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# The Effects of Crossbreeding and Low Fish Meal Diets on Growth-Related Traits in Chinook Salmon (*Oncorhynchus tshawytscha*)

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Graduate Program in Biology

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## Abstract

Growth rate is the most important trait that can be manipulated to create more efficient aquaculture. Crossbreeding, where different populations are bred, has the potential to increase performance through release from inbreeding depression. I crossed a farm population of Chinook salmon (*Oncorhynchus tshawytscha*) with seven wild populations, then compared growth rate, feed conversion efficiency, swimming speed and metabolic rate between the crossbred and original farmed lines. Crossbreeding resulted in increased growth rates, but had no effect on the other traits. I next evaluated the feasibility of using a diet that replaced fish meal with corn gluten meal and poultry meal. The alternative diet had no effect on growth rate or survival, but led to increased fat content and decreased tissue pigmentation. My thesis supports using crossbreeding in salmon aquaculture to increase growth rate, but found a low fish meal diet was not viable due to its effects on tissue colour.

## Keywords

Chinook salmon, aquaculture, crossbreeding, heterosis, alternative feed, growth rate, tissue quality, swimming speed, metabolic rate

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# Table of Contents

Abstract .....	i
Acknowledgments.....	ii
Table of Contents .....	iii
List of Tables .....	iv
List of Figures .....	v
List of Abbreviations .....	<b>Error! Bookmark not defined.</b>
Animal care protocol.....	<b>Error! Bookmark not defined.</b>
1 Introduction .....	1
2 Methods.....	9
2.1 Creating experimental lines .....	9
2.2 Crossbreeding study.....	11
2.3 Alternative diet study.....	14
2.4 Statistical analysis.....	18
3 Results.....	20
3.1 Crossbreeding study.....	20
3.2 Alternative diet study.....	30
4 Discussion .....	36
4.1 Summary and Conclusions .....	42
5 References .....	43
Curriculum Vitae .....	57

## List of Tables

Table 1. Ingredients and nutrient composition of the high fish meal diet (High FM) and low fish meal diet (Low FM). .....	15
Table 2. Summary of eight Chinook salmon ( <i>Oncorhynchus tshawytscha</i> ) populations used to create the hybrid lines. Location is provided for the corresponding hatcheries. YIAL is a cross of Big Qualicum and Robertson Creek populations, and therefore does not have a location. Pairwise $F_{ST}$ values for each population relative to YIAL are included. ....	10
<b>Table 3.</b> Average body mass ( $g \pm SE$ ) of eight lines of Chinook salmon ( <i>Oncorhynchus tshawytscha</i> ) during both short-term hatchery and long-term net pen intervals. Test statistics (degrees of freedom, F statistic, P value) are included for the ANOVA comparing body mass among lines at each measurement point. ....	23
Table 4. Body mass and total body length of Chinook salmon ( <i>Oncorhynchus tshawytscha</i> ) fed either the control high fish meal (High FM) or low fish meal (Low FM) diets at four sampling points. Test statistics (F statistic, degrees of freedom, P value) are included. ....	31
Table 5. Survivorship of Chinook salmon ( <i>Oncorhynchus tshawytscha</i> ) fed either the high fish meal (high FM) or the low fish meal (low FM) diet during three intervals. Survival (%) is calculated for each interval. Significance is reported as Fisher's exact test. ....	32

## List of Figures

Figure 1. Specific growth rate (% body mass/day) of eight lines of Chinook salmon (*Oncorhynchus tshawytscha*) measured in the hatchery. The eight lines are in order of relatedness to YIAL based on FST values. Panel A shows data from September 23<sup>rd</sup>-October 14<sup>th</sup>, 2014, panel B from October 14<sup>th</sup>–29<sup>th</sup> 2014, panel C from October 29<sup>th</sup> – November 12<sup>th</sup>, 2014, and panel D from November 12<sup>th</sup> – 26<sup>th</sup> 2014. Boxes show the 25<sup>th</sup> and 75<sup>th</sup> percentile with a line indicating the median. Whiskers show the minimum and maximum values within 1.5 lengths of the box. Outliers are designated with a black dot. Asterisks indicate significant differences from the YIAL line according to Tukey's HSD..... 25

Figure 2. Specific growth rate (% body mass/day) of eight lines of Chinook salmon (*Oncorhynchus tshawytscha*) measured in the net pens. The eight lines are in order of relatedness to YIAL based on FST values. Panel A) occurred from June – November 2014, panel B) from November 2014 – February 2015, panel C) from February – May 2015, and panel D) occurred from May – November 2015. Boxes show the 25<sup>th</sup> and 75<sup>th</sup> percentile with a line indicating the median. Whiskers show the minimum and maximum values within 1.5 lengths of the box. Outliers are designated with a black dot. Asterisks indicate significant differences from the YIAL line according to Tukey's HSD. .... 27

Figure 3. Conversion efficiency (g body mass/ g of feed), average feed eaten (% eaten of feed given), volitional swimming speed (body lengths/s) and metabolic rates (mgO<sub>2</sub>/kg/hr) of eight lines of Chinook salmon (*Oncorhynchus tshawytscha*). The hybrid lines are in order of relatedness to YIAL based on FST values. Boxes shows the 25<sup>th</sup> and 75<sup>th</sup> percentile with a line indicating the median. Whiskers show the minimum and maximum values within 1.5 lengths of the box. Outliers are designated with a black dot. Letters indicate significant differences according to Tukey's HSD. .... 29

Figure 4. Specific growth rate (% body mass/day) of Chinook salmon (*Oncorhynchus tshawytscha*) fed either the high fish meal (High FM) diet or the low fish meal (Low FM) diet. The box shows the 25<sup>th</sup> and 75<sup>th</sup> percentile with a line indicating the median. Whiskers show the minimum and maximum values within 1.5 lengths of the box. Outliers are designated with a black dot. Panel A) shows interval 1, which occurred from November 2014

– June 2015, and panel B) shows interval 2 which occurred from July 2015 – March 2016. Growth rate did not differ significantly between diets for either interval. .... 33

Figure 5. Total body fat (% body composition) of Chinook salmon (*Oncorhynchus tshawytscha*) fed either the high fish meal diet (High FM) or the low fish meal diet (Low FM). Boxes show the 25th and 75th percentile with a line indicating the median. Whiskers show the minimum and maximum values within 1.5 lengths of the box. Outliers are indicated with a dot. Fish fed the low fish meal diet had significantly higher body fat than those fed the high fish meal diet..... 34

Figure 6. Tissue colour analysis for Chinook salmon (*Oncorhynchus tshawytscha*) fed either the high fish meal diet (High FM) or the low fish meal diet (Low FM). Boxes show the 25th and 75th percentile, with a line indicating the median. Whiskers show the minimum and maximum values within 1.5 lengths of the box. Outliers are shown with a dot. Panel A) is the average Salmofan colour card measurement on a scale of 20 (lightest colouration) to 30 (darkest colouration) and panel B) is the average peak area of Astaxanthin content in the tissue sample (g). The low fishmeal diet was significantly lower for both colour card values and astaxanthin concentration measurements. .... 35

# 1 Introduction

Capture fishery yields have been static since the 1980's, and aquaculture is increasingly supplying the seafood demand of a growing human population (FAO, 2016). Indeed, with the decline of capture fisheries and continuing growth of the human population, aquaculture is predicted to supply the majority of fish for human consumption by 2030 (WBR, 2013). Finfish make up 65% of global aquaculture production, of which most are carnivorous species (FAO, 2010; 2014). One of the major concerns with aquaculture of carnivorous species is the reliance on capture fisheries to supply fish meal and fish oil for aquaculture feeds, which has led to overfishing and declines in lower trophic level fishes such as mackerel (*Scomber scombrus*), herring (*Clupea harengus*) and whiting (*Merlangius merlangus*) (Pauly *et al.*, 2002, Parés-Sierra *et al.*, 2014). The proportion of fish meal used for aquaculture production has increased by 20% in the last decade, suggesting continued aquaculture production will be a contributor to the collapse of fish stocks worldwide (Martinez-Porchas & Martinez-Cordova, 2012). Additionally, despite the expectation that the amount of fish produced through aquaculture will exceed the amount of beef, pork and poultry produced through agriculture within the next decade, fish domestication, breeding and husbandry are comparatively poorly understood (Lien, 2015). There are thus substantial opportunities to improve the production efficiency of aquaculture, including optimizing breeding programs to create high-performing aquaculture stocks and modifying aquaculture feed composition to relieve pressure on capture fisheries.

Of the 362 species of finfish farmed globally, salmonids make up 30% of total finfish production. Canada is the world's fourth largest producer of farmed salmon, producing Atlantic salmon (*Salmo salar*), Chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) (FAO, 2016; DFO, 2010; 2015). Within Canada, Chinook salmon are farmed predominantly in British Columbia, where they are native, and make up approximately 40% of farmed salmon (Bryden *et al.*, 2004). Chinook salmon is the largest of the salmonid species, a mature (4 years old) Chinook salmon weighs an average of 18 kg, more than three times the size of an Atlantic salmon of the



same age (NOAA, 2016). In the wild, Chinook salmon are anadromous and have a single spawning event in which they return to their natal streams to lay eggs before dying (Groot and Margolis, 1991). It is this anadromy and strong homing tendency that lead to local adaptations to natal streams, which has led to reproductively isolated sub-populations on relatively small geographic scales (Groot and Margolis, 1991). This reproductive life history trait makes Chinook salmon an excellent model species to conduct studies on how breeding design can affect fitness and performance traits as a result of crossbreeding between populations of differing genetic relatedness. To date, however, studies on the effectiveness of crossbreeding and diet are focused on Atlantic salmon, coho salmon and rainbow trout (*Oncorhynchus mykiss*; e.g. Bryden *et al.*, 2004; Gatlin *et al.*, 2007).

Domesticated high-performing lines of salmon have been developed via both long-term natural selection to captivity, and accumulation of genetic gain, such as beneficial mutations and allele combinations, from selective breeding (Bentsen & Thodesen, 2005; Bryden *et al.*, 2004). For example, selective breeding has led to improved growth rate in rainbow trout (Gjerde, 1986), Atlantic salmon (Gjerde, 1986; Gjerde & Korsvoll, 1999; Gjedrem, 2000) and coho salmon (Hershberger *et al.*, 1990; Myers *et al.*, 2001). However, selective breeding is dependent on the traits of interest expressing additive genetic variance (Gjedrem, 1997). Additionally, intense directional selection can lead to inbreeding depression, which is common in aquaculture stocks that are already derived from relatively few fish and bred within a small closed population (Gjøen and Bentsen, 1997). Crossbreeding, a technique in which different lines or populations of the same species are bred, has the potential to improve performance in aquaculture (Bentsen and Thodesen, 2005). Crossbreeding is an alternative approach that can improve performance and counteract the effects of inbreeding depression via genetic rescue. Genetic rescue restores genetic diversity in a population thereby increasing fitness, which occurs via heterosis (Whiteley *et al.*, 2015). Heterosis is thought to occur through two mechanisms, heterozygote advantage, where heterozygosity at loci provides greater fitness than either homozygote, or dominance effects, where one parent's dominant allele masks the other parent's deleterious recessive allele (Edmands, 2007; Lynch, 1991). However, crossbreeding can alternatively lead to reduced performance in offspring, characterized as

outbreeding depression, due to genetic incompatibilities between the parental strains (Lynch, 1991a; Edmands, 2007). Outbreeding is a threat to environmentally adapted populations, such as aquaculture production populations, as it can swamp local adaptation removing fixed beneficial mutations or allele combinations, leading to poorer performance in a specific environment (Edmands, 2007; Frankham *et al.*, 2011). Crossbreeding may thus be an effective way to increase performance and yield in aquaculture, but there is still uncertainty about implementation and appropriate populations or strains to be used to achieve the desired results.

