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Population and family effects on gene transcriptional profiles of eight hybrid Chinook salmon (Oncorhynchus tshawytscha) populations: implications for conservation and aquaculture

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POPULATION AND FAMILY EFFECTS ON GENE TRANSCRIPTIONAL PROFILES OF EIGHT HYBRID CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) POPULATIONS: IMPLICATIONS FOR CONSERVATION AND AQUACULTURE

by

Shelby Toews

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I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research, as follows: Chapter 2 of this thesis is co-authored with Dr. Kyle Wellband and my co-supervisors, Dr. Daniel Heath and Dr. Brian Dixon. Chapter 3 of this thesis is co-authored with my co-supervisors, Dr. Daniel Heath and Dr. Brian Dixon. I am the sole author of Chapter 1 and Chapter 4. In all cases, the key ideas, primary contributions, experimental designs, data analysis and interpretation, were performed by the author, and the contributions of co-authors was primarily through help with experimental design, interpretation, editing, and providing funding and logistical support.

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ABSTRACT

Aquaculture has been the fastest growing food industry worldwide since 1985 and has contributed significantly to the Canadian economy. Thus, methods for increasing aquaculture production are currently being investigated, such as selective breeding programs. Here I developed transcriptional profiles of eight hybrid half-sibling populations of Chinook salmon (*Oncorhynchus tshawytscha*) and examined them for evidence of local adaptation and as a potential marker for marker-assisted selection method. I found evidence of local adaptation among the populations, further supporting this as a driving force behind the large variation witnessed in Chinook salmon life history variation. I used the transcriptional profiles developed for each population in the freshwater (juvenile) stage and tested for correlations with saltwater performance (growth rate and survival). I found significant correlations between saltwater performance factors and juvenile transcription, suggesting that saltwater performance in Chinook salmon can be predicted using freshwater transcription patterns. There were also significant correlations between freshwater and saltwater transcription, indicating possible mechanisms behind the correlation between freshwater and saltwater traits. Freshwater Chinook salmon transcriptional profiles are a promising novel marker for application in marker-assisted selection breeding programs in aquaculture. Overall, transcriptional profiling using selected known-function genes provide the ability to study both local adaptation and performance in Chinook salmon populations.

DEDICATION

To my parents, Belinda and John Toews, and my fiancé, Seth Mackie, for their undying love and support.

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CHAPTER I

GENERAL INTRODUCTION

The human population has been growing exponentially since the early 1900s (Lutz and Qiang, 2002) and the current population as of May, 2017 is well over 7.3 billion people (US Census, 2016), resulting in an increased demand for food, especially protein. In 2009, 800 million people were considered to be malnourished, which translates to a lack of protein intake (Aiking, 2011). A possible solution to this need for protein is an increase in fish consumption as they are a good source of nutrients, comparable to beef, and fish are also more efficient at converting feed into flesh (Gjedrem et al., 2012). With aquaculture leading the food industry as of 1985 (Diana, 2009) one way to increase global fish availability would be to increase productivity through methods such as selective breeding (Bostock et al., 2010). Selective breeding can be based on many different traits, including the underlying genetic structure of the genome. If it is successful, this will not only lead to increased protein availability, but will benefit Canada's economy because aquaculture activity is common on both the Atlantic and Pacific coasts. Between 1986 and 2006 the economic benefit of aquaculture in Canada increased from $35 million to $912 million, and has continued to rise (CAIA, 2015). Therefore if genetic-based methods are developed to increase productivity (i.e., growth rate and survival) without increasing costs, they can be employed in Canada and worldwide based on genetic conservation among species. This will benefit the Canadian economy and aid in decreasing the gap between the growing human population and the food resources available.

Aquaculture

Aquaculture is defined as "the controlled growing of some type of aquatic crop, mainly for food" (Diana, 2009) and is the fastest-growing food industry in the world (Gjedrem et al., 2012). This increase in aquaculture production is due to the decrease of commercial and wild stocks from overharvesting (Bostock et al., 2010). It is estimated that by the year 2020, the annual per person seafood consumption will be approximately 1.5 kg, resulting in a need for 10 million metric tons of seafood each year, disregarding the growing population (Diana, 2009). Within Canada, aquaculture began in the 1980s, growing economically from a GDP of $35 million in 1986 to $912 million in 2006 (Marshall, 2003). This is in part due to the fact that there are fish farms on both the Atlantic and Pacific coasts, highlighting the growing need for increased fish growth and survival rates (Lamaze et al., 2014).

The aquaculture industry on the west coast of Canada focuses primarily on salmon, mostly Atlantic salmon (*Salmo salar*), but also to a lesser extent, Chinook salmon (*Oncorhynchus tshawytscha*). However, there are some significant limitations to the productivity of salmon aquaculture because of their long life cycle. The time period from fertilization to harvest can be up to three years (or longer), and all of the eggs that are fertilized will not survive until harvest because of disease or other mortality factors (Wheatley et al., 1995). The most costly factor in aquaculture is the time it takes for them to reach harvest size because of the associated costs of fish feed (Cook et al., 2000). Their growth is made up of factors that can be controlled, such as feeding habits, and others that cannot, such as seasonal temperature changes (Asche and Bjørndal, 2011). One method to decrease production cost would be to grow native species wherever possible,

because they are likely to reach maximum growth in their home environment. However, native species (i.e., Chinook salmon) do not always have the growth rates of other species (i.e., Atlantic salmon) therefore selection of stocks with faster growth rates is crucial to increasing aquaculture production. Yellow Island Aquaculture Ltd. (YIAL) is one example of an aquaculture company on the west coast of Canada that grows only native Chinook salmon. They are an organic salmon hatchery, meaning no antibiotics or hormones are used, and they supply salmon for both consumers and researchers (YIAL, 2008). Thus by studying salmon reared at YIAL, transcriptional profiles with faster growth rates and better survival, under intensive culture conditions, can be selected as a part of a selective breeding program.

Gene Expression

Gene expression is the production of functional proteins in a specific tissue after its conversion from the original DNA strand to protein, by means of RNA transcription and translation (Hedge and Kang, 2008). Transcription and translation are the two main rate-determining steps for gene expression; however multiple studies have shown that transcription has, on average, a greater influence on gene expression, than its counterpart, translation (Platt, 1986; McAdams and Arkin, 1997; Nguyen et al., 2003). The transcription process is tissue-specific and is regulated by microRNAs (miRNA) and transcription factors. Transcription factors are proteins that have the ability to alter the rate at which RNA is created from DNA, by either up-regulating or down-regulating the activity of the RNA polymerase (Martinez and Walhout, 2009). Sladek et al., (2007) reported that both genetic (familial) traits and the environment have influences on transcription rates, ultimately affecting gene expression. Therefore gene expression can

vary by population, and at the genetic level, this can be due to either random genetic drift (Greuber et al., 2013) or local adaptation (Vitti et al., 2013).

An organism's gene expression level also varies greatly for different genes, tissues, and conditions (Schmittgen and Livak, 2008). For example, in Chinook salmon, phenotypic variation in growth can occur in captive uniform environments, indicating potential variation in the genome (Cutts et al., 1998). A study by Heath et al., (1999) found that genetic influences on a fish's traits increases through development, signifying that gene expression may be a major contributor to variation in observed growth rates. Thus by measuring gene expression of specific functional genes among salmon populations and comparing those data with growth and survival rates, conclusions can be made as to which genes have a larger impact on the overall performance of Chinook salmon.

Chinook Salmon

Chinook salmon are an anadromous fish found along the Western coast of North America, from Northern Mexico to the Arctic Ocean, as well as the Eastern coast of Asia from Taiwan to the Arctic Ocean (Healey, 1991). Chinook are the largest of the Pacific salmon species, with the largest Chinook on record weighing 57 kg (Government of Canada, 2013). Chinook salmon were selected as the study species for this project because salmonids are one of the most studied fish species, resulting in an extensive amount of available genomic information (Olsvik et al., 2005), critical to gene expression studies, such as this one. Chinook salmon are extremely valuable to the Canadian economy, both for their use as sportfish and as a food source (Lamaze et al., 2014). However, Chinook salmon are also known to be highly susceptible to stressors, such as

handling stress (Heath et al., 1993) and immune challenges (Quinn, 2005), resulting in negative health issues, slow growth and potentially death (Bostock et al., 2010). The stressors and immune challenges that Chinook salmon face in aquaculture may have a role in their growth rate and survival, by altering their genetic expression of specific genes. Therefore measuring the effects of stress and disease at the genetic level in Chinook salmon may be a key component in increasing productivity and, ultimately, improving the likelihood of the success of salmon aquaculture.

Quantitative Real-Time PCR

Quantitative real time-polymerase chain reaction (qRT-PCR) is a form of PCR that allows an estimate of the number of copies (i.e., transcripts) of a gene to be calculated. For quantifying gene expression, it is the most sensitive and reliable method available (Pfaffl et al., 2002). qRT-PCR works by designing two primers (one forward and one reverse) as well as a probe for a specific gene sequence. The probe is a fluorescent molecule that sends a signal each time it binds to a transcript (Bustin and Mueller, 2005). This results in an actual count of the number of transcripts that are present in a sample, for a specific gene. These transcript counts can then be used to determine and analyse gene expression among individuals.

Gene expression can be calculated from qRT-PCR through two different methods; absolute quantification and relative quantification. Absolute quantification determines the exact number of transcripts present in a sample through linear regression of a standard curve. In contrast, relative quantification compares the expression level of a target gene to that of an internal control gene (Livak and Schmittgen, 2001; Bustin and Mueller, 2005). This method also accounts for any differences in tissue used for RNA extraction

among samples (Bustin and Mueller, 2005). For the purposes of this study, relative quantification will be used, because it allows the change in expression to be determined by comparing the transcript level to a reference gene and grants the ability to develop transcriptional profiles compared to the literature-suggested reference genes. The calculation method that is generally used to quantify the relative gene expression is the $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen, 2001). This is based on the "critical threshold" of a qRT-PCR, which is the point at which the number of transcripts in the sample reaches a fixed threshold and is based on many factors (i.e. fluorescent dye, efficiency, etc.). Therefore the more transcribed copies there are of the gene, the sooner it will reach this threshold and produce a peak (Livak and Schmittgen, 2001; Bustin and Mueller, 2005). The method compares the C_T value of the target gene to that of the reference gene, and then compares that value between a control individual and a challenged individual to get the relative expression change in the target gene for the treated individual (Livak and Schmittgen, 2001).

Thesis Objectives

The main objective of this thesis was to determine whether different Chinook salmon stocks have different genetic potentials for increased productivity in aquaculture, and whether this can be predicted based on their transcriptional profiles. A total of 80 families (composing 8 populations) were created in the Fall of 2013, using highly inbred females and males from one domestic stock and seven wild stocks from local rivers (Big Qualicum River, Capilano River, Chilliwack River, Nitinat River, Puntledge River, Quinsam River, and Robertson Creek), as indicated in Figure 1.1. I attempted to identify which of the experimental stocks would be the best candidate for a selective breeding

program to increase the production at YIAL. This was done by determining each population's average growth rate and survival in the salt water, and comparing it to their underlying gene transcription profiles in both the freshwater and saltwater phase to determine if gene transcription can be used to predict growth and survival. A schematic diagram of the breeding design can be seen in Figure 1.2.

Chapter 2 used juvenile Chinook salmon (approximately 6-7 months old) reared at YIAL that were split into three categories: control, immune stimulus, and handling stress (two challenges that salmon face in aquaculture). Half-siblings from eight different experimental populations (seven families from each stock) were used to produce transcriptional profiles for a suite of 26 candidate genes (Appendix A1). The candidate gene list was composed of immune-, growth-, metabolic-, and stress-related genes, along with two reference genes, *B-actin* and *EF-1a*. The main objective of this chapter was to determine if there were population-level differences in gene transcription and whether these differences were likely adaptive (i.e., due to local adaptation) or neutral (i.e., due to random genetic drift).

My objective in Chapter 3 was to determine whether the performance of Chinook salmon in salt water (i.e. growth and survival) could be predicted based on gene transcription from the freshwater phase. The fish used in this chapter were composed of four families from each of the eight populations (N = 32). These families were selected from the same subset of families used in Chapter 2. Correlations were performed between the transcriptional profiles created in Chapter 2 and the growth and survival rates of the saltwater salmon to see if performance could be predicted using gene transcription. I then also created transcriptional profiles for the saltwater fish to correlate with the freshwater

transcriptional profiles. Therefore, if growth and survival could be predicting using juvenile transcription patterns, this would allow me to determine the underlying mechanism controlling these predictions.

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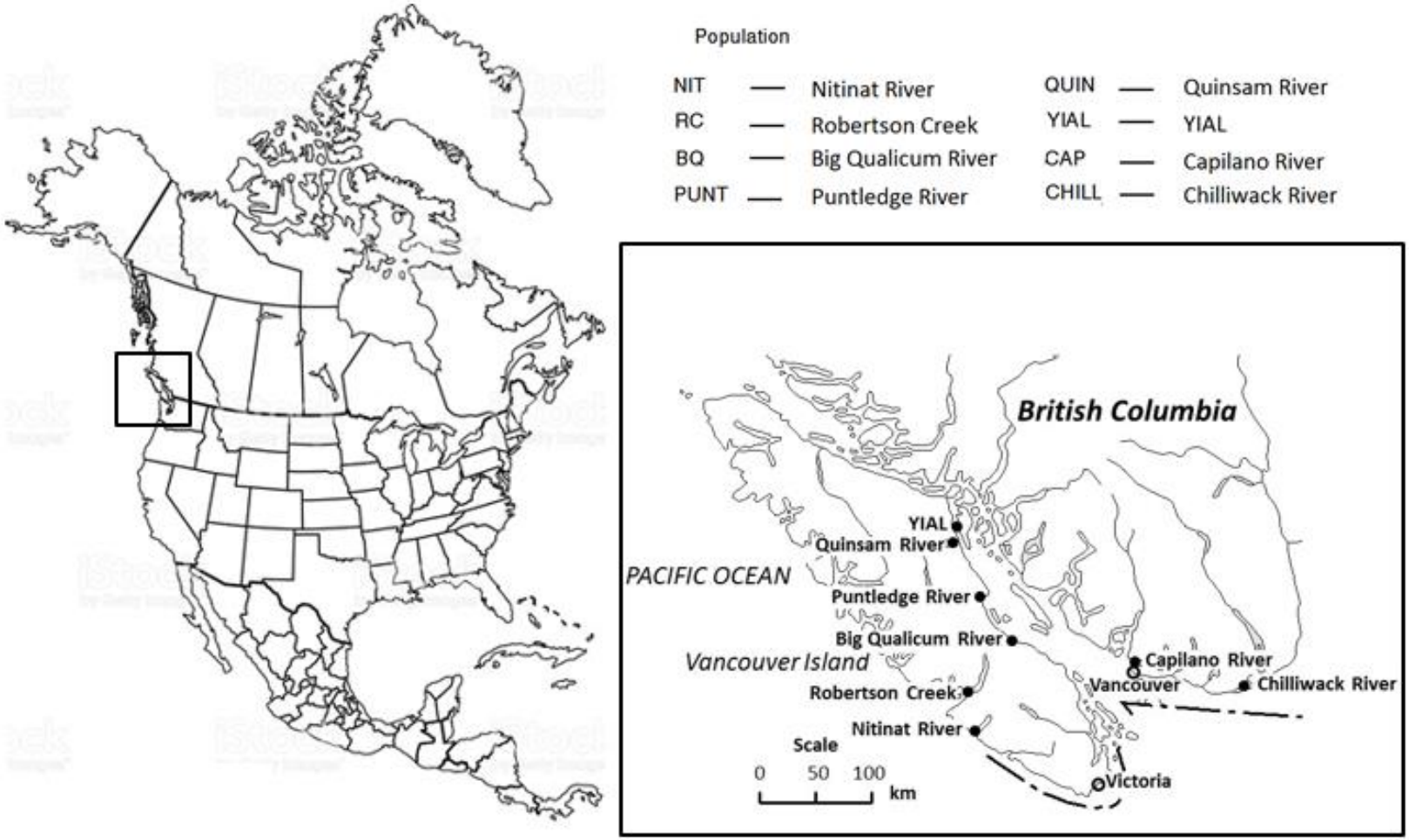

Figure 1: Map of southwestern British Columbia, showing the locations of the study populations.

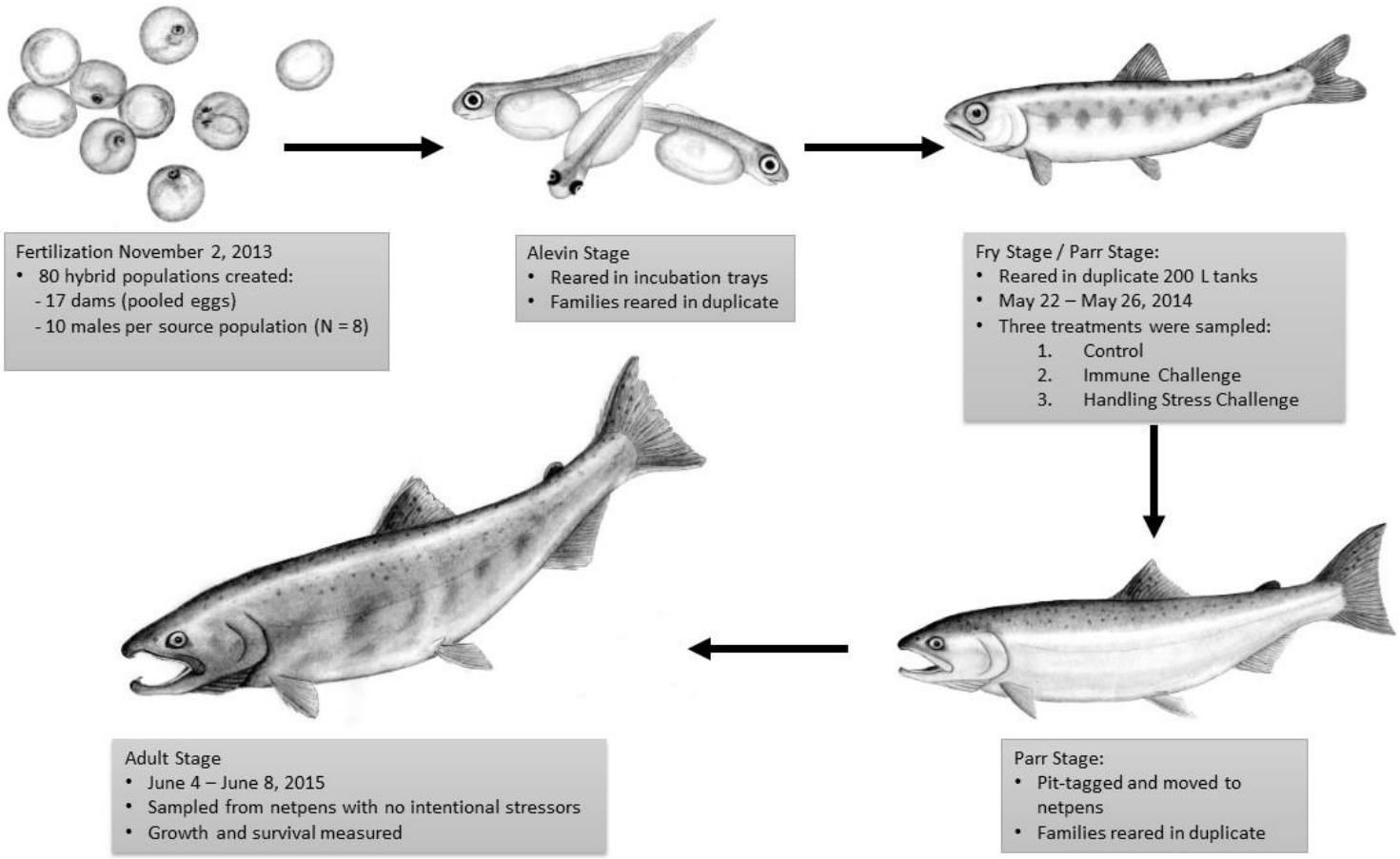

Figure 1.2: Schematic diagram of rearing and sampling design for Chapter 2 and Chapter 3.

CHAPTER II

VARIATION IN JUVENILE CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) TRANSCRIPTION AMONG AND WITHIN EIGHT POPULATION CROSSES FROM BRITISH COLUMBIA, CANADA

Introduction

Phenotypic differences among populations are common in nature, and have been reported in many taxa, from bacteria (Torsvik et al., 1990), plants (Bossdorf et al., 2005), daphnia (Wolf and Mort, 1986) to vertebrates, such as fish (Mittelbach et al., 1999) and other animals (Charmantier et al., 2008). Among-population phenotypic variation can be attributed to environmental effects, heritable genetic differences, and genetic-by-environment interactions, or a combination of these factors (Lande, 1975). Environmental effects on population-level phenotypic variation are common, and are most often investigated where anthropogenic impacts are known or suspected (Allendorf and Hard, 2009). Genetic effects contributing to among-population phenotypic differences are also widely reported (Armbruster and Schwaegerle, 1996), and the genetic divergence among populations may be due to genetic drift and/or natural selection (Lacy, 1987). Generally, genetic drift is most important for population divergence in small, or newly colonized, populations (Gratten et al., 2012; Greuber et al., 2013; Vitti et al., 2013). Natural selection, on the other hand, drives adaptive population divergence, and is generally thought to be more rapid and is the basis for local adaptation. Among-population phenotypic differences may thus reflect both ecological and evolutionary effects, and the systematic analysis of population phenotypic diversity can help define conservation priorities and improve our understanding of the ecological and evolutionary factors that contribute to biodiversity.

