Effects of outbreeding on farmed Chinook salmon product quality

Celine Marie-Emanuelle Lajoie

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EFFECTS OF OUTBREEING ON FARMED CHINOOK SALMON PRODUCT QUALITY

By

Celine M.E. Lajoie

A Thesis
Submitted to the Faculty of Graduate Studies
through the Great Lakes Institute for Environmental Research
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the Degree of Master of Science
at the University of Windsor

Windsor, Ontario, Canada

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Effects of outbreeding on farmed Chinook salmon product quality

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DECLARATION OF ORIGINALITY

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Approximately 20% of British Columbia's salmon farming industry is represented by native Pacific Chinook salmon (*Oncorhynchus tshawytscha*). Few commercial facilities rear Chinook salmon, limiting their breeding stocks which may allow for inbreeding and can potentially lead to downstream effects on product quality. As consumers refuse to pay for low quality products even when prices are reduced, product quality metrics have become increasingly important to the aquaculture industry. As such, there is a need to determine whether product quality of farmed Chinook salmon can be improved through hybridization between wild and farmed populations. Product quality metrics were assessed in adult Chinook salmon generated from hybrids between six wild populations and one inbred commercial population to determine the impact of hybridization on product quality. Assessed quality metrics included slaughter yield, fillet yield, condition factor, colour, and lipid content. Overall, I found that fillet quality metrics differed across populations, and that hybrid populations did not outperform the farmed control in most metrics except for colour. I further aimed to examine the relationship between growth rate and product quality. I found that growth had significant relationships with traits of commercial interest such as, colour, fat content, jacking rate, and condition factor. Although hybrid populations did not outperform the farmed population, this thesis provides evidence that hybrid chinook salmon may be competitive commercially as they exceed or perform at desired market values for traits.
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CHAPTER ONE-GENERAL INTRODUCTION

Aquaculture

The earth’s population is expected to reach 9.7 billion people by 2050 and the demand for more nutritious high-quality foods is expected to double as the population, urbanization and average income increases (Diouf, 2009; FAO, 2016). As such, it is expected that humanity’s dependence on protein cannot be met solely by land-based production or marine-based production but instead a combination of both (Duarte et al., 2009; Gjedrem et al., 2012). Therefore, there is a need to increase production of aquatic protein sources (Marra, 2005; Gjedrem et al., 2012). Aquaculture, which refers to the cultivation and rearing of aquatic species, has become one the fastest growing food producing sectors worldwide as it now accounts for ~50% of the world’s food fish and has recently surpassed capture fisheries in terms of production (FAO, 2016). There are numerous advantages associated with the consumption of fish as a source of protein. For example, compared to terrestrial farm animals, such as pigs and poultry, captive salmon are more efficient at converting feed into meat (Smith, 1992; Gjedrem et al., 2012), have improved protein retention rates in captivity, and show improvements in energy allocation (Thodesen et al., 1999; Neely et al., 2008; Gjedrem et al., 2012). Not only are fish important sources of protein, they also contain other nutrients like amino acids, long chain omega 3-fatty acids, vitamins (D, A, and B) and minerals (calcium, iodine, zinc, iron and selenium) (FAO, 2016). However, aquaculture can have potentially negative environmental effects. Studies have shown that escapes led to the establishment of some exotic species such as the Pacific oyster (Magallana gigas; Shatkin et al., 1997) and black carp (Mylopharyngodon piceus) (Nico and Williams, 2001). Studies have further shown that fish farms may affect species habitat use as they can overlap with native
species (Markowitz et al., 2004; Gaitan-Espitia et al., 2017). Also, there is a possibility for disease and parasite transfer to occur between farmed and wild populations as studies have found in oysters (Burreson et al., 2000) and koi fish (Dixon, 2009). Nonetheless, the rise of Aquaculture is inevitable and therefore the need for sustainable aquaculture has grown due to its potential to reduce pressure on fisheries (Frankic and Hershner, 2003). Recent progress includes a shift from using wild fish sourced feed to a soybean cake or corn gluten protein sourced diet (Morikawa, 1999), regulations for number of parasites on fish and restrictions on farming in areas that may negatively affect wild populations (Taranger et al., 2015), and the use of triploid fish in farms to prevent breeding between escapees and wild populations (Benfey, 2001). Furthermore, organic aquaculture may be able to reduce the documented negative effects of current aquaculture practices (Georgakopoulos and Thomson, 2007).

**Canadian salmon aquaculture**

In Canada, aquaculture is an important industry as it accounts for 20% of total seafood production and its value has increased by 63% in the past ten years (DFO, 2013). The Canadian aquaculture industry can be separated into three sectors: shellfish, seaweed and finfish (Chopin and Bastarache, 2004). The finfish sector is the most dominant and accounts for over 60% of Canadian aquaculture products, 98% of which can be attributed to salmon production (DFO, 2012). Canada is ranked fourth in the world for salmon production and the industry is responsible for over 10,000 jobs, most located in New Brunswick and British Columbia (BC) (Pinfold, 2013). Farmed Atlantic salmon, considered to be Canada’s top aquaculture export (Pinfold, 2013), began production in BC in the 1980s and now account for approximately 80% of salmon production. However, the introduction of Atlantic salmon farming to Pacific waters has been
controversial (Gross, 1998), and has led to a risk of disease transfer and potential ecological interactions between escaped Atlantic salmon and wild Pacific salmon (Noakes et al., 2000). Native Pacific salmonids such as Coho salmon and Chinook salmon (*Oncorhynchus tshawytscha*) are also farmed in BC but only represent 20% of production. The culture of Pacific salmon species has great economic potential because these fishes are native to the areas in which they are reared and are more tolerant of diseases, such as Infectious salmon anemia (ISA) (Rolland and Winton, 2003), and have a greater resistance to parasites such as sea lice (*Lepeoptheirus salmonid copepodids*) (Jones et al., 2007). As such, it is possible that farming Pacific salmon could result in fewer environmental impacts. Currently, only a few commercial facilities raise Chinook and therefore limiting the availability of producers to optimize stocks for captive rearing.

**Domesticated salmon product quality and growth rate**

Domesticated populations are often different from their wild counterparts as they have undergone physiological, morphological and behavioural changes. Traits which may be advantageous in the wild may be less so in captive settings and traits that may be disadvantageous in wild settings may be able to persist in captive populations (Weber and Fausch, 2003; Huntingford, 2004; Harvey et al., 2016). Farmed habitats provide a relaxed environment with a lack of predators, no intra-species competition, and excess food to promote growth (Einum and Fleming, 2000; Jonsson and Jonsson, 2006; Harvey et al., 2016). However, even with selective pressures mostly relaxed, fish may still be influenced by density related factors such as increased aggression, stress due to fish transport and handling, as well as a greater likelihood for disease (Barton and Iwama, 1991). Nonetheless, fish farms provide the ideal environment for producers to maximize
the trait of highest economic importance: growth rate (Quinton et al., 2005). Fish exhibiting high growth rates are more likely to have higher feed conversion efficiency and greater body weight at harvest; traits that are directly related to the price of the fillet (Quinton et al., 2005). Not only this, but other important quality metrics have shown positive relationships with growth rate such as lipid content and colour (Quinton et al., 2005). However, it is possible that a quality-quantity trade-off may exist between growth rate and product quality as rapid growth rate may lead to undesirable effects on product quality. Therefore, it is important that a producer is able to quickly generate a large product without compromising product quality traits such as fillet colour, lipid content, body shape, slaughter and fillet yield, and fillet shape.

In-order to compete on both global and local markets salmon farmers wish to produce late maturing healthy fish that not only have elevated growth rates but also have a deep pink flesh colour, optimal lipid content and good body conformation with high yields (Camara and Symonds, 2014). Salmon flesh colour is due to the accumulation of carotenoids through their diet which are deposited in their flesh (Garner, 2010). Carotenoids are thought to be important for human health and disease prevention (Yeum and Russell, 2002; Rajasingh et al., 2006) and have shown to be play a role in vision, development and immune functions (Schiedt et al., 1985; Stephensen, 2001; Palace and Werner, 2006). Consumers associate high levels of carotenoids and a deep pink flesh colour with high-quality fish and as such, commercial salmon producers supplement fish feed with carotenoids such as synthetic astaxanthin (Rajasingh et al., 2006; Johnston et al., 2006). The carotenoid compositions of feeds can represent 15-20% of total feed production costs; however, retention of dietary carotenoids in salmonids ranges from 2-
22% (Torrissen, 1995; Rajasingh et al., 2006). However, studies have shown that selection for growth rate can lead to indirect favourable improvements in fillet colour (Quinton et al., 2005; Johnston et al., 2006).

Unlike terrestrial animal meat, fish lipids are beneficial to human health and have been known to decrease cardiovascular and inflammatory diseases (Klor et al., 1997). Lipids are essential for the colour, flavour and texture of salmon (Rasmussen, 2001), and can greatly vary between populations of fish (Morkore et al., 2001) Farmed salmon are fed high fat diets to increase growth rate (Shearer, 1994). Production losses are possible due to increased level of fat deposition in the body cavity (Jobling et al., 1998; Jobling, 2001). When salmon fed high fat diets (39%) were compared to fish fed medium fat diets (32%), they had higher carcass lipid levels and larger fat deposits in the muscle (Bjerkeng et al., 1997). It is strongly suggested that fat content in salmonids should not exceed 18% (Gjedrem et al., 1997). Not only can high fat diets lead to a product that is oily in texture, and susceptible to rancidity, they can also result in a poorly pigmented product (Bell et al., 1998). It is possible that selection for elevated growth in salmonids may lead to a trade-off between product quality and growth rate due to elevated fat content in salmonids.

Selection for rapid growth in farmed salmonids can lead to a “domesticated phenotype” which is characterized by a rotund body shape (Gjerde and Shaeffer, 1989; Kause et al., 2003; Colihueque and Aranaeda, 2014). Consumer preference for body shape varies depending on product purchased and market; if large fillets are desired, fish exhibiting rotund body shapes would be favoured (Røra et al., 2001), but when salmon is being sold whole consumers prefer a more streamlined body resembling wild salmon.
It is possible to evaluate fish body form through calculating condition factor (Fulton’s K), as it has been correlated to body depth in Atlantic salmon (*Salmo salar*) and Rainbow trout (*Oncorhynchus mykiss*) (Petersson et al., 1996). Fillet yield increases with increasing condition factor implying that larger and wider fish produce greater yields (Røra et al., 2001). Condition factor is also associated with the well-being or fitness of the fish (Jones et al., 1999) and can be used to assess health of fish stocks and compare feeding activity of populations in different environmental conditions (Le-cren, 1951; Lizama and Ambrosia, 2002; Ighwela et al., 2011).

Producing fish with high slaughter and fillet yields is desirable as less flesh is wasted and a larger percent of the fish’s body is edible and able to be sold to consumers (Gjedrem, 1997). Also, increased growth rate is directly related to increased yields.

Slaughter yield or dressing percentage of the fish is important when fish are being sold whole and refers to the proportion of gutted weight to full body weight of the fish (Røra et al., 2001). As fillets are the most economically valuable portion of the fish, fillet yield, the portion of fillet weight to full body weight, is an important trait. Fillet shape, the relationship between length and weight of the fillets (length/weight²) is also an important economic trait as thinner fillets are correlated to lower fillet yields when compared to more voluminous shaped fillets (Mørkore et al., 2001).