Genetic distance between populations is a potential predictor of performance in crossbred offspring (Edmands & Timmerman 2014). Though there are many tools to determine genetic similarity between populations,  $F_{ST}$  is the most widely used descriptive statistic in population genetics, and offers a metric to compare genetic dissimilarity of populations using neutral markers (Holsinger and Weir, 2009).  $F_{ST}$  is a measure of population differentiation based on the variance of allele frequencies and the probability of identity by descent, allowing the interpretation of genetic diversity shared between two populations (Holsinger and Weir, 2009). It is widely assumed that increased genetic distance between parental lines, or higher  $F_{ST}$  values, results in increased performance of offspring (Waser, 1993) and this trend has been exhibited in multiple species such as the freshwater snail (*Physa acuta*; Escobar *et al.*, 2008) and the clam (*Meretrix meretrix*, Lu *et al.*, 2012). However, the evidence from fish, specifically salmonids, is less clear as most studies on crossbreeding have not assessed parental genetic similarity. A study on intraspecific crosses between different populations of Atlantic salmon with  $F_{ST}$  values between 0.035 and 0.095 (Houde *et al.*, 2011) and another study on crossbred lines of coho salmon with  $F_{ST}$  values between 0.026-0.032 (Dann *et al.*, 2010) did not find any effects of crossbreeding in a number of fitness-related traits. Further studies of the relationship between genetic distance and performance traits are needed to assess the usefulness of this approach for salmonids. It is necessary to assess the use of genetic distance as a predictor of performance in Chinook salmon crossbred progeny, to justify its use as a basis for aquaculture breeding programs.

Growth rate is generally considered the most important trait in aquaculture, so crossbreeding will be most valuable if it improves this trait. Crossbreeding has been shown to increase growth rate in species such as carp (*Cyprinus carpi*; Wohlfarth, 1993; Hulata, 1995), tilapia (*Oreochromis niloticus*; e.g. Earnst *et al.*, 1991; Hulata *et al.*, 1993), and catfish (*Clarias* spp.; e.g. Hulata, 2001). Crossbreeding is less common in salmonid aquaculture, in large part due to the uncertainty about the effects of crossbreeding on growth rate. Studies of Atlantic salmon (Einum and Fleming, 1997) and rainbow trout (Wangila and Dick, 1996) found that crossbreeding between farmed and wild lines led to significant increases in growth rate, although similar studies of Chinook salmon failed to replicate these results (Cheng *et al.*, 1987; Bryden *et al.*, 2004). Thus, there is uncertainty about the effects of crossbreeding on growth rate in salmon, as well as the underlying mechanisms through which crossbreeding might alter growth rate (Einum and Fleming, 1997; Wangila and Dick, 1996).

Four traits that may underlie differences in growth rate are feed consumption, feed conversion efficiency, volitional swimming speed and metabolic rate. Feed consumption determines the total amount of energy available to an individual for growth and other processes, and has previously been shown to be positively associated with growth rate (e.g. Cook *et al.*, 2000). Feed conversion efficiency, an individual's ability to convert feed into biomass, may increase growth rate by allowing individuals fed a fixed ration to allocate a greater proportion of their diet to growth, or alternatively may reduce aquaculture costs by allowing fish to achieve a similar growth rate with less food. Additionally, studies on Atlantic salmon found a genetic relationship between growth rate and feed consumption and utilization, where farm lines selected over multiple generations for increased growth had an associated increase in feed conversion efficiency (Thodesen *et al.*, 1999). Daily swimming activity uses up to 52% of salmon's expendable energy and is thus a major determinant of metabolic rate (Brown and Geist, 2002). Previous studies on salmonids have reported that lower swimming speeds result in reduced metabolic rate (Linnér and Brännäs, 2001; Jobling, 1994; Carter *et al.*, 2001), potentially leading to more energy that can be allocated to growth. Swimming speed has exhibited high heritability in Atlantic salmon and the strong association between swimming speed and metabolic rate suggests both could be affected by crossbreeding (Hurley and Schom,

1984; Ohlberger et al. 2005). However, the effects of crossbreeding have not been assessed for these traits.

Breeding designs to harness the effects of non-additive genetic variation will likely result in increased performance of offspring, but other methods, such as modifying feeds, can also improve aquaculture efficiency. Salmonid aquaculture is dependent on the availability of high protein feeds (typically 40-50% protein by mass) (Hardy, 1996). Feed is the most costly component in salmon aquaculture production, making up 51% of the total production cost (DFO, 2010). This cost comes from the requirement of high levels of fish meal and fish oil, which are used due to their high protein content (60-70%) and balanced composition of amino acids required by salmonids (Gatlin *et al.*, 2007).

However, many fisheries that support fish meal production are in decline, leading to decreased availability and increasing costs of fish meal and fish oil (Gatlin *et al.*, 2007). With the prediction that aquaculture will double in the next 10 years (WBR, 2013), the need to determine alternative protein sources for salmonid diets is critical to lower costs, alleviate pressure on capture fisheries and ensure sustained aquaculture production into the future. There is thus a need to identify alternative protein sources that can serve as a replacement for fish meal in salmonid feeds. These alternative protein sources will need to provide similar essential nutrients, including protein and fat levels and amino acid and fatty acid composition (Gatlin *et al.*, 2007). Ultimately, the feasibility of incorporating alternative protein sources into salmonid feeds will depend on the effects of those feeds on key aquaculture performance traits that include growth rate, fat content and tissue colour (Koteng, 1992).

As with crossbreeding programs growth rate is the one of the traits of highest importance when determining the efficacy of alternative protein sources for aquaculture. Salmonid feeds that include plant protein sources, such as soybean meal or corn gluten meal, have previously been shown to lead to reduced growth rate in Atlantic salmon and rainbow trout (e.g. Mambrini *et al.*, 1999; Mundheim *et al.*, 2004). Plant sources are thought to cause reduced growth rates due to their amino acid composition and low digestible protein/energy ratio (Gatlin *et al.*, 2007). Digestible protein/energy ratio is the fraction of protein that was not damaged (denatured) during processing and the remaining fraction

which the individual possesses the appropriate enzymes to break down (Gatlin *et al.*, 2007). Due to the less than favourable results of plant protein studies, poultry based protein sources have become a topic of focus due to their high protein content, comparatively higher digestible protein/energy ratio and amino acid composition similar to that of fish meal (Hatlen *et al.*, 2015; Sealey *et al.*, 2011). However, salmonid feeds that include poultry protein sources have shown mixed effects on growth where replacing 40-50% of fish meal with poultry meal had no effect on growth in rainbow trout (Steffens, 1994; Sealey *et al.*, 2011), but replacing 30% of fish meal with poultry meal in a Chinook salmon diet led to significantly lower growth rates (Fowler, 1991). Fish meal substitution with poultry meal likely results in reduced growth rates due to a methionine deficiency which can cause immune suppression leading to decreased growth and survival (Sealey *et al.*, 2011). Studies on Atlantic salmon diets that replaced 50% of fish meal with soy and poultry meals (Hatlen *et al.*, 2015) or rainbow trout diets that replaced 75% of fish meal with soy, corn and poultry meals (Lu *et al.*, 2015) led to growth rates equal to the control diets. In these cases, the amino acid composition deficiencies in plant and poultry sources appear to compensate for each other. Based on these results, salmonid diets that include a mixture of plant and poultry proteins sources have the potential to maintain normal growth rates in aquaculture.

In addition to supplying energy required for growth rate, salmonid diets must also ensure maintenance of adequate flesh quality (e.g. fat composition, tissue colour), especially when assessing alternative protein sources. Replacing fish meal with alternative protein sources can result in altered body composition, specifically fat content, which influences tissue texture, flavour and therefore marketability (Bjerkeng *et al.*, 1997; Gatlin *et al.*, 2007). Plant protein sources have a low crude lipid content, which must be supplemented, commonly using fish oil, to create a balanced fatty acid profile (Gatlin *et al.*, 2007; Parés-Sierra *et al.*, 2014). In contrast, poultry meal has a high crude fat content, and its inclusion in alternative aquaculture diets at levels as low as 25% has led to increased body fat in Atlantic salmon and rainbow trout (Sealey *et al.*, 2011; Steffens, 1994). Both plant and poultry meal are extremely low in omega-3 fatty acids (<1%), which is concerning due to the role fatty acids play in cell membrane composition and growth in salmonids (NRC, 1993; Parés-Sierra *et al.*, 2014). Generally, salmonids have an essential

omega-3 fatty acid requirement of 1% of the diet, which can be met by including additional dietary fatty acids, commonly acquired from fish oil (NRC, 1993). Though studies using a combination of plant and animal protein sources have not been assessed for body fat content, the use of plant meal may mitigate the high levels of crude fat in poultry meal, and the low crude fat content in plant sources will leave a margin to add essential fatty acids (fish oil) without creating a high fat diet.

Another major concern in diet formulation for salmonids is the maintenance of red flesh colouration, as this is the most important factor for the consumer. This colouration results from the uptake of carotenoid pigments from the diet followed by deposition in the muscle fibers. Astaxanthin is the primary carotenoid responsible for this red colouration and is also the major precursor to vitamin A (Anderson *et al.*, 2000; Saez *et al.*, 2016). In previous studies of rainbow trout, diets that included plant protein sources, mainly corn gluten meal, were associated with reduced tissue colouration relative to fish meal diets (Saez *et al.*, 2014; 2016). This difference may occur because corn gluten meal contains high levels of yellow xanthophylls (lutein and zeaxanthin; 200–500 mg/kg) (Park *et al.*, 1997; Skonberg *et al.*, 1998), which do not impart the same red flesh colouration and could limit astaxanthin uptake due to passive competition during digestion (Furr and Clark, 1997; Olsen and Baker, 2006). The effects of diets containing poultry meal on salmonid flesh colouration have not been assessed, but little effect on tissue colouration is predicted because poultry meal contains little carotenoids (Moreno *et al.*, 2016). The effects of diets containing both corn gluten meal and poultry meal on flesh colouration are uncertain.

Due to the relative infancy of the aquaculture industry, domestication of fish is poorly understood so I assessed both the effects of crossbreeding and an alternative diet on Chinook salmon. For this study, I first crossed seven wild paternal populations of Chinook salmon with females from a farm production population of Chinook salmon, creating 7 hybrid lines, to assess crossbreeding effects on growth and growth related traits when compared to the original farmed line. I hypothesized that crossbreeding between a farm line and wild lines of Chinook salmon will affect growth rate and performance in growth-related traits through genetic rescue from inbreeding depression in the farm line

Using a subset of these offspring I then assessed the feasibility of using an alternative feed that replaced fish meal with animal and plant protein sources. I also hypothesized that replacing standard fishmeal with poultry meal and corn gluten meal will meet all nutritional requirements of Chinook salmon. Several lines of evidence indicate that the farm line (Yellow Island Aquaculture Ltd) is inbred, including small breeding population size, high incidence of juvenile deformities, high homozygosity at seven microsatellite loci and low egg to smolt survival (Heath, 2002, unpublished data). The seven populations used to create the lines were located across Vancouver Island and mainland British Columbia, offering a span of genetic relatedness's between the hybrid lines produced. This is the first study to assess crossbreeding on performance across a range of genetic distances between parents, and at multiple life stages in the offspring. Five performance traits (growth rate, feed consumption, feed conversion, swimming speed and metabolic rate) were measured and compared between the farm line and crossbred lines. I predicted that there would be increased performance in crossbred offspring from intermediate genetic relatedness across the lines used. If crossbreeding at these genetic distances produces increased growth rate, I predicted there would be an associated increase in feed conversion efficiency due to the expected genetic relationship between these traits. Additionally, due to the heritability of swimming speed and metabolic rate, I predicted crossbreeding would affect these traits. Currently there are no studies assessing the efficacy of a combination protein source diet for Chinook salmon aquaculture. My study examined the effects of using a combination of corn gluten meal (47%) and poultry meal (27%) to replace 74% of the fish meal in an aquaculture diet fed to Chinook salmon. Four traits (growth rate, survival, fat content and colouration) were analyzed and compared between fish fed the alternative diet or a conventional fish meal based diet. I predict that poultry meal will offset any differences in amino acid composition in the corn gluten meal leading to normal growth rate, while the low level of poultry meal (>30%) will limit any excessive fat accumulation. The level of corn gluten meal in the diet is lower than previous studies, suggesting fewer novel xanthophylls to interfere with colour deposition in the muscle, so I predicted that tissue colour would not be affected.

## 2 Methods

### 2.1 Creating experimental lines

Experiments were conducted at Yellow Island Aquaculture Ltd. (YIAL), a Chinook salmon farm located on Quadra Island, British Columbia. The YIAL population was founded between 1988 and 1990 with females from the Robertson Creek (RC) population and males from the Big Qualicum River (BQ) population. In October 2013, ten males (n=80) were collected from the Chilliwack River (Chil), Puntledge River (Punt), Nitinat River (Nit), Big Qualicum River (BQ), Robertson Creek (RC), Capilano River (Cap), Quinsam River (Quin) by department of fisheries and oceans (DFO) employees and sacrificed for their milt (Table 1). The milt was brought to YIAL, where YIAL employees also captured and sacrificed 10 YIAL males for their milt. YIAL employees then captured 16 YIAL females which were sacrificed for their eggs, eggs were collected and pooled, then divided into eight equal portions. Collaborators from university of Windsor then pooled the milt from ten males from a single location and used it to fertilize one portion of the eggs. To create a control line, collaborators from the university of Windsor fertilized a portion of eggs with the milt of ten YIAL males. This design resulted in a total of eight lines (7 crossbred, 1 control).