Phenotypic differences among populations are especially prevalent in salmonid species, where natal homing leads to reproductive isolation among populations that can result in divergence, due to both genetic drift and natural selection (Quinn et al., 2000). Much of the variation observed among salmonid populations, even within a few kilometers from one another, has been speculated or shown to be due to local adaptation (Taylor, 1991). Chinook salmon (*Oncorhynchus tshawytscha*), the largest of Pacific salmon species (Healey, 1991), are highly philopatric which may contribute to rapid evolution of locally adaptive traits due to population isolation and segregation (Quinn, 1993). Chinook salmon also exhibit remarkable among-population phenotypic variation in growth, behaviour, and life history, suggestive of adaptive response to selection and local adaptation (Cutts et al., 1998; Vasemägi et al., 2005). However, there is debate as to whether these differences in Chinook salmon populations are due to genetic drift (Quinn et al., 2000) or local adaptation (Adkison, 1995). One class of phenotypic variation that is at the base of most phenotypic differences among populations is gene transcription.

Gene transcription is the first step in gene expression and has been identified as a rate-determining step in the process of protein synthesis (Platt, 1986; Hedge and Kang, 2008). However, gene transcription is not the only factor affecting the regulation of functional proteins, for example, Calmodulin (*CAL*) is always present in the cell, but is only active when it binds with a calcium ion (Cheung, 1980). Therefore the number of transcripts in the cell is not directly related to the expression of the gene, but they do represent the potential maximum amount of protein that can be produced (McAdams and Arkin, 1997). Gene transcription has been used to show evidence for local adaptation among populations in many species, including Chinook salmon (Fraser et al., 2011).

Transcription can vary among individuals, families, and populations, generally depending on the candidate gene and the tissue being sampled (Livak and Schmittgen, 2001). There have been many studies that have examined gene transcription differences among salmonid populations (Domínguez-Cuevas et al., 2006; Wellband and Heath, 2013; Lamaze et al., 2014; He et al., 2015), and population-level variation in transcription has been proposed as a mechanism for local adaptation. For example, a study by Debes et al. (2012) examined *MHC* transcription across wild and domesticated Atlantic salmon (*Salmo salar*) populations. They found differences in the amount of *MHC* transcripts present between the two populations, which is suggesting that the transcription of *MHC* is locally-adapted in the wild and in farmed populations. Differences in gene transcription are often believed to be central to local adaptation as they are generally heritable and variable (Ferea et al., 1999) allowing for evolutionary responses. Wellband and Heath, (2013) partitioned transcriptional variation in Rainbow Trout (*Oncorhynchus mykiss*) and found patterns that supported natural selection processes over genetic drift in the majority of the genes they studied. However, Roberge et al., (2006) reported that population differences in gene transcription could also be due to genetic drift, rather than local adaptation. Roberge et al., (2006) found that genes not under direct selection were altering their transcription patterns in parallel with the loci under selection, suggestive of genetic drift. Examining gene transcription as a phenotype and partitioning phenotypic variance for transcription can help quantify the genetic basis for population-level variation in transcription. This is important for both successful commercial aquaculture breeding programs and conservation efforts, because transcription is the basis for phenotypic variation (Gibson and Weir, 2005) and we need to fully understand the nature

of population-level differences at the genetic level to be able to understand them at the phenotypic level.

Here I used Chinook salmon as a model system to explore gene transcription at 26 known-function gene loci, spanning immune, growth, metabolic, and stress-related functions. I selected this wide group of genes so that I could study the overall transcriptional profile of each fish at rest, and in response to challenges, rather than at the individual gene level. I used a hierarchical breeding design to test for population differences in gene transcription at the 26 selected loci across eight half-sibling populations. This design minimized environmental and maternal effects, and I was therefore able to partition the variance in gene expression into population and additive genetic (sire) effects. I analysed transcriptional profiles for juvenile (freshwater stage) Chinook salmon at rest and in response to two defined and ecologically relevant challenges; immune stimulation and handling stress. I expected to find population-level transcriptional differences at many of my fitness-related loci due to local selection pressures and genetic drift, but weak sire effects acting on gene transcription (and hence low heritability estimates) due to strong local selection pressures. While such a pattern of transcriptional variation would be consistent with local adaptation, additional work would be needed to confirm transcriptional local adaptation among my study populations. By studying fitness related genes at rest and in response to ecologically relevant challenges, we will be able to better understand what drives phenotypic differences among populations and whether it is likely due to genetic drift of local adaptation.

Materials and Methods

Breeding and Rearing

The salmon used in this study were juvenile half-siblings (approximately 6-7 months old from fertilization), reared from crosses between highly inbred females and males from one farmed (Yellow Island Aquaculture Ltd., YIAL) and seven wild populations taken from rivers around southwestern BC (Big Qualicum River, Capilano River, Chilliwack River, Nitinat River, Puntledge River, Quinsam River, and Robertson Creek; Figure 1.1). The eggs came from 17 females who were the offspring of self-fertilization of one functional hermaphrodite Chinook salmon. The hermaphrodite was produced through hormonal (alpha-methyl testosterone) manipulation of female embryos (Komsa, 2012). These females were thus highly inbred, with an average inbreeding coefficient (F) of 0.50. By combining the eggs of these females, I minimized possible maternal effects and variation due to dam component genetic variance. Thus variation among half-sib families within each population is primarily due to sire effect, or additive genetic variance. Within each population (seven wild and one domestic), sperm from ten males was used to create ten half-sib families. After fertilization, eggs from each cross were divided and incubated in two replicate cells. At the onset of exogenous feeding, fry from the two replicates in each family were combined, and 120 fry were haphazardly removed and transferred to each of two replicate rearing tanks (200 L). Fish were fed to satiation three times per day and the tanks were cleaned on a rotating schedule every 3-4 days. Due to logistical constraints, only seven of the ten families from each population were sampled for this study; the seven families were selected at random.

Sampling

Fry from all replicate tanks were sampled from May 22 to May 26, 2014, when the fish were 2.8g (±0.73 SE) in mean wet weight across all families and population crosses. Five fish were dip netted from each replicate tank and humanely euthanized immediately in an overdose solution of clove oil; these were the untreated controls. Care was taken to ensure that the control fish experienced no stress, to avoid possible gene transcriptional response. The sampled control fish were immediately weighed, dissected to expose the body cavity, and placed in a highly-concentrated salt buffer (ammonium sulfate, 1M sodium citrate, 0.5M EDTA, H_2SO_4 to bring the pH to 5.2) for preservation for later RNA extraction.

Two challenge treatments were used in this study; immune stimulus and handling stress. For the immune stimulation, five fish were dip netted from each tank and placed in a commercially available vaccine bath (prepared following manufacturer's protocol) for 60 seconds (Vibrogen 2: *Vibrio anguillarum-ordalii*; Novartis Animal Health Canada, Inc. Charlottetown, PEI). This vaccination method was selected based on work by Aykanat et al. (2012) that demonstrated a significant transcriptional response in the interleukin genes (*IL-1*, *IL-8*, *L-8R*, and *TNF*) in Chinook salmon fry 24 hours after a one minute Vibrogen bath. Following exposure, the fish were placed in cages (30cm sections of 15cm PVC pipes with netting on either end) and returned to the tank to recover for 24 hours. While in the recovery tanks, the fish were kept at a density of one fish per 0.94 L of water compared to one fish per 0.6 L in the rearing tanks at the time of sampling, minimizing the chance of eliciting a novel stress response related to crowding. While I cannot rule out the possibility of initial handing stress, or confinement stress contributing to the transcriptional response in these fish, the immune stimulus is likely the dominant

challenge driving transcriptional response at 24 hours post challenge. After 24 hours, the fish were euthanized in an overdose of clove oil, dissected to expose the body cavity, and preserved in a salt solution (as above). Lastly, the handling stress fish (5) were dip netted from each replicate tank and placed in a shallow pan lined with netting. The netting was lifted out of the water to expose the fish to the air (air emersion) for 30 seconds. Following the exposure, fish were placed into recovery cages (30cm sections of 15cm PVC pipe with netting on either end, at the same density as the immune stimulus fish) and returned to the tank for two hours. After the two hour recovery time, the fish were euthanized in an overdose solution of clove oil, dissected to expose body cavity, and preserved in a salt solution as above. All preserved fish were initially stored at -20°C for 3-7 days, then transferred to a -80°C freezer.

RNA Extraction

A total of 672 fish were used for gene transcription analysis; these comprised two fish per (replicate) tank, three treatments, seven families, and eight populations (2 fish X 2 tanks X 3 treatments X 7 families X 8 populations). RNA extraction from liver tissue (approximately 8.0 mg) was performed using Isol-RNA Lysis Reagent (5 Prime) following the manufacturer's protocol, and the extracted RNA was stored at -80°C. RNA quality was tested on a random subset of the samples both on the 2100 Bioanalyzer to determine the RNA Integrity Number (RIN) and on 2% agarose gels (Schroeder et al., 2006). All RIN values were between 6.4 and 7.7, indicative of high RNA quality and minimal degradation, while gel images showed the expected rRNA bands, indicative of RNA integrity. The RNA concentration for each sample was estimated by means of spectrophotometry on a NanoVue spectrophotometer (General Electric Company). The

concentrations ranged from 1000 ng/μL to 4000 ng/μL, all samples lower than 1000 ng/μL were re-extracted. If there was no liver tissue remaining for that sample, another individual from the same family was selected for analysis, as only two of the original five fish were initially used for extraction. High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Burlington, ON, Canada) were used to synthesize cDNA for all RNA samples, following the manufacturer's protocol. The amount of ddH_2O added to the cDNA was dependent on the concentration of mRNA that was calculated as above and was selected to achieve a uniform final concentration. cDNA samples were stored at -20°C until further analysis.

Primer and Probe Optimization

Two endogenous control genes (*B-actin* and *EF-1a*) were selected to normalise the expression profiles of the 26 candidate genes. These two genes were selected because previous work had demonstrated stable expression across Chinook salmon tissue and had been used as reference genes in previous studies (Lee, 2000; Olsvik et al., 2005). Each set of primers for the candidate genes were designed using Geneious Software v7.1.5 (http://www.geneious.com, Kearse et al., 2012) and optimized on DNA from Chinook salmon eyed eggs. After PCR optimization, the primers were tested on a subset of my cDNA samples with SyBr® Green Dye I (Thermo Fisher Scientific) following the manufacturer's protocol on the QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific). After testing positive for amplification of the expected sized fragment using SyBr® Green assays, quantitative real-time PCR (qRT-PCR) primers and Taqman® probes were developed using Primer Express® Software v3.0.1 (Thermo Fisher

Scientific) for all 26 candidate genes and the two endogenous controls. The qRT-PCR primers were developed around intron-exon boundaries, to reduce the chance of replicating DNA or pseudogene RNA (Ye et al., 2012) and for a low amplicon length (50-100bp). The Taqman® probe was designed for a melting temperature between 58-60°C.

Quantitative Real-Time PCR

TaqMan® OpenArray® chips from Applied Biosystems (Burlington, ON, Canada) were used to quantify transcription on a QuantStudio 12K Flex Real-Time PCR System following the manufacturer's protocol. Each chip contained 64 through-holes in a 56x48 format, thus 48 cDNA samples were run in duplicate for each of the 28 genes (26 candidate and 2 endogenous controls) on each chip. A 5μL solution of: cDNA (1.2μL per sample), ddH_2O (1.3μL), and 2.5μL of TaqMan® OpenArray® Real-Time PCR Master Mix (Applied Biosystems, Burlington, ON, Canada) was prepared, distributed across a 384-well plate and then loaded onto the TaqMan® OpenArray® chips using the OpenArray® AccuFill System to reduce inter-assay variation. The through-holes on the chips were pre-loaded with the primer and probe sequences for each of the 28 genes by the manufacturer. A total of 14 chips were used for 672 cDNA samples (2 fish X 2 tanks X 3 treatments X 7 families X 8 populations).

Expression Analysis

ExpressionSuite Software v1.0.3 (Applied Biosystems, Burlington, ON, Canada) was used to calculate raw critical threshold (C_T) and ΔC_T (C_T values normalized to endogenous controls) for each gene. Only one of the two endogenous control genes (*EF-*

1a) was used to normalize my transcription data due to high variation in the *B-actin* gene transcription. ExpressionSuite Software calculates variation in C_T values across all samples and ranks genes based on their variation: *EF-1a* had a variation score of 3.7, whereas *B-actin* had a much higher variation score of 13.8. The average ΔC_T value for each candidate gene across all populations can be seen in Figure 2.1.

I used $\Delta\Delta C_T$ values to quantify gene transcription response to a challenge. $\Delta\Delta C_T$ was calculated by subtracting the average ΔC_T for the control fish in each family from the ΔC_T values from each challenged fish in that respective family (Livak and Schmittgen, 2001). I calculated $\Delta\Delta C_T$ for only those genes that showed a significant treatment effect. I did not analyse treatment response transcription data for genes that did not show a significant treatment effect to avoid analysing a "response" when there is no evidence for a treatment effect.

Statistical Analysis

Statistical analyses were performed using JMP Statistical Software v12.0.1 and R Statistical Software v3.2.5. All False Discovery Rate (FDR) significance corrections were completed in R.

Treatment response for each of the 26 candidate genes was tested to determine which genes demonstrated a difference in transcription in response to either of the environmental challenges (i.e. immune stimulus and handling stress). General linear models (GLMs) were performed using the lme4 package in R, with main effects for Population, Treatment, and Population-by-Treatment interaction that were fitted to the dependent variable (gene transcription, ΔC_T). Fish weight and replicate tank were added as covariates and the analyses were performed for each candidate gene separately within

each treatment group, comparing the immune or stress transcription response to the control transcription. A False Discovery Rate (FDR) correction was done for each treatment to reduce Type I errors. This analysis allowed me to identify genes that exhibited a significant transcriptional response to the treatments. Only those genes that exhibited a significant response to the challenges were used to calculate $\Delta\Delta C_T$ and were further analysed for population and sire level effects. I identified a gene as exhibiting a significant transcriptional response to a challenge if treatment or population-by-treatment factors were significant at the 0.05 alpha level post-FDR correction.

Population and sire effects for each gene were tested for by including tank nested within sire, sire nested within population, and population in a GLM within JMP Statistical Software, with weight as a covariate. Dependant variables included ΔC_T for all genes in the control fish and $\Delta\Delta C_T$ for all the genes that showed a significant treatment response for both treatments. FDR corrections were implemented within each treatment (and control) to reduce Type I errors. A Tukey HSD analysis was used to determine which populations exhibited significantly different at-rest or challenge transcriptional response from one another.

Narrow-sense heritability (h^2) was calculated by partitioning the transcriptional variance of the ΔC_T for the control fish and the $\Delta\Delta C_T$ of the challenged fish (only for the genes with a significant treatment effect) into: tank (nested within sire), sire, and weight (as a random effect) within each population (performed in JMP Statistical Software). This allowed me to quantify the relative contributions of the sire effects within each population for each gene at rest and in response to a challenge. Additive genetic variance (V_A) was estimated by multiplying the sire variance component by four and heritability

was estimated by dividing the V_A by the total phenotypic variance (Falconer & Mackay, 1996).

Results

Treatment Effects

The immune stimulus had a significant treatment effect on the transcriptional response of 11 of the 26 candidate genes, whereas the stress challenge had a significant effect on the transcriptional response of two of the 26 genes (Appendix B1). Interestingly, the immune stimulus had no effect on the stress genes, but did have a significant effect on at least two genes in the other three functional groupings. The largest impact of the immune stimulus was on the immune-related and growth-related genes, where five of eight immune genes and four of eight growth genes showed a significant response, whereas only two of the six metabolic genes demonstrated a significant change. The handling stress only elicited a significant transcriptional response in one immune gene (*IL*-8) and one stress gene (*hsp90a*). Of the genes that demonstrated a significant treatment effect in response to the immune stimulus, three were up-regulated (*IL-1B*, *LEPTIN*, and *PK*) and the rest were down-regulated (Figure 2.2). The genes that were significantly different in transcription in response to the handling stress treatment were both down-regulated (Figure 2.3). Overall, the immune stimulus had a greater effect on gene transcription than the handling stress, and I found very little evidence for population-by-treatment effects (Appendix B1).

Population and Sire Effects

Population and sire effects were tested to determine whether the differences observed among individual transcription was due to their population of origin or heritable influences from their sire. To determine the degree of population and sire effects on gene transcription, I also included tank (nested within sire) and weight (as a random variable) to correct for variation from other sources. The effects of body weight were more substantial than tank effects, and were observed in both treatment groups and in the control fish (Table 2.1). At rest, *MHBIIB*, *NKEF*, *IGFBP2b*, *THR-b*, *CYP1a*, and *metA* all exhibited significant effects of fish weight on gene transcription (Table 2.1). I found *EGR-1*, *IGF-1*, *LEPTIN*, and *CYP1a* experienced significant effects of fish weight on transcriptional response to the immune stimulus, while there were no significant effects on the fish subjected to a handling stress (Table 2.1). The tank effect on gene transcription was only observed for five genes; *CK-1* and *IL-8* at rest, and *MHCIIB*, *CYP1a*, and *PK* in response to the immune stimulus. No stress challenge genes exhibited a significant tank effect.

There were significant population effects on gene transcription across all three treatments, after FDR corrections (Table 2.1). The control group fish demonstrated a significant population effect at nine of the 26 candidate genes (Figure 2.4) including loci from all functional gene groups (i.e. immune, growth, metabolic, and stress). Only one growth gene (*GH-R*), two metabolic genes (*CYP1a* and *FAS*), and one stress gene (*hsp70*) exhibited significant population effects for resting transcription, whereas five of the eight immune genes had a significant population effect (Table 2.1). Of the 11 genes that showed a significant treatment effect in the immune stimulus, five exhibited a population effect on the transcriptional response (Figure 2.5); two immune genes (*CAL* and *NKEF*),

two growth genes (*GH-R*, and *IGF-1*), and one metabolic gene (*CYP1a*). Only one of the two genes that had a significant treatment response to the handling stress challenge (*hsp90a*, a stress gene) exhibited a significant population effect (Table 2.1).

In this analysis, family, or sire, effects reflect additive genetic variance averaged across all populations. Significant sire effects on gene transcription were seen in seven of the 26 candidate genes at rest (Table 2.1). However, within those genes, only three of the four functional gene categories demonstrated a significant sire effect; three immune genes (*CAL*, *IL-8*, *SAA*), two growth genes (*GH-R* and *THR-B*), and two metabolic genes (*CYP1a* and *FAS*) (Table 2.1). The immune stimulus fish experienced a significant sire effect for only four of the 12 genes tested (Table 2.1), these included three of the four functional groups; two immune genes (*CAL* and *NKEF*), one growth gene (*EGR-1*), and one metabolic gene (*CYP1a*). For the stress-treatment fish, only the stress gene, *hsp90a*, exhibited a significant sire effect.

Heritability

The average population narrow-sense heritability (h^2) across all genes and for all treatments combined, ranged from $h^2 = 0.12$ for Chilliwack River to $h^2 = 0.38$ for Nitinat River, with the domestic stock, YIAL, falling in the middle ($h^2 = 0.28$) (Appendix B2). The average heritability of gene transcription in response to a handling stress challenge ($h^2 = 0.22$) was lower that of the fish at rest ($h^2 = 0.26$) and the fish responding to an immune stimulus ($h^2 = 0.33$). Overall, the immune genes had relatively low h^2 for all three treatment groups; control ($h^2 = 0.23$), immune stimulus ($h^2 = 0.25$), and handling stress ($h^2 = 0.04$). The metabolic function genes generally had higher h^2; control ($h^2 = 0.38$) and immune stimulus ($h^2 = 0.38$), handling stress had no significant treatment effect

on the transcription of metabolic genes so they were not included in this analysis (Appendix B2). When examining individual genes, the metabolic gene, *FAS*, followed by the metabolic gene, *CYP1a*, had the highest h^2 across all populations at rest (Figure 2.6). Whereas for the treatment groups, the growth gene, *IGF-1* in response to the immune stimulus had the highest average heritability ($h^2 = 0.52$) across all populations (Figure 2.7).

Discussion

Population level phenotypic variation is important as it reflects local adaptive potential, which can lead to population divergence and ultimately, species evolution (Grueber et al., 2013). While gene transcription differences at the population level can be due to either genetic or environmental factors (Zaidi et al., 2004; Amaral et al., 2008; Hedge and Kang, 2008; Martinez and Walhout, 2009), my experimental design allowed me to minimize and partition environmental and/or maternal effects on transcriptional variation. Thus, the transcription differences I report are likely due to genetic differences among populations. However, among-population genetic differences may be the result of genetic drift and/or natural selection (Gratten et al., 2012). Genetic drift is random allele frequency change usually associated with small population size, and is thought to have little effect on functional loci that are expected to be under strong selection, unless the population has experienced severe and recurrent bottlenecks or recent founder effects (Vitti et al., 2013). My experiment included only genes with functions known to contribute to organism survival and reproduction and thus, while I cannot rule out genetic drift as a potential contributor to my observed among-population variation, it is unlikely. Furthermore, my estimates of the within-population additive genetic variance of gene

transcription indicate that the transcriptional profiles among the source populations include heritable variation. Given that the population-level transcriptional variation I observed is heritable, it likely reflects local adaptation among the sampled populations (He et al., 2015).

