Relating the Sockeye Salmon (*Oncorhynchus nerka*) Spawning Migration Experience with Offspring Fitness: A Study of Intergenerational Effects.

by

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THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

in the Department
of
Biological Sciences

SIMON FRASER UNIVERSITY
March 2004

APPROVAL

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Degree: Master of Science

Title of Thesis:

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ABSTRACT

Adult Pacific salmon arrive at the Fraser River with a finite supply of energy to allocate between reproductive development and river migration, yet it is unknown if environmental conditions experienced by parents cause energetic trade-offs that ultimately affect offspring fitness. This thesis examined populations of Fraser River sockeye salmon (*Oncorhynchus nerka*) that differed in their migration distance (Weaver - 100 km, Gates - 363 km, and Early Stuart - 1086 km) to assess the hypothesis that migratory stress exerts an intergenerational effect on offspring fitness.

When compared among three year classes of Early Stuart sockeye salmon, metrics for ovarian development at six locations along the migration route revealed no evidence of facultative adjustments of either egg number or egg size en route. In contrast, significant interannual variation existed for final ovary mass, egg size and egg number over a 16-year period, with reductions in ovary mass and egg size associated with years of high river discharge rate during the migration. Selection against maternal phenotypes with a high ovarian investment strategy was postulated as a mechanism to reconcile both data sets.

Maternal and paternal gamete origin significantly influenced offspring survival. However, egg viability did not correlate with phenotypic variation in maternal energetic condition, osmoregulatory status, reproductive hormonal state, egg composition, stress, or moribund condition. Nevertheless, at the population level, migration severity may have

impacted overall egg quality because (a) the two populations that experienced more severe migration conditions in 1999 and 2000 had the lowest overall embryo survival (Gates = 77%; Early Stuart = 81%; Weaver = 94%; - artificial fertilizations), and (b) a poor maternal condition (using pre-spawn mortality as surrogate of poor condition and adverse migration conditions) was positively correlated with low egg to fry survival in Early Stuart sockeye salmon over a 15 year period.

Given that changes in egg size and the number of surviving offspring associated with parental influences are clear examples of intergenerational effects in sockeye salmon, the weight of evidence suggests that migratory stress associated with the parental spawning migration can contribute to an alteration in intergenerational gene flow and offspring size.

DEDICATION

I dedicate this work to my mum for believing in me and supporting me with unconditional love.

ACKNOWLEDGEMENTS

Funding for this study was provided by a grant from Forest Renewal British Columbia to J.S. Macdonald and the Fisheries and Oceans Canada (DFO) Stuart-Takla Fish Forestry Interaction Project, National Science and Engineering Research Council (NSERC) grant to M.C. Healey, S.G. Hinch, and A.P. Farrell, and the DFO Environmental Watch Program. Invaluable field assistance was provided by David Barnes, Janine Beckett, Kerry Mullen, Cher King, Jennifer Mackie, Brigit Jenson, Yale First Nations, D'Arcy First Nations, Pacific Salmon Commission staff, Cultus Lake Laboratory staff, and Middle River DFO Stock Assessment crew. Tissue composition and plasma analysis would not have been possible without Janice Oakes, Glenn Crossin, David Higgs, Ed Donaldson, and Jack Smith. A.P. Farrell, J.S. Macdonald, E.A. MacIsaac, and T.W. Williams willing supplied scientific expertise, advice, personal encouragement, and support for this thesis. I will be forever indebted to Herb Herunter and Ryan Galbraith who assisted in every way imaginable throughout this project. A heartfelt thank you to Leslie Learmonth for providing me with the latitude and the perspective to complete this study.

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INTRODUCTION

The non-feeding freshwater migration of adult Pacific salmon (*Oncorhynchus spp.*) for reproduction involves many remarkable feats. One such physiological feat is balancing the distribution of finite stored energy resources among the competing demands of migration, routine metabolism, and reproductive development. The consequences of these allocation decisions made by adults during their return migration and spawning may have dramatic implications to offspring fitness, i.e. an intergenerational effect. This thesis uses different populations of Fraser River sockeye salmon (*Oncorhynchus nerka*) to explore the intergenerational connection between migratory experience and offspring fitness.

Species and population specific adaptations have evolved to ensure migrational and reproductive success in salmonids. Nevertheless, not all salmon successfully complete their migration, in part due to variable and adverse environmental conditions either that exist in a given year, or that are emerging with changing climate regimes and increased land use activities (Bradford and Irvine 2000; Morrison et al. 2002). Adverse environmental conditions experienced during the spawning migration that result in mortality prior to spawning or offspring mortality will obviously affect intergenerational gene flow within a population, and this is the general topic of this thesis. In fact, understanding the connection between how adult salmonids adapt, acclimate, or respond to adverse conditions and their resultant reproductive success is paramount to predicting the full implications of freshwater environmental change on the future success of salmon.

This thesis will study the evidence, mechanisms, and implications of these intergenerational effects in salmon by examining aspects of the ecology and physiology of reproductive development during spawning migrations and the resultant offspring fitness.

Intergenerational Effects

Intergenerational effects have not been explicitly defined in the scientific literature. Therefore, I provide my own broad definition that includes both genetic and epigenetic intergenerational effects that can influence the next generation at both the individual and population level. Thus, an intergenerational effect is defined as any change in the fitness of an individual that resulted from their parents' phenotypic response to environmental conditions. This definition encompasses other terms such as crossgenerational or transgenerational (Heath and Blouw 1998; Lozano and Ydenberg 2002). Intergenerational effects can be adaptive, non-adaptive, or maladaptive, but making these distinctions is extremely difficult (Mousseau and Fox 1998).

Epigenetic intergenerational effects include those effects that result from environmental conditions experienced by the parent that affect the phenotypic traits expressed by the offspring independent of offspring genetic make-up. This broad category includes most types of maternal effects. Common examples of maternal effects considered adaptive include mate choice, nest site selection, egg provisioning and maternal care (Mousseau and Fox 1998). Genetic intergenerational effects arise when the parental environment affects offspring genotype, such as genotoxic effects (Hoffman 1991). However, this also includes the situation where environmental conditions select for or against certain phenotypes that have an associated genotype. Within a population the genetic effect will be to alter the proportion of a given genotype in the next generation. A change in gene frequency, as the result of differential reproductive success and the associated phenotypic variation are the foundations for natural selection to proceed. However, the study of

natural selection is more often concerned with direct environmental effects and phenotypic variation within a generation, rather than across generations.

At the individual level, an intergenerational effect is a change in offspring fitness resulting from an environmental influence that their parents experienced. For example, alterations in maternal growth patterns are associated with changes in egg size (Morita et al. 1999). If a change in egg size occurs in a high proportion of individuals, the intergenerational effect is felt at the population level. Similarly, if egg numbers are reduced, any density dependent responses in offspring fitness could shift population dynamics for the next generation (Benton et al. 2001).

Maternal effects are likely the most common form of intergenerational effects (Mousseau and Fox 1998). A maternal effect occurs when the mothers' phenotype, or her environmental experience, causes a phenotypic change in her offspring (Roff 1998). Maternal effects can have a genetic basis in those instances where the mothers' phenotype, such as size, is inherited. However, it is difficult to quantify the genetic basis for these traits and hence their adaptive significance. For example, while significant correlations between female body size and egg size exist (Einum and Fleming 2002), it has yet to be directly demonstrated that these maternal effects, in fish, are actually adaptive (Heath and Blouw 1998).

Salmonid Spawning Migration and Intergenerational Effects

Environmental factors can alter salmonid reproductive development (Donaldson 1990; Schreck et al. 2001). However, there is very little empirical data relating a particular parental phenotypic response to an environmental condition with offspring fitness. The ensuing general review, focussed on salmon, explores what is known about the intergenerational link between adult migratory stress and offspring fitness at three levels. At level one, I examine the background information on the migration, maintenance, and reproductive costs associated with freshwater adult migrations to provide a knowledge base for this thesis. I highlight those particular environmental factors, such as water temperature and discharge, which may impact migration ability. At the second level, reproductive development, I examine ovarian development and the changes in egg size and fecundity that may take place during the adult freshwater migration. At the third level, reproductive output, I describe current information available on relationships among egg size, number, quality and viability. For reproductive development and output, I emphasize knowledge gaps with respect to how migrational stress could create intergenerational effects. These knowledge gaps will help form the rationale for this thesis.

Adult Freshwater Migration and Energy Expenditures

Migration plays a central role in the salmonid life history. All salmonids are categorized as either non-anadromous (spends entire life in freshwater) or anadromous (migrations to and then from marine environment). Some of the necessary adaptations that anadromous

salmon employ to overcome the final freshwater migration phase to reproduce have been well documented (Groot and Margolis 1991; Groot et al. 1995; Høgåsen 1998). Beyond the issues of the switch from life in seawater and the switch from an anabolic to a catabolic physiological state, a critical problem facing salmon is to balance the energetic cost of migration with the on-going development cost of reproductive maturation, while maintaining homeostasis. Failure at either task would mean either no reproduction or compromised reproduction. A highly variable freshwater environment increases the potential for intergenerational effects to occur. Therefore, it is important to understand how these migration, maintenance, and reproduction costs in salmon change during the freshwater period and how these costs vary with environmental conditions.

Migration Costs

The cost of migration in salmonids, which varies in response to changing aboitic and biotic factors, can be inferred in several ways but not assessed directly. The simplest method is to estimate how body energy status changes during the migration (Gilhousen 1980). Body compositional analysis has been used to estimate gross energy expenditures associated with different stages of migration (Gilhousen 1980; Jonsson et al. 1997) and to partition energy allocation between migration and reproduction (Crossin et al. 2003; Patterson et al. 2004). For example, Crossin (2003) found that Fraser River sockeye salmon populations with more difficult migrations had higher total energy and % lipid reserves at the beginning of migration, expended more energy during the migration, but partitioned less overall energy into ovary development than short distance migrators. However, releasing individual salmon from the energy expense of the natural migration

does not result in an increased reallocation of energy to reproduction (Patterson et al. 2004).

More complex methods include measurements of oxygen consumption rates at different temperatures and swim speeds (Brett and Glass 1973) and modelling swimming behaviours during the migration with the associated energetic costs (Hinch and Rand 1998). The cost of swimming can be estimated in swim tunnels by measuring maximum prolonged swimming speed (critical swimming speed = U_{crit}) and maximum oxygen consumption ($MO_{2\ max}$) (Brett 1995). This involves periodically increasing water velocity and observing the maximum speed a fish can swim for a set period of time while continuously recording oxygen consumption. This work has demonstrated that swimming ability or U_{crit} values will increase with water temperature up to optimum value (~ 15 °C Brett and Glass 1973; Lee et al. 2003c). Post-exercise oxygen consumption can also be recorded to estimate delayed costs due to factors such as anaerobic contributions to swimming performance and disturbed ionic status (Lee et al. 2003b). These types of measurements can calibrate telemetry readings of the muscle contractions associated with tail beats (electromyograms or EMG), as salmon migrate through a river to estimate energy expenditures (Hinch and Rand 1998). The EMG studies have shown that salmon have gender and species specific differences in energetic swimming efficiencies. Factors that reduce swimming ability and/or increase the cost of swimming include advanced maturation state (Williams et al. 1986), poor fish health (Jain et al. 1998), sub-optimal swimming behaviours (Hinch et al. 2002), and genetics (Lee et al. 2003a).

Adult salmon appear to migrate at an optimal swim speed of 1 body length per second (bl • s^{-1}) in terms of cost of transport (Quinn 1988; Webb 1995), which is typically well below the maximum prolonged swimming speed or U_{crit} (e.g., 2.5 bl • s^{-1} for adult sockeye salmon at 15 °C; Brett and Glass 1973) for most of their migration (Hinch et al. 2002). The cost of transport (COT), or energy expended per kilometre of migration, is the sum of energy expended on locomotion and energy expended on standard metabolism as a function of swim speed (Webb 1995). COT has a U-shaped relation with swimming speed. Thus, while increasing swimming speed increases net COT (COT – routine metabolic rate or RMR), the overall COT is increased when migration is delayed. Of course, overall COT is what draws down the fish's energy stores. Clearly, environmental changes such as increases in river discharge, which might increase encountered water velocities, can increase the cost of transport if fish are forced to swim outside of their optimal U. In addition, any delay in travel incurs additional routine metabolic costs.

Maintenance Costs

Migration costs are inextricably linked to routine maintenance costs (RMR) and routine metabolic rates of salmon increase exponentially with water temperature (Brett 1995; Lee et al. 2003c). Thus, salmon migrating at a low water temperature will save energy due to a lower RMR but suffer a decrease in maximum swimming performance at temperatures below an optimum of approximately 15 °C (Brett and Glass 1973). This could result in delayed migration if water velocity was elevated. The on-going reproductive development and morphological remodelling will increase RMR costs for adult salmon

above the standard metabolic rate (SMR) (Hendry 1998), but there are no costs associated with digestion because fish ceased to feed. Nevertheless, the situation becomes more complex as adult fish begin to senesce. It appears that individual physiological systems may be progressively turned off. For example, upon arrival at the spawning grounds, osmotic homeostasis is only partially maintained, as measured by kidney histology (Robertson and Wexler 1957) and plasma osmolality (Fisheries and Oceans Canada or DFO, unpublished data). In fact, in anadromous Pacific salmon kidney degeneration is greater in semelparous sockeye salmon than iteroparous (repeat spawners) steelhead salmon (*O. mykiss)* (Clarke and Hirano 1995).

Reproduction Costs

On the surface, energy allocation to reproduction during the final freshwater phase is probably the easiest to quantify of the three major costs, at least for female salmon. Ovarian development in freshwater essentially involves the mobilizing and re-packaging of protein and lipids to the ovaries from other body tissues (Wiegand 1996). Ovarian mass and energy content can be measured at freshwater entry and compared with the levels at the spawning grounds to determine the gross energy changes with migration (Hendry and Berg 1999). This oversimplification fails to address the energetic efficiency of this process, or whether efficiency is affected by environmental conditions. Moreover, the cost of packaging is seldom discussed; it is assumed that making ten eggs weighing 10 g is energetically equivalent to making 100 eggs at 1 g. Furthermore, it would be unwise to continue to hold to the assumption that male gonadal development costs are negligible (Brett 1995) simply because there is no change in gonadal mass during their

freshwater migration (Idler and Clemens 1959). Clearly, the costs of ripening the testes cannot be ignored, even if they are small relative to maternal ovarian investment.

Reproductive Development

The following summarizes the information regarding ovarian reproductive development in female salmon. The focus is on those aspects of female reproductive physiology that may respond to environmental change and affect egg size and egg number. This information is then used to outline the potential connections between parental environmental experience with changes in final egg size, egg number, egg quality and ultimately offspring fitness.

Ovarian Development

Ovarian development in salmonids is classified as synchronous, as all eggs are recruited from a homogenous population of developing oocytes (Tyler and Sumpter 1996). The six major development events for oocytes are oogenesis, primary oocyte growth, cortical alveolis stage, vitellogenesis, maturation, and ovulation (Tyler and Sumpter 1996). The latter three events occur primarily after freshwater entry. Therefore, they are potentially susceptible to environmental conditions encountered during migration and are considered further.

The major portion of oocyte growth occurs during vitellogenesis for salmonids. Hormonal control of vitellogenesis involves the release of gonadotropins (GtH_I and GtH_{II}), homologous to follicle stimulating hormone (FSH) and lutinizing hormone (LH) in mammals, which stimulates steroidogenesis in the ovary. The steroid 17-β Estradiol (E_2) is released from the ovary and initiates the production of the major yolk precursor,

vitellogenin (Vtg), in the liver. Plasma Vtg, a glycolipophosphoprotein, is then taken up by oocytes. During maximum ovarian growth plasma Vtg can comprise up to half the total blood protein (Tyler et al. 1990). Therefore, Vtg represents the major means by which energy is moved from the body stores to salmon eggs. It is unknown whether such high concentrations of Vtg represent maximal rates of Vtg synthesis and/or uptake for salmon, or more importantly if these rates could be modulated in response to changes in migratory conditions. Thus, we know very little about the specific flux rates in relation to egg size.

The lack of specific information regarding the control of oocyte growth and development makes it difficult to determine a mechanism whereby vitellogenesis could be disrupted during a stressful freshwater migration. The type and duration of stress is important as acute stressors can increase plasma concentrations of gonadotropins in salmonids (Sumpter et al. 1987), whereas prolonged stress may cause gonadotropins to decrease (Zohar 1980). Stress can suppress plasma concentrations of E_2 in salmonids (Pickering et al. 1987; Macdonald 2000), which could affect Vtg production. Both temperature and Vtg concentrations in the surrounding media have been demonstrated to affect the rate of Vtg uptake by oocytes (Tyler and Sumpter 1996), which could then influence egg size. However, complete linkages between stress, hormone suppression, lower plasma Vtg concentrations, and oocyte growth are lacking at present (Schreck et al. 2001; King et al. 2003).

Oocytes develop at different rates for different stages of vitellogenesis (Tyler et al. 1996). Nevertheless, final egg size typically shows very little variance within each individual salmon (Berg et al. 2001). In contrast, within a population or species, individuals can show marked differences, illustrating important genetic and phenotypic differences on egg size. There are likely maximum constraints on egg size that are set during early, or mid-vitellogenesis. Most studies have demonstrated either no change in egg size or a reduction in egg size for any intervention work during vitellogenesis, especially in cases involving stress (Contreras-Sanchez et al. 1998; Schreck et al. 2001). An exception reported by Tyler et al. (1994) was an increase in egg diameter for some but not all rainbow trout unilaterally ovariectomized at mid-vitellogenesis. Campbell et al. (1992) found no appreciable change in egg size, but found a delay in maturation associated with repeated acute stress applied during early vitellogenesis. This implies that the mean rate of oocyte growth can change during early to mid-vitellogenesis. Confinement stress applied during late-vitellogenesis in sockeye salmon resulted in a delay in maturation, but no difference in ovulated egg size, hence a slower rate of yolk deposition was inferred from the data (Patterson et al. 2004). The degree of phenotypic plasticity in egg size during late stages of vitellogenesis is unknown in salmon.

The number of follicles recruited is normally set at the beginning of vitellogenesis (Tyler et al. 1990). This conclusion is based on experimental work with iteroparous rainbow trout (*O. mykiss*) unilaterally ovariectomized at different stages of ovarian development (Tyler et al. 1994; Tyler et al. 1996). Full compensatory ovarian hypertrophy was possible up to early-vitellogenesis and by mid-vitellogenesis (4 months prior to

ovulation) only partial (approximate 75%) compensation occurred. Compensation in rainbow trout occurred due to the existence of a second smaller pool of primary oocytes, which might not be present in semelparous Pacific salmon. Regardless, maximum fecundity and the commitment to vitellogenesis are likely set long before freshwater entry for most Pacific salmon; they enter freshwater in mid to late stages of vitellogenesis (DFO unpublished data). Therefore, any change in fecundity associated with the adult freshwater period would likely be a reduction and not an increase in recruited oocyte numbers. Recent experimental evidence supports this contention, as sockeye salmon, in late-vitellogenesis, intercepted at the beginning of a long migration and allowed to mature in a no flow environment did not increase egg number, despite a two-fold saving in energy expenditure (Patterson et al. 2004).

Reduction in egg number would involve follicle atresia. In mammals apoptosis, or programmed cell death, is the molecular mechanism responsible for atresia. However, a direct link between apoptosis and atresia in teleosts has not been found (Wood and Van Der Kraak 2001). In theory, any caloric value gained from re-absorbed egg material, associated with a reduction in egg number, could potentially be available for re-allocation to migration or maintenance costs. However, there are also no published data to suggest the number of ovulated eggs is reduced during the latter stages of maturation in association with freshwater conditions.

Reproductive Output

At the culmination of a successful freshwater migration and reproductive maturation female salmon release a set number of ovulated eggs of a given size and quality, termed reproductive output. Egg number, size, quality, and viability are likely under strong selective pressure as these attributes are the major non-genetic contribution a female salmon will confer to her offspring; to optimize maternal fitness (Einum and Fleming 2000a).

Egg Size versus Egg Number

It is presumed that egg size and number are an optimal combination based on evolutionary biology and life history theory (Smith and Fretwell 1974; Roff 1992), as well as physiology and anatomy. In general, such optimality models follow these rules:

a) a fixed level of ovarian investment requires a trade-off between egg size and number;

b) a positive relationship exists between egg size and survival;

c) maternal fitness is the product of average egg survival and number; and

d) consequently, optimal size and number are determined by the combination that maximizes maternal fitness.

At the population level, clear evidence exists for a trade off between egg size and number in a wide variety of fish (Elgar 1990), particularly salmon (Beacham and Murray 1993). For example, Fleming and Gross (1990) found a trade-off between egg number and size that changed with latitude in coho salmon (*O. kisutch*). To explain this finding, they proposed a local optimum for egg size, which decreased with latitude (in response to

environmental conditions associated with latitudinal temperature gradients) and was associated with increased fecundity. Beacham and Murray (1993), in their extensive review of all Pacific salmon, confirmed the trade-off between egg size and number, but disputed a clinal change in rearing temperature as being the proximate factor shaping egg size. Instead, they postulated selection for higher fecundity (and smaller eggs) to compensate for the higher juvenile mortality rates observed in the cooler northern latitudes. However, most work supports selection for an optimal egg size, rather than fecundity, in response to environmental conditions (Jonsson and Jonsson 1999; Hendry et al. 2001). For example, population differences in mean egg size among adult sockeye salmon have been correlated with the incubation environment (Quinn et al. 1995) and juvenile feeding opportunities (Linley 1993). Similarly, Atlantic salmon appear to increase egg size and correspondingly decrease fecundity in response to reduction in juvenile resource abundance (Jonsson et al. 1996), which suggests that variation in optimal egg sizes could be an adaptive phenotypically plastic response to anticipated changes in juvenile rearing environments. However, it is unclear how such a response would work mechanistically. It is not known whether an adaptive phenotypically plastic response occurs latter in life, which would allow adult Pacific salmon to alter egg size in response to migration conditions.

At the level of the individual fish, empirical support for some of the above rules is equivocal. Ovarian investment may not be fixed investment, but may be facultatively adjusted in response environmental conditions (Hutchings 1996; Schreck et al. 2001). Also, the relationship between egg size and survival changes as a function of the

environment (Hutchings 1991) and as function of the development stage of the individual (Hendry et al. 2001). In partial response to large natural variation in egg size and number within a population, these models have evolved to include maternal condition as a predictive factor that can influence this trade-off (Parker and Begon 1986).

Egg Quality and Egg Viability

An increase in egg size is often directly equated with an increase in egg quality (Einum and Fleming 2000a). While the benefits of increased egg size have been clearly demonstrated, the relationship is simplistic and ignores intrinsic egg factors that may contribute to a successful egg fertilization and embryo development (Brooks et al. 1997). For example, all eggs of a given size are not created equal. Comparisons between wild and captive salmon show that wild fish had a consistently higher fertilization and hatching success, even though egg size was similar (Brooks et al. 1997; Patterson et al. 2004). Such differences can only be attributed to egg quality and have been partially related to differences in the egg composition itself (Craik and Harvey 1984). In fact, the relative composition of both protein and lipid content can vary significantly among families (Herunter et al. 2000; Berg et al. 2001). Eggs are composed of many additional components and so the degree to which environmental influences can alter the overall egg quality remains unclear (Brooks et al. 1997).

An essential component of any assessment of egg quality is the ability of an egg to become fertilized. Successful egg fertilization requires a single sperm cell to penetrate through the egg micropyle and contact the egg plasma membrane (Yanagimachi et al.

1992). Preceding this event, a female salmon must dig a nest (redd), be involved in courtship, and synchronously release gametes with a selected male. Because the micropyle closes and sperm fertilizability declines within seconds after water contact (Hoysak and Liley 2001), gametes must be released in close proximity of each other. Despite the detailed information on the fertilization event itself, there is almost no information relating egg composition to egg survival at fertilization or any stage of embryonic development (Brooks et al. 1997; Ketola et al. 2000). Therefore, even if it is possible to document a change in egg constituents associated with parental migrational stress, at present we cannot relate a compositional change to offspring survival. Previous attempts have demonstrated that chronic and acute stress applied under controlled conditions to parents reduced gamete viability (Campbell et al. 1992, 1994), but they could not determine what specific egg composition factors reduced survival.

Study Approach

The above overview demonstrates a fertile ground to study the impact of parental migration experience on the fitness of subsequent offspring. Anadromous sockeye salmon, being both semelparous and capital breeders, are a useful model to study the intergenerational effects of the parental migrational experience on offspring fitness because reproductive energy expenditures associated with freshwater migration can be isolated. Furthermore, Fraser River sockeye salmon populations (termed Fraser sockeye) spawn in a wide variety of locations, have very different spawning migrations and can experience high interannual variability in freshwater environmental conditions within a specific stock (Burgner 1991; Quinn and Adams 1996; Macdonald 2000; Macdonald et al. 2000; Hodgson and Quinn 2002). In this thesis, I take advantage of the intraspecific and interannual variation in migration severity to examine the role of adult migratory stress on offspring fitness. The following section provides general information on Fraser sockeye with a particular focus on differences in spawning migration conditions for the populations that I used. In addition, I highlight three current stock assessment methods (en route loss, pre-spawn mortality, and egg to fry survival) used by fisheries managers to link migratory stress to offspring fitness. En route loss is defined as an estimate of fish mortality that occurs during the freshwater spawning migration, but not including the spawning grounds. En route loss is calculated by subtracting the number of fish estimated to have reached the natal spawning grounds from the number estimated to have entered the Fraser River after compensating for losses from in river fisheries (Macdonald 2000). The term pre-spawn mortality (PSM) is the percentage of egg retention for a population and is based solely on those females that successfully reached their natal

spawning area (Gilhousen 1990). Egg to fry survival is the estimated percentage of over winter survival based on the estimated number of eggs deposited and the total number of fry exiting the natal incubation stream the following spring (Bradford 1995).

Fraser River Sockeye Salmon: Populations and Spawning Migrations

The Fraser River produces the greatest abundance of salmonine fishes of any single river in the world (Northcote and Larkin 1989). Historically, returns of Fraser sockeye average over 10 million per annum (Cass 2003) and periodically exceed 20 million (Gilhousen 1992). Indigenous people along the Fraser River have relied on this abundant and predictable return of sockeye as a primary food source for centuries. The more recent exploitation of Fraser sockeye has been a mainstay of the large multi-million dollar commercial fishing industry in BC southern coast. Recent and dramatic declines in some populations of Fraser sockeye have raised the consciousness of the plight of salmon in the public arena and led to the listing of one population, Cultus sockeye, as endangered by COSEWIC (DFO 2003; Cooke et al. 2004). Thus, sockeye salmon have become an icon for the cultural, economic, and ecological vitality of the Fraser River basin.

Fraser sockeye are comprised of over 150 reproductively isolated spawning populations distributed throughout the Fraser River basin (stocks and populations will be considered synonymous throughout this thesis). Every year they begin entering the river in late June and continue to enter until late October. Spawning occurs from late July through November. Distinct spawning populations are aggregated into four distinct management groups based on the run timing of their historical entry into the Fraser River. See Table 1

for details on these four run timing groups and Figure 1 for their associated major spawning locations. Most Fraser sockeye spawn in streams and rivers associated with major lake systems. Eggs incubate over winter and fry emerge in the spring, when they migrate to their associated lake environment. Fry spend a full year rearing in the lake environment before embarking on their seaward migration the following spring as smolts. Sockeye then spend two years feeding and growing in the North Pacific before returning to the Fraser River. While this life history is applicable to over 90% of Fraser sockeye salmon, a large degree of life history variation exists among sockeye salmon populations (Burgner 1991). Alternate life history strategies will be identified in this thesis whenever they may have a bearing on the results.

