microsatellite single locus variability in *Salmo salar* L. (Atlantic salmon). Heredity 77:423-432.

Sankoff D. 1999. Comparative Mapping and Genome Rearrangement. (Dekkers *et al.*Eds.) Proc. of the Conf. "From Jay L. Lush to genomics: visions for animal breeding and genetics.

Schechert A.W., H.G. Welz and H.H. Geiger. 1999. QTL for resistance to *Setosphaeria turcica* in tropical African maize. Crop Science. 39, 514-523.

Schneider S., D. Roessli and L. Excoffier. 2000. Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Schrooten C., H. Bovenhuis, W. Coppieters and J.A. M. Van Arendonk. 2000. Whole genome scan to detect quantitative trait loci for confirmation ad fucntional traits in dairy cattle. J. Dairy Sci. 83: 795-806.

Schutz J.R. and R. M. Harrell. 1998. use of sex reversal in striped bass to create an all-male population. North American journal of Aquaculture 61: 97-106.

Schutz J.R. and R.M. Harrell. 1999. Use of sex reversal in striped bass to create an all-male population. North American Jn. of Aquaculture 61: 97-106.

Scriber K.T., J.R. Gust and R.L. Fields. 1996. Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. CJFAS 53: 833-841.

Selvamani M.J.P., S.M. Degnan and B.M. Degnan. 2001. Microsatellite genotyping of individual Abalone larvae: parentage assignment in aquaculture. Marine Biotech., 3:478-485.

Shavorskaya. O.A. 2004. Identification of genes affecting flowering time variation in Brassica species. Doctoral thesis. Swedish University of Agricultural Sciences, Uppsala.

Shelton W.L. 1986. Broodstock development for monosex productin of grass carp. Aquaculture 57: 311-319.

Shimoda N., E.W. Knapik, J. Ziniti, C. Sim, E. Yamada, S. Kaplan, D. Jackson, F. de Sauvage, H. Jacob and M.C. Fishman. 1999. Zebrafish genetic map with 2000 microsatellite markers. Genomics 58:219-232.

Silverstein J.T. and W.K. Hershberger. 1992. Precocious maturation in coho salmon (*Oncorhynchus kisutch*): estimation of heritability. Bull. Aquaculture. Assoc. Can., 92: 34-36.

Simianer H. 1994. Derivation of Single-locus Relationship Coefficients Conditional on Marker Information. Theor. Appl. Genet., 88: 548-556.

Slate J., P.M. Visscher, S. MacGregor, D. Stevens, M.L. Tate and J.M. Pemberton. 2002. A genome scan for quantitative trait loci in a wild red deer (*Cervus elaphus*). Genetics 162: 1863-1873.

Slettan A., I. Olsaker and O. Lie. 1997. Segregation studies and linkage analysis of Atlantic salmon microsatellites using haploid genetics. Heredity 78:620-627.

Smitherman R.O., R. A. Dunham and D. Tave. 1983. Review of catfish breeding research 1969-1981 at Auburn university. Aquaculture 33: 197–205.

Soller M. and I. Medjugorac. 1999. A successful marriage: making the transition from QTL mapping to marker assisted selection. Dekkers et al.)Eds.) Proc. of the Conf. "From Jay L. Lush to genomics: visions for animal breeding and genetics.

Soller M., A. Genizi and T. Brody. 1976. On the power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. Theor. Appl. Genet. 47: 35-59.

Somorjai I.M.L. 2001. Quantitative trait loci for fitness trait in Arctic charr: conservation in rainbow trout and correlation among traits. Masters Thesis. University of Guelph, Ontario.

Somorjai I.M.L., R.G. Danzmann and M.M. Ferguson. 2003. Distribution of temperature tolerance quantitative trait loci in Arctic charr (*Salvelinus alpinus*) and inferred homologies in rainbow trout (*Oncorhynchus mykiss*). Genetics 165: 1443-1456.

Sonesson A.K. 2003. Possibilities for marker assisted selection in fish breeding schemes. Paper presented at the workshop entitled "Marker assisted selection: A fast track to increase genetic gain in plant and animal breeding?", held during a conference on "Molecular marker assisted selection as a potential tool for genetic improvement of crops, forest trees, livestock and fish in developing countries". 17 November to 12 December 2003, Turin, Italy.

Sonesson A.K. and T.H.E. Meuwissen. 2000. Mating schemes for optimum contribution selection with constrained rates of inbreeding. Genet. Sel. Evol. 32: 231-248.

Sorgeloos P. 2001. Technologies for sustainable aquaculture development, Plenary Lecture II. In R.P. Subasinghe, P. Bueno, M.J. Phillips, C. Hough, S.E. McGladdery & J.R. Arthur, eds. Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20-25 February 2000. pp. 23-28. NACA, Bangkok and FAO, Rome.

Spielman R.S., R. E. McGinnis and W.J. Ewens. 1993. Transmission test for linkage-disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am. J. Hum. Genet., 52:506-516.

Staurnes M., T. Sigholt, O.A. Gulseth and R. Eliassen. 1994. Effects of maturation on seawater tolerance on anadromous Arctic char. Trans. Am. Fish. Soc., 123: 402-407.

Streelman J. and T.D. Kocher. 2002. microsatellite variation associated with prolactin expression and growth of salt-challenged tilapia. Physiological genomics 9:1-4.

Su G.-S., L.-E. Liljedahl and G.A.E. Gall. 1996. Effects of inbreeding on growth and reproductive traits in rainbow trout *Oncorhynchus mykiss* . Aquaculture 142, 139–148.

Sullivan K., D.J. Martin, R.D. Cardwell, J.E. Toll and S. Duke. 2000. An analysis of the effects of temperature on salmonids of the Pacific Northwest with implications for selecting temperature criteria. Sustainable Ecosystems Institute. Portland, OR, USA.

Swick R.A. and M.C. Cremer. 2001. Livestock production: a model for aquaculture?, Guest Lecture. In R.P. Subasinghe, P. Bueno, M.J. Phillips, C. Hough, S.E. McGladdery & J.R. Arthur, eds. Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20-25 February 2000. pp.49-60. NACA, Bangkok and FAO, Rome.

Taniguchi N., M. Takagi and K. Mikita. 1999. Microsatellite DNA markers for monitoring genetic changes in hatchery stocks of Red Sea Bream (Pagrus major): a case study. In Mustafa S. Genetics in sustainable Fisheries Management.

Tanksley S.D. 1993. Mapping polygenes. Annu. Rev. Genet., 27:205-233.

Tao W.J. and E.G. Boulding. 2003. Association between single nucleotide polymorphism in candidate genes and growth rate in Arctic charr (*Salvelinus alpinus* L.). Heredity 91: 60-69.

Tautz D. 1989. Hypervariability of simple sequences as a general source of polymorphic DNA markers. Nucl. Acids Res, 17: 6463- 6471.

Tave, D. 1993. Genetics for Fish Hatchery Managers, 2nd ed. Van Nostrand Reinhold, New York, New York, USA.

Tessier N., L. Bernatchez and J.M. Wright. 1997. Population structure and impact of supportive breeding inferred from mitochondrial and microsatellite DNA analysis in land-locked Atlantic salmon *Salmo salar* L. Mol. Ecol. 6: 735-750.

Thumma B.R., B.P. Naidu, A. Chandra, D.F. Cameron, L.M. Bahnisch and C. Liu. 2001. Identification of causal relationship among traits related to drought resistance in Stylosanthes scabra using QTL analysis. Jn. of Experimental Botany 52: 203-214.

Trikka D, Z. Fang, A. Renwick, S. H. Jones, R. Chakraborty, M. Kimmel and D. L. Nelson. 2002. Complex SNP-Based Haplotypes in Three Human Helicases: Implications for Cancer Association Studies. Genome Res., 12(4): 627- 639.

Van Tassell C.P., M.S. Ashwell and T.S. Sonstegard. 2000. Detection of putative loci affecting milk, healthm and conformation traits in a US Holstein population using 105 microsatellite markers. J. Dairy Sci. 83: 1865-1872.

Vannuccini S. 2003. Overview of fish production, utilization, consumption and trade (based on 2001 data). Fishery Information, Data and Statistics Unit, FAO, Rome.

Vaughn T.Y.T., L.S. Pletscher, A Peripato, K. King-Ellison, E. Adams, C. Erikson and J.M. Cheverud. 1999. Mapping quantitative trait loci for murine growth: a closer look at genetic architechture. Genet Res., Cambridge, 74: 313-322.

Walter R. 1997. Consumer Perspective. NABC Report 9. Resource Management in Challenged Environments. New York: National Agricultural Biotechnology Council.

Waples R. S. 1991. Genetic Interactions between Hatchery and Wild Salmonids: Lessons from the Pacific Northwest. CJFAS 48 (Suppl. 1): 124-133.

Ward R.D. and P.M. Grewe. 1994. Appraisal of molecular genetic techniques in fisheries. In Carvalho G. R. and Pitcher T. J. (Ed) 1994. Reviews in Fish Biology and Fisheries, 4 (3), pp. 300-325.

Weiss K.M. 1998. In Search of Human Variation. Genome Res. 8: 691-697.

Weller J.I. 2001. Quantitative trait loci analysis in animals. CABI publishing, Oxon, UK.

Whittaker J.C., R.N. Curnow, C.S. Haley and R. Thompson. 1995. Using marker-maps in marker-assisted selection. Genetical Res., Cambridge 66: 255-265.

Wilson C.C. and L. Bernatchez. 1998. The ghost of hybrids past: fixation of arctic charr (*Salvelinus alpins*) mitochondrial DNA in an introgressed population of lake trout (*S. namaycush*). Molecular Ecology 7: 127-132.

Woods L. C., B. Ely, G. Leclerc and R.M. Harrell. 1996. Evidence for genetic purity of captive and domestic striped bass broodstocks. In Doyle, R. W., C.M. Herbinger, M. Ball and G. A. E. Gall (ED). Genetics in Aquaculture V: 41-44.

Woram R.A. 2001. Construction of a genetic linkage map for Arctic charr (*Salvelinus alpinus* L.) and comparative mapping with Atlantic salmon, brown trout and rainbow trout. Masters Thesis. University of Guelph.

Woram R.A., C. McGowan, J.A. Stout, K. Gharbi, M.M. Ferguson, B. Hoyheim, E.A. Davidson, W.S. Davidson, C. Rexroad and R.G. Danzmann. 2004. A genetic linkage map for Arctic char (*Salvelinus alpinus*): evidence for higher recombination rates and segregation distortion in hybrid versus pure strain mapping parents. Genome, 47: 304-315.

Woram R.A., K. Gharbi, T. Sakamoto, B. Hoyheim, L.-E. Holm, K. Naish, C. McGowan, M.M. Ferguson, R.B. Phillips, J. Stein, R. Guyomard, M. Cairney, J.B. Taggart, R. Powell, W. Davidson and R.G. Danzmann. 2003. Comparative Genome Analysis of the Primary Sex-Determining Locus in Salmonid Fishes. Genome Res. 13: 272-280.

Wright J.M. and P. Bentzen. 1994. Microsatellites: genetic markers for the future. In Carvalho G. R. and Pitcher T. J. (Ed) 1994. Reviews in Fish Biology and Fisheries, Vol. 4, No. 3, pp. 384-388.

Wu R., C.-X. Ma, J. Zhu and G. Casella. 2002b. Mapping epigenetic QTLs affecting developmental trajectories. Genome, 45: 28-33.

Wu R., C.-X. Ma, M. Chang, R. C. Littell, S.M. Wu, T.M. Yin, M. Huang, M.Wang and G Casella. 2002a. A logistic mixture model for characterizing genetic determinants causing differentiation in growth trajectories. Genet. Res., Cambridge, 79: 235-245.

Xie C. and S. Xu. 1998. Efficiency of multistage marker-assisted selection in the improvement of multiple quantitative traits. Heredity. 80:489-498.

Yin X., P. Stam, M.J. Kropff and Ad H.C.M. Schapendonk. 2003. Crop modeling, QTL mapping, and their complementary role in plant breeding. Agronomy Journal 95: 90-98.

Youngson A.F., A.I. Mitchell, P.T. Noack and L.M. Laird. 1997. Carotenoid pigment profiles distinguish anadromous and nonanadromous brown trout (*Salmo trutta*). CJFAS 54:1064-1066.

