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Sarah Lehnert

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REPRODUCTIVE INTERACTIONS BETWEEN WILD AND FARmed CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA): CONSERVATION AND ECOLOGICAL IMPLICATIONS

By

Sarah Lehnert

A Thesis
Submitted to the Faculty of Graduate Studies through the Great Lakes Institute for Environmental Research in partial fulfillment of the requirements for the degree of Master of Science at the University of Windsor

Windsor, Ontario, Canada

2012

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REPRODUCTIVE INTERACTIONS BETWEEN WILD AND FARMED CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA): CONSERVATION AND ECOLOGICAL IMPLICATIONS

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August 30, 2012
CO-AUTHORSHIP STATEMENT

I hereby declare that this thesis incorporates material that is result of joint research from co-authored and submitted journal articles undertaken by the supervision of my supervisor Dr. Daniel Heath (University of Windsor). The primary contributions, data collections, laboratory work, and interpretation of the data was performed by the author, with additional input on data analysis, interpretation of data, and written discussion by co-authors. I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-author(s) to include the above material(s) in my thesis.

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ABSTRACT

Wild Chinook salmon (*Oncorhynchus tshawytscha*) populations could be adversely affected through reproductive interactions with escaping farmed salmon. To determine the reproductive ability of farmed Chinook salmon relative to wild, I compared sperm traits, as well as fertilization and reproductive success in competitive spawning channels. Farmed Chinook salmon males had greater sperm performance relative to wild males, and they were equally successful at competing for mates and fertilizing eggs. However, farm-sired offspring experienced lower survival to the fry stage, which could mediate any impact on the wild populations. Given that hybridization can lead to negative genetic effects via outbreeding, I also tested the theory of outbreeding depression in backcrossed hybrid (F$_2$) Chinook salmon using fitness related traits. I found no evidence of outbreeding depression in Chinook salmon, which further suggests that the introgression of farmed genes into the wild would not result in negative fitness consequences for wild salmon populations.
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1.0 GENERAL INTRODUCTION

Salmonid aquaculture

Aquaculture, the farming of aquatic organisms, is an economically significant industry that continues to expand throughout the world. The rapid increase in global salmonid aquaculture production has raised concerns about the effect of domestication on fish (Naylor et al. 2005). The aquaculture setting provides a very different environment for fish compared to the wild, resulting in changes in the selective pressures that can lead to fundamental genetic changes at the population level (Skaala et al. 2004; Jonsson and Jonsson 2006). Farming practices often result in a reduction in genetic diversity due to genetic bottlenecks, as well as the divergence of farmed stocks from wild populations as a result of novel selective pressures associated with domestication (Einum and Fleming 1997; Norris et al. 1999; Skaala et al. 2004).

Genetic effects of aquaculture

The loss of genetic diversity has been demonstrated by the lower allelic diversity of farmed salmon populations compared to wild salmon (Norris et al. 1999; Skaala et al. 2004), which can result from large numbers of offspring being produced from only a small number of breeding individuals. Small numbers of breeding individuals results in reduced effective population size (Ne) and can lead to increased incidences of inbreeding (Bentsen and Olesen 2002). The loss of heterozygosity associated with inbreeding is unfavorable, as homozygosity at a locus can cause deleterious recessive alleles to be expressed, and thus inbreeding is commonly associated with a loss of fitness (Allendorf and Leary 1986). Here I can define fitness as the extent to which individuals contribute genes to future generations (Endler 1986).
Farming practices can also lead to genetic changes that result in farmed populations becoming genetically divergent from their wild counterparts (Einum and Fleming 1997). Traits artificially selected for in the aquaculture environment, whether intentional or unintentional, may provide an advantage under culture conditions, but may be maladaptive in the wild (Einum and Fleming 1997; Hutchings and Fraser 2008). Under culture, individuals are often selectively bred for specific traits, for example salmon producers frequently select for high growth rates, delayed age at maturation, bright flesh color, and high disease resistance (Gjøen and Bentsen 1997). Previous research suggests that farmed salmon commonly differ in growth rates (Einum and Fleming 1997; Saikkonen et al. 2011), body shape (Fleming et al. 1994), predator avoidance behavior (Einum and Fleming 1997), and various other traits that can affect fitness of farmed fish in the wild.

Escapes from aquaculture

The diverging gene pools of farmed fish relative to that of the wild populations pose a serious threat to the genetic structure and diversity of the wild populations if hybridization occurs between wild and farmed fish (Fleming et al. 2000; McGinnity et al. 2003). Reproductive interactions between wild and farmed fish are possible when fish escape from aquaculture sites, and escapes can occur chronically, as small-scale losses, or sporadically, as large-scale events often resulting due to catastrophic infrastructure failure (Naylor et al. 2005). Escaped farmed salmon can directly impact the wild population, as gene flow between wild and farmed fish may lead to heterosis or outbreeding depression depending on the nature of stocks (Waples 1991). Hybridization may produce offspring
with reduced fitness and disrupt local adaptation, thus putting the wild stock at risk (McGinnity et al. 2003; Fraser et al. 2008).

To achieve hybridization, a farmed fish must first escape from an aquaculture site, survive and migrate to spawning grounds and successfully mate in the wild, during which reproductive behavior will be a key factor influencing its breeding success (Fleming et al. 1996). Much research exists on the reproductive success of hatchery salmon in competition with wild salmon (Fleming and Gross 1993; Berejikian et al. 2001; Berejikian et al. 2009), and more recent studies examine reproductive interactions between wild and transgenic salmon (Fitzpatrick et al. 2011; Moreau et al. 2011). Furthermore, other research has focused on the reproductive success of wild and farmed Atlantic salmon in competition (Fleming et al. 1996; Weir et al. 2004), however, few studies have focused on farmed Pacific salmon. All studies on the reproductive interactions between farmed and wild salmonids have shown that artificial rearing practices have adverse effects on reproductive behavior, as those studies have shown that cultured salmon have lower reproductive success relative to wild salmon (Fleming and Gross 1993; Fleming et al. 1996; Berejikian et al. 2001; Weir et al. 2004; Berejikian et al. 2009; Fitzpatrick et al. 2011; Moreau et al. 2011).

**Salmonid mating system**

The reproductive success of farmed salmon often differs from that of wild because, under culture conditions, natural selective pressures are lost and reproduction often involves artificial fertilization and no sexual selection. In nature, female salmon are choosy, as females must compete for and defend high quality nest sites and they produce only a small number of larger gametes relative to males, thus females experience greater
selective pressure when choosing a mate (Foote 1990; Quinn 2005). In the salmon mating system males provide few resources, leading females to preferentially select certain males over others based on indirect benefits that include “good genes” and/or “compatible genes” (reviewed in Neff and Pitcher 2005). Another significant component of reproductive interactions among salmonids is male-male competition, and it is an important factor shaping sexual selection in the salmonid mating system in general, where males will compete among themselves to gain access to ovipositing females (Fleming et al. 1996). Male salmonids can compete and gain social dominance through different phenotypic traits such as body size and spawning coloration (Fleming and Gross 1994). In addition to pre-spawning competition, post-spawning competition in male salmon includes sperm competition, as salmon are external fertilizers where several males may simultaneously fertilize the eggs of a single female (Parker 1970).

**Sperm competition**

Sperm competition occurs when sperm from two or more males compete for fertilization of an egg (Parker 1970). Under intense sperm competition, theory predicts that sperm swim speed should be favored at the expense of sperm longevity (Ball and Parker 1996). This is because sperm swim speed increases with sperm length, and longer sperm can swim faster but require more energy thus resulting in a trade off for longevity (Ball and Parker 1996). Although faster sperm speed may also result in a trade off for sperm density, in which an individual can either have fewer fast-swimming sperm or more numerous slower sperm (Parker 1982), and evidence suggests that sperm length decreases with increasing sperm competition intensity in fishes (Stockley et al. 1997). However, given that fertilization happens so quickly after egg and sperm association in
salmonids (Hoysak and Liley 2001), faster sperm swim speed may offset any
disadvantage in sperm number. This is demonstrated by Gage et al. (2004), as Atlantic
salmon males with greater sperm velocity fertilized more eggs even when competing
male had more numerous sperm. Relative sperm performance will thus be an important
contributing factor to the reproductive success of salmonid males (Gage et al. 2004).
Outside of this thesis, no studies have focused on the sperm performance of farmed
salmonids relative to their wild counterparts, although Skjæraasen et al. (2009) and Butts
et al. (2011) reported reduced sperm performance in farmed cod (*Gadus morhua*), and
Rideout et al. (2004) demonstrated equal sperm performance in farmed haddock
(*Melanogrammus aeglefinus*). Although farming practices could lead to adverse affects
on sperm quality, farm fertilization protocols (i.e., mixed-milt spawning) could
potentially enhance sperm performance, as it can lead to sperm competition (Campton et
al. 2004). Given that farmed salmon generally display behavioral inferiority in
reproduction compared to wild salmon (see above), it is possible that farmed males could
achieve reproductive success in competition with wild males through enhanced sperm
traits (Birkhead and Møller 1998; Hutchings and Myers 1988), thus providing one
mechanism by which farmed genes could introgress into wild populations.

**Outcome of hybridization**

Despite the lower reproductive success reported for farmed salmon, escaped fish
do successfully reproduce and hybridize with wild fish (Crozier 2000; Lura and Sægrov
1991). Gene flow between spatially separated and isolated populations may result in
heterosis by increasing the genetic diversity within a population through the introduction
of novel genes, or by masking the effects deleterious recessive alleles (Whitlock et al.
Heterosis occurs when first generation hybrid offspring exhibit superiority in fitness compared to their parents (Lynch 1991). However, evolution has worked to reduce gene flow between Pacific salmon populations and can thus foster local adaptation, and this is displayed through strong natal philopatry with generally low straying rates (Taylor 1991). Escapes from aquaculture sites to nearby rivers may pose a threat to local adaptation (Bourret et al. 2011), and when successful hybridization occurs it may have considerable implications for the conservation of wild stocks. When gene flow occurs between two genetically divergent populations outbreeding depression may result (Templeton 1986; Lynch 1991). Outbreeding depression is a reduction in fitness by means of additive and/or nonadditive genetic effects of hybridization between genetically divergent populations (Lynch 1991). First generation hybrids may experience reduced fitness in both parental environments as a result of additive genetic effects that occurs when the hybrid displays an intermediate phenotype to both parents (Lynch 1991).

Nonadditive genetic effects of outbreeding depression are expected to occur in the second or later generations when coadapted gene complexes are disrupted by introgression of a novel genotype and subsequent recombination (Templeton 1986; Lynch 1991). Outbreeding presents consequences not only for wild-farmed hybridization, but also for conservation programs trying to increase genetic diversity by mixing stocks (Neff et al. 2011).

**Study Species: Chinook salmon**

Chinook salmon (*Oncorhynchus tshawytscha*) are a Pacific salmonid species found along both northern coasts of the Pacific Ocean, as well as introduced populations throughout the world. Chinook salmon life-history includes semelparity and anadromity,
and similar to other salmonid species, Chinook display very specific homing behaviors as adults return to natal streams during spawning migration (Quinn and Dittmann 1990). Natal philopatry tends to result in subdivided populations, and limits the extent of gene flow between populations, which, across a diversity of environments, establishes ideal conditions for local adaptation (Taylor 1991). Chinook salmon stocks have been declining since the 1990s along the west coast of Canada (Noakes et al. 2000), and declining Chinook stocks have resulted in extensive re-stocking programs as well as farming of the species. The potential for local adaptation in this species and inevitable escapes from aquaculture sites pose a risk to natural stocks as escapes may alter locally adapted gene pools of wild populations. With Chinook salmon being a species of conservation interest, it is important that we understand the potential impacts that farm escapes can have on wild populations.

1.1 THESIS OBJECTIVES

The goal of this thesis is to examine the reproductive ability of farmed Chinook salmon males relative to wild males, and determine the genetic impacts of outbreeding in the species. Understanding the reproductive ability of escaped farmed salmon in the wild can help quantify risks associated with escapes and establish proper management strategies.

Chapter 2 objective

Relative sperm performance can provide insight into the competitive ability of salmonid males, thus my objective was to identify differences in sperm traits (including sperm velocity, motility, longevity and density) between wild and farmed Chinook salmon males. Examining sperm traits between wild and farmed salmon allows me to
determine if one male type would have a competitive advantage under sperm competition, as well as provides understanding as to how farming practices affect sperm quality in fishes.

Chapter 3 objectives

Differences in sperm performance allowed predictions about the outcomes of competitive mating, however spawning channel experiments provide quantification of the actual success that wild and farmed Chinook salmon males would experience when in competition. My objective was to determine differences in fertilization and reproductive success between wild and farmed males under competition for female mates in semi-natural spawning channels. Fertilization success and reproductive success was measured by the paternity of eggs and fry, respectively. The results provide insight into the success of an escaped farmed salmon in the wild, and thus highlight the risks associated with farming an indigenous species. Additionally, examining both the early (eggs) and late (fry) stages of development allow me to determine effects of sexual and natural selection, respectively, on farmed and wild Chinook salmon success.

Chapter 4 objectives

I examine the impact of outbreeding in Chinook salmon by testing the theory of outbreeding depression using a multigenerational approach. My objective was to determine the effects of outbreeding in Chinook salmon by comparing performance traits between backcrossed hybrids (F2) and purebred offspring. This approach provides novel knowledge on the effects of outbreeding in Chinook salmon, as well as highlights potential genetic effects associated with wild-farmed hybridization.

1.2 REFERENCES


2.0 SPERM TRAIT DIFFERENCES BETWEEN WILD AND FARmed CHINook SALMON (ONCORHYNCHUS Tshawytscha)1

2.1 INTRODUCTION

Salmon aquaculture is an economically important industry; however, there are increasing concerns about the potential impacts of interactions between farmed and wild fish (Hindar et al. 1991; Naylor et al. 2005; Skaala et al. 1990). These interactions are of major concern when considering escapes from aquaculture sites, because the unnatural and controlled aquaculture setting provides an especially different environment for fish to evolve in compared to the wild, resulting in phenotypic and genetic differences in the farmed populations (Heath et al. 2003; Skaala et al. 1990). The genetic changes occurring in aquaculture involve the loss of genetic diversity as well as the divergence of farmed stocks from the original wild population (Hindar et al. 1991; Skaala et al. 1990). Additionally, homogametic male fish (XX males) are used for commercial production of all female stocks, and if such fish escape and reproduce successfully in the wild they would skew the sex ratio in the wild population. Hybridization through reproductive interactions between escaped farmed and wild salmon is an immediate threat to the fitness and genetic composition of natural populations (Hindar et al. 1991; McGinnity et al. 2003; Naylor et al. 2005). For example, McGinnity et al. (2003) showed that farmed-wild hybrid offspring have lower survival compared to wild offspring, and that competition from farmed and hybrid offspring reduces wild smolt production in Atlantic salmon (Salmo salar).

