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Jacking in Chinook salmon (Oncorhynchus tshawytscha): environmental and genotypic effects on life history strategy

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Jacking in Chinook salmon (*Oncorhynchus tshawytscha*): environmental and genotypic effects on life history strategy

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Abstract

Jacking in Chinook salmon (*Oncorhynchus tshawytscha*), is defined as precocious sexual maturation of males after at least 1 year in sea water, and occurring 1 year prior to females of the same cohort. Substantial evidence supports genetic, environmental, and genetic by environmental effects on precocious maturation in Chinook salmon, however the underlying mechanisms remain unclear.

Passive integrated transponder tagged fish were followed through fresh and salt water growth to the sexual maturation of the jacks. Growth data was recorded to examine the relationship between size/growth effects in freshwater rearing on subsequent precocious sexual maturation of the jacks. No effect of size/growth in freshwater was detected. Reanalysis of data from a previous study showed an effect of developmental rate on jacking.

Sequencing tested the possibility of a relationship between jacking and Major Histocompatibility (MH) genes at the MH class II β 1 locus. One genotype positively affected the likelihood of jacking.

Dedication

This thesis is dedicated to:

- A, T, G, C, without whom none of this would have been possible.
- Rita Young, a Mohawk princess who taught me the wonders and inherent value of the natural world, and to respect it always.
- My children, Rylee Young and Clayton Young, for helping me to remember the importance of curiosity and exploration.
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Chapter 1

General Introduction

Chinook salmon (*Oncorhynchus tshawytscha*) spawn in freshwater and typically smolt and migrate to sea during spring of their first year (Healey 1991). Adult Chinook salmon return to the spawning grounds after 1-6 years at sea (Healey 1991). The term "jacking" is applied to sea-run males in Chinook salmon who exhibit precocious sexual maturation after at least one year in sea water, usually one year prior to maturation of females within the same cohort (Healey 1991). Male Chinook salmon may also sexually mature in the freshwater prior to smolting as "precocious parr", however this is a relatively rare phenomenon (Taylor 1989; Foote et al. 1991; Bernier et al. 1992), and is more common among Atlantic salmon (*Salmo salar*) stocks. Jacking has been described in Chinook salmon (Healey 1991; Bocking and Nass 1992), Coho salmon, *Oncorhynchus kisutch* (Bilton 1980; Sandercock 1991), and Sockeye salmon, *Oncorhynchus nerka* (Burgner 1991). Jacks may comprise up to 90% of the males from a given Chinook salmon stock (Hard et al. 1985), however, typically most stocks exhibit jacking rates of 5-15 % of the returning population (Ricker 1972; Healey 1991; Bocking and Nass 1992; Mullan et al. 1992).

Precocious maturation in male salmonids constitutes an alternative male mating strategy; the younger, smaller, cryptically colored jacks gain access to females for reproduction via a "sneaking" behavior facilitated by their small size, whereas the older, larger "hooknose" males guard females and fight to defend territory (Gross 1984, 1985; Bohlin et al 1990; Taborsky 1994). Alternative mating strategies have been formalized into models of the evolutionarily stable strategy (ESS) using game theory (Smith 1982).

A strategy is defined as a genetically-based life history plan (Gross 1996). Tactics are the proximate methods, or phenotype used to achieve the life history strategy. Each individual possesses only one strategy, but one or more tactics may be employed to execute the strategy. Gross (1996) defined alternative strategies as two or more strategies with equal average fitnesses, and each strategy is determined by a genetic polymorphism. A mixed strategy is defined as one strategy with alternative tactics having equal average fitnesses under frequency-dependent selection and exhibiting genetic monomorphism resulting in an Evolutionarily Stable Strategy frequency. This differs from a conditional strategy wherein the alternative tactics are determined by status-dependent selection (with or without frequency-dependent selection), and the tactics have unequal average fitnesses, possess a genetic monomorphism, and exhibit an Evolutionarily Stable Strategy switch-point. The strategy is affected through a mechanism (physiological, neurological, or developmental) that detects appropriate cues and engages the strategies decision rule, such as fight when larger than X and sneak when smaller (conditional strategy), or fight with probability 0.2 and sneak with probability 0.8 (mixed strategy). In Chinook salmon the alternative male mating strategy is hard to classify since the decision to engage the jack life history strategy creates a phenotype that results in a morphologically distinct form and is not defined exclusively by behavior. Furthermore, the strategy exhibits components of both a true alternative strategy due to the high heritability of jacking shown by Heath et al. (2002), and also seems conditional upon reaching a threshold size, growth, or developmental rate (e.g. Heath et al. 1991, 1996).

The alternative male mating phenotypes in chinook salmon represent a type of phenotypic plasticity that produces two discreet morphotypes. Phenotypic plasticity is

the capacity of a genotype to produce different phenotypes in response to changing environmental conditions (Pigliucci 2001). The environment does not merely "permit" development, but rather guides it, or even induces it (Gilbert 2001, 2005). This complex interaction between an individual's genetic makeup and the environmental conditions it experiences during growth and maturation creates the opportunity for a variety of phenotypes to arise from the same genotype (West-Eberhard 2003). Substantial evidence supports both genetic (Iwamoto et al. 1984; Silverstein and Hershberger 1992; Heath et al. 1994, 2002) environmental (Heath et al. 1991, 1994; Silverstein et al. 1998; Vollestad et al. 2004; Larsen et al. 2006; among others) and genetic by environmental interaction (Heath et al. 1996) effects on precocious maturation in Pacific salmon.

While age at sexual maturity in salmonids has been shown to be affected by both genetic and environmental factors, the underlying mechanisms remain unclear. The use of a threshold trait model for alternative male mating strategies in salmonids has allowed for the integration of genetic and environmental effects (Myers and Hutchings 1986; Hazel et al. 1990; Hutchings and Myers 1994; Hutchings 2002). Substantial evidence supports large size, fast growth, and high lipid content as important threshold criteria for initiation of sexual maturation (Rowe et al. 1991, Silverstein and Shimma 1994; Heath et al. 1996; Silverstein et al. 1998; Shearer and Swanson 2000, among others). An inherent weakness of threshold models is several underlying physiological processes may contribute to particular thresholds, which confounds identification of determining factors. An example is found in studies showing that when freshwater developmental rates are temperature accelerated, increased rates of precocious male maturation resulted (Bilton 1978, 1980; Bilton et al. 1982; Crandall and Gall 1993; Heath et al 1994; Shearer et al.

2002). In these studies and others, it is uncertain if the observed effects on the rate of precocious maturation resulted from increased developmental rates or were a response to increased food consumption and higher growth rates at increased temperature. However, the proximate mechanisms affecting precocious male sexual maturation must be characterized if we are to predict the response of salmon life history patterns to changes in the environment.

In vertebrates, genes of the Major Histocompatibility Complex (MHC) are involved in the specific immune response by encoding cell-surface proteins that bind peptide fragments derived from pathogens and present those fragments to T-cells that elicit an appropriate immune response (Klein 1986). The genes of the MHC are among the most polymorphic in vertebrates, and the majority of this variation is contained within the peptide-binding region (PBR) involved in pathogen's binding (Hughes and Yeager 1998). There are two classes of MHC molecules, class I and class II. Class I MHC molecules are heterodimers, comprising a transmembrane peptide (class I heavy chain), and three extracellular domains ($\alpha 1$, $\alpha 2$, $\alpha 3$) each of which is encoded by a different exon from a single gene. The Class I PBR consists of two α-helices next to a β-pleated sheet, formed by the $\alpha 1$ and $\alpha 2$ domains of the class I heavy chain (Jeffery and Bangham 2000). The MHC class II molecule is a heterodimer that consists of two transmembrane proteins, an α and a β chain, which are encoded by two separate genes. Specific sites within both chains form the Class II PBR. In Class I molecules the PBR binds peptides derived from cytosolic proteins which are typically 8-9 amino acids in length. Conversely, in Class II molecules the PBR binds peptides derived from extracellular proteins which are generally 15-24 amino acids long. Substitution of only one or two amino acids in the PBR can

result in large differences in the range of peptides bound, and hence to pathogen resistance (Frank 2002). Indeed, studies have conferred support for a positive relationship between MHC diversity and disease resistance (reviewed in: Jeffery and Bangham 2000; Sommer 2005). At the individual level, certain alleles have been shown to confer resistance to diseases such as malaria in humans (Hill et al. 1991), Marek's disease in chickens (Briles et al. 1977), and furunculosis and infectious salmon anaemia virus in Atlantic salmon (Grimholt et al. 2003; Kjoglum et al. 2006). At the population level, high MHC diversity is beneficial for conferring resistance to a broad changing array of pathogens on an evolutionary time scale, and this diversity is hypothesized to be maintained by balancing selection, heterozygote advantage, frequency-dependent selection, or variable chronological/environmental selection (Nei and Hughes 1991; Hedrick 2002).

