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**Genetic effects on phenotypic traits throughout ontogeny in Chinook salmon
(*Oncorhynchus tshawytscha*)**

by

Britney K. Falica

A Thesis
Submitted to the Faculty of Graduate Studies
through Biological Sciences
in Partial Fulfillment of the Requirements for
the Degree of Master of Science at the
University of Windsor

Windsor, Ontario, Canada

2011

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Genetic effects on phenotypic traits throughout ontogeny in Chinook salmon

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ABSTRACT

While genetic effects on offspring phenotypic traits are well studied in fish, examining all genetic components to variation in traits across developmental stages has been rarely explored. Using a full factorial breeding design, I investigated additive and nonadditive genetic effects and maternal effects on offspring length, survival and swimming ability throughout ontogeny in Chinook salmon (*Oncorhynchus tshawytscha*), a species with a nonresource-based mating system. I also used existing ‘high-survival’ and ‘low-survival’ lines of Chinook salmon to determine if these two lines still show differences in survival and length, and if the two lines show differences in swimming ability. Genetic variation was found for offspring length, survival, and swimming ability, where results varied depending on the phenotypic trait examined and developmental stage. Future research should continue to follow the genetic architecture of phenotypic traits within species throughout ontogeny, and could compare populations to further improve conservation efforts of this species.

DEDICATION

I dedicate this thesis to my mother, who has never doubted my abilities for a second, and was there for me every step of the way.

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TABLE OF CONTENTS

DECLARATION OF ORIGINALITY iii

ABSTRACT iiv

DEDICATION v

ACKNOWLEDGEMENTS vi

LIST OF TABLES x

LIST OF FIGURES xiv

CHAPTER

I. INTRODUCTION..... 1

1.1 Genetic Architecture of Performance Traits..... 1

1.2 Study Species - Biology & Status..... 5

1.3 Thesis Objectives..... 7

1.3.1 Overview of Chinook salmon used for thesis objectives... 7

1.3.2 Chapter II Objectives..... 8

1.3.3 Chapter III Objectives..... 8

1.4 References..... 9

**II. PATERNAL IDENTITY AFFECTS OFFSPRING SWIMMING
PERFORMANCE IN OLDER JUVENILE CHINOOK SALMON,
ONCORHYNCHUS TSHA WHYTSCHA..... 14**

2.1 Introduction..... 14

2.2 Methods..... 18

2.3 Results..... 23

2.4 Discussion..... 26

2.5 References..... 33

III.	VARIATION IN CONTRIBUTIONS OF ADDITIVE AND NONADDITIVE GENETIC EFFECTS AND MATERNAL EFFECTS ON LENGTH AND SURVIVAL THROUGHOUT ONTOGENY IN CHINOOK SALMON (<i>ONCORHYNCHUS TSHA WYTSCHA</i>).....	52
	3.1 Introduction.....	52
	3.2 Methods.....	55
	3.3 Results.....	63
	3.4 Discussion.....	68
	3.5 References.....	77
IV.	GENERAL CONCLUSIONS.....	99
	4.1 Summary of findings.....	99
	4.2 Future directions.....	102
	4.3 References.....	104
	VITA AUCTORIS	107

LIST OF TABLES

Table 2.1 Summary of the 7 studies to my knowledge that exist on parental genetic effects on offspring swimming ability of fishes. The reference, stage of ontogeny – species, if parental care occurred and by who, what the authors used as a measure of swimming performance, and the results for each study are given. For the result, if maternal, paternal, or interaction effects are not mentioned, it means they were not tested for..... **38**

Table 2.2 6x6 Breeding design with ‘performance crosses’ identified. Dam identification numbers and their corresponding performance crosses are displayed vertically and bolded; sire identification numbers and their corresponding performance crosses are displayed horizontally and bolded. The resulting offsprings’ performance cross for all 36 families is shown..... **41**

Table 2.3 Velocity of water at each rheostat voltage for swim trials..... **42**

Table 2.4 Summary of the one-way random factor ANOVA results for family effects on offspring U-crit10s and U-crit 30s, for both rounds of swimming. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), F statistic, *P*-value, and the variance component (σ^2) with the percent of total variance (% total var) explained. Significant values ($P < 0.05$) are indicated in bold..... **43**

Table 2.5 Summary of the two-way random factor ANOVA results for parental effects (dam, sire, and their interaction) on offspring U-crit10s and U-crit30s for both rounds of swimming. The table includes the source of variation, degrees of freedom (DF,

with the numerator and denominator values where appropriate), sum of squares (SS), F statistic, *P*-value, and variance component (σ^2) with the percent of total variance (% total var) explained by each source (negative variance components are treated as zero). The table also includes the percent of phenotypic variance (% phenotypic var) from maternal effects, and additive and nonadditive genetic effects. Significant values ($P < 0.05$) are indicated in bold..... **44**

Table 2.6 Summary of the one-way fixed factor ANOVA results for performance cross effects on offspring U-crit10s and U-crit30s for both rounds of swimming. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), F statistic, *P*-value, and the variance component (σ^2) with the percent of total variance (% total var) explained (negative variance components are treated as zero). Significant values ($P < 0.05$) are indicated in bold..... **46**

Table 3.1 Summary of survival measurements, taken 4 times throughout ontogeny. The table shows for each date the offspring’s current stage of development, dates included, important notes concerning whether fish removal had occurred or not, and how survival was calculated (per family) at that date..... **83**

Table 3.2 Summary of the one-way random factor ANOVA results for family effects on offspring length, for all 5 dates measured. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), *F* statistic, *P*-value, and the variance component (σ^2) with the percent of total variance (% total var) explained. Significant values (adjusted alpha level of $P < 0.013$) are indicated in bold..... **84**

Table 3.3 Summary of the two-way random factor ANOVA results for parental effects (dam, sire, and their interaction) on offspring length, for all 5 dates measured. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), *F* statistic, *P*-value, and variance component (σ^2) with the percent of total variance (% total var) explained by each source (negative variance components are treated as zero). The table also includes the percent of phenotypic variance (% phenotypic var) from maternal effects, and additive and nonadditive genetic effects. Significant values (adjusted alpha level of $P < 0.013$) are indicated in bold..... **85**

Table 3.4 Summary of the one-way fixed factor ANOVA results for performance cross effects on offspring length, for all 5 dates measured. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), *F* statistic, *P*-value, and the variance component (σ^2) with the percent of total variance (% total var) explained (negative variance components are treated as zero). Significant values (adjusted alpha level of $P < 0.013$) are indicated in bold..... **87**

Table 3.5 Summary of the two-way random factor ANOVA results for parental effects (dam and sire) on offspring survival, for all 4 dates measured. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), *F* or Wald statistic (*W*), *P*-value, and variance component (σ^2) with the percent of total variance (% total var) explained by each source (negative variance components are treated as zero). The table also includes the percent of phenotypic variance (% phenotypic var) from maternal effects, and

additive and nonadditive genetic effects. Significant values (adjusted alpha level of $P < 0.017$) are indicated in bold. For *Date A* and *Date B*, the P -values are from the two-way random factor ANOVA, and for *Date C* and *Date D*, the P -values are from logistic regression (as explained in the text)..... **88**

Table 3.6 Summary of the one-way fixed factor ANOVA results for performance cross effects on offspring survival, for all 4 dates measured. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), F or Wald statistic (W), P -value, and the variance component (σ^2) with the percent of total variance (% total var) explained (negative variance components are treated as zero). Significant values (adjusted alpha level of $P < 0.017$) are indicated in bold. For *Date A* and *Date B*, the P -values are from the one-way fixed factor ANOVA, and for *Date C* and *Date D*, the P -values are from logistic regression (as explained in the text)..... **89**

LIST OF FIGURES

Fig. 2.1 Critical swimming speeds (U-crit) significantly improved from *round 1* to *round 2* of swimming, for both U-crit10s and U-crit30s. The filled circles represent U-crit10s, and the open circles U-crit30s..... **47**

Fig. 2.2 Variation in offspring mean critical swimming speed (U-crit) due to family for *round 2* of swimming. The filled circles and solid line represent U-crit10s, and the open circles and dashed line U-crit30s..... **48**

Fig. 2.3 Paternal effect on offspring mean critical swimming speed (U-crit). *Round 1* of swimming for (a) U-crit10s and (b) for U-crit30s. *Round 2* of swimming for (c) U-crit10s and (d) for U-crit30s. The pattern is non-significant for *round 1* of swimming but is significant for *round 2* for both U-crit10s and U-crit30s, with the letters ‘a’, ‘b’, and ‘c’ denoting the homogenous subsets..... **49**

Fig. 2.4 Maternal effect on offspring mean critical swimming speed (U-crit). *Round 1* of swimming for (a) U-crit10s and (b) for U-crit30s. *Round 2* of swimming for (c) U-crit10s and (d) for U-crit30s. The patterns are non-significant..... **50**

Fig. 2.5 Effect of performance cross on offspring mean critical swimming speed (U-crit) for both U-crit10s and U-crit30s for (a) *round 1* of swimming and for (b) *round 2* of swimming. The filled circles represent U-crit10s, and the open circles U-crit30s. For the performance crosses, the first letter indicates the performance line of the dam, and the second letter indicates the performance line of the sire. The trend is significant for *round 2* U-crit30s, and the letters ‘a’ and ‘b’ denote the homogenous subsets..... **51**

Fig. 3.1 Dam identity vs. mean fork length (cm) for a) *Date 1*: March 2009, b) *Date 2*: June 2009, c) *Date 3*: November 2009, d) *Date 4*: June 2010, e) *Date 5*: June 2011 and f) all dates. The trend is significant for all dates, with the letters ‘a’, ‘b’, ‘c’, ‘d’ and ‘e’ denoting the homogenous subsets..... **90**

Fig. 3.2 Sire identity vs. mean fork length (cm) for a) *Date 1*: March 2009, b) *Date 2*: June 2009, c) *Date 3*: November 2009, d) *Date 4*: June 2010, e) *Date 5*: June 2011 and f) all dates. The trend is significant for *Date 2*, *Date 3* and *Date 5*, with the letters ‘a’, ‘b’ and ‘c’ denoting the homogenous subsets..... **92**

Fig. 3.3 Performance cross vs. mean fork length (cm) for a) *Date 1*: March 2009, b) *Date 2*: June 2009, c) *Date 3*: November 2009, d) *Date 4*: June 2010, e) *Date 5*: June 2011 and f) all dates. For the performance crosses, the first letter indicates the performance line of the dam, and the second letter indicates the performance line of the sire. The trend is significant for *Date 1*, *Date 2*, *Date 3* and *Date 4*, with the letters ‘a’, ‘b’ and ‘c’ denoting the homogenous subsets..... **94**

Fig. 3.4 Dam identity vs. mean percent survival for a) *Date A*: March 2009, b) *Date B*: June 2009, c) *Date C*: June 2010, and d) *Date D*: June 2011. The trend is significant for *Date A*, *Date C*, and *Date D*, with the letters ‘a’ and ‘b’ denoting the homogenous subsets for *Date A*. Logistic regression was used for *Date C* and *Date D*, and asterisks for those dates indicate dams that produced offspring with significantly higher survival than the dam with the lowest survival (dam # 8). Figures were plotted using the untransformed percent survival data..... **96**

Fig. 3.5 Sire identity vs. mean percent survival for a) *Date A*: March 2009, b) *Date B*: June 2009, c) *Date C*: June 2010, and d) *Date D*: June 2011. The trend is significant

for *Date A* only, with the letters ‘a’ and ‘b’ denoting the homogenous subset. Figures were plotted using the untransformed percent survival data..... 97

Fig. 3.6 Performance cross vs. mean percent survival for a) *Date A*: March 2009, b) *Date B*: June 2009, c) *Date C*: June 2010, and d) *Date D*: June 2011. For the performance crosses, the first letter indicates the performance line of the dam, and the second letter indicates the performance line of the sire. The trend is significant for *Date D*, with asterisks indicating the performance crosses that produced offspring with significantly higher survival than the performance cross with the lowest survival (L/H). Figures were plotted using the untransformed percent survival data..... 98

CHAPTER I

INTRODUCTION

1.1 Genetic Architecture of Performance Traits

Investigations of parental effects on offspring fitness-related traits have been of ongoing interest to evolutionary biologists, with research across a variety of taxa and offspring traits, such as on viability in pygmy grasshoppers (*Tetrix subulata*) (Caesar & Forsman, 2009), on immunocompetence in bluethroats (*Luscinia svecica*) (Johnsen *et al.*, 2000), on mortality in two mice strains (*Mus musculus*) (Gyekis *et al.*, 2011), on reproductive performance in bank voles (*Clethrionomys glareolus*) (Klemme *et al.*, 2008), and on hatching success in Atlantic halibut (*Hippoglossus hippoglossus* L.) (Ottesen & Babiak, 2007). Recently, there has been an increased focus on genetic effects of offspring traits, specifically on investigating whether the genetic quality of parents influences intraspecific variation in offspring fitness characteristics (e.g. Wedekind *et al.*, 2008; Rodriguez-Munoz & Tregenza, 2009). ‘Genetic quality,’ or the genetic architecture of fitness has two components, ‘good genes’ and ‘compatible genes’ (reviewed in Neff & Pitcher, 2005; and in Neff *et al.*, 2011). Good genes are alleles that increase fitness separately from the remaining genome, and will show additive genetic variation. When good genes cause variation in fitness in a population, the population will respond to directional selection. In the good genes model, many females prefer to mate with the same male (in species where females are choosy), usually the male with the most elaborate trait which may reflect superior alleles (Neff & Pitcher, 2005). Compatible genes on the other hand, are alleles that increase fitness only when in combination with other alleles, such as through heterozygote advantage (overdominance) or epistasis.

Compatible genes will show nonadditive genetic variation, i.e. some dam-sire genetic combinations will have higher fitness than other combinations. When compatible genes cause variation in fitness in a population, the mechanisms responsible for acquiring compatible genes (and not the population itself) will respond to directional selection. In the compatible genes model, females do not prefer the same male as her choice depends on her own genotype (Neff & Pitcher, 2005).

Studying both good genes and compatible genes components of genetic quality simultaneously on offspring traits is achieved most effectively by performing the North Carolina Design II (Lynch & Walsh, 1998) method of breeding. The North Carolina Design II method involves the most comprehensive artificial fertilization method, where the gametes of a set number of dams and sires are crossed in every pair-wise combination. To effectively isolate genetic effects from other factors that affect offspring fitness (such as from direct benefits like parental care), investigations on genetic architecture of traits must be employed using animals that have nonresource-based mating systems (i.e. when only genes are provided to the offspring, and parental care is absent) (Neff & Pitcher, 2005; Hettyey *et al.*, 2010). Fishes for instance, are ideal. Although fish have the widest variety of reproductive modes of the vertebrates (Green, 2008), many species of fish have nonresource-based mating systems. Furthermore, many fishes are oviparous and fertilize gametes externally, which is convenient for the North Carolina Design II as gametes can be extracted and then artificially fertilized. After quantifying a desired phenotypic trait in the offspring, a two-way ANOVA can be used to determine dam, sire, and dam x sire effects on that trait (Lynch & Walsh, 1998). Many studies on parental genetic effects on offspring however have ended at this step (Bang *et*

al., 2006) which is unfortunate as the North Carolina Design II method offers further benefits. The design offers further investigation by allowing one to partition the variation among additive genetic effects, nonadditive genetic effects and true maternal effects (e.g. Wedekind *et al.*, 2008). Assuming one selects a species with a nonresource-based mating system, the contributions to phenotypic variance can be calculated as follows. Since sires provide only genes to their offspring, additive genetic effects are estimated by calculating four times the sire component of variance. Similarly, nonadditive genetic effects are calculated from four times the dam x sire component of variance (Lynch & Walsh, 1998). The dam component of variance (i.e. female effect) encompasses both maternal additive genetic effects and maternal non-genetic effects; this is why studies that end their investigation after dam, sire and dam x sire components of variance are achieved are hindered in that any significant dam effects can be due to either her genetics or non-genetic effects, and not segregating the two leaves questions unanswered. ‘Maternal effects’ in fish are defined as ‘the non-genetic contribution of a female to the phenotype of her offspring’ (reviewed in Green, 2008) and include items such as the amount of nutrients provisioned in her eggs, hormones and cytoplasm, and where she chooses to deposit her eggs (Green, 2008). The genetic contributions of dams and sires to the zygote can be considered equal, and so the contribution of true maternal effects to phenotypic variation to a trait can be calculated by the difference between the dam and sire components of variance (Lynch & Walsh, 1998; reviewed in Neff & Pitcher, 2005). Studies on the contributions of additive and nonadditive genetic effects and maternal effects to offspring fitness-related traits in fishes show a wide range of results, which can be attributed to the variety of species, populations, stages of ontogeny, and the

phenotypic traits examined; such as embryo survival (e.g. Wedekind *et al.*, 2008; Rodriguez-Munoz & Tregenza, 2009; Kekalainen *et al.*, 2010a), disease resistance (e.g. Balfry *et al.*, 1997), anti-predator behaviour (e.g. Evans *et al.*, 2004), larval spinal deformity, (e.g. Evans & Neff, 2009), and growth (e.g. Bang *et al.*, 2006; Pitcher & Neff, 2007; Evans *et al.*, 2010; Janhunen *et al.*, 2011). Although through analysis of previous studies researchers are becoming increasingly aware that the genetic architecture of traits vary intra-individually across time (Heath & Blouw, 1998; see Evans *et al.*, 2010 and references therein), only a few studies in fish have actually attempted to follow all genetic components (additive and nonadditive genetic effects and maternal effects) of performance traits across developmental stages (Wedekind *et al.*, 2001; Wedekind *et al.*, 2008; Evans *et al.*, 2010). To my knowledge, no one study has followed the contributions of additive and nonadditive genetic effects and maternal effects to variation in a phenotypic trait from the larval stage through to the adult stage of development.

Of the phenotypic traits that have been chosen by researchers for examining the effects of genetic quality, one trait that has surprisingly received little attention is offspring swimming ability, a trait that is clearly important for the survival of fishes (see Plaut, 2001 and references therein). To my knowledge, only seven studies exist on genetic effects on swimming (Nicoletto, 1995; Garenc *et al.*, 1998; Evans *et al.*, 2004; Green & McCormick, 2005; Huuskonen *et al.*, 2009; Nadeau *et al.*, 2009; and Kekalainen *et al.*, 2010b). Furthermore, in those seven studies, not all used species with nonresource-based mating systems or studied all components of genetic quality and true maternal effects. One way to quantify swimming ability is to measure the critical speed (U_{crit}) (Fisher and Leis, 2009) which is relevant for investigating maximum aerobic capabilities,

especially in fishes that travel upstream or against currents such as the Chinook salmon, *Oncorhynchus tshawytscha* (Plaut, 2001). U-crit can be obtained by swimming a fish against water current and incrementally increasing the water speed until fatigue occurs (i.e. when the fish can no longer hold its station) (Brett, 1964). Given that variation in many offspring fitness-related traits has been attributed to differences in the genetic quality of parents, it is likely that genetics influence offspring swimming ability as well.

1.2 Study Species - Biology & Status

Chinook salmon (*Oncorhynchus tshawytscha*), the largest of the Pacific salmon, are anadromous (i.e. breed in fresh water but spend much of their lives growing at sea) and semelparous (i.e. have one breeding season and die shortly after) (Healey, 1991). Chinook are external fertilizers and have a nonresource-based mating system, which makes them a suitable species for investigating the genetic architecture of offspring phenotypic traits (Healey, 1991; Lynch & Walsh, 1998). Female Chinook prepare and guard nest sites (Quinn, 2005), provide genes to their offspring as well as other contents provisioned into the egg (Quinn, 2005; Green, 2009). Male Chinook compete among each other for access to ripe females, and provide only genes to their offspring (Quinn, 2005). After the endogenous feeding stage, young ocean-type Chinook offspring will leave their freshwater natal stream, and begin their downstream migration towards the sea, which can be actively directed (Healey, 1991). The now exogenously feeding juvenile Chinook can obtain drifting food by holding their station in the water current (Childerhose & Trim, 1979). After spending one to seven years at sea, mature Chinook will begin a long migration (some even travel thousands of kilometers) using mainly

olfaction to return back to their freshwater natal streams where they were born, in order to spawn (Childerhose & Trim, 1979; Healey, 1991). Due to their strong sense of homing to their natal streams, many sub-populations of Chinook, referred to as ‘stocks,’ have formed (Healey, 1991).

