Phenotypic and genetic evaluation of Fraser strain Arctic charr (*Salvelinus alpinus*) in brackish and freshwater

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ABSTRACT

PHENOTYPIC AND GENETIC EVALUATION OF FRASER STRAIN ARCTIC CHARR (*SALVELINUS ALPINUS*) IN BRACKISH AND FRESHWATER

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I examined phenotypic and genetic variation in growth traits in 30 families of commercial Fraser strain Arctic charr (*Salvelinus alpinus*) reared in freshwater (FRW) and brackish water (BRW) in Eastern Canada. I detected family by treatment interactions for all traits [body weight (BW), condition factor (K) and specific growth rate (SGR)] across all measurement dates and growth intervals, however, mean family BW in FRW was correlated phenotypically with BRW BW. In addition, FRW fish showed significantly greater survival than those transferred to BRW and fish which survived until the conclusion of the experiment were significantly heavier in BW at the baseline assessment than their full-sibs that died. These observations suggest that BW in FRW and BW in BRW should be analyzed as separate but correlated traits in Arctic charr breeding programs. I then tested the potential for genetic improvement in this species by calculating genetic parameters for BW and K, and tested if previously identified quantitative trait loci (QTL) for these traits were detectable across the broodstock. QTL with experiment-wide and chromosome-wide significance for body size and condition factor were detected on multiple linkage groups. Heritability for BW and K was moderate in FRW (0.29-0.38) but lower in BRW (0.14-0.17). Genetic correlations for BW across environments were positive and moderate (0.33-0.67), however equivalent K correlations were weaker (0.24-0.37). This information was then used to predict the rate of genetic

change following one generation of selection for BW using phenotypic selection and genomic methodologies including marker-only selection and marker assisted selection. The greatest response in the rate of genetic change was achieved by selecting only from families in which significant BW QTL had been identified. As such, marker assisted selection showed the greatest gain in genetic response with 5.4% in FRW and 4.3% in BRW. These results have applications to commercial aquaculture as the Canadian aquaculture industry is attempting to diversify with alternative species. Such genetic improvement strategies will aid in developing a strain of Arctic charr characterised by increased BW.

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GENERAL INTRODUCTION

As the productivity of traditional wild fisheries either plateaus or declines and the demand for fish and shellfish products increases, Canada has the potential to increase the production of such products in its commercial aquaculture industry. In terms of world aquaculture production, Canada ranks 23rd and only accounts for 0.3% of the total world production (FAO, 2010). Since the origins of commercial aquaculture in the 1970's, practices in Canada have not changed greatly. In recent years, the finfish industry has focused on the production of a single species, the Atlantic salmon (*Salmo salar*) and as a consequence, the market value of this commercial aquaculture species has decreased due to overproduction and global competition. Overall, the aquaculture industry has been limited in terms of growth due to various factors and the expansion of the industry resides in the ability to develop new technology to enhance the efficiency of breeding programs and in the development of alternative aquaculture species.

The Arctic charr (*Salvelinus alpinus*) is one such alternative species currently being examined for commercial culture in Canada (CCFAM, 2010). The Arctic charr is a salmonid fish with a northern hemispheric, circumpolar distribution that includes regions of Canada, Alaska, Greenland, Iceland, Scandinavia, Russia and the Arctic seas (Scott and Crossman, 1998). This species is found in many different environments including rivers, lakes and inshore marine waters and wild populations are currently in decline throughout much of their natural range (Scott and Crossman, 1998; Johnston, 2002). Although the large variability in life history makes the Arctic charr a more challenging species for culture due to the lack of "domesticated" stains, this species is becoming an increasingly important aquaculture product in Canada. Commercially important traits

such as growth rates and maturation timing are highly variable; however, this species exhibits many other characteristics which make it appealing for culture. For example, Arctic charr are tolerant to cold temperatures and perform well under high density growing conditions (Johnston, 2002). Additionally, Arctic charr are considered a "luxury" food item and therefore, fetch a much high market price than competitors such as Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*).

Iceland is the world's largest producer of aquaculture Arctic charr (FAO, 2010). The selective breeding program for Icelandic Arctic charr originated at Hólar University College in 1992 and incorporated wild fish collections from both anadromous and freshwater sources into a national broodstock development program. Through selective breeding, the Icelandic breeding program has successfully increased harvest weight to double what it was when the program originated while reducing the incidence of early maturation to a near negligible level (Kuettner, 2011). Following closely behind Iceland in Arctic charr production is Sweden. Arctic charr have been reared in Swedish hatcheries since the 1950s using techniques developed for salmon and trout for the purpose of stocking local rivers and lakes (Eriksson et al. 2010). Aquaculture production of charr for the market began in the 1980s and a program was established to evaluate suitable populations for intensive farming and a subsequent selective breeding program was established. As a result, following seven generations of pedigree-based selective breeding, the Swedish Arctic charr breeding program has doubled growth performance and has reduced of the incidence of precocious maturation to less than 5% in fish weighing less than 1 kg. In comparison to Iceland and Sweden, the Canadian Arctic charr aquaculture industry is fragmented in terms of production and multiple charr strains from

various sources are currently being cultured nationwide. The Arctic charr being cultured in Eastern Canada originated from the Fraser River in Labrador whereas many of the Arctic charr farms in Western Canada are using strains originating from Nauyuk Lake and the Tree River system in Nunavut (De March and Baker, 1990; De March, 1991; Lundrigan et al. 2005). These charr strains were established from small numbers of founders and subsequent pedigree information on these strains are limited. As the industry in Canada lacks a national broodstock development program, little selective breeding has occurred within these strains to enhance economically important traits of interest.

The New Brunswick department of Agriculture, Fisheries and Aquaculture began an Arctic charr breeding program in 1996 as a pilot project to assess the potential of this species for commercial culture. The responsibility of the Arctic charr aquaculture project was handed over to the Coastal Zones Research Institute (CZRI) in 2002. The CZRI is a private non-profit institution affiliated with the Université de Moncton, based in Shippagan, New Brunswick, Canada. The CZRI Arctic charr aquaculture program is a partnership of private and government researchers that aim to develop a genetically-improved strain of Arctic charr for commercial aquaculture in Atlantic Canada. The goal of the breeding program is to develop Arctic charr with an optimal combination of fast growth rate and low incidence of early sexual maturation. As CZRI maintains the only certified “disease free” Arctic charr hatchery in Eastern Canada, it is imperative that selection and breeding for genetic improvement must occur within this strain.

Many salmonid breeding programs select based solely on the mass selection for body weight. Because Arctic charr show marked size heterogeneity, mass selection does

not always maximize genetic potential as environmental effects are often confounded with genetic effects. Genetic analyses of family performance for traits of economic importance, including growth and age at maturation are needed for the development of a genetic improvement plan. Since the development of the breeding program in 1996, the Arctic charr broodstock at CZRI has operated as a within-family sequential selection program based primarily on body weight and to a lesser extent to reduce precocious maturation. The population pedigree has been tracked since 1996 and as a result, inbreeding has been largely minimized and the genetic variability is being conserved. Although animal models are being employed in an attempt to estimate breeding values for the CZRI broodstock, the characterization of the genetics of this broodstock will aid in the selection of families for future development.

Compared to terrestrial agriculture, aquaculture is still in its infancy of development. Although there are a number of aquaculture successes (Svavarsson, 2007; Nilsson et al. 2010), there are still issues plaguing further development of the industry. One potential issue is that aquaculture breeding programs often involve the rearing of aquatic animals in diverse environments and production systems (i.e., Sundstrom et al. 2007; Khaw et al. 2009; Navarro et al. 2009; Dupont-Nivet et al. 2008; Dupont-Nivet et al. 2010). This varied husbandry may lead to family by environment interactions characterized by detectable differences across environments in the proportion of the phenotypic variation that is controlled by genetic and environmental components and/or a re-ranking of the best performing genotypes (Falconer and Mackay, 1996). One important consequence of these interactions is that selection within one environment may lead to lower than expected performance and economic gains under other environmental

conditions. As a result, the estimation of family by environment interactions (Sae-Lim et al. 2010) and their economic impact (Ponzoni et al. 2008) is an important consideration in any selective breeding program.

Arctic charr breeding programs (e.g., Nilsson et al. 2010) typically evaluate and select broodstock based on individual performance under hatchery rearing conditions in freshwater. Due to the challenges associated with spawning Arctic charr in ocean net pens and the high value of broodstock (Johnston, 2002), producers maintain their breeding stock in land-based, freshwater facilities. Such contained, land based aquaculture systems offer protection from disease and facilitate population monitoring. However, limited and costly freshwater water availability have led to the reduced dependence on freshwater grow-out systems and increased interest in brackish and salt water rearing tanks. As current broodstock selection for increased growth is based on performance in freshwater rearing conditions, an increase in brackish water rearing may impact Arctic charr selection programs given the potential for family by environment interactions. The relationship between the growth of Arctic charr in brackish and freshwater is unclear. By rearing full-sib families from a commercial strain of charr in both environments, genetic analyses will help to determine how these freshwater broodstock might be genetically improved for economically-important brackish water performance traits.

In chapter 2, I investigated whether Fraser strain Arctic charr reared in freshwater differed in survival and growth from full siblings transferred to brackish water and whether family performance was consistent throughout one year of commercial growth. Fish were grown communally in either FW or BW from May 2008 to May 2009 and were

measured at three dates during the growth experiment. My goals were to determine if fish show significant differences in four performance traits (survival, body weight, condition factor and specific growth) between fresh water and brackish water environments, whether family performance in freshwater is a significant predictor of performance in brackish water and the extent of family by environment interactions.

Traits of economic importance such as growth rate, generally have moderate heritability for aquaculture species (Carlson and Seamons, 2008) such that positive responses to selection have been achieved (Gjedrem, 1992; Gjoen and Bentsen, 1997; Quinton et al. 2005). Arctic charr have low to moderate heritability for growth traits depending on the strain and rearing environment. For example, Swedish charr have been documented to have heritability estimates for weight ranging from 0.34 to 0.65 at 2 years of age and 0.38 to 0.52 at 3 years of age. Estimates of heritability for condition factor have been reported to range from 0.32 to 0.56. Additionally, phenotypic correlations between dams and sires have been reported as strong and positive (Nilsson, 1990; Nilsson, 1992). Compared to breeding programs for livestock, the potential for genetic gain in aquaculture breeding programs is increased because phenotypic variation is often high, the generation interval is relatively short and fertility is high. However, the varied response of phenotypes to heterogeneous environments in fishes (Costa et al. 2010) requires that reliable estimates of genetic parameters for production traits must be made in a wide range of rearing systems and environmental conditions before being included in a selective breeding program.

Rapid advances in molecular techniques and the availability of genetic marker technology is leading towards a shift from “classical” selection schemes in aquaculture

breeding programs to a more contemporary approach which involves using information about the phenotype of the organism, as well as the genotype of the individual (Sonesson, 2007; Lo Presti et al. 2009; Piyasatian et al. 2009). The genetic control underlying growth related traits are often complex and studies of the genetic architecture of these traits in commercial aquaculture is now possible through the mapping of quantitative trait loci (QTL) onto genetic linkage maps composed of highly variable genetic markers. QTL are genomic regions that are closely linked to one or more genes that affect a quantitative trait. Currently, microsatellite markers (tandem repeats of genomic sequences which demonstrate high levels of allele polymorphism) are the most commonly used marker in salmonid aquaculture research and these markers have been used for constructing linkage maps and mapping economically important traits to QTL regions (Chistiakov et al. 2006).

The first step in QTL detection is to construct a genomic linkage map. In Arctic charr, a moderately dense, sex-specific linkage map has been developed for the Fraser strain by arranging polymorphic microsatellite markers in chromosomal segments based on their segregation relationships (Woram et al. 2003; Danzmann et al. 2005). Secondly, QTL for traits of interest in Arctic charr must be identified and screened across the entire genome using evenly spaced markers (Somorjai et al. 2003; Moghadam et al. 2007; Kuettner et al. 2011). By using this "genome scan" approach, trait-linked markers can be narrowed down to a particular chromosomal region and placed on the linkage map. The mapping of specific QTL related to traits of interest in Arctic charr can be greatly accelerated when coupled through comparative genomics to model species in which the entire genome has been sequenced (Liu and Cordes, 2004).

Knowledge of QTL for growth related traits in salmonid fishes (e.g., Reid et al. 2005; Moghadam et al. 2007; McClelland and Naish, 2010; Wringe et al. 2010; Kuettner et al. 2011; Norman et al. 2011; Gutierrez et al. 2012) is in the early stages of being integrated into selective breeding programs because the consistency and magnitude of QTL effects across multiple families within a broodstock and across environments and rearing systems are generally unknown. Recent work has detected QTL for growth-related traits in Arctic charr based on genome scans in a limited number of families from the Fraser strain from eastern Canada (Moghadam et al. 2007). Specifically, a QTL of large effect was detected on Arctic charr linkage group 8 (AC-8) which accounted for more than 34% of the variation in body weight. Significant body weight QTL were also revealed on AC-13 and -25 and a single significant condition factor QTL on AC-36 accounting for 13% of the observed variation. More recently, genome-wide significant QTL were detected on AC-1, -19, -20 and -28 for body size traits in the same strain (Norman et al. 2010). Similar studies with Icelandic Arctic charr (Kuettner et al. 2011) showed some overlap in body weight QTL location with the Canadian fish (AC-4) but also some differences (AC-20) indicating that the different evolutionary and culture histories of these strains could have led to differential fixation of QTL.

In chapter 3, I investigated the potential for genetic improvement in Fraser strain Arctic charr by calculating genetic parameters such as estimates of heritability and the degree of genetic correlation for body weight and condition factor. Additionally, I tested if previously identified quantitative trait loci (QTL) for these traits were detectable across a commercial broodstock and whether these effects were consistent in both freshwater and brackish water rearing environments.

National breeding programs for Arctic charr as realized in Norway, Iceland and Sweden (Svavarsson, 2007; Eriksson et al. 2010; Nilsson et al. 2010), indicate that a coordinated effort including multiple strains from multiple sources can result in desirable changes in domesticated traits such as increased growth, larger body size and decreased incidence of precocious maturation. To optimize Arctic charr aquaculture in Canada, a national breeding program should be established. Specifically, a breeding program for Canadian Arctic charr should incorporate detailed phenotypic records in multiple rearing environments, combined with genetic parameters such as estimates of heritability, and QTL information for traits of interest. Compared to classical phenotype-based selection, marker assisted selection (MAS) exploits linkage disequilibrium between genetic markers and QTL. MAS can improve the intensity of selection when the heritability of the trait is low, when the trait is expressed later in the life cycle and when traits can only be measured on siblings rather than the broodstock (Dekkers, 2007; Piyasatian et al. 2007; Hospital, 2009; Piyasatian et al. 2009). Although gains have already been seen in aquatic animal breeding, further selection to increase growth is possible and substantial gains could be achieved if genetic parameters are combined with the identification of QTL for growth traits in a form of MAS. MAS is currently being used in the breeding programs of livestock species (Solberg et al. 2008; Ibanez-Escriche and Gonzalez-Recio, 2011) and recent approaches are based on SNP (single nucleotide polymorphism) markers distributed throughout the genome (genomic selection) (Van Raden and Sullivan, 2010).

In chapter 4, I investigated the possibility of using microsatellite markers linked to QTL for variation in body weight among multiple families of Fraser strain Arctic charr to develop a selective breeding scheme to increase body weight in future generations of

charr. Specifically, based on phenotypic and genetic information collected in chapters 2 and 3, I tested the effect of differing selection strategies (phenotype-only selection, marker-only selection and marker assisted selection) using selection index theory that was developed for MAS by Lande and Thompson (1990) for freshwater and brackish water rearing environments following one generation of selection.

The present study is one of the few to investigate brackish water as a possible rearing environment for Arctic charr and to investigate the potential for family x environment interactions in the growth and survival of Arctic charr in a commercial rearing environment. This is also one of the first studies to investigate the effect of QTL across multiple families in a commercial broodstock, to evaluate the linkage phase of the QTL effects and to combine genetic parameters and QTL into a single investigative framework. Additionally, when data collected from this study were utilized in a theoretical breeding program, the results indicated favorable increases in genetic gain are possible using MAS. Importantly, there is potential to implement this information into a selective breeding scheme for CZRI broodstock at the conclusion of the study. The result of this research has applications to commercial aquaculture and also adds to the basic knowledge of the genome organization of Arctic charr.