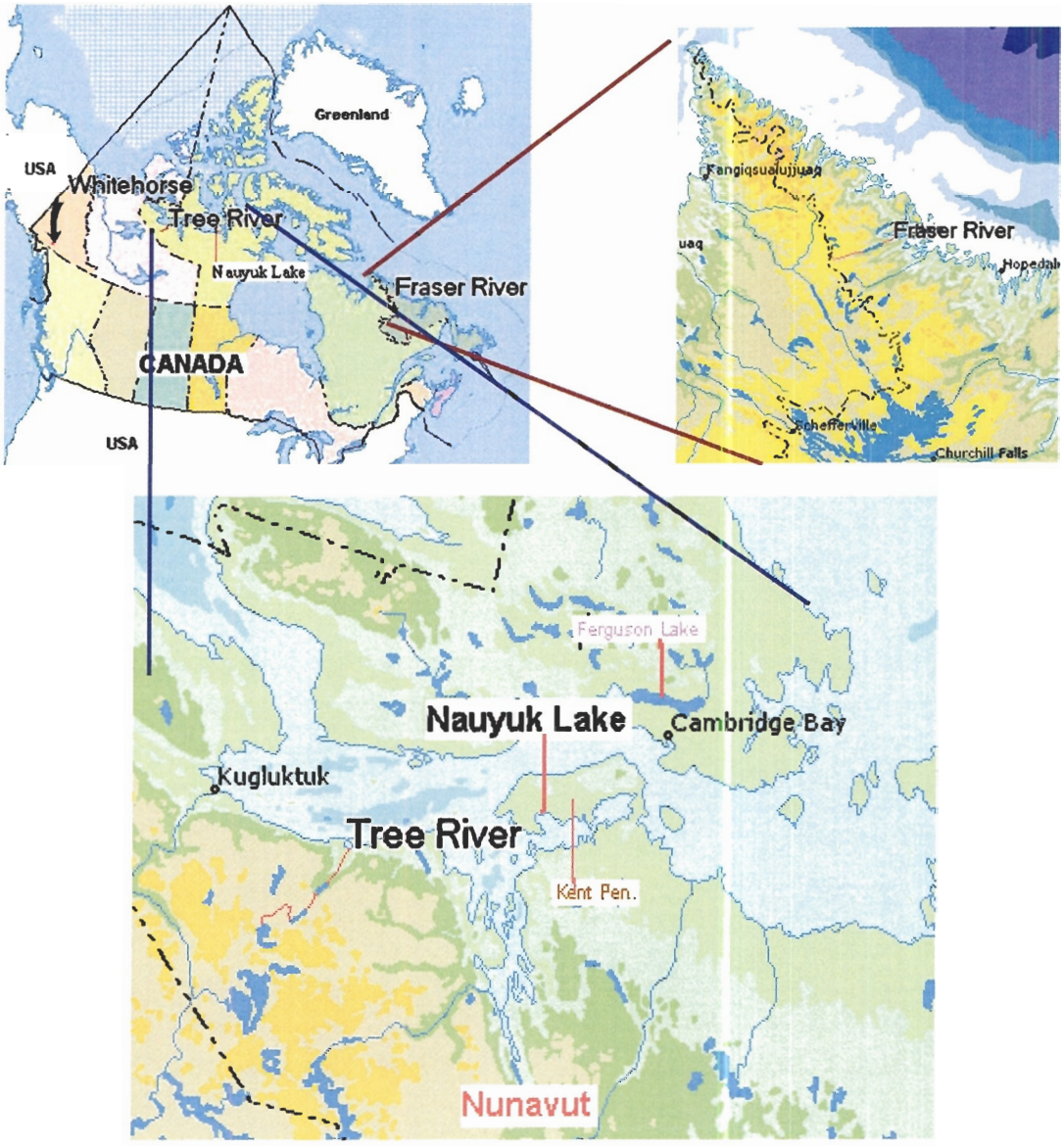
Yu Z. and X. Guo. 2003. Genetic Linkage Map of the Eastern Oyster *Crassostrea virginica* Gmelin. Biol. Bull. 204(3): 327 - 338.

Zheng L., S. Wang, P. Romans, H. Zhao, C. Luna and M.Q. Benedict. 2003. Quantitative trait loci in Anopheles gambiae controlling the encapsulation response against *Plasmodium cynomolgi* Ceylon. BMC Genetics 4(16).

Appendices

Appendix I

Map of Canada, showing geographical locations of the Fraser River strain, Fraser River (Newfoundland and Labrador) at 56°62′N & 62°25′W, Yukon Gold strain, Nauyuk Lake (Nunavut) at 68°22′N & 107°35′W and the Tree River strain, Tree River (Nunavut) at 67°38′N & 111°53′W.



Appendix II

Results of all the microsatellite markers used in the Arctic charr project. Alleles were visualized using radioactive ($\gamma^{32}P$) genotyping technique. 2; duplicated loci as reported in Woram *et al.* 2004, NA; No amplicon observed, UA; unsuitable amplicon. DP; differentially polymorphic, DM; differentially monomorphic, P; polymorphic across the two Arctic charr strains at Icy Waters Ltd., M; monomorphic.

No.	Marker	Linkage Group (Woram *et al.* 2004)	Optimum annealing Temp (^{O}C)	Amplicon size range in bp (approx.)	Test screening in Icy Waters Ltd. stocks				Refrence or genbank accession number
					No. of alleles				
					Nauyuk Lake (N=6)	Tree River (N=6)	Total (N=12)	Polymorphism	
1	As1.10NUIG	unassigned	NA	-	-	-	-	-	AF020846
2	As1.14NUIG	unassigned	60	UA	-	-	-	-	AF020847
3	As1.22NUIG	unassigned	45	160	1	1	2	DM	AF020848
4	BRFO001	unassigned	NA	-	-	-	-	-	U90327
5	BS131	unassigned	NA	-	-	-	-	-	unpublished
6	Cocl3LAV	26	UA	-	-	-	-	-	Bernatchez 1996
7	MBO024	unassigned	60	UA	-	-	-	too weak	unpublished
8	MBOA72	unassigned	UA	-	-	-	-	-	unpublished
9	MBOB83	unassigned	47	UA	-	-	-	several amplicons	unpublished
10	MBOC43	unassigned	45	200	1	1	1	M	unpublished
11	MBOJ50	unassigned	UA	<100	-	-	-	-	unpublished
12	MST15	unassigned	NA	-	-	-	-	-	AB001058
13	MST3	unassigned	50/55/60	220	1	1	1	M	AB001060

14	MST43	unassigned	NA	-	-	-	-	-	AB001069
15	MST543	unassigned	NA	-	-	-	-	-	AB001062
16	MST591	unassigned	NA	-	-	-	-	-	AB001064
17	MST60	unassigned	NA	-	-	-	-	-	AB001057
18	MST79	unassigned	50-55	150	1	1	1	M	AB001063
19	MST85[2]	unassigned	55	160-220 & 320-400	2	3	5	DP	AB001059
20	Ocl1UW	45	UA	smeary	-	-	-	-	Condrey and Bentzen 1998
21	Ogo1UW	unassigned	55	220	1	1	1	M	AF007827
22	Ogo2UW	unassigned	55-60	100-300	2	2	2	M	AF009794
23	Ogo3UW	unassigned	NA	-	-	-	-	-	AF009795
24	Ogo4UW	12 & 27	60	220	5	4	8	P	AF009796
25	Ogo5UW	unassigned	53-55	180	1	1	1	M	AF009797
26	Ogo6UW	unassigned	NA	-	-	-	-	-	AF009798
27	Ogo7UW	unassigned	NA	-	-	-	-	-	AF009799
28	Ogo8UW	16	60	100-250	5	4	8	P	AF009800
29	OMM1007	unassigned	53-57	200	1	2	2	P	AF346669
30	OMM1008	unassigned	60	250-400	1	1	1	M	AF346670
31	OMM1012	unassigned	57	240	2	2	3	P	AF346673
32	OMM1013	unassigned	57-60	260-320	1	1	2	DM	AF346674
33	OMM1025	unassigned	55	350	1	1	1	M	AF346682
34	OMM1035	unassigned	60	300	1	1	2	DM	AF346685
35	OMM1037	unassigned	51-53	150	2	3	4	P	AF346687

36	OMM1040	unassigned	NA	-	-	-	-	-	AF346690
37	OMM1122	unassigned	NA	-	-	-	-	-	AF352773
38	OMM1184	25	52-58	120	2	3	3	DP	AF469969
39	OMM1228	4	60	240	2	2	3	P	AF470009
40	OMM1365	unassigned	60	700	-	-	-	Too big amplicon	BV005164
41	Omy10INRA	7	60	>400	-	-	-	Too big amplicon	unpublished
42	Omy11DIAS	17	60	180	1	1	1	M	unpublished
43	Omy13INRA	31	50-55	200-270	1	3	4	DP	unpublished
44	Omy18INRA	16	55-60	200	2	2	3	P	unpublished
45	Omy1UG	21	50	300	2	2	3	P	unpublished
46	Omy207UG	unassigned	UA	<100	-	-	-	-	O'Connell *et al.* 1997
47	Omy278UG	unassigned	55	120	1	1	1	M	O'Connell *et al.* 1997
48	Omy287UG	unassigned	55	280	1	1	1	M	O'Connell *et al.* 1997
49	Omy301UG	7	55	70-150	2	2	4	DP	Jackson *et al.* 1998
50	Omy325UG	7	UA	several amplicons	-	-	-	several amplicons	O'Connell *et al.* 1997
51	Omy38DU	10	60	172-194 & 207-263	2	3	4	P	unpublished
52	Omy6DIAS	4	50/ 55	320	4	4	8	DP	AF239042
53	Omy77DU	12	60	120-240	2	1	3	DP	Morris *et al.* 1996
54	Omy7INRA	23	55-60	250	1	1	1	M	unpublished
55	OmyFGT11TUF	unassigned	UA	smeary	-	-	-	-	T. Sakamoto, 1997, Ph.D. Thesis, Tokyo University of Fisheries
56	OmyFGT19TUF	27	50	150	2	2	3	P	T. Sakamoto, 1997, Ph.D. Thesis, Tokyo University of Fisheries
57	OmyFGT1TUF	27	NA	-	-	-	-	-	T. Sakamoto, 1997, Ph.D. Thesis, Tokyo University of Fisheries

58	OmyFGT25TUF	unassigned	60	180	1	1	1	M	T. Sakamoto, 1997, Ph.D. Thesis, Tokyo University of Fisheries
59	OmyFGT8TUF	unassigned	53	150	1	1	1	M	T. Sakamoto, 1997, Ph.D. Thesis, Tokyo University of Fisheries
60	OmyJTUF	19	50-55	130	1	1	1	M	T. Sakamoto, 1997, Ph.D. Thesis, Tokyo University of Fisheries
61	OmyPuPuPyDU	13	UA	smeary	-	-	-	-	Morris *et al.* 1996
62	OmyRGT15TUF	unassigned	50	200	1	1	1	M	unpublished
63	OmyRGT19TUF	unassigned	NA	-	-	-	-	-	AB087595
64	OmyRGT1TUF	2	53	100	1	1	1	M	AB087586
65	OmyRGT39TUF	25	55-60	150	3	3	6	DP	AB087607
66	OmyRGT43TUF	unassigned	54	NA	-	-	-	-	AB087610
67	OmyRGT44TUF	unassigned	55-60	NA	1	1	1	M	AB087611
68	OmyRGT46TUF	19	57	180	1	1	1	M	AB087612
69	OmyRGT47TUF	unassigned	55-60	200	1	1	1	M	AB031200
70	OmyRGT4TUF	20	60	140	2	2	4	DP	AB087589
71	OmyRGT9TUF	unassigned	60	120	1	1	1	M	AB087590
72	OmyRT8TUF	4 & 28	NA	-	-	-	-	-	unpublished
73	One10ASC	26	50	160	1	3	4	DP	U56710
74	One11ASC	14	50-52	120	2	2	3	P	U56711
75	One12ASC	unassigned	NA	-	-	-	-	-	U56712
76	One13ASC	unassigned	NA	-	-	-	-	-	U56713
77	One14ASC	unassigned	NA	-	-	-	-	-	U56714
78	One15ASC	unassigned	50-55	110	1	1	1	M	U56715
79	One17ASC	unassigned	UA	smeary	-	-	-	-	U56717

80	One18ASC	unassigned	55	180-220	4	4	6	P	U56718
81	One1ASC	24	60	120-220	3	3	4	P	U56699
82	One20ASC	unassigned	60	>400	-	-	-	Too big amplicon	U56720
83	One21ASC	unassigned	50	120	1	1	1	M	U56721
84	One22ASC	unassigned	NA	-	-	-	-	-	U56722
85	One2ASC	unassigned	UA	-	-	-	-	too weak	U56700
86	One3ASC	unassigned	57	200	1	1	1	M	Scribner *et al.* 1996
87	One4ASC	unassigned	55-60	250	2	unsuitable amplicon	-	-	U56703
88	One5ASC	unassigned	50-55	180	2	1	2	P	Scribner *et al.* 1996
89	One6ASC	unassigned	50-55	350	2	5	7	DP	U56706
90	One7ASC	8	UA	several amplicons	-	-	-	-	U56707
91	One8ASC	6	55-60	148-160	2	3	4	P	U56708
92	One9ASC	unassigned	60	250	1	2	3	DP	U56709
93	Ots3BML	7	UA	<100	-	-	-	-	Banks *et al.* 1999 (AF107031)
94	Ots500NWFSC	4	55/ 60	200	3	4	6	P	AY042690
95	Ots510NWFSC	24	55	200	1	1	2	DM	AY042700
96	Ots516NWFSC	5 & 23	50	150	2	3	4	P	AY042706
97	Ots517NWFSC	5	NA	smeary	-	-	-	-	AY042707
98	Ots523NWFSC	1 & 35	NA	smeary	-	-	-	-	AY042713
99	Ots531NWFSC	26	NA	smeary	-	-	-	-	AY042721
100	Sal16UG	1	NA	smeary	-	-	-	-	Unpublished; Danzmann R., U. of Guelph
101	Sal5UG	36	50	190-275	7	7	11	DP	Unpublished; Danzmann R., U. of Guelph

