

**THE RELATIONSHIP BETWEEN GENETIC VARIATION AND FITNESS IN
CHINOOK SALMON, *ONCORHYNCHUS TSHAWYTSCHA* (WALBAUM):
IMPLICATIONS FOR AQUACULTURE AND EVOLUTIONARY THEORY**

by

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B.Sc., The University of Victoria, 1983

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Abstract

The main objectives of this study were to assess the performance of chinook salmon hybrids for evidence of heterosis (Chapter 1) and to describe the relationship between genetic variation and fitness in chinook salmon (Chapter 2). Multiple traits from freshwater and saltwater life stages were measured for a study population composed of wild and domestic purebreeds and their reciprocal hybrids. The study population was reared at a commercial salmon farm. Microsatellite DNA analysis was used to generate genetic variation estimates. There was favourable but low heterosis for 36% of the performance traits evaluated. There was little evidence that the degree of favourable heterosis may be predicted from the genetic similarity of the parents. Strong maternal effects, particularly from wild dams, may have inflated or masked heterosis for certain traits. There was no demonstration of 'useful' heterosis, that is, heterosis that exceeds the performance of the best parental lineage; however, crossbreeding as a possible breeding strategy for chinook salmon is not dismissed. The contention that genetic variation-fitness relationships are weak was supported by this study. Freshwater and saltwater growth were the only traits for which significant relationships with genetic variation were detected. Associative overdominance is identified as a mechanistic explanation for genetic variation-fitness relationships in chinook salmon. Hybrids appear to drive the overall pattern of these relationships. Heterosis and genetic variation-fitness relationships are empirical observations that may or may not have the same cause so, in this sense, the chapters stand alone, but together they clearly illustrate differences among the hybrids and purebreeds that suggest the need for further research. Finally, there are two interesting features of salmonid evolution and biology – tetraploidy and philopatry – that may, in the future, initiate reinterpretation of many current theories regarding genetic variation and fitness/performance in this group.

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General Introduction

Evolution by natural selection and breed improvement by artificial selection both require genetic variation. However, selection reduces genetic variation by driving some alleles to fixation and eliminating others (Freeman and Herron 1998). How then is genetic variation maintained in populations? Heterozygote advantage (overdominance) is one of several mechanisms believed to preserve genetic variation in populations (Roff 1997; Freeman and Herron 1998). Overdominance is defined as the higher fitness of a heterozygote at a locus, relative to either of the corresponding homozygotes, thus the fitness of a genotype increases with the number of heterozygous loci it contains (Mitton and Grant 1984). Heterozygosity presumably broadens the range of physiological function and tolerance (Samollow and Soulé 1983) while homozygosity increases expression of deleterious recessive alleles which results in inbreeding depression (reduced fitness) (Falconer and Mackay 1996). Heterosis ('hybrid vigour') is the reversal of inbreeding depression through outbreeding. The generally accepted mechanistic explanation for heterosis is that increased heterozygosity, as a result of outbreeding, masks the deleterious recessive alleles in the F_1 generation ('dominance', Shields 1982 but see Hedgecock et al. 1995). However, there may be an optimum degree of outbreeding beyond which fitness decreases, and outbreeding depression would be observed (Lynch 1991). That is, mating between individuals from divergent populations may break up co-adapted gene complexes (Lynch 1991), resulting in hybrid performance (typically F_2) that is significantly reduced relative to the original parental lines (Lynch and Walsh 1998). There is, to summarize, a continuum between inbreeding depression and outbreeding depression with heterosis in the middle (Figure i.1). Along this continuum is a shift in emphasis from interactions within loci (dominance) to those among loci (epistasis) (Lynch and Walsh 1998).

The relationship between genetic variation and fitness is a complex topic in evolutionary biology (Britten 1996). Understanding why there are (or are not) genetic variation-fitness relationships is further complicated by the concept of fitness itself. The merits of one fitness definition over another are beyond the scope of this study (see reviews by Ollason 1991; Beatty

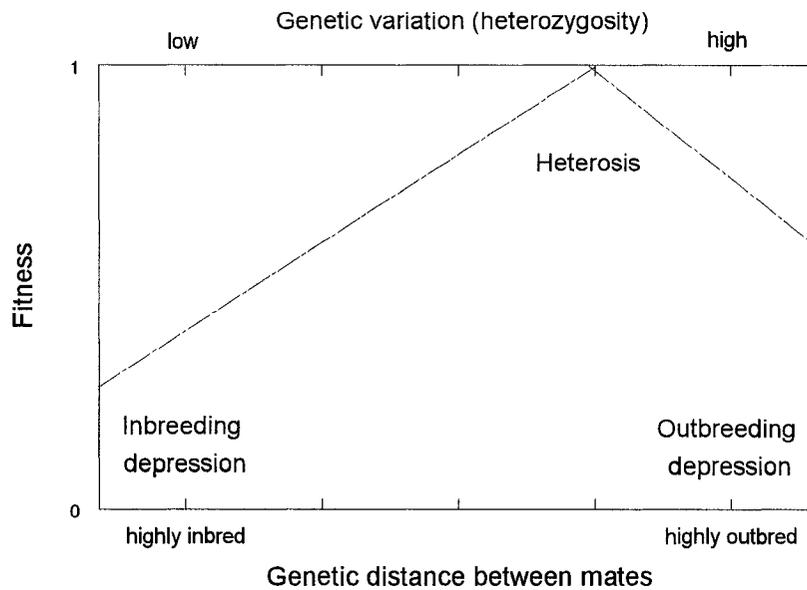


Figure i.1. The fitness continuum from inbreeding depression to outbreeding depression (modified from Shields 1982; Mitton 1993; Waples 1994).

1992). Theoretically, fitness is the extent to which an individual contributes genes to future generations, but, in practice, it is an individual's score for a measure of performance expected to correlate with genetic contribution to future generations (Freeman and Herron 1998). The fitness of an individual is the final outcome of all its developmental, physiological and behavioural traits (Falconer and Mackay 1996). Variation in these traits ('fitness components', Endler 1986) reflects more or less, depending on their place in a hierarchy (Figure i.2), the variation in fitness. Direct measurement of fitness is uncommon, particularly for natural systems, because of the logistics and time commitment necessary to collect the appropriate data. Indirect measures of fitness (e.g., body size) that are correlated with components of fitness (e.g., fecundity) or direct measures of fitness components (e.g., fecundity, fertility, survival, mating ability, fertilizing ability) are more typically reported (Endler 1986). Offspring and parental fitness are inseparable and any distinction between the two is arbitrary (Falconer and Mackay 1996). For example, offspring survival, a function of viability as illustrated in Figure i.2, is a factor in parental fitness, but it is also a direct measure of an offspring fitness

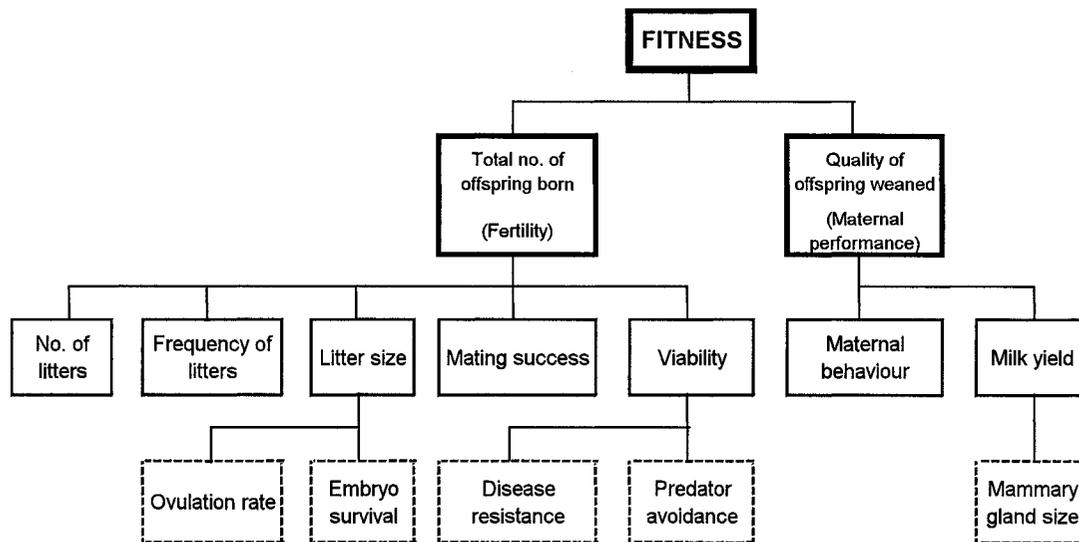


Figure i.2. Example of a fitness component hierarchy. Modified from Falconer and Mackay (1996).

component. Performance traits may be the same as some fitness components (e.g., survival) or they may be traits specific to production or culture (e.g., harvest weight) and unrelated to reproductive contribution.

The focus of this research is the detection and characterization of the relationship between genetic variation and fitness or performance in captive-reared wild and domestic chinook salmon (*Oncorhynchus tshawytscha*). The search for this relationship was approached from two perspectives. Chapter 1 assesses the performance of intraspecific hybrids for evidence of heterosis that could be of value in an aquaculture breeding program, that is, genetic variation examined from an applied perspective. Chapter 2 generates empirical observations on genetic variation-fitness relationships and discusses these relationships in an evolutionary context.

Chapter 1

PERFORMANCE OF HYBRIDS BETWEEN WILD AND DOMESTIC CHINOOK SALMON: EVIDENCE OF HETEROSIS

1.0 Abstract

This study examined the performance of chinook salmon hybrids, reared at a commercial salmon farm, for evidence of heterosis (hybrid vigour). The study population consisted of wild and domestic purebreeds and their reciprocal hybrids. Twenty-eight traits were measured in several areas of performance (survival, growth, saltwater tolerance and stress response). Mid-parent heterosis was calculated for all traits. Additionally, performance differences between wild and domestic fish were assessed, maternal effects were described and heritabilities were estimated for selected traits. Favourable but low heterosis was demonstrated for 36% of the performance traits. None of this was 'useful' heterosis, that is, heterosis that exceeds the performance of the best parental lineage. There appeared to be strong maternal effects, particularly from wild dams, that may have inflated or masked heterosis for certain traits (e.g., freshwater size-at-age). There is little evidence that the degree of favourable heterosis may be predicted from the genetic similarity of the parents. However, there is some suggestion of outbreeding depression. There is evidence of decreased genetic variation and inbreeding depression among the domestic fish and past breeding practices appear to have affected their performance. Heritability estimates indicate that a selection program based on weight at 615 days post-fertilization or stress recovery could elicit a selection response. The lack of favourable heterosis for many traits, and its generally small effect when present, seem to suggest that crossbreeding is of limited utility in chinook salmon culture. However, several reasons are discussed as to why it should not be ruled out as a viable breeding strategy. Finally, regardless of the degree of heterosis, the domestic population appears to have benefited from the influx of new genes.

1.1 Introduction

Quantitative genetics principles, as traditionally applied to livestock and food crop species, were first considered for salmon farming in Norway in the 1970s (e.g., Gjedrem 1976; Ihssen 1976) and, are now, an integral part of Atlantic salmon (*Salmo salar*) culture in many countries (Sattaur 1989; Gjøen and Bentsen 1997). Atlantic salmon is the principle species farmed in British Columbia. The remaining production (~20% in 1999) is primarily chinook salmon (*Oncorhynchus tshawytscha*), and there are six or seven farms in B.C. that breed chinook salmon for commercial purposes (R. Deegan, B.C. Ministry of Agriculture, Food and Fisheries, pers. comm., Jan. 2001). Mass selection for size and survival, conducted in an informal manner with minimal assessment of its efficacy, appears to be the only breeding method employed. Chinook salmon culture in B.C. would likely benefit from more structured breeding strategies designed to improve production (e.g., faster growth) or reduce losses (e.g., increased disease resistance). Essential to the successful development of such breeding programs is reliable and species-specific information on phenotypic variation, non-additive genetic variation (e.g., heterosis) and additive genetic variation (i.e. heritability) (Gjerde 1986; Gjøen and Bentsen 1997).

Heterosis resulting from crosses between inbred lines or between different races or varieties is well known, and is an important component of breed improvement in plants and animals (Falconer and Mackay 1996). Crossbreeding in fish culture, with the exception of the common carp and catfish, has not been widely applied. Crossbreeding to exploit heterosis does not appear to be part of breeding programs in Atlantic salmon culture – although Gjøen and Bentsen (1997), commenting on Norway's sophisticated commercial breeding system, suggest that crossing captive sub-populations may be a future consideration if genetic variation is lost. Chevassus and Dorson (1990) also state that the use of non-additive genetic variation in fish culture has been little investigated and may be promising, for example, when the low heritability of traits closely associated with fitness (e.g., disease resistance) renders selection less effective.

Heterosis for growth has been shown repeatedly in one of the first fish to be cultured, the common carp (*Cyprinus carpio*) (Wohlfarth 1995). Heterosis has also been documented in this species for survival, disease tolerance (e.g., Hines et al. 1974; Sövényi et al. 1988) and feed conversion efficiency (Suzuki and Yamaguchi 1980). Heterosis for disease resistance has been shown in poeciliids (Clayton and Price 1994). Heterosis for a number of traits, including growth and survival, has been demonstrated for interspecific catfish (*Clarias* spp.) crosses (Rahman et al. 1995). There are examples of heterosis in salmonids for: a) growth (Hershberger 1978 [coho salmon, *O. kisutch*]; Klupp 1979 [rainbow trout, *O. mykiss*]; Ayles and Baker 1983 [rainbow trout]; Einum and Fleming 1997 [Atlantic salmon]); b) survival (Ayles and Baker 1983 [rainbow trout]); c) behaviour (Einum and Fleming 1997 [Atlantic salmon]); d) rate of return (review by Wohlfarth 1986 [various salmonids]; and e) developmental traits (Ferguson et al. 1985 [rainbow trout]; Ferguson et al. 1988 [cutthroat trout, *O. clarki*]). However, Cheng et al. (1987) report no significant heterosis for growth or survival in chinook salmon and other studies have found heterosis for some traits but not for others (e.g., Reisenbichler and McIntyre 1977 [steelhead trout, *O. mykiss*]; Gjerde and Refstie 1984 [Atlantic salmon]; Ferguson et al. 1985 [rainbow trout]).

Heterosis may be predictable from the genetic distance between parental lineages (e.g., Meng et al. 1996; Xiao et al. 1996; Figure i.1). The theory is that the probability of divergent fixation of interacting alleles increases with genetic distance and, thus, heterosis is more likely to occur (Bentsen et al. 1998). However, genetic incompatibilities also become more likely as the genetic distance between parental stocks increases (Waples 1991; Figure i.1), for example, when populations differentiate through adaptation to local conditions (Falconer and Mackay 1996) or when crosses are between different species or distantly related populations (Lynch and Walsh 1998). The resultant outbreeding depression is typically manifested in the F₂ generation; therefore, it is not a concern in commercial culture (crossbreeding to exploit heterosis by definition occurs anew each generation). However, outbreeding depression is often raised as a conservation concern in fish management (e.g., Phillip and Whitt 1991;

Leberg 1993; Waples 1994; Phillip and Claussen 1995). Salmonids, particularly Pacific and Atlantic salmon, show a pronounced tendency to form local populations distinct from one another for a variety of traits (Taylor 1991), thus outbreeding depression is a potential outcome of certain management practices (e.g., stock transfers) and farmed fish escapement (Emlen 1991; Waples, 1994). The topic of outbreeding depression has not been fully explored in salmonids (e.g., Ferguson et al. 1985; Gharrett and Smoker 1991; Johnsson and Abrahams 1991; Waples 1991; Einum and Fleming 1997; Gharrett et al. 1999).

Demonstrating heterosis for performance in chinook salmon under culture conditions was the main goal of this study but additional parameters relevant to breeding programs, such as heritability and maternal effects, were also evaluated. Population-specific estimates of heritability and characterization of maternal effects are important. Selection for growth rate, stress response and disease resistance have shown positive results in salmonids (e.g., Gjedrem 1983; Gjerde 1986; Fevolden et al. 1991; Fleming and Einum 1997) but heritabilities should be determined for the same environment in which selection will take place (Gjedrem 1983). Also, the efficacy of breeding strategies may be compromised by the degree to which maternal effects are responsible for phenotypic variation in traits of interest (Chambers and Leggett 1996).

The performance traits measured in this study were selected based on their importance to fish culture. Obviously, good survival and growth are essential in an industry where animals are harvested for profit. However, attainment of good growth and survival is not simple – stress, disease and variation in adaptive physiological processes (i.e. smoltification) can affect both. For example, once fish reach the saltwater ('grow-out') stage superior survival is particularly desirable, but it is threatened by disease (Price 1985; Chevassus and Dorson 1990). Improved disease resistance would increase survival with additional benefits, such as reduced drug costs and less risk of development of antibiotic resistant pathogens (Inglis et al. 1993). Stress in cultured salmonids appears to have detrimental effects on growth (reviewed by Sumpter 1993), and it is a poorly understood factor in disease resistance (e.g., Möck and

Peters 1990; Fevolden et al. 1992). Stress response differs according to stressor type, environment, species, life stage and prior history (e.g., Barton et al. 1980; Pickering et al. 1982; Barton et al. 1986; Salonius and Iwama 1993). Consequently, 'good' performance for stress response cannot be generalized without simplification and evaluation in context – but it is probably better to be a 'weak responder' (and 'fast recoverer') in a farm setting (Fevolden et al. 1993). Finally, the readiness of a smolt for saltwater entry will impact parameters such as survival and growth (e.g., Folmar and Dickhoff 1981; Clarke and Shelbourn 1985; Hoar 1988). Successful and early smoltification has economic benefits produced by maximizing time spent in the more favourable growing conditions of the ocean (Hoar 1988).

The main objective of this study was to examine the performance of hybrids between wild and domestic chinook salmon for evidence of heterosis. Twenty-eight traits were measured in four areas of performance (survival, growth, saltwater tolerance and stress response). Hybrid performance was evaluated relative to wild and domestic purebred crosses. All crosses were reared in a commercial salmon culture environment. Additional objectives were to estimate heritabilities for selected performance traits; to describe maternal effects; to assess the performance differences between wild and domestic fish; and to compile evidence for the presence of outbreeding depression. The results of this study are discussed primarily with respect to their application to commercial chinook salmon farming. The conservation implications of outbreeding depression and wild-domestic fish interactions are also briefly discussed.

1.2 Methods

1.2.1 Breeding Program

Study Populations

Chinook salmon from two sources were used as parental stock. Half of the parents came from a commercial salmon farm (Yellow Island Aquaculture Ltd., Quadra Island, B.C.). These fish were four generations removed from eyed eggs obtained from the Robertson Creek Salmonid Enhancement Project hatchery, Port Alberni, B.C. in 1985. Hereafter, the

commercially farmed fish will be referred to as 'domestic'. The rest of the parents came from Big Qualicum River, Qualicum Beach, B.C.. A chinook salmon enhancement program has been active on this river since 1967 (G. Ladouceur, Dept. of Fisheries and Oceans [DFO], pers. comm., April 2000). Annual hatchery production has been 4 million smolts for the past four years – a major contribution to the total production of the river (G. Ladouceur, DFO, pers. comm., April 2000). Hereafter, the Big Qualicum fish will be referred to as 'wild', with the understanding that some individuals may have been hatchery-reared.

Breeding

One hundred and four parent fish (26 domestic sires, 26 domestic dams, 26 wild sires, 26 wild dams) were artificially spawned on October 23, 1997. The domestic fish were spawned at Yellow Island Aquaculture Ltd. (YIAL) and the wild fish were spawned by DFO personnel at the Big Qualicum River Salmonid Enhancement Project hatchery (BQSEP). All domestic parents were 3-year-olds. Wild parents were of unknown age; however, reference to age and length data collected by BQSEP during the 1997 spawning period suggests that the majority of the sires were 3-year-olds and that the dams were mainly 3- and 4-year-olds. Parent fish were measured (length) and weighed (Appendix I). A blood or liver sample was taken from each fish for DNA extraction. A sample of unfertilized eggs was collected from each dam (26-127 eggs per dam) for calculation of fecundity and to determine mean egg weight.

A partial diallel mating design (dams nested in sires) was used to generate a total of 104 crosses ('families') in 26 'diallel sets' (Figure 1.1). Eggs were 'dry fertilized' (milt and eggs mixed together followed by the addition of water, Leitritz and Lewis 1980). Fertilization occurred 3 to 18.5 hours after gamete collection, depending on the cross. Eggs were transferred to stacked incubation trays (Heath Tecna Corp., Kent, WA and Marisource, Tacoma, WA) within 30 minutes of fertilization. Each tray held one diallel set. Families within a diallel set were randomly assigned to a 30.5 cm × 40.6 cm × 5.1 cm compartment (four compartments per tray). Fertilized eggs were surface disinfected with a povidone-iodine solution (Dynamic Aqua-Supply Ltd., Surrey, B.C.).

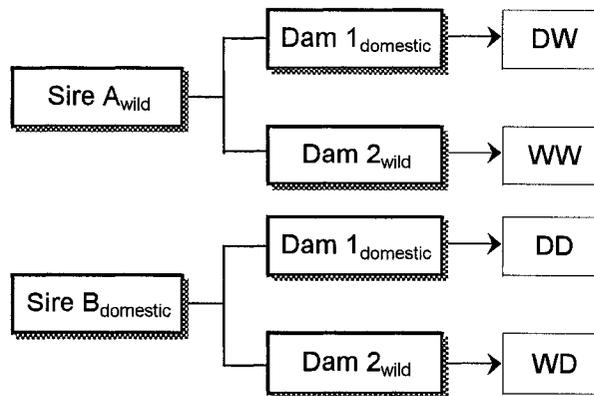


Figure 1.1. Partial diallel mating design with dams nested in sires (single 'diallel set' shown). 104 chinook salmon families were generated from 26 wild dams, 26 wild sires, 26 domestic dams and 26 domestic sires (in 26 diallel sets). Two-letter codes on the right are 'cross type' designations used throughout the text.