Currently, the socio-economically important Chinook salmon are designated as endangered and threatened (depending on population) under the U.S. Endangered Species Act (Fullerton *et al.*, 2011). Pacific salmon stocks have been declining drastically, mainly due to anthropogenic activities (for reviews see: Araki *et al.*, 2008; Carlson & Seamons 2008; Fraser, 2008; Waples & Hendry, 2008). Consequently, a great deal has been invested into supportive breeding programs which produce salmonids extensively in attempts to replenish the depleting stocks (Araki *et al.*, 2008; Swanson *et al.*, 2008). What is still uncertain, however, is if such breeding programs can maintain biodiversity, fitness and ultimately generate populations in the wild that are successful and independent from our intervention (Fraser, 2008). Although with good intentions, the outcome of captive-reared progeny for supplementation can be highly unfavorable, resulting in both phenotypic and genetic changes (e.g. Reisenbichler & Rubin, 1999; Blanchet *et al.*, 2008). As wild populations are locally adapted, captive-reared fish have been shown to be inferior to native individuals when released into the wild, suffering from maladaptive behaviours like increased aggression (Blanchet *et al.*, 2008), increased risk-taking behavior causing higher predation (Kekalainen *et al.*, 2008), and abnormal timing of spawning (Swanson *et al.*, 2008). Captive-reared individuals may therefore threaten the fitness of wild populations when these inferior counterparts breed with wild fish (Swanson *et al.*, 2008), and have even been shown to have carry-over effects on

wild-born descendants of captive reared parents (Araki *et al.*, 2009). Although numerous studies have shown that genetics play an important role in offspring fitness-related traits, and that genetics even play a role in phenotypic plasticity (i.e. the ability to respond to changing environments) (Evans *et al.*, 2010), artificial breeding programs have mostly ignored the genetic architecture of fitness-related traits and that wild individuals possess genetic adaptations to their dynamic environment (Wedekind, 2002; Fraser *et al.*, 2011; Neff *et al.*, 2011).

1.3 Thesis Objectives

1.3.1 Overview of Chinook salmon used for thesis objectives

The Chinook salmon dams and sires used to accomplish the thesis objectives are either 8th generation descendants (Chapter 2) or 7th generation descendants (Chapter 3) from crosses between wild females taken from the Robertson Creek Hatchery (Port Alberni, B.C.) and wild males taken from Big Qualicum River Hatchery (Qualicum Beach, B.C.) in 1985. The study species have since been raised at the Yellow Island Aquaculture Ltd (YIAL) hatchery and netcage site on Quadra Island, British Columbia. Using these descendants, YIAL initiated a marker-assisted broodstock selection program in 1997, creating a ‘high-survival line’ and a ‘low-survival line’ based on variation in growth- and survival-related gene markers (Docker & Heath, 2002). Although these two lines have been selected for differences in growth and survival, it is unclear when in development these differences manifest, and whether the two lines still show differences in growth and survival at the present time. Using the North Carolina Design II method to cross a set number of dams and sires (some dams and sires from the high-survival line,

and some dams and sires from the low survival line), offspring were developed that were purebred and hybrid for the high- and low- survival lines. This portion of my thesis has applications to aquaculture, if the offspring show differences in size and/or survival. It also, however, is interesting for further investigating parental genetic effects on offspring fitness-related traits, concerning whether or not the high- and low- survival lines will differ in their influence on the offspring traits depending on whether the dam or the sire is of the high- or low-survival line.

1.3.2 Chapter 2 Objectives

Chapter 2 has two objectives. The first is to employ the North Carolina Design II method using 6 dams and 6 sires in Chinook salmon. This allowed me to determine dam, sire, and dam x sire effects on offspring swimming performance (measured as U-crit), and to partition the variance in offspring swimming ability to additive and nonadditive genetic effects as well as maternal effects. U-crit was measured at two different time-points during the parr stage of development, to determine if and how the genetic architecture of this fitness-related trait changes with age. The second objective is to use the same 6 dams and 6 sires which are from the existing ‘high-survival’ and ‘low-survival’ lines of Chinook salmon to determine if these two lines show differences in swimming ability (U-crit).

1.3.3 Chapter 3 Objectives

Chapter 3 also has two objectives. The first is to follow the genetic architecture of two performance traits (body size and survival) throughout ontogeny in Chinook salmon.

I used 7 dams and 7 sires crossed in a North Carolina Design II breeding design to determine dam, sire, and dam x sire effects on offspring length and survival, and the variance in these traits was partitioned into additive and nonadditive genetic effects as well as maternal effects. Length was measured at five time-points throughout ontogeny, during the larval, parr, juvenile, and twice at the adult stage of development. Survivorship was calculated at 4 time-points throughout ontogeny, during the larval, parr, juvenile, and adult stages of development. The second objective is to use the same 7 dams and 7 sires which are from the existing ‘high-survival’ and ‘low-survival’ lines of Chinook salmon to determine if these two lines still show differences in length and survival, and to determine when any differences manifest.

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CHAPTER II
PATERNAL IDENTITY AFFECTS OFFSPRING SWIMMING PERFORMANCE
IN OLDER JUVENILE CHINOOK SALMON, *ONCORHYNCHUS*
TSHAWHYTSCHA

2.1 Introduction

Investigating maternal and paternal effects on offspring fitness-related traits has been an ongoing interest for evolutionary ecologists, with maternal and paternal effects being caused by environmental influences (e.g. Freeman-Gallant, 1998; Gruebler & Naef-Daenzer, 2010), genetic differences (e.g. Johnsen *et al.*, 2000; Klemme *et al.*, 2008), and combinations of the two (e.g. Caesar & Forsman, 2009; Evans *et al.*, 2010). Fitness effects have been seen in situations where females choose mates for direct benefits like food and care for her offspring (i.e. resource-based mating systems, Neff & Pitcher, 2005) and also in situations where parents provide no care but only genes to their offspring (i.e. nonresource-based mating systems, Neff & Pitcher, 2005), providing systems in which genetic and environmental contributions can be separately assessed. In the latter mating system — where offspring receive only genes — the effects of parental genetic quality on a particular trait in offspring can be assessed to ask (i) are some dams or sires of “better” genetic quality than others (i.e. does the trait under consideration show additive genetic variance from good genes), and (ii) are certain parental genomic combinations “better” than others (i.e. does the trait in consideration show nonadditive genetic variance from compatible genes) (reviewed in Neff & Pitcher, 2005)? In fishes, a large variety of mating systems exist, and the roles of dam and sire influences have been well studied in offspring traits such as embryo survival (e.g. Wedekind *et al.*, 2008;

Rodriguez-Munoz & Tregenza, 2009; Kekalainen *et al.*, 2010a), disease resistance (e.g. Balfry *et al.*, 1997), anti-predator behaviour (e.g. Evans *et al.*, 2004), larval spinal deformity, (e.g. Evans & Neff, 2009), and growth (e.g. Bang *et al.*, 2006; Pitcher & Neff, 2007; Evans *et al.*, 2010; Janhunen *et al.*, 2011), where the results among the studies vary, not surprisingly considering the different species, developmental stages, and phenotypic traits examined.

Surprisingly, parental genetic effects on offspring swimming ability of fishes, a rather essential characteristic for survival (e.g. Bailey, 1984; Fisher & Leis, 2009; also see Plaut, 2001 and references therein), has rarely been explored, with only seven studies to our knowledge on this topic (Table 2.1). Two (Garenc *et al.*, 1998; Green & McCormick, 2005) of those seven studies involved offspring that were subjected to parental care, which means their results are more likely to be confounded by non-genetic parental effects. Furthermore, only four studies (Nicoletto, 1995; Green & McCormick, 2005; Huuskonen *et al.*, 2009; Kekalainen *et al.*, 2010b) actually examined contributions from both good genes and compatible genes, whereas the remaining studies (Garenc *et al.*, 1998; Evans *et al.*, 2004; Nadeau *et al.*, 2009) investigated only one component of genetic quality. Thus, the role of parental genetic effects on a critical aspect of fish survival remains woefully understudied, and more analyses are needed on both additive and nonadditive genetic contributions of parents to this trait to fully understand the fitness consequences of both natural and aquacultural mating decisions.

While knowledge of genetic quality on swimming ability is lacking, other aspects of fish swimming (e.g. physiology, types and functions, temperature effects, oxygen consumption) and the tools for investigating swim performance are well established, as

the swimming performance of fishes has been repeatedly documented in the last half-century beginning with design of the respirometer by Brett (1964) (for some reviews see: Hammer, 1995; Blake 2004; Farrell, 2007; Fisher & Leis, 2009; Kieffer, 2010). One of the most common measures of swimming ability of fishes is the critical speed (U-crit) (Fisher and Leis, 2009) which is relevant for investigating maximum aerobic capabilities, especially in fishes that travel upstream or against currents such as the Chinook salmon, *Oncorhynchus tshawytscha* (Plaut, 2001). The process of measuring U-crit consists of swimming a fish against water current and incrementally increasing the water speed until fatigue occurs where the fish can no longer hold its station (Brett, 1964). Although there is currently no proof that U-crit is *directly* correlated with fitness, U-crit is associated with the swimming capacities of fishes and other ecologically relevant traits (reviewed in Plaut, 2001). Thus, it is plausible to infer that U-crit is a measure of a fish's ability to perform during activities that involve swimming and is thus linked to survival (Plaut, 2001). Therefore, investigating additive and nonadditive genetic contributions on swimming ability could provide valuable information on how this trait could respond to selection. Additionally, because only four studies to our knowledge have explored contributions from both good genes and compatible genes on swimming performance (Table 2.1), investigating the role of genetic quality will add to the limited knowledge on the genetic architecture of this trait, and also on whether swimming ability has a heritable component in this group.

In the current study, I used a fully crossed breeding design to investigate the roles of genetic quality and non-genetic maternal effects on offspring swimming performance in Chinook salmon. The design allowed me to separate genetic variance in offspring

swimming ability into additive and nonadditive genetic effects as well as environmental maternal effects (Neff and Pitcher, 2005). The fish used in the current study are also part of a ‘high-survival line’ and ‘low-survival line’ of Chinook salmon created from a marker-assisted broodstock selection program that identified two separate salmon lines based on variation in growth- and survival-related gene markers (Docker & Heath, 2002). The two lines were thus also part of the fully crossed breeding design which created offspring that were purebred and hybrid for the high- and low-survival lines, which allowed me to determine if the different survival lines also possess different swimming abilities. Chinook salmon provide an ideal system to investigate parental genetic effects on offspring fitness-related traits because they are external fertilizers and have a nonresource-based mating system (Healey, 1991). Male Chinook mate with many females and provide no parental care, only genes to their offspring (Healey, 1991), thus presenting the opportunity to study ‘good genes’ effects (i.e. additive genetic variation) on offspring swimming (Neff and Pitcher, 2005). Female Chinook provide genes, but also provision nutrients and other contents to the egg (Healey, 1991; Green 2008), thus presenting the opportunity to study ‘good genes’ effects (i.e. additive genetic variation) and maternal effects respectively on offspring swimming (Neff and Pitcher, 2005). The full factorial breeding system crosses all dams and sires in every pair-wise combination, which allowed me to also examine ‘compatible genes’ effects (i.e. nonadditive genetic variation) on offspring swimming (Neff and Pitcher, 2005).

2.2 Methods

Study Species, Breeding Design and Rearing Conditions

Study Species: The Chinook salmon parents I used for the current study are 8th generation descendants from crosses between wild females taken from the Robertson Creek Hatchery (Port Alberni, B.C.) and wild males taken from Big Qualicum River Hatchery (Qualicum Beach, B.C.) in 1985. These fish have since been raised at the Yellow Island Aquaculture Ltd (YIAL) hatchery and netcage site on Quadra Island, British Columbia. Descendants are also part of a marker-assisted broodstock selection program initiated at YIAL in 1997, where a ‘high-survival line’ and a ‘low-survival line’ were created based on variation in growth and survival related gene markers, and these two lines have since been maintained at YIAL (Docker & Heath, 2002). All procedures were approved by the University of Windsor Animal Use and Care Committee.

Breeding Design and Fish Rearing: In the fall of 2009 I haphazardly selected a sample of sexually mature adult salmon until I had 6 females and 6 males; 3 females and 3 males were of the ‘high-survival line’ and 3 females and 3 males of the ‘low-survival line’ identified from previously implanted coded wire tags inserted into the nose of each fish. I sacrificed the parents via cerebral concussion and extracted eggs and milt for a 6x6 quantitative genetic breeding design (North Carolina Design II; Lynch & Walsh, 1998) creating 36 half- and full-sib families. Eggs from each female were fertilized by each male, and fertilized eggs were split into two cells per family in Heath trays to account for location effects. All of the parents I used in the full factorial breeding design were purebred for either the high-survival line (H/H) or the low-survival line (L/L), hereon referred to as their ‘performance cross’. Thus, the offspring will be one of the following

four performance crosses: ‘H/H’ where both parents were of the high-survival line, ‘H/L’ where the dam is of the high-survival line and the sire is of the low-survival line, ‘L/H’ which is the opposite of the previous, or ‘L/L’ where both parents are of the low-survival line (Table 2.2). The incubation trays were exposed to natural, untreated fresh water that ranged from 7°C to 9°C. For 30 minutes a day, 3 times a week until hatching, UV treated salt water was pumped through the trays to reduce fungus growth. Every other day until the end of the endogenous feeding stage, the incubation trays were checked and all unfertilized eggs and dead offspring were removed. At the end of the endogenous feeding stage, the offspring were transferred to 36 individual 200 L barrels, one family per barrel. Using a similar rearing design, Heath *et al.* (1999) did not find a correlation between rearing density (which could be different due to differences in survival among families) and growth. The barrels were reared in a common environment given flow-through fresh water ranging from 7°C to 10.5°C, aeration, and light from 7am – 5pm. The fish were fed daily, barrels were vacuumed every 5 days, and any dead fish were removed.

Swim Flume Design and Protocol

Flume Design: I quantified swimming performance by measuring U-crit in a Plexiglass flume, based on the design of Stobutzki & Bellwood (1994), with dimensions of 63 x 33 x 8.8 cm subdivided into three swimming channels each 45 x 2.5 x 5 cm. I used a removable lid (51 x 31 x 1.2 cm) with an opaque cover to allow placement of fish into the flume. I secured plastic drinking straws of 5mm outer diameter and approximately 6cm in length with silicone at the upstream end of each swim chamber to act as flow straighteners providing laminar flow (Stobutzki & Bellwood, 1994). I placed mesh screen behind the straws and at the downstream end of each chamber to contain the

fish in the swimming channels. With the addition of the straws and screen each channel length was reduced to 30 cm. Natural untreated fresh water, at the same temperature as the parr barrels, recirculated through the flume pumped by a submersible effluent pump. In addition to the recirculating water, an external source of water flowed into the flume reservoir to prevent water temperature from increasing due to heat from the submersible pump. I manipulated water velocity by using a rheostat (Staco Co., Dayton, Ohio) to manipulate voltage applied to the pump. Water velocity at each rheostat voltage was subsequently determined by measuring transport time of food dye in the chamber over a defined length (Table 2.3), since the chamber was too small to allow use of a current meter. The maximum cross-sectional areas of all fish used were less than 10% of the cross-sectional area of the channels, and so I did not need to adjust for blocking effect (Smit *et al.*, 1971).

Protocol: For each swimming trial, I used a sample of 3 offspring haphazardly selected from one of the 36 families; 1 fish placed into each channel. Before each trial began, I let the fish acclimate to the flume for 30 minutes with the water velocity set at 16.95 cm/s (60 volts) to normalize swimming behaviour (duration and velocity selected during preliminary trials). After the acclimation period, I started the trial by increasing the water velocity by 5 volts every 15 minutes until the fish fatigued, with ‘fatigue’ defined as a fish ceasing swimming and its entire body remaining on the downstream mesh screen. I noted the time and voltage when a fish was impinged for 10 seconds, but continued the trial increasing water velocity every 15 minutes until impinged for 30 seconds. This allowed me to determine a value for the critical swimming speed for both 10 seconds fatigued (U-crit10s) and 30 seconds fatigued (U-crit30s). When all 3 fish

fatigued for 30 seconds I ended the trial, removed them from the flume and into individual buckets filled with natural fresh water with an airstone and let them recover for 5 minutes. After the recovery period, I placed the fish in an anaesthetic bath (comprised of 0.3mL of clove oil in 5mL of ethanol for every 16L of water) to measure fork length and wet weight of each fish. Following this, I provided another recovery period in oxygenated fresh water, and then returned the fish to their respective family barrel. I repeated the above protocol for all families and considered it ‘*round 1*’ of swimming which occurred at approximately 15 weeks post-hatch.

After I swam all 36 families, I conducted the entire flow challenge experiment again and considered it ‘*round 2*’ of swimming, which occurred at approximately 18 weeks post-hatch. Thus, I swam a sample from every family on two instances. On the day of a families’ swim trial, I did not feed them, as being fed versus fasted has been shown to affect a fish’s critical swimming speed (Thorarensen & Farrell, 2006). I calculated U_{crit} for each fish according to Brett (1964) and as explained by Fisher & Leis (2009): $U_{crit} = U + (t/t_i * U_i)$, where U is the penultimate speed, U_i is the velocity increment, t is the time swum in the final velocity increment and t_i is the set time interval for each velocity increment.

Statistical Analysis

If a fish did not start swimming during the 30 minute acclimation period it was regarded as ‘disqualified’ (even if it started swimming when the actual test began), and thus was not included in the analysis. In addition, if a family only had 1 fish swim during its trial the family was not included in the analysis since there would not be any ‘within group’ variation for the ANOVA. To determine if there were parental genetic effects on

the number of fish that swam per family (i.e. willingness to swim), I used two-sided Fisher's exact test (Field, 2009). I chose this test instead of the Pearson's chi-square because the maximum number of fish that could swim per family is 3, thus the expected frequencies are less than 5 (Field, 2009). The Kolmogorov-Smirnov test confirmed that all U-crit10s and U-crit30s data for both rounds of swimming were normal. To determine if critical swimming speeds differed among rounds of swimming, I used one-way fixed factor ANOVA.

Parental genetic effects on offspring swimming performance

I first used two one-way fixed factor ANOVAs to determine if offspring body size (fork length and wet weight) affected U-crit; it did not (for neither U-crit10s nor U-crit30s, for both rounds) and so I removed offspring body size from the analysis (all *P*-values greater than or equal to 0.09). I used a one-way random factor ANOVA to determine family effects on critical swimming speed for both U-crit10s and U-crit30s for both rounds of swimming. To calculate the variance components I followed formulas given in Table 1 from Graham & Edwards (2001), and used the average for the sample size since they were unequal due to having to remove individuals from the analysis (as detailed above). I followed this by using one-tailed independent *t*-tests for significant results to determine which families were different from one another, with Bonferroni correction for multiple *t*-tests where appropriate (Field, 2009).

To further differentiate parental effects on offspring swimming performance (U-crit), I used two-way random factor ANOVA (for both U-crit10s and U-crit30s, for both rounds), to partition variance in offspring swimming to female identity (dam), male identity (sire), and their interaction (dam x sire). The variance components were

calculated as mentioned above. The contribution of additive genetic effects to offspring U-crit was calculated from four times the sire component of variance, the nonadditive genetic effects were calculated from four times the dam x sire component of variance, and the maternal effects were calculated from the difference between the dam and sire components of variance (reviewed in Neff & Pitcher, 2005). I used Tukey's posthoc on paternal results to determine which males sired better performing offspring.

Effect of performance cross on offspring swimming performance

To determine if the differential performance crosses affect U-crit, I used a one-way ANOVA (for both U-crit10s and U-crit30s, for both rounds) with performance cross entered as a fixed factor. The variance components were calculated as mentioned above. I then used Tukey's posthoc on significant results to determine which performance crosses differed in swimming ability.

2.3 Results

For *round 1* of swimming, 83 out of a possible 108 individuals (76.9%) actually swam and included individuals from all 36 families. However, since families where only 1 individual swam had to be taken out of the analysis (due to no variation for the ANOVA), the analysis includes 76 individuals representing 29 families. For *round 2* of swimming, 87 out of a possible 108 individuals (80.6%) actually swam but 4 families had to be excluded, again due to having only one swimming member. Thus, the analysis for *round 2* includes 83 individuals representing 32 families. Two-sided Fisher's exact test on the number of fish that swam per family (i.e. willingness to swim) revealed that there was no effect of family (*round 1*: $P = 0.295$; *round 2*: $P = 0.577$), female (*round 1*: $P =$

0.153; *round 2*: $P = 0.767$) or male (*round 1*: $P = 0.683$; *round 2*: $P = 0.369$) on the number of fish that swam. The critical swimming speeds did significantly improve from *round 1* to *round 2* of swimming for U-crit10s (*Round 1*: $\mu = 27.2 \pm 0.71$ cm/s, $n = 76$; *Round 2*: $\mu = 36.2 \pm 1.24$ cm/s, $n = 83$; ANOVA: $F(1, 157) = 37.42$, $P < 0.001$) and for U-crit30s (*Round 1*: $\mu = 30.9 \pm 0.87$ cm/s, $n = 76$; *Round 2*: $\mu = 40.3 \pm 1.27$ cm/s, $n = 83$; ANOVA: $F(1, 157) = 36.39$, $P < 0.001$) (Fig. 2.1).