102	Sal7UG	23	57-60	300-370	2	3	4	P	Unpublished; Danzmann R., U. of Guelph
103	Sal9UG	12	60	220-320	3	1	4	DP	Unpublished; Danzmann R., U. of Guelph
104	SalD100SFU	20	55-60	200	3	4	5	P	AF537308
105	SalD25SFU	26	UA	smeary	-	-	-	-	AF537305
106	SalD30SFU	unassigned	57-60	300	3	6	9	DP	AF537311
107	SalD39SFU	25	55	190-290	7	7	11	DP	AF537310
108	SalE38SFU	18	55	110-220	7	8	11	DP	AF537309
109	SalF41SFU	3 & 24	55-60	240	3	1	3	P	AF537306
110	SalF56 SFU	11	50-60	280	3	5	8	DP	AF537307
111	SalJ81SFU	3	55	150-220	2	6	8	DP	AF537304
112	SalP61SFU	14	55	135-195	7	7	11	P	AF537312
113	SapA49SFU	unassigned	55/ 60		1	1	1	M	unpublished
114	SapA67SFU	unassigned	UA	several amplicons	-	-	-	-	unpublished
115	SapA69SFU	unassigned	45	380	2	2	3	P	unpublished
116	SapA8SFU	unassigned	55/60	380	1	1	1	M	unpublished
117	SapB16SFU	unassigned	55	250	1	1	1	M	unpublished
118	SapC37SFU	unassigned	60	>400	-	-	-	Too big amplicon	unpublished
119	SapD63aSFU	unassigned	55/60	120	1	1	1	M	unpublished
120	SapD63bSFU	unassigned	NA	-	-	-	-	-	unpublished
121	SapF32SFU	unassigned	NA	-	-	-	-	-	unpublished
122	SapF41SFU	3 & 24	55-60	250	6	5	8	P	unpublished
123	SapG19SFU	unassigned	NA	-	-	-	-	-	unpublished

124	SapG37SFU	unassigned	NA	-	-	-	-	-	unpublished
125	SapH67SFU	unassigned	45	250	1	1	1	M	unpublished
126	SapI26SFU	unassigned	45-50	70 & 300	2	1	2	P	unpublished
127	SapI37SFU	unassigned	NA	-	-	-	-	-	unpublished
128	SapN91SFU	unassigned	NA	-	-	-	-	-	unpublished
129	Sco19UBC	14	55	>400	-	-	-	Too big amplicon	unpublished
130	Sfo11LAV	unassigned	50-55	120	1	1	1	M	U50306
131	Sfo23LAV	unassigned	55	175-350	8	8	12	P	U50304
132	Sfo8LAV	unassigned	55	250-310	10	10	12	P	U50305
133	SL/i(INRA)	22	50	250-320	3	2	4	P	unpublished
134	SmaBFRO1	21	50-55	290	4	2	4	P	unpublished
135	Sox9-ms	20	55-60	250	3 (dupli.)	2	3	DP	unpublished
136	Ssa107NUIG	unassigned	NA	-	-	-	-	-	unpublished
137	Ssa119DU	unassigned	60	320	1	1	1	M	U58892
138	Ssa120DU	unassigned	NA	-	-	-	-	-	U58896
139	Ssa14DU	9	50	160	1	3	3	P	McConnell *el al.*1995
140	Ssa16NUIG	4	NA	-	-	-	-	-	unpublished
141	Ssa171DU	unassigned	55	100	1	2	2	P	U43693
142	Ssa19.29NUIG	unassigned	NA	-	-	-	-	-	U37489
143	Ssa197DU	unassigned	57	120	1	2	2	P	U43694
144	Ssa20.19NUIG	16	55-57	75-180	1	2	2	P	U37494
145	Ssa202DU	unassigned	NA	-	-	-	-	-	U43695

146	Ssa208DU[2]	unassigned	55-60	200-360	5	5	8	P	AF019161
147	Ssa289	unassigned	45-47	120	1	2	3	DP	unpublished
148	Ssa293DU	unassigned	NA	-	-	-	-	-	O'Connell *et al.* 1997
149	Ssa3NUIG	32 & 34	60	UA	-	-	-	redesign	unpublished
150	Ssa46	unassigned	50	100	1	1	1	M	unpublished
151	Ssa4DU	unassigned	55	>400	-	-	-	Too big amplicon	McConnell *el al.*1995
152	Ssa5.27NUIG	unassigned	55	140	3	1	3	DP	U37491
153	Ssa52	unassigned	NA	-	-	-	-	-	unpublished
154	Ssa57DU	unassigned	NA	-	-	-	-	-	AF019179
155	Ssa58DU	unassigned	60	380	3	5	8	DP	AF019180
156	Ssa73DU	unassigned	46/ 50	380	1	1	1	M	AF019186
157	Ssa77NUIG	1	60	180	1	4	4	DP	unpublished
158	Ssa79NUIG	unassigned	NA	-	-	-	-	-	unpublished
159	Ssa85DU	13	55	130-230	6	6	8	P	U43692
160	Ssa86DU	unassigned	45-47	120	1	1	1	M	AF019191
161	Ssa9DU	unassigned	NA	-	-	-	-	-	AF019197
162	BHMS121♦	25	50	120	1	2	3	DP	AF256769
163	BHMS130♦	28	50	350	1	1	1	M	AF256663
164	BHMS142♦	37	UA	smeary	-	-	-	-	AF256772
165	BHMS206♦	6 & 8	58	220	3	3	5	DP	AF256680
166	BHMS217♦	15	50/ 55	250	1	3	4	DP	AF256786
167	BHMS330♦	8	60	250	1	1	1	M	AF256748

168	BHMS356*	15	53	200	2	3	4	P	AF256751
169	BHMS409*	1	50-60	280	1	1	2	DM	AF256811
170	BHMS411*	19	UA	<100	-	-	-	-	AF256761
171	BHMS429*	30	50-60	280	3	3	4	P	AF256719
172	BHMS431*	4	53	120	1	1	1	M	AF256720
173	BHMS490*	4	54	120	2	4	4	DP	AF256724
174	BHMS540.1*	45	50	UA	-	-	-	-	AF257059
175	BHMS546*	38	60	250	1	1	2	DM	AF256829
176	BHMS7.030*	4	46	150 & 250	2	2	3	DP	AF256838
177	BHMS7.033*	4	NA	-	-	-	-	-	AF256658
178	SsaF43NUIG	26	NA	-	-	-	-	-	Sanchez *et al.* 1996
179	SsaF48NUIG	unassigned	NA	-	-	-	-	-	Sanchez *et al.* 1996
180	SsaF49NUIG	unassigned	NA	-	-	-	-	-	U37496
181	SsaH24NUIG	unassigned	NA	-	-	-	-	-	Sanchez *et al.* 1996
182	SSLEEL53	unassigned	UA	smeary	-	-	-	-	U86704
183	SSLEEN17	unassigned	45-46	300	1	1	1	M	U86705
184	SSLEEN82	unassigned	45-47	200	1	1	1	M	U86706
185	SSLEEP96	unassigned	50-55	300	1	1	1	M	U86707
186	SSLEER15	33	50	400	1	1	2	DM	U86708
187	SSLEET47	unassigned	50-60	200	1	1	1	M	U86709
188	SSOSL25	unassigned	UA	smeary	-	-	-	-	Slettan *et al.* 1993 (Z48581)
189	SSOSL311	unassigned	50-53	150	1	1	1	M	Slettan *et al.* 1993 (Z48597)

190	SSOSL32(i)	4	62	140	2	2	2	P	Z69642
191	SSOSL34	unassigned	NA	-	-	-	-	-	Z69643
192	SSOSL417	unassigned	58-60	200	1	1	1	M	Slettan *et al.* 1993 (Z48598)
193	SSOSL436	unassigned	NA	-	-	-	-	-	Z49118
194	SSOSL438	unassigned	NA	-	-	-	-	-	Z49134
195	SSOSL446	unassigned	45	350	1	1	1	M	Z69644
196	SSOSL456	29	50-60	170-190	3	3	5	P	Z69645
197	SSOSL85	unassigned	50-53	280	1	2	2	P	Slettan *et al.* 1993 (Z48596)
198	Str7INRA	25	50/53/55	280	-	-	-	incomplete	unpublished

*BHMS markers have been renamed, for details see Table 2.2.

Appendix III

Description of eight microsatellite markers used for parentage assignment in this study. The number of alleles and allele size range are based on the results obtained from semi-automated fluorescent genotyping technique.

Marker	**Arctic charr linkage group**	**Primer sequences (5'-3'), forward and reverse.**	**Annealing temp. (°C)**	**Number of alleles**	**Allele size range (bp)**	**Reference or genbank accession number**
Ssa85 DU	AC-13	AGG TGG GTC CTC CAA GCT AC ACC CGC TCC TCA CTT AAT C	55	8	130-223	O'Reilly *et al.* 1996 (U43692)
SalE38 SFU	AC-18	CGC CTT GTC ATA CAT TAC ACC AGC CTA CAG AAA CAG GAG AAA G	55	11	114-213	AF537309
Sfo8 LAV	Unassig ned	CAA CGA GCA CAG AAC AGG CTT CCC CTG GAG AGG AAA	55	12	250-308	Angers *et al.* 1995
Sfo23 LAV	Unassig ned	GTG TTC TTT TCT CAG CCC AAT GAG CGT TAC GAG AGG	55	12	176-300	Angers *et al.* 1995
One18 ASC	Unassig ned	ATG GCT GCA TCT AAT GGA GAG TAA AAA CCA CAC ACA CTG TAC GCC AA	55	6	180-220	Scribner *et al.* 1996
Sal5 UG	AC-36	TTT GCA TTG AGC CTC TGT TGT TTC AGC TGC TAT TAG GAA AT	50	11	196-272	Unpublished; Danzmann R., U. of Guelph
SalP61 SFU	AC-3 or AC-14	CAC TTA TTA ACG CCC ACT CCC TTC ACA ACC ACA GGA AAG AAC TC	55	10	139-193	AF537312
SalD39 SFU	AC-25	GGG GAG TCT GTG TTA AGT TGA ATG GAC GTT CCT CTG AC	55	11	194-290	AF537310
			Total	81		

Appendix IV

Allele frequencies for the eight microsatellites tested on the four Arctic charr groups at Icy Waters Ltd. TR; Tree River, GY; Nauyuk Lake (Yukon Gold™), H1; Hybrid TR_fxYG_m, H2; Hybrid YG_fxTR_m.