The potential for hybridization between wild and farmed salmon will depend on numerous factors, although primarily on the reproductive success of escaped farmed individuals in the wild (Fleming et al. 1996). The effect of artificial rearing on salmon reproductive behavior and success has been widely studied showing, under experimental conditions, farm-raised, transgenic and hatchery salmon have reduced competitive and reproductive success compared to wild salmon (Berejikian et al. 2001; Fitzpatrick et al. 2011; Fleming and Gross 1993; Fleming et al. 1996; Moreau et al. 2011; Weir et al. 2004). Although artificially reared males and females both experience lower reproductive success when in competition with wild fish, the lower reproductive success is more pronounced in males relative to females (Fleming and Gross 1993; Fleming et al. 1996). Specifically, males show less aggression and partake in fewer spawning events than wild males; as well, they display inappropriate mating behavior resulting in females denying access to the oviposition site (Fleming and Gross 1993; Fleming et al. 1996). In addition to those behaviors, Webb et al. (1991) reported that escaped farmed and wild Atlantic salmon spawn in different reaches of the river, further reducing the likelihood of hybridization. Nevertheless, escaped farmed salmon do successfully reproduce and hybridize with wild fish (Crozier 2000; Lura and Sægrov 1991). In a study of 16 Scottish rivers, escaped Atlantic salmon females contributed up to 7% of the fry in some rivers (Webb et al. 1993), furthermore the experimental release of farmed Atlantic salmon in a Norwegian river revealed that 55% of farm escapes contributed 19% of the genes to the next generation of adult salmon (Fleming et al. 2000). While behavioral interactions play a key role in breeding success, salmonids are external fertilizers allowing several males to simultaneously fertilize the eggs of a single female. Consequently, relative sperm
performance will also be an important contributing factor to the reproductive success of farmed salmon in the wild (Gage et al. 2004). This is because subdominant males can offset behavioral inferiority through enhanced sperm traits (Birkhead and Møller 1998; Hutchings and Myers 1988). Farmed males could achieve higher fertilization success by having faster swimming sperm, as Gage et al. (2004) found males with higher sperm velocity had greater fertilization success even when competing male had a greater number of sperm.

Gamete quality is an important factor in evaluating the risk associated with farm escapes and it is also important to ensure high fertilization rates under farm production breeding, yet few studies have tested the effects of farm rearing on sperm traits in fishes. The effect of farming on reproductive traits in penaeid prawns has been extensively studied (Alfaro and Lozano 1993; Pratoomchat et al. 1993; Rendon Rodriguez et al. 2007). Research shows captive rearing can negatively impact sperm traits in prawns, including an increased percentage of abnormal spermatozoa, reduced number of sperm in spermatophores, reduced percentage of viable sperm (Leung-Trujillo and Lawrence 1987), and the degeneration of the male reproductive tract (Talbot et al. 1989). The effect of farming on sperm traits in fishes has been studied by Skjæraasen et al. (2009) where sperm traits were compared between wild and farmed cod (Gadus morhua). They showed that wild males had a higher percentage of motile sperm, sperm velocity and spermatoocrit compared to farmed males at the beginning of the spawning season; whereas, at the end of the spawning season sperm velocity was still higher in wild males, but there were no differences in other traits. Greater sperm velocity observed in wild cod relative to farmed was also shown in a second study (Butts et al. 2011) indicating that higher sperm quality
in wild males may be a common phenomenon in this species. On the other hand, a study on haddock (*Melanogrammus aeglefinus*) found no difference in sperm velocity or spermatocrit between wild and farmed males throughout the spawning season (Rideout et al. 2004). All of those studies examined farmed fish populations only one generation removed from the wild, thus highlighting the need for studies examining sperm traits in a more intensively farmed species, several generations removed from the wild, to assess the true impacts of farming on sperm traits in fishes.

A common practice used in salmonid aquaculture to reduce the early maturation of males is the hormonal sex-reversal of females to create homogametic (XX) males (Heath et al. 2002). XX males produce sperm that only bears the X chromosome and milt from these males can be used to fertilize eggs and produce all female production stock (Devlin et al. 1991). The hormonal manipulation associated with sex-reversal can have negative impacts on testes development and sperm traits in teleosts, including a decrease in sperm density and motility in *Betta splendens* (Kirankumar and Pandian 2002), deformed testis in Eurasian perch (*Perca fluviatilis*) (Rougeot et al. 2002), and incomplete sperm duct development in salmonids (Johnstone et al. 1979; Geffen and Evans 2000). However, normal gonadal development and sperm duct formation have been demonstrated in XX males from various species, including northern pike (*Esox lucius*) (Luczynski et al. 2003) and Chinook salmon (*O. tshawytscha*) (Heath et al. 2002). As well, studies report no difference in sperm traits between XX and XY males for Eurasian perch (Rougeot et al. 2004) and Coho salmon (*Oncorhynchus kisutch*) (Fitzpatrick et al. 2005), and no difference in testicular sperm density or ATP concentrations between XX and XY male rainbow trout (*O. mykiss*) (Geffen and Evans...
2000). Although sex-reversal is prevalent in aquaculture, few comparative studies on sperm traits of XX and XY males exist for salmonids (Fitzpatrick et al. 2005; Geffen and Evans 2000), particularly for species with morphologically normal gonads and functional sperm ducts.

Given that large numbers of farmed salmonids are known to escape from aquaculture sites (Naylor et al. 2005), studying sperm traits in wild and farmed salmon will provide insight into the potential for escaped males to hybridize with the wild population. Through the examination of sperm motility, velocity, longevity and density, I evaluate sperm performance of farmed fish relative to wild fish in Chinook salmon. In this study I compare sperm traits between XX farmed, XY farmed and wild (XY) males, allowing me to determine the impact of farming as well as sex-reversal on sperm traits in salmon. Additionally, competitive fertilization success is positively correlated with sperm velocity in salmonids (Gage et al. 2004; Lahnsteiner et al. 1998; Liljedal et al. 2008; Pitcher et al. unpublished data), allowing me to assess the potential reproductive success of escaping farmed male salmon in the wild based on their sperm characteristics.

2.2 MATERIALS AND METHODS

Fish type and origin

All Chinook salmon used in this study originate from river systems on Vancouver Island, British Columbia, Canada. Farmed salmon were obtained from an organic Chinook salmon farm, Yellow Island Aquaculture Ltd. (YIAL), Quadra Island, BC. The organic farming practices involve no use of pesticides or antibiotics and the fish are fed a diet that mimics that of wild salmon, which includes offshore fish protein and naturally derived carotenoid pigment. The farmed salmon males included both homogametic (XX)
and heterogametic (XY) males. YIAL began producing homogametic males in 1985 from XX milt acquired from the Big Qualicum hatchery, Vancouver Island. In the years following, XX males were spawned with YIAL broodstock to create a monosex population. At YIAL, XX males are generated through the exogenous treatment with the androgen 17α-methyltestosterone (400μg L⁻¹) for 2h at 520 ATUs (accumulated thermal units) and at 620 ATUs of development (Heath et al. 2002). All XX males in this study were 6 to 7 generations domesticated at YIAL and were bred in either the fall of 2005 or 2006 through mixed-milt spawning and were thus 4 or 5 years of age at time of sampling. All XY males at YIAL were descendant from gametes obtained from Robertson Creek and Big Qualicum hatcheries in 1985, and 4 generations later (1997), fish were mated in a full factorial cross with wild fish from Big Qualicum River (Bryden et al. 2004). All XY males used in this study are therefore up to 7 generations domesticated at YIAL but introgressed with wild genes 3 generations removed from the wild Big Qualicum stocks. The XY stock has been maintained by single male and single female crosses, and all XY males used in this study were bred in the fall of 2006 and were thus 4 years of age at the time of sampling. Both farmed male types were hatched and reared in fresh water until smolting when they were transferred to saltwater pens until sexual maturation. Mature XX and XY males were seined from saltwater pens and transferred to fresh water from October 4 to October 13 and October 14 to 18, 2010, respectively. Wild Chinook salmon were seined from the Quinsam River on October 21, anesthetized with CO₂ and transported approximately 1.5-hours by vehicle to YIAL in 700-L of oxygenated river water. No mortalities occurred as a result of transport. Wild males were presumed to be individuals spawned in the fall of 2007 and were thus 3 years of age at time of sampling.
All farmed and wild males were kept in 2500-L freshwater holding tanks and sampled between October 14 and 22. Fish were anesthetized with buffered MS222, then weight (± 10 g) and fork length measurements (± 1 mm) were recorded.

**Sperm collection and measurements**

After weight (mean weight ± S.E., 4.41 ± 0.16 kg) and length (mean length ± S.E., 71.0 ± 0.9 cm) measurements were taken, milt (sperm and seminal plasma) was stripped from individual males by applying gentle pressure to the abdomen. Any milt in contact with urine, water or other contaminants was not used. Milt was collected in plastic bags, stored at approximately 4°C and analyzed immediately in the on-site laboratory. Sperm activated with 10 µL of fresh water were video recorded through a microscope and assessed with sperm-tracking software (see Pitcher et al. 2009). Video recordings were conducted using a negative phase-contrast microscope (CX41 Olympus) with 10X magnification objective mounted with a CCD B/W video camera (at 50Hz vertical frequency). Sperm motility and velocity were measured at 5, 10 and 15 s post-activation using HTM-CEROS sperm analysis system (CEROS version 12, Hamilton Thorne Research, Beverly, MA, USA), an objective method for studying sperm motility in fish (Kime et al. 2001). The image analyzer was used with the following settings: number of frames = 60, minimum contrast = 20-30, and minimum cell size = 3 pixels. Sperm motility was defined as the percentage of motile sperm cells which was determined using this software by dividing the number of progressively motile sperm cells by the total number of sperm cells in the field of view at 5, 10 and 15 seconds post-activation. For each individual, three measures of sperm velocity were evaluated: The average path velocity (VAP in µm s⁻¹, defined as the average velocity along a smoothed
cell path), the straight line velocity (VSL in \( \mu \text{m s}^{-1} \), defined as the average velocity along a straight line connecting the start and end points of the cell’s path) and the curvilinear velocity (VCL in \( \mu \text{m s}^{-1} \), defined as the average velocity along the actual path that the cell travels). Velocity estimates represent the mean velocity of all individual motile sperm cells. All three sperm velocity measures described above, which are VAP, VSL and VCL, were significantly positively correlated at all time periods after activation (\( r^2 \) ranged from 0.20 to 0.88, all \( p < 0.003, N = 43 \)), pooling male types. Given that all sperm velocity measures were correlated and yielded qualitatively similar results, all further velocity results will be based on VAP, which is commonly used in Chinook salmon and other \textit{Oncorhynchus} spp. studies to represent sperm velocity (e.g. Lahnsteiner et al. 1998; Rosengrave et al. 2008) as it describes the smoothed path by which the sperm cell travels. Sperm longevity was also estimated from video tracks, and was considered the time from activation until approximately 95% of sperm cells within the field of view had ceased forward movement (see Gage et al. 2004). When assessing sperm motility, and sperm velocity and longevity, the total number of sperm cells in the field of view was on average (± S.E.): 79.3 ± 5.4, 70.7 ± 5.0 and 55.5 ± 4.8 at 5, 10 and 15 seconds post-activation, respectively.

An “improved Neubauer chamber” haemocytometer under 400X magnification was used to estimate sperm density (Pitcher et al. 2007; Pitcher et al. 2009). Briefly, the number of sperm cells in 5 of 25 larger squares was counted (each square subdivided for simplified counting). This count was used to estimate the number of sperm cells in all 25 squares, which was then multiplied by the depth of the chamber (10 \( \mu \text{m} \)) and then again
by the initial volume of the sample. The estimated densities were expressed as the number of sperm cells per milliliter of stripped milt.

Statistical Analyses

Temporal changes (5, 10 and 15 s post-activation) in sperm motility and velocity between XX, XY and wild males were analyzed using repeated measures ANOVAs followed by Tukey’s test for post-hoc pairwise comparisons. The model was further decomposed into individual one-way ANOVAs coupled with Tukey’s post hoc test at each time period to determine significant interactions. Sperm longevity, sperm density and Fulton’s condition factor between XX, XY and wild males were analyzed using one-way ANOVAs followed by Tukey’s post-hoc test to examine all pairwise comparisons.

All means are reported ± S.E. Data were tested for normality. Transformation of sperm motility and velocity data failed to improve normality, however, although assumptions of parametric tests were not fully met, the ANOVA is known to be robust enough to deal with these issues (Underwood 1981). To verify this, non-parametric tests (Kruskal-Wallis) were also performed and yielded qualitatively similar results as parametric tests. Fish sample size varied across sperm performance metrics (XX N = 15-17, XY N = 8-11, Wild N = 20-26), as not all samples were usable for each trait examined due to video tracks displaying water flow causing inaccurate readings, or milt samples contaminated with water, blood and/or urine.

2.3 RESULTS

Sperm Motility

Percentage of motile sperm cells decreased significantly over time and differed significantly among male types (Fig. 2.1A; Repeated Measures ANOVA, F = 2.84, p =
XX and XY farmed males had significantly greater percentage of motile sperm compared to wild males (p = 0.0002 and p = 0.003, respectively), and there was no difference between XX and XY farmed males in percent motility (p = 0.99).

**Sperm Velocity**

Sperm velocity decreased significantly over time and differed significantly among male types (Fig. 2.1B; Repeated Measures ANOVA, F = 4.38, p = 0.008). Post-hoc tests revealed that XX and XY farmed male sperm velocity was significantly greater than that of wild males (p = 0.03 and p = 0.04, respectively), however no significant difference existed between XX and XY farmed males in sperm velocity (p = 0.45).

**Sperm Longevity**

Sperm longevity differed significantly among male types (Fig. 2.1C; ANOVA; F= 4.10, p = 0.02). Post-hoc tests of sperm longevity showed significant differences between XX farmed and wild males (p = 0.03), but no significant difference in sperm longevity between XX and XY farmed males (p = 0.97) or XY farmed and wild males (p = 0.12).

**Sperm Density**

Sperm density differed significantly among male types (Fig. 2.1D; ANOVA; F= 6.39, p = 0.003), with XY farmed males having the greatest density of sperm cells per milliliter of milt. Post-hoc tests of sperm density showed significant differences between XY farmed and wild males (p = 0.003) and XX and XY farmed males (p = 0.015), but no significant differences between XX farmed and wild males (p = 0.94).