In teleosts genes of the MHC do not form a single complex; they are thus properly referred to as "MH" genes in teleosts (Stet et al. 2002). In contrast to most other teleosts, Chinook salmon are characterized by low copy number of MH class II loci, and hence relatively low allelic diversity; previous studies have documented only 3-6 alleles per population (Miller and Withler 1996; Kim et al. 1999; Docker and Heath 2002; Pitcher and Neff 2006). In contrast, other species, for example cichlids, may have up to 17 class II loci and much higher allelic diversity (Malaga-Trillo et al. 1989). In other vertebrates, for example humans, there are 9 classical genes, so an individual can posses a maximum of 18 MHC I or II alleles, and some of the class I and class II genes have over 200 allelic variants (The MHC sequencing consortium 1999). Dionne et al. (2007) suggested that directional selection may replace balancing selection in environments

where only one or a few pathogens can survive for a specific host, and thus reduce overall diversity at MH genes in salmon. This may partly explain the apparent directional selection observed in some populations of sockeye salmon (*Oncorhynchus nerka*) at MH genes (Miller et al. 2001). Despite the low diversity at MH class II in Chinook salmon, MH class II has been shown to significantly affect disease resistance. Chinook salmon MH class II heterozygotes had a significantly higher survival rate versus homozygotes when exposed to an infectious haematopoietic necrosis virus (IHNV) in a study by Arkush et al. (2002). Chinook salmon have also been shown to have both additive and non-additive genetic contributions to early survival from MH class II alleles (Pitcher and Neff 2006). Additionally, evidence for habitat and climate specific local adaptation in amino acid substitution patterns in the functionally important PBR has been shown in both Chinook (Heath et al. 2006), and sockeye salmon (Miller et al. 2001). Thus, despite the inherently low diversity at the MH II locus in Pacific salmon relative to other taxa, MH genotype has significant effects on survival in salmonids, and in Chinook salmon in particular.

Previous studies have shown that jacks are the fastest growing fish in the salt water prior to sexual maturation (Heath et al. 1996; Chapter 2). Possibly the faster growth of the jacks reflects overall good or compatible genes (Neff and Pitcher 2005), for instance those at MH loci that conferred a reduced pathogen load and allowed more resources to be directed to growth, reproductive efforts, and ultimately developmental rate. The relationship between MH diversity and life history in Chinook salmon has not previously been investigated.

This thesis is primarily focused on an investigation of environmental and genetic factors affecting precocious sexual maturation of male Chinook salmon, and has been organized into two sections that address different factors potentially affecting jacking rates in Chinook salmon. The first section, Chapter 2, examines growth/weight and developmental trajectories of individual fish in the freshwater-rearing period. To achieve this, a cohort of 4 full sib families were passive integrated transponder (PIT) tagged and individual level growth/weight data was recorded at regular intervals through to sexual maturation of the jacks. Additionally, data from an earlier experiment with differing rearing temperatures was normalized for ATU (acquired thermal units, also known as degree days) to allow comparison of mean mass at equivalent developmental stage and subsequent jacking rates between temperature accelerated and non-accelerated fish. This allowed examination of the freshwater growth rate and body mass trajectories effects on life history strategy. The second section, Chapter 3, examines Major Histocompatability (MH) variation between 40 pairs of jack and non-jack siblings from within 11 families, to detect any correlation between MH heterozygosity and male mating phenotype. Previous studies have shown that an "optimal" MH genotype confers up to a 3% body size increase over sub-optimal MH genotypes in Chinook salmon (Pitcher and Neff 2007). Based on the well established relationship between size/growth and precocious sexual maturation in Chinook salmon, I hypothesize that jacks will be more diverse at MH class II B1 loci relative to their non-mature brothers. To achieve this, a 294 bp fragment of the MH class II B1 peptide binding region was amplified, cloned, and subsequently sequenced. Allele and genotypic frequencies were then compared between jacks and non-mature siblings.

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Chapter 2

Freshwater Initiation of Precocious Male Sexual Maturation in Chinook Salmon: Effects of Developmental Rates, Body Size and Growth.

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2.1 Introduction

Fish in general, and salmonids in particular, exhibit remarkable life history variation among species, populations and individuals within populations (Groot and Margolis 1991). This life history variation is particularly evident in sexual maturation traits (Gross 1984; Taborsky 1994). For example, the age of maturation in male Chinook salmon *Oncorhynchus tshawytscha* is very plastic, occurring from 1-6 years after fertilization (Healey 1991). Life history variation in salmon has been extensively studied and numerous models have been developed to explain the observed variation in traits such as age and size at maturity or alternative reproductive strategies (e.g, Bohlin et al. 1990; Hutchings and Myers 1994). However, such models focus on the fitness consequences of alternative life history strategies, but do not generally deal with the mechanisms driving the variation at the individual level. However, those mechanisms must be characterized if we are to predict the response of salmon life history patterns to changes in the environment. Genetic (e.g. Iwamoto et al. 1984; Hard et al. 1985; Silverstein and Hershberger 1992; Heath et al. 1994, 2002), environmental (e.g. Alm 1959; Taylor 1989; Thorpe 1991; Heath et al. 1994), and genetic-by-environmental interaction (Heath et al. 1994) effects on the incidence of early male maturation in salmonid species have been demonstrated. All studies have reported a strong environmental component, but the exact nature of that effect remains elusive. Precocious

male maturation (the term 'jacking' is applied in Chinook and Coho salmon) in particular has received much attention since jacks are of little commercial value due to the small size and loss of body condition associated with maturation. Thus studies designed to provide an understanding of jacking in commercial stocks have been the focus of applied research in attempts to minimize or eliminate jacking (e.g., Heath et al. 1991).

Mechanistic approaches to modeling alternative male mating strategies in salmonids have used threshold trait models to integrate the genetic and environmental effects (Myers and Hutchings 1986; Hazel et al. 1990; Hutchings and Myers 1994; Hutchings 2002). The hypothesis is that a threshold for size, growth rate, or stored energy (eg. lipid content) must be exceeded during some critical period for precocious sexual development to proceed (Rowe and Thorpe 1990a,b; Rowe et al. 1991; Clarke and Blackburn 1994; Silverstein and Shimma 1994; Hopkins and Unwin 1997; Silverstein et al. 1997,1998; Shearer and Swanson 2000). One weakness of threshold models is that several underlying physiological processes may contribute to particular thresholds, making the identification of determining factors difficult.

Temperature is a critical environmental factor that affects physiological processes in fish such as; development rate, growth and body condition, and ultimately, behavior and life history (Le Cren 1958; Atkinson 1994; Fox and Crivelli 2001). Several studies have used water temperature to manipulate freshwater developmental rates, generating fish of differing size and growth, and subsequently examined precocious male sexual maturation rates (e.g. Crandall and Gall 1993; Heath et al. 1994; Shearer et al. 2002). A number of studies have demonstrated that when freshwater developmental rates are accelerated, increased rates of precocious male maturation result (Bilton 1978, 1980;

Bilton et al. 1982; Crandall and Gall 1993; Heath et al. 1994; Shearer et al. 2002). However, in those studies and others, it is unclear whether the observed effects on the rate of precocious male maturation were due to increased developmental rates or a response to increased food consumption and higher growth rates at increased temperatures. Recent studies have shown the effects of temperature (i.e., development rate) on maturation rates of fish, independent of size or growth rate (Fox and Crivelli 2001; Dembski et al. 2006; Dhillon and Fox 2004). Dhillon and Fox (2004) equalized growth rates (via ration manipulation) among replicates of Japanese Medaka (*Oryzias latipes*) experiencing four different rearing temperatures. They showed that both age and size at maturity decrease with an increase in temperature even when growth rate is controlled (Dhillon and Fox 2004).

Heath et al. (1994) showed that in Chinook salmon, the incidence of jacking increased with elevated incubation temperature, but that it appeared to be independent of body size or growth. Here I test for an effect of individual freshwater growth and body size on subsequent jacking rates in Chinook salmon. Four full-sib families of individually PIT-tagged (passive integrated transponder) juvenile Chinook salmon were reared and each individual weighed at regular intervals through to maturation of the jacks. Size at age data for individually tagged fish provides higher resolution for estimating the effect of body size and growth-trajectories on the incidence of jacking than is possible in population- or family-level investigations (Juanes et al. 2000). I also reanalyze data from Heath et al.'s (1994) study of 12 full sib families of juvenile Chinook salmon raised under accelerated and ambient incubation temperatures to normalize developmental stage to equivalent acquired thermal units. I then explicitly test for the effects of body size

versus incubation temperature on jacking incidence. This approach provides a powerful test of the competing hypotheses of body size or growth rate thresholds versus developmental acceleration as early life determinants of jacking in Chinook salmon.