Locus	Allele (bp)	Frequency				
		TR (n=250)	YG (n=210)	H1 (n=185)	H2 (n=203)	total (n=848)
Ssa85	130	0.16	0.00	0.01	0.00	0.05
DU	136	0.33	0.00	0.25	0.07	0.17
	138	0.10	0.00	0.04	0.01	0.04
	156	0.07	0.00	0.01	0.00	0.02
	187	0.22	0.98	0.61	0.69	0.61
	219	0.12	0.01	0.09	0.22	0.11
SalE38	114	0.00	0.21	0.03	0.04	0.14
SFU	118	0.00	0.44	0.28	0.26	0.47
	124	0.00	0.09	0.16	0.12	0.17
	130	0.42	0.00	0.25	0.04	0.38
	136	0.08	0.00	0.04	0.36	0.24
	144	0.28	0.26	0.23	0.18	0.48
	162	0.09	0.00	0.00	0.00	0.05
	213	0.13	0.00	0.01	0.00	0.08
Sfo8	250	0.00	0.25	0.06	0.03	0.08
LAV	256	0.00	0.41	0.05	0.35	0.20
	264	0.00	0.01	0.17	0.11	0.07
	271	0.05	0.32	0.22	0.01	0.14
	278	0.21	0.00	0.06	0.00	0.07
	290	0.58	0.00	0.43	0.38	0.36
	308	0.16	0.00	0.01	0.12	0.08
Sfo23	176	0.00	0.08	0.06	0.20	0.00
LAV	178	0.00	0.08	0.15	0.06	0.08
	180	0.00	0.50	0.27	0.07	0.22
	199	0.04	0.33	0.02	0.16	0.15
	203	0.00	0.00	0.00	0.00	0.00
	222	0.33	0.00	0.03	0.28	0.19
	275	0.13	0.00	0.20	0.19	0.14
	283	0.00	0.00	0.00	0.00	0.00
	300	0.49	0.00	0.27	0.03	0.23

Locus	Allele (bp)	Frequency				
		TR (n=250)	YG (n=210)	H1 (n=185)	H2 (n=203)	total (n=848)
One18	180	0.00	0.06	0.02	0.01	0.02
ASC	185	0.00	0.24	0.21	0.14	0.14
	189	0.01	0.32	0.07	0.15	0.13
	191	0.00	0.26	0.03	0.09	0.09
	195	0.00	0.10	0.21	0.12	0.10
	204	0.13	0.00	0.04	0.19	0.09
	220	0.86	0.02	0.43	0.30	0.42
Sal5	196	0.44	0.00	0.05	0.22	0.19
UG	203	0.00	0.10	0.06	0.12	0.07
	205	0.00	0.70	0.22	0.30	0.29
	214	0.02	0.00	0.00	0.00	0.01
	218	0.04	0.00	0.20	0.00	0.06
	225	0.08	0.00	0.00	0.00	0.03
	230	0.02	0.00	0.00	0.21	0.06
	245	0.00	0.19	0.23	0.08	0.12
	258	0.22	0.00	0.20	0.03	0.12
	268	0.11	0.00	0.02	0.03	0.04
	272	0.06	0.00	0.00	0.00	0.02
SalP61	139	0.04	0.00	0.01	0.24	0.07
SFU	152	0.04	0.01	0.00	0.02	0.02
	157	0.00	0.30	0.09	0.03	0.10
	164	0.06	0.00	0.23	0.01	0.07
	170	0.06	0.16	0.20	0.18	0.14
	174	0.62	0.10	0.40	0.09	0.32
	178	0.08	0.41	0.06	0.43	0.24
	180	0.02	0.00	0.00	0.00	0.01
	184	0.07	0.00	0.01	0.00	0.02
	193	0.02	0.00	0.00	0.00	0.01
SalD39	194	0.04	0.00	0.00	0.20	0.06
SFU	196	0.25	0.00	0.02	0.05	0.09
	238	0.00	0.00	0.02	0.01	0.01
	243	0.00	0.20	0.02	0.08	0.07
	245	0.13	0.01	0.24	0.04	0.10
	255	0.17	0.57	0.31	0.46	0.37
	259	0.00	0.22	0.18	0.11	0.12
	268	0.02	0.00	0.01	0.00	0.01
	272	0.24	0.00	0.18	0.05	0.12
	280	0.05	0.00	0.01	0.00	0.02
	290	0.08	0.00	0.02	0.00	0.03

Appendix V

Juvenile fish probability estimates for between group differences in weight (above diagonal) and length (below diagonal).

		Weight											
		TRfxTRm	YGfxYGm	YGfxTRm	TRfxYGm	(YGfxTRm)fxYGm	(TRfxYGm)fxYGm	YGfx(TRfxYGm)m	YGfx(YGfxTRm)m	(YGfxTRm)fxTRm	(TRfxYGm)fxTRm	TRfx(TRfxYGm)m	TRfx(YGfxTRm)m
Length	TRfxTRm		7.57E-34	0.0076**ns**	0.046**ns**	4.34E-17	5.16E-13	0.000173	9.15E-36	4.45E-11	2.86E-16	1.55E-31	0.332**ns**
	YGfxYGm	9.30E-37		5.88E-48	1.08E-39	5.53E-06	5.23E-07	1.13E-14	0.0193**ns**	5.70E-50	4.10E-65	2.89E-66	5.04E-23
	YGfxTRm	1.01E-09	1.40E-73		0.257**ns**	1.24E-27	7.92E-22	2.42E-09	5.30E-49	1.58E-06	3.10E-10	2.66E-25	0.0052**ns**
	TRfxYGm	0.334**ns**	1.47E-35	4.95E-08		1.73E-22	8.27E-18	2.26E-07	1.21E-41	2.30E-07	3.38E-11	2.88E-26	0.028**ns**
	(YGfxTRm)fxYGm	2.13E-23	0.00199**ns**	1.83E-54	2.63E-23		0.193**ns**	0.0000221	1.28E-08	1.43E-36	9.19E-49	1.50E-56	8.40E-12
	(TRfxYGm)fxYGm	1.49E-11	3.10E-09	1.25E-34	5.15E-12	0.00108**ns**		0.00083**ns**	1.31E-09	3.65E-32	9.29E-43	3.37E-53	3.19E-09
	YGfx(TRfxYGm)m	0.00874**ns**	1.11E-20	2.47E-15	0.00353**ns**	5.24E-12	0.000054		2.42E-17	1.05E-19	9.76E-27	3.05E-41	0.00276**ns**
	YGfx(YGfxTRm)m	5.34E-34	0.0994**ns**	4.66E-65	1.33E-33	0.000176	5.96E-10	1.45E-20		5.77E-53	7.42E-68	5.52E-70	1.10E-25
	(YGfxTRm)fxTRm	1.08E-17	1.48E-70	0.000147	1.52E-15	7.71E-58	4.68E-42	1.47E-23	3.29E-68		0.165**ns**	4.27E-10	1.46E-10
	(TRfxYGm)fxTRm	3.42E-27	7.77E-94	4.87E-09	6.52E-24	1.96E-77	5.14E-57	1.93E-33	3.27E-87	0.057**ns**		9.52E-09	6.98E-15
	TRfx(TRfxYGm)m	3.78E-29	6.99E-82	4.03E-12	2.37E-26	1.52E-70	3.89E-55	2.98E-35	1.14E-80	0.000923**ns**	0.0345**ns**		5.27E-30
	TRfx(YGfxTRm)m	0.0514**ns**	1.33E-35	0.000112	0.116**ns**	7.37E-25	3.92E-14	0.000122	1.22E-34	8.28E-11	5.93E-17	6.04E-20	

Null rejected at P<0.00076 (Bonferroni 0.05/66). Ns; indicates not significant

Appendix VI

Results of all the microsatellite markers used for the genome wide scan in family 6-10 of the Icy Waters Arctic charr. For details see Appendix II. *; Locus mapped as duplicated in Arctic charr (Woram *et. al.* 2004). $; observed duplicated in Arctic charr in this study.

No.	Marker	Linkage Group (Woram *et al.* 2004)	Primer sequences (5'-->3') Forward	Revers	Annealing Temp (^{O}C)	Amplicon size range (approx.)	Results Test Screening (N=12; YG=6, TR=6)	Screening Family 6-10	Genotyping technique
1	As1.22NUIG	unassigned	unpublished	unpublished	45	160	differentially Monomorphic	informative	32p
2	BHMS121	25	CTT CTC TGT CGT ATG AAA TC	TAA CAC CCT TAC CCG TCC	50	120	differentially Biallelic	informative	HEX
3	BHMS206*	8 & 6	CCA AAT AAC TGA CAA GTG AG	CAG AGG TTG ATA ATG GGG	60	220	Shared Polymorphic	informative	FAM
4	BHMS330	8	CTA GAT CAC TCA CCC AGG	GTG CTT TTG GCT TAT GTT AG	60	250	differentially Monomorphic	*uninformative*	32p
5	BHMS356	15	CCA ATG ATG TAT ATG GCG	TTT GTG AAT GGG AGA CCG	53	200	Shared Polymorphic	informative	FAM
6	BHMS409	1	CCT GTT GTT TGG GTG TCG	AGT TGA TCT TAC CTG GGG	55	280	differentially Monomorphic	informative	32p
7	BHMS429	30	CCC CTG TCA AAC GTC TTC	AGC ACA CTG GAT TCA AGG	55	280	Shared Polymorphic	informative	TAM
8	BHMS490*	4 & 25	TCA AAT CTG TGT GAC TGC	ATG GAA CAA ATC ACC CTC	54	120	Shared Polymorphic	informative	FAM
9	BHMS546	38	GGG ACA CTC ATC TTG AAT G	GGT AAG CAT TTC ACA GTA AG	60	250	Shared Polymorphic	informative	HEX
10	BHMS7.030	4	ATG GTG AGT AGA TCC CCC	ATG ACT CCA GTT CCA CCC	46	150 & 250	Shared Polymorphic	*uninformative*	HEX
11	MST85[5]	unassigned	GGA AGG AAG GGA GAA AGG T	GGA AAA TCA ATA CTA ACA A	55	160-220 & 320-400	differentially Polymorphic	informative	FAM
12	Ogo4UW*	12 & 27	GTC GTC ACT GGC ATC AGC TA	GAG TGG AGA TGC AGC CAA AG	60	220	Shared Polymorphic	informative	HEX
13	OMM1012	unassigned	TGC ACT TCC GCT TCT	ATA GGA CAG GGT AAT GGG	55	240	Shared Polymorphic	*uninformative*	32p
14	OMM1013	unassigned	GAG GGA GCC AGA GAT ATT GAG	GGT CCT CCA TCG ATT CAA A	57-60	260-320	differentially Monomorphic	*uninformative*	TAM
15	OMM1035	unassigned	CTG CTG CTG AGA GAT GTG TTT	CGC CTC ATT TCA ACT ACT GT	60	300	differentially Monomorphic	*uninformative*	32p

No.	Marker	Linkage Group (Woram *et al.* 2004)	Primer sequences (5'-->3') Forward	Revers	Annealing Temp (^{O}C)	Amplicon size range (approx.)	Results Test Screening (N=12; YG=6, TR=6)	Screening Family 6-10	Genotyping technique
16	OMM1037	unassigned	GCG ACT GGA TTT AAT ACT GC	TCC TCT GAC TGC CAT TAC ATC	51-53	150	Shared Polymorphic	informative	HEX
17	OMM1184	25	TGT ATG TGT CCT TTA TGG G	GGA TGG ATG ATA TGG CTA TAC	55	120	Shared Polymorphic	*uninformative*	32p
18	OMM1228	4	CCC TTC CTG TGT GTC GTT GTT	CAG GAG TCA CTT GGC AGT AGG AG	60	240	Shared Polymorphic	informative	FAM
19	Omy13INRA	31	GTA AAC ATG TGT TTA CCA TGC C	GAA TCC TGC ACT ATA ATC GCC	50-55	200-270	differentially Polymorphic	informative	HEX
20	Omy18INRA	16	CGG GCT CAG ATT TCA CAG	GAT GCA TGT ACT CTG TAG GTA GC	55-60	200	Shared Polymorphic	*uninformative*	FAM
21	Omy301UG	7	ACT TAA GAC TGG CAA CCT T	CTA CAC GGC CTT CGG GTG AGA	55	70-150	differentially Polymorphic	informative	FAM
22	Omy38DU[$]	10	TGG TTG TTG CCA TTT GTC TC	GCC TGT ATT GTC TGA GAA GG	60	172-194 & 207-263	Shared Polymorphic	informative	TAM
23	Omy6DIAS	4	CCA CCA ACT TCT TAC ATG AT	CTA TGG GGA CAG CCG AAT AA	55	320	differentially Polymorphic	informative	HEX
24	Omy77DU	12	CGT TCT CTA CTG AGT CAT	GTC TTT AAG GCT TCA CTG CA	60	120-240	differentially Polymorphic	informative	HEX
25	OmyRGT39TUF	25	TAA GCG CAT GAC TGA ACA GG	TAT GTG ACC CCG ACC AAA TT	55-60	150	differentially Polymorphic	informative	TAM
26	OmyRGT4TUF	20	GGA ACA CTG AGA ATT CCT CCC	TCG CTC AGC CAC TAC AAG TG	60	140	differentially Polymorphic	informative	HEX
27	One10ASC	26	ATG GGG AAC AGA AGA GGA AT	CTG TAG GTG TGA AAT GTA TTT AAA	50	180	differentially Polymorphic	informative	TAM
28	One11ASC	14	GTT TGG ATG ACT CAG ATG GGA CT	TCT ATC TTT CCT GTC AAC TTC CA	50-52	120	Shared Polymorphic	informative	FAM
29	One18ASC	unassigned	ATG GCT GCA TCT AAT GGA GAG TAA	AAA CCA CAC ACA CTG TAC GCC AA	55	180-220	Shared Polymorphic	informative	TAM
30	One1ASC	24	GTC TTA CCA AAT GTC TTC CTC CT	GCC ATT TAG CAT ACG ATT TTA TC	60	120-220	Shared Polymorphic	informative	HEX
31	One8ASC	6	AAC ATT CTG GGA TGA CAG GGG TA	CTG TTC TGC TCC AGT GAA GTG GA	55-60	148-160	Shared Polymorphic	informative	FAM
32	Ots500NWFSC	4	AAC TCC TGG ACA AAC CTC G	TGA CCC TGC CCA TAA CAC	60		Shared Polymorphic	*uninformative*	FAM
33	Ots510NWFSC	24	ACT GGG AGC TTA TTG TTC AC	ACG ATA AGA GGC AAA GGA C	55	200	differentially Monomorphic	*uninformative*	HEX
34	Sal5UG	36	TTT GCA TTG AGC CTC TGT TG	TGT TTC AGC TGC TAT TAG GAA AT	50	190-275	Shared Polymorphic	informative	FAM