All successfully spawning Fraser sockeye have faced a common experience, a freshwater upstream migration in the Fraser River. The physiological, behavioural, and ecological adaptations that different populations have evolved must deal with the wide variation and interaction of the physical and biotic factors at play and be specific to the portions of the Fraser River that each stock encounters. Migration distance is likely the most important factor shaping these adaptations because of the associated changes in freshwater migration time, freshwater migration dates, geography, climate, and water conditions with distance travelled upstream. Fish migrating longer distances will need more energy and are likely to encounter a wider range of temperatures and water velocities. Water temperatures range from 10 to 20 $^{\circ}$C in the main stem of the Fraser River and associated tributaries throughout the migration period from July to October for Fraser sockeye

(Macdonald 2000; et al. 2000; Figure 2a). Main stem Fraser River discharge rates vary 10-fold, declining from 11000 to 1000 $m^3 \cdot s^{-1}$ throughout this same period (Figure 2b).

All interior stocks are separated from coastal stocks by their migration through the hydraulic tempests of the lower Fraser Canyon, such as Hells Gate. Interior stocks tend to enter the system earlier and as such experience higher average water temperatures and discharges. Other more localized factors that sockeye salmon have to contend with include high elevation gains, glacial run-off, physical constrictions, periodic barriers (e.g., low flows; beaver dams), lake turnovers, and pollution. Minor and more recently major changes in the date of entry into the Fraser River (Cooke et al. 2004) will alter the probability of encountering specific river discharges or temperatures.

Water flow through the Fraser Canyon decreases substantially during the summer. This means that discharge rates experienced by each of the four run timing groups decreases considerably with date of entry (Figure 2b). In fact, the first group of sockeye salmon to enter the river, the Early Stuart sockeye salmon (termed Early Stuart sockeye), experience discharge rates in the lower river three times greater than the last run timing group, the Late run sockeye salmon (Late run sockeye). In terms of annual variability, the historical average discharge experienced by Late run sockeye (2200 $m^3 \cdot s^{-1}$) is less than twice the standard deviation (SD) of discharge (2900 $m^3 \cdot s^{-1}$) historically experienced by Early Stuart sockeye. Therefore, it is not surprising that maximum sustained swimming ability of Early Stuart sockeye is greater than Late run sockeye (Lee et al. 2003c). Historically, high discharge rates have negatively affected Fraser sockeye

migrations by increasing encountered water velocity beyond their sprint swimming capabilities, effectively halting migration. Documented cases where Fraser sockeye were held up by high water velocities and showed signs of exhaustion occurred in 1911, 1913, and 1997 (Thomspon 1945; Macdonald et al. 2000).

En route Loss

In certain years many individual sockeye fail in their attempt to successfully migrate through Fraser River (Cooper and Henry 1962) resulting in estimates of en route loss in some years to be in excess of a million fish (Macdonald et al. *In Revision*). However, rarely has there been physical evidence of a million or even thousands of dead fish in the Fraser River (Thompson 1945), the exception being the creation of an abnormal hydraulic barrier at Hells Gate in 1911 and 1913 when a rockslide, triggered by railroad construction, closed off part of the river. The lack of physical verification for en route losses is due in part to the fact that sockeye salmon are negatively buoyant in freshwater, and that carcasses may not resurface in the Fraser River due to low water temperatures (D. Patterson unpublished data). The most compelling argument for recurrent en route losses comes from the close association between adverse environmental river conditions (high flow and high temperature) and the high estimates of en route loss (Macdonald et al. *In Revision*).

Large en route losses of sockeye from a single spawning population will reduce the number of offspring in proportion to the % mortality and population size. In addition, the genotype and phenotype in the offspring will reflect parents that could successfully

negotiate migration challenges, such as higher temperature tolerance, better swimming behaviours, faster swimmers or better run timing. This assumes that part of this mortality is not random and that certain phenotypes will be selected against. Certainly individual variation exists among and within populations for both swimming ability and tolerance to high temperature (Servizi and Jensen 1977; Lee et al. 2003c). If these traits have genetic component, the genetic make-up of subsequent generations will have been affected by the particular set of environmental conditions their parents experienced during migration.

Pre-Spawn Mortality

The annual population mean for egg retention, i.e. = PSM, can range from 0% to 90% for Fraser sockeye (Gilhousen 1990). PSM has also been positively correlated with adverse environment conditions experienced by the adult Fraser sockeye in freshwater (Gilhousen 1990). Fisheries managers currently incorporate the PSM estimates into future return forecasts by adjusting the egg deposition numbers (Cass 2003). However, it is not known whether these successfully deposited eggs have an equal chance of survival independent of parental experience.

Egg to Fry Survival

There is a paucity of information on egg to fry survival estimates for most Fraser sockeye populations (Bradford 1995). The limited information comes mainly from spawning channel operations with controlled incubation environments. Annual over winter survival estimates from Fraser River spawning channels have ranged from 10% to 90%

(DFO unpublished data). These survival estimates are considerably higher than the long term average of 20% for Early Stuart sockeye (DFO unpublished data). Although, interannual variation in egg to fry survival has been documented, an intergenerational connection between adverse adult migration experiences and low egg to fry survival has not been made. This type of intergenerational effect on offspring survival is poorly understood and even less is known about the non-lethal effects of maternal stress during migration may have on performance of surviving fry.

Thesis Objectives

Fraser River sockeye salmon stocks are a suitable model for testing the intergenerational effect of migratory stress on offspring fitness in part because variations in en route loss and PSM show that clear correlations exist between adverse parental migration experiences and future reductions in population recruitment. However, there is almost no information on the mechanisms that may connect adult migratory stress to a specific intergenerational effect. In fact, the legacy of the parents' migration is seldom explored beyond the reduction in the total number of egg deposited for a population. Intergenerational effects emphasise a broader recognition from fisheries managers and scientists that a salmon's fitness is not just dependent on the environment it currently occupies but also past experiences of their parents, especially the parental spawning migration experience. Therefore, the objectives of this thesis are to elucidate this intergenerational connection in certain Fraser sockeye populations at three levels. The central questions and approach taken in each chapter are as follows:

Chapter 1: Does migratory stress affect reproductive development?

- a) document interannual variation in egg development during migration
- b) relate interannual migration severity to reproductive investment (ovary mass, egg size, & fecundity).

Chapter 2: Does migratory stress affect gamete viability?

- a) determine if embryo survival is related to parental origin of gametes.
- b) relate population embryo survival with degree of migration severity.

Chapter 3: Does migratory stress affect offspring fitness?

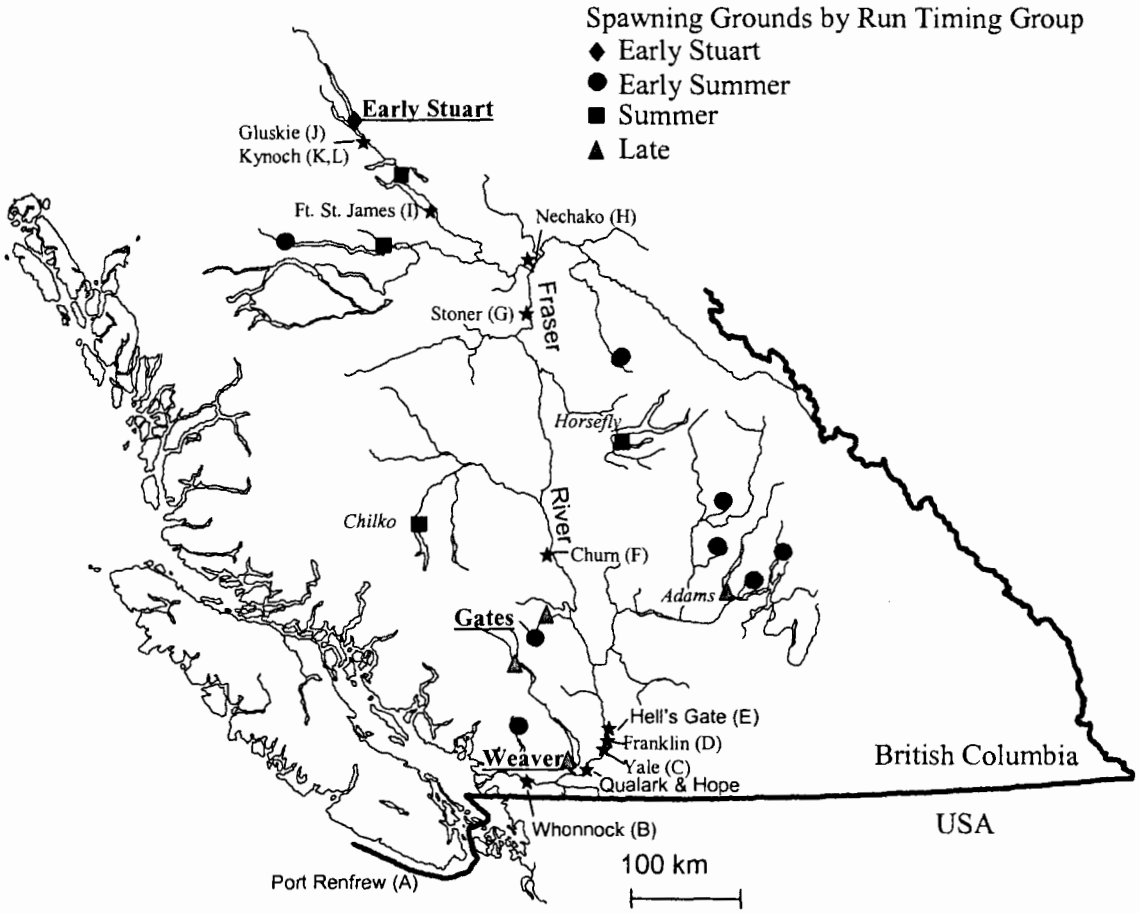
- a) relate maternal condition (as an indication of migratory stress) and egg composition to offspring survival
- b) compare interannual egg to fry survival using PSM as a surrogate of migratory stress among three populations.

The different approaches within each chapter will focus on (a) individual, and (b) population level intergenerational effects.

Study Populations

To complete my objectives, three Fraser sockeye populations were examined from three different run timing groups (Figure 1). These populations are termed Early Stuart sockeye (the Early Stuart timing group), Gates sockeye (an Early Summer run timing group), and Weaver sockeye (a Late run timing group). Early Stuart sockeye spawn in over 40 tributaries in the Stuart-Takla drainage. In this study, only two creeks were sampled terminally, Gluskie Creek and Kynoch Creek. Gates sockeye spawn in both Gates Creek and Gates Creek spawning channel. Weaver sockeye spawn in both Weaver Creek and the Weaver Creek spawning channel.

Figure 1: Fraser River watershed with en route sample collection locations (letters – match with Table 2), the locations of major spawning populations associated with each of the four run timing groups, location of Early Stuart, Weaver, and Gates spawning populations (all bolded) used is this study, and location of temperature and discharge collection site (Qualark & Hope) in relation to Hells Gate.



These three stocks represent a broad range of possible migration difficulties among Fraser sockeye populations. The interior Early Stuart sockeye population have arguably the most difficulty migration of any sockeye salmon. They must navigate through the Fraser Canyon, traverse 1086 km and gain an elevation of 670 m. Gates sockeye must also pass through the lower Fraser Canyon but only travel a third of the distance and less than half the elevation (363 km and 250 m). The coastal Weaver sockeye migrate 100 km, gain 10 m in elevation, and do not enter the Fraser Canyon. The three populations

also encounter very different temperature and discharge patterns (see Figure 2) because of their different river entry time (i.e., run timing). As a result Early Stuart sockeye typically face the highest annual river discharges, Gates sockeye swim through the warmer river temperatures and Weaver sockeye experience low discharge levels and moderate temperatures (Table 1). Furthermore, all are small stream spawners and populations are non-threatened with annual run sizes in excess of 65000 (Cass 2003). Because annual adult enumeration programs have been performed by DFO, there are historical data available for en route loss, PSM, and egg to fry survival. In addition, there has been considerable supplemental information for these stock regarding interspecific differences in swimming ability (Lee et al. 2003c; Hinch et al. 2002), spawning behaviour (Healey et al. 2002), and gross energy utilization (Crossin 2003). Table 1 summarizes some the differences and similarities among the three run timing groups for the respective populations, and Figures 1 and 2 act as references for migration conditions.

Figure 2: (a) The 25-year average Fraser River (a) daily water temperatures (solid circles; ± 1 SD dashed lines) at Hells Gates. The range of median and average (spikes) passage date past Hells Gate for all run timing groups is shown in the inset between graphs. (b) The 90-year average (solid line) daily discharge for the lower Fraser Canyon, measured at Hope. The daily discharge patterns for the three years used in this study are also given, 1999 (diamonds), 2000 (circles), and 2001 (squares).

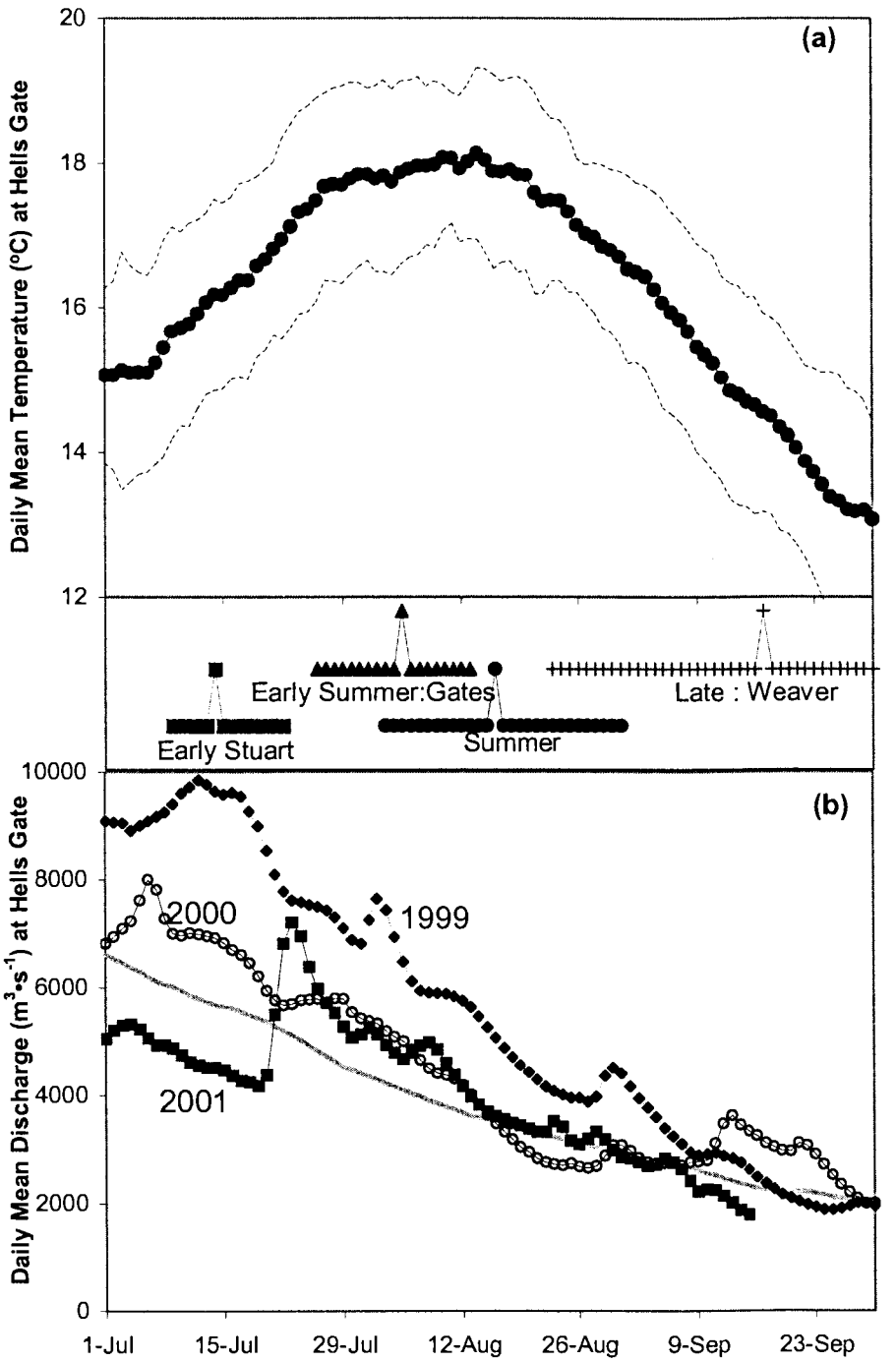


Table 1: Summary information for the mean migrational conditions experienced by Fraser sockeye salmon based on the four major run timing groups; Early Stuart, Early Summer, Summer, and Late run. All data including rankings are based on 25-year averages.

Major Run Timing Group	Year	Median River Entry Date	Mean 19 Day Temperature °C	Mean 19 Day Discharge cms	25 Year Ranking of Discharge	Pre-Spawn Mortality %
Early Stuart	Average	12-Jul	16.1	5369		13%
	1999	12-Jul	13.7	9134	1st	16%
	2000	7-Jul	14.6	7051	5th	11%
	2001	7-Jul	16.4	4803		
Early Summer (Gates)	Average	3-Aug	17.7	4043		12%
	1999	3-Aug	16.4	6512	1st	5%
	2000	27-Jul	17.3	5484	3rd	30%
Summer	Average	15-Aug	17.6	3402		
Lates (Weaver)	Average	13-Sep	15.1	2232		8%
	1999	3-Sep	15.3	3502	1st	25%
	2000	18-Aug	17.4	3193	3rd	13%

CHAPTER 1: INTERANNUAL VARIATION IN EGG DEVELOPMENT AND REPRODUCTIVE INVESTMENT IN EARLY STUART SOCKEYE SALMON

Abstract

Interannual variation in ovarian investment and the associated variation in egg size and fecundity of a long distance migrating population of Fraser sockeye (1100 km) provides clear evidence for an intergenerational effect passed from parent to offspring that directly affects both the individual offspring size and potentially the future recruitment of the population. By comparing interannual differences in migration severity with reproductive investment, I showed that smaller egg size and ovary mass were associated with years of high discharge and low temperatures in the Fraser River during their migration period. In contrast, detailed egg measurements along the migration route during two difficult migration years (1999 and 2000) and a benign migration year (2001), as well as constant plasma vitellogenin levels throughout migration in 2000, suggest a fixed rate of increase in egg size for the threefold increase in egg mass that occurs from river entry to spawning. This occurs despite that fact that stress levels were high and reproductive hormone levels suppressed at certain locations with difficult passage. Furthermore, fecundity was unchanged during the freshwater migration. Therefore, the facultative in-river adjustments of egg size and/or fecundity in response to environmental conditions in the river were not supported by this research. Therefore, the hypothesis that final egg size and fecundity are set prior to freshwater entry cannot be rejected. Instead, the interannual differences in reproductive investment are likely a reflection of direct selection against certain phenotypes in the river (i.e. in river mortality). However, direct selection cannot be assessed by sampling only 'survivors', the Achilles heel for this kind of study.

Introduction

The evolution of energy allocation strategies among different physiological processes should support those strategies that maximize fitness. Semelparous Pacific salmon have a single opportunity to optimize the allocation of energy between reproduction and adult migration. A trade-off between ovarian development and energy for swimming may occur if the energy requirements of migration itself change during the limited energy budget period (Kinnison et al. 2001).

The ovary mass of some Fraser River sockeye salmon triples during their upstream non-feeding migration (Gilhousen 1980). In competition with reproductive development for the finite supply of energy during their final phase are the energy demands of migration and routine maintenance. Migration and maintenance costs vary on an annual basis depending on the environmental conditions the fish encounter in the river. If these were correlated with interannual variations in either egg size or fecundity, they would constitute an intergenerational effect in response to the maternal reallocation of energy resulting from the migrational experience. This chapter looks for evidence of such intergenerational effects by correlating interannual variations in ovary mass, egg size, and fecundity with migration severity and by documenting the physiological and morphological changes in ovarian development throughout the freshwater migration experience.

Energy allocation to ovary development and migration is governed by physiological controls (Weigand 1996). The extent to which two these systems are interrelated during

spawning migrations is unknown. Theoretically, adaptive physiological mechanisms could link the two systems and their respective energy allocations during spawning migrations (Kinnison et al. 2001). For example, release of stress hormones, associated with extreme migration conditions (Macdonald et al. 2000), could interfere with production of reproductive hormones necessary for optimal egg development (Pottinger and Pickering 1990; Kubokawa et al. 1999; Schreck et al. 2001). Similarly, excessive blood flow requirement by locomotory muscles during upstream migration (Thorarensen et al. 1993) could divert blood flow away from the ovary, reducing nutrient depositional rates to the eggs and impacting egg size. A more extreme possibility is egg reabsorption, or atresia, which has been documented in rainbow trout during the late phase of maturation (Donaldson 1990), potentially providing additional energy stores for migration at a cost to egg number.

Potential trade-offs in reproduction versus migration within an individual fish are predicated on the assumption that either egg size and/or number can be facultatively adjusted during upstream migration. Currently, no evidence exists to suggest this mechanism is available for Pacific salmon during their final freshwater migration. Instead, the vast literature on Pacific salmon reproduction has focussed on the trade-off within reproductive energy supply between egg size and number (see Introduction) at the population level. Most of these papers have mentioned the existence of a large variation in egg size and number, but they still present the results based on optimum value, obfuscating individual variation. At the population level, explanations for the interannual variation in egg size and number include changes in ocean productivity (Healey 1986),

age class structure (Healey 1986), migration distance (Kinnison et al. 2001), and early juvenile growth history (Hutchings 1991). There have been no previous studies relating interannual variation in migrational conditions with interannual variation in reproductive investment, which is the purpose of Chapter 1.

Early Stuart sockeye salmon arguable face the most difficult freshwater migrational conditions of any sockeye salmon population in the world during their upstream passage. Their annual ascent each July takes place when water conditions for both discharge and temperature in the Fraser River are at there most variable both within and among years (Figure 2; Morrison et al. 2002). Discharge values in the main stem range from 11000 $m^{-3} \cdot s^{-1}$ to 3000 $m^{-3} \cdot s^{-1}$ and temperature values range from 10 to 20 °C during this month (Figure 2). Discharge rates > 7000 $m^{-3} \cdot s^{-1}$ have been known to slow migration and while those > 9000 $m^{-3} \cdot s^{-1}$ can act as a hydraulic barrier and stop migration (Macdonald 2000). Similarly, water temperatures above 18 °C have increased both en route loss and PSM estimates (Gilhousen 1990; Macdonald et al. *In Revision*). Therefore, Early Stuart female sockeye salmon are a particularly good model when it comes to interannual variation in parental migration experience.

The broad objective of this chapter is to determine if migratory stress affects reproductive development in female sockeye salmon. Specifically, two approaches were taken. The first approach examine the evidence on whether migrating salmon can facultatively adjust the reallocation of a finite energy supply in response to environmental conditions experienced en route. To do this ovarian development (ovary constituents, egg size, and

fecundity) and associated physiology changes in Early Stuart sockeye were documented throughout the freshwater migration for 1999, 2000, and 2001. The second approach tested the null hypothesis that there is no interannual variation (1977-2002) in final ovary mass, egg mass, and fecundity. However, if interannual variation did exist I predicted it would be related to the severity of the migratory conditions experienced, as measured by Fraser River discharge and temperature data. The implications of both these approaches on population and individual fitness are discussed.

Materials & Methods

Reproductive Development En Route

Early Stuart female sockeye salmon were collected at different locations along their spawning migration route in 1999, 2000, and 2001 and sampled for body size, ovaries, and blood plasma (Figure 1). Catch information is summarized in Table 2. Every attempt was made to sample the peak portion of the Early Stuart run based on historical timing information and in season lower river escapement estimates (Pacific Salmon Commission; i.e., PSC). Scale analysis, run time information and sample location were used for racial identification (Mike Lapointe, PSC).

Body Size

The standard length (SL), distance from tip of snout to hypural plate, was used to record length. The wet mass (M) of the whole fish (including ovaries) was measured fresh. The ovaries were removed and weighed fresh. The gonadal somatic index (I_G) was calculated by dividing the ovarian mass (G) by the total body mass (includes ovaries). One ovary, or skein, was chosen at random and kept on ice for detailed egg measurements and the remaining ovary was kept frozen at -20 °C for proximate analysis.

Table 2: Catch summary information for Early Stuart females used for blood and ovary analysis, organized by year, sample location (letters match with Figure 1), gear type, sample date, and sample size.

			1999		2000		2001	
Sample Location	Distance from Mouth of Fraser (km)	Gear type	Dates	Sample Sizes	Dates	Sample Sizes	Dates	Sample Sizes
Port Renfrew (A)	-250	Purse Seine	July 9	10	June 28	10	June 29	12
Whonnock (B)	50	Gill Net - Boat	July 9-11	10	July 4	10	July 5-6	22
Yale (C)	170	Angled	July 20	8	July 6	12	July 10	4
Franklin Rock (D)	175	Dip net	July 15-16	11				
Hells Gate (E)	200	Dip net	July 22	10	July 10	10	July 13-14	10
Churn Creek (F)	440	Dip net	July 30	9	July 16-17	10	July 18	11
Stoner (G)	693	Gill Net - Hand			July 27	5	July 25	8
Nechako (H)	773	Gill Net - Hand	Aug 6-7	10	July 26	5		
Ft. St. James (I)	967	Gill Net - Hand	Aug 9-10	9				
Gluskie Arrival (J)	1086	Fence dip net			Aug 5	10	July 30	10
Kynoch Arrival (K)	1076	Fence dip net	Aug 12	10				
Kynoch Spawner (L)	1076	Dip net	Aug 14-15	16	Aug 4-8	39		

Ovary Analysis

The skein retained for egg measurements was weighed to the nearest 0.1 g. Measurements were made on 3 sets of 10 fresh eggs taken at random from the skein. Egg diameter was measured by lining up 10 eggs in a row, just touching, and recording total length with callipers to the nearest 0.05 mm (measurement error is ~ 1 % of individual egg diameter). These eggs were then placed on pre-weighed 43 mm aluminium weigh boats and wet mass was recorded to the nearest 0.0001 g (measurement error is ~ 0.5 % of individual egg mass). The eggs were then dried (48 hours at 60 ^{o}C) to determine egg dry mass (E). Fecundity (F) was estimated by dividing the average egg wet mass based

on the three samples by the total ovary mass. This method likely overestimates the fecundity of the samples because ovary mass includes both eggs and non-egg portions of the ovary, but this would be a systematic error among the three years. In 2001, egg wet mass values were estimated by single wet mass value of 10 eggs. Egg dry mass and egg diameter were measured in 2001.

Ovary constituent analysis was used to assess changes in relative composition by sample location and year. A skein for each female was thawed at 4° C before being completely homogenized in a food processor. Duplicate homogenates from each fish were analysed for % moisture, % dry matter, % ash, % protein, and % lipid content according to the procedures of Higgs et al. (2000). Caloric equivalents were used to convert from % composition of lipid (36.4 kJ • g^{-1}) and protein (23.6 kJ • g^{-1}) to energy density (MJ • kg^{-1}) (Higgs et al., 1995). The homogenates from spawning fish did not include the supporting ovarian integument, but consisted solely of loose eggs.

Plasma Analysis

Blood plasma analysis can provide an integrated measure of the physiological state of individual fish, with respect to acute stress and gametogenesis at the time of sampling (Donaldson 1990; Pickering 1992). Blood samples were taken within five minutes of capture from the caudal vein using a needle attached to heparinized vacutainer. The plasma was partitioned using a refrigerated centrifuge and stored at –80° C. Plasma

samples were subsequently analysed for cortisol, 17-β estradiol (E_2), and vitellogenin (Vtg) according to the methods described by Donaldson et al. (2000).