Cross Type Designation

There are two purebred cross types (one domestic and one wild) and two reciprocal hybrid cross types (domestic with wild) in this study. Each cross type is identified by a two letter code – the first letter designates the dam (D = domestic, W = wild), and the second letter designates the sire. Thus, there are four cross types codes: DD, DW, WD and WW (Figure 1.1). There were initially 26 families per cross type.

Freshwater Husbandry

The families were reared at YIAL hatchery facilities on Quadra Island, B.C.. The hatchery's water supply originates from an artesian well. Flow rate in the incubation trays was 12-15 L/minute and the mean temperature was 9.2°C (range 7.5-10.0°C). Water temperature was monitored with a data logger (Onset Computer Corp., Pocasset, MA) to allow for an accurate calculation of accumulated temperature units (ATUs), a standard descriptor for early developmental stages in fish. One temperature unit equals the number of degrees Celsius (above freezing) for a period of 24 hours (Becker et al. 1982).

When the eggs reached the 'eyed' stage (eye pigmentation clearly visible through

the eggshell) they were mechanically shocked (poured from one container to another from a height of approximately 20 cm and then swirled) and, 24 hours later, all eggs that had turned white were removed and counted. This is a modification of a standard hatchery practice used to identify and remove unfertilized eggs (e.g., Leitritz and Lewis 1980). The remaining live eggs were counted. Eggs were randomly culled from larger families so that no family had more than 424 eggs (\bar{x} = 349, range 82-424). The entire process (from mechanical shocking to culling) took place over five days (Nov. 30 to Dec. 4, 1997) when the eggs ranged from 340-386 ATUs. Eighty-eight percent of the families were fully hatched by December 22, 1997 (543-552 ATUs).

Alevins were transferred ('ponded') to rearing tanks January 29-30, 1998 (892-911 ATUs). Ponding time was determined based on elapsed ATUs (Becker et al. 1982) and the physical appearance of the alevins (degree of yolk sac absorption). Ponding is timed to coincide with the commencement of exogenous feeding. A total of 94 families were ponded to individual rearing tanks. Ten families were omitted from further study – six families had less than 100 fry each (too few to fulfil future sampling criteria) and four families from one tray had been inadvertently mixed.

Mean rearing tank volume was 121 L (range 101-135 L) at ponding. Initial mean stocking density was 0.89 g/L (range 0.27-1.47 g/L). Stocking density was monitored for each rearing tank and when it reached 10 g/L the volume of water in that tank was increased. Stocking density was no longer monitored once intensive sampling for performance evaluation began. Flow rate for the rearing tanks was 3.2 L/minute and the mean temperature (January 29, 1998 to June 10, 1998) was 8.3°C (range 7.5-9.9°C). Fry were maintained indoors under dim artificial (incandescent) light (initially 10 hrs light:14 hrs dark, increasing to 15 hrs light:9 hours dark by late April) and hand-fed commercial feed (Ewos Canada Ltd., Surrey, B.C.) multiple times per day. Tanks were cleaned (vacuum siphoned) twice a week.

Fish were nose-tagged with an automatic tagging unit (Northwest Marine Technology, Inc., Shaw Island, WA) over three days between May 21-25, 1998. The nose-tags were colour

coded and each family was assigned a unique code. A maximum of 100 fish per family (range 10-100) were tagged. Ten nose-tagged fish from each of 91 families were retained for the saltwater tolerance trial. The remaining nose-tagged fish were transferred to a 12000 L outdoor tank pending transfer to saltwater holding facilities. Nose-tags can only be recovered via post-mortem dissection with the aid of a portable sampling detector (Northwest Marine Technology, Inc.).

Saltwater Husbandry

Chinook salmon are physiologically ready for transfer to saltwater following the process of smoltification (Hoar 1988). Physical characteristics (silver body, colourless fins) and past experience regarding the expected timing of smoltification at YIAL, were used as indicators of smolting in the study fish. All fish appeared to have smolted by mid to late May 1998.

Smolts were immersion vaccinated for vibriosis (Microvib™, Microtek International Ltd, Saanichton, B.C.) on June 21, 1998 (241 days post-fertilization). They were transferred to YIAL's saltwater holding facilities on July 6, 1998 (256 days post-fertilization). These facilities are located on the east side of Discovery Passage, 10 km north of Campbell River, B.C.. All smolts (representing 82 families) were housed together in a 5 m × 10 m netpen (10 m deep) until January 6, 1999 when they were transferred to a full-size netpen (10 m × 10 m, 10 m deep) where they remained for the rest of the study. Fish were hand-fed to satiation with commercial feed (Taplow Grower, Taplow Ventures Ltd., North Vancouver, B.C.) twice per day. The local mean ocean temperature was 10°C for the period July 7, 1998 to July 6, 1999.

1.2.2 Cross Type Performance

1.2.2.1 Performance Traits

All sampled fish were euthanized by immersion in 250 mg/L tricaine methanesulfonate (TMS) (Aqualife TMS™, Syndel Laboratories Ltd., Vancouver, B.C.) and anaesthetized by immersion in 50-100 mg/L TMS, unless noted otherwise. All study protocols were approved by the Animal Care and Use Committee of the University of Northern British Columbia.

Survival

Freshwater Survival:-

Mortalities were counted and removed, at least weekly, through all freshwater stages. Dead eggs were identified when they turned white, and, during the vulnerable pre-eyed stage, were removed with forceps to minimize disturbance to the live eggs. Freshwater survival was calculated for three stages: a) eyed egg (from the eyed stage until hatching); b) alevin (from hatching until ponding); and c) fry (from ponding to May 20, 1998).

Disease Resistance:-

Vibriosis is one of several diseases affecting saltwater survival of farmed salmon in B.C. (Beacham and Evelyn 1992a). Resistance to vibriosis was assessed in two ways:

Outbreak survival:- A vibriosis outbreak occurred at the study site in the spring-summer of 1999 (9-12 months post-transfer to saltwater). Dead fish were retrieved, counted and retained for nose-tag recovery (i.e. family identification). The number of fish in each family at the start of the outbreak (mid-April) was determined from family proportions at saltwater transfer (July 1998) extrapolated to the actual (counted) population size as of January 6, 1999. Mortality between this date and the start of the outbreak was <2%. Implicit in the outbreak survival estimate are the assumptions that dead fish are recovered with equal probability, regardless of cross type, and that mortality before the outbreak was randomly distributed among families.

Disease challenge:- The bacterial pathogen *Vibrio anguillarum*, the causative agent of vibriosis (Inglis et al. 1993), was used in a disease challenge trial. The source culture came from Dr. G. Iwama's laboratory at the University of British Columbia. The trial was conducted over four days (June 2-5, 1998: 222-225 days post-fertilization). The source culture was subcultured onto TSA-salt media (20 g Tryptic Soy Agar, 7.5 g NaCl, 500 mL dH₂O), and incubated at room temperature (20-25°C) for 20 hours prior to the start of each treatment day. On the treatment day, *V. anguillarum* from the subculture was suspended in 5 mL of peptone saline (1 g peptone, 8.5 g NaCl, 1 L distilled H₂O). The optical density of this suspension at

540 nm (OD_{540}) was determined. Peptone saline was added as necessary to adjust the OD_{540} to 1.0. A concentration of approximately 1×10^6 colony-forming units (cfu) per mL was obtained by adding 0.1 mL of the 1.0 OD_{540} suspension to 100 mL of peptone saline. This was the suspension injected into the treatment fish. Tuberculin syringes (1 cc) were loaded with the suspension and kept on ice until used. The actual challenge dose (cfu/mL) in the 1.0 OD_{540} suspension was determined using the standard plate count method (Harley and Prescott 1996). The concentration of *V. anguillarum* administered on each treatment day was: Day 1 – 1.24×10^6 cfu/mL; Day 2 – 1.20×10^6 cfu/mL; Day 3 – 1.28×10^6 cfu/mL; and Day 4 – 1.06×10^6 cfu/mL.

Eighty-seven families were included in the trial. There were 25 treated fish and 10 control fish per family. The average weight of these fish was approximately 7 g. Fish were anaesthetized in TMS buffered with sodium bicarbonate. Treated fish were injected intraperitoneally with 0.1 mL of the *V. anguillarum* suspension. Control fish were injected in the same manner but with 0.1 mL of peptone saline only. Processing time for each family was approximately 10 minutes and occurred during daylight hours (10:30-18:00). Treated and control fish were returned to their tank of origin and housed together with unhandled fish. Differential pelvic fin clipping was used to distinguish the treated and control fish.

Tanks were inspected for mortalities every 3 hours (except between 24:00 and 06:00) for ten days post-challenge. Time of death was considered to be the mid-point of the monitoring period in which a fish died (e.g., 03:00). The first monitoring period of each new treatment day began at 18:00. Mortalities were collected and identified (as control, treated or unhandled). Monitoring finished on June 15, 1998 at midnight. Mean water temperature during this period was 9.1°C (range 8.4 - 9.9°C).

The number of hours post-treatment at which the first fish died and at which 12 of 25 fish (48%) died were chosen as indicators of disease resistance, referred to as *hours*₁ and *hours*₁₂, respectively.

Growth

Freshwater Size-at-Age and Growth Rate:-

Ten alevins from each family were weighed (as a group) on January 25, 1998 (~856 ATUs, 93 days post-fertilization), 4-5 days (depending on family) before ponding. Fry were live weighed on 14 occasions (at 5-15 day intervals) between February 9, 1998 (108 days post-fertilization) and May 19, 1998 (207 days post-fertilization). Initially, 30 fry from each family were weighed as a group. Later, when they were larger, 15 fry from each family were weighed as a group. Growth rate is usually exponential over intervals of a year or less and should be expressed as an instantaneous rate (i.e. specific growth rate) (Busacker et al. 1990). Specific growth rate (% change in body weight per day) was calculated as:

$$\{[\ln(\text{weight } 2) - \ln(\text{weight } 1)]/\text{number of days}\} \times 100$$

for the period from 108 (weight 1) to 207 (weight 2) days post-fertilization (99 days). Fry were not identified individually so specific growth rate was based on family means (Cheng et al. 1987).

Saltwater Size-at-Age and Growth Rate:-

Fish were sampled twice to obtain size-at-age data in saltwater. The first sample (n = 745) was collected December 16-21, 1998 (~420 days post-fertilization and ~6.5 months post-transfer to saltwater). A seine net was set in the netpen to confine the fish and a brailer was used to remove fish and transfer them to the euthanizing solution. Fish were wet weighed and the heads retained for nose-tag recovery. The second sample (n = 900) was taken July 11-14, 1999 (~615 days post-fertilization and just over 1 year post-transfer to saltwater) following the same methodology. Repeat measurement of individual fish was not possible so growth rate was determined from family means. Specific growth rate was calculated for the period from 420 (weight 1) to 615 (weight 2) days post-fertilization (205 days).

Stress Response

The elevation of plasma cortisol levels as an indicator of stress resulting from various capture, handling, and holding practices is well established for salmonids (Barton and Iwama

1991). A stress response trial was conducted from May 25-29, 1998 (214-218 days post-fertilization) during daylight hours (07:00-19:30). Mean water temperature was 8.6°C during this period (range 8.2-9.8°C). Fish were not fed on the day of their trial (last feeding at approximately 17:00 the previous day). Eighty-six families were included in the trial. Mean weight of the trial fish was 5.7 g (± 0.1 S.E.).

Ten fish were sampled at each of three sampling times: T_0 , before application of the stressor (i.e. basal); T_1 , 1 hour after application of stressor; and T_2 , 4 hours post-stressor. Fish were captured using a dip net. T_0 fish were netted first and transferred directly to the euthanizing solution. Next, T_1 and T_2 fish were netted together and placed on a screen held out of the water ('aerial emersion' stressor *sensu* Heath et al. 1993; Salonijs and Iwama 1993) for 60-120 seconds. They were transferred to separate holding cages (stocking density ~4.5 g/L) suspended in their tank of origin. Transfer to holding cages occurred as part of the aerial emersion stressor. The entire holding cage was removed and immersed directly in the euthanizing solution (250 mg/L TMS buffered with an equal concentration of sodium bicarbonate) at the appropriate sampling interval. Blood was collected from the severed caudal peduncle with heparinized microhaematocrit tubes. Processing time for each group of ten was 10-15 minutes. Blood was centrifuged (3 minutes at ~4000 rpm) and the plasma removed and stored frozen at -20°C for three weeks and then at -40°C until assayed.

Plasma cortisol levels were determined using the GammaCoat™ [¹²⁵I] Cortisol Radioimmunoassay Kit (DiaSorin, Inc., Stillwater, MN). Plasma samples with marked haemolysis or that appeared cloudy were excluded from the analysis. Counts per minute (CPM) were converted to µg/dL based on the competitive binding principle and using standard curve formulae described in Shrimpton and McCormick (1999). Two variables were used to describe stress response: a) response – percent change in mean family cortisol concentration from T_0 to T_1 ; and b) recovery – percent difference in mean family cortisol concentration between T_0 to T_2 .

Saltwater Tolerance

A saltwater tolerance trial was conducted from June 10-14, 1998 (230-234 days post-fertilization). The fish used in the trial were nose-tagged in late May 1998 (see Section 1.2.1) and transferred to a 720 L indoor holding tank (stocking density ~10 g/L) where they remained until the start of the trial. Ninety-one families (10 fish per family) were included in the trial. Mean weight of the trial fish was 8.1 g (\pm 0.1 S.E.).

Fifteen fish were transferred at half hour intervals (between 08:00-18:30) from the freshwater holding tank to holding cages (stocking density ~9.6-16.0 g/L) suspended in a 3000 L indoor tank supplied with natural seawater. Mean saltwater temperature was 11.9°C during the trial (range (11.2-13.5°C). The mean salinity was 27.7 ppt (measured with a Hach C0150 Conductivity Meter at 21°C) and did not change appreciably over the course of the trial (range 27.5 to 27.9 ppt). The freshwater temperature was not monitored hourly during this period but daily morning temperatures were 8.1-8.5°C.

Twenty-four hours after transfer to saltwater the fish were sampled. Fish were euthanized and blood was collected from the severed caudal peduncle with heparinized microhaematocrit tubes. Blood was centrifuged (3 min. at ~4000 rpm) and the plasma was removed and stored frozen at -20°C for one week and then at -40°C until assayed. Carcasses were retained for nose-tag recovery.

Plasma chloride ion concentrations (mEq/L) were determined with a digital chloridometer (Haake Buchler Instruments Inc., Saddle Brook, NJ). All samples were assayed in duplicate. Plasma samples with marked haemolysis or cloudiness were excluded from the analysis. Studies have found plasma chloride ion concentrations to be generally less than 170 mEq/L after one day in saltwater (Folmar and Dickhoff 1981 [coho salmon: ~168 mEq/L]; Johnston and Cheverie 1985 [rainbow trout: ~145-165 mEq/L]; McGeer et al. 1991 [coho salmon, six stocks: 160-180 mEq/L]; Brauner et al. 1992 [coho salmon: ~150 mEq/L]) and, after a month or more, to range from 128-155 mEq/L (Wagner et al. 1969 [chinook salmon]; Folmar and Dickhoff 1981 [coho salmon]; Bernier et al. 1993 [chinook salmon]). Therefore, a plasma

chloride ion concentration of 170 mEq/L was chosen as an appropriate upper boundary for indication of successful smoltification.

1.2.2.2 Statistical Analyses

Data normality was assessed by visual examination of normal probability plots for evidence of skewness or kurtosis (Zar 1996). Levene's test was used to determine whether variance was homogeneous among cross types for each performance trait. Generally, one-way fixed-effects analyses of variance (ANOVA) were used to test for the effect of cross type on performance traits. The non-parametric Kruskal-Wallis ANOVA by ranks was used if assumptions were violated (i.e. non-normality, heterogeneity of variance), and could not be corrected with standard data transformations. Either a one-way ANOVA or a Kruskal-Wallis ANOVA was used to determine the effect of density, trial date, treatment date or rearing location (e.g., tray position) on various fitness measures. The Tukey Honest Significant Difference (HSD) post-hoc comparison of means was applied when required. All analyses were done with STATISTICA (Release 5.1, 1996, StatSoft Inc., Tulsa, OK). A significance level of $\alpha = 0.05$ was used for all tests.

1.2.3 Genetic Characteristics

Microsatellite DNA data from Chapter 2 (see Section 2.2.2 for details of methodology) were used to compare the genetic characteristics of the domestic and wild populations. The DNA came from the parental fish ($n = 52$ for each population). The program TFPGA (Tools for Population Genetic Analyses Version 1.3, M.P. Miller, 1997, Northern Arizona University, Flagstaff, AZ) was used to calculate heterozygosity estimates and Nei's unbiased genetic distance measure and to test for differences in allele frequencies with exact probability tests (for individual loci) and Fisher's Combined Probability test (for all loci combined). Fifty batches of 2000 permutations were used to estimate the P -value for each locus. The sequential Bonferroni adjustment was used to control for group-wide Type I error rate when required (Rice 1989; Palmer 1994).

1.2.4 Heterosis

Heterosis was calculated as the difference between the mean performance of the hybrids (DW and WD), combined and separately, and the mean performance of their purebred parent lines (DD and WW), expressed as a percentage of the mean performance of the parent lines ('mid-parent heterosis', Bourdon 1997). Mid-parent heterosis (MPH) was calculated for all performance traits.

A similarity index, based on the sharing of alleles (Section 1.2.3) between the sire and dam of each family ('parental similarity index'), was calculated according to Equation 1 in Lynch (1990). The relationships between degree of MPH for each trait and parental similarity indices were tested using either simple linear regression analysis or the non-parametric Spearman rank correlation. Analyses were done with STATISTICA (Release 5.1, 1996, StatSoft Inc., Tulsa, OK) and a significance level of $\alpha = 0.05$ was used. The sequential Bonferroni adjustment was used to control for group-wide Type I error rate (Rice 1989; Palmer 1994).

1.2.5 Heritability

Narrow sense heritabilities (h^2) were estimated using half sib and full sib variance estimates (Falconer and Mackay 1996; Lynch and Walsh 1998). The diallel mating design allowed the calculation of h^2 components for the entire population and for wild and domestic paternal and maternal half sibs. Mean square values were generated using the General Linear Model in SYSTAT (Version 7.0.1, 1997, SPSS Inc., Chicago, IL). The model for the paternal half sib analysis was:

$$z_{ijk} = \mu + s_i + d_{ij} + e_{ijk},$$

where z_{ijk} is the performance trait measure for the k^{th} offspring from the family of the i^{th} sire and j^{th} dam, μ is the population mean, s_i is the effect of the i^{th} sire, d_{ij} is the effect of the j^{th} dam nested within the i^{th} sire, and e_{ijk} is the residual deviation (Lynch and Walsh 1998). Similarly, the model for the maternal sib analysis was:

$$z_{ijk} = \mu + d_i + s_{ij} + e_{ijk},$$

where z_{ijk} is the performance trait measure for the k^{th} offspring from the family of the i^{th} dam

and j^{th} sire, μ is the population mean, d_i is the effect of the i^{th} dam, s_{ij} is the effect of the j^{th} sire nested within the i^{th} dam, and e_{ijk} is the residual deviation. All effects are random. Variance components were estimated from the mean square values, and the sire- and dam-component estimates of h^2 were calculated, according to standard formulae (Falconer and Mackay 1996; Lynch and Walsh 1998). There were unequal numbers of offspring per dam/sire so the mean number was used as suggested by Falconer and Mackay (1996). Standard errors of the h^2 estimates were calculated according to Roff (1997). The sire-component (h^2_s) is the best estimate of h^2 when the dam-component (h^2_D) is much larger than the sire-component for paternal half sib analysis (vice versa for maternal half sib analysis), otherwise the average of the sire- and dam-components (h^2_{s+D}) is the most reliable estimate (Falconer and Mackay 1996). Paternal half sib analysis yields the most reliable estimates of h^2 (Lynch and Walsh 1998).

Heritabilities were determined for performance traits that were measured on an individual basis (i.e. saltwater size-at-age and saltwater tolerance). Additionally, stress response and recovery were recalculated as the change in individual cortisol concentration at one hour (T_1) and 4 hours (T_2) post-stressor relative to mean family basal (T_0) cortisol concentration (rather than mean family T_1 and T_2 concentration relative to mean family T_0 concentration as in Section 1.2.2.1). Heritabilities were not estimated for traits described by family values (i.e. freshwater size-at-age, growth rate, survival, disease resistance) because the half sib family sizes would be very small ($n = 2$) and result in unacceptably large standard errors (Lynch and Walsh 1998). An estimate of h^2 for weight at 216 days post-fertilization, based on individual weights taken during the stress response trial, was included for comparative purposes.

1.3 Results

1.3.1 Cross Type Performance

Survival

Freshwater Survival:-

The 104 families were distributed among 26 incubation trays (each subdivided into four compartments) in four stacks (up to seven trays per stack). There was no significant effect of tray position, compartment position or stack on eyed egg survival. However, there was a significant effect of tray position on alevin survival ($H_{6,97} = 17.838$, $P = 0.007$). Consequently, alevin survival was standardized by dividing mean family values by the overall mean for their tray position. Each family was maintained in their own rearing tank (no replication) during the fry stage, so a test for tank effects on fry survival was not possible. The generalized location of the rearing tanks in the hatchery (i.e. aisle assignment) did not have a significant effect on fry survival.

There was a significant effect of cross type on freshwater survival (eyed eggs: $H_{3,104} = 50.863$, $P = 0.000$; alevins: $H_{3,97} = 22.064$, $P = 0.000$; fry: $H_{3,94} = 16.224$, $P = 0.001$) (Figure 1.2). Families with wild dams had higher survival than those with domestic dams (eyed eggs: $H_{1,104} = 50.112$, $P = 0.000$; alevins: $H_{1,97} = 20.956$, $P = 0.000$; fry $H_{1,94} = 16.131$, $P = 0.000$). There was no significant effect of sire origin on freshwater survival.