**Fulton’s Condition Factor**

A post-hoc examination of Fulton’s condition factor for each of the groups was conducted, calculated as \( K = (WL^{-3}) \times 10^5 \), where \( W \) is weight (g) and \( L \) is fork length.
Condition factor was significantly different among male types (Fig. 2.2; ANOVA; $F = 6.68, p = 0.003$). XX and XY males had significantly higher condition factor than wild males ($p = 0.021$ and $p = 0.007$, respectively).

**2.4 DISCUSSION**

For the sperm traits examined, wild males generally had lower performance values than XX and XY farmed males, and no difference existed in sperm traits between XX and XY males, except in sperm density. Many sperm traits can be good indicators of fertilizing capacity, however, sperm velocity is known to be the primary variable affecting competitive fertilization success in salmonids, including Atlantic salmon (Gage et al. 2004), rainbow trout (Lahnsteiner et al. 1998), Arctic charr (*Salvelinus alpinus*) (Liljedal et al. 2008), Coho salmon (Pitcher et al. unpublished data) and Chinook salmon (Flannery 2011). Sperm density can also be important in sperm competition, and sperm number is shown to increase with increasing intensity of sperm competition in fishes (Stockley et al. 1997). However, Gage et al. (2004) demonstrate the importance of sperm velocity in Atlantic salmon, as males with faster sperm had greater fertilization success even when competing males had more numerous sperm. Thus I suggest that my findings indicate XX and XY farmed males would have greater fertilization success when in sperm competition with wild males from the Quinsam River. Higher competitive fertilization success of farmed males may lead to a higher level of hybridization between escaping farmed fish and wild fish than expected based on the numbers of fish alone. Hybridization will allow gene flow from farmed stocks to the wild, likely resulting in a reduction of fitness in the wild population (McGinnity et al. 2003), perhaps increasing the likelihood for local population extirpation. However, the extent of hybridization may be
reduced through behavioral inferiority in the farmed males, as many studies show that cultured salmon have reduced reproductive success when in competition with wild salmon (Berejikian et al. 2001; Fitzpatrick et al. 2011; Fleming and Gross, 1993; Fleming et al. 1996; Moreau et al. 2011; Weir et al. 2004).

My finding of little or no difference in sperm performance between XX and XY farmed males is consistent with other studies examining the effect of sex-reversing on sperm traits in closely related species such as Coho salmon (Fitzpatrick et al. 2005) and rainbow trout (Geffen and Evans 2000). Unlike those species, all XX Chinook salmon have morphologically normal gonads and sperm ducts (Heath et al. 2002). Although my analyses should be replicated in other Chinook salmon broodstocks, I suggest that, based on my findings, there are no negative implications for fertilization success resulting from using sperm from XX males to fertilize production eggs.

Only a few studies have examined the effect of farming on sperm traits in fishes. Skjæraasen et al. (2009) and Butts et al. (2011) reported that wild male cod had greater sperm performance compared to farmed cod, whereas Rideout et al. (2004) observed no difference in sperm traits between wild and farmed haddock. My study provides the first sperm performance data for a farmed fish population several generations removed from the original wild stocks, which may provide an explanation as to why my results differ from previous studies. The greater sperm performance found in farmed Chinook salmon males may result from selective pressure on sperm competition from mixed-milt spawning in the aquaculture environment. The pooling of milt from several males to fertilize eggs can lead to a loss of genetic diversity in the population due to differences in sperm competitive ability among males being pooled (Campton 2004; Neff et al. 2011).
Mixed-milt spawning in Chinook salmon (Withler and Beacham 1994) showed extreme variation in fertilization success of individual males, ranging between 5% and 88% when milt from three males was pooled. However, this only provides an explanation for the greater sperm performance observed in XX males, as XY males were not subjected to mixed-milt spawning at YIAL.

The greater sperm performance of XX and XY farmed males may also be a consequence of differences in the relative spawning condition of the fish from each group. Fulton’s condition factor ($K$), which reflects differences in fish body mass for a given body length such that higher values are presumed to indicate better condition, was greater for XX and XY farmed males compared to wild males (Fig. 2.2). Although the higher condition factor of farmed fish in comparison to wild fish can be attributed to diet, condition factor and sperm performance may also be a reflection of the male’s spawning stage. During the spawning season, fish, especially anadromous species, are subjected to energetic costs that result in weight loss (Jonsson et al. 1997) and thus a reduction in condition factor, as well, the aging of sperm in fishes during the spawning season affects the quality of sperm (Rana 1995). In many fish species, the spawning season is marked by a gradual increase followed by a gradual decrease in sperm motility (Munkittrick and Moccia 1987; Suquet et al. 1998) and sperm density (Aas et al. 1991; Büyükhatipoglu and Holtz 1984). However, other studies have shown an increase in sperm density or spermatocrit at the end of the spawning season (Rakitin et al. 1999; Rideout et al. 2004; Skjæraasen et al. 2009; Suquet et al. 1998). Although the pattern of changes in sperm traits over the spawning season is not known for Chinook salmon, the difference between farmed and wild males in condition and sperm performance may be an indication of their
stage in the spawning process. However, I found no significant correlation between sperm velocity and condition factor ($p = 0.35$, $N = 43$), indicating that higher condition does not predict faster sperm. This suggests that my results are not an artifact of condition factor or spawning stage, but reflect fundamental differences in sperm performance between the Chinook salmon populations.

The differences observed between male types could be also attributed to the age of the individual males, as wild males were presumed to be younger than farmed males. It is possible that older males have greater sperm performance in Chinook salmon; however, previous studies of Pacific salmon species have found that younger males have similar or better sperm performance (Hoysak and Liley 2001; Liley et al. 2002; Pitcher et al. unpublished data). Stress due to transportation may have also affected sperm performance of wild males, as a study on white bass, Morone chrysops, showed reduced motility in stressed individuals (Allyn et al. 2001), although these effects have not been examined in salmonids. Milt collection was completed immediately after transport for approximately half of the wild males, whereas the remaining wild males had 20-hours to recover prior to sampling. However, sperm velocity and sperm motility of wild males did not differ between sampling times (T-test; $p = 0.59$ and $p = 0.97$, respectively). Finally, I included only one wild and one farmed population in my analyses, thus raising the possibility of pseudoreplication (Hurlbert 1984). Ideally, future studies should include multiple farmed and wild Chinook salmon populations to increase the generality of my results; however my study provides a valuable starting point for quantifying the hybridization risks associated with escaped farmed Chinook salmon on the spawning grounds.
In conclusion, my study shows that farmed males had greater sperm performance compared to wild males. Irrespective of condition factor, spawning stage and age, my data shows that if escaping farmed salmon males entered nearby rivers during the spawning season they would have an advantage in sperm competition with wild salmon. From an ecological perspective, the ability of farmed males to outcompete wild males can have significant impacts on natural populations, ranging from outbreeding depression and loss of genetic diversity to extirpation (Fleming et al. 2000; Hindar et al. 1991; McGinnity et al. 2003). However, despite sperm competition playing an important role in male-male interactions in salmonids, behavioral interactions are also critical for reproductive success (Fleming et al. 1996). While farmed Chinook salmon males may have greater sperm performance, it is possible that these farmed males have lost much of their behavioral ability to compete for mates and gain access to females due to domestication, and thus would not be reproductively successful in the wild. Currently, I am examining the semi-natural spawning competitions between wild and farmed Chinook salmon to test this possibility.

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Figure 2.1. Means (± standard error) of XX farmed, XY farmed and wild Chinook salmon (Oncorhynchus tshawytscha) males for sperm traits: (A) percent motility, (B) sperm velocity (VAP, see Materials and methods), (C) sperm longevity and (D) sperm density. Asterisks (*) over time periods and different letters over bars indicate significant differences between male types (p < 0.05). Sample size varied over sperm traits (see Materials and methods for details).
Figure 2.2. Fulton’s condition factor (mean ± standard error) of XX farmed (N = 18), XY farmed (N = 10) and wild (N = 27) Chinook salmon (*Oncorhynchus tshawytscha*) males. Fulton’s condition factor was calculated as $K = (W/L^3) \times 10^5$, where $W$ is weight (g) and $L$ is fork length (mm).
3.0 REPRODUCTIVE INTERACTIONS BETWEEN WILD AND FARmed
CHINOOK SALMON: COMBINED EFFECTS OF SEXUAL AND NATURAL
SELECTION1

3.1 INTRODUCTION

As the salmon aquaculture industry continues to expand throughout the world, domestication will lead to increasing genetic consequences for farmed salmon populations. Artificial rearing in aquaculture leads to genetic changes that can result in a loss of genetic diversity due to bottlenecks, and genetic divergence of farmed populations from their wild counterparts due to different selection regimes and genetic drift (Hindar et al. 1991; Skaala et al. 2006; Fraser et al. 2010). These changes are particularly troublesome considering the potential for reproductive interactions between wild and farmed populations that result when farmed salmon escape and hybridize with wild salmon (Noakes et al. 2000). Such hybridization can pose serious threats to the genetic structure and diversity of wild populations (Hindar et al. 1991). The disruption of locally adapted genes through hybridization between wild and farmed salmon can lead to a reduction in fitness of the wild stocks (Fleming et al. 2000; McGinnity et al. 2003). Previous studies on Atlantic salmon (Salmo salar) have reported that wild-farmed hybrid offspring have lower survival relative to wild offspring, and that wild smolt production can be negatively impacted by competition from hybrid and farmed offspring (McGinnity et al. 2003).

1 Lehnert, S.J., Heath, J.W., Heath, D.D. Reproductive interactions between wild and farmed Chinook salmon: combined effects of sexual and natural selection. (Submitted to Evolutionary Applications, June 2012)
The probability of wild-farm hybridization events will be dependent on various factors, including the ability of the farmed fish to escape, survive, migrate to a spawning site and reproduce in the wild, where reproductive behavior will be essential to successful hybridization (Fleming et al. 1996). Once the spawning grounds are reached, male success can be defined in several ways, including mating success based on behavioral observation, fertilization success based on the paternity of the eggs, and reproductive success based on the paternity of the fry. Artificial rearing practices can have negative impacts on reproductive behavior, and studies have shown that, under experimental conditions, cultured salmon (including farm-raised, transgenic and hatchery salmon) all experience lower competitive mating success relative to wild salmon (Fleming et al. 1996; Weir et al. 2004; Berejikian et al. 2009; Fitzpatrick et al. 2011; Moreau et al. 2011). The impact of artificial culture on reproductive behavior may be more pronounced in males compared to females, as Fleming et al. (1996) reported that, based on mating observations and embryo viability, farmed Atlantic salmon females achieved 20-40% the fertilization success of wild females, whereas farmed males achieved only 1-3% of the success of wild males. The low mating success reported for farmed males may be attributed to their limited competitive ability, given that they show less aggression, display inappropriate mating behavior and participate in less courting and spawning activity (Fleming et al. 1996). The ability of males to compete for females (or their gametes) is the main driver of sexual selection in the salmonid mating system, in which dominance hierarchies are formed by males to determine which males gain access to ovipositing females (Fleming et al. 1996). Salmonid males frequently establish dominance hierarchies through phenotypic traits such as body size and spawning
coloration (Fleming and Gross 1994), additionally, males may directly compete, albeit cryptically, through sperm competition mechanisms (Taborsky 1998; Pitcher et al. 2009). Despite the lower reproductive success of farmed salmon, there are documented cases of escaped Atlantic and Pacific salmon successfully reproducing in the wild (Lura and Sægrov 1991; Crozier 2000; Correa and Gross 2008). Furthermore, recent work has shown that farmed Chinook salmon (*Oncorhynchus tshawytscha*) have higher sperm performance (e.g., greater sperm velocity, motility, longevity and density) compared to wild salmon (Lehnert et al. 2012), which may enhance their competitive ability and compensate for behavioral inferiority in reproduction (Hutchings and Myers 1988; Lehnert et al. 2012).

Although a number of studies examine the fertilization or reproductive success of hatchery versus wild salmon (McLean et al. 2003; Berejikian et al. 2009; Schroder et al. 2010), fewer studies compare fertilization or reproductive success between farmed (aquaculture) salmon and wild salmon (Fleming et al. 1996; Weir et al. 2004; McGinnity et al. 2003). The few studies that focused on farmed males do not incorporate the practice of finfish aquaculture in which females are hormonally sex-reversed to create homogametic (XX) males (Heath et al. 2002). Monosex (all female) populations are used to reduce the impacts of early maturation in males before they attain a marketable size (Devlin et al. 1991). XX males produce sperm that bears only the X chromosome (Devlin et al. 1991), thus escapes from monosex farm populations could lead to a female-biased sex ratio in the wild if the escaped males successfully hybridize with wild females. To date, no studies examine the ability of farmed XX males to compete and spawn in the presence of wild males, although Garner et al. (2010) observed that XX male Chinook
salmon displayed normal spawning behavior and experienced mating success similar to that of control farmed XY males under semi-natural conditions.

Aside from differences in reproductive behavior in farmed and wild adult salmon, the offspring from wild and farmed origins often diverge in behavioral and life history traits that can result in differences in selection pressures when offspring are exposed to the same environment (Weir and Grant 2005; Jonsson and Jonsson 2006). For example, under the same conditions, farmed Atlantic salmon experienced lower egg survival (Fleming et al. 1996) and lower survival to the smolt stage (McGinnity et al. 1997) relative to wild salmon. Kostow (2004) reported that cultured steelhead (O. mykiss) offspring were larger and experienced lower smolt-to-adult survival in comparison to their wild counterparts. Fleming and Einum (1997) observed that farmed Atlantic salmon offspring exhibited higher growth rates compared to wild offspring, although their growth was suppressed when in competition with wild offspring. Domestication also resulted in the divergence of ecologically relevant behavioral traits such as aggression and predator avoidance (Fleming and Einum 1997). However the difference in aggressive behavior varies among studies, as some report aggression is higher in wild salmon compared to cultured (Berejikian et al. 1996), whereas other studies report the reverse (Fleming and Einum 1997; Houde et al. 2010). Additionally, predator avoidance is generally lower in cultured individuals relative to wild, as they more frequently display behavior that put them at greater risk of predation (Johnsson et al. 1996; Fleming and Einum 1997). The various changes resulting from domestication can lead to wild and farmed offspring experiencing differences in selection when in the same environment.
Many studies of reproductive interactions between cultured and wild salmon use molecular genetic pedigree reconstruction to compare the reproductive success of individuals (Berejikian et al. 2009; Schroder et al. 2010; Fitzpatrick et al. 2011; Moreau et al. 2011); however, those studies evaluate reproductive fitness at only one stage in offspring development. Examining success of individuals at the egg stage may provide an unrealistic measure of reproductive success in a natural setting, as natural selection can have a substantial effect on survival of offspring from the egg to fry stage (Einum and Fleming 2000b; McGinnity et al. 2003) as described above. Furthermore, given the high mortality between the egg and fry stages, examining reproductive fitness at the fry stage will be highly dependant on the specific selection regime present in the experimental site. Thus such an approach would not allow the determination of the role of sexual selection in the relative spawning success of farmed and wild male salmon. Given that farmed offspring have lower freshwater survival relative to wild offspring (McGinnity et al. 1997; McGinnity et al. 2003), it is essential that reproductive fitness studies include both the egg (fertilization success = sexual selection) and fry (survival = natural selection) stages to properly quantify the differential fitness of wild and farmed salmon.