Interannual Variation in Ovarian Investment

Historical Data

Historical information on ovary mass, egg size, fecundity, and fish size for Early Stuart sockeye were provided by Stock Assessment Division (STAD) of DFO (Keri Benner, DFO-Kamloops). Within each year approximately 25 gravid females sockeye were collected from each of three different Early Stuart spawning streams, Gluskie, Forfar, and Kynoch during peak arrival (Schubert and Fanos 1997). The standard length was recorded and scales and otoliths were removed for age classification. Both tight skeins were removed and fixed in 10% formalin. Skeins were then thoroughly rinsed in freshwater, air dried and weighed. An approximately one third sub-sample of the total ovarian mass is removed and weighed. A complete egg count was made on the sub-sample. Total fecundity was estimated by dividing the total ovary mass by sub-sample mass and multiplying by number of eggs counted in the sub-sample. A full count of all the eggs from both skeins was done on approximately 1 in 5 females. The absolute discrepancy between full counts and calculated counts is 1 egg (i.e. no directional bias), and standard deviation for the discrepancy is 64 eggs (i.e. < 2% of average fecundity). A sub-sample of 10 eggs from 10 females from each creek are removed and dried at 60 °C for 48 hours to estimate individual egg dry mass. Individual egg dry mass, ovary mass, and standard length measurements for 1985 and 1987 were taken from Linley (1993).

Egg dry weights taken from Linley (1993) were taken from spawning or spawned out females and as such they are not identical in development stage to of those taken by STAD (tight females). To account for the continue growth in egg mass at the spawning ground, 6% reduction in egg mass was subtracted from all ripe egg values used. This was based on the regression equation between egg size from tight arrival females (STAD samples) and spawning fish collected in 1999 and 2000, after correcting for length.

Annual Migration Conditions:

Daily discharge data for the Fraser River at Hope was provided by Water Survey of Canada. Fraser River water temperatures at Hope were provided by Dave Barnes (DFO-Cultus Lake). Daily discharge value at Hope, for 1999, 2000, and 2001, for the duration of the Early Stuart migration are shown in Figure 2b.

Statistical Analysis

All tests were deemed statistically significant at an alpha value < 0.05.

Egg Development:

The pair wise correlation coefficients for egg dry mass, egg wet mass, and egg diameter for individual females were all above 0.9. Therefore, to avoid the confounding effect of multicollinearity, a single variable of egg size was used. Egg dry mass was chosen as the single variable to test egg development with location and year, because of the following: lowest variance of the three variables; egg dry mass also represents the gross amount of energy deposited in the yolk and is therefore a better reflection of the energetic cost to the

female (Berg et al. 2001); and egg dry mass is not subject to changes caused by formalin fixation and preservation (Fleming and Ng 1987). However, this necessitated transforming the 2001 data from wet mass to dry mass using average egg moisture content for each site and location based on 1999 and 2000 data. Egg moisture content did not differ between years (ANOVA $P>0.05$).

Ovary mass, fecundity, and egg dry mass were standardized to a common fish length using the standardization equation derived from the historical data (see historical data statistics). This assumes there was no change in these allometric relationships during freshwater migration. Interannual variations in gonad mass, fecundity, and dry egg mass, by distance and time in freshwater were tested using an analysis of variance model (GLM) with location nested within year (Minitab Ver. 13). Time in freshwater is reported as the number of days past the initial sample date at Whonnock for each respective year (Table 2). To increase sample sizes Yale, Franklin Rock and Hells Gate samples were pooled, as were Stoner and Nechako samples. Egg size and ovary mass growth rates were calculated using the slope from the linear regression of each variable against both distance from the Fraser River mouth and time in freshwater (Whonnock date equal day 0). The slopes for year specific equations were tested to determine if growth rates were similar among years (test equal slopes). Power analysis was used to determine the probability of detecting a significant difference for some of the comparisons made (Minitab Vers. 13).

Ovary Constituents

Changes in ovary protein and lipid content with location were tested using a GLM for both 1999 and 2000. The overall influence of year and location, on the response variables, ovary moisture, protein, and ovary lipid, for ovary constituents were examined using a nested MANOVA design (Sokal and Rohlf 2000; Minitab Vers. 13). Location was nested within year to look for interannual changes by site. If an overall treatment effect was established, a single ANOVA was used to test the treatment effect on an individual response variable. In those cases involving a significant treatment effect for an individual response variable, a Tukey-Kramer multiple means test (i.e., Tukey) was applied (Minitab Ver. 13).

Plasma analysis

The overall change in plasma variables, cortisol, E_2, and Vtg, with sample location was tested using a MANOVA. Site specific differences for each plasma variable were then determined using a single ANOVA and a Tukey test. To satisfy the statistical requirements of normality and homoscedasticity, the plasma data were log-transformed when necessary. To examine the relationship between cortisol and estradiol, and between estradiol and Vtg within individuals, correlation coefficients were calculated for each comparison at two locations Whonnock and Hells Gate.

Historical Data

To compare ovary mass (G), fecundity (F) and egg size (E) among the different years, each variable was standardized to common standard length (SL_s) (McGurk 2000). The majority of Early Stuart sockeye return as 4 year olds, however, a small but variable portion (typically 10% but can reach up 50% annually) return as 5 year olds to the three collection streams. Therefore, prior to standardization for length, the assumption that creek and age at maturity had no significant influence on the ovary mass versus length relationship was tested. There was no difference in the relationship between SL and G based on age (ANCOVA $P > 0.05$) or creek (ANCOVA $P > 0.05$) using log transformed values. Ovary mass, fecundity and egg size all have an allometric relationship with fish length (i.e $\boldsymbol{b} \neq 1$, whereby $y=ax^{\boldsymbol{b}}$). This was confirmed by calculating $\boldsymbol{b}$ for each variable (G,F,E) using a log transformed linear model (e.g., $\log(G) = \log(a) + \boldsymbol{b}\log(SL)$). All log(G) values were plotted against log(SL) for each year to confirm that the slopes were similar among years. The logarithmic transformation is preferred to maintain equal variance over the full range of values. Standardized values for G, F, and E were calculated using the following equations respectively:

$$G_s = G_o(SL_s \bullet SL_o^{-1})^b$$

$$F_s = F_o(SL_s \bullet SL_o^{-1})^b$$

$$E_s = E_o(SL_s \bullet SL_o^{-1})^b$$

For example, where G_s = length standardize ovary mass, SL_s = average length for all sockeye salmon tested, SL_o = observed standard length, G_o = observed ovary mass, and $\boldsymbol{b}$ ovary specific scaling coefficient. Using length standardized values, interannual variation in overall reproductive output, ovary mass, fecundity, and egg size was assessed

using an MANOVA. Changes in individual response variables among years were assessed using a single ANOVA.

Migration Conditions

Annual migration conditions values were calculated based on a 19-day average discharge (Q_{19}) and a 19-day average temperature (T_{19}) experienced by Early Stuart sockeye in the Fraser Canyon. The date centred on the annual median migration date for Early Stuart sockeye passing Hells Gate for each given year (I. Guthrie, PSC, personal communication). The choice for using these particular predictor variables was based on previous work that found significant correlation between these variables and en route loss for Early Stuart sockeye (Macdonald et al. *In Revision*). Predictor variable choice and selection methods are clearly outlined in Macdonald et al. (*In Revision*). Six single linear regressions were made between the three response variables (G_s, F_s, and E_s) and the two predictor variables (Q_{19} and T_{19}). The two predictor variables were highly correlated (R^2 = -0.89) as were two of the response variables (G_s and E_s; R^2 = 0.69). Therefore, sequential Bonferroni corrections were made to control for the type I error rate for the number of regressions equations tested using non-independent predictors and response variables (Sokal and Rohlf 2000).

Results

Reproductive Development.

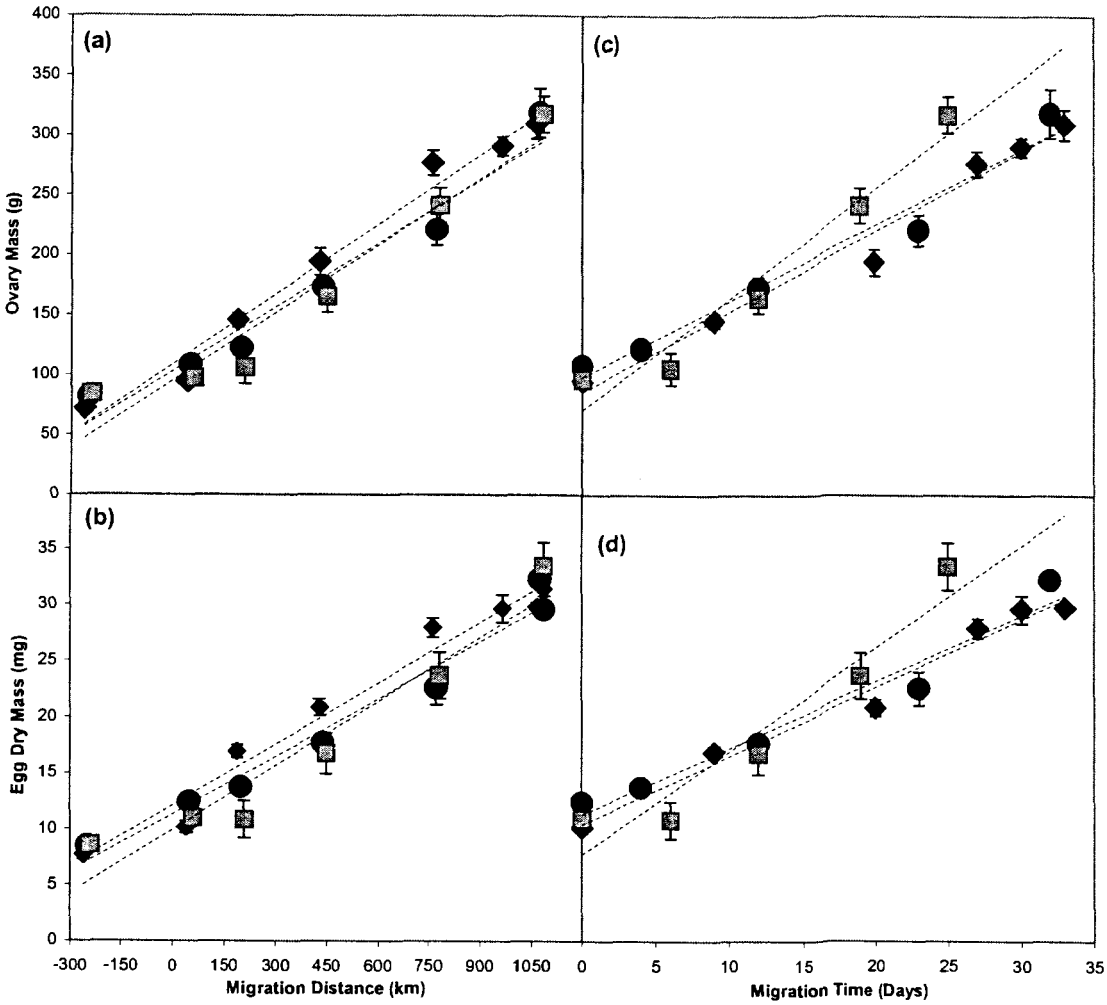
Egg Size and Egg Number

There was no difference in fecundity either by location or by year for Early Stuart sockeye salmon (GLM P>0.05). However, the average fecundity and standard deviation values that I calculated for all locations (4171 ± 660 SD, n = 10 per location) were consistently higher and more variable than those calculated by STAD for the same three year period (3591 ± 388 SD ~ n = 75 per year). This systematic bias in average egg number was expected based on my inclusion of non-egg portions of ovary mass in my fecundity calculations. The variability is likely a limitation of the methodology I used and that more complete counts or large sample sizes are recommended. For example, the power of detecting even a difference of 200 eggs between river entry and the spawning ground, based on the samples size of 10 and SD of 660, was low (β= 0.10) (Minitab Ver. 13).

There was significant growth in ovary mass during freshwater migration in all three years (regressions P<0.05; equal slopes; Figure 3a) that was driven by the concomitant increase in egg mass (regressions P<0.05; equal slopes; Figure 3b). In fact, migration distance accounted for 85%, 79%, and 86% of the variance in ovary mass, and 86%, 88%, and 75 % of the variance in egg mass for the 1999, 2000, and 2001, respectively. On average egg mass and ovary mass were significantly greater in 1999 throughout the migration

than in either 2000 or 2001 (regression intercept test $P<0.05$; equal slopes). However, there was no significant difference in egg mass or ovary mass in the marine approach area (Port Renfrew), freshwater entry (Whonnock), or spawning ground arrival for the 3 years (GLM $P>0.05$). A paired comparison at a common sample locations in river showed that ovary and egg mass were significant greater at Hells Gate in 1999 than both 2000 and 2001 (GLM & Tukey $P<0.05$). Similarly, ovary and egg mass samples in 1999 were greater than 2000 samples taken from Nechako river (GLM & Tukey $P<0.05$). There were no significant differences between 2000 and 2001. The excellent fit of the linear regression of ovary and egg mass versus distance likely reflects that fact sockeye once they enter the river progress directly to spawning grounds independent of entry date (DFO unpublished data). Therefore, migration distance is largely a reflection of migration time. In fact, when adjustments were made for differences in time in freshwater, based on sample days from river entry (i.e., Whonnock), the variance explained by sample date on ovary mass (85%, 79% & 83%; Figure 3c) and egg mass (88%, 88% & 72%; Figure 3d) for the 3 years, is almost equal to the variance explained by migration distance. However, unlike migration distance, there were significant differences in slope among the years for migration time versus both ovary and egg mass (unequal slopes regression), suggesting that egg deposition rates were almost 50% higher in 2001 (0.89 mg • day^{-1}) than 1999 (0.59 mg • day^{-1}) and 2000 (0.57 mg • day^{-1}) during in river migration (Figure 3d).

Figure 3: Increases in (a) ovary mass and (b) egg dry mass by distance migrated upstream (km 0 = mouth of Fraser River) and increases in (c) ovary mass and (d) egg mass by migration time (time 0 = freshwater entry) during spawning migrations for Early Stuart sockeye salmon sampled in 1999 (diamonds), 2000 (circles), and 2001 (squares). En route egg mass and ovary mass was significant greater with distance migrated upstream during 1999 than 2000 and 2001 (regression intercept; slopes equal). En route egg mass and ovary mass appeared to increase at a higher rate in 2001 than 2000 or 1999 (regression unequal slopes), but final egg size and mass did not change (ANOVA; P>0.05). Values are standardized for length and error bars represent ± 1 S.E. Sample sizes, locations, and distances are given Table 2.



The small differences (< 1.0 mg) in absolute values between the individual egg mass values I calculated and those provided by STAD for the same three year period, are likely based on differences in methodology used. For example, they used larger sample sizes (n=30 versus n=10) and their measurements were based on eggs fixed in formalin instead of fresh eggs.

Ovary Constituents

Ovary protein content averaged 24% by wet mass (Table 3) and did not vary with sample location or between 1999 and 2000 (GLM $P>0.05$). However, in both 1999 and 2000, lipid and moisture content of ovary varied significantly with sample location (MANOVA & ANOVA $P<0.05$), but only lipid content varied significantly between year and site (GLM $P<0.05$; Figure 4a). In both years, lipid content was approximately 14.5 % at river entry, but decreased significantly during migration and was approximately 11.0 % at the spawning grounds (Tukey $P<0.05$). Nevertheless, this decrease occurred at a significantly shorter distance upstream in 1999 (Churn – 440 km) than 2000 (Nechako – 773 km) (Tukey $P<0.05$; Figure 4a). Interestingly, these site differences disappear when ovary mass is compared against migration time (ANOVA $P>0.05$; Figure 4b).

Figure 4: The mean % lipid content of ovaries for Early Stuart sockeye salmon by (a) location (km 0 = mouth of Fraser River) and (b) by migration time (time 0 = river entry) highlighting the significant decline in % lipid content from river entry to spawning grounds in 1999 (diamonds) and 2000 (circles) (ANOVA $P<0.05$). Significant differences by location and year are denoted by an "*" (GLM $P<0.05$). Values represent location means and error bars are ± 1 S.E.

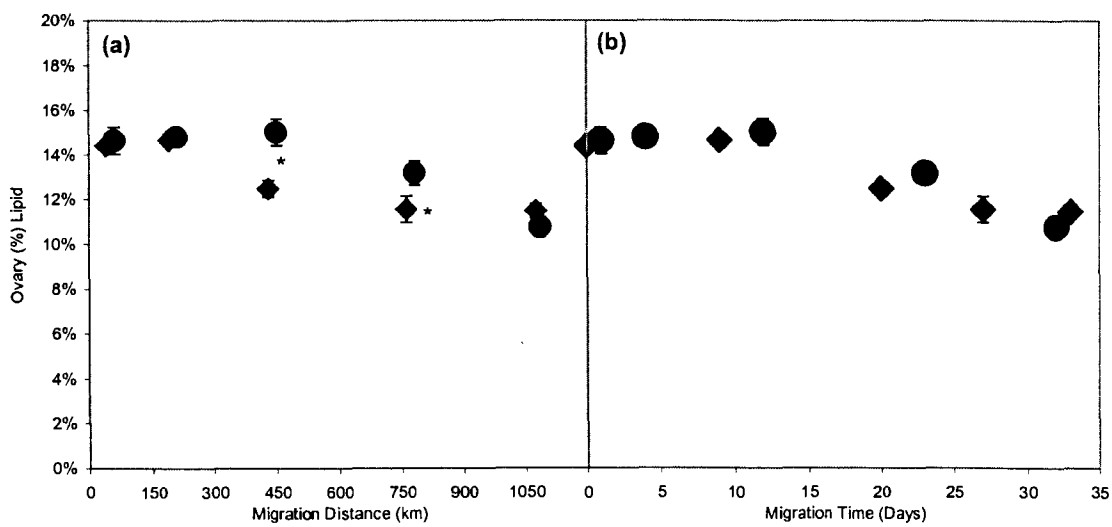


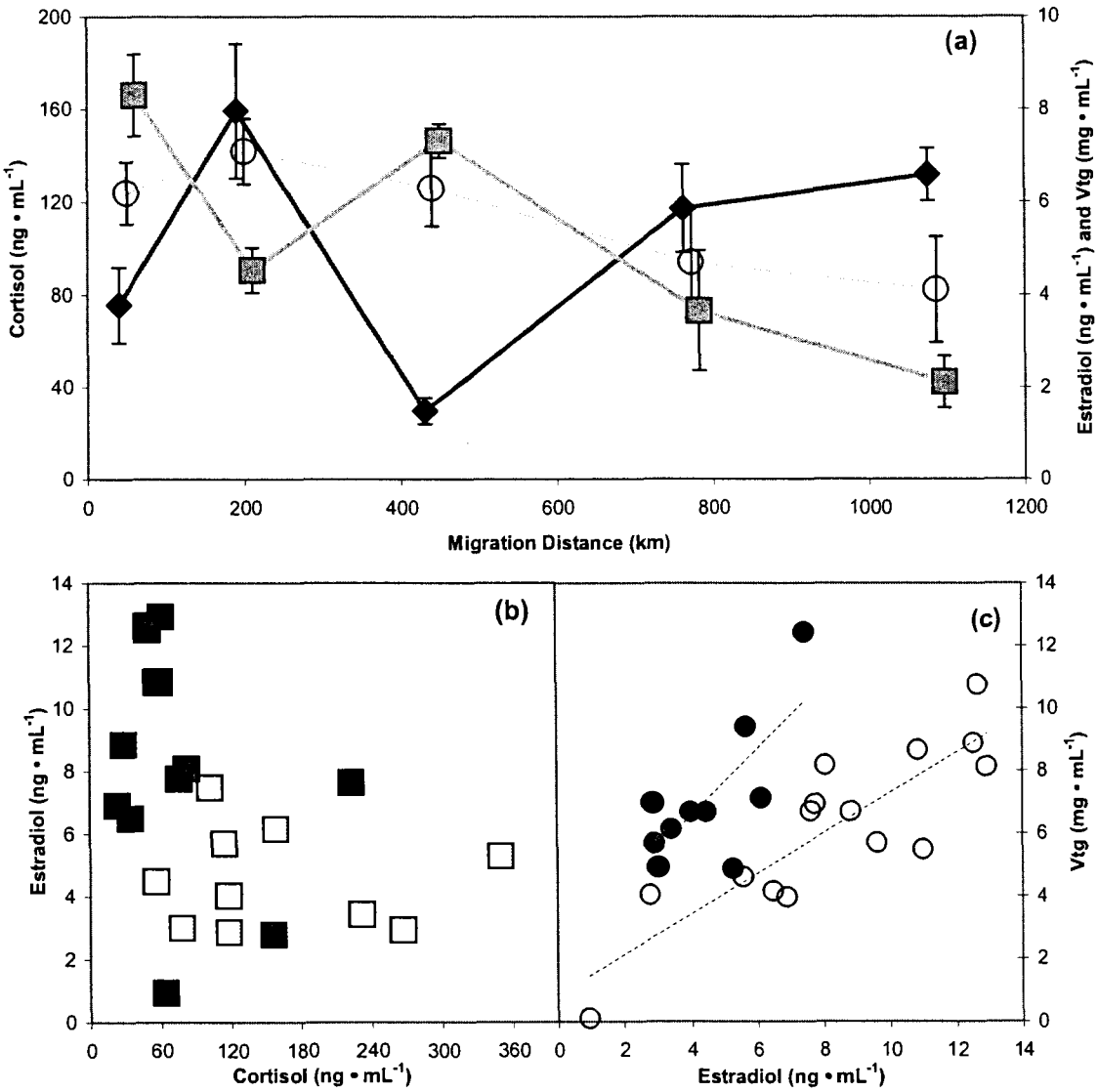
Table 3: Ovary change in mass, energy density and composition (% moisture, dry matter, ash, protein, and lipid by wet mass) during upstream migration for Early Stuart sockeye collected during 2000.

	Port Renfrew	Whonnock	Hells Gate	Churn Creek	Nechako	Gluskie Arrival	Kynoch Spawners
Ovary Mass (g)	74.7	104.2	122.7	172.9	224.1	287.1	210.9
(± S.D.)	11.9	23.1	16.0	37.9	49.3	57.7	109.4
Moisture (%)	56.4	58.2	56.4	57.0	58.6	63.8	64.5
(± S.D.)	1.2	0.8	0.6	0.9	2.0	2.2	2.3
Ash (%)	2.7	2.1	2.9	2.8	2.6	2.6	2.1
(± S.D.)	0.6	0.4	0.6	0.3	0.6	0.6	0.4
Protein (%)	23.5	24.4	25.7	25.5	25.8	22.7	22.9
(± S.D.)	0.7	1.0	0.6	0.6	0.9	1.3	2.1
Lipid (%)	16.8	14.6	14.8	15.0	13.2	10.8	11.1
(± S.D.)	0.7	1.0	0.6	1.0	0.9	0.7	0.8
Energy (MJ • kg^{-1})	12.2	11.5	11.9	12.0	11.3	9.6	9.8
(± S.D.)	0.4	0.3	0.2	0.3	0.5	0.5	0.8
Sample Size	10	10	10	10	10	12	34

Plasma Analysis

Cortisol and E_2 levels fluctuated significantly with sample location (MANOVA & ANOVA $P<0.05$). For example, plasma levels from Hells Gate samples, had significantly high cortisol levels (160 ng • mL^{-1}) and suppressed E_2 (4.5 ng • mL^{-1}) values than adjacent locations (25-75 ng • mL^{-1} cortisol & 7.5-8.5 ng • mL^{-1} E_2; Tukey $P<0.05$). This covariance pattern among these two variables continued throughout the spawning migration (Figure 5a). However, E_2 was expected to decline with reproductive maturity independent of cortisol (Dye et al. 1986). Interestingly, and consistent with the constant egg growth rate, plasma levels of Vtg did not vary with sample location (ANOVA $P>0.05$) and averaged 4-7 mg • mL^{-1} throughout the migration.

Figure 5: a) Changes in female plasma levels of the stress hormone cortisol (diamonds), reproductive hormone estradiol (squares), and vitellogenin (Vtg; circles) by location for Early Stuart female sockeye salmon sampled during upstream spawning migration in 2000 (0 km = mouth of Fraser River). There was an overall location effect on these plasma variables (MANOVA $P<0.05$), but only cortisol and estradiol exhibited significant changes among locations (ANOVA $P<0.05$). Error bars represent ± 1 S.E.. b) There was no relationship (correlation test $P>0.05$) within individuals between cortisol and estradiol at either Whonnock (50 km – hollow) or Hells Gate (200 km- solid), but c) there was a significant positive relationship (correlations $P<0.05$) between estradiol and Vtg at both locations.



At the individual level, there was no relationship between an increase cortisol and E_2 (correlations $P>0.05$; Figure 5 b). In comparison, the expected positive relationship between plasma E_2 and Vtg within individuals did occur (correlations $P<0.05$; Figure 5c).

Interannual Variation in Ovarian Investment

There was significant interannual differences for annual mean values of ovary mass, egg dry mass and fecundity (MANOVA $P<0.05$; ANOVA $P<0.05$; Figure 6), thus I reject the null hypothesis of no interannual variation in reproductive investment. The annual mean value (± SD & range among years) for ovary mass was 292 g (± 14 g; 259 to 315 g), fecundity was 3577 g (± 109 mg; 3372 to 3850), and dry egg mass was 30.8 mg (± 1.4 mg; 28.4 to 33.4 mg), standardized to 53.4 cm fish length (SL). The following pair wise correlations and P-values among the three variables based on annual means are as follows: $R = 0.28$ ovary mass and fecundity ($P = 0.35$); $R = 0.69$ for ovary mass and dry egg mass ($P = 0.01$); $R = -0.30$ for fecundity and dry egg mass ($P=0.33$). The years with the lowest ovary mass, fecundity, and egg mass were 12.6 %, 5.7 %, and 8.1 %, respectively, below the historical mean values. The interannual variation in reproductive investment could be explained in part by the ambient migration conditions, as 4 of the 6 regressions were significant (regressions $P<0.05$; sequential Bonferroni correction). The four significant regression equations (slope, intercept, and R^2 values) were as follows: ovary mass and discharge (-0.0049 g • m^{-3}• s^{-1}, 320, 33.1; Figure 7a); egg size and discharge (-0.00053 g • m^{-3}• s^{-1}, 33, 38; Figure 7b); ovary mass and temperature (6.79 g • $^{o}C^{-1}$, 182, 44; Figure 7c); and egg mass and temperature (0.63 mg • $^{o}C^{-1}$, 20.1, 34; Figure 7d). The consistent direction between reproductive investment and migration severity is not surprising given positive correlation between two measures of reproductive investment (ovary mass and egg mass) and the strong negative correlation between the migration severity indices (Q_{19} and T_{19} $R = -0.89$), suggesting a common mechanism linking migration conditions to reproductive investment.

Figure 6: Interannual variation (a) ovary mass, (b) fecundity, and (c) dry egg mass of Early Stuart sockeye sampled at the spawning grounds with tight skeins (all ANOVAs $P<0.05$). Solid horizontal bar represents historic average value for each parameter. All values have been standardize to common length (53.4 cm; see data analysis) and each data point represents annual mean value with errors bars representing 95 % confidence limits.