**The improvement of aquaculture stocks using outbreeding**

In order to meet the growing protein demands of the world’s population, the aquaculture industry has focused on increasing and improving production efficiency through selective breeding and domestication (Keys et al., 2004). Often, aquaculture
populations are established using a small number of founder families which are subject to selection for a single trait (Preston and Clifford, 2002). Previous research has shown that small breeding populations are vulnerable to inbreeding, mating between related individuals, which can lead to a decrease in genetic variation of the population and negative effects on performance traits (Falconer and Mackay, 1996; Bierne et al., 2000; Keys et al., 2004). Effects of inbreeding include the limitation of inbred individuals to respond to selection and can lead to inbreeding depression. Inbreeding depression is defined as the reduction in expected performance in offspring from inbred individuals when compared to a non-inbred population (Keys et al., 2004). Fortunately, outbreeding, introducing new genetic material to a population, may lead to heterosis (hybrid vigor), the creation of a genotype that is superior to either parent, allowing hybrid offspring to have higher performance than their parents (Baranwal, 2012). Heterosis has been previously observed in economically important aquaculture species such as farmed carp (Cyprinus carpio) (eg. Wohlfarth, 1994; Bakos and Gorda, 1995), tilapia (Oreochromis niloticus) (Bentsen et al., 1998), and catfish (Clarias sp.) (Suresh, 1991). However, when genetic distance between parental populations is too great, outbreeding depression, defined as reduced performance in hybrids, may occur. Outbreeding depression has been previously observed in fish species for traits such as homing ability in pink salmon (Oncorhynchus gorbuscha) (Bams, 1976), salinity tolerance in kokanee salmon (Oncorhynchus nerka) (Foote et al., 1992) and physiological performance in largemouth bass (Micropterus salmoides) (Cooke et al., 2001). Although heterosis may occur in the first generation (F1), it may be followed by outbreeding depression in the second (F2) (Edmands, 1999; Marr et al., 2002). Nonetheless, outbreeding farmed populations with wild populations may
allow for the opportunity to identify and select traits of high economic value in hybrids such as growth rate, survivorship and product quality (Newkirk and Haley, 1983; Gjedrem, 1985). In salmonid species like Atlantic salmon and Rainbow trout (*Oncorhynchus mykiss*), heterosis has been previously observed for in aquaculture performance traits such as growth, behavior and survival (Ayles and Baker, 1983; Einum and Fleming, 1997). However, few studies have examined the effects of outbreeding on fillet quality: Glover et al. (2009) found that F1 hybrid Atlantic salmon exhibited intermediate growth and quality when compared to wild and farmed parental strains. As such, not much is known about the ability of outbreeding to improve the viability/production capacity of smaller-scale pacific salmonids such as Chinook salmon and its effect on important fillet quality metrics.

**Thesis Objectives**  
The main objective of this thesis was to determine how outbreeding product quality in seven populations of hybrid Chinook salmon and its effects on the relationship between growth rate and fillet quality metrics. In the Fall of 2013, seven hybrid populations of Chinook salmon were created at an organic fish farm located in BC called Yellow Island Aquaculture Ltd (YIAL), through breeding females from one domesticated farmed stock (YIAL) (see Chapter 2) with males from six wild populations from nearby rivers (Big Qualicum River, Chilliwack River, Nitinat River, Puntledge River, Quinsam River, and Robertson Creek). My goal was to determine whether production metrics such as body shape, yield, lipid content and colour scores were improved in wild-farmed hybrid salmon when compared to offspring from a purely domesticated stock. The relationship between these traits and growth rate was also investigated to determine
whether a trade-off existed in product quality in a domesticated population that has undergone years of selection for growth when compared to farmed hybrids.

In **Chapter 2**, three-year-old adult Chinook salmon hybrids reared at YIAL were harvested for product quality metrics. Using both industry standard and more scientific measures, we examined traits such as body shape, yield parameters, lipid content and colour scores to determine the effects of outbreeding on fillet quality. We compared traits across hybrid populations to see how populations varied and how they compared to the YIAL population to determine whether outbreeding led to an increase in quality. The overall objective was to determine whether Chinook salmon aquaculture performance could be increased by the influx of wild genes to a small domesticated population.

In **Chapter 3**, we aimed to expand upon results from the previous chapter by evaluating the relationship between product quality metrics, survivorship, precocious sexual maturity, and growth rate between hybrid populations and a domesticated stock. Due to industry preference for fish with high conversion feed efficiency and elevated growth rate to maximize yield, it is possible that trade-offs may exist between the production quality of the fish and traits commonly associated with elevated growth rate such as high lipid content. First, we aimed to determine whether survivorship in freshwater was correlated to growth rate. Next, we evaluated whether elevated growth rate was related to precocious sexual maturity in hybrid populations and how rates would compare to the farmed population. We then looked at the relationships between growth rate and fillet colour and lipid content. These investigated relationships may lead to insight on how domestication may modify these relationships through the comparison of hybrid stocks to YIAL.
References


CHAPTER TWO-IMPACT OF HYBRIDIZATION ON FARMED CHINOOK SALMON PRODUCT QUALITY

Introduction

Producers of farmed animals and plants often seek to improve yield and product quality through selection and breeding (Gjedrem, 1985). The goal of most aquaculture breeding programmes is to select for individuals that have the highest genetic performance for a phenotypic trait of interest (Gjøen and Bentsen, 1997). Breeding programmes are expected to generate continuous genetic gain in commercial traits but are vulnerable to inbreeding as captive populations have few broodstock individuals and small effective population sizes. (Kincaid, 1983). However, it may be possible to increase genetic diversity in an inbred population through introducing genetically distinct individuals to a population (outbreeding) (Edmands, 1999). Producers can then develop an improved line from one or multiple local populations, wild or farmed, by crossing with their original stock (Brummett and Pozoni, 2009). Wild populations are often more genetically diverse than captive populations and may be able to enhance the performance of captive populations with low effective population sizes (Brummett and Pozoni, 2009). Some studies have found increased performance in offspring of captive populations that were crossed with wild populations (Bentsena et al., 1998), although others have found intermediate performance when compared to parental strains (Glover et al., 2009). Crossing with multiple populations and comparing stock performance allows for producers to identify potentially profitable strains as different populations may exhibit variability in performance as they originate from different geographic locations and are differentially locally adapted to their environment (Knibb, 2000). Outbreeding may also lead to heterosis (hybrid vigor): when hybrid offspring outperform either parent (Edmands, 1999). Although heterosis has been previously observed in aquaculture
species (Wohlfarth, 1993; Bakos and Gorda, 1995; Bentsena et al., 1998; Suresh, 1991), outbreeding depression, when hybrid offspring have lower fitness than the parental stock, can negatively impact traits/fitness, has also been observed (Gharrett et al., 1999; Tymchuk et al., 2007). Outbreeding farmed populations (homogenous stocks) with multiple wild populations (heterogenous stocks) may also allow for the identification and selection of traits with high economic value such as high growth rate, survivorship and product quality (Newkirk and Haley, 1983; Gjedrem, 1985).

In Canada, both Atlantic salmon and Chinook salmon (*Oncorhynchus tshawytscha*) are farmed, although the former represents the bulk of Canada’s aquaculture production, most of which occurs in British Columbia (Pinfeld, 2013). The rearing of Atlantic salmon on the west coast of Canada remains controversial because of perceived negative effects of their farms, such as the risk of disease transfer (Gross, 1998) and potential interactions of escaped individuals and native Pacific salmon (Oakes et al., 2000). The culture of Pacific salmonids has great economic potential because these fishes are native to the areas in which they are reared and are more tolerant of diseases, such as Infectious Salmon Anemia (ISA) (Rolland and Winton, 2003), and have a greater resistance to parasites such as sea lice (*Lepeophtheirus salmonis*) (Jones et al., 2008). Few breeding programs exist for Chinook salmon limiting the ability of producers to optimize stocks for captive rearing. As such, it is possible that current farmed stocks of Chinook salmon may be vulnerable to inbreeding and that outbreeding may increase the viability/production capacity of these stocks. Heterosis has been previously observed in salmonids such as Rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*), for various traits such as growth, behaviour, and survival (Ayles and Baker, 1983;
Einum and Fleming, 1997). Few studies on heterosis in Chinook salmon exist (see Bryden et al., 2004; Wessel et al., 2006) and none examine product quality, specifically fillet quality metrics.

The purpose of this study was to determine whether outbreeding a farmed domesticated population of Chinook salmon with wild populations would improve product quality metrics. We crossed a farmed population with six different wild populations and measured performance in market-sized offspring for industry-relevant quality metrics, including: body shape, slaughter yield, fillet yield and shape, lipid content and colour. We measured both fillet and slaughter yield as fish production is costly and high yields indicate less of the fish is wasted (Røra et al., 2001). Body shape was also measured as consumers often prefer more streamlined body types when buying fish whole and producers prefer more rotund bodies when selling fillets because they are associated with higher yields (Gjøen and Bentsen, 1997, Røra et al., 2001; Kause et al., 2003). We also measured lipid content as levels above 18% could lead to production losses and discoloration of the flesh (Gjedrem et al., 1997). Fillet colour was measured because it is an important trait economically as carotenoid compositions of feeds represent 15-20% of total production costs, and consumers associate a deep pink colour with superior flesh quality, freshness and flavour (Johnston et al., 2006). Overall, we hypothesized that the addition of new wild genetic material to the farmed population would lead to increased performance in hybrid populations, in this case higher product quality, when compared to a farmed control population. We expected that hybrids perform well in the farmed environment as they are progeny of farmed fish previously reared in that environment. Specifically, we expected to increase population level
variation in fillet quality metrics that would enable producers to maximize culture
efficiency and stock performance

**Materials and Methods**

*Facility and generation of hybrids stocks*

Research took place at Yellow Island Aquaculture Ltd. (YIAL), an organic Chinook salmon farm, located on Quadra Island, British Columbia, Canada (50° 07’N, 125°19’W). The domesticated stock of Chinook salmon at YIAL have been in production since 1985 and are descendants of crosses between two wild source populations located on Vancouver Island: Big Qualicum (49°23’N, 124°36’W) and Robertson Creek (49°20’N, 124°58’W). In this study, half-siblings were generated through adding milt from males collected from six wild populations (Figure 2.1) to eggs from hermaphrodite females from YIAL. These females were used to purposely reduce maternal effects so that variation observed among populations could be more directed at sire effects. We generated ten-half sib families within each population (six wild and one farmed) by using milt from 10 males from each of the six wild populations: Big Qualicum River (49°23’N, 124°36’W), Nitinat River (48°51’N, 124°39’W), Puntledge River (49°41’N, 125°02’W), Quinsam River (50°01’N, 125°18’W), Robertson Creek (49°20’N, 124°58’W), and Chilliwack River (49°04’N, 121°42’W) (Figure 2.1). These wild populations were chosen due to their proximity to YIAL and the possible variation in traits that may impact product quality that may arise due to population differences. Fertilized eggs from each of the 70 families were then divided into incubation trays in two replicate cells. On March 14, 2014 fry from replicate cells were combined and were randomly assigned to each of two 200L replicate tanks. Populations were randomly subsampled and fish were counted and weighed on 19 March 2014, 22 April 2014, 8 May 2014, 20 May 2014, 4 June 2014.
and 14 June 2014. On July 7, 2014, once fish had reached 3-5g wet mass, they were PIT tagged to allow for individual identification. From 11-12 August 2014, fish were moved to 16 population-specific and replicated saltwater sea pens (dimensions: 15ft × 15ft × 10ft deep). In November 2015, salmon in sea pens were weighed and early sexually maturing individuals (i.e., jacks) were removed, and remaining individuals from each population were combined into 8 sea pens by population. From 30-31 October 2017, 3-year old market-sized Chinook salmon (n=206) were collected from sea pens and euthanized for the collection of quality metrics.

**Measurement of fillet quality metrics**

*Body shape analysis and product yield*

Fish were weighed to the nearest gram using an electric scale (OHAUS Valor™ 4000 Series) and fork length was measured to the nearest cm. Condition factor (Fulton’s K) was calculated for each fish (K= Body weight (g) x 100/ (length cm)³) as a higher condition factor can be associated with a deeper body shape and greater yield (Petersson et al., 1995; Kause et al., 2003). To determine body shape, fish were photographed on their right side in a light box (D 60.71cm × H 76.84cm × W 81.915 cm) with a digital single-lens reflex (DSLR) camera (Nikon D3300) using an Aputure light emitting diode (LED) ring flash (AHL-HN100 Nikon) as a constant light source (see Figure 2.2). Twelve homologous landmarks were placed on the photographs of each salmon in TpsDig Software (Rohlf, 2008) and partial warp analysis was used to compare morphological shape variation among the 7 populations (see Figure 2.2) (Zelditch et al., 2004; Colborne et al., 2011). Thin-plate analysis was performed on the scores from the DFA to visualize body shapes associated with each DFA axis. In-order to determine differences among
populations, a MANOVA was performed on DFA1 (scores related to body depth) and DFA2 (scores related to caudal peduncle depth and head shape).