The fertilized eggs were reared separately, by line, in Heath incubation trays until emergence, at which time they were transferred to 190L cylindrical tanks in the hatchery. Water was continuously aerated, and temperature was maintained at 7-9°C by a flow through system. Fish were kept on a 12h light:12h dark photoperiod and fed to satiation twice daily using Chinook grower feed (Table 2; Taplow Feeds Inc., Vancouver, British Columbia). Once fish reached a mass of 3 g collaborators from the university of Windsor injected fish in the stomach cavity with a passive integrated transponder (PIT) tag to identify individuals (BioMark Ltd. HPT12, 1mm tags). Tags were implanted using a MK7 implanter and read with a 601 reader, to identify individuals (BioMark Ltd.).



**Table 1.** Summary of eight Chinook salmon (*Oncorhynchus tshawytscha*) populations used to create the hybrid lines. Location is provided for the corresponding hatcheries. YIAL is a cross of Big Qualicum and Robertson Creek populations, and therefore does not have a location. Pairwise  $F_{ST}$  values for each population relative to YIAL are included.

Population	Location		$F_{ST}$ from YIAL
	Latitude (°)	Longitude (°)	
YIAL			0
Big Qualicum	49.30	-124.55	0.0053
Robertson Creek	49.31	-124.96	0.0054
Capilano	49.34	-123.11	0.0089
Quinsam	50.01	-125.30	0.0128
Puntledge	49.68	-125.03	0.0151
Chilliwack	53.41	-122.57	0.0226
Nitinat	48.84	-125.63	0.0235

## 2.2 Crossbreeding study

### Calculating genetic distance

To examine the genetic similarity among lines, I calculated pairwise  $F_{ST}$  between each of the 7 paternal wild populations and the YIAL population. Pairwise  $F_{ST}$  was calculated using allele frequency data from thirteen microsatellite loci for each population, collected by DFO (DFO, 2006). Allele frequencies in the YIAL population were assumed to be intermediate between the Robertson Creek and Big Qualicum populations, its original founders. I estimated pairwise  $F_{ST}$  indices by analysis of variance using the equations of Weir and Cockerham (1984).

### Growth rate and feed utilization

On August 11, 2014, 56 fish from each line were divided into eight replicate tanks (7 fish per tank; 448 fish total; 64 total tanks) in a hatchery by YIAL employees. On September 23-24, October 13-14, October 28-29, November 11-12 and November 25-26, 2014, I seined the fish from the tanks, identified them by PIT tag and anesthetized them with clove oil (40mg/L) to measure length and body mass. Growth measurements were collected every 2 weeks to minimize handling stress while still allowing me to measure short term feed conversion efficiency. During two periods (October 30 – November 10, and November 13 – November 24), I also measured food consumption to calculate two feed conversion efficiency intervals. During these periods, all tanks were vacuumed prior to feeding to remove any waste. I provided feed as 2% of the total biomass of the tank, as determined from the most recent mass measurements. Fish were fed once a day over a 10-minute time period, 5 small feedings during the 10-minute period, between 9:00 and 15:00. After the 10-minute period the uneaten feed was removed by vacuum (Helland, 1996). The uneaten feed was then placed in a dehydrator at 45°C for two hours' time, based on preliminary trials, to return feed to its original dry mass. Feed consumption was calculated as the mass of feed provided minus the mass of uneaten feed. Feed conversion efficiency was calculated over each period by dividing the increase in body mass by the amount of feed eaten (Helland, 1996). Each tank provided a single measurement of

conversion efficiency (i.e. the tank is the unit of replication) for a total of 8 units of measurement per line.

Long term growth rate was measured using the remaining individuals that were moved to net pens on August 11, 2014. 8091 fish were moved to 16 net pens ( $5 \times 5 \times 5$  m; 2 net pens per line) for an average of 1000 fish from each line divided equally into two net pens (density of  $1\text{kg/m}^3$ ), ensuring stocking density was below the maximum value of  $20\text{kg/m}^3$ . Individuals in the net pens were fed twice a day to satiation using Chinook grower feed (Taplow feeds Inc.) by YIAL employees. On June 2014, November 2014, February 2015, May 2015, and November 2015, the fish were seined from the pens, identified by PIT tag and anesthetized with clove oil to measure length and body mass. The data collection at these measurement points were a team effort between myself, and collaborators from GLIER and the University of Windsor. The June 2014 and November 2015 data collection were conducted solely by collaborators from GLIER, and the University of Windsor. Individuals were placed back in the same net pen immediately after measurements, except following the May 2015 measurements, when the two replicate net pens from each line were combined into one net pen per line.

### Volitional swimming speed measurements

I measured volitional swimming speed between April 19 and May 21, 2015.

Measurements were taken by placing a Hero3 underwater camera (GoPro Inc.) 2.3 meters deep into the net pens. The camera was placed 1.5 meters away from the net pen wall, facing the closest wall, to record a section of the school from a linear plane (Boucher, 1999). The camera was placed in each net pen for 30 minutes before recording to allow fish time to acclimate to its presence and resume schooling. Four recordings were taken per net pen, with two net pens per line, for a total of 64 videos. One recording was taken in the morning prior to feeding and one in the afternoon, an hour after feeding one two different days.

I generated 20 time points randomly within 1 hour for each video. The first fish at each time point that appeared to be swimming in a linear plane was chosen and speed was measured by tagging the location of the individual's head, tracing its trajectory for one

second and determining the number of pixels travelled in that time using jRuler Pro V3.1 (Informer Technologies Inc., 2015). Fish body length in pixels was measured at the beginning and end of each trajectory, and if the fish was within 5% of the number of pixels at the start and end point it was considered to be swimming perpendicular relative to the camera and its measurement was kept. Swim speed was measured for 20 random individuals per video, for a total of 80 individuals per line. Speed was converted to body lengths/second (bl/s) using mean length measurements taken on May 25, 2015.

## Metabolic rate measurements

I measured metabolic rate for seven lines (all but RC) from July – September 2015, using a randomly chosen subset of fish from the net pens. Metabolic rates were taken at each hybrid line's specific volitional swimming speed: 0.29 m/s (Puntledge), 0.32 m/s (YIAL), 0.35 m/s (Chilliwack), 0.35 m/s (Capilano), 0.32 m/s (Quinsam), 0.36 m/s (Nitinat), and 0.36 m/s (Big Qualicum). This allowed me to obtain a more accurate measurement of average metabolic rate of each line in the net pen environment. A 5L swim tunnel with an oxygen spot sensor connected to the AutoResp respiration measurement software was used to measure oxygen levels and calculate metabolic rate (Loligo Systems). The water bath had a flow through design with water being pumped from the Pacific Ocean (depth >1m), to maintain constant temperature (7-8°C), salinity and pH. Water velocity within the swim chamber was calibrated prior to experimentation using a Geopacks impeller flowmeter (Map Marketing Ltd. 2014). The oxygen sensor was calibrated every third fish using a two-point calibration technique (Loligo Systems). Prior to the metabolic rate trials an individual fish was weighed, placed in the chamber and given 1 additional hour to acclimate at a water speed of 0.01m/s and 1 hour to acclimate at a water speed of 0.15m/s (approximately 50% of volitional swim speed). After the acclimation period the speed was changed to the individual's line specific volitional swim speed and each individual completed two trials. For each trial the chamber was closed and oxygen concentrations were measured every second for 25 minutes or until the oxygen concentration dropped below 6.5 mgO<sub>2</sub>/L. The individual was then given a 10-minute rest period at 0.01m/s, while the chamber was flushed with oxygenated water, before a second trial at its volitional swim speed. Metabolic rate was calculated using the mass specific

oxygen consumption rate formula,  $VO_2 = (SO_2) \times V/t \times 1/BW$ , where  $VO_2$  is oxygen consumption rate (mg  $O_2$ /kg/hour),  $SO_2$  is the slope of oxygen consumption over time (mg $O_2$ /hr),  $V$  is the respirometer volume minus volume of experimental animal (liter),  $t$  is time (hour), and  $BW$  is body weight of experimental animal (kg) (Loligo Systems). Metabolic rate for each individual was calculated as the average of its two trials.

## 2.3 Alternative diet study

### Creating experimental diets

I created two diets in collaboration with Taplow Feeds. The control diet was a high fish meal diet that is the standard Chinook grower feed produced by Taplow Feeds. The experimental diet was a low fish meal diet designed to match the control diet apart from replacing 74% of the fish meal with poultry meal and corn gluten meal (Table 2). The low fish meal diet also removed 25% of the fish oil and increased wheat content by 25%. To obtain a crude analysis of diet composition, a sample of each diet was ground to a fine powder and analyzed in a Spectrastar 2400 NIR Spectrometer (Unity Scientific Inc.) The crude analysis showed that fat, protein and fiber content of both diets were similar (Table 2). Vitamin A and vitamin E contents were higher and vitamin B content was lower in the high fish meal diet compared to the low fish meal diet (Table 2).

**Table 2.** Ingredients and nutrient composition of the high fish meal diet (High FM) and low fish meal diet (Low FM).

	High FM Diet	Low FM Diet
<i>Ingredients</i>		
Fish meal (%)	59	15
Fish oil (%)	20	15
Wheat (%)	20	25
Poultry meal (%)	0	16
Corn gluten meal (%)	0	28
Astaxanthin (%)	0.006	0.006
Vitamin Premix (%)	1	1
<i>Proximate composition</i>		
Protein (%)	40	40
Fat (%)	24	25
Fiber (%)	3	2
Vitamin A (IU/Kg)	8000	3000
Vitamin B (IU/Kg)	2000	3000
Vitamin E (IU/Kg)	200	135
Astaxanthin (peak area/g tissue)	1.0	1.5

## Growth and survival measurements

On November 23<sup>rd</sup>, 2014, I moved the individuals held in the hatchery from August – November 2014 into two saltwater net pens, mixing lines, with 184 fish in the first pen and 196 fish in the second. Individuals length and mass were measured to begin interval 1 growth measurements. Both pens remained on the Chinook grower feed (Taplow Feeds Inc.) and were fed twice daily. Over the course of two weeks (December 1 – 14<sup>th</sup>, 2014) one pen was slowly transitioned onto the low fishmeal diet, via incorporation of this diet with the Chinook grower feed at increasing concentrations. On December 15<sup>th</sup>, 2014, the second net pen was completely switched to the low fish meal diet and these individuals remained on this diet for the duration of the study. On June 25 and 26 of 2015, I moved the fish from the net pens to the 190L tanks in the hatchery, at which point total body length and body mass measurements were repeated for the end of interval 1. Fish were held in the hatchery for 4 weeks for a separate study of feed conversion efficiency, during which time they continued to be fed the same diet but growth rate was not calculated. I took total body length and body mass measurements on July 26 to start interval 2 and fish were moved back to the net pens, switching the location of the pens of the fish receiving the high and low fish meal diets. On March 17 and 18 of 2016 I completed the final total body length and body mass measurements for the end of interval 2 and fish were euthanized, with an overdose of clove oil (~400mg/L), for tissue analysis. Mortalities were collected three times a week for the duration of the study by YIAL employees. Mortality within each diet treatment was calculated for interval 1 and 2, and the hatchery interval from June 2015 – July 2015.

## Tissue quality analysis

After euthanization, I removed full body fillets, filleting over the rib bones. Total body fat percentage was measured with a Distell fish fat meter (FFM 692) (Distell, Scotland, United Kingdom). The “Salmon-1” protocol was used, which took the average of four readings on the skin side of each fillet, along the lateral line (Distell, 2010). This gave the average of eight readings for each individual, and 50 individuals were analyzed for each diet treatment. Tissue colour analysis was performed using the DSM Salmofan™(© 2011 Koninklijke DSM N.V.). The colour was analyzed using the 20-30 colour index (20 being

low red values and 30 being high red values). Fillets were placed skin side down, on a white non-reflective baseboard and observed under indirect sunlight. The fan was closed then fanned directly above the center of the fillet in three sections (anterior, dorsal and posterior), and the number corresponding to the most similar colour chip was recorded. This process was repeated for the second fillet from the same fish. Two observers performed independent colour readings for each fish, blind to both treatment and hybrid line. These colour readings were averaged to obtain whole fillet colouration. Finally, I took a portion of tissue from the right fillet of each fish, directly in front of the dorsal fin, and immediately frozen for subsequent carotenoid analysis.