Aquaculture continues to expand globally and we know large numbers of farmed salmon have escaped from aquaculture sites (Noakes et al. 2000; Naylor et al. 2005), yet reproductive interactions between wild and farmed salmon have been investigated only rarely using experimental spawning competition studies (Fleming et al. 1996; Weir et al. 2004) or larger scale studies in natural settings (Fleming et al. 2000; McGinnity et al. 2003). In a natural experiment, Fleming et al. (2000) reported that 55% of farmed escapes accounted for 19% of the genes in the following generation of adult Atlantic salmon.
However, there is very little data on the survival or reproductive success of escaped farmed Pacific salmon, making it difficult to predict and estimate risks. Farmed and wild reproductive interactions will involve more than simple competition for mates, as the salmonid mating system is complex and involves dynamics such as cryptic competition (e.g., sperm competition), and natural selection in addition to sexual selection. My study investigates the competitive reproductive interactions between wild and farmed Chinook salmon males, with experimental controls, with measures of success taken at two different developmental stages to quantify sexual and natural selection. My study examines the difference in fertilization success (eggs) and reproductive success (fry) between wild and farmed XX and XY Chinook salmon males on semi-natural spawning channels using molecular genetic pedigree analysis. I also explore the role of difference in sperm performance and body size on fertilization and reproductive success. Chinook salmon populations continue to decline in their native range (Noakes et al. 2000) with many populations considered threatened or endangered (COSEWIC 2006; ESA 1973), my study will contribute to the understanding of reproductive interactions between wild and farmed salmon, as well as help estimate, and if necessary manage, the risk of escaped farmed salmon in the wild.

3.2 MATERIALS AND METHODS

Fish origin and collection

In this study, all of the Chinook salmon used originated from river systems on Vancouver Island, British Columbia, Canada. The farmed salmon were from Yellow Island Aquaculture Ltd. (YIAL), Quadra Island, BC, an organic Chinook salmon farm that does not use pesticides or antibiotics. The YIAL population was founded and
maintained with gametes obtained from Robertson Creek and Big Qualicum hatcheries, on Vancouver Island in 1985. YIAL rears two types of male Chinook salmon, homogametic (XX) and heterogametic (XY) males, which grow to a similar size and mass, and have comparable levels of circulating testosterone and 17ß-estradiol (Heath et al. 2002; for history of male salmon in this study see Lehnert et al. (2012)). Briefly, YIAL began producing homogametic males in 1987 from XX milt acquired from the Big Qualicum hatchery. At YIAL, XX males are generated through exogenous treatment of the developing embryos with the androgen 17 α-methyltestosterone (400 µg/L) for 2h at 520 ATUs (accumulated thermal units) and at 620 ATUs of development (Heath et al. 2002). All XX males in this study were 6 to 7 generations domesticated at YIAL and were 4 or 5 years of age at the time of sampling. All XY males at YIAL were 7 generations domesticated at YIAL but introgressed with wild genes 3 generations removed from the wild Big Qualicum stocks (Bryden et al. 2004), and were 4 years of age at the time of the project.

All mature farmed salmon used here were hatched and reared in fresh water at the YIAL hatchery until smolting when they were transferred to saltwater pens until sexual maturation. Fish were fed a diet formulation that mimics that of wild salmon, which includes offshore fish protein and naturally derived carotenoid pigment. Mature XX and XY males were seined from saltwater pens between October 4 and 18, 2010, and transferred to freshwater tanks at YIAL. Mature females were transferred from saltwater pens to fresh water between October 1 and 13.

Wild Chinook salmon from the Quinsam River population were seined on October 21, 2010. Individuals were anesthetized with CO₂, held in 700-L of oxygenated
river water and transported approximately 1.5-hours by vehicle to YIAL. No mortalities occurred as a result of capture and transport. Wild males were presumed to be individuals that spawned in the fall of 2007 (based on body size) and were thus 3 years of age at time of sampling.

All fish were kept in 2500-L freshwater holding tanks, sampled between October 8 and 22 and subsequently moved to spawning channels. Fish were anaesthetized with buffered MS222, then wet weight (± 10 g) and fork length measurements (± 1 mm) were recorded, and sperm samples were taken from all males for sperm performance analysis. I analyzed sperm traits, including sperm velocity (average path velocity, VAP), longevity and density as described in Lehnert et al. (2012). Tissue was taken from the adipose fin and preserved in 95% ethanol for later genetic analysis. A coded passive integrated transponder (PIT) tag was then injected into the dorsal musculature of each fish, allowing permanent identification of individuals as every tag has a unique 16-digit numerical code.

**Spawning channel trial design**

Fish were transferred to six freshwater spawning channels, with females being transferred between October 8 and 13, 2010, and allowed to acclimate for at least 12 days prior to the addition of the males. Males were added between October 20 and 22, 2010. All channels contained 12 farmed females, with four females of each age class (4-, 5- and 6-year-old) allocated to each channel. All channels contained 8 males: four channels received equal numbers of wild and farmed males (4 wild, 2 XX farmed and 2 XY farmed; “competition channels”). The two remaining channels were control channels with one containing only wild males (N = 8) with farmed females, and the other channel containing only farmed males (4 XX, 4 XY) with farmed females. Each channel measured 15 x 3.5 m with approximately 1.0 m water depth, and a partially recirculated
flow of approximately 300 L/min. The substrate in the channels consisted of 0.5-1.0 m (depth) of gravel approximately 3-6 cm in diameter, which is comparable to natural stream size composition. The channels were outdoors and thus subject to natural light and temperature regimes, but were enclosed with netting to deter land predators and reduce the likelihood of fish jumping between channels. Fish were left to spawn without interference, and were removed from channels after they died. At the end of the trial, I determined that 2 females had jumped into a neighboring channel, and one fish identified as a female was actually male (this individual had no fertilization success). These changes resulted in Channel 1 (competition channel) having 13 females and 9 males (4 wild, 2 XY farmed, and 3 XX farmed), and Channel 2 (farmed control channel) having 10 females and 8 farmed males.

**Offspring collection**

Hydraulic sampling for egg collection was conducted January 10 to 14, 2011, when eggs were expected to be between the eyed- and hatching-stages (250-500 ATU), based on the water temperature during the previous months. Hydraulic sampling involves the forceful injection of air into the gravel bed to release eggs from the nest into the water column, thus allowing eggs to be collected by net. The ability of eggs to survive the mechanical shock of hydraulic sampling is dependent on the age of the eggs, as studies have shown that newly fertilized eggs will experience high sampling mortality, however one month after spawning, 92-98% of salmon eggs will be resistant to mechanical shock (Collins et al. 2000; Thedinga et al. 2005). Thus we collected eggs at the eyed stage to avoid sampling related mortality. Hydraulic sampling was conducted in a grid pattern equally spaced over the entire channel and provided equal sampling effort across all channels. Although sampling was conducted equally across channels, there is potential
for sampling bias to occur if female nest construction varied and resulted in differential nest depth in the gravel bed. Given that all females in our study originate from the same farm population, I assumed no difference in nest building. All eggs were netted and sorted, and all live eggs were preserved in 95% ethanol for genetic analysis while dead eggs were counted and discarded. Although I cannot discriminate between unfertilized and fertilized dead eggs, it is possible the egg survival to the eyed stage differed between males (García-González 2008), however I assumed equal egg survival among individual males. Eggs not hydraulically sampled were left to develop in the channels, and on April 7 and 8, 2011, fry that survived and emerged from the substrate were collected by electrofishing and seining. Channels were subsequently drained on May 19 and 20 to collect all fry that escaped previous sampling methods. All collected fry were humanely euthanized in clove oil and fin tissue was stored in 95% ethanol for later genetic analysis.

**Microsatellite and parentage assignment**

Parental and offspring DNA was extracted from fin tissue and egg samples using an automated plate-based extraction protocol (Elphinstone et al. 2003). Parents and offspring were genotyped at 6 previously described tetranucleotide microsatellite loci: Ots107 (Nelsen and Beacham 1999), RT212 (Spies et al. 2005), Ots209, Ots211, Ots204, Ots213 (Greig et al. 2003) and if further genotyping was necessary to assign parentage, RT191 (Spies et al. 2005) and a dinucleotide microsatellite, Omy325 (O’Connell et al. 1997) were used. DNA was amplified using polymerase chain reaction (PCR) at the microsatellite loci with fluorescent dye-labeled forward primers and fragment sizes were visualized using a LiCor 4300 DNA analyzer (LiCor Biosciences, Inc.). Fragment sizes
were scored using GENE IMAGIR 4.05 software (Scanalytics Inc.) to generate individual genotypes.

Parentage was assigned using maximum likelihood methods in CERVUS version 3.0 (Marshall et al. 1998; Kalinowski et al. 2007) using only offspring genotyped at 2 or more loci with allele typing error set to 1%. I assigned parentage at a strict confidence level of 95% to minimize type B error of assigning a false parent (Hitoshi and Blouin, 2005). The number of successfully assigned eggs and fry varied across channels with a total of 1262 offspring assigned.

Statistical Analysis:

I present only results of the combined “farmed” type males, as no differences (t-test, p > 0.05) were found between XX and XY farmed males for fertilization and reproductive success. As well, in the absence of competition from wild males (i.e. control channel), XX and XY farmed males did not differ in mean number or percentage of egg and fry sired (t-test, p > 0.09) or in the mean number of mates (t-test, p = 0.51).

Through parentage assignment, I measured both fertilization and reproductive success based on the eggs and fry produced, respectively. I determined the percentage of males that were able to achieve any degree (eggs ≥1 and fry ≥1) of fertilization and reproductive success. I then excluded males who achieved no success from the remaining analyses. Individual fertilization and reproductive success were measured as the number of offspring sired per individual and calculated as a percentage of the total number of offspring produced in the respective channel. Percentage data were log-transformed to meet the assumptions of parametric analysis, and all data were analyzed in SPSS 20.0 (SPSS, Chicago, Illinois, USA). I compared mean individual fertilization and reproductive success between male types using an independent t-test or a two-way analysis of variance (ANOVA) to control for channel effects, where appropriate. All analyses were repeated for the control
channel (i.e., pure wild or pure farmed) fish to compare the effect of the presence and absence of farmed-wild interactions.

I used Pearson correlation analyses to examine the relationship between the number of mates versus fertilization and reproductive success. I used multivariate linear regression analyses to test the predictive relationship of traits that are expected to contribute to fertilization and reproductive success. The model included the variables channel, male type and trait, as well two-way interactions of trait with channel and male type. Traits tested included sperm velocity, body weight and days spent in channel. I tested sperm velocity (average path velocity, VAP) because it is an important aspect of male-male competition as sperm velocity is considered the primary determinant of competitive fertilization success for salmonid species, as observed in Atlantic salmon (Gage et al. 2004). Body weight was tested because various studies conclude that male size is important for fertilization or spawning success in salmonids (Fleming and Gross 1994; Fleming et al. 1996; Jones and Hutchings 2002). Finally, the number of days spent in the spawning channel was included, as longer-lived males would have more opportunities to engage in mating events (Dickerson et al. 2005) and thus fertilization and reproductive success may be affected by this trait.

Egg survival was calculated as the number of live eggs divided by the total number of eggs collected within a channel. Since dead eggs could not be assigned parentage, I only present data for egg survival per channel (not per individual or male type).

The focus of my study is male-male interactions; however, I do consider female effects and I conducted comparisons between female egg and fry parentage using one-way ANOVA to determine differences between channels. I calculated percentage of eggs
and fry produced based on the total number of offspring in the respective channel, and data were log-transformed to meet the assumptions of parametric analyses. Pearson correlation analyses were used to examine the correlation between the number of mates and the percentage of eggs and fry produced.

3.3 RESULTS

Fertilization success (egg)

Egg fertilization success in competition: Approximately 83% of live eggs were assigned parentage with 95% confidence. The number of eggs assigned per channel ranged from 23 to 134 eggs, and channels varied in the number of eggs sired by wild and farmed males with a significant difference overall (Table 3.1). The proportions of wild and farmed males that were successful in fertilizing at least one egg were 75% and 65%, respectively. For these successful males, the number of eggs fertilized ranged from 1 to 72 per male (Fig. 3.1). Mean (± S.E.) male fertilization success (for only those males who fertilized at least one egg) was 17.4 ± 4.2% (N= 23), and ranged from 0.75-73.9%.

Fertilization success was not significantly different between wild and farmed males when channel effects were included (Fig. 3.2A; Two-way ANOVA; N = 23; Male type F_{1,15} = 0.15, p = 0.71; Channel F_{3,15} = 1.14, p = 0.36; Male type*Channel F_{3,15} = 0.68, p = 0.58).

Correlates with fertilization success: The number of female mates each male had was significantly correlated with his egg fertilization success (Fig 3.3A; r = 0.53, N = 23, p = 0.009) across all male types. The mean (± S.E.) number of mates per male was 2.9 ± 0.4 mates with a range of 1-9 and was not significantly different between wild and farmed males (t_{21} = 0.21, N = 23, p = 0.84). No male trait significantly predicted egg fertilization success based on the multivariate linear models with male type and channel as covariates.
The male traits examined included sperm velocity ($F_{5,13} = 0.59; p = 0.71$), body weight ($F_{5,17} = 0.19; p = 0.96$), and days spent in spawning channel ($F_{5,17} = 0.81; p = 0.56$).