An experienced worker from a neighbouring fish processing plant (with no prior knowledge of origin populations) gutted, filleted, and assessed colour of fish. This was done to ensure that fish would be processed similarly to methods used in a fish processing plant. By using one worker, we ensured that filleting and colour evaluation would be constant across all fish. However, using only one worker may bias the results to his filleting technique and his perception of colour. Therefore, to reduce this potential bias we used other techniques such as computer evaluation of colour and body shape analysis. Whole fish were gutted and were weighed to nearest gram using an electronic scale (OHAUS Valor™ 4000W Series). Slaughter yield (proportion of gutted weight to body weight) \( \text{(Slaughter yield (\%) = Gutted weight (g) x 100/ Body weight (g))} \) was calculated for each fish as fish exhibiting higher yields are more desirable as they generate less waste. After fish were gutted, they were filleted, rinsed with seawater and trimmed according to aquaculture standards. More voluminous shaped fillets are correlated to higher fillet yields and are more economically viable. Fillet yield and fillet shape were calculated as follows. The left fillet length (cm) was measured and weighed on an electronic scale to calculate fillet mass index \( \text{((FMI= fillet weight (g) x 100/ fillet length (cm}}^2) \) \( \text{)) (Mørkøre et al., 2001) \) Left and right-side fillets collected from each fish were weighed to the nearest gram with an electronic scale to calculate fillet yield \( \text{(Fillet Yield (\%)= (fillet weight (g) x 100)/ (body weight (g)) (Rørå et al., 2001).} \)
Lipid content

Lipid content was measured because if it is too high (exceeds 18%) it can lead to diminished product quality (Gjedrem et al., 1997). Lipid content was assessed on newly euthanized whole fish using an industry standard hand-held micro-wave fat probe, considered to be a fast, easy to use and portable method (Distell Fish Fatmeter model 692, Distell Inc, West Lothian Scotland, U.K) (Vogt et al., 2002). The fat probe determines lipid content of somatic tissues by emitting a low powered wave (frequency, 2GHz ± 2000 MHz; power, 2mW) that measures the inverse relationship between water and lipid content, and converts water concentration to lipid concentration (Cooke et al., 2005). The probe was placed on the right side of the salmon and measurements were taken from 4 different locations to determine the fat content of each fish.

To confirm the accuracy of the industry standard fat meter, we also conducted lipid extraction analyses via the Soxhlet technique (Chin et al., 2009). When compared to fat probe results, Soxhlet lipid extraction is considered to be a more accurate method (Kortschal et al., 2011), and often reveals higher fat content values than fat-probes (Vogt et al., 2002). Muscle samples (~5g) were collected from each fillet, were flash frozen, and stored in a -80°C for lipid content analysis. Samples were thawed, placed in pre-weighed tubes, reweighed, and then stored in a -80°C freezer for 24 hours. Next, samples were placed on a freeze dryer for 48 hours. After, samples were placed in a dessicator and weighed to determine moisture content. They were then put in pre-dried Whatcom filter paper envelopes, weighed and then stored in a drying oven at 60°C for 7 days. Afterwards, samples were reweighed and extracted through Soxhlet lipid extraction with 750mL of Petroleum ether for 8 hours in a three-funnel glass Soxhlet apparatus. After 8 hours, samples were removed from the apparatus and stored in a fume hood overnight.
The following day, they were placed in a dry oven at 60°C for 7 days. Samples were then weighed to determine lipid concentration of muscle samples. Results from chemical lipid extraction were compared to values obtained from the standard method, the fat-probe, to determine if they generated similar results. Accuracy of a fish fat-probe, when compared to chemical methods for fat content are ± 1% for low fat fillets (<25% lipid content) and ± 3% for fatty fillets (>25% lipid content) (Distell, 2011). However, results vary depending on scale thickness, fat distribution and muscle type (light vs dark), water content levels, how quickly the fat-probe is moved from location to location on the fish and the level of expertise of the person using the fat-probe (Sigurgisladottir et al., 1997).

**Fillet colour**

As it is important and costly for the industry to meet consumer preferences for deep pink/red coloured fillets, fillet colour was assessed using two methods. First, fillet colour was graded immediately post slaughter using the industry standard DSM SalmoFan™ Lineal colour card (DSM, 2014). Specifically, colour was assessed in four different locations on the fillet: the ventral part of the fillet, below the dorsal fin, on the caudal peduncle and the gut. Scores on the colour card ranged from 20 (palest colour; light pink) to 34 (darkest colour; dark red) and values obtained from fillets were averaged to obtain overall colour score of the fillet. To confirm the capability of the industry standard SalmoFan™, we also conducted colour analysis via computer vision analysis (Léon et al., 2006). Fillets were photographed in a light box next to a DSM SalmoFan™ Lineal colour card with a digital single-lens reflex (DSLR) camera (Nikon D3300) using an Aputure light emitting diode (LED) ring flash (AHL-HN100 Nikon) as a constant light source.
source (see Figure 2.2). Colour was also quantified through digital photography and computer vision (Léon et al, 2006). Colour spaces are used to visualize and represent colour in two and three-dimensional space (Trussel et al., 2005). When measuring colour, the L*a*b colour space is most common as it is the closest to human colour perception. Commission Internationale d’Éclairage (CIE) L*a*b* is an international standard to measure colour where L* represents the lightness component (0 to 100), a* (redness) and b* (yellowness) are chromatic components (-120 to 120) (Yam and Papadakis, 2004).

Images (JPEG) of fillets were uploaded to Adobe PhotoShop CC 2015 for colour analysis where the DSM SalmoFan™ colour scores 22 and 32 were used as colour standards. Grids (2.5cm X 2.5cm) were placed in four different locations on the fillet (the ventral part of the fillet, below the dorsal fin, on the caudal peduncle and the gut) and the “blur” function was used to obtain average CIE L*A*B* values in those areas (see Figure 2.3) (adapted from Hu et al. 2015).

**Statistical analyses**

Data are presented as mean ± standard error. Assumptions for normality were tested prior to data analysis. Differences in mean population slaughter yield, colour score, fillet yield, fillet shape (FMI) were analyzed using one-way ANOVAs. Population differences in fat content were analyzed using ANCOVAs, with fish weight as a covariate because lipid content is known to be influenced by fish weight. Post hoc analyses were conducted using a Tukey HSD test to determine population differences in quality metrics. Pearson’s correlations were conducted to determine relationship between fat-probe and soxhlet lipid extraction values. Population differences for redness (a*) value from computer vision were analyzed using an ANCOVA with a colour standard used as a covariate. Pearson’s correlations were conducted to determine relationship between
colour scores and computer vision values. Data were analyzed using SPSS statistical software analysis software (IBM Corp. Released 2013. IBM SPSS Statistic for Windows, Version 22.0. Armonk, NY: IBM Corp).

**Results**

Condition factors (K) of hybrids and domesticated YIAL stocks were not significantly different (One-way ANOVA, F_{6,200}= 0.591, p=0.74, Figure 2.4). Body shape differed significantly among populations (MANOVA, Wilks’ λ=0.38, P<0.0001), with 56% of the variation in body shape explained by body depth (DFA 1; 30%) and head shape and caudal peduncle size (DFA 2; 26%). Significant differences among populations were found when examining DFA 1 (body depth) scores (One-way ANOVA, F_{6,200}=2.2, p<0.0001). Thin-plate spline analysis of body depth (DFA 1) showed that Puntledge fish had more streamlined bodies than Big Qualicum, Chilliwack, Robertson Creek, Nitinat and YIAL, which were more rotund, and Quinsam was more streamlined than Robertson Creek (Figure 2.5). DFA 2 (head shape and caudal peduncle depth) revealed significant differences among populations (One-way ANOVA, F_{6,200}=6.2, p<0.001). Thin-plate spline analysis of DFA 2 showed that YIAL fish had shorter heads and smaller caudal peduncles than Chilliwack, Quinsam and Robertson Creek (Figure 2.5). Slaughter yield (%) varied significantly across populations (One-way ANOVA, F_{6,200}=6.2, p=0.002) (Figure 2.6). Post-hoc analysis revealed Chilliwack had a significantly lower slaughter yield than Robertson Creek, YIAL and Quinsam (all p<0.02). Fillet Mass Index (FMI) did not differ significantly across populations (ANOVA, F_{6,200}=1.1 p=0.35) (Figure 2.7). However, fillet yield differed significantly across populations (ANOVA F_{6,200}=6.3, p<0.001; Figure 2.8). Post-hoc analysis revealed that Chilliwack had significantly lower yield than all populations (all p<0.02).
Correlational analyses confirmed a significant relationship between the fat meter and Sohxlet extraction methods (Pearson Correlation: $r=0.78$, $p<0.001$) (Figure 2.9). However, neither lipid content (%) measured using the fat meter or extractions varied significantly among populations (fat meter: $F_{6,198}=1.001$, $P=0.43$; Figure 2.10; Sohxlet extraction: $F_{6,197}=0.54$, $P=0.78$; Figure 2.11). Fillet colour differed significantly among populations (ANOVA, $F_{6,200}=10.5$, $P<0.001$; Figure 2.12). Post-hoc analysis revealed that Chilliwack had significantly lower colour scores than all populations (all $<0.03$); when Big Qualicum was compared to YIAL it had a significantly higher colour score ($p<0.01$).

When comparing fillet colour scores from the salmofan to colour space values (lightness, redness and yellowness) significant correlations were found. Correlation analysis revealed that lightness was negatively correlated with salmofan score ($p<0.0001$, $r=-0.46$) (Figure 2.14a), and differed significantly across populations (ANCOVA, $F_{6,197}=0.2.8$, $p=0.012$). Fillet colour analysis in Photoshop™ showed that redness differed significantly among populations (ANCOVA, $F_{6,197}=8.7$, $P<0.0001$), and was positively correlated with Salmofan score ($p<0.0001$, $r=0.72$) (Figure 2.14b). A post hoc test of computer vision scores for redness showed similar population performance to colour score evaluation by an experienced worker. Yellowness ($b^*$) also differed significantly among populations (ANCOVA, $F_{6,197}=4.714$, $p<0.001$), and was positively correlated with salmofan score ($R=0.33$, $p<0.001$) (Figure 2.14c).

**Discussion**

In this study, six populations of first generation farmed hybrid Chinook salmon (wild-farmed hybrids) and a farmed domestic population were compared across multiple fillet quality traits deemed important for aquaculture. Contrary our predictions, hybrid populations did not outperform the domestic farmed population in any fillet quality
metric except for colour. Although population variation was observed for some traits (body shape, slaughter yield, fillet yield), there were no population differences for other traits (fat content, condition factor and fillet shape). Previous studies have similarly found population variation in product quality metrics for Atlantic salmon (Johnston et al., 2006; Powell et al., 2008). Also, when comparing fillet quality metrics across multiple European Sea Bass populations, heterosis was not observed in hybrid populations for any fillet quality trait measured (Vandeputte et al., 2014). Glover et al. (2009) compared fillet quality in wild, farmed and hybrid (Wild x Farmed) populations of Atlantic salmon. One wild and one farmed population was used to produce hybrids and evidence for heterosis was not found. Studies in salmonids comparing first generation (F1) hybrid performance to wild and farmed parental strains have often found that hybrids performed similarly to parental strains or displayed intermediate values for performance in traits such as colour, fat content, yield, growth (Glover et al., 2009), and survivorship (McGinnity et al., 1997; Fleming et al., 2000; Bryden et al., 2004).