## Carotenoid analysis

Carotenoid content was measured in both diets and in a subset of fish from each diet treatment. I extracted carotenoids per Garner *et al.*, (2010), as described below. All solvents were cooled on ice prior to extraction to prevent retinoid and carotenoid degradation. Three to nine individuals were used from each line for each diet, for a total of 48 individuals analyzed for the low fish meal diet and 43 individuals for the high fish meal diet. Two replicate extractions were used for four low fish meal individuals and five high fish meal individuals to quantify technical repeatability. Three replicates were used for each of the high fish meal feed and low fish meal feed. Tissue samples were thawed and homogenized, then retinoic acid was included as an internal standard to correct for extraction efficiency. The tissue samples were extracted three times in acetone and concentrated, then extracted six times with methyl tert-butyl ether (MtBE), concentrated and sealed in an amber glass vial.

With the aid of Dr. Mark Bernards, I carried out HPLC-MS analysis on an Agilent 1260 HPLC coupled with a 6230 TOF-MS (Agilent technologies, USA). Shortly after extraction, samples (25  $\mu$ L) were injected onto a Poroshell 120 EC-C18 column (3.0  $\times$  50 mm, 2.7  $\mu$ m, Agilent technologies, USA) and eluted with a solvent gradient as follows: an isocratic flow of solvent “A” of (methanol-water, 8:1) was maintained at 100% from time 0 – 4 minutes, followed by a linear gradient to 100% Solvent “B” (Methanol-hexanes, 4:1) over 2.5 minutes followed by an isocratic flow at 100% for 10 minutes before the mobile phase was switched over to 100% A and maintained for an additional



seven minutes for equilibration before the next injection. The HPLC flowrate was 0.5 mL/min and the column temperature was maintained at 25°C. Retinoic acid and astaxanthin were detected by UV at 325 nm and 480 nm, respectively, prior to infusion into the TOF. For LC-MS measurements the LC was coupled to the TOF-MS with an atmospheric-pressure chemical interface (APCI), operating in positive ionization mode. Nitrogen was used as the drying gas at a flowrate of 5 L/min, and was maintained at 350°C with a nebulizer pressure of 60 psi. The Corona needle was operated at 4µA (5235V). The capillary was operated at 6.1µA with a cone voltage of 3500V. The vaporizer chamber temperature was set to 350°C, and dry gas temperature was held at 350°C. Astaxanthin in sample extracts was identified and compared using the chromatographic retention times and full-scan mass spectra derived from an astaxanthin reference standard (Sigma 0982). Retinoic acid was included in sample extracts as an internal standard, and was identified and compared alongside a reference standard (Sigma R2625). Both standards were prepared and diluted to 0.01mg/mL and run alongside the samples under the same conditions. Retinoic acid ( $C_{20}H_{28}O_2$ , exact mass 300.4351) reference standard was found at 301.2171 m/z ( $[M+H]^+$ ) while the astaxanthin ( $C_{40}H_{52}O_4$ , exact mass 596.3865) reference standard was found at 597.3847 m/z ( $[M+H]^+$ ).

## 2.4 Statistical analysis

Growth rate for each interval was calculated as  $GR = (\ln(W_1) - \ln(W_0)) / T \times 100$ , where  $W_0$  is the individual's initial body mass,  $W_1$  is the individual's final body mass, and  $T$  is the number of days between body mass measurements. Growth rate was analyzed using ANOVA with line as a fixed factor in the crossbreeding study, and diet as the fixed factor in the diet study. A Bonferroni correction was applied to growth rate and body mass measurements to correct for multiple tests. Tank was included as a random factor in the hatchery growth rate ANOVA. Conversion efficiency, swimming speed, and metabolic rate were analyzed using ANOVA with line as a fixed factor. Net pen was included as a random factor for the crossbreeding growth rate, swimming speed and metabolic rate ANOVAs. Time of day was included as a random factor in the swimming speed ANOVA. Salmofan colour values, carotenoid concentrations and tissue fat percentages

were each analyzed using an ANOVA with diet as a fixed factor. Hybrid line was included as a random factor in the ANOVAs, and the interaction between line and diet was included for all traits in the diet study. Survivorship was analyzed using a Fisher's exact test to compare the number of survivors and mortalities between the two diet treatments within each sampling period. Pearson's correlation was used to compare the colour card values with corresponding astaxanthin tissue values, as well as specific growth rate with swimming speed, metabolic rate and feed conversion efficiency. When a random factor was significant, it was stated and the proportion of variance explained by that factor was estimated using  $\text{Eta}^2$  (Kirk, 1982). All statistical analyses were performed using R (V.0.99.484, R Development Core Team, 2008).

## 3 Results

### 3.1 Crossbreeding study

$F_{ST}$  values were calculated for each of the hybrid lines in relation to the YIAL population. The founding hybrid lines (RC and BQ) were equally distant from the YIAL line and had the smallest  $F_{ST}$  values (Table 1). The most northern Vancouver Island lines (Quin and Punt) grouped together with similar  $F_{ST}$  values, and southern lines (Nit, and Chil) grouped together, with the exception of Cap, which had a much smaller  $F_{ST}$  value than was hypothesized based on location (Table 1).

Body mass differed significantly between lines across all measurement points in the study (Table 3). However, most lines did not differ from the YIAL lines during the hatchery interval (September – November 2014), except for Chil and Nit (Table 3). The Chil line was significantly smaller than the YIAL line at the initial measurement point and the first two measurement points during the hatchery interval (Table 3). The Nit line was smaller than the YIAL line at the first four measurement points during the hatchery interval (Table 3). The net pen interval (November 2014 – March 2016) had four lines that were significantly smaller (RC, Quin, Chil, Nit) and one which was significantly larger (Punt) than the YIAL line at the initial measurement point (Table 3). No hybrid line differed from the YIAL line at the November 2014 measurement point (Table 3). At the May 2015 measurement point Punt, RC, Cap, Quin, and Chil were significantly larger than YIAL line (Table 3). Three lines (Punt, Cap, and Quin) remained significantly larger than the YIAL line at the final measurement point (Table 3).

Specific growth rate differed significantly, albeit inconsistently, during both the short-term hatchery and long term net pen intervals. For the initial hatchery growth rate measurement interval from September 23 – October 14, 2014, Cap, Quin and Chil had significantly higher growth rates than the YIAL line ( $p < 0.004$ ,  $F_{1,389} = 7.3$ ; Figure 1). Tank had a significant effect on the model ( $p < 0.001$ ,  $F_{1,48} = 2.98$ ) and accounted for 27% of the variation in the model. Only the Chil line continued to have a significantly higher growth

rate than the YIAL line from October 14<sup>th</sup> – 29<sup>th</sup>, while the Nit line had a significantly lower growth rate ( $p < 0.004$ ,  $F_{1,381} = 15.87$ ; Figure 1). Tank had a significant effect ( $p < 0.001$ ,  $F_{1,48} = 2.61$ ) and accounted for 22% of the variation in the model. During the third measurement interval, the Chil line remained significantly higher than the YIAL line, BQ, RC and Cap also had significantly higher growth rates ( $p < 0.004$ ,  $F_{1,374} = 22.14$ ; Figure 1). Tank had a significant effect ( $p = 0.01$ ,  $F_{1,48} = 1.6$ ) and accounted for 14% of the total variation in the model. During the final measurement interval, the same 4 lines from the previous interval with the addition of Quin were all significantly higher than the YIAL line ( $p < 0.004$ ,  $F_{1,369} = 4.28$ ; Figure 1). Tank had a significant effect ( $p = 0.001$ ,  $F_{1,48} = 1.84$ ) and accounted for 20% of the total variation in the model.

The long-term net pen growth rate measurements showed similar results to those taken in the hatchery. The June – November 2014 growth rate interval indicated that all lines, except the RC line, had significantly higher growth rates than the YIAL line ( $p < 0.004$ ,  $F_{1,1557} = 30.23$ ; Figure 2). Pen had a significant effect ( $F_{1,16} = 4.88$ ,  $p < 0.001$ ) and accounted for 2.1% of the total variation in the model. During the November 2014 – February 2015 interval, BQ, Cap, Quin, Chil and Nit all had significantly lower growth rates than the YIAL line ( $p < 0.004$ ,  $F_{1,1407} = 21.35$ ; Figure 2). Net pen had a significant effect ( $F_{1,16} = 6.85$ ,  $p < 0.001$ ) and accounted for 3.4% of the variation in the model. During the February – May 2015 interval the four lines from the previous interval (Cap, Quin, Chil, and Nit), as well as the RC line, had significantly higher growth rates than the YIAL line ( $p < 0.004$ ,  $F_{1,1472} = 80.14$ ; Figure 2). Net pen had a significant effect ( $p < 0.001$ ,  $F_{1,8} = 18.15$ ) and accounted for 5% of the total variation in the model. For the final interval, only the Quin line continued to have a significantly higher growth rate than the YIAL line ( $p < 0.004$ ,  $F_{1,739} = 4.68$ ; Figure 2).

Three lines (BQ, RC, Chil) had significantly higher feed conversion efficiency than the YIAL line ( $p = 0.003$ ,  $F_{1,47} = 3.72$ ; Figure 3). Conversion efficiency was significantly correlated with the average growth rate during the same experimental period (September – November 2014) ( $r^2 = 0.77$ ,  $p = 0.004$ ,  $F_{1,8} = 19.5$ ). Feed consumption was significantly higher for the RC, Cap and Quin lines than the Nit line ( $p = 0.02$ ,  $F_{1,47} = 2.49$ ), and did not differ between the YIAL line and any of the other lines (Figure 3). There was no

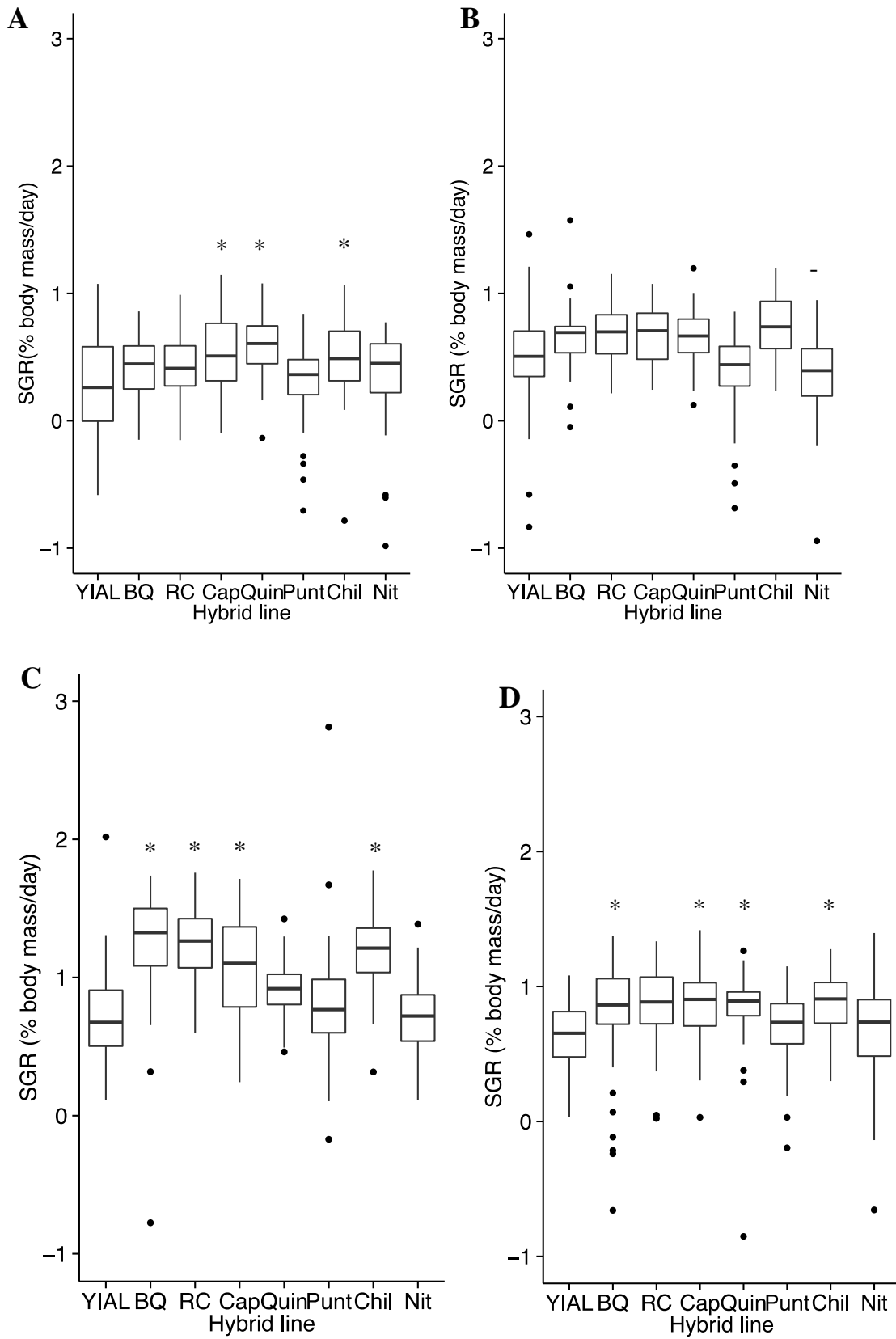
correlation between the amount of feed consumed and growth rate during the September – November 2014 experimental period ( $r^2=0.16$ ,  $p=0.3$ ,  $F_{1,8}=1.11$ ).