Parental genetic effects on offspring swimming performance

For *round 1* of swimming, there was no significant family effect on either U-crit10s or U-crit30s (Table 2.4). There was however, a significant family effect on both U-crit10s and U-crit30s for *round 2* of swimming, which explained 23% and 24% of the variation in U-crit respectively (Table 2.4) (Fig. 2.2). One-tailed independent *t*-tests for *round 2* of swimming showed that for U-crit10s the family with the highest U-crit ($\mu = 54.8 \pm 0.18$ cm/s) was different from the family with the lowest U-crit ($\mu = 21.1 \pm 2.13$ cm/s) ($t(1) = -15.74$, $P = 0.02$). Also, for U-crit30s the family with the highest U-crit ($\mu = 57.8 \pm 3.23$ cm/s) was different from the family with the lowest U-crit ($\mu = 22.9 \pm 0.21$ cm/s) ($t(1) = -10.79$, $P = 0.02$). No other U-crit pairs were statistically different from one another.

A two-way random factor ANOVA for *round 1* of swimming revealed that none of the three effects (dam, sire, and dam x sire) significantly affected offspring swimming performance for either U-crit10s or U-crit30s (Table 2.5) (Fig. 2.3a and b; Fig. 2.4a and b). However for *round 2* of swimming, the sire effect (additive genetic effect) became significant for U-crit10s and even more so for U-crit30s (Fig. 2.3c and d), explaining 14% and 25% of the variation respectively, but the dam (Fig. 2.4c and d) and interaction

effects were not significant (Table 2.5). For *round 2* of swimming, I estimated that additive genetic effects represented 56% of the total phenotypic variance in U-crit10s, and 100% for U-crit30s (Table 2.5). Tukey's posthoc for *round 2* for U-crit10s showed that offspring sired by male 511 had a significantly higher mean U-crit ($\mu = 41.4 \pm 3.19$ cm/s, $n = 16$) than offspring sired by male 504 ($\mu = 27.0 \pm 1.75$ cm/s, $n = 13$) ($P = 0.006$) (Fig.2.3c). Tukey's posthoc for *round 2* for U-crit30s showed that again offspring sired by male 511 had a significantly higher mean U-crit ($\mu = 46.4 \pm 3.37$ cm/s, $n = 16$) than offspring sired by male 504 ($\mu = 30.1 \pm 1.93$ cm/s, $n = 13$) ($P = 0.001$). The posthoc for *round 2* for U-crit30s also revealed that there is more divergence among sires (3 homogenous subsets) than U-crit10s where males 504, 506, and 507 are clustered together, and males 502, 503, 507 and 511 are clustered together, with some overlap between these two groups. (Fig. 2.3d).

Effect of performance cross on offspring swimming performance

For *round 1* of swimming, there was no effect of performance cross on either U-crit10s or U-crit30s (Table 2.6) (Fig. 2.5a). For *round 2* of swimming there was no effect for U-crit10s, but a significant effect for U-crit30s which explained 61% of the variation (Table 2.6) (Fig. 2.5b). Tukey's posthoc tests for *round 2* U-crit30s showed that offspring of the H/H performance cross had a significantly higher mean U-crit ($\mu = 46.1 \pm 1.84$ cm/s, $n = 23$) than offspring of the L/L performance cross ($\mu = 35.7 \pm 2.44$ cm/s, $n = 21$) ($P = 0.011$). It also revealed 2 homogenous subsets where crosses H/H and L/H are clustered together and crosses L/H, H/L, and L/L are clustered together (Fig. 2.5b).

2.4 Discussion

My study examined the role of genetic quality and maternal effects on offspring swimming performance in Chinook salmon, a species that demonstrates a nonresource-based mating system. I determined the offsprings' U-crit as a method to compare swimming ability among families at two time points for each family, once at approx. 15 weeks post-hatch ('*round 1*') and then again at approx. 18 weeks post-hatch ('*round 2*'). Values of U-crit significantly improved from *round 1* to *round 2* of swimming, indicating that even being only approximately 3 weeks older allows Chinook to have enhanced swimming ability. Interestingly, U-crit was not affected by offspring body size, similar to previous studies outlined in Table 2.1 (Huuskonen *et al.*, 2009; Nadeau *et al.*, 2009; Kekalainen *et al.*, 2010b). As Kekalainen *et al.* (2010b) mention, this could indicate that the offspring body size traits measured in our study (fork length and wet weight) and swimming performance (U-crit) are separate measures of fitness. However, it is known that rapidly growing larvae may benefit from having their sense organs and swimming ability more developed, assisting in predator detection and escape (Bailey, 1984; Bailey & Batty, 1984; Fuiman *et al.*, 2004). The fact that I found that U-crit was not dependent on body size could be due to there not being enough variation in offspring body size to detect an effect, or possibly the measure of swimming ability I chose (U-crit), but perhaps the two offspring fitness-related traits are in fact independent of each other.

The sire component of variance (and not dam or dam x sire effects) was the only factor explaining genetic variation in U-crit. Furthermore, and perhaps most interesting is that the paternal effect was only significant for *round 2* of swimming, suggesting an increasing role of paternal effects (i.e. additive genetic effects) on offspring swimming.

The fact that paternal identity only had a significant effect on swimming for *round 2* supports the notion that it is important to consider the current age of the offspring when examining the genetic architecture of traits (Heath & Blouw, 1998; Wilson & Réale, 2006; Kruuk *et al.*, 2008; Evans *et al.*, 2010), especially when comparing results between studies. The paternal effect only explained 14% of the variation in U-crit10s and 25% of the variation in U-crit30s. That I was only able to explain a small amount of variation could be due to low sample size creating too much variation with groups. However, since the contribution of additive genetic effects to phenotypic variation increased from *round 1* to *round 2* of swimming (from 0% to 56% for U-crit10s and from 27% to 100% for U-crit30s), it would be interesting to see if this trend continues as the offspring age, and indicates the necessity of future research on genetic quality throughout ontogeny. The only study from Table 2.1 that I can compare my results to is Nadeau *et al.* (2009), as they tested for maternal and paternal effects in offspring of a similar age to ours. They did not find a paternal effect on juvenile burst swimming, however their design involved four blocks where each block represented one of four males, and so it is possible that the effect could have been confounded by block. Evidence for paternal effects later in life exists on other traits in offspring, such as on parasite resistance in 3 month old sticklebacks (Barber *et al.*, 2001), and on length in juvenile brown trout (Serbezov *et al.*, 2010). However, it is important to recognize that paternal effects have also been shown to play a role on various traits in very young offspring (e.g. Wedekind *et al.*, 2001; Bang *et al.*, 2006; Polacik & Reichard, 2009; Huuskonen *et al.*, 2011). Evans *et al.* (2010) followed the survival and growth of Chinook salmon offspring from larval to juvenile stage, showing a shift from maternal to additive genetic effects on survival across those

developmental stages, and strong additive effects on juvenile length, adding to the importance of considering age when examining and comparing the genetic architecture of traits.

That I did not find any dam effects (neither genetic nor maternal environmental) is most likely due to the age of the fish when measured, as maternal effects on offspring phenotypic traits are known to decrease over time (reviewed in Heath & Blouw, 1998). Interestingly, the dam component of variance was actually lower than the sire component of variance (for all except for *round 1*, Ucrit10s), which is theoretically not possible since the dam component of variance includes both maternal additive genetic effects and maternal environmental effects (Lynch & Walsh, 1998). This finding is therefore indicative of negative maternal effects as was found by Heath *et al.* (1999), which are masking additive genetic effects from dams. In Chinook salmon, Heath *et al.* (1999) found that maternal effects were high initially on offspring size during the larval stage of development, but then decreased to the point of becoming negative during the next developmental stage (fry stage). In their study, the negative maternal effects occurring at the fry stage corresponded to a change in offspring growth rate, where offspring at this age that hatched from smaller eggs actually had faster growth than offspring from larger eggs (Heath *et al.*, 1999). That I found that the dam component of variance was less than the sire component of variance at the parr stage of development supports the notion by Heath *et al.* (1999) that the decrease in maternal effects is not always a steady decrease to zero, but instead can have a negative effect before finally having no effect. Previous genetic studies on offspring swimming (see Table 2.1) that found maternal effects were conducted on newly-hatched larvae (Green & McCormick, 2005; Huuskonen *et al.*, 2009;

Kekalainen *et al.*, 2010b), whereas I swam my fish at approx. 3.5 and 4 months after hatching. Furthermore, Nadeau *et al.* (2009), which also used older juvenile salmon, did not find maternal effects on offspring burst swimming and report that they too may have seen maternal influences had they conducted tests sooner after hatching. I also suspect, given previous research mentioned above, that I would have seen maternal effects had I measured newly-hatched larvae. By reviewing previous research (Table 2.1) and combining my results, it seems that dams play the prominent role influencing offspring swimming ability at early ages, which switches to sires being the more prominent influence later in life. An exception to the idea of maternal effects early on is Nicoletto's (1995) study on guppies (*Poecilia reticulata*), where they found maternal effects on U-crit in adult offspring. A possible explanation for this finding is that guppies are live-bearers and thus life history (such as oviparity versus viviparity) can affect the role of non-genetic maternal effects (Heath & Blouw, 1998). As such, it is likely that when comparing the genetic architecture of offspring traits of fishes with varying life histories (e.g. an ovoviviparous guppy and an egg laying, semelparous salmonid), the findings could be quite diverse. Overall, considering that I found a paternal influence on U-crit when older, I suggest that during *round 1* I swam the offspring at an 'intermediate' age where the genetic architecture on this trait was shifting from predominantly maternal influences to paternal influences, revealing additive genetic variation from 'good genes' that are finally detected during *round 2* of swimming. Further analyses throughout ontogeny are necessary to confirm this given that no one study currently exists that follows the genetic architecture of offspring swimming ability throughout life-stages (Table 2.1).

My finding of a paternal effect on U-crit at a later age ties into my investigation on the effect of performance cross on offspring swimming performance. There was no effect of performance cross on U-crit for *round 1* of swimming, however there was a significant effect for *round 2*, with offspring of the H/H cross having a significantly higher mean U-crit than offspring of the L/L performance cross. Interestingly, the L/H performance cross (when sires were high performance) had a higher mean U-crit than the H/L performance cross (when dams were high performance), providing further evidence that additive genetic variation from sires seems to be playing a more important role (on U-crit, at this age) than additive genetic variation from dams. Overall, the posthoc tests indicate that if offspring acquire an ‘L’ (from either parent) their mean U-crit is no better than the ‘L/L’s, but if offspring acquire an ‘H’ from their father they will have a higher mean U-crit. This section of my study indicates that indeed, the existing ‘high-survival line’ and ‘low-survival line’ of Chinook salmon also show differences in swimming ability, and provides further evidence that there is a genetic component to swimming ability and that sires have a stronger influence on parr swimming.

As part of my methodology for measuring swimming performance, I chose to measure U-crit when an individual was fatigued for 10 seconds, but continued the test and measured U-crit again for that same fish when fatigued for 30 seconds. The purpose of this was to evaluate if 10 seconds of rest was enough to identify that an individual was fatigued, since I did not use an electrified grid (e.g. Anglea *et al.*, 2004) on the downstream screen to stimulate swimming. Other U-crit tests using salmon that did not use an electrified grid have used varying criteria for ‘fatigue’, such as being impinged for longer than 5 seconds (Peake *et al.*, 1997), for 30 seconds (Wagner *et al.*, 2003), or

ceasing to swim after providing decreases in velocity, and then returning to the fatigue speed (Alsop & Wood, 1997; Katzman & Cech Jr, 2001). During my swim trials, after resting for only 10 seconds, many fish would continue to swim for much longer, indicating that 30 seconds of rest is needed to identify actual fatigue. However, all of the results for parental genetic effects on swimming were similar for U-crit10s vs. U-crit30s. This indicates that 10 seconds of rest may be enough to identify initial fatigue if experimental time is limited, however 30 seconds of fatigue might be a more accurate estimation of true U-crit. It is also worthwhile to note that the mean U-crit values were higher for U-crit30s than for U-crit10s. Therefore, researchers should be cautious when comparing the critical swimming speeds of fishes if different methodology is used and I suggest that future studies on genetic effects on swimming use a fatigue cut-off closer to 30 seconds for a more robust indicator of effects.

Specifically, U-crit is relevant for investigating endurance and aerobic capacity (Fisher and Leis, 2009), and as mentioned earlier, is associated with the swimming abilities of fishes and thus linked to their survival (Plaut, 2001). One might therefore propose the following question; are those individuals with higher critical speeds more fit to withstand selection pressures? I swam my study fish (which are ocean-type Chinook) at approx. 3.5 and 4 months after hatching. Around this time in the wild, some juvenile (ocean-type) Chinook salmon are leaving their freshwater natal stream where they were born, and are migrating to the ocean (Healey, 1991). Downstream migration can be actively directed in Chinook parr (Healey, 1991), and young Chinook hold their station in the water current to obtain drifting food (Childerhose & Trim, 1979). Therefore, measuring U-crit for Chinook salmon at the age I did may be an indication of their ability

to remain against the current, and thus feed. Perhaps better swimming offspring would have higher survival rates and so those genes would be passed on, but this remains to be explicitly tested.

In conclusion, I found increasing paternal effects (additive genetic variation) on offspring swimming performance in older juvenile Chinook salmon. Overall, there is a severe gap in the knowledge of genetic effects on offspring swimming performance, despite the critical importance of swimming to survival. In the few studies that have been done (Table 2.1), effects range from no genetic contribution up to and including full maternal and paternal effects and both additive and nonadditive genetic variation. Given the different life-stages studied and measures of swimming ability used, the range of genetic effects seen is perhaps not surprising but highlights that expanded research effort is needed in this area. In the future, I would like to see research that explores genetic quality on offspring swimming performance throughout ontogeny, from newly-hatched larvae through maturation. This would allow one to fully map the changing patterns in the genetic architecture of this trait which is so important for the survival of fishes and ultimately to explain variation in fitness. Additional future research could also incorporate sexual selection by conducting a mate choice experiment, to determine for example if female Chinook are able to ‘detect’ the higher quality sires. If so, these females could acquire indirect benefits from ‘good genes’ by producing better swimming offspring.

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Table 2.1 Summary of the 7 studies to my knowledge that exist on parental genetic effects on offspring swimming ability of fishes. The reference, stage of ontogeny – species, if parental care occurred and by who, what the authors used as a measure of swimming performance, and the results for each study are given. For the result, if maternal, paternal, or interaction effects are not mentioned, it means they were not tested for.

Reference	Stage - Species	Parental Care?	Measure of Swim Performance	Result
Nicoletto, 1995	Sexually mature adults -guppy (<i>Poecilia reticulata</i>)	No	Critical swimming speed (U-crit)	Maternal effects (No paternal or interaction effects)
Garenc <i>et al.</i> , 1998	Juveniles – threespine sticklebacks (<i>Gasterosteus aculeatus</i>)	Yes – by males during egg incubation and early post-hatch	Burst-swimming	Inter-family differences significant at 2 months but NOT at 3.6 months
Evans <i>et al.</i> , 2004	Newborns -guppy (<i>Poecilia reticulata</i>)	No	Anti-predator behaviour (schooling,	Paternal effects on capture time only

			swimming speed response, & ability to evade capture	
Green & McCormick, 2005	Newly-hatched larvae – tropical clownfish <i>(Amphiprion melanopus)</i>	Yes – paternal egg care	Critical swimming speed (U-crit)	Maternal effects (No paternal or interaction effects)
Huuskonen <i>et al.</i> , 2009	Newly-hatched, yolk-sac larvae – whitefish <i>(Coregonus lavaretus)</i>	No	Time of fatigue against gravity- driven flow	Maternal and family effects (No paternal or interaction effects)
Nadeau <i>et al.</i> , 2009	Juveniles – sockeye salmon <i>(Oncorhynchus nerka)</i>	No	Burst-swimming	No maternal or paternal effects
Kekalainen <i>et al.</i> , 2010b	Newly-hatched, yolk-sac larvae - whitefish <i>(Coregonus</i>	No	Time of fatigue against gravity- driven flow	All 3 parental effects significant: Maternal,

lavaretus)

paternal, and

interaction

effects

Table 2.2 6x6 Breeding design with ‘performance crosses’ identified. Dam identification numbers and their corresponding performance crosses are displayed vertically and bolded; sire identification numbers and their corresponding performance crosses are displayed horizontally and bolded. The resulting offsprings’ performance cross for all 36 families is shown.

		L	H	L	L	H	H
	Dam/Sire	502	503	504	506	507	511
L	15	L/L	L/H	L/L	L/L	L/H	L/H
L	26	L/L	L/H	L/L	L/L	L/H	L/H
L	38	L/L	L/H	L/L	L/L	L/H	L/H
H	41	H/L	H/H	H/L	H/L	H/H	H/H
H	46	H/L	H/H	H/L	H/L	H/H	H/H
H	49	H/L	H/H	H/L	H/L	H/H	H/H

Table 2.3 Velocity of water at each rheostat voltage for swim trials.

Voltage	Water velocity (cm/s)	Velocity increment (cm/s)
60	16.95	
		6.13
65	23.08	
		5.22
70	28.30	
		4.67
75	32.97	
		5.49
80	38.46	
		2.64
85	41.10	
		10.62
90	51.72	
		10.78
95	62.50	
		-1.28
100	61.22	
		-3.53
105	57.69	

Table 2.4 Summary of the one-way random factor ANOVA results for family effects on offspring U-crit10s and U-crit 30s, for both rounds of swimming. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), F statistic, *P*-value, and the variance component (σ^2) with the percent of total variance (% total var) explained. Significant values ($P < 0.05$) are indicated in bold.

Source of variation	DF	SS	MS	<i>F</i>	<i>P</i>	σ^2 (% total var)
ROUND 1						
<i>U-crit10s</i>						
Family	28, 47	1225.4	43.8	1.225	0.264	3.1 (8)
Residual	47					35.7 (92)
<i>U-crit30s</i>						
Family	28, 47	2009.6	71.8	1.488	0.112	9.0 (16)
Residual	47					48.2 (84)
ROUND 2						
<i>U-crit10s</i>						
Family	31, 51	5400.1	174.2	1.765	0.035	29.0 (23)
Residual	51					98.7 (77)
<i>U-crit30s</i>						
Family	31, 51	5745.5	185.3	1.818	0.029	32.1 (24)
Residual	51					101.9 (76)

Table 2.5 Summary of the two-way random factor ANOVA results for parental effects (dam, sire, and their interaction) on offspring U-crit10s and U-crit30s for both rounds of swimming. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), F statistic, *P*-value, and variance component (σ^2) with the percent of total variance (% total var) explained by each source (negative variance components are treated as zero). The table also includes the percent of phenotypic variance (% phenotypic var) from maternal effects, and additive and nonadditive genetic effects. Significant values ($P < 0.05$) are indicated in bold.

Source of variation	DF	SS	MS	<i>F</i>	<i>P</i>	σ^2 (% total var)	% phenotypic var
ROUND 1							
<i>U-crit10s</i>							
Dam	5, 18.2	186.5	37.3	0.729	0.610	-0.9 (0)	Maternal 0
Sire	5, 18.6	108.4	21.7	0.425	0.825	-1.9 (0)	Additive 0
Dam x Sire	18, 47	922.9	51.3	1.435	0.160	6.0 (14)	Nonadditive 57
Residual	47					35.7 (86)	
<i>U-crit30s</i>							
Dam	5, 18.3	357.1	71.4	1.303	0.306	1.1 (2)	Maternal 0
Sire	5, 18.7	569.9	114.0	2.083	0.113	3.8 (7)	Additive 27
Dam x Sire	18, 47	987.7	54.9	1.138	0.349	2.5 (4)	Nonadditive 18
Residual	47					48.2 (87)	

Table 2.5 continued

ROUND 2

U-crit10s

Dam	5, 21.8	477.2	95.4	0.661	0.656	-3.2 (0)	Maternal	0
Sire	5, 21.8	2213.2	442.6	3.069	0.030	19.0 (14)	Additive	56
Dam x Sire	21, 51	3055.3	145.5	1.474	0.130	18.0 (13)	Nonadditive	53
Residual	51					98.7 (73)		

U-crit30s

Dam	5, 22.2	769.2	153.8	1.596	0.202	3.7 (3)	Maternal	0
Sire	5, 22.2	3235.4	647.1	6.713	0.001	35.3 (25)	Additive	100
Dam x Sire	21, 51	2021.0	96.2	0.944	0.541	-2.2 (0)	Nonadditive	0
Residual	51					101.9 (72)		

Table 2.6 Summary of the one-way fixed factor ANOVA results for performance cross effects on offspring U-crit10s and U-crit30s for both rounds of swimming. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), F statistic, *P*-value, and the variance component (σ^2) with the percent of total variance (% total var) explained (negative variance components are treated as zero). Significant values ($P < 0.05$) are indicated in bold.