**Colour Score**

As predicted, colour scores differed significantly among populations with Big Qualicum as the only population to significantly outperform the control YIAL. Steine et al. (2005) found that a consumer’s willingness to spend more money on a fillet increased when comparing salmon scoring a 32 on a the Salmofan™ to those scoring 27 or lower, but found no difference in preference when comparing fish scoring from 27-29 on the fan (Steine et al., 2005). These results may indicate that if faced with a choice between populations, consumers may be willing to pay higher prices for Big Qualicum, lower prices for YIAL, Robertson Creek, Quinsam and Nitinat and even less for Chilliwack (the population with the lowest score). Unlike our study, which found most hybrid
populations’ colour performance to match or surpass (i.e: Big Qualicum) our benchmark farmed population, Glover et al. (2009) found that farmed Atlantic salmon were redder than both hybrid and wild individuals, and hybrid individuals outperformed wild fish reared in a farmed setting. Astaxanthin content in flesh has been linked to selection for elevated growth rate indicating that offspring of different populations of farmed fish may exhibit redder colours when reared in the same environment (Quinton et al., 2005; Glover et al., 2009). In the wild, Chinook salmon exist in both red and white pigmented morphs due to a genetic color polymorphism (Tyndale et al., 2008; Lehnert et al., 2016a).

Chinook salmon populations found in the Chilliwack river have large numbers of white fleshed salmon which may explain why Chilliwack had lower colour scores (DFO, 1999). The observed variation in colour scores among populations is consistent with previous work done on flesh pigmentation in Chinook salmon that found that when offspring of white and red flesh individuals were reared in the same environment and fed the same diet, there was still variation in colour scores (McCallum et al., 1987). Also, a recent genome-wide association study has identified several genes associated with Chinook salmon pigmentation (Lehnert, 2016b), indicating that population variation in colour scores may be due to genetics. This indicates that future crossbreeding with Big Qualicum may result in higher colour scores in future generations.

**Body shape**

Condition factor (Fulton’s K) did not differ significantly across populations. It was uniformly high for all populations, which may indicate that fish experienced isometric growth and good health overall (Ighwela et al., 2011). From an economic standpoint, our results indicate that outbreeding had little effect on this trait and all populations would be above industry average (0.9) (Røra et al. 2001). A reason for such
high values may be due to fish not being starved prior to slaughter. Body shape varied significantly among populations with Puntledge hybrids having significantly more streamlined bodies than Big Qualicum, Chilliwack, Robertson Creek, Nitinat and YIAL, which showed a more rotund shape overall. Markets selling whole salmon may prefer Puntledge fish as they had streamlined bodies which resemble wild phenotypes (Gjøen and Bentsen, 1997, Røra et al., 2001; Kause et al., 2003). Farmed salmon tend to have deeper body depths than wild fish because they are fed to satiation, face less strenuous swimming than wild fish, and grow more quickly overall (Swain et al., 1991). Not surprisingly, salmon culturing has greatly affected salmonid morphology with cultured salmon generally having smaller heads and deeper body depth than wild fish (Fleming et al., 1994). A study by Wessel et al., (2006) comparing morphometrics from three lines of hatchery reared Chinook salmon (a wild line derived from founding population, a hatchery line, and a hybrid population formed through crossing hatchery and wild fish), found that that fish from the hatchery line were much more streamlined and had shorter heads and narrower caudal peduncles than wild fish and differed significantly. Moreover, hybrids were significantly different from both wild and hatchery fish and were morphologically intermediate from parental strains, indicating a genetic component to differences in wild and hatchery derived fish (Wessel et al., 2006). It is well known that wild Chinook salmon populations exhibit different body morphology depending on migration timing and habitat differences (Currens et al., 1989; Hard et al., 1999). Chinook salmon from larger rivers tend to be larger overall, and have larger heads, caudal peduncles and fins than those from smaller rivers (Kinnison et al., 1998). In our study, thin-plate spline analysis indicated that the YIAL domesticated population had shorter
heads and smaller caudal peduncles than Chilliwack, Quinsam and Robertson Creek. Reasons for observed differences include adaptation to the captive environment, morphological plasticity, and genetic differences (Swain et al., 1991). The establishment of a general breeding goal for appearance is difficult because consumers and fish farmers do not always desire the same characteristics in their fish and it is difficult to produce streamlined fish that grow quickly and that are large enough for market sale without being rotund, as mass is more easily added to the girth and not the length of the fish (Kause et al., 2003). Furthermore, other studies have found that more rotund fish are sometimes preferred as they produce higher fillet yields (Røra et al., 2001).

Slaughter Yield
Slaughter yield varied significantly across populations and Chilliwack had a significantly lower yield than Quinsam, Robertson Creek, and YIAL. All other hybrid populations did not differ significantly from YIAL. Slaughter yield values of Atlantic salmon range from 82-96% and values above 90% are desired (Røra et al., 2001). Although Chilliwack had a significantly lower slaughter yield than the farmed control, all populations had slaughter yields above 90% and performed above the desired value for that trait. Although differences were statistically significant, all populations performed around the same value (90-91%) and therefore small differences of 1% may not matter to producers. Heritability can be low for slaughter yield as this trait is based on intestine weight, it may be difficult to select for smaller intestines and has been suggested to instead focus on reducing visceral fat percentage (Gjedrem, 1997).

Fillet shape and yield
Fillet shape (fillet mass index; FMI) did not differ significantly across populations which differ from results from a previous study that found population differences for
populations of free living and farmed Atlantic salmon (Mokore et al., 2001). Fillets that are more voluminous (have a higher FMI) have been shown to be less vulnerable to weight loss when trimmed (Mokore et al., 2001). In our study, fillet yield varied significantly across populations and Chilliwack had significantly lower fillet yield than all other populations. On average, Chilliwack had a 4-5% lower yield than all populations which could greatly affect production value of that stock as a few percent differences in yield can have a considerable economic impact when fish are processed (Peterman and Phelps, 2012). The average range of fillet yields of all populations (53-59%) was below the average range of fillet yield for Atlantic salmon (60-68%) (Einen et al., 1999).

Population differences in fillet yield (%) observed in our study are similar to studies done in rainbow trout (6%) (Smith et al., 1988), and Atlantic salmon (7%) (Einen et al., 1999). Factors that can affect fillet yield include feed ration (Einem et al., 1999), diet composition (Rasmussen, 2001), sexual maturity (Paaver et al., 2004), genetic line (Smith et al., 1988), differences in muscle mass and adipose tissues (Dunajski, 1979; Bugeon et al., 2010).

**Lipid content**

Lipid content did not vary across populations for either method (atmeter or chemical extraction). Also, there was a significant relationship between fat content obtained from the fat-probe and lipid extraction which is consistent with values from previous studies (Distell 2011; Vogt et al., 2002) indicating that a fat-probe is a reliable tool for predicting fat content. Mean fat-probe value was higher than the average mean lipid content extraction value which is consistent with other studies that have found that values from extraction are often higher than those found with fat probes (Vogt et al., 2002). Fillets with lower fat content are more desirable as production losses can occur
due to increased level of fat deposition, leading to a product with an oily texture, poorly pigmented, susceptible to rancidity and strongly flavoured (Rørå et al., 2003). Selection for elevated growth rate may indirectly lead to undesirable increases in fat deposition in salmon flesh. This is problematic for economic value as fat percentage in salmonids should not exceed 16-18% (Gjedrem et al., 1997). Mean fat content, determined through Soxhlet extraction exceeded the aquaculture desired value of 18%, for Atlantic salmon. However, due to the fat-probe being the industry standard, and high correlation between the probe and Soxhlet, it may be more accurate to the industry to use fat-probe results. Also, Chinook salmon are considered a fattier fish than Atlantic salmon and higher fat values are expected (Exler, 1987).

**Conclusion**

Overall, quality metrics varied in hybrid populations although no clear evidence for heterosis was found. It is possible that Chilliwack exhibited outbreeding depression, as colour and fillet yield scores were low, however this could simply be due to the existence of white fleshed salmon existing in high numbers in the natural population and maladaptation of the hybrids to the cultured environment. However, most hybrid populations performed similarly to YIAL demonstrating values associated with high quality fish. Although differences were found among populations for traits such as slaughter and fillet yield, these differences may not be large enough to greatly affect fillet value. In-order to determine the best performing stock, future work should focus on whether a correlation exists between fillet quality metrics and growth and whether a trade-off exists between traits and survivorship (see Chapter 3). Future studies should be conducted on a second generation of hybrids using populations such as Big Qualicum and Robertson Creek who performed well when compared to YIAL.
References


versus genetic origin. Canadian Journal of Fisheries and Aquatic Sciences 48, 1783–1791.


**Figure Captions**

**Figure 2.1** Map of southern British Columbia, Canada, showing the location of six wild populations of Chinook salmon (*Oncorhynchus tshawytscha*) (Big Qualicum, Chilliwack River, Nitinat, Puntledge, Quinsam, Robertson Creek) where wild males were selected for outbreeding in this study. The location of Yellow Island Aquaculture on Quadra Island, BC where hybrid stocks and an domesticated farmed stock (YIAL) were reared in an aquaculture setting to assess fillet quality metrics.

**Figure 2.2**: A) A typical image of a Chinook salmon (*Oncorhynchus tshawytscha*) photographed in a light box with a digital single-lens reflex camera for morphometric body shape analysis. B) Homologous landmarks (12) are placed on salmon for relative warp analysis to determine differences in body shape C) A typical image of a Chinook salmon fillet used to assess fillet colour image analysis (see methods for details).

**Figure 2.3** Images of Chinook salmon (*Oncorhynchus tshawytscha*) fillets uploaded to Adobe PhotoShop CC 2015 for colour analysis where the DSM SalmoFan™ colour scores 22 and 32 were used as colour standards. Grids (2.5cm X 2.5cm) were placed in four different locations on the fillet (the ventral part of the fillet, below the dorsal fin, on the caudal peduncle and the gut) and the “blur” function was used to obtain average lightness (L*), redness (a*) and yellowness (b*) values in those areas.

**Figure 2.4** Violin plots of condition factor (K) of six outbred farmed populations; Big Qualicum (BQ), Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin), Robertson Creek(RC) and one farmed inbred stock, Yellow Island Aquaculture (YIAL) of Chinook salmon (*Oncorhynchus tshawytscha*) at Yellow Island Aquaculture, British Columbia. Width of shaded areas of violin plots represent distribution of data with area
and horizontal lines within each inner box plots represents the population median, top and bottom boundaries of each box represent the 25th and 75th-percentile respectively, top and bottom whiskers represent the 5th and 95th percentile. Surrounding bullet points represent data outliers that fall outside the 95% confidence interval. Condition factor (K) is the relationship between body length and total weight of an individual (K= Body weight (g) *100/ (length cm)³).

**Figure 2.5** Discriminant Function Analysis (DFA) of body shape of six outbred farmed; Big Qualicum (BQ), Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin), Robertson Creek (RC) and one farmed inbred stock, Yellow Island Aquaculture Ltd.(YIAL) of Chinook salmon (*Oncorhynchus tshawytscha*). Thin-plate spines were used to visualize body shape associated with values for DFA 1(body shape, x axis) and DFA 2 (head shape, caudal peduncle depth, y axis) (see methods for details).