2.2 Methods and Materials:

Breeding & rearing: Fourth generation domestic stock originally derived from the Robertson Creek Salmon Enhancement Facility (Department of Fisheries and Oceans, Canada), Vancouver Island, were used to create four jack-sired families with 3 year-old females in a 1:1 mating scheme at the commercial hatchery facilities of Yellow Island Aquaculture Ltd. (YIAL-Quadra Island, B.C., Canada). On 17 May 2000, 150 fry from each family were anesthetized with clove oil (10 ppm.) and injected intraperitoneally with a Passive Integrated Transponder (PIT) tag (Bio-mark Inc). This facilitated the collection of weight data as the fish developed through to the eventual sexual maturation of the jacks, and subsequently allowed an analysis of freshwater size/growth correlations and ultimate male phenotype. The 600 tagged fry were held in a common 3000-liter freshwater tank at an average temperature of approximately 8°C to 30 July 2000, when they were transferred to a 3.3m X 3.3m X 3.3m seawater cage. The fish were reared to sexual maturity of the jacks under standard commercial rearing conditions at YIAL. Fish were feed to satiation one or two times daily with a commercial fish diet (Taplow Feeds, Campbell River, British Columbia).

Sampling and analysis: Fish were anesthetized and individually weighed at the initial PIT tagging 17 May 2000 (approximately 210 days post fertilization) and on; 30 July 2000, 7

October 2000, 3 February 2001, 20 April 2001, 6 June 2001, and 17 November 2001. On November 17, 2001, all the fish were euthanised and jacks were identified based on their well-developed testes, production of milt, and secondary sexual characteristics (body morphology and skin color). Specific growth rate (SGR), was calculated over each of the time intervals between sampling events for each fish as:

$$SGR = [\ln(Wt_{final}) - \ln(Wt_{initial})] \cdot 100 / d \quad (1)$$

Where $Wt_{initial}$ was the weight of an individual fish at the beginning of the interval, Wt_{final} was the weight at the end of the interval, and d is the number of days within the interval. One-way ANOVA was used to test for differences in SGR and wet weight between fish that eventually became jacks versus later-maturing fish at each sampling interval. I also used a correlation analysis to test whether freshwater body size or SGR predicts body size later in life prior to sexual maturation of the jacks.

Incubation acceleration effects: Data from Heath et al. (1994) was reanalyzed to normalize body size to equivalent accumulated thermal units (ATU`s) in developmentally accelerated and non-accelerated Chinook salmon. Mating design, incubation, rearing protocols, sampling, and analyses of the previous study are described in Heath et al. (1994). Briefly, six 3-year-old females were spawned, and the eggs from each female were divided into 2 batches and fertilized by a 2-year-old male (jack), and by a 3-year-old male (non-jack). The resultant 12 families were again divided in half to create two sub-groups, one group being reared at an accelerated incubation temperature (c. 10.2°) and the

other at a non-accelerated incubation temperature (c. 8.0°), and temperature was measured daily. Upon yolk-sac depletion, swim-up fry were reared in tanks with a common water source and jacks were identified based on body morphology and body colour in the following year. Specific jacking rate (SJR) was estimated for each family (Heath et al. 1994).

Using daily incubation water temperature data, I calculated ATUs at each sampling time for the accelerated and non-accelerated families. The accelerated fish acquired ATUs at a faster rate than the non-accelerated fish during incubation; hence, at a given point in time an accelerated fish was further along the developmental trajectory vs. a non-accelerated fish. To normalize for developmental stage I plotted mean body weight at each sampling time against ATUs for the two treatments. Rather than use regression to estimate mean body size for each family at common ATUs, I choose an ATU value where real data existed for each family at common ATUs (1576.5 ± 4.5 ATU). This point represented a difference of 27 days growth between sample dates for the accelerated vs. the non-accelerated fish to acquire 1576.5 ATUs (accelerated fish: April 16, non-accelerated fish: May 22). A T-test (SPSS) was then used to test for significant differences in mean family weight at this common number of ATUs. The estimated normalized body size was then plotted against SJR to test for developmentally-corrected body size effects on eventual jacking rate.

2.3 Results

Growth rates and precocious maturation

The total jacking rates in each of the four families in the PIT tag study were: 22.2%, 17.2%, 25.8%, and 22.9%. The combined jacking rate over all four families was

22.0%. No significant difference in body size ($t = 0.045$, $df = 264$, $p = 0.964$) or SGR ($t = -1.621$, $df = 264$, $p = 0.106$) in the freshwater rearing stage was found between jacks and non-mature fish in any of the four replicate families (Figure 2.1, Figure 2.2). Jack specific growth rate did become significantly higher than non-mature fish in Feb 2001 (in salt water), and remained so up to the point of gonadal growth and sexual maturation (June 2001) at which time non-maturing fish had significantly higher growth rates (as the maturing fish reduced somatic growth; Figure 2.2b). There was no significant correlation between freshwater growth rate on 7/30/00 and saltwater growth rate on 6/6/01, in jacks ($r = 0.062$, $p = 0.627$) or non-mature males ($r = -0.134$, $p = 0.057$).

Developmental effects on precocious maturation

Although the accelerated families exhibited a statistically significantly higher jacking rate compared to the non-accelerated families (Heath et al. 1994), the accelerated families consistently weighed less than their non-accelerated counterparts at equivalent ATUs in the fresh water (Figure 2.3a &b). Furthermore, I found no correlation between freshwater mean body size or growth rates normalized for ATUs and jacking rates (Figure 2.3b; accelerated: $r^2 = 0.001$, $p=0.939$; non-accelerated: $r^2 = 0.119$, $p=0.272$).

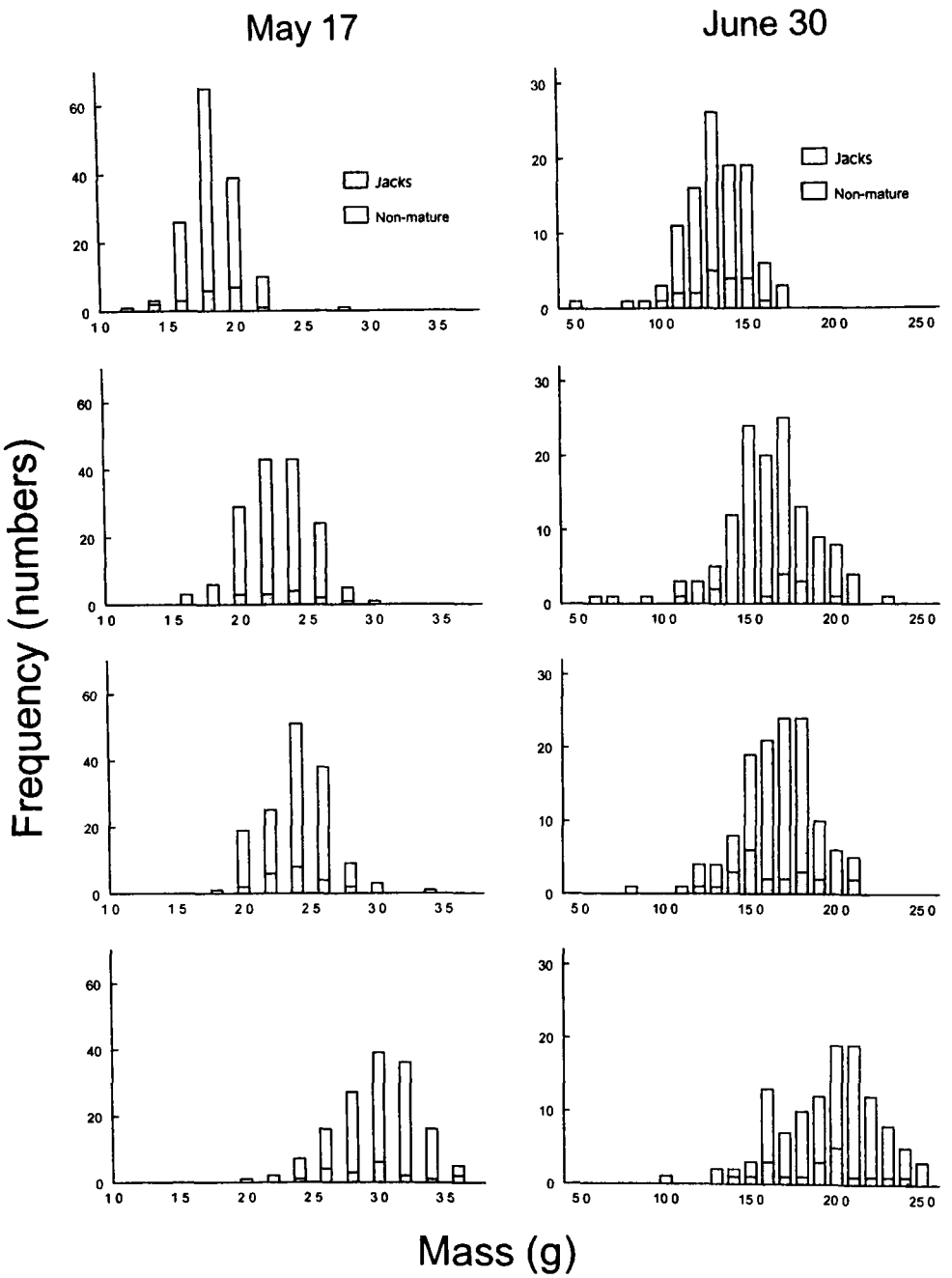

Figure 2.1. Mass-frequency distributions of jacks vs. non-mature fish by family for the two freshwater sampling dates (5/17/00, 7/30/00). There are no significant differences ($P > 0.05$) between the size distributions of jacks and non-mature fish during freshwater rearing.