Source of variation	DF	SS	MS	<i>F</i>	<i>P</i>	σ^2 (% total var)
ROUND 1						
<i>U-crit10s</i>						
Performance cross	3, 72	45.4	15.1	0.381	0.767	-9.4 (0)
Residual	72					9.7 (100)
<i>U-crit30s</i>						
Performance cross	3, 72	51.7	17.2	0.293	0.830	-15.9 (0)
Residual	72					56.7 (100)
ROUND 2						
<i>U-crit10s</i>						
Performance cross	3, 79	838.9	279.6	2.302	0.083	60.8 (33)
Residual	79					121.5 (66)
<i>U-crit30s</i>						
Performance cross	3, 79	1754.0	584.7	5.026	0.003	180.1 (61)
Residual	79					116.3 (39)

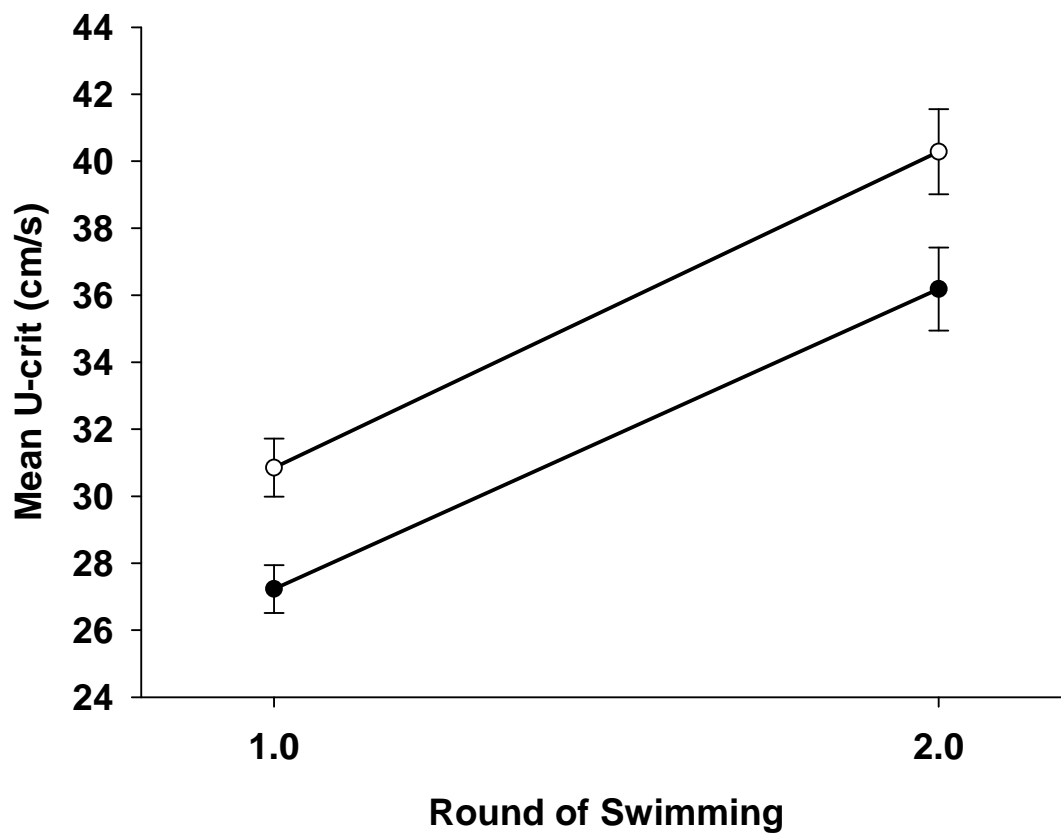


Fig. 2.1 Critical swimming speeds (U-crit) significantly improved from *round 1* to *round 2* of swimming, for both U-crit10s and U-crit30s. The filled circles represent U-crit10s, and the open circles U-crit30s.

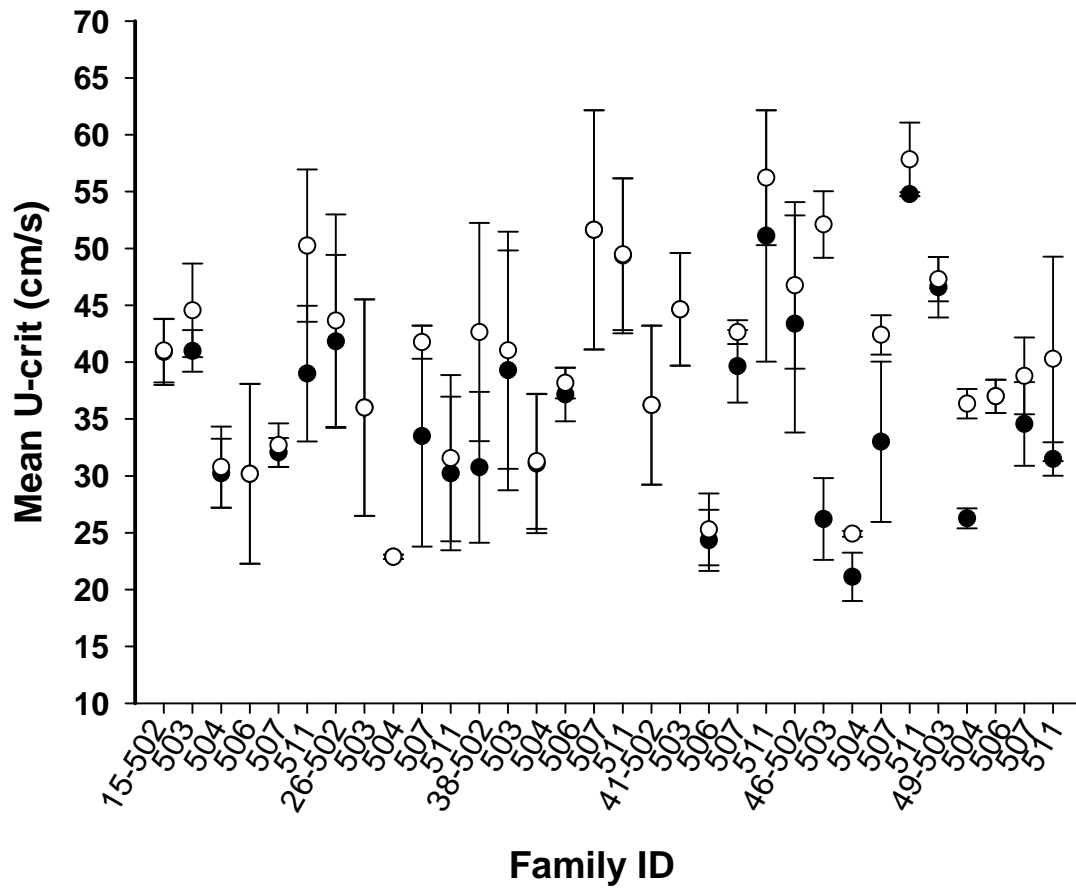


Fig. 2.2 Variation in offspring mean critical swimming speed (U-crit) due to family for *round 2* of swimming. The filled circles and solid line represent U-crit10s, and the open circles and dashed line U-crit30s.

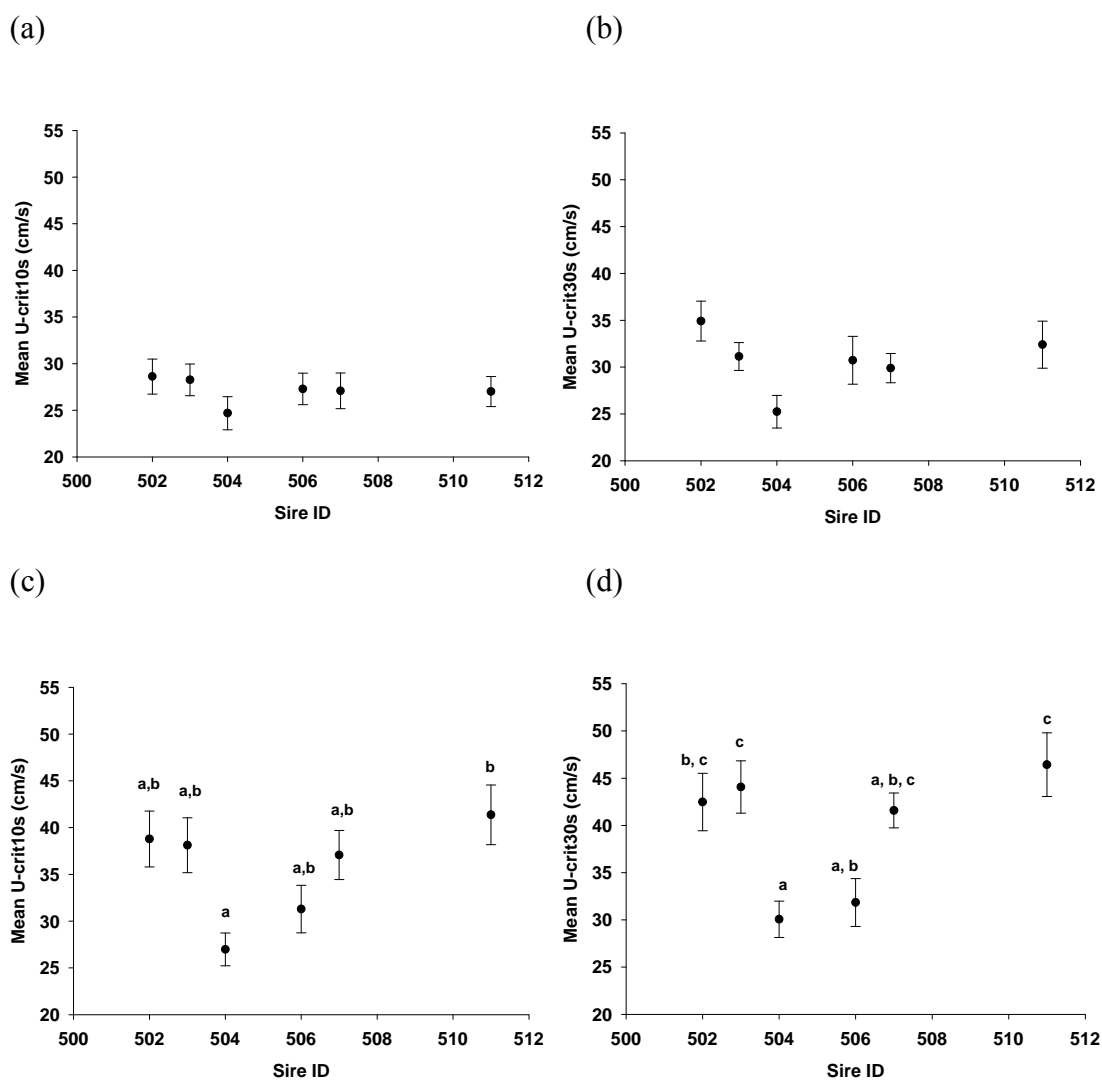


Fig. 2.3 Sire effect on offspring mean critical swimming speed (U-crit). *Round 1* of swimming for (a) U-crit10s and (b) for U-crit30s. *Round 2* of swimming for (c) U-crit10s and (d) for U-crit30s. The pattern is non-significant for *round 1* of swimming but is significant for *round 2* for both U-crit10s and U-crit30s, with the letters ‘a’, ‘b’, and ‘c’ denoting the homogenous subsets.

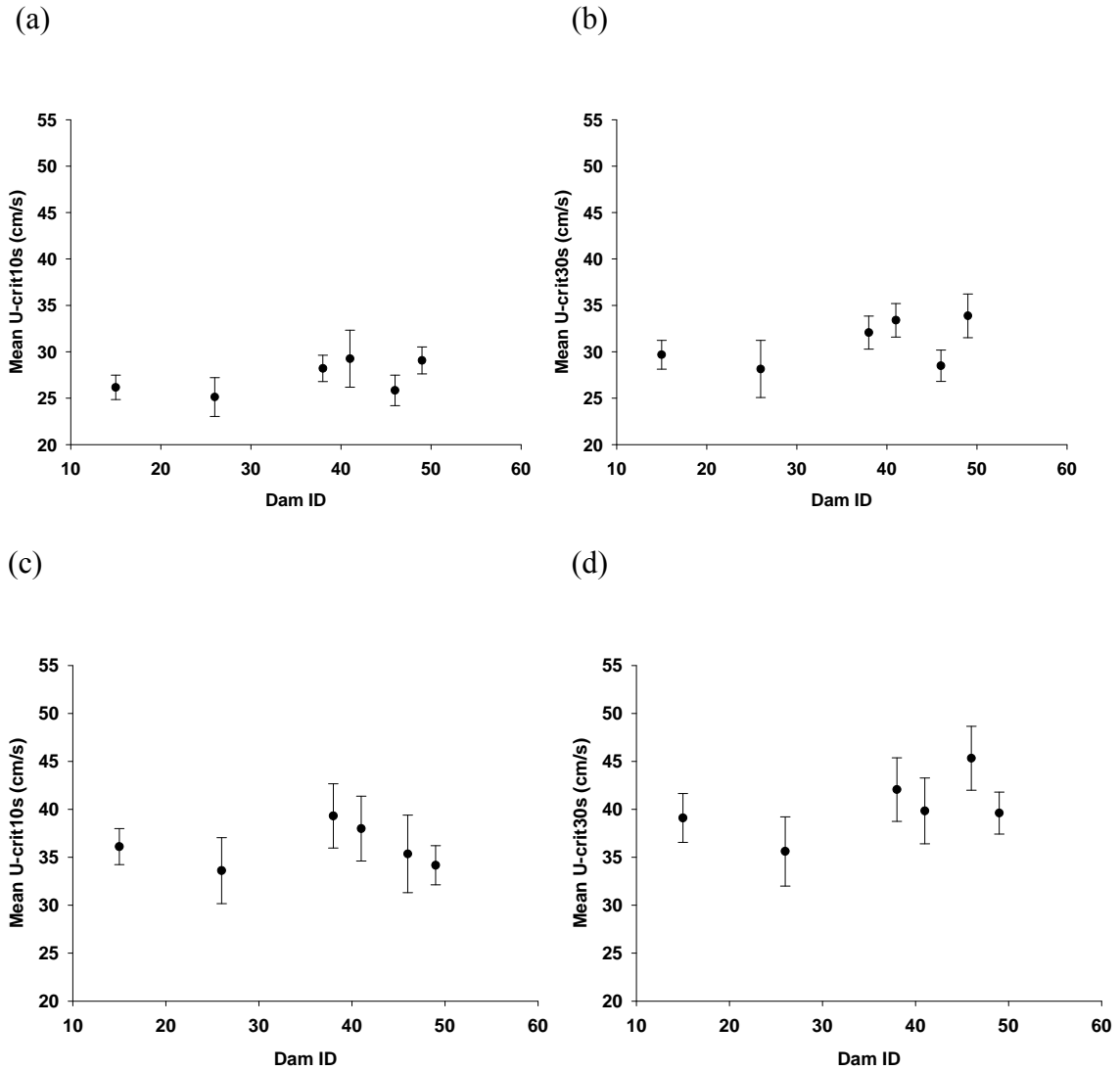


Fig. 2.4 Dam effect on offspring mean critical swimming speed (U-crit). *Round 1* of swimming for (a) U-crit10s and (b) for U-crit30s. *Round 2* of swimming for (c) U-crit10s and (d) for U-crit30s. The patterns are non-significant.

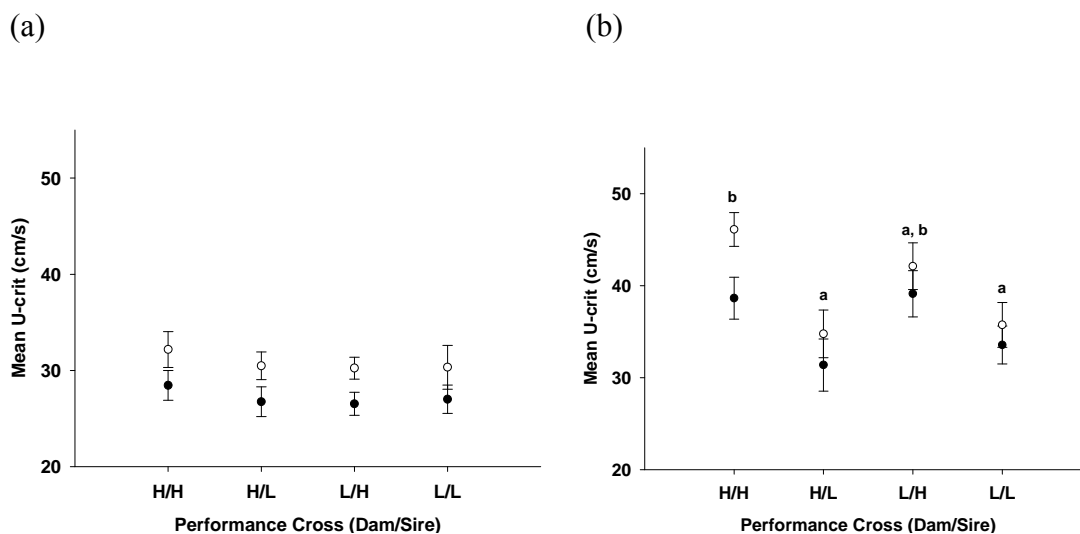


Fig. 2.5 Effect of performance cross on offspring mean critical swimming speed (U-crit) for both U-crit10s and U-crit30s for (a) *round 1* of swimming and for (b) *round 2* of swimming. The filled circles represent U-crit10s, and the open circles U-crit30s. For the performance crosses, the first letter indicates the performance line of the dam, and the second letter indicates the performance line of the sire. The trend is significant for *round 2* U-crit30s, and the letters ‘a’ and ‘b’ denote the homogenous subsets.

CHAPTER III

**VARIATION IN CONTRIBUTIONS OF ADDITIVE AND NONADDITIVE
GENETIC EFFECTS AND MATERNAL EFFECTS ON LENGTH AND
SURVIVAL THROUGHOUT ONTOGENY IN CHINOOK SALMON
(*ONCORHYNCHUS TSHAWYTSCHA*)**

3.1 Introduction

Phenotypic traits in offspring may show inter-individual variation due to differences in the genetic quality of their parents (e.g. Bilde *et al.*, 2008; Klemme *et al.*, 2008). ‘Genetic quality’ is comprised of two components, both ‘good genes’ and ‘compatible genes’, whereby offspring traits (e.g. disease resistance) may show additive genetic variation from good genes and/or nonadditive genetic variation from compatible genes (reviewed in Neff & Pitcher, 2005). Research on either one or both of these components of genetic quality are most ideally studied in mating systems where only genes are provided to offspring, known as ‘nonresource-based mating systems’ as opposed to ‘resource-based mating systems.’ In resource-based mating systems, offspring receive not only genes but additional parental care such as food, shelter and protection from predators, which can also affect offspring characteristics (Neff & Pitcher, 2005). Nonresource-based mating systems are therefore better suited for investigating the effects of genetic quality on a desired trait in offspring, as other confounding factors like direct benefits influencing intra-specific variation among offspring can be avoided (Neff & Pitcher, 2005; Wedekind *et al.*, 2008; Hettyey *et al.*, 2010).

Studies on parental genetic effects on offspring performance traits are plentiful in fishes (e.g. Wedekind *et al.*, 2001, Wedekind *et al.*, 2008; Huuskonen *et al.*, 2009; Polacik & Reichard, 2009; Rodriguez-Munoz & Tregenza, 2009; Jacob *et al.*, 2010), perhaps because not only do many species have nonresource-based mating systems, but many are also external fertilizers (Hettyey *et al.*, 2010). Thus, investigators can extract and artificially cross the gametes of dams and sires to produce full factorial breeding designs (i.e. North Carolina Design II) (Lynch & Walsh, 1998). In fishes with no parental care, investigators must also however consider maternal (i.e. non-genetic) effects on offspring. Although there may be no parental care, dams can influence the quality of her offspring by the amount of nutrients provisioned in her eggs, hormones and cytoplasm, and by where she chooses to deposit her eggs (reviewed in Green, 2008). Although numerous studies exist on parental effects on offspring performance traits in fishes (e.g. in whitefish, *Coregonus* sp., Wedekind *et al.*, 2001; in striped bass, *Morone chrysops* x *Morone saxatilis*, Wang *et al.*, 2006; in sockeye salmon, *Oncorhynchus nerka*, Nadeau *et al.*, 2009), not all used species with nonresource-based mating systems; thus results are likely confounded by parental non-genetic effects (Neff & Pitcher, 2005; Hettyey *et al.*, 2010). Additionally, not all previous studies further partitioned the observed parental effects (dam, sire, and dam x sire) into contributions of additive and nonadditive genetic effects and maternal effects to the phenotypic variance in the trait measured (Neff & Pitcher, 2005; Bang *et al.*, 2006). Since the ‘dam’ factor includes both genetic and maternal effects, failure to partition genetic effects further only allows assessment of ‘female effects’, a broader term which encompasses both genetic and maternal influences (Green, 2008). Thus without further partitioning one cannot determine if significant

female effects are because of the mothers' genetics, non-genetic contributions, or both (Bang *et al.*, 2006; Green, 2008). Using the North Carolina Design II to its full potential is advantageous, as one can separate the dam component of variance into its two elements (Lynch & Walsh, 1998; for an example on larval spinal deformity see Evans & Neff, 2009). By performing a full factorial breeding design, all possible crosses are carried out between a group of dams and a group of sires. Thus, variance in the measured phenotypic trait can be partitioned among additive genetic effects, nonadditive genetic effects, and true maternal (non-genetic) effects (Lynch & Walsh, 1998; reviewed in Neff & Pitcher, 2005; Puurtinen *et al.*, 2009).