If the among-population transcriptional variation I observed is due to local adaptation, then I would predict that immune function transcriptional response would show a spatial pattern, as pathogen communities tend to be spatially clustered (Murray et al., 1995). I considered three spatial clusters among my river populations; the west coast of Vancouver Island (Robertson Creek and Nitinat River), the east coast of Vancouver Island (Big Qualicum River, Puntledge River, Quinsam River, and YIAL), and the mainland populations (Capilano River and Chilliwack River). When I examined my immune genes at rest and in response to the immune stimulus, most genes demonstrated some level of spatial similarity (Figures 2.4 and 2.5). There were no immune genes that exhibited significantly different transcription patterns at the population-level for the handling stress challenge, therefore that treatment was not considered. Though the spatial clusterings at the population level based on the Tukey results demonstrate weak clustering, it is important to note that these populations were reared form the same mothers and in the same environments. Therefore the spatial clustering I do witness, is only from the sire effect on gene transcription. My spatial similarity in the transcriptional patterns of the immune genes at rest and in response to the immune stimulus are supported by work from Kent, (2011) which determined that individual populations of sockeye salmon (*Oncorhynchus nerka*) had adapted to immunological stressors in their own environments. The concept of the salmonid immune system as a very locally adapted

trait with environmental dependent effects is gaining wider acceptance (Evans et al., 2010). Similar to the spatial patterns in the immune-related genes, I also found some evidence for spatial clustering in my selected metabolic-related genes; however, the growth and stress-related genes showed no spatial pattern in their transcription patterns.

The metabolic genes, *CYP1a* and *FAS*, at rest demonstrated spatial clustering, but not between the three defined geographical clusters, but between the more northern populations (Quinsam River, Puntledge River, Big Qualicum River, and YIAL) and the more southern populations (Robertson Creek, Nitinat River, Capilano River, and Chilliwack River) (Figure 2.4). However these spatially-clustered metabolic function genes did not exhibit any evidence for spatial clustering in response to the immune stimulus. The one stress gene, *hsp90a,* that demonstrated a handling stress treatment effect, showed no evidence for spatial clustering for any of the populations (Figure 2.5). Overall, the gene transcription at rest showed the highest level of spatial clustering in regards to transcriptional patterns, and the immune genes had the most apparent population divergence patterns. Growth, metabolic, and stress genes play a smaller role in natural selection than immune function in natural populations (Eizaguirre et al., 2009), therefore any spatial clustering witnessed in those genes may be due to "soft" selection, rather than direct survival based selection.

My ad hoc inspection of the patterns of transcription among the sampled population leads me to the speculation that the eight populations may have experienced local selection associated with pathogen community differences. If this is correct, I would expect the overall pattern of gene transcription to reflect spatial distribution of the sampled population. To test this, I performed a Principal Coordinate Analysis (PCoA)

across all genes within each functional category and for each treatment separately, generating 12 PCoAs (i.e. four gene groups by three treatments). This allowed me the ability to visualize any population clusters for each of the four gene categories. The PCoA results demonstrated strong evidence for spatial clustering in the immune and metabolic-related genes at rest (Figures 9A and 9B). In both gene groups (immune and metabolic) there is a clustering of the east Vancouver Island populations. However, only three of the four east Vancouver Island populations (Quinsam River, Big Qualicum River, and Puntledge River) clustered together for the immune genes (Figure 2.8 A). YIAL, the domestic stock, was the only east Vancouver Island population to not group with the others, which is not surprising since these fish should not similar to the other east Vancouver Island populations. The west Vancouver Island populations, on the other hand, demonstrated strong spatial clustering for the metabolic genes at rest (Figure 2.8 B), and weak spatial clustering for the immune genes at rest (Figure 2.8 A). The only geographic group to not show strong spatial clustering in gene transcription were the mainland populations (Capilano River and Chilliwack River) and this could be due to the high urbanization at Chilliwack River when compared to Capilano River. Therefore these two mainland rivers may be geographically close to one another, but salmon migrating through the rivers experience different environments, leading to differing abilities for local adaptation. This pattern of transcriptional profiles is highly suggestive of locally adaptive differences, as genetic drift would be expected to generate spatially unrelated divergence. Generally, spatial patterns are more obvious for at-rest transcriptional profiles, and thus transcription at the resting state may reflect past divergent selection pressures, while transcriptional response to acute challenges may have evolved across all

of the study populations similarly. Due to their anadromous life history and wide distributional range, Chinook salmon are expected to exhibit high levels of local adaptation to their freshwater habitats (Taylor, 1991; Quinn et al., 2000; Fraser et al., 2011). It is therefore perhaps not surprising that I observed such pronounced population differences in fitness-related gene transcription at rest.

My study population crosses were generated by fertilizing highly-inbred (hermaphrodite self-crossed) eggs with sires from eight different populations (seven wild and one domestic). Thus the transcriptional differences I detected among "populations" reflect only sire contributions to population-level variation. Had I used crosses where the dams were also from the study populations, the among-population transcriptional differences would be expected to be even greater than what I observed due to dam effects (maternal and dam additive genetic effects). Thus my breeding design results in a conservative test of population-level transcription divergence. My one pure cross population, YIAL, did not differ dramatically from the other crosses, indicating that it is unlikely my crosses experienced large outbreeding depression, nor heterosis.

My breeding design allowed the calculation of sire effects within each population cross to determine an estimate of additive genetic variance (and thus heritability) underlying the population-level variation in gene transcription at rest and in response to a challenge. Evidence for local adaptation within a population can be found by examining variation in heritability, since Fisher (1930) proposed that low levels of heritability are a result of high selection pressures (i.e. local adaptation). Therefore I expected to see low heritability in gene transcription of my candidate genes if the populations had undergone strong selection associated with local adaptation (e.g., Mousseau & Roff, 1987),

especially in the immune-related genes, due to pathogen-driven "hard" selection (Murray et al., 1995). When examining candidate genes across all three treatments, the average h^2 for immune stimulus transcription response was the lowest, perhaps indicative of high selection pressure on the immune response within my Chinook salmon study populations. This is also consistent with previous work that has shown that pathogen communities can drive strong selection within a species (Murray et al., 1995; Miller et al., 1997). Overall, metabolic gene transcription had the highest h^2 values, suggesting that selection pressure on metabolic function in salmonids is not as great as for immune, growth, and stress-related genes. However, high variation in transcriptional h^2 was observed among all genes, across all populations, perhaps reflecting variation in the strength of selection acting at specific gene loci among populations. For example, the immune gene *CAL* had high h^2 in the Puntledge River (0.87) and Robertson Creek (0.86) crosses, but low h^2 in the Quinsam River (0.00) and Nitinat River (0.00) crosses.

While many studies have reported transcriptional profile comparisons among populations across diverse taxa (Wolf and Mort, 1986; Torsvik et al., 1990; Bossdorf et al., 2005; Charmantier et al., 2008), few have explored the genetic basis of this variation using quantitative genetic breeding designs. Here I used a unique breeding design that combined highly inbred females with sires from one domestic and seven wild populations to explore the nature of transcriptional variation among and within populations of Chinook salmon. I found patterns of response consistent with local adaptation at selected fitness-related genes, both at rest and in response to ecologically relevant challenges. I also found high levels of additive genetic variance within the eight populations for selected genes, suggesting high heritability of transcriptional profiles, at least within

these Chinook salmon crosses. However, the magnitude of the heritability estimates varied substantially among the study populations for individual gene transcription, highlighting the need to include population of origin as a factor in all evolutionary or ecological gene transcription studies (He et al., 2016). My study contributes to our understanding of the role of among-population transcriptional variation and local adaptation in salmonids using Chinook salmon as a model. My use of fitness-related gene transcription profiling is also valuable for both salmon aquaculture and hatchery rearing for management/conservation applications.

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Table 2.1: Population, sire, tank, and weight effects for each gene at rest across all 8 populations were tested running a General Linear Mixed Model (GLMM) with tank, nested within sire, nested within population as fixed effects and weight added as a random effect. The independent variable tested was the resting ΔC_T of each gene. The values given are the raw p-values from the GLMM but the significance is based on the post-FDR corrections, where *** p<0.0001, ** p<0.001, * p<0.05.

Gene	Treatment Response	Population Effect	Sire Effect	Tank Effect	Weight Effect
CAL	Control	**<0.0001 ****	**<0.0001 ****	0.14	0.30
CK-1	Control	**0.0002 ****	0.19	**0.0064 ***	0.95
IL-1B	Control	0.41	0.22	0.073	0.95
IL-8	Control	**0.0045 ***	**<0.0001 ****	**<0.0001 ****	0.014
MHCIIB	Control	**0.0039 ***	0.21	0.12	**0.0004 ****
NKEF	Control	**0.0018 ***	0.029	0.13	**0.0049 ***
SAA	Control	0.12	**0.0035 ***	0.028	0.017
TNF-a	Control	0.14	0.29	0.038	1.00
EGR-1	Control	0.017	0.023	0.059	0.070
GH-R	Control	**0.0038 ***	**0.0005 ****	0.052	0.53
IGF-1	Control	0.95	0.024	0.22	0.063
IGFBP2b	Control	0.059	0.058	0.30	**0.0013 ****
LEPTIN	Control	0.022	0.080	0.28	1.00
MYO1a	Control	0.059	0.033	0.75	0.037
p53	Control	0.35	0.14	0.46	0.14
THR-b	Control	0.12	**0.011 ***	0.69	**<0.0001 ****
COI	Control	0.075	0.022	0.021	0.19
CPT1	Control	0.025	0.075	0.74	0.32
CYP1a	Control	**<0.0001 ****	**<0.0001 ****	0.017	**0.0017 ***
FAS	Control	**<0.0001 ****	**<0.0001 ****	0.18	0.088
PEPCK	Control	0.48	0.084	0.14	0.65
PK	Control	0.50	0.047	0.015	0.50
GR2	Control	0.70	0.73	0.31	0.93

hsp70	Control	**0.0079 ***	0.16	0.12	0.28
hsp90A	Control	0.49	0.28	0.89	0.76
metA	Control	0.075	0.045	0.034	**0.0007 ****
CAL	Immune	**<0.0001 *****	**<0.0001 *****	0.15	0.12
CK-1	Immune	0.097	0.12	0.073	0.091
IL-1B	Immune	1	0.02	0.37	0.91
MHCIIB	Immune	0.79	0.035	**0.0002 *****	0.45
NKEF	Immune	**0.022 ***	**0.025 ***	0.074	0.52
EGR-1	Immune	0.22	**0.018 ***	0.65	**0.0011 ****
GH-R	Immune	**0.018 ***	0.32	0.28	0.47
IGF-1	Immune	**0.021 ***	0.48	0.40	**<0.0001 *****
LEPTIN	Immune	0.26	0.43	0.54	**0.011 ***
p53	Immune	1.0	0.090	0.020	1.0
CYP1a	Immune	**<0.0001 *****	**<0.0001 *****	**0.0005 ****	**<0.0001 *****
PK	Immune	0.50	0.047	**0.015 ***	0.50
IL-8	Stress	0.44	0.043	0.069	0.18
hsp90a	Stress	**<0.0001 *****	**0.0017 ***	0.13	0.23

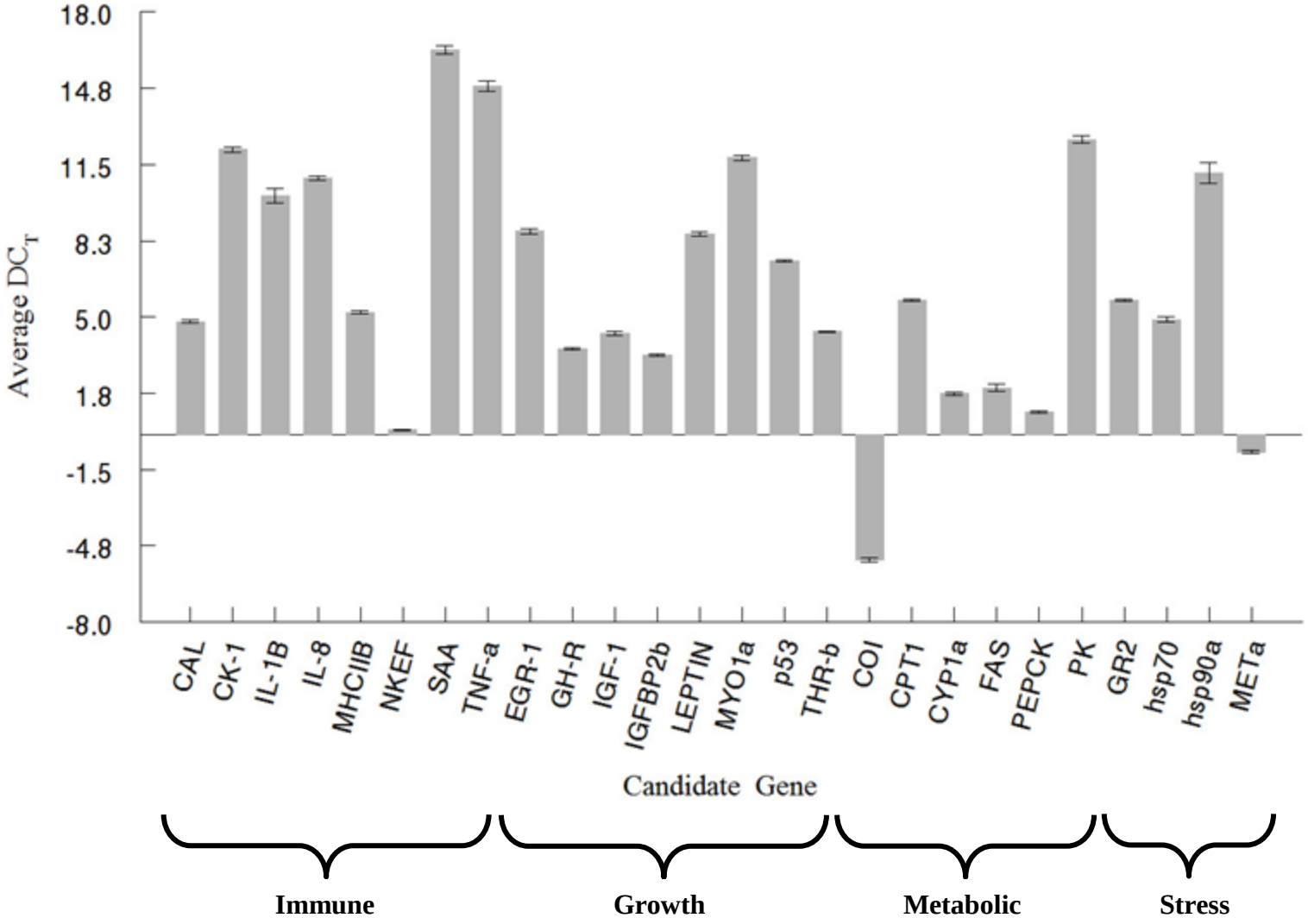

Figure 2.1: Average control ΔC_T for each candidate gene across all 8 populations. The average ΔC_T for each family (within each population) was used in the calculation of the $\Delta\Delta C_T$ fold change in response to a treatment in Figures 3 and 4.

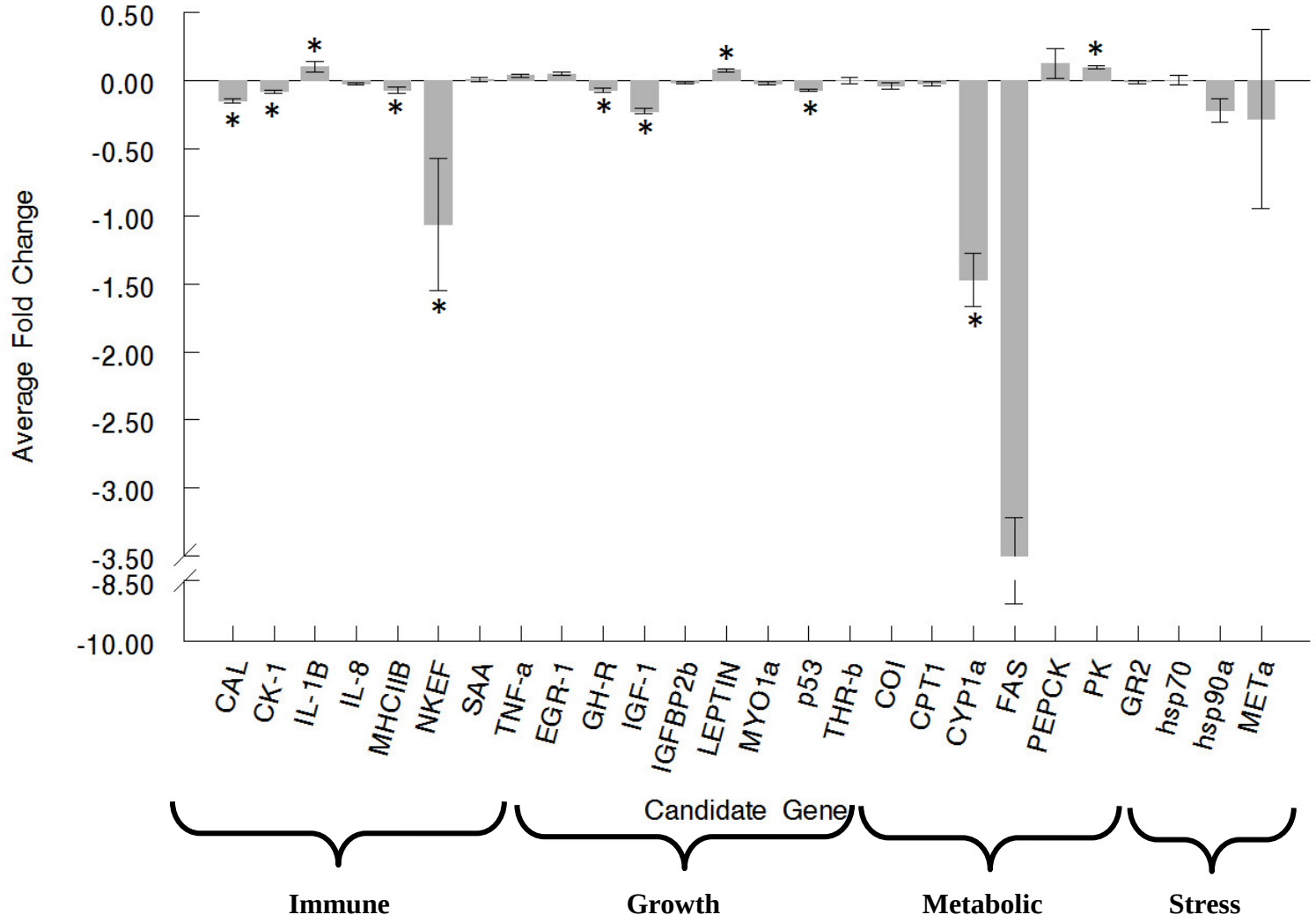

Figure 2.2: Average transcriptional response (fold change; $2^{-\Delta\Delta Ct}$) to the immune stimulus averaged across all 8 populations. Genes that demonstrated a significantly different ($p < 0.05$) treatment response in transcription are denoted with an asterisk.

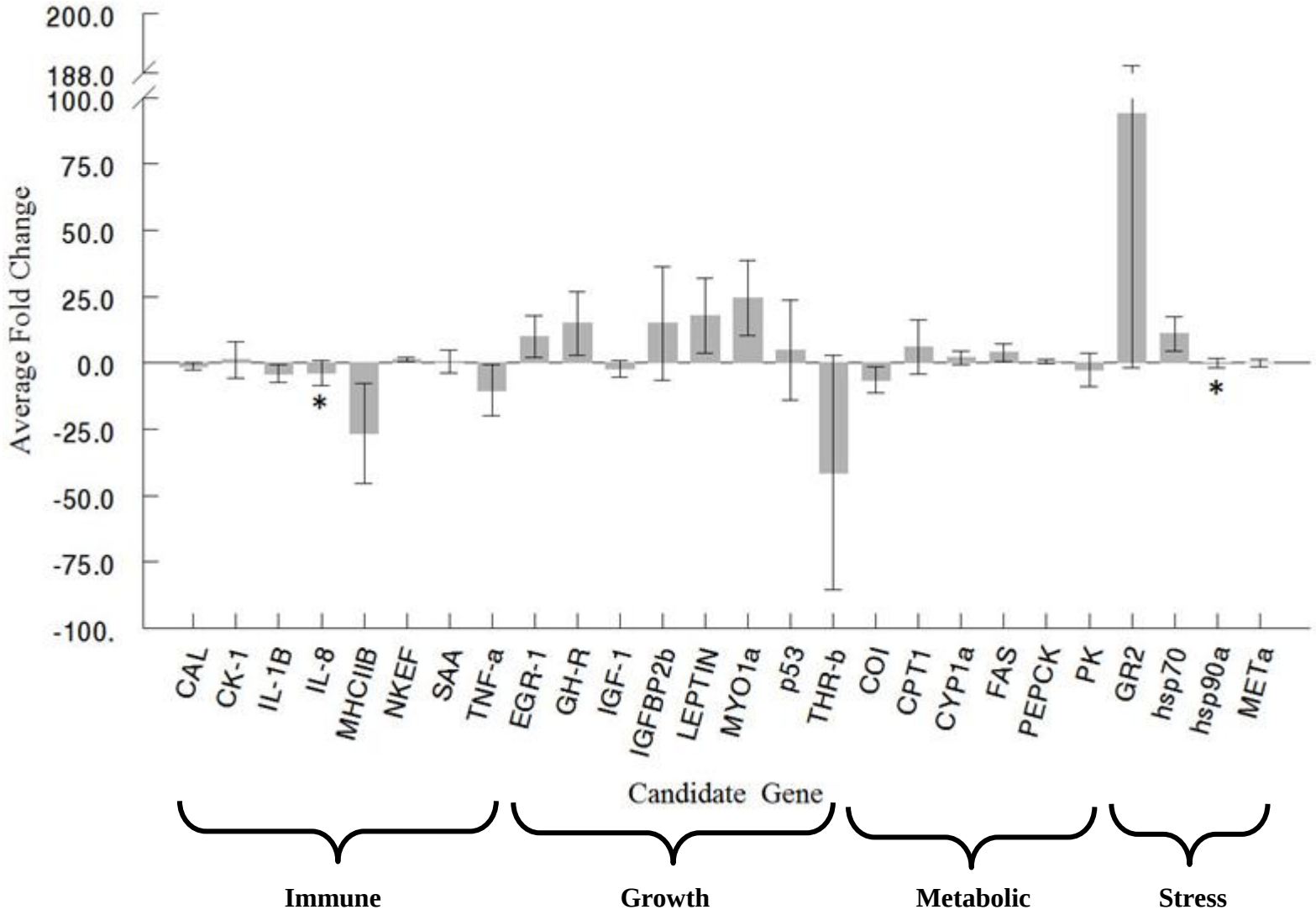

Figure 2.3: Average transcriptional response (fold change; $2^{-\Delta\Delta Ct}$) to the handling stress challenge averaged across all 8 populations. Genes that demonstrated a significantly different ($p < 0.05$) treatment response in transcription are denoted with an asterisk.