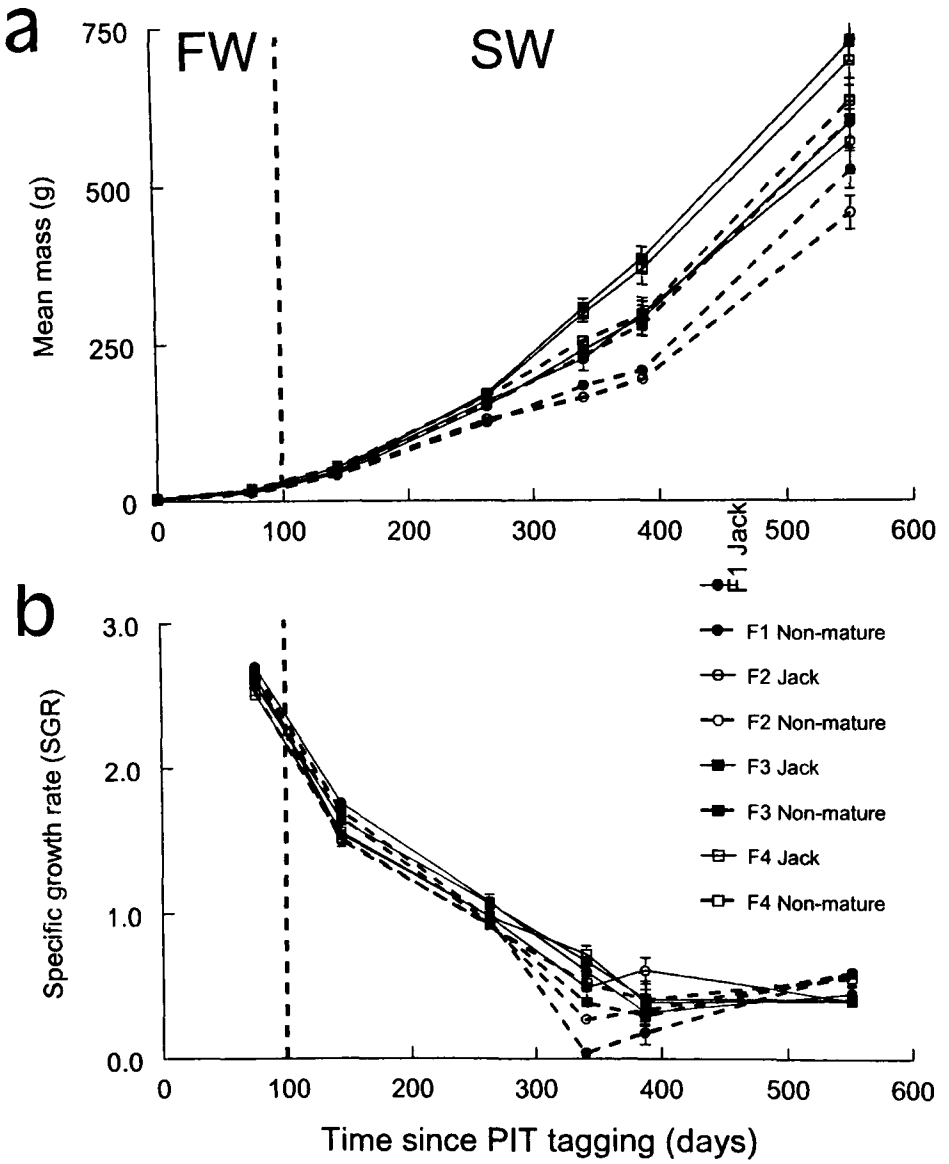

Figure 2.2: Mean family mass (± 1 se) and specific growth (± 1 se) rate for jacks and non-mature fish through early freshwater rearing to maturation of the jacks in salt water (fish were PIT tagged approximately 210 days post fertilization). Jacks are indicated by solid lines, non-mature by dashed. The first two data points represent the freshwater rearing stage (FW), and exhibit no significant difference in growth rate or mean mass during this period, while significant differences do develop by the salt water (SW) life history phase.

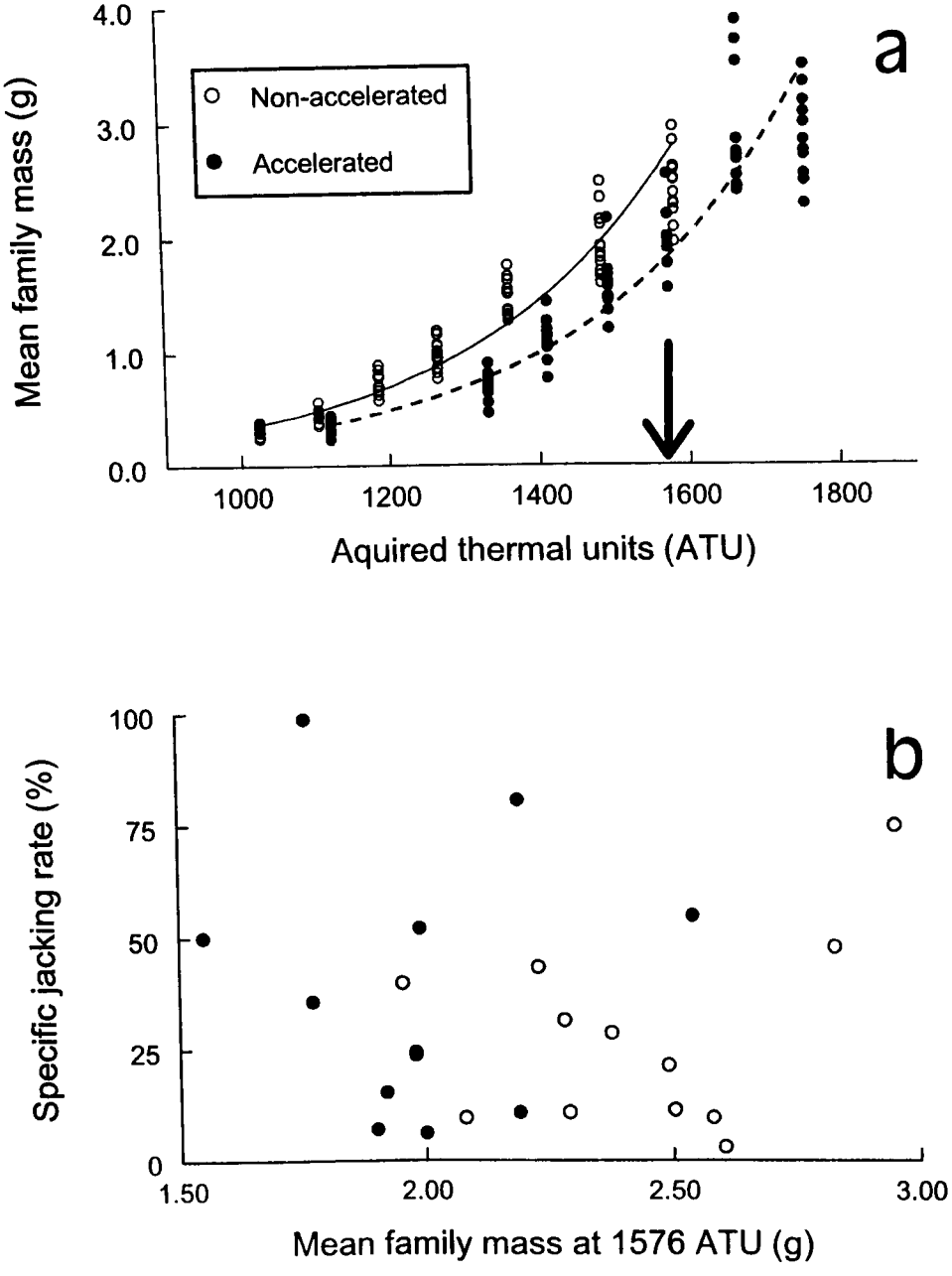

Figure 2.3: Relationship between mean family mass and specific jacking rate (SJR) corrected for ATUs. Panel (a): mean family mass at ATUs for the accelerated families (filled circles) and non-accelerated families (open circles). The accelerated families weigh less at equivalent ATUs, despite having significantly higher jacking rates. Panel (b): specific jacking rate by mean family mass at 1576 ATU± 4.5 ATU (indicated by arrow in panel a). The graph shows no correlation between SJR and mean family mass.