Swimming speed differed significantly between lines, where RC and Nit were the only lines with significantly faster swimming speeds than the YIAL line ( $p<0.001$ ,  $F_{1,8}=7.32$ ; Figure 3). Net pen accounted for 7% of the total variation in the model ( $\eta^2=0.07$ ). There was no correlation between swimming speed and growth rate ( $r^2=0.05$ ,  $p=0.6$ ,  $F_{1,8}=0.31$ ). Metabolic rates measured at volitional swimming speeds did not differ between lines ( $F_{1,70}=1.69$ ,  $p=0.136$ ; Figure 3). There was no correlation between metabolic rate and growth rate ( $r^2=0.27$ ,  $p=0.22$ ,  $F_{1,7}=1.9$ ).

**Table 2.** Average body mass (g  $\pm$ SE) of eight lines of Chinook salmon (*Oncorhynchus tshawytscha*) during both short-term hatchery and long-term net pen intervals. Test statistics (degrees of freedom, F statistic, P value) are included for the ANOVA comparing body mass among lines at each measurement point.

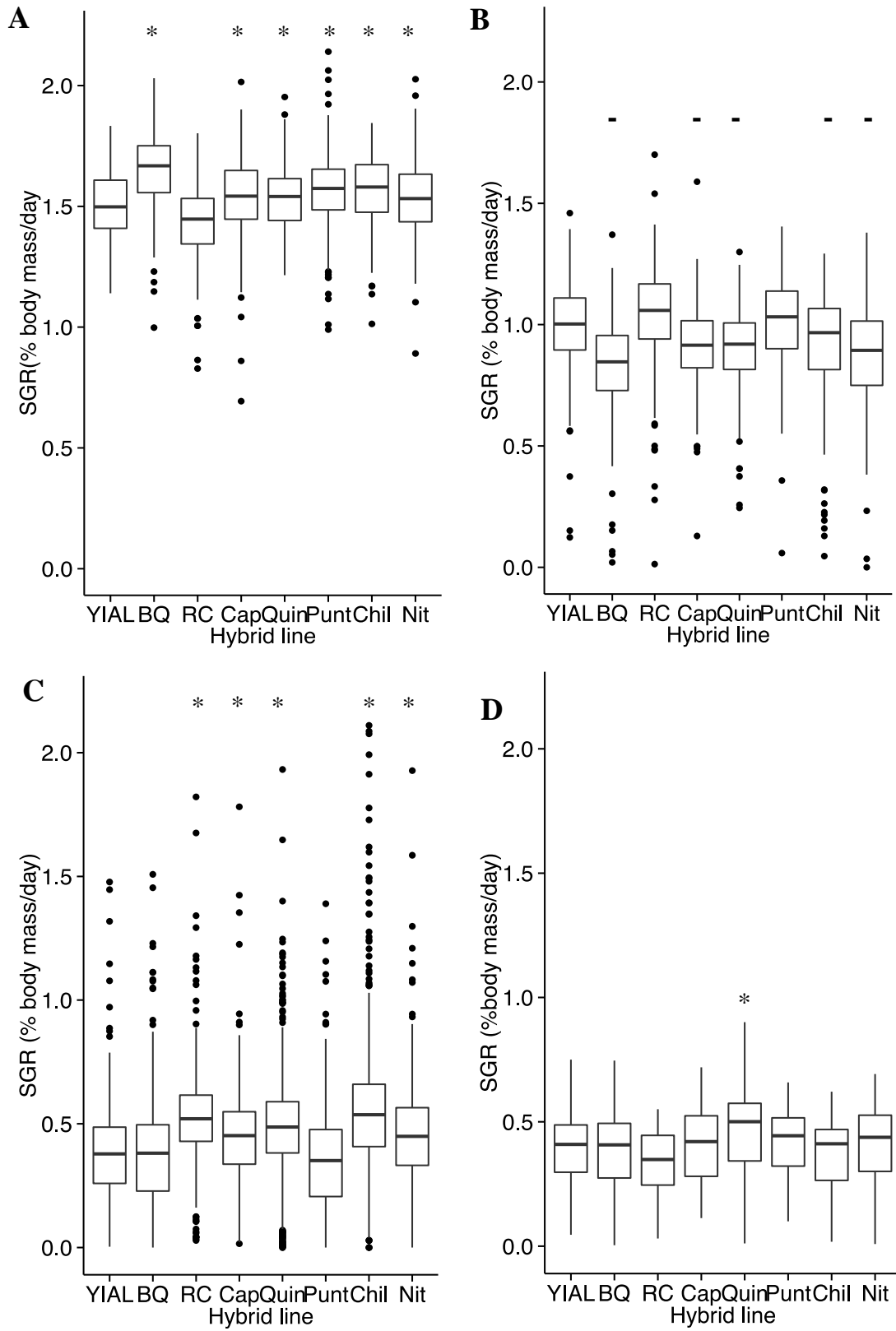
	YIAL	BQ	RC	Cap	Quin	Punt	Chil	Nit	Significance
<i>Hatchery individuals</i>									
June 14 2014	4.48 $\pm$ 0.31	3.94 $\pm$ 0.11	4.60 $\pm$ 0.13	4.07 $\pm$ 0.15	4.16 $\pm$ 0.13	4.28 $\pm$ 0.12	<b>3.58<math>\pm</math>0.14<sup>-</sup></b>	4.03 $\pm$ 0.11	P=0.006 F <sub>1,432</sub> =4.7
September 23 2014	18.6 $\pm$ 1.0	18.3 $\pm$ 0.6	18.7 $\pm$ 0.6	17.0 $\pm$ 0.7	17.5 $\pm$ 0.8	17.2 $\pm$ 0.9	<b>15.1<math>\pm</math>0.7<sup>-</sup></b>	<b>15.8<math>\pm</math>0.8<sup>-</sup></b>	P=0.006 F <sub>1,412</sub> =6.2
October 14 2014	20.4 $\pm$ 1.3	20.4 $\pm$ 0.9	20.7 $\pm$ 0.9	18.9 $\pm$ 1.1	19.8 $\pm$ 1.2	18.5 $\pm$ 1.1	<b>17.0<math>\pm</math>0.9<sup>-</sup></b>	<b>17.2<math>\pm</math>0.9<sup>-</sup></b>	P=0.006 F <sub>1,394</sub> =5.9
October 29 2014	21.9 $\pm$ 1.6	22.4 $\pm$ 1.0	22.7 $\pm$ 1.1	21.1 $\pm$ 1.3	21.7 $\pm$ 1.3	19.8 $\pm$ 1.2	19.2 $\pm$ 1.2	<b>18.4<math>\pm</math>1.0<sup>-</sup></b>	P=0.006 F <sub>1,382</sub> =5.8
November 12 2014	24.7 $\pm$ 1.9	26.7 $\pm$ 1.2	26.9 $\pm$ 1.1	24.2 $\pm$ 1.2	24.5 $\pm$ 1.2	22.8 $\pm$ 1.4	22.9 $\pm$ 1.5	<b>20.9<math>\pm</math>1.4<sup>-</sup></b>	P=0.006 F <sub>1,382</sub> =7.2
November 26 2014	26.9 $\pm$ 2.1	30.2 $\pm$ 1.4	30.6 $\pm$ 1.5	27.5 $\pm$ 1.4	27.6 $\pm$ 1.3	25.7 $\pm$ 1.6	25.6 $\pm$ 1.7	23.4 $\pm$ 1.6	P=0.006 F <sub>1,374</sub> =7.0
<i>Net pen individuals</i>									
June 2014	5.40 $\pm$ 0.03	5.02 $\pm$ 0.04	<b>5.30<math>\pm</math>0.04<sup>-</sup></b>	5.61 $\pm$ 0.04	5.40 $\pm$ 0.04	<b>5.26<math>\pm</math>0.04<sup>+</sup></b>	<b>4.79<math>\pm</math>0.05<sup>-</sup></b>	<b>4.93<math>\pm</math>0.05<sup>-</sup></b>	P=0.005 F <sub>1,8050</sub> =30.3
November 2014	54.2 $\pm$ 0.9	63.9 $\pm$ 1.3	48.6 $\pm$ 0.9	57.995 $\pm$ 0.9	55.2375 $\pm$ 0.8	58.2725 $\pm$ 1.0	50.975 $\pm$ 0.8	51.9364 $\pm$ 0.8	P=0.04 F <sub>1,1557</sub> =6.2
February 2015	137.2 $\pm$ 0.9	139.1 $\pm$ 1.2	<b>131.2<math>\pm</math>0.9<sup>-</sup></b>	135.3 $\pm$ 1.2	<b>129.6<math>\pm</math>0.9<sup>-</sup></b>	<b>150.7<math>\pm</math>1.2<sup>+</sup></b>	<b>119.3<math>\pm</math>1.3<sup>-</sup></b>	<b>122.1<math>\pm</math>1.0<sup>-</sup></b>	P=0.005 F <sub>1,1407</sub> =21.4
May 2015	182.6 $\pm$ 1.9	189.5 $\pm$ 2.5	<b>208.3<math>\pm</math>2.2<sup>+</sup></b>	<b>202.0<math>\pm</math>2.4<sup>+</sup></b>	<b>195.4<math>\pm</math>1.8<sup>+</sup></b>	<b>195.8<math>\pm</math>2.2<sup>+</sup></b>	<b>193.7<math>\pm</math>2.4<sup>+</sup></b>	180.3 $\pm$ 2.3	P=0.005 F <sub>1,1472</sub> =80.4
November 2015	415.5 $\pm$ 6.8	410.1 $\pm$ 12.4	408.8 $\pm$ 8.2	<b>479.9<math>\pm</math>15.7<sup>+</sup></b>	<b>502.5<math>\pm</math>14.5<sup>+</sup></b>	<b>459.4<math>\pm</math>11.7<sup>+</sup></b>	416.2 $\pm$ 10.5	434.5 $\pm$ 13.0	P=0.005 F <sub>1,739</sub> =4.68

*Note:* Bolded numbers with + and – symbols indicate either larger, or smaller significant differences, compared to the YIAL line according to Tukey HSD.