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CHAPTER 2

Family x Environment interactions in the growth and survival of Arctic charr (*Salvelinus alpinus*) grown in brackish and freshwater

Abstract

I investigated whether Fraser strain Arctic charr *Salvelinus alpinus* L. reared entirely in freshwater (FRW) differed in survival and growth from their full siblings transferred to brackish water (BRW) and whether family performance was consistent throughout one year of commercial growth. Fish were grown communally (n = 3600) in either FRW or BRW tanks from May 2008 to May 2009. Arctic charr in FRW showed significantly greater survival than those transferred to BRW. Mean family survival in BRW was moderately correlated with mean family survival in FRW ($r = 0.342$; $P = 0.032$). Fish which survived until the conclusion of the experiment were significantly heavier in body weight at the baseline assessment than their full-sibs which died. Family x treatment interactions were detected for body weight, condition factor and specific growth across all measurement dates (May 2008, Oct. 2008 and May 2009). Despite the varied response of families to treatment, mean family body weight in FRW was correlated phenotypically with BRW body weight in October 2008 ($r = 0.633$, $P < 0.0001$) and marginally correlated in May 2009 ($r = 0.289$, $P = 0.061$). These data suggest that body weight in FRW and body weight in BRW should be analyzed as separate but correlated traits in Arctic charr breeding programs.

Introduction

Aquaculture breeding programs often involve the rearing of aquatic animals in diverse environments and production systems (i.e., Sundstrom et al. 2007; Khaw et al. 2009; Navarro et al. 2009; Dupont-Nivet et al. 2008; Dupont-Nivet et al. 2010). This varied husbandry may lead to family by environment interactions characterized by detectable differences across environments in the proportion of the phenotypic variation that is controlled by genetic and environmental components and/or a re-ranking of the best performing genotypes (Falconer and Mackay, 1996). One important consequence of these interactions is that selection within one environment may lead to lower than expected performance and economic gains under other environmental conditions. As a result, the estimation of family by environment interactions (Sae-Lim et al. 2010) and their economic impact (Ponzoni et al. 2008) is an important consideration in any selective breeding program.

As Canada's commercial aquaculture industry continues to decline and as the market value of Atlantic salmon *Salmo salar* L. decreases due to overproduction and global competition, alternative aquaculture species, such as Arctic charr are receiving increased attention (Rogers and Davidson, 2001; Johnston, 2002; CCFAM, 2010). Fisheries and Oceans Canada has identified Arctic charr as an emerging species with the greatest potential for economic viability in freshwater aquaculture (CCFAM, 2010). At present, the industry is fragmented in terms of production and multiple charr strains from various sources are currently being cultured nationwide. The Arctic charr being cultured in Eastern Canada originated from the Fraser River in Labrador whereas many of the Arctic charr farms in Western Canada are using strains originating from Nauyuk Lake

and the Tree River system in Nunavut (Lundrigan et al. 2005). These charr strains were established from small numbers of founders and subsequent pedigree information on these strains is limited (Lundrigan et al. 2005). As the industry lacks a national broodstock development program, little selective breeding has occurred within these strains to enhance economically important traits of interest. Therefore, major opportunities exist for the improvement of Arctic charr aquaculture stocks in Canada through broodstock development and the application of selective breeding programs as realized in Sweden (Eriksson et al. 2010; Nilsson et al. 2010) and Iceland (Svavarsson, 2007).

Arctic charr breeding programs (e.g., Nilsson et al. 2010) typically evaluate and select broodstock based on individual performance under hatchery rearing conditions in freshwater. Due to the challenges associated with spawning Arctic charr in ocean net pens and the high value of broodstock (Johnston, 2002), producers maintain their breeding stock in land-based, freshwater facilities. Such contained aquaculture systems offer protection from disease and facilitate monitoring, but limited water supply and economic reasons have led to the reduced dependence on freshwater grow-out systems and increased use of brackish and salt water. Current broodstock selection for increased growth is based on performance in freshwater rearing conditions, and therefore, an increase in brackish water rearing may impact Arctic charr selection programs given the potential for family by environment interactions.

Growth performance of charr in seawater is typically inferior to that of cultured charr in freshwater (Arnesen et al. 1994; Duston et al. 2007). Although offspring of anadromous Arctic charr undergo a parr–smolt transformation and exhibit endocrine

changes and salinity tolerance similar to that of Atlantic salmon (Jørgensen et al. 2007), seawater trials have indicated that growth rate decreases and mortality increases post-seawater transfer (Gjedrem, 1975; Gjedrem and Gunnes, 1978; Wandsvik and Jobling, 1982; Refstie, 1983; Arnesen et al. 1993a; Arnesen et al. 1993b; Arnesen et al. 1994). Growth in the brackish water environment is less understood but evidence suggests that it may be similar to that in freshwater (Arnesen et al. 1994; Duston et al. 2007).

The present study compared the growth and survival of individually tagged Fraser strain Arctic charr from thirty full-sib families reared in both freshwater and brackish water for 12 months at a commercial site. My goals were to determine if fish show significant differences in four performance traits (survival, body size, condition factor and growth) between freshwater and brackish water environments, whether family performance in freshwater is a significant predictor of performance in brackish water and the extent of family by environment interactions. The results of this research will have implications to Arctic charr breeding programs and facilitate the development of stocks with efficient performance in different environments and production systems.

Material and Methods

Population and experimental design

The Arctic charr in this experiment were members of the F4 generation from the breeding program established at the Coastal Zones Research Institute (CZRI; Shippagan, New Brunswick, Canada) in 1996. Thirty sires were mated with 30 dams to create 30 full-sib families in October 2006. The genetic relationships among the sires and dams were unknown. The CZRI broodstock was founded from fish obtained from the Rockwood Aquaculture Research Center (RARC; Gunton, Manitoba). The RARC broodstock was founded by mating a small number of wild males and females from the Fraser River in Labrador (Lundrigan et al. 2005).

Embryos were incubated under low light conditions in circular incubation chambers supplied with re-circulated freshwater with temperatures ranging from 4.5 to 6^{o}C. After hatching, the embryos remained in the incubation chambers until yolk sac absorption was complete. At the onset of exogenous feeding, 1500 - 2000 individuals from each family were transferred to 150 L tanks for first feeding at 10 to 12^{o}C. Families were reared separately using standard husbandry practices until the average weight of the fish reached 4.6 g (June 2007). At this time, a small piece of pelvic or pectoral fin (various combinations of left and/or right fin clips) were removed from each fish with scissors to identify family. Arctic charr were distributed among five 4 m^3 tanks (6 families in each tank). Fish were fed a commercial salmon diet (EWOS Canada Ltd., Surrey, British Columbia, Canada) based on the ration recommendations of the feed manufacturer at 4% of body weight per day. Light was supplied from fluorescent fixtures

timed to an artificial photoperiod based on day length predictions at 50^{o} N. When the fish reached an approximate body size of 230 g (January 2008), 120 individuals from each family were randomly selected for the study (n = 3600). Each fish was anesthetized in 0.02% Tricaine-S (Western Chemical Inc., Ferndale, Washington, USA) for 2 minutes and a passive integrated transponder (PIT-tag; AVID, Norco, California, USA) was implanted in the dorsal muscle with a syringe. At this time, the wet weight (g) (BW) and fork length (cm) (FL) of the fish was measured.

Growth study

In February of 2008, the 3600 PIT-tagged fish were transported at a stocking density of 75 kg/m^3 under constant oxygen aeration to a commercial rearing site (CanAqua Seafoods Ltd., Advocate Harbour, Nova Scotia, Canada) where they were placed in four 16 m^3 tanks. Each tank housed 30 fish from each family for a total of 900 individuals per tank. All tanks were outdoors and initially fed by freshwater (FRW). In May 2008, salinity in two of the four tanks was gradually increased from 0.0 ppt to approximately 20 ppt brackish water (BRW) over a 14 day period while the incoming water for the other two tanks remained fresh water. All experimental tanks were exposed to a natural photoperiod and temperature regime resulting in ice build up on the surface of the tanks during winter. Therefore, for a few weeks after the fish were transferred to the commercial site (February 2008) and during the following winter, the ice buildup prevented feeding. Fish were maintained using standard husbandry practices and were fed to satiation using the EWOS commercial salmon diet. The growth study ran from May 2008 to May 2009.

Traits recorded

At each sampling date (May 2008, October 2008 and May 2009), the water level in each tank was decreased to allow the fish to be captured using dip nets. Each fish was anesthetized in 0.02% Tricaine-S (Western Chemical Inc., Ferndale, Washington, USA), scanned with a PIT-tag reader (AVID Power Tracker, Norco, California, USA) and the individual number was found in the data base. The BW was measured to the nearest gram and FL was measured to the nearest 0.5 cm. Following measurements, fish were placed in a recovery tank under constant oxygen aeration until they could be returned to the experimental tanks.

Throughout the experiment, mortalities were collected from the bottom of the experimental tanks and the PIT-tag was removed from the carcass. Only individuals whose PIT-tag was recovered were considered "confirmed mortalities". Fish not found in the tanks at a given sampling date and not recovered as a confirmed mortality were classified as "missing". "Missing" individuals were assumed to be mortality due to predation. The sum of confirmed mortalities and missing fish was designated total mortalities.

At the conclusion of the study, specific growth rates for body weight (SGR) at each sampling interval were calculated for each individual using the equation:

$$SGR = 100[\ln(M_f) - \ln(M_i)] / T,$$

where M_f is the final measurement, M_i is the initial measurement and T is the time interval between measurements (in days).

Fulton's condition factor (K) of individual fish was calculated for each sampling date using the equation:

$$K = 100\ (BW\ L^{-3}),$$

where *Wt* is the wet weight (g) and *L* the fork length (cm).

Statistical analysis

Total and confirmed mortality was calculated for each environment as described above. As there were no statistical differences between replicate tanks, replicates were pooled and a paired *t*-test was used to compare family mortality (total and confirmed) between environmental treatments. Pearson's *r* was used to test for a significant association between the number of confirmed mortalities for each family in the two environments.

We next tested if individuals that survived to the end of the experiment (May 2009) had greater initial body weights and condition factor (January 2008) compared to the confirmed mortality group that perished during the growth study. The body weights and condition factor of individuals in January 2008 that survived until May 2009 or died were compared across all families using a two-way ANOVA with family and mortality status as factors.

The SAS *proc* mixed model analysis (SAS Institute Inc., Cary, North Carolina, USA) was used to test for differences in the BW, SGR and K of fish reared in fresh and brackish water for each sampling date and interval using the following equation:

$$y_{ijkl} = R_i + E(T)_j + F_k + T_l + F_k*T_l + e_{ijkl}$$

where R_i is the random effect of rearing tank, $E(T)_j$ is the random effect of experimental tank nested in treatment, F_k is the fixed effect of family, T_l is the fixed effect of treatment (fresh or brackish water) and $F_k * T_l$ is the interaction of family and treatment. Preliminary checks for homogeneity of variance using the modified Levene test indicated the variances differed. As the model would not converge using one residual variance, separate residual variances were used for each treatment.

I tested for significant associations between mean family BW, SGR and K in fresh water and brackish water with Pearson's *r*. Prior to this, a two-sample *t*-test was used to test if the mean body weight of individual families differed between replicate tanks and replicate tank means were pooled if significant differences were not observed between the tank means. Families where mean body weight differed significantly among replicates (families 3, 7, 9 & 13 in October 2008 and families 7, 9, 14, 16, 26 & 30 in May 2009) were initially removed from the analysis. The correlation analysis was then rerun with the entire data set and no differences were determined from the selected family analysis. Thus, the findings from all 30 families are presented below.

Results

Survival

After 12 months of commercial rearing, the mean number of confirmed and total mortalities was significantly greater in the brackish water environment compared to the freshwater environment (P = 0.041 and 0.002, respectively) (Fig. 1). Mean family survival in BRW was moderately correlated with mean family survival in FRW (r = 0.342; P = 0.032). In both environmental treatments, individuals who survived to the end of the experiment had significantly higher body weights at the beginning of the experiment (May 2008) than their full siblings who perished throughout the study (Table 2-1). Initial condition factor was not significantly affected by survivorship (Table 2-1).

Body weight, growth and condition factor

The effect of treatment on BW was significant at the conclusion of the study in May 2009 but not in October 2008 (Table 2-2). At the family level, fish reared in FRW were heavier in May 2009 than their full siblings reared in BRW in 23 cases and of these six were significantly so (Table 2-3). There were no significant effects of treatment on K and SGR on any sampling date or interval between sampling dates, respectively (Table 2-2). The treatment by family interaction was highly significant (P > 0.0001) for every trait at each sample date (Table 2-2). This effect is further illustrated at the family level where certain families showed significantly higher trait values in FRW whereas others showed the opposite effect (Tables 2-3 to 2-5).

Mean family body weight in FRW was significantly associated with body weight in BRW in October 2008 (r = 0.633, P < 0.0001) and marginally correlated in May 2009

(r = 0.289, P = 0.061). Condition factor in FRW was not significantly associated with K in BRW (October 2008 r = - 0.047, P = 0.403; May 2009 r = - 0.037, P = 0.423). Similarly, specific growth rate in FRW was not correlated with SGR in BRW in October 2008 (r = 0.027, P = 0.444) or May 2009 (r = 0.048, P = 0.401).

Discussion

Effect of salinity

The modest differences in confirmed mortalities coupled with the effects of environment on growth traits suggests that although Fraser strain Arctic charr can be grown in BRW with some success, there is evidence to suggest that separate breeding programs may be required for optimal growth in both environments. Although the effect of treatment is not significant for each trait at each sampling date, the evidence of significant treatment x family interaction for each trait suggests that BRW does influence growth performance among families differentially. Nevertheless, BRW fish did not show reduced SGR in either measurement interval which suggests that acclimation to BRW does not present a major physiological challenge. These observations are consistent with the anadromous life history of the Fraser strain's origin (Dempson and Green, 1985). However, because some families reared in brackish water outperformed their freshwater counterparts, the degree of pre-adaptation to salinity differs between families.

It is difficult to evaluate my results on the performance of Arctic charr in BRW to other studies because of inconsistencies in initial body size and duration of experiments. Results from the present study indicate that early growth in brackish water is similar to that of freshwater as significant differences in body weight due to treatment were not observed until one year post-transfer. Similarly, Arnesen et al. (1994) did not observe statistical differences among growth rates of Hammerfest strain Arctic charr reared in FRW and BRW. Duston et al. (2007), on the other hand, reported that body weight and condition factor of immature Fraser strain Arctic charr in FRW was significantly greater

than siblings reared in BRW. These findings support the results of the present study which indicate there are modest differences in body weight due to BRW treatment.

The effect of salinity on mortality of Arctic charr is highly variable. In the present study, total mortalities were significantly increased in the BRW environment whereas the effect of salinity on confirmed mortalities was weak. However, significant differences in mortality in FRW and BRW have not been reported in the literature (Arnesen et al. 1993b; Duston et al. 2007). The observation of highly significant differences between treatments in the present study could be explained by predation by birds. High levels of predation were observed in both freshwater and brackish water tanks due to the fact that tanks were outdoors, uncovered and fish were often close to the surface for feeding. Mortality due to predation may be a secondary response to the brackish water treatment. For example, exposure to the brackish water environment may result in physiological stress such as elevated levels of cortisol. Alternatively, behavioral changes such as decreased feeding resulting in lower energy reserves, or individual fish spending more time near the surface, may make the fish more susceptible to predation.

Results of the present study support the theory that body size at transfer is a major factor dictating survival in saline environments (Dempson, 1993). It is generally accepted that mortality in salmonid fishes is inversely related with body size after direct transfer to sea water (Johnsson and Clarke, 1988). Although the minimum size threshold for survival in salt water has been reported as approximately 15 - 18 cm fork length for Labrador Arctic charr (Dempson, 1993), the results of the present study suggest that the size threshold may be even greater for cultured charr. Prior to transfer to brackish water, the mean fork length of charr in the present study was 26 cm and mean body weight was 228

g. Results of the present study suggest that initial body weight < 220 g significantly increased mortality post-transfer to BRW. Similarly, Duston et al. (2007) reported that survival in greater salinity was significantly reduced for individuals with an initial body weight < 300 g compared to those fish that were > 300 g even though the fork lengths of fish in their study greatly exceed the minimum threshold as suggested by Dempson (1993).

Differential response of families to treatments

Arctic charr families in the present study showed marked heterogeneity in their response to the environmental treatments. Although significant differences in growth between families in the two environments were observed, mean family body weight in BRW was moderately correlated with the same trait in FRW early on. The effect of family on growth-related traits has been previously documented in Fraser strain Arctic charr. Duston et al. (2007) reported that the effect of family on final body size was highly significant when comparing the growth of Fraser strain charr at varying salinity levels however, the interaction of family and salinity was not significant.