2.4 Discussion

I found that weight and/or growth rates during the freshwater life-history phase of Chinook salmon do not directly affect the likelihood of jacking. This result was evident at the individual fish level in four replicate full-sib families, as well as among the families in the developmental acceleration study (Heath et al. 1994). This is in contrast to other studies that have concluded that early weight or growth rates are primary contributing factors to subsequent salt-water precocious sexual maturation rates in salmon (Bilton 1978, 1980; Bilton et al. 1982; Lamont 1990; Shearer et al. 2002; Vollestad et al. 2004), but supports the conclusions in Heath et al. (1994). For example, a recent study by Vollestad et al. (2004) concluded that Chinook salmon smolt size prior to salt water entry was the predominant factor influencing subsequent probability of precocious maturation based on average smolt weight among cohorts (over 30 years) as an index of freshwater growth rate. Shearer et al. (2002) also concluded that freshwater body size was a contributing factor to the incidence of jacking in Chinook salmon based on a temperature manipulated growth treatment - unfortunately, the experimental design did not allow separate examination of the confounded effects of developmental acceleration and size. Other studies have demonstrated only weak effects of early juvenile size and subsequent rates of precocious maturation (e.g. Crandall and Gall 1993; Tripping et al. 2003), yet have generally proposed early growth rate as an important factor in the subsequent incidence of precocious male sexual maturation. For example, Crandall and Gall (1993) showed that rainbow trout reared in a high temperature rearing treatment (11.3 – 17.9°C) produced almost three times as many precocious males as those held at a low temperature treatment (9.0 – 17.9°C). However, precocious males were present in every weight

category prior to maturation (i.e. large and small body size), indicating the lack of a consistent early body size threshold affecting maturation (Crandall and Gall 1993). Clearly the correlated effects of developmental rate, growth and body size confound many published studies designed to test for freshwater effects on precocious sexual maturation in Pacific salmonids.

I am the first to show that the rate of precocious maturation is independent of individual freshwater size and growth-rate in Chinook salmon. Additionally, my study shows that developmental acceleration can have significant effects on male maturation life-history at a much earlier life stage than is generally accepted; that is, in the pre-feeding egg and larval incubation stage. My results are in agreement with previous studies demonstrating a significant effect of temperature on maturation rates irrespective of growth effects (Fox and Crivelli 2001; Dembski et al. 2006; Dhillon and Fox 2004). Fox and Crivelli (2001) offered empirical evidence that temperature regime can affect life-history traits independently of a growth effect in field trials. Their study demonstrated that pumpkinseed sunfish (*Lepomis gibbosus*) from warmer environments in southern France matured earlier and at a smaller size than those found in cooler Canadian lakes, under conditions where no significant difference in growth rates existed between the two environments (Fox and Crivelli 2001). Dembski et al. (2006) also demonstrated that an increase in water temperature increased rates of precocious maturation in pumpkinseed sunfish, and also reduced the size of the maturing fish. Dhillon and Fox (2004) equalized growth rates and body size via diet manipulation among replicates of Medaka (*Oryzias latipes*) experiencing four different temperature regimes. Age and size at maturity decreased as rearing temperature increased, even

though somatic growth rates were held equivalent among all treatments (Dhillon and Fox 2004). In my study freshwater growth rates did not differ significantly between developmental acceleration and non-acceleration treatments. However, the rate of precocious sexual maturation was significantly higher in the paired families that experienced early life developmental acceleration (Heath et al. 1994).

In the developmentally accelerated fish from Heath et al. (1994), the mean size at a fixed ATU was actually smaller than the mean size of a non-accelerated fish. Thus, the accelerated group, while exhibiting significantly higher rates of precocious maturation, had a smaller mean body size throughout the early freshwater rearing period. Since developmental acceleration increases standard metabolic rate (Metcalfe 1998), the lower weight at equivalent ATUs of the accelerated families is likely due to the higher energetic cost associated with an elevated standard metabolic rate during the pre-feeding incubation phase of development (see Metcalfe 1998). Studies have also shown that an increase in environmental temperature elevates growth rates but affects catabolism more than anabolic rate, and thus body size at a given stage of development is reduced (van der Have and de Jong 1996). McCarthy (2000) showed that relative standard metabolic rate of Atlantic salmon fry 5 weeks after first feeding was correlated with relative standard metabolic rate 113 days later; despite the fact the fish were 20 times larger and had developed a bimodal weight frequency distribution. McCarthy's (2000) results demonstrate that early developmental rate may be predictive of long-term metabolic rate, that is, metabolic rate is primed by early life developmental rates. Since freshwater body size and growth is generally not correlated with saltwater size or growth rate in fish (this study; Crandell & Gall 1993; see Heath & Blouw 1998), early body size or growth would

not provide a reliable prediction of body size at the initiation of precocious sexual maturation (see Heath et al. 1991, 1997). Freshwater developmental rate, on the other hand, may plausibly predict future body size via metabolic rate effects, and hence may indirectly shift maturation thresholds in the salt water. I propose that pre-feeding incubation developmental rate may be an underlying factor contributing to subsequent saltwater maturation schedules in Pacific salmon (*Oncorhynchus spp.*), and that observed earlier age of returning salmon (Ricker 1981) may in fact reflect increased egg and larval incubation temperatures resulting from climate change.

In this study, rates of jacking in Chinook salmon were independent of body size or growth parameters in the freshwater life history stage. The lack of size and growth effects is counter to published studies suggesting the importance of those factors on the subsequent incidence of jacking. Furthermore, I show that the effect of pre-feeding incubation temperature on jacking rates was significant, independent of size and growth effects, and hence I push initiating factors of maturation back to an earlier stage in ontogeny than that generally reported for salmonids. Finally, increasing stream water temperatures are expected to favour earlier sexual maturation trajectories in salmon populations (Mote et al. 2003), hence climate change effects on salmon streams may drive an increase in the frequency of jacks.

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Chapter 3
MH Diversity and Life History Strategy in Male Chinook Salmon

3.1 Introduction

Salmonid fishes exhibit considerable intra- and inter-population variation in age at sexual maturity reflecting their general high degree of plasticity in life history traits. This plasticity results in Pacific salmon species reaching sexual maturity from 1 to 7 years of age. For example, coastal Chinook salmon (*Oncorhynchus tshawytscha*) males mature at 3-5 years of age; however, some males sexually mature and return to spawn after only one year in sea water, before any females of the same year class (Healey 1991). This precocious sexual maturation of Chinook salmon males is referred to as "jacking", and the jacks display an alternative male mating phenotype (Healy 1991; Heath et al., 1991). The smaller, cryptically colored, and younger Chinook salmon jacks gain access to fertilize female gametes by utilizing "sneaking" behavior, while the larger, older males guard females and fight amongst themselves for dominance (Bohlin et al. 1990). Precocious sexual maturation of salmonid males has been modeled as a threshold trait; the fish will mature if the rate of surplus energy acquisition exceeds a genetically determined threshold during a critical period (e.g. Atlantic salmon: Thorpe et al. 1998). The heritability and a Y-chromosome influence on jacking have also been modeled as a threshold trait (Heath et al. 2002).

While age at sexual maturity in salmonids has been shown to be influenced by both genetic and environmental factors (Rowe and Thorpe 1990; Silverstein and Hershberger 1992; Heath et al. 1994, 2002), the underlying mechanisms remain unclear. Considerable evidence exists for large size, fast growth, and high lipid content as important criteria for initiation of sexual maturation (Rowe et al. 1991. Silverstein and

Shimma 1994; Silverstein et al. 1997; Shearer and Swanson 2000, among others). Experimental studies in Atlantic salmon (*Salmo salar*) have linked intraspecific variation in male maturation strategy to physiological mechanisms such as developmental rate (Metcalfe and Thorpe 1992), metabolic rate (Metcalfe et al. 1995; McCarthy 2000), and variation at protein-coding loci (Jordan et al. 1990; Pollard et al. 1994; Blanco et al. 1998). Developmental rate is accepted as a key factor affecting life history variation in Atlantic salmon (Metcalfe 1998; Thorpe et al. 1998), and its effects have been reported on increased jacking rates in Chinook salmon (Heath et al. 1994; Chapter 2). A positive relationship between allozyme heterozygosity, embryonic developmental rate (i.e. relative date of first feeding) and subsequent life history strategy in Atlantic salmon was shown by McCarthy et al. (2003), while other studies have shown a positive association between multilocus heterozygosity and developmental rate (Danzmann et al. 1989; DiMichele and Powers 1991). Thus, previous studies have begun to challenge the paradigm that the smaller precociously mature male salmon are inferior, or "making the best of a bad situation" (Davies 1982) compared to older, larger, males. Instead there is a growing realization that jacks may be higher quality males with more resources available to allocate to early maturation.