No.	Marker	Linkage Group (Woram *et al.* 2004)	Primer sequences (5'-->3') Forward	Revers	Annealing Temp (^{O}C)	Amplicon size range (approx.)	Results Test Screening (N=12; YG=6, TR=6)	Screening Family 6-10	Genotyping technique
35	Sal7UG	23	CTA CAG GTT TCC CTG TGG GG	AGC CTG AAC ATT ACC CCT GA	57-60	300-370	Shared Polymorphic	informative	FAM
36	Sal9UG	12	TCA CTG CTT CAA GGT ATT TTT ACT T	AAT TAG AGC TGC TAG GTC AGT GAG	60	220-320	differentially Polymorphic	informative	FAM
37	SalD100SFU*	20	TGA CTA TAA CTA CCA AAC TGC TTA C	AGA CAC AGA CAA GCA TTC G	55-60	200	Shared Polymorphic	informative	32p
38	SalD30SFU	unassigned	TTT GGT GTT ATG ACT CTG CG	CAA GCA GAA TCG TTT GGT C	57-60	300	differentially Polymorphic	*strange alleles*	TAM
39	SalD39SFU	25	GG GAG TCT GTG TTA AGT TGG	TGA ATG GAC GTT CCT CTG AC	55	190-290	Shared Polymorphic	informative	TAM
40	SalE38SFU	18	CGC CTT GTC ATA CAT TAC ACC	ACG CTA CAG AAA CAG GAG AAA G	55	110-220	Shared Polymorphic	informative	TAM
41	SalF41SFU*	3 or 24	ATC CGC TAT GAA CCA CAG G	ACT GCT CCG GCA ACT ACA G	60	240	differentially Monomorphic	informative	HEX
42	SalF56SFU	11	TGC AGT TCC ACA ATA TAT CCC	AAG GGCACA CTC AGA TTT TG	55	280	differentially Polymorphic	informative	TAM
43	SalJ81SFU	3	CAG CAT AAT CAC TCC CGC	GAA AGC TAC CTT GCG TGC	55	150-220	differentially Polymorphic	informative	FAM
44	SalP61SFU	14	CAC TTA TTA ACG CCC ACT CCC	TTC ACA ACC ACA GGA AAG AAC TC	55	135-195	Shared Polymorphic	informative	HEX
45	SapA69SFU	unassigned	TTT GAG CAG TGT ATA ACG TTT TAA C	AGA GTG TCA GAT GTC AAT CAC CTA C	45	380	Shared Polymorphic	*uninformative*	TAM
46	SapI26SFU[$]	unassigned	ACA TAC TCT GCC TTA TTT CAT ACG	CAT TGG TGA TCC TTT CTT CAG	45-50	70 & 300	Shared Polymorphic	informative	32p
47	Sfo23LAV	unassigned	GTG TTC TTT TCT CAG CCC	AAT GAG CGT TAC GAG AGG	55	175-350	Shared Polymorphic	informative	HEX
48	Sfo8LAV	unassigned	CAA CGA GCA CAG AAC AGG	CTT CCC CTG GAG AGG AAA	55	250-310	Shared Polymorphic	informative	FAM
49	SL/i(INRA)	22	GAA AAT AAC TAT AGA CAT TGC TGG	CGT CCT TAC ACT CCA GAG GG	50	250-320	Shared Polymorphic	informative	TAM
50	Ssa14DU	9	CCT TTT GAC AGA TTT AGG ATT TC	CAA ACC AAA CAT ACC TAA AGC C	50	160	Shared Polymorphic	informative	FAM
51	Ssa171	unassigned	TTA TTA TCC AAA GGG GTC AAA A	GAG GTC GCT GGG GTT TAC TAT	55	100	Shared Polymorphic	informative	FAM
52	Ssa197	unassigned	GGG TTG AGT AGG GAG GCT TG	TGG CAG GGA TTT GAC ATA AC	57	120	Shared Polymorphic	*uninformative*	HEX
53	Ssa20.19NUIG[$]	16	TCA ACC TGG TCT GGC TTC GAC	CTA GTT TCC CCA GCA CAG CC	55-57	75-180	Shared Polymorphic	informative	HEX

No.	Marker	Linkage Group (Woram *et al.* 2004)	Primer sequences (5'-->3') Forward	Revers	Annealing Temp (^{o}C)	Amplicon size range (approx.)	Results Test Screening (N=12; YG=6, TR=6)	Screening Family 6-10	Genotyping technique
54	Ssa208[$]	unassigned	AAA CCT GTG AGC TGG AAC A	CCA CAA CAG AGT ATC AGC TG	55-60	200 & 360	Shared Polymorphic	informative	FAM
55	Ssa289	unassigned	unpublished	unpublished	45- 47	120	Shared Polymorphic	informative	32p
56	Ssa77NUIG	1	GCC ATC ACC TCA CTG TGT GG	CTC GTG CTT TTT CCT GGT CC	60	180	Shared Polymorphic	informative	HEX
57	Ssa85DU	13	AGG TGG GTC CTC CAA GCT AC	ACC CGC TCC TCA CTT AAT C	55	130-230	Shared Polymorphic	informative	FAM
58	SsaR15LEE	33	CAC CAC TGT CGC TGT GTC C	TGC TGC GTT TTA TTT AAG CC	50	400	differentially Monomorphic	*uninformative*	32p
59	SSOSL32*	4 & 25	CTG TAT ACT ATG GTG GTG GCT	TTA TGT CAG ACC AGG TGG CTA	62	140	Shared Polymorphic	informative	FAM
60	SSOSL456	29	CTT CCC AGG AGT CAT CAT AAA TCT	TAA ACC CCA CTG CTT GTT GAG TGT	55	170-190	Shared Polymorphic	informative	HEX
61	Str7INRA	25	GGA TCA CCC CTA CTA AAT GGG	TGC TGT AAG TGA ACA TTA AGG C	55-60	280	Shared Polymorphic	*uninformative*	32p
62	U5.27NUIG	unassigned	GTT ACC TTG CTC CTA G	CCA GTG TGC CAC CCC	55	140	Shared Polymorphic	informative	TAM

Appendix VII

Putative QTL for total length (LT), body weight (WT), and Fulton's condition factor (K_{TL}) in family 6-10 of Icy Waters Arctic charr. Values in **bold** indicate significant allele effects at $p<0.05$, while values in ***bold-italics*** indicate marginal effects at $0.06>p>0.05$ for the transmission disequilibrium test (TDT).

No.	Locus	Linkage Group (Woram et al. 2004)	N	Parental Sex	Allele (freq.)	Total Length (cm)		Weight (gm)		Fulton's Condition Factor (K_{TL})	
						Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*
1	As1.22NUIG	unassigned	36	f	a(25) - b(11)	14.9(1.8) - 14.6(2.4)	0.918	32.3(12.9) - 30.9(14.5)	0.710	0.92(0.06) - 0.91(0.04)	0.398
				m	Homozygous(a/a)	-		-		-	
2	BHMS121*	25	36	f	123(20) - 131(16)	14.2(1.9) - 15.6(1.9)	**0.052**	27.7(10.5) - 37.1(14.7)	**0.047**	0.91(0.05) - 0.92(0.06)	0.346
				m	131(23) - 135(13)	15.4(1.8) - 13.9(2.0)	**0.041**	35.0(13.4) - 26.3(11.4)	**0.052**	0.91(0.05) - 0.92(0.05)	0.779
3	BHMS206*	8 & 6	36	f	174(24) - 180(12)	15.3(2.0) - 14.0(1.7)	**0.056**	35.0(14.0) - 25.6(9.1)	**0.048**	0.92(0.05) - 0.90(0.05)	0.207
				m	Homozygous(209)	-		-		-	
5	BHMS356*	15	36	f	189(17) - 202(19)	15.2(1.8) - 14.5(2.1)	0.194	34.4(12.1) - 29.6(14.1)	0.178	0.93(0.06) - 0.90(0.04)	**0.043**
				m	Homozygous(202)	-	-	-	-	-	-
6	BHMS409*	1	35	f	A(21) - b(14)	14.7(2.2) - 15.2(1.6)	0.409	31.815.2) - 32.8(10.2)	0.479	0.92(0.05) - 0.91(0.06)	0.600
				m	Homozygous(b/b)	-		-		-	
7	BHMS429*	30	36	f	217(19) - 223(17)	14.7(2.0) - 15.0(2.0)	0.680	31.1(13.3) - 32.7(13.5)	0.635	0.92(0.04) - 0.91(0.06)	0.987
				m	213(21) - 217(15)	14.9(2.2) - 14.8(1.8)	0.923	32.3(15.0) - 31.2(10.8)	0.885	0.91(0.05) - 0.92(0.06)	0.499

No.	Locus	Linkage Group (Woram et al. 2004)	N	Paretal Sex	Allele (freq.)	Total Length (cm)		Weight (gm)		Fulton's Condition Factor (K_n)	
						Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*
8	BHMS490*	4 & 25	36	f	111(22) - 115(14)	14.5(2.3) - 15.4(1.3)	0.188	29.6(14.5) - 35.4(10.4)	0.123	0.90(0.05) - 0.94(0.04)	**0.008**
				m	109(19) - 111(17)	15.8(1.7) - 13.8(1.8)	**0.002**	38.2(13.3) - 24.7(9.1)	**0.002**	0.93(0.050 - 0.90(0.04)	**0.016**
9	BHMS546*	38	36	f	190(18) - 213(18)	15.4(1.5) - 14.3(2.3)	0.090	34.4(10.2) - 29.4(15.6)	0.100	0.92(0.04) - 0.91(0.06)	0.775
				m	Homozygous(190)	-	-	-	-	-	-
11(a)	MST85(a)	unassigned	35	f	174(22) - 208(13)	14.9(2.1) - 14.4(1.6)	0.384	32.5(13.6) - 28.7(10.8)	0.403	0.92(0.05) - 0.91(0.05)	0.607
				m	174(16) - 182(19)	14.6(1.7) - 14.9(2.20	0.596	30.0(10.6) - 32.0(10.6)	0.667	0.92(0.05) - 0.91(0.06)	0.816
11(b)	MST85(b)	unassigned	36	f	329(21) - 383(15)	15.1(2.1) - 14.4(1.8)	0.344	33.9(14.8) - 29.1(10.5)	0.451	0.91(0.06) - 0.92(0.05)	0.784
				m	Homozygous(383)	-	-	-	-	-	-
12	Ogo4UW	12 & 27	36	f	170(17) - 209(19)	15.0(1.8) - 14.7(2.2)	0.775	32.2(12.3) - 31.5(14.3)	0.800	0.91(0.05) - 0.92(0.05)	0.787
				m	174(21) - 209(15)	14.7(2.1) - 15.1(1.9)	0.760	30.6(12.6) - 33.6(14.3)	0.688	0.91(0.05) - 0.93(0.05)	0.273
16	OMM1037	unassigned	36	f	124(18) - 128(18)	15.6(1.9) - 14.1(1.8)	**0.019**	37.1(14.6) - 26.6(9.5)	**0.019**	0.92(0.06) - 0.91(0.05)	0.309
				m	Homozygous(124)	-		-		-	
18	OMM1228	4	36	f	239(17) - 242(19)	14.8(2.1) - 14.9(1.9)	0.862	32.0(13.2) - 31.7(13.6)	0.763	0.92(0.05) - 0.91(0.05)	0.340
				m	226(19) - 239(17)	14.6(2.2) - 15.1(1.7)	0.657	30.5(13.2) - 33.4(13.5)	0.646	0.91(0.05) - 0.92(0.05)	0.750
19	Omy13INRA	31	36	f	227(17) - 237(19)	15.1(1.9) - 14.6(2.1)	0.557	33.3(13.3) - 30.5(13.4)	0.537	0.92(0.05) - 0.91(0.05)	0.763
				m	222(16) - 237(20)	15.2(1.7) - 14.6(2.2)	0.279	32.9(11.0) - 31.0(15.0)	0.308	0.90(0.0) - 0.92(0.05)	0.544