Disease Resistance:-

Outbreak survival:- There was a significant effect ($F_{3,78} = 9.052$, $P = 0.000$) of cross type on survival during the vibriosis outbreak – DD had significantly higher survival (92.4%) than the other cross types. Hybrids were not significantly different from WW (DW: 86.8%, WD: 85.9%, WW: 83.1%). Both dam origin and sire origin had a significant effect on survival ($F_{1,80} = 14.294$, $P = 0.000$; $F_{1,80} = 9.092$, $P = 0.003$, respectively).

Disease challenge:- The challenge was severe – mortality was 76-100% in all families at the end of the ten day monitoring period (0.5% mortality for control fish during the same period). Cumulative mortality curves (Figure 1.3) were also indicative of an extreme challenge (short incubation time and steep mortality curve – GjØen et al. 1997). The majority of the mortality occurred 3-5 days after treatment. Treatment day had a significant effect on $hours_1$ ($H_{3,86} = 23.359$, $P = 0.000$) and $hours_{12}$ ($H_{3,86} = 30.969$, $P = 0.000$). The *V. anguillarum* challenge dose varied with treatment day (Section 1.2.2.1) but was not obviously related to

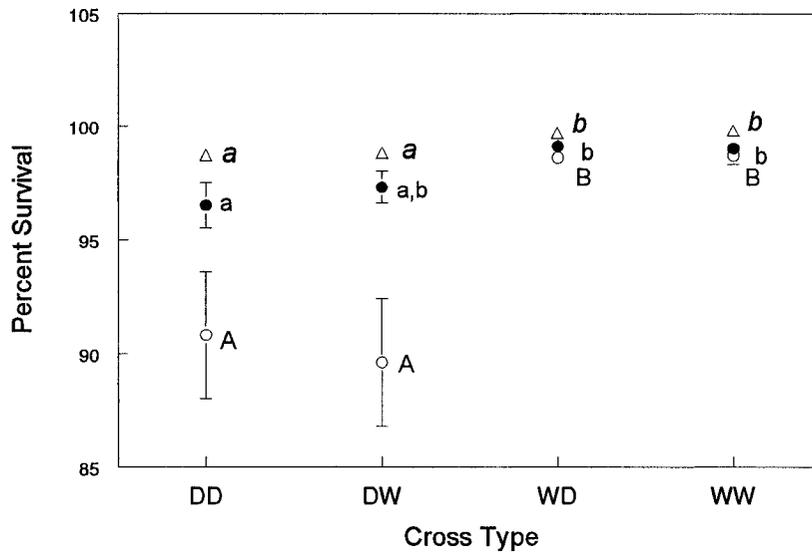


Figure 1.2. Survival of eyed eggs (open circle), alevins (filled circle) and fry (open triangle). Values with matching letters (eyed eggs: upper case; alevins: italics; fry: lower case) are not significantly different ($P > 0.05$, Tukey HSD post-hoc comparison of means). Error bars represent 1 standard error. Alevin survival unstandardized in this presentation (see text). Sample sizes (no. of families) for eyed eggs: DD = 26, DW = 26, WD = 26, WW = 26; alevins: DD = 24, DW = 24, WD = 24, WW = 25; and fry: DD = 24, DW = 22, WD = 24, WW = 24.

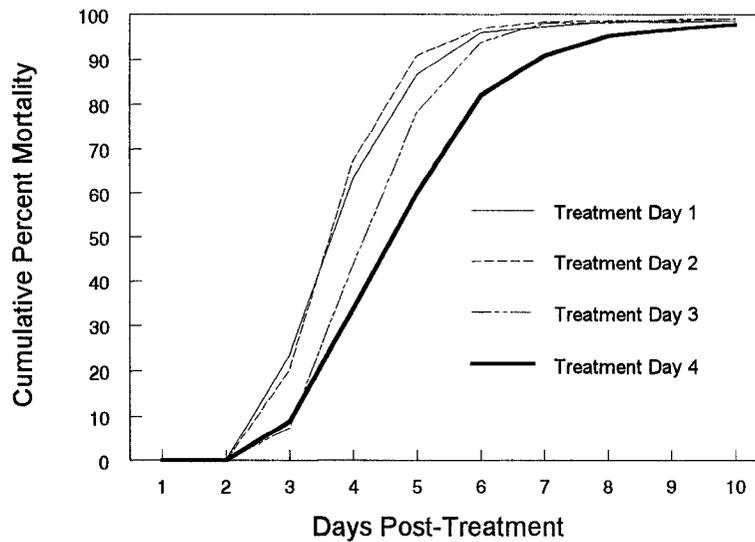


Figure 1.3. Cumulative percent mortality (all families combined) over a ten day period following a *Vibrio anguillarum* disease challenge. The challenge dose of bacteria (cfu/mL) differed among treatment days (see Section 1.2.2.1).

the treatment day effect. $hours_1$ and $hours_{12}$ were each standardized by dividing mean family values by the overall mean for the treatment day. One family was dropped from the trial because of a hypoxia incident early in the post-challenge monitoring period.

There was no significant effect of cross type on $hours_1$ or $hours_{12}$. Families with wild dams had a significantly ($F_{1,84} = 4.897$, $P = 0.030$) greater $hours_{12}$ than those with domestic dams (Table 1.1). There was no significant effect of sire origin on $hours_1$ or $hours_{12}$ or dam origin on $hours_1$ (Table 1.1).

Table 1.1. Mean hours to death (± 1 standard error) following a disease challenge with *Vibrio anguillarum*, summarized for families according to their sire/dam origin. Unstandardized for the effect of treatment day in this presentation (see text).

Parent Origin	Mean hours to death of 1 st fish ($hours_1$)	Mean hours to death of 12 th fish ($hours_{12}$)
Wild dam (WW + WD)	79.5 \pm 1.5	106.7 \pm 2.5
Domestic dam (DD + DW)	76.9 \pm 1.2	100.4 \pm 2.2
Wild sire (WW + DW)	78.1 \pm 1.6	102.3 \pm 2.3
Domestic sire (DD + WD)	78.3 \pm 1.2	104.7 \pm 2.5

Growth

Freshwater Size-at-Age and Growth Rate:-

There was no significant effect of position (tray, stack or compartment) on alevin pre-ponding weight (93 days post-fertilization) or of aisle assignment on fry size-at-age and freshwater specific growth rate. There was also no significant effect of fry density (individuals/L) on any size-at-age measures. The effect of density on freshwater specific growth rate could not be satisfactorily tested.

There was a significant effect ($F_{3,87} = 48.360$, $P = 0.000$) of cross type on alevin weight at 93 days post-fertilization (Figure 1.4). There was also a significant effect ($P \leq 0.001$) of cross type on fry weight for all 14 sampling times (Figure 1.4) and on freshwater specific growth rate ($F_{3,90} = 6.816$, $P = 0.000$). Mean specific growth rates (% change in body weight per day) were: DD = 2.26 \pm 0.04; DW = 2.29 \pm 0.04; WD = 2.07 \pm 0.03; and WW = 2.16 \pm 0.04. WD

had a significantly lower ($P \leq 0.004$) specific growth rate than DD and DW. Reciprocal hybrids were no longer significantly different in weight after 172 days post-fertilization (Figure 1.4). Families with wild dams had significantly ($P \leq 0.002$) greater size-at-age than those with domestic dams throughout the freshwater phase; however, their specific growth rate was significantly lower ($F_{1,92} = 17.123$, $P = 0.000$) than families with domestic dams. There was no significant effect of sire origin on freshwater size-at-age and specific growth rate.

Saltwater Size-at-Age and Growth Rate:-

There was a significant effect of cross type on weight at both 420 and 615 days post-fertilization ($F_{3,690} = 33.036$, $P = 0.000$; $H_{3,807} = 59.108$, $P = 0.000$, respectively) (Figure 1.5). There was no significant effect of cross type on saltwater specific growth rate. Dam and sire origin had a significant effect ($P < 0.05$) on both sizes-at-age. There was no significant effect of dam or sire origin on specific growth rate.

Stress Response

Stress response was significantly affected by trial day ($H_{4,80} = 13.986$, $P = 0.007$). Therefore, stress response for each family was standardized by dividing the mean family value by the overall mean response for each trial day. There were no significant correlations between weight and stress response or recovery. There was no significant effect of cross type on stress response or recovery (Figure 1.6). There was also no significant effect of sire or dam origin on stress response or recovery. Mean family stress response ranged from 12% to 603% ($\bar{x} = 177\%$) above basal cortisol levels. Mean family recovery ranged from -30% to 332% ($\bar{x} = 70\%$) relative to basal cortisol levels.

Saltwater Tolerance

Seventy-seven percent of the challenged fish had plasma chloride ion concentrations ($[Cl^-]$) that were ≤ 170 mEq/L and the mean plasma $[Cl^-]$ values for each cross type were also below this threshold (Figure 1.7). Plasma $[Cl^-]$ was significantly negatively correlated with weight ($r = -0.34$, $P = 0.000$), and there was a significant difference ($H_{3,758} = 83.054$, $P = 0.000$) in weight among cross types (Figure 1.7). Consequently, the residuals generated from the

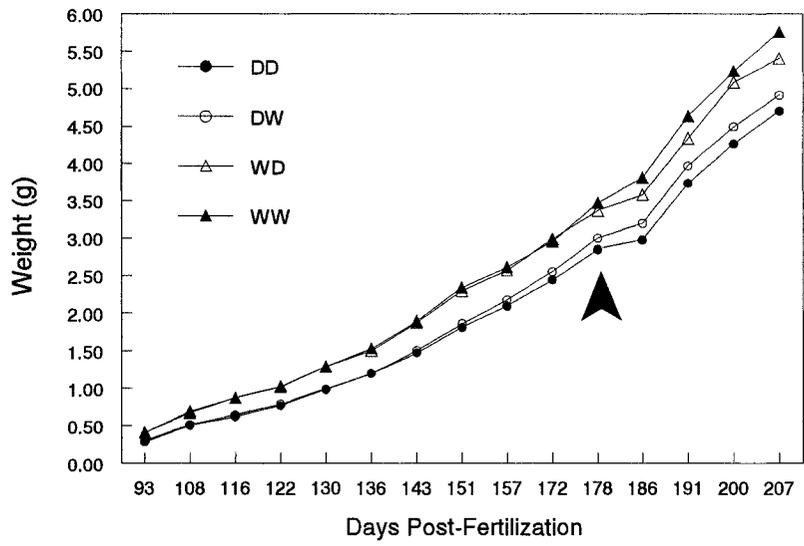


Figure 1.4. Size-at-age (body weight) for each cross type during the freshwater phase. Significant effect ($P > 0.05$) of cross type at all sampling times. Arrowhead indicates point at which reciprocal hybrids were no longer significantly different in size. Sample sizes (no. of families) at 93 days: DD = 23, DW = 21, WD = 23, WW = 24; and from 108-207 days: DD = 24, DW = 22, WD = 24, WW = 24.

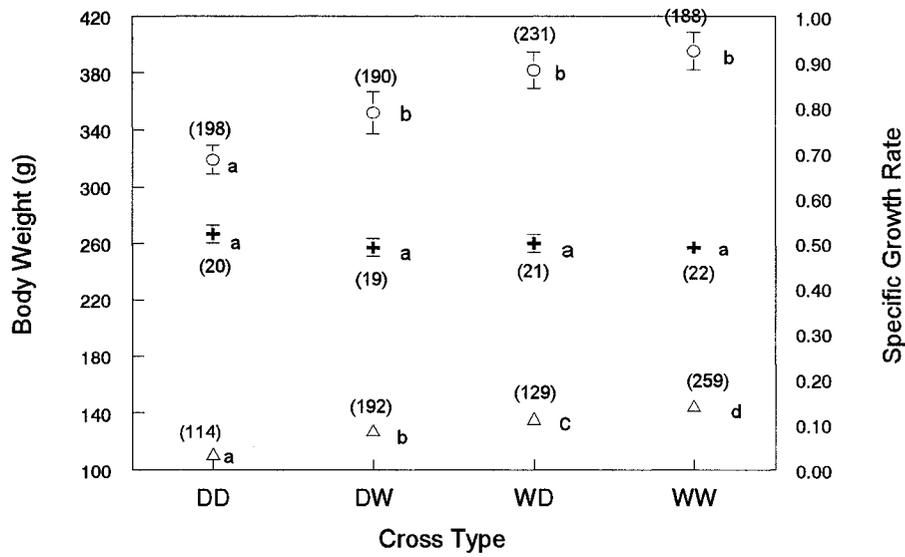


Figure 1.5. Size-at-age (body weight) and specific growth rate for each cross type during saltwater residency. Weight determined twice: a) 420 days post-fertilization (triangle); and b) 615 days post-fertilization (circle). Specific growth rate calculated over the 205 day period between these samples. Values with matching letters are not significantly different ($P > 0.05$, Tukey HSD post-hoc comparison of means). Error bars represent 1 standard error. Sample sizes in brackets (no. of individuals for size-at-age, no. of families for specific growth rate).

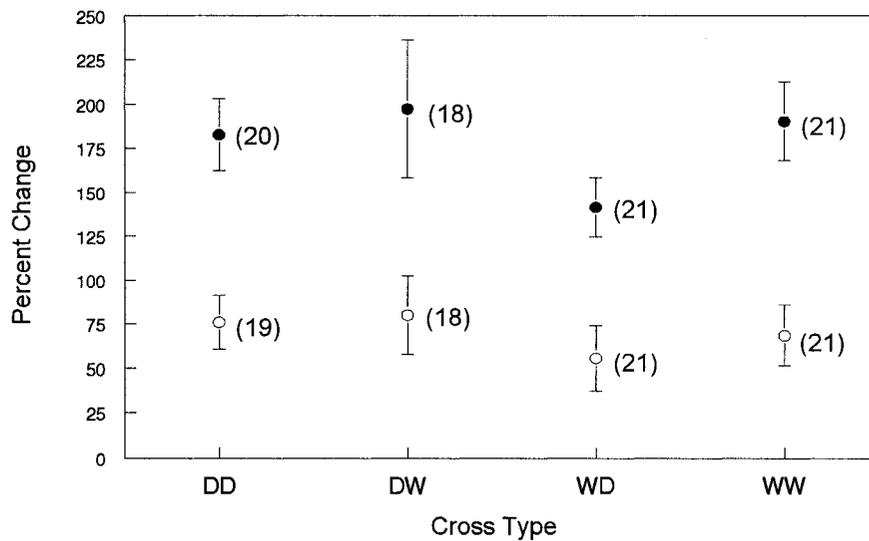


Figure 1.6. Percent change in plasma cortisol relative to basal levels 1 hour post-stressor ('stress response', filled circle) and 4 hours post-stressor ('stress recovery', open circle). Values for stress response unstandardized in this presentation (see text). Cross types not significantly different ($P > 0.05$, Tukey HSD post-hoc comparison of means). Error bars represent 1 standard error. Sample sizes in brackets (no. of families).

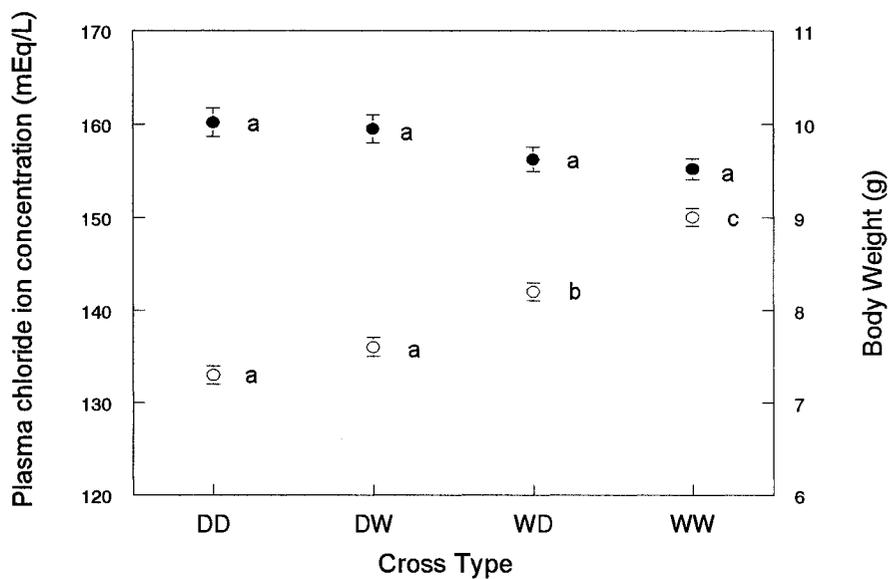


Figure 1.7. Mean plasma chloride ion concentration (mEq/L) following a saltwater tolerance trial (filled circle) and mean weight of sampled fish (open circle) for each cross type. Values with matching letters are not significantly different ($P > 0.05$, Tukey HSD post-hoc comparison of means). Note that residuals from the regression of plasma [Cl⁻] on body weight were used to test for differences in saltwater tolerance among cross types (see text). Error bars represent 1 standard error. No. of individuals sampled: DD = 183, DW = 174, WD = 189, WW = 212.

simple linear regression of individual plasma [Cl⁻] on body weight were used to test for differences in saltwater tolerance among cross types and between domestic and wild parents. No significant effects of either cross type or parental origin on saltwater tolerance were found.

1.3.2 Genetic Characteristics

Heterozygosity estimates for the wild and domestic stocks were similar (Table 1.2). However, there were differences among the wild and domestic fish for levels of polymorphism and allele identity (Table 1.2). Allele distributions were significantly different between wild and domestic fish for five of eight loci and over all loci combined (Table 1.2). Nei's unbiased genetic distance between the wild and domestic stocks was 0.0846.

Table 1.2. Summary of genetic characteristics for the wild and domestic chinook salmon populations. Data generated from microsatellite DNA loci for 52 individuals from each population (26 males, 26 females). *P*-values ($\alpha = 0.05$) are for the results of exact tests for population differentiation (based on allele distribution).

Locus	Heterozygosity		No. of alleles		<i>P</i> -value
	Wild	Domestic	Wild	Domestic	
<i>Ots4</i>	0.83	0.81	10	8	0.0000
<i>Ssa197</i>	0.92	0.81	31	21	0.0000
<i>Ots3</i>	0.77	0.85	10	10	0.0294 ¹
<i>Ots107</i>	0.81	0.90	23	16	0.0000
<i>Ots1</i>	0.46	0.52	10	7	0.0012
<i>Omy325</i>	0.87	0.87	11	11	0.2268
<i>Sfo8</i>	0.79	0.85	18	18	0.0000
<i>Oneμ3</i>	0.44	0.48	2	2	0.6718
Mean	0.74	0.76	14.4	11.6	0.0000 ²

¹ non-significant after sequential Bonferroni adjustment

² results for population differentiation, based on allele distribution, over all loci

1.3.3 Heterosis

Only 18 of the 28 performance traits evaluated for evidence of heterosis are included in the figures and tables presented in this section. Ten freshwater size-at-age measures were omitted only for conciseness of presentation and they are included in the results reported in the text.

Estimates of mid-parent heterosis (MPH) for the performance traits ranged from -8.1% to 7.2% for all hybrids combined (figures 1.8 and 1.9). Note that negative values of MPH do not necessarily indicate poorer performance (e.g., stress response). MPH was notable (>1%) and favourable for 10 of 28 traits with an absolute mean of 3.6%. Hybrid performance was not significantly different from either purebred cross type for freshwater survival, weight from 186-207 days post-fertilization, freshwater and saltwater specific growth rate, stress response and recovery, and disease resistance (Table 1.3). Hybrid performance was sometimes as good as, but never better than, the best purebreed for all other traits (Table 1.3). WW was the best performing purebreed for all traits except outbreak survival and freshwater specific growth rate.

Estimates of MPH for the performance traits ranged from -15.4% to 11.1% for DW and from -22.7% to 17.7% for WD (figures 1.8 and 1.9). MPH was notable and favourable for only 2 of 28 traits for DW ($|\bar{x}| = 3.5\%$) and for 21 of 28 traits for WD ($|\bar{x}| = 10.3\%$) (figures 1.8 and 1.9). Neither hybrid's performance was significantly different from either purebred cross type for saltwater specific growth rate, stress response and recovery, and disease resistance (Table 1.4). DW and WD were sometimes as good as, but never outperformed, the best purebred cross type for the remaining traits (Table 1.4).

Mid-Parent Heterosis and Parental Similarity:-

There was only a single significant relationship between MPH and parental similarity index (tables 1.3 and 1.4). MPH for eyed egg survival was positively correlated with parental similarity index ($R = 0.574$, $P = 0.002$) for the WD cross type (Table 1.4).

1.3.4 Heritability

There was appreciable h^2 for body weight at 615 days (0.48 ± 0.30) and stress recovery (0.43 ± 0.30) when all cross types were considered together ('E', paternal half sib analysis for entire population, Figure 1.10). All other estimates of h^2 were negative or included zero within one standard error margin ('E', Figure 1.10). Estimates of h^2 calculated according to dam or sire origin were highly variable with indications of strong maternal effects (i.e. large [>1.0] dam-component) and possible paternal effects (i.e. large sire-component) ('A-D', Figure 1.10).