Conclusions

The results of this study indicate that Arctic charr siblings reared in fresh and brackish water exhibit similar growth and survival during the first six months of commercial rearing. As the environmental treatment showed only modest effects on confirmed mortality and growth traits, we may conclude that performance in FRW is a general predictor of performance in BRW during early commercial rearing. The predictability of BRW performance is strongest early on and declines over time as the

effect of family and family by environment interactions increase. Growth trajectories in a given environment may remain consistent providing the environmental conditions are not altered and growth advantages or constraints during early growth in freshwater will not necessarily carry on throughout the grow-out phase in brackish water.

The differential response of families observed in BW, K and SGR in the present study among Arctic charr families grown in different environments suggest that family effects should be taken into account during broodstock selection at the farm level. Individual analyses indicated that certain families outperformed others at particular measurement dates and some families showed significantly higher trait values in FRW whereas others showed the opposite effect. As such, selection for growth traits is possible, but the response due to selection will not be consistent across all families. The results indicate that selection of individuals for brackish water culture needs to be evaluated at the family level and that separate breeding programs may be required for optimal growth in both environments.

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Table 2-1: Effect of initial body weight, condition factor and family on long-term survivorship of Arctic charr (*Salvelinus alpinus*) families grown communally in freshwater and brackish water from May 2008 to May 2009.

Treatment	Trait	Effect	df	F-ratio	*P*-value
FRW[1]	BW[3]	Family	29.0	5.91	< 0.001
		Survivorship	1.0	24.79	< 0.001
		Survivorship*Family	29.0	4.96	< 0.001
		Error	1711.0		
BRW[2]	BW	Family	29.0	19.03	< 0.001
		Survivorship	1.0	6.78	0.009
		Survivorship*Family	29.0	1.64	0.017
		Error	1738.0		
FRW	K[4]	Family	29.0	16.37	< 0.001
		Survivorship	1.0	2.42	0.120
		Survivorship*Family	29.0	1.17	0.240
		Error	1463.0		
BRW	K	Family	29.0	17.87	< 0.001
		Survivorship	1.0	0.24	0.627
		Survivorship*Family	29.0	1.56	0.029
		Error	1738.0		

[1] Freshwater

[2] Brackish water

[3] Body weight

[4] Condition factor

Table 2-2: Effect of family, treatment and family by treatment interaction on body weight, condition factor and specific growth rate of Arctic charr (*Salvelinus alpinus*) families grown communally in freshwater and brackish water from May 2008 to May 2009.

Date	Trait	Effect	Degrees of Freedom (numerator, denominator)	F-statistic	*P*-value
Oct. 2008	BW[1]	Family	29.0, 2448.00	10.77	<0.001
		Treatment	1.0, 1.84	0.81	0.473
		Treatment*Family	29.0, 2448.00	2.69	<0.001
May 2009	BW	Family	29.0, 1952.00	7.23	<0.001
		Treatment	1.0, 2.55	14.72	0.044
		Treatment*Family	29.0, 1952.00	4.15	<0.001
Oct. 2008	K[2]	Family	29.0, 2430.00	12.95	<0.001
		Treatment	1.0, 2.05	11.85	0.073
		Treatment*Family	29.0, 2430.00	2.71	<0.001
May 2009	K	Family	29.0, 2037.00	15.97	<0.001
		Treatment	1.0, 2.26	0.03	0.872
		Treatment*Family	29.0, 2037.00	2.28	<0.001
Oct. 2008	SGR[3]	Family	29.0, 2505.00	22.55	<0.001
		Treatment	1.0, 2.11	0.02	0.394
		Treatment*Family	29.0, 2505.00	2.11	<0.001
May 2009	SGR	Family	29.0, 1933.00	20.57	0.172
		Treatment	1.0, 2.07	1.09	0.315
		Treatment*Family	29.0, 1933.00	3.87	<0.001

[1] Body weight

[2] Condition factor

[3] Specific growth rate

Table 2-3: Family specific differences in body weight measured in grams, plus or minus the standard error of Arctic charr (*Salvelinus alpinus*) families grown communally in freshwater and brackish water from May 2008 to May 2009.

	January 2008		May 2008		October 2008					May 2009				
Family	FRW[1]	n	FRW	n	FRW	n_{FRW}	BRW[2]	n_{BW}	$P \leq 0.05$	FRW	n_{FRW}	BRW	n_{BRW}	$P \leq 0.05$
1	212.3 ± 4.5	120	232.2 ± 4.6	117	423.7 ± 13.8	58	415.3 ± 20.7	46		920.3 ± 29.1	48	836.8 ± 47.8	40	
2	224.3 ± 4.0	120	261.4 ± 4.9	120	531.9 ± 15.3	53	535.1 ± 23.9	54		1137.1 ± 29.1	50	1068.9 ± 45.8	54	
3	209.2 ± 5.1	120	229.8 ± 5.6	119	448.1 ± 14.1	58	376.2 ± 29.2	44	*	1066.1 ± 33.1	38	882.5 ± 76.0	33	*
5	273.4 ± 5.1	120	280.8 ± 5.0	119	383.3 ± 21.8	55	482.2 ± 25.9	38	*	725.2 ± 39.9	39	859.2 ± 61.5	35	*
7	231.2 ± 4.5	120	251.4 ± 4.8	120	411.2 ± 18.1	47	438.7 ± 58.5	48		961.5 ± 45.9	45	612.3 ± 59.8	46	*
8	214.4 ± 3.5	120	253.2 ± 4.2	120	503.9 ± 23.0	55	487.7 ± 15.6	54		991.6 ± 30.2	50	1026.0 ± 36.8	53	
9	166.4 ± 3.4	120	202.6 ± 4.2	118	474.4 ± 13.3	57	465.8 ± 15.5	54		948.3 ± 32.5	46	926.6 ± 43.2	47	
10	233.5 ± 3.9	120	258.5 ± 3.7	119	479.3 ± 15.2	55	479.6 ± 22.3	51		919.2 ± 33.4	52	870.3 ± 46.5	45	
11	193.5 ± 4.1	120	230.6 ± 5.0	120	550.5 ± 13.2	55	488.9 ± 16.9	56	*	1133.6 ± 41.4	49	831.1 ± 52.5	53	*
12	174.1 ± 3.6	120	204.8 ± 4.1	118	488.5 ± 15.6	55	463.8 ± 25.8	44		1082.8 ± 34.0	52	878.8 ± 61.2	42	
13	237.9 ± 4.9	120	247.7 ± 4.8	119	368.6 ± 17.2	55	435.4 ± 26.3	42	*	883.1 ± 25.6	52	949.1 ± 71.8	30	
14	192.4 ± 4.0	120	216.3 ± 4.8	119	440.3 ± 17.1	55	439.1 ± 22.6	45		1002.8 ± 27.2	48	897.1 ± 50.9	46	
15	285.1 ± 4.8	120	306.0 ± 5.2	119	444.8 ± 18.6	51	496.5 ± 19.6	53		889.8 ± 39.9	40	963.2 ± 50.5	44	
16	251.7 ± 6.1	120	271.8 ± 5.5	120	458.6 ± 15.7	54	457.8 ± 19.7	53		937.5 ± 33.0	43	968.2 ± 40.4	45	
17	186.6 ± 4.6	120	219.5 ± 5.4	119	461.1 ± 17.5	53	370.2 ± 30.5	32	*	962.2 ± 39.2	41	757.7 ± 78.4	27	*
18	260.6 ± 5.9	120	258.5 ± 5.6	120	373.6 ± 19.1	54	375.9 ± 28.2	42		880.5 ± 33.4	50	837.5 ± 61.8	37	
19	242.3 ± 4.6	120	273.2 ± 5.7	119	523.2 ± 12.5	57	441.1 ± 22.6	55	*	1055.5 ± 30.2	53	873.2 ± 54.8	50	*
20	230.3 ± 4.9	120	242.6 ± 5.2	119	412.2 ± 20.9	49	396.4 ± 21.1	40		869.1 ± 37.8	41	658.7 ± 57.4	36	
22	210.4 ± 4.1	120	231.1 ± 5.1	120	395.4 ± 21.6	55	290.7 ± 25.5	46	*	938.2 ± 42.8	43	513.3 ± 67.0	36	*
23	210.4 ± 4.3	120	224.5 ± 4.7	120	468.6 ± 16.7	57	455.5 ± 29.3	45		949.5 ± 28.5	51	929.0 ± 61.9	43	
24	263.7 ± 5.3	120	241.1 ± 5.6	118	376.3 ± 13.2	55	482.3 ± 22.7	48	*	748.2 ± 22.1	50	847.1 ± 52.7	44	

25	312.1 ± 6.6	120	338.5 ± 6.7	120	530.5 ± 16.0	57	512.2 ± 24.4	53		982.4 ± 36.9	52	975.2 ± 58.5	50	
26	241.0 ± 5.2	120	251.7 ± 5.6	120	494.2 ± 15.7	55	498.5 ± 24.6	55		1076.8 ± 31.3	49	950.2 ± 56.7	52	
27	198.0 ± 4.4	120	233.6 ± 4.7	119	470.0 ± 14.1	48	462.9 ± 20.0	48		991.0 ± 30.6	40	830.7 ± 49.4	45	
28	269.9 ± 6.3	120	280.7 ± 5.5	118	502.2 ± 15.7	51	439.8 ± 24.6	46		1107.2 ± 29.6	44	862.6 ± 58.4	43	
30	184.2 ± 3.1	120	205.3 ± 3.8	120	451.9 ± 15.0	55	472.8 ± 17.6	52		974.2 ± 31.1	46	1029.1 ± 43.6	51	
31	218.6 ± 4.9	120	229.2 ± 5.7	118	338.3 ± 17.3	48	339.5 ± 23.5	30		892.3 ± 30.0	40	591.4 ± 67.1	26	*
32	254.5 ± 4.7	120	271.6 ± 4.5	119	448.9 ± 13.4	58	504.2 ± 20.8	49		1026.3 ± 33.0	52	944.1 ± 46.8	44	
33	227.9 ± 6.3	120	235.3 ± 6.1	118	406.3 ± 21.9	48	313.0 ± 33.0	27	*	899.5 ± 45.4	38	839.1 ± 81.6	17	
35	239.5 ± 5.5	120	245.2 ± 5.3	119	492.7 ± 19.4	55	439.3 ± 23.3	53		1115.2 ± 29.0	49	931.3 ± 49.6	49	
Mean	228.4 ± 1.5	3600	248.9 ± 1.38	3573	455.4 ± 4.5	1618	447.9 ± 4.3	1403		976.1 ± 6.4	1391	904.8 ± 10.5	1263	*

[1] Freshwater

[2] Brackish water

Table 2-4: Family specific differences in condition factor plus or minus the standard error of Arctic charr (*Salvelinus alpinus*) families grown communally in freshwater and brackish water from May 2008 to May 2009.

	January 2008		May 2008		October 2008					May 2009				
Family	FRW[1]	n	FRW	n	FRW	n_{FRW}	BRW[2]	n_{BRW}	$P \leq 0.05$	FRW	n_{FRW}	BRW	n_{BRW}	$P \leq 0.05$
1	1.16 ± 0.03	120	1.08 ± 0.03	118	1.13 ± 0.01	58	1.08 ± 0.12	46		1.30 ± 0.02	48	1.29 ± 0.18	40	
2	1.34 ± 0.03	120	1.33 ± 0.03	117	1.37 ± 0.02	53	1.31 ± 0.11	54		1.59 ± 0.03	50	1.44 ± 0.12	54	
3	1.26 ± 0.04	120	1.19 ± 0.04	119	1.23 ± 0.02	58	0.80 ± 0.12	44	*	1.45 ± 0.04	38	1.35 ± 0.18	33	
5	1.49 ± 0.04	120	1.30 ± 0.03	120	1.10 ± 0.02	55	0.95 ± 0.15	38		1.36 ± 0.04	39	0.91 ± 0.20	35	*
7	1.25 ± 0.02	120	1.17 ± 0.02	119	1.18 ± 0.04	47	1.13 ± 0.13	48		1.45 ± 0.04	45	1.28 ± 0.22	46	
8	1.23 ± 0.03	120	1.24 ± 0.03	120	1.26 ± 0.04	55	1.33 ± 0.11	54		1.39 ± 0.03	50	1.89 ± 0.19	53	*
9	1.03 ± 0.02	120	1.05 ± 0.02	118	1.20 ± 0.01	57	1.20 ± 0.08	54		1.31 ± 0.02	46	1.18 ± 0.11	47	
10	1.38 ± 0.03	120	1.29 ± 0.03	119	1.21 ± 0.01	55	1.31 ± 0.12	51		1.29 ± 0.03	52	1.10 ± 0.15	45	*
11	1.17 ± 0.02	120	1.16 ± 0.02	120	1.24 ± 0.02	55	1.34 ± 0.09	56		1.35 ± 0.05	49	1.24 ± 0.10	53	
12	1.03 ± 0.02	120	1.01 ± 0.02	120	1.10 ± 0.01	55	0.99 ± 0.10	44		1.35 ± 0.03	52	1.25 ± 0.22	42	
13	1.40 ± 0.03	120	1.23 ± 0.02	119	1.26 ± 0.03	55	1.28 ± 0.09	42		1.61 ± 0.03	52	1.73 ± 0.17	30	
14	1.16 ± 0.02	120	1.20 ± 0.03	120	1.23 ± 0.02	55	0.87 ± 0.10	45	*	1.41 ± 0.02	48	0.98 ± 0.14	46	*
15	1.52 ± 0.04	120	1.35 ± 0.03	117	1.21 ± 0.02	51	1.34 ± 0.11	53		1.35 ± 0.06	40	1.22 ± 0.15	44	
16	1.49 ± 0.05	120	1.35 ± 0.04	119	1.24 ± 0.02	54	1.22 ± 0.10	53		1.45 ± 0.05	43	1.34 ± 0.19	45	
17	1.14 ± 0.04	120	1.09 ± 0.03	120	1.30 ± 0.02	53	0.99 ± 0.10	32	*	1.35 ± 0.03	41	1.17 ± 0.12	27	
18	1.41 ± 0.04	120	1.23 ± 0.03	120	1.16 ± 0.02	54	0.85 ± 0.09	42	*	1.39 ± 0.04	50	1.42 ± 0.12	37	
19	1.38 ± 0.03	120	1.29 ± 0.04	119	1.19 ± 0.02	57	1.31 ± 0.13	55		1.31 ± 0.03	53	1.55 ± 0.23	50	
20	1.17 ± 0.02	120	1.06 ± 0.02	119	1.11 ± 0.04	49	0.99 ± 0.07	40		1.32 ± 0.05	41	1.35 ± 0.32	36	
22	1.22 ± 0.03	120	1.13 ± 0.03	120	1.26 ± 0.03	55	0.86 ± 0.08	46	*	1.49 ± 0.04	43	0.94 ± 0.14	36	*
23	1.30 ± 0.04	120	1.18 ± 0.03	119	1.20 ± 0.02	57	1.18 ± 0.20	45		1.38 ± 0.02	51	1.37 ± 0.32	43	
24	1.44 ± 0.04	120	1.33 ± 0.04	119	1.41 ± 0.03	55	1.14 ± 0.11	48	*	1.67 ± 0.04	50	1.57 ± 0.21	44	

25	1.49 ± 0.04	120	1.38 ± 0.03	120	1.31 ± 0.03	57	1.23 ± 0.08	53		1.47 ± 0.03	52	1.43 ± 0.16	50
26	1.29 ± 0.03	120	1.20 ± 0.03	120	1.32 ± 0.02	55	1.42 ± 0.18	55		1.55 ± 0.03	49	1.73 ± 0.20	52
27	1.20 ± 0.03	120	1.20 ± 0.03	119	1.26 ± 0.02	48	1.35 ± 0.14	48		1.39 ± 0.02	40	1.28 ± 0.19	45
28	1.48 ± 0.04	120	1.31 ± 0.03	119	1.32 ± 0.02	51	1.16 ± 0.12	46	*	1.54 ± 0.04	44	1.75 ± 0.22	43
30	1.14 ± 0.03	120	1.09 ± 0.03	120	1.16 ± 0.01	55	1.33 ± 0.15	52		1.32 ± 0.02	46	1.55 ± 0.14	51
31	1.22 ± 0.02	120	1.12 ± 0.02	118	1.21 ± 0.02	48	0.86 ± 0.06	30	*	1.46 ± 0.03	40	1.38 ± 0.18	26
32	1.32 ± 0.02	120	1.23 ± 0.02	118	1.34 ± 0.02	58	1.29 ± 0.05	49		1.51 ± 0.03	52	1.39 ± 0.11	44
33	1.27 ± 0.03	120	1.16 ± 0.03	118	1.27 ± 0.04	48	0.94 ± 0.08	27	*	1.52 ± 0.04	38	1.63 ± 0.20	17
35	1.28 ± 0.02	120	1.16 ± 0.02	119	1.29 ± 0.02	55	1.13 ± 0.05	53	*	1.54 ± 0.03	49	1.51 ± 0.11	49
Mean	1.29 ± 0.01	3600	1.20 ± 0.01	3572	1.36 ± 0.01	1618	1.59 ± 0.02	1403		1.34 ± 0.01	1391	1.55 ± 0.02	1263

[1] Freshwater

[2] Brackish water

Table 2-5: Family specific differences in specific growth rate plus or minus the standard error of Arctic charr (*Salvelinus alpinus*) families grown communally in freshwater and brackish water from May 2008 to May 2009.