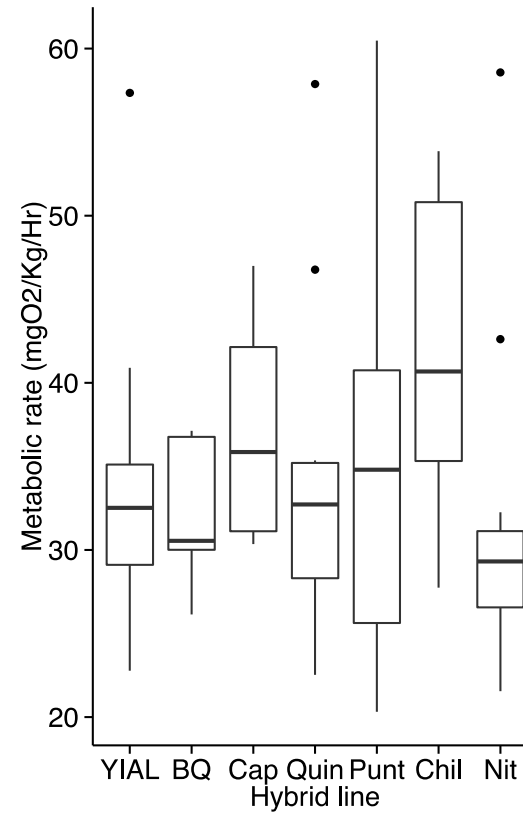
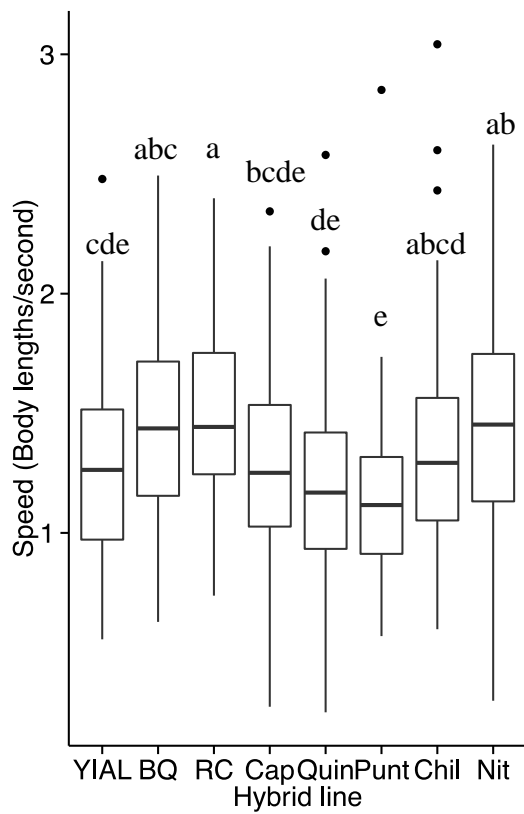
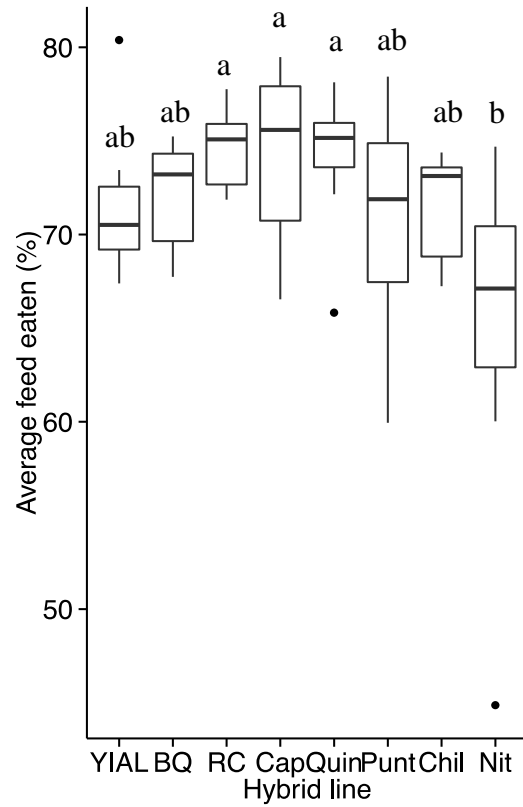
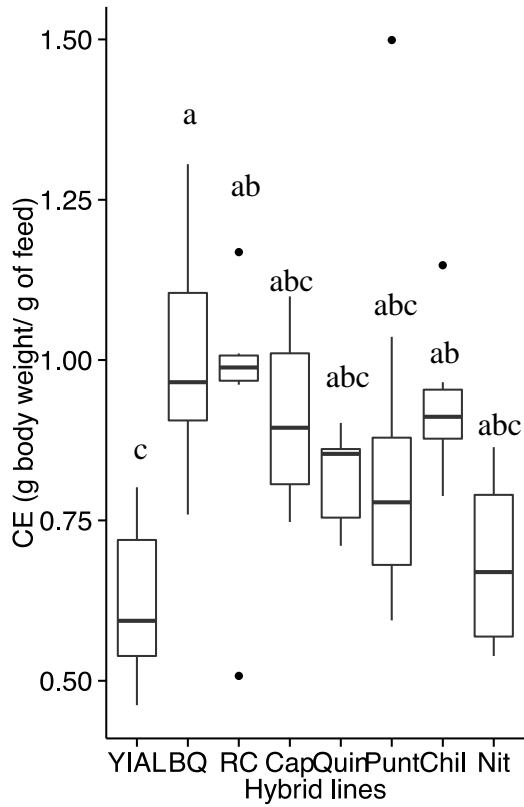


**Figure 1.** Specific growth rate (% body mass/day) of eight lines of Chinook salmon (*Oncorhynchus tshawytscha*) measured in the hatchery. The eight lines are in order of relatedness to YIAL based on  $F_{ST}$  values. Panel A shows data from September 23<sup>rd</sup>-October 14<sup>th</sup> 2014, panel B from October 14<sup>th</sup>-29<sup>th</sup> 2014, panel C from October 29<sup>th</sup>-November 12<sup>th</sup> 2014, and panel D from November 12<sup>th</sup>-26<sup>th</sup> 2014. Boxes show the 25<sup>th</sup> and 75<sup>th</sup> percentile with a line indicating the median. Whiskers show the minimum and maximum values within 1.5 lengths of the box. Outliers are designated with a black dot. Asterisks indicate significantly different hybrid lines from the YIAL line according to Tukey's HSD.





**Figure 2.** Specific growth rate (% body mass/day) of eight lines of Chinook salmon (*Oncorhynchus tshawytscha*) measured in the net pens. The eight lines are in order of relatedness to YIAL based on  $F_{ST}$  values. Panel A) occurred from June – November 2014, panel B) from November 2014 – February 2015, panel C) from February – May 2015, and panel D) occurred from May – November 2015. Boxes show the 25th and 75th percentile with a line indicating the median. Whiskers show the minimum and maximum values within 1.5 lengths of the box. Outliers are designated with a black dot. Asterisks indicate significantly different hybrid lines from the YIAL line according to Tukey's HSD.



**Figure 3.** Conversion efficiency (g body mass/ g of feed), average feed eaten (% eaten of feed given), volitional swimming speed (body lengths/s) and metabolic rates (mgO<sub>2</sub>/kg/hr) of eight lines of Chinook salmon (*Oncorhynchus tshawytscha*). The hybrid lines are in order of relatedness to YIAL based on  $F_{ST}$  values. Boxes shows the 25th and 75th percentile with a line indicating the median. Whiskers show the minimum and maximum values within 1.5 lengths of the box. Outliers are designated with a black dot. Letters indicate significant differences according to Tukey's HSD.

### 3.2 Alternative diet study

At the start of the study, neither total body length nor body mass differed between the fish assigned to the two diet treatments (Table 4). At the following measurement point (June 2015) individuals on the high fish meal diet were 3% longer than individuals on the low fish meal diet (Table 4). There was no significant difference in body length for the remaining measurement points in the study (Table 4). Body mass did not differ between diet treatments during the entirety of the study (all  $p > 0.05$ ) (Table 4). There was no significant difference in growth rate between fish from the two diet treatments during the first interval ( $F_{1,311} = 3.62$ ,  $p = 0.058$ ; Figure 4) or the second interval ( $F_{1,91} = 1.48$ ,  $p = 0.226$ ; Figure 4). Line was included as a random effect in the model, there was no interaction between line and diet and line did not have a significant effect for mass, length or growth rate. There was no significant difference in survival during any of the measurement intervals (all  $p > 0.05$ ) (Table 5).

The diets had a significant effect on multiple aspects of salmon tissue quality (tissue fat and tissue colouration). Individuals fed the low fish meal diet had 25% higher total body fat percentage than those fed the high fish meal diet ( $F_{1,99} = 4.58$ ,  $p = 0.035$ ; Figure 5). Individuals on the low fish meal diet also had lighter coloured flesh than those on the high fish meal diet ( $F_{15,99} = 48.82$ ,  $p < 0.001$ ; Figure 6). Hybrid line had a significant effect on flesh colouration ( $F_{15,99} = 4.39$ ,  $p = 0.003$ ) and accounted for 18% of the total variation in the model.

Carotenoid analysis was consistent with the tissue colour results, as astaxanthin content in tissue from salmon fed the low fish meal diet was significantly lower than those fed the high fish meal diet ( $F_{1,83} = 14.29$ ,  $p < 0.001$ ; Figure 6). Hybrid line had a significant effect on carotenoid content ( $F_{1,83} = 3.17$ ,  $p = 0.005$ ) and accounted for 20% of the total variation in the model. As expected, there was a significant positive correlation between red colouration and astaxanthin content in the flesh ( $F_{1,70} = 9.52$ ,  $p = 0.003$ ;  $r^2 = 0.35$ ).

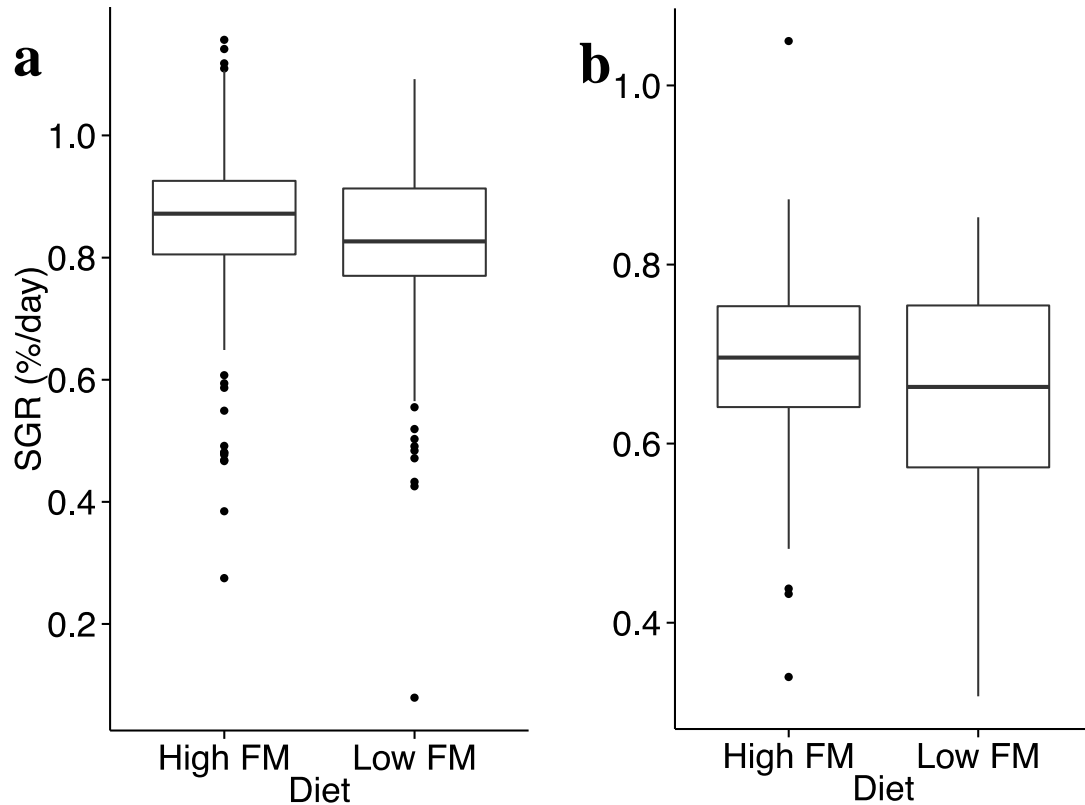
Astaxanthin was added to both high and low fish meal feeds in equal quantities (Table 1) and LC-MS analysis of carotenoid content in feed pellets determined that there was no significant difference in astaxanthin levels between the high and low fish meal diets ( $F_{1,4} = 2.77$ ,  $p = 0.171$ ; Table 1).

**Table 3.** Body mass and total body length of Chinook salmon (*Oncorhynchus tshawytscha*) fed either the control high fish meal (High FM) or low fish meal (Low FM) diets at four sampling points. Test statistics (F statistic, degrees of freedom, P value) are included.