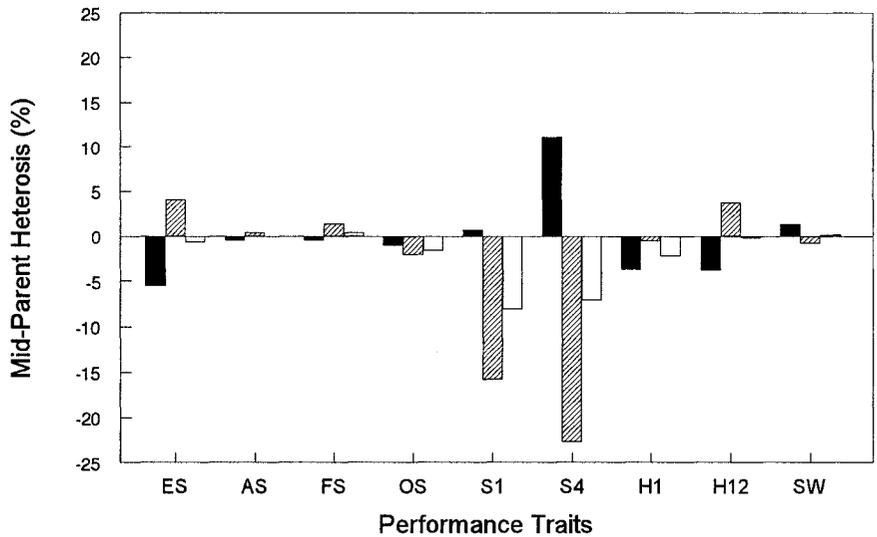


Figure 1.8. Mid-parent heterosis (MPH) for survival, stress response and saltwater tolerance. DW = filled bars, WD = striped bars, hybrid mean = open bars. ES = eyed egg survival; AS = alevin survival; FS = fry survival; OS = outbreak survival; H1 = disease resistance - *hours*₁; H12 = disease resistance - *hours*₁₂; S1 = stress response; S4 = stress recovery; and SW = saltwater tolerance. Standardized values used to determine MPH for AS, S1, H1 and H12 (see text).

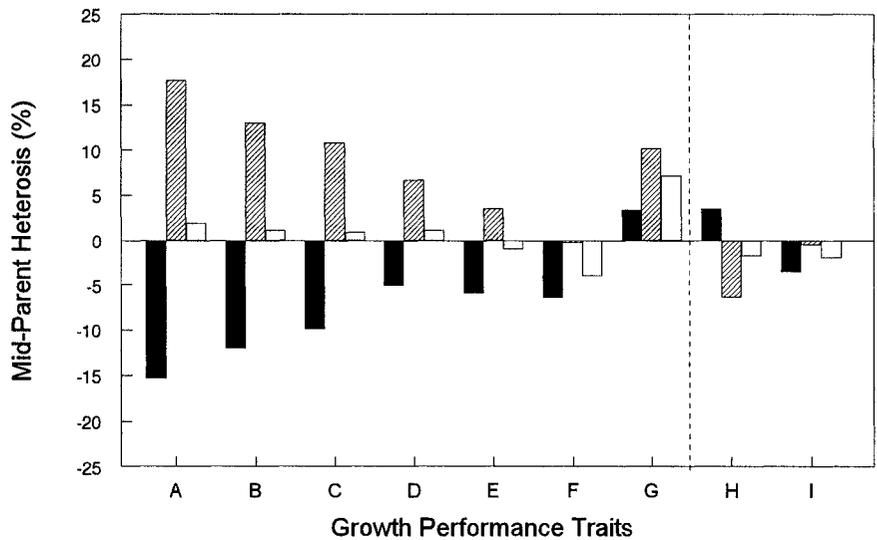


Figure 1.9. Mid-parent heterosis for growth. DW = filled bars, WD = striped bars, hybrid mean = open bars. Size-at-age and growth rate histograms are separated by a dotted line. A = 93 day wgt; B = 122 day wgt; C = 151 day wgt; D = 178 day wgt; E = 207 day wgt; F = 420 day wgt; G = 615 day wgt; H = freshwater specific growth rate; I = saltwater specific growth rate.

Table 1.3. Comparison of the performance of all hybrids combined relative to purebred cross types and the correlation of mid-parent heterosis (MPH) with parental similarity indices. Correlation coefficients flagged by '*' were rendered non-significant after sequential Bonferroni correction – all others were non-significant before Bonferroni correction.

Performance Trait	Performance of Hybrids relative to: ¹		Correlation coefficient ²
	DD	WW	
Eyed egg survival	S	S	0.26
Alevin survival	S ³	S ³	-0.04 ³
Fry survival	S	S	0.16
Outbreak survival	P	S	0.13
<i>Hours</i> ₁	S ³	S ³	0.00 ³
<i>Hours</i> ₁₂	S ³	S ³	-0.01 ³
93 day weight	B	P	0.10
122 day weight	B	P	0.10
151 day weight	B	P	0.15
178 day weight	B	S	0.34*
207 day weight	S	S	0.27
420 day weight	B	P	0.06
615 day weight	B	S	0.02
Freshwater specific growth rate	S	S	0.25
Saltwater specific growth rate	S	S	-0.15
Stress response	S ³	S ³	0.08 ³
Stress recovery	S	S	0.03
Saltwater tolerance	S ³	S ³	-0.03

¹ determined by Tukey HSD post-hoc comparison of means: B = significantly better performance; P = significantly poorer performance; S = performances not significantly different

² correlation coefficient may be generated from Spearman rank correlation or simple linear regression analyses

³ used standardized values (see text)

Table 1.4. Comparison of the performance of each hybrid cross type relative to purebred cross types and the correlation of mid-parent heterosis (MPH) with parental similarity indices for each hybrid type. Correlation coefficients are flagged by ‘***’ when they are still significant after sequential Bonferroni correction or by ‘*’ when rendered non-significant after Bonferroni correction.

Performance Trait	Performance of DW relative to: ¹		Correlation Coefficient ²	Performance of WD relative to: ¹		Correlation Coefficient ²
	DD	WW		DD	WW	
Eyed egg survival	S	P	0.18	B	S	0.57**
Alevin survival	S ³	P ³	-0.13 ³	B ³	S ³	-0.07 ³
Fry survival	S	S	0.06	B	S	0.24
Outbreak survival	P	S	0.01	P	S	0.20
Hours ₁	S ³	S ³	-0.12 ³	S ³	S ³	0.10 ³
Hours ₁₂	S ³	S ³	-0.04 ³	S ³	S ³	0.02 ³
93 day weight	S	P	-0.08	B	S	0.33
122 day weight	S	P	0.16	B	S	0.12
151 day weight	S	P	0.25	B	S	0.15
178 day weight	S	P	0.44*	B	S	0.32
207 day weight	S	P	0.43*	S	S	0.18
420 day weight	B	P	-0.07	B	P	0.21*
615 day weight	B	S	-0.08	B	S	0.11
Freshwater specific growth rate	S	S	0.42	P	S	0.20
Saltwater specific growth rate	S	S	-0.25	S	S	-0.06
Stress response	S ³	S ³	0.24 ³	S ³	S ³	0.0 ³
Stress recovery	S	S	0.09	S	S	-0.01
Saltwater tolerance	S ³	S ³	-0.03	S ³	S ³	-0.03

¹ determined by Tukey HSD post-hoc comparison of means: B = significantly better performance; P = significantly poorer performance; S = performances not significantly different

² correlation coefficient may be generated from Spearman rank correlation or simple linear regression analyses

³ used standardized values (see text)

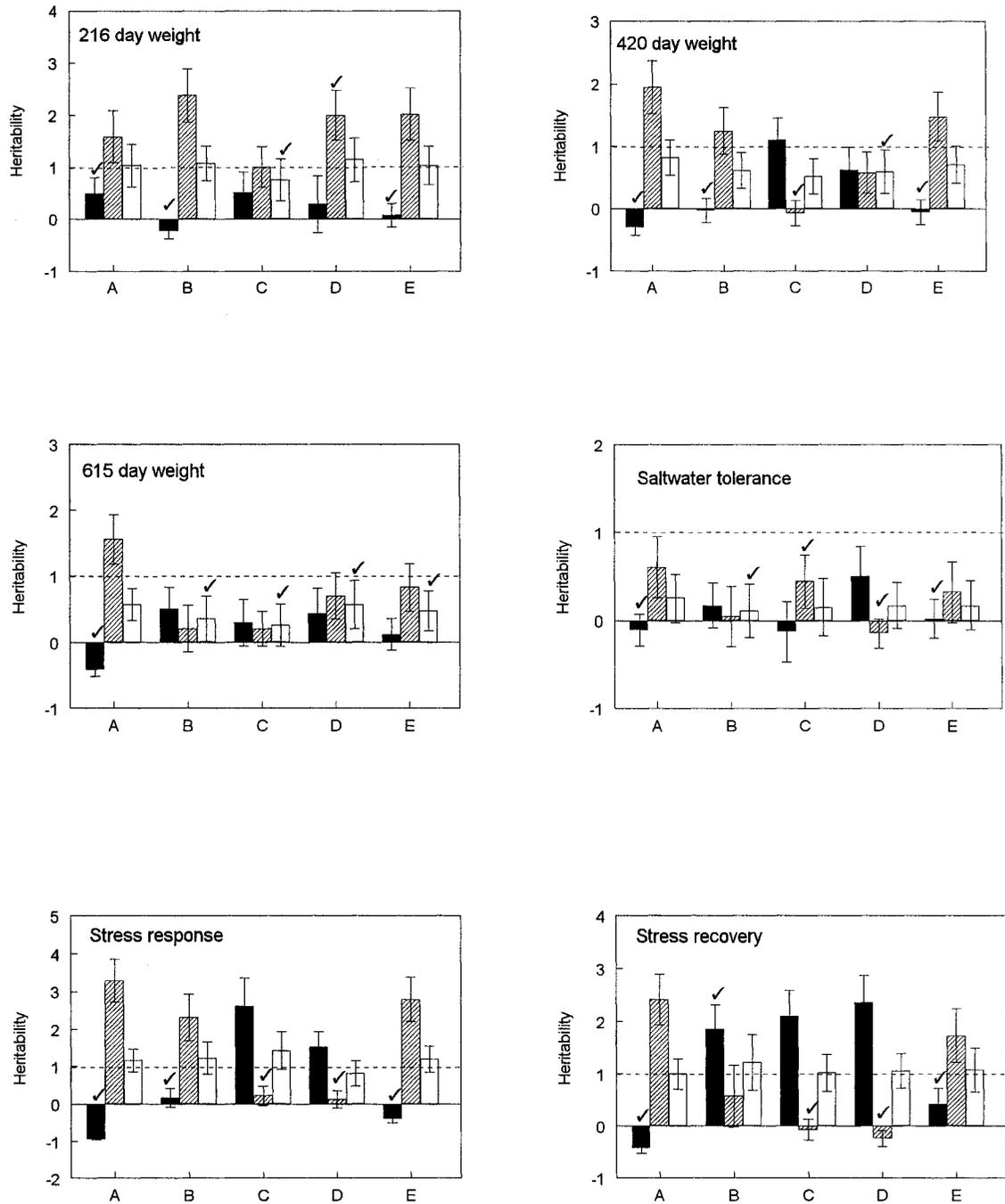


Figure 1.10. Narrow sense heritability (h^2) estimates for selected performance traits. Filled bars = $h^2_{S_i}$; striped bars = $h^2_{D_i}$; open bars = h^2_{S+D} . Bars marked with '✓' are best estimates of h^2 (see Section 1.2.5). A = domestic paternal half sib (PHS) analysis; B = wild PHS analysis; C = domestic maternal half sib (MHS) analysis; D = wild MHS analysis; E = PHS analysis for entire population. Error bars represent 1 standard error.

1.4 Discussion

There was notable (>1%) mid-parent heterosis for 54% of the performance traits examined for hybrids between wild and domestic chinook salmon. Sixty-seven percent of those traits (i.e. 36% of all traits) demonstrated favourable heterosis (mean |MPH| = 3.6%). The remainder showed unfavourable heterosis (mean |MPH| = 2.3%). However, favourable heterosis is not the predominant outcome if MPH estimates for traits categorized in the same area of performance (e.g., multiple freshwater size-at-age measures) are averaged together. In that case, 70% of the MPH estimates are notable and, of those, only 29% are favourable. Reported values for heterosis in fish vary widely (Table 1.5; Wohlfarth 1993); however, the favourable MPH estimates in this study are considered low (Bentsen et al. 1998). A previous study on chinook salmon reported no significant heterosis for body weight, specific growth rate and survival in freshwater and saltwater (Cheng et al. 1987); however, when mid-parent heterosis is calculated and summarized from their published data as it was in this study, similar levels of MPH are found (mean favourable |MPH| = 5.5%; mean unfavourable |MPH| = 3.5%).

The lack of favourable heterosis for many traits and its generally small effect when present, suggest that crossbreeding for improvement of performance in cultured chinook salmon may be of limited utility. None of the heterosis detected in this study, for the hybrids combined or separately, was 'useful' heterosis, that is, heterosis that exceeds the performance of the best parental lineage (Falconer and Mackay 1996). Effective crossbreeding requires the maintenance of pure, preferably inbred, lines. This could be a difficult or costly undertaking, particularly for animals, as inbred lineages often suffer from low offspring viability and other problems (Lacy et al. 1993; Waldman and McKinnon 1993; Falconer and Mackay 1996). Hybrid performance must, therefore, be significantly superior to the best lineage to justify a crossbreeding program (Kinghorn 1983; Gjerde 1993; Bourdon 1997). The purebred wild fish were clearly the superior parental lineage in this study for all but outbreak survival and freshwater specific growth rate. Obviously, in commercial culture, lineages maintained or obtained for crossbreeding would be domestic not wild. Interestingly, although there was no

Table 1.5. Mid-parent heterosis (MPH) estimates for various performance traits in plants and animals.

Organism	Trait	Average MPH (%)	MPH (%) Range	Reference
Rainbow trout:	100 day weight ¹	32	--	Klupp 1979
	150 day weight ¹	0.5	--	Klupp 1979
	282 day weight ¹	-2	--	Klupp 1979
	Harvest weight ²	--	-2.8 to 49.8	Ayles and Baker 1983
	Survival ²	--	2.7 to 24.3	Ayles and Baker 1983
	Harvest weight ³	--	-9 to 36	Gjerde 1988
Atlantic salmon:	Harvest weight ⁴	-1.6	-9.0 to 5.9	Gjerde 1993
	Ocean survival ⁴	2.5 ⁵	-33.6 to 37.6 ⁵	Gjerde 1993
Common carp:	8 month weight ⁶	--	-26.1 to 23.9 ⁵	Suzuki and Yamaguchi 1980
Nile tilapia (<i>Oreochromis niloticus</i>):	Harvest weight ⁷	4.3	--	Bentsen et al. 1998
Platies (<i>Xiphophorus</i> spp.):	White spot infection ⁸	16.2 ⁹	--	Clayton and Price 1994
Beef cattle:	Yearling weight	6.0	--	Bourdon 1997
Dairy cattle:	Mature weight	5.0	--	Bourdon 1997
	Calf survival	15.5	--	Bourdon 1997
Swine:	Days to 230 lbs	-7.0	--	Bourdon 1997
Sheep:	Mature ewe weight	5.0	--	Bourdon 1997
Poultry:	Body weight	3.0	--	Bourdon 1997
	Egg production	12.0	--	Bourdon 1997
Rice:	Yield per plant ¹⁰	57.0	-8.5 to 130.0	Zhang et al. 1994
	Seeds per panicle ¹⁰	34.8	-10.2 to 88.7	Zhang et al. 1994
	Kernel weight ¹⁰	3.7	-5.1 to 12.8	Zhang et al. 1994
	Days to maturity ¹¹	-3.3	-20.1 to 19.8	Xiao et al. 1996
	Yield potential ¹¹	68.5	-8.0 to 205.0	Xiao et al. 1996
	Panicles per plant ¹¹	6.8	-30.0 to 45.1	Xiao et al. 1996

¹ 3 hybrid crosses

² range for average values from each of six hybrid crosses

³ 15 hybrid crosses

⁴ 10 hybrid crosses

⁵ recalculated for this presentation as per Section 1.2.4

⁶ 4 hybrid crosses

⁷ least square mean heterosis of both reciprocals of all strains (n = 8) across all test environments

⁸ 2 hybrid crosses

⁹ for lower parasite count

¹⁰ 28 hybrid crosses

¹¹ 43 hybrid crosses

useful heterosis at the population level, there were individual hybrid families that exhibited useful heterosis (38% of hybrid families, averaged over 18 traits). This information and the following discussion suggest that crossbreeding between suitable chinook salmon lineages should not be ruled out as a viable breeding strategy.

Breeding programs that routinely incorporate crossbreeding generally use lineages that are inbred to some degree (Sprague 1983; Falconer and Mackay 1996; Lynch and Walsh 1998). Lower heterosis might be expected if relatively non-inbred lineages are crossed. There is evidence that at least one of the lineages used in this study was inbred. Heterozygosity estimates for the purebred wild and domestic populations are not significantly different (0.74 and 0.76, respectively) and are similar to other microsatellite DNA-derived values for salmonids (e.g., DeWoody and Avise 2000; Withler et al. 2000). However, a heterozygosity estimate for this domestic population after one generation of captive breeding was 0.86 (Heath et al. 1994), indicating that genetic diversity has declined over time. Further, there is evidence for erosion of additive genetic variance in the domestic population, as might be expected with inbreeding (Falconer and Mackay 1996). That is, when averaged over the six traits, h^2 was much lower for domestic sires (-0.28) than for wild sires (0.40) ('A' vs. 'B', Figure 1.10).

Additionally, there is evidence of inbreeding depression among the domestic fish. First, their performance for most traits measured in this study was inferior to the other cross types, although this could be due to factors other than inbreeding (e.g., maternal effects). Second, DD fry had a significantly higher incidence of gross deformities than did WW fry (17.7% vs. 1.6%). An increased frequency of deformities is a common result of inbreeding in fish (Kincaid 1976; Waldman and McKinnon 1993). The domestic fish were also smaller than the wild fish contrary to studies that have found domesticated salmonids to be larger than their wild counterparts under artificial conditions (e.g., Vincent 1960; Fleming and Eium 1997). Again, this may be the result of factors other than inbreeding – the domestic population used in this study is subject to mass selection for vibriosis resistance and against early male maturation (jacking). Reduced growth may be an unintentional or correlated response to this otherwise

successful selection regime (note the superior performance of the purebred domestic fish for vibriosis outbreak survival, p. 21). Regardless of the degree of heterosis, the domestic population appears to have benefited from the influx of new genes, although to adequately describe the effect it would be necessary to compare important performance traits (e.g., harvest weight) between the study population and an equivalent cohort of YIAL's regular production fish.

The genetic distance between the wild and domestic populations was not large (< 0.15 , Nei 1978) which has implications for the degree of heterosis expected, or alternately, for the likelihood of outbreeding depression occurring. This study crossed only two lineages, domestic (YIAL) and wild (Big Qualicum River), producing two different hybrids, DW and WD. Thus, an analysis of the relationship between genetic distance and heterosis comparable to studies that have utilized large numbers of hybrids (e.g., Zhang et al. 1994 [43 hybrids]) was not possible. However, using the same theoretical basis, this study examined the effects of decreasing similarity index (i.e. increasing genetic distance) between individual dam-sire matings (families) on mid-parent heterosis values. The results lend little support to the idea that the degree of heterosis (favourable) may be predicted from the genetic similarity of the parents or parental lineages as reported in other studies (Levin and Bulinska-Radomska 1988; Xiao et al. 1996; Meng et al. 1996 but see Bentsen et al. 1998). However, there was some evidence suggestive of outbreeding depression that will be discussed later.

Zhang et al. (1994) observed that yield had much greater heterosis than did yield component traits, as would fit a model of yield as a multiplicative function of its components. Weight at 615 days post-fertilization is the most 'cumulative' performance trait in this study and it does have one of the highest favourable MPH values (7.2%), rivalled only by stress response and recovery (8.1% and 7.1%, respectively). Heterosis for harvest weight could potentially be higher than that for any of the performance traits examined in this study – another reason not to discount the utility of crossbreeding to commercial culture.

The degree of heterosis also appears to be a function of the quality of the environment

(Wohlfarth 1993; Marks 1995). For example, there is evidence for greater heterosis for growth rate under conditions that cause lower growth rates in the purebreeds (Wohlfarth 1993 but see Bentsen et al. 1998). The conditions the study fish were reared under are similar to those used for commercial production at the same facilities, so, presumably, would be conducive to good growth. Therefore, heterosis may be less pronounced due to favourable genotype-environment interactions for all cross types.

It is quite common for the performance of reciprocal hybrids to be different (Clayton and Price 1994). The performances of the reciprocal hybrids in this study were statistically different for eyed egg and alevin survival, early freshwater size-at-age, freshwater specific growth rate, 420 day weight, and saltwater tolerance. WD performed better than DW for all these traits except freshwater specific growth rate. Ninety-one percent of the traits with notable MPH for WD showed favourable heterosis while only 8% did for DW. The reciprocal hybrids in this study were no longer significantly different in freshwater size after 172 days post-fertilization. Other studies have found this to be the approximate time at which maternal effects decline in salmonids (e.g., Silverstein and Hershberger 1992; Heath et al. 1993; Heath and Blouw 1998). Differences in reciprocal effects may indicate that lineages differ in maternal effects (Bentsen et al. 1998). This possibility will be discussed later. Other possible sources of reciprocal effects are sex-linked or cytoplasmic inheritance or paternal effects (Bentsen et al. 1998). Differences in reciprocal hybrid performance may justify the use of particular sire or dam lines for a more effective crossbreeding program (Bentsen et al. 1998).

The heritability estimates in this study indicate that a selection program based on saltwater size-at-age (615 days post-fertilization) and stress recovery could generate a selection response in this population. The heritabilities estimated for size (body weight) fall within the range of h^2 values found in the literature (Table 1.6; Gjedrem 1983; Kinghorn 1983). The heritability estimate for stress recovery is higher than h^2 values reported for other stress-related parameters (Table 1.6); however, it is important to note that these values are the only

Table 1.6. Narrow sense heritability (h^2) estimates for body weight and stress response in salmonids. Estimation methods vary. Standard error included if available.