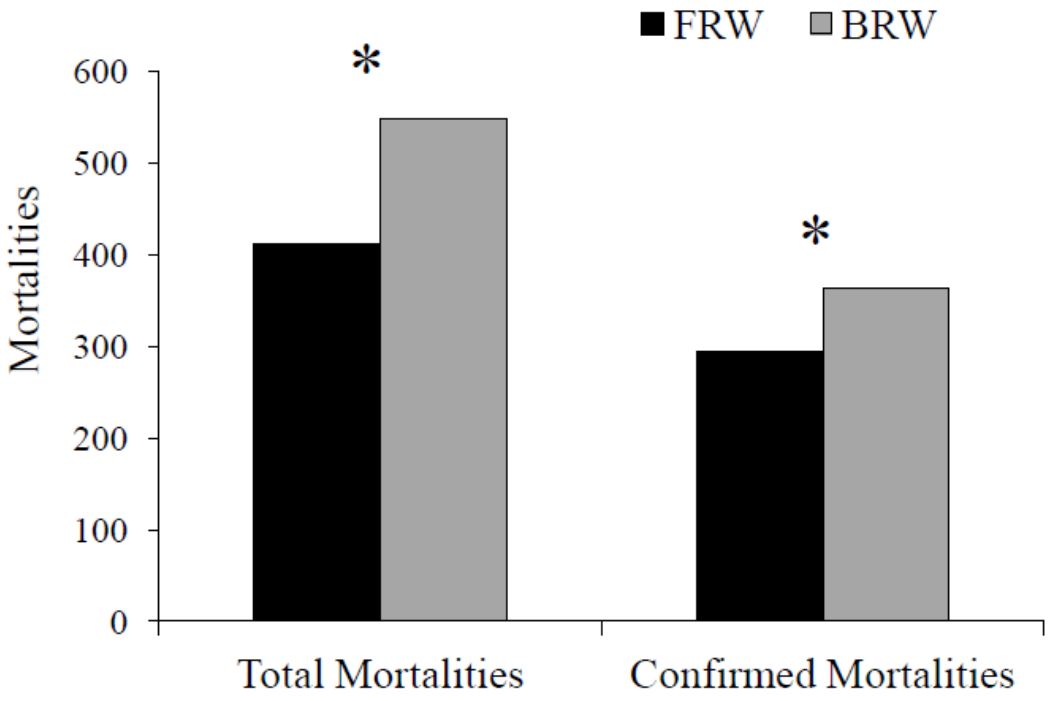
	January - May 2008		May - October 2008					October 2008 - May 2009				
Family	FRW[1]	n	FRW	n_{FRW}	BRW[2]	n_{BRW}	$P \leq 0.05$	FRW	n_{FRW}	BRW	n_{BRW}	$P \leq 0.05$
1	0.10 ± 0.01	117	0.35 ± 0.03	51	0.47 ± 0.02	53		0.32 ± 0.02	48	0.39 ± 0.01	49	
2	0.16 ± 0.01	120	0.39 ± 0.02	53	0.49 ± 0.02	56		0.36 ± 0.01	51	0.32 ± 0.01	48	
3	0.10 ± 0.02	119	0.55 ± 0.03	47	0.45 ± 0.03	53		0.39 ± 0.01	45	0.41 ± 0.02	50	
5	0.03 ± 0.01	119	0.28 ± 0.03	52	0.40 ± 0.03	50	*	0.37 ± 0.02	45	0.35 ± 0.02	41	
7	0.09 ± 0.01	120	0.43 ± 0.02	57	0.37 ± 0.03	54		0.35 ± 0.01	55	0.41 ± 0.02	48	*
8	0.17 ± 0.01	120	0.31 ± 0.03	52	0.35 ± 0.02	57		0.36 ± 0.02	47	0.31 ± 0.01	52	
9	0.20 ± 0.01	118	0.39 ± 0.02	52	0.35 ± 0.03	52		0.36 ± 0.01	48	0.42 ± 0.02	46	
10	0.11 ± 0.01	119	0.45 ± 0.03	46	0.40 ± 0.04	48		0.33 ± 0.02	43	0.38 ± 0.02	47	
11	0.19 ± 0.01	120	0.43 ± 0.04	48	0.27 ± 0.05	38	*	0.31 ± 0.03	46	0.18 ± 0.04	43	*
12	0.17 ± 0.02	118	0.56 ± 0.03	48	0.49 ± 0.03	49		0.34 ± 0.02	44	0.23 ± 0.03	46	
13	0.03 ± 0.01	119	0.34 ± 0.03	45	0.32 ± 0.04	44		0.38 ± 0.03	41	0.30 ± 0.03	38	
14	0.13 ± 0.02	119	0.31 ± 0.04	48	0.21 ± 0.04	50	*	0.21 ± 0.04	42	0.33 ± 0.03	41	
15	0.07 ± 0.01	119	0.20 ± 0.04	47	0.34 ± 0.05	47		0.27 ± 0.04	43	0.30 ± 0.03	42	
16	0.08 ± 0.01	118	0.48 ± 0.03	55	0.41 ± 0.03	45		0.30 ± 0.04	50	0.27 ± 0.02	42	
17	0.17 ± 0.02	119	0.34 ± 0.05	46	0.24 ± 0.06	40	*	0.28 ± 0.03	43	0.29 ± 0.03	41	
18	-0.08 ± 0.01	120	0.30 ± 0.04	45	0.22 ± 0.05	50		0.26 ± 0.03	41	0.30 ± 0.03	38	
19	0.11 ± 0.01	119	0.43 ± 0.03	52	0.24 ± 0.04	56	*	0.27 ± 0.02	41	0.33 ± 0.03	41	
20	0.05 ± 0.01	119	0.41 ± 0.04	55	0.35 ± 0.05	46		0.28 ± 0.03	48	0.30 ± 0.03	42	
22	0.09 ± 0.01	120	0.21 ± 0.04	51	0.26 ± 0.04	48		0.31 ± 0.03	40	0.27 ± 0.02	42	*
23	0.07 ± 0.01	118	0.32 ± 0.04	48	0.14 ± 0.05	43		0.33 ± 0.03	43	0.29 ± 0.03	41	
24	0.03 ± 0.01	118	0.36 ± 0.04	49	0.30 ± 0.04	51	*	0.27 ± 0.02	48	0.36 ± 0.02	41	

25	0.08 ± 0.01	120	0.27 ± 0.04	48	0.35 ± 0.05	41		0.31 ± 0.02	45	0.36 ± 0.02	51	
26	0.05 ± 0.01	120	0.38 ± 0.04	46	0.38 ± 0.03	55		0.41 ± 0.02	44	0.32 ± 0.03	41	
27	0.18 ± 0.01	119	0.38 ± 0.03	56	0.11 ± 0.04	53		0.41 ± 0.02	46	0.31 ± 0.02	40	
28	0.04 ± 0.01	118	0.57 ± 0.02	54	0.49 ± 0.02	59		0.36 ± 0.03	50	0.28 ± 0.03	43	
30	0.10 ± 0.01	119	0.27 ± 0.03	57	0.32 ± 0.04	57		0.46 ± 0.02	50	0.44 ± 0.02	46	
31	0.04 ± 0.01	118	0.35 ± 0.04	52	0.33 ± 0.04	53		0.37 ± 0.03	43	0.45 ± 0.02	46	*
32	0.07 ± 0.01	119	0.25 ± 0.03	53	0.37 ± 0.03	56	*	0.36 ± 0.02	40	0.37 ± 0.02	47	
33	0.04 ± 0.01	118	0.50 ± 0.03	55	0.45 ± 0.02	54		0.42 ± 0.02	46	0.38 ± 0.02	46	
35	0.04 ± 0.01	119	0.35 ± 0.03	54	0.34 ± 0.03	55		0.47 ± 0.02	43	0.37 ± 0.02	44	
Mean	0.09 ± 0.01	3568	0.33 ± 0.01	1522	0.28 ± 0.02	1513		0.59 ± 0.01	1359	0.47 ± 0.03	1323	

[1] Freshwater

[2] Brackish water

Figure 2-1: Number of confirmed and total (includes predation) Arctic charr (*Salvelinus alpinus*) mortalities at the conclusion of a 12 month growth study where fish were exposed to two different environmental treatments, freshwater (FRW) and brackish water (BRW). Fish were reared under commercial rearing conditions, from May 2008 to May 2009. * denotes significant at $P \leq 0.05$.



CHAPTER 3

Comparative analysis of genetic parameters and quantitative trait loci for growth traits in Fraser strain Arctic charr (*Salvelinus alpinus*) reared in freshwater and brackish water environments

Abstract

To determine the potential for genetic improvement in Fraser strain Arctic charr (*Salvelinus alpinus*), I calculated genetic parameters for body weight (BW) and condition factor (K) and tested if previously identified quantitative trait loci (QTL) for these traits were detectable across a commercial broodstock reared in both freshwater (FRW) and brackish water (BRW). Individuals from 30 full-sib families were reared up to 29 month of age in FRW and BRW tanks at a commercial facility. Heritability for BW and K was moderate in FRW (0.29-0.38) but lower in BRW (0.14-0.17). Genetic correlations for BW across environments were positive and moderate (0.33-0.67), however equivalent K correlations were very weak (0.24-0.37). I identified a single BW QTL with experiment-wide effects on linkage group AC-8, as well as 4 BW QTL (AC-4, -13, -14, -19) and 3 K QTL (AC-4, -5, -20i) with chromosome-wide effects across families. Notably, the QTL on AC-8 had significant effects with BW at 3 out of 4 sampling dates in FRW and had significant allelic phase disequilibrium with BW across families, suggesting a tight coupling of the marker region to the QTL in this population. Body weight QTL were identified on AC-4 in both FRW and BRW environments and AC-4 was the only linkage group with a detectable QTL for both K and BW. Modest consistency of some QTL effects as well as moderate heritability in both environments suggests that there is some potential for genetic improvement of growth in this species even though gene by environment interactions are high.

Introduction

The rapid advances in the availability of genetic marker technology is leading to a shift from "classical" selection schemes in breeding programs to a more contemporary approach which involves using information about the phenotype, as well as the genotype of the individual at specific loci (Sonesson, 2007; Lo Presti et al. 2009; Piyasatian et al. 2009). Compared to phenotype based selection, marker assisted selection (MAS) exploits linkage disequilibrium between genetic markers and quantitative trait loci (QTL) and can improve the intensity of selection when the heritability of the trait is low, when the trait is expressed later in the life cycle and for carcass traits such as fillet weight that can only be measured on siblings rather than the broodstock (Dekkers, 2007; Piyasatian et al. 2007; Hospital, 2009; Piyasatian et al. 2009). MAS is currently being used in the breeding programs of livestock species (Solberg et al. 2008; Ibanez-Escriche and Gonzalez-Recio, 2011) and most recent approaches are based on SNP markers distributed throughout the genome (genomic selection) (Van Raden and Sullivan, 2010).

Selective breeding programs in aquaculture species, such as salmonids, are mostly based on the phenotype combined with knowledge of quantitative genetic parameters calculated from individuals evaluated in a single environment (Gjedrem and Thodesen, 2005; Fjalestad, 2005; Gjerde, 2006; Ibanez-Escriche and Gonzalez-Recio, 2011). Traits of economic importance such as growth rate generally have moderate heritability (Carlson and Seamons, 2008) such that positive responses to selection have been achieved (Gjedrem, 1992; Gjoen and Bentsen, 1997; Quinton et al. 2005). Compared to breeding programs for livestock, the potential for genetic gain in aquaculture breeding programs is increased because phenotypic variation is often high, the generation interval

is relatively short and fertility is high. However, the varied response of phenotypes to heterogeneous environments in fishes (Costa et al. 2010) requires that reliable estimates of genetic parameters for production traits must be made in a wide range of rearing systems and environmental conditions before being included in a selective breeding program. Knowledge of QTL for growth related traits in salmonid fishes (e.g., Reid et al. 2005; Moghadam et al. 2007; McClelland and Naish, 2010; Wringe et al. 2010; Kuettner et al. 2011; Norman et al. 2011) is in the early stages of being integrated into selective breeding programs because the consistency and magnitude of QTL effects across multiple families within a broodstock and across environments and rearing systems are generally unknown.

Canada's aquaculture industry is increasingly looking to alternative finfish species other than Atlantic salmon (*Salmo salar*) for culture (CCFAM, 2010). Arctic charr (*Salvelinus alpinus*) is a desirable species for culture in Canada as it is tolerant to cold water temperatures and grows well under high rearing densities (CCFAM, 2010). At present, the aquaculture potential of Arctic charr is greatly reduced by large variation in growth, early maturity and limited salinity tolerance, resulting in low output (Duston et al. 2007). Increasing cost associated with rearing fish in freshwater have also led to an interest in rearing these fish in brackish water environments (Beveridge, 2004). Most selective breeding programs for this species in Canada have primarily focused on increased growth rates and later maturation outside of a formal quantitative genetic framework. In contrast, more significant gains have been achieved in Iceland (Svavarsson, 2007) and Sweden (Nilsson et al. 2010) through selective breeding programs where low to moderate heritability for growth traits have been estimated

depending on the strain and rearing environment (Nilsson, 1990; Nilsson, 1992; Montañez et al. 2007).

Recent work has detected QTL for growth-related traits in Arctic charr based on genome scans in a limited number of families from the Fraser strain from eastern Canada (Moghadam et al. 2007). Specifically, a QTL of large effect was detected on Arctic charr linkage group 8 (AC-8) which accounted for more than 34% of the variation in body weight. Significant body weight QTL were also revealed on AC-13 and -25 and a single significant condition factor QTL on AC-36 accounting for 13% of the observed variation. More recently, genome-wide significant QTL were detected on AC-1, -19, -20 and -28 for body size traits in the same strain (Norman et al. 2010). Similar studies with Icelandic Arctic charr (Kuettner et al. 2011) showed some overlap in body weight QTL location with the Canadian fish (AC-4) but also some differences (AC-20) indicating that the different evolutionary and culture histories of these strains could have led to differential fixation of QTL.

Results from studies on closely related species such as Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) can also aid in classifying growth-related QTL regions in Arctic charr as large regions of homology have now been identified among these species (Timusk et al. 2011). Significant QTL for body weight, for example, have been identified on Atlantic salmon linkage group 10 (AS-10) and AS-17 (Reid et al. 2005) which are homologous with chromosomal regions on AC- 25 and -28, respectively. The most complete study to date has identified body weight QTL on rainbow trout linkage groups RT-3, -6, -8, -9, -10, -12, -13, -22, -24, -27 (Wringe et al. 2010), and all of these regions share homology with the QTL locations in Arctic charr described above,

with the exception of AC-1. AC-1 is metacentric chromosome in Arctic charr which appears homologous to the RT-29 metacentric in rainbow trout. Although QTL effects have not previously been identified on RT-29, a recent study on compensatory growth revealed strong QTL growth effects on this linkage group in cyclically fed fish, but not control fish (Magee, 2011).

I estimated the narrow sense heritability and genetic correlations of body weight in two rearing environments and searched for QTL effects on body weight in 30 families from a broodstock of Fraser strain Arctic charr. Specifically, we aimed to test if the genetic parameters differed between full sibs reared in fresh and brackish water environments and if QTL for growth traits identified in genome scans from the same strain (Moghadam et al. 2007) and other salmonids (O'Malley et al. 2003 and Haidle et al. 2007) had detectable effects across a commercial strain. A select subset of microsatellite markers previously associated with growth QTL, were used in this study. Additionally, we aimed to test the consistency of QTL effects across multiple families and rearing environments to evaluate the degree to which marker assisted selection could potentially benefit the breeding program for Fraser strain Arctic charr.

Methods

Experimental Fish

Fraser strain Arctic charr originated from gamete collections made by Fisheries and Oceans Canada from the Fraser River, Labrador, in 1981 and 1984 and fish were originally reared at the former Rockwood Aquaculture Research Centre (RARC; Gunton, Manitoba). This charr strain was established from small numbers of founders and subsequent pedigree information is not available. A subset of the RARC broodstock were transferred to The New Brunswick department of Agriculture, Fisheries and Aquaculture (NBAFA) who began an Arctic charr breeding program in 1996 as a pilot project to assess the potential of this species for commercial culture. The NBAFA selected individuals for mating based solely on the observed phenotype, normally body weight, and pedigrees are not available. The Coastal Zones Research Institute (CZRI; Shippagan, New Brunswick) inherited the NBAFA broodstock in 2002.