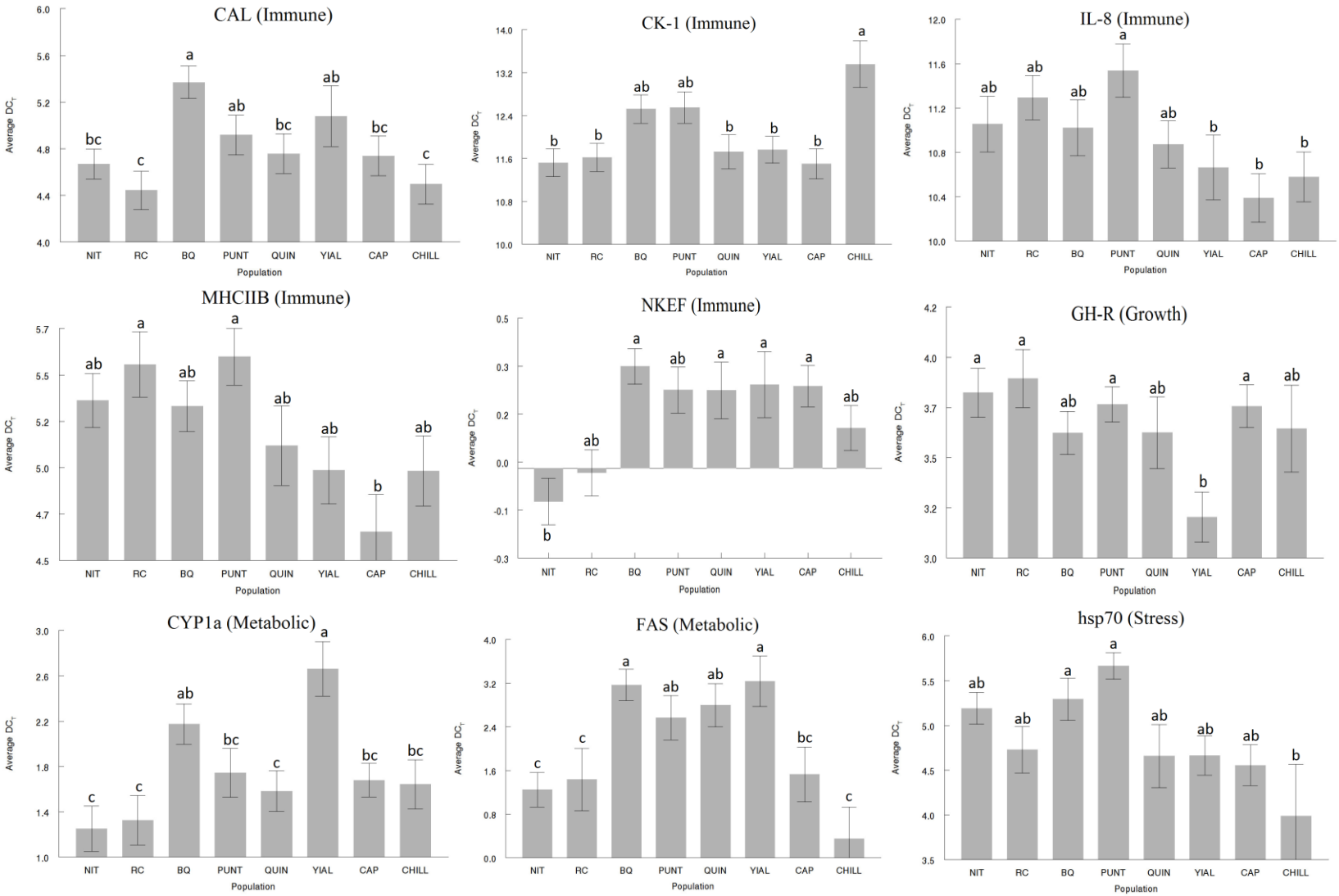

Figure 2.4: Bar graph showing mean transcription (± 1 SE) by population for each candidate gene at rest that exhibited a significant population effect, post-FDR Correction. The letters above each bar show population differences in transcription

based on a post-hoc Tukey's test. The putative function for each candidate gene is in brackets. Population abbreviations as in Figure 1.1.

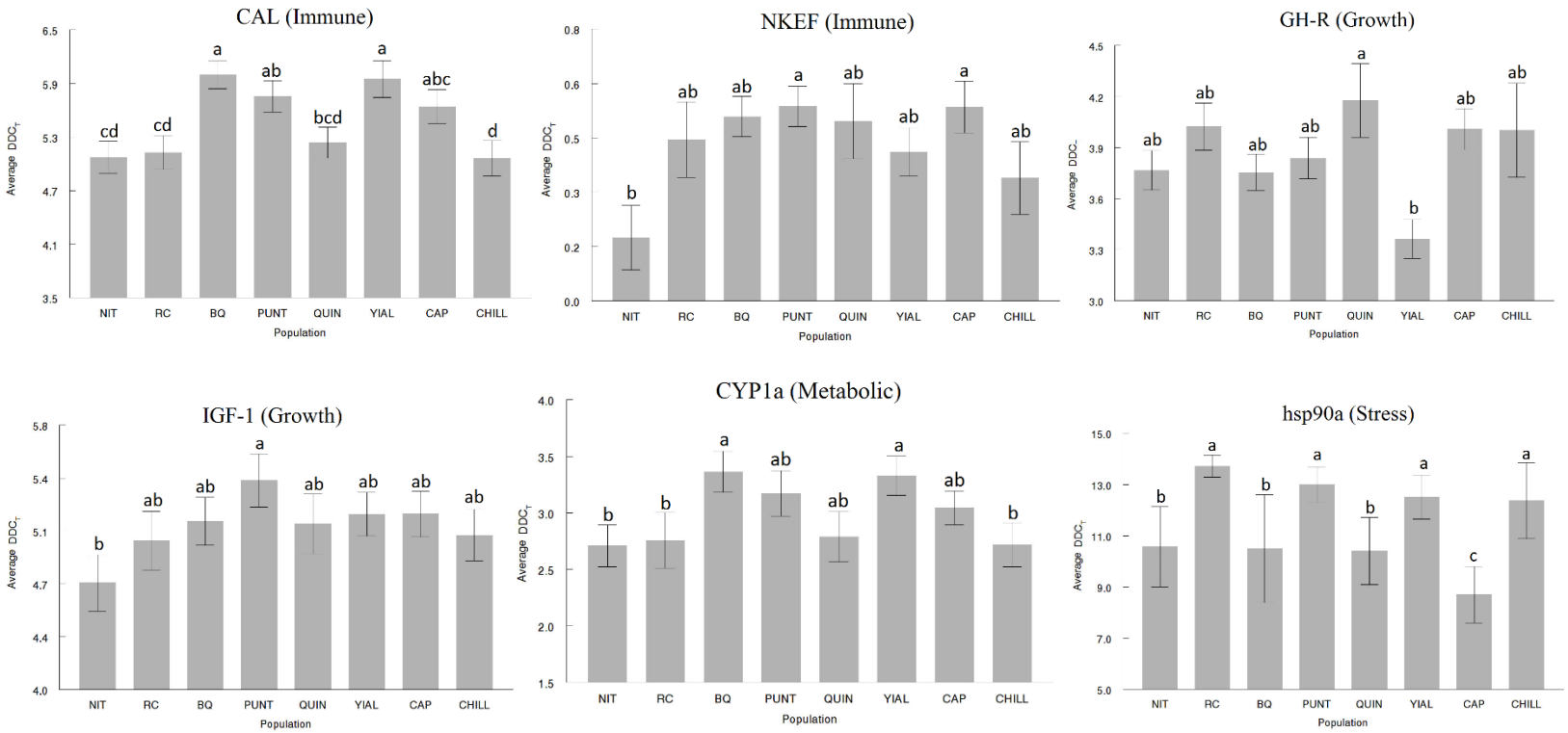

Figure 2.5: Bar graph showing mean transcription (± 1 SE) by population for each candidate gene that was significantly different in response to a challenge and that also exhibited a significant population effect, post-FDR Correction. The letters above each bar show population differences in transcription based on a post-hoc Tukey's test. Putative function is in brackets for each candidate gene and the population abbreviations are based on Figure 1.1. The significant genes in response to the immune stimulus were *CAL*, *NKEF*, *GH-R*, *IGF-1*, and *CYP1a* and only *hsp90a* was significantly different at the population level for the handling stress challenge.

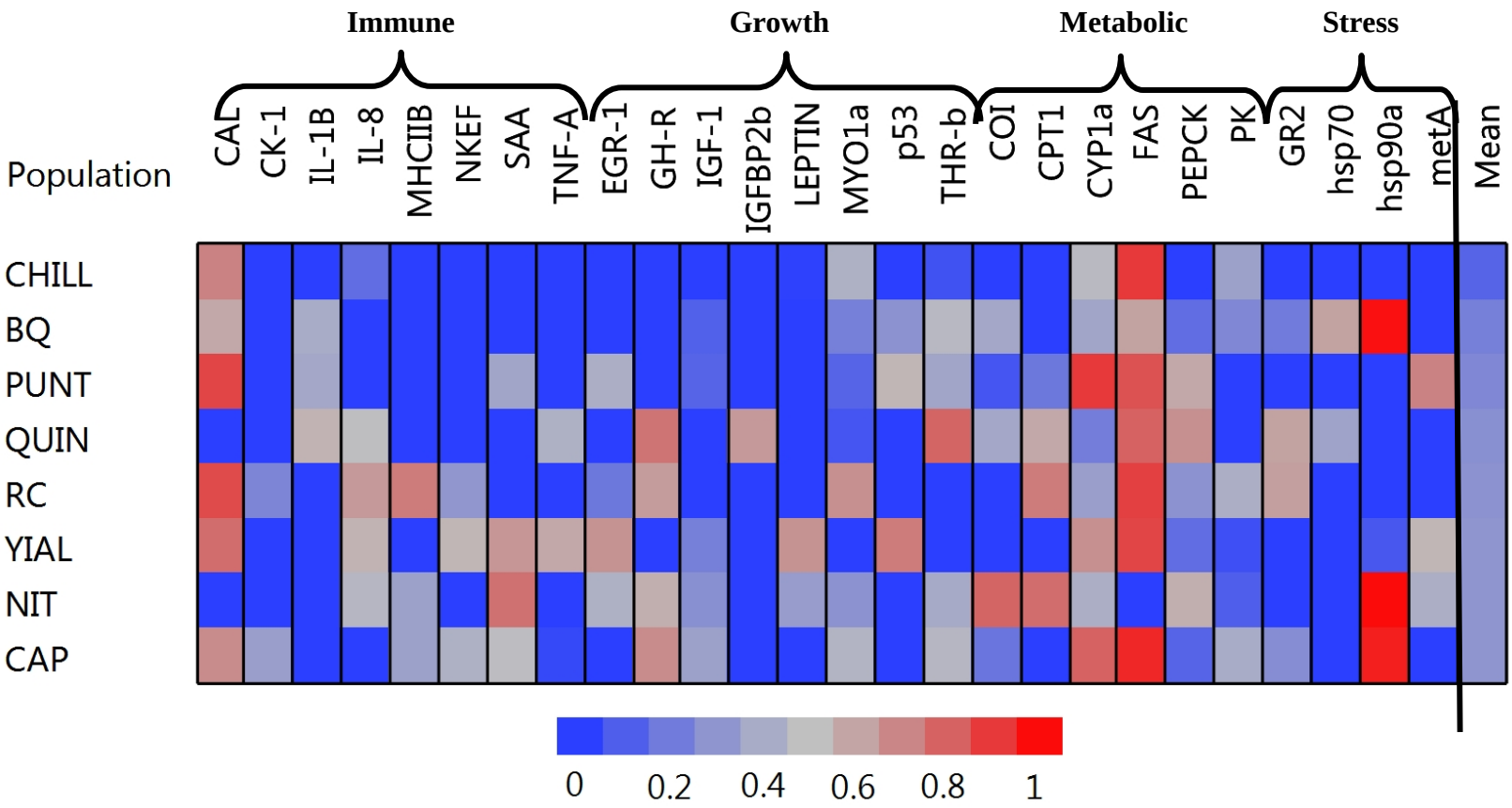

Figure 2.6: Heatplot of the at-rest (control) mean narrow-sense heritability (h^2) values for each candidate gene for each population. The populations are listed in order of lowest average h^2 (Chilliwack River) to the highest average h^2 (Capilano River), with the mean population h^2 across all candidate genes shown at the right. The genes are listed in alphabetical order based on functional group.

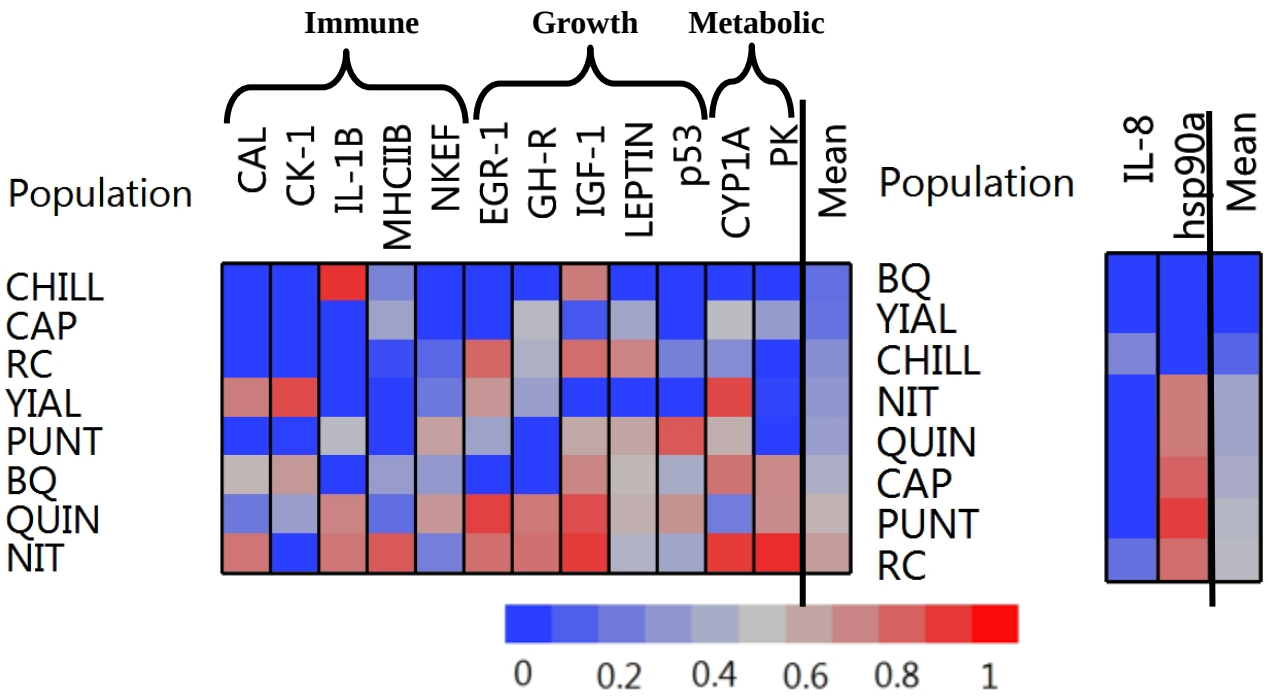

Figure 2.7: Heatplot of the average immune response narrow-sense heritability (h^2) (left) and handling stress response h^2 (right) for each population. the populations are listed in each plot in order from the lowest average h^2 for that treatment, to the largest, with the mean h2 across all candidate genes of each population on the right. The handling stress response was only significant for two candidate genes, *IL-8*, an immune gene, and *hsp90a*, a stress gene.

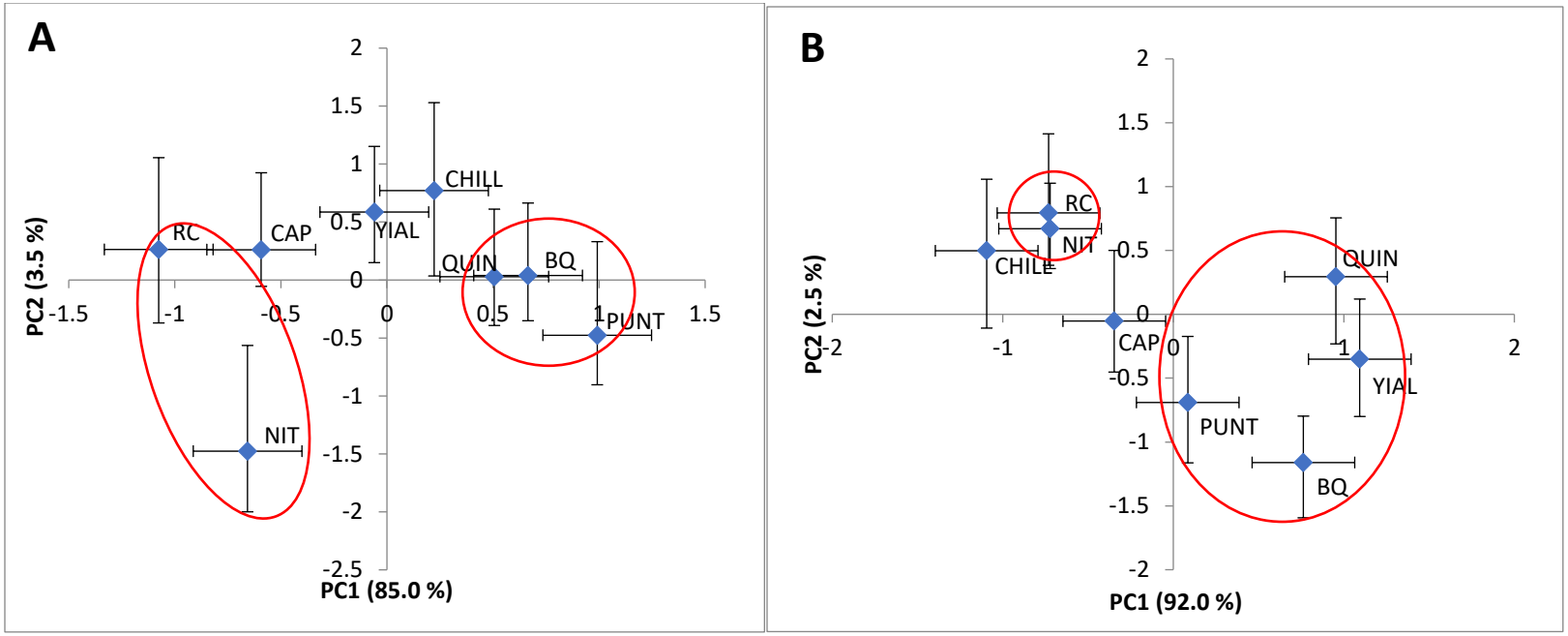

Figure 2.8: Scatter plot of mean Principal Coordinates (± 1 SE) for immune gene (A) and metabolic genes (B) ΔC_T for each population at rest to test for evidence of spatial clustering of transcriptional profiles among the eight experimental populations. Population abbreviations as in Figure 1. Spatial clusters are circled with ellipses.

CHAPTER III

PREDICTING THE PERFORMANCE OF CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) IN THE SALTWATER PHASE USING FRESHWATER PHASE GENE TRANSCRIPTION

Introduction

Aquaculture has been the fastest growing food industry in the world since 1985 and is expected to increase with the growing human population (Diana, 2009). Salmon aquaculture is economically important to Canada, with extensive saltwater farms on both the Atlantic and Pacific coasts (Pinfold, 2013). The two most important factors in aquaculture production are growth and survival because commercial production requires uniformly large fish that will survive from fertilization to harvest (Lamaze et al., 2014). However, both growth and survival are most important later in life, after considerable investment has been made (Asche and Bjørndal, 2011). Therefore, late life growth and survival should be selected for early in life as less investment will go into unsuccessful fish strains, thus maximizing aquaculture productivity. Gjredrem et al., (2012) found that by selectively breeding over one generation in Atlantic salmon (*Salmo salar*), growth rates increased by 12%, indicative of the gains possible through focussed selection plans. Marker assisted selection that focusses on early life indicators of saltwater performance would provide an opportunity for rapid performance gains in salmon aquaculture. This can be done through marker-assisted selection by breeding fish based on a specific marker that varies among individuals, such as morphology or DNA sequences that are linked to desired phenotypes, such as disease resistance and growth rate (Yue, 2013).