**Figure 2.6** Violin plot of Slaughter Yield (%) in six outbred farmed, Big Qualicum (BQ), Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin), Robertson Creek (RC) and one farmed inbred stock, Yellow Island Aquaculture Ltd. (YIAL) of Chinook salmon (*Oncorhynchus tshawytscha*). Width of shaded areas of violin plots represent distribution of data with area and horizontal lines within each inner box plots represents the population median, top and bottom boundaries of each box represent the 25th and 75th-percentile respectively, top and bottom whiskers represent the 5th and 95th percentile. Surrounding bullet points represent data outliers that fall outside the 95% confidence interval. Different letters (a-b) correspond to significant differences between populations (p<0.005).
Figure 2.7 Violin plot of fillet shape (fillet mass index, FMI) in six outbred farmed, Big Qualicum (BQ), Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin), Robertson Creek (RC) and one farmed inbred stock, Yellow Island Aquaculture Ltd. (YIAL) of Chinook salmon (*Oncorhynchus tshawytscha*). Width of shaded areas of violin plots represent distribution of data with area and horizontal lines within each inner box plots represents the population median, top and bottom boundaries of each box represent the 25th and 75th-percentile respectively, top and bottom whiskers represent the 5th and 95th percentile. Surrounding bullet points represent data outliers that fall outside the 95% confidence interval.

Figure 2.8 Violin plot of Fillet Yield (%) in six outbred farmed, Big Qualicum (BQ), Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin), Robertson Creek (RC) and one farmed inbred stock, Yellow Island Aquaculture Ltd. (YIAL) of Chinook salmon (*Oncorhynchus tshawytscha*). Width of shaded areas of violin plots represent distribution of data with area and horizontal lines within each inner box plots represents the population median, top and bottom boundaries of each box represent the 25th and 75th-percentile respectively, top and bottom whiskers represent the 5th and 95th percentile. Surrounding bullet points represent data outliers that fall outside the 95% confidence interval. Significant differences correspond to different letters (a-b) (P<0.005).

Figure 2.9 Relationship between lipid content (%) obtained from a handheld microwave fat probe placed on the skin of a Chinook salmon (*Oncorhynchus tshawytscha*) and lipid extracted (%) from muscle (Pearson’s, P<0.0001, r=0.78) in six outbred farmed, Big Qualicum (BQ), Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin)
(y=3.25 + 0.25x, r²=0.48), Robertson Creek (RC), and one farmed domesticated stock, Yellow Island Aquaculture Ltd. (YIAL) of Chinook salmon.

**Figure 2.10** Violin plot of lipid content (%) in six outbred farmed, Big Qualicum (BQ), Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin), Robertson Creek (RC) and one farmed inbred stock, Yellow Island Aquaculture Ltd. (YIAL) of Chinook salmon (Oncorhynchus tshawytscha). Width of shaded areas of violin plots represent distribution of data with area and horizontal lines within each inner box plots represents the population median, top and bottom boundaries of each box represent the 25th and 75th-percentile respectively, top and bottom whiskers represent the 5th and 95th percentile. Surrounding bullet points represent data outliers that fall outside the 95% confidence interval.

**Figure 2.11** Violin plot of lipid extraction (%) in six outbred farmed, Big Qualicum (BQ), Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin), Robertson Creek (RC) and one farmed inbred stock, Yellow Island Aquaculture Ltd. (YIAL) of Chinook salmon (Oncorhynchus tshawytscha). Width of shaded areas of violin plots represent distribution of data with area and horizontal lines within each inner box plots represents the population median, top and bottom boundaries of each box represent the 25th and 75th-percentile respectively, top and bottom whiskers represent the 5th and 95th percentile. Surrounding bullet points represent data outliers that fall outside the 95% confidence interval.
Figure 2.12 Relationship between lipid content (%) obtained from a handheld microwave fat probe placed on the skin of a Chinook salmon (*Oncorhynchus tshawytscha*) and lipid extracted (%) from muscle (Pearson’s, P<0.0001, r=0.78) in six outbred farmed, Big Qualicum (BQ), Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin) (y=3.25 + 0.25x, r²=0.48), Robertson Creek (RC), and one farmed domesticated stock, Yellow Island Aquaculture Ltd. (YIAL) of Chinook salmon.

Figure 2.13 Violin plot of colour scores in six outbred farmed, Big Qualicum (BQ), Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin), Robertson Creek (RC) and one farmed inbred stock, Yellow Island Aquaculture Ltd. (YIAL) of Chinook salmon (*Oncorhynchus tshawytscha*). Width of shaded areas of violin plots represent distribution of data with area and horizontal lines within each inner box plots represents the population median, top and bottom boundaries of each box represent the 25th and 75th-percentile respectively, top and bottom whiskers represent the 5th and 95th percentile. Surrounding bullet points represent data outliers that fall outside the 95% confidence interval. Different letters (a-c) correspond to significant differences between populations (p<0.005).

Figure 2.14 A) Relationship between lightness (L*) obtained from computer analysis of salmon fillets and colour scores assigned from a DSM Salmofan™ in six hybrid farmed Chinook salmon (*Oncorhynchus tshawytscha*) populations, Big Qualicum (BQ), Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin), Robertson Creek (RC), and one farmed inbred population, Yellow Island Aquaculture Ltd. (YIAL) of Chinook salmon. B) Relationship between redness (a*) obtained from computer analysis
of salmon fillets and colour scores assigned from a DSM Salmofan™ in six hybrid
farmed Chinook salmon (Oncorhynchus tshawytscha) populations, Big Qualicum (BQ),
Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin), Robertson Creek
(RC), and one farmed inbred population, Yellow Island Aquaculture Ltd. (YIAL) of
Chinook salmon. C) Relationship between yellowness (b*) obtained from computer
analysis of salmon fillets and colour scores assigned from a Salmofan™ in six hybrid
farmed Chinook salmon (Oncorhynchus tshawytscha) populations, Big Qualicum (BQ),
Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin), Robertson Creek
(RC), and one farmed inbred population, Yellow Island Aquaculture Ltd. (YIAL) of
Chinook salmon
Figure 2.1
Figure 2.2

A)

B)

C)
Figure 2.3
Figure 2.4

Condition Factor

BQ  Chil  Nit  Punt  Quin  RC  YIAL

Population
Figure 2.5
Figure 2.6

![Box plot showing slaughter yield (%) for different populations (BQ, Chil, Nit, Punt, Quin, RC, YIAL). The populations are differentiated by letters (a, b, ab), indicating significant differences in slaughter yield.](figure2_6.png)
Figure 2.7

![Box plot showing fillet shape (g/cm²) for different populations (BQ, Chil, Nit, Punt, Quin, RC, YIAL).]
Figure 2.8
Figure 2.9

![Figure 2.9: Scatter plot showing the relationship between Fat Probe Lipid content (%) and Extraction lipid content (%). The plot includes various symbols representing different samples: BQ, Chil, Nit, Punt, Quin, RC, and YIAL.](image_url)
Figure 2.12
CHAPTER THREE: An examination of potential trade-offs between product quality and quantity driven by growth in hybrid Chinook salmon

Introduction

Life history trade-offs are defined as the fitness costs that occur when a beneficial change in one trait leads to a negative change in another (Stearns, 1989). Rapid growth in the wild can offer numerous ecological and evolutionary advantages, with elevated growth rate and larger body size showing positive relationships with survival, reproductive investment and even predator avoidance (Fleming and Gross, 1990; Jobling, 1994; Shearer and Swanson, 2000). Therefore, selection for individuals with maximized net energy acquisition, large body size and quick growth rates should be favoured (Biro et al., 2006; Stearns 1992). However, the extent to which individuals can maximize growth rate benefits may be limited by numerous factors such as accessibility to resources (Stearns, 1992), number of competitors (Mazur et al., 1993; Werner and Anholt, 1993), environmental stressors (Barton, 2002), reproduction (Smith and Fretwell, 1974), and predation (Persson et al., 1996). Limited growth rate can in turn lead to trade-offs with traits that would have otherwise been favourably influenced by growth including tissue quality (Monaghan et al., 2009), aggression (Vøllestad and Quinn, 2003), reproductive investment (Fleming and Gross, 1990), swimming performance (Farrell et al., 1997) and survival (Werner and Anholt, 1993).

In contrast to wild settings where access to resources are often limited, captive (i.e., agricultural) systems generally provide feed to growing animals daily to promote growth and have reduced conspecific competition and a lack of predation risk (Gjedrem, 2000). The goal of agricultural production of livestock is the selection of traits that
maximize final body size/yield in the least amount of time while improving feed conversion efficiency; there are numerous examples within classic agricultural systems of selection on growth rate to achieve this goal (Emmerson, 1997; Mangel and Stamps, 2001; Schenkel et al., 2004). Even within the relatively younger aquaculture industry, producers are primarily interested in maximizing stock performance (i.e., final fish size, overall biomass production) by selecting for traits such as increased growth rate and increased feed conversion efficiency (Gjøen et al., 1997; Harvey et al., 2016). For example, in Atlantic salmon (*Salmo salar*) domestication has led to an estimated 10-15% increase in growth rate per generation compared to wild stocks (Gjedrem, 2000; Glover et al., 2009; Solberg et al., 2013ab). Nonetheless, although seemingly ideal resource conditions in captivity would presumably relax growth constraints (and relieve growth dependent trade-offs), aquaculture producers may still face trade-offs driven by the impact of selection for maximal growth rates on commercially important quality based traits, ultimately decreasing the final production value. Paradoxically, if captive organisms experience enough of a relaxation in the trade-off between protein quantity and quality due to being provided with excess resources (Emmerson, 1997), some quality traits may even show a positive relationship with growth rate (Quinton et al., 2005; Johnston et al., 2006). Also, although selective pressures are relaxed in captive environments, other density related factors may still affect quality-quantity trade-offs such as increased aggression, stress related to handling and transport, and the possibility for increased disease transmission (Barton and Iwama, 1991). As such, it is important to investigate potential trade-offs between growth and product quality in farmed fishes.
To compete in the market place, commercial aquaculture farmers must avoid (or at least reduce) product quality-quantity trade-offs. For example, within commercial salmonid fish farms, a producer’s goal should ideally be to generate a large product quickly, without compromising additional quantity traits such as slaughter yield (Røra et al., 2001), or significantly impacting highly desirable quality traits such as optimal body shape (optimal condition factor; Gjerde and Shaeffer, 1989; Kause et al., 2003; Ighewela et al., 2011; Colihueque and Aranaeda 2014), deep pink flesh colour (Quinton et al., 2005) and low lipid content (Camara and Symonds, 2014). Although feed supplementation, including feeding fish high quality diets supplemented with carotenoids, can help diminish some of these trade-offs, supplementation does not always improve product quality (e.g., only 2-22% of feed supplemented carotenoids are retained in flesh; Torrissen, 1989; Storebakken and No, 1992). Supplementation is also costly to producers (representing upwards of 15-20% of total production costs (Torrissen, 1995; Nickell and Bromage, 1998ab; Johnston et al., 2006), and high fat diets which promote rapid growth can also lead to undesired fat deposition in muscle and modify fillet quality leading to production losses (Shearer, 1994; Regost 2001). Since high incidences of inbreeding and domestication in farmed populations may exacerbate some of these issues and potential trade-offs, farmers often look to outbreeding (infusing new unrelated individuals from a different population to an inbred population) captive fish with those from wild populations since these are not under the same selection pressures as farmed fish and may have traits deemed more desirable to consumers (e.g Johnston et al., 2006). Also, moderate heritability estimates (colour $h^2$=0.13-0.14; fat $h^2$=0.19; harvest weight $h^2$= 0.10-0.21; condition factor $h^2$=0.30-0.39, slaughter yield $h^2$=0.10) have been found.
for product quality metrics, indicating that these traits may respond to selection (Rye and Refstie, 1995; Gjedrem, 1997; Quinton et al., 2005). Outbreeding can lead to heterosis, or hybrid vigor, when offspring performance exceeds that of either parent (Debes et al., 2014). As such, evaluating the relationship between growth rate and fillet quality metrics following outbreeding will help better quantify and therefore predict how further selection on growth traits may generate trade-offs that ultimately could increase product quality.

In this study, we investigated the relationship between growth rate and multiple product quality metrics in farmed Chinook salmon. We examined the effect of outbreeding on these relationships by crossing a captive farmed population, with six different wild populations. We focused on resultant traits of interest to the salmon aquaculture industry including condition factor, slaughter yield, lipid content and fillet colour. We also examined whether survivorship differed among populations when compared to the control farmed aquaculture stock. Classic quantity-quality trade-off theory from studies of wild vertebrates would predict a simple negative relationship between growth rate and quality metrics (Figure 3.1A). However, despite selection on higher growth rates in captivity, an abundance of resources available for investment in growth and quality-related traits may relax the strength of these trade-offs, and even perhaps reverse these trait associations (Figure 3.1B). Our goal was to examine the direction and potential strength of these trade-offs across hybrid stocks and quality traits.