Species	h^2	Reference
<i>Post-stress cortisol levels:</i>		
Atlantic salmon	0.05 ± 0.03	Fevolden et al. 1993
Rainbow trout	0.27 ± 0.10	Fevolden et al. 1993
<i>Body weight less than 1 year post-fertilization:</i>		
Chinook salmon	0.0 ± 0.27	Withler et al. 1987
	0.57 ± 0.28	Heath et al. 1999
Brook trout	0.08 ± 0.13; 0.60 ± 0.27	Robison and Luempert 1984
Rainbow trout	0.73 ± 0.35; 0.74 ± 0.34; 0.82 ± 0.38; 1.06 ± 0.49	Klupp 1979
	0.10 ± 0.05; 0.22 ± 0.15; 0.29 ± 0.15	McKay et al. 1986
	0.52 ± 0.15	Gall and Huang 1988
<i>Body weight one year or more post-fertilization:</i>		
Chinook salmon	0.25 ± 0.10; 0.39 ± 0.08	Winkelman and Peterson 1994
	0.20 ± 0.30	Mousseau et al. 1998
Atlantic salmon	0.38 ± 0.10	Gjerde and Gjedrem 1984
	0.41-0.60 (S.E. ≤ 0.19)	Fjalestad et al. 1996
Rainbow trout	0.32 ± 0.11	Gjerde and Gjedrem 1984
	0.17 ± 0.12; 0.20 ± 0.12	McKay et al. 1986
	0.18 ± 0.12; 0.20 ± 0.11; 0.20 ± 0.10	Gall and Huang 1988
	0.21 (S.E. ≤ 0.10)	Gjerde and Schaeffer 1989
	0.14	Su et al. 1997

published h^2 estimates for post-stressor cortisol levels in salmonids. Heritability estimates may be biased by the combination of individuals from two different stocks (wild and domestic). Selection to improve an economically important trait such as harvest weight should be based directly on performance for harvest weight (Winkelman and Peterson 1994); however, when such data is unavailable selection could be based on other (presumably correlated) traits (e.g., 615 day weight).

All areas of evaluation (cross type performance, heterosis and heritability) indicate, not unexpectedly, the presence of strong maternal effects. There was a significant effect of dam origin (solely) on freshwater survival, freshwater size and specific growth rate, and one measure of disease resistance ($hours_{12}$). Generally, families with wild dams performed better than those with domestic dams, that is, maternal effects from the wild fish appear to be

stronger. The difference in the performance of the reciprocal hybrids, as discussed above, is also indicative of a wild dam advantage. Egg quality was most likely a factor in the difference between wild dam and domestic dam families observed in this study. Egg weight was significantly different ($t = 6.809$, 49 df, $P = 0.000$) between wild ($\bar{x} = 279$ mg) and domestic dams ($\bar{x} = 210$ mg). There was one exception to the apparent wild dam advantage – freshwater specific growth rate was higher for domestic dam families. This may be an example of negative or compensatory maternal effects (Heath and Blouw 1998; Heath et al. 1999).

Maternal effects can be estimated as the difference between the dam-component of variance and sire-component of variance divided by the total phenotypic variance (Beacham 1990; Section 1.2.5 [paternal sib analysis for entire population]). Maternal effects on body weight were 40% in freshwater (216 day weight), declining to 38% at 420 days and then to 18% at 615 days. Such a decline has been reported before, varying in magnitude and timing (e.g., McKay et al. 1986 [rainbow trout]; Heath et al. 1999 [chinook salmon]). Similarly, the dam-component of heritability for weight decreased in magnitude over time (Figure 1.10). Maternal effects on saltwater tolerance were low (7%). Maternal effects on stress response and recovery were surprisingly high, 80% and 33%, respectively. The sire-component of heritability was also notably large for stress recovery. Sire effects are not unknown in fish (e.g., Smoker 1986), and have been reported previously for stress parameters in salmonids (e.g., Fevolden et al. 1991).

Maternal effects can complicate estimation of the additive and non-additive genetic variance available for breed improvement strategies. Selection for improved growth performance would be more effective at later ages (Gall and Huang 1988) when maternal effects have subsided and the degree of additive genetic variance can be more reliably determined. For example, there is a general tendency for heritability of weight to increase with age in salmonids (Kingham 1983) as maternal effects decline. Maternal effects may inflate the degree of heterosis for certain performance traits. Heterosis for growth appears to be more pronounced in younger fish (e.g., Klupp 1979; Wohlfarth 1993). This pattern is evident for WD

in this study (see Figure 1.9). A decrease in heterosis for freshwater size over time is probably related to a concurrent decrease in maternal effects. Differences in maternal effects could be exploited in a breeding program, for example, selection for larger egg size could improve early performance, or, in this study, the preferential use of wild dams over domestic dams would lead to better hybrid performance.

Outbreeding depression can occur in the F_1 generation if hybrids are inferior to the locally adapted population but is more likely in the F_2 generation and beyond due to the breakup of coadapted gene complexes (Lynch and Walsh 1998). There was no strong evidence for outbreeding depression in its strictest sense (i.e. hybrid performance inferior to both purebreeds). The 'locally adapted' population in this study (DD) was, in fact, generally outperformed by one or both of the hybrids. However, at the level of individual hybrid families, there were cases in which hybrid performance was poorer than both purebred lineages (31% of hybrid families, averaged over 18 traits). As previously mentioned, the analysis of the relationship between parental similarity index and mid-parent heterosis provided some evidence for outbreeding depression. There were marginally significant positive correlations with size-at-age for all hybrids combined (178, 186 and 191 days post-fertilization) and DW separately (172-207 and 420 days post-fertilization). Families with less similar parents produced more unfavourable (i.e. negative) MPH in all cases (4 of 10 relationships presented, Figure 1.11). This contrasts with the significant positive correlation between MPH for eyed survival (WD only) and parental similarity index in which, even at the lowest degree of similarity, heterosis was still favourable (Figure 1.12). Finally, an increase in trait variance in F_1 hybrids relative to parental types has been suggested as another indicator of outbreeding depression (Gharrett and Smoker 1991 but see Edmands 1999) – there was no evidence of that in this study (data not presented).

The differences in cross type performance described in this study have only speculative relevance to the conservation and management of wild salmonid stocks. Collectively, hybrids between domestic and wild chinook salmon generally performed as well as purebred wild fish.

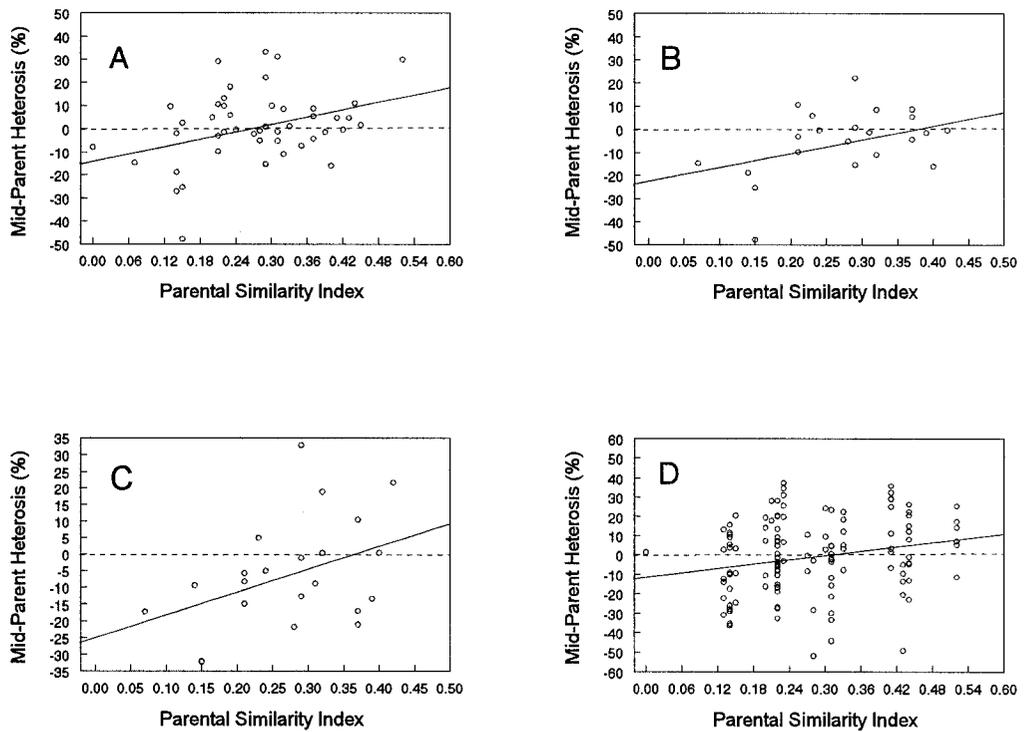


Figure 1.11. Linear regression of mid-parent heterosis on parental similarity index for various size-at-age measures. A: 178 day weight all hybrids combined; B: 178 day weight DW only; C: 207 day weight DW only; D: 420 day weight WD only. Regressions were 'marginally significant' (rendered non-significant after sequential Bonferroni adjustment). Note that positive MPH is favourable for these performance traits.

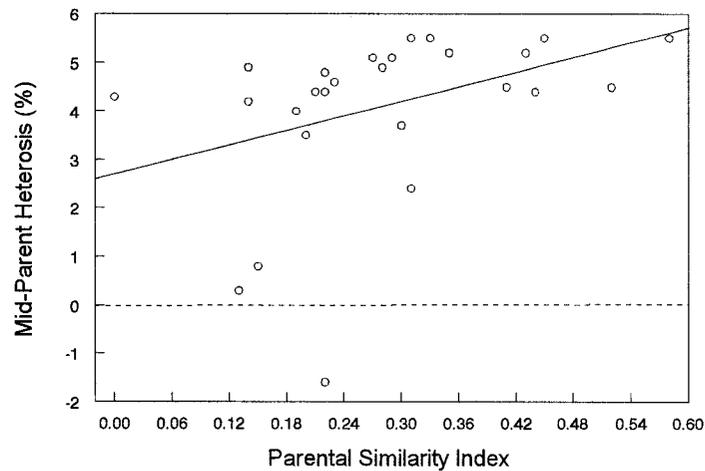


Figure 1.12. Spearman rank correlation between mid-parent heterosis for eyed egg survival (WD only) and parental similarity index. Correlation was significant ($R = 0.574$, $P = 0.002$). Note that positive MPH is favourable for this performance trait.

However, when specific hybrids (i.e. DW) were considered, there were some traits (e.g., size-at-age) for which performance was poorer, relative to wild fish. That this was true in an artificial environment, might suggest the possibility of even more pronounced differences in nature. However, no legitimate inferences about impacts of domestic fish on wild fish can be drawn unless hybrid performance, including the F₂ generation and beyond, is actually evaluated in the natural environment (Emlen 1991).

Some authors suggest that crossbreeding is not a viable approach (logistically or economically) in salmonid culture (Kinghorn 1983; Gjerde 1993). However, this study concludes that the exploitation of heterosis through crossbreeding should not be dismissed as a potential breeding strategy in chinook salmon. That the effects of inbreeding may be somewhat mitigated in salmonids (see Conclusion) allows for speculation – the viability of inbred lines, and hence the feasibility of their maintenance, may be better than is generally found for many farmed animals. A small aquaculture operation may be more successful at implementing a crossbreeding program as the maintenance of parental lineages and the monitoring of performance may be logistically simpler on a small scale. Further research is required to bolster the proposed application of crossbreeding in chinook salmon culture. For example, the performance of hybrids between different lineages of domestic chinook salmon should be assessed for evidence of heterosis, and more performance traits should be monitored including harvest weight, bacterial kidney disease resistance, and timing of sexual maturation.

Chapter 2

THE RELATIONSHIP BETWEEN MICROSATELLITE DNA GENETIC VARIATION AND FITNESS IN CHINOOK SALMON

2.0 Abstract

This study used data from eight microsatellite DNA loci to generate three genetic variation measures (direct count heterozygosity, genetic distance [d^2], similarity index) for each of 104 families of chinook salmon. The captive-reared study population consisted of purebred wild families, purebred domestic families and wild-domestic reciprocal hybrids. Twenty-six traits were examined in three offspring fitness categories (survival, growth and developmental stability), and two traits were evaluated in one parental fitness category (reproduction). Two types of genetic variation analyses were used – multilocus and locus-specific. Results support other studies that indicate genetic variation-fitness relationships are typically weak and suggest that associative overdominance cannot be ruled out as a mechanistic explanation for genetic variation-fitness relationships. Some general patterns emerged that warrant further investigation. Freshwater and saltwater size-at-age were the only fitness-related traits for which significant relationship with genetic variation were detected. There were both negative and positive relationships. Multilocus analysis, indicative of the general effects form of associative overdominance, detected significant relationships only for size-at-age late in the freshwater phase. Locus-specific analysis, indicative of the local effects form of associative overdominance, detected significant relationships for size-at-age in the early freshwater phase and in the saltwater phase. The locus-specific analysis identified several loci that were significantly related to saltwater size-at-age, while only single loci were notable for freshwater size-at-age. Finally, a separate analysis of purebred and hybrid families suggests that hybrids drive the overall pattern of the genetic variation-fitness relationships found in this study, that is, the relationships for the hybrids tend to reflect those detected for all families combined.

2.1 Introduction

Relationships between genetic variation and various fitness measures have been demonstrated for a variety of organisms (e.g., Garton 1984 [gastropod, *Thais haemastoma*]; Danzmann et al. 1986 [rainbow trout, *Oncorhynchus mykiss*]; Wildt et al. 1987 [lion, *Panthera leo*]; Pemberton et al. 1988 [red deer, *Cervus elaphus*]; Quattro and Vrijenhoek 1989 [Sonoran topminnow, *Poeciliopsis occidentalis*]; Borsa et al. 1992 [marine bivalve, *Ruditapes decussatus*]; Zhang et al. 1994 [rice, *Oryza sativa*]; Oostermeijer et al. 1995 [marsh gentian, *Gentiana pneumonanthe*]; Coltman et al. 1998 [harbour seal, *Phoca vitulina*]; Pogson and Fevolden 1998 [Balsfjord population of Atlantic cod, *Gadus morhua*]; Bierne et al. 2000 [shrimp, *Penaeus stylirostris*). However, other studies have found no relationships (e.g., Booth et al. 1990 [land snail, *Cerion*]; Elliott and Pierce 1992 [land snail, *Otala lactea*]; Foltz et al. 1993 [marine snail, *Littorina littorea*]; Whitlock 1993 [fungus beetle, *Bolitotherus cornutus*]; Gardner 1994 [mussels, *Mytilus* spp.]; Savolainen and Hedrick 1995 [Scots pine, *Pinus sylvestris*]; Ferguson 1996 [rainbow trout]; Sheffer et al. 1997 [Gila topminnow, *Poeciliopsis o. occidentalis*]; Pogson and Fevolden 1998 [Barents Sea population of Atlantic cod]). A meta-analysis of published correlation coefficients between heterozygosity and two fitness measures (growth rate and fluctuating asymmetry) suggests that genetic variation-fitness relationships are generally weak (Britten 1996), but the data, in general, do not indicate a universality of such relationships (Zouros et al. 1988; David 1998).

Two mechanisms have been proposed for the detection of relationships between genetic variation and fitness. First, marker loci may be under direct selection (i.e. functional, as is the case with allozymes) and overdominance produces a genetic variation-fitness relationship (David 1998; see also 'true overdominance hypothesis', Roff 1997 and 'selection hypothesis', Pogson and Fevolden 1998). Second, marker loci may reflect genetic variation at other loci through either linkage disequilibrium or identity disequilibrium (David 1998). This is termed 'associative overdominance' (David 1998) although dominance effects may also be involved in the generation of the genetic variation-fitness relationships (Roff 1997; Deng and Fu

1998). David (1998) describes two forms of associative overdominance: local effects and general effects. The local effects form is characterized by neutral marker loci that are in linkage disequilibrium with functional loci (David 1998; see also 'associative overdominance hypothesis', Roff 1997). The general effects form is characterized by marker loci (neutral or functional) that reflect heterozygosity at the genome level, as a result of identity disequilibrium (inbreeding) (David 1998; see also 'inbreeding depression hypothesis', Roff 1997 and 'genotypic association', Savolainen and Hedrick 1995).

An overwhelming majority of published genetic variation-fitness studies have used allozymes to estimate heterozygosity. Microsatellite and other non-coding DNA markers have seldom been used in the study of genetic variation-fitness relationships, despite their high profile in population studies. To date, there are published studies that have used Restriction Fragment Length Polymorphisms (RFLPs) (Pogson and Zouros 1994 [scallop, *Placopecten magellanicus*]; Zhang et al. 1994 [rice]; Pogson and Fevolden 1998 [Atlantic cod]); minisatellite DNA (Hitchings and Beebee 1998 [common toad, *Bufo bufo*]); and microsatellite DNA (Zhang et al. 1994 [rice]; Coltman et al. 1998 [harbour seal]; Coulson et al. 1998 [red deer]; Coltman et al. 1999 [Soay sheep, *Ovis aries*]; Coulson et al. 1999 [red deer]; Bieme et al. 2000 [shrimp]; Slate et al. 2000 [red deer]). Microsatellite DNA markers have several advantages over allozymes in the description of genetic variation-fitness relationships. First, from a practical standpoint, because microsatellite DNA can be amplified by polymerase chain reaction (PCR), very small, degraded or archived samples can be used and the sacrifice of the study organisms is typically not required (Nielsen 1996; David 1998). Secondly, microsatellites are much more polymorphic than allozymes – heterozygosities are often greater than 0.5 (Estoup et al. 1998), thus they may be a more sensitive measure of genetic variation (Coltman et al. 1998; Coulson et al. 1998 but see David 1998). Non-coding marker loci, such as microsatellites, can also assist in the elucidation of the genetic mechanism responsible for genetic variation-fitness relationships when contrasted with allozyme analyses (Pogson and Zouros 1994; David 1998). Finally, in addition to their role as marker loci, microsatellites can provide estimates of the

degree of divergence between two alleles (genetic distance) – information unavailable from traditional estimates of heterozygosity (Coltman et al. 1998; David 1998).

The main objective of this chapter was to examine the relationship between genetic variation and fitness in chinook salmon (*Oncorhynchus tshawytscha*) in an evolutionary context. Genetic variation was estimated from microsatellite DNA analysis at eight loci for 104 families. Three genetic variation measures were calculated – heterozygosity, genetic distance (d^2 , Coltman et al. 1998) and similarity index. Heterozygosity and genetic distance at individual loci were also calculated. Twenty-six fitness-related traits were examined in three offspring fitness categories (survival, growth, developmental stability), and two traits (relative egg weight and relative fecundity) were evaluated in one parental fitness category (reproduction). Relative fecundity and survival are direct measures of fitness components while relative egg weight, growth and developmental stability are indirect measures correlated to fitness components (e.g., survival, fecundity). Wild and domestic chinook salmon and their hybrids were used to maximize the range of genetic variation and to provide an opportunity to contrast genetic variation-fitness relationships in purebreeds and hybrids. Heterozygosity and genetic distance should be positively related to fitness and similarity index should be inversely related to fitness. Alternately, if outbreeding depression is present, then these relationships should be reversed or, perhaps, non-linear (see Lynch 1991).

The findings of this research are discussed with respect to: a) the contribution to existing knowledge regarding genetic variation-fitness relationships, particularly among salmonids; b) the nature of the genetic mechanism behind the relationships (e.g., local versus general effects forms of associative overdominance); and c) the utility of microsatellite DNA data in studies of genetic variation-fitness relationships.

2.2 Methods

2.2.1 Breeding Program

Chinook salmon from two sources were used as parental stock. Domestic parents (26 sires, 26 dams) came from a commercial salmon farm (Yellow Island Aquaculture Ltd., Quadra

Island, B.C.) and wild parents (26 sires, 26 dams) came from the Big Qualicum River Salmonid Enhancement Project hatchery, Qualicum Beach, B.C.. The domestic fish are descended from stock that originated from Robertson Creek (Port Alberni, B.C.) in 1985. Additional information on these source populations is provided in Section 1.2.1. Fish were artificially spawned on October 23, 1997 and a partial diallel mating design (dams nested in sires) was used to generate a total of 104 crosses ('families') (Figure 2.1). There were two purebred family types (wild and domestic) and two hybrid family types (wild dam-domestic sire, domestic dam-wild sire). Offspring were incubated and reared to smoltification at hatchery facilities provided by Yellow Island Aquaculture Ltd. (YIAL). Families were maintained in separate rearing tanks throughout the freshwater stage. Saltwater rearing was at YIAL's netpen site on the west coast of Quadra Island. Offspring were housed together at this stage, but families were identifiable (post-mortem) by colour-coded nose-tags (Northwest Marine Technology Inc., Shaw Island, WA). Section 1.2.1 describes specifics of incubation conditions, freshwater and saltwater husbandry, and sample collection.

2.2.2 Genetic Variation

DNA Extraction

A modified proteinase K digestion method (Devlin et al. 1991) was used to extract DNA from whole blood samples collected from the parent fish. Briefly, 5 μ L of blood was digested in 475 μ L of buffer (10 mM Tris [pH 8.0], 10 mM ethylenediaminetetraacetic acid [EDTA], 1% sodium dodecylsulphate [SDS]) with 25 μ L (10 mg/mL) of proteinase K incubated overnight at 36°C with gentle rocking. The digestion was extracted twice with phenol:chloroform:isoamyl alcohol (24:24:1); and precipitated with a 0.1 volume of 3 M NaOAc and a 0.6 volume of isopropanol. The pellet was washed with 70% ethanol, dried, re-dissolved in 100 μ L of Tris-EDTA (10 mM Tris [pH 8.0], 1 mM EDTA) and incubated at 36°C for one hour. Lastly, 1.5 μ L of RNase was added to the DNA solution and it was incubated an additional two hours at 36°C. These stock DNA solutions were stored at -30°C. The same protocol was followed for the extraction of DNA from liver (approximately 5 mg of tissue was used in the initial digestion).