Chinook salmon are the largest of the Pacific salmon species (Healey, 1991) and are native along the entire west coast of North America, from Northern Mexico to the

Arctic Ocean, as well as the east coast of Asia from Taiwan to the Arctic Ocean. They are now being commercially farmed along the west coast of North America, primarily in British Columbia, Canada (Bryden et al., 2004). They represent a valuable niche farmed fish market in Canada and the USA because they are a large salmonid species that is native to the Pacific Ocean, unlike Atlantic Salmon (*Salmo salar*) which are the most commonly farmed salmon species on the west coast (Volpe et al., 2000). Farming Chinook salmon instead of Atlantic salmon along the west coast serves provides benefits, such as reduced environmental concerns of escape, and they are less susceptible to local pathogens, potentially increasing aquaculture productivity (Naylor et al., 2005). However, Chinook salmon have not been systematically domesticated, and therefore there is a need to improve their growth and survival to enhance their commercial viability. There is thus a need to apply effective and rapid selection methods to improve Chinook salmon growth and survival to make them a competitive aquaculture species.

Traditional selection approaches are likely too inefficient for Chinook salmon because of their long production cycle time. Marker-assisted selection that can focus on early life markers to predict Chinook salmon performance in the saltwater phase are promising for increasing aquaculture production. Marker-assisted selection is usually based on DNA polymorphisms such as single nucleotide polymorphisms (Wang et al., 1998) or microsatellite DNA repeat numbers (Weber, 1990); however, gene expression patterns can also be used (Zabel and Acord, 2004). Specifically, I focus on gene transcription, which is the first of two rate-determining steps (transcription and translation) in gene expression, which is known to drive phenotypic variation (Platt, 1986; Hedge and Wang, 2008). Many studies have examined the relationship between

gene transcription and desired phenotypes in aquaculture. For example, Waldbieser et al., (2001) suggested gene transcription as a powerful marker in the selective breeding of channel catfish (*Ictalurus punctatus*), while Liao et al., (2013) used transcription as a performance marker in Crucian Carp (*Carassius carassiusus*) aquaculture. Gene transcription has been studied in Atlantic salmon for aquaculture production, where Roberge et al., (2007) used a microarray approach to assess gene transcription in a farmed and control Atlantic salmon populations and found that their transcriptional profiles had evolved in six generations under direct selection, providing further evidence for the benefit of using transcription for aquaculture breeding programs. The idea of selective breeding based on genetic markers, rather than phenotypes, is still relatively new (Fjalestad et al., 2003), yet this approach may provide novel opportunities for selective breeding in Chinook salmon.

My goal was to predict saltwater performance of Chinook salmon based on freshwater transcription profiles as markers of growth and survival. To accomplish this, I measured growth rate and survival in the saltwater stage in eight hybrid crosses, bred from highly inbred dams and males from seven wild and one domestic population to generate four families within each hybrid cross. I also measured gene transcription at 26 candidate genes selected from four functional groups; immune function, growth, metabolic function, and stress response in juveniles from the same families in the freshwater phase (approximately 6-7 months old). My prediction was that transcriptional profiles at key performance-related genes during the freshwater rearing stage would be good predictors of later performance in the saltwater grow-out stage. If saltwater performance can be predicted using freshwater transcription profiles, then a marker-

assisted selective breeding program could be implemented to increase aquaculture performance, prior to substantial investments in rearing less-productive crosses through to harvest. This study aims to predict saltwater growth and survival in Chinook salmon using freshwater transcription profiles that are resistant to the two most common stressors in aquaculture, immune and handling stress challenges (Quinn et al., 2005).

Materials and Methods

Breeding and Rearing

The fish used in this study were bred at Yellow Island Aquaculture Ltd. (YIAL) in November, 2013 by crossing the mixed eggs of 17 dams with milt from 10 males from 7 wild populations (Big Qualicum River, Capilano River, Chilliwack River, Nitinat River, Puntledge River, Quinsam River, and Robertson Creek) and one domestic population (YIAL) (Figure 1.1). A total of 80 families were created, however due to logistical constraints, only 32 families were sampled in this study (four per hybrid cross). The dams were the offspring of a self-fertilized hermaphrodite Chinook salmon that was produced through hormonal manipulation of female embryos using alpha-methyl testosterone (Hunter and Donaldson, 1983; Komsa, 2012). Using the mixed eggs from these highly inbred female fish minimizes maternal and dam effects and provides the opportunity for heterosis to improve offspring performance. The fish were bred in November, 2013 and reared in replicate tanks prior to being transferred to salt water (Chapter 2). The fish were PIT-tagged in June, 2014 for permanent identification and transferred to replicate netpens (4.1m x 4.1m x 3.0m deep) in the Georgia Strait August 11-12, 2014. While in the netpens, the fish were all fed the same amount of feed twice daily to satiation and the mortalities were removed once weekly.

Sampling

The fish used in this study were sampled for liver tissue for gene transcription measurements at YIAL at two times; once while the hybrid crosses were in the freshwater rearing phase (approximately 6-7 months old) and once in the saltwater phase (approximately 20 months old). The first sampling period occurred May 22-26, 2014 and the fish were split into three treatments; immune stimulus (fish were subjected to Vibrogen2: *Vibrio anguillarum-ordalii*; Novartis Animal Health Canada, Inc. Charlottetown, PEI for 60 seconds and then allowed to recover for 24 hours before euthanization), handling stress challenge (fish were subjected to a 30 second air emersion and allowed to recover for two hours before euthanization), and a control group (no intentional stressors placed upon them). Ten fish per family (5 per replicate tank) were dip-netted from each treatment and humanely euthanized in an overdose solution of clove oil. Body cavities were exposed and they were placed in a highly-concentrated salt buffer (ammonium sulfate, 1M sodium citrate, 0.5M EDTA, H_2SO_4 to bring the pH to 5.2) and stored at -80°C. The full detailed protocol can be read in Chapter 2.

The second sampling period took place at YIAL between June 4, 2015 and June 8, 2015 when the fish were in the saltwater grow-out phase. Each day between 8am and 12pm salmon were collected from their netpens using a brail net. After capture, the fish were immediately humanely euthanized in an overdose solution of clove oil, and liver tissue was removed and stored at room temperature in a highly-concentrated salt buffer (ammonium sulfate, 1M sodium citrate, 0.5M EDTA, H_2SO_4 to bring the pH to 5.2) until the samples were shipped to Windsor, Ontario (June 8, 2015). No more than 20 fish were sampled at one time so that the entire process, per fish, was less than 10 minutes, minimizing the chance of RNA degradation. Upon arrival in Windsor, the samples were

stored at -20°C prior to RNA extraction. There were no intentional stressors placed upon the fish during the saltwater sampling. A total of 183 fish were used in this study (two to three fish per netpen X 4 families X 8 hybrid crosses), the number of fish per hybrid cross was dependent on whether 2 or 3 fish were captured from the replicate netpens for each family.

RNA Extraction

RNA was extracted from the liver of both the freshwater and saltwater phase fish using Isol-RNA Lysis Solution (5 Prime) following the manufacturer's protocol. Whole liver was used for the freshwater phase fish (8.0 mg) and approximately 10 mg of tissue was taken from each saltwater phase individual. Once extracted, the RNA was stored at -80°C. RNA integrity (RNA Integrity Number; RIN) was determined on a sub-set of samples on the 2100 Bionanalyzer (Schroeder et al., 2006) and all samples showed minimal degradation. Spectrophotometry was used to estimate RNA concentration in each sample on a NanoVue spectrophotometer (General Electric Company). Target sample concentrations were between 1000 ng/μL and 4000 ng/μL and any samples below 1000 ng/μL were re-extracted. cDNA was synthesized using High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Burlington, ON, Canada), following the manufacturer's protocol and the amount of RNA and ddH_2O added was dependent on the RNA concentrations, as above. The cDNA samples were stored at -20°C prior to qRT-PCR.

qRT-PCR

Quantitative Real-Time PCR (qRT-PCR) was completed using TaqMan® OpenArray® assays from Applied Biosystems (Burlington, ON, Canada) on a QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific) following the manufacturer's protocol. 5.0µL reactions were prepared using 2.5µL of TaqMan® OpenArray® Real-Time PCR Master Mix (Applied Biosystems), 1.2µL of cDNA (per sample), and 1.3µL of ddH_2O, and loaded onto the TaqMan® OpenArray® assays using the OpenArray® AccuFill System which reduces inter-assay variation. Each assay contained 64 through-holes in a 56x48 format that was pre-loaded with the qRT-PCR primers and probes for each of the 26 candidate genes and 2 endogenous control genes. I chose my candidate genes based on four functional groups that are related to performance; immune function, growth, metabolic function, and stress response. The primers and probes for the 28 genes (all from published sequences on GenBank - https://www.ncbi.nlm.nih. gov/genbank/) were developed on Primer Express® v3.0.1 (Thermo Fisher Scientific) using previously optimized DNA Chinook salmon primers as a guide (Appendix A1). The full protocol for the primer and probe development can be found in Chapter 2.

Transcription Analysis

The raw C_T (critical threshold) and ΔC_T values were calculated using ExpressionSuite Software v1.0.3 (Applied Biosystems, Burlington, ON, Canada) for both freshwater and saltwater transcription. The ΔC_T provides a normalization of each candidate gene's transcript number to that of the endogenous reference gene (Heid et al., 1996) to account for differences in the amount of tissue (mRNA) per sample. Initially

two reference genes were selected, *EF-1a* and *B-actin*; however, after normalization analyses in ExpressionSuite for both the freshwater and saltwater transcription, only *EF-1a* was stable enough to be used as a reference gene. The test calculates the variation in C_T across all samples and ranks each candidate gene based on the variation. *B-actin* had the highest variation score in both life stages and was thus eliminated as a reference gene. The OpenArray® assay generates two estimates of ΔC_T for each gene/fish, I used the mean value for each candidate gene per individual for the following analyses.

Statistical Analysis

Statistical analyses were completed in JMP Statistical Software v12.0.1 and R Statistical Software v3.2.5.

Percent survival, average growth rate, and biomass for each population in the saltwater phase were calculated using the 4 families within each hybrid cross. The percent survival was calculated by dividing the number of remaining fish in each hybrid cross in June, 2015 by the number of fish originally transferred into the netpens. The average growth rate was based on saltwater growth and was calculated as:

$$Growth\ Rate = \frac{(b-a)}{300}$$

where *b* was the weight of the fish at sampling in June, 2015, *a* was the weight of the fish when they were placed in the netpens in August, 2014, and 300 was the approximate number of days each fish was in the netpens. The population biomass was calculated by multiplying the number of survivors in each of the four families by the average weight of the family.

To characterise the transcriptional profile for each fish, I used Principal Component Analyses (PCAs) in R using the prcomp function for the transcription data for all of the genes within each of the 4 gene categories (i.e. immune function, growth, metabolic function, and stress response) and across all 3 freshwater treatment groups (control, immune stimulus, and handling stress). This was done instead of at the individual gene level, because I was more interested in the overall transcriptional profile, rather than how each individual gene was affecting saltwater performance. The initial eigenvalues for all 12 PCAs (Appendix C1) were used to determine which principal components (PCs) would be used for correlations to average growth rate and survival of salmon in the saltwater phase. The first 2 or 3 PCs (the first PCs that explained at least 70 % of the overall variance combined) within each of the gene function categories in the freshwater phase were used to test for correlations with average saltwater growth rate and saltwater survival. One of the stress genes, *hsp90a*, was not included in the analyses because of the large number of missing values in the transcription data.

Correlations were tested between the family growth rate and survival in the netpens, and the average freshwater transcription principal components (for all four gene categories under the three treatment groups; control, immune stimulus, and handling stress) using the "Fit Y by X" function in JMP Statistical Software. Correlations were deemed significant if the fit was significant at the 0.05 alpha level. I labelled genes as main factors for each PC if their was > |0.3|.

To explore the mechanism behind significant correlations between freshwater transcription and saltwater performance, I tested for relationships between gene transcription in the fresh versus salt water. Individual gene transcription was averaged

across all individuals within a hybrid cross at the freshwater and saltwater phase. Correlation analyses were then performed at the individual gene level within each hybrid cross (between the freshwater and saltwater transcription). This allowed me to determine if the transcription profiles at the two separate life phases were correlated with one another.

Results

Saltwater Growth Rate, Survival, and Biomass

The average saltwater growth rate (Figure 3.1) across all eight hybrid crosses varied between 0.46 g/day and 0.56 g/day, with an average of 0.50 g/day. Quinsam River had the lowest saltwater growth rate and Puntledge River had the highest (Figure 3.1). The full domestic cross, YIAL, had a growth rate of 0.49 g/day, placing it fifth out of the 8 hybrid crosses. The hybrid cross with the lowest saltwater survival (Figure 3.1) was Nitinat River, with 68.5% surviving from June, 2014 to June, 2015, while the highest survival was in the Chilliwack River cross (90.1%). The average saltwater survival was 80.6%, just below YIAL's survival of 80.9%. Since there wasn't a single population with a high survivorship and a high growth rate, the biomass for each population was calculated. The smallest biomass was 11.7 kg for Nitinat River and the largest was 16.6 kg for Robertson Creek. These performance values were then tested for correlations with juvenile transcription at rest, in response to an immune stimulus, and in response to a handling stress challenge.

Predicting Saltwater Performance

The number of PCs used for each gene function group, across the three treatments, were selected based on the amount of combined variance they explained (Appendix C1). The first two PCs of each functional category for the immune stimulus and handling stress response transcription were used in the correlational analyses with saltwater growth and survival. At rest, the first two PCs were used for the growth, metabolic, and stress gene groups, however the first three PCs of the immune genes were used (they explained 51.0%, 19.5%, and 16.1% of the variance, respectively).

A total of five significant correlations were found between freshwater transcription PCs and saltwater performance in Chinook salmon (Figure 3.2). At rest, PC2 (*IL-1B*, *TNF-a*, and *IL-8*) of the immune genes was positively correlated to average growth rate in the saltwater phase. However, the at rest PC1 (*hsp70*) of the stress genes was negatively correlated to the average growth rate, and positively correlated to the survival of the 8 hybrid crosses (Table 3.1). The immune stimulus freshwater fish had a negative correlation between the metabolic gene transcription PC2 (*FAS*, *PEPCK*, *COI*, and *PK*) and survival of the saltwater fish (Table 3.2). The handling stress fish also had a single significant (negative) correlation between the immune gene transcription PC1 (*IL-1B*) and survival in the saltwater (Table 3.3). There were no significant correlations between freshwater transcription and biomass.

When I compared freshwater transcription to saltwater transcription at the individual gene level, all 8 hybrid crosses exhibited highly correlated transcription patterns (Figure 3.3). The lowest R^2 value for these correlations was 0.80 for Chilliwack River, and all correlations had a p-value less than 0.0001 (Table 3.4).

Discussion

There was variation witnessed in growth rate, survival, and biomass among the crosses and among the families within the crosses I studied. This was expected since salmonids are known to have considerable variation in growth rate and other life history patterns among populations (Schaffer and Elson, 1975; Hutchings and Marris, 1985; Lahti, et al., 2001). This variation in life history traits, such as growth and survival has been witnessed in both wild salmon and farmed salmon populations (Norris et al., 1999). Given the large variation I observed in performance variables (i.e. growth rate, survival, and biomass), artificial selection should be effective to improve the performance of production fish by selecting among families and crosses. However artificial selection methods previously used have been based on phenotypic traits (Meuwissen et al., 2001). While this is a widely-used method, Chinook salmon require a long rearing-time in salt water, which is costly for aquaculture production. This, coupled with the environmental variation reported for Chinook salmon (Fuhrman et al., 2017), suggests the need for a selection method that uses juvenile-stage markers to predict saltwater performance. Novel methods using marker-assisted selection at the genomic level are now being implemented in plants and other animals (Neira et al., 2006; Lorenzana and Bernardo, 2009) and they represent a promising alternative for rapid performance improvement in Chinook salmon.

Saltwater survival is critical for commercial aquaculture, because low saltwater survival is a primary source of economic loss due to the loss of investment that has already gone into rearing the fish to that point (Sveier and Lied, 1998). This mortality can be due to pathogens (Johnson et al., 2004), rearing density (Wedermeyer, 1997), water quality (Ellis et al., 2002), food availability (Robel and Fisher, 1999), and many other

sources (Turnbull et al., 2005). I found a total of three freshwater gene PCs correlated with survival in the saltwater phase, one positive and two negative. At rest, stress gene PC1 was positively correlated with saltwater survival and the only gene comprising this PC was *hsp70* (-0.99 factor loading). Therefore although it is a positive correlation, it is a lower number of *hsp70* transcripts in relation to higher transcripts in the other stress genes that is dominating this correlation. *hsp70* is a highly-conserved protein and is essential for cell survival under stressful conditions (Smith et al., 1999) and therefore lower transcription of this protein at rest is correlating with a higher likelihood of survival. Since this protein is involved in the stress response, it does not need to be expressed at elevated levels while the fish are at rest, saving energy within the individual. The other two significant correlations with saltwater survival, the immune stimulus response in the metabolic gene group (PC2) and the stress challenge response in the immune gene group (PC1) were both negatively correlated with saltwater survival. The genes comprising the metabolic PC2 under immune stimulus were *COI* (0.61 factor loading), *PK* (0.64 factor loading), *FAS* (-0.35 factor loading), and *PEPCK* (0.28 factor loading). The largest two factors of this PC (*COI* and *PK*) are both involved in the production of ATP (Burke et al., 1983; Qin et al., 2006), suggesting that a higher level of transcription could be correlated to an energy trade-off (Copeland et al., 2011), increasing the risk of mortality. The same can be said for the immune gene, *IL-1B*, because it is the only gene to correlate with saltwater survival under a handling stress challenge (*IL-1β* had a factor loading of 0.96). This gene is involved with inflammation and is one of the first responders to infection (Huising et al., 2004), therefore an increase in transcription is likely associated with an energy trade-off and is hence associated with lower survival. It

is also well-documented that the stress response in organisms can suppress immune function (Bonga, 1997), therefore when subjected to a handling stress, fish immune response will be suppressed and all energy will be put toward the stress response. In general, I expected to find significant correlations between the immune gene PCs and survival because the immune system is critical to survival.

Growth rate is another important component of commercial rearing of salmon in aquaculture because of the high cost of salmon feed (Bostock et al., 2010), especially during the saltwater phase. Considering that all fish were reared from the same dams and were fed the same ration, there must be an underlying reason as to why there was variation in growth rates. This variation could be due to differences in feed conversion, efficiency among families and crosses (Sveier and Lied, 1998). Thus variation in the transcription of genes linked to feed conversion can be selected for, leading to more feed conversion efficient fish for aquaculture. I found a positive correlation between immune gene transcription at rest and growth rate, whereas stress gene transcription at rest had a negative correlation with growth rate. The three immune genes that comprised the immune PC that correlated with growth rate in the saltwater phase were *IL-1β*, *IL-8*, and *TNF-a*. All three genes are involved in inflammation (Huising et al., 2004; Seppola et al., 2008), and *TNF-a* can also regulate the expression of itself, *IL-1β*, and *IL-8* (Hong et al., 2013), supporting the grouping of these three genes in a PCA of transcription. However, when the factor loadings are considered (Appendix C2), it is evident that *IL-1β* is negatively correlated with *IL-8* and *TNF-a* within the PC. Therefore, as the number of transcripts for both *IL-8* and *TNF-a* increase, the number of *IL-1β* transcripts decrease. *IL-1β* is also regulated by receptor proteins that stop it from attacking healthy cells

(Buchs et al., 2001), therefore this lower number of transcripts is likely due to internal mechanisms keeping *IL-1β* at healthy levels. *IL-1B* is also a regulator of muscle mass in salmonids (Zou and Secombes, 2016), therefore it is not surprising that a lower number of *IL-1B* was correlated with a lower average growth rate in the saltwater phase. The fact that these immune genes at rest in the freshwater phase are positively correlated with growth rate in the saltwater phase suggests that higher levels of immune gene transcripts can lead to an increase in fish size. However, this could be an indirect effect associated with the immune function of these genes and surviving until harvest. This transcriptional profile may not lead to the largest growth in salt water, but it may be the largest growth rate of the fish that survive through the first year in salt water. Therefore, fish with higher growth rates were likely putting more energy into their growth than into their immune function, and were more likely to die off and not make it to their maximum size (Visse et al., 2015). The first stress gene PC at rest, *hsp70* (with a factor loading of -0.99), was the only other PC to correlate with average growth rate. It was a negative correlation, however due to the negative loading of *hsp70*, higher transcription levels of this gene were correlated to a lower growth rate. This finding, as well as the fact that none of the treatment response gene transcription PCs significantly correlated with growth rate support the idea of an energy trade-off between gene transcription and individual growth rate (Vøllestad and Quinn, 2003; Copeland et al., 2011).

Although both growth and survival are critical variables for aquaculture, the ultimate goal for aquaculture is to maximise biomass for a given level of resource investment. I thus calculated biomass and tested for significant freshwater transcription PC correlates. Interestingly, I found no significant correlations between freshwater gene

transcription PCs and biomass despite the fact that both survival and growth rate had significant correlations with freshwater transcription PCs. This could be due to an energy trade-off between growth and survival in Chinook salmon. Energy trade offs between growth and survival are well documented (Bassu et al., 2017) and have been studied in many species, from insects (Nijhout and Emlen, 1998) and birds (Gustafsson et al., 1994), to mammals (Weiner, 1992), and salmonids (Finstad et al., 2010). Many of these studies have found that immunity investment has led to another trait, such as growth, being compromised (Zuk and Stoehr, 2002; Schmid-Hempel, 2003). The idea of an energy trade-off is further supported by my findings of the at-rest stress gene PC1 (*hsp70*) that was significantly correlated with both growth and survival, positively with survival and negatively with growth. Therefore, the fish that had higher transcription of the stress gene PC1 at rest are putting more energy toward protecting themselves from environmental stressors (Healy et al., 2010), than towards their growth rate. Since there were no significant correlations between transcription and biomass, transcriptional profiles that increase survivorship should be selected first for aquaculture breeding programs. Then once populations with high survivorship in salt water have been produced, growth rates can be selected for using a marker-assisted method to maximize the overall biomass.