When considering the genetic architecture of phenotypic traits, recent research shows that the stage of ontogeny is influential, as the genetic architecture of traits can vary within individuals across their life stages (Heath & Blouw, 1998; see Evans *et al.*, 2010 and references therein). In Chinook salmon (*Oncorhynchus tshawytscha*), during the larval stage maternal effects are the prominent factor affecting survival but by the parr stage, additive genetic effects affect survival and maternal effects no longer do, presenting evidence for a shift from maternal to genetic influences on offspring survival (Evans *et al.*, 2010). Similarly, variation in genetic architecture with age was found in early embryo mortality vs. late embryo mortality in Alpine whitefish (*Coregonus zugensis*) (Wedekind *et al.*, 2008). Previous studies have shown significant heritabilities and genetic components to performance traits like body size and survival (e.g. Gjerde & Schaeffer, 1989; Silverstein & Hershberger, 1995; Choe & Yamazaki, 1998; Hard *et al.*, 1999; Funk *et al.*, 2005; Bang *et al.*, 2006; Ma *et al.*, 2008; Nielsen *et al.*, 2010). To my knowledge however, no one study currently exists that has followed the contributions of

additive and nonadditive genetic effects and maternal effects to variation in a phenotypic trait in fish from larva through to sexual maturation. In the current study I used Chinook salmon, an externally fertilizing species with a nonresource-based mating system (Healey, 1991), in a North Carolina Design II method performing all possible crosses between 7 dams and 7 sires to examine dam, sire, and dam x sire effects on offspring body size and survival. I followed the two performance traits in the offspring for 3 years (from hatching through to the adult stage). I also estimated the contributions of additive genetic effects, nonadditive genetic effects, and true maternal effects to offspring length and survival at several time points throughout ontogeny. The second part of the current study takes advantage of the fact that the dams and sires utilized belong to either a ‘high-survival line’ or a ‘low-survival line,’ created from a marker-assisted broodstock selection program initiated in 1997 that identified two separate salmon lines based on variation in growth- and survival-related gene markers (Docker & Heath, 2002). The fully crossed breeding design therefore created offspring that were purebred or hybrid for the high- and low-survival lines, allowing me to determine if the different survival lines currently still possess differences in size and survival. Additionally, since our study spans larval stages through to adulthood, I was able to determine at what stage of development any differences in survival and size between the two lines manifested.

3.2 Methods

Study Species, Breeding Design and Rearing Conditions

Study Species and Breeding Design: In the fall of 2008, I haphazardly selected 7 female and 7 male sexually mature (4 year old) Chinook salmon to create 49 half- and

full-sib families in a North Carolina Design II, which crosses the gametes of all dams and sires in every pair-wise combination (Lynch & Walsh, 1998). The dams and sires used in the current study were 7th generation descendants originating from crosses between wild females taken from the Robertson Creek Hatchery (Port Alberni, B.C.) and wild males taken from Big Qualicum River Hatchery (Qualicum Beach, B.C.) in 1985, and raised at the Yellow Island Aquaculture Ltd (YIAL) hatchery and netcage site on Quadra Island, British Columbia. In 1997, YIAL began a marker-assisted broodstock selection program creating two differential survival lines (termed a ‘high-survival line’ and a ‘low-survival line’) based on variation in growth and survival related gene markers (Docker & Heath, 2002). The descendants I used in the current study were also from these two lines. The dams and sires for the current study were haphazardly selected until 3 of the dams were from the high-survival line and 4 were from the low-survival line (and same for the sires), with identity established from previously implanted coded wire tags inserted into the nose of each fish. From here on, the high-survival line (H) and the low-survival line (L) will be referred to as ‘performance crosses’. The full factorial breeding design created offspring that were one of the following four performance crosses: ‘H/H’ where both parents were of the high-survival line, ‘H/L’ where the dam is of the high-survival line and the sire is of the low-survival line, ‘L/H’ which is the opposite of the previous, or ‘L/L’ where both parents are of the low-survival line. All procedures were approved by the University of Windsor Animal Use and Care Committee.

Rearing Conditions: The selected adult salmon were sacrificed via cerebral concussion, and gametes were extracted for artificial fertilizations, where an approximately equal amount of eggs from each female were fertilized by each male. I

split fertilized eggs from a family into two cells in Heath trays to account for location effects, therefore requiring 98 cells (2 per family). During incubation the Heath trays were exposed to natural, untreated fresh water (from an artesian well) that ranged from 7°C to 9°C. UV-treated salt water was pumped through the trays for 30 minutes a day, 3 times a week to reduce fungus growth until hatching. The incubation trays were checked every other day until the end of the endogenous feeding stage to remove all unfertilized eggs and dead offspring.

At the end of the endogenous feeding stage in March 2009, all larvae from a family were transferred to a 200 L barrel, therefore requiring 49 barrels. However, if the offspring count in a family barrel exceeded 150 individuals, the remainder were transferred to a new barrel, but only the original barrels were considered for this study. Heath *et al.* (1999) which used a similar rearing design did not find a correlation between rearing density (which could be different due to mortality differences among families) and growth. All barrels were cared for equally with flow-through fresh water ranging from 7°C to 10.5°C, aeration, and light from 7am – 5pm. Fish care consisted of feeding the offspring daily with EWOS feed (EWOS Canada Ltd.), vacuuming the barrels every 5 days, and removing any dead offspring.

In June 2009, a sample of 30 parr (unless there were fewer remaining individuals) from each family were anaesthetized with clove oil and injected with Passive Integrated Transponder (PIT) tags to allow individual identification. All tagged offspring from every family were then transferred to one 15 x 15 x 20 ft netpen at YIAL in the Pacific ocean. Offspring were reared to adulthood, where in June 2010 all individuals were transferred to a bigger netpen 15 x 30 x 30 ft, and then later transferred once again to a new netpen

(15 x 30 x 30) in June 2011. In November 2010, any males that had become ‘Jacks’ were removed from the netpen; the removal of Jacks is only relevant for this study in terms of survival, meaning that I must take into account that fish were removed and thus should not be considered ‘dead’. During ocean life, fish were fed twice a day (Taplow Grower, Taplow Ventures Ltd.). Any mortalities were retrieved and scanned for their PIT tag, to identify their dam and sire.

Body size measurements

As indicators of body size, I measured fork length and wet weight. I measured offspring body size 5 times throughout ontogeny as follows: ‘*Date 1*’ = March 2009/End of larval stage; ‘*Date 2*’ = June 2009/Parr stage; ‘*Date 3*’ = November 2009/Juvenile stage; ‘*Date 4*’ = June 2010/Adult stage; and ‘*Date 5*’ = June 2011/Adult stage. For *Date 1*, I measured a sample of 20 fish per family. For *Date 2* I measured all PIT-tagged fish, which was 30 fish per family unless there were fewer remaining individuals. For *Dates 3, 4* and *5* I measured all PIT-tagged fish that were still alive at the sample date.

Survival measurements

To calculate survival, it was important that I accounted for fish that were removed artificially during rearing so that they were not considered fish that were lost due to natural death. Thus, I calculated offspring percent survival as the # of individuals alive per family/ total # of individuals per family initially (after any removal). The denominator in this calculation accounts for when individuals were artificially removed which occurred at barreling, PIT tagging, and when Jacks were removed as explained above. I calculated offspring percent survival for each family at 4 times throughout

ontogeny, referred to as ‘*Dates A-D*’: ‘*Date A*’ = March 2009; ‘*Date B*’ = June 2009; ‘*Date C*’ = June 2010; and ‘*Date D*’ = June 2011 (Table 3.1).

Statistical Analysis

Body size

I was able to collect length data at all sample dates, but I was not able to collect weight data at *Date 4* when the fish were living in netpens in the ocean. This was due to technical difficulties of the scale I had available at the time not being able to tare in rough conditions. Thus, I chose to analyze only the length data since I had data for all sample dates, and since the length and weight data were highly correlated (see below). When testing the fork length and wet weight data for normality, the Kolmogorov-Smirnov test confirmed that the data were statistically not normal. However, Field (2009; pg. 144) states that a limitation of the K-S test is that it is very easy to get significant results from small deviation from normality when there are large sample sizes. Thus, a significant K-S test does not necessarily mean that the ‘deviation’ from normality will bias the results when analyzing the data and that one should examine the normality plots to view the scope of any non-normality (Field, 2009). The current study does indeed have very large sample sizes at each date length data were collected, and so I followed up the K-S test by viewing histograms and Q-Q plots of the data. Upon inspection of the fork length data, histograms showed bell-shaped curves and Q-Q plots revealed observed values that fell exactly along the straight line (except for a only a few points at the ends) indicating that the data were normal. This was not the case for some of the wet weight data indicating deviations from normality.

To confirm that I could use only the fork length data for my analyses, I performed a correlation test to determine if the length and weight data were correlated with one another. I chose to use the non-parametric Spearman's correlation coefficient since the weight data were not normal. The test confirmed that fork length and wet weight were highly correlated, $r = .99$, p (one-tailed) $< .001$. Thus, I used only fork length as an indicator of body size.

Parental genetic effects on offspring length

I used 5 one-way random factor ANOVAs (one for each sample date) to determine family effects on offspring fork length. I adjusted the alpha level to 0.013 (0.05/4) for all dates to account for the same individuals being measured throughout this study. To calculate the variance components I followed formulas given in Table 1 from Graham & Edwards (2001), and used the average for the sample size since they were unequal due to differences in mortality among families.

To further differentiate parental effects (from overall family effects) on offspring length, I used 5 two-way random factor ANOVAs (one for each date) using the adjusted alpha level of 0.013, to partition variance in offspring fork length to female identity (dam), male identity (sire), and their interaction (dam x sire). The variance components were calculated as mentioned above. The contribution of additive genetic effects to offspring fork length was calculated from four times the sire component of variance, the nonadditive genetic effects were calculated from four times the dam x sire component of variance, and the maternal effects were calculated from the difference between the dam and sire components of variance (reviewed in Neff & Pitcher, 2005). I used Tukey's

posthoc on significant results to determine which dams and sires differed in offspring length.

Performance cross effects on offspring length

To determine if the differential performance crosses affect offspring length, I used 5 one-way fixed factor ANOVAs (one for each date, using the adjusted alpha level of 0.013) with performance cross as the main effect. The variance components were calculated as mentioned above. I then used Tukey's posthoc on significant results to determine which performance crosses differed in length.

Survival

I first transformed the percent survival data using the arcsine square-root transformation, and tested the survival data for all dates for normality using the K-S test. All data were normal.

Parental genetic effects on offspring survival

To differentiate parental effects on offspring survival, I used 4 two-way random factor ANOVAs (one for each date) which allowed me to partition variance in offspring survival to female identity (dam) and male identity (sire). I was unable to obtain the interaction effect (dam x sire) for *Date A* and *Date B* since there is only one percent survival value per family and thus no variation for the ANOVA. I adjusted the alpha level to 0.017 (0.05/3) for all dates to account for the same individuals being measured throughout this study. Variance components were calculated in the same way as described above for length. Because the offspring were PIT tagged in June 2009, I was able to follow the survival of *each individual* from here on and could include this for analysis of survival at *Date C* and *Date D*. Thus, I performed logistic regression on those

last two dates as it is more powerful. Since I have many individuals per family, I can now obtain the interaction effect (dam x sire) for *Date C* and *Date D*. I used the adjusted alpha level of 0.017 (0.05/3) to be conservative.

In summary for '*parental genetic effects on offspring survival*,' I used a two-way random factor ANOVA for *Date A* and *Date B* and then logistic regression for *Date C* and *Date D*. However, I also performed a two-way random factor ANOVA for *Date C* and for *Date D* to obtain the mean squares to calculate the variance components. I used Tukey's posthoc for *Date A* and *Date B* on results to determine which dams and sires produced better performing offspring (denoting homogenous subsets in figures using letters). For *Date C* and *Date D* logistic regression provided which dams and sires produced offspring with significantly higher survival than the dam and sire with the lowest offspring survival (denoted by asterisks in figures; $P < 0.05$).

Performance cross effects on offspring survival

To determine if the differential performance crosses affect offspring survival at *Dates A-D*, I followed the same statistical procedures as explained immediately above for '*parental genetic effects on offspring survival*,' using both ANOVA and logistic regression and the adjusted alpha level of 0.017 (0.05/3). The only difference here is that performance cross effects required a one-way fixed factor ANOVA as opposed to a two-way random factor ANOVA for parental effects.

3.3 Results

Body size

Parental genetic effects on offspring length

Family significantly affected offspring fork length for all dates examined, explaining 48% of the variation in length for *Date 1*, 19% for *Date 2*, 17% for *Date 3*, 10% for *Date 4*, and 6% of the variation in length for *Date 5* (Table 3.2).

For *Date 1* (end of larval stage), dam and dam x sire significantly affected offspring fork length, explaining 38% and 12% of the variation respectively, but the sire effect was non-significant (Table 3.3). Maternal effects represented 38% of the total phenotypic variance in length while nonadditive genetic effects represented 49% of the total phenotypic variance in length. Tukey's posthoc revealed 5 homogenous subsets for dam effects. Dam ID # 7 produced offspring with the highest mean length ($\mu = 4.27 \pm 0.01$ cm, $n = 115$) and dam ID # 12 produced offspring with the lowest mean length ($\mu = 3.94 \pm 0.01$ cm, $n = 140$) (Table 3.3) (Fig. 3.1a; Fig. 3.2a).

For *Date 2* (parr stage), all three factors (dam, sire and dam x sire) significantly affected offspring fork length, explaining 15%, 2% and 4% of the variation respectively (Table 3.3). I estimated that maternal effects represented 13% of the total phenotypic variance in length. Additive genetic effects represented 8% of the total phenotypic variance in length, and nonadditive genetic effects represented 14% of the total phenotypic variance in length. Tukey's posthoc revealed 5 homogenous subsets for dam effects and 3 homogenous subsets for sire effects. Dam ID # 7 produced offspring with the highest mean length ($\mu = 8.02 \pm 0.04$ cm, $n = 121$) and dam ID # 12 produced offspring with the lowest mean length ($\mu = 7.36 \pm 0.03$ cm, $n = 210$). Sire ID # 226

produced offspring with the highest mean length ($\mu = 7.93 \pm 0.03$ cm, $n = 203$) and sire ID # 227 produced offspring with the lowest mean length ($\mu = 7.67 \pm 0.07$ cm, $n = 190$) (Table 3.3) (Fig. 3.1b; Fig. 3.2b).

For *Date 3* (juvenile stage), dam and sire significantly affected offspring fork length, explaining 13% and 4% of the variation respectively, and the interaction of dam x sire became non-significant (Table 3.3). I estimated that maternal effects represented 9% of the total phenotypic variance in length, and additive genetic effects represented 15% of the total phenotypic variance in length. Tukey's posthoc revealed 5 homogenous subsets for dam effects and 3 homogenous subsets for sire effects. Dam ID # 9 produced offspring with the highest mean length ($\mu = 16.5 \pm 0.06$ cm, $n = 173$) and dam ID # 12 produced offspring with the lowest mean length ($\mu = 15.5 \pm 0.08$ cm, $n = 155$). Sire ID # 226 produced offspring with the highest mean length ($\mu = 16.2 \pm 0.08$ cm, $n = 171$) and sire ID # 230 produced offspring with the lowest mean length ($\mu = 15.6 \pm 0.08$ cm, $n = 144$) (Table 3.3) (Fig. 3.1c; Fig. 3.2c).

For *Date 4* (adult stage), dam was the only factor that significantly affected offspring fork length explaining 11% of the variation (Table 3.3). I estimated that maternal effects represented 10% of the total phenotypic variance in length. Tukey's posthoc revealed 5 homogenous subsets for dam effects. Dam ID # 9 produced offspring with the highest mean length ($\mu = 23.3 \pm 0.19$ cm, $n = 138$) and dam ID # 12 produced offspring with the lowest mean length ($\mu = 20.9 \pm 0.22$ cm, $n = 114$) (Table 3.3) (Fig. 3.1d; Fig. 3.2d).

For *Date 5* (adult stage), dam and again sire significantly affected offspring fork length explaining 7% and 2% of the variation respectively, and the interaction of dam x

sire was non-significant (Table 3.3). I estimated that maternal effects represented 5% of the total phenotypic variance in length, and additive genetic effects represented 7% of the total phenotypic variance in length. Tukey's posthoc revealed 3 homogenous subsets for dam effects and 2 homogenous subsets for sire effects. Dam ID # 9 produced offspring with the highest mean length ($\mu = 43.7 \pm 0.42$ cm, $n = 82$) and dam ID # 12 produced offspring with the lowest mean length ($\mu = 40.5 \pm 0.42$ cm, $n = 69$). Sire ID # 227 produced offspring with the highest mean length ($\mu = 43.2 \pm 0.42$ cm, $n = 90$) and sire ID # 230 produced offspring with the lowest mean length ($\mu = 41.0 \pm 0.33$ cm, $n = 84$) (Table 3.3) (Fig. 3.1e; Fig. 3.2e).

Performance cross effects on offspring length

Performance cross significantly affected offspring fork length for *Date 1*, *Date 2*, *Date 3* and *Date 4* explaining 55%, 22%, 25%, and 16% of the variation respectively, but did not significantly affect length for *Date 5* (Table 3.4). Tukey's posthoc revealed 2 homogenous subsets for *Date 1*, *Date 2*, and *Date 4*, and 3 homogenous subsets for *Date 3*. For *Date 1*, the L/H performance cross produced offspring with the highest mean length ($\mu = 4.14 \pm 0.01$ cm, $n = 233$) and the H/H performance cross produced offspring with the lowest mean length ($\mu = 4.03 \pm 0.01$ cm, $n = 180$). For *Date 2*, the H/H performance cross produced offspring with the highest mean length ($\mu = 7.89 \pm 0.02$ cm, $n = 270$) and the L/L performance cross produced offspring with the lowest mean length ($\mu = 7.68 \pm 0.03$ cm, $n = 414$). For *Date 3*, the L/H performance cross produced offspring with the highest mean length ($\mu = 16.1 \pm 0.06$ cm, $n = 260$) and the H/H performance cross produced offspring with the lowest mean length ($\mu = 15.7 \pm 0.06$ cm, $n = 212$). For *Date 4*, the L/H performance cross produced offspring with the highest mean length ($\mu =$

22.3 ± 0.18 cm, n = 204) and the H/H performance cross produced offspring with the lowest mean length ($\mu = 21.6 \pm 0.14$ cm, n = 169) (Table 3.4) (Fig. 3.3).

Survival

Parental genetic effects on offspring survival

For *Date A* (end of larval stage), dam and sire both significantly affected offspring survival explaining 61% and 10% of the variation respectively, with mean survival from dams ranging from 13 – 78% and from sires ranging from 46 – 75%. I could not test the interaction effect (dam x sire). From the variance components, I estimated that maternal effects represented 51% of the total phenotypic variance in survival, and that additive genetic effects represented 40% of the total phenotypic variance in survival. Tukey's posthoc revealed 2 homogenous subsets for dam effects and 2 homogenous subsets for sire effects (Table 3.5) (Fig. 3.4a; Fig. 3.5a).

For *Date B* (parr stage), neither dam nor sire significantly affected offspring survival. Although non-significant, mean survival from dams ranged from 85 – 96% and from sires ranging from 84 – 95%. I could not test the interaction effect (dam x sire) (Table 3.5) (Fig. 3.4b; Fig. 3.5b).

For *Date C* (adult stage), logistic regression revealed that dam and dam x sire significantly affected offspring survival, and the sire effect was non-significant. By using the mean squares from the two-way random factor ANOVA, I calculated that the dam effect explained 12% of the variation in survival. I estimated that maternal effects represented 12% of the total phenotypic variance in survival. As mentioned previously, I could not obtain a value for the mean square for the interaction (dam x sire) effect. Thus, I could not calculate that variance component for this effect, or determine what

percentage of the total phenotypic variance that the nonadditive genetic effects represent. The logistic regression revealed that four of the dams produced offspring that had statistically ($P < 0.05$) higher survival than the dam that produced offspring with the lowest survival. The dams produced offspring with survival ranging from 55 – 72%. Although non-significant, sires produced offspring with survival ranging from 59 – 68% (Table 3.5) (Fig. 3.4c; Fig. 3.5c).