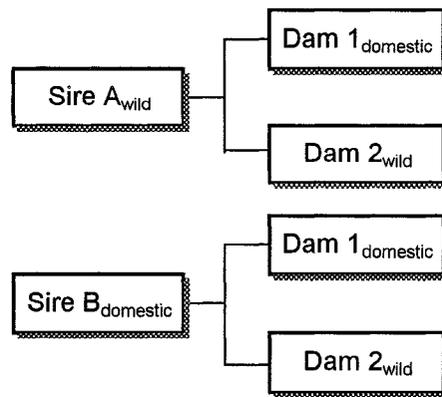


Figure 2.1. Partial diallel mating design with dams nested in sires. 104 chinook salmon families were generated from 26 wild dams, 26 wild sires, 26 domestic dams and 26 domestic sires.

Microsatellites – Laboratory Analysis

Eight microsatellite loci were used to determine genetic diversity: *Ots1*, *Ots3*, *Ots4*, *Ots107*, *Sfo8*, *Ssa197*, *Omy325* and *Oneμ3*. *Ots107* and *Ssa197* are compound dinucleotide-tetranucleotide repeat arrays. The others are dinucleotide repeat arrays. Loci were chosen based on the following criteria: a) reliability of PCR amplification, b) opportunity for multiplexing during fragment analysis, and c) unambiguous and reliable allele recognition.

Each polymerase chain reaction (PCR) included: 0.12 μL (100 ng/μL) of the forward and reverse microsatellite primer; 1.5 μL of 10X mM PCR buffer (GIBCO-BRL, Canadian Life Technologies, Inc., Burlington, Ont.); 0.9 μL of 25 mM MgCl₂; 0.3 μL of deoxyribosenucleoside triphosphates (dNTPs) (contains 10 mM of each dNTP); 0.03 μL (0.3 U) of *Taq* polymerase (GIBCO-BRL); 0.8 μL of extracted DNA (10 μL stock DNA diluted in 90 μL ddH₂O); and ddH₂O to make up to a 15 μL reaction volume. PCR reactions were run with primer-specific conditions (Table 2.1) on a PTC-100™ Programmable Thermal Controller (MJ Research, Inc., Watertown, MA). The forward primer for all loci was dye-labeled and the resulting dye-labeled fragments were run on an automated sequencer (Visible Genetics, Toronto, Ont.) with appropriate size standards. All fragments (alleles) were determined to the nearest 0.3 base pair and rounded to the nearest whole odd or even (depending on microsatellite) base pair size.

Table 2.1. Primer-specific polymerase chain reaction (PCR) conditions applied in this study. Also presented are sources for the initial description and use of these microsatellite DNA loci.

Locus	Source	Annealing temp. (°C)	No. of cycles ¹
<i>Omy325</i>	Olsen et al. 1996	62-56 ²	35
<i>Onep3</i>	Scribner et al. 1996	56-52 ³	35
<i>Ots1</i>	Banks et al. 1999	56-52 ³	35
<i>Ots3</i>	Banks et al. 1999	50	35
<i>Ots4</i>	Banks et al. 1999	56-52 ³	35
<i>Ots107</i>	Nelson and Beacham 1999	58	35
<i>Sfo8</i>	Olsen et al. 1996	50	40
<i>Ssa197</i>	Olsen et al. 1996; O'Reilly et al. 1996	56-52 ³	35

¹ each cycle: 1 min. denaturing (94°C) step, a 1 min. annealing step, and a 1.5 min. extension (72°C) step

² includes a 'touchdown' step with 5 cycles of -1°C per cycle until final annealing temp. of 56°C is reached

³ includes a 'touchdown' step with 4 cycles of -1°C per cycle until final annealing temp. of 52°C is reached

Microsatellites – Data Analysis

Three measures of genetic variation were derived from the microsatellite DNA data:

Heterozygosity:- Individual (sire and dam) heterozygosity (H) at a locus was either 0 (homozygote) or 1 (heterozygote). Mean individual H was the average of these values across all eight loci. Family (i.e. offspring) H was estimated using Mendelian inheritance rules applied to the sire and dam genotypes at each locus. Equal gamete viability was assumed. Mean family H was the average of these estimates across all loci.

D-squared (d^2):- The measure d^2 , described in Coulson et al. (1998), was used as an estimate of genetic distance. The rationale for d^2 as a measure of genetic distance is the stepwise model of microsatellite evolution (Valdes et al. 1993 but see Banks et al. 2000) which hypothesizes that the distance (in repeat units) between two alleles is related to the time since their divergence (Goldstein et al. 1995; Coulson et al. 1998). Individual d^2 is the squared difference in repeat units between alleles at a locus, as determined for each parent. Mean individual d^2 is the average of these values over all loci. Family d^2 was estimated using Mendelian inheritance rules applied to the sire and dam genotypes at each locus with the

assumption of equal gamete viability. Mean family d^2 is the average of these estimates across all loci.

Similarity Index:- A 'parental similarity index', based on the sharing of alleles (for all eight loci) between the sire and dam of each family, was calculated according to Equation 1 in Lynch (1990).

These genetic variation estimators were used in two types of analyses – multilocus and locus-specific. Both types of analyses have the potential to detect genetic variation-fitness relationships generated by associative overdominance; however, the multilocus analysis is expected to be sensitive to the general effects form and the locus-specific analysis is expected to be sensitive to the local effects form (see Introduction, p. 44-45). Mean family H, mean individual H, mean family d^2 , mean individual d^2 and parental similarity index are genetic variation estimators used in the multilocus analysis. Individual and family H, and individual and family d^2 are genetic variation estimators used in the locus-specific analysis. Table 2.2 presents a summary of the genetic variation estimators used in this study.

Table 2.2. Summary of genetic variation estimators used in this study.

Measure of Genetic Variation	Estimator	Describes:	Analysis Type
Heterozygosity	Individual H	Sire or dam; each locus	Locus-specific
	Mean individual H	Sire or dam; all loci	Multilocus
	Family H	Offspring; each locus	Locus-specific
	Mean family H	Offspring; all loci	Multilocus
D-squared (d^2)	Individual d^2	Sire or dam; each locus	Locus-specific
	Mean individual d^2	Sire or dam; all loci	Multilocus
	Family d^2	Offspring; each locus	Locus-specific
	Mean family d^2	Offspring; all loci	Multilocus
Similarity Index	Parental similarity	Sire + dam; all loci	Multilocus

The program TFPGA (Tools for Population Genetic Analyses Version 1.3, M.P. Miller, 1997, Northern Arizona University, Flagstaff, AZ) was used to test for Hardy-Weinberg Equilibrium using the exact test with approximation of the true exact probability generated by Monte Carlo simulation (1000 permutations), and to calculate the inbreeding coefficient f (equivalent to Wright's F_{IS} , see Weir 1990).

2.2.3 Fitness Traits

Three categories of offspring fitness (survival, growth and developmental stability) and one category of parental fitness (reproduction) were selected for evaluation. A total of twenty-eight fitness traits were examined. Detailed methodologies for determination of some of these traits have been described previously in Section 1.2.2.1.

Survival

Freshwater Survival:-

Mortalities were counted and removed, at least weekly, through all freshwater stages. Freshwater survival was calculated for three stages: a) eyed egg (from the eyed stage until hatching); b) alevin (from hatching until ponding); and c) fry (from ponding to May 20, 1998).

Disease Resistance:-

Resistance to vibriosis, a common disease of wild and domestic salmonids (Inglis et al. 1993), was assessed in two ways. The methodologies are summarized briefly below – refer to Section 1.2.2.1 for details.

Outbreak survival:- A vibriosis outbreak occurred at the study site in the spring-summer of 1999 (9-12 months post-transfer to saltwater). Dead fish were retrieved, counted and retained for nose-tag recovery (i.e. family identification).

Disease challenge:- The bacterial pathogen *Vibrio anguillarum*, causative agent of vibriosis, was chosen for a disease challenge trial. Eighty-seven families were included in the trial which was conducted in June 1998 (222+ days post-fertilization). Twenty-five fish per family were anaesthetized and injected intraperitoneally with 0.1 mL of 1×10^6 colony-forming units of bacteria suspended in peptone saline. Controls (10 per family) were injected in the

same manner but with peptone saline only. Treated and control fish were differentially fin-clipped and housed together. Fish were monitored for 10 days post-treatment. Tanks were inspected every 3 hours (except between 24:00 and 06:00) for mortalities. Time of death was considered to be the mid-point of the monitoring period in which the fish died (e.g., 03:00). The number of hours post-treatment at which the first fish died and at which 12 of 25 fish (48%) died were chosen as indicators of disease resistance, referred to as *hours₁* and *hours₁₂*, respectively.

Growth

Freshwater Size-at-Age and Growth Rate:-

A sample of alevins from each family was weighed at 93 days post-fertilization, that is, 4-5 days (depending on family) before ponding. Fry from each family were live weighed (as a group) on 14 occasions (at 5-15 day intervals) between 108 and 207 days post-fertilization. Freshwater growth rate was calculated as the change in mean family weight (mg) per day over this period (99 days). Diehl and Audo (1995) found that this calculation of growth rate (i.e. relative growth rate) was better for detecting relationships with genetic variation than specific growth rate.

Saltwater Size-at-Age and Growth Rate:-

Fish were sampled twice to obtain size-at-age data in saltwater. The first sample (n = 745) was taken 420 days post-fertilization (~6.5 months post-transfer to saltwater). Fish were euthanized, weighed, and the heads retained for nose-tag recovery. A second sample (n = 900) was taken 615 days post-fertilization (just over 1 year post-transfer to saltwater). Growth rate was calculated (from family means) as the change in weight (g) per day for the period between the two samples (205 days).

Developmental Stability

Fluctuating Asymmetry:-

Fluctuating asymmetry (FA) is characterized by random differences between right and left trait measurements that result in a normal distribution around a mean of zero (Van Valen

1962). These random differences between sides are assumed to reflect errors in development (Whitlock 1996), that is, FA is an observable effect of developmental instability (Palmer 1996). Greater developmental stability is typically associated with increased fitness (e.g., Naugler and Leech 1994; Ueno 1994 [adult beetle longevity]; Møller 1997 but see Ueno 1994 [male beetle mating success]; Clarke 1995; Leung and Forbes 1997).

Fry from 80 families were collected in July 1998 (278-279 days post-fertilization). A total of 240 fish (three per family) were measured for seven bilateral traits: 1) head length (measured from snout to posterior edge of operculum); 2) maxillary length; 3) eye diameter (anterio-posterior axis); 4) pelvic fin ray number; 5) pectoral fin ray number; 6) gill raker number on upper first gill arch; and 7) gill raker number on lower first gill arch. A single observer took all measurements (left and right side) and all measurements on an individual fish were completed on the same day. Meristic traits were counted once and metric traits were measured twice (first measurement of all traits completed before repeat measurements done). Metric trait measurements were standardized by dividing by fork length. These data also form part of a study on the heritability of fluctuating asymmetry (Bryden and Heath 2000).

A series of analytical steps, as described in Palmer (1994), were followed before identifying any asymmetry as fluctuating asymmetry, and a regression of $|R-L|$ against fork length was used to determine if FA was independent of body size (Palmer 1994). A standardized composite FA index was calculated as the sum of $(|R-L|/\text{standard deviation})$ for all traits exhibiting fluctuating asymmetry.

Reproduction

Relative Fecundity and Relative Egg Weight:-

Relative fecundity is the number of eggs produced per gram of dam body weight. Relative egg weight is the amount (mg) of an average individual egg produced per kilogram of dam body weight. Total egg number and mean egg weight were extrapolated from a sample of unfertilized eggs from each dam (Section 1.2.1).

2.2.4 Statistical Analyses

Data normality was assessed by visual examination of normal probability plots for evidence of skewness or kurtosis (Zar 1996). Generally, simple linear regression analyses were used to test for relationships between measures of fitness and the genetic variation estimators. The appropriateness of linear regression analysis was assessed by visual examination of residuals for evidence of non-normality, heteroscedasticity or non-linearity (Chatterjee and Price 1977). The non-parametric Spearman Rank Correlation was used if assumptions were violated (e.g., non-normality), and could not be corrected with standard data transformations. Multilocus and locus-specific analyses with offspring fitness traits were done for all families combined and for purebred and hybrid families separately. Multilocus and locus-specific analyses with reproductive traits were done for all individuals (dams) combined. Either a one-way ANOVA or Kruskal-Wallis ANOVA by ranks was used, when required, to determine the effect of trial date, treatment date, density or rearing location (e.g., stack) on various fitness measures (e.g., eyed egg survival). All statistical analyses were done with STATISTICA (Release 5.1, 1996, StatSoft Inc., Tulsa, OK). A significance level of $\alpha = 0.05$ was used for all tests. The sequential Bonferroni adjustment was used to control for group-wide Type I error rate when required (Rice 1989; Palmer 1994).

2.3 Results

2.3.1 Genetic Variation

The microsatellite loci exhibited considerable polymorphism with one exception (*One μ 3*) (Figure 2.2, Table 2.3). Overall, the study population was in Hardy-Weinburg Equilibrium (HWE) although four of the eight loci deviated significantly from HWE (Table 2.3). There were heterozygote deficiencies ($f > 0$) at three of these loci (Table 2.3). The mean heterozygosity and mean number of alleles per locus for the parent fish (Table 2.3) are comparable to other studies of anadromous fish (DeWoody and Avise 2000: mean heterozygosity = 0.68, mean number of alleles per locus = 11.3). Family heterozygosity and d^2 , averaged over all families ($n = 104$) for each loci, are shown in Table 2.4. Parental similarity index, averaged over all

families, was 0.28. The frequency distributions of mean family heterozygosity, mean family d^2 and parental similarity index are presented in Figure 2.3.

2.3.2 Genetic Variation and Fitness Traits

Mean values and sample sizes for the fitness traits are presented in Appendix II.

Survival

Freshwater Survival:-

There was a significant effect of incubation tray position on alevin survival (Section 1.3.1). This variable was standardized by dividing the mean family value by the overall mean for the appropriate tray position. Tank effects on fry survival could not be tested directly; however, there was no significant effect of aisle assignment on fry survival (Section 1.3.1).

None of the freshwater survival variables were significantly correlated with genetic variation when all families were considered together (Table 2.5) or when purebred and hybrid families were considered separately (tables 2.6 and 2.7).

Disease Resistance:-

Outbreak survival:- There were no significant relationships between survival during a vibriosis outbreak and genetic variation when all families were considered together (Table 2.5), or, when purebreeds and hybrids were considered separately (tables 2.6 and 2.7).

Disease Challenge:- The challenge was severe (see Chapter 1). Mortality was 76-100% in all families at the end of the ten day monitoring period. Treatment day had a significant effect on $hours_1$ and $hours_{12}$, so these traits were standardized by dividing mean family values by the overall mean for the treatment day (Section 1.3.1).

There were no significant regressions of $hours_1$ and $hours_{12}$ with genetic variation when all families were considered together (Table 2.5). There were also no significant regressions with genetic variation when purebreeds and hybrids were considered separately (tables 2.6 and 2.7).

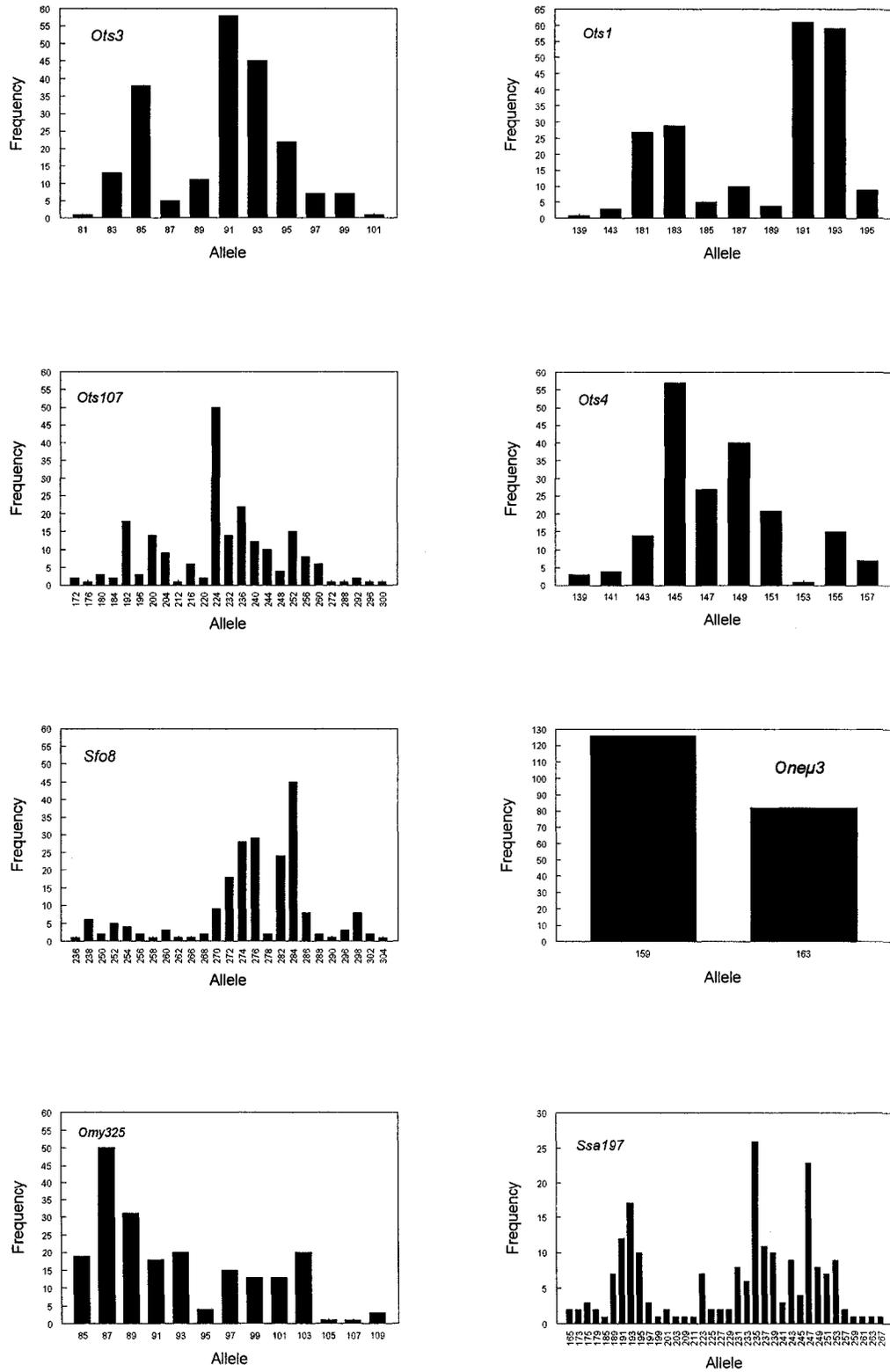


Figure 2.2. Frequency distribution of alleles for eight microsatellite DNA loci.

Table 2.3. Genetic characteristics of the parent population (n = 104) determined from analysis of eight microsatellite loci. HWE = Hardy-Weinburg Equilibrium.

Locus	Size range (bp)	No. of alleles	Heterozygosity ¹	f^2	H ₀ : HWE (P-value)
<i>Omy325</i>	85-109	13	0.87	0.010	0.000 ³
<i>Oneμ3</i>	159, 163	2	0.46	0.051	0.834
<i>Ots1</i>	137-195	10	0.49	0.356	0.000 ³
<i>Ots3</i>	81-107	11	0.81	0.005	0.111
<i>Ots4</i>	139-157	10	0.82	-0.053	0.002 ³
<i>Ots107</i>	172-300	25	0.86	0.037	0.331
<i>Sfo8</i>	236-304	25	0.82	0.072	0.248
<i>Ssa197</i>	169-269	36	0.87	0.062	0.000 ³
All loci	--	16.5 ⁴	0.75 ⁴	0.066	>0.10

¹ direct count generated by TFPGA

² equivalent to Wright's F_{IS}

³ significant after sequential Bonferroni adjustment

⁴ mean

Table 2.4. Genetic variation in the offspring population. Family H and family d^2 averaged across all families (n = 104) for each locus.

Locus	Family H	Family d^2
<i>Omy325</i>	0.86	20.7
<i>Oneμ3</i>	0.46	1.8
<i>Ots1</i>	0.85	33.5
<i>Ots3</i>	0.82	8.5
<i>Ots4</i>	0.80	7.9
<i>Ots107</i>	0.87	73.7 ¹
<i>Sfo8</i>	0.88	79.5
<i>Ssa197</i>	0.95	306.9 ²
Mean	0.81	66.6

¹ approximate, based on 100% tetranucleotide repeat units

² approximate, based on 100% dinucleotide repeat units

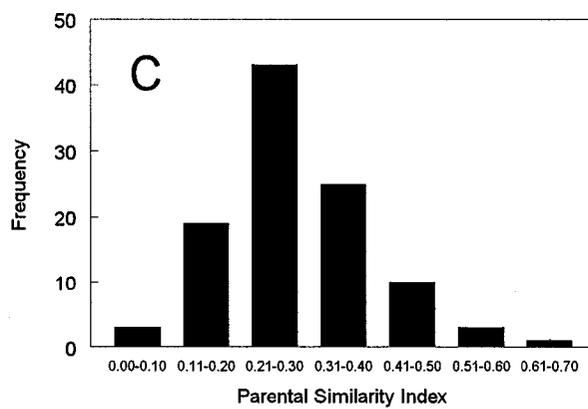
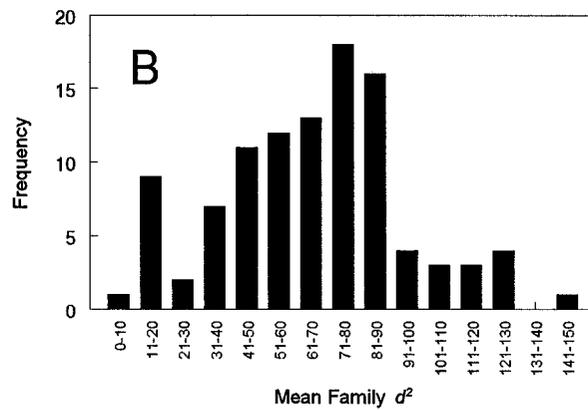
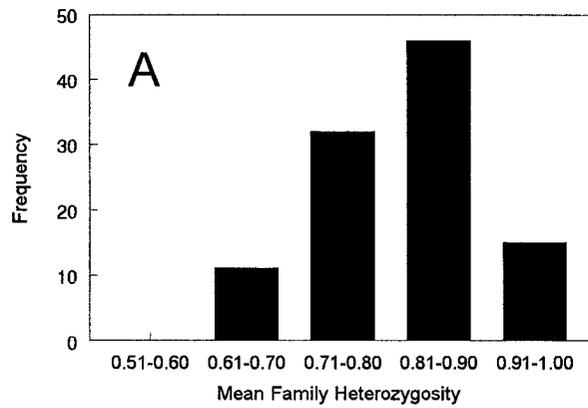


Figure 2.3. Frequency distributions of: A. mean family heterozygosity; B. mean family d^2 ; and C. parental similarity index. Sample sizes: A = 104 families; B = 104 families; C = 104 dam-sire pairs.