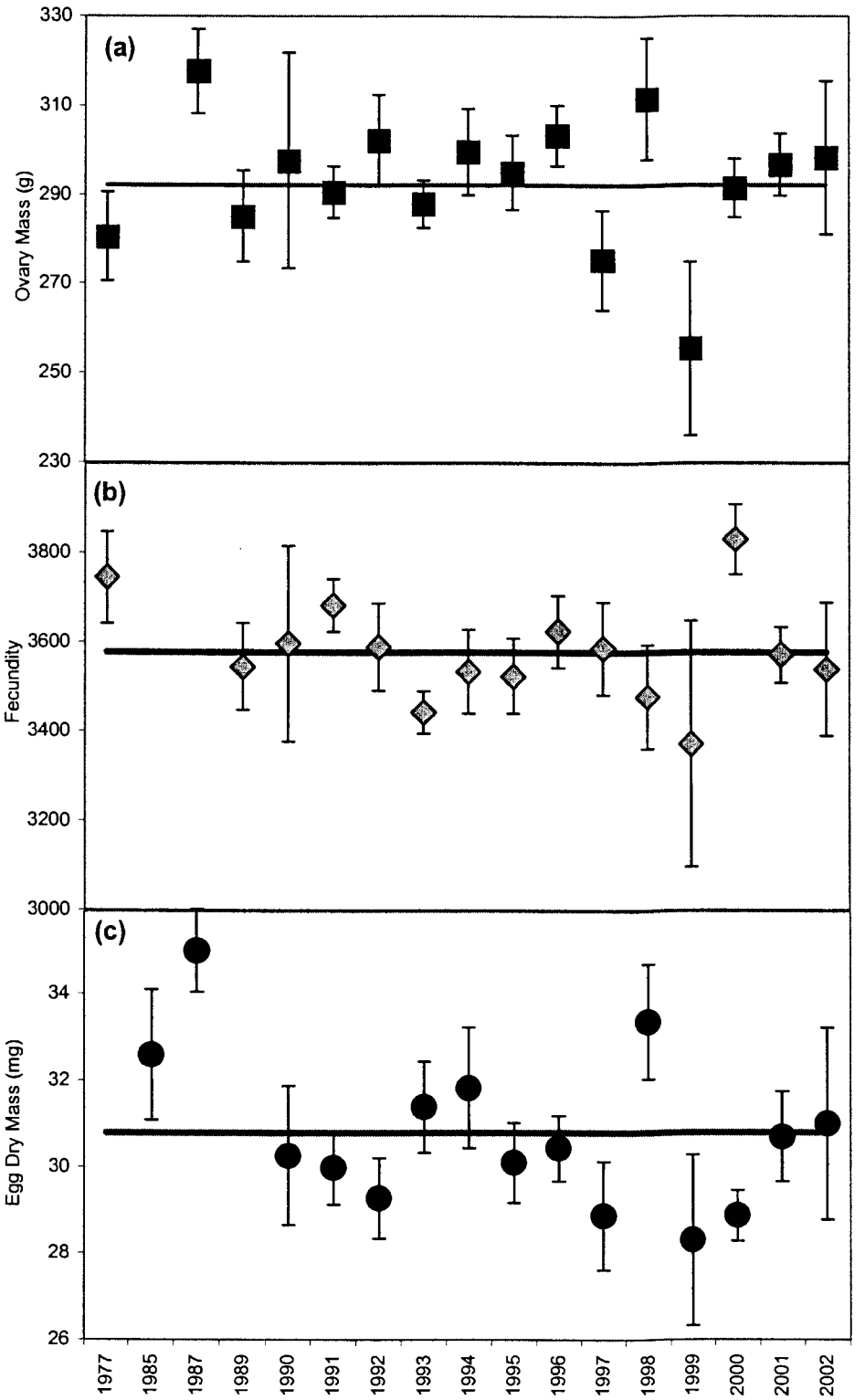
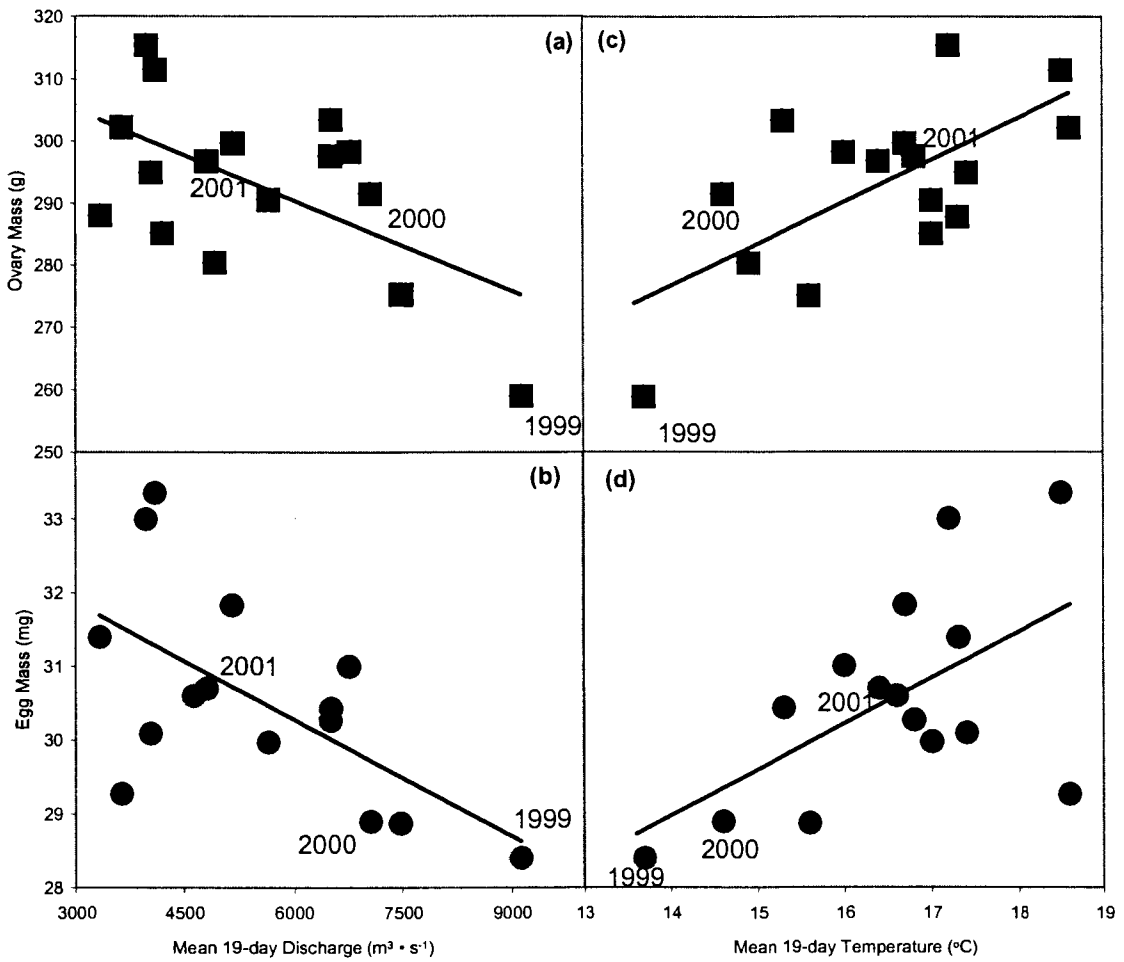


Figure 7: Interannnual comparison of migration severity and reproductive investment for Early Stuart sockeye (all regressions $P<0.05$), using (a) ovary mass versus 19-day mean discharge (b) egg dry mass versus discharge, (c) ovary mass versus temperature and (d) egg dry mass versus temperature. Migration severity represents the 19 day mean discharge and 19-day mean temperature during the peak migration period past Hells Gate. Each data point represents the annual mean value for each corresponding year standardized for length. Years 1999, 2000, and 2001 are noted.



Discussion

Reproductive Development

There was no evidence to suggest that sockeye salmon can facultatively adjust egg size or number during natural spawning migrations. This statement is supported by the following five results: 1) There was no change in fecundity either with location or time; 2) There was no difference in egg size at the spawning grounds across 3 years of observations; 3) There was a constant increase in egg size with distance for all 3 years; 4) The decrease in ovarian lipid content was similar over time for 1999 and 2000; and 5) The plasma levels of Vtg were unchanged throughout migration in 2000. This is despite the large variation in migration conditions experienced across the three years, as Q_{19} in 1999 was 44% and 2000 was 32 % higher than 2001 (see Table 1 & Figure 2). Patterson et al. (2004) came to similar conclusion with regards to points 1) and 2) in that final egg size and fecundity did not change in captive versus naturally spawning Early Stuart sockeye, despite significant modulations to energy expenditure associated with migration under controlled conditions. However, they also surmised that egg development rate was plastic in that two groups of captive fish took longer on average to reach full egg size.

I am unaware of data from sockeye salmon to suggest that egg development rate and constant plasma Vtg levels are constant during spawning migrations. In contrast, a similar survey style study on pink salmon (*O. gorbuscha*) showed a clear decline in plasma Vtg with migration distance upstream (Dye et al. 1986), Early Stuart female plasma Vtg levels were approximately 5.5 mg • mL^{-1} at all locations, whereas the average

pink salmon plasma Vtg levels sampled at river entry (Whonnock) were 8.0 mg • mL^{-1}, spawning ground arrival females were 2.0 mg • mL^{-1} and the spawning ground levels were near zero. Pink salmon, however, differ from Early Stuart sockeye by entering the Fraser River with an ovarian mass very close to the full maturation size (Williams et al. 1986) despite having similar GSI at spawning ground arrival (~ 16%).

Environmental stressors can elevate plasma cortisol levels and suppress plasma concentrations of E_2 (Pickering et al. 1987), and E_2 can stimulate Vtg production (Tyler and Sumpter 1996). In this study, at the population level, the migration through a difficult passage reach (i.e. Hells Gate) did elevate a cortisol levels (stress response) and suppress E_2 levels relative to adjacent locations (with lower water velocities), but overall Vtg levels remained the constant at each site. However, at the individual level the results are very different. The predicted inverse relationship between cortisol and E_2 did not occur but there was positive relationship between E_2 and Vtg at both locations. Therefore, the predicted functional response between stress and regulation of reproductive development was inconsistent between the population and individual level. This individual variability needs to be explored further.

There have been several studies directly examining the influence of migration difficulty on ovarian investment (Gilhousen 1980; Beacham and Murray 1993; Kinnison et al. 2001), but few have looked at the relative composition change associated with the freshwater migration (Crossin et al. 2003). Hendry and Berg (1999) provided % lipid content at coastal entry (5.1%) and on the spawning grounds (4.1 %). Both of these

values are lower than those reported here for comparable locations (Port Renfrew = 16.8% & Kynoch spawners = 11.1%). Speculation as to why such differences exist, especially given the similarity in analytical method, likely revolves around different energy requirements for the developing embryos. The relative proportions of protein and lipid contents are a maternal influence that will affect the development of their offspring (Berg et al. 2001). Interestingly, there was very little variation among individuals (years pooled) with respect to protein (SD = 1.3%) and lipid (SD = 0.6%) contents, suggesting that an optimal combination of these maternal resources exists (Berg et al. 2001; Patterson et al. 2004).

Critique of Approach

A recurrent problem with a survey style study is that they only survivors are sampled, and yet inferences are drawn for the population as a whole. Therefore, some cautions should be made with regards to the possible selection against certain phenotypes during migration. Based on personal observations it is likely that a large portion of the 1999 Early Stuart run that entered the lower Canyon prior to July 20th 1999, were held up at Hells Gate and subsequently many may not have made it all the way to the spawning grounds. The en route loss estimate for Early Stuarts in 1999 was 81% (Ian Guthrie PSC). Therefore, any temporal component across the population, with respect to reproductive investment (Woody et al. 2000), would obscure the results based on the individual sampling of survivors from an unknown timing segment of the population. It is important to determine whether those fish that were sampled at en route locations would be expected to survive and spawn. In fact, closer examination of the energetic

body condition of the fish reveals that it is unlikely that any of the 1999 fish sampled at Nechako (4.6 MJ • kg^{-1}), or Ft. St. James (4.3 MJ• kg^{-1}) would have made it to spawn (DFO unpublished data), given that the critical energy level for life and death in spawning sockeye is postulated to be 4.0 MJ• kg^{-1} (Crossin 2003; see Chapter 3 results). Even based on conservative energy expenditures of 0.15 MJ • kg^{-1} per day (Lee et al. 2003c) and estimated travel times of 5 and 2 days, respectively, most of the Nechako and Ft. St. James fish would be dead prior to reaching the spawning grounds. The few remaining fish would likely not have enough energy to spawn (Healey et al. 2002). Predicting en route loss based on energetic modelling has been documented for Early Stuart sockeye (Rand and Hinch 1998), however, this is the first time energetic state relative to reproductive state has been linked to the likelihood of surviving to spawn. This is novel aspect of this thesis and area that should be further explored.

Reinterpretation of the egg growth rates and egg mass at given locations need to be done based on the possibility that mortality and selection may have occurred en route. For example, in 1999, the individual egg mass at Nechako and Ft. St. James locations were similar to values at the spawning ground (Tukey $P>0.05$), suggesting that these fish had reached approximately full egg size while en route. This is also supported by the fact that their energy condition is more indicative of fish on the spawning ground (Crossin 2003), than an en route migrator (Higgs et al. 2000). If fish in 1999 reached full egg size before reaching the spawning grounds, then estimates of egg growth rates would be higher and more akin to values estimated for 2001. Therefore, I conclude that the differences seen in egg growth rate seen in Figure 4 can easily be interpreted as either minor differences in

timing of samples relative to peak migration, which is unknown, and/or selection against different timing components within a run. This is consistent with my statement that egg growth rate is fixed during natural spawning migrations.

Interannual Variation in Ovary Investment

The results support my original prediction that migration severity has a negative impact on reproductive investment, based on the consistent association between high discharge (or low temperature) and reduced ovary and egg mass. Therefore, at the population level, the severity of migration experienced by the parents can potentially influence average phenotypic size of the progeny in the next generation. Previous studies on maternal effects on ovarian investment fall into two categories, environmental factors that directly affect maternal size (e.g., Jonsson and Jonsson 1997) or stressors applied during captive studies (e.g., Contreras-Sanchez et al. 1998). It is possible the reductions in reproductive investment are the result of migrational stress. However, as mentioned previously, there is no evidence that egg size or number can be adjusted en route (see stress values Chapter 3). It is unlikely the differences in ovary investment are related to maternal size because each variable was standardized for length. Based on the assumption of a predetermined ovarian investment before river entry, I speculate that interannual differences in ovary mass and egg size are likely the result of selection against maternal phenotypes that had expressed high ovarian investment during years of difficult migration. It seems plausible that a range of different reproductive strategies exists within the population with regards to energy allocation for reproduction and the success of each strategy would be contingent on environmental conditions encountered. In support of these two statements,

Fraser sockeye exhibit interannual variation and intraspecific variation in the amount of somatic energy at river entry (Crossin 2003), there exists wide variation in migration conditions that are associated with en route loss (Macdonald 2000; Macdonald et al. *In Revision*), and interannual differences and intraspecific variation in final reproductive output (this study). This hypothesized intergenerational effect, namely reproductive investment is influenced by migration severity, should be tested against other Fraser sockeye populations.

Fecundity has a heritable component (Su et al. 1997; Smoker et al. 2000). Therefore, if highly fecund individuals are being selected against, then the genotype proportion will change in the next generation. The population level fitness consequences of a reduced egg number will likely include a reduction in future recruitment and changes in offspring survival rates that are based density dependent relationships. For salmonids, density dependent responses have been documented in both the competition for intergravel oxygen (Einum et al. 2002) and predation risks post-emergence (Brio et al. 2003). However, in this study there is no evidence of a migration selection force that may be underlining the documented interannual variation in fecundity. This area remains unexplained.

I feel some level of perspective should be made with regards the above results. The relative difference in fecundity between an average fecundity year and low fecundity year is less than 5% (e.g. 1998 only 150 eggs less than average). While this modest interannual difference may be statistically significant (although, it was not correlated with

migration severity), the biological relevance of such a decline needs some context. In the same year, 1998, that fecundity was low, 78% of an estimated 154 000 Early Stuart sockeye entering the Fraser River did not arrive at the spawning grounds and a further 42% succumbed to pre-spawn mortality (PSC & DFO unpublished data). In other words of 277 200 000 eggs that females brought into the Fraser River in 1998 (assume 50:50 sex ratio and an average 3600 eggs per female) the 5 % reduction in egg number seen on the spawning grounds accounted for less than 0.5 % of the total number of eggs lost. Therefore, the above discussions regarding potential changes in genotype proportions reflect to a greater degree phenotype loss associated with en route loss and PSM estimates rather than decreased fecundity *per se*.

The fitness consequences of a reduce egg size in salmon include smaller size at emergence (Hutchings 1991), higher predation (West and Larkin 1987), lower swimming performance (Bams 1967), and slower initial growth rates (Heath et al. 1999). However, it is hard to imagine the small difference in egg size (an 8% decline or 2 mg from the historical average) would have serious fitness consequences at the population level, especially given that this value is less than the standard deviation in egg size (3 mg) among individuals of the same size within a year. Moreover, an average 2 mg savings translates into approximately 76 kJ of energy, which is only enough energy to sustain the average female in a population for just under one day (Healey et al. 2002). However, the 95% confidence interval for egg sizes (12 mg) among individuals is large by comparison. This would translate into a wider scope of energy available (432 kJ) across those individuals and this would translate into approximately 8 days of energy on the spawning

ground (Healey et al. 2002). Therefore, the hypothesized difference in individual allocation strategies, based in part on the observed phenotypic plasticity in egg size, may make the difference between successfully spawning or not. In addition, the success of individual phenotypes is likely conditional on the migrational energy expenditures during a given year as evidenced by the decline in egg size with high discharge levels. Moreover, egg size has been shown to be heritable in some salmonids (Su et al. 1997), therefore another consequence of changes in egg size may involve changes in the intergenerational gene flow itself.

In summary, the reproductive development of migrating sockeye appears to be highly conserved, based on a 3-year detailed analysis. Fecundity, egg growth, changes in relative egg composition, and plasma Vtg levels are all fixed during the spawning migration from the mouth of the Fraser River to the spawning grounds. Therefore, there is no evidence to reject the hypothesis that egg size and egg number are set prior to freshwater entry. Severe migration conditions were significantly correlated with reductions in reproductive investment at the population level, but changes were small (<10%) over the 25 year study period. Although, this is evidence of an intergenerational effect at the population level, I argue that the mechanism causing the decline is better explained by selection against maternal survival en route than individual adjustment of reproductive investment during migration. Fitness implications of a reduction in egg size for individuals will be explored in Chapter 3. The next chapter will focus of estimating whether the full compliment of eggs produced by each female spawner are of equal

viability, with emphasis on the implications of differential survival and the associated changes in intergenerational gene flow.

CHAPTER 2: PARENTAL INFLUENCES ON EMBRYO SURVIVAL FROM THREE FRASER RIVER SOCKEYE SALMON POPULATIONS.

Abstract

In 1999 and 2000, a novel and robust method was designed and implemented to assess gamete viability from individual male and female parents from three sockeye salmon populations with different spawning migrations (Weaver - 100 km; Gates – 363 km; Early Stuart -1086 km). Repeatable and high levels of fertilization success (>90 %) were obtained for most individuals and all of the 259 unique pairings of 87 females and 39 males produced viable offspring. By comparing embryo survival to the eyed stage among different full-sib and half-sib broodlines, an individual offspring's chance of survival was shown to be related to the parental origin of the gametes. This non-random gamete viability is a potential mechanism underpinning the intergenerational effect of differential embryo survival observed among populations and between years. There was no evidence that certain combinations of males and females were more or less viable, and variance in embryo survival was more likely to be associated with the maternal rather than the paternal gamete origin. Unlike Weaver sockeye, parents from the Early Stuart and Gates sockeye stocks were exposed to difficult migration conditions en route and they both had significantly lower and more variable gamete viability. This result suggests an intergenerational effect associated with migration experience. Thus, parental variation in gamete viability in wild populations has the potential to reduce future recruitment and affect the genetic make-up of the next generation.

Introduction

In chapter 1 of this thesis I concluded that migratory severity is correlated with a decrease in egg mass. Prior to an assessment of fitness consequences associated with a given egg mass, the egg must first become successfully fertilized. Therefore, this chapter focuses on a direct measure of offspring fitness, differential survival of developing embryos.

The genotype of the surviving offspring is the direct result of a successful fertilization and early embryo survival. The overall gene pool is determined by the relative genotype proportion of unsuccessful to successful fertilizations. Thus, parental exposure to chronic and acute stress during the late stages of maturation may have a profound effect on offspring survival in salmonids (Campbell et al. 1992). Unequal contributions of parental gametes will change the genetic variation within a population, especially since multiple partners for both males and females occur during the breeding season (Foote et al. 1997). Therefore, fertilization success and early embryo survival are likely crucial steps in determining alterations in intergenerational gene flow. However, these statements rely on the assumption that differential survival exists between the gametes produced by individuals within a population. Therefore, the focus of this chapter to determine to what degree differential embryo survival is influenced by maternal and paternal origin of the contributing gametes in wild sockeye salmon.

Maternal influences on embryo survival have been well documented in cultured rainbow trout (Nagler et al. 2000). Differences in fertilization rates among males of both cultured

rainbow trout and Atlantic salmon (*Salmo salar*) have also been well established (Aas et al. 1991; Gile and Ferguson 1995). Similarly, fertilization experiments using wild sockeye salmon, that were not specifically designed to test parental influences, have found both a maternal (Herunter et al. 2000; Hoysak and Liley 2001) and parental influence (Galbraith 2003) on embryo survival. In this thesis parental influence (either maternal or paternal) is synonymous with parental effects (e.g., maternal effects), unless otherwise stated.

Under natural fertilization and incubation conditions, interannual variation in over winter egg to fry survival for Early Stuart sockeye range from 8% to 70% (Timber Whitehouse DFO unpublished data). The source of this wide variation in fry survival is likely a combination of incubation environment, parental gamete quality, and measurement error. In wild salmon, the multitude of factors that contribute to gamete viability cannot be controlled because individual adults are phenotypically plastic in their response to a common environmental influence (Schreck et al. 2001). This same plasticity may also apply to gamete viability. Laboratory and field experiments have examined the individual factors and combinations of factors that contribute to egg mortality in sockeye salmon (Cope 1996). However, these experiments sometimes obscure parental effects by pooling gametes (Herunter et al. 2000; Galbraith 2003), non-random selection for healthy individuals (Hoysak and Liley 2001), and controlling adult rearing environments (Nagler et al. 2000). Uncontrolled incubation environments (Cope 1996) and small sample sizes (Novales Flamarique and Harrower 1999) further confound matters. Therefore, the variation that exists among individuals in their ability to produce viable offspring needs

to be established before correlation analysis and intervention experiments can accurately be undertaken to identify parental factors that may contribute to poor fertilization success and egg mortality. Similarly, population variation in embryo survival needs to be established, prior to inferring a relationship between migration severity and offspring survival at the population level.

In this Chapter I test the null hypothesis that all gametes have equal probability of producing viable progeny, irrespective of parental and population origin. A novel method was developed for assessing gamete viability that proved to be robust under both field and laboratory conditions. Three populations of Fraser sockeye (Early Stuart, Gates, and Weaver) were used to test the hypothesis. Because these populations differ widely in the difficulty of their respective spawning migrations (see Introduction), I could examine the prediction that overall population viability decreases with increased migratory difficulty encountered by their parents during abnormal migration condition year.

Materials & Methods

Study Design

Gametes from spawning sockeye salmon were collected from three populations at their different natal spawning locations Kynoch (Early Stuart population), Gates, and Weaver Creeks in both 1999 and 2000 (see details in Table 1).

General Protocol

The general protocol section describes the methods that apply to all cross fertilization experiments, incubation conditions, and data analysis. Deviations from these methods will be noted in the respective experiments

Gamete Collection:

Live adults were selected at random from stream reaches where active spawning was underway. Most fish were already paired and were actively spawning based on visual observations. Two exceptions were the last fish collection at Kynoch Creek in 2000 and all Weaver sockeye collected in 2000. These fish were removed at random from holding areas below enumeration fences. It was not possible to visually determine if pairing or spawning was taking place below the fences.

Adult sockeye salmon were caught with a dipnet and checked for ripeness. Gentle abdominal pressure was applied from the dorsal to ventral direction and easy extrusion of

single eggs or milt signified a sufficient degree of ripeness. Ripe fish were sacrificed with a sharp blow to the head. Gametes removed from each individual were kept separate in dry plastic containers, which were kept in a cooler (~ 4°C). Gamete storage at lower temperatures prolongs viability (Jensen and Alderdice 1984). The total egg wet mass, to the nearest 0.1 g, and the approximate volume of milt, to the nearest 1 mL, was recorded for individual fish.

Fertilization Technique

A dry fertilization technique (Scott and Baynes 1980) was applied to all crosses. An individual cross involved approximately 100 eggs being placed in a 120 mL glass jar. Egg number was estimated based on average wet mass per egg using the following conservative estimates for each population: Early Stuart 10 g = 100 eggs; Gates 12 g = 100 eggs; Weaver 15 g = 100 eggs. Approximately 0.1 mL of milt per 10 g of eggs, was added to the eggs using a 1 mL syringe. The sperm were then activated by adding 15 mL of water to the jar, gently swirling the mixture, and adding an additional 30 mL of water. After 2 minutes the excess sperm were rinsed off by replacing the original water with 60 mL of fresh water. The eggs were kept for minimum of 45 minutes in the glass jars to allow for water hardening and then placed in incubation containers. Because all sperm should be dead after 45 minutes water exposure (Liley et al. 2002), the time delay in transferring eggs prevented cross fertilization in common incubation chambers.

In 1999, all gametes collected were fertilized within 4 hours. Fertilizations for Gates and Early Stuart sockeye took place streamside and Weaver sockeye were fertilized at Cultus

Lake Laboratory (CLL). From August 4th to August 7th 2000, Early Stuart gametes were fertilized on site. Gametes collected on August 8th from Kynoch Creek, and all Gates and Weaver fish collected in 2000, were placed in plastic containers filled with 99.99% pure oxygen and transported overnight to CLL (Billard 1981). Fertilizations took place at CLL approximately 24 hours after fish capture.

Incubation

Two different incubation environments were used. Weaver and Gates progeny were incubated at Cultus Lake Laboratory in both 1999 and 2000. All 1999 Early Stuart and the majority of 2000 Early Stuart progeny were incubated in the Kynoch Creek, with exception of those transported to CLL.

Kynoch Creek

On August 14th 1999, visible mortality (opaque eggs) occurred between fertilization and placement of 30 eggs into egg incubation capsule. Therefore, 90 eggs were randomly selected from each jar post-fertilization on August 15th 1999 and equally distributed among 3 capsules. The field incubation capsules were a perforated stainless steel cylinder (3.7 cm X 12 cm) with a tight fitting test cap (Scrivener 1988; Cope 1996). The eggs were interspersed with pre-sorted gravel (1.3 to 5.1 cm diameter) within each capsule, closed and placed to a mean depth of 20 cm in artificial redds dug with a shovel located in glides within the sample location area. Redds were progressively dug downstream of the first redd to avoid mechanical shock (Jensen and Alderdice 1989). In 2000, 100 eggs from each cross were divided into two capsules, which were buried in

separate redds, as insurance against the localized mortality in 1999. Clusters of redds were protected from disturbance using fencing. A Vemco© datalogger was buried 20 cm in one of the artificial redds to record intergravel water temperatures. The egg capsules were left undisturbed until they reached the eyed stage (Jensen and Alderdice 1989). The contents of each capsule were removed and eggs were assessed as either live eyed, or clear (live but no evidence of fertilization or embryo development), or dead opaque.