I also tested for the ability to predict saltwater performance based on freshwater transcription at the individual gene level to determine if the same patterns were reflected. I found a total of 12 significant correlations (out of a possible 52); eight for growth rate in salt water and four for survival in salt water (Appendix C3). Interestingly, all of the correlations between individual gene transcription and saltwater growth rate were

positive and all of the correlations with saltwater survival were negative. There was also at least one gene from each functional category (i.e. immune, growth, metabolic, and stress) that had a significant correlation with either of the saltwater performance factors (biomass was not included in this analysis since it was not correlated with any of the transcriptional profile PCs). Of the genes that positively correlated with saltwater growth rate, there were four immune genes (*CAL*, *IL-8*, *MHCIIB*, *TNF-a*), two growth genes (*IGF-1* and *IGFPB2b*), one metabolic gene (*CYP1a*), and one stress gene (*hsp70*). Whereas there was one immune gene (*SAA*), one growth gene (IGFBP2b), one metabolic gene (*COI*), and one stress gene (hsp70) that were negatively correlated with saltwater survival. This broad variety of functional genes correlating with saltwater performance, (across all gene groups at the individual gene level), provide further support for the use of transcriptional profiles for a marker-assisted selective breeding program. I also witnessed similar patterns at the individual gene level in terms of correlations with saltwater performance. There were no individual genes that positively correlated with both saltwater growth rate and survival, thus even at the individual gene level, there does not seem to be an ability to select for both a high growth rate and survivorship using wither individual gene transcription or transcriptional profiles.

Lastly, I explored the mechanisms behind the correlations between freshwater transcription and saltwater performance through testing for correlation between freshwater and saltwater transcription and found very strong correlations across the transcriptional profile used in this study (Figure 3.3). These correlations suggest that transcription patterns do not change drastically in the candidate genes I tested throughout Chinook salmon life, highlighting how freshwater transcription profiles can be correlated

with saltwater phenotype. The ability to predict saltwater performance using early-life transcription offers promising selection opportunities for aquaculture. This will allow aquaculture productions to sample potential source populations for a specific transcriptional profile before they have been reared to harvest size in salt water, which can take up to five years (Wheatley et al., 1995). The development of a marker-assisted selection method that uses transcriptional profiles serves as a viable marker-assisted selection method for selective breeding in salmon aquaculture.

In conclusion, Chinook salmon are a promising farm species and by farming them in preference to non-native species, they are less of a concern for environmental damage (Naylor et al., 2005). I found transcription predictors for growth and survival in salt water that explained between 19.5 % and 58.1 % of the variance in saltwater performance among families of Chinook salmon crosses. The at-rest stress genes that positively correlated with survival and negatively correlated with growth rate explained 58.1 % of the variation among the families I studied. The difference in correlation between stress gene transcription and growth and survival present evidence of an energy trade-off in Chinook salmon (Zera and Harshman, 2001). This trade-off could present limitations to the possible advances generated by selection because they may not allow for overall biomass to be selected for using transcriptional profiles. However, this limitation can be minimized by selecting first for survivorship, and then for growth rate. By using freshwater transcription as a predictor for growth and survival, time and effort will not be spent on rearing less-profitable stocks. For the implementation of this method, salmon farms should sample the transcriptional profile of potential source populations and select the profile that is highly correlated with increased survivorship. After rearing populations

with high survivorship, a transcriptional marker related to average growth within those populations can then be selected for to increase the overall biomass. I believe this marker-assisted approach to selective breeding is a novel idea and should be implemented in not only Chinook salmon, but in other aquaculture species as well.

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Table 3.1: Control freshwater transcription Principal Component (PC) correlations with the saltwater growth rate and survival across all 8 hybrid crosses. The p-values and R^2 values for the correlations are given, and in bold font if they are significant ($p < 0.05$). The gene groups are listed in the order of immune, growth, metabolic, and stress. The percent variance explained by each PC that was used is given in brackets.

Gene Group	Principal Component	Performance Factor	R^2 Value	p-value
Immune	PC1 (50.1 %)	Survival	0.095	0.086
		Growth Rate	0.0074	0.64
	PC2 (19.5 %)	Survival	0.082	0.11
		Growth Rate	0.24	**0.004 ***
	PC3 (16.1 %)	Survival	0.065	0.16
		Growth Rate	0.49	0.22
Growth	PC1 (40.0 %)	Survival	$1.4*10^{-5}$	0.98
		Growth Rate	0.049	0.22
	PC2 (29.6 %)	Survival	0.00029	0.93
		Growth Rate	0.094	0.088
Metabolic	PC1 (65.1 %)	Survival	0.0044	0.72
		Growth Rate	0.076	0.13
	PC2 (22.6 %)	Survival	0.026	0.38
		Growth Rate	0.078	0.12
Stress	PC1 (58.6 %)	Survival	0.19	**0.013 ***
		Growth Rate	0.22	**0.0062 ***
	PC2 (26.3 %)	Survival	0.0071	0.65
		Growth Rate	0.030	0.34

Table 3.2: The immune stimulus transcriptional response was correlated with saltwater performance factors (i.e. survival and growth rate). The p-values and R^2 values for the correlations are given, and those that were deemed significant at the 0.05 alpha level are in bold face. The percent variance explained by each PC used in the analyses is given in brackets.

Gene Group	Principal Component	Performance Factor	R^2 Value	p-value
Immune	PC1 (62.5 %)	Survival	0.066	0.15
		Growth Rate	0.0018	0.82
	PC2 (15.9 %)	Survival	0.0036	0.75
		Growth Rate	0.011	0.57
Growth	PC1 (41.6 %)	Survival	0.063	0.17
		Growth Rate	0.0032	0.80
	PC2 (35.6 %)	Survival	0.056	0.19
		Growth Rate	0.036	0.30
Metabolic	PC1 (49.9 %)	Survival	0.025	0.39
		Growth Rate	0.013	0.53
	PC2 (26.7 %)	Survival	0.17	**0.020 ***
		Growth Rate	0.00	0.99
Stress	PC1 (65.6 %)	Survival	0.11	0.070
		Growth Rate	0.00084	0.88
	PC2 (30.5 %)	Survival	0.027	0.37
		Growth Rate	0.0041	0.73

Table 3.3: The handling stress transcriptional response was correlated with saltwater performance factors (i.e. survival and growth rate). The p-values and R^2 values for the correlations are given, and those that were deemed significant at the 0.05 alpha level are in bold face. The percent variance explained by each PC used in the analyses is given in brackets.

Gene Group	Principal Component	Performance Factor	R^2 Value	p-value
Immune	PC1 (58.1 %)	Survival	0.17	**0.020 ***
		Growth Rate	0.00017	0.94
	PC2 (23.5 %)	Survival	0.066	0.16
		Growth Rate	0.057	0.19
Growth	PC1 (53.8 %)	Survival	0.035	0.31
		Growth Rate	0.013	0.54
	PC2 (19.7 %)	Survival	0.011	0.57
		Growth Rate	0.0017	0.82
Metabolic	PC1 (70.6 %)	Survival	0.0042	0.72
		Growth Rate	0.0012	0.85
	PC2 (18.8 %)	Survival	$8.3*10^{-5}$	0.96
		Growth Rate	0.018	0.47
Stress	PC1 (63.4 %)	Survival	0.10	0.072
		Growth Rate	0.0092	0.60
	PC2 (27.5 %)	Survival	0.071	0.14
		Growth Rate	0.023	0.40

Table 3.4: The results of the correlation between at-rest freshwater transcription and saltwater transcription for each hybrid cross. The individual gene transcription was averaged for each hybrid cross at both the freshwater and saltwater phases. The hybrid crosses are listed in geographical order of source river from the southwest coast of Vancouver Island to the mainland. All p-values were significant.

Population	**R^2 Value**	**p-value**
Nitinat River	0.84	$3.5*10^{-11}$
Robertson Creek	0.83	$9.6*10^{-11}$
Big Qualicum River	0.93	$1.9*10^{-15}$
Puntledge River	0.92	$8.2*10^{-15}$
Quinsam River	0.82	$1.7*10^{-10}$
YIAL	0.93	$1.4*10^{-15}$
Capilano River	0.94	$1.4*10^{-16}$
Chilliwack River	0.80	$5.5*10^{-10}$

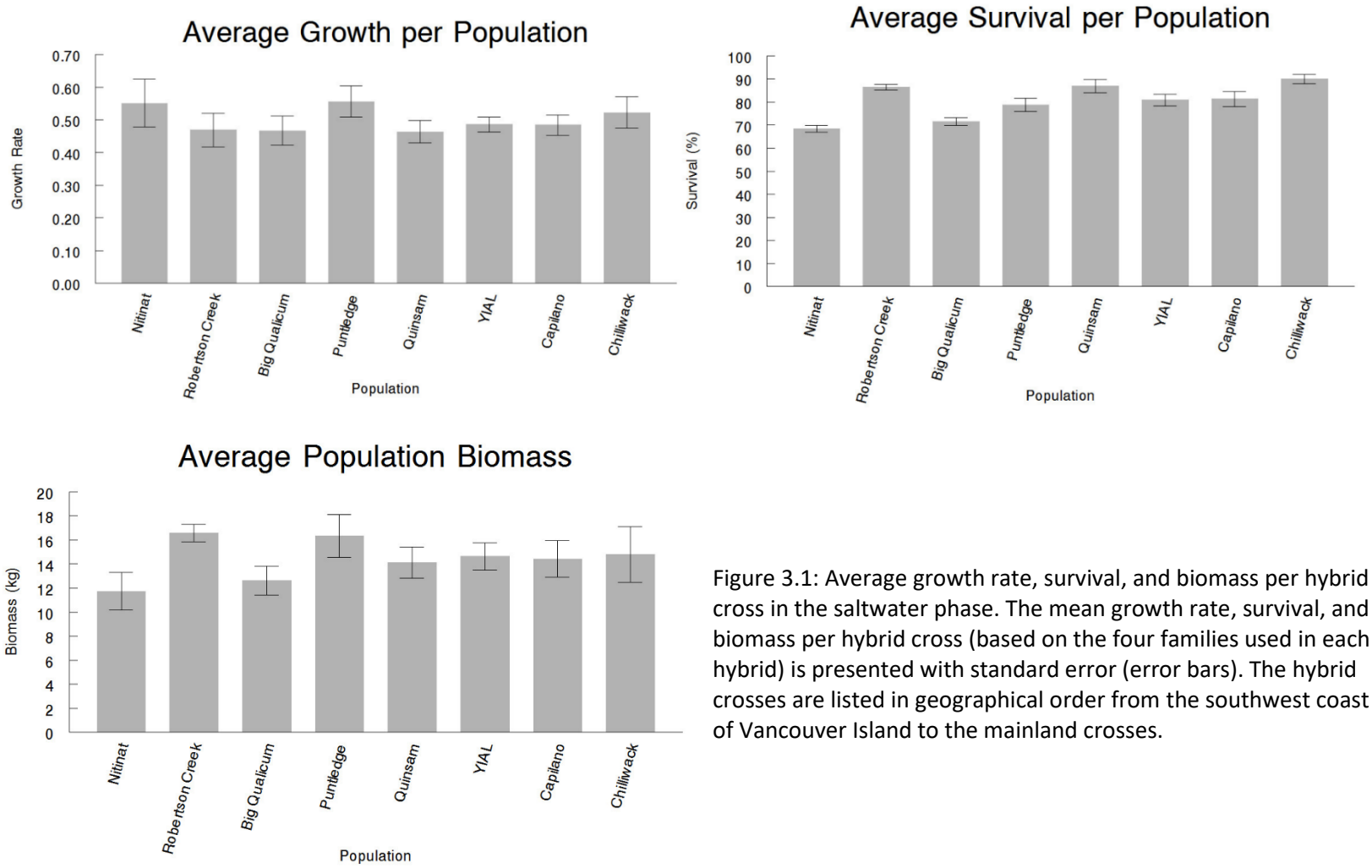


Figure 3.1: Average growth rate, survival, and biomass per hybrid cross in the saltwater phase. The mean growth rate, survival, and biomass per hybrid cross (based on the four families used in each hybrid) is presented with standard error (error bars). The hybrid crosses are listed in geographical order from the southwest coast of Vancouver Island to the mainland crosses.

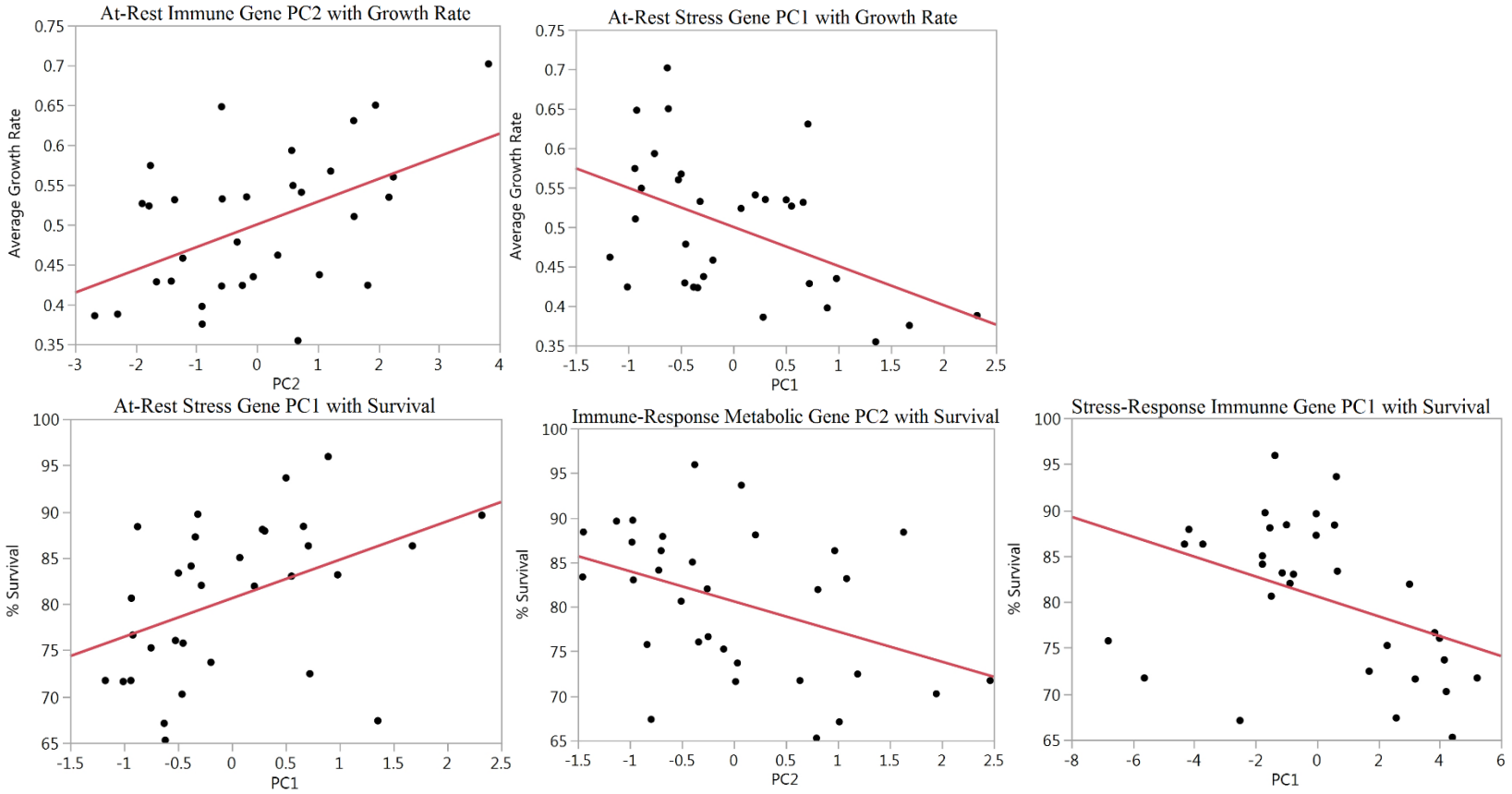
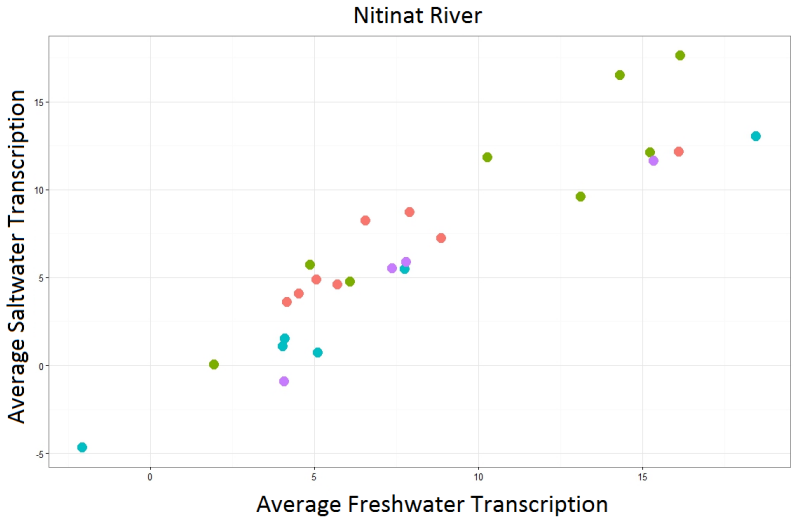
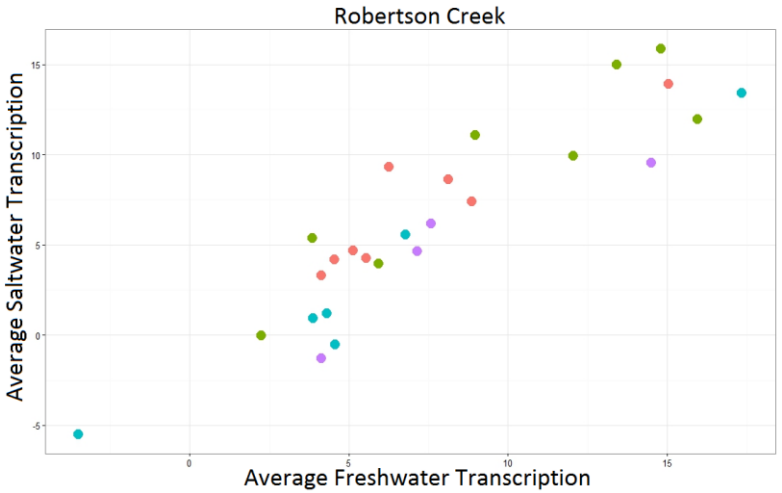
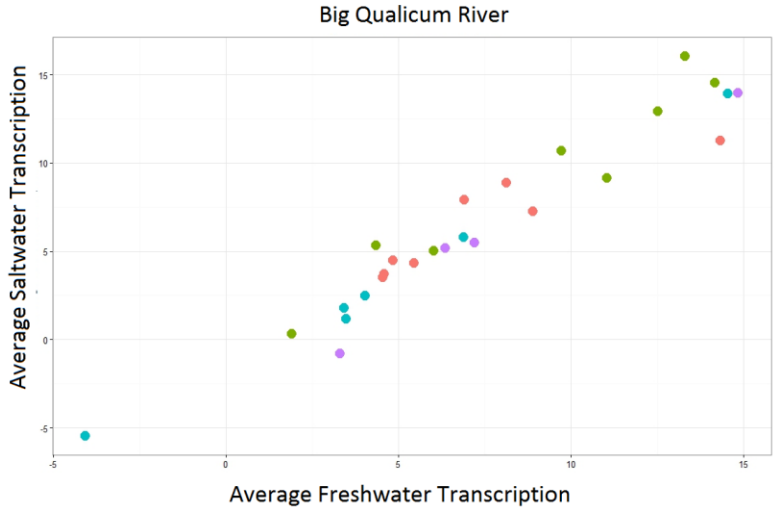
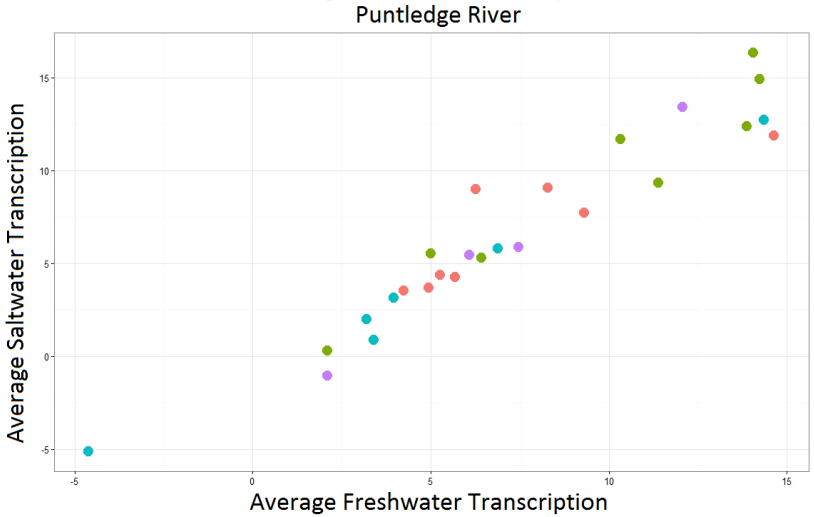


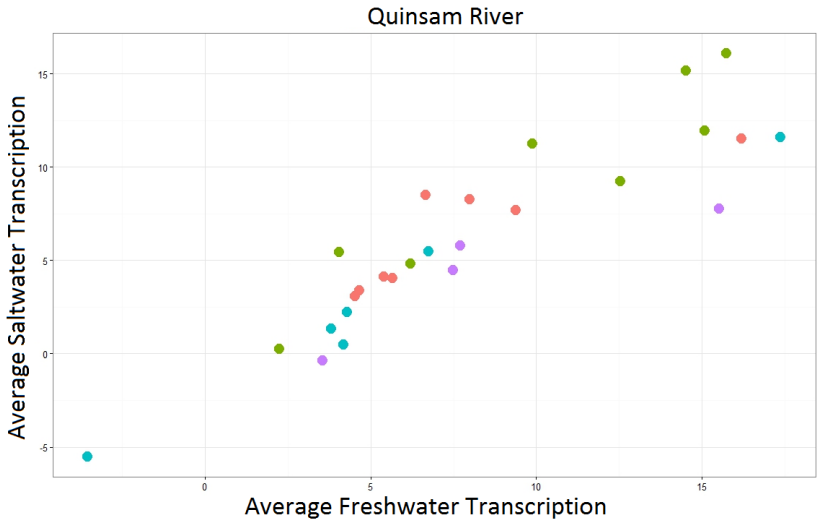
Figure 3.2: Freshwater gene transcription principal components (PC) correlated with saltwater performance (i.e. growth and survival). The fit line is depicted in each graph, demonstrating the relationship of the correlation.