Table 2.5. Summary of relationships between genetic variation and fitness traits for all families combined. Genetic variation estimators: family heterozygosity = FH; mean family heterozygosity = MFH; individual heterozygosity = IH; mean individual heterozygosity = MIH; family d^2 = FD; mean family d^2 = MFD; individual d^2 = ID; mean individual d^2 = MID; and parental similarity index = PS. Non-significant results are coded as n.s..

Fitness Category	No. of traits	Multilocus Analysis ¹	Locus-specific Analysis ²
Reproduction ³	2	n.s.	• ID: <i>Ots107</i> ; 0.376 ⁴ ; 0.006; n.a.; rel. egg wgt
Freshwater Survival	3	n.s.	n.s.
Disease Resistance	3	n.s.	n.s.
Freshwater Size-at-Age and Growth Rate	16	<ul style="list-style-type: none"> • MFH: -0.250; 0.016; 0.061; 172 days⁵ • MFH: -0.340; 0.001; 0.119; 191 days⁵ • PS: 0.259; 0.012; 0.067; 191 days⁵ 	<ul style="list-style-type: none"> • FH: <i>Sfo8</i>; -0.300; 0.003; 0.090; 108 days⁵ • FH: <i>Sfo8</i>; -0.330; 0.001; 0.109; 130 days⁵ • FH: <i>Sfo8</i>; -0.280; 0.005; 0.081; 136 days⁵ • FH: <i>Sfo8</i>; -0.320; 0.002; 0.103; 143 days⁵ • FH: <i>Sfo8</i>; -0.280; 0.005; 0.081; 191 days⁵
Saltwater Size-at-Age and Growth Rate	3	n.s.	<ul style="list-style-type: none"> • FD: <i>Ots1</i>; 0.192; 0.000; 0.037; 420 days⁵ • FD: <i>Ots107</i>; -0.130; 0.000; 0.018; 420 days⁵ • FD: <i>Ots1</i>; 0.183; 0.000; 0.034; 615 days⁵ • FD: <i>Ots3</i>; -0.09; 0.007; 0.009; 615 days⁵ • FD: <i>Ots107</i>; -0.100; 0.005; 0.010; 615 days⁵ • FH: <i>Sfo8</i>; -0.110; 0.002; 0.012; 615 days⁵
Fluctuating Asymmetry	1	n.s.	n.s.

¹ significant results reported as: genetic variation estimator; regression/correlation coefficient; *P*-value; r^2 value; trait

² significant results reported as: genetic variation estimator; locus; regression/correlation coefficient; *P*-value; r^2 value; trait

³ only two estimators used – mean individual H and mean individual d^2

⁴ Spearman Rank Correlation coefficient

⁵ weight at x days post-fertilization

Table 2.6. Summary of relationships for the multilocus genetic variation analysis with fitness traits for purebred and hybrid families. Genetic variation estimators: mean family heterozygosity = MFH; mean family d^2 = MFD; and parental similarity index = PS. Non-significant results are coded as n.s..

Fitness Category	Purebred Families ¹	Hybrid Families ¹
Freshwater Survival	n.s.	n.s.
Disease Resistance	n.s.	n.s.
Freshwater Size-at-Age and Growth Rate	n.s.	<ul style="list-style-type: none"> • MFD: 0.381; 0.011; 0.145; 93 days² • MFD: 0.432; 0.003; 0.187; 108 days² • MFD: 0.447; 0.002; 0.200; 122 days² • MFD: 0.414; 0.004; 0.172; 130 days² • MFD: 0.412; 0.004; 0.170; 136 days² • MFD: 0.377; 0.010; 0.142; 151 days² • MFH: -0.390; 0.007; 0.154; 172 days² • MFH: -0.340; 0.020; 0.117; 178 days² • MFH: -0.430; 0.003; 0.189; 191 days² • PS: 0.364; 0.013; 0.132; 172 days² • PS: 0.341; 0.020; 0.116; 178 days²
Saltwater Size-at-Age and Growth Rate	<ul style="list-style-type: none"> • MFH: -0.140; 0.006; 0.020; 615 days² • PS: 0.129; 0.011; 0.017; 615 days² 	<ul style="list-style-type: none"> • MFD: 0.236; 0.000; 0.056; 420 days²
Fluctuating Asymmetry	n.s.	n.s.

¹ significant results reported as: genetic variation estimator; regression/correlation coefficient; *P*-value; r^2 value; trait

² weight at x days post-fertilization

Table 2.7. Summary of relationships for the locus-specific genetic variation analysis with fitness traits for purebred and hybrid families. Genetic variation estimators: family heterozygosity = FH; family d^2 = FD. Non-significant results are coded as n.s..

Fitness Category	Purebred Families ¹	Hybrid Families ¹
Freshwater Survival	n.s.	n.s.
Disease Resistance	n.s.	n.s.
Freshwater Size-at-Age and Growth Rate	<ul style="list-style-type: none"> • FD: <i>Ssa197</i>; -0.440; 0.02; 0.190; 93 days² • FD: <i>Ssa197</i>; -0.410; 0.003; 0.172; 108 days² • FD: <i>Ssa197</i>; -0.460; 0.001; 0.216; 116 days² • FD: <i>Ssa197</i>; -0.420; 0.003; 0.179; 122 days² • FD: <i>Ssa197</i>; -0.440; 0.002; 0.192; 130 days² • FD: <i>Ssa197</i>; -0.410; 0.004; 0.171; 136 days² • FD: <i>Ssa197</i>; -0.390; 0.006; 0.155; 143 days² • FD: <i>Ssa197</i>; -0.400; 0.005; 0.159; 157 days² 	n.s.
Saltwater Size-at-Age and Growth Rate	<ul style="list-style-type: none"> • FD: <i>Omy325</i>; -0.180; 0.001; 0.032; 420 days² • FD: <i>Ots1</i>; 0.189; 0.000; 0.036; 420 days² • FD: <i>Ots1</i>; 0.238; 0.000; 0.057; 615 days² • FD: <i>Ots3</i>; -0.170; 0.001; 0.029; 615 days² • FH: <i>Sfo8</i>; -0.150; 0.004; 0.022; 420 days² • FH: <i>Sfo8</i>; -0.180; 0.000; 0.029; 615 days² 	<ul style="list-style-type: none"> • FD: <i>Ots1</i>; 0.207; 0.000; 0.043; 420 days² • FD: <i>Ots3</i>; 0.164; 0.003; 0.027; 420 days² • FD: <i>Ots107</i>; -0.170; 0.003; 0.028; 420 days² • FD: <i>Ssa197</i>; 0.217; 0.000; 0.047; 420 days² • FD: <i>Omy325</i>; 0.143; 0.003; 0.021; 615 days² • FH: <i>Ots1</i>; -0.200; 0.000; 0.039; 420 days²
Fluctuating Asymmetry	n.s.	n.s.

¹ significant results reported as: genetic variation estimator; locus; regression/correlation coefficient; *P*-value; *r*² value; trait
² weight at x days post-fertilization

Growth

Freshwater Size-at-Age and Growth Rate:-

There was no significant effect of position (tray, stack or compartment) on alevin pre-ponding weight (93 days post-fertilization) or of hatchery aisle location on fry size-at-age and growth rate. There was also no significant effect of fry density (individuals/L) on any size-at-age measures.

Freshwater size-at-age was not significantly related to mean family d^2 at any time, but there were some significant regressions between size-at-age and parental similarity index (positive) and mean family H (negative) after 157 days post-fertilization, for all families combined (Table 2.5, Figure 2.4). Only hybrids had any significant multilocus relationships with freshwater size-at-age when analyzed separately from purebreeds (Table 2.6). Similar to the findings for all families together, there were positive regressions with parental similarity index

and negative regressions with mean family H, after 157 days (Table 2.6). Additionally, there were multiple positive regressions with mean family d^2 from 93 to 151 days post-fertilization (Table 2.6).

Family H at *Sfo8* was significantly negatively related to five of 15 size-at-age measures for all families combined (Table 2.5). Four of the five were from the period before 150 days post-fertilization (Table 2.5). Family d^2 at *Ssa197* was significantly negatively related to 8 of 15 size-at-age measures for purebreeds (Table 2.7). All eight were from the period up to and including 157 days post-fertilization (Table 2.7). Freshwater size-at-age was not significantly related to genetic variation at any other loci.

There were no significant relationships between freshwater growth rate and genetic variation when all families were considered together (Table 2.5) or when purebred and hybrid families were considered separately (tables 2.6 and 2.7).

When the family types were examined separately the magnitude and sign of the regression coefficients from the multilocus relationships between size-at-age and genetic variation varied considerably (Figure 2.5). The difference between the wild and domestic purebreeds for mean family d^2 is particularly striking (Figure 2.5). Each family type had a least one significant relationship between genetic variation and size-at-age but the total number of significant and marginally significant relationships for the two hybrids was greater than for the two purebreeds (Figure 2.5).

Saltwater Size-at-Age and Growth Rate:-

Multilocus analysis detected no significant regressions between genetic variation and saltwater size-at-age or growth rate when all families were combined (Table 2.5). However, when purebred and hybrid families were considered separately, there were significant relationships between mean family H (negative) and parental similarity index (positive) and 615 day weight for the purebreeds and between mean family d^2 (positive) and 420 day weight (positive) for the hybrids (Table 2.6). The magnitude of the regression coefficients between mean family H and parental similarity index and size-at-age were considerably lower in the

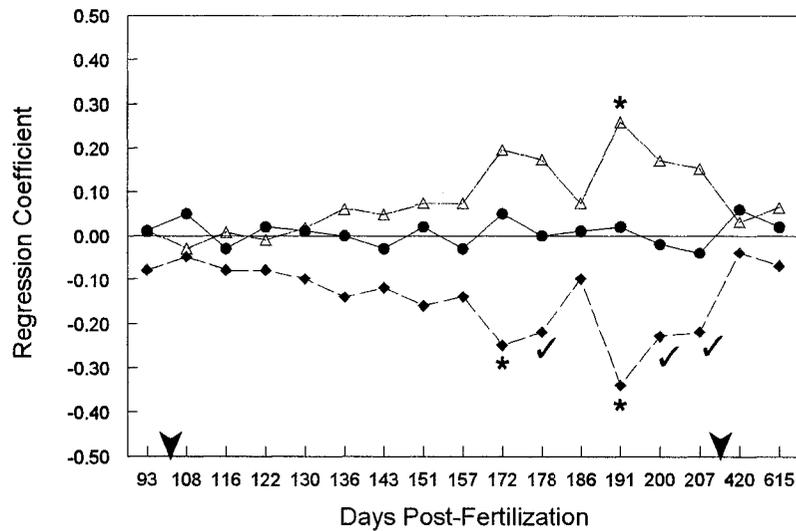


Figure 2.4. Change in magnitude of linear regression coefficients from multilocus analysis (all families combined) for relationships between size-at-age and genetic variation in freshwater and saltwater. Mean family H = filled diamond; mean family d^2 = filled circle; parental similarity index = open triangle. Left arrowhead indicates ponding, right arrowhead indicates transfer to saltwater. Significant relationships after sequential Bonferroni adjustment are indicated by "*". Relationships rendered non-significant after Bonferroni adjustment are indicated by '✓'.

saltwater phase than they had been in the late freshwater phase (Figure 2.4).

Family d^2 at *Ots1* was significantly positively related to 420 and 615 day weight while family d^2 at *Ots107* was significantly negatively related to the same traits (Table 2.6). Family d^2 at *Ots3* was significantly negatively related to 615 day weight as was family H at *Sfo8* (Table 2.6). Separate analyses of the hybrids and purebreds also found that several loci (*Ots1*, *Ots3*, *Ots107*, *Sfo8*, *Ssa197* and *Omy325*) were significantly related to 420 and 615 day weight (Table 2.7). There were no significant regressions between growth rate and genetic variation.

The magnitude of the regression coefficients between saltwater size-at-age and genetic variation were more similar among the family types than was the case for freshwater size-at-age (Figure 2.5). Four of the six significant multilocus relationships were for the purebred wild and wild dam-domestic sire family types (Figure 2.5).

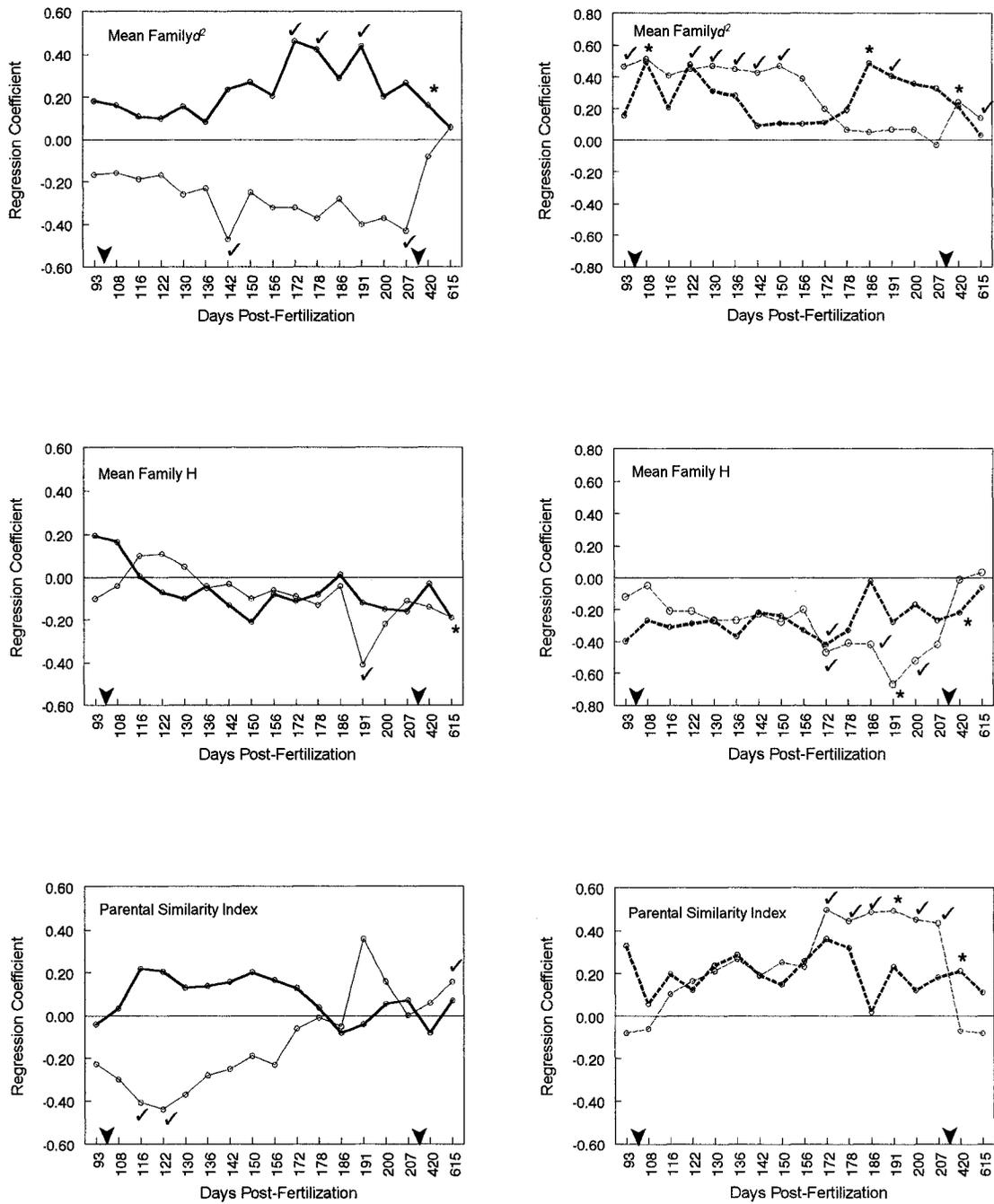


Figure 2.5. Change in magnitude of linear regression coefficients over time from multilocus analysis for relationships between genetic variation and size-at-age, by family type. Thin solid line = purebred domestic; thick solid line = purebred wild; thin broken line = hybrid: domestic dam/wild sire; thick broken line = hybrid: wild dam/domestic sire. Left arrowhead indicates ponding, right arrowhead indicates transfer to saltwater. Significant relationships after sequential Bonferroni adjustment are indicated by '*'. Relationships rendered non-significant after Bonferroni adjustment are indicated by '✓'.

Developmental Stability

Fluctuating Asymmetry:-

Maxillary length and head length exhibited directional asymmetry. The asymmetry identified in the other five traits (eye diameter, upper and lower gill raker number, pectoral and pelvic fin ray number) was confirmed as fluctuating asymmetry (see Bryden and Heath 2000) and these traits were used to calculate the composite FA index.

There were no correlations between fluctuating asymmetry and trait size so size-scaling was not required. There were no significant relationships between composite FA index and genetic variation when all families were considered together (Table 2.5) or when purebreeds and hybrids were analyzed separately (tables 2.6 and 2.7).

Reproduction

There were no significant regressions between relative fecundity and genetic variation (Table 2.5). Relative egg weight was significantly positively correlated with individual d^2 at *Ots107* (Table 2.5). All other correlations between relative egg weight and individual female genetic variation were non-significant.

2.4 Discussion

Genetic Variation-Fitness Relationships – Generalities and Patterns:-

An important prediction of the associative overdominance hypothesis is that genetic variation-fitness relationships can be detected by non-coding (neutral) markers such as microsatellite loci (Pogson and Fevolden 1998). Three percent (15 of 530) of the analyses performed for all families together were significant after sequential Bonferroni adjustment. Inclusion of relationships that were significant before the adjustment ('marginally significant', Ferguson 1996) increased this result to 11%. Despite this low number of significant results, their occurrence suggests that associative overdominance may be a component of the relationship between genetic variation and fitness in chinook salmon. This is consistent with what is generally accepted – that definitive support for the existence of associative overdominance is elusive but more likely to be resolved with the increasing use of molecular

markers (David 1998; Lynch and Walsh 1998; Pogson and Zouros 1998; Pogson and Fevolden 1998).

Some patterns emerge from the results of this study. First, freshwater and saltwater size-at-age are essentially the only (one exception) fitness-related traits for which significant relationships with genetic variation were detected, although there were marginally significant relationships in all fitness categories except fluctuating asymmetry. Second, multilocus analysis, indicative of the general effects form of associative overdominance, detected significant relationships only for size-at-age late in the freshwater phase. Third, locus-specific analysis, indicative of the local effects form of associative overdominance, detected significant relationships for size-at-age in the early freshwater phase and in the saltwater phase (there was also a single significant relationship with relative egg weight). A mix of local and general effects is not surprising and the relative importance of the different mechanisms may vary depending on the trait (Roff 1997). Finally, the locus-specific analysis identified several loci that were significantly related to saltwater size-at-age, while only a single locus was significantly related to early freshwater size-at-age.

The separate analysis of purebred and hybrid families also suggests some general patterns. First, as for the combined analysis, freshwater and saltwater size-at-age are the only fitness-related traits for which there were any significant relationships. Again, there were marginally significant results for all but fluctuating asymmetry. Second, saltwater size-at-age is the only trait for which purebreeds and hybrids share a similar set of significant relationships – several loci are of significance and there are significant multilocus relationships. Third, purebred families have significant locus-specific relationships with early freshwater size-at-age and, as for the combined analysis, only a single (although different) locus is implicated (but see discussion on p. 68). Interestingly, if marginally significant results for the hybrids are considered, a single locus is also of note for early freshwater size-at-age and it is the same as that detected for all families together (*Sfo8*). Fourth, hybrids have significant multilocus relationships with size-at-age throughout the freshwater phase. Finally, hybrids appear to drive

the overall pattern of the genetic variation-fitness relationships in this study. That is, the significant and marginally significant relationships for the hybrids tend to reflect those detected for all families combined.

Genetic Variation-Fitness Relationships – Direction:-

The direction (sign) of the significant multilocus relationships detected in this study for all families combined are counter to expectations that genetic variation and size (fitness) will be positively correlated. Other studies (e.g., Alvarez et al. 1989; Ferguson 1990; Ferguson 1992; Savolainen and Hedrick 1995) have also found negative relationships, although their meaning and origins have not been adequately addressed (Deng and Fu 1998).