Cultus Lake Laboratory

To minimize handling, eggs at CLL were not counted prior to incubation. Incubation baskets were a rectangular clear plastic box (10 X 10 X 5 cm) with nylon fly screen attached to the top and bottom. Twenty incubation baskets were placed in each Heath tray, and a Heath stack consisted of 7 trays all supplied with Cultus Lake water drawn from a depth of 15 m at a flow rate of 5 L $\bullet$ min^{-1}. Dissolved oxygen levels were above 8.0 mg $\bullet$ mL^{-1}, based on periodical checks in the top and bottom Heath trays using an Oxyguard© meter. Water temperature was recorded in each stack using a Vemco© datalogger. Water temperatures ranged from 9 to 11 ^{o}C from fertilization through to the eyed stage. The stacks were clothed in black plastic to maintain darkness (Novales Flamarique and Harrower 1999). Dead eggs were picked from the baskets weekly. Again, eggs were assessed as either live eyed, clear or dead.

Data Analysis:

Percent survival to eyed stage was calculated for each cross and % survival data was arcsine square root transformed prior to statistical analyses. Minitab Ver. 13 statistical

software was used for all statistical tests. Data were presented based on untransformed values, but all statistical tests were performed on transformed data. An alpha level of <0.05 was considered significant.

Method Development

Method development comprised of six separate experiments designed to test specific factors that may confound the results from the individual and population variation experiments. Each experiment consisted of one or more trials, and the results from some of the individual trials were used in more than one experiment. Trials were defined as set of one or more cross fertilizations that occurred on the same day using the same set of gametes. A cross is a set of fertilizations that occur at the same time. For each trial the assignments of eggs and milt to each jar and fertilization order were randomized. Table 4 summarizes the gamete origin and the number of parents used in each trial, and the experiment to which the results were applied.

Individual male and female sockeye salmon may have 1 to 4 partners during the breeding season (Healey et al. 2002; Mehranvar et al. *Submitted*). Even within a single natural spawning event, multiple males may attempt to fertilize a single release of eggs (Foote et al. 1997). Therefore, the eggs from each female were crossed with the milt from a minimum of 3 males and each male was crossed with eggs from a minimum of 5 females.

Experiment 1: Test for Interaction between Unique Pairings

To test whether certain combinations of male and female gametes were more or less successful than others, I tested for interaction using triplicate and duplicate crosses of 8 females with 3 males from both Early Stuart trial **A** and Weaver trial **D**, respectively. The following analysis of variance model (GLM) was applied:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha*\beta + \varepsilon_{ijk}$$

Where Y_{ijk} = embryo survival for "i" dam, cross with "j" sire for "k" replicate, μ = constant, α_i = dam, β_j = sire, $\alpha*\beta$ interaction term between dam and sire, and ε_{ijk} = error term.

Experiment 2: Repeatability Estimates for Single Pair Matings

Duplicate crosses of unique pairings were made at random within each trial to assess repeatability of the fertilization technique. Regression analysis was used to determine if the first replicate was a significant predictor of embryo survival in the second replicate. Separate regressions were used for the 19 duplicate crosses from Early Stuart fish incubated at Kynoch (from trials **F**, **G**, **H**, **I**), 10 Early Stuart duplicate crosses incubated at CLL (trial **J**), 12 duplicate Gates crosses at CLL (trial **C**, **K**), and the 24 duplicate Weaver crosses at CLL.

Experiment 3: Repeatability Estimates of Incubation Environment

Eggs were incubated at either Kynoch Creek or CLL. Cope (1996) documented large variation in egg survival among different artificial redds in Kynoch Creek. To determine

if redd location had a significant effect on embryo survival, redd # was added to the GLM model for parental effects (see Experiment 8). The 2000 Early Stuart eggs from trials **E**, **F**, **H**, and **I** (placed in separate redds) were used for this analysis. CLL environment was assessed using Gates eggs from each unique pairing in 1999 (Trial **B**). Eggs from the Gates fertilizations were divided into two baskets and randomly placed within Heath trays. Separate pair wise comparisons of survivals from Gates baskets and Kynoch capsules were made using linear regression. In addition, overall embryo survival in Kynoch versus CLL was compared using single ANOVA and Tukey test on mean individual survivals from each Early Stuart trial completed in 2000.

Experiment 4: Influence of Dry versus Wet Fertilization on Embryo Survival

A dry fertilization technique is the preferred method for most Pacific salmon broodstock programs because of the high overall fertilization success achieved. However, there is no evidence that relative survival among different single pair matings using a more natural wet method is comparable to a dry method for sockeye salmon fertilization. In other words, it was deemed necessary to eliminate the possibility that some gametes were differentially successful in dry fertilization versus more natural spawning conditions. Linear regression analysis was used to compare embryo survival from the 11 unique single pairings using a dry fertilization technique and a wet fertilization technique from a concurrent study (Galbraith 2003). The wet fertilization technique consisted of placing unfertilized eggs in flowing water and then injecting milt into the water column. The fertilization process was the only difference between the two groups (Galbraith 2003).

Experiment 5. Influence of Collection order on Fertilization Success

The viability of milt and eggs decreases with time after stripping (Jensen and Alderdice (1984). Collection of broodstock in a natural environment is a difficult endeavour. The many fruitless forays with a dipnet increase the possibility that collection order influenced subsequent embryo survival. Therefore, within each Early Stuart trial completed in 2000, the rank order of collection was compared with the fertilization success for both males and females (linear regression). The prediction was that gametes from individuals collected closer to fertilization time (higher rank number) would have higher survival (i.e., a positive slope).

Experiment 6: Influence of Ovulation Date on Fertility

Egg fertility is known to decline with date from ovulation, based on repeat stripping of rainbow trout (Springate et al. 1984) and holding experiments with Atlantic salmon (De Gaudemer and Beall 1998). The only surrogate for date of initial ovulation was the ratio of egg mass present at the time of sampling relative to the expected initial egg mass at ovulation. This assumes that GSI would decrease with time from initial ovulation with successive spawning acts (akin to repeat stripping). An expected GSI of ~ 16% for Early Stuart sockeye is based values from Ballantyne et al. (1996) and Patterson et al. (2004). If ovulation date had an effect on egg viability, the prediction would be a decrease in embryo survival with decreasing GSI. This was tested using linear regression of GSI and embryo survival for all Early Stuart females collected in 2000.

Individual Variation (Experiment 7)

I used individual variation associated with fertilizations with different potential partners, and not the variation associated with repeat fertilizations of the same unique pair (See results for experiment 2). Given that multiple pairings have been well documented for sockeye salmon (Foote et al. 1997), it is necessary to assess gamete viability by cross fertilizations with multiple partners. Moreover, in artificially assorted mating designs, such as this, it is not possible to predict which pairing would be natural, because mate choice is removed. Individual variations in the ability to produce surviving embryos were based on results from the unique pairing within all 13 trials. For data analysis, a single average fertilization was determined for each unique pair. In cases with replicate crosses for the same unique pair, only the first unique pairing was used, unless all crosses within the trial were balanced (i.e., trial **A** and trial **D**). Based on the results from experiment 3, there were significant differences in embryo survival associated with capsule location within a particular redd. Therefore, maximum capsule survival was used to minimize the bias associated with the variability of the natural incubation environment (see results experiment 3). The same GLM model applied in experiment 1 was used, with the interaction term removed. All females were crossed with 3 males, therefore sample size was n=3 for females. Male sample size ranged from n=3 unique females (trial **I**) to n=9 unique females (trial **E**).

Population Differences (Experiment 8)

Population survival was based on the average embryo survival for each individual from the respective populations within that year. In 1999, trial **A** was excluded for population comparison because of the difference in methodology used. Population differences within a year were determined using a single ANOVA. A statistical comparison between years was not made due to the limited degrees of freedom available.

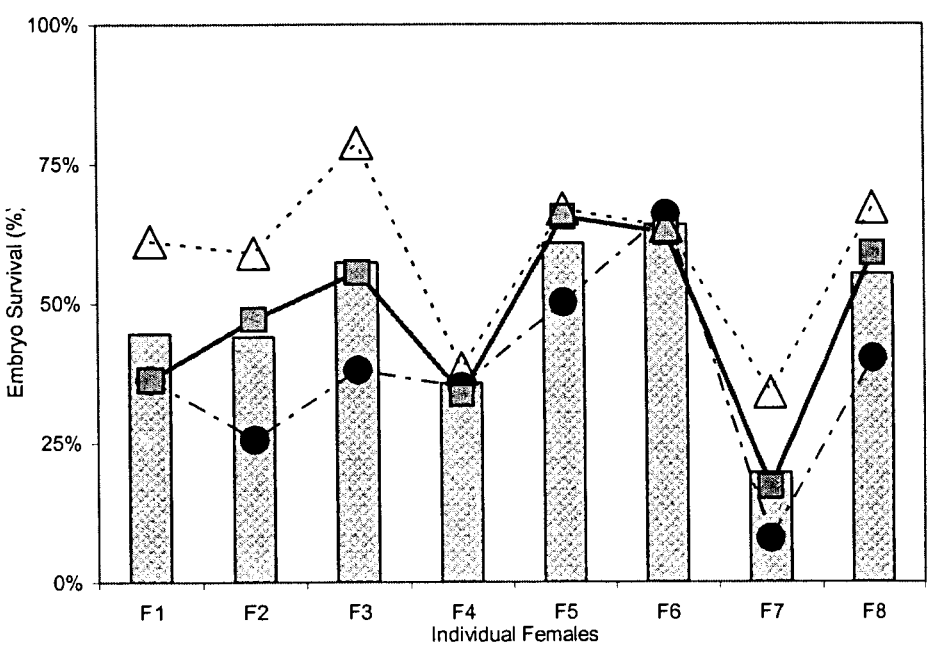
Results

Method Development

Experiment 1: Test for Interaction between Unique Pairings

There was no evidence of interaction between specific male-female pairing from either Early Stuart 1999 or Weaver 2000 (GLM P>0.05). For example, male Z in trial **A** had consistently either the same or lower fertilization successes than both male X, and male Y, regardless of female partner (Figure 8). Therefore, it is possible to determine gamete viability from an individual by random selection of partners, without fear that certain combinations will be differentially successful.

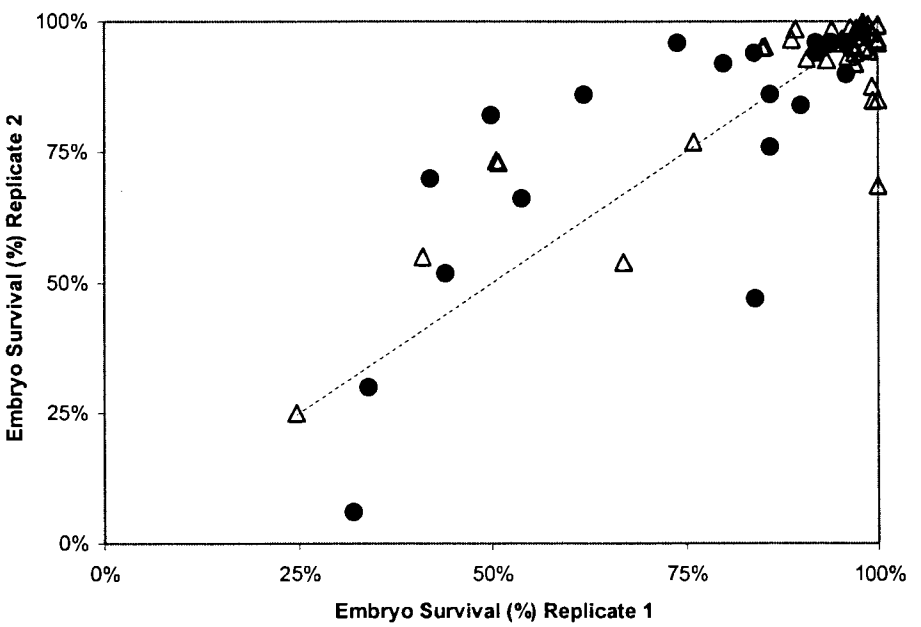
Figure 8: Embryo survival (% eyed) from dam-sire crosses (n=24) of 1999 Early Stuart sockeye, based on male and female gamete origin from trial **A** (see Table 4). Each data bar for a single dam represents the average embryo survival from 3 replicates crosses with three sires. Each triangle (Male X), square (Male Y) and circle (Male Z), represents the mean embryo survival for 3 replicate crosses with the corresponding female. There was no evidence of interaction (GLM P<0.05), but there was a significant maternal (GLM P<0.05) and paternal (GLM P<0.05) influence on embryo survival. [Experiment 1].



Experiment 2: Repeatability Estimates for Single Pair Matings:

There was a significant relationship between replicate crosses of same unique pairings in all 3 populations and at two incubation environments (all regressions $P<0.05$). The overall correlation among all pairs was 0.82, within CLL crosses it was 0.81, and for Kynoch Creek crosses it was 0.74 (Figure 9). Therefore, this method for assessing embryo survival of unique pairings was robust under two incubation environments.

Figure 9: Repeatability of embryo survival (% eyed), based on replicate crosses performed streamside at Kynoch (circles) Creek and at Cultus Lake Laboratory (triangles) in 2000 for Early Stuart, Gates, and Weaver sockeye populations. [Experiment 2]

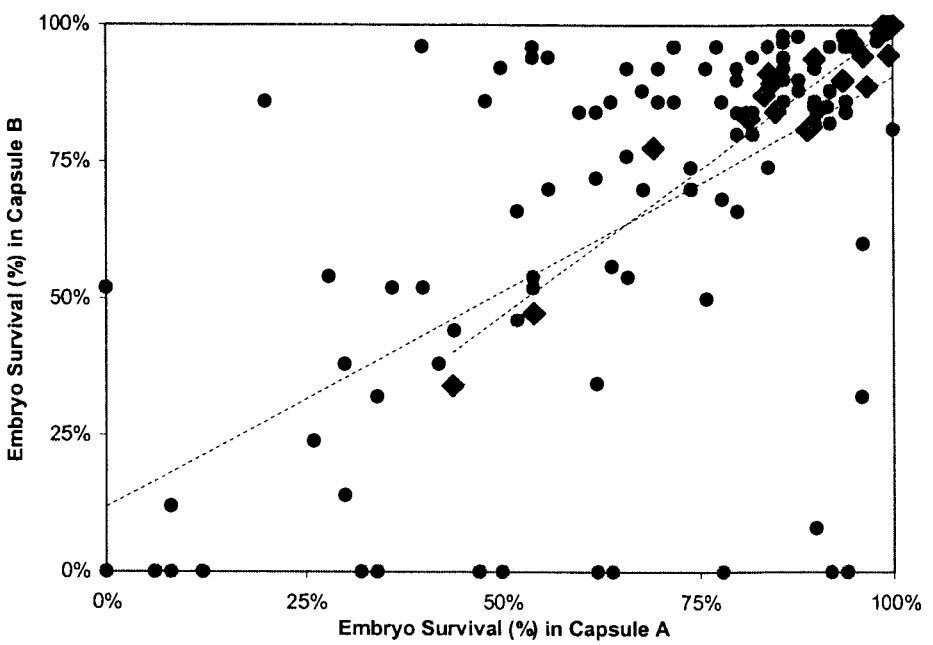


Experiment 3: Repeatability Estimates of Incubation Environment

There was no evidence that survival was different between eggs reared in different locations within the Health stacks at CLL (regression $P<0.05$, $R^2 = 0.91$; Figure 10). On average there was a significant relationship between eggs from single fertilization cross placed in two different locations within Kynoch creek, but only 35% of the variance for egg survival within a capsule was explained by the replicate cross (regression $P<0.05$; R^2

= 0.35; Figure 10). Some of the remaining 65% variance in survival could be explained by the significant effect of specific redd location in 3 of the 4 trials (**E,G,I**) in 2000 (GLM $P<0.05$; Table 4). In most cases mortality was associated with high infiltration of fine sediment and was highly localized within each redd (personal observation), suggesting poor water exchange and suffocation from low oxygen levels. There were significant differences in the average embryo survival attained among the 6 trials of Early Stuart sockeye in 2000 (GLM $P<0.05$). However, embryo survival from those Early Stuart eggs that were transported and then fertilized and reared at CLL (Trial **J**) were not significantly different than 4 of the 5 spawning ground trials (**F,G,H,I**) (Tukey test; $P>0.05$). Thus, fertilization results from streamside experiments were comparable to those controlled under laboratory conditions and overnight transportation of gametes did not adversely affect embryo survival.

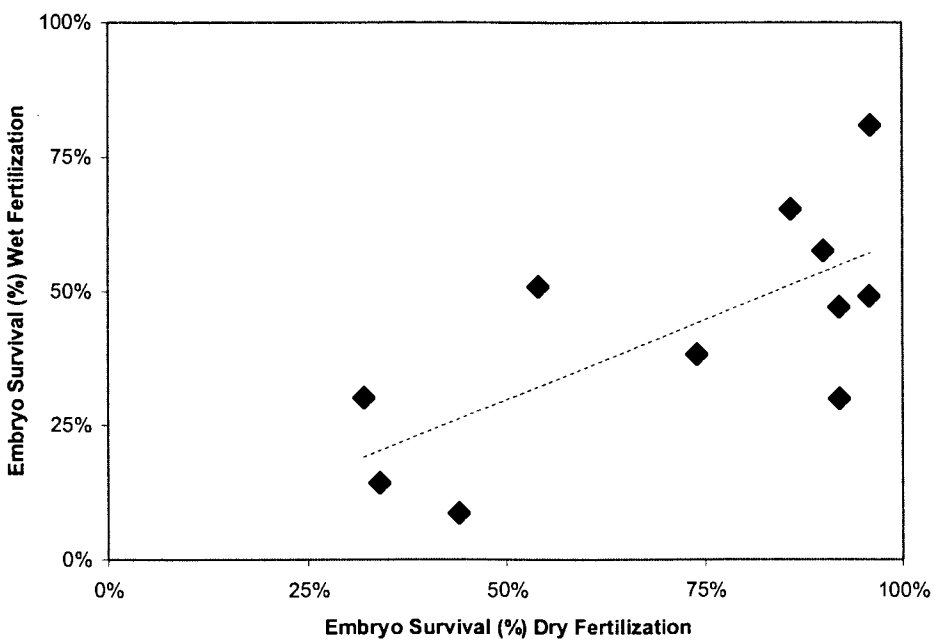
Figure 10: Repeatability of embryo survival (% eyed) separated post-fertilization, for eggs incubated in Kynoch Creek (circles) and Cultus Lake Laboratory (diamonds). [Experiment 3]



Experiment 4: Influence of Dry versus Wet Fertilization on Embryo Survival

Pair wise comparisons among the 11 unique pairings found that dry fertilization success was significantly related to wet fertilization success and was 60% higher on average (regression $P<0.05$; $R^2 = 0.50$, slope = 0.59). Therefore, dry fertilization success was a predictor of wet fertilization success (Figure 11), and can be used a surrogate for wild fertilization success.

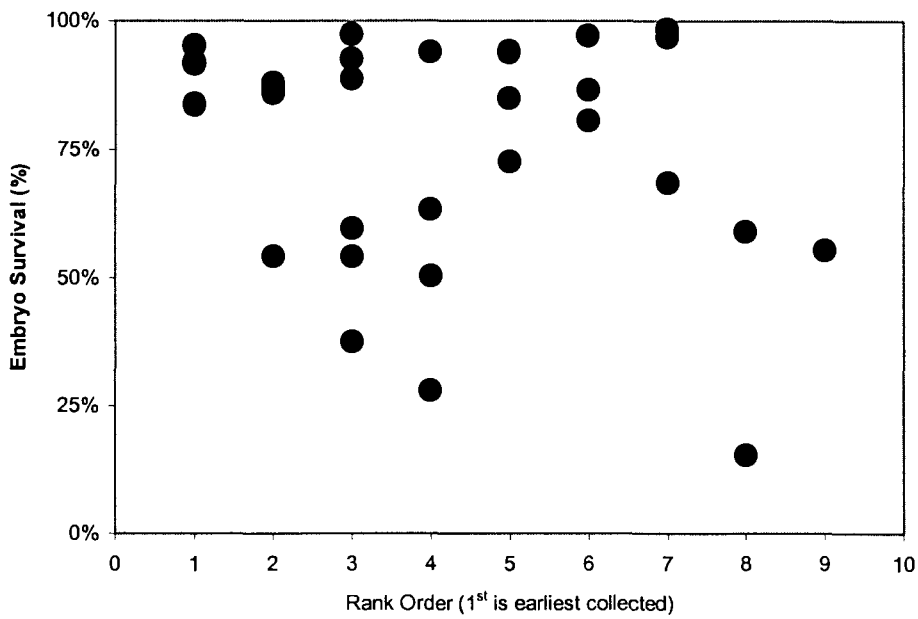
Figure 11: Based on 11 unique pairings dry fertilization success (% eyed) was a significant predictor (regression $P<0.05$) of wet fertilization success (% eyed). Each data point represents a single unique dam-sire combination. [Experiment 4]



Experiment 5. Influence of Collection order on Fertilization Success

There was no evidence of decline in gamete viability with time from collection (linear regression P>0.05). Contrary to the prediction, the first caught fish did not have lower fertilization success, and therefore, the timelines used to collect fish in the field experiment (within 4 hours) did not affect fertilization success (Figure 12).

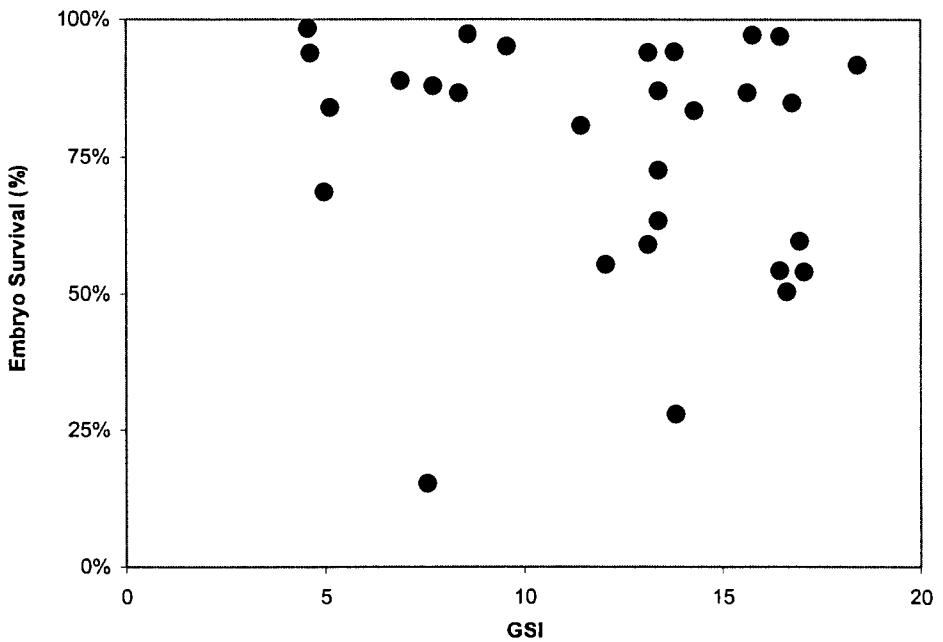
Figure 12: Collection order, based on rank order, did not influence (regression P>0.05) embryo survival (% eyed). Each value represents mean embryo survival for a single female crossed with three separate males. [Experiment 5]



Experiment 6: Influence of Ovulation Date on Fertility

There was no relationship between GSI and embryo survival for Kynoch 2000 crosses (Figure 13). Therefore, there is no evidence to suggest that time since ovulation was a confounding factor influencing embryo survival in this experiment (regression P>0.05).

Figure 13: Embryo survival (% eyed) for Early Stuart sockeye salmon sample at the spawning ground in 2000 was not related (regression P>0.05) to the time since ovulation, based on the relative egg mass at the time of sampling (GSI – ovary mass • total body mass -1). Each value represents mean embryo survival for a single female crossed with three males. [Experiment 6]



Individual Variation (Experiment 7)

In 10 of the 13 trials there was a significant maternal influence on embryo survival (GLM $P<0.05$; Table 4). Therefore, I must reject the original null hypothesis that stated the probability of particular embryo surviving is not based on the parental origin of the egg. Significant maternal effects were found for eggs from Early Stuart and Gates sockeye in both 1999 and 2000. Figure 14 provides a clear example of maternal influence on embryo survival for two Early Stuart trials incubated in both a natural (trial **E**; Fig.14a) and laboratory environment (trial **J**; Fig. 14b). A paternal influence on embryo survival was detected in 4 of the 12 trials (GLM $P<0.05$), 2 trials from Early Stuart and 2 Gates sockeye trials. For example, as shown in Figure 8, a significant paternal influence as Male Z was consistently lower than Male X, and Y (GLM $P<0.05$).

Figure 14: Mean embryo survival (% eyed) for unique pairings among Early Stuart sockeye in 2000 organized by incubation environment (a) trial **E** Kynoch creek and (b) trial **J** Cultus Lake Laboratory (see Table 4). Each data point represents the average embryo survival of different female crosses with 3 different males. There are significant maternal influences on embryo survival (GLM $P<0.05$), but not paternal (GLM $P>0.05$) in both trials. Common letters indicate no significant difference among means (Tukey test). Error bars, representing 1 SD, and indicate that overall variance is lower under controlled incubation conditions in CLL than natural incubation condition in Kynoch creek. [Experiment 7]

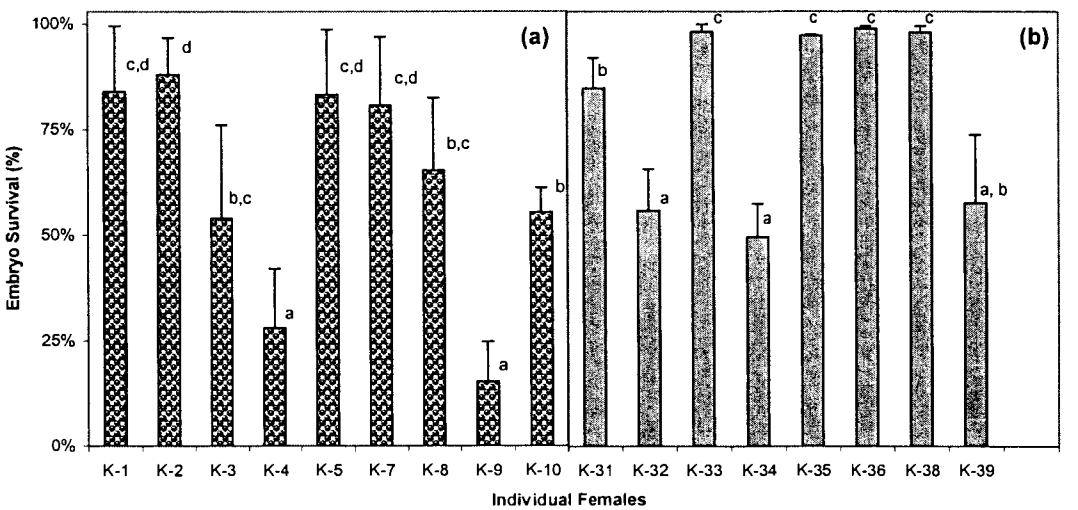


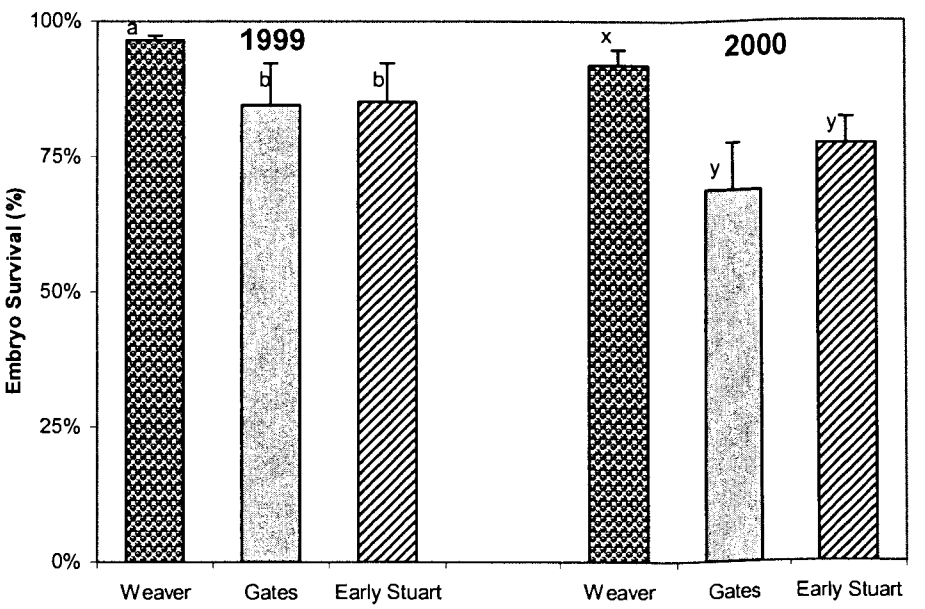
Table 4: Summary information for each trial including dam and sire sample sizes, collection dates and P-values for dam, sire, and incubation (redd) effects (GLM see text for details). The experiment column indicates which experiments (#'s match Material and Methods) the corresponding trial results were applied to.