For the present study, 30 males and females were spawned in October 2006 to create 30 full-sib families of Arctic charr at CZRI. Post-spawning, a small piece of pectoral fin was removed from each parent fish and stored in 70% ethanol. Charr embryos were incubated under low light conditions using standard husbandry practices in tanks supplied with freshwater at 4.5 to 6^{o}C. At the onset of exogenous feeding, 1,500 to 2,000 individuals from each family were transferred to 150 L tanks and were reared separately until the fish reached a mean weight of 6 g (June 2007). Fish were identified to family using fin clips, distributed among five 4 m^3 rearing tanks (6 families / tank) and fed a commercial salmon diet (EWOS Canada Ltd., Surrey, British Columbia, Canada) at 4% of body weight per day. At a mean body weight of 230 g (12 months post-hatch), 120

individuals from each family were implanted with a passive integrated transponder (PIT-tag; AVID Canada) and measured for wet weight (g) and fork length (cm). Progeny tissue samples were collected at this time by removing a small section of pectoral fin tissue.

In February of 2008, the fish were transported to a commercial rearing site (CanAqua Seafoods Ltd., Advocate Harbour, Nova Scotia, Canada) where they were placed in four 16 m^3 experimental tanks. Each tank housed 30 fish from each family for a total of 900 individuals per tank. All tanks were outdoors and initially fed by freshwater. In May 2008, salinity in 2 of the experimental tanks was gradually increased from 0 ppt to 20 ppt over a 14 day period to create brackish water (BRW) conditions, while the other 2 tanks remained supplied by freshwater (FRW). Charr were exposed to a natural photoperiod and temperature regime and were fed to satiation using a commercial salmon diet (EWOS Canada Ltd., Surrey, British Columbia, Canada). The growth study ran from May 2008 to May 2009.

Phenotypic Measurements

At the commercial site, individual phenotypic observations on all fish were made at 16, 22, and 29 months post-hatch (May 2008, October 2008 and May 2009). Fish were captured using dip nets and anesthetized in 0.02% MS-222. Body weight (BW) was measured to the nearest gram and fork length was measured to the nearest 0.5 cm. Individual sex and maturity status were identified by the presence of external sexual characteristics. Maturity status was classified numerically as female = 1, male = 2, immature = 3, and unknown = 4. Following measurements, fish were placed in a recovery tank under constant oxygen aeration until they could be returned to the experimental

tanks. Fulton's condition factor (K) of individual fish was calculated for each sampling date using the equation:

$$K = 100\ (BW \times L^{-3}),$$

where *BW* is the wet weight (g) and *L* the fork length (cm). Measurement times and trait descriptive statics including the number of fish per family are presented in Table 3-1. Phenotypic comparisons of BW, K and survival in the two environments are reported in Chapter 2.

Molecular Analysis

Genomic DNA was extracted from parental tissue samples (n = 60) using commercially available DNA extraction kits (Sigma-Aldrich Co.) and each fish was genotyped for variation at 23 microsatellite loci. The 23 microsatellite markers linked to QTL for BW were selected for investigation based on previous work in Arctic charr (Moghadam et al. 2007), rainbow trout (Haidle, 2007; O'Malley et al. 2003), Atlantic salmon (Reid et al. 2005) and information from ongoing research using the Woram et al. (2004) Arctic charr mapping panels. Specifically, microsatellite markers on Arctic charr (AC) linkage groups AC-1, -4, -5, -8, -11, -13, -14, -18, -19, and -20 were targeted in the present study. Marker selection was completed prior to the publication of the most complete QTL study to date in rainbow trout (Wringe et al. 2010) and as such the choice of markers was not consistent with the most recent data in one instance. I used a marker on AC-19 based on data in Haidle (2007), which was not confirmed by Wringe et al. (2010). When markers selected from rainbow trout and Atlantic salmon had either not been mapped in Arctic charr or were not polymorphic in one of the Arctic charr parents, other markers found in a nearby location on the same linkage group (Timusk et al. 2011)

were selected for genotyping. A final selection of 10 microsatellite markers (1 being duplicated = 11 markers) was made to represent the most polymorphic markers in the strain associated with strongest previously identified growth QTL regions (Appendix A).

Microsatellite genetic markers were amplified using standard procedures as outlined in Moghadam et al. (2007). Polymerase chain reactions (PCR) were performed in 11 µL reaction volumes with either the forward or reverse marker primer being 5'-fluorescently end-labelled using tetrachloro–6-carboxy-fluorescein (TET). The PCR mixture was then placed in a thermocycler for initial denaturation (95^{o}C for 3 min), followed by 34 amplification cycles (94^{o}C for 30 s), annealing (50 to 58^{o}C for 40 s), extension (72^{o}C for 50 s) and concluded with final extension (72^{o}C for 5 min). To prepare the samples for genotyping, PCR products were mixed with 10 µL of loading dye (95% formamide, 10 mM NaOH and 0.25% bromophenol blue), followed by denaturation at 95^{o}C for 5 min. Amplified PCR products were detected using polyacrylamide gel electrophoresis (6% polyacrylamide gel, 19:1 ratio of acrylamide to bisacrylamide, 8 M urea, 0.5x TBE buffer). Electrophoresis was performed under denaturing conditions for a minimum of 1.5 h using a constant voltage of 1,600 V. Scanning and visualizations were performed with a FMBIO III fluorescence scanner and Image Analysis Software (MiraiBio Inc.). Following preliminary screening, the most polymorphic markers were selected for genotyping in the progeny (Table 3-2). None of the markers from Atlantic salmon were polymorphic in the Arctic charr families so the final list of markers used in the study included 6 markers from rainbow trout and 5 markers from Arctic charr sources.

Progeny pectoral fin tissue samples were processed and genomic DNA extracted (n = 3,600) as described above. In total, 11 microsatellite loci were genotyped, including one duplicated marker (Table 3-2). Microsatellite markers Omm5018 and Omm5056 were genotyped using procedures as outlined above. The other 8 markers were genotyped using a multiplex technique using an ABI 3770 sequencer (Appendix A). Markers selected for the ABI analysis were designated one of 4 possible fluorescent colors, blue (6-Fam), green (VIC), yellow (NED) and red (PET). Markers that did not have overlapping size ranges were assigned the same color. Custom order 5'-fluorescent labeled/unlabeled di-repeat primers were obtained from Applied Biosystems Canada (www3.appliedbiosystems.com/index.htm). Loci were individually amplified using PCR reaction as outlined above. Following amplification, PCR products from each of the 8 markers were pooled and 2 µL of pooled products was added to 18 µL of distilled water prior to being separated with the ABI 3770 sequencer (Appendix B). Fragments were visualized and scored using GeneMapper 4.0 software (Applied Biosystems).

Genetic Parameters

To calculate the genetic parameters, the measures of BW and K recorded in FRW and BRW were treated as separate traits. Because no relationship or phenotypic information was available on parents, sires and dams were assumed to be unrelated and analyses used only progeny data. Phenotypic and genetic parameters of BW and K were estimated using multiple-trait animal models with DMU Version 6 software (Madsen and Jensen, 2002). Body weight and K at times 2, 3 and 4 were analysed with the following model:

$$Y_{ijklm} = RT_{ij} + ExpT_{ik} + SEX_{il} + A_{im} + e_{ijklm},$$

where i represents the trait, Y_{ijklm} is the observation of trait i for individual m, RT_{ij} is the fixed effect of rearing tank j on trait i, $ExpT_{ik}$ is the fixed effect of experimental tank k on trait i, SEX_{il} is the fixed effect of sex and maturity status l on trait i, A_{im} is the random animal genetic effect of trait i for individual m, and e_{ijklm} is the random residual error for trait i for animal m. The models for BW1 and K1 did not contain the rearing tank effect, but all other effects were as described above. Because only full-sib families were used in this study, the individual genetic effect included additive and potential dominance genetic effects. Heritability (h^2) was calculated as the ratio of genetic variance to total phenotypic variance.

To examine the effect of gene by environment interactions (G x E) on BW and K, 4 trivariate models were run containing traits: 1) BW1, BW3-FRW and BW3-BRW; 2) BW1, BW4-FRW and BW4-BRW; 3) K1, K3-FRW and K3-BRW; and 4) K1, K4-FRW and K4-BRW. For all G x E models, residual covariances between FRW and BRW traits (measured on different individuals) were set to 0 and phenotypic covariances were non-estimable. Only G x E results from these analyses are presented here, but similar models using BW2 and K2 were also run with very similar results.

Genetic and phenotypic correlations between traits measured at different ages have been frequently studied in salmonids (e.g. Elvingson and Johansson, 1993; Bonnet et al. 1999; Kause et al. 2003a) and as such were not the main focus of this study. To study G x E in the present study, the correlations between BW and K within each environment were compared. To examine the relationships between BW and K for a given age within each environment, another set of models were run with the traits: 5) BW1 and K1; 6) BW1, BW2 and K2; 7) BW1, BW3 and K3; and 8) BW1, BW4 and K4.

Quantitative Trait Loci Detection

Individual body weights were *z*-standardized to account for observed differences due to the rearing tank environment for each sampling date with the exception of January 2008. A population wide analysis that included the effects of male and female parents and a full-sib model was used to test for significant QTL with chromosome- and experiment-wide effects using GridQTL software (Seaton et al. 2006; http://www.gridqtl.org.uk). Putative QTL were revealed by calculating *P*-values for all trait-by-linkage group combinations with the significance of the *F*-statistic estimated after 1000 permutations. Estimation of the proportion of variation associated with each male- or female-specific QTL region were obtained using MultiQTL (http://www.multiqtl.com). *P*-values were generated with 1,000 permutations of the phenotypic values for BW and K against the genetic data for each marker.

Specific microsatellite alleles at a given locus were tested across all parents in combination with all other alleles co-occurring with that given allele using a paired Wilcoxon signed ranks test. This test assessed whether a given allele was consistently associated with either larger or smaller BW across a range of families in the strain. Thus if 4 alleles were detected in the strain for a given marker, then 4 independent sets of contrasts (i.e., paired Wilcoxon signed rank tests) were conducted, provided that the allele was present in 5 or more parents. Data points were taken as the mean size of the progeny with each allelic type within a family. Significant effects obtained with the test would be indicative of strong gametic phase disequilibrium of the allele with either large or small BW or K in the fish from the population dependent upon the direction of the allelic means.

Results

Genetic Parameters

Body weight showed moderate heritabilities in both brackish and freshwater environments with BRW estimates (h^2 = 0.12, 0.15; Table 3-3) being approximately half those for FRW (h^2 = 0.28 to 0.38). Body weight genetic variances were similar in both environments (CV_G = 0.13-0.16), but BW had proportionately greater phenotypic variance in BRW (CV_P = 0.37) than in FRW (CV_P = 0.0.24-0.29). Genetic correlations (r_G) between BW in BRW and FRW at a given age were positive but decreased in strength from October 2008 (r_G = 0.67; Table 3-4) to May 2009 (r_G = 0.33).

Condition factor generally showed lower heritability than BW, with a similar trend of lower heritability in BRW (h^2 = 0.10, 0.07; Table 3-3) compared with FRW (h^2 = 0.10-0.18). Genetic variances for K were slightly lower than for BW (CV_G = 0.06-0.12). Freshwater K had noticeably lower phenotypic variance in October 2008 and May 2009 (CV_P = 0.18, 0.21) compared to other measurements. Cross-environment r_G estimates for K were positive but weak (r_G = 0.37, 0.24; Table 3-4).

Within-environment genetic correlations between BW and K were positive and strong at the beginning of the study and later in BRW (r_G = 0.80-0.85; Table 3-4), but surprisingly weak for later Oct 2008 and May 2009 FRW measurements (r_G = 0.13, 0.23).

Quantitative Trait Loci

The location of QTL was inconsistent between the freshwater and brackish water environments and across sampling dates (Table 3-5). A significant BW QTL with an experiment-wide effect was detected on AC-8 in May 2008 while chromosome-wide

significant QTL were identified on AC-4, -8, -13 in the FRW environment. Significant QTL for BW were identified on AC-8 at three of the four sampling dates and two sampling dates for AC-13. No BW QTL with experiment-wide effects were detected in the BRW environment. Chromosome-wide significant QTL were identified on AC-4, -14 and -19 on at least one sample date in fish reared in BRW and all three linkage groups had significant effects in fish sampled on October 2008. Condition factor QTL with chromosome-wide effects were identified on AC-4, -5, and -20i in the FRW environment but only on the January 2008 and May 2009 sample dates. No K QTL were detected in the BRW environment on either date.

The within family QTL analysis detected a limited number of QTL for markers that showed effects across families (Table 3-6). For example, 7 families showed significant QTL effects on AC-8 for the January 2008 sampling period. Consistency in phase between the marker and QTL alleles was detected for markers on AC-8 and AC-13 in the January and May 2008 samplings. The 150 base pair (bp) allele at marker Omi26TUF on AC-8 was significantly associated with larger BW while the 148 bp allele was significantly associated with smaller BW in FRW across families for each of the January and May 2008 sampling dates (Table 3-7). The 319 bp allele for marker Omm1211 on AC-13 was significantly associated with larger BW. No significant differences were detected with the other loci that had yielded significant effects across families.

Discussion

The detection of significant QTL and estimated full-sib heritability for growth related traits in the Fraser strain broodstock of Arctic charr reared in freshwater and brackish water suggests there is additive genetic variation controlling their phenotypic expression. Therefore, there is potential for genetic improvement in this species and selection for growth traits will show marked improvement under varying environmental conditions. However, the differential detection of QTL across families and environments as well as weak genetic correlations and differential heritability across environments provide evidence that G x E should be taken into account during broodstock selection.

Heritability and Quantitative Trait Loci of Growth Traits

The heritability estimates for BW and K found in the current study are generally in accordance with previous studies on Arctic charr (Nilsson, 1990; Nilsson, 1992; Nilsson et al. 2010; Gjedrem, 2000). The heritability estimates in this study may be inflated as the lack of half-sibs in the experimental design made it impossible correct for the bias introduced by dominance and common environment (Falconer and Mackay, 1996). However, the current results suggest that genetic selection will continue to improve growth in Fraser strain Arctic charr, as in other charr and salmonid breeding programs (Gjedrem, 2000; Nilsson et al. 2010).

Body weight QTL previously located in a limited number of families in the Fraser strain of Arctic charr (Moghadam et al. 2007) were detectable across the broodstock. I detected BW QTL with experiment-wide effects on AC-8, BW QTL with chromosome-wide effects on AC-4, -8, -13, -14 and -19 and K QTL with chromosome-wide effects on AC-4, -5, and -20 across all families and environments. This result also supports previous

findings that these regions explain a significant proportion of phenotypic variation in BW (Moghadam et al. 2007). Several of the identified regions are not only consistent with previous work on this species (Moghadam et al. 2007) but also show homologies to the identified BW and K QTL regions in Atlantic salmon and rainbow trout (O'Malley et al. 2003; Reid et al. 2005; Moghadam et al. 2007; Haidle, 2007; Wringe et al. 2010). This supports the finding that these QTL regions house one or more genes regulating growth performance in salmonids.

The strongest and most repeatable BW QTL in the present study was found on AC-8 and supports previous genome scans with this strain (Moghadam et al. 2007). Moreover, the QTL on AC-8 had significant allelic phase disequilibrium with BW across families, suggesting a tight coupling of the marker region to the QTL in this population. Specifically, the 150 bp allele and 148 bp allele at Omi26TUF were consistently associated with larger and smaller BW, respectively. The BW QTL on AC-8 detected at the first sampling date remained consistent throughout the study while all other QTL regions detected varied depending on the age of the fish. This is not unexpected as QTL regions are differentially expressed with age (Martyniuk et al. 2003; Kuettner et al. 2011). The repeatability of BW QTL on this linkage group and the experiment-wide effects found in the present study suggests that this chromosomal region affects growth throughout the life cycle while other regions of the genome having an effect on growth may be restricted to specific ontogenetic periods.