The genes of the Major Histocompatability Complex (MHC) have become one of the most prominent gene classes employed to study natural selection in wild populations (e.g., Bernatchez and Landry 2003; Sommer 2005). Genes of the MHC are among the most polymorphic in the vertebrate genome and are fundamental to the initiation of the specific immune response in vertebrates (Klein 1986; Hughes 1999). The genes of the MHC encode cell surface glycoproteins that bind non-self antigens derived from parasites

or pathogens and present them to T-cells, and this interaction triggers the specific immune response. Two classes of MHC genes exist in the vertebrate genome: MHC class I genes provide defense against intracellular pathogens by binding peptides and are expressed on the surface of all nucleated somatic cells. MHC class II genes differ in that they are predominantly involved in monitoring the extracellular environment via presentation of peptides primarily derived from extracellular parasites and pathogens (e.g. bacteria, cestodes, nematodes; Klein and Horejsi, 1997). The amino-acid sequence of the peptide-binding region (PBR) within the MHC molecules determines the specificity of antigen-binding and pathogen recognition (Brown et al. 1988). Diversity at MHC loci should therefore confer resistance to a greater array of pathogens (Doherty and Zinkernagel 1975; Hughes and Nei 1988). Indeed numerous studies provide evidence for a positive relationship between MHC diversity and disease resistance (reviewed in: Jeffery and Bangham 2000; Sommer 2005).

MHC genes in teleosts do not form a single complex, they are therefore properly known as "MH" genes in teleosts (Stet et al. 2002). In contrast to most other fish, Chinook salmon are characterized by a low copy number of MH class II loci, which translates into relatively low allelic diversity; previous studies have reported only 3-6 alleles per population (Miller and Withler 1996; Kim et al. 1999; Docker and Heath 2002; Pitcher and Neff 2006), whereas other species, for example cichlids, may have up to 17 class I loci and much higher allelic diversity (Malaga-Trillo et al. 1998). Despite the low diversity at in Chinook salmon, MH class II has been shown to significantly affect disease resistance. Arkush et al. (2002) showed that MH class II heterozygotes had significantly higher survival rate versus homozygotes in Chinook salmon exposed to an

infectious haematopoietic necrosis virus (IHNV). In Atlantic salmon, specific MH alleles have been associated with resistance to IHNV (Miller et al. 2004), infectious salmon anaemia (ISA) (Kjoglum 2006; Grimholt et al. 2003), and *Aeromonas salmonicida*, the bacteria causing furunculosis (Langefors et al. 2001; Grimholt et al. 2003). A link between MH genotype and susceptibility to sea lice has also been shown in Atlantic salmon (Glover et al. 2007). Chinook salmon have been shown to have both additive and non-additive genetic contributions to early survival from MH class II alleles (Pitcher and Neff 2006). Thus, in spite of the inherently low diversity at the MH II locus in Pacific and Atlantic salmonids relative to other taxa, MH genotype has significant effects on survival in salmonids, and in Chinook salmon in particular.

The reported effects of MH genotype on disease resistance and survival should confer superior growth and development in salmon with more MH diversity, as the cost of infection slows growth and development in genotypes with inferior pathogen resistance. The well-established relationship between high growth/development rates and subsequent jacking rates in Chinook salmon (Heath et al. 1991) may thus be related to diversity at MH PBR regions. Indeed, jack sired families have been found to exhibit higher survival relative to non-jack-sired families through severe outbreaks of Vibriosis (a common saltwater pathogen) under culture conditions in netcages (D.D. Heath, unpublished data). However, to my knowledge, no formal test of the possibility of a relationship between alternative male mating strategies and MH genotype has been made. Here I present the first study to compare MH class IIB heterozygosity, allelic diversity and genotype frequencies between alternative male maturation life histories in Chinook salmon.

3.2 Materials and Methods

Samples

Domestic Chinook salmon used in this project were originally from the Robertson Creek stock and were part of a larger breeding project at Yellow Island Aquaculture Ltd. (YIAL; see Bryden et al. 2004). The study used a partial diallel mating design to generate a total of 104 families; however, some were lost during the course of the study so 94 families remained (Bryden et al. 2004). All fry were identified to family by colour-coded wire nose tags (Northwest Marine Technology, Shaw Island, WA) (Bryden et al. 2004). Before transfer to saltwater, smolts were immersion vaccinated for vibriosis (Microvibk, Microtek International, Saanichton, BC), and at approximately 500 days post-fertilization the fish were humanely euthanized, blood taken for DNA extraction, and the fish were identified as jack or non-mature. Jacks were distinguished from non-mature fish based on secondary sexual characteristics, gonad inspection and body mass (e.g., Heath et al. 2002). For this project, a total of 2-8 offspring from 11 families were used (determined by existing numbers of jack and non-mature males that could be paired within families). Within each family, I selected paired jacks and non-mature male siblings, where fish from each pair had similar weight, and sex of non-mature fish was identified using sex-specific PCR probes for Chinook salmon (Devlin et al. 1991).

MH genotyping

DNA was extracted from 40 jacks and 40 male non-maturing male siblings using a standard plate-based extraction method (Elphinstone et al. 2003). The peptide binding

region B1 of MH class II was PCR amplified with primers developed by Docker and Heath (2002), producing a 294 bp fragment. The PCR consisted of: 1μL of extracted DNA, 0.5μL of each primer (100ng/μL), 2.5 μL of 10x reaction buffer, 3.0μl of $MgCl_2$ (25mM), 0.2 U of Taq polymerase, and ddH_20 to make a 25μl reaction. Reactions were run in a PTC-200 Thermocycler (MJ Research) for 30 cycles consisting of denaturation at 95° for 30 sec, primer annealing at 52° for 30 sec, and extension at 72° for 1 min; the 30 cycles were proceeded by an initial denaturation at 95° for 2 min, and followed by a final 10 min extension at 72°. The PCR products were cloned into the pGEM (Promega) vector following the manufacturer's protocol. White colonies were selected, grown overnight in LB broth, vortexed, and boiled for 2 minutes. The insert was PCR amplified using the M13 forward primer and reverse primers. PCR products were purified using AMPure (Agencourt) purification system and then sequencing reactions were performed using the M13 forward primer along with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). After purification using CLEANseq (Agencourt), sequencing was performed using ABI BigDye Terminator version 3.1 on an ABI 3130xl sequencer.

Data Analysis

A saturation curve was employed to determine the minimum number of clones required to detect both alleles in individual fish. Plotting the number of new alleles detected by the number of clones sequenced, the number of sub-clones necessary to detect all alleles was determined by the point where the curve leveled off. On average, the second allele was discovered after sequencing the 4^{th} sub-clone in non-mature males, and the 3^{rd} sub-clone in jacks, thus, to score both alleles, a minimum of 5 sub-clones in

each fish were sequenced (maximum 13 sub-clones). MH class II B1 alleles were identified by aligning them with MH alleles reported by Docker and Heath (2002). Sequences were aligned with Geneious Pro 3.6.2 (Biomatters Limited) software.

Tools for population genetic analyses (TFPGA) 1.3 (Miller 1997) was used to calculate individual allele frequencies within the jack and the non-mature groups, and to compute genotypic frequencies. An exact test was done to test for allelic frequency differences between jack and non-mature males. A Fishers exact test was performed on a 2 X 2 contingency table to test whether or not phenotype (jack or non-mature male) significantly affected genotypic frequency distribution (heterozygous or homozygous). A contingency test was employed to test for significant differences in genotypic frequencies for selected genotypes between jacks and non-mature males.

3.3 Results

A total of 4 alleles were identified: 3 common alleles (OtsMHB1-1, OtsMHB1-3, OtsMHB1-4), and 1 rare allele (OtsMHB1-5) (Figure 1). The allelic frequency distributions were almost identical for jack and non-mature male siblings (Table 1), the only differences occurring due to 2 heterozygous jacks possessing the rare OtsMHB1-5 allele vs. only 1 heterozygous non-mature male with this allele. An exact test (TFPGA, Miller 1997) confirmed there were no significant difference in allelic frequencies between the jack and non-mature male groups ($X^2 = 5.657$, df = 4, P = 0.2267). Nonetheless, observed versus expected numbers of heterozygote genotypes differed between jacks and non-mature males, a contingency table test showed that the difference in heterozygote frequency between the two groups was statistically significant ($X^2 =$

4.219; df = 1, one-tailed P = 0.02), with jacks being heterozygous at the MH B I locus more often than their non-mature brothers. Examination of individual genotype frequencies between the two groups via Chi-square analysis revealed that the significantly higher heterozygosity in jacks was driven primarily by a single genotype: the OtsMHBI-1,4 heterozygote genotype differed significantly in frequency between the jacks and non-mature males, 19 jacks possessed this genotype compared to 10 non-mature males ($X^2 = 4.381$; df = 1, one-tailed P = 0.036, Figure 3). Interestingly, OtsMHBI-1,4 heterozygotes are polymorphic at 4 variable amino acid sites in the putative peptide binding region (Figure 2).