Trial Code	Year	Stock	Date	Sample Location	Dams	Sires	Dam Effect P-Value	Sire Effect P-Value	Redd Effect P-Value	Experiment
A	1999	Early Stuart	14-Aug	Creek	8	3	**0.001**	**0.001**		1 & 7
B	1999	Early Stuart	15-Aug	Creek	8	3	0.059	0.704		7 & 8
C	1999	Gates	30-Aug	Channel	6	3	**0.002**	0.312		3, 7 & 8
D	1999	Weaver	13-Oct	Creek	8	3	0.378	0.207		1, 2, 7 & 8
E	2000	Early Stuart	4-Aug	Creek	9	3	**0.001**	0.481	**0.001**	2 to 8
F	2000	Early Stuart	5-Aug	Creek	7	3	**0.003**	0.328	0.800	2 to 8
G	2000	Early Stuart	5-Aug	Creek	3	3	**0.048**	n.a.	**0.020**	2 to 8
H	2000	Early Stuart	6-Aug	Creek	5	3	**0.001**	**0.001**		2, 5 to 8
I	2000	Early Stuart	7-Aug	Creek	3	3	**0.030**	0.701	**0.001**	2 to 8
J	2000	Early Stuart	8-Aug	Fence	8	3	**0.001**	0.171		2, 3, 5 to 8
K	2000	Gates	22-Aug	Channel	11	3	**0.003**	**0.001**		7 & 8
L	2000	Gates	22-Aug	Channel	3	3	**0.005**	**0.032**		7 & 8
M	2000	Weaver	12-Oct	Fence	8	3	0.219	0.275		7 & 8
				Totals	87	39				

Population Differences (Experiment 8)

There were significant difference in embryo survival among the three populations in both 1999 and 2000 (ANOVA $P<0.05$). In both years, the Weaver sockeye had significantly higher embryo survival than both Early Stuart and Gates sockeye (Tukey $P<0.05$; Figure 15). This result is consistent with original prediction that overall population viability will decrease with increased migratory difficulty.

Figure 15: Overall mean embryo survival (% eyed) for individual parents from Early Stuart, Gates, and Weaver sockeye salmon populations in 1999 and 2000. Common letters indicate no significant differences among population within a year (Tukey $P<0.05$) and the error bars represent 95% confidence intervals. [Experiment 8]



Discussion

Method Development

The assessment of the repeatability estimates for both the unique fertilizations and incubation environment clearly demonstrate some of the strengths and weaknesses of the methods. The strengths of the method include (a) the consistently high fertilization success with replicate crosses when overall fertilization success is high, and (b) the overall comparability in embryo survival rates between natural and laboratory environments. However, the ability to detect an individual difference in fertility was a function of the overall mean survival. A 10% reduction in survival is easier to detect when overall embryo survival exceeds 95%. Conversely, the power of detecting a 10% in fertilization success when the overall survival was 50% is lower due to the increase in variance. The variance in embryo survival rates is very much a function of incubation environment (Cope 1996). A recommendation is that sample sizes, including number of partners, number of fertilizations, and redd locations be increased if incubation is to be completed on the spawning grounds.

The lack of interaction between specific male-female pairs is consistent with work on other salmonids (Gile and Ferguson 1995; Hawkins and Foote 1998). It also supports conclusions from previous cross fertilizations with sockeye salmon that detected parental effects, but did not test specifically for possible interaction effects (e.g., Herunter et al. 2000; Hoysak and Liley 2001). In addition, the lack of interaction reduces the number

fertilizations necessary to determine individual differences in fertilization success. However, this result only applies to intrastock comparisons completed at the same location and time. It is still necessary to determine whether interaction plays a role with cross fertilizations among stocks.

The confidence in performing assessments of viability in naturally spawning populations is increased given the results from the dry versus wet fertilization and the lack of an effect of capture time on fertility. The fact dry fertilization rates can be used as a surrogate for a more natural wet fertilization technique reaffirms prior work and suggests that using a dry technique would be appropriate future work. Fertility rates did not change with capture order, despite some of the incumbent difficulties in capture time. These results are supported by Bencic et al. (2000) in that they did not detect a difference in sperm motility when stored in air over a 24 hour period, perhaps suggesting that the oxygenation used for the overnight transport may not have been necessary.

In contrast to Springate et al. (1984) and De Gaudemer and Beall (1998), I did not find a relationship between the inferred time since ovulation and egg viability. A plausible explanation is that average longevity on the spawning ground for a ripe female sockeye is approximate 8 days for Early Stuart sockeye (Healey et al. 2002). This is less than the 10-day period found necessary to see decline in eyed embryo survival below 90% (Springate et al. 1984). Therefore, in the case of healthy natural spawners, it is unlikely egg viability is dependent on time since ovulation.

Intergenerational Effects of Individuals

Differential embryo survival based on parental origin is clear evidence of an intergenerational effect. Understanding natural selection requires studying various factors that can cause changes in the genetic makeup of a population because of differential reproductive success (Dodson and Dodson 1985). This study has established differential offspring survival based on parental gamete origin and therefore intergenerational gene flow is affected. However, direct proof that the parental migration experience can cause changes in intergenerational gene flow is still lacking.

The documented male influence on gamete viability provides empirical support to the theoretical argument that female salmon may use multiple partners to mitigate against a particular male having low viability. The paternal effect on embryo survival is less pronounced if a highly potent male compensates for a poor quality sperm of others in single spawning event. At low population densities paternal effects could be more significant, contributing to dispensatory survival, because the number of potential multiple partners is likely an inverse function of the density of spawners and the overall sex ratio (Fleming 1998; Healey et al. 2002). The above inferences are based on the assumption that differences in male reproductive success in this study are reflective of differences in success at the fertilization event (not post-fertilization); a conclusion supported by Giles and Ferguson (1995) using rainbow trout.

A novel finding of this study is that both maternal and paternal gamete origin affected embryo survival in sockeye salmon. The first attempt to establish both maternal and

paternal influences on embryo survival in rainbow trout used 4X4 and 3X3 crosses (Nagler et al. 2000) and found only a maternal influence. In the present study, using 39 males and 87 females over 13 trials, only 4 of the trials detected a paternal influence, suggesting that previously the small sample sizes (10 males & 10 females – 3 trials) may have hampered the effort to reveal male influence on embryo survival. Moreover, the differences among females (8 of 13 trials) may seem more dramatic than differences among sires (4 of 12 trials), but twice as many females were tested. Future work could concentrate on applying the appropriate power analysis to determine the samples sizes required to detect parental effects. It should also be re-iterated that most of these crosses were incubated under optimal conditions. It is recommended that attempts be made to replicate crosses and incubate them at sub-optimal conditions to determine if interaction between environment and gamete origin occurs.

Population Differences

The lower overall embryo survival despite similar fertilization techniques for Early Stuart (81%) and Gates (77%) versus Weaver (94%) is consistent with the prediction that increased migratory difficulty of adults decreases embryo survival in sockeye salmon. Gates and Early Stuart adult sockeye, unlike Weaver sockeye, must negotiate the Fraser Canyon. In addition, particularly difficult migration conditions existed in the Fraser Canyon in 1999 and 2000, based on historical comparisons (see Table 1 and Figure 2b). While direct comparisons with the results of Macdonald et al. (2000) are difficult because of the dissimilarity in design (they used pooled gametes, a single dry fertilization, and a non-random selection of fish), the results from this study and the first attempt to compare

population level fertilization success in Fraser sockeye salmon are similar. They used a controlled mating design and chose to compare two populations of Fraser sockeye that had also experienced extreme migration conditions. They also made comparisons of embryo survival of Early Stuart sockeye for 1997 (second highest discharge on record) and 1998 (highest temperature year on record for all stocks) with Horsefly embryo survivals from 1998. The embryo survivals for all three groups were < 80%, which is similar to the present study embryo survival attained for Gates and Early Stuart sockeye. In addition, all values are less than the 90% survival that we now know is possible based on Weaver results.

The change in embryo survival associated with parental influence will alter both number and density of fry emerging in the spring. Any resulting density-dependent responses to fitness, such as dispensatory survival, would constitute a population level intergenerational effect. For an individual sockeye fry it is not easy to predict the consequences of changes in the density of conspecifics. For example, the lack of con-specifics could decrease fitness through processes such as density dependent predation (Cartwright et al. 1998), or increase fitness by greater foraging opportunities (Brio et al. 2003) and reduced oxygen demand within a redd during incubation (Einum et al. 2002).

The consistently high egg survival for Weaver sockeye (97% in 1999 and 92% in 2000) provided an important benchmark for future intergenerational studies at the population level. Based on these results, an egg viability < 90% could potentially be used a threshold to detect population level effects in gamete viability. A cautionary note is that

the overall sample sizes (11 to 55 individuals per population per year) is small in comparison to the total spawning populations (>30000; Cass 2003). Based on the average standard deviation for largest sample size group (SD = 18.5 % Early Stuart 2000), the population sample size required to detect a population level difference of 10%, at an β= 0.80 (power) and using 3 populations would be 67 individuals per population (Minitab Vers. 13).

In summary, the methodology developed here proved to be robust and can be used to assess gamete quality in sockeye salmon populations under natural and controlled laboratory environments. Establishing the evidence of individual and population level variation in embryo success lays the foundation for future work on intergenerational effects. Some of the previously unexplained large natural variation in interannual egg to fry survival can be attributed in part to the differential gamete viability of the contributing parents (Bradford 1995; Cope 1996). The next chapter will examine physiological condition of these same parents in relation to their offspring survival.

CHAPTER 3: MATERNAL CONDITION, EGG SIZE, AND OFFSPRING FITNESS IN SOCKEYE SALMON

Abstract

Using correlational analysis on a suite of physiological, reproductive and morphological parameters, no single parameter of maternal condition was found to be a good predictor of offspring survival. For three populations of Fraser River sockeye salmon large females produced large eggs, which in turn produced large hatchlings and fry. However, there was no correlation between initial egg mass and embryo survival for either laboratory (optimal) or natural (variable) incubation conditions. There was very little variation in relative egg composition among females and populations, and no single egg attribute was related to subsequent offspring survival or maternal condition. In addition, gamete viability assessment showed that individual moribund females, i.e. those that had failed to spawn successfully (high egg retention females), could produce viable offspring with survival rates comparable to active spawners in the population with artificial fertilization. In fact, relating migrational experience to maternal condition and then predicting which maternally transferred attributes relate to offspring survival still remains elusive. Adverse migration conditions have been linked to high levels of egg retention, or unsuccessful spawners. Historical analysis within populations revealed that high rates of egg retention were correlated with poor egg to fry survival in Early Stuart sockeye, that utilize natural spawning streams, but not in Weaver and Gates sockeye utilizing spawning channels. This suggests a possible interaction between poor egg equality and incubation environment that needs to be further explored at a more sophisticated level.

Introduction

Differential survival of embryos based on maternal gamete origin was a major conclusion from Chapter 2. This result provides strong evidence that a link may exist between maternal condition and offspring fitness. Maternal condition at the time of spawning is determined in part by the series of morphological, physiological and reproductive changes during their reproductive migration that salmon must undergo (Groot et al. 1995). At the same time, sockeye salmon must perform an upstream migration that can be extremely stressful (Macdonald et al. 2000; Macdonald 2000). The direct physiological effects of acute stress on salmonids have been well studied (Fagerlund et al. 1995), but individuals can vary in their response to a common environmental stressor (Schreck et al. 2001). The degree to which a senescing salmon will invest a limited energy supply to compensate for a migratory stressor will likely involve trade-offs with other physiological systems. However, it is not known whether these trade-offs involve changes in egg quality parameters that may ultimately affect offspring fitness. Therefore, the focus of this chapter is to explore the connection between the variation in maternal condition and offspring fitness.

Variation in egg size is the most common mechanism whereby variation among females will affect the phenotype of the offspring (Heath et al. 1999). Egg size has also become synonymous with egg quality, although this is rarely tested directly (Berg et al. 2001), and is commonly used as a surrogate for offspring fitness (Heath et al. 2003). Moreover, there is a strong suggestion that increase migratory difficulty can have a negative influence on the average population egg size in a given year (see Chapter 1). The results

from studies concerning egg size and survival to fry stage have been contradictory (e.g., van den Berghe and Gross 1989 & Quinn et al. 1995 versus Einum et al. 2002). However, most suggest that survival advantages for increase fry size are greater after fry emergence (West and Larkin 1987; Einum and Fleming 2000b), but this survival relationship maybe conditional on the environment the offspring encounter (Good et al. 2001).

The physical condition of female sockeye salmon ready to spawn varies considerably (Patterson et al. 2004). This is especially true in years sockeye salmon encounter difficult migration conditions (Macdonald et al. 2000; Macdonald 2000). Macdonald et al. (2000) speculated that poor maternal condition of Horsefly females in 1998 was the consequence of a migration that encountered high water temperatures. In addition, the group that experienced the highest temperatures and arrived early produced eggs with a lower hatching success than late arriving fish. Fish with extremely poor physical condition are classified as moribund and do not spawn successfully. Moribund females laden with eggs likely contribute to the PSM estimates. However, it is not known whether the egg viability of unspawned moribund females is comparable to healthy spawners. Thus differences in maternal condition and egg viability may indicate different energy allocation strategies within the population.

The maternal transfer of proteins, lipids, enzymes, hormones, antibodies, and other factors are essential to embryonic development (Brooks et al. 1997). Yet, very little is known about how yolk composition is influenced by environmental factors, with the

possible exception of diet (Wiegand 1996). Temperature for example is likely to influence lipid composition, especially membranes, which in turn can affect larval viability, but there is little information for fish (Wiegand 1996). There are some studies relating egg composition to fry survival (Olin and von der Decken 1990; Srivastava and Brown 1991; Washburn et al. 1990) and linking maternal condition to egg viability (Campbell et al. 1992, 1994). Moreover, the relative composition of protein or lipid content within an egg can vary significantly among families (Herunter et al. 2000; Berg et al. 2001), as does egg viability among maternal broodlines (Chapter 2). But I have found no comprehensive studies that have examined maternal condition, egg composition and viability in Pacific salmon.

There are two sources of stock assessment information on Fraser sockeye salmon, collected by DFO that can potentially be used to infer a connection between migration severity, maternal condition, and egg viability. PSM surveys are conducted annually on the spawning grounds, and indirectly assess maternal condition by examining the proportion of the population that did not successfully deposit eggs. High PSM has been correlated with adverse migration conditions (Gilhousen 1990). Therefore, I propose that PSM can be used as a surrogate for both poor maternal condition and adverse migrational experience on the population level. In addition to PSM estimates, annual assessments of egg to fry survival are also made on some Fraser sockeye populations, including Early Stuart, Gates, and Weaver. Therefore, it may be possible to correlate PSM with egg to fry survival estimates, and thus link maternal condition with offspring survival on an interannual basis.

In this chapter, I tested three null hypotheses. The first hypothesis is that female sockeye salmon with larger eggs will produce on average larger embryos. The second hypothesis is that smaller eggs have lower chance of survival. The third hypothesis is that moribund (unspawned) females at the spawning ground have lower egg viability than active spawners. In addition, I tested the following three predictions: The first prediction is that there are significant correlations between maternal condition and egg composition, and between maternal condition and embryo survival among individual spawning females; The second prediction is that population differences in overall maternal condition are related to the population differences in embryo survival; The third prediction is that high PSM does have an intergenerational affect by reducing egg to fry survival.

Materials & Methods

Assessment of Maternal Condition

The key parameters I chose to reflect the overall condition of spawning female salmon, based on Clarke and Hirano (1995), fall into the 5 main categories: body condition, stress, osmoregulation, reproductive hormones, and egg composition. Body condition is a general assessment of body size and energy status. Both variables may influence such factors as mate choice, site selection, and longevity on the spawning grounds (increase nest defence time) (Hendry 1998). Acute stress during ovarian development has been demonstrated to interfere in reproduction (Campbell et al. 1992; Schreck et al. 2001). Osmoregulatory state provides an integrated measure of physiological status of a fish's ability to maintain ionic homeostasis (Clarke and Hirano 1995). The preparedness of individual to spawn is in part determined by its' reproductive hormonal status (Donaldson 1990). However, it is not clear how the rapid hormonal changes that occur on the spawning grounds affect egg quality. Egg composition includes both individual egg size parameters and the constituent make-up of the eggs. Both factors have been implicated as possibly influencing offspring fitness (Olin and von der Decker 1990; Brooks et al. 1997).

The associated parameters for each category are listed in Table 5. For most of these parameters the analysis methods have been laid out in Chapter 1. Plasma lactate, glucose, and 17α, 20β dihydroxy-4-pregnen-3-one (17,20P), testosterone, and 11- keto testosterone were analyzed according to Donaldson et al. (2000). The methods for

analyzing plasma ions (Na^+, K^+, Cl^-) and osmolality followed (Farrell et al. 2001). Body constituent analysis and energy calculations were based on Higgs et al. (2000).

Egg Size, Embryo Size and Survival

The offspring survival for each maternal broodline is based on the same cross fertilizations used in Chapter 2. Ten individuals from each maternal broodline were randomly selected upon reaching the 100% hatch stage, and emergent fry stage. Fry emergence was estimated based on the number of thermal units accrued using Jensen et al. (1992) sockeye egg development model. Each fish was killed, blot dried and weighed to the nearest 0. 1 mg.

Moribund Females

In 1999, only 3 of the 30 female spawners assessed using comparable techniques had a mean offspring embryo survival less than 80% (Chapter 2). Therefore, it was deemed necessary to look for more extreme examples of unhealthy females to test whether egg viability could be compromised by maternal condition. In 2000, 3 moribund females from the Early Stuart and 5 from Weaver sockeye population were non-randomly selected as examples of poor condition females. Moribund fish were selected based on their lethargic non-aggressive behaviour, ease of capture, and unspawned condition. To determine that previous spawning had not taken place, fish were checked for both a full compliment of eggs and no evidence of digging behaviour (i.e., no erosion of ventral

fins). The moribund females from Weaver, but not Early Stuart, were sampled for the same suite of morphological and physiological variables as spawning fish.

Pre-Spawn Mortality & Egg to Fry Survival.

Annual assessments of PSM are made by Fisheries and Oceans stock assessment as part of their carcass recovery programs (Gilhousen 1990; Schubert and Fanos 1997). Each female carcass was examined for retention of eggs and scored as 100%, 50% or 0% spawned. PSM is a % estimate of the average amount of egg retention of all carcasses examined. Egg to fry survival is a % estimate that is calculated by dividing the estimated number of fry emigrating from the stream from the estimated total egg deposition. Egg deposition numbers are calculated by multiplying the number of females by the average population fecundity (see Chapter 1 for methods) by the percent success of spawn (100% - PSM). Therefore, egg deposition numbers have already been corrected for a reduction in fry production associated with PSM. Total fry emigration is based on standard mark recapture techniques (Macdonald et al. 1992). Historical data on PSM and egg to fry survival for Gates sockeye (1968 to 1999) and Weaver sockeye (1965 to 2000) are based on spawning channel values provided by Jeff Grout (DFO, Annacis Island). PSM and egg to fry survival data for Early Stuart sockeye (1988 to 1999) were provided for Kynoch, Forfar, and Gluskie Creeks by Keri Benner (DFO, Kamloops).

Data Analysis

Linear regression was used to determine the effect of egg size on embryo size at hatch and fry emergence. The effect of initial egg size on embryo survival to eyed stage was assessed using a linear regression for all eggs combined, as well as separate regressions for Gates eggs and Early Stuarts eggs incubated at CLL, and Early Stuart eggs incubated at Kynoch Creek. A single estimate of egg size, i.e. egg dry mass, was chosen because of the high multicollinearity among the different measures of egg size, egg dry mass, diameter, and egg wet mass (see Chapter 1).

The connection between maternal condition and egg composition in 2000 was assessed using correlation analysis. Correlation analysis was used to compare individual parameters of maternal condition and egg composition with egg survival. The maternal parameters that were significantly correlated with egg survival (no initial adjustment for experiment wise error rate) were then compared among the populations to determine if these results were consistent across stocks. In addition, principal component analysis (PCA) was run for both Gates and Early Stuart 2000 data. Weaver 2000 data were excluded because of the lack of maternal influence on embryo survival (see Chapter 2). PCA results were used to look for patterns of variable association to determine if embryo survival was associated with set of variables.

Population level differences in female condition were assessed to determine if differences in maternal condition are consistent with the pattern of population embryo survival found in Chapter 2 (see Figure 15). The prediction is that Early Stuart and Gates sockeye

would be more similar than Weaver, given that they both had significantly lower embryo survival (see Chapter 2). The effect of population on the individual maternal response variables within each of the 5 categories was examined using a MANOVA. Response variables were reduced within a category if they were highly correlated (i.e., $R > |0.8|$). Variable selection among the highly correlated variables was based in part on the PCA results. Single ANOVA's were used for each response variable if an overall population effect was established within a category (i.e., MANOVA $P<0.05$). This same statistical approach was used to determine if the maternal condition of a moribund female was different from a healthy spawner. Only Weaver females captured in 2000 were used for this analysis and each female was classified as either a Spawner or a Moribund fish. Survival of embryos from moribund females was compared to embryo survival from spawning females within their respective populations using an single ANOVA.

Results

Egg Size, Embryo Size and Survival

The initial egg dry mass was a significant predictor of embryo size at hatch ($P<0.05$; $R^2 = 0.97$) and fry size at emergence ($P<0.05$; $R^2 = 0.95$) (Figure 16). Therefore, I cannot reject the first hypothesis that maternal effect of egg mass was a good predictor of subsequent embryo size. However, for both populations, across both incubation conditions and among all females, egg size was not a significant predictor of embryo survival (all 4 regressions $P>0.05$; Figure 17), despite the wide range in egg size (21.4 mg to 45.7 mg) and embryo survival (15 to 99 %). Therefore, I reject the second hypothesis that egg size will influence embryo survival.

Figure 16: The positive relationship between mean initial egg dry mass (mg) and subsequent offspring size (mg wet mass) at (a) hatching and (b) fry emergence, organized by maternal broodlines (regressions $P<0.05$). Each value represents the average size value of 10 offspring at each stage for all three populations, Early Stuart (circles), Gates (square), and Weaver (diamonds) in 1999.

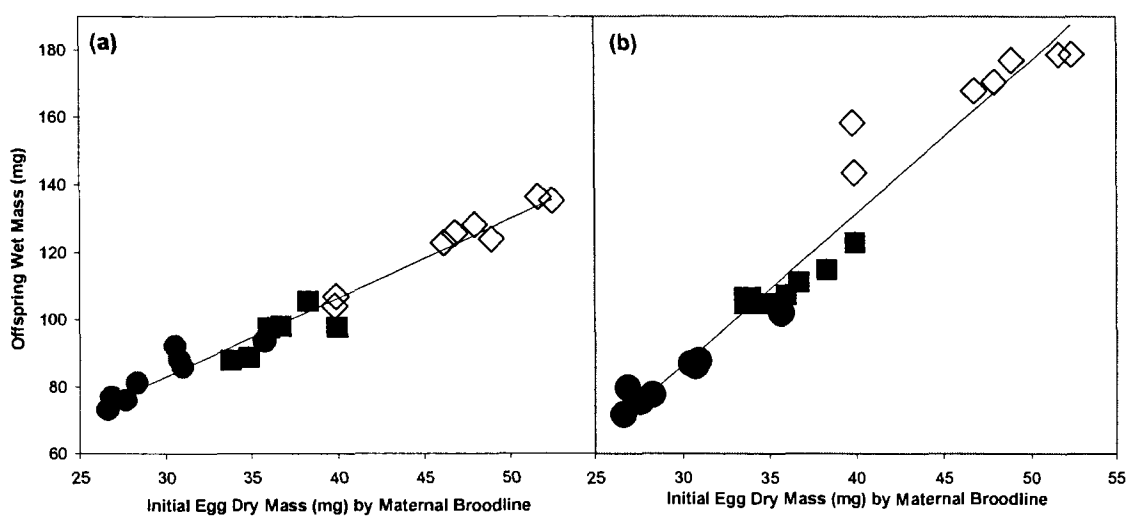
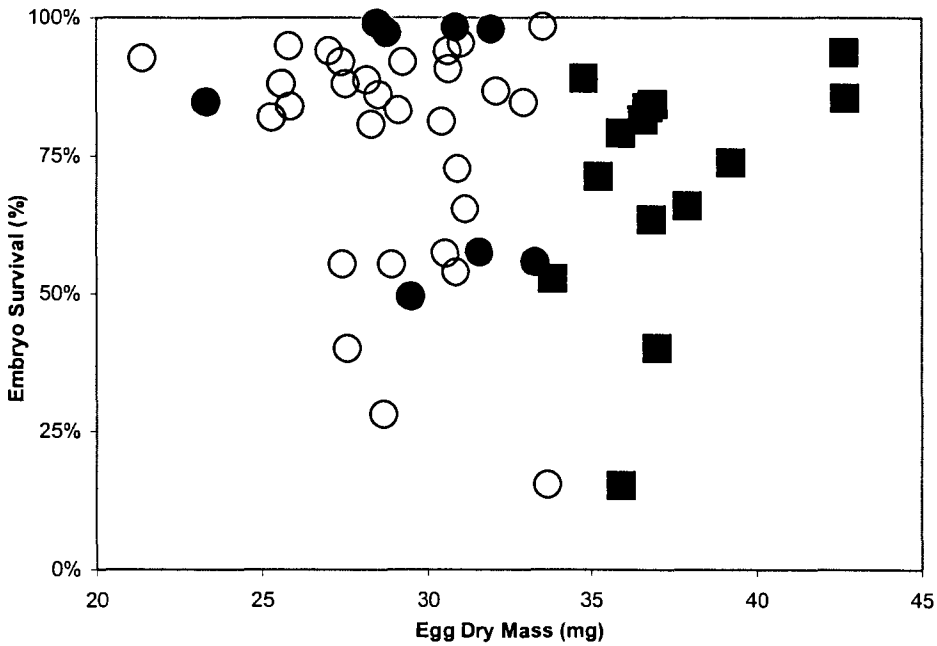


Figure 17: There was no relationship (all regressions P>0.05) between initial egg dry mass and embryo survival (% eyed) under natural incubation conditions, Early Stuart 2000 (hollow circles), and optimal conditions at Cultus Lake Laboratory for Early Stuart 2000 (solid circles) and Gates 2000 (squares). Each data point represents mean embryo survival for a single female crossed with 3 males.