Quantitative trait loci detected on selected linkage groups other than AC-8 also build on genome scan data from Fraser strain charr and Icelandic charr as well as other salmonid species. As mentioned previously, BW and K QTL have been detected on AC-

4, -13, -19 and -20 (Moghadam et al. 2007; Norman et al. 2011). Similarly, Kuettner et al. (2011) found significant BW QTL on AC-4 and K QTL on AC-20 in Icelandic fish. Comparative analyses indicate that the homologous regions in other salmonid species have similar effects. AC-4 is homologous to RT-27 where both BW and K QTL were identified by Wringe et al. (2010). Additionally, AC-13 is homologous to BW QTL regions on RT-24 (Wringe et al. 2010), while the major QTL region detected on AC-8 appears homologous to segments of RT-17p. While a major growth related QTL has not been described on RT-17, this linkage group arm shares homeology with RT-22, where growth-related QTL have been identified (Wringe et al. 2010). AC-20 is homologous to regions of linkage groups RT-9 and AS-11 in rainbow trout and Atlantic salmon, respectively, where QTL for both BW and K have been detected (Reid et al. 2005; Wringe et al. 2010).

Considering K is a trait derived from both BW and length, it might be expected that chromosomal regions controlling the traits would be similar. However, aside from AC-4, there is little overlap in QTL location for both BW and K found in this study. My findings differ from earlier work with the Fraser strain (Moghadam et al. 2007) where co-localization of BW and K QTL were observed on 4 Arctic charr linkage groups, 3 of which were examined in the current study. Results from the present study suggest that the combination of genes influencing the phenotypic expression of K may be different from the combination of genes influencing the phenotypic expression BW. This observation is consistent with Fishback et al. (2002) and Martyniuk et al. (2003) who found that K is independent of both length and BW. The current data also suggest that the interaction of genes influencing the phenotypic expression of K may differ to a greater extent

temporally, compared to BW trait genes, as more variation was found in the distribution of K QTL across sampling dates compared to BW QTL. Three of the 5 QTL regions detected with BW showed consistent effects in either the freshwater or brackish water environments across both temporal measurement periods, whereas none of the 3 K QTL regions provided evidence for the repeatability of this association between measurement times.

Gene by Environment Interactions

Although wild, anadromous Arctic charr undergo endocrine changes and salinity tolerance similar to that of Atlantic salmon (Jørgensen et al. 2007) there are limited examples with varying results of Arctic charr being reared in saline conditions. The QTL and genetic parameter results of this study give evidence of strong G x E interactions affecting growth in Fraser strain Arctic charr reared in fresh and brackish water.

In this study, the location of QTL for BW and K were inconsistent between the freshwater and brackish water environments. These results are in contrast with an earlier study by Norman et al. (2011) in which full-sibs from 6 of the 30 families used in the present study were tested for QTL relating to salinity tolerance traits. The previous study showed that the same QTL were often detected in both environments, suggesting that the occurrence of body size QTL was not affected by either salt water or freshwater exposure (Norman et al. 2011). It is possible that this discrepancy between Norman et al. (2011) and the present study could be due to differences in experimental design. Individuals from the Norman et al. (2011) study were reared in full seawater for only 4 months compared to the one year duration in the present study. In addition, Norman et al. (2011) solely exposed experimental fish to seawater and did not include siblings

reared in freshwater tanks for comparison. It is also important to note that the current trial was conducted in brackish water rather than full strength sea-water and thus the results presented here may not be directly comparable due to differences in the environmental treatment.

Body weight and K measured in the different environments exhibited disparate heritability and weak cross-environment genetic correlations, which further indicate G x E interaction. Although the heritability values for BW in FRW are comparable to previous reports with this species (Nilsson, 1990), the BRW estimate is approximately half that in FRW. The smaller heritability value for both BW and K in brackish water appears to be the result of increased phenotypic variation in the trait. Weak cross-environment genetic correlations in this study indicate G x E interactions for growth that may be even stronger than those previously found in rainbow trout (Kause et al. 2003; Kause et al. 2004) and Atlantic salmon (Quinton, 2005). Another study testing for salinity tolerance transferred Fraser strain Arctic charr to salinities of either 0 ppt, 10 ppt, 20 ppt, or 30 ppt (Duston et al. 2007). Duston et al. (2007) reported that both family and salinity had significant effects on final body weight indicating that body size was indeed affected by varying salinities. Although Duston et al. (2007) did not detect a family x salinity interaction; the potential importance of such an interaction was not dismissed by the authors. Duston et al. (2007) used the same environmental treatment (20 ppt brackish water) as the current study but the duration of brackish water exposure was only five months compared to the 12 months of the present study.

Gene by environment interactions are expected in novel environments or stressful conditions (Cote et al. 2007) and initial observations of significantly lower body weight

and higher mortality at the conclusion of 12 months of brackish water rearing demonstrated that the brackish water environment is physiologically challenging for Arctic charr (Dumas et al. 1995; Duston et al. 2007). The weaker genetic correlations between BW and K in the freshwater environment at times 3 and 4 are therefore unexpected as genetic correlations are expected to decrease in more stressful environments. More studies are required to test if this is a true genetic effect. Considering there are few studies examining the effect of exposure to brackish water environment on Arctic charr and the corresponding results are variable, it seems as though G x E interactions need to be evaluated on the level of the individual farm.

Considerations for Selective Breeding

This study is one of the first to evaluate the consistency of QTL across a commercial broodstock of salmonid fishes. I have identified variation in BW QTL across multiple families of Arctic charr and that combined with moderate heritability and genetic correlations between full-sibs reared in fresh and brackish water environments indicates indicate there is some potential for genetic improvement of growth in this species in both environments. Despite the strong G x E interactions, positive cross-environment genetic correlations, overlap of BW QTL in both environments, and overlap of BW and K QTL on AC-4 suggests that improved brackish water growth can be achieved to some degree through selection on broodstock reared in freshwater. This is consistent with the finding that phenotypic performance in freshwater is a general predictor of performance in brackish water (Chapter 2). However, it appears as though the predictability of the results depends on the length of time reared in brackish water as G x E increases with time. In the current study, evidence of strong G x E interactions for

growth indicates that certain genotypes may be better adapted to culture in a brackish water environment. If brackish water rearing is the future of Arctic charr aquaculture then brackish water performance records for broodstock or their relatives will be required to achieve greater selection accuracy and faster genetic progress. In the long term, it may be necessary to select and develop a new saline-adapted strain of Arctic charr.

Although the sensitivity of a single-marker approach in detecting QTL employed in the present study is less than using interval mapping, this approach has enabled us to detect BW and K QTL across multiple families with moderate consistency compared to other work on this species. For QTL to be a reliable tool for selective breeding schemes, the QTL should be detected at the same chromosomal region in different experimental families. Quantitative trait loci studies based on only a few families are a necessary platform from which marker-assisted selective breeding studies may begin but should be interpreted with caution as significant effects may be confused with family effects. Although the present study has identified some consistency across families, not all significant QTL and significant allelic associations are consistent across all families. Therefore, selection programs must use caution when adding these alleles into the selection process. Although the most consistent allele effects were identified on AC-8 and AC-13, QTL identified on AC-4, -14 and -19 may also be incorporated into a selective breeding program providing the family specific phase of the allele is known.

To implement this QTL information into a marker-assisted breeding program for Arctic charr, molecular markers such as Omi26TUF and Omm1211 must be incorporated into a framework of breeding value estimation. For marker-assisted selection to be as effective as possible, several QTL for the trait should be incorporated into the breeding

framework to maintain large genetic variance in the trait for genetic selection. Due to the potential for recombination due to the pairing of homologous chromosomes in meiosis, further research must be conducted to identify markers that are closely linked to the genes controlling the trait of interest so that the markers can be used for more than one generation of selection. Finally, the molecular markers will need to be re-evaluated in a few generations to ensure the markers are still efficient at predicting relative QTL effects.

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Table 3-1: Numbers of observations and numbers per family, means and standard deviations for body weight (BW) condition factor (K) measured on Fraser strain Arctic charr (*Salvelinus alpinus*) reared in freshwater (FRW) and brackish water (BRW) environments.

Measurement Date	Age (Months)	Trait	N	Mean n per family (min, max)	Mean	SD
Jan. 2008	12	BW1	3600	120 (119, 120)	228.25	62.28
		K1	3600	120 (119, 120)	1.28	0.34
May 2008	16	BW2	3572	119 (117, 121)	248.82	62.76
		K2	3572	119 (117, 121)	1.20	0.31
Oct. 2008	22	BW3 – FRW	1618	54 (47, 58)	455.19	135.72
		BW3 – BRW[1]	1403	47 (27, 53)	447.13	166.23
		K3 – FRW	1618	54 (47, 58)	1.24	0.18
		K3 – BRW[1]	1403	47 (27, 52)	1.26	0.53
May 2009	29	BW4 – FRW	1391	46 (38, 58)	973.75	245.35
		BW4 – BRW[2]	1263	42 (27, 51)	877.86	374.36
		K4 – FRW	1110	37 (22, 48)	1.44	0.22
		K4 – BRW[2]	889	30 (15, 43)	1.49	0.65

[1] Measurements correspond to 5 months of brackish water rearing

[2] Measurements correspond to 12 months of brackish water rearing

Table 3-2: Microsatellite markers selected for the current study to investigate body weight QTL in Arctic charr (*Salvelinus alpinus*). Markers were selected prior to the start of the study using previously reported QTL relationships from the literature.

Linkage Group	Marker	Source Species[1]	Source Marker	Reference
AC-1	Omm5155	AC-1	BX311884	Moghadam et al. 2007
AC-4	Omm1228	RT-27	OmyRGT31TUF	O'Malley et al. 2003
AC-5	Omi179TUF	RT-1	BX076085	Haidle, 2007
AC-8	Omi26TUF	AC-8	Omi26TUF	Moghadam et al. 2007
AC-11	Omm5018	RT-19	Omm5018	Haidle, 2007
AC-13	Omm1211	AC-13	Omm1211	Moghadam et al. 2007
AC-14	Sco19UBC	RT-24	OMM1320	Haidle, 2007
AC-18	Omm5056	RT-22	BHMS422a	O'Malley et al. 2003
AC-19	OmyRGT46TUF	RT-13	Ocl4UW	O'Malley et al. 2003
AC-20	CL25555i	AC-20	OMM1274	Moghadam et al. 2007
AC-20	CL25555ii	AC-20	OMM1274	Moghadam et al. 2007

[1] AC = Arctic charr; RT = rainbow trout

Table 3-3: Genetic (SD_G) and phenotypic standard deviation (SD_P), coefficients of genetic (CV_G) and phenotypic variation (CV_P), and heritability ($h^2 \pm$ SE) estimates for body weight (BW) and condition factor (K) measured on Arctic charr reared in freshwater (FRW) and brackish water (BRW) environments.

Trait	SD_G	SD_P	CV_G	CV_P	h^2	$\pm$ SE
BW1	36.21	58.89	0.16	0.26	0.38	$\pm$ 0.09
BW2	31.88	59.98	0.13	0.24	0.28	$\pm$ 0.07
BW3 – FRW	74.37	131.75	0.16	0.29	0.32	$\pm$ 0.08
BW3 – BRW[1]	57.72	163.76	0.13	0.36	0.12	$\pm$ 0.04
BW4 – FRW	128.46	232.59	0.13	0.24	0.31	$\pm$ 0.08
BW4 – BRW[2]	130.39	331.72	0.15	0.38	0.15	$\pm$ 0.05
K1	0.14	0.33	0.11	0.26	0.18	$\pm$ 0.05
K2	0.09	0.30	0.08	0.25	0.10	$\pm$ 0.03
K3 – FRW	0.08	0.22	0.06	0.18	0.12	$\pm$ 0.04
K3 – BRW[1]	0.15	0.49	0.12	0.39	0.10	$\pm$ 0.04
K4 – FRW	0.11	0.30	0.08	0.21	0.14	$\pm$ 0.04
K4 – BRW[2]	0.16	0.61	0.11	0.41	0.07	$\pm$ 0.04

[1] Measurements correspond to 5 months of brackish water rearing

[2] Measurements correspond to 12 months of brackish water rearing

Table 3-4: Cross- and within-environment genetic ($r_G \pm$ SE) and phenotypic correlations (r_P) among body weights (BW) and condition factors (K), for Fraser strain Arctic charr (*Salvelinus alpinus*) reared in freshwater (FRW) and brackish water (BRW) environments at different measurement dates.

	Cross-environment		Within-environment	
Date	r_G BW $\pm$ SE	r_G K $\pm$ SE	r_G BW:K $\pm$ SE	r_P BW:K
Jan. 2008	-	-	0.85 $\pm$ 0.06	0.56
May 2008	-	-	0.80 $\pm$ 0.09	0.55
Oct. 2008	0.67 $\pm$ 0.15	0.37 $\pm$ 0.25	FRW 0.23 $\pm$ 0.21	FRW 0.44
			BRW[1] 0.82 $\pm$ 0.11	BRW[1] 0.65
May 2009	0.33 $\pm$ 0.22	0.24 $\pm$ 0.29	FRW 0.13 $\pm$ 0.22	FRW 0.26
			BRW[2] 0.85 $\pm$ 0.14	BRW[2] 0.57

[1] Measurements correspond to 5 months of brackish water rearing

[2] Measurements correspond to 12 months of brackish water rearing

Table 3-5: Significant QTL for body weight (BW) and condition factor (K) detected for Fraser strain Arctic charr (*Salvelinus alpinus*) linkage groups across sampling dates. Arctic charr were grown communally in two rearing environments, brackish and fresh water.

	Linkage Group										
Trait	**1**	**4**	**5**	**8**	**11**	**13**	**14**	**18**	**19**	**20i**	**20ii**
BW1				*		*					
BW2				**		*					
BW3		*					*		*		
BW4		*	*	*							
K1		*								*	
K2											
K3											
K4			*								

Freshwater

Brackish water

* Chromosome-wide significance

** Population-wide significance

Table 3-6: Results of the MultiQTL single marker analysis identifying family specific body weight (BW) QTL. Compares the number of families in which a particular allele, measured in base pairs (bp), is segregating and the number of families in which significant BW QTL ($P \leq 0.05$) have been identified. Fraser strain Arctic charr (*Salvelinus alpinus*) families were grown communally in brackish (BRW) and freshwater (FRW).

Trait	Linkage Group	Marker	Env.	Segregating Allele, bp[1]	# Families Segregating	# Families Significant QTL
BW1	AC-8	Omi26TUF	FRW	150	19	7
	AC-13	Omm1211	FRW	362	20	5
BW2	AC-8	Omi26TUF	FRW	150	19	8
	AC-13	Omm1211	FRW	319	9	4
BW3	AC-4	Omm1228	BRW	269	11	5
	AC-14	Sco19UBC	BRW	-	-	4
	AC-19	OmyRGT46TUF	BRW	-	-	2
BW4	AC-4	Omm1228	FRW	269	11	3
		Omm1228	BRW	269	11	3
	AC-8	Omi26TUF	FRW	150	19	4

[1] The segregating allele is the allele which is most often associated with significant BW QTL and"-" indicates that no one allele is associated with the BW QTL

Table 3-7: Significant associations of mean body weight for each allele, measured in base pairs, where significant ($P \leq 0.05$) QTL were identified in individual Fraser strain Arctic charr (*Salvelinus alpinus*) families. Arctic charr families were grown communally in brackish (BRW) and freshwater (FRW).