**Egg fertilization success without competition:** The number of eggs fertilized by farmed and wild males in absence of competition from the other male type (i.e., in the control channels) was 108 and 112 eggs, respectively (Table 3.1). In the absence of competition, 62% of wild males and 75% of farmed males were successful in fertilizing eggs. For successful males, the number of eggs fertilized ranged from 1 to 42 for wild males, and 9 to 35 for farmed males. There was no significant difference in individual fertilization success between wild males with and without competition from farmed males (Fig. 3.2A; $t_{15} = 0.014$, N=17, $p = 0.99$). As well, there was no significant difference between egg fertilization success for farmed males with and without competition from wild males (Fig. 3.2A; $t_{15} = -1.09$, N = 17, $p = 0.29$).

**Egg Survival:** Egg survival ranged from 9% to 49% (Table 3.2) and was significantly different among channels ($\chi^2 = 198$, $p < 0.001$), with the highest survival observed in the wild control channel.

**Reproductive success (fry)**

**Reproductive success in competition:** Approximately 64% of fry were assigned parentage with 95% confidence. The number of fry assigned per channel ranged from 0 to 270 fry, and channels varied significantly in the number of fry sired by wild and farmed males ($p < 0.001$; Table 3.3). Two channels (Channel 3 and 6) had only 0 and 6 fry assigned, respectively, and I thus exclude those channels from further analyses. The proportion of wild and farmed males that contributed to fry production (i.e., those males that sired $\geq 1$ fry) were 63% and 22%, respectively. For these successful males, the
number of fry sired ranged from 1 to 226 fry. Mean (± S.E.) individual reproductive success was 28.6 ± 14.1% (N = 7) and ranged from 0.37 - 83.7%. Wild male reproductive success was significantly higher than farmed male success (Fig. 3.2B; \( t_5 = 3.37, N = 7, p = 0.02 \)).

**Correlates with reproductive success:** The number of female mates was significantly correlated to individual male reproductive success (Fig. 3.3B; \( r = 0.86, N = 7, p = 0.013 \)). For males who contributed to fry production, the mean (± S.E.) number of mates was 2.9 ± 0.7 with a range of 1-6 (N = 7) and mean number of mates was not significantly different between wild and farmed males (\( t_5 = 0.11, N = 7, p = 0.09 \)). Linear regression model revealed male type significantly predicted individual reproductive success (\( r^2 = 0.69, F_{1,5} = 11.3, N = 7, p = 0.02 \)). Multivariate linear regression models revealed that no other traits examined could predict reproductive success including sperm velocity (\( F_{4,1} = 22.2; p = 0.16 \)), body weight (\( F_{5,1} = 0.76; p = 0.70 \)), and days spent in channel (\( F_{5,1} = 1.74; p = 0.52 \)).

**Reproductive success without competition:** The number of fry produced by farmed and wild males in the absence of competition from the other male type was 139 and 38 fry, respectively (Table 3.3). Only 37% of wild males contributed to fry production when there was no competition from farmed males, whereas 62% of farmed males contributed to fry production when there was no competition from wild males. Of the successful males, the number of fry ranged from 6 to 24 fry per wild males, and 1 to 111 fry per farmed males. There was no significant difference in reproductive success between wild males with and without competition from farmed males (Fig. 3.2B; \( t_6 = -0.29, N = 10, p = 0.78 \)). As well, there was no significant difference in reproductive success between
farmed males with and without competition from wild males (Fig. 3.2B; $t_5 = -1.83$, $N = 7$, $p = 0.13$).

**Female effects**

Mean female body weight did not differ among channels (ANOVA, $F_{5,65} = 0.31$, $N = 71$, $p = 0.90$), nor did the mean number of days spent in the channel ($F_{5,65} = 0.37$, $N = 71$, $p = 0.87$). Approximately 65% of females were successful in producing live eggs, and the number of male mates was positively correlated with proportion of eggs produced ($r = 0.40$, $N = 46$, $p = 0.006$), but was not correlated with proportion of fry produced ($r = 0.33$, $N = 24$, $p = 0.12$) by females. Mean number of mates acquired by females differed significantly among channels ($F_{5,65} = 4.79$, $N = 71$, $p = 0.001$).

**3.4 DISCUSSION**

In my study, male Chinook salmon egg fertilization success was equal for wild and farmed fish (independent of wild-farmed competitive interactions) based on the paternity of eggs collected. These results are inconsistent with other experimental studies on the breeding success of wild and farmed salmon (Fleming et al. 1996; Fleming et al. 2000; Weir et al. 2004). Fleming et al. (1996; 2000) reported that farmed Atlantic salmon males had lower mating success based on behavioral observations with and without competition from wild males. Additionally, Fleming et al. (1996; 2000) estimated fertilization success based on the number of fertilized embryos recovered from nests where males were observed spawning (i.e. inferred behavioral parentage), and both studies concluded that farmed males experienced reduced fertilization success relative to wild males. Weir et al. (2004) reported that farmed Atlantic salmon males did not effectively establish dominance hierarchies, and even though they did spawn with
females, they were often unsuccessful in fertilizing eggs due to their inability to release sperm. Furthermore, Berejikian et al. (1997) found that captive-reared Coho salmon (*O. kisutch*) were reproductively inferior to wild salmon based on observed spawning behavior. However, all of those studies assess fertilization success based on behavioral observations alone, while I estimated male fertilization success as genetic paternity assignment of the eggs. This difference in the methodology of fertilization success estimation may be the reason why my results do not agree with previous work, as Mehranvar et al. (2004) observed that behavior would underestimate the actual success of subordinate males. Further difference in methodology include that those studies (Fleming et al. 1996; Fleming et al. 2000; Weir et al. 2004) incorporated both wild and farmed females, whereas I use only farmed females. Additionally, sperm competition, important in the salmonid mating system, may have contributed to the success of farmed Chinook salmon males in my study, as the farmed males in my study had significantly greater sperm performance relative to wild males used in competition (Lehnert et al. 2012). Particularly, the higher sperm velocity exhibited by farmed males in my study fish (Lehnert et al. 2012) is likely an important factor in the pattern of egg fertilization (Gage et al. 2004). While I did not observe spawning behavior, greater sperm performance may be one mechanism by which farmed males may compensate for their presumed behavioral inferiority (Hutchings and Myers 1988).

The two studies that did use genetic analysis to determine fertilization success differences between wild and farmed salmon focused only on the Atlantic salmon alternative reproductive phenotype, or “precocious parr” (Garant et al. 2003; Weir et al. 2005). Farmed male precocious parr have been suggested as a possible vehicle for
increasing introgression of farmed genes into wild populations (Garant et al. 2003; Weir et al. 2005). Garant et al. (2003) found that farmed Atlantic salmon precocious parr had higher fertilization success relative to wild precocious parr, and Weir et al. (2005) reported that wild-farm hybrid precocious parr had greater fertilization success relative to wild and farmed precocious parr. However, in both those studies, the wild salmon precocious parr were reared in a hatchery environment to eliminate environmental effects, which would not be representative of actual wild-farmed interactions on natural spawning grounds.

Although fertilization success is an important element of salmonid reproduction, fertilization alone will not be representative of the realized reproductive success due to high mortality during the egg to fry development period (Einum and Fleming 1997; García-González 2008). As García-González (2008) reports, there may be an inequality in fertilization success and post-hatch paternity success, thus highlighting the importance of studying both egg and fry stages as paternal effects can influence embryo viability. In my study, reproductive success, based on genotype paternity assignment of fry, of farmed males was significantly lower than the reproductive success of wild males. There was no significant difference in reproductive success with and without competition from the other male type, for both wild and farmed males, respectively, thus indicating behavioral interactions are not likely contributing to the observed differences. Strikingly, in competition, farmed salmon sired only 1.7% of the fry relative to the wild salmon. My results are consistent with McGinnity et al. (2003), where no differential survival between wild and farmed Atlantic salmon offspring at the eyed-egg stage was observed, while farmed offspring experienced significantly greater mortality during the freshwater
fry stage. However, McGinnity et al. (2003) eliminated differences in reproductive behavior by artificially fertilizing eggs and rearing the eggs in a hatchery until hatch when they were transferred to a stream environment. Fleming et al. (2000) conducted a study allowing reproductive interactions between wild and farmed Atlantic salmon in an experimental river and found, as I did, that wild salmon contributed significantly more fry than farmed salmon; however they did not assay parentage assignment at the egg stage.

The difference observed between fertilization and reproductive success indicates substantial differences in egg to fry survival for wild and farmed sired salmon fry. Male’s individual fertilization success was not significantly correlated to his reproductive success (N = 23, p = 0.11), suggesting differential survival of offspring. This difference in early survival is an important conservation consideration, as the interbreeding of wild and escaped farmed salmon will result in the introgression of genes that may be maladaptive in the wild environment (Einum and Fleming 1997; Fleming and Einum 1997). As various studies suggest, aquaculture practices can result in dramatic intentional and unintentional genetic changes in the farmed population (Fleming and Einum 1997; Skaala et al. 2006; Fraser et al. 2010), in which many traits advantageous in the farmed environment will provide no advantage in nature. Previous research suggest that differences in wild and farmed post-hatch survival are often a consequence of maternal effects, as egg size can affect offspring survival (Einum and Fleming 2000a) and, generally, farmed females will have smaller eggs relative to wild females (Fleming et al. 2000; Heath et al. 2003). However, in my study maternal effects are not a factor because all females were of farmed origin and I found farm-wild male effects on
incubation and fry survival. Although differences in survival between wild and farmed offspring can be attributed to differences in anti-predator behavior, this is also not likely a factor affecting survival in this study, as predators (at least large ones) were excluded by fencing and nets. Finally, differences in survival may be related to the ability of offspring to acquire resources, as Berejikian et al. (1996) reported that farmed steelhead fry would need an advantage in size to compete with wild fry for food. Additionally, increased aggression, which is often observed in farmed offspring, could be a disadvantage in environments where resources cannot be monopolized, since it incurs higher metabolic costs (Vøllestad and Quinn 2003). Fleming and Einum (1997) observed that wild Atlantic salmon fry performed significantly better relative to farmed fry in a semi-natural stream, although farmed fry often dominate in tank environments, indicating that performance of farmed fry is context-dependent. Thus, the tendency for farmed offspring to exhibit greater activity and aggression relative to wild offspring (Einum and Fleming 1997; Fleming and Einum 1997), combined with possible reduced foraging success may elevate energetic costs (Vøllestad and Quinn 2003), and ultimately, reduce survival.

As expected, the number of females each male spawned with was significantly correlated with fertilization success and reproductive success, consistent with other studies examining either fertilization or reproductive success (Garant et al. 2001; Mehranvar et al. 2004; Neff et al. 2008). Males and females mated polygamously, which is not unusual in the salmonid mating system (Garant et al. 2005). As sexual selection theory predicts, males should maximize fitness by mating with multiple females since male investment in offspring is often lower relative to females (Trivers 1972). I found females had fewer mates relative to males, and although the average number of mates for
Chinook salmon in the wild has not been reported, other studies have found that female Chinook salmon spawn with fewer mates than males and exhibit mate choice (Neff et al. 2008). Females and males can gain fitness benefits through multiple mating, as individuals can increase their probability of having heterozygous offspring through mating with multiple individuals (Brown 1997). Multiple mating can maximize reproductive success through increased offspring genetic diversity and thus may reduce inbreeding (Brown 1997; Tregenza and Wedell 2002), provide kin-selection benefits (Griffiths and Armstrong 2002) and allow offspring to thrive under a broader range of environmental conditions (Yasui 1998; Fox and Rauter 2003). Garant et al. (2005) observed that multiple mates in wild Atlantic salmon resulted in more outbred offspring, which in turn contributed to greater reproductive success, although the genetic benefit of multiple mates was only significant for females. While I observed that for males both fertilization and reproductive success were significantly correlated with number of mates, a female’s number of mates was also only correlated (positively) to fertilization success. Although females may mate with multiple males for reasons discussed above, perhaps females invest more in better quality males (Trivers 1972) resulting in a greater percentage of offspring surviving from a single male mate.

No factors other than the number of mates significantly predicted fertilization success. While sperm performance is known to be important in salmonid fertilization success, I found no correlation between individual fertilization success and sperm velocity. Additionally, there was no correlation between fertilization success and sperm velocity when wild and farmed males were analyzed separately, in fact wild males exhibited a negative (but not significant) relationship. However, it should be noted that
due to unusable samples (i.e., contamination or water flow causing inaccurate readings) not all males could be measured for sperm velocity (missing 25% of males, N=12), and perhaps the missing data affected these results. Other than sperm performance, various studies demonstrate the importance of male size for fertilization or spawning success of salmonids (Fleming and Gross 1994; Fleming et al. 1996; Jones and Hutchings 2002), likely due to male dominance and access to females. Fleming et al. (1996) observed that male size was related to fertilization success only for wild males, and not for farmed males. However, I observe no relationship between body size and fertilization success for all males, as well as for wild and farmed males separately. My study has a female-biased operational sex ratio, which may relax the intensity of male intrasexual competition (Kvarnemo and Ahnesjö 1996), and perhaps male size, among other phenotypic characteristics, provides no mating advantage under such conditions. In nature, the operational sex ratio of salmon is highly variable (Beacham 1984; Fleming 1998), and even though I only tested a female biased operational sex ratio, Neff et al. (2008) demonstrated that sex ratio has no effect on the intensity of sexual selection in Chinook salmon. Additionally, sexual selection may depend on factors other than phenotypic traits, as female Chinook salmon may choose mates to increase diversity of offspring at the major histocompatibility (MH) genes (Neff et al. 2008), however MH genotypes were not determined in my study.

Although no factors significantly predicted fertilization success (egg parentage), I do find that male origin was able to predict reproductive success (fry parentage), as a result of differential survival between wild and farmed sired offspring. I did not find any other predictors of reproductive success, and although body weight is often deemed an
important contributor to male success (Fleming et al. 1996), other studies have also found that body size does not significantly predict reproductive success in salmonids (Garant et al. 2001; McLean et al. 2004; Dickerson et al. 2005), including Chinook salmon at YIAL (Garner et al. 2010).