**Methods and Materials**

*Generation of hybrid stocks*

Research took place at Yellow Island Aquaculture Ltd. (YIAL), an organic Chinook salmon farm, located on Quadra Island, British Columbia, Canada (50°07’N,125
The domesticated stock of Chinook salmon at YIAL have been in production since 1985, and are descendants of crosses between two wild source populations located on Vancouver Island: Big Qualicum (49°23’N, 124°36’W) and Robertson Creek (49°20’N, 124°58’W). In this study, we generated ten-half sibling families by crossing milt from 10 males within each of six wild populations: Big Qualicum River (49°23’N, 124°36’W), Nitinat River (48°51’N, 124°39’W), Puntledge River (49°41’N, 125°02’W), Quinsam River (50°01’N, 125°18’W), Robertson Creek (49°20’N, 124°58’W), and Chilliwack River (49°04’N, 121°42’W) (see Chapter 2; Figure 2.1), with eggs from hermaphrodite females from YIAL. These females were used to purposely reduce maternal effects so that variation and therefore phenotypic differences observed across populations could be more directed at sire effects. Male populations were chosen for their accessibility and expected genetic divergence and variability across populations.

Fertilized eggs from the 70 families were then divided into incubation trays in two replicate cells. On March 14, 2014 fry from replicate cells were combined and assigned to each of the 200L replicate tanks.

**Survivorship, early sexual maturation and specific growth rate**

Once fish had reached 3-5g wet mass, each fish was tagged (passive integrated transponder-PIT) to allow for individual identification. From 11-12 August 2014, fish were moved to 16 population-specific and replicated saltwater sea pens. From 18-21 May 2015, salmon were weighed to the nearest gram to help determine saltwater growth rate. In November 2015, early sexually maturing males (known as “jack” males) were counted, weighed, and removed from the populations to determine the jacking rate (proportion of males in a population that sexually mature at least one year prior to females after spending a year in sea water; Heath et al., 1994). Jacking rate was
calculated at the population level by dividing the number of jacks in each population by the total number of fish. Remaining fish were combined into 8 net pens separated by population. From 29-30 October 2017, 3-year old Chinook salmon (n=204) were harvested from sea pens and evaluated for fillet quality metrics (see below). Throughout rearing, fish that died in saltwater had been identified to allow for survivorship calculations, calculated as the percent of fish in each population that survived from November 2014 until harvest. In this calculation, fish that were used for other research projects as well as fish that reached sexual maturation were removed from the calculation as their mortality was non-random. Biomass was calculated by multiplying average mass per population by saltwater survivorship.

*Product quality metrics*

At harvest, fish were weighed to the nearest gram using an electric scale (OHAUS Valor™ 4000 series) and fork length was measured to the nearest cm. Whole fish were gutted by an experienced worker from a neighbouring fish processing plant, and weighed to the nearest gram for the calculation of slaughter yield (proportion of gutted weight to body weight). Lipid content was measured since high levels (exceeding 16-18%) can lead to a diminished product quality and production losses (Gjedrem et al. 1997). Lipid content was assessed using an industry standard hand-held micro-wave fat probe (Distell Fish Fatmeter model 692, Distell Inc, West Lothian Scotland, U.K). The fat probe determines lipid content of somatic tissues by emitting a low powered wave (frequency, 2GHz ± 2000 MHz; power, 2mW) that measures the inverse relationship between water and lipid content, and converts water concentration to lipid concentration (Cooke et al.,
The probe was placed on the right side of the salmon and measurements were taken from 4 different locations in-order to determine the fat content of each fish. After fish were gutted, they were filleted, rinsed with seawater and trimmed according to aquaculture standards. Fillet colour was subsequently assessed because a deep red coloured fillet is associated with high production value. Fillet colour was graded immediately post slaughter using the industry standard DSM SalmoFan™ Lineal colour card (DSM, 2014). Specifically, colour was assessed in four different locations on the fillet: the ventral part of the fillet, below the dorsal fin, on the caudal peduncle and the gut. Scores on the colour card ranged from 20 (palest colour; light pink) to 34 (darkest colour; dark red) and values obtained from fillets were averaged to obtain overall colour score of the fillet.

**Statistical analysis**

Specific growth rate (SGR= \(\frac{\log \text{(body weight at harvest)} - \log \text{(initial body weight)}}{\text{days}}\)) in saltwater was calculated using weights of fish obtained at harvest (30-31 October 2016) and on 19 May 2015. A linear regression was used to determine if a relationship existed between growth rate and final biomass. For jacking rate calculations, specific growth rate was also calculated using weights of fish sampled on 11 November 2015 and 19 May 2015. Condition factor (Fulton’s K) was calculated for each fish each (K= \(\frac{\text{Body weight (g)} \times 100}{\text{(length cm)}^3}\)), as a higher condition factor can be associated with a more desirable body shape. To determine the proportion of fish remaining for production after gutting, slaughter yield was calculated (slaughter yield (%)= \(\frac{\text{Gutted weight (g)} \times 100}{\text{Body weight (g)}}\)). Statistical analyses were performed using JMP Statistical Software V12.01. Linear mixed model (LMM) were performed to determine if specific growth rate differed across populations, with saltwater net pen and
family identify added as random effects nested within population. Responses for quality metrics, colour, lipid content, yield and condition factor were tested to determine whether growth rate had a significant impact on traits. Linear mixed models (LMMs) were used to examine population specific differences in quality metrics, as well as relationships between specific growth rate, with main effects for Population, Specific Growth Rate and Population-by-Growth Rate interaction that were fitted to the dependent variable (colour, lipid content, yield, condition factor). Saltwater net pen and family identity were added as random factors and nested within population. Pair-wise comparisons (Tukey HSD) were performed to test for differences between populations in quality metrics. A binomial general linear mixed effects model (GLMM), with a logit link function, was used to measure population differences in saltwater survivorship and jacking rate (weight and net pen added as a random factor for jacking rate). Pearson’s correlations were then performed to examine the relationship between survivorship, jacking rate and specific growth rate among populations.

**Results**

A binomial generalized mixed model revealed significant differences in saltwater survivorship across population ($X^2=99.6$, $p<0.001$) (Table 3.1). Saltwater survivorship was low for all populations (1.3%-9.3%), and was not predicted by growth rate (Pearson’s correlation: $R=0.41$, $p=0.36$) (Figure 3.2). Specific growth rate did not differ significantly across populations ($F_{6,45}=0.9$, $p=0.49$), and family identity did not predict variation in specific growth rate ($p=0.09$), but net pen did ($p=0.006$). There was no significant relationship between final biomass and growth rate (Linear regression,
R=0.009, p=0.844) (Figure 3.3). When examining the relationship between growth rate and colour score, we found a significant interaction between specific growth rate and population (F_{6,168.4}=5.9, p<0.001), with both SGR and population showing significance in the model (F_{1,170.6}=11.9, p=0.007 and F_{6,58.3}=11.9, p=0.002, respectively) (Figure 3.4). Family effects (p=0.18) and net pen effects (p=0.43) were not significant for colour score (Table 3.1). Growth rate showed a significant positive relationship with fat content (F_{1,182.7}=5.6, p=0.02), although the effects of growth rate on fat did not differ across populations (F_{6,82.4}=0.24, p=0.95), and the interaction was not significant (F_{6,177.7}=0.25, p=0.96) (Table 3.1) Neither family (p=0.90) or net pen (p=0.43) predicted variation in lipid content (Figure 3.5). Slaughter yield was not significantly related to specific growth rate (p=0.47), although it did vary across populations (p=0.03), the interaction was not significant (p=0.58) and both family (p=0.76) and net pen (0.28) were unable to predict yield (Figure 3.6; Table 3.1) Condition factor did not vary across population (F_{5,58.0}=0.26, p=0.95), but it was significantly positively affected by specific growth rate (F_{1,165}=14, p=0.003), the interaction was not significant (F_{6,162.7}=1.08p=0.42) and family (p=0.88) and net pen (p=0.09) did not have an influence (Figure 3.7; Table 3.1). When examining the relationship between growth rate and jacking rate, we found a significant relationship (p<0.01), SGR showed significance in the model (X^2=15.5, p<0.001), but not population (X^2=9.3, p=0.15) and the interaction between the two was not significant (X^2=7.8, p=0.25).
Discussion

Domestication of farmed fishes has led to phenotypes that are significantly different from wild populations as selection for increased growth rate leads to indirect selection on other quality traits which may reduce product quality and hence value. Most farmed populations are subjected to high levels of inbreeding and as such, it is important to determine whether the addition of new genetic material through outbreeding will influence product quality, growth, survival and rates of sexual maturity. Overall, we did not find consistent quality-quantity trade-offs across populations, but instead that growth showed a significant positive relationship with fillet quality metrics including lipid content, onset of early sexual maturity, condition factor and the onset of early sexual maturity (Quinton et al., 2005; Glover et al., 2009). Colour and specific growth rate had a more complex relationship as the relationship between the two traits differed across populations. In contrast to previous work, we did not find links between high growth rate and slaughter yield (Rasmussen, 2001) or survival across populations (Ayles and Baker, 1983; Ng et al., 2009). It is likely that due to the ideal resource conditions of captive populations (e.g., lack of predators, less competition, daily feedings), we were unable to detect any quality-quantity trade-offs.

Survivorship differed significantly across populations and YIAL had significantly greater survivorship than all hybrid crosses. Our results contrast with those of previous work reporting higher survival in hybrid fishes (Ayles and Baker, 1983; Ng et al., 2009). The YIAL fish may have displayed higher survivorship as they may be better adapted to the captive environment and have been selected for traits that allow them to perform well in those conditions (Harvey et al., 2016). Although we found no significant relationship between biomass and growth rate, YIAL had a higher biomass than all hybrid crosses.
We found no significant differences across populations for specific growth rate, although we did find a net pen effect indicating that growth rate differed across net pens, most likely due to differences in density or environmental differences among pens. Our results differ from previous studies that found that farmed fishes outgrew hybrid populations in farmed settings (Glover et al., 2009; Solberg et al., 2013a; Harvey et al., 2016). It has been suggested that the effects of outbreeding are dependent on the rearing environment of hybrids (Burton, 1987; Waser et al., 2000). Hybrid stocks may have performed similarly due to GxE interactions as they shared genes with the farmed stock, were reared in the same environment and fed the same diet (McClelland et al., 2005). It has also been suggested that domesticated stocks have become adapted to their culture diets, which is why they grow quickly (Glover et al., 2009; Solberg et al. 2013a; Skaala et al., 2012; Harvey et al., 2016). Growth rate in the wild is limited by energy expenditure on predator avoidance, foraging behaviour, territory defence and influence by growth rate (Orlov et al., 2006; Jonsson and Jonsson, 2011; Harvey et al., 2016). Rearing fish in a relaxed captive setting may have allowed for hybrids to perform similarly to the domesticated control.