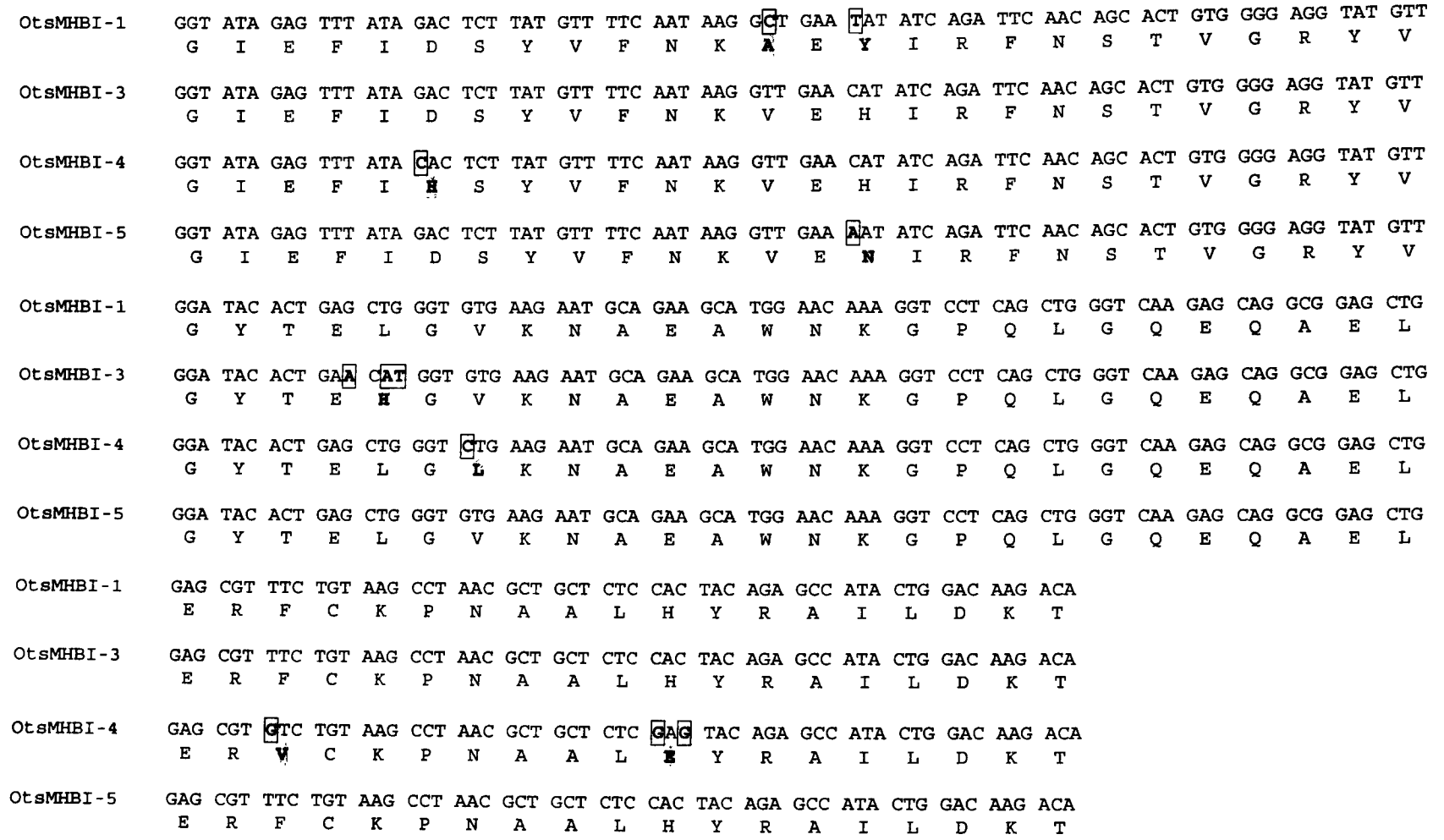

Figure 3.1 Combined DNA and Amino Acid Sequences of the Peptide Binding Region β1 of MH Class II in Four Detected Alleles from 80 Chinook Salmon (40 Jacks and 40 Non-Mature Males). 6/7 Variable Sites are Functional Changes

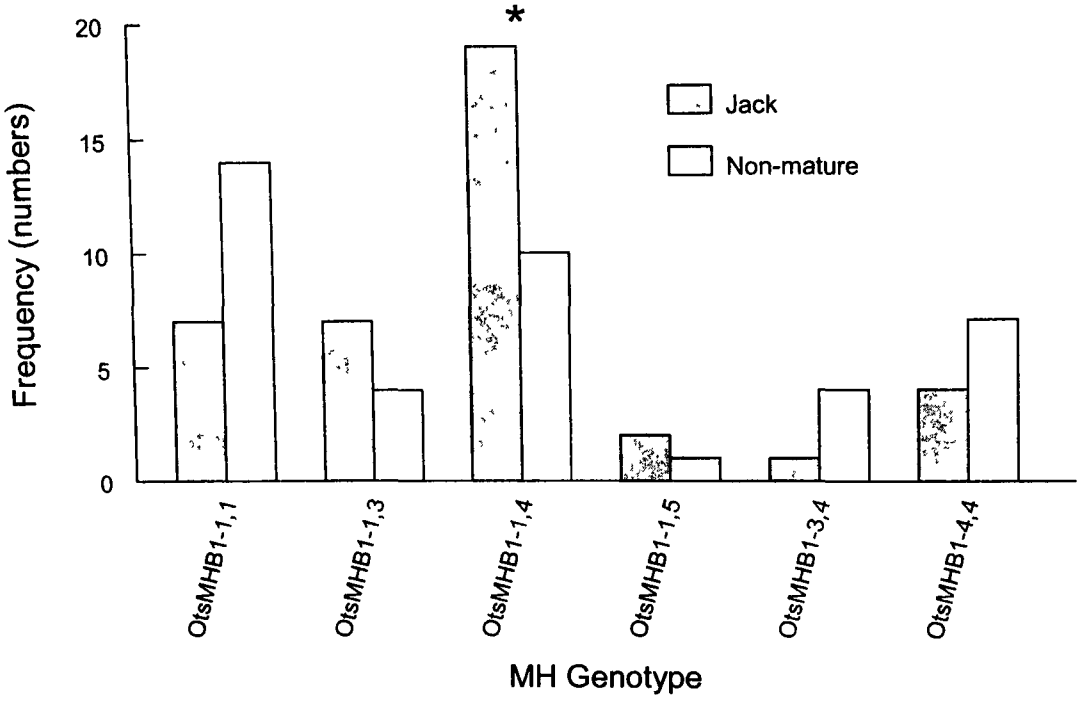

Figure 3.2: Genotypic frequencies for 40 jacks and 40 non-mature males. Only the OtsMHBI-1/OtsMHBI-4 genotype differs significantly in frequency between the two male groups. The asterisk denotes the significant frequency difference.

Table 3.1: Allelic frequencies for MH-BI Locus in Chinook salmon for 40 paired jack and non-mature male siblings.

Group	Alleles				Total
	OtsMHBI-1	OtsMHBI-3	OtsMHBI-4	OtsMHBI-5	n
Jacks	0.525	0.100	0.350	0.025	80
Non-mature	0.538	0.100	0.350	0.012	80

3.4 Discussion

The most salient result of this study was that heterozygosity at the MH class II B1 PBR differed significantly between jacks and non-mature males. Thus, in spite of the fact that each jack had a non-jack sibling counterpart in the study, i.e. common parentage (11 families), fish that were heterozygous at the MH class II B1 PBR were significantly more likely to develop into a jack. This indicates a relationship between heterozygosity at MH loci and likelihood of jacking. One mechanism for this relationship may be increased metabolic efficiency of MH heterozygous genotypes, particularly when exposed to pathogens. Resistant genotypes will have more energy available for growth and reproduction, hence, the observed correlations between growth, size, developmental rate, and precocious sexual maturation in Chinook salmon (e.g. Chapter 1; Heath et al 1997), may be driven by more MH diverse fish exhibiting lower pathogen load.