In conclusion, my results suggest that farmed Chinook salmon males may achieve equal fertilization success relative to wild males if they successfully escape and migrate to river spawning grounds, but offspring of the farmed salmon males may exhibit lower incubation and fry survival relative to wild salmon. It is generally accepted that farmed salmon males have low fertilization success relative to their wild counterparts; however, my results show that the potential impact of escaped farmed salmon may be greater than previously realized (Fleming et al. 1996; Weir et al. 2004; Fleming et al. 2000). Although the fertilization success achieved by the farmed males in my trials was offset by lower offspring survival, farmed males remove reproductive opportunities from wild salmon males, and will drive the overall fitness of the population down (since their offspring exhibit lower post-fertilization survival). Many studies suggest that farmed females mating with wild males will be the primary means by which farm genes will introgress into wild gene pools (Fleming et al. 1996). My results suggest that, for Chinook salmon, escaped farmed males may also be an important vector for farm gene introgression into wild populations, however future studies should include wild and farmed female interactions as female escapements are likely to exceed male escapements given current farming practices. Furthermore, in my study, the survival of offspring sired by farmed males may have been higher had I incorporated wild females into the design. Additionally, conclusions based on the competitive spawning interactions of farmed
Chinook salmon will vary depending on which stage in the reproductive process is assessed. Although escapes from Chinook salmon farms may be limited given global aquaculture production of Chinook salmon is low (13,541 tonnes), less than 1% of global Atlantic salmon production (FAO 2010), my research is relevant to other areas farming indigenous species. Nevertheless, escaped farmed Chinook salmon have the potential for impacting the fitness of wild salmon stocks. Although impacts associated with farmed-wild hybridization are generally considered negative, introgression of farmed genes into declining wild stocks may provide an infusion of genetic diversity and potentially contribute positively to the population in the long term (Peterson 1999).

3.5 REFERENCES


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Table 3.1. Number of eggs successfully assigned to wild and farmed Chinook salmon (*Oncorhynchus tshawytscha*) sires (with total number of eggs collected) in six spawning channels. The channels consisted of four “competition” channels with equal numbers of competing wild and farmed males, and two control channels.

<table>
<thead>
<tr>
<th>Channel</th>
<th>Type</th>
<th>Assigned number of eggs</th>
<th>Total eggs collected</th>
<th>( \chi^2 )</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wild</td>
<td>Farmed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Competition</td>
<td>85</td>
<td>16</td>
<td>105</td>
<td>47.1</td>
</tr>
<tr>
<td>3</td>
<td>Competition</td>
<td>4</td>
<td>19</td>
<td>46</td>
<td>9.8</td>
</tr>
<tr>
<td>5</td>
<td>Competition</td>
<td>85</td>
<td>40</td>
<td>150</td>
<td>16.2</td>
</tr>
<tr>
<td>6</td>
<td>Competition</td>
<td>39</td>
<td>95</td>
<td>156</td>
<td>23.4</td>
</tr>
<tr>
<td>Overall</td>
<td>Competition</td>
<td>213</td>
<td>170</td>
<td>457</td>
<td>4.8</td>
</tr>
<tr>
<td>2</td>
<td>Farm Control</td>
<td>-</td>
<td>108</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Wild Control</td>
<td>112</td>
<td>-</td>
<td>129</td>
<td></td>
</tr>
</tbody>
</table>

*Notes:* Chi-square tests were used to determine significant differences (at \( \alpha \) level of 0.05 and indicated by asterisks, *) between the number of farmed and wild eggs sired within a channel and overall.
Table 3.2. Percent egg survival and number of live eggs per channel for six spawning channels, including four competition channels with equal numbers of wild and farmed Chinook salmon (*Oncorhynchus tshawytscha*) males, and two control channels (see Methods). Percent egg survival was calculated as the number of live eggs divided by the total number of eggs in each respective channel.

<table>
<thead>
<tr>
<th>Channel</th>
<th>Type</th>
<th>Egg Survival</th>
<th>Live eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Competition</td>
<td>16.6 %</td>
<td>105</td>
</tr>
<tr>
<td>3</td>
<td>Competition</td>
<td>9.2 %</td>
<td>46</td>
</tr>
<tr>
<td>5</td>
<td>Competition</td>
<td>29.4 %</td>
<td>150</td>
</tr>
<tr>
<td>6</td>
<td>Competition</td>
<td>29.7 %</td>
<td>156</td>
</tr>
<tr>
<td>2</td>
<td>Farm Control</td>
<td>34.1 %</td>
<td>137</td>
</tr>
<tr>
<td>4</td>
<td>Wild Control</td>
<td>49.2 %</td>
<td>129</td>
</tr>
</tbody>
</table>
Table 3.3. Number of fry successfully assigned to wild and farmed Chinook salmon (*Oncorhynchus tshawytscha*) sires (with total number of fry collected) in six spawning channels. The channels consisted of three “competition” channels with equal numbers of competing wild and farmed males, and two control channels.

<table>
<thead>
<tr>
<th>Channel</th>
<th>Type</th>
<th>Assigned number of fry</th>
<th>Total fry collected</th>
<th>$\chi^2$</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wild</td>
<td>Farmed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Competition</td>
<td>253</td>
<td>0</td>
<td>377</td>
<td>253</td>
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<tr>
<td>5</td>
<td>Competition</td>
<td>267</td>
<td>3</td>
<td>392</td>
<td>258.1</td>
</tr>
<tr>
<td>6</td>
<td>Competition</td>
<td>0</td>
<td>6</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Overall</td>
<td>Competition</td>
<td>520</td>
<td>9</td>
<td>777</td>
<td>493.6</td>
</tr>
<tr>
<td>2</td>
<td>Farm Control</td>
<td>-</td>
<td>139</td>
<td>255</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Wild Control</td>
<td>38</td>
<td>-</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>

Notes: Chi-square tests were used to determine significant differences (at $\alpha$ level of 0.05 and indicated by asterisks, *) between the number of farmed and wild fry sired within a channel and overall. Channel 3 produced no fry.
Figure 3.1. Histogram of the distribution of eggs fertilized by individual wild (black) and farmed (gray) Chinook salmon (*Oncorhynchus tshawytscha*) males in competition (N=23), and includes only successful males (eggs fertilized ≥ 1).
Figure 3.2. Mean (± S.E.) individual (A) egg fertilization success and (B) reproductive success of wild and farmed Chinook salmon (*Oncorhynchus tshawytscha*) males in competition (gray) and without competition (white), and includes only males which were successful in (A) achieving fertilization (eggs fertilized ≥ 1) and (B) contributing to fry (fry contribution ≥ 1).
Figure 3.3. Number of mates vs. (A) fertilization success and (B) reproductive success of wild (□) and farmed (▲) Chinook salmon (Oncorhynchus tshawytscha) males in competition, and includes only males which were successful in (A) achieving fertilization (eggs fertilized ≥ 1) and (B) contributing to fry (fry contribution ≥ 1). Individual (A) fertilization and (B) reproductive success are represented as the percentage of (A) eggs and (B) fry sired by the male within his respective channel.
4.0 OUTBREEDING EFFECTS ON GROWTH, SURVIVAL AND STRESS RESPONSE IN SECOND GENERATION BACKCROSSED CHINOOK SALMON

4.1 INTRODUCTION

Salmonid conservation management is often confronted with the challenge of whether to inbreed or outbreed populations in order to either maintain local adaptation or increase genetic diversity (Edmands 2007). The outbreeding of populations for conservation purposes is a relatively recent strategy that suggests imperiled populations could be “genetically rescued” by the infusion of new genes into the population (Tallmon et al. 2004). The theory of genetic rescue is based on the idea that small populations would likely suffer from inbreeding and the resulting inbreeding depression, but that the introgression of novel genotypes could add diversity to the population, increasing fitness, and thus “rescue” the population from extirpation (Tallmon et al. 2004; Edmands 2007). The infusion of novel alleles is expected to provide fitness benefits due to heterosis (where heterozygous offspring experience greater fitness relative to their parents) as well as the masking of recessive deleterious alleles (genetic load) (Lynch 1991). However with outbreeding there is also the potential for outbreeding depression to occur, depending on the nature of the hybridizing stocks (Lynch 1991; Edmands 2007). Outbreeding is of particular concern since salmon populations are generally thought to be locally adapted to their natal streams (Taylor 1991), and thus outbreeding could disrupt gene interactions contributing to local adaptation (Emlen 1991; Edmands 2007).

Outbreeding depression results from both additive and non-additive genetic effects when genetically divergent populations interbreed and backcross (Lynch 1991).
Additive genetic effects are often observed in the first generation hybrid, and result when hybrid offspring possess a phenotype intermediate to both parental populations that can lead to a reduction in fitness in either parental environment (Templeton 1986). Non-additive genetic effects of outbreeding are expected to arise when the hybridizing populations have genes that have coevolved (Templeton 1986) and thus there is an interaction of alleles at multiple loci (e.g., epistasis). The effects of outbreeding, through the disruption of coadapted gene complex through the introgression of novel alleles and genotypes, will not be apparent until the second or later generations, when divergent parental genomes undergo recombination (Lynch 1991). Thus it is important for studies of outbreeding to be multi-generational, as the first generation hybrid may even experience heterosis (greater fitness relative to parents), and subsequently exhibit outbreeding depression in later generations as previously documented in copepods (Edmands 1999) and birds (Marr et al. 2002).

Outbreeding depression has been detected in fish species affecting fitness-related traits such as survival (Gharrett et al 1999; Gilk et al. 2004; Tymchuk et al. 2007) and gill morphology (Gharrett and Smoker 1991). Gilk et al. (2004) observed that hybridization reduced survival in second-generation offspring of pink salmon (*Oncorhynchus gorbuscha*) indicative of the non-additive genetic model of outbreeding depression. Although many other studies have found that outbreeding does not always have negative effects on various physical performance traits (Sheffer et al. 1999; Fraser et al. 2008; Houde et al. 2011b), outbreeding depression may have more detectable effects on physiology. Cooke and Phillip (2005) demonstrated that hybridization negatively affected cardiovascular performance in largemouth bass (*Micropterus salmoides*), as well as swim
performance (Cooke et al. 2001). Negative effects on physiological characteristics can equate to potential fitness reduction, as many physiological responses are important for local adaptation. For example, stress response is considered an important and adaptive physiological response in fishes, allowing the fish to cope with environmental stressors and re-establish homeostasis (Barton 2002). The stress response is also important for aquaculture practices, as reducing stress in fish can ultimately improve health and reduce mortality. As well, the effect of outbreeding on stress response can inform risk evaluation of unintentional escapes from aquaculture. Farmed salmon populations often experience a loss of genetic diversity and become genetically divergent from their wild counterpart (Norris et al. 1999; Skaala et al. 2006) thus the mating between wild and escaped farmed salmon may produce hybrid offspring that experience outbreeding depression (Templeton 1986). If outbreeding between wild and farmed salmon negatively affects the stress response, it may have serious fitness consequences for wild salmon populations that must deal with various natural and anthropogenic stressors on a regular basis. Outbreeding depression is also important for hatchery programs that intentionally release cultured salmon for conservation programs, and like farmed salmon, hatchery salmon can also diverge genetically from wild populations (Fraser 2008).

I test for outbreeding depression through growth, survival and stress response in outbred farmed Chinook salmon (*O. tshawytscha*). I use a multi-generational approach to properly quantify the effects of outbreeding inbred lines of Chinook salmon by comparing performance traits between backcrossed hybrids (F2) from inbred lines and the pure inbred line. Understanding outbreeding can be valuable for hatchery, conservation
and aquaculture breeding programs, as well as for estimating the impacts of escaped farmed salmon on wild populations.

4.2 MATERIALS AND METHODS

**Breeding design**

The Chinook salmon that were used were provided by Yellow Island Aquaculture Ltd. (YIAL), an organic Chinook salmon farm located on Quadra Island, British Columbia, Canada. Salmon have been maintained at YIAL since 1985, and originated from Roberson Creek and Big Qualicum hatchery on Vancouver Island, BC. Specific inbred lines have been maintained at YIAL since 1997, in which fish were selected for high growth rate and high survival (HH), as well as low growth rate and low survival (LL). YIAL started HH and LL lines through selection based on variation in growth- and survival-related gene markers (Docker and Heath 2002). A recent study at YIAL has shown that HH individuals still maintain significantly higher survival rates relative to LL individuals, although there was no difference detected in their fork length at 1.5-years of age (Falica 2011). The first letter in the cross denotes the dam (female) and the second letter denotes the sire (male). In November 2010, sexually mature fish (10 males and 10 females) were seined from saltwater net pens and artificially spawned in a full factorial breeding design resulting in 100 crosses (families). All females in the breeding design were purebred HH to minimize potential maternal effects. Males in the study included hybrids (HL and LH), as well as purebred HH. The breeding design thus resulted in 60 families of backcrossed hybrids (30 HH x HL and 30 HH x LH) and 40 purebred (HH x HH) families, although some individual crosses were lost during the study.

**Husbandry and sampling**
Eggs were incubated in vertical stack incubation (Heath) trays, and dead eggs were counted and removed to determine egg survival. Eggs were counted between December 17, 2010 and March 2, 2011 on 14 occasions at intervals of less than 2 weeks. After hatch in March 2011, approximately 70-100 fish from each surviving cross (66 families) were transferred to 200-L holding tanks, and fish were fed daily. On March 24-25, 2011, a subsample of 20 fish per family were weighed and measured. On June 14-15, 2011, a subsample of 20 fish per family were injected with a passive integrated transponder (PIT) tag, and weighed and measured. PIT tagging allowed for individual identification of each fish as each tag has a unique 16 digit numeric code. On July 1, 2011, all tagged fish were immersion vaccinated for vibriosis, and transferred to saltwater net pens on July 14. PIT tagging of fish allowed accurate survival and growth records for each individual, and fish were weighed and measured on two more occasions in the saltwater, October 29, 2011 and April 18, 2012. Saltwater survival data were coded by individual fish as a binominal data point of “0” for mortality or “1” for survival, and all mortalities were recorded over the course of the experiment from entry into saltwater July 14, 2011 to June 4, 2012.

**Stress Response**

To measure stress response, 36 families with 3-6 individuals per family were chosen to collect baseline and 1-hour post-stress plasma cortisol concentration data. Experimental design included the families of 6 females x 6 males, which equated to 12 purebred families and 24 backcrossed hybrid families. Fish from those families were randomly selected during sampling on April 18 2012, and 195 fish were transferred to a 15 x 15 ft net pen to acclimate for at least 40 hours. On April 20 between the hours of
9:00-18:00, fish were netted and anesthetized in a clove oil bath, and blood was collected from the caudal vein of fish by ventral insertion of a 1-cc heparinized syringe with a 22-gauge needle. Fish were sampled in groups of 10-15 individuals to ensure that sampling occurred within a short time frame, less than 6 minutes after capture. Fish recovered in 1000-L totes for 1-hour, and then blood was taken again to obtain the stress-induced sample. Time of day and time from capture to blood sampling were recorded for all fish. Syringes were kept cool after sampling, transferred to heparinized microcentrifuge tubes on ice and subsequently stored at 4 degrees Celsius for up to 12 hours. Microcentrifuge tubes were centrifuged at 13000 rpm for 2 minutes to separate red blood cells and plasma. Plasma was transferred to 1.5 ml tube and frozen for later laboratory analysis. After the trial, I monitored survival of the sampled (stressed) individuals for 3-weeks post-treatment.