No.	Locus	Linkage Group (Woram et al. 2004)	N	Paretal Sex	Allele (freq.)	Total Length (cm)		Weight (gm)		Fulton's Condition Factor (K_{TL})	
						Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*
21	Omy301UoG	7	36	f	70(17) - 134(19)	14.9(2.1) - 14.8(1.9)	0.862	32.4(14.4) - 31.3(12.4)	0.800	0.91(0.04) - 0.92(0.06)	0.356
				m	70(14) - 79(22)	14.5(2.3) - 15.1(1.8)	0.527	30.6(14.8) - 32.7(12.4)	0.615	0.91(0.06) - 0.92(0.05)	0.961
22(a)	Omy38DU(a)	10	14	genotypic	178/178(5)-187/187(9)	16.2(2.1) - 14.6(1.4)	0.204	41.3(18.7) - 29.3(9.0)	0.205	0.91(0.09) - 0.91(0.05)	0.688
			27		178/178(5)-178/187(22)	16.2(2.1) - 14.6(2.1)	0.208	41.3(18.7) - 30.8(13.0)	0.257	0.91(0.09) - 0.92(0.04)	0.976
			31		187/187(9)-178/187(22)	14.6(1.4) - 14.6(2.1)	0.915	29.3(9.0) - 30.8(13.0)	0.881	0.91(0.05) - 0.92(0.04)	0.593
22(b)	Omy38DU(b)	10	36	f	207(25) - 263(11)	15.0(1.9) - 14.6(2.1)	0.757	32.6(13.6) - 30.3(12.8)	0.680	0.92(0.05) - 0.91(0.05)	0.501
				m	Homozygous(263)	-		-		-	
23	Omy6DIAS	4	35	f	213(21) - 275(14)	15.2(1.8) - 14.2(2.2)	0.195	33.9(13.5) - 28.1(12.8)	0.245	0.91(0.05) - 0.91(0.06)	1.000
				m	213(19) - 229(16)	14.5(2.20 - 15.2(1.7)	0.436	29.9(13.4) - 33.6(13.4)	0.487	0.91(0.050 - 0.91(0.05)	0.702
24	Omy77DU	12	20	genotypic	146/146(7)-235/235(13)	15.1(1.7) - 14.7(2.5)	0.937	32.8(10.3) - 31.4(14.6)	0.721	0.93(0.03) - 0.92(0.06)	0.633
			23		146/146(7)-146/235(16)	15.1(1.7) - 14.9(1.7)	0.579	32.8(10.3) - 31.9(13.9)	0.413	0.93(0.03) - 0.91(0.06)	0.278
			29		235/235(13)-146/235(16)	14.7(2.5) - 14.9(1.7)	0.983	31.4(14.6) - 31.9(13.9)	0.983	0.92(0.06) - 0.91(0.06)	0.619
25	OmyRGT39TUF	25	36	f	106(15) - 118(21)	15.5(1.3) - 14.4(2.2)	0.072	36.1(10.5) - 28.8(14.3)	**0.047**	0.94(0.04) - 0.90(0.05)	**0.009**
				m	116(18) - 118(18)	14.0(1.9) - 15.7(1.7)	**0.010**	26.0(10.3) - 37.7(13.5)	**0.007**	0.90(0.04) - 0.93(0.06)	**0.016**
26	OmyRGT4TUF	20	36	f	140(18) - 142(18)	14.2(1.0) - 15.5(1.7)	**0.060**	27.5(11.7) - 36.3(13.4)	**0.052**	0.90(0.04) - 0.93(0.06)	**0.044**
				m	132(22) - 140(14)	15.2(1.8) - 14.3(2.2)	0.194	33.8(12.4) - 28.9(14.4)	0.200	0.92(0.05) - 0.91(0.06)	0.782

No.	Locus	Linkage Group (Woram et al. 2004)	N	Paretal Sex	Allele (freq.)	Total Length (cm)		Weight (gm)		Fulton's Condition Factor (K_{TL})	
						Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*
27	One10ASC	26	36	f	166(15) - 172(21)	14.8(2.1) - 14.9(1.9)	0.822	31.5(14.6) - 32.1(12.5)	0.630	0.91(0.04) - 0.92(0.06)	0.384
				m	160(17) - 172(19)	14.6(1.8) - 15.1(2.1)	0.516	29.4(9.8) - 34.0(15.6)	0.358	0.91(0.05) - 0.92(0.05)	0.715
28	One11ASC	14	36	f	Homozygous(124)	-	-	-	-	-	-
				m	124(16) - 137(20)	15.3(1.8) - 14.5(2.1)	0.399	34.4(14.3) - 29.8(12.3)	0.484	0.91(0.05) - 0.92(0.05)	0.786
29	One18ASC	unassigned	36	f	195(19) - 204(17)	15.2(2.2) - 14.5(1.8)	0.303	34.6(15.0)- 28.8(10.5)	0.254	0.92(0.04) - 0.91(0.06)	0.390
				m	Homozygous(220)	-		-		-	
30	One1ASC	24	36	f	Null(22) - 137(14)	14.5(2.1) - 15.5(1.6)	0.200	29.7(13.2) - 35.2(13.0)	0.256	0.92(0.06) - 0.91(0.04)	0.794
				m	Null(18) - 133(18)	14.8(2.3) - 14.9(1.6)	0.962	32.1(15.7) - 31.7(10.6)	0.950	0.91(0.06) - 0.92(0.04)	0.400
31	One8ASC	6	17	genotypic	148/148(10) - 158/158(7)	14.4(1.7) - 15.2(1.5)	0.406	27.8(10.1) - 32.9(10.4)	0.380	0.89(0.04) - 0.91(0.06)	0.351
			29		148/148(10)-148/158(19)	14.4(1.7) - 14.9(2.4)	0.396	27.8(10.1) - 33.6(16.0)	0.371	0.89(0.04) - 0.93(0.05)	**0.045**
			26		158/158(7) -148/158(19)	15.2(1.5) - 14.9(2.4)	1.000	32.9(10.4) - 33.6(16.0)	0.862	0.91(0.06) - 0.93(0.05)	0.505
34	Sal5UG	36	36	f	230(20) - 245(16)	14.9(2.2) - 14.6(1.8)	0.679	31.7(15.2) - 32.0(10.8)	0.567	090(0.05) - 0.93(0.05)	**0.053**
				m	196(21) - 258(15)	14.8(1.7) - 15.0(2.30	0.700	31.2(11.4) - 32.8(15.8)	0.797	0.92(0.04) - 0.91(0.06)	0.573
35	Sal7UG	23	36	f	Homozygous(344)	-	-	-	-	-	-
				m	308(16) - 344(20)	15.1(1.8) - 14.7(2.1)	0.679	33.5(13.8) - 30.5(12.9)	0.679	0.93(0.05) - 0.91(0.05)	0.346
36	Sal9UG	12	36	f	Null(21) - 306(15)	14.5(2.1) - 15.3(1.7)	0.344	29.9(12.8) - 34.5(13.8)	0.336	0.91(0.05) - 0.92(0.06)	0.551

No.	Locus	Linkage Group (Woram et al. 2004)	N	Paretal Sex	Allele (freq.)	Total Length (cm)		Weight (gm)		Fulton's Condition Factor (K_{TL})	
						Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*
				m	Homozygous(243)	-	-	-	-	-	-
37	SalD100SFU*	20	24	genotyp ic	a/a(13) - b/b(11)	15.1(1.2) - 14.9(2.6)	0.642	32.1(8.6) - 33.7(15.0)	0.505	0.90(0.05) - 0.94(0.04)	0.103
			25		a/a(13) - a/b(12)	15.1(1.2) - 14.5(2.2)	0.270	32.1(8.6) - 29.9(16.2)	0.270	0.90(0.05) - 0.90(0.06)	0.769
			23		b/b(11) - a/b(12)	14.9(2.6) - 14.5(2.2)	0.449	33.7(15.0) - 29.9(16.2)	0.347	0.94(0.04) - 0.90(0.06)	0.118
39	SalD39SFU	25	36	f	255(13) - 259(23)	14.8(2.7) - 14.9(1.6)	0.947	32.7(17.7) - 31.4(10.4)	0.961	0.90(0.07) - 0.92(0.04)	0.428
				m	272(24) - 290(12)	15.2(1.9) - 14.1(1.9)	0.127	34.1(13.8) - 27.5(11.2)	0.154	0.91(0.05) - 0.93(0.05)	0.578
40	SalE38SFU	18	36	f	124(21) - 142(15)	15.0(1.8) - 14.6(2.3)	0.760	32.8(13.5) - 30.5(13.2)	0.700	0.92(0.05) - 0.91(0.06)	0.489
				m	130(20) - 140(16)	15.2(2.1) - 14.5(1.9)	0.279	34.0(14.7) - 29.2(11.0)	0.390	0.91(0.06) - 0.92(0.05)	0.835
41	SalF41SFU*	3 or 24	36	f	182(18) - 204(18)	14.4(2.2) < 15.3(1.6)	0.211	29.6(13.7) < 34.1(12.7)	0.268	0.92(0.05) < 0.91(0.05)	0.668
				m	Unsuitable to score						
42	SalF56SFU	11	36	f	179(23) - 273(13)	14.9(2.6) > 14.8(1.5)	0.947	32.4(15.2) > 30.9(9.1)	0.974	0.91(0.06) > 0.92(0.05)	0.679
				m	167(17) - 211(19)	15.0(1.8) - 14.7(2.1)	0.646	32.2(12.2) - 31.6(14.4)	0.716	0.91(0.05) - 0.92(0.05)	0.567
43	SalJ81SFU	3	36	f	140(23) - 211(13)	14.9(1.82) - 14.7(2.3)	0.921	32.2(13.1) - 31.3(14.0)	0.805	0.92(0.06) - 0.91(0.04)	0.467
				m	Homozygous(200)	-		-		-	
44	SalP61SFU	14	36	f	139(13) - 178(23)	14.9(2.0) - 14.9(2.0)	0.895	32.3(12.8) - 31.6(13.7)	0.754	0.93(0.04) - 0.91(0.05)	0.390
				m	174(25) - 178(11)	15.0(1.9) - 14.4(2.2)	0.757	32.8(13.8) - 29.6(12.2)	0.744	0.91(0.06) - 0.92(0.04)	0.654

No.	Locus	Linkage Group (Woram et al. 2004)	N	Paretal Sex	Allele (freq.)	Total Length (cm)		Weight (gm)		Fulton's Condition Factor (K_{TL})	
						Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*
46	Sapl26SFU[$]	unassigned	36	f	a(15) - c(21)	14.6(1.7) - 15.1(2.2)	0.490	29.4(10.5) - 33.6(14.9)	0.531	0.91(0.04) - 0.92(0.06)	0.359
				m	a(17) - b(19)	14.6(2.3) - 15.1(1.7)	0.590	30.5(13.9) - 33.1(12.8)	0.646	0.91(0.05) - 0.92(0.05)	0.750
47	Sfo23LAV	unassigned	36	f	199(20) - 222(16)	14.9(1.9) - 14.8(2.2)	1.000	31.8(12.3) - 32.0(14.7)	0.949	0.92(0.05) - 0.91(0.06)	0.798
				m	290(22) - 300(14)	15.2(1.8) - 14.3(2.2)	0.194	33.8(12.4) - 28.9(14.4)	0.206	0.92(0.05) - 0.91(0.06)	0.782
48	Sfo8LAV	unassigned	36	f	256(20) - 286(16)	14.9(1.8) - 14.8(2.2)	0.848	31.7(13.2) - 32.0(13.6)	0.750	0.91(0.05) - 0.92(0.05)	0.620
				m	278(19) - 308(17)	14.5(2.2) - 15.3(1.7)	0.358	29.9(13.4) - 34.1(13.1)	0.383	0.91(0.05) - 0.92(0.05)	0.600
49	SL/i(INRA)	22	36	f	256(16) - 271(20)	15.2(2.1) - 14.6(1.9)	0.147	33.9(13.1) - 30.2(13.4)	0.245	0.91(0.06) - 0.92(0.05)	0.415
				m	Homozygous(271)	-	-	-		-	
50	Ssa14DU	9	36	f	140(19) - 148(17)	14.6(1.7) - 15.2(2.3)	0.419	29.8(10.2) - 34.1(16.0)	0.590	0.92(0.05) - 0.91(0.06)	0.215
				m	143(16) - 148(20)	14.6(2.0) - 15.1(2.0)	0.323	30.6(14.3) - 32.9(12.6)	0.484	0.92(0.05) - 0.91(0.05)	0.502
51	Ssa171	unassigned	36	f	96(18) - 99(18)	14.2(2.0) - 15.5(1.7)	**0.055**	27.3(11.6) - 36.4(13.5)	**0.041**	0.9(0.04) - 0.93(0.06)	**0.022**
				m	Homozygous(99)	-		-		-	
53(a)	Ssa20.19NUIG(a)	16	36	f	79(17) - 87(19)	14.7(1.9) - 14.9(2.1)	0.729	31.1(12.8) - 32.1(14.2)	0.961	0.93(0.05) - 0.91(0.05)	0.670
				m	Homozygous(79/79)	-		-		-	
53(b)	Ssa20.19NUIG(b)	16	35	f	null(20) - 93(15)	14.7(1.6) - 14.9(2.4)	0.652	30.3(11.2) - 33.4(16.0)	0.571	0.91(0.05) - 0.93(0.05)	0.192
				m	Homozygous(113)	-		-		-	