For *Date D* (adult stage), logistic regression revealed that the dam effect was the only factor significantly affecting offspring survival, explaining 27% of the variation. I estimated that maternal effects represented 26% of the total phenotypic variance in survival. Four of the dams produced offspring that had statistically ($P < 0.05$) higher survival than the dam that produced offspring with the lowest survival. The dams produced offspring with survival ranging from 34 – 57%. Although non-significant, sires produced offspring with survival ranging from 35 – 49% (Table 3.5) (Fig. 3.4d; Fig. 3.5d).

Performance cross effects on offspring survival

Performance cross significantly affected offspring survival for *Date D* only explaining 80% of the variation. Logistic regression revealed that for *Date D*, two of the performance crosses (H/H and H/L) produced offspring that had statistically ($P < 0.05$) higher survival than the performance cross that produced offspring with the lowest survival (L/H). For *Date D* due to performance cross, offspring survival ranged from 36 – 53% (Table 3.5) (Fig. 3.6).

3.4 Discussion

The current study presents the first investigation to follow the contributions of additive and nonadditive genetic effects and maternal effects to variation in phenotypic traits in fish from larval stages through to adulthood. I used the North Carolina Design II (Lynch & Walsh, 1998) breeding design to cross all dams and sires in every pair-wise combination, and followed the body size and survival of the offspring for three years in Chinook salmon. I determined offspring size (fork length) at five times throughout ontogeny, and offspring survivorship at four times throughout ontogeny, and partitioned the variation to additive and nonadditive genetic effects, and maternal effects. In previous studies that used species with nonresource-based mating systems to determine all three contributions (additive, nonadditive and maternal effects) to offspring size and survival, the results among studies were variable. For instance, effects on offspring growth range from both maternal and additive effects (in Chinook salmon fry, Evans *et al.*, 2010), to nonadditive and maternal effects but no additive effects (in larval Lake Ontario Chinook salmon, Pitcher & Neff, 2007), to additive effects on length but not on weight showing variation due to what measure of size was used (in larval Atlantic herring, Bang *et al.*, 2006). Similarly, effects on offspring survival range from nonadditive and maternal effects (in embryonic sea lamprey, Rodriguez-Munoz & Tregenza, 2009), to all three (additive, nonadditive and maternal) (in larval Lake Ontario Chinook salmon, Pitcher & Neff, 2007). The differences among the studies in contributions to phenotypic variation are likely due to environmental variation, various species used, and developmental stage when measured.

Body Size

In the current study, I found that results varied across development, with the factors explaining variation in length generally decreasing over time. Interestingly, the dam component of variance remained significant on offspring length for all five dates measured which at first seems to contradict the statement that maternal effects decrease over time (reviewed in Heath & Blouw, 1998; and in Green, 2008). However, when the dam component was separated and the maternal effects were estimated, the maternal effect went from representing 38% of the phenotypic variation at the larval stage, but decreased to 13% by the parr stage and continued to decrease for the remaining dates. That I found higher maternal effects early in development supports the established concept that maternal effects decrease over time, due to other factors like offspring genome and environmental quality increasing in their influence (e.g. Heath *et al.*, 1999; reviewed in Heath & Blouw, 1998; and in Green, 2008). The nonadditive (dam x sire) effects on length in the current study also decreased over time, suggesting that genetic compatibility does affect length but the effects are life-stage specific. Similarly, in a previous study on Chinook salmon, maternal and nonadditive effects contributed to larval growth (comparable to my *Date 1* measurement), which represented 11% and 73%, respectively, of the phenotypic variation (Pitcher & Neff, 2007). In the current study, I found that nonadditive effects were higher than maternal effects (by 11%) for larval length, which was also seen in Pitcher and Neff (2007), although their nonadditive effects were much larger than the maternal effects. The sire component of variance was not significant (i.e. no additive effects) for larval length, but was significant at the parr stage, juvenile stage, and adult stages of development, although the additive effects represented

a small portion of phenotypic variance. Additive effects on length have also been found at the fry stage in Chinook previously (Evans *et al.* 2010), however their additive effects were much stronger representing 39% for one population of Chinook salmon, and 33% for another population of Chinook. That I found no additive effects on larval length is also consistent with previous work on larval Lake Ontario Chinook (Pitcher and Neff 2007). Previous studies have shown that additive genetic effects are important for body size in fish and explain for example, 62% and 27% of the variation in early and late mortality respectively, in whitefish (*Coregonus* sp.) (Wedekind *et al.*, 2001); 14% of the variation in alevin length in brook charr (*Salvelinus fontinalis*) (Perry *et al.*, 2004); 57% of the variation in larval size in Chinook salmon (*Oncorhynchus tshawytscha*) (Heath *et al.*, 1999); 65% of the variation in larval standard length in Atlantic herring (*Clupea harengus* L.) (Bang *et al.*, 2006) and 39% and 33% of the variation in fry length in two populations of Chinook salmon (*Oncorhynchus tshawytscha*) (Evans *et al.* 2010). Overall, the current study shows changes in contributions of additive and nonadditive effects and maternal effects throughout developmental stages. By reviewing our results and the findings of similar studies, it seems as though maternal effects and nonadditive effects contribute to larval length, which switches to additive effects playing a role when older.

Although the differences in offspring length among dams and among sires seems small, it is well known that the size of offspring, especially in the early stages of development is a major influence for survival and recruitment (e.g. Jenkins & King, 2006; Fontes *et al.*, 2011; reviewed in Chambers & Leggett, 1996). For instance, being larger at hatching offers several benefits such as, having more time to find food sources

thus being more resistant to starvation (Miller et al. 1988), being too big for smaller predators to handle and consume (Bailey, 1984), and having sense organs and swimming ability more developed assisting in predator detection and escape (Bailey, 1984; Bailey and Batty, 1984; Fuiman et al. 2004). In Pacific salmon specifically, larger smolts also possess several advantages including better escape from predators and ability to catch prey due to enhanced swimming ability, and ultimately greater survival when migrating to and entering the sea (Beckman et al. 2003). In steelhead trout (*Oncorhynchus mykiss*), smolt-to-adult survival had a positive relationship with length (Ward et al. 1989). Thus, it would be interesting to see if the differences in offspring length among dams and sires seen in the current study would influence the fitness of the offspring if they were in the wild.

Survival

For survival, I also found that results varied throughout ontogeny, with the factors explaining variation generally decreasing over time similar to length. Evans *et al.* (2010) who also used Chinook salmon, found analogous results, as they found that maternal effects were high in larval survival for both populations they examined (55% and 61% of the phenotypic variation) which decreased drastically when older at the fry stage (4% and 0%). In the current study, maternal effects contributed more than additive effects to larval survival (51% and 40%, respectively), and then decreased drastically with age. My finding of maternal effects and additive effects on survival at *Date 1* (near the end of the endogenous feeding stage) is consistent with Pitcher & Neff's (2007) study that used Lake Ontario Chinook salmon. They found in larvae, which is comparable to our *Date 1* measurement, that maternal effects represented 51% and additive effects represented 56%

of the phenotypic variance in survival (and also found that nonadditive effects represented 54%), the difference in their study being that additive effects were actually slightly higher than maternal effects (Pitcher & Neff, 2007). Unlike Evans et al. (2010) however, I did not find that additive effects played a stronger role in older fish, as the sire component was nonsignificant for *Dates B, C, and D*. I could only test the dam x sire component for *Dates C and D*. Dam x sire was significant for *Date C*, but I was unable to determine how much of the phenotypic variation nonadditive effects represented. It is unfortunate that I could not test nonadditive effects at *Dates A and B*, as nonadditive effects and maternal effects together represented 80% of the phenotypic variation in hatching success (additive effects were zero) in sea lamprey (*Petromyzon marinus*) (Rodriguez-Munoz & Tregenza, 2009). Interestingly, in their study nonadditive effects played a much larger role than maternal effects, as nonadditive effects represented 65.5% whereas maternal effects represented only 14.8% of the phenotypic variation (Rodriguez-Munoz & Tregenza, 2009). Overall, it seems as though all three effects (additive, nonadditive and maternal) contribute to larval survival. Although I did not find additive effects later on, Evans *et al.* (2010) did and it is possible that had I used wild fish I may have seen similar results. By comparing the highest quality dam to the lowest for dates that the dam effect was significant, survival was increased by 65% for *Date A*, 16% for *Date C*, and 23% for *Date D*. By comparing the highest quality sire to the lowest for *Date A* (the only date the sire effect was significant), survival was increased by 29%. Since offspring mortality is high in the early stages of development, these genetic influences early on may impact subsequent recruitment to the population.

Performance cross effects on length and survival

That the performance crosses affected offspring length early on in the larval stages, decreasing through to adult stage means that differences among the high- and low-survival lines manifest early on, but decrease throughout development. The L/H performance cross produced the longest offspring for all dates except for *Date 2*. That the L/H performance cross (versus the H/L performance cross) produced longer offspring, it means that when sires are from the high-survival line, they produce bigger offspring than when dams are from the high-survival line, suggesting that sires, or additive genetic variance plays a more important role on length, across all stages of ontogeny. Depending on stage of development, either the H/H or the L/L performance crosses produced the shortest offspring. It is surprising that the H/H performance cross sometimes produced the shortest offspring, which indicates that the two survival lines have not maintained their integrity in terms of length.

For survival, results varied throughout ontogeny but unlike for length, the variation explained did not decrease over time but in fact increased. Performance cross explained 32% of survival for *Date A* (end of larval stage), which increased to 58% by *Date B* (parr stage), which decreased to 0% for *Date C* (adult), but then increased to 80% of the variation in survival by *Date D* (adult) and was only significant for *Date D*. This finding indicates that differences among the lines in terms of survival do not manifest themselves until older in the adult stage of development, which may be due to that the survival lines were selected based on survival to adulthood. At *Date D*, the H/L performance cross produced offspring with the highest survival, and increased survival by 17% compared to the lowest surviving cross (L/H). That the H/L performance cross

produced offspring with higher survival indicates that the dam is more important for survival. Although I cannot be certain if dams produce offspring with higher survival due to her genetic contribution or maternal effects (since dam identity includes both), it is likely due to her genetic contribution (additive effects) since maternal effects are known to decrease over time (reviewed in Heath & Blouw, 1998; and in Green, 2008), and since by *Date D* the offspring were almost 3 year-old adult salmon. The L/L performance cross never produced offspring with the highest survival, and the H/H performance cross never produced offspring with the lowest survival indicating that the two survival lines have maintained their integrity in terms of survival.

For both length and survival, the hybrid performances crosses (either H/L or L/H) most often produced offspring that were the longest and had the best survival (Fig. 3.3 & Fig. 3.6). This finding suggests that overdominance (i.e. the heterozygotes have higher fitness than either homozygote) is occurring, which has thus maintained the genetic diversity in terms of the high- and low-performance genotypes throughout the past seven generations. Heterozygosity has been positively correlated with several performance traits in salmonids, such as body size, disease resistance, viability, egg size, and egg number (as reviewed in Wang *et al.*, 2002).

In the current study, the offspring were reared in a common environment given the same amount of food, where predators were absent and where other factors that normally influence survival (e.g. competition for resources) were likely minimal due to the hatchery setting. This was done to minimize confounding factors so that any differences in size or survival seen among the offspring could be attributed to differences in genetic quality (and maternal effects). Thus, perhaps it is possible that had the

offspring been reared in the wild where selection pressures (e.g. due to risk of starvation or predation) exist more heavily, differences in size and/or survival among the offspring could be more pronounced. In a tropical damselfish (*Acanthochromis polyacanthus*), parental condition affected juvenile survival when offspring were reared in a low-food environment, but not when reared in a high-food environment (Donelson *et al.*, 2009). It is also important to mention that if genotype x environment interaction exists, then the different genotypes in the current study would respond differently to changes in the environment (Lynch & Walsh, 1998). Heath *et al.* (1993) found significant dam-by-incubation temperature and sire-by-incubation temperature (i.e. genotype-by-environment) effects on growth- and stress-related traits in Chinook salmon fry. Evans *et al.* (2010) also found genotype-by-environment effects on larval and fry survival and on fry length in Chinook salmon, whereas Wang *et al.* (2009) did not find genotype-by-environment effects on growth performance in yellow perch (*Perca flavescens*). In European seabass (*Dicentrarchus labrax*), genotype-by-environment effects were found for growth rate, but not for weight (Dupont-Nivet *et al.*, 2010). Genotype-by-environment interactions have also been reported in other fishes such as coho salmon (*Oncorhynchus kisutch*) (Devlin *et al.*, 2004), and Atlantic salmon (*Salmo salar*) (Darwish & Hutchings, 2009). However genotype-by-environment interactions have been shown to be weak for growth traits and timing of maturity in rainbow trout (*Oncorhynchus mykiss*) (Fishback *et al.*, 2002; Kause *et al.*, 2003), for visceral fat in gilthead seabream (*Sparus auratus* L.) (Navarro *et al.*, 2009) and absent for disease resistance in Chinook salmon (Balfry *et al.*, 1997). If genotype-by-environment interactions exist, then the results in the current study could be specific to their rearing

environment, and thus future studies could examine several heterogeneous environments. Differences in size and/or survival may have been more evident had I selected dams and sires that were obtained directly from the wild or less domesticated. The dams and sires in the current study are 7th generation descendants raised in a hatchery setting since 1985. Therefore, it is possible that mechanisms of selection that may maintain differences in genetic quality of individuals in the wild are lacking in the more domesticated fish used in my study. I used these descendants for the purpose of studying the high- and low-survival lines. However, since hatchery-reared and wild salmon have been shown to have many morphological, ecological and behavioural differences between them (e.g. Blanchet *et al.*, 2008; Anttila & Manttari, 2009; reviewed by Reisenbichler & Rubin, 1999; and in Flagg *et al.* 2000), future studies could use dams and sires from the wild to better understand the genetic architecture of traits naturally occurring. Additionally, it is likely that differences in survival may have been more prominent, even in the fish used in the current study, if I did not have to ‘reset’ the number of individuals per family due to logistical constraints of rearing and amount I could PIT-tag. Ideally, I would have preferred to obtain an estimate of survival throughout ontogeny without ever having to remove individuals, but it was not feasible.

In conclusion, my study adds to growing evidence that genetic architecture of traits varies within individuals across development. No other study has followed contributions of additive and nonadditive genetic effects and maternal effects to variation in a phenotypic trait in offspring from larval to adult stages. I found that genetic and maternal effects play an important role in larval length and survival, and that these effects decrease with age, possibly due to environmental variation masking genetic effects. My

study also adds to evidence of nonadditive genetic effects (i.e. genetic compatibility) affecting larval length, suggesting that ‘compatible genes’ can play very important roles in larval survival. This also suggests that individuals who get to choose their mates may benefit by having offspring with higher fitness if they find these more genetically compatible individuals. Finally, my study has various applications to conservation. Artificial breeding programs for depleting stocks of Pacific salmon currently exist (Swanson *et al.*, 2008), although it is unclear how successful the individuals are in the wild and if they will be self-sustaining (Araki *et al.*, 2008; Fraser, 2008). My study indicates that individuals do differ in their genetic quality, which may cause differences in fitness among individuals. Future breeding programs that mate individuals randomly may want to consider this. Allowing female Chinook to mate with many males may increase her chances of her offspring gaining additive genetic benefits from ‘good genes’, and/or finding more genetically compatible mates which would provide her offspring with nonadditive genetic benefits from ‘compatible genes.’

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Table 3.1 Summary of survival measurements, taken 4 times throughout ontogeny. The table shows for each date the offspring's current stage of development, dates included, important notes concerning whether fish removal had occurred or not, and how survival was calculated (per family) at that date.

	Stage of Development	Date Included	Details	Calculation
<i>Date A</i>	larval stage	Dec. 08-Mar. 09	<i>before</i> fish were removed from barrels	# of individuals alive at Mar.09/ total # of individuals at Dec. 08
<i>Date B</i>	from larval to parr	Mar. 09-Jun. 09	<i>after</i> fish were removed from barrels	# of individuals alive at Jun. 09/ new # of individuals after fish removal
<i>Date C</i>	from parr to adult	Jun. 09-Jun. 10	<i>after</i> PIT tagging occurred	# of individuals alive at Jun. 10/ # of individuals PIT tagged
<i>Date D</i>	adult	Jun. 10-Jun. 11	<i>after</i> Jacks were removed	# of individuals alive at Jun. 11/ new # of individuals after fish removal

Table 3.2 Summary of the one-way random factor ANOVA results for family effects on offspring length, for all 5 dates measured. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), *F* statistic, *P*-value, and the variance component (σ^2) with the percent of total variance (% total var) explained. Significant values (adjusted alpha level of $P < 0.013$) are indicated in bold.

Source of variation	DF	SS	MS	<i>F</i>	<i>P</i>	σ^2 (% total var)
<i>Date 1</i>						
Family	48, 906	14.7	0.307	18.89	< 0.001	1.5 x 10 ⁻² (48)
Residual	906					1.6 x 10 ⁻² (52)
<i>Date 2</i>						
Family	48, 1330	93.1	1.940	7.564	< 0.001	6.0 x 10 ⁻² (19)
Residual	1330					2.6 x 10 ⁻¹ (81)
<i>Date 3</i>						
Family	48, 1044	236.7	4.931	5.606	< 0.001	1.8 x 10 ⁻¹ (17)
Residual	1044					8.8 x 10 ⁻¹ (83)
<i>Date 4</i>						
Family	48, 812	714.0	14.88	3.060	< 0.001	5.6 x 10 ⁻¹ (10)
Residual	812					4.9 (90)
<i>Date 5</i>						
Family	48, 513	1116.2	23.25	1.670	0.004	8.5 x 10 ⁻¹ (6)
Residual	513					13.92 (94)

Table 3.3 Summary of the two-way random factor ANOVA results for parental effects (dam, sire, and their interaction) on offspring length, for all 5 dates measured. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), *F* statistic, *P*-value, and variance component (σ^2) with the percent of total variance (% total var) explained by each source (negative variance components are treated as zero). The table also includes the percent of phenotypic variance (% phenotypic var) from maternal effects, and additive and nonadditive genetic effects. Significant values (adjusted alpha level of $P < 0.013$) are indicated in bold.

Source of variation	DF	SS	MS	<i>F</i>	<i>P</i>	σ^2 (% total var)	% phenotypic var
<i>Date 1</i>							
Dam	6, 36.0	10.6	1.761	19.26	< 0.001	1.2×10^{-2} (38)	Maternal 38
Sire	6, 36.1	0.35	0.058	0.641	0.696	-2.5×10^{-4} (0)	Additive 0
Dam x Sire	36, 906	3.30	0.092	5.635	< 0.001	3.9×10^{-3} (12)	Nonadditive 49
Residual	906					1.6×10^{-2} (50)	
<i>Date 2</i>							
Dam	6, 36.2	58.4	9.739	16.74	< 0.001	4.9×10^{-2} (15)	Maternal 13
Sire	6, 37.6	11.0	1.830	3.220	0.012	6.4×10^{-3} (2)	Additive 8
Dam x Sire	36, 1330	21.0	0.584	2.277	< 0.001	1.2×10^{-2} (4)	Nonadditive 14
Residual	1330					2.6×10^{-1} (79)	

Table 3.3 Continued***Date 3***

Dam	6, 37.7	137.9	22.99	19.03	< 0.001	1.4×10^{-1} (13)	Maternal	9
Sire	6, 39.0	45.4	7.569	6.307	< 0.001	4.1×10^{-2} (4)	Additive	15
Dam x Sire	36, 1044	43.9	1.219	1.386	0.066	1.5×10^{-2} (1)	Nonadditive	6
Residual	1044					8.8×10^{-1} (82)		

Date 4

Dam	6, 40.0	464.4	77.41	18.40	< 0.001	5.8×10^{-1} (11)	Maternal	10
Sire	6, 42.5	66.3	11.05	2.615	0.030	5.5×10^{-2} (1)	Additive	4
Dam x Sire	36, 812	150.4	4.178	0.859	0.706	-3.8×10^{-2} (0)	Nonadditive	0
Residual	812					4.9 (88)		

Date 5

Dam	6, 45.5	526.5	87.76	9.270	< 0.001	1.0 (7)	Maternal	5
Sire	6, 65.0	186.0	31.00	3.100	0.010	2.8×10^{-1} (2)	Additive	7
Dam x Sire	36, 513	327.7	9.103	0.654	0.941	-4.4×10^{-1} (0)	Nonadditive	0
Residual	513					13.9 (91)		

Table 3.4 Summary of the one-way fixed factor ANOVA results for performance cross effects on offspring length, for all 5 dates measured. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), *F* statistic, *P*-value, and the variance component (σ^2) with the percent of total variance (% total var) explained (negative variance components are treated as zero). Significant values (adjusted alpha level of $P < 0.013$) are indicated in bold.