	<b>November 2014</b>	<b>June 2015</b>	<b>July 2015</b>	<b>March 2016</b>
<b><i>Body mass (g±SD)</i></b>				
High FM	27.3±5.9	184.6±49.8	184.3±54.0	498.5±118.0
Low FM	27.5±7.0	172.9±50.9	190.2±62.7	467.4±131.2
<i>Test statistics</i>	F <sub>1,366</sub> =1.05	F <sub>1,309</sub> =1.14	F <sub>1,189</sub> =0.11	F <sub>1,99</sub> =3.01
	p=0.51	p=0.28	p=0.93	p=0.15
<b><i>Body length (mm±SD)</i></b>				
High FM	140 ±9	262 ±27	268 ±24	355 ±34
Low FM	140±10	255±30	261±27	341±50
<i>Test statistics</i>	F <sub>1,366</sub> =0.05	F <sub>1,309</sub> =4.56	F <sub>1,189</sub> =3.01	F <sub>1,99</sub> =2.70
	p=0.97	p=0.06	p=0.17	p=0.22

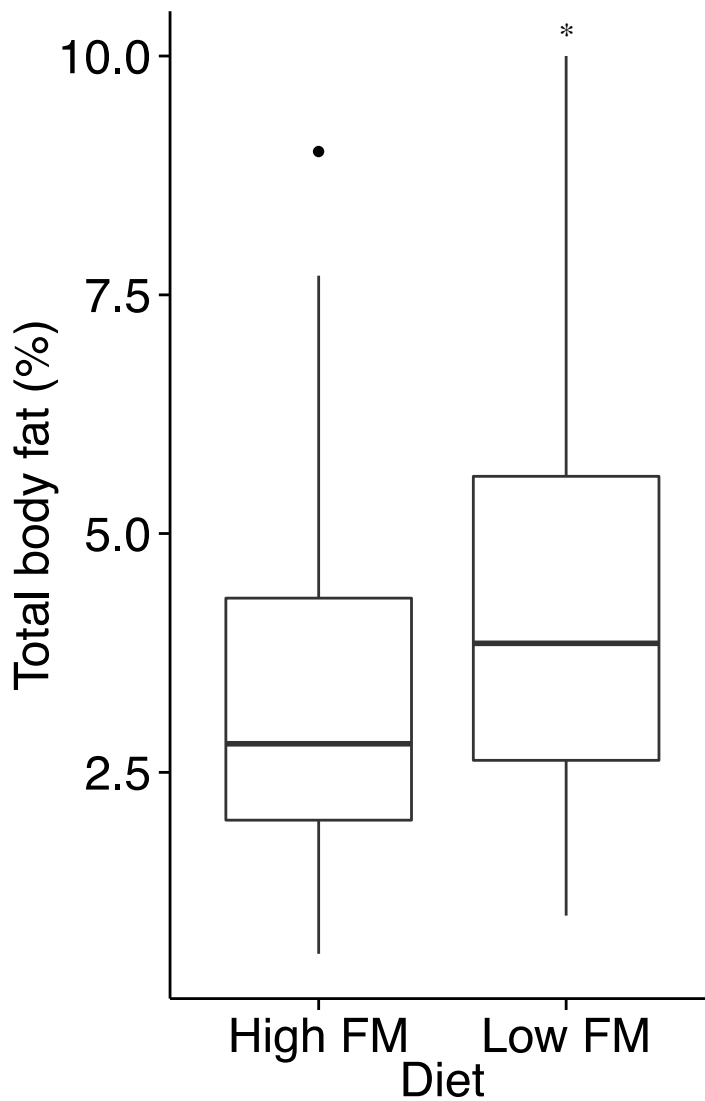
**Table 4.** Survivorship of Chinook salmon (*Oncorhynchus tshawytscha*) fed either the high fish meal (high FM) or the low fish meal (low FM) diet during three intervals. Survival (%) is calculated for each interval. Significance is reported as Fisher's exact test.

	<b>Diet</b>	<b>Total</b>	<b>Survivors</b>	<b>Mortalities</b>	<b>% Survival</b>	<b>Significance</b>
November 2014 – June 2015	High FM	192	154	38	80.2	0.89
	Low FM	185	150	35	81	
June 2015 – July 2015	High FM	154	82	72	52.9	0.08
	Low FM	150	65	85	42.9	
July 2015 – March 2016	High FM	82	50	32	60.5	0.11
	Low FM	65	48	17	73.4	
<b>Total</b>	High FM	192	50	142	26.0	1.00
	Low FM	185	48	136	25.9	

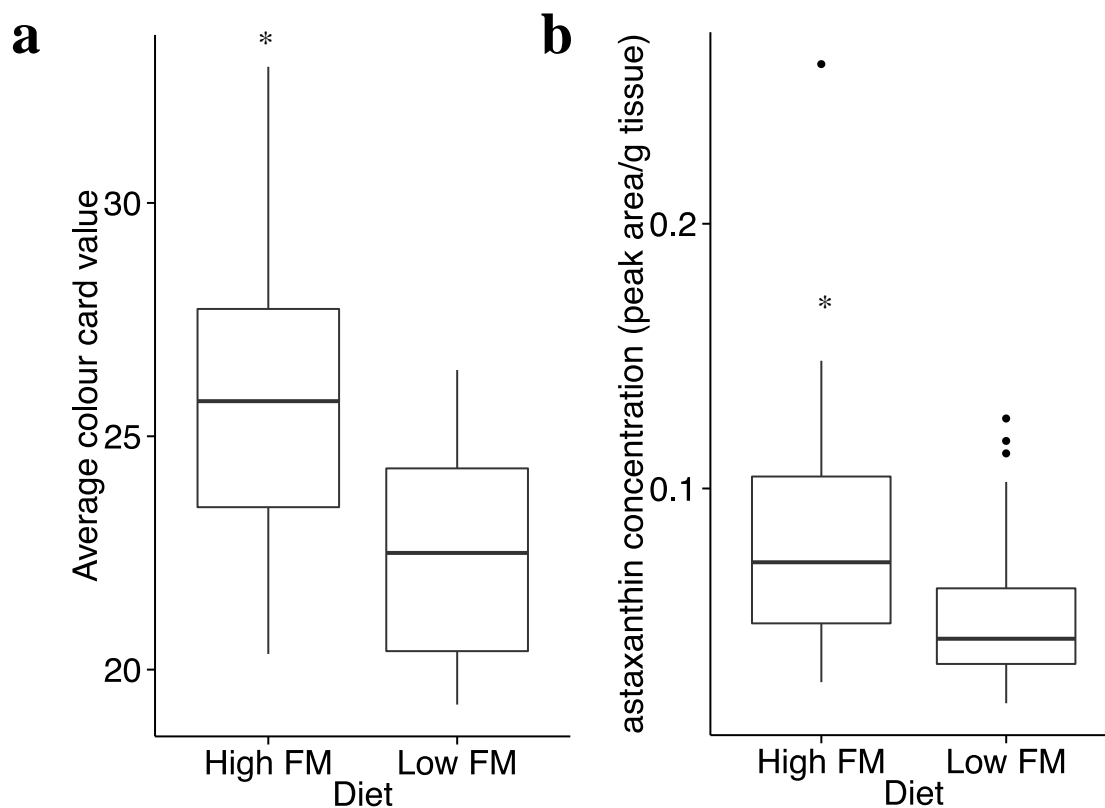


**Figure 4.** Specific growth rate (% body mass/day) of Chinook salmon (*Oncorhynchus tshawytscha*) fed either the high fish meal (High FM) diet or the low fish meal (Low FM) diet. The box shows the 25th and 75th percentile with a line indicating the median. Whiskers show the minimum and maximum values within 1.5 lengths of the box. Outliers are designated with a black dot. Panel A) shows interval 1, which occurred from November 2014 – June 2015, and panel B) shows interval 2 which occurred from July 2015 – March 2016. Growth rate did not differ significantly between diets for either interval.





**Figure 5.** Total body fat (% body composition) of Chinook salmon (*Oncorhynchus tshawytscha*) fed either the high fish meal diet (High FM) or the low fish meal diet (Low FM). Boxes show the 25th and 75th percentile with a line indicating the median. Whiskers show the minimum and maximum values within 1.5 lengths of the box. Outliers are indicated with a dot. Fish fed the low fish meal diet had significantly higher body fat than those fed the high fish meal diet.



**Figure 6.** Tissue colour analysis for Chinook salmon (*Oncorhynchus tshawytscha*) fed either the high fish meal diet (High FM) or the low fish meal diet (Low FM). Boxes show the 25th and 75th percentile, with a line indicating the median. Whiskers show the minimum and maximum values within 1.5 lengths of the box. Outliers are shown with a dot. Panel A) is the average Salmofan colour card measurement on a scale of 20 (lightest colouration) to 30 (darkest colouration) and panel B) is the average peak area of Astaxanthin content in the tissue sample (g). The low fishmeal diet was significantly lower for both colour card values and astaxanthin concentration measurements.

## 4 Discussion

I found that hybrid lines created from populations with  $F_{ST}$  values ranging from 0.005 – 0.02 showed significant differences in growth rate, but there was no clear level of genetic distance that exhibited offspring with the highest growth rate. Additionally,  $F_{ST}$  did not predict offspring with superior feed consumption, feed conversion or swimming speed. In other studies,  $F_{ST}$  did not predict crossbreeding effects, as at larger  $F_{ST}$  values than those observed in my study no effect of crossbreeding was observed for growth or survival in Chinook salmon ( $F_{ST} = 0.13$ ; Lehnert *et al.* 2014), or for survival in Atlantic salmon ( $F_{ST} = 0.95$ ; Houde *et al.* 2011). The lack of a relationship in results for salmonids could be caused by population specific factors which cannot be captured by genetic distance measures (Kawecki and Ebert 2004; Edmands 2007). Fixed beneficial mutations arising from local adaptation can be lost in hybrids; this effect may be especially important if the fixed mutations are leading to high performance or survival in the aquaculture environment (Kawecki and Ebert, 2004). Additionally, sire and dam effects can influence crossbreeding, as Granier *et al.* (2011) found exogenous feeding and growth rate of crossbred offspring were significantly affected by the maternal line in the first 15 weeks' post hatching. The strong dam effects seen in Granier's study suggest that it is not only certain lines that are genotypically appropriate for crossbreeding but within these lines the maternal or paternal genotype is also important (Gjerde, 2005; Granier *et al.* 2011). The lack of support in my study and in other studies suggest that genetic distance between populations or lines is a poor indicator of the effects of crossbreeding on growth and growth-related traits assessed in experimental settings in salmonids.

Crossbreeding has the potential to improve aquaculture yields through increased growth rate. In my study crossbreeding had significant, albeit inconsistent, effects on growth rate. Generally, the crossbred lines had similar or significantly higher growth rates than the YIAL line. In particular, three hybrid lines showed promising growth rate results when compared to the YIAL line. The Capilano line had significantly higher growth rate than the YIAL line for three of the four hatchery measurement points (average difference = +46%) and two of the four net pen measurement points (average difference = +17%). The Quinsam line had significantly higher growth rate than the YIAL line for two of the four

hatchery measurement points (average difference= +54%) and three of the four net pen measurement points (average difference= +21%). Finally, the Chilliwack line had significantly higher growth rate than the YIAL line for three of the four hatchery measurement points (average difference= +39%) and two of the four net pen measurement points (average difference= +27%). More importantly at the final measurement point three lines (Cap, Quin, Chil) were significantly larger, having significantly higher body mass, than the YIAL line. The observed increase in growth rate probably occurred due to a release from inbreeding depression in the farm line, as there is evidence that the YIAL line is experiencing inbreeding depression (Bryden et al, 2004; Heath et al. 2002), and studies have found that increases in performance of crossbred offspring are most pronounced when one of the lines is inbred (Whiteley *et al.* 2015). Despite this possible mechanism, differences in growth rate were variable among lines and over time. The drop in growth rates seen in the November 2014 – February 2015 interval in the net pens may be due to sampling error, where only 200 of the ~800 fish in each line was recorded and used for SGR calculations. Additionally, the February – May 2015 interval is likely the most indicative of later life stage growth trends and should be the focus of conclusions for crossbreeding for growth in Chinook salmon. This is because for the final interval (May – November 2015) many individuals were removed from the study to be used in separate supplementary studies, dropping the sample size from a range of 600 – 900 down to 30 – 200. The lack of differences between YIAL and other hybrid lines in the final measurement point may simply be due to the low sample size, or strong selection for certain body sizes with the equipment used to remove fish from the pens. Based on the overall growth rate trends, I suggest there is a need to evaluate multiple lines when attempting to use crossbreeding to improve growth rate in a salmonid line (Whiteley *et al.*, 2015). However, crossbreeding for growth rate is effective in the improvement of a domesticated line of Chinook salmon, and should be further implemented in breeding programs.