Trait	Linkage Group	Marker	Env.	Allele, bp	Effect[1]	*P*-value	PEV[2]
BW1	AC-8	Omi26TUF	FRW	148	<	0.007	0.062-0.166
				150	>	0.005	
				156	-	0.638	
				158	-	0.128	
	AC-13	Omm1211	FRW	296	-	0.286	0.043-0.084
				300	-	0.130	
				304	-	0.139	
				319	>	0.009	
				354	-	0.161	
				362	-	0.055	
BW2	AC-8	Omi26TUF	FRW	148	<	0.022	0.055-0.242
				150	>	0.014	
				156	-	0.594	
				158	-	0.063	
	AC-13	Omm1211	FRW	296	-	0.213	0.041-0.053
				300	-	0.116	
				304	-	0.114	
				319	>	0.004	
				354	-	0.161	
				362	-	0.158	
BW3	AC-4	Omm1228	BRW	247	-	0.715	0.092-0.157
				250	-	0.649	
				265	-	0.126	
				269	-	0.308	
	AC-14	Sco19UBC	BRW	204	-	0.685	0.111-0.196
				215	-	0.201	
				226	-	0.260	
				247	-	0.326	
				255	-	0.884	
	AC-19	OmyRGT46TUF	BRW	146	-	0.099	0.063-0.145
				166	-	0.159	
				168	-	0.332	
				172	-	0.176	

BW4	AC-4	Omm1228	FRW	247	-	0.465	0.064-0.151
				250	-	0.820	
				265	-	0.737	
				269	-	0.875	
	AC-4	Omm1228	BRW	247	-	0.465	0.084-0.163
				250	-	0.785	
				265	-	0.073	
				269	-	0.239	
	AC-8	Omi26TUF	FRW	148	-	0.548	0.073-0.262
				150	-	0.520	
				156	-	0.826	
				158	-	1.000	

[1] Effect was determined to either be associated with large (>) or small (<) body size, or have no effect (-)

[2] PEV = Proportion of experimental variation (%) in individual families

CHAPTER 4

Genetic improvement of Arctic charr (*Salvelinus alpinus*) using phenotypic and genomic selection strategies

Abstract

The Canadian aquaculture industry is increasingly looking towards the development of alternative species for culture, such as the Arctic charr. To develop a national breeding program, desirable phenotypes and genetic parameters must be measured in diverse environments. A total of 3600 fish from 30 families of Arctic charr were reared in either fresh (FRW) or brackish water (BRW) and individual body weight was recorded. I estimated the rate of genetic change for each environment based on one generation of selection using (i) phenotype-only selection (PS), (ii) marker only selection (MS) and (iii) marker assisted selection (MAS) in two different scenarios. In scenario A, selection occurred within the top 20% of individuals across all families in the study where as in scenario B, the top 20% of individuals were selected only from families where QTL for body weight was identified. In both environments, predicted genetic response was more favorable in the next generation. Selection for increased body weight was increased using marker assisted selection versus phenotypic selection alone. The greatest response in the rate of genetic change was achieved by selecting only from families in which significant BW QTL had been identified. As such, marker assisted selection showed the greatest gain in genetic response with 5.4% in FRW and 4.3% in BRW. These results have applications to commercial aquaculture as the Canadian aquaculture industry is attempting to diversify with alternative species. Such genetic improvement strategies will aid in developing a strain of Arctic charr characterised by increased body weight.

Introduction

Nearly half of the marketed fish for human consumption comes from aquaculture and salmonids make up the greatest proportion of aquaculture products (FAO, 2010). As the global population continues to increase and wild fisheries catches continue to decline, the number of aquaculture products are expected to increase to make up for the difference. Considering that resources such as food, water and energy are finite, existing aquaculture operations must strive to increase production efficiently through selective breeding. In comparison to agriculture species, selection programs based on a formal quantitative genetic framework are only available for a few aquaculture species (e.g. salmonids and tilapia). The majority of selection programs for aquaculture species traditionally select for growth traits based on mass selection for phenotype alone (Gjedrem and Thodesen, 2005; Fjalestad, 2005; Ibanez-Escriche and Gonzalez-Recio, 2011; Gjedrem et al. 2012; Lind et al. 2012).

The goal of selective breeding is to develop a more productive and better suited animal for farming. In particular, selective breeding is used to change the population mean for a trait under selection, most often growth-related traits and results in the changing of the frequencies of the desired allele (Gjedrem and Thodesen, 2005; Lind et al. 2012). To do this, breeding programs commonly use one or a combination of the following selection methods to identify parents for the next generation (Fjalestad, 2005): (1) Individual selection is the most common method where selection is based on an individuals' observed phenotype. (2) Pedigree selection involves the selection of broodstock based on the breeding values from the parent organisms (Spangler et al. 2008). (3) Family selection occurs when family groups are ranked in order of best mean performance for the trait of interest (Gjedrem, 2010). (4) Within-family selection

involves the selection of individuals who deviate positively from the family mean (Sonesson, 2007). (5) Progeny testing offers the best estimate of an individuals' breeding value because parents are selected based on the performance of their offspring (Pszczola et al. 2012). The majority of commercial aquaculture operations use individual selection as the sole means to identify parents for the next generation because it is relatively simple and often produces the desired response (Ibanez-Escriche and Gonzalez-Recio, 2011). However, breeding programs utilizing this kind of selection may experience inbreeding if breeding records are not kept and the mass selection of individuals may not be useful if there are large changes in environmental conditions (Fjalestad, 2005; Gjedrem et al. 2012; Lind et al. 2012).

In recent years, the development of moderate to high density linkage maps for agriculture species, the identification of quantitative trait loci (QTL) for traits of economic importance and high-throughput chips for genotyping thousands of single nucleotide polymorphisms (SNPs) has lead to rapid advancements in animal breeding when integrated with traditional methods of phenotypic selection (Van Raden and Sullivan, 2009; Ibanez-Escriche and Gonzalez-Recio, 2011). Most traits of economic significance are controlled by many loci that are inherited quantitatively (Chistiakov et al. 2006). Regions of the genome which control quantitative traits are known as QTL. Genomic selection, the estimation of genome wide breeding values based on SNP genotyping is the current state of the art in animal breeding (Ibanez-Escriche and Gonzalez-Recio, 2011; Solberg et al. 2008). Genomic selection is a reality in the dairy cattle industry (Van Raden and Sullivan, 2009) and pilot evaluations have been

conducted in swine (Cleveland et al. 2010; Lillehammer et al. 2011), sheep (Banks and Van der Werf, 2009) and poultry (Long et al. 2007; Preisinger, 2012).

The application of genomic selection in aquaculture is not feasible at this time as few genetic maps with high marker density and high-throughput SNP chips are available for fish species (Ibanez-Escriche and Gonzalez-Recio, 2011; Lo Presti et al. 2009; Tong and Chu, 2002). However, the potential for genomic selection in aquaculture has been established through simulation studies (Sonesson, 2007; Sonesson and Meuwissen, 2009; Nirea et al. 2012). Genetic maps of moderate density and the development of SNPs (Gutierrez et al. 2012) for aquaculture species are becoming increasingly available. Knowledge of associations between microsatellite markers and commercially important traits and additionally, knowledge of homologies among species has allowed the identification of QTL for traits of economic importance in aquaculture such as growth, condition, age at maturation, flesh color, upper thermal tolerance and salinity tolerance (O'Malley et al. 2003; Perry et al. 2005; Reid et al. 2005; Moghadam et al. 2007; Baranski et al. 2010; McClelland and Naish, 2010; Wringe et al. 2010; Kuettner et al. 2011; Norman et al. 2011). The identification of QTL in aquaculture breeding programs are providing the foundations for a shift from traditional phenotypic selection towards breeding programs which incorporate a combination of phenotype and genetic information which has the potential to significantly increase the rate of genetic improvement through marker assisted selection (MAS). MAS may be of particular importance when trying to select for traits which have low heritability, are difficult to measure, occur late in the life cycle, or are measured post-harvest. The application of

MAS in aquaculture has the potential to significantly increase the intensity of selection for economically important traits.

As a pilot project to assess genetic improvement under differing selection strategies, I used data collected from juvenile full-sib Fraser strain Arctic charr families from the CZRI broodstock reared typical commercial land-based tanks. The rate of genetic change following one generation of selection was predicted in response to alternative strategies for increased body weight in either fresh or brackish water environments. In particular, I estimated genetic change using traditional phenotypic selection and genomic methodologies including marker-only selection and marker assisted selection.

Methods

Thirty Fraser strain Arctic charr males and females from the Coastal Zones Research Institute's (CZRI; Shippagan, New Brunswick, Canada) broodstock were used to create 30 full-sib families in October 2006. Fish were reared at CZRI until February 2008 when they were transferred to a commercial grow-out facility (CanAqua Seafoods Ltd., Advocate Harbour, NS) where fish were exposed to two different rearing environments (freshwater and brackish water). Small pieces of pectoral fin tissue were collected from both broodstock and progeny for genetic analysis with 11 microsatellite markers previously linked to QTL for body weight in salmonid fishes. Thirty fish from each family were reared in duplicate tanks of freshwater and brackish water. Each fish was measured for body weight (g) and fork length (cm) in October 2008 at the age of 2 years. Data collected at this time were used for the calculation of genetic parameters. Details of the growth study, data collection and genetic analysis were described in detail in the previous chapters.

Genetic Parameters

To calculate the genetic parameters, observations of body weight recorded under each environmental treatment were treated as separate traits in freshwater and brackish water. Phenotypic and genetic parameters of body weight were estimated using the multiple-trait animal model with DMU6 software (Madsen and Jensen, 2002) as outlined in the previous chapter. Heritability (h^2) was calculated as the ratio of genetic variance to total phenotypic variance. Full-sib family effect (c^2) was calculated as the ratio of full-sib family variance to total phenotypic variance.

Prediction of Genetic Responses to Selection

The rate of genetic gain was predicted using three different selection strategies, (i) phenotype-only selection (PS), (ii) marker-only selection (MS) and (iii) marker assisted selection (MAS) for each environment based on one generation of selection based on selection index theory that was developed for MAS by Lande and Thompson (1990). Finally, the correlated response to selection was calculated to predict the body weight of fish in brackish water due to phenotypic selection for body weight in freshwater.

The responses of body weight in both freshwater and brackish water environments due to one generation of phenotype-only selection were calculated using the equation:

$$\frac{\Delta_{BV}}{t} = \frac{h^2 \, i \, \sigma_P}{L},$$

where Δ_{BV}/t represents the rate of genetic change per unit of time (t) due to phenotypic selection for body weight, h^2 is the heritability of body weight, i is the selection, σ_P is the phenotypic variation for body weight and L is the generation interval in years. Estimates of h^2 and phenotypic variation are as calculated in the previous chapter. The selection intensity for body weight when the top 20% of individuals are kept as broodstock is assumed to be 1.4 and the generation interval is three years. The rate of genetic change, measured in grams/year, was converted to the overall percent change in mean value of the phenotype for each environment.

To predict the responses of body weight in both freshwater and brackish water environments due to one generation of marker-only selection, the relative efficiency (r) of selection on only the marker loci was found using the equation:

$$r = \sqrt{p/h^2},$$

where p is the proportion of genetic variance explained by the marker loci and h^2 is the heritability of the trait. Marker loci were selected based on the population-wide results reported in the previous chapter. The proportion of genetic variance explained by the marker loci was calculated by summing the mean *PEV* (proportion of explained variation) of loci where significant ($p < 0.05$) QTL were found. QTL for the trait were identified using a family specific, single marker analysis (MultiQTL; http://www.multiqtl.com) to reveal within family linkage disequilibrium. *P*-values were generated with 1000 permutations of the phenotypic values for body weight against the genetic data set for each microsatellite marker as outlined in the previous chapter. The rate of genetic change was then calculated by multiplying the relative efficiency (r) of selection on only the marker loci by the response of body weight due to phenotypic selection (Δ_{BV}/t).

To predict the responses of body weight in both freshwater and brackish water environments due to one generation of marker assisted selection, the relative efficiency (r_{MAS}) of selection on the marker loci and the phenotype was found using the equation:

$$r_{MAS} = \sqrt{\ p/h^2 \ + \ \frac{(1-p)^2}{1-h^2p}},$$

where p is the proportion of genetic variance explained by the marker loci and h^2 is the heritability of the trait. The proportion of genetic variance explained by the marker loci was calculated by summing the mean *PEV* (proportion of explained variation) of loci where significant QTL ($p < 0.01$) had been identified. The rate of genetic change was then calculated by multiplying the relative efficiency (r) of selection on only the marker loci by the response of body weight due to phenotypic selection (Δ_{BV}/t). Genetic responses in the traits for both environmental treatments were calculated as percentage change relative to the original trait mean in the data.

Because Arctic charr are primarily grown in freshwater, correlated response calculations were used to predict body weight in brackish water from phenotypic selection for body weight in freshwater. The correlated response of one generation of selection was calculated by:

$$\frac{\Delta_{BVy|x}}{t} = \frac{r_{BVx,BVy}\, h_x\, h_y\, i_x\, \sigma_{Py}}{L},$$

where trait x is body weight in freshwater and trait y is body weight in brackish water, $\Delta_{BVy|x}/t$ represents the rate of genetic change in trait y per unit of time (t) due to phenotypic selection for trait x, $r_{BVx,BVy}$ is the genetic correlation between traits x and y, h_x is the square root of heritability for trait x, h_y is the square root of heritability for trait y, i_x is the selection intensity for trait x when the top 20% of individuals are kept as broodstock, σ_{Py} is the phenotypic variation for trait y and L is the generation interval in years.

The rate of genetic change for each environment based on one generation of selection

using (i) phenotype-only selection (PS), (ii) marker only selection (MS) and (iii) marker assisted selection (MAS) was then tested in two different scenarios. In scenario A, selection occurred within the top 20% of individuals across all families in the study where as in scenario B, the top 20% of individuals were selected only from families where QTL for body weight was identified.

Results

The PEV for body weight in families where significant QTL were identified ranged from 0.06 to 0.26 in freshwater and 0.08 to 0.20 in brackish water (Table 4-1). When selecting across all families in the CZRI broodstock, the rate of genetic change in the trait body weight due to phenotype-only selection was 4.2% and 2.5% in freshwater and brackish water, respectively (Fig. 4-1). The predicted response of body weight due to one generation of marker only selection was 2.2% and 1.9% in freshwater and brackish water, respectively (Fig. 4-1). Marker assisted selection showed the greatest gain in genetic response with 4.5% in freshwater and 3.0% in brackish water (Fig. 1). The correlated response of body weight in brackish water due to phenotypic selection for body weight in freshwater was low at 2.5% (Fig. 4-1).

When selecting only specific families in which significant QTL for body weight had been identified, the response in the rate of genetic change in the trait body weight due to marker-only selection was 4.6% and 4.0% in freshwater and brackish water, respectively (Fig.4- 2). Marker assisted selection showed the greatest gain in genetic response with 5.4% in freshwater and 4.3% in brackish water (Fig. 4-2).

Discussion

This study has provided some of the first results on the incorporation of marker information into traditional selective breeding programs for Fraser strain Arctic charr. Specifically, the results indicate that selection index calculations can be used to evaluate a range of selection strategies for the CZRI breeding program when marker information is available.

The correlated responses of body weight in brackish water to phenotypic selection for body weight in freshwater shows no advantage over phenotypic selection for brackish water performance. The results indicate that separate breeding programs may be required for optimal growth in both environments. As CZRI's Arctic charr broodstock are kept in freshwater, land based tanks (Claude Pelletier, personal communication), this will require the collection of body weight measurements on siblings housed in both fresh and brackish water environments and selecting with-in families which have favorable performance in brackish water.

The addition of molecular marker information into the selection program for Arctic charr was expected to increase the genetic response after one generation of selection. This was not the case when the selection of broodstock occurred within the top 20% of individual fish across all families. When selection included all families, genetic response with MS was reduced and was similar with MAS compared to PS. Considering this study found many QTL of small effects with only few QTL of large effect, that QTL were not significant in all families tested and the fact that allelic phase differed between families (Chapter 3) selection across all families was not expected to result in large gains in genetic response after one generation of selection. Predicted genetic response in the

next generation for both MS and MAS was increased compared to PS when within-family selection was practiced. This is similar to the results of Sonesson (2007) who found that total genetic gain was increased by 15% after one generation of selection with marker information for MAS schemes compared with the non-MAS. As these simulations are based on only one generation of phenotypic data without and pedigree information, the accuracy is relatively low. As such, the addition of marker information into such a breeding program should be used in conjunction with available phenotypic data (Dekkers, 2007).

In the present study, only one marker per linkage group was used to estimate QTL effects and the genetic map for Arctic charr is much more sparse compared to other salmonid fishes (Palti et al. 2011).For marker assisted selection to be as effective as possible, several QTL for the trait should be incorporated into the breeding framework to maintain large genetic variance in the trait and to increase the accuracy of selection. A suggested method to increase marker density is the addition of markers flanking the QTL (Sonesson, 2007). By adding two flanking markers on either side of the QTL, Sonesson (2007) increased the total genetic gain by 2% in generation two and 11% in generation three, thus making the molecular markers more informative and increasing the accuracy of selection. Complementary to this, more than one generation of phenotypic records is needed to obtain a high accuracy of selection. Nielsen et al. (2009) found that there is an upper limit to the increase in accuracy with increasing marker density if the number of phenotypic records is not increased.

Over time, recombination between markers and QTL will reduce the accuracy of selection. The predicted genetic response in the present study is based on only one

generation of selection. In a simulation study, Nielsen et al. (2009) documented a 14.7% decrease in the accuracy of selection from the first to the second generation, followed by a 10.5 % decrease from the second generation to the third. This suggests that molecular markers used to identify QTL for economically important traits will need to be re-evaluated in a few generations to ensure the markers are still efficient at predicting relative QTL effects. Additionally, further research must be conducted to identify the specific polymorphisms within the actual candidate genes influencing the trait and use these markers directly in the breeding program.