Cortisol Assay

Plasma levels of cortisol were measured using a commercial enzyme immunoassay (EIA; Enzo Life Sciences, Inc.) following the supplied kit protocol. Optimization of plasma pooled from several individuals was used to determine optimal plasma dilution prior to assays. Optimal plasma dilution for baseline and stress-induced samples was 1:100, and triplicates for each sample were used in the assay.

Genetic differentiation

DNA was extracted from fin tissue of 32 individuals from each parental line (HH and LL) using an automated plate-based extraction protocol (Elphinstone et al. 2003). Individuals were genotyped at 10 previously described microsatellite loci: Ots107 (Nelsen and Beacham 1999), RT212, RT191 (Spies et al. 2005), Ots209, Ots211, Ots204,
Ots213 (Greig et al. 2003), Omy325 (O’Connell et al. 1997), OtsG67, and OtsG432 (Williamson et al. 2002). Polymerase chain reaction (PCR) was used to amplify DNA at the microsatellite loci with fluorescent dye-labeled forward primers and fragment sizes were visualized using a LiCor 4300 DNA analyzer (LiCor Biosciences, Inc.). Individual genotypes were generated based on fragment sizes scored using GENE IMAGIR 4.05 software (Scanalytics Inc.). Genetic differentiation between the two inbred lines was estimated by calculating pair-wise $F_{ST}$ values (Weir and Cockerham 1984) between HH and LL groups using ARLEQUIN at 10000 permutations.

**Statistical analysis**

All data were tested for normality and homogeneity of variance. Proportional egg survival data were arcsine square root transformed to improve normality. When necessary, length, weight, and condition factor data were log transformed to improve normality. Specific growth rate (SGR) was calculated for the course of the experiment (310 days), using the equation:

$$SGR = \left(\ln W_1 - \ln W_0\right) \frac{x 100}{t} \quad [1]$$

where $W_1$ is the final weight and $W_0$ is the initial weight, and $t$ is the number of days in the growth period. SGR data met assumptions of normality when statistical outliers with low SGR (> 0.75 g/day) were excluded, which accounted for only 1% of the sample. Cortisol measures included baseline and stress-induced plasma cortisol as well as the stress response measured as the change in cortisol from baseline to 1-hour post stress for individual PIT-tagged fish. For cortisol data, I controlled for time of day by using...
standardized residuals of the regression between sampling group time and cortisol measures. Residuals met assumptions of parametric tests.

Performance (egg survival, morphology, growth and stress response) measures were compared between two cross types (purebred and outbred). All data were analyzed to compare cross type (purebred HH or backcross) effects using a mixed effect model which included cross type as a fixed factor, with random factors of dam, sire nested within type, and the interaction of sire and dam. I report when significant results were observed for any of the random factors. Saltwater survival and 3-weeks post-stress survival were compared using a contingency table with Pearson chi-square test, as the data did not meet the assumptions of parametric analyses.

Sample size varied over the course of the experiment as a result of mortalities. Initial sample size (at time of tagging, June 2011) was n = 1318, which decreased to n = 1121 by the last sampling period (April 2012).

4.3 RESULTS

Egg Survival

Three females produced non-viable eggs that resulted in the number of families being reduced to 70 families immediately after incubation began, however this did not affect relative proportions of cross types as all 10 females were purebred HH. Eggs produced by those three females were excluded from eggs survival analysis and remaining experiments. Mean family egg survival did not differ significantly between cross types (Table 4.1; F= 0.02, p = 0.90).

Measurements and Growth
Fork length and wet weight did not differ between outbred and purebred lines across all sampling times (Table 4.1; p > 0.58 and p > 0.59, respectively). Additionally, Fulton’s condition factor did not differ between types at any sampling time (Table 4.1; p > 0.70). Significant nonadditive genetic variance (dam by sire interactions) was observed for fork length (p = 0.033), wet weight (p = 0.007) and condition (p = 0.004) at the first sampling (March 2011), and for wet weight (p = 0.04) and condition (p = 0.004) at the second sampling period (June 2011). Specific growth rate did not differ between outbred and purebred crosses over the course of the experiment (Table 4.1; F = 2.04, p = 0.19).

**Saltwater Survival**

Saltwater survival (July 2011 to June 2012) was not significantly different between cross types (Table 4.1; $\chi^2 = 0.49$, p = 0.48).

**Stress Response**

Baseline and stress induced plasma cortisol did not differ significantly between outbred and purebred cross types (Table 4.1; p = 0.53 and p = 0.91 respectively, n = 194). As well, there was no significant difference in stress response between outbred and purebred cross types (Table 4.1; F = 0.24, p = 0.65).

Percent mortality 3-weeks post-stress for outbred cross type was more than double that experienced by purebred cross type, although this difference in their survival was not significant (Table 4.2; $\chi^2 = 3.29$, p = 0.07).

**Genetic differentiation**

Genetic divergence between parental HH and LL inbred lines was highly significant (p < 0.001), with a pair-wise F$_{ST}$ of 0.129 between inbred lines.

4.4 DISCUSSION
The effects of outbreeding are important considerations for developing proper management protocols for salmonid conservation, as well as for predicting risks associated with the hybridization between hatchery/farmed salmon and wild salmon. My study found no evidence of outbreeding depression in Chinook salmon for fitness-related traits of growth, survival and stress response. Although my results are consistent with some studies (Sheffer et al. 1999; Fraser et al. 2008; Dann et al. 2010; Houde et al. 2011a,b), other studies have reported evidence for outbreeding depression in fishes (Cooke et al. 2001; Cooke and Phillip 2005), including salmonids (Gharrett and Smoker 1999; Gilk et al. 2004; Tymchuk et al. 2007). For example, outbreeding depression has been reported for pink salmon (Gilk et al. 2004) where outbreeding significantly reduced F2 survival, although other fitness-related traits, such as homing ability and variance in family size, were unaffected.

The extent of the outbreeding effects are expected to be dependent on the genetic differentiation between the parental populations; however, the level of genetic differentiation which results in outbreeding depression is difficult to predict as it generally varies among species (Edmands 2007). It is understood that outbreeding between two populations will have greater fitness consequences as the genetic distance between the populations increases (Edmands 2007). As a result of artificial selection, the neutral genetic differentiation (FST) between the parental lines used in my study was high (0.129), suggesting that the parental lines are genetically differentiated. Houde et al. (2011b) found that Atlantic salmon populations ranging in FST value from 0.0353 to 0.0953 did not experience outbreeding depression in backcrossed hybrids in the wild. Additionally, Sheffer et al. (1999) found no evidence of outbreeding depression in the
endangered Gila topminnow (*Poeciliopsis o. occidentalis*) at high levels of genetic differentiation with $F_{ST}$ values ranging from 0.223-0.712 (Parker et al. 1999). However, Leberg (1993) reported that mosquitofish (*Gambusia holbrooki*) populations with $F_{ST}$ values between 0.016 to 0.032 found evidence of small (but non-significant) outbreeding effects, and similarly, low genetic differentiation was observed for pink salmon where outbreeding depression resulted in reduced survival (Gharrett et al. 1999; Beacham et al. 1988). Clearly, the relationship between $F_{ST}$ and outbreeding depression is not straightforward, making it difficult to predict outbreeding depression based on simple genetic differentiation, likely due to species- and possibly population–specific effects (Edmands et al. 2007; Houde et al. 2011b). Although predictions of outbreeding depression based on measures of neutral genetic differentiation (e.g. microsatellite $F_{ST}$) may be problematic, McClelland and Naish (2007) suggest that genetic differentiation based on quantitative traits ($Q_{ST}$) may be more informative for predicting outbreeding depression. Additionally, differentiation based on functional gene markers would be useful for outbreeding studies, as genes acted on by natural selection, such as MHC class II genes, would provide more concise information about locally adaptive differences between populations (Heath et al. 2006).

Outbreeding depression is expected to occur when hybrid offspring experience a reduction of fitness in their parental environment through the loss of locally-adapted traits, either due to the expression of an intermediate parental phenotype or the disruption of coadapted genes (Templeton 1986; Lynch 1991). The fish from HH and LL inbred lines maintained at YIAL have experienced the same rearing and environmental conditions for a number of generations. Given that the inbred lines are adapted to the
same local environment, outbreeding may not result in any negative consequences under these circumstances. However, given that many natural salmon populations have been spatially isolated in environmentally heterogeneous habitats for numerous generations, outbreeding depression may be more likely to occur if wild populations are outbred. Additionally, when comparing wild with hatchery or farmed salmon, artificially cultured populations have generally undergone changes due to genetic bottlenecks, genetic drift and artificial selection (Roberge et al. 2006). Studies have reported outbreeding depression through hybridization of Atlantic salmon as wild-farmed hybrid offspring experienced lower survival relative to wild offspring (McGinnity et al. 2003). Furthermore, Roberge et al. (2008) found that nonadditive gene interactions resulted in significantly different expression of 298 genes in F$_2$ wild-farmed hybrids compared to the wild strain of Atlantic salmon, with many genes having physiological and morphological importance. Hatchery programs are another important consideration, as outbreeding populations of different strains may risk outbreeding depression given adaptations to different parental stream environments.

It is possible that the aquaculture environment was not suitable for inducing outbreeding effects as selection pressures are often relaxed and the detection of outbreeding depression will be dependent on the environment (Tymchuk et al. 2007). Gilk et al. (2004) and Gharrett and Smoker (1991) found outbreeding depression when pink salmon were subjected to natural conditions in the wild, whereas, Houde et al. (2011a) found no evidence of outbreeding depression in Atlantic salmon when reared under experimental conditions. Additionally, I found no difference in the stress response between outbred and purebred groups as a result of acute handling stress. Interestingly,
survival 3-weeks post-stress was not significantly different between outbred and purebred cross types, however outbred individuals experienced double the mortality rate of purebred individuals, and differences approached statistical significance. Perhaps chronic stress would have resulted in greater observed differences, as the genetic effects of outbreeding may be considered minimal under benign conditions. However, heightened detrimental effects may be observed under more stressful conditions, as demonstrated in Drosophila (Kondrashov and Houle 1994). Furthermore, I measured fitness related traits in fish less than 2 years of age, and it is possible that outbreeding may have fitness consequences later in the life cycle such as during sexual maturation. As well, McClelland and Naish (2007) suggest that outbreeding depression may not be apparent until the F3 generation in salmonids, as they are residual tetraploids with low recombination rates. Tymchuk et al. (2007) found that, under the risk of predation, outbreeding depression for survival was not detected until the F3 generation in rainbow trout (O. mykiss). Investigating three generations or more of outbreeding may prove beneficial to studies of outbreeding depression, particularly in salmonid species.

Finally, it should be acknowledged that all the females in my study were purebred (HH) whereas the different cross types were dependent on males (LH, HL and HH). My results may have differed had I included reciprocal crosses in the breeding design, as reciprocal crosses can differ, presumably due to maternal, paternal or sex-linked gene effects (Bentsen et al. 1998). The design thus eliminated maternal cross-type effects, and I also used a mixed effect model to control for maternal and paternal effects in the analysis. Houde et al. (2011a) examined outbreeding in Atlantic salmon and found significant maternal and paternal cross type effects, but no significant effects of
outbreeding when controlling for those effects, thus highlighting the importance of incorporating parental effects into models for outbreeding studies.

In conclusion, I found no evidence for outbreeding depression in Chinook salmon despite a high level of genetic divergence between the lines. I thus suggest that outbreeding of moderately genetically differentiated Chinook salmon populations would result in little or no negative fitness consequences for the offspring. Although this may be positive from a conservation perspective, my results should be evaluated with caution given rearing occurred under experimental conditions, and the fish were adapted to the benign environment for several generations. Future work should include three or more generations and populations from different environments under natural conditions to provide a better understanding of the risks associated with salmonid outbreeding.

4.5 REFERENCES


Table 4.1. Means (± standard error) for all traits examined between outbred (F$_2$ backcrossed hybrids) and purebred Chinook salmon (*Oncorhynchus tshawytscha*) with significance (p) of comparisons. Comparisons were made using a mixed effect model for all traits with type as a fixed effect and dam, sire nested within type and their interaction as random effects. Pearson chi-square tests were used to compare between types where indicated by *.

<table>
<thead>
<tr>
<th>Trait (units)</th>
<th>Outbred HH x HL</th>
<th>Outbred HH x LH</th>
<th>Purebred HH x HL</th>
<th>Purebred HH x LH</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg survival (%)</td>
<td>68.8 ± 3.0</td>
<td>69.6 ± 2.9</td>
<td>67.1 ± 4.4</td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>Length (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.47</td>
</tr>
<tr>
<td>March 2011</td>
<td>3.6 ± 0.006</td>
<td>3.6 ± 0.006</td>
<td>3.6 ± 0.005</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>June 2011</td>
<td>7.7 ± 0.02</td>
<td>7.8 ± 0.02</td>
<td>7.8 ± 0.02</td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>October 2011</td>
<td>15.4 ± 0.05</td>
<td>15.4 ± 0.05</td>
<td>15.5 ± 0.04</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>April 2012</td>
<td>22.2 ± 0.09</td>
<td>22.2 ± 0.10</td>
<td>22.0 ± 0.08</td>
<td></td>
<td>0.11</td>
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<tr>
<td>Weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.72</td>
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<tr>
<td>March 2011</td>
<td>0.44 ± 0.003</td>
<td>0.45 ± 0.003</td>
<td>0.45 ± 0.003</td>
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<td>0.09</td>
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<tr>
<td>June 2011</td>
<td>5.26 ± 0.05</td>
<td>5.31 ± 0.04</td>
<td>5.45 ± 0.04</td>
<td></td>
<td>0.09</td>
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<tr>
<td>October 2011</td>
<td>41.6 ± 0.5</td>
<td>41.2 ± 0.4</td>
<td>41.8 ± 0.4</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>April 2012</td>
<td>136.8 ± 1.7</td>
<td>134.0 ± 1.9</td>
<td>131.8 ± 1.5</td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>Condition (g/cm³)</td>
<td>March 2011</td>
<td>June 2011</td>
<td>October 2011</td>
<td>April 2012</td>
<td></td>
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<tr>
<td></td>
<td>0.94 ± 0.006</td>
<td>1.14 ± 0.003</td>
<td>1.12 ± 0.003</td>
<td>1.23 ± 0.004</td>
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<tr>
<td></td>
<td>0.96 ± 0.005</td>
<td>1.13 ± 0.003</td>
<td>1.12 ± 0.003</td>
<td>1.21 ± 0.004</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.96 ± 0.005</td>
<td>1.14 ± 0.003</td>
<td>1.12 ± 0.003</td>
<td>1.21 ± 0.004</td>
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</tr>
<tr>
<td></td>
<td>0.21</td>
<td>0.16</td>
<td>0.09</td>
<td>0.10</td>
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<td></td>
<td>0.29</td>
<td>0.08</td>
<td>0.05</td>
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<tr>
<td></td>
<td>0.004*</td>
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<tr>
<td></td>
<td>0.89</td>
<td>0.70</td>
<td>0.90</td>
<td>0.91</td>
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</table>