Maternal Condition, Egg Composition, and Embryo Survival

Individual Level

With the exception of larger females producing on average larger eggs, there was no significant pair wise correlation between maternal condition and egg composition that was consistent across all populations. In addition, there was no single parameter of either maternal condition or egg composition that was a consistent predictor of embryo survival in all populations. The only significant correlations with embryo survival among the 36 variables were as follows: Early Stuart % ovary ash by wet mass (R = -0.41) and plasma potassium (K^+) (R = 0.48); Gates plasma cortisol (R = 0.71); Weaver % body ash (R = 0.81); and for all females combined plasma K^+ (R = 0.45). PCA results for both Gates

and Early Stuart confirmed that the variables within a major category will co-vary within a principal component. For example, the first principal component for Early Stuart sockeye loaded heavily on those variables associated with maturity and senescence (i.e., reproductive hormones, GSI, plasma ions) and the second principal component was associated with maternal size (i.e., SL, body mass, egg size, ovary mass). However, the relative loadings for embryo survival were low for the first 3 principal components in both populations. The PCA results support the pair wise results suggesting that the variables used in this study as surrogates of maternal condition in spawning females are not good predictors of embryo survival. Therefore, without any corroborating evidence from other populations, or other experiments, the few significant pair wise correlations between maternal condition and embryo survival will be treated a type I errors (Sokal and Rohlf 2000). This is contrary to the original prediction that maternal condition is correlated to offspring survival.

Population Differences in Maternal Condition

There were significant differences in maternal condition among the three populations with respect to body condition, osmoregulatory parameters, reproductive hormones and egg composition (all MANOVA's $P<0.05$). The only category that was not different were the stress parameters (MANOVA $P>0.05$). Table 3 indicates which univariates were significantly different among the populations (ANOVA $P<0.05$). Contrary to the original prediction of similarity among maternal parameters from Gates and Early Stuart females versus Weaver (based on Figure 15); there was no consistent pattern with respect to those univariates that were significantly different among the populations (Table 5).

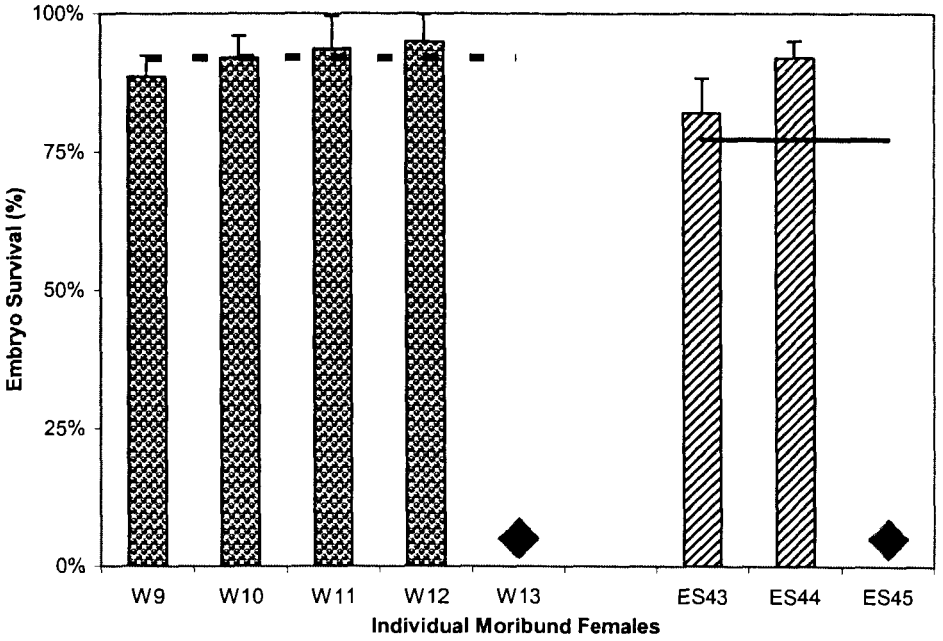
Table 5: List of categories (**bolded**) and associate parameters for maternal condition and egg size parameters. The mean values ± 1 SD for each spawning population, Early Stuart, Gates, and Weaver and for moribund females from Weaver, all collected in 2000. An "*" indicates a significant difference among the three populations for a given parameter, after first determining a significant MANOVA for the associated category. A "#" indicates a significant difference between Weaver spawners and Weaver moribund for a given parameter.

Categories / Parameters	Early Stuart	Gates	Weaver	Weaver Moribund	
Body Condition					
* Standard Length (cm)	53.8 ± 1.9	52.1 ± 2.1	56.4 ± 2.8	57.4 ± 3.1	
Whole Body Mass (g)	1952.4 ± 277.1	1964.4 ± 333.7	2903.5 ± 288.8	2881.2 ± 738.4	
Gonad Weight (g)	234.5 ± 93.9	291.6 ± 137.4	539.2 ± 68.7	413.0 ± 210.2	
Condition Factor - K	1.3 ± 0.1	1.4 ± 0.2	1.6 ± 0.1	1.5 ± 0.2	
GSI	12% ± 4.3	14% ± 0.1	19% ± 0.0	14% ± 0.1	
Body Constituents					
% Protein	16.3 ± 1.5	15.4 ± 1.0	15.8 ± 0.8	14.4 ± 0.7	#
* % Lipid	2.0 ± 0.7	2.7 ± 1.2	2.2 ± 0.5	1.6 ± 0.4	
% Moisture	79.6 ± 1.7	78.9 ± 1.7	79.3 ± 1.5	81.3 ± 1.3	
% Ash	2.5 ± 0.3	2.5 ± 0.3	2.4 ± 0.6	2.4 ± 0.6	
Energy (MJ • kg^{-1})	4.6 ± 0.5	4.7 ± 0.6	4.6 ± 0.3	4.0 ± 0.3	#
Stress					
Cortisol (ng • mL^{-1})	186.1 ± 103.8	130.2 ± 44.6	176.3 ± 55.3	355.3 ± 91.7	#
Lactate (mMol • L^{-1})	5.2 ± 3.8	5.2 ± 4.4	4.2 ± 1.2	10.2 ± 4.9	#
Glucose (mMol • L^{-1})	11.6 ± 5.9	10.9 ± 3.3	6.7 ± 2.0	7.1 ± 4.4	
Osmoregulation					
* Sodium (mMol • L^{-1})	129.8 ± 13.9	129.8 ± 11.3	142.8 ± 8.9	102.9 ± 12.2	#
* Potassium (mMol • L^{-1})	2.1 ± 1.5	2.3 ± 1.6	4.0 ± 1.3	4.2 ± 1.2	
* Chloride (mMol • L^{-1})	101.1 ± 15.7	107.3 ± 13.6	120.1 ± 8.5	80.5 ± 8.6	#
Osmolality (mMol • L^{-1})	275.9 ± 28.4	280.3 ± 19.0	296.0 ± 16.0	228.4 ± 33.3	#
Reproductive Hormones					
* Estradiol (ng • mL^{-1})	0.5 ± 0.3	1.3 ± 0.6	1.0 ± 0.5	0.9 ± 0.5	
* Testosterone	56.4 ± 30.6	54.8 ± 34.7	109.4 ± 31.5	24.9 ± 11.5	#
* 17,20bP (ng • mL^{-1})	69.1 ± 33.7	45.6 ± 34.1	83.7 ± 21.1	52.0 ± 28.3	#
* Vitellogenin (mg • mL^{-1})	4.9 ± 5.6	0.9 ± 0.7	4.4 ± 4.6	0.9 ± 0.6	
Egg Composition					
* Egg Dry Weight (mg)	29.6 ± 2.4	37.3 ± 2.6	47.2 ± 3.7	44.2 ± 9.4	
Egg Diameter (mm)	4.6 ± 0.2	5.0 ± 0.1	5.4 ± 0.2	5.4 ± 0.4	
Egg Wet Weight (mg)	79.6 ± 7.1	104.8 ± 6.5	125.3 ± 10.9	121.8 ± 21.7	
% Egg Moisture	62.8 ± 1.2	64.4 ± 1.7	62.3 ± 1.3	63.8 ± 2.7	
Egg Constituents					
% Protein	22.6 ± 2.0	23.3 ± 1.9	22.6 ± 0.9	21.9 ± 1.9	
% Lipid	11.1 ± 0.8	10.6 ± 1.2	10.9 ± 0.6	10.4 ± 0.8	
% Moisture	64.8 ± 2.3	64.7 ± 2.6	63.0 ± 1.6	65.2 ± 2.8	
% Ash	2.0 ± 0.4	2.5 ± 0.7	2.3 ± 0.3	1.9 ± 0.5	
Energy (MJ • kg^{-1})	9.7 ± 0.7	8.9 ± 2.7	9.6 ± 0.4	9.3 ± 0.8	
* Embryo Survival	76% ± 22%	70% ± 22%	92% ± 5%	92% ± 3%	
Sample Size	28	14	8	5	

Moribund Females

The six moribund females produced viable eggs and contrary to the original hypothesis, they had egg survivals that were comparable to healthy spawners (ANOVA $P<0.05$; Figure 18). Observations made prior to fertilization for the two moribund females that did not produce any viable offspring, indicated that these eggs had already showed signs of water hardening, possibly indicating over ripening (Barnes et al. 2003). The maternal condition of moribund females was significantly different than the healthy spawners for all categories (MANOVA $P<0.05$) except egg composition (MANOVA $P>0.05$) (Table 5). Moribund females had significantly less body energy (~ 0.6 MJ • kg^{-1}) than healthy spawners (ANOVA $P<0.05$). The indicators of acute stress, cortisol and lactate, were elevated in moribund fish (ANOVA $P<0.05$). Conversely, plasma levels of testosterone and Vtg levels were significantly depressed in moribund females, remained within the range of values seen in successfully spawning Early Stuart and Gates fish. In addition, in appears that moribund fish have lost their ability to maintain osmotic homeostasis as plasma osmolality, plasma Na^{+1} and Cl^{-1} ions were low (ANOVAs $P<0.05$). The univariate parameters of maternal condition are consistent with the behavioural observations that moribund females are in fact close to death, but there are no data to suggest that egg composition or viability was compromised.

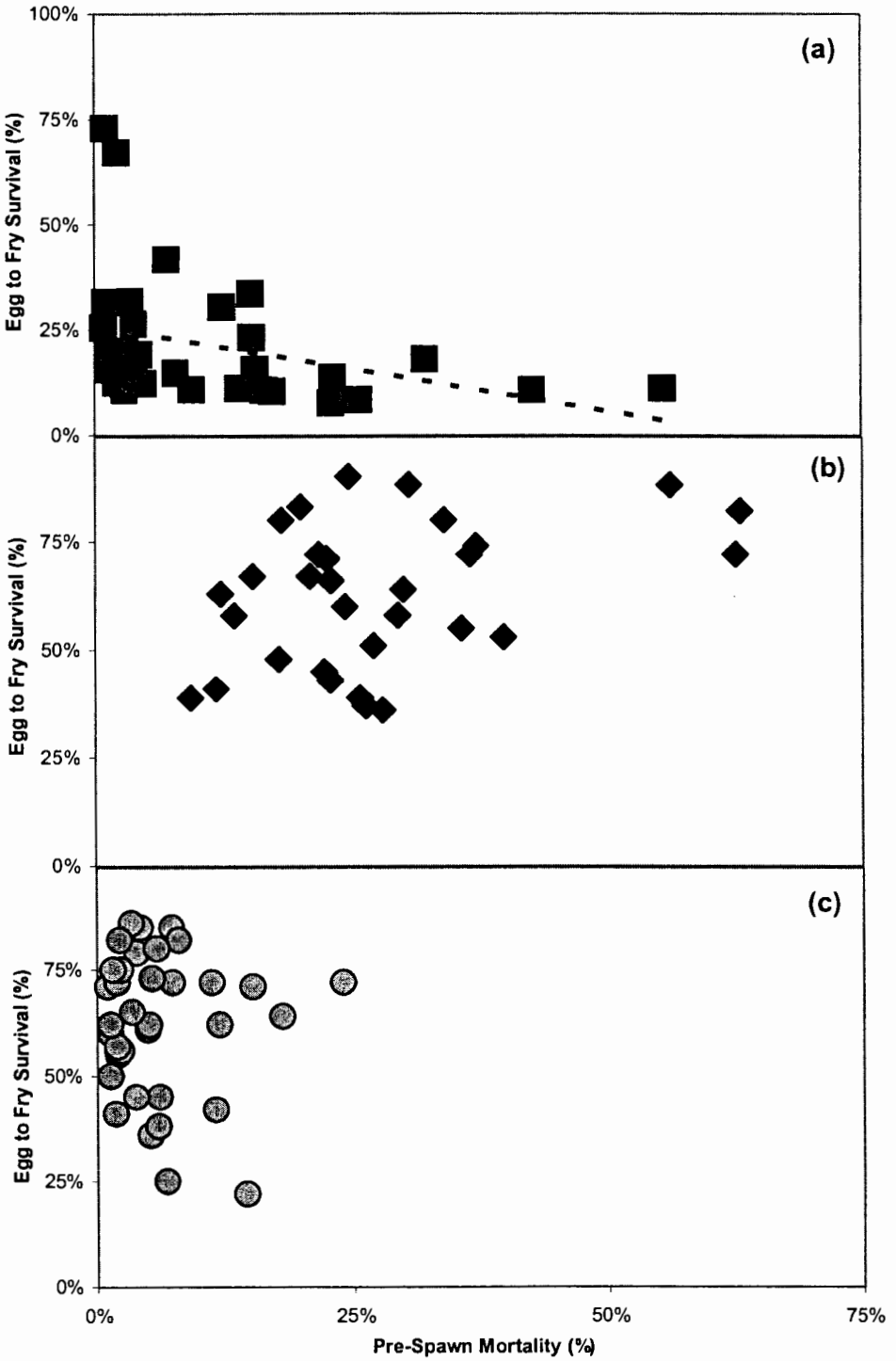
Figure 18: Embryo survival of individual moribund females from Weaver 2000 (bubbles) and Early Stuart 2000 (diagonal lines) contrasted against the population average for Early Stuart 2000 (——) and Weaver 2000 (- - - - -) spawners. Each column represents the average embryo survival to eyed stage of a moribund female crossed separately with 3 males. Errors bars are 1 SD. For female W13 and ES45 the diamonds represent zero survival, both females appeared to have water hardened eggs prior to fertilization.



PSM & Egg to Fry Survival

Egg to fry survival was negatively correlated with PSM in Early Stuart sockeye (regression $P<0.05$; Figure 19a), suggesting an intergenerational effect. In addition, Early Stuart sockeye over-winter survival ranged from 8% to 73% (mean = 21%), but PSM only explained 17% of the variance in survival. The mean over-winter survivals were higher for Gates (63%) and Weaver (62%) than Early Stuart, but there no indication that high PSM were correlated with low egg to fry survivals for either population (regressions $P>0.05$; Figure 19bc). Therefore, only the Early Stuart results are consistent with my original prediction that high PSM is indicative of condition that leads to an intergenerational effect, through reductions in egg to fry survival.

Figure 19: Pre-spawn mortality, used as a surrogate for adverse migrational experience, was a significant predictor of overall egg to fry survival for (a) Early Stuart sockeye (regression $P<0.05$) spawning in natural spawning environment but not for (b) Gates, and (c) Weaver sockeye spawning in controlled channels.



Discussion

Egg Size, Embryo Size, and Survival

The maternal effect on egg size and subsequent embryo size under a natural and hatchery incubation environment found in this study is compatible with earlier work on salmonids incubated in hatchery environments (Hutchings 1991; Berg et al. 2001). Sockeye salmon offspring phenotype, namely embryo size at hatch and emergence, was clearly influenced by the original amount of dry egg matter conferred by the mother. Therefore, any reduction in egg size associated with migration conditions (Chapter 1) will reduce offspring size at two ecologically important life history stages (Wootton 1998). I suggest that my surrogate of offspring fitness (offspring size) can be influenced by maternal migrational experience, but this does not test whether offspring size is in fact a good surrogate of offspring fitness.

I found no relationship between embryo size and embryo survival, this result was contrary to original hypotheses and some previous studies (Heath et al. 1999; Einum et al. 2002; however see Hutchings 1991). Equivocal results can arise because of small sample sizes and/or a lack of variation in either the predictor or response variable. In this study, samples sizes were large (51 females), there was large variation both within and among populations in egg mass, and in the large range in embryo survivals (see Figure 17). Therefore, I reject the hypothesis that smaller eggs have lower chance of survival during embryonic development. This would also imply that the theoretical fitness benefit or cost

associated with egg size is most likely to be conferred after fry emerge from the gravel (Hutchings 1991; Ojanguren et al. 1996).

Earlier, it was assumed that larger eggs suffered higher mortality under poor incubations environments due to an oxygen limitation associated with their lower surface to volume ratio compared with smaller eggs (Quinn et al. 1995). However, recent empirical evidence suggests that large eggs within a maternal broodline (n = 10) have higher survival under low oxygen conditions (Einum et al. 2002). Although they found a difference within a maternal broodline, it was not evident from the data presented that mean maternal broodline egg size was related to survival under low oxygen or normal oxygen levels. In this study, egg size conferred no survival advantage when eggs were reared in either a natural, variable oxygen environment (Cope 1996), or a well aerated laboratory incubation environment. Therefore, based on the results from this study and Einum et al. (2002), the oxygen limitation theory for individual egg survival should be re-evaluated. Some of the concerns are as follows: a) Most of the variation in embryo size pre-hatch is the result of differences in yolk mass (Beacham and Murray 1985), with a low oxygen demand, and not in the developing body tissue, with a high oxygen demand; b) Body tissue development occurs just below the surface of the egg where oxygen diffusion distances would be considerable less than the average calculated diffusion distance of a sphere; c) Larger embryos have less mass specific oxygen consumption than smaller embryos, based on allometric scaling coefficient of 0.44 (Einum et al. 2002).

Maternal Condition, Egg Composition, and Embryo Survival

Contrary to the original prediction, the energetic and physiological status of individual female spawners is not a good predictor of the ability to produce viable offspring. In fact, even moribund females, which are an extreme example of a poor maternal condition, were capable of producing viable eggs. These equivocal results concerning maternal condition and egg survival are consistent with many previous attempts to link maternal stress and egg viability (see review Brooks 1997; Schreck 2001). For example, mild stress applied to female rainbow trout (Contreras-Sanchez et al. 1998) and Atlantic salmon (Booth et al. 1995) at late stages of vitellogenesis did not result in a decrease in egg viability compared to untreated fish. In contrast, rainbow trout (Campbell et al. 1994) and sockeye salmon (Patterson et al. 2004) exposed to stress during late vitellogenesis had lower progeny survival than control groups. Although, all of these studies reported a significant stress response in the individual parents they did not correlate individual variation in maternal stress variables with progeny survival, with exception of Patterson et al. (2004). Patterson et al. (1994) found significant differences in progeny survival based on the parental gamete origin of captive sockeye salmon (stressed group), but as with this study the individual variability in progeny survival was not correlated with any morphological, physiological, or reproductive variable recorded for each fish.

It has been long recognized in aquaculture that the relative combination of lipid and proteins in broodstock diet formulations can influence maternal tissue composition, egg composition and subsequent egg survival in fish (Washburn et al. 1990; Izquierdo et al.

2001). However, in this study using wild sockeye salmon I did not find any correlation between maternal body composition (lipid and protein contents) and egg composition or between egg composition and embryo survival. These results are supported in part by previous work by using 17 female rainbow trout (Craik and Harvey 1984). They found significant differences in both the relative lipid and protein contents of eggs and in embryo survival, but they did not find any correlation between egg composition and viability. I recommend that future work assessing egg viability based on egg composition should include other factors that have been identified as potentially important to embryo survival, such as essential amino acids, fatty acids, enzymes, and hormones levels (Olin and Von Der Decken 1990; Brooks et al. 1997; Izquierdo et al. 2001). Moreover, individual egg composition analysis should also be performed to determine the range of variability within a female, because increased heterogeneity may be indicative of maternal stress (Contreras et al. 1998) and could have individual fitness consequences (Berg et al. 2001).

This study supports the claim that salmon will protect those systems required for successful reproduction, such as reproductive hormones, egg composition, and egg quality (Schreck et al. 2001; Patterson et al. 2004), potentially to the detriment of their own survival (i.e., PSM). For example, the reproductive hormone levels and egg composition of moribund females were similar to active spawners, even though moribund females would not have spawned successfully. There was also no difference in the % proximate egg composition of the three Fraser sockeye salmon populations, suggesting that egg quality is highly conserved even under conditions of natural stress (Patterson et

al. 2004). The high egg viability of some poor condition females provides empirical support for Schreck et al.'s (2001) conceptual model that describes the possible buffering mechanisms whereby mothers may protect their eggs in the face of environmental stress. The individual maternal plasticity in the ability to mitigate against stress (Schreck et al. 2001) is a possible source for some of the variation in maternal influence on embryo survival. Moreover, it may explain the lack of association at the individual level between particular maternal phenotype and embryo survival, as physiologically healthy and unhealthy mothers produced both good and poor quality eggs.

A novel aspect of this work is that it evaluated the energetic state, osmoregulatory function, stress response, reproductive hormonal status, egg composition, and egg viability for individual spawners. This information provides some of the first baseline values for the natural range of physiological and energetic states that are compatible with producing viable offspring in wild sockeye salmon (Table 5). In addition, the results from the integrative approach provide insight into the sequential decline of physiological systems as death approaches and may also be used determine physiological threshold values to predict successful spawning behaviour. For example, the energetic condition of active spawners from all populations was approximately 4.6 MJ • kg^{-1}, whereas moribund females had lower energy densities of 4.0 MJ • kg^{-1}. This results confirm a previous proposal that 4.0 MJ • kg^{-1} is the critical threshold value for separating successful from unsuccessful spawners (Crossin 2003) and supports previous work correlating energy expenditure to PSM (Gilhousen 1990). Similarly, the low plasma ions and osmolality values of moribund females could be used as threshold values to indicate the inability to

maintain osmoregulatory homeostasis. In contrast, the highly variable reproductive hormones levels and stress values seen in both active and moribund females, suggests that both categories would be poor choices for determining behavioural reproductive success. The high stress levels exhibited by all fish are consistent with final maturation and natural spawning being a stressful event (Kubokawa et al. 1999; Carruth et al. 2000), and reproductive hormones levels being conserved (Patterson et al. 2004).

Intergenerational Effect of PSM on Over Winter Survival

The reduction in over-winter embryo survival when parents likely experienced migrational difficulty in Early Stuart sockeye is an example of an intergenerational effect. In this case, the population dynamics of the next generation are influenced by the environmental experience of their parents, i.e. those females that were able to complete the migration and spawning were the only genetic contributors to future populations. If true, this is an empirical example of time-lag in populations dynamics created by a parental effect (Rossiter 1996), and therefore this information has the potential to be used in future stock recruitment estimates.

The connection between high PSM and low egg to fry survival found in the natural spawning population is likely based on both poor egg viability and an overestimate of successful egg deposition. The egg viability for Early Stuart active spawners that experienced two difficult migration years was approximately 10% lower (see Figure 15) than the 90% benchmark level for a healthy spawner postulated in Chapter 2. Egg deposition was likely overestimated because carcasses without any eggs are all assumed

to have been successful spawners, and it is possible that some of the fish (i.e., those with low energy) released eggs independent of any associated spawning act. In addition, females that did spawn may have insufficient energy to dig deep nests or defend their nests, increasing the likelihood of egg superimposition (Essington et al. 2000) and/or predation.

The lack of association between high PSM and low egg to fry survival for Gates and Weaver sockeye is consistent with the result that poor condition females produce viable eggs, and thus, no decrease in egg survival associated with parental condition would be expected. This would also imply that the association between PSM and egg to fry survival in Early Stuart sockeye was a spurious result. However, another plausible explanation involves the potential interaction that may exist between incubation environment and egg quality. The high overall survival estimates for the Gates (62%) and Weaver (61%) spawning channels, versus Early Stuart (21%), suggest that controlled spawning channel environments may mitigate against some of the mortality factors associated with natural spawning environments and/or low egg quality (Essington et al. 2000). For example, the incubation factors that might increase mortality, such as low oxygen content (Einum et al. 2002), high sediment levels (Chapman 1988), freezing (Cope and Macdonald 1998), increased predation (Foote and Brown 1998), poor water quality (Lacroix 1985), and egg superimposition (i.e., spawner densities) (Essington et al. 2000) can be controlled in spawning channels. Low quality eggs may have comparable survival to high quality eggs in controlled incubation environments, but not in less hospitable natural environments (Cope and Macdonald 1998; Heath et al. 2003). This

explains why PSM was not correlated to egg to fry survival under the better incubation conditions created in Gates and Weaver spawning channels. In addition, my original prediction hinged on the assumption that PSM was a good surrogate of environmental condition(s) experienced by the parents (Gilhousen 1990), this may not have been the case for Gates and Weaver sockeye. Unlike Early Stuart sockeye, previous attempts to correlate PSM with migration severity have not concentrated specifically on Gates and Weaver sockeye (Gilhousen 1990). The above explanations are not mutually exclusive, further analysis and future experimentation may help partition the large interannual variation in over winter egg to fry survival (>60%) exhibited in all three populations.

In summary, at the individual level the large degree of phenotypic plasticity in maternal condition is not matched with the differential survival of progeny, making it impossible predict embryo survival, even using the most extreme cases of poor maternal condition. However, at the population level, I found an association between the severity of the migratory experience of the parents and the % survival of the progeny for Early Stuart sockeye. Migratory stress may influence population level offspring fitness, but an attempt to find a causal mechanism by examining the predicted trade-offs in energy allocations within individuals has proved difficult. The phenotypic plasticity in maternal condition and egg viability that exist among individual sockeye and the incubation conditions may obfuscate any causal link between migratory stress and offspring fitness within an individual. In fact, if different individual strategies existed for energy allocation between maternal health and egg quality this would be consistent with the documented individual variation in both traits. I predict that future attempts to survey

population level differences in spawner condition and then link these differences to embryo survival will have a low probability of success, unless prior connections between maternal condition and egg composition, and between egg composition and embryo survival can be made at the individual level. At present we have no reliable estimate of future embryo survival based on the maternal health of spawners.

This study only looked at few measures of offspring fitness namely embryo survival, offspring number (or densities), egg size, and proximate egg composition. I recommend future assessments of different performance measures that may be associated with offspring fitness in sockeye salmon, such as, swimming performance (Bams 1967), metabolic capacities (Patterson et al. *In Press*), developmental stability (Campbell et al. 1998), and growth rates (Einum 2003).

CONCLUSION

In this thesis, I documented some clear examples of intergenerational effects in sockeye salmon. The main intergenerational effect at the population level was the change in the number of offspring produced and the associated change in intergenerational gene flow. Direct evidence for a change in offspring number is based on the interannual variation in fecundity (Chapter 1) and differential embryo survival (Chapter 2). Changes in offspring size were the main intergenerational effect for an individual progeny. Offspring size was affected by the original egg size (Chapter 3), which varied among generations (Chapter 1). I propose that these intergenerational effects were the direct result of migratory stress experienced by the parents, but this still needs to be determined experimentally. However to support this contention, I have summarized the major findings in this thesis. These results are organized by the 3 original questions posed in the introduction:

Chapter 1: Does migratory stress affect reproductive development?

- There was no supporting evidence to indicate that female sockeye can adjust egg size or fecundity during their upstream migration. In contrast, there was significant interannual variation in final reproductive investment that was correlated to migration conditions. These results were reconciled by proposing that selection against maternal phenotypes occurred en route.

Chapter 2: Does migratory stress affect gamete viability?

- The parental and population origin of gametes has a significant effect on embryo survival. The population with easiest migration conditions had the highest overall survival.

Chapter 3: Does migratory stress affect offspring fitness?

- The phenotypic variation in maternal condition did not correlate with offspring survival. However, years of high migratory stress, using PSM as a surrogate, were correlated with low offspring survival in the population with the most difficult migration experience, Early Stuart sockeye.