Source of variation	DF	SS	MS	<i>F</i>	<i>P</i>	σ^2 (% total var)
<i>Date 1</i>						
Performance cross	3, 951	2.194	0.731	25.49	< 0.001	3.6×10^{-2} (55)
Residual	951					2.9×10^{-2} (45)
<i>Date 2</i>						
Performance cross	3, 1375	8.355	2.785	8.991	< 0.001	8.8×10^{-2} (22)
Residual	1375					3.1×10^{-1} (78)
<i>Date 3</i>						
Performance cross	3, 1089	25.39	8.464	8.160	< 0.001	3.4×10^{-1} (25)
Residual	1089					1.0 (75)
<i>Date 4</i>						
Performance cross	3, 857	69.62	23.21	4.331	0.005	9.9×10^{-1} (16)
Residual	857					5.4 (84)
<i>Date 5</i>						
Performance cross	3, 558	55.18	18.39	1.251	0.290	3.3×10^{-1} (2)
Residual	558					14.7 (98)

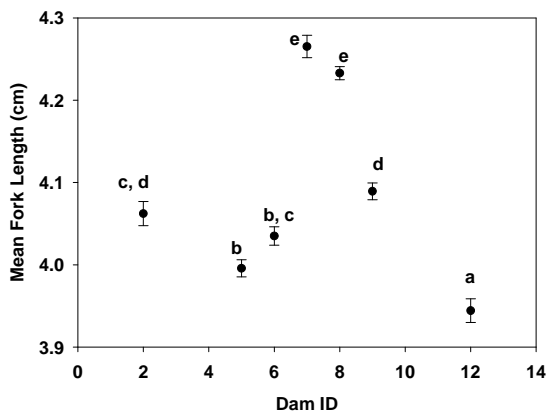
Table 3.5 Summary of the two-way random factor ANOVA results for parental effects (dam and sire) on offspring survival, for all 4 dates measured. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), *F* or Wald statistic (*W*), *P*-value, and variance component (σ^2) with the percent of total variance (% total var) explained by each source (negative variance components are treated as zero). The table also includes the percent of phenotypic variance (% phenotypic var) from maternal effects, and additive and nonadditive genetic effects. Significant values (adjusted alpha level of $P < 0.017$) are indicated in bold. For *Date A* and *Date B*, the *P*-values are from the two-way random factor ANOVA, and for *Date C* and *Date D*, the *P*-values are from logistic regression (as explained in the text).

Source of variation	DF	SS	MS	<i>F</i> or Wald	<i>P</i>	σ^2 (% total var)	% phenotypic var
<i>Date A</i>							
Dam	6, 36	2.647	0.441	<i>F</i> = 15.64	< 0.001	5.9×10^{-2} (61)	Maternal 51
Sire	6, 36	0.579	0.096	<i>F</i> = 3.419	0.009	9.7×10^{-3} (10)	Additive 40
Residual	36					2.8×10^{-2} (29)	
<i>Date B</i>							
Dam	6, 36	0.262	0.044	<i>F</i> = 2.144	0.072	3.4×10^{-3} (14)	Maternal 10
Sire	6, 36	0.163	0.027	<i>F</i> = 1.331	0.269	1.0×10^{-3} (4)	Additive 16
Residual	36					2.0×10^{-2} (82)	
<i>Date C</i>							
Dam	6, 36	0.202	0.034	<i>W</i> = 20.91	0.002	2.4×10^{-3} (12)	Maternal 12
Sire	6, 36	0.042	0.007	<i>W</i> = 5.636	0.465	-1.4×10^{-3} (0)	Additive 0
Dam x Sire	36			<i>W</i> = 56.39	0.016		
Residual	48					1.7×10^{-2} (88)	
<i>Date D</i>							
Dam	6, 36	0.322	0.054	<i>W</i> = 44.62	< 0.001	5.6×10^{-3} (27)	Maternal 26
Sire	6, 36	0.096	0.016	<i>W</i> = 12.34	0.055	1.4×10^{-4} (1)	Additive 3
Dam x Sire	36			<i>W</i> = 38.94	0.339		
Residual	48					1.5×10^{-2} (72)	

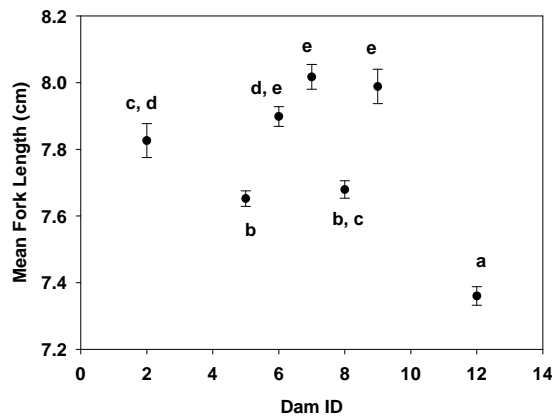
Table 3.6 Summary of the one-way fixed factor ANOVA results for performance cross effects on offspring survival, for all 4 dates measured. The table includes the source of variation, degrees of freedom (DF, with the numerator and denominator values where appropriate), sum of squares (SS), *F* or Wald statistic (*W*), *P*-value, and the variance component (σ^2) with the percent of total variance (% total var) explained (negative variance components are treated as zero). Significant values (adjusted alpha level of $P < 0.017$) are indicated in bold. For *Date A* and *Date B*, the *P*-values are from the one-way fixed factor ANOVA, and for *Date C* and *Date D*, the *P*-values are from logistic regression (as explained in the text).

Source of variation	DF	SS	MS	<i>F</i> or Wald	<i>P</i>	σ^2 (% total var)
<i>Date A</i>						
Performance cross	3, 45	0.380	0.127	<i>F</i> = 1.474	0.234	4.1×10^{-2} (32)
Residual	45					8.6×10^{-2} (68)
<i>Date B</i>						
Performance cross	3, 45	0.160	0.053	<i>F</i> = 2.397	0.081	3.1×10^{-2} (58)
Residual	45					2.2×10^{-2} (42)
<i>Date C</i>						
Performance cross	3, 45	0.051	0.017	<i>W</i> = 8.010	0.046	-1.0×10^{-3} (0)
Residual	45					1.8×10^{-2} (100)
<i>Date D</i>						
Performance cross	3, 45	0.240	0.080	<i>W</i> = 37.33	< 0.001	6.4×10^{-2} (80)
Residual	45					1.6×10^{-2} (20)

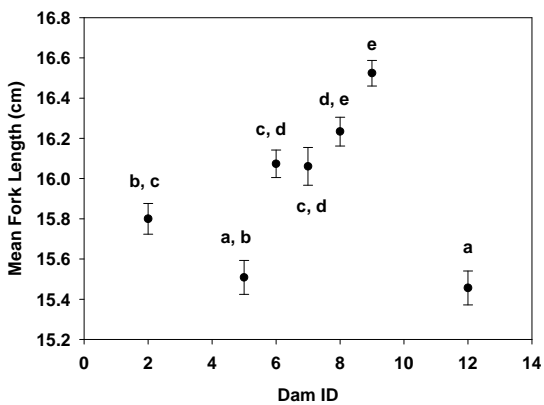
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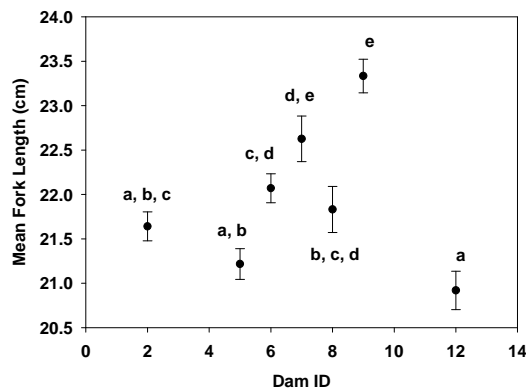
b)



c)



d)



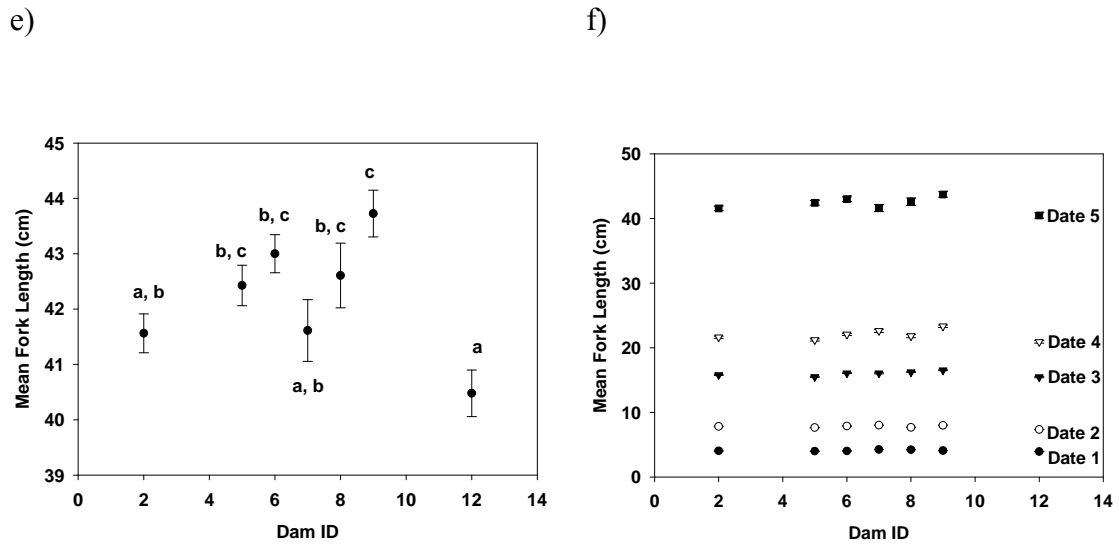
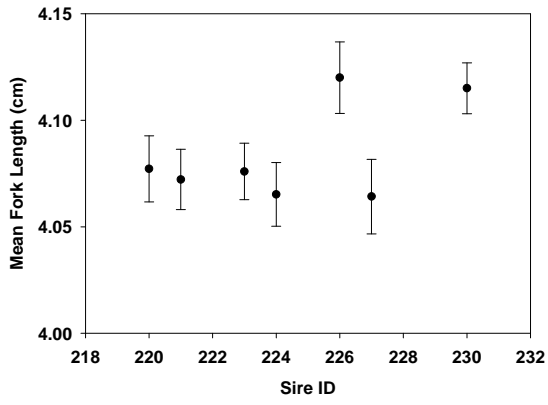
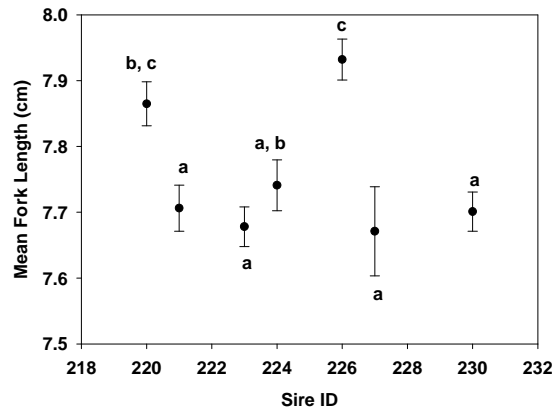


Fig. 3.1 Dam identity vs. mean fork length (cm) for a) *Date 1*: March 2009, b) *Date 2*: June 2009, c) *Date 3*: November 2009, d) *Date 4*: June 2010, e) *Date 5*: June 2011 and f) all dates. The trend is significant for all dates, with the letters ‘a’, ‘b’, ‘c’, ‘d’ and ‘e’ denoting the homogenous subsets.

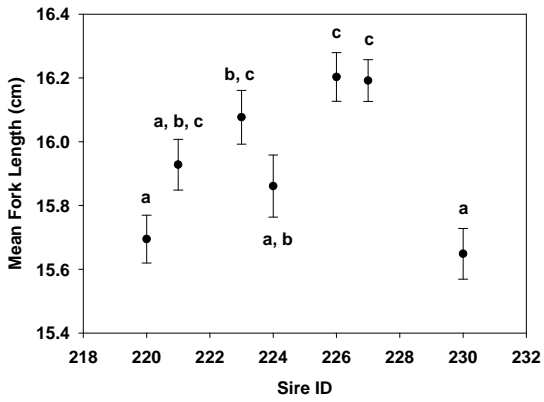
a)



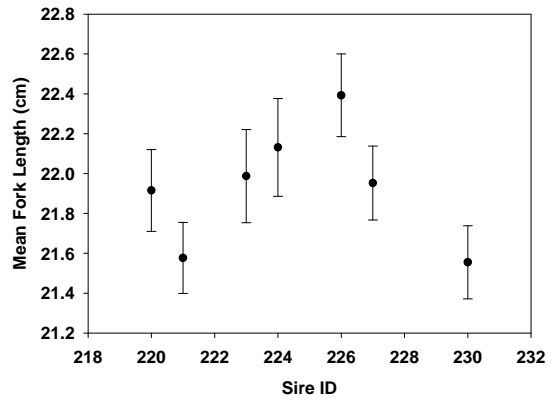
b)



c)



d)



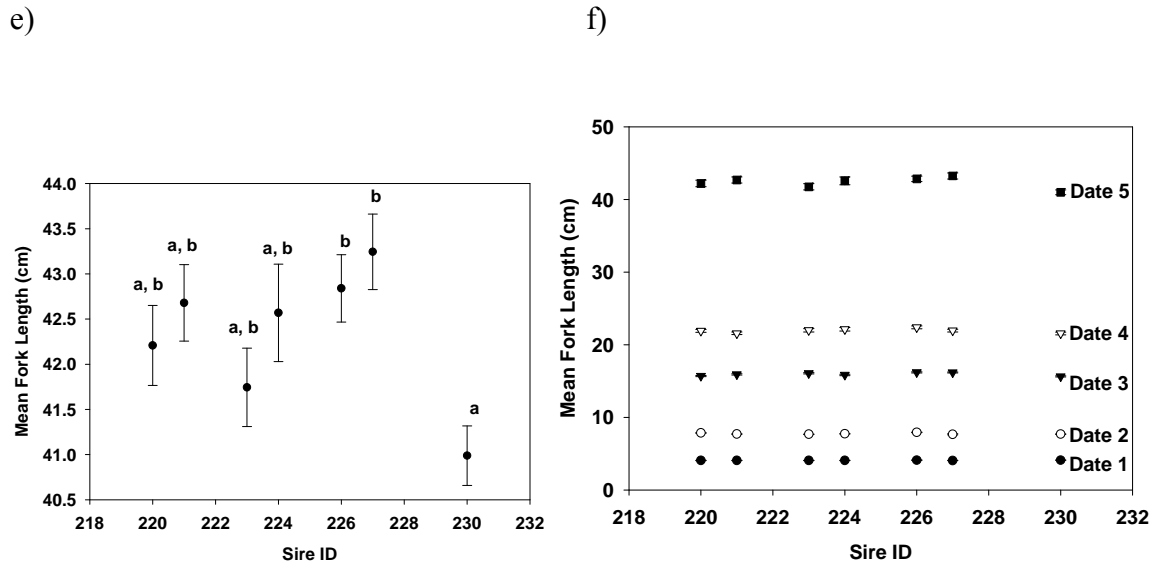
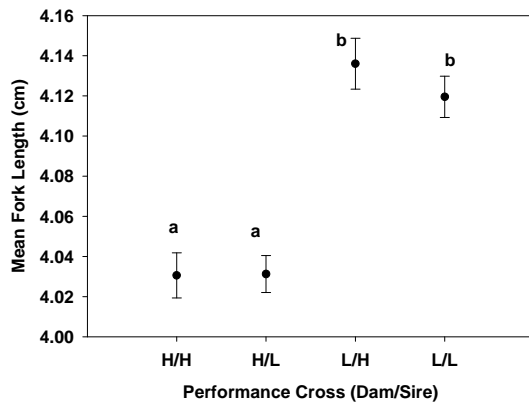
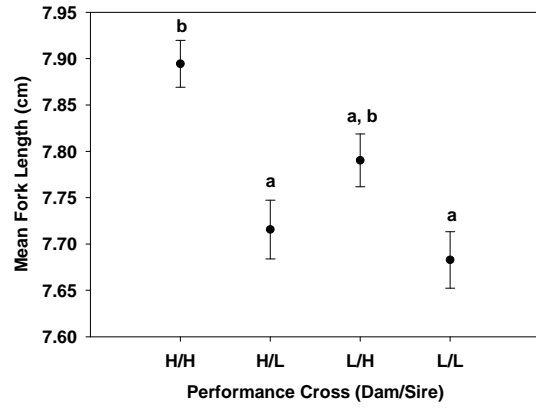


Fig. 3.2 Sire identity vs. mean fork length (cm) for a) *Date 1*: March 2009, b) *Date 2*: June 2009, c) *Date 3*: November 2009, d) *Date 4*: June 2010, e) *Date 5*: June 2011 and f) all dates. The trend is significant for *Date 2*, *Date 3* and *Date 5*, with the letters ‘a’, ‘b’ and ‘c’ denoting the homogenous subsets.

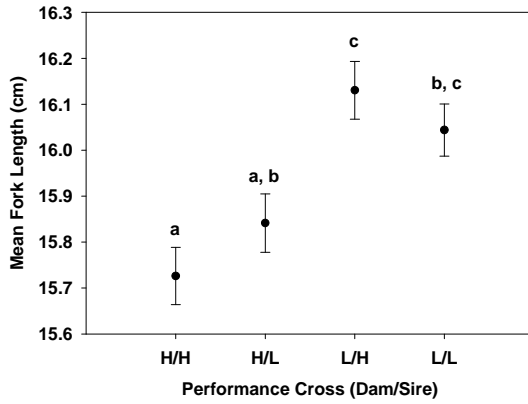
a)



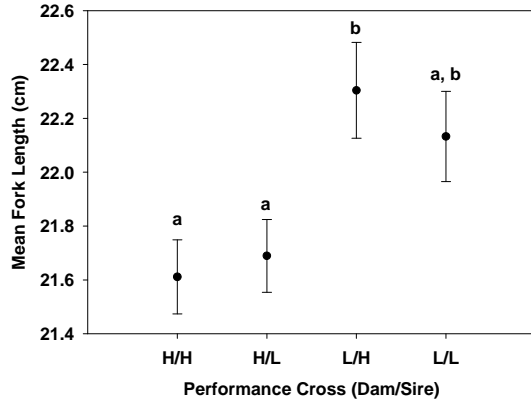
b)



c)



d)



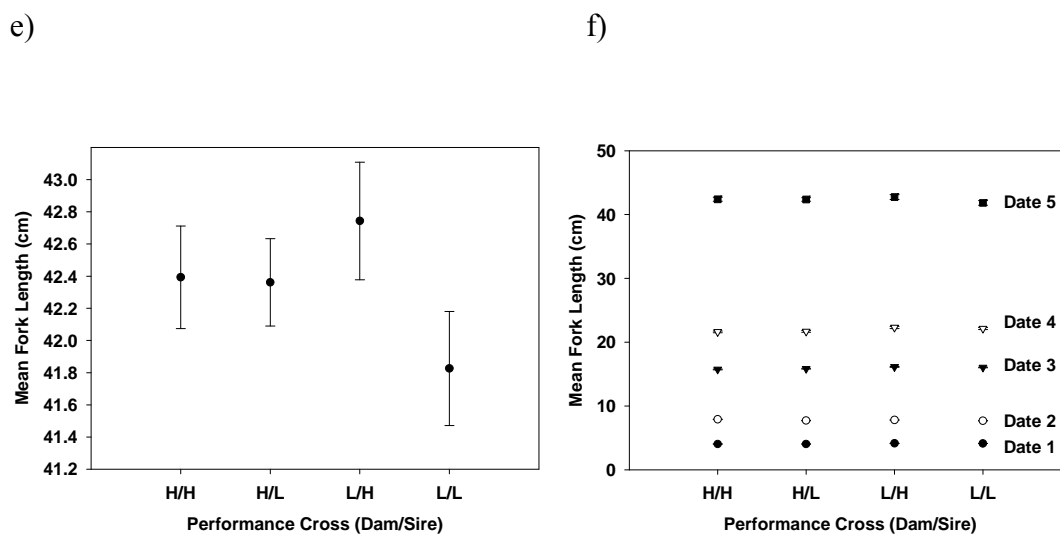


Fig. 3.3 Performance cross vs. mean fork length (cm) for a) *Date 1*: March 2009, b) *Date 2*: June 2009, c) *Date 3*: November 2009, d) *Date 4*: June 2010, e) *Date 5*: June 2011 and f) all dates. For the performance crosses, the first letter indicates the performance line of the dam, and the second letter indicates the performance line of the sire. The trend is significant for *Date 1*, *Date 2*, *Date 3* and *Date 4*, with the letters ‘a’, ‘b’ and ‘c’ denoting the homogenous subsets.