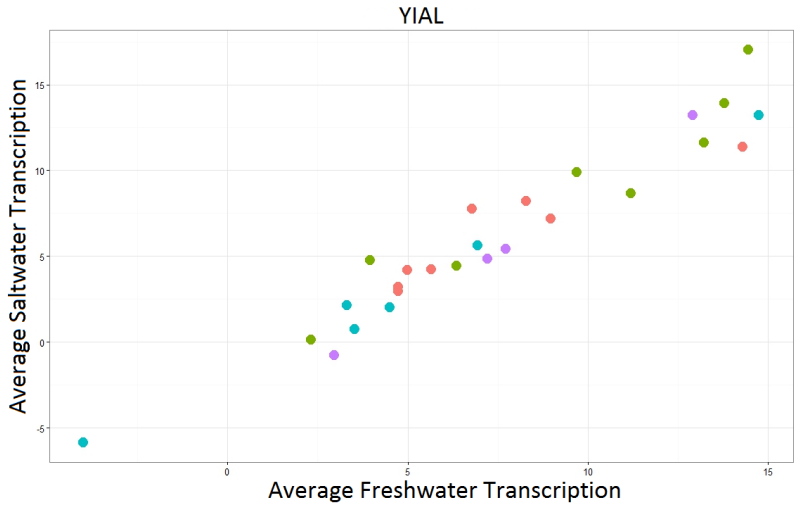

Nitinat River
Average Saltwater Transcription
Average Freshwater Transcription

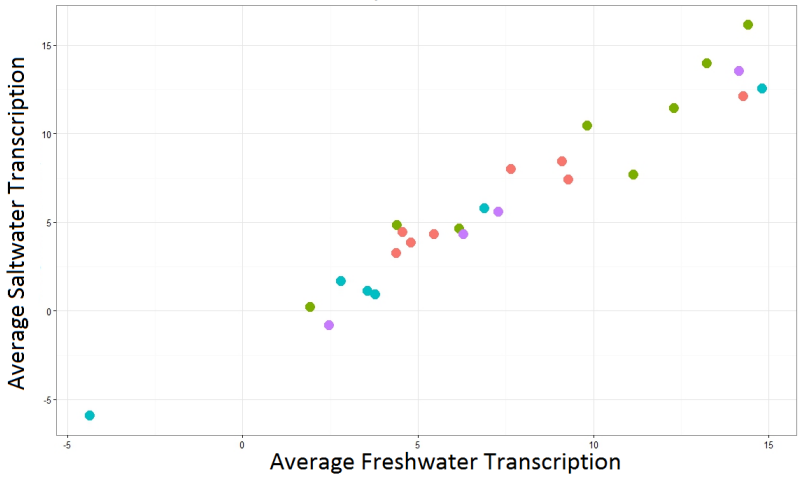

Robertson Creek
Average Saltwater Transcription
Average Freshwater Transcription

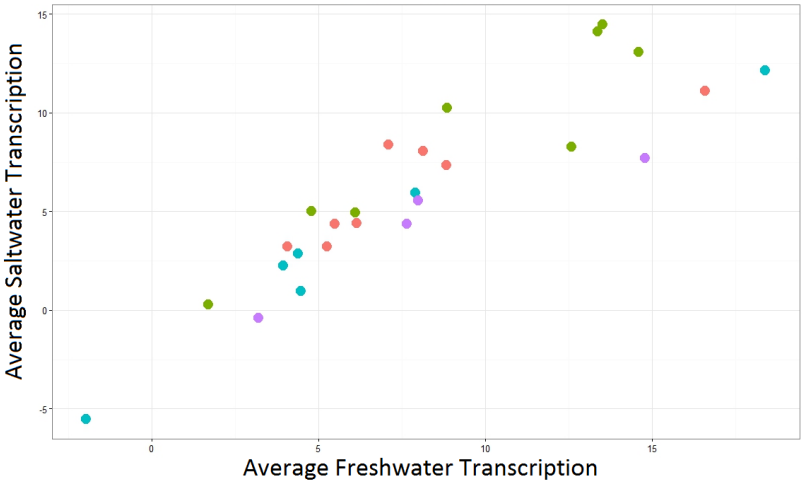

Big Qualicum River
Average Saltwater Transcription
Average Freshwater Transcription


Puntledge River
Average Saltwater Transcription
Average Freshwater Transcription


Quinsam River
Average Saltwater Transcription
Average Freshwater Transcription


YIAL
Average Saltwater Transcription
Average Freshwater Transcription


Capilano River
Average Saltwater Transcription
Average Freshwater Transcription


Chilliwack River
Average Saltwater Transcription
Average Freshwater Transcription

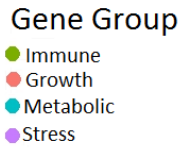


Figure 3.3: Freshwater to saltwater transcriptional correlations for all 8 hybrid crosses. The average transcription was found for each individual candidate gene in both life phases; freshwater and saltwater. The average of each candidate gene in the freshwater and saltwater phase was then plotted for each hybrid cross. Positive correlations were found among all hybrid crosses.

CHAPTER IV

CONCLUSION

Aquaculture has been the fastest growing food industry worldwide since 1985 (Diana, 2009) and is expected to rise with the growing human population and overexploitation of wild-catch fisheries (Bostock et al., 2010). It is also extremely important to the Canadian economy (Pinfold, 2013), especially along the west coast where Atlantic salmon (*Salmo salar*), a non-native salmon species make up 80% of farmed species (Volpe et al., 2001). Atlantic salmon are the primary farmed salmon because of their consistent growth rates and previous domestication efforts (Noakes et al., 2000); however, Chinook salmon (*Oncorhynchus tshawytscha*), a west coast native species are also now being farmed (Bryden et al., 2004). Since Chinook salmon are a relatively new aquaculture species, they have not yet been domesticated and they are suffering from immune and handling stress challenges in aquaculture settings (Espelid et al., 1996). The overall goal of this thesis was to explore marker-assisted selective breeding opportunities for Chinook aquaculture by studying the mechanisms behind Chinook salmon population differences at the genotypic and phenotypic levels. More specifically, I aimed to develop a marker-assisted selection program of heritable gene transcription patterns to predict the survival and growth rate of Chinook salmon in the saltwater life-phase. By using native populations of Chinook salmon and studying their gene transcription and associated performance, I was able to identify markers that can be used to predict both the size and survival of Chinook salmon populations in aquaculture.

The goal of Chapter 2 was to study population and family-level differences in gene transcription of 26 fitness-related genes in juvenile Chinook salmon at rest and in response to two ecologically-relevant challenges; an immune stimulus (Heath et al., 1993) and a handling stress challenge (Quinn et al., 2005). My results demonstrated significant differences in gene transcription at the population-level, as well as evidence for local adaptation among the selected Chinook salmon populations. I found spatial clustering in some transcriptional patterns (i.e. west coast of Vancouver Island, east coast of Vancouver Island, and mainland populations). By minimizing maternal effects, I was also able to determine narrow-sense heritability (h^2) for gene transcription within each population for each gene. In doing so, I found indirect evidence for high selection pressures acting on select genes within some of the experimental populations, highlighting the fundamental differences among local populations of Chinook salmon.

In Chapter 3, I aimed to determine whether the freshwater transcriptional profiles (from Chapter 2) could predict the performance of Chinook salmon in salt water. I defined performance as growth rate and survival of Chinook salmon, since they are the two most important factors of performance in aquaculture (Barton & Iwama, 1991). The gene transcription data from Chapter 2 was used to test for correlations with average growth rate and survival in the saltwater phase. I found significant correlations between freshwater transcription and saltwater performance, indicating the potential to develop commercial marker-assisted selection breeding programs for Chinook salmon performance. I also ran correlational analyses between freshwater and saltwater transcription and found highly significant correlations within all eight populations. Therefore the mechanism driving the correlations between freshwater transcription

profiles and saltwater performance is likely the similarity of gene transcription in the freshwater and saltwater phases, resulting in predictable phenotypes.

Overall, the two data chapters I present examine gene transcription within eight half-sibling Chinook salmon populations at two different life phases; fresh water and salt water. When integrated, these chapters explore the ability to predict performance of Chinook salmon in the saltwater phase based on likely locally-adapted patterns in gene transcription at the freshwater phase. Since Chinook salmon only spend 8-20 months in their natal streams (Healey, 1991), the potential for them to be locally adapted lies in the correlation between freshwater and saltwater transcription. Therefore, Chinook salmon transcription profiles appear to be developed at an early age, and maintained throughout their growth in salt water. Chinook salmon are also known to exhibit considerable variation in phenotype (Cutts et al., 1998; Vasemägi et al., 2005) and genotype (Armbruster and Schwaegerle, 1996) both within and among populations, supporting the likelihood of local adaptation as the cause of these phenotypic variations.

Numerous studies have postulated local adaptation as the driving force behind phenotypic differences in Chinook salmon (Adkinson, 1995; Crozier et al., 1998; Dionne et al., 2007; Evans et al., 2010; Taylor, 1991) and the results presented in Chapter 2 provide further evidence. The combined evidence of local adaptation and the variation of Chinook salmon phenotypes within and among populations, make them a prime candidate for a selective breeding program. The populations and sire effects I found in Chapter 2, gave rise to the ability to select specific Chinook salmon populations for a marker-assisted selective breeding program using transcriptional profiles. Therefore, salmon from potential source populations can be tested for their transcriptional profile

before they are reared in aquaculture. This will save considerable time and money because the salmon will not have to be reared for three years before a beneficial phenotype can be selected (Cook et al., 2000; Lamaze et al., 2014). Since both the freshwater and saltwater transcriptional profiles correlated in the eight river populations (Chapter 3), salmon could be sampled from source rivers at any life stage for selective breeding purposes.

As of 2012, there were only two documented reports of selective breeding programs in Chinook salmon aquaculture (Gjedrem et al., 2012). This lack of focussed breeding programs is a potential reason that Chinook are not being widely farmed in their native region. Thus, this thesis not only introduces a new marker-assisted selective breeding program, but provides evidence that Chinook salmon performance could be improved by selection. Using the information provided, Chinook salmon should become a more commonly farmed salmon on the British Columbia coast, reducing the ecological risks of escaped non-native species, such as the Atlantic salmon. This thesis provides a novel marker-assisted selective breeding approach that should be implemented in aquaculture to increase production and decrease the associated costs.

Future Directions

The goal of this thesis was to examine the underlying causes of phenotypic variation in Chinook salmon populations and to determine if a genetic marker (i.e. transcription) could be used to predict saltwater growth and survival for aquaculture. Chinook salmon are the largest of the Pacific salmon (Healey, 1991), and yet they only make up 18% of British Columbia aquaculture (Noakes et al., 2000). I found strong evidence of local

adaptation in Chinook salmon as well as the possibility to predict growth rate and survival in the saltwater phase based on freshwater transcriptional profiles. These results shed light on many aspects of Chinook salmon genomics, but result in some unanswered questions. For example, the immune genes I studied did not correlate with survival in the saltwater phase across all three treatments. Further, my MHC gene was not one of the immune genes to correlate with survival, and it is a well-documented immune gene that plays a critical role in disease-resistance and survival (Arkush et al., 2002), therefore I expected it to significantly correlate with survival. This is likely due to the sampling occurring less than 96 hours after the immune stimulus (Mjaaland et al., 2005), however studies have witnessed changes in transcription as early as 24 hours (Mackenzie et al., 2008). This finding suggest that further studies should be completed using different immune-related genes to determine the genes responsible for the variation in saltwater survival.

This thesis was also completed using eight half-sibling hybrid families that were all bred using inbred dams. This allowed me to minimize maternal effects; however, as a result I was not able to study the full range of population level differences in gene transcription. Thus, other studies may examine these same questions with full crosses (where the dam and sire come from the same source river) to determine if the population-level effects are even stronger than the ones I found.

While I suggest local adaptation as the mechanism behind these population-level differences in gene transcription, local adaptation is difficult to test because of the many factors involved in population divergence; i.e. gene flow, population history, etc. (Kawecki and Ebert, 2004). One method to test for local adaptation in Chinook salmon,

would be to perform a reciprocal transplant study, where native fish from one location are reared in another river system to test for "home vs away" fitness differences (Stelkens et al., 2012). However, due to logistical reasons, common garden experiments can be performed instead, where the same principles are applied, but the fish are reared in a laboratory or hatchery setting, controlling for specific environmental effects (Harvey et al., 2015). This type of study has been done in brown trout (*Salmo trutta*) to determine adaptation to early rearing environment (Stelkens et al., 2012), Atlantic salmon to study cardiac performance (Gradil et al., 2016), and in Chinook salmon to test for thermal tolerance in differentially adapted populations (Fuhrman et al., 2017). This approach appears more practical for Chinook salmon and therefore should be applied to these Chinook salmon populations to confirm that the differences I observed at the population are in fact due to local adaptation, rather than genetic drift.

The marker-assisted selective breeding program suggested here should be further studied using other saltwater performance traits, such as pathogen resistance (Robertsen et al., 1990), feed conversion (Storebakken and Austreng, 1987), and other aquaculture-desired phenotypes (Gjedrem et al., 2012). By studying these other factors and their relationship to juvenile transcription, a full transcriptional profile marker-assisted selection protocol could be developed to select salmonids, and other species, for aquaculture production. Current selective breeding programs in salmonid aquaculture consist of phenotypic selection (Meuwissen et al., 2001), and marker-assisted selection using sequence polymorphisms (Hayes et al., 2007; Neira et al., 2006), but none that use transcriptional profiles as a predictive tool, making this a novel method.

In conclusion, local adaptation appears to play a pivotal role in phenotypic differences among Chinook salmon populations. This underlying ability to adapt to a given environment, makes Chinook salmon a prime candidate for aquaculture, especially with the ability to predict their performance using early-life transcriptional profiles.

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APPENDICES

Appendix A1 – Candidate gene list

The candidate genes used throughout Chapter 2 and Chapter 3 for qRT-PCR assays of gene transcription (TaqMan® OpenArray® chips (Applied Biosystems, Burlington, ON)), including: gene name, functional grouping, primers and probes used in the analysis, the amplicon length, and the accession number for reference.

Gene	F'	R'	FAM	Amplic-on Length	Accession Number
Endogenous Control Genes					
Beta-Actin (*B-actin*)	gacccagatcatgtttgagacctt	tccatgacgataccggtggta	caggccgtgttgtc	105 bp	FJ890357.1
Elongation Factor-1a (*EF-1a*)	aataccctcctcttggtcgtttc	cttgtcgacggccttgatg	tgcgtgacatgaggc	80 bp	AF498320.1
Immune Genes					
Calmodulin (*CAL*)	cagacagcgaggaggagatca	taaccgttcccatccttgtca	agaagcgttccgtgtct	61 bp	BT074280.1
Chemokine 1 (*CK-1*)	tcctggctgctctgttctctct	acagcagtccgctgattgtg	ctcatcatcaccctcatt	68 bp	AF093810.1
Interleukin 1B (*IL-1B*)	ccagggaggcagcagcta	cgggcgtgacgtacgaa	acaaagtgcatttgaac	59 bp	DQ778946.1
Interleukin 8 (*IL-8*)	cgcactgcagagacactga	acaaatctcctgaccgctcttg	tcagagtggcaatgatc	59 bp	DQ778949.1
Major histocompatibility complex 2B (*MHCIIB*)	gccatactggacaagacagttgag	tcataggcgctgcacatcag	cccatgtcagactgag	98 bp	U34718.1
Natural killer enhancing factor (*NKEF*)	tgaggtcattggtgcctctgt	gaggtgtgttggtccaagca	attcccacttctgccatc	92 bp	AF250193.1
Serum amyloid-A (*SAA*)	agctgctcaaggtgctaaagacat	ccagttggcgtccttcatg	tggcgtgcatatgg	61 bp	NM_001124436.1
Tumor necrosis factor-α (*TNF-α*)	cccaccatacattgaagcagatt	ggattgtattcaccctctaaatgga	ccggcaatgcaaaa	70 bp	DQ778945.1
Growth Genes					
Early growth response protein 1 (*EGR-1*)	cgaacatctgaatggagatacattacc	caggctccagggtgaacct	ctatcggctgtgacaagt	128 bp	NM_001141824.1
Growth hormone receptor (*GH-R*)	ccccactaaagagtcccgatt	ctaaacccaaggcagcaaaga	ccagttactgtcctgctt	62 bp	NM_001124731.1
Insulin-like growth factor 1 (*IGF-1*)	atttcagtaaaccaacgggctatg	cgtccacaataccacggttatg	ccagttcacgacggtc	66 bp	U14536.1
Insulin-like growth factor binding protein (*IGFBP2b*)	caactgtcccgaggaacctaag	ctccagctcctgtgcacaag	cccagcagcccatga	64 bp	HM358881.1
Leptin 1 (*LEPTIN*)	ggtgattaggatcaaaaagctggat	cagccgcagggagaaatg	acctgatcgagggcat	74 bp	NM_001145890.1
Myostatin-1A (*MYO1a*)	gggaaatgatctggccgtta	tctgaaatcgtcacctccatga	agaaggactgcaaccc	75 bp	EU009952.1
Tumor Suppressor p53 (*p53*)	cagtccagcacagccaagtc	cgccaactggcagaacaact	acttgcacatactcgc	72 bp	AF071574.1
Thyroid Hormone Receptor-b (*THR-b*)	gctctgctacaggccgtcat	gttcaaaggccagaaggaactc	tcctccgaccgtccg	108 bp	AB303988.1
Metabolic Genes					
Carnitine Palmytol Transferase 1 (*CPT1*)	gaagggcctgatcaaaaagtgt	tccccttgtccctgaagtga	cttcatccagatcgc	86 bp	AJ620357.1
Cytochrome p450 Family 1A (*CYP1a*)	tcttccttcctgccgttcac	gaagtagccattgagggatgtgt	ccacactgcacgatc	66 bp	M21310.1
Cytochrome Oxidase 1 (*COI*)	ggcagcaggcattactatgttactc	gcctgccgggtcaaaga	cggaccgaaatcta	67 bp	KP720599.1
Fatty Acid Synthase (*FAS*)	ccaggtctgtacggtcttcca	cgaaccggctgatgtcctt	agaggaacggcaagct	58 bp	XM_014179800.1
Phosphoenolpyruvate Carboxykinase (*PEPCK*)	acaaaggcaaggttatcatgca	accgaagttgtagccgaagaag	acccctcgccatgc	65 bp	AF246149.1
Pyruvate Kinase (*PK*)	gcaaaaacatcaagatcatatccaa	atgccatcgctagcttcca	ccgcagattcgacg	85 bp	NM_001141703.1
Stress Genes					
Glucocorticoid Receptor 2 (*GR-2*)	agcaccgtgccaaaagatg	gccttccccaactccttga	ctcatcaaacactgcctg	83 bp	AY495372.1
Heat Shock Protein 70 (*hsp70*)	tcaacgatcaggtcgtgcaa	cgtcgctgaccaccttgaa	ccgacatgaagcactg	60 bp	U35064.1
Heat Shock Protein 90a (*hsp90a*)	agatcttccttagggagctcatctc	tgtcaagctctcgtatctgatcttg	aactcttcagatgctttgg	71 bp	U89945.1
Metallothionein A (*metA*)	gctccaaactggatcttgcaa	tggtgcatgcgcagttg	tgcggtggatcctg	62 bp	DQ139342.1

Appendix B1 – Significant treatment effect to the immune and handling stress challenges

Treatment, population, population-by-treatment, tank, and weight for each candidate gene in response to both the immune stimulus and the handling stress treatment across all 8 population crosses. Results are based on a General Linear Mixed Model (GLMM) with treatment, population, and population-by-treatment as fixed effects, and tank and random added as random effects. The independent variable was the ΔC_T at rest and for the challenged fish. The values given are the raw p-values from the GLMM, but the significance (asterisks) is based on the post-FDR corrections, where *** p<0.0001, ** p<0.001, * p<0.05.