<table>
<thead>
<tr>
<th>Saltwater survival (%)</th>
<th>79.3 ± 2.0</th>
<th>76.7 ± 2.1</th>
<th>79.6 ± 1.8</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific growth rate (g/day)</td>
<td>1.05 ± 0.004</td>
<td>1.04 ± 0.004</td>
<td>1.03 ± 0.003</td>
<td>1.03 ± 0.003</td>
</tr>
<tr>
<td>Baseline cortisol (residual)</td>
<td>0.18 ± 0.13</td>
<td>-0.11 ± 0.12</td>
<td>-0.079 ± 0.12</td>
<td>0.22</td>
</tr>
<tr>
<td>Stress induced cortisol (residual)</td>
<td>-0.093 ± 0.12</td>
<td>0.12 ± 0.13</td>
<td>-0.019 ± 0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>Stress response (residual)</td>
<td>-0.28 ± 0.12</td>
<td>0.23 ± 0.12</td>
<td>0.075 ± 0.12</td>
<td>0.46</td>
</tr>
<tr>
<td>3-weeks post stress survival (%)</td>
<td>79.1 ± 5.0</td>
<td>87.1 ± 4.3</td>
<td>92.4 ± 3.0</td>
<td>-</td>
</tr>
</tbody>
</table>

ns: variance associated with the parameter is 0 as this covariance parameter is redundant in the model.
Asterisk (*) represents significance at the alpha level of 0.05.
Table 4.2. Contingency table for 3 weeks post-stress survival between outbred (F$_2$ backcrossed hybrids) and purebred Chinook salmon (*Oncorhynchus tshawytscha*) compared using Pearson chi-square test.

<table>
<thead>
<tr>
<th>Type</th>
<th>Alive</th>
<th>Dead</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outbred</td>
<td>107</td>
<td>22</td>
<td>129</td>
</tr>
<tr>
<td>Purebred</td>
<td>61</td>
<td>5</td>
<td>66</td>
</tr>
<tr>
<td>Total</td>
<td>168</td>
<td>27</td>
<td>195</td>
</tr>
</tbody>
</table>

*p = 0.07*
5.0 GENERAL DISCUSSION

Escapes of domesticated animals can have negative impacts on their wild conspecifics through means of competition, parasite transfer and genetic interactions. Domesticated escapes may not only have effects within their own species (Fleming et al. 2000; McGinnity et al. 2003), but may also affect overall biodiversity in an ecosystem (Manchester and Bullock 2000). Great concern exists about the impacts of farmed salmon on wild populations, and escapes can pose significant threats if reproductive interactions take place, but many steps must be accomplished for interactions to occur (Fig. 5.1). Current knowledge on fish escapes and subsequent impacts are best documented in Atlantic salmon (*Salmo salar*), and the following example is based on data taken for Atlantic salmon. For reproductive interactions to occur, a fish must first escape from the aquaculture site, and in the North Atlantic it is estimated that two million salmon may escape each year, which represents about 0.01% of total aquaculture production (McGinnity et al. 2003). Secondly, a fish must survive in the wild, where survival at sea of escaped farm salmon can average between 16-44% (Whoriskey et al. 2006). Next, a fish must successfully migrate to fresh water, and the percentage of escaped salmon that make it to spawning grounds have been reported to range from 0.3-11% (Morris et al. 2008). After entering fresh water a fish must successfully compete for mates. Fleming et al. (2000) found that farmed males and females experienced 24% and 32%, respectively, the success of wild males and females. Furthermore, at those levels of mating success, farmed genes constituted 19% of the genes in the next generation of adults (Fleming et al. 2000). Finally, Hindar et al. (2006) estimated that if farmed salmon represent 20% of the spawning individual it would have significant negative impacts on a wild population
within 10 generations. Although much is known about Atlantic salmon, numbers show that in British Columbia Chinook salmon (*Oncorhynchus tshawytscha*) farm escapes have ranged from 0 – 390,165 per year since 1987 (Escape Statistics, Province of BC Fisheries and Aquaculture). Even though the farming of Atlantic salmon is more prevalent than that of Chinook salmon on the west coast, escapes from Chinook salmon farms in BC can pose a greater risk to the wild as they can interbreed with wild conspecifics, whereas Atlantic salmon have failed to establish populations in BC even after intentional introductions and they are not likely to produce viable offspring through interbreeding with wild Pacific salmon (Bisson 2006). As well, there are efforts to report and recapture escaped farmed Atlantic salmon in BC (i.e., DFO Atlantic Salmon Watch Program), while farmed Chinook salmon are not easily discriminated from wild Chinook salmon. Although Chinook escapes present a larger threat to the wild population in the Pacific Ocean, little has been investigated on the impacts of Chinook salmon escapes until now.

In this thesis, I have provided novel insight into the impacts of salmon farming practices on the reproductive ability of Chinook salmon, and the effects of outbreeding in the species. Firstly, I have shown that farmed Chinook salmon males have greater sperm performance relative to wild males. Additionally, I concluded that farmed XY male and XX male Chinook salmon are equal in sperm performance, thus indicating no negative implications of using XY or XX male sperm for broodstock production. Furthermore, I showed that farmed XX and XY male Chinook salmon have an advantage in sperm competition with wild males given their higher sperm velocity (Gage et al. 2004), which could potentially lead to a higher rate of farmed gene introgression into the wild population than previously expected. In fact, this is the first study to demonstrate that
farming practices can actually improve sperm performance in fishes, whereas previous work (although limited to cod and haddock) had demonstrated the opposite or no effect (Rideout et al. 2004; Skjæraasen et al. 2009). Furthermore, although XX and XY males pose the same risk of gene introgression, XX males also present a novel threat given that the hybridization of farmed XX males and wild females could skew sex ratios in the wild.

In addition to establishing differences in sperm traits between wild and farmed Chinook salmon, I also determined the actual fertilization and reproductive success of wild and farmed salmon under competition in semi-natural spawning channels. The study was different from many other competitive mating experiments, as I was able to determine paternity of offspring at two different stages in development (eggs and fry) allowing me to assess both fertilization (eggs) and reproductive (fry) success, which provided information about sexual and natural selection on wild versus farmed salmon. I found that farmed and wild Chinook salmon males were equally successful at fertilizing eggs, however, wild males achieved significantly higher reproductive success due to the low survival of farm-sired offspring, providing evidence of natural selection acting against the farmed male offspring in the spawning channels. I also determined that there was no evidence of sexual selection based on sperm velocity, body size and spawning longevity from the paternity of the eggs from the spawning channels. Although my work suggests that farmed Chinook salmon males have the ability to compete for females and successfully fertilize eggs when competing with wild males, it also demonstrates that the impacts on the wild population would be mediated by the low survival of farm-sired offspring. Thus male escapes from aquaculture do not pose a significant genetic threat to
the wild population, however this can still lead to lost opportunities for wild males, and subsequently affect wild salmon production.

I determined that farmed salmon males are capable of successfully mating in the presence of wild salmon, which led me to investigate the effects of outbreeding which could arise if farmed-wild hybrid offspring survived and reproduced in the wild. I tested for effects of outbreeding in Chinook salmon, and I found no evidence of outbreeding depression in the species. I tested a suite of fitness-related traits for outbreeding effects, including growth, survival and stress response and I concluded that outbreeding has no negative consequences in Chinook salmon between the egg stage and the subsequent year of life. This research demonstrates that outbreeding in Chinook salmon, either intentional for conservation purposes (e.g., “genetic rescue”) or unintentional through the hybridization of wild and farmed/hatchery salmon, does not result in large negative fitness consequences. However, my study was conducted under culture conditions, and different outcomes are possible under more natural (and stressful) conditions. That is, outbreeding effects are species specific, and they are most likely situation specific as well (Edmands 2007; Houde et al. 2011).

In this thesis, I have demonstrated that there is significant potential for farmed Chinook salmon genes to be introgressed into the wild population. If Chinook salmon escape from aquaculture and migrate to spawning grounds, farmed males will have a competitive advantage in sperm competition with wild males. Additionally, farmed males can gain access to females and successfully fertilize eggs. However, the genetic impacts of these reproductive interactions are immediately reduced, as farm-sired offspring will have significantly lower survival relative to wild-sired offspring likely as a result of
natural selection acting on the maladaptive traits of farmed salmon in natural settings. Furthermore, I found that outbreeding in Chinook salmon does not have negative fitness consequences and thus farmed genes introgressed into the wild may be diluted through backcrossing with the wild population, however farmed genes will likely not be selected against by means of outbreeding depression.

It should be acknowledged that my thesis only included one wild and one farmed population, however I believe this data is representative of other wild and farmed populations, especially given that numerous studies have found that farming practices adversely affect the reproductive success of farmed salmon (Fleming et al. 1996; Fleming et al. 2000; McGinnity et al. 2003). Investigating other wild and farmed Chinook salmon populations would be valuable in the future, but artificial culture will ultimately reduce the fitness of farmed individuals under wild conditions (McGinnity et al. 2003), and thus I would expect similar results given the very different selective pressures experienced by wild and farmed populations of any aquacultured species.

Finally, although salmon aquaculture is regarded negatively by some, and even though escapes from salmon aquaculture can be abundant (Naylor et al. 2005), current improvements in containment technology will likely reduce the numbers of escapes, as well as improve management and reporting (e.g., BC Fisheries Act: Aquaculture Regulation). While escapes are generally viewed as harmful, it is also possible that the introgression of novel farmed genes into endangered salmon populations could provide fitness benefits (Peterson 1999; Tallmon et al. 2004). Of course, large numbers of farmed salmon reproducing in the wild could genetically swamp the wild population and lead to serious fitness declines. Although, based on Atlantic salmon data (see above), I could
assume 0.01% of Chinook escape, and 0.3-11% of escapes migrate to freshwater. With these numbers the likelihood that a Chinook salmon will escape and then migrate to spawning grounds will be 0.00003 - 0.000011%. The subsequent reproductive success (fry) of farmed male escapes on spawning grounds is 0 - 0.02% (according to my data). This suggests that the likelihood of Chinook escaping, migrating to spawning grounds and contributing fry to the next generation is extremely low (< 2 x 10⁻⁸%). The introgression of farmed genes from a small number of migrants may provide fitness benefits in the long term, as natural selection should act to either immediately reduce the frequency of maladaptive genes or potentially increase frequency of genes if they prove beneficial to the wild population (Peterson 1999).

5.1 FUTURE DIRECTIONS

This thesis has substantially contributed to the knowledge of reproductive abilities of farmed Chinook salmon relative to wild salmon, and provided new insight into potential impacts of farmed Chinook salmon escapes. My project has helped shed light on aspects of farmed-wild interactions that previous research has sometimes ignored. For example, my approach for studying both egg and fry using genetic markers sets a new standard for spawning experiments, especially given the implications of interpreting results with only one of these life stages. Additionally, including sperm characteristics in spawning experiments also represent a sometimes neglected but important factor. Although this thesis addresses important questions regarding farmed-wild interactions, it also set the framework for new and important areas of research. Future experiments building on my own work that would be valuable for this field include:
• Experiment 1: An experiment to estimate the reproductive success of farmed Chinook salmon females relative to wild females using similar experimental design. My thesis demonstrated that farmed male fertilization success was greater than previously realized from farmed-wild studies of other salmon species (Fleming et al. 1996). Understanding the success of farmed Chinook salmon females will provide further information on the impacts of escapes, and allow me to determine if one sex poses a greater risk to the wild population. This research would be particularly valuable given that escapes from aquaculture are more likely to be female.

• Experiment 2: I have determined that farmed males are successful at competing for mates and fertilizing eggs in competition with wild males under semi-natural conditions. Future work to expand on this project would be to introduce farmed salmon (males and females) into natural spawning grounds with wild salmon and use the same genetic approach to determine fertilization and reproductive success. Furthermore, later life stages could also be examined through genetic techniques to determine the overall lifetime success of farmed, wild and hybrid offspring.

• Experiment 3: Tagging and tracking of released farmed Chinook salmon in order to estimate sea survival in the wild and subsequent migration to spawning grounds. Given that Chinook salmon males can successfully spawn in the presence of wild males, understanding the migration patterns of farmed Chinook salmon in the wild could provide critical data as to which river systems are at greater risk of invasion by farmed salmon. This would also help predict the
relative ratio of farmed and wild salmon on spawning grounds given a known number of escapes, which could be used for management purposes.

- **Experiment 4:** Although my project did not detect outbreeding depression during the egg stage and subsequent year of life, continuing to monitor these individuals throughout the next year and into sexual maturation may show evidence of outbreeding depression. Later life stages may prove more stressful and the ultimate measure of fitness is reproductive success. Additionally, at sexual maturation, these individuals could be used to create the F$_3$ generation that may be required for salmonids to show outbreeding depression owing to their tetraploid ancestry.

- **Experiment 5:** In addition to continuing to monitor the crosses at YIAL, I would also suggest an experiment using wild and farmed Chinook salmon studied under both culture and natural conditions. An experiment that crosses wild and farmed Chinook to create first generation hybrids, with later F$_2$ backcrosses, would expand further on my research, and add valuable data for estimating the impacts of escapes from Chinook salmon farms.

In conclusion, although my research has contributed greatly to the field of farmed-wild interactions, particularly for Chinook salmon in which previous research was virtually non-existent, my project also sets the stage for new research directions in this exciting field of science. With the aquaculture industry continuing to grow globally, it is important that we continue to expand our knowledge about the impacts of farmed salmon on the wild populations. Salmon are important not only economically, but also as an essential part of the ecosystem, as they provide a significant influx of marine derived
nutrients annually to freshwater systems that radiates throughout trophic levels from bacteria and insects to birds and mammals (Gende et al. 2002). Consequences for salmon populations would equate to consequences for aquatic and terrestrial ecosystems alike.

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Figure 5.1. Steps required for successful farm-wild hybridization.

1. Escape
2. Survive
3. Homing to freshwater stream
4. Spawning success
   - Mate competition + Gamete competition
5. Offspring survival
APPENDICES

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