Feed consumption and feed conversion efficiency are important in aquaculture as traits that potentially affect growth rate, but the effects of crossbreeding on these traits is not well-understood. In my study, the YIAL line did not differ in feed consumption from any of the hybrid lines. Additionally, feed consumption did not correlate with growth rate.

The similar feed intake across hybrid lines is likely due to the feeding regime implemented. Fish were fed twice a day on a restricted regime; without continuous access to food, variation in appetite (voluntary feed intake) may not have been fully expressed (Henryon *et al.*, 2002). Given that restricted feeding regimes are standard practise in salmonid aquaculture, it is unlikely that consumption rate can be effectively manipulated to enhance growth rate. Conversion efficiency, on the other hand, was higher in crossbred lines, with Big Qualicum, Robertson Creek and Chilliwack lines being significantly higher. Additionally, feed conversion efficiency correlated with growth rate in my study, where lines with higher growth rate had higher feed conversion efficiencies. Similar results were seen with crossbred lines of rainbow trout, where lines with high growth rate also had high feed conversion efficiencies (Henryon *et al.* 2002). These findings are also consistent with studies that have identified genetic correlations between feed conversion efficiency and growth rate (Cameron and Curran, 1994; Kuhlert *et al.*, 2003). My study thus adds to a growing body of literature suggesting that crossbreeding can enhance feed conversion efficiency, and that this trait may mediate differences in growth rate.

Energy utilization associated with activity is another factor that may underlie differences in growth rates seen between hybrid lines. Swimming is an energetically costly activity, so slower swimming fish would be expected to expend less energy, leading to more energy available to allocate to growth. In my study two hybrid lines (Robertson Creek, Nitinat) had significantly higher swimming speeds than the YIAL line, and no line had a significantly lower swimming speed than the YIAL line. Contrary to my hypothesis, however, swimming speed did not correlate with growth rate and slower swimming fish did not have higher growth rates. Indeed, of the three fastest growing lines (Capilano, Quinsam, Chilliwack), none had a swimming speed that differed significantly from the YIAL line. Though there were significant differences in swimming speeds, metabolic rates measured at each line's swimming speed did not differ among lines. The similarity of metabolic rates across lines may be due to the relatively narrow range of speeds recorded among lines in my study (1.1-1.4 bl/s), which cover only a narrow range of the speeds at which Chinook salmon are known to swim (0.6-2.2 bl/s; Brown and Geist, 2002). Overall, there was no evidence that swimming speed or metabolic rate drive the observed differences in growth rate in my study.

Alternative aquaculture diets that replace fish meal with plant or animal meal could influence the health and survival of fishes if they do not provide an adequate source of all essential nutrients. In previous studies, alternative diets that replaced 75-100% of fish meal with poultry and corn gluten meal were associated with no change in survival in either Atlantic salmon (Burr *et al.*, 2012) or rainbow trout (Burr *et al.*, 2012; Lu *et al.*, 2015). In my study, Chinook salmon were fed a diet that replaced 74% of fish meal with a combination of corn gluten meal and poultry meal, and I similarly found no difference in survival between diets. It is likely that alternative aquaculture diets do not affect survival because the diets are specifically formulated to provide all essential nutrients. Indeed, the alternative diet in my study had the same composition of protein, fat, and fiber as the high fish meal feed, which is formulated based on the needs of salmonids (NRC, 1993). Overall, reduced survival does not appear to be a barrier to the inclusion of plant or animal meal in alternative aquaculture diets for salmon.

Alternative aquaculture diets that include plant or animal meal could lead to differences in growth rate. Several studies have shown that replacing 25% or more of fish meal with plant meal (soy, pea, corn gluten meal) results in reduced growth rate (e.g. Mundheim *et al.*, 2004; Pratoomyot *et al.*, 2011; Torstensen *et al.*, 2008). Plant protein sources have high protein digestibility when compared to fish meal (Lu *et al.*, 2015), so the difference in growth rate likely occurs instead because plant protein sources have a different amino acid composition—generally lower levels of lysine, methionine, arginine and tryptophan—compared to fish meal (Mente, 2003; NRC, 2011). Deficiencies of these amino acids are known to be associated with impaired growth rate in fishes (Davies *et al.*, 1997; Gaylord and Barrows, 2009). In contrast, poultry meal has a similar amino acid composition to fish meal (Dong, 1993; Lu *et al.*, 2015). However, replacement of 25% or more of fish meal with poultry meal has been observed to reduce growth rates in salmon (Steffens, 1994; Fowler, 1991). Generally, salmon diets replacing fish meal solely with poultry meal result in reduced growth rates (Fowler, 1991; Rawles *et al.*, 2006). However, combining poultry meal with a combination of other plant or animal sources result in growth rates comparable to standard fish meal diets (e.g. Hatlen *et al.*, 2015; Burr *et al.*, 2012). My study found no difference in growth rates in Chinook salmon fed an alternative diet with 74% of fish meal replaced with a combination of poultry meal

(27%) and corn gluten meal (47%). The success of these diets that combine poultry meal and plant protein sources likely occurs because combinations of protein meals produce a more balanced amino acid composition and increased digestible protein/energy ratio than either plant or animal protein meal on its own. Together these data suggest that poultry meal in combination with corn gluten meal can be incorporated at high levels into alternative aquaculture diets without affecting growth rate in salmonids.

Corn gluten meal and poultry meal differ from fish meal in fat content and fatty acid composition, which could influence total body fat in fishes that are fed these items. Inclusion of poultry meal in salmonid diets leads to fish with higher body fat (Bjerkeng *et al.*, 1997; Sheehan, 1996), whereas the inclusion of corn gluten meal has had no reported effects on body fat content (Sato *et al.*, 2003; Mundheim *et al.*, 2004). My study found that fish fed a diet that included poultry and corn gluten meal had 25% higher total body fat than fish fed the fish meal diet. This difference in body fat occurred despite the similar fat content of the two diets (High FM = 24%, Low FM = 25%). However, the diets differed in fatty acid composition: poultry meal is low in Omega-3 fatty acids and rich in saturated and monounsaturated fatty acids, whereas fish meal is rich in Omega-3 and polyunsaturated fatty acids (Nates, 2011; NRC, 2011). Salmon require high levels of polyunsaturated fatty acids in their diets and comparatively low levels of saturated fatty acids (Parés-Sierra *et al.*, 2014; Sargent, 1998). Instead of utilizing saturated fatty acids, salmon accumulate these in muscle tissue, around the organs and in the liver (NIFES, 2015). It is thus likely that the differences in fatty acid composition led to increased fat storage in individuals fed the diet containing poultry meal. Regardless, the total body fat of Chinook salmon fed both diets were within the range accepted by consumers (Exler and Pehrsson, 2007), which suggests that the alternative aquaculture diet did not lead to a reduction in tissue quality related to total body fat.

In salmonids, diets containing corn gluten meal have been associated with reduced concentrations of astaxanthin and the associated red tissue colouration (Seaz *et al.*, 2014; 2016). Consistent with these findings, in my study, the individuals fed the alternative diet that contained corn gluten meal had a significant reduction in tissue redness and tissue astaxanthin concentration relative to the high fish meal diet. Astaxanthin content did not

differ between the two diets in my study, so dietary astaxanthin levels cannot explain this result. Instead, this difference in tissue colouration may occur because of competition from novel xanthophylls present in the alternative feed. Similar to astaxanthin, these novel xanthophylls are absorbed in the gut and deposited in the flesh, but do not impart the same distinctive red colour (Furr and Clark, 1997; Olsen and Baker, 2006). Based on previously reported concentrations of xanthophylls in corn gluten meal (Heuzé *et al.*, 2015), the alternative diet in my study contained xanthophylls (lutein and zeaxanthin; 9 mg/kg) at a sixth of the concentration of astaxanthin (60 mg/kg). A study by Olsen and Baker (2006) in Atlantic salmon found that a diet with 23 mg/kg of lutein and 55 mg/kg of astaxanthin did not significantly affect astaxanthin deposition or colouration of tissue, so passive interference by other xanthophylls was unlikely to be the major factor causing reduced astaxanthin concentrations in my study. Another explanation for decreased tissue colouration could be that the two diets led to differences in astaxanthin utilization. Up to half of the astaxanthin absorbed by salmon may be metabolized to Vitamin A (Torrissen, 1989; Ytrestøyl *et al.*, 2005), especially when dietary levels of vitamin A are low (Goodwin, 1986; Matsuno, 1991). In my study the alternative diet had 60% less vitamin A (3000IU/kg) compared to the high fish meal diet (8000IU/kg), likely because the Vitamin A content of corn gluten meal is much lower than that of fish meal (USDA, 2016; IFOMA, 1993). Studies on salmonid vitamin requirements are inconclusive, and suggest that the vitamin A requirement of pacific salmon may range anywhere from 30 – 25 000 IU/kg (Halver, 1972; Woodall, 1964). It is thus possible, although not certain, that the alternative diet provided insufficient Vitamin A, leading to greater conversion of astaxanthin to Vitamin A and reduced astaxanthin concentrations in muscle tissue. Corn gluten meal is likely responsible for the reduced redness and astaxanthin concentrations in the tissue of fish fed alternative diets due to both competition with novel carotenoids and reduced vitamin A levels.



## 4.1 Summary and Conclusions

My study shows that inclusion of novel genetic material, from wild salmon populations, can increase performance of a farm brood stock line. Crossbreeding led to increased growth rates in multiple lines in this study, though these differences were not driven solely by associated increases in conversion efficiency, swimming speed, or metabolic rate. Ongoing research with my industrial partner will also incorporate data on health and immunocompetence, traits that have exhibited positive results due to crossbreeding and have associated positive genetic correlations with growth rate (Nielsen *et al.*, 2010; Henryon *et al.*, 2002). My results support the idea that crossbreeding effects depend on the specific lines being mixed, and do not correspond with neutral genetic markers, suggesting genetic distance between populations does not provide a baseline to estimate the effects of crossbreeding (Houde *et al.*, 2011; Tymchuk *et al.*, 2010). The best choice to increase growth rate of this farmed line would be to use either the Capilano or Quinsam lines, though introducing any novel genetic material into the farmed line increases the probability of obtaining high performing offspring with no indication of negative performance effects.

Due to the importance of tissue colouration in the marketability of salmon, a corn gluten and poultry meal diet cannot be used in its present formulation in salmon aquaculture. Corn gluten meal is a cost-effective and readily available ingredient, but appears to be the cause of reduced tissue astaxanthin concentrations and colouration. Given the likely role of astaxanthin consumption to fuel Vitamin A synthesis in this difference, further study is warranted to determine if the addition of greater concentrations of vitamin A to the diet can mitigate this effect. Despite this effect, our study adds to the growing evidence that poultry meal, in combination with another plant protein source, is a viable alternative to high concentrations of fish meal in salmonid diets.

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## Curriculum Vitae

Katarina Doughty

### Education

**University of Western Ontario.** London, Ontario, Canada

M.Sc. Biology (September 2014- April 2017)

B.Sc. Biology with Honors (2010-2014)

### Teaching Experience

Biology 2290: Research Methods in Biology (2015 Winter Semester, 2016 Winter Semester)

Biology 3436: Animal Behaviour (2015 Fall Semester)

### Research Experience

**M.Sc. research project** **2014 - present**

University of Western Ontario

**Aquatic Science Technician** **Summer 2013 and 2014**

Department of Fisheries and Oceans, Canada

**Undergraduate Research Project** **2013-2014**

University of Western Ontario

### Awards

Deans Honor List 2014

Western Alumni "Go Global" Awards in Science (Value \$2000) 2012

### Presentations

Ontario Ecology, Ethology and Evolution Meeting (OE3C) May 2016

Fallona Family Showcase Interdisciplinary conference November 2015

Ontario Biology Day Conference April 2014

Western Undergraduate research symposium April 2014



**Pending publications from MSc. thesis work:**

Effects of replacing fish meal with poultry meal and corn gluten meal on growth rate and tissue quality for Chinook salmon (*Oncorhynchus tshawytscha*) aquaculture. *In progress*.

Assessing outbreeding across a range of genetic relatedness on performance-related traits in eight outcrossed populations of Chinook salmon. *In progress*.

**Qualifications**

PADI Open Water Diver Certification

SVOP: Small Vessel Operators Proficiency (Commercial boaters licence)

Pleasure craft Operators licence (Recreational boaters licence)

Marine VHF Radio Operator

ROC-M (Restricted Operator Certificate – Maritime)

First Aid and CPR