Gene	Treatment	Population	Treatment	Population-by-Treatment	Tank	Weight
CAL	Immune	**0.003 ***	**<0.0001 *****	0.67	**<0.0001 *****	1
	Stress	**0.00039 ***	0.46	0.44	**<0.0001 *****	1
CK-1	Immune	**0.0023 ***	**<0.0001 *****	0.47	0.04	0.63
	Stress	**0.0012 ***	0.61	0.21	**<0.0001 ****	1
IL-1B	Immune	0.42	**0.0016 ***	0.18	**0.0004 ***	0.6
	Stress	0.47	0.4	0.4	**<0.0001 ****	0.6
IL-8	Immune	0.024	0.082	0.98	0.02	0.51
	Stress	0.4	**<0.0001 ****	0.61	**<0.0001 *****	0.2
MHCIIB	Immune	0.34	**0.0058 ***	0.64	0.2	0.8
	Stress	0.014	0.88	0.96	0.07	0.24
NKEF	Immune	**0.0053 ***	**<0.0001 *****	0.51	**<0.0001 ****	0.2
	Stress	**<0.0001 ****	0.074	0.68	0.06	0.32
SAA	Immune	0.3	0.16	0.075	1	1
	Stress	0.54	0.94	0.19	0.1	0.5
TNF-A	Immune	0.28	0.017	0.82	0.1	1
	Stress	0.012	0.014	0.76	0.9	1
EGR-1	Immune	0.083	0.00038	0.49	0.02	0.03
	Stress	0.015	0.032	0.98	**0.002 ***	**0.001 ***
GH-R	Immune	0.024	**0.0021 ***	0.49	**0.008 ***	0.47
	Stress	**0.0024 ***	0.53	0.11	**<0.0001 *****	0.3
IGF-1	Immune	0.95	**<0.0001 *****	0.86	0.18	**0.005 ***
	Stress	0.92	0.31	0.75	**0.004 ***	0.051

IGFBP2b	Immune	**0.0024 ***	0.53	0.4	0.2	0.1
	Stress	0.47	0.65	0.049	**0.007 ***	0.11
LEPTIN	Immune	0.063	**<0.0001 *****	0.53	0.24	0.02
	Stress	0.033	0.55	0.77	**0.01 ***	0.56
MYO1a	Immune	0.6	0.27	0.012	0.08	1
	Stress	0.36	0.81	0.37	0.5	0.5
p53	Immune	0.022	**<0.0001 *****	0.37	**0.008 ***	0.18
	Stress	0.063	0.21	0.86	**0.0003 ***	1
THR-b	Immune	0.44	0.83	0.71	1	0.7
	Stress	0.47	0.48	0.53	0.3	1
COI	Immune	0.55	0.21	0.66	**0.0008 ***	0.1
	Stress	0.069	0.26	0.96	0.07	1
CPT1	Immune	0.65	0.22	0.51	0.2	1
	Stress	**0.004 ***	0.83	0.99	0.1	1
CYP1a	Immune	0.032	**<0.0001 *****	0.35	**<0.0001 *****	0.04
	Stress	**0.00011 ***	0.59	0.22	**<0.0001 *****	0.1
FAS	Immune	**0.0015 ***	0.096	0.059	**<0.0001 *****	1
	Stress	**0.0013 ***	0.18	0.75	**<0.0001 *****	1
PEPCK	Immune	0.12	0.55	0.98	**0.0002 ***	**0.0007 ***
	Stress	0.44	0.91	0.88	0.09	0.88
PK	Immune	0.15	**<0.0001 *****	0.85	0.7	0.2
	Stress	**<0.0001 *****	0.16	0.28	**0.0006 ***	0.02
GR2	Immune	0.74	0.84	0.45	0.49	0.05
	Stress	0.064	0.88	0.81	1	1
hsp70	Immune	**0.011 ***	0.021	0.68	**0.001 ***	0.45
	Stress	**0.00037 ***	0.012	0.95	0.4	1
hsp90a	Immune	0.023	0.93	0.2	1	1
	Stress	0.45	0.55	**0.007 ***	0.9	0.6
metA	Immune	0.22	0.13	0.83	1	0.5
	Stress	**0.0093 ***	0.28	0.29	**0.006 ***	1

Appendix B2 – Narrow-sense heritability across all populations for each gene

The narrow-sense heritability (h^2) for all eight populations across all 26 genes. The h^2 was calculated by multiplying the sire variance by four and dividing by the total phenotypic variance. h^2 was calculated for all candidate genes at rest, however only the genes that demonstrated a significant treatment effect were included for the immune stimulus and handling stress challenged fish.

Gene	Treatment	Big Qualicum River	Capilano River	Chilliwack River	Nitinat River	Puntledge River	Quinsam River	Robertson Creek	YIAL
CAL	Control	0.59	0.68	0.71	0.00	0.87	0.00	0.86	0.77
CK-1	Control	0.00	0.34	0.00	0.00	0.00	0.00	0.24	0.00
IL-1B	Control	0.40	0.00	0.00	0.00	0.38	0.55	0.00	0.00
IL-8	Control	0.00	0.00	0.15	0.45	0.00	0.49	0.64	0.55
MHCIIB	Control	0.00	0.35	0.00	0.35	0.00	0.00	0.73	0.00
NKEF	Control	0.00	0.42	0.00	0.00	0.00	0.00	0.31	0.54
SAA	Control	0.00	0.48	0.00	0.76	0.37	0.00	0.00	0.65
TNF-a	Control	0.00	0.03	0.00	0.00	0.00	0.42	0.00	0.59
EGR-1	Control	0.00	0.00	0.00	0.42	0.41	0.00	0.19	0.66
GH-R	Control	0.00	0.68	0.00	0.57	0.00	0.75	0.63	0.00
IGF-1	Control	0.11	0.35	0.00	0.28	0.12	0.00	0.00	0.22
IGFBP2b	Control	0.00	0.00	0.00	0.00	0.00	0.64	0.00	0.00
LEPTIN	Control	0.00	0.00	0.01	0.33	0.00	0.00	0.00	0.66
MYO1a	Control	0.22	0.44	0.42	0.29	0.12	0.07	0.67	0.00
p53	Control	0.29	0.00	0.00	0.00	0.54	0.00	0.00	0.73
THR-b	Control	0.46	0.45	0.06	0.39	0.37	0.79	0.00	0.00
COI	Control	0.38	0.18	0.00	0.79	0.07	0.38	0.00	0.00
CPT1	Control	0.00	0.00	0.00	0.77	0.19	0.59	0.73	0.00
CYP1a	Control	0.37	0.80	0.47	0.41	0.90	0.21	0.34	0.67
FAS	Control	0.61	0.94	0.90	0.00	0.84	0.80	0.88	0.87
PEPCK	Control	0.15	0.12	0.00	0.57	0.59	0.67	0.29	0.15
PK	Control	0.25	0.40	0.35	0.10	0.00	0.00	0.41	0.05
GR2	Control	0.20	0.27	0.00	0.00	0.00	0.61	0.62	0.00
hsp70	Control	0.61	0.00	0.00	0.00	0.00	0.36	0.00	0.00

hsp90a	Control	0.99	0.96	0.00	1.00	0.00	0.00	0.00	0.08
metA	Control	0.00	0.00	0.00	0.41	0.71	0.00	0.00	0.54
CAL	Immune	0.49	0.00	0.00	0.67	0.00	0.17	0.00	0.65
CK-1	Immune	0.58	0.00	0.00	0.00	0.00	0.30	0.00	0.77
IL-1B	Immune	0.00	0.00	0.82	0.67	0.42	0.64	0.00	0.00
MHCIIB	Immune	0.30	0.32	0.21	0.74	0.00	0.14	0.04	0.00
NKEF	Immune	0.28	0.00	0.00	0.19	0.56	0.59	0.12	0.17
EGR-1	Immune	0.00	0.00	0.00	0.69	0.32	0.79	0.71	0.59
GH-R	Immune	0.00	0.41	0.00	0.69	0.00	0.67	0.38	0.30
IGF-1	Immune	0.63	0.07	0.66	0.81	0.54	0.76	0.69	0.00
LEPTIN	Immune	0.48	0.33	0.00	0.39	0.55	0.51	0.63	0.00
p53	Immune	0.36	0.00	0.00	0.33	0.74	0.59	0.20	0.00
CYP1a	Immune	0.68	0.43	0.00	0.81	0.51	0.18	0.24	0.78
PK	Immune	0.62	0.29	0.00	0.84	0.00	0.62	0.00	0.02
IL-8	Stress	0.00	0.00	0.19	0.00	0.00	0.00	0.13	0.00
hsp90a	Stress	0.00	0.64	0.00	0.58	0.71	0.59	0.62	0.00

Appendix C1 – Percent variance explained by each principal component

The variance explained for each principal component (PC) across all 12 principal component analyses (three treatments x four gene groups). The first two PCs were used in each group for correlational analyses with saltwater performance, except for the control group immune genes where the first three PCs were used.

Treatment	Gene Group	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Control	Immune	50.1 %	19.5 %	16.1 %	6.6 %	4.3 %	2.6 %	0.80 %	0.097 %
	Growth	40.0 %	29.6 %	12.8 %	7.2 %	3.4 %	2.9 %	1.7 %	0.50 %
	Metabolic	65.1 %	22.6 %	7.0 %	3.2 %	1.6 %	0.54 %	---	---
	Stress	58.6 %	26.3 %	14.9 %	---	---	---	---	---
Immune Treatment	Immune	62.5 %	15.9 %	11.6 %	4.1 %	2.9 %	2.0 %	0.98 %	0.062 %
	Growth	41.6 %	35.6 %	7.5 %	5.3 %	4.6 %	2.8 %	1.6 %	1.00 %
	Metabolic	49.9 %	26.7 %	13.8 %	6.0 %	2.5 %	1.2 %	---	---
	Stress	65.6 %	30.5 %	4.0 %	---	---	---	---	---
Handling Stress Treatment	Immune	58.1 %	23.5 %	6.4 %	5.9 %	2.8 %	1.6 %	1.4 %	0.31 %
	Growth	53.8 %	19.7 %	13.3 %	6.2 %	3.5 %	1.9 %	0.99 %	0.57 %
	Metabolic	70.6 %	18.8 %	6.8 %	1.9 %	1.3 %	0.56 %	---	---
	Stress	63.4 %	27.5 %	9.1 %	---	---	---	---	---

Appendix C2 – The factor loading for each PCA

The factor loading of each PCA is given, those in bold were considered to make up a significant amount of the variance for that PC. Genes were considered significant if the factor loading was > 0.3 or < -0.3.

Treatment Group	Gene Group	Gene	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
Control	Immune	CAL	-0.030	0.17	-0.018	**0.54**	-0.11	**0.75**	-0.035	**0.31**
		CK-1	0.020	0.053	-0.24	**0.70**	**0.49**	**-0.46**	-0.037	0.0074
		IL-1B	**-0.73**	**-0.50**	**-0.47**	-0.0079	-0.069	0.067	-0.016	-0.0013
		IL-8	-0.13	**0.40**	-0.15	0.14	**-0.68**	**-0.63**	**-0.42**	0.10
		MHCIIB	-0.089	0.22	-0.090	0.17	**-0.32**	-0.090	**0.87**	-0.22
		NKEF	0.013	0.013	0.013	0.18	-0.050	0.22	**-0.27**	**-0.92**
		SAA	**-0.59**	0.096	**0.78**	0.13	0.093	-0.11	-0.0067	0.00025
		TNF-a	**-0.31**	**0.71**	**-0.30**	**-0.34**	**0.41**	0.14	-0.025	-0.045
	Growth	EGR-1	**-0.42**	**0.58**	**0.69**	-0.065	-0.074	0.0049	0.011	0.018
		GH-R	-0.23	-0.20	0.11	0.20	**0.67**	**0.57**	-0.16	0.23
		IGF-1	-0.074	**-0.58**	**0.46**	**0.46**	**-0.26**	-0.11	**0.38**	0.074
		IGFBP2b	-0.067	-0.083	0.11	**0.32**	0.18	-0.21	**-0.45**	**-0.77**
		LEPTIN	**-0.41**	**0.35**	**-0.49**	**0.63**	-0.046	-0.075	0.24	0.058
		MYO1a	**-0.77**	**-0.38**	-0.20	**-0.43**	-0.14	-0.086	-0.069	-0.069
		p53	-0.00051	-0.024	-0.026	0.21	**-0.63**	**0.44**	**-0.58**	0.16
		THR-b	-0.025	-0.049	0.054	0.12	0.17	**-0.64**	-0.048	**0.56**
	Metabolic	COI	0.017	-0.054	**-0.989**	0.086	-0.060	-0.099	---	---
		CPT1	-0.041	0.030	0.030	**-0.30**	**0.44**	**-0.85**	---	---
		CYP1a	**-0.30**	-0.14	-0.063	**-0.86**	**-0.36**	0.12	---	---
		FAS	**-0.94**	-0.11	0.0077	**0.29**	0.13	0.0041	---	---
		PEPCK	0.043	-0.12	-0.11	**-0.26**	**0.81**	**0.50**	---	---
		PK	0.14	**-0.98**	0.078	0.11	-0.046	-0.10	---	---
	Stress	GR2	-0.12	0.23	**0.97**	---	---	---	---	---
		hsp70	**-0.99**	-0.021	-0.12	---	---	---	---	---

		metA	-0.0070	**-0.97**	0.23	---	---	---	---	---
Immune Treatment	Immune	CAL	-0.023	0.17	0.0020	0.12	**-0.58**	**0.71**	-0.24	-0.24
		CK-1	-0.098	0.089	0.089	**0.51**	**-0.60**	**-0.58**	0.11	-0.034
		IL-1B	**-0.95**	0.0024	**0.25**	-0.18	0.029	-0.0074	-0.087	0.0022
		IL-8	-0.10	0.24	0.0092	**0.76**	**0.51**	0.14	**-0.27**	0.013
		MHCIIB	-0.044	-0.080	**0.30**	0.22	0.074	**0.32**	**0.86**	-0.050
		NKEF	-0.0041	0.057	0.028	0.043	-0.17	0.17	-0.0080	**0.97**
		SAA	0.11	**0.92**	0.24	**-0.26**	0.056	-0.11	0.11	-0.020
		TNF-a	**-0.26**	0.24	**-0.89**	0.013	-0.015	0.026	**0.30**	0.0058
	Growth	EGR-1	0.011	**-0.95**	0.15	0.032	-0.10	-0.24	0.028	-0.11
		GH-R	-0.13	-0.23	-0.15	-0.21	**-0.37**	**0.70**	-0.15	**0.47**
		IGF-1	-0.045	0.083	0.20	**-0.66**	-0.097	**-0.43**	**0.31**	**0.47**
		IGFBP2b	-0.055	0.017	-0.033	**-0.34**	-0.21	**0.32**	**0.60**	**-0.61**
		LEPTIN	-0.12	-0.17	**-0.83**	**-0.25**	**0.45**	-0.091	0.030	0.011
		MYO1a	**-0.97**	0.037	0.075	0.18	-0.029	-0.098	0.050	-0.0071
		p53	-0.025	0.12	**-0.30**	-0.20	**-0.64**	**-0.34**	**-0.49**	**-0.30**
		THR-b	-0.13	-0.037	**0.37**	**-0.52**	**0.42**	0.19	**-0.53**	**-0.29**
	Metabolic	COI	**0.29**	**0.61**	**-0.59**	-0.078	**-0.40**	0.15	---	---
		CPT1	-0.022	0.045	-0.23	0.24	**0.61**	**0.71**	---	---
		CYP1a	**0.36**	-0.10	-0.12	**0.86**	0.0037	**-0.32**	---	---
		FAS	**0.85**	**-0.35**	0.073	**-0.35**	0.085	0.12	---	---
		PEPCK	0.053	**0.28**	**-0.27**	**-0.25**	**0.67**	**-0.59**	---	---
		PK	0.23	**0.64**	**0.72**	0.11	0.092	0.085	---	---
	Stress	GR2	**-0.30**	**0.33**	**0.90**	---	---	---	---	---
		hsp70	**-0.76**	**-0.65**	-0.022	---	---	---	---	---
		metA	**-0.57**	**0.69**	**-0.44**	---	---	---	---	---
Handling Stress Treatment	Immune	CAL	0.016	-0.015	**0.34**	**-0.69**	0.11	**0.49**	-0.24	**0.32**
		CK-1	0.10	0.19	-0.12	-0.062	**-0.94**	0.21	0.069	0.069
		IL-1B	**0.96**	-0.086	0.20	0.13	0.061	0.044	0.058	-0.023
		IL-8	0.13	0.014	-0.00053	**-0.41**	-0.16	**-0.73**	**-0.50**	-0.093

		MHCIIB	0.017	0.0016	0.053	**-0.45**	0.017	**-0.33**	**0.82**	0.061
		NKEF	-0.027	-0.0078	0.11	-0.23	-0.020	0.23	0.028	**-0.94**
		SAA	0.060	**0.98**	0.064	0.023	0.19	-0.020	-0.0051	-0.017
		TNF-a	0.21	0.017	**-0.90**	**-0.28**	0.20	0.17	-0.033	-0.014
	Growth	EGR-1	**0.89**	-0.080	**0.29**	**-0.25**	0.11	-0.13	0.14	-0.092
		GH-R	0.21	-0.17	**-0.30**	**-0.31**	**-0.45**	**0.43**	**-0.59**	0.10
		IGF-1	-0.066	-0.23	**0.48**	**-0.44**	-0.24	-0.095	**0.66**	0.11
		IGFBP2b	-0.019	0.0070	**-0.38**	-0.12	0.070	**-0.46**	**-0.27**	**-0.75**
		LEPTIN	**0.39**	0.040	**-0.56**	**0.71**	-0.017	0.055	0.11	0.088
		MYO1a	-0.083	**-0.95**	0.14	0.23	0.097	-0.017	-0.045	-0.078
		p53	-0.0022	-0.041	**-0.34**	**-0.26**	**0.84**	0.19	-0.14	0.22
		THR-b	0.022	-0.046	-0.060	-0.027	-0.078	**-0.74**	**-0.31**	**0.59**
	Metabolic	COI	0.095	0.048	**-0.85**	**0.40**	-0.24	0.21	---	---
		CPT1	-0.020	0.012	0.090	0.24	**0.67**	**0.69**	---	---
		CYP1a	**-0.35**	-0.020	**0.26**	**0.83**	0.028	**-0.35**	---	---
		FAS	**-0.93**	-0.061	-0.20	**-0.28**	-0.027	0.12	---	---
		PEPCK	0.033	0.059	**-0.40**	-0.13	**0.70**	**-0.58**	---	---
		PK	0.067	**-0.99**	-0.0096	0.030	-0.043	0.052	---	---
	Stress	GR2	0.055	**-0.37**	**0.93**	---	---	---	---	---
		hsp70	**-0.95**	**-0.30**	-0.064	---	---	---	---	---
		metA	**0.31**	**-0.88**	**-0.37**	---	---	---	---	---

Appendix C3 – Individual gene correlations with saltwater performance

The individual gene transcription was averaged for each family (N = 4) across all 8 populations and correlated with the average growth rate and survival in the saltwater phase. Significant correlations are in bold face and denoted with a *.

Gene Group	Candidate Gene	Performance Factor	R^2	p-value
Immune	CAL	Survival	0.011	0.56
		Growth Rate	0.15	**0.031 ***
	CK-1	Survival	0.015	0.50
		Growth Rate	0.072	0.14
	IL-1B	Survival	0.0057	0.68
		Growth Rate	0.0013	0.84
	IL-8	Survival	0.090	0.10
		Growth Rate	0.22	**0.0065 ***
	MHCIIB	Survival	0.037	0.29
		Growth Rate	0.23	**0.005 ***
	NKEF	Survival	0.011	0.57
		Growth Rate	0.011	0.57
	SAA	Survival	0.19	**0.012 ***
		Growth Rate	0.00015	0.95
	TNF-a	Survival	0.076	0.13
		Growth Rate	0.17	**0.018 ***
Growth	EGR-1	Survival	0.060	0.18
		Growth Rate	0.059	0.18
	GH-R	Survival	0.067	0.15
		Growth Rate	0.00018	0.94
	IGF-1	Survival	0.032	0.33
		Growth Rate	0.24	**0.005 ***
	IGFBP2b	Survival	0.19	**0.013 ***
		Growth Rate	0.15	**0.030 ***
	LEPTIN	Survival	0.056	0.19

		Growth Rate	0.024	0.40
	MYO1a	Survival	0.0059	0.68
		Growth Rate	0.043	0.26
	p53	Survival	0.12	0.054
		Growth Rate	0.010	0.59
	THR-b	Survival	0.054	0.20
		Growth Rate	0.091	0.093
Metabolic	COI	Survival	0.12	**0.047 ***
		Growth Rate	0.036	0.30
	CPT1	Survival	0.033	0.32
		Growth Rate	0.017	0.48
	CYP1a	Survival	0.00	0.99
		Growth Rate	0.15	**0.030 ***
	FAS	Survival	0.0031	0.76
		Growth Rate	0.079	0.12
	PEPCK	Survival	0.029	0.36
		Growth Rate	0.0084	0.62
	PK	Survival	0.025	0.39
		Growth Rate	0.036	0.30
Stress	GR2	Survival	0.0054	0.69
		Growth Rate	0.044	0.25
	hsp70	Survival	0.20	**0.011 ***
		Growth Rate	0.22	**0.007 ***
	hsp90a	Survival	0.18	0.079
		Growth Rate	0.099	0.20
	metA	Survival	0.013	0.53
		Growth Rate	0.042	0.26

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