In the present study, the proportion of variation in body weight explained by the microsatellite loci is less than 25% which is similar to published reports for this species (Moghadam et al. 2007). The QTL on AC-8 accounted for the highest PEV for body weight in my study which is consistent with Moghadam et al. (2007) who found a QTL accounting for 34% PEV localized to the same linkage group. Increased marker density is expected to increase the accuracy of selection (Solberg et al. 2008; Nielsen et al. 2009). The increase in genetic response observed when MS and MAS were applied to targeted families where significant QTL for body weight were located indicates that the trait can be improved using marker information compared to traditional PS. In the present study the increase in genetic response after one generation of selection was only 1.2% and 1.8% in fresh and brackish water, respectively. Specifically, MAS for increased gain in body weight does not seem to result in significantly greater genetic response compared to PS. Based on the present gains in genetic response, the cost of genotyping must be evaluated prior to the inclusion of marker information in a breeding program as resulting gains in genetic response may not make up for the cost of genotyping. This suggests that although

small gains in body weight are possible with the present MAS simulation, these gains can be improved through increasing the accuracy of selection by the discovery of additional QTL and markers which are more tightly linked to the trait. In current Arctic charr breeding schemes, MAS will be especially useful for traits that can only be measured on the siblings of the selection candidates such as disease tolerance, traits which cannot be measured pre-harvest such as fillet traits, or traits which have low heritability/low genetic response in current phenotype based selection.

Although the application of genomic selection for Arctic charr is not feasible at this time as genetic maps with high marker density and high-throughput SNP chips are not presently available for this species, this simulation study indicates favorable increases in genetic gain are possible if MAS was applied to CZRI's Fraser strain Arctic charr breeding program based on selection index theory that was developed by Lande and Thompson (1990). In the future, Arctic charr breeding programs should follow the successful lead of other commercial livestock (Van Raden and Sullivan, 2009; Lillehammer et al. 2011; Preisinger, 2012) and QTL for growth performance should be incorporated into a greater framework of MAS which includes other traits such as disease tolerance, fillet traits and sex-limited traits. The incorporation of MAS in selective breeding programs for Arctic charr is now within the realm of possibility as high density maps can be created through new and less expensive methods of high-throughput sequencing that is becoming more and more available (Gutierrez, et al. 2012; Huang et al. 2012). This could even lead to the possibility of whole genome selection.

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Table 4-1: Proportion of variation in the trait body weight explained by the microsatellite loci (PEV) in families where significant QTL ($P < 0.05$) were identified using a population-wide analysis. Arctic charr (*Salvelinus alpinus*) families were grown communally in freshwater (FRW) and brackish water (BRW) environments.

			Families where significant QTL ($P < 0.05$) were identified																	
Treatment	LG	Marker	1	2	3	5	7	11	15	16	17	19	20	22	23	24	25	27	28	32	35
FW	AC-4	Omm1228				0.14	0.15	0.09							0.06						0.09
	AC-8	Omi26TUF	0.10				0.26	0.13									0.07				
	AC-13	Omm1211								0.12		0.10							0.12		
BW	AC-4	Omm1228				0.14	0.08								0.16	0.09			0.09		
	AC-14	Sco19UBC		0.20	0.11						0.14		0.11					0.11			
	AC-19	OmyRGT46TUF							0.08			0.14		0.06						0.11	

Figure 4-1: Predicted rate of genetic gain in percent (%) for the trait body weight using three different selection strategies, (i) phenotype-only selection (PS), (ii) marker only selection (MS) and (iii) marker assisted selection (MAS) for Arctic charr grown communally in freshwater (FRW) and brackish water (BRW) based on one generation of selection across all families (Scenario A) in the Costal Zones Research Institutes Fraser strain Arctic charr broodstock. Additionally, correlated response (FRW|BRW) of body weight for fish grown in brackish water due to phenotypic selection for body weight in freshwater.

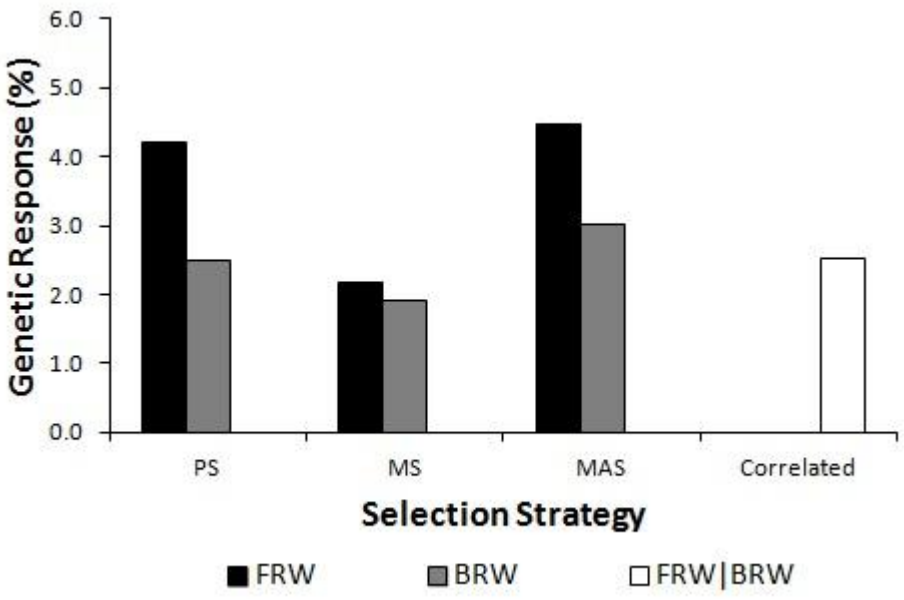
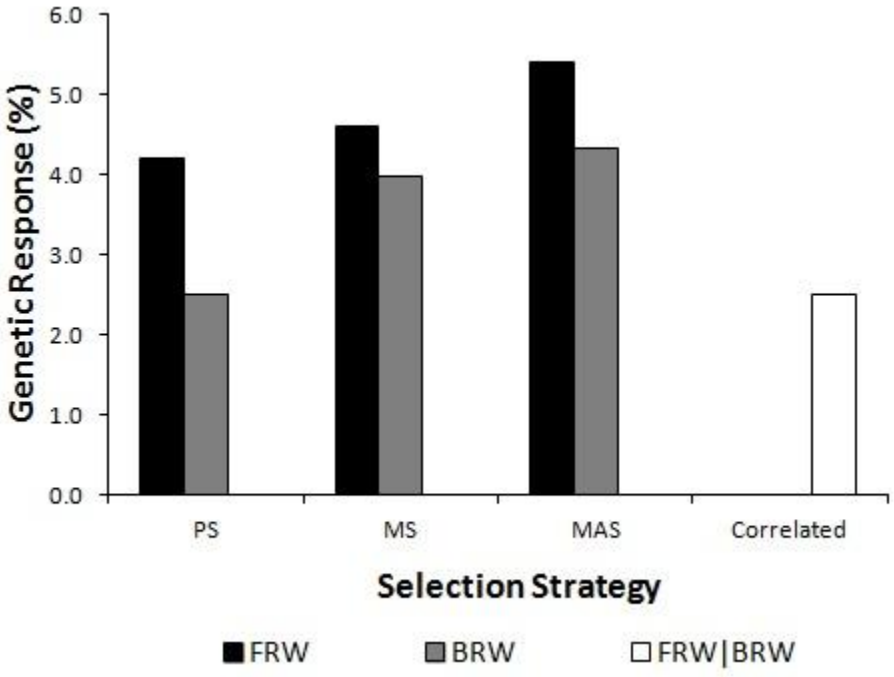


Figure 4-2: Predicted rate of genetic gain in percent (%) for the trait body weight using three different selection strategies, (i) phenotype-only selection (PS), (ii) marker only selection (MS) and (iii) marker assisted selection (MAS) for Arctic charr grown communally in freshwater (FRW) and brackish water (BRW) based on one generation of selection in select families where significant QTL for body weight were identified (Scenario B) from the Costal Zones Research Institutes Fraser strain Arctic charr broodstock. Additionally, correlated response (FRW|BRW) of body weight for fish grown in brackish water due to phenotypic selection for body weight in freshwater.



GENERAL CONCLUSIONS AND RECOMMENDATIONS

My thesis contributes to the understanding of the genetic variation of growth traits in cultured populations of Fraser strain Arctic charr. The work presented offers insight into the future of animal breeding of anadromous salmonid fishes. Specifically, the present study evaluated the relative phenotypic performance of Arctic charr broodstock families in freshwater and brackish water and used microsatellite markers to identify QTL related to variation in body weight and condition factor. This is the first study to investigate the effect of BW and K QTL across multiple families in a commercial broodstock. This is the first study to evaluate the linkage phase of the QTL effect which indicated whether a given allele was consistently associated with either larger or smaller BW across a range of families in the strain. Additionally, this is the first study to include both genetic parameters and QTL information into a single investigative framework to simulate the genetic response after one generation of selection using marker assisted selection.

My work on evaluating phenotypic response of full-sibling charr in parallel rearing environments has provided evidence that the effect of environment needs to be considered in a selective breeding program. Specifically, in the present study when the phenotypic performance of Arctic charr siblings was compared across environments, similar growth and survival was observed during the first six months of commercial rearing regardless of treatment. The predictability of brackish water performance based on observations on freshwater siblings was strongest at the onset of the study but declined over time as the effect of family by environment interactions increased. The results indicate that growth trajectories in a given environment remain consistent providing the

environmental conditions are not altered and that any growth advantages or constraints observed during early rearing in freshwater will not necessarily continue throughout the grow-out phase in brackish water. As CZRI is the provider of progeny for many Arctic charr producers in Atlantic Canada, future studies should be expanded to evaluate the phenotypic performance of Arctic charr families at multiple farms and in multiple environments to test if environmental differences observed in the present study are consistent. Additionally, the differential response of Arctic charr families observed in body weight, condition factor and specific growth rate suggest that family effects must be considered during broodstock selection at the farm level. Individual analyses in the present study indicated that certain families outperformed others at particular measurement dates and some families showed significantly higher trait values in one environment while other families showed the opposite effect, indicating that the response due to selection will not be consistent across all families. Based on such results, I suggest that separate breeding programs may be required for optimal growth in both environments.

The detection of significant QTL and estimated full-sib heritability for growth related traits in Arctic charr reared in freshwater and brackish water suggests there is additive genetic variation controlling their phenotypic expression. Using targeted markers linked to QTL for growth traits in previous studies my results provide some evidence that these regions have repeatable effects in multiple families of charr. Although no single QTL was found to have repeatable effects across the population studied, these results have allowed me to narrow down the genomic regions of interest which should be investigated further. Specifically, regions of AC-4, -8 and -13 which showed repeatable

effects throughout the study should be extensively mapped to increase the marker density. Increasing the marker density will enable fine scale mapping and the identification of the underlying genes controlling these traits.

The present study provides evidence that although the QTL regions evaluated here exhibit repeatable effects among many families, that these markers are not tightly linked to the genes underlying growth traits. Specifically, as the direction of the QTL effect was witnessed to change, the allelic phase of the QTL must be known for each family to be useful in a breeding program. By increasing the marker density and identifying candidate genes for growth traits, the future of selective breeding for this species can be realized by using such information to select broodstock candidates based on genetic markers. The simulation study presented in this thesis suggests that under present conditions, small gains in body weight are possible using marker assisted selection and these gains can be improved through increasing the accuracy of selection by the discovery of additional QTL and markers which are more tightly linked to the trait. However, traits which are influenced by environmental conditions, such as body weight in the present study, will likely have a greater response to selection when a combination of phenotypic and marker assisted selection is applied. For the purposes of a complete selective breeding program in a broader framework, further research should be conducted to identify QTL regions for other economically important traits such as disease resistance, flesh color and age at maturation. The addition of such traits into a selective breeding framework including selection for growth traits would improve selection for traits that appear later in life and carcass traits which can only be measured on the siblings of the broodstock candidates.

In conclusion, by identifying the genetic variation within aquaculture populations for economically important traits, I have increased understanding of the genetic architecture of this population while under strong selection pressure. Using the Fraser strain broodstock available from the CZRI as a model provides the opportunity to speculate regarding the possibility of a national Arctic charr selective breeding program and the possible gains which could be achieved through MAS. There are however, several issues which still need to be evaluated prior to applying these results in practice.

APPENDIX A

Table A-1: Forward (F-) and reverse (R-) primer sequences for each marker investigated.

Linkage Group	Marker	Sequence
AC-1	Omm5155	F-GGACAGAACTGCCACTAAGTGTG
		R-GAGGAGACAGGGAAGAGCTATTG
AC-4	Omm1228	F-CCCTTCCTGTGTGTCGTTGTT
		R-CAGGAGTCACTTGGCAGTAGGAG
AC-5	Omi179TUF	F-TTATCCTAGTGCCGGGTCTG
		R-ATGCAGCTTTTCAGTGGCTT
AC-8	Omi26TUF	F-AGGATTAGACCTGCTCTCCTCA
		R-CGGTCAGACAGAAACATCCA
AC-11	Omm5018	F-GAAGGAACGGAACAGAGTGGTAATCAC
		R-TCGGACAGGTAACTGGAACGGAT
AC-13	Omm1211	F-ACCCACTCTCCCACTCAGTATT
		R-GAAGGAGGCTTGGAAGTGTATC
AC-14	Sco19UBC	F-CTTGAAATTAGTTAAACAGC
		R-CCAAACTACCCAATAATC
AC-18	Omm5056	F-TCACCATCACCTTCATCGCCT
		R-ACATGCTGCCCTTTGACGGAG
AC-19	OmyRGT46TUF	F-TCAGAAATCCAGCCAAAACC
		R-GACGCAAAGAGAGTTCAGTGG
AC-20i/ AC-20ii	CL25555i	F-GAACTGTAAAGTGATGGAAAA
		R-GACATACACTCATACAGGTGG

APPENDIX B

Screen possible microsatellite markers using standard acrylamide gels. Select markers that have strong polymorphic bands, relatively free from stutters and not duplicated. Determine the size range of the markers of interest and find the sequence information (this is required to order custom primers).

The ABI has 4 fluorescent labeled colors that can be attached to the primer. Blue (6-Fam), Green (VIC), Yellow (NED) and Red (PET). After selecting your markers for the ABI, assign each marker a color. More than one marker can have a color if their size ranges do not overlap.

Custom order primers are available at Applied Biosystems Canada (www3.appliedbiosystems.com/index.htm). Primers are shipped dry as 10 000 pmols. Once in the lab, the primers need to be diluted to make a stock solution. The stock solution should be 100 pmol/µl. Keep primers frozen and out of the light.

For a PCR reaction, the primers need to be diluted. Some screening tests will be required to determine the appropriate primer concentration for the given markers. (Note: begin by screening individual markers prior to pooling). Normally, the primers can be used at 4-10 pmol/µl. Often, 6-Fam & PET colors are weaker and may require a greater concentration of primer. Once diluted, the primers can be used in the 11µl PCR reaction (Table B-1) using the standard PCR reaction program with marker specific annealing temperatures. After each marker is optimized individually, pool the markers together and run on the ABI. When working with primers, attempt to keep primers cool and out of direct light.

A PCR reaction is completed for each marker. After the PCR is complete, pool the products together. Usually begin with 2µl of NED & VIC, 3µl of 6-Fam and 4µl of PET. Do some test screens on the ABI and adjust as necessary. Once the appropriate pooling volumes have been established, determine the appropriate dilution factor. Once all the optimizing is complete, begin with large scale genotyping.

NOTE: The volume in the PCR reaction was increased from the standard 7µl to 11µl to control for evaporation during the PCR reaction.

Table B-1: Volumes used for one PCR reaction (to use, multiply by the number of reactions you want to complete).

Order	Reagent	1 RXN Volume (µl)
1	dd H2O	2.05
2	5X Buffer	2.31
3	25 mM Mg	0.63
4	5 mM dNTP	0.26
5	10 uM Primer-F	0.30
6	10 uM Primer-R	0.30
7	10 mg/ml BSA	0.11
8	5 u/ul Taq	0.04
	TOTAL	6.00

add 5µl DNA

After mixing all the reagents, pipette 6ul to each reaction well and then add 5ul of DNA. Do not add oil as it will clog the capillaries of the ABI sequencer. Seal plates and place in the thermocycler.

Table B-2: Thermocycler program for DNA amplification.

Step	Temperature (^{o}C)	Time (mins)
1	95	3:00
2	94	0:30
3	annealing temp	0:40
4	72	0:50
5	5 = Go to 2	34 X
6	72	5:00
7	10	10:00
8	END	

Following all the PCR reactions, the products need to be pooled in one plate. Markers labelled with the 4 fluorescent tags were pooled as followed: 2µl of NED (yellow) & VIC (green), 3µl of 6-Fam (blue) and 4µl of PET (red). This plate of "pooled products" must be diluted prior to being run on the ABI – therefore, add 2ul of your pooled products to another plate with 18ul of ddH2O (1:10 dilution). Plates may now be run on the ABI.