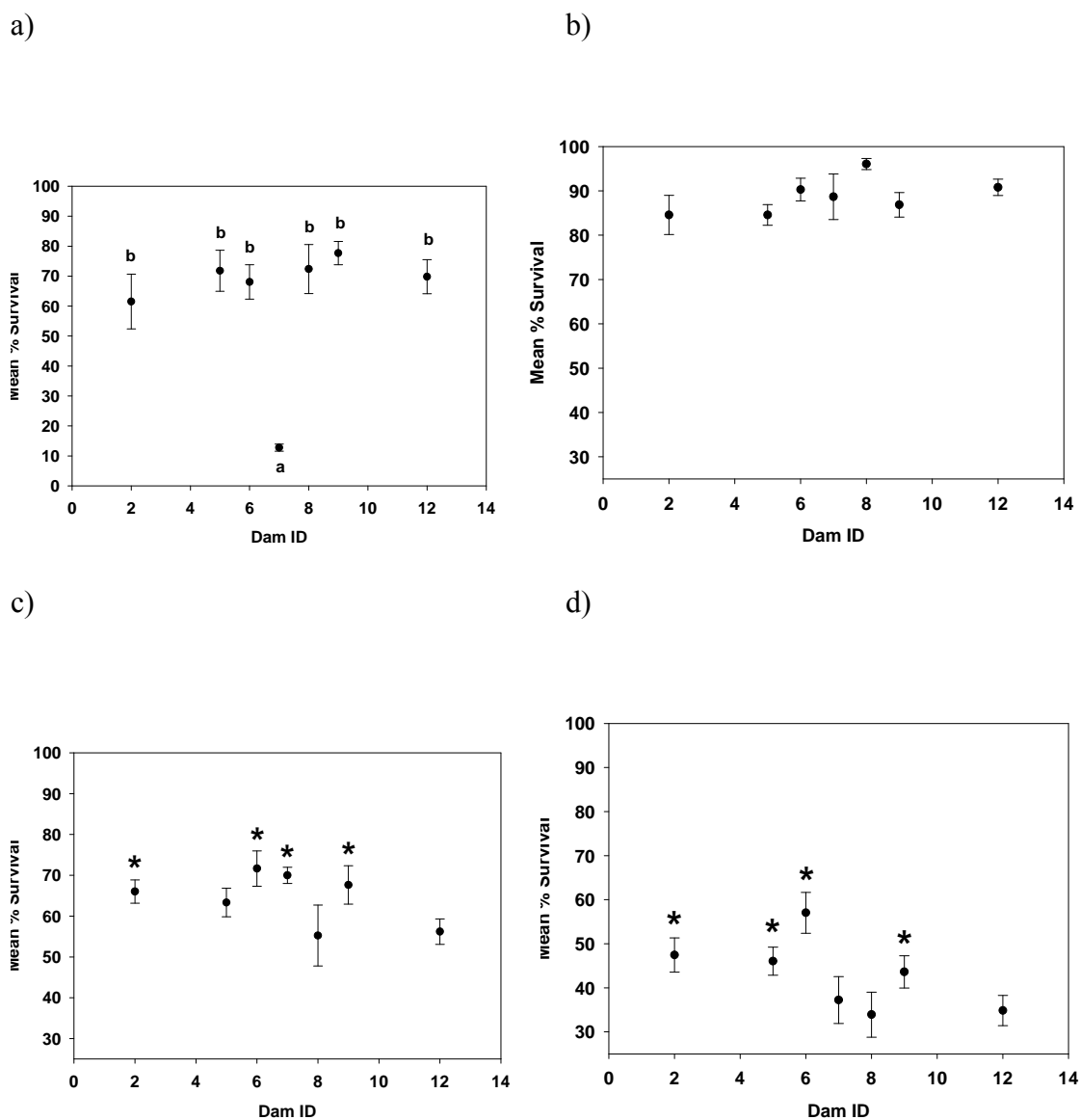


Fig. 3.4 Dam identity vs. mean percent survival for a) *Date A*: March 2009, b) *Date B*: June 2009, c) *Date C*: June 2010, and d) *Date D*: June 2011. The trend is significant for *Date A*, *Date C*, and *Date D*, with the letters 'a' and 'b' denoting the homogenous subsets for *Date A*. Logistic regression was used for *Date C* and *Date D*, and asterisks for those dates indicate dams that produced offspring with significantly higher survival than the dam with the lowest survival (dam # 8). Figures were plotted using the untransformed percent survival data.

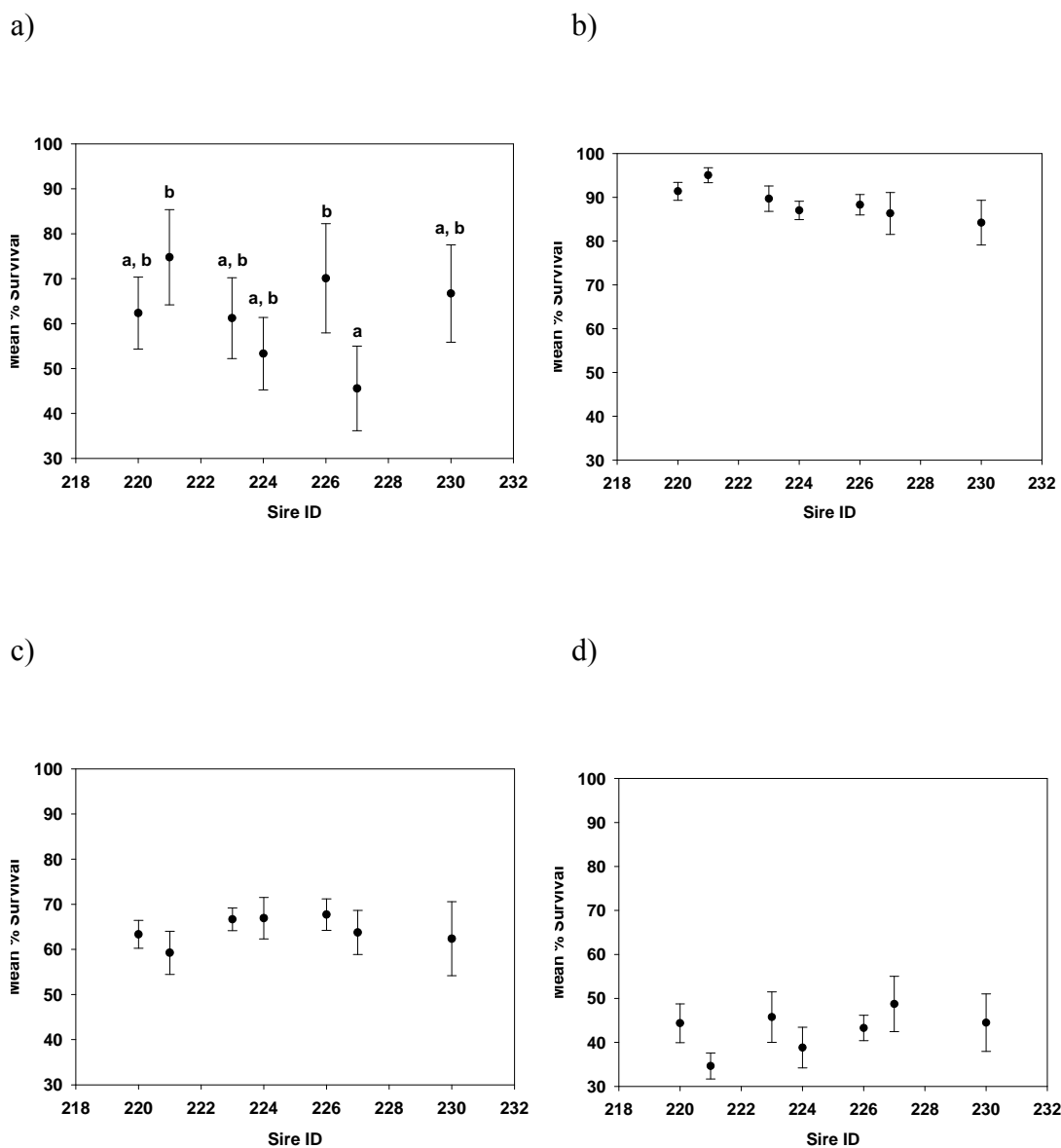


Fig. 3.5 Sire identity vs. mean percent survival for a) *Date A*: March 2009, b) *Date B*: June 2009, c) *Date C*: June 2010, and d) *Date D*: June 2011. The trend is significant for *Date A* only, with the letters ‘a’ and ‘b’ denoting the homogenous subset. Figures were plotted using the untransformed percent survival data.

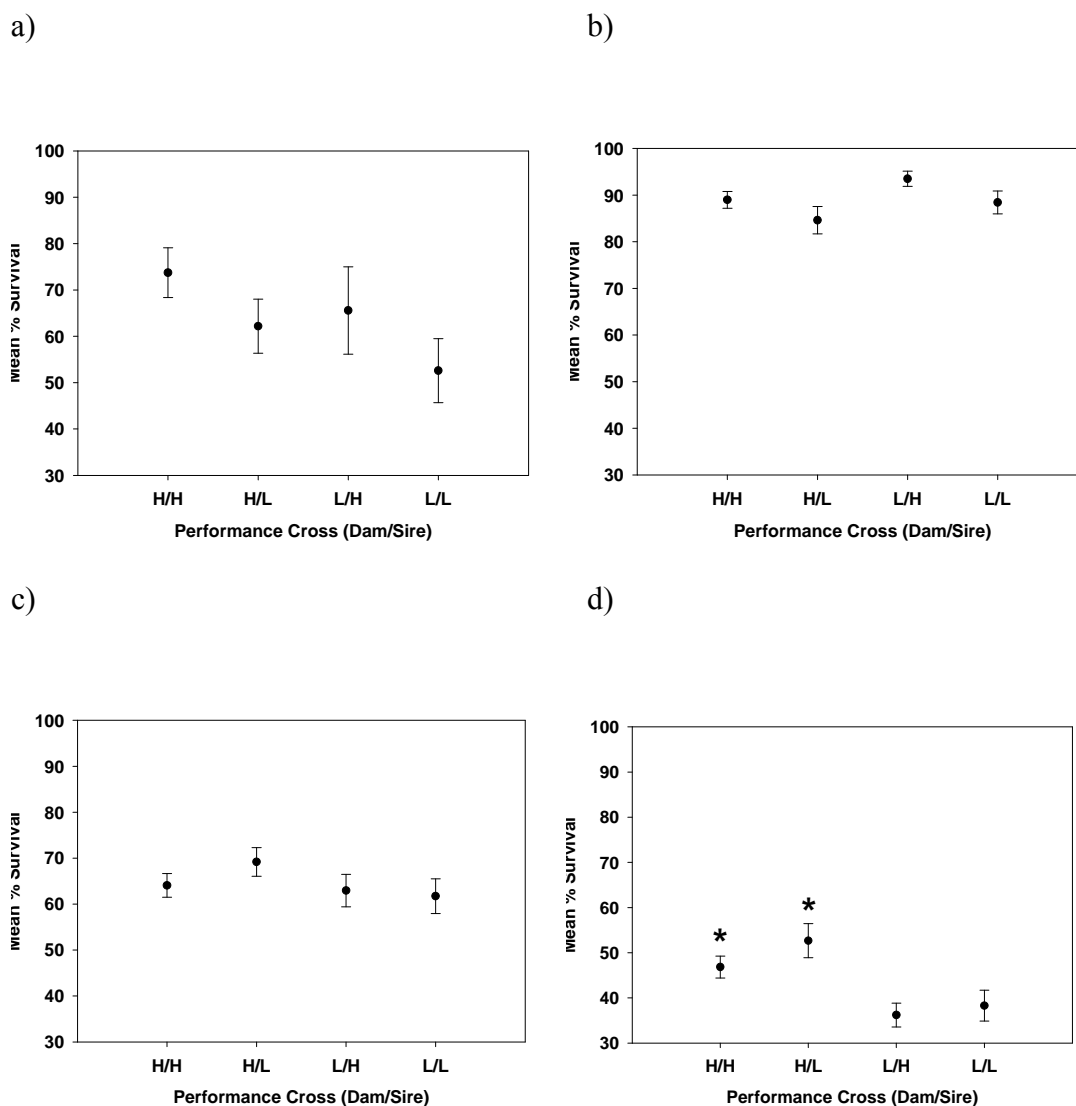


Fig. 3.6 Performance cross vs. mean percent survival for a) *Date A*: March 2009, b) *Date B*: June 2009, c) *Date C*: June 2010, and d) *Date D*: June 2011. For the performance crosses, the first letter indicates the performance line of the dam, and the second letter indicates the performance line of the sire. The trend is significant for *Date D*, with asterisks indicating the performance crosses that produced offspring with significantly higher survival than the performance cross with the lowest survival (L/H). Figures were plotted using the untransformed percent survival data.

CHAPTER IV

GENERAL CONCLUSIONS

4.1 Summary of findings

In my thesis, I investigated dam, sire, and dam x sire components of variance and the roles of additive and nonadditive genetic effects and maternal effects in three fitness-related traits (length, survival, and swimming) in Chinook salmon. My thesis is unique in that it followed the contributions of additive and nonadditive genetic effects and maternal effects to variation in phenotypic traits throughout ontogeny (Chapter 3), a task that has not yet been undertaken in fish. My thesis also contributes to knowledge of parental genetic contributions to offspring swimming ability (Chapter 2). Previous research has shown variation in the roles that good genes, compatible genes and maternal effects play within the same trait and within species. Although the estimated contributions of additive, nonadditive and maternal effects on traits might differ, there have been some common themes among species. Maternal effects (i.e. non-genetic effects) have been widely studied in fish, and it is recognized that maternal effects typically play a more important role in the early life history stages of fish, and decrease with age (e.g. Heath *et al.*, 1999; Perry *et al.*, 2004; for reviews see Heath & Blouw, 1998; Green, 2008; Marshall *et al.*, 2008). In Chapter 2 on offspring swimming ability, I did not find any maternal effects, which is consistent with this theme as I did not swim larvae but swam older individuals near the end of their parr stage, meaning that maternal effects that could have existed initially, were no longer present by the parr stage of development. For length (Chapter 3), maternal effects were most evident at the first sample taken near the end of the larval stage, and decreased to hardly any contribution at later stages of development. Similarly

for survival (Chapter 3), maternal effects were much more important at the larval stage, and decreased drastically throughout development.

The importance of sire effects are becoming more recognized in the literature (see Rideout *et al.*, 2004 and references therein), and have been reported to be important in both the early stages of development (e.g. Wedekind *et al.*, 2001; Bang *et al.*, 2006; Polacik & Reichard, 2009; Huuskonen *et al.*, 2011), and also in later stages (e.g. Barber *et al.*, 2001; Serbezov *et al.*, 2010). Due to being masked by maternal effects (such as by differences in egg size; Rideout *et al.*, 2004), additive genetic effects have sometimes been shown to be more prominent in later stages of development (e.g. Evans *et al.*, 2010). This is what I found in Chapter 2 on offspring swimming ability; the sire effects were significant only for older parr, and the estimated contribution of additive genetic effects increased then as well. The dam effect was not significant, therefore attributing all additive genetic variation to paternity. I found a similar trend for offspring length in Chapter 3, where additive genetic effects were not present in the larval stages, but became evident for the parr, juvenile, and adult stages. However, additive genetic effects contributed much less to the phenotypic variation in length, than for swimming. Also, because the dam component of variance was significant at all dates for length, it means that dam additive genetic variation also played a role, as opposed to only sire additive genetic variation playing a role in offspring swimming. The theme of additive genetic effects not becoming more important than maternal effects until later stages of development does not apply to my analysis on survival (Chapter 3). For phenotypic variance in larval survival, maternal and additive genetic effects contributed almost equally, and I did not find any additive effects on older offspring survival. In summary,

my thesis shows that additive genetic effects can be important in both early and later stages of development, depending on the phenotypic trait examined.

Nonadditive effects have also shown to be an important component of offspring fitness (e.g. Wedekind *et al.*, 2001; Evans & Neff, 2009; Kekalainen *et al.*, 2010a). My analysis on parr swimming ability (Chapter 2) showed some contributions of nonadditive effects, but the effect was non-significant. From the analysis on offspring length (Chapter 3), I found nonadditive effects during the larval stage, which were slightly higher than maternal effects. By the parr stage, nonadditive effects were still significant on length but decreased to representing only a small amount of the phenotypic variation, and then were non-significant for the remaining dates. For survival, I found significant dam x sire effects in the adult stage, but I could not estimate the contribution of nonadditive effects. Unfortunately, I could not determine dam x sire effects for the larval and parr stages of development. Overall, my thesis adds to evidence of the importance of genetic compatibility on offspring traits, and that the contribution is life-stage specific.

The other theme of my thesis was whether two different salmon lines (referred to as performance crosses) selected for differential growth and survival in 1997, have presently maintained differences in growth and survival, and if the two lines show differences in swimming ability. I also determined at which stages of development any differences among the lines manifested. Performance cross (i.e. whether the offspring were H/H, H/L, L/H or L/L) affected offspring swimming ability only in older parr (and not in younger parr). For offspring length, performance cross effects were significant for four out of the five dates measured spanning larval, parr, juvenile, and adult stages of development. For survival however, performance cross effects were only significant in

the adult stage. Interestingly, whether the dam or sire was from the high-survival line influenced offspring performance. For instance, the L/H performance cross (when sires were high performance) produced longer and better swimming offspring than the H/L performance cross (when dams were high performance) indicating the importance of additive genetic variation on those traits. For survival however, the opposite trend was found, where the H/L performance cross produced offspring with the highest survival, emphasizing the role of dam effects. That the two salmon lines created in 1997 still show differences in size and survival (and swimming), provides further evidence for heritability of these traits. My results showed that the integrity of the two lines have been maintained for survival, but not necessarily for length since the H/H performance line produced the shortest offspring at some stages of development. Regardless, the results indicate that any artificial breeders (such as for aquaculture), could implement a similar broodstock selection program if their goal is to increase offspring survival. The broodstock selection program would be especially useful for hatchery managers who wish to engage in organic farming to diminish the use of harmful substances, which also benefits surrounding wild fish.

4.2 Future Directions

Taken together, the cumulative results of my thesis provide evidence for genetic variation in offspring length, survival, and swimming ability in Chinook salmon. My thesis also displays how the contributions of additive, nonadditive and maternal effects can vary depending on the phenotypic trait examined, and stage of ontogeny. The next question might therefore ask, do Chinook dams and sires ‘recognize’ this genetic

variation, and sexually select the most appropriate individuals? Since female Chinook spawn multiple times in a series of nests (Healey, 1991), her eggs are presumably fertilized by more than one male, which may maximize her chances of receiving indirect benefits from good genes, and/or also finding more compatible mates. For instance, embryonic mortality is lower in polyandrously fertilized offspring (allowing sperm competition) than in monandrous fertilizations in a species (Arctic charr, *Salvelinus alpinus*) with a nonresource-based mating system (Kekalainen *et al.*, 2010b).

Additionally, evidence exists for female Chinook salmon exhibiting mate choice (by delaying spawning in the presence of smaller males) (Berejikian *et al.*, 2000) which may be another mechanism for females to obtain indirect benefits, and thus increase the fitness of their offspring. Offspring mortality is high in fishes, especially during the transitional period from larval to juvenile stage (Caley *et al.*, 1996). Since my thesis and previous research shows genetic variation in performance traits like body size and swimming performance, wild female Chinook may be able to increase the survival of her offspring by ‘choosing’ the best mate, since both additive and nonadditive genetic effects play important roles.

Although there were some common themes for the genetic architecture of traits in Chinook, differences among the literature in contributions of good genes and compatible genes effects also exist. Thus, another future direction from my thesis might be to investigate many wild populations within a species, to determine how similar or different the roles of additive and nonadditive effects and maternal effects are on those traits. As stated by Sanford & Kelly (2011), local adaptation “results in resident genotypes that have a higher fitness in their native habitat than do foreign genotypes from more distant

populations.” Subpopulations within the same species of salmon exist (due to natal homing) which have shown to exhibit local adaptation (reviewed in Fraser *et al.*, 2011). Therefore, following the genetic architecture of fitness-related traits within subpopulations seems necessary to capture the genetic influences on the fitness of those individuals, as which genes and genotypes are important in one population may not necessarily be the case in others. Population-specific knowledge might therefore be critical for improving the success of artificial propagation programs for endangered and threatened wild Chinook salmon.

4.3 References

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