Correlational links between MH genes and resistance or susceptibility to major salmonid diseases have been reported in the literature (Langefors et al. 2001; Grimholt et

al. 2003; Arkush et al. 2002; Miller et al. 2004; Kjoglum 2006). A recent study by de Eyto et al. (2007) compared MH genotypic frequencies of Atlantic salmon surviving in a river six months after their introduction as eggs with frequencies expected from parental crosses. After comparing the changes in MH genotype frequencies to those at eight microsatellite loci they concluded that the observed selection at the MH locus was due to disease-mediated natural selection, rather than a demographic event (de Eyto et al. 2007). Another study also in Atlantic salmon found that offspring of wild naturally mating salmon were more MH dissimilar and exhibited a significantly lower parasite load (marine nematode) compared to artificially bred salmon (Consuegra and Leaniz 2008). In fact, offspring of artificially crossed salmon were almost four times as likely to be infected as free-mating salmon, irrespective of the similar levels of MH allelic diversity in both groups. Thus, the evidence that MH genotype affects fitness via pathogen-directed selection in salmonids is substantial and the importance of dissimilarity over diversity suggests an allelic interaction (a form of dominance) that may be driving function.

The fact that OtsMHBI-1,4 heterozygotes are polymorphic at 6 of 7 variable amino acid sites in the PBR means that they are also the most diverse genotype found in our study population. This may allow a wider spectrum of pathogens to be recognized to facilitate lower pathogen loads in these fish, allowing more resources to be directed to growth and reproductive efforts, and hence, a condition-dependent threshold for early maturation would be exceeded. Previous work has shown that jacks are the fastest growing fish in the salt water prior to maturation (Heath et al. 1996). Maybe the faster growth of the jacks reflects overall good or compatible genes (Neff and Pitcher 2005),

such as MH loci that served to reduce pathogen load and lower the threshold for jacking. A study by Balfry et al. (1997) showed that jack-sired families (with higher incidence of jacks) exhibited greater survival during a vibrio outbreak relative to non-jack-sired families, providing support for the idea that jacks may benefit from superior disease resistance. Thus, MH diversity may be contributing to life history variation in Chinook salmon via the strong selection pressure exerted by disease.

Studies have shown mate choice based on MH compliment occurs in brown trout (*Salmo trutta*, Forsberg et al. 2007), Atlantic salmon (Landry et al. 2001), and in Chinook salmon (Neff et al. 2008). Those studies all found that females chose their mates to increase heterozygosity of their offspring at the MH, and specifically, at the peptide-binding region. This general mating pattern is in agreement with the good genes as heterozygosity hypothesis that predicts, in relation to MH genes, mate choice should function to enhance offspring fitness by conferring the optimum immune defence (Brown 1997). If jacks generally exhibit more diverse MH genes, why do females not prefer to mate with the jacks? Several factors underlie what on the surface appears to be paradoxical. First, to our knowledge no direct test of Chinook salmon mate choice for jacks has ever been done, in the study by Neff et al. (2008) jacks were not included. Additionally, a study by Watters (2005) showed that in Coho salmon (*Oncorhynchus kisutch*) females performed more digging behaviour (a measure of mate choice) when accompanied by jacks. Female Coho salmon also appeared to favor mating with jacks to avoid the costs associated with mating with the larger more aggressive older males (Watters 2005). Second, females are choosing for offspring fitness, not the fitness of the fertilizing male *per se*, thus the choice of mate will depend on the female's own genotype

at the MH locus and how her chosen mate compliments her genotype. Published studies demonstrate female choice for allelic dissimilarity over simple diversity (Neff et al. 2008), and a heterozygous jack will not always provide maximum dissimilarity. Third, it is possible that limited opportunity for MH-dependent mate choice occurs when other reproductive mechanisms are active such as male-male competition for fertilizations (Bernatchez and Landry 2003). There is some evidence that when early arriving jacks are the only males on the spawning grounds, females will refrain from spawning until the larger later-maturing males arrive (Foote 1989; Berejikian et al. 2000). This female delay of reproduction may ensure high levels of competition among males that would increase the probability of successful fertilization of her eggs, in addition to selection for MH compatible genotypes.

Rapid unintentional evolution has been shown to occur in response to captive breeding and hatchery programs (Heath et al. 2003; Araki et al 2007; Blanchet et al. 2008). Captive breeding programs are particularly susceptible to erosion of genetic diversity (Wang et al. 2002), yet genetic diversity is important for population fitness, especially given environmental perturbation (Frankham 2008). Ironically, jacks have been excluded from hatchery supportive breeding programs due to concerns that they would increase the frequency of unwanted jacks in the supplemented population. Exclusion of jacks reduces effective population size (N_e; Waples 1990), and this is exacerbated by systematic selection of particular phenotypes (e.g. large fish) increasing the incidence of positive assortive mating, both of which contribute to loss of genetic diversity (Waples and Do 1994). Thus, selection against jacks in hatchery and captive breeding programs will not only reduce the effective population size, but also ultimately

reduce fitness in the population via removal of unique diversity at the immunologically important MH loci in jacks.

I found that Chinook salmon jacks had significantly higher levels of heterozygosity at the MH II B1 locus than their non-mature male siblings. This was due to one particular genotype OtsMHBI-1/OtsMHBI-4 occurring at a significantly higher frequency in the jacks. The higher levels of functional heterozygosity in the jack phenotype contradict the paradigm that jacks are less fit than older, later maturing males, and that they are merely "making the best of a bad situation" (Gross 1996). Theory predicts that local pathogen-driven selective forces will result in habitat- and climate-specific local adaptation in amino acid substitution patterns in the functionally important PBR (Sommer 2005; Heath et al. 2006). Thus, MH diversity in jacking Chinook salmon may be population–specific, and thus important for local adaptation, as well as future evolutionary potential.

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Chapter 4
Conclusions

4.1 Key Findings

The key results of this research on the incidence of jacking in Chinook salmon were:

1) Developmental acceleration during early incubation exerts a positive effect on jacking rates.
2) Mean family growth and/or size in the freshwater rearing period does not predict jacking rate.
3) Jacks are more likely to be heterozygous at MH class II loci versus older, larger males, and this is primarily driven by the otsMHB1-1,4 genotype.

4.2 General Discussion

The results of the PIT-tagging study showed that, body size, and growth rate in the freshwater life-history phase of Chinook salmon, does not directly affect the likelihood of jacking. Re-analysis of a previously published developmental acceleration study (Heath et al. 1994) further supported this finding, as the accelerated fish actually exhibited a consistently smaller mean mass at equivalent ATU relative to non-accelerated fish. At the same time, developmentally accelerated fish families had significantly higher jacking rates, demonstrating the profound effect of temperature accelerated development on precocious sexual maturation of male Chinook salmon. This contrasts with other studies that have concluded that early weight or growth rates are the primary factors contributing to subsequent salt-water precocious sexual maturation rates in salmon

(Bilton 1978, 1980; Lamont 1990; Shearer et al. 2002; Vollestad et al. 2004), but is in agreement with the conclusions in Heath et al. (1994).

The implications of temperature accelerated effects on life history traits in salmonids are particularly relevant to management and conservation efforts in light of the current predictions of continued global warming (Mote et al. 2003). Higher water temperature will mean higher metabolic rates for juvenile salmon, thus, increasing global temperatures are predicted to decrease size and age at maturity of Pacific salmonids (Mote et al. 2003). An understanding of the relationship between temperature, developmental acceleration, and subsequent effects on jacking rates, will enable managers and scientists to better predict changes in stock composition and adjust management strategies accordingly.

The results of MH genotyping study showed a positive relationship between diversity at the MH class II locus and likelihood of jacking. This is the first study to show a relationship between MH diversity and life history traits in Pacific salmon. Furthermore, the higher heterozygosity at MH in Chinook jacks was driven by one particular allelic combination that was the most diverse genotype occurring in the study population. This may allow a greater repertoire of pathogens to be recognized, facilitating lower pathogen loads in these fish and allowing more resources to be directed to growth and reproductive efforts, and hence, a condition-dependent threshold for early maturation to be exceeded. Pitcher and Neff (2007) provide evidence supporting a link between MH genotype and subsequent body size in Chinook salmon; they found that optimal MH genotypes could increase offspring body length by up to 3%.

Diversity at the MH genes is also increasingly important in a global warming regime as temperature also plays a role in determining the infectivity and virulence of pathogens (Griffiths 1991). In most pathogens, infectivity and virulence increase with temperature, as demonstrated for bacterial pathogens in fish (e.g. - Nordmo and Ramstad 1999; Larsen et al. 2004). Hence, maintenance of the inherent MH diversity of jacks within salmon populations should enable the population to respond more effectively to changes in pathogen diversity or virulence via a greater repertoire of PBR variants. The MH diversity in salmon is predicted to be driven by local pathogen compliment that is both habitat and climate specific (Sommer 2005; Heath et al. 2006). Thus, the MH diversity in jacking Chinook salmon may be population specific, and thus is important for local adaptation, as well as future evolutionary potential, particularly in context of climate change.

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Vita Auctoris

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