Overall, there was a significant effect of growth rate on colour scores and these effects differed across populations. When comparing YIAL to hybrid populations we found populations performed differently at similar growth rates (Figure 3.2). For example, Big Qualicum, the population with the highest mean colour score, showed a negative relationship with growth rate. Conversely, regardless of variation in growth rates Big Qualicum produced individuals with consistently high colour scores (30-34). Therefore, if farmers were concerned with producing fish with high colour scores and had
difficulty maintaining consistent growth rates, Big Qualicum would still produce consistently high fillet colour quality and may be an ideal population. In contrast, populations such as Robertson Creek and Chilliwack exhibited positive relationships between growth rate and colour indicating that they may not be ideal populations for producers to choose due to their poor colour performances at low growth rates. Interestingly, YIAL performed consistently across a variety of growth rates, yet still yielded lower colour scores on average than Big Qualicum, when at low growth rates indicating again that Big Qualicum may be a better population for growth and colour. Most studies examining the effects of outbreeding on farmed fishes compare hybrid performance to both wild and farmed parental strains and find that farmed fishes outperform both hybrid and wild fishes, and hybrids display intermediate growth and fillet quality traits (Glover et al., 2009; Harvey et al., 2016). Observed positive relationships between growth and colour, in populations such as Robertson Creek and Chilliwack, may be due to feed supplementation of astaxanthin as previous supplementation studies have found that salmon exhibit superior growth (Torrissen, 1984; Christiansen and Torrissen, 1996). Alternatively, it has also been suggested that selection for growth may have led to indirect selection for improved colour (Quinton et al., 2005; Glover et al., 2009). Recent work on salmonid colour genetics has identified genes that may be responsible for carotenoid pigmentation, indicating that population variation in colour observed may be due to genetics (Lehnert et al., 2016).

Although lipid content did not differ significantly across populations, it was significantly and positively related to growth rate. This is consistent with other studies that have shown that domesticated fishes undergoing selection for growth rate will also
respond with increases in lipid content (Quinton et al., 2005; Johnston et al., 2006). As mentioned previously, all fish were fed the same aquaculture standard diet to promote growth rate and this may explain why no differences across populations were observed (Harvey et al., 2016). Also, all populations fell below the desired market ceiling of 16-18% lipid content indicating that they all had market desired lipid levels, suggesting there was no trade-off generated by growth rate.

We found that although growth rate showed a positive relationship with condition factor this trait did not differ across populations. These results are consistent with a study on Coho salmon (Oncorhynchus kisutch) that found no difference in condition factor across hybrid and farmed populations (Chitteden et al., 2010), yet differed from studies on crosses of striped bass (Morone saxatilis) and yellow bass (Morone mississippiensis) that found decreased condition factor across populations (Wolters and Demay, 1996). Work on Nile Tilapia (Oreochromis niloticus) found that condition factor was influenced by growth rate and inferred that high condition factors, such as those observed in this study, are good indicators of a population’s performance (Ighwela et al., 2011). Similar to other product quality traits, condition can be highly influenced by the captive environment (e.g., population density, water temperature, food availability), and may explain why observable differences did not occur when fish were reared in similar environments (Ritcher, 2007; Ndiaye, 2015). Conversely, slaughter yield differed across populations, but there appeared to be no significant effect of growth. These results differ from other studies that have found direct influences of growth (Dunham et al., 2002) and heterosis on yield (Argue et al., 2002). Nonetheless, across populations Chilliwack had
the lowest yield resulting from a greater viscera weight and subsequent greater waste in this population (Souza et al., 2015).

We found relatively high incidences of jacking rate in our populations and we found a positive relationship between early onset of sexual maturity and growth rate across the populations. Research conducted on hatchery-reared salmon showed significant influences of growth rate on jacking rate (Heath et al., 1994; Berejikian et al., 2010). However, we found no difference across populations for onset of early sexual maturity. All Chinook salmon in our study were essentially half-siblings as they were descendants from hermaphrodite females; other studies have found that performance of maternal line affects jacking rate of Chinook salmon (Forest et al., 2016). It is also possible that high incidences of jacking occurred because farmed fish are fed frequently (compared to wild) which could lead to early sexual maturity (Vainikka et al., 2012). Also, it is important to note that Salmon farms are unable to sell early sexually maturing individuals as they have small body sizes and diminished flesh quality. As such, most Pacific salmon farms rear only female or infertile salmon (i.e., triploids). However, this does not negate the problematic effects that high jacking rates could have for aquaculture broodstock maintenance and development (see Forest et al., 2016).

The overall lack of observed quality-quantity trade-offs in this study may be due to the lack of difference in rearing conditions between populations and the consistent and daily provision of resources. Other studies have found that heterosis in salmonids was dependent on the growth stage and traits measured (Granier et al., 2011), by neglecting the freshwater phase or sampling product quality metrics solely prior to slaughter, it is possible that trade-offs had occurred at earlier stages, but were not measured. As survival
was different across populations, it is possible that other factors not measured in this study, such as superior immune function in the farmed stock affected results, or behavioural differences across populations may have contributed to these results. Due to study constraints, it was impossible to measure fillet texture, an important product quality metric which has been shown to be influenced by growth rate and can be negatively impacted by increased fat content (Johnston et al., 2006). It may be difficult to observe heterosis or outbreeding depression in the first generation (Nilsson 1993; Hulata 2001; Bryden et al. 2004; Gunther et al. 2005), as losses in local adaption are not usually observable, until the second or later generations of outbreeding allow for full recombination of the parental genome (Edmands, 2007; Houde et al., 2011). Therefore, future directions of this study would include measuring product quality-quantity trade-offs in freshwater stages, the examination of future generations and comparisons of hybrids to wild and farmed strains to better understand trade-offs that may occur.
References


Table 3.1 Summary table of average product quality, survivorship, precocious sexual maturity rates (Jacking Rate) values calculated for seven outbred Chinook salmon (*Oncorhynchus tshawytscha*) populations: Big Qualicum (BQ), Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin), Robertson Creek (RC) and one farmed domesticated stock, Yellow Island Aquaculture Ltd (YIAL) (farmed domesticated stock). Population, family and netpen effects tested for product quality metrics (colour score, slaughter yield, lipid content and condition factor) across all 7 populations “Pop” were tested running linear mixed models (LMM) with “population” as a fixed effect and “netpen” nested within “family” with population as random effects. Differences in survivorship and the effect of growth on jacking rate were tested with bimodial generalized linear mixed models (GLM). Comparisons in model were made to farmed domesticated stock, YIAL. Bolded terms are significant at the level of alpha <0.05.

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<th>Quality</th>
<th>Nov 2015 N=795</th>
<th>Oct 2016 N=204</th>
<th>Fixed effects</th>
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<tr>
<td></td>
<td>BQ  Chil  Nit  Punt  Quin  RC  YIAL</td>
<td>SGR  Pop  SGR*Pop</td>
<td></td>
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<tr>
<td>Quality</td>
<td>N1  112  108  82  92  80  121  200</td>
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<tr>
<td>Jacking Rate (%)</td>
<td>25±4.1  25±4.1  37±5.3  33±4.7  27±5.0  34±4.3  26±3.1</td>
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<td>Survival (%)</td>
<td>5.1±0.8  3.1±0.7  1.3±0.4  1.6±0.4  2.8±0.5  2.3±0.5  9.3±0.1</td>
<td>-  0.001  -</td>
<td></td>
</tr>
<tr>
<td>Colour Score</td>
<td>31.3±0.3  25.8±1.0  29.2±0.7  30.5±0.5  30.9±0.4  29.7±0.6  29.7±0.6</td>
<td>&lt;0.001  0.002  &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Fat content (%)</td>
<td>8.3±0.5  6.2±0.8  8.5±0.7  7.9±0.7  9.0±0.8  8.5±0.5  9.1±1.1</td>
<td>0.02  0.95  0.96</td>
<td></td>
</tr>
<tr>
<td>Slaughter yield</td>
<td>90.7±0.3  89.9±0.3  91.2±0.4  90.9±0.3  91.2±0.3  91.4±0.2  91.4±0.1</td>
<td>0.47  0.03  0.58</td>
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<td>Condition Factor</td>
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<td>0.03  0.95  0.42</td>
<td></td>
</tr>
</tbody>
</table>
Figure captions

**Figure 3.1** Predicted relationship between relative quality (fillet quality) and relative quantity (growth rate) in salmon from two different environments: A) Free living (limited resources) environment similar to what would be seen in the wild where quality should be dependent on resource availability and therefore trade-off with quantity, and B) Unlimited resources (presumably similar to an aquaculture setting) where animals are fed at or close to satiation, and quality may not trade-off with quantity due to plentiful resources.

**Figure 3.2** Non-significant relationship between saltwater survivorship (%) and specific growth rate (SGR) (with standard error) across seven outbred Chinook salmon (*Oncorhynchus tshawytscha*) populations: Big Qualicum, Chilliwack, Nitinat, Puntledge, Quinsam, Robertson Creek, and one farmed domesticated stock, Yellow Island Aquaculture Ltd (farmed domesticated stock).

**Figure 3.3** Non-significant relationship between biomass and specific growth rate (SGR) (with standard error) across seven outbred Chinook salmon (*Oncorhynchus tshawytscha*) populations: Big Qualicum, Chilliwack, Nitinat, Puntledge, Quinsam, Robertson Creek, and one farmed domesticated stock, Yellow Island Aquaculture Ltd (farmed domesticated stock).

**Figure 3.4** Significant interaction between population and specific growth rate (SGR) on fillet colour score across seven outbred Chinook salmon (*Oncorhynchus tshawytscha*) populations: Big Qualicum, Chilliwack, Nitinat, Puntledge, Quinsam, Robertson Creek, Yellow Island Aquaculture Ltd. (farmed domesticated stock).
Figure 3.5 Positive significant relationship between lipid content (%) and specific growth rate (SGR) across seven outbred Chinook salmon (Oncorhynchus tshawytscha) populations: Big Qualicum, Chilliwack, Nitinat, Puntledge, Quinsam, Robertson Creek and one farmed domesticated stock, Yellow Island Aquaculture Ltd. (farmed domesticated stock).

Figure 3.6 Non-significant relationship between Slaughter yield (%) and specific growth rate (SGR) across seven outbred Chinook salmon (Oncorhynchus tshawytscha) populations: Big Qualicum, Chilliwack, Nitinat, Puntledge, Quinsam, Robertson Creek and one farmed domesticated stock, Yellow Island Aquaculture Ltd (farmed domesticated stock). A significant population effect was observed.

Figure 3.7 Positive significant relationship between condition factor and specific growth rate (SGR) across seven outbred Chinook salmon (Oncorhynchus tshawytscha) populations: Big Qualicum, Chilliwack, Nitinat, Puntledge, Quinsam, Robertson Creek, and one farmed domesticated stock, Yellow Island Aquaculture Ltd (farmed domesticated stock).
Figure 3.2

Survivorship (%) vs. Saltwater SGR (%BW/day)

- Big Qualicum
- Chilliwack
- Nitinat
- Puntledge
- Quinsam
- Robertson Creek
- YIAL
Figure 3.3

- Big Qualicum
- Chilliwack
- Nitinat
- Puntledge
- Quinsam
- Robertson Creek
- YIAL

Biomass vs. Saltwater SGR (%BW/day)
Figure 3.6

Slaughter Yield (%) vs. Saltwater SGR (%BW/day)

- Big Qualicum
- Chilliwack
- Nitinat
- Puntledge
- Quinsam
- Robertson Creek
- YIAL
Figure 3.7

The figure shows a scatter plot with the following variables:
- **Condition Factor** on the y-axis.
- **Saltwater SGR (%BW/day)** on the x-axis.

Different markers and colors represent different locations:
- Big Qualicum
- Chilliwack
- Nitinat
- Puntledge
- Quinsam
- Robertson Creek
- YIAL
CHAPTER 4: GENERAL DISCUSSION

The overall goal of this thesis was to evaluate the potential implications of outbreeding on product quality in a farmed Pacific salmon by combining ecological principles with applied science and industry. To do so, we outcrossed males from six wild Chinook salmon (*Oncorhynchus tshawytscha*) populations with hermaphrodite females from an inbred farmed domesticated population (Yellow Island Aquaculture Ltd.; YIAL), and we evaluated the performance of offspring using industry relevant metrics (*Chapter 2*). By evaluating performance across population for multiple traits of economic importance, we were able to infer how outbreeding with wild populations may be influenced by growth rate (*Chapter 3*). This chapter’s purpose is to summarize my research while discussing its implications for aquaculture and conservation as well as suggest future research that may solidify and expand upon these findings.