Negative relationships between genetic variation and fitness are problematic with respect to the overdominance hypothesis. Computer simulations by Deng and Fu (1998) suggest that both positive and negative relationships can theoretically exist in a population, but there are simpler explanations, for example, outbreeding depression. However, outbreeding depression is not strongly expressed in the F_1 generation, and Figure 2.6 demonstrates that regression coefficients generated from mean family d^2 with various traits, while differently distributed between purebreeds and hybrids, are positive for hybrids, not negative as would be expected if there was outbreeding depression. Possibly, the range of genetic variation measured in this study was not large enough to fully define its relationship with fitness. That is, if the relationship is non-linear (see Figure i.1) then one range of genetic variation may describe a different part of the curve than another range of genetic variation. For example, heterozygosity estimates from this study ranged from 0.61-1.00 (see Figure 2.3a) and, thus, may describe a negative relationship, whereas, a lower range of heterozygosity may describe a positive relationship.

The direction of the genetic variation-fitness relationships differs among the four family types that constitute the study population (see Figure 2.5). Combining these family types could potentially create artificial counterintuitive relationships if, for example, purebred wild fish had lower mean heterozygosity but higher mean body weight than purebred domestic fish. This

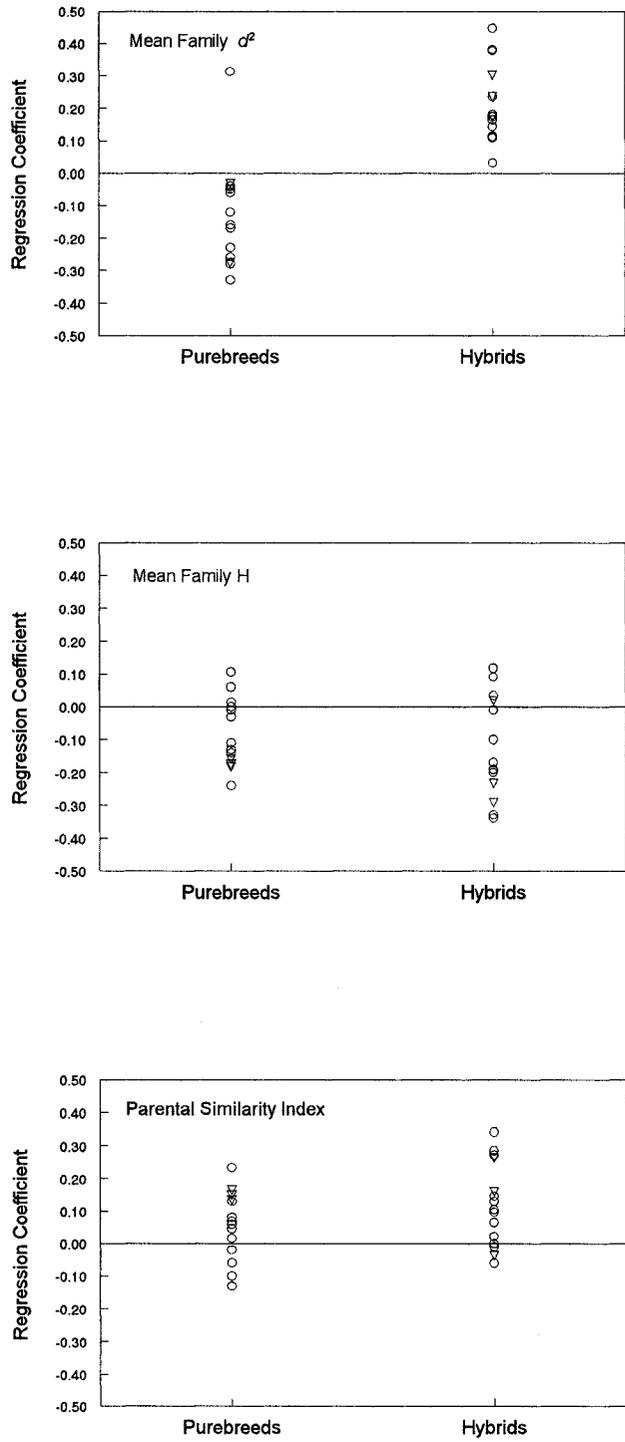


Figure 2.6. Scatterplot of linear regression coefficients (circle) and Spearman Rank correlation coefficients (triangle) generated from the analysis of genetic variation-fitness relationships for purebred versus hybrid families. The majority of these relationships are non-significant (Table 7). Data from 15 traits presented (FA omitted and only a subset of freshwater size-at-age measures included: 93 days, 122 days, 151 days, 178 days and 207 days).

does not appear to be the general case in this study as the mean values for the multilocus and locus-specific genetic variation estimators do not differ significantly among family types (data not presented). There is one interesting exception – there was a significant effect ($H_{3,104} = 17.996$, $P = 0.000$) of family type on mean d^2 for *Ssa197*. As discussed previously, purebred families have significant negative relationships between freshwater size-at-age and mean d^2 for this locus. It appears that these relationships may be spurious (see Table 2.7) as domestic purebreeds have higher mean d^2 for *Ssa197* but significantly lower freshwater size-at-age than wild purebreeds (see Chapter 1). Combining these two family types would presumably generate a negative relationship. There are, however, family types (e.g., purebred domestic) that clearly exhibit genetic variation-fitness relationships of counterintuitive direction that cannot be explained in the same way.

Genetic Variation-Fitness Relationships – Trait-specific Patterns:-

Saltwater and freshwater size-at-age were essentially the only traits that were significantly related to genetic variation. That these relationships were found is not a surprise since such relationships are relatively common in the literature (see reviews in Mitton 1993; Britten 1996). Perhaps size (weight) is more sensitive to inbreeding and overdominance than any of the other traits measured. Interestingly, the locus-specific analysis identified several loci that were significantly related to saltwater size-at-age (in apparent contrast to the case for freshwater). This may indicate that saltwater size is affected by more factors (e.g., sexual maturation, competition, disease tolerance) than is freshwater size.

An interesting pattern is apparent for the multilocus relationships with freshwater size-at-age for the hybrids. Early in the freshwater stage (up to 151 days post-fertilization) there are significant positive regressions between size-at-age and mean family d^2 . Later (172+ days post-fertilization) there are significant but counterintuitive regressions with mean family heterozygosity (negative) and parental similarity index (positive). Maternal effects or certain properties of the genetic variation estimators may be part of an explanation for this observation.

The lack of significant relationships for the rest of the fitness categories examined in

this study is not unexpected. However, the almost complete absence of significant relationships between genetic variation and female reproductive parameters is surprising. The parameters selected for analysis appear to be excellent candidates for detecting the presence of genetic variation-fitness relationships because both the genetic variation and fitness component measures are the most direct of any used in this study. However, other studies have also reported only weak evidence for such relationships (e.g., Danzmann et al. 1988; Liskauskas and Ferguson 1990; Heath et al. 2001). The non-significant relationship between FA and genetic variation is not remarkable as the literature is divided – some studies have found a relationship (e.g., Leary et al. 1983; Leary et al. 1984; Graham and Felley 1985; Danzmann et al. 1986; Blanco et al. 1990; Mulvey et al. 1994), and others have not (e.g., Beacham and Withler 1987; Beacham 1991; Whitlock 1993; Moran et al. 1997). Freshwater survival was so high (mean >94%, see Appendix II) and strongly influenced by maternal effects that lack of any significant relationships with genetic variation is not surprising, although other salmonid studies have found evidence for such relationships (e.g., Beacham 1991). The disease challenge was extreme which may have prevented any effective individual resistance and might have masked any variation in resistance among families (Beacham and Evelyn 1992b). The absence of a relationship between genetic variation and vibriosis outbreak survival is compatible with the findings from the disease challenge trial.

Genetic Variation-Fitness Relationships – Loci of Interest:-

Sfo8 was significantly related to early freshwater size-at-age for all families and *Ssa197* was significantly related to early freshwater size-at-age for purebreeds (but see previous discussion). Later in the freshwater phase, there were marginally significant relationships for purebreeds that were mainly due to *Ots1*. The locus-specific analysis for the hybrids generated many marginally significant relationships (primarily for *Sfo8*). *Sfo8* and *Ots1* would be potential candidates for quantitative trait loci (QTL) analysis for freshwater growth. *Ots107* generated the only significant result for a trait other than size-at-age (i.e. relative egg weight). Generally, it does not appear that strong results for specific loci were affecting multilocus relationships,

although, significant and marginally significant *Sfo8* relationships may be responsible for the significant multilocus relationships for late freshwater size-at-age (see Table 2.5).

Detection of Genetic Variation-Fitness Relationships – Theory and Technicalities:-

There are several reasons why genetic variation and fitness relationships might be non-detectable or non-existent. First, because studies, by necessity, use indirect measures of both genetic variation and fitness to examine these relationships, they will, if present, be unavoidably weak. This weakness combined with study designs of low power may prevent detection (David 1998). The generally large sample sizes used in this study are an improvement over many previous studies on salmonids (e.g., Leary et al. 1984; Danzmann et al. 1986; Blanco et al. 1990; Ferguson 1990; Liskauskas and Ferguson 1990). However, David (1998) suggests that sample sizes of thousands may be required, a target that may be logistically impossible for many species or environments.

Second, and related to the above point, the genetic variation estimators available may not adequately describe genetic variation. Chakraborty (1981) suggests that multilocus genetic variation estimators may be poor representatives of the entire genome, although simulations by Pamilo and Pálsson (1998) demonstrate that variation at neutral loci can predict genomic heterozygosity. Further, and specific to this study design, the offspring genetic variation estimates are the expected, not observed, values for each family and, as such, may be less appropriate than assumed.

Third, experimental conditions might not induce responses that are dependent on the level of heterozygosity (Vázquez-Domínguez et al. 1998). There is considerable evidence to suggest that genetic variation and fitness relationships may be more apparent under stressful conditions (Samollow and Soulé 1983; Mitton and Grant 1984; Gentili and Beaumont 1988; Ferguson 1990; Borsa et al. 1992; Audo and Diehl 1995; Pogson and Fevolden 1998; Vázquez-Domínguez et al. 1998), although too much stress has also been shown to suppress such relationships (Audo and Diehl 1995). Whether the fish in this study existed under 'stressful' conditions is unknown; however, various hatchery practices are known to induce

stress responses (e.g., Sharpe et al. 1998). Interestingly, during a obviously stressful event (the vibriosis outbreak), there was still no significant relationship between survival and genetic variation. Age-specific (e.g., Ferguson 1992 [rainbow trout]; David et al. [marine bivalve, *Spisula ovalis*]), season-specific (e.g., Samollow and Soulé 1983 [western toad, *Bufo boreas*]), and population-specific (e.g., Pogson and Fevolden [Atlantic cod]) effects on genetic variation-fitness relationships have also been demonstrated.

Fourth, non-genetic maternal effects could mask genetic variation-fitness relationships. For example, it appears that the relationship between genetic variation and freshwater size-at-age became stronger (more detectable) over time (see Figure 2.4). This pattern may be related to decreasing maternal effects, although stress associated with smoltification could also account for it. Interestingly, this pattern is not particularly apparent when the family types are considered separately (Figure 2.5), despite differences in the strength of wild and domestic maternal effects (Chapter 1).

David (1998) suggests that moderate inbreeding increases the likelihood of detecting genetic variation-fitness relationships (but see Gentili and Beaumont 1988). The study population does not appear to be inbred – it is in Hardy-Weinburg Equilibrium (HWE) and mean heterozygosity (0.75) is relatively high. Three loci had significant heterozygote deficiencies but only one was substantial (>30%). Large departures from HWE tend to suggest inbreeding (Freeman and Herron 1998) but, in that case, similar deficiencies at other loci would be expected. There are other possible explanations for heterozygote deficiencies, in particular, the Wahlund effect may be responsible for smaller magnitude deficiencies such as observed for *Ssa197* (Gaffney et al. 1990) and misidentification of heterozygotes as homozygotes may have occurred for certain loci. Speculation regarding the level of inbreeding in the study population as a whole is somewhat misleading as it actually consists of two different populations and their hybrids. There is some evidence to suggest that the domestic population may be inbred and exhibiting signs of inbreeding depression (Section 1.4) but significant genetic variation-fitness relationships do not appear to be any more prevalent among the

domestic purebreeds than among the (probably) less inbred wild purebreeds (see Figure 2.5).

Genetic Variation-Fitness Relationships:- Microsatellite DNA and D-squared:-

There are some possible problems with the use of microsatellite DNA in the examination of genetic variation-fitness relationships. First and most basically, there is still too little known about microsatellites to make definitive statements about their utility in these analyses. For example, Pogson and Zouros (1994) suggest that VNTR loci (such as microsatellites) may turn out to be inappropriate markers to test the associative overdominance hypothesis. Second, the power of genetic variation-fitness analyses may be reduced when very polymorphic markers, such as microsatellites, are used (Lynch and Walsh 1998), but, at the same time, there is also valuable information available from polymorphic microsatellites regarding genetic divergence (David 1998).

Alternate measures of genetic variation, such as genetic distance and genetic similarity, reflect the relatedness of an individual's parents in a different way than heterozygosity and might be expected to be more sensitive to inbreeding (Goldstein et al. 1995; Coulson et al. 1998). However, the percentages of significant results detected by the different genetic variation measures in this study were similar (heterozygosity: 3%, d^2 : 4%, similarity index: 5%; or, with marginally significant results included, 10%, 12% and 8%, respectively).

There is a striking difference between hybrid and purebred regression coefficients generated from the d^2 analysis that is not mirrored by the other genetic variation measures (Figure 2.6). *D-squared* was also the most consistent estimator of the genetic variation-fitness relationship for size over time (see Figure 2.4). These may be indications (see also p. 68) that d^2 has characteristics that makes it particularly useful in the analysis of genetic variation-fitness relationships and Coltman et al. (1998) did find d^2 to be a better measure of individual genetic variation than heterozygosity for microsatellite data.

Genetic Variation-Fitness Relationships – Implications and Future Research:-

Given the apparently weak relationship between genetic variation and fitness, what are the consequences for evolutionary theory when a mechanism for the preservation of genetic

variation is not particularly strong or universal? Fortunately, overdominance (heterozygote advantage) is not the only, and perhaps not even the most important, mechanism for preserving genetic variation (Roff 1997). Other mechanisms believed to maintain genetic variation are environmental heterogeneity, frequency dependent selection, antagonistic pleiotropy, and mutation-selection balance (Roff 1997).

Since genetic variation appears to have such a small effect on fitness, are genetic variation-fitness relationships really that important? The r^2 values for the significant relationships reported in this study are low ($\bar{x} = 0.090$), that is, little of the variation in fitness is explained by genetic variation per se, just as others have noted (e.g., David 1998; Pogson and Fevolden 1998). However, for species under threat of inbreeding or population isolation (i.e. reduced genetic variation), genetic variation-fitness relationships, even of a small magnitude, could have a population-level impact, particularly if the effect is on more direct measures of fitness such as survival or fecundity. R-squared values from freshwater size-at-age relationships were higher ($\bar{x} = 0.138$) than those from saltwater size-at-age relationships ($\bar{x} = 0.030$) in this study. Thus, hypothetically, decreasing genetic variation over time could have a particularly notable impact on chinook salmon growth in freshwater, with ramifications for a variety of parameters such as smoltification and, subsequently, survival. A scenario like this would have greater impact on a k-selected species as opposed to a highly fecund species such as the chinook salmon.

There are presently no published studies that have used microsatellite DNA to examine the relationship between genetic variation and fitness in fish. Thus, opportunities for future research are vast. This study identifies four areas, in particular, that would benefit from further research. First, the counterintuitive direction of many of the genetic variation-fitness relationships requires clarification. Second, characterization of these relationships in hybrids of varying degrees of divergence would be interesting from a conservation perspective. Third, comparison of fitness measures and genetic variation estimates between the wild and domestic purebreeds in this study suggests that examination of these parameters at another level might

be revealing. That is – what is the relationship between population genetic variation and population fitness within a species or metapopulation? Finally, the relevance and utility of the different genetic variation estimators to the resolution of genetic variation-fitness relationships requires further exploration.

Conclusion

Basic and applied research are concerned with the discovery of new knowledge and not with immediate applicability – the distinction between the two is in the choice of questions (Ben-David 1991). Questions in basic research arise from purely intellectual interests (Ben-David 1991) and the unpredictability and uncertainty inherent in the answers to these questions are significant (Geiger 1993; Callon 1994). Questions in applied research, on the other hand, are ‘problem-oriented’ – the assumption being that the results will eventually be of practical value (Ben-David 1971; Ben-David 1991). The two chapters in this thesis approach essentially the same data set in two different manners that embody the definitions of applied (Chapter 1) and basic (Chapter 2) research.

The chapters are linked in several ways. First, using different analytical approaches, the chapters together find little convincing evidence for the existence of outbreeding depression in the study population. This is consistent with much of the current empirical database on outbreeding depression in fish (Waldman and McKinnon 1993) but could be improved with further research. Second, while the practical assessment of hybrid performance is central to Chapter 1 the second chapter draws attention to the same hybrids but from a different perspective. Third, both chapters clearly illustrate differences among the four cross (family) types that suggest the need for further research, some of which could have conservation implications regarding wild and domestic/hatchery fish interactions.

Heterosis and genetic variation-fitness relationships are empirical observations that may or may not have the same cause (David 1998) and, in this sense, the chapters stand alone. The general conclusions from Chapter 2 do not have any immediate application to commercial salmon farming – the apparently small effects of genetic variation on fitness (performance) are relatively unimportant in this artificial setting. Alternately, the conclusions from the first chapter are strongly applied, but data on hybrid performance fits easily into an evolutionary context with implications for topics such as hybrid zone stability and speciation.

There are two interesting features of salmonid evolution and biology that may, in the

future, initiate reinterpretation of many current theories regarding genetic variation and fitness in this group. First, salmonids have an unusual genetic system and its ramifications for quantitative genetic analyses and genetic variation-fitness relationships are poorly understood, particularly regarding microsatellites, and are seldom addressed in conventional studies. The Salmonidae are one of only two fish families that have a tetraploid origin (Allendorf and Thorgaard 1984). This polyploid genetic system may minimize effects of inbreeding depression and exacerbate problems of outbreeding depression (Allendorf and Waples 1996). Second, salmonid reproductive characteristics – low fecundity (relative to marine fish), demersal eggs, pair-bonding, territoriality, and strong natal philopatry – all tend to promote inbreeding (Shields 1982). Shields (1982) suggests that inbreeding has an adaptive advantage in philopatric species because it preserves coadapted gene complexes and that this benefit may outweigh the costs associated with inbreeding.

For these reasons, salmonids may be able to tolerate more inbreeding than other organisms. The fitness cost of inbreeding is known to differ widely among species, with some taxa more susceptible than others (Avisé 1994). There is, however, little evidence that a history of inbreeding in a population reduces the deleterious effects of further inbreeding in that population (Waldman and McKinnon 1993) although the possibility has relevance to the detection of genetic variation-fitness relationships.

This study used an expansive design and new technology to address a basic but controversial question in biology that has relevance to aquaculture and evolutionary theory.

Specifically, this research makes the following contributions to science:

- expands the knowledge base on chinook salmon, a species of management and conservation concern;
- assesses heterosis observed in chinook salmon hybrids for potential application in commercial salmon farming;
- supports a large and complex body of research on genetic variation-fitness relationships;
- uses microsatellite DNA technology and data analyses methods to elucidate genetic variation-fitness relationships – one of the first studies to do so for salmonids;

- presents data relevant to a theoretical discussion of the impact of escaped domestic chinook salmon on wild populations;
- reports the first heritability estimate for saltwater tolerance (osmoregulatory ability) in salmonids;
- presents one of the few known heritability estimates for stress response in salmonids;
- identifies loci that may be of value in QTL analyses for freshwater growth in salmonids; and
- provides data integral to another scientific paper – Bryden and Heath (2000).

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Appendix I

Mean weight and fork length (± 1 standard error) of chinook salmon artificially spawned October 23, 1997. Domestic fish are from Yellow Island Aquaculture Ltd. (Quadra Island, B.C.) and wild fish are from Big Qualicum River (Vancouver Island, B.C.).

Fish	N	Mean Weight (kg)	Mean Fork Length (cm)¹
Wild dams	26	7.53 \pm 0.39	84.8 \pm 1.5
Domestic dams	26	2.35 \pm 0.08	56.8 \pm 0.7
Wild sires	26	6.89 \pm 0.37	79.7 \pm 1.4
Domestic sires	26	2.07 \pm 0.15	54.7 \pm 1.4

¹ Length of wild fish was measured as post-orbital to hypural plate (POH) length. Converted to fork length (FL) using the formula derived by Healey and Heard (1984): $POH = 0.761FL + 47.2$ (all lengths in mm).

Appendix II

Summary of mean values of fitness/performance traits for chinook salmon (all families combined). Only three of the 15 freshwater size-at-age measures are presented.

Trait	n ¹	Mean ± 1 S.E.	Units
Relative fecundity	51*	0.79 ± 0.02	no. eggs per g body weight
Relative egg weight	52*	65 ± 4	mg egg per kg body weight
Eyed egg survival	104	94.5 ± 1.1	%
Alevin survival ²	97	99.3 ± 0.1	%
Fry survival	94	98.0 ± 0.3	%
Outbreak survival	82	87.0 ± 0.7	%
<i>Hours</i> ₁ ²	86	78.2 ± 1.0	hrs
<i>Hours</i> ₁₂ ²	86	103.5 ± 1.7	hrs
93 day weight ³	91	0.35 ± 0.08	g
108 day weight ³	94	0.59 ± 0.01	g
207 day weight ³	94	5.20 ± 0.11	g
420 day weight ³	694*	135.2 ± 1.1	g
615 day weight ³	807*	365.6 ± 3.7	g
Freshwater specific growth rate	94	2.19 ± 0.02	% change in body weight per day
Saltwater specific growth rate	82	0.50 ± 0.01	% change in body weight per day
Composite FA index	214*	2.62 ± 0.14	Standard deviations

¹ number of families unless noted otherwise: * = number of individuals

² unstandardized

³ number of days post-fertilization