No.	Locus	Linkage Group (Woram et al. 2004)	N	Paretal Sex	Allele (freq.)	Total Length (cm)		Weight (gm)		Fulton's Condition Factor (K_{TL})	
						Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*	Respective Means (Std. Dev.)	Test-Stat.*
54(a)	Ssa208(a)	unassigned	36	f	230(17) - 258(19)	14.9(1.8) - 14.8(2.2)	0.788	31.4(11.4) - 32.3(15.0)	0.862	0.90(0.06) - 0.93(0.05)	0.373
				m	230(20) - 242(16)	15.0(2.0) - 14.7(2.0)	0.911	33.0(14.40 - 14.7(11.9)	0.949	0.92(0.06) - 0.90(0.04)	0.379
54(b)	Ssa208(b)	unassigned	36	f	310(17) - 340(19)	15.5(1.9) - 14.3(1.9)	**0.064**	35.9(13.7) - 28.2(12.0)	**0.053**	0.93(0.06) - 0.91(0.04)	0.089
				m	280(19) - 300(17)	14.7(1.9) - 15.0(2.1)	0.428	29.9(12.2) - 34.0(14.3)	0.254	0.89(0.05) - 0.94(0.04)	**0.003**
55	Ssa289	unassigned	36	f	a(18) - b(18)	14.7(1.7) - 15.0(2.3)	0.476	30.1(11.50 - 33.6(14.6)	0.467	0.90(0.05) - 0.93(0.05)	0.227
				m	Homozygous(b/b)	-		-		-	
56	Ssa77NUIG	1	36	f	162(15) - 166(21)	14.7(1.7) - 15.0(2.2)	0.785	29.6(10.3) - 33.5(15.0)	0.585	0.89(0.05) - 0.93(0.05)	**0.031**
				m	Homozygous(166)	-		-		-	
57	Ssa85DU	13	36	f	Homozygous(187)	-		-		-	
				m	187(18) - 223(18)	14.9(2.0) - 14.9(2.0)	0.658	31.5(14.2) - 32.3(12.6)	0.681	0.90(0.04) - 0.93(0.05)	0.070
59	SSOSL32(i)	4 & 25	36	f	000(15) - 100(21)	14.3(2.0) - 15.3(1.9)	0.261	27.9(10.9) - 34.7(14.2)	0.223	0.90(0.04) - 0.92(0.06)	0.303
				m	000(13) - 085(23)	14.7(1.6) - 14.9(2.2)	0.609	30.2(10.3) - 32.8(14.7)	0.767	0.91(0.05) - 0.92(0.06)	0.753
60	SSOSL456	29	36	f	179(20) - 185(16)	14.6(2.1) - 15.2(1.9)	0.445	30.3(12.7) - 33.8(14.0)	0.435	0.92(0.05) - 0.91(0.05)	0.811
				m	177(19) - 179(17)	14.5(1.9) - 15.3(2.0)	0.234	29.3(11.6) - 34.7(14.7)	0.254	0.91(0.03) - 0.92(0.07)	0.455
62	U5.27NUIG	unassigned	36	f	130(160 - 132(20)	14.9(2.2) - 14.8(1.9)	0.962	32.4(14.9) - 31.4(12.1)	0.863	0.91(0.04) - 0.92(0.06)	0.440
				m	Homozygous(130)	-		-		-	

* Mann-Whitney U-rank test was performed. *BHMS markers have been renamed, for details see Table 2.2.

Appendix VIII

Results of linear regression analysis for total length (R^2 log LT), body weight (R^2 log WT), and Fulton's condition factor (R^2 logK_{TL}) in family 6-10.

locus	parental sex	R^2 log LT	R^2 log WT	R^2 log K_{TL}
As1.22NUIG	f	0.008	0.009	0.009
BHMS121*	f	0.124	0.113	0.007
	m	0.146	0.116	0.014
BHMS206*	f	0.102	0.105	0.041
BHMS356*	f	0.039	0.047	0.080
BHMS409*	f	0.016	0.009	0.030
BHMS429*	f	0.005	0.004	0.000
	m	0.000	0.000	0.009
BHMS490*	f	0.063	0.080	0.151
	m	0.255	0.263	0.119
BHMS546*	f	0.086	0.077	0.001
MST85a	f	0.012	0.012	0.004
	m	0.004	0.001	0.018
MST85b	f	0.028	0.024	0.000
Ogo4UW	f	0.006	0.004	0.001
	m	0.011	0.016	0.049
OMM1037	f	0.147	0.136	0.010
OMM1228	f	0.001	0.000	0.027
	m	0.022	0.019	0.000
Omy13INRA	f	0.020	0.019	0.002
	m	0.023	0.014	0.034
Omy301UG	f	0.001	0.000	0.002
	m	0.020	0.018	0.000
Omy38aDU	genotypic	0.072	0.060	0.011
Omy38bDU	f	0.008	0.008	0.003
Omy6DIAS	f	0.072	0.063	0.000
	m	0.043	0.039	0.001

locus	parental sex	R^2 log LT	R^2 log WT	R^2 log K_{TL}
Omy77DU	genotypic	0.011	0.009	0.030
OmyRGT39TUF	f	0.099	0.116	0.145
	m	0.192	0.203	0.121
OmyRGT4TUF	f	0.123	0.132	0.081
	m	0.056	0.050	0.001
One10ASC	f	0.001	0.002	0.012
	m	0.015	0.015	0.006
One11ASC	m	0.043	0.035	0.001
One18ASC	f	0.024	0.028	0.030
One1ASC	f	0.073	0.062	0.002
	m	0.002	0.004	0.029
One8ASC	genotypic	0.020	0.027	0.157
Sal5UG	f	0.000	0.003	0.099
	m	0.001	0.000	0.015
Sal7UG	m	0.015	0.020	0.042
Sal9UG	f	0.041	0.036	0.000
SalD100SFU	genotypic	0.025	0.023	0.092
SalD39SFU	f	0.002	0.005	0.031
	m	0.076	0.056	0.023
SalE38SFU	f	0.011	0.013	0.018
	m	0.032	0.024	0.008
SalF41SFU	f	0.062	0.048	0.013
SalF56SFU	f	0.000	0.000	0.001
	m	0.007	0.003	0.026
SalJ81SFU	f	0.005	0.005	0.002
SalP61SFU	f	0.000	0.001	0.045
	m	0.025	0.018	0.007
SapI26SFU	f	0.013	0.014	0.003
	m	0.022	0.020	0.001
Sfo23LAV	f	0.000	0.000	0.003

locus	parental sex	R^2 log LT	R^2 log WT	R^2 log K_{TL}
	m	0.056	0.050	0.001
Sfo8LAV	f	0.001	0.000	0.007
	m	0.043	0.039	0.001
SL/i(INRA)	f	0.024	0.015	0.031
Ssa14DU	f	0.017	0.011	0.022
	m	0.015	0.008	0.037
Ssa171	f	0.125	0.140	0.121
Ssa20.19aNUIG	f	0.002	0.000	0.055
Ssa20.19bNUIG	f	0.000	0.003	0.062
Ssa208a	f	0.001	0.000	0.046
	m	0.006	0.009	0.035
Ssa208b	f	0.081	0.084	0.035
	m	0.005	0.017	0.244
Ssa289	f	0.004	0.008	0.041
Ssa77NUIG	f	0.002	0.009	0.152
Ssa85DU	m	0.000	0.001	0.100
SSOSL32i	f	0.070	0.072	0.028
	m	0.001	0.001	0.000
SSOSL456	f	0.025	0.020	0.004
	m	0.037	0.035	0.003
U5.27NUIG	f	0.000	0.000	0.001

♦BHMS markers have been renamed, for details see Table 2.2.

Appendix IX

The computer code for the regression analysis for the total length (logTL) on 78 possible permutations in the family 6-10 of Icy Waters Arctic charr. The code was modified for the analysis of body weight (logWT) and Fulton's condition factor ($logK_{TL}$) in the same family. The analysis was performed using SAS (version 8.0) software from the SAS Institute, Cary, North Carolina, USA.

```
/* following is the code for "loglen" */
 proc iml;
  use tem1 var _all_;
  read all var _all_ into z;
  N=36*78;
  varnum=j(N,1,0);
  ylt=j(N,1,0);
  x=j(N,1,0);
  do i=1 to 78;
   do j=1 to 36;
     k=j+36*(i-1);
      varnum[k]=i;
      x[k]=z[j,i+6];
      ylt[k]=z[j,4];
     end;
  end;
 create loglen var{varnum ylt x};
 append;
 close loglen;
 run;
quit;
 ods output FitStatistics=outlen;
 ods listing close;
  proc glm data=loglen;
  by varnum;
  class x;
  model ylt=x/ss1;
 run;
 ods listing;
 proc print data=outlen;
 run;
```

Appendix X

Recombination frequencies (male), chi-square test values and individual genotypes at two male-specific loci in the three Tree River backcross families (3-10, 4-10, and 6-10) for the Sfo8LAV marker (allele 308) and Omy6DIAS locus (allele 229).

Family 3-10

		Chi Sq.
213/278	15	6.78
213/308	0	7.75
229/278	0	7.75
229/308	16	8.78
N=	31	RF(r)= 0.00

Family 4-10

		Chi Sq.
213/278	15	7.5
213/308	0	7.5
229/278	0	7.5
229/308	15	7.5
N=	30	RF(r)= 0.00

Family 6-10

		Chi Sq.
213/278	19	11.11
213/308	0	9.00
229/278	0	9.00
229/308	17	7.11
N=	36	RF(r)= 0.00

Individual genotypes for Sfo8LAV and Omy6DIAS in the three Tree River backcross families.

Family 3-10

		Sfo8LAV	Omy6 DIAS
Parents	**Female**	**256/286**	**213/275**
	Male	**278/308**	**213/229**
Progeny	14	256/278	213/213
	18	278/286	213/275
	29	256/278	213/213
	72	256/278	213/275
	73	278/286	213/275
	106	278/286	213/213
	144	278/286	213/213
	148	256/278	213/275
	343	278/286	213/213
	349	256/278	213/275
	387	278/286	213/213
	392	278/286	213/213
	421	256/278	213/275
	457	256/278	213/213
	492	256/278	213/213
	13	256/308	229/275
	16	256/308	229/275
	86	256/308	229/275
	96	256/308	213/229
	120	256/308	229/275
	252	286/308	229/275
	291	286/308	229/275
	298	256/308	229/275
	320	286/308	213/229
	331	286/308	213/229
	340	256/308	213/229
	355	256/308	213/229
	406	256/308	213/229
	416	256/308	213/229
	424	256/308	213/229
	482	286/308	213/229

Family 4-10

		Sfo8LAV	Omy6 DIAS
Parents	**Female**	**256/286**	**213/307**
	Male	**278/308**	**213/229**
Progeny	22	278/286	213/307
	61	278/286	213/307
	62	256/278	213/307
	71	278/286	213/307
	180	278/286	213/307
	247	256/278	213/307
	265	256/278	213/213
	323	278/286	213/307
	338	278/286	213/213
	357	256/278	213/307
	373	278/286	213/307
	425	256/278	213/213
	436	256/278	213/307
	485	278/286	213/307
	496	278/286	213/213
	3	286/308	229/307
	24	286/308	229/307
	66	286/308	229/307
	101	256/308	213/229
	105	256/308	213/229
	115	256/308	213/229
	136	286/308	213/229
	255	256/308	229/307
	278	286/308	229/307
	287	256/308	229/307
	301	286/308	229/307
	330	286/308	213/229
	405	256/308	213/229
	412	256/308	229/307
	487	256/308	229/307

Family 6-10

		Sfo8LAV	Omy6 DIAS
Parents	**Female**	**256/286**	**213/275**
	Male	**278/308**	**213/229**
Progeny	85	256/278	213/213
	91	256/278	213/213
	95	278/286	213/213
	98	278/286	213/275
	111	278/286	213/213
	114	278/286	213/213
	143	256/278	213/213
	150	278/286	213/213
	152	278/286	213/275
	229	278/286	213/213
	274	278/286	213/275
	325	256/278	213/275
	334	278/286	213/213
	337	256/278	213/275
	389	278/286	213/275
	404	278/286	213/213
	408	256/278	213/213
	434	278/286	213/275
	458	256/278	213/213
	26	256/308	213/229
	80	256/308	213/229
	92	256/308	213/229
	94	256/308	213/229
	163	286/308	229/275
	277	256/308	213/229
	308	256/308	229/275
	364	256/308	229/275
	385	256/308	213/229
	388	256/308	229/275
	395	256/308	229/275
	437	286/308	213/229
	474	256/308	213/229
	476	286/308	213/229
	481	256/308	229/275
	489	256/308	null/229
	490	286/308	229/275

Appendix XI

Summary of comparisons between male and female juvenile fish for three growth parameters in the ten full-sib families from cross 10; $(YG_fxTR_m)_fxTR_m$ (February 2003). Sex of the progeny was decided based on the presence or absence of the male specific marker-allele (Sfo8LAV-308). Test statistics failed to reject the null hypothesis at $p<0.05$ for any of the family.