In Chapter two, I aimed to determine the effects of outbreeding on salmon product quality metrics of hybrid Chinook salmon populations in comparison to a farmed inbred population. We hypothesized that the addition of new wild genetic material, outbreeding, to a farmed inbred population would increase performance of hybrids. Domestication of small farmed aquaculture populations and selective breeding for production traits has been shown to lead to inbreeding and declines in performance traits (Falconer and Mackay, 1996; Bierne et al., 2000; Keys et al., 2004). Intra-species and inter-species outbreeding in aquaculture species has yielded successful performing hybrid populations in aquaculture species such as farmed carp (*Cyprinus carpio*) (eg. Wohlfarth 1993; Bakos and Gorda, 1995), tilapia (*Oreochromis niloticus*) (Bentsen et al. 1998), and catfish
In aquaculture, studies on outcrossing multiple wild populations with domesticated populations are rare (Vandeputte et al., 2014) and studies examining the product quality performance of hybrid fishes, particularly economically important salmonids, are rarer (Glover et al., 2009). Overall, I found no evidence for heterosis as hybrid populations performed similarly to our control farmed population for most traits, and were generally considered high-quality fish. In fact, the only trait that a hybrid population (Big Qualicum) surpassed our farmed stock in was colour. We may not have detected heterosis as the effects of outbreeding are generally difficult to detect in salmonids in the first generation, because full recombination of the parental genome does not occur until the second generation or later (Edmands, 2007; Houde et al., 2011).

Salmon are residual tetraploids and have low recombination rates, meaning that outbreeding effects may not be observable until an F3 generation or later (Allendorf and Thorgard, 1984; Lehnert et al., 2014). Also, it is possible that YIAL’s performance was influenced by generations of selection in captivity for increased growth rates and quality metrics which are often positively affected by growth rate, as well as adaptation to culture environment (Quinton et al., 2005; Glover et al., 2009). This study would have benefited from more sampling points to determine whether quality of farmed fishes differed over time and if early product quality metrics could determine future quality metrics. Finally, this study would have benefited from having monetary values associated with product quality metrics as doing so would better highlight which populations would have been the most profitable.

In Chapter three, I aimed to determine the relationship between growth rate and product quality metrics to better understand the quantity-quality trade-offs that may occur
in hybrid Chinook salmon. Growth rate is often considered the most important trait of
most breeding goals for farmed salmonids (Gjoen and Bentsen, 1997). However,
increases in growth rate have been linked to unfavourable increases in fat content and
onset of early sexual maturity that may deter product quality (Quinton et al., 2005;
Johnston et al., 2006). As such, it was necessary to determine whether there was a trade-
off between desired product quality metrics and growth rate in hybrid populations to fully
understand the usefulness of outbreeding on improving hybrid performance. Overall, we
found that specific growth rate did not differ across populations although relationships
between growth rate and product quality metrics did. As expected, condition factor and
fat content responded positively to increased growth rates and colour responded
differently across populations. Therefore, the way in which populations responded to
growth rate should impact decisions on which population to rear to optimize
economically viable traits such as growth and colour. Slaughter yield was not affected by
growth rate but did differ significantly across populations. Survival was not correlated
with growth yet differed significantly across populations as the farmed domesticated
stock YIAL (9.3% survivorship) had the highest amount of remaining fish at the time of
harvest. Onset of sexual maturity was high in all populations, did not differ significantly
across populations, and was positively related to growth rate which is consistent with
previous work done in hatcheries (Heath et al., 1994). The lack of observable differences
among most populations for growth and product quality relationships may be resultant
from the hybrid and farmed populations being reared in the same environment as most of
these traits are influenced by diet, temperature and population density (Ndiaye, 2015;
Harvey et al., 2016). Also, other potential trade-offs, such as the potential negative
relationship between fillet texture and fat content (Johnston et al., 2006), were not investigated for this study and should be in future studies as it may allow for the identification of a quality-quantity trade-off. Also, the inclusion of more product quality traits will give a better understanding of the overall aquaculture performance of Chinook salmon, especially when compared to research on behaviour (Dender et al., 2017) and immune function (Toews et al., 2017).

**Ideal population for future outbreeding with YIAL**

A goal of this thesis was to identify the highest performing hybrid population, when comparing hybrid populations to the domesticated farmed population. In order to accomplish this, I ranked populations based on performance traits such as survival and final weight (biomass), and average colour score (Figure 4.1). Overall, YIAL had the greatest product quality when compared to the hybrid populations and Big Qualicum had the second highest ranking. Based on YIAL’s performance, it would be beneficial for producers to continue using YIAL stock for future production. This research is similar to other work comparing product quality between farmed and hybrid individuals that found that farmed salmon outperformed hybrids (Glover et al., 2009). In Chapter 2, almost all populations performed similarly to YIAL for important commercial traits such as condition factor, slaughter yield, fillet yield, fillet shape and lipid content (Chilliwack scores lower in fillet and slaughter yield). Although YIAL was out performed for average colour score by Big Qualicum, YIAL still had the greatest biomass indicating greater survival and therefore more fish to sell. However, if producers are looking to outcross YIAL with a hybrid population, I would recommend Big Qualicum as they had consistently high colour scores (30-34), at both low and high growth rates and the second largest biomass. Recent work has identified multiple single nucleotide polymorphisms
SNPs) on salmon chromosomes that are linked to carotenoid pigment, indicating that there are multiple genes responsible for salmon colour (Lehnert et al., 2016), therefore future outcrossing with Big Qualicum may improve colour score. Also, because we saw variation in colour (and other traits) among hybrid populations, it would be beneficial to survey more wild populations to find those with the traits of highest economic value and consider them for outbreeding.

**Future directions and implications**

My research complements other work done in domesticated salmonids that has found hybrid performance to be comparable to farmed strains (Refstie, 1983; Gjerde and Refstie, 1984; Glover et al., 2009). The results from Chapter two, combined with the lack of research on hybrid performance for aquaculture quality product metrics, increases the importance for future studies to investigate performance of F2 generations. Future studies aiming to improve production efficiency of salmonids would benefit from a comparison of hybrid individuals to both farmed and wild parental populations. In doing so, it may be possible to predict F2 (and future generation) performance by evaluating how well wild populations perform, especially when compared to their offspring. As mentioned previously, it may take multiple generations to fully observe the effects of outbreeding in salmonids (Edmands 1997; Houde et al., 2011), and as such evaluating wild population performance may help infer how future hybrid generations will perform. Also, these populations should be reared in an aquaculture setting to make comparisons more applicable to future hybrid generations. It may also be beneficial to perform similar studies at multiple fish farms (Gjerde and Refstie, 1984), as results may assist in further understanding how outbreeding effects may differ geographically, and provide a better understanding of its utility to the industry. Fraser et al. (2011) found that differences in
fitness in hybrid population performances in the wild, may be due to small spatial scales.
As such, expanding the number and distances of populations we study may lead to a
better understanding of an outbreeding gradient (see Walpes, 1995) and hybrid
performance for aquaculture populations.

It remains controversial to rear non-native Atlantic salmon in British Columbia
due to perceived negative effects of their farms, such as the risk of disease transfer
(Gross, 1998) and potential interactions of escaped individuals and native Pacific salmon
(Oakes et al., 2000). Furthermore, other studies have shown that Pacific salmonids are
better able to deal with stressors such as diseases (Infectious salmon anemia; Rolland and
Winton, 2003) and parasites (sea lice; Jones et al., 2008) when compared to Atlantics.
Due to the wealth of knowledge on Atlantic salmon farming, most of the “high quality”
values in this chapter were derived from Atlantic salmon research (Gjedrem et al., 1997;
Einen, 1999; Røra et al., 2001; Steine et al., 2005). In this study, with the exception of
fillet yield, (Chinook, 58%; Atlantic, 60-68%), hybrid populations of Chinook salmon
met or exceeded the criteria, for high quality salmon regardless of whether or not they
outperformed YIAL. For example, it is uncommon for Atlantic salmon (25-27; Steine et
al., 2005) colour values to find themselves ranging as high as our Chinook salmon
populations did (~30). Therefore, this study may provide support for a transition from
farming the more commonly reared Atlantic salmon (~80%) to the less commonly farmed
Chinook salmon (~16%). Not only does this study provide evidence that Chinook salmon
may surpass Atlantic salmon quality, it is also possible that fillet yield may be improved
through selection for body size traits (Quinton et al., 2005). Atlantic salmon have shown
increases of 10-15% genetic gain of growth rate per generation (Gjedrem, 2000; Glover
et al., 2009; Solberg et al., 2013ab); as Chinook salmon are the largest salmon species it is possible that continued selection for optimized growth rate in domesticated stocks will allow for Chinook salmon to surpass Atlantic salmon for both fillet yield and growth rate. Also, Chapter two revealed no significant quality-quantity trade-offs in hybrid and farmed populations, potentially demonstrating that Chinook salmon may be more robust to quality trade-offs that can occur in Atlantic salmon (Johnston et al., 2006), although further traits (ie. texture) should be tested to further test this statement. However, to better test these hypotheses future work is needed not only on selection for product quality traits in domesticated Chinook salmon but for consumer preference tests between Pacific and Atlantic salmonid species.

This thesis also has implications for understanding potential interactions between wild and farmed Pacific salmonids. Wild Pacific salmon populations are locally adapted to their natal streams and are therefore threatened by potential outbreeding depression (Taylor 1991; Fraser 2008; Neff et al., 2011; Lehnert et al., 2014). If farmed Chinook salmon were to escape and interbreed with native populations, it is possible that genes associated with local adaption in wild salmon would be disrupted and offspring would suffer outbreeding depression (Lehnert et al., 2014). Most product quality metrics we measured in Chapter three are associated with fitness in the wild such as growth rate, final body weight, lipid content, survival in saltwater and early onset of sexual maturity. Through population comparisons of these traits, it may be possible to infer how diploid hybrid individuals would perform if they were to escape and mate with wild Chinook salmon. Similar work has been done in Atlantic salmon and has found that both hybrids have higher growth and lipid content than wild individuals (Glover et al., 2009), reduced
predator avoidance responses (Houde et al., 2010); and F1 and F2 hybrids experience reduced survival which could lead to fitness depression within wild populations (Mcginnity et al, 2003). Future work would entail rearing multiple generations of hybrids under semi natural conditions to better understand how they may impact wild populations if they were to escape.

Conclusions

Overall, my thesis has provided evidence that outbreeding between wild and farmed Chinook salmon populations can lead to hybrids exhibiting similar performance for product quality traits when compared to a domesticated population, although more work is needed to evaluate future generations’ performance. Not only this, but through the lack of the identification of a trade-off due to growth rate and product quality, it is possible that rearing Chinook salmon may be ideal when faced with Atlantic salmon as an alternative. This work suggests that rearing Pacific salmonids may yield fish exhibiting high product quality overall. This work, alongside future work identifying hybrid Chinook salmon production lines with elevated fillet quality will contribute to the expansion of Chinook salmon farming in B.C. and will be vital to the success of Canada's growing role in Pacific salmon farming internationally.
References


Toews, S., 2017., "Population and family effects on gene transcriptional profiles of eight hybrid Chinook salmon (Oncorhynchus tshawytscha) populations: implications for conservation and aquaculture" Electronic Theses and Dissertations. 6020.


Figure Captions

Figure 4.1 Bar graph of expected rankings of six outbred farmed populations; Big Qualicum (BQ), Chilliwack (Chil), Nitinat (Nit), Puntledge (Punt), Quinsam (Quin), Robertson Creek (RC) and one farmed inbred stock, Yellow Island Aquaculture (YIAL) of Chinook salmon (*Oncorhynchus tshawytscha*) at Yellow Island Aquaculture, British Columbia, based on product quality. Product quality was determined by multiplying population biomass by average fillet colour score.
Figure 4.1

Product Quality (Colour Score/Biomass)

Population

BQ  Chil  Nitinat  Punt  Quin  RC  YIAL
VITA AUCTORIS

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Year of Birth: 1992

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