Family	No. of Juvenile fish	No. of females & males	Total Length (cm)		Weight (gm)		Fulton's Condition Factor (K_{TL})	
			Respective Means (Std. Dev.)	Test-Stat. ($p<0.05$)*	Respective Means (Std. Dev.)	Test-Stat. ($p<0.05$)*	Respective Means (Std. Dev.)	Test-Stat. ($p<0.05$)*
1-10	16	7 & 9	13.3(2.4) - 11.5(1.4)	0.089	22.0(13.7) - 13.9(5.7)	0.125	0.85(0.08) - 0.86(0.08)	0.925
2-10	47	24 & 23	12.3(1.7) - 12.0(2.0)	0.626	17.5(8.6) - 16.5(9.1)	0.704	0.88(0.06) - 0.87(0.06)	0.552
3-10	35	16 & 19	13.1(2.0) - 13.2(1.8)	0.777	21.9(10.3) - 22.0(10.1)	0.987	0.91(0.06) - 0.89(0.06)	0.412
4-10	36	16 & 20	12.1(1.8) - 12.8(1.7)	0.217	17.9(10.3) - 20.3(8.4)	0.425	0.91(0.09) - 0.91(0.07)	0.995
5-10	46	24 & 22	12.1(1.6) - 12.7(1.8)	0.261	15.3(7.3) - 18.6(8.5)	0.140	0.81(0.1) - 0.85(0.07)	0.154
6-10	36	19 & 17	14.4(2.3) - 15.1(1.8)	0.355	29.8(14.0) - 33.0(13.5)	0.482	0.92(0.05) - 0.91(0.05)	0.705
7-10	33	13 & 20	13.9(1.4) - 14.3(2.0)	0.529	26.0(8.0) - 28.2(11.6)	0.555	0.92(0.03) - 0.91(0.05)	0.644
8-10	20	10 & 10	13.1(2.1) - 12.3(1.7)	0.318	20.8(10.3) - 16.9(6.2)	0.267	0.86(0.05) - 0.85(0.06)	0.864
9-10	40	21 & 19	10.4(1.2) - 10.1(1.4)	0.468	9.9(3.5) - 9.4(4.1)	0.616	0.85(0.08) - 0.85(0.07)	0.996
10-10	12	9 & 3	13.3(1.8) - 14.3(2.1)	0.458	22.7(11.7) - 27.4(11.7)	0.561	0.90(0.07) - 0.89(0.10)	0.956

*one-way ANOVA was performed

Appendix XII

Average weight and length (Fall 2001), and between groups differences in weight (above diagonal) and length (below diagonal) for the four groups of 1996 Arctic charr broodstock Icy Waters Ltd.

	Weight (Kg)			Length (cm)		
	Avg.	Std. Dev	Var.	Avg.	Std. Dev	Var.
YG	2.78	0.66	0.44	55.70	3.35	11.18
TR	3.20	0.57	0.34	60.20	3.32	11.15
Hybrid 1: TRfxYGm	2.78	0.65	0.42	57.00	4.42	19.50
Hybrid 2: YGfxTRm	3.20	0.51	0.26	58.49	2.86	8.17

	YG	TR	Hybrid 1	Hybrid 2	
YG		xxx	ns	x	Weight
TR	xx		xxx	ns	
Hybrid 1: TRfxYGm	ns	x		xx	
Hybrid 2: YGfxTRm	xx	x	xx		
	Length				

x; P<.05 but >.01, xx; P<.01 but >.001 and xxx; P<.001

Appendix XIII

Recombination frequencies (male) and chi-square test values at four polymorphic loci (AC-25) in the Tree River backcross family 6-10.

SalD39SFU & BHMS121

		Chi Sq.
272/131	23	21.78
272/135	1	7.11
290/131	0	9.00
290/135	12	1.00
N=	36	
RF(r)=	0.028	

SalD39SFU & BHMS490

		Chi Sq.
272/109	15	4.00
272/111	9	0.00
290/109	4	2.78
290/111	8	0.11
N=	36	
RF(r)=	0.361	

SalD39SFU & OmyRGT39TUF

		Chi Sq.
272/116	10	0.11
272/118	14	2.78
290/116	8	0.11
290/118	4	2.78
N=	36	
RF(r)=	0.389	

BHMS121 & BHMS490

		Chi Sq.
131/109	15	4.00
131/111	8	0.11
135/109	4	2.78
135/111	9	0.00
N=	36	
RF(r)=	0.333	

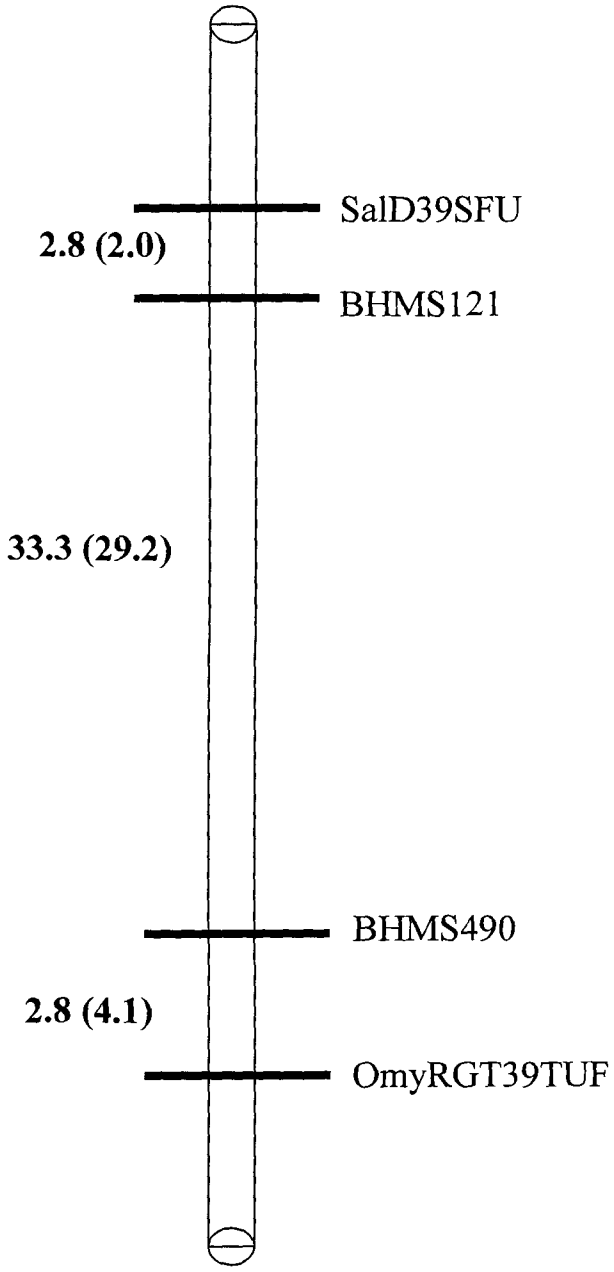
BHMS121 & OmyRGT39TUF

		Chi Sq.
131/116	9	0.00
131/118	14	2.78
135/116	9	0.00
135/118	4	2.78
N=	36	
RF(r)=	0.361	

BHMS490 & OmyRGT39TUF

		Chi Sq.
109/116	1	7.11
109/118	18	9.00
111/116	17	7.11
111/118	0	9.00
N=	36	
RF(r)=	0.028	

Marker order and estimated map distances (cM) between two closely linked markers in family 6-10. Values in parentheses are distances estimated by Woram *et al.* 2004. Marker order determined here is consistent with estimated provided by Woram *et al.* 2004 (Figure 4.3 & 4.4).



Appendix XIV

Weekly batch-weight data over 32 week period collected for twelve lines of juvenile Arctic char produced in the Fall 2001.

Date	Week	Cross1 $(YG_fxTR_m)xYG_m$	Cross2 TR_fxTR_m	Cross3 $YG_fx(TR_fxYG_m)_m$	Cross4 $(TR_fxYG_m)xYG_m$	Cross5 TR_fxYG_m	Cross6 YG_fxYG_m	Cross7 $TR_fx(TR_fxYG_m)_m$	Cross8 $TR_fx(YG_fxTR_m)_m$	Cross9 $YG_fx(YG_fxTR_m)_m$	Cross10 $(YG_fxTR_m)xTR_m$	Cross11 YG_fxTR_m	Cross12 $(TR_fxYG_m)xTR_m$
28-Feb-02	3	0.14	0.16	0.13	0.13	0.15	0.12	0.19	0.16	0.11	0.16	0.15	0.13
7-Mar-02	4	0.15	0.18	0.14	0.16	0.19	0.15	0.19	0.17	0.13	0.18	0.17	0.15
14-Mar-02	5	0.18	0.21	0.16	0.17	0.19	0.16	0.22	0.19	0.14	0.20	0.21	0.18
20-Mar-02	6	0.20	0.24	0.18	0.18	0.23	0.18	0.26	0.21	0.16	0.23	0.21	0.21
1-Apr-02	7	0.26	0.31	0.24	0.25	0.29	0.22	0.34	0.31	0.28	0.30	0.29	0.28
9-Apr-02	8	0.30	0.37	0.28	0.30	0.35	0.26	0.41	0.35		0.37	0.34	0.36
16-Apr-02	9	0.35	0.42	0.32	0.37	0.41	0.32	0.49	0.42	0.28*	0.45	0.38	0.42
25-Apr-02	10	0.44	0.51	0.38	0.40	0.52	0.38	0.60	0.51	0.33	0.53	0.48	0.51
1-May-02	11	0.47	0.64	0.42	0.47	0.56	0.40	0.67	0.55	0.36	0.59	0.54	0.57
10-May-02	12	0.52	0.68	0.44	0.50	0.63	0.44	0.92	0.71	0.38	0.74	0.66	0.67
17-May-02	13	0.55	0.73	0.53	0.56	0.70	0.48	0.88*	0.74	0.42	0.76	0.73	0.74
27-May-02	14	0.59	0.86	0.61	0.65	0.82	0.56	1.02	0.90	0.48	0.95	0.98	0.94
5-Jun-02	15	0.64	0.80	0.74	0.72	0.83	0.63	1.21	0.98	0.51	1.05	0.90*	1.03
11-Jun-02	16	0.72	0.93	0.80	0.79	0.97	0.67	1.30	1.07	0.64	1.16	1.04	1.17
18-Jun-02	17	0.82	1.03	0.92	0.90	1.04	0.83	1.37	1.23	0.72	1.26	1.13	1.19
26-Jun-02	18	0.94	1.15	1.05	0.95	1.17	0.92	1.56	1.56	0.89	1.45	1.22	1.48
2-Jul-02	19	1.02	1.27	1.17	1.12	1.65	1.01	1.87	1.21	1.06	1.61	1.32	1.59
9-Jul-02	20	1.09	1.29	1.20	1.11	1.34	1.02	1.75	1.28	0.98	1.50	1.35	1.53
17-Jul-02	21	1.18	1.52	1.35	1.28	1.51	1.21	1.95	1.36	1.23	1.82	1.49	1.76
24-Jul-02	22	1.42	1.58	1.52	1.45	1.60	1.31	2.44	1.64	1.50	1.86	1.71	2.17
30-Jul-02	23	1.54	1.70	1.66	1.57	1.58	1.41	2.44	1.56	1.73	2.23	2.36	2.39
6-Aug-02	24	1.57	2.02	2.06	1.69	1.89	1.61	2.61	1.78	1.64	2.49	2.46	2.75
13-Aug-02	25	1.81	2.05	1.94	1.89	2.08	1.62	3.36	2.06	2.28	2.81	2.50	3.22
20-Aug-02	26	1.98	2.54	2.55	1.86	2.40	1.84	3.62	2.37	2.44	2.90	2.83	3.36
27-Aug-02	27	2.44	2.52	2.97	2.24	2.43	2.25	3.78	2.80	2.45	3.03	2.95	3.35
3-Sep-02	28	2.66	2.82	2.96	2.80	2.89	2.40	4.42	2.74	3.01	3.97	3.46	4.45
10-Sep-02	29	2.88	3.26	3.35	2.99	3.16	2.68	4.84	3.18	3.01	4.46	4.19	3.74
17-Sep-02	30	3.26	3.42	3.71	3.18	3.39	3.00	5.73	3.08	3.49	4.81	4.52	4.83
24-Sep-02	31	3.44	4.49	3.98	3.19	3.65	3.52	6.37	3.60	3.91	5.20	4.97	5.23
1-Oct-02	32	4.02	4.93	4.94	3.98	4.28	3.65	6.59	4.33	3.47	6.40	6.17	6.49
8-Oct-02	33	4.06		5.02	4.39	4.55	4.04	7.20	4.17	3.73	7.05	6.67	7.33
15-Oct-02	34	4.85	5.22	4.94	4.86	5.49	4.60	6.81	4.62	3.71	6.17	7.27	6.98
22-Oct-02	35	5.68	6.19	5.88	4.93	5.95	5.19	8.88	4.92	4.09	7.25	8.08	8.21

*Values in grey boxes are lighter than the previous week.