Based on the weight of evidence presented from the indirect approach used, I feel that the answer to all three questions is a conditional yes, and thus parental migratory stress does result in intergenerational effects. The answer is conditional in so far as the experimental design used could not directly test each question as a hypothesis. Future work should concentrate on directly manipulating the migratory conditions fish experience, while being aware of and controlling for the confounding effects that captive experiments may present (Patterson et al. 2004).

REFERENCES

Aas, G.H., Refstie, T., and Gjerde, B. 1991. Evaluation of milt quality in Atlantic salmon. Aquaculture, **95**: 125-132.

Ballantyne, J.S., Mercure, F., Gerrits, M.F., Van Der Kraak, G., McKinley, S., Martens, D.W., Hinch, S.G., and Diewert, R.E. 1996. Plasma nonesterified fatty acid profiles in male and female sockeye salmon, *Oncorhynchus nerka*, during the spawning migration. Can. J. Fish. Aquat. Sci. **53**: 1418-1426.

Bams, R.A. 1967. Differences in performance of naturally and artificially propagated sockeye salmon migrant fry, as measured with swimming and predation tests. J. Fish. Res. Bd. Can. **25**: 1117-1153.

Barnes, M.E., Sayler, W.A., Cordes, R.J., and R.P. Hanten. 2003. Potential indicators of egg viability in landlocked fall Chinook salmon spawn with or without the presence of overripe eggs. N. Am. J. Aquacult. **65**: 49-55.

Beacham, T.D., and Murray, C.B. 1985. Effect of female size, egg size, and water temperature on developmental biology of chum salmon (*Oncorhynchus keta*) from the Nitinat River, British Columbia. Can. J. Fish. Aquat. Sci. **42**: 1755-1765.

Beacham, T.D., and Murray, C.B. 1993. Fecundity and egg size variation in North American Pacific salmon (*Oncorhynchus*). J. Fish. Biol. **42**: 485-508.

Bencic, D.C., Krisfalusi, M., Cloud, J.G., and Ingermann, R.L. 2000. Short-term storage of salmonid sperm in air versus oxygen. N. Am. J. Fish. Manage. **62**: 19-25.

Benton, T.G., Ranta, E., Kaitala, V., and Beckerman, A.P. 2001. Maternal effects and the stability of population dynamics in noisy environments. J. Anim. Ecol. **70**: 590-599.

Berg, O. K., Hendry, A. P., Svendsen, B., Bech, C., Arnekleiv, J.V., and Lohrmann, A. 2001. Maternal provisioning of offspring and the use of those resources during ontogeny: variation within and between Atlantic Salmon families. Func. Ecol. **15**: 13-23.

Billard, R. 1981. Short-term preservation of sperm under oxygen atmosphere in rainbow trout (*Salmo gairdneri*). Aquaculture, **23**: 287-293.

Booth, R.K., J.D.Kieffer, K. Davidson, A. T. Bielak, and B. L. Tufts. 1995. Effects of late-season catch and release on anaerobic metabolism, acid-base status, survival, and gamete viability in wild Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **52**: 283-290.

Bradford, M.J. 1995. Comparative review of Pacific salmon survival rates. Can. J. Fish. Aquat. Sci. **52**: 1327-1338.

Bradford, M.J. and Irvine, J.R. 2000. Land use, fishing, climate change, and the decline of Thompson River, British Columbia, coho salmon. Can. J. Fish. Aquat. Sci. **57**: 13-16.

Brett, J.R. 1995. Energetics. *In* Physiological ecology of Pacific Salmon. *Edited by* C.L. Groot, L. Margolis and W.C. Clarke. UBC Press, Vancouver. pp 1-68.

Brett, J.R., and Glass, N.R. 1973. Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. J. Fish. Res. Board Can. **30**: 379-387.

Brio, P.A., Post, J.R., Parkinson, E.A. 2003. Density-dependent mortality is mediated by foraging activity for prey fish in whole-lake experiments. J. Anim. Ecol. **72**: 546-555.

Brooks, S., Tyler, C.R., and Sumpter, J.P. 1997. Egg quality in fish: what makes a good egg? Rev. Fish Biol. Fish. **7**: 387-416.

Burgner, R.L. 1991. Life history of sockeye salmon (*Oncorhynchus nerka*). *In* Pacific Salmon Life Histories. *Edited by* C. Groot and L. Margolis. UBC Press. Vancouver. pp. 1-118.

Campbell, P.M., Pottinger, T.G. and Sumpter, J.P. 1992. Stress reduces the quality of gametes produced by rainbow trout. Biol. Reprod. **47**: 1140-1150.

Campbell, P.M., Pottinger, T.G. and Sumpter, J.P. 1994. Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. Aquaculture, **120**: 151-169.

Campbell, W.B., Emlen, J.M. and Hershberger, W.K. 1998. Thermally induced chronic development stress in coho salmon: integrating measures of mortality, early growth, and developmental instability. Oikos, **81**: 398-410.

Carruth, L.L., Dores, R.M., Maldonado, T.A., Norris, D.O., Ruth, T. and Jones, R.E. 2000. Elevation of plasma cortisol during the spawning migration of landlocked kokanee salmon (*Oncorhynchus nerka kennerlyi*). Comp. Biochem. Physiol. C **127:** 123-131.

Cartwright, M.A., Beauchamp, D.A., and Bryant, M.D. 1998. Quantifying cutthroat (*Oncorhynchus clarki*) predation of sockeye salmon (*Oncorhynchus nerka*) fry using a bioenergetics approach. Can. J. Fish. Aquat. Sci. **55**: 1285-1295.

Cass, A. 2003. Pre-season run size forecasts for Fraser River sockeye and pink salmon in 2003. Can. Science Advice Secret. No. 2002-116.

Chapman, D.W. 1988. Critical review of variables used to define effects of fines in redds of large salmonids. Trans. Am. Fish. Soc. **117**: 1-21.

Clarke, W.C. and Hirano, T. 1995. Osmoregulation. *In* Physiological ecology of Pacific Salmon. *Edited by* C., Groot, L. Margolis and W.C. Clarke. University of British Columbia Press, Vancouver pp. 317-377.

Contreras-Sanchez, W.M., Schreck, C.B., Fitzpatrick, M.S., and Pereira, C.B. 1998. Effects of stress on the reproductive performance of rainbow trout (*Oncorhynchus mykiss*). Biol. Reprod. **58**: 439-447.

Cooke, S.J., Hinch, S.G., Farrell, A.P., Lapointe, M.F., Macdonald, J.S., Patterson, D.A., Healey, M.C., and Van Der Kraak, G. 2004. Abnormal migration timing and high

en route mortality of sockeye salmon in the Fraser River, British Columbia. Fisheries, **29**: 22-33

Cooper, A.C., and Henry, K.A. 1962. The history of Early Stuart sockeye run. Int. Pac. Salmon Fish. Comm. Prog. Rep. **10**.

Cope, R.S. 1996. Responses of sockeye salmon (*Oncorhynchus nerka*) embryos to intragravel incubation environments in selected streams within the Stuart-Takla watershed. M.Sc. thesis. University of British Columbia. Vancouver.

Cope, R.S., and Macdonald, J.S. 1998. Responses of sockeye salmon (*Oncorhynchus nerka*) embryos to intragravel incubation environments in selected streams within the Stuart-Takla watershed. *In* Forest-fish Conference: Land Management Practices Affecting Aquatic Ecosystems. *Edited by* M.K. Brewin, and D.M.A. Monita. Nat. Resour. Can. Inf. Rep. NOR-X-**356**: pp.283-294.

Craik, J.C.A., and Harvey, S.M. 1984. Egg quality in rainbow trout: the relation between egg viability, selected aspects of egg composition, and time of stripping. Aquaculture, **40**: 115-134.

Crossin, G.T. 2003. Effects of ocean climate and upriver migratory constraints on the bioenergetics, fecundity, and morphology of wild, Fraser River salmon. M.Sc. thesis, University of British Columbia, Vancouver.

Crossin, G.T., Hinch, S.G., Farrell, A.P., Whelly, M.P., and Healey, M.C. 2003. Pink salmon (*Oncorhynchus gorbuscha*) migratory energetics: response to migratory difficulty and comparisons with sockeye salmon (*Oncorhynchus nerka*). Can. J. Zool. **81**: 1986-1995.

De Gaudemer, B., and Beall, E. 1998. Effects of overripening on spawning behaviour and reproductive success of Atlantic salmon females spawning in a controlled flow channel. J. Fish. Biol. **53**: 434-446.

Dodson, E.O., and Dodson, P. 1985. Evolution process and product. Wadsworth Inc. Belmont, California.

DFO. 2003. Cultus Lake sockeye salmon. Sci. Advis. Sec. Stock Status Rep. 2003/024.

Donaldson, E. M. 1990. Reproductive indices as measures of the effects of environmental stressors in fish. Am. Fish. Soc. Symp. **8**: 109-122

Donaldson, E.M., Smith, J., Barnes, D., Clarke, W.C., Gordon, R., and Martens, D. 2000. Physiological and endocrine changes during the anadromous migration of Early Stuart sockeye salmon (*Oncorhynchus nerka*) in 1997. *In* Mortality during the migration of Fraser River sockeye salmon (*Oncorhynchus nerka*): a study of the effect of ocean and river environmental conditions in 1997. Edited by J.S. Macdonald. Can. Tech. Rep. Fish. Aquat. Sci. **2315:** pp.67-90.

Dye, H.M., Sumpter, J.P., Fagerlund, U.H.M., and Donaldson, E.M. 1986. Changes in reproductive parameters during the spawning migration of pink salmon, *Oncorhynchus gorbuscha* (Walbaum). J. Fish. Biol. **29**: 167-176.

Einum, S. 2003. Atlantic salmon growth in strongly food-limited environments: effects of egg size and paternal phenotype? Environ. Biol. Fish. **67**: 263-268.

Einum, S., and Fleming, I.A. 2000a. Highly fecund mothers sacrifice offspring survival to maximize fitness. Nature, **405**: 565-567.

Einum, S., and Fleming, I.A. 2000b. Selection against late emergence and small offspring size in Atlantic salmon (*Salmo salar*). Evolution, **54**: 628-639.

Einum, S., and Fleming, I.A. 2002. Does within-population variation in fish egg size reflect maternal influences on optimal values? Am. Nat. **160**: 756-765.

Einum, S., Hendry, A.P., and Fleming, I.A. 2002. Egg size evolution in aquatic environments: does oxygen availability constrain size? Proc. R. Soc. Lond. B **269**: 2325-2330.

Elgar, M.A. 1990. Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. Oikos, **59**: 283-287.

Essington, T.E., Quinn, T.P., and Ewert, V.E. 2000. Intra- and inter-specific competition and the reproductive success of sympatric Pacific salmon. Can. J. Fish. Aquat. Sci. **57**: 205-203.

Fagerlund, U.H.M., McBride, J.R., and Williams, I.V. 1995. Stress and tolerance. *In* Physiological Ecology of Pacific Salmon. *Edited by* C. Groot, L. Margolis, L. and W.C. Clarke. UBC Press, Vancouver. pp. 459-503.

Farrell, A.P., Gallaugher, P.E. and Routledge, R. 2001. Rapid recovery of exhausted adult coho salmon after commercial capture by troll fishing. Can. J. Fish. Aquat. Sci. **58**: 2319-2324.

Fleming, I.A. 1998. Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. Can. J. Fish. Aquat. Sci. **55**(Suppl. 1): 59-76.

Fleming, I.A., and Ng, S. 1987. Evaluation of techniques for fixing, preserving, and measuring salmon eggs. Can. J. Fish. Aquat. Sci. **44**: 1957-1962.

Fleming, I.A., and Gross, M.R. 1990. Latitudinal clines: a trade-off between egg number and size in Pacific salmon. Ecology, **71**: 1-11.

Foote, C.J., and Brown, G.S. 1998. Ecological relationships between freshwater sculpins (genus *Cottus*) and beach-spawning sockeye salmon (*Oncorhynchus nerka*) in Iliamna Lake, Alaska. Can. J. Fish. Aquat. Sci. **55**: 1524-1533.

Foote, C.J., Brown, G.S., and Wood, C.C. 1997. Spawning success of males using alternative mating tactics in sockeye salmon, *Oncorhynchus nerka*. Can. J. Fish. Aquat. Sci. **54**: 1785-1795.

Galbraith, R.V. 2003. Suspended sediment effects on fertilization success in Pacific Salmon (*Oncorhynchus spp.*). MSc. Thesis. Simon Fraser University, Burnaby.

Gile, S.R., and Ferguson, M.M., 1995. Factors affecting male potency in pooled gamete crosses of rainbow trout, Oncorhynchus mykiss. Environ. Biol. Fishes, **42**: 267-275.

Gilhousen, P. 1980. Energy sources and expenditures in Fraser River sockeye salmon during their spawning migration. Int. Pac. Salmon. Fish. Comm. Bull. **22**.

Gilhousen, P. 1990. Prespawning mortalities of sockeye salmon in the Fraser River system and possible causes. Int. Pac. Salmon. Fish. Comm. Bull. **26**.

Gilhousen, P. 1992. Estimation of Fraser River sockeye escapements from commercial harvests data, 1892-1944. Int. Pac. Salmon Fish. Comm. Bull. **27**.

Good, S.P., Dodson, J.J., Meekan, M.J., and Ryan, D.A.J. 2001. Annual variation in size-selective mortality of Atlantic salmon (*Salmo salar*) fry. Can. J. Fish. Aquat. Sci. **58**:1187-1195.

Groot, C., and L. Margolis (*Editors*). 1991. Pacific Salmon Life Histories. UBC Press, Vancouver.

Groot, C. L. Margolis, and W.C. Clarke (*Editors*). 1995. Physiological ecology of Pacific Salmon. UBC Press Vancouver.

Hawkins, D.K., and Foote, C.J. 1998. Early Survival and development of coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead (*Oncorhynchus mykiss*), and reciprocal hybrids. Can. J. Fish. Aquat. Sci. **55**: 2097-2104.

Healey, M.C. 1986. Optimum size and age at maturity in Pacific salmon and effects of size-selective fisheries. *In* Salmonid age at maturity. *Edited by* D.J. Meerburg. Can. Spec. Publ. Fish. Aquat. Sci. **89**: 39-52.

Healey, M.C., Lake, R., and Hinch, S.G. 2002. Energy expenditures during reproduction by sockeye salmon (Oncorhynchus nerka). Behaviour, **140:** 161-182.

Heath D.D. and Blouw, D.M. 1998. Are maternal effects in fish adaptive or merely physiological side effects? *In* Maternal Effects as Adaptations. *Edited by* T.A. Mousseau and C.W. Fox. Oxford University Press, Oxford. pp. 178-201.

Heath, D.D., Fox, C.W., and Heath, J.W. 1999. Maternal effects on offspring size: variation through early development of chinook salmon. Evolution, **53**: 1605-1611.

Heath, D.D., Heath, J.W., Bryden, C.A., Johnson, R.M., and Fox, C.W. 2003. Rapid evolution of egg size in captive salmon. Science, **299**: 1738-1740.

Hendry, A.P. 1998. Reproductive energetics of Pacific salmon: strategies, tactics, and trade-offs. Ph.D. Thesis, University of Washington, Seattle.

Hendry, A.P., and Berg, O.K. 1999. Secondary sexual characters, energy use, senescence, and the cost of reproduction in sockeye salmon. Can. J. Zool. 77: 1663-1675.

Hendry, A.P., Day, T., and Cooper, A.B. 2001. Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. Am. Nat. **157**: 387-407

Herunter, H. E., Pedersen, L. F., Patterson, D.A., and Galbraith, R.V. 2000. Early Stuart sockeye salmon (*Oncorhynchus nerka*) egg quality and egg-to-alevin survival for the 1997 escapement. *In* Mortality during the migration of Fraser River sockeye salmon (*Oncorhynchus nerka*): a study of the effect of ocean and river environmental conditions in 1997. *Edited by* J.S. Macdonald. Can. Tech. Rep. Fish. Aquat. Sci. **2315**: pp. 105-116.

Higgs, D.A., Macdonald, J.S., Levings, C.D., and Dosanjh, B.S. 1995. Nutrition and feeding habits in relation to life history stage. *In* Physiological ecology of Pacific Salmon Edited by C. Groot, L. Margolis, and W.C. Clarke. UBC Press, Vancouver. pp. 159-315.

Higgs, D.A., Rowshandeli, M., Greig, M., Barnes, D.P. and Macdonald, J. S. 2000. Changes in the proximate constituents and gross energy in the muscle (fillet) of 4-year old Early Stuart sockeye salmon (*Oncorhynchus nerka*) during the 1997 upstream Fraser River spawning migration. *In* Mortality during the migration of Fraser River sockeye salmon (*Oncorhynchus nerka*): a study of the effect of ocean and river environmental conditions in 1997. *Edited by* J.S. Macdonald. Can. Tech. Rep. Fish. Aquat. Sci. **2315**. pp. 91-104.

Hinch, S.G. and Rand, P.S. 1998. Swim speeds and energy use of upriver-migrating sockeye salmon (*Oncorhynchus nerka*): role of local environment and fish characteristics. Can. J. Fish. Aquat. Sci. **55:** 1821-1831.

Hinch, S.G., Standen, E.M., Healey, M.C., and Farrell, A.P. 2002. Swimming patterns and behaviour of upriver-migrating adult pink salmon (*Oncorhynchus gorbuscha*) and sockeye (*O. nerka*) salmon as assessed by EMG telemetry in the Fraser River, British Columbia, Canada. Hydrobiologia, **483**: 147-160.

Hodgson, S., and Quinn, T.P. 2002. The timing of adult sockeye salmon migrations into fresh water: adaptations by populations to prevailing thermal regimes. Can. J. Zool. **80**: 542-555.

Hoffman, G.R. 1991. Genetic toxicology. *In* Casarett and Doull's Toxicology: The Basic Science of Poison. *Edited by* M.O. Amdur, J. Doull, and C.D. Klaassen. Pergamon Press, New York. pp. 201-225.

Høgåsen, R. H. 1998. Physiological changes associated with the diadromous migration of salmonids. Can. Spec. Publ. Fish. Aquat. Sci. **127**.

Hoysak, D.J., and Liley, N.R. 2001. Fertilization dynamics in sockeye salmon and a comparison of alternative male phenotypes. J. Fish. Biol. **58**: 1286-1300.

Hutchings, J.A. 1991. Fitness consequences of variation in egg size and food abundance in Brook trout *Salvelinus fontinalis.* Evolution, **45:** 1162-1168.

Hutchings, J.A. 1996. Adapative phenotypic plasticity in brook trout, *Salvelinus fontinalis*, life histories. Ecoscience, **3**: 25-32.

Idler, D.R., and Clemens, W.A. 1959. The energy expenditures of Fraser River sockeye salmon during the spawning migration to Chilko and Stuart Lakes. Int. Pac. Salmon Fish. Comm. Prog. Rep. **6**.

Izquierdo, M.S., Fernandez-Palacios, H., and Tacon, A.G.J. 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture, **197**: 25-42.

Jain, K.E., Birtwell, I.K., and Farrell, A. P. 1998. Repeat swimming performance of mature sockeye salmon following a brief recovery period: a proposed measure of fish health and water quality. Can. J. Zool. **76**: 1488-1496.

Jensen, J.O.T., and Alderdice, D.F. 1984. Effect of temperature on short-term storage of eggs and sperm of chum salmon (*Oncorhynchus keta*). Aquaculture, **37**: 251-265.

Jensen, J.O.T., and Alderdice, D.F. 1989. Comparison of mechanical shock sensitivity of eggs of five Pacific salmon (*Oncorhynchus*) species and steelhead trout (*Salmo gairdneri*). Aquaculture, **78**:163-181.

Jensen, J.O.T., McLean, W.E., Rombough, P.J., and Septav, T. 1992. S.I.R.P. user manual. Salmonid incubation rearing programs for IBM compatible microcomputers. Can. Tech. Report. Fish. Aquat. Sci. **1878**.

Jonsson, N., and Jonsson, B. 1997. Energy allocation in polymorphic Brown trout. Funct, Ecol. **11**: 310-317.

Jonsson, N., and Jonsson, B. 1999. Trade-off between egg mass and egg number in brown trout. J. Fish. Biol. **55**: 767-783.

Jonsson, N., Jonsson, B., and Fleming, I.A. 1996. Does early growth cause a phenotypically plastic response in egg production of Atlantic salmon? Funct. Ecol. **10**: 89-96.

Jonsson, N., Jonsson, B., and Hansen, L.P. 1997. Changes in proximate composition and estimates of energetic costs during upstream migration and spawning in Atlantic salmon *Salmo salar*. J. Anim. Ecol. **66**: 425-436.

Ketola, H.G., Bowser, P.R., Wooster, G.A., Wedge, L.R., and Hurst, S.S. 2000. Effects of thiamine on reproduction of Atlantic salmon and a new hypothesis for their extirpation in Lake Ontario. Trans. Am. Fish. Soc. **129**: 607-612.

King, H.R., Pankhurst, N.W., Watts, M., and Pankhurst, P.M. 2003. Effect of elevated summer temperatures on gonadal steroid production, vitellogenesis and egg quality in female Atlantic salmon. J. Fish. Biol. **63**: 153-167.

Kinnison, M.T., Unwin, M.J., Hendry, A.P., and Quinn, T.P. 2001. Migratory costs and the evolution of egg size and number in introduced and indigenous salmon populations. Evolution, **55:** 1656-1667.

Kubokawa, K., Watanabe. T., Yoshioka, M., and Iwata, M. 1999. Effects of acute stress on plasma cortisol, sex steriod and glucose levels in male and female sockeye salmon during the breeding season. Aquaculture, **172**: 335-349.

Lacroix, G.L. 1985. Survival of eggs and alevins of Atlantic salmon (*Salmo salar*) in relation to the chemistry of interstitial water in redds in some acidic streams of Atlantic Canada. Can. J. Fish. Aquat. Sci. **42**: 292-299

Lee, C.G., Devlin, R.H., and Farrell, A.P. 2003a. Swimming performance, oxygen consumption in adult transgenic and ocean-ranched coho salmon (*Oncorhynchus kisutch*). J. Fish. Biol. **62**: 753-766.

Lee, C.G., Farrell, A.P., Lotto, A., Hinch, S.G., and Healey, M.C. 2003b. Excess post-exercise consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon following critical speed swimming. J. Exp. Biol. **206**: 3253-3260.

Lee, C.G., Farrell, A.P., Lotto, A., MacNutt, M.J., Hinch, S.G., and Healey, M.C. 2003c. The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon stocks. J. Exp. Biol. **203**: 3239-3251.

Liley, N.R., Tamkee, P., Tsai, R., and Hoysak, D.J. 2002. Fertilization dynamics in rainbow trout (*Oncorhynchus mykiss*): effect of male age, social experience, and sperm concentration and motility on in vitro fertilization. Can. J. Fish. Aquat. Sci. **59**: 144-152.

Linley, T.J. 1993. Patterns of life history variation among sockeye salmon (*Oncorhynchus nerka*) in the Fraser River, British Columbia. Ph.D. thesis, University of Washington, Seattle. Thesis No. 41086.

Lozano, G.A., and Ydenberg, R.C. 2002. Transgenerational effects of maternal immune challenge in tree swallows (*Tachycineta bicolor*). Can. J. Zool. **80**: 918-925.

Macdonald, J.S. (*Editor*). 2000. Mortality during the migration of Fraser River sockeye salmon (*Oncorhynchus nerka*): a study of the effect of ocean and river environmental conditions in 1997. Can. Tech. Rep. Fish. Aquat. Sci.. **2315**.

Macdonald, J.S., Scrivener, J.C., and Smith, G. 1992. The Stuart-Takla Fishery/Forestry Interaction Project: Study Description and Design. Can. Tech. Rep. Fish. Aquat. Sci. **1899**.

Macdonald, J.S., Foreman, M.G.G., Farrell, T., Williams, I.V., Grout, J., Cass, A., Woodey, J.C., Enzenhofer, H., Clarke, W.C., Houtman, R., Donaldson, E.M., and Barnes, D. 2000. The influence of extreme water temperatures on migrating Fraser River sockeye salmon (*Oncorhynchus nerka*) during the 1998 spawning season. Can. Tech. Rep. Fish. Aquat. Sci. **2326**.

Macdonald, J.S., Guthrie, I.C., and Patterson, D.A. *In Revision*. Models to adjust spawning escapement targets for Fraser River sockeye salmon (*Oncorhynchus nerka*) to compensate for the influence of environmental conditions on migration success. Submitted to Can. J. Fish. Aquat. Sci.

McGurk, M.D. 2000. Comparison of fecundity-length-latitude relationships between nonanadromous (kokanee) and anadromous sockeye salmon (*Oncorhynchus nerka*). Can. J. Zool. **78**: 1791-1805.

Mehranvar, L., Healey, M.C., Farrell, A.P., and Hinch, S.G. *Submitted.* Social versus genetic makers of reproductive success in sockeye salmon, Oncorhynchus nerka. *Submitted to* Evolutionary Ecology Research.

Minitab. Ver. 13. Stats Software. 2000. Minitab Inc. USA.

Morita, K, Yamamoto, S., Taksahima, Y., Matsuishi, T., Kanno, Y., and Nishimura, K. 1999. Effect of maternal growth history on egg number and size in wild white-spotted char (*Salvelinus leucomaenis*). Can. J. Fish. Aquat. Sci. **56**: 1585-1589.

Morrison, J., Quick, M.C., and Foreman, M.G.G. 2002. Climate change in the Fraser River watershed: flow and temperature predictions. J. Hydrology. **263**: 230-244.

Mousseau, T.A.M., and Fox, C.W. (*Editors*) 1998. Maternal effects as adaptations. Oxford University Press. Oxford.

Nagler, J.J., Parsons, J.E., and Cloud, J.G. 2000. Single pair mating indicates maternal effects on embryo survival in rainbow trout, *Oncorhynchus mykiss*. Aquaculture, **184**: 177-183.

Northcote, T.G., and Larkin, P.A. 1989. The Fraser River: A major salmonine production system. *In* Proceedings of the International Large River Symposium. *Edited by* D.P. Dodge. Can. Spec. Publ. Fish. Aquat. **106**: 172-204.

Novales Flamarique, I. and Harrower, W.L. 1999. Mortality of sockeye salmon raised under light backgrounds of different spectral composition. Environ. Biol. Fishes, **55:** 279-293.

Olin, T., and Von Der Decken, A. 1990. Yolk proteins in salmon (*Salmo salar*) oocytes, eyed eggs, and alevins differing in viability. Can. J. Zool. **68**: 895-900.

Ojanguren, A.F., Reyes-Gavilan, F.G., and Brana, F. 1996. Effect of egg size on offspring development and fitness in brown trout, *Salmo trutta* L. Aquaculture, **147**: 9-20.

Parker, G.A., and Begon, M. 1986. Optimal egg size and clutch size: effects of environment and maternal phenotype. Am. Nat. **128**: 573-592.

Patterson, D.A., Macdonald, J.S., Hinch, S.G., Healey, M.C., and Farrell, A.P. 2004. The effect of exercise and captivity on energy partitioning, reproductive maturation, and fertilization success in adult sockeye salmon. J. Fish. Biol. **64**: 1039-1059.

Patterson, D.A., Guderley, H., Bouchard P., Macdonald, J.S., and Farrell, A.P. *In Press*. Maternal influence and population differences in activities of mitochondrial and gylcolytic enzymes in emergent sockeye salmon (*Oncorhynchus nerka*) fry. Can. J. Fish. Aquat. Sci.

Pickering, A.D., Pottinger, T.G., Carragher, J., and Sumpter, J.P. 1987. The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. Gen. Comp. Endocrinol. **68**: 249-259.

Pickering, A.D. 1992. Rainbow trout husbandry: management of the stress response. Aquaculture, **100**: 125-139.

Pottinger, T.G., and Pickering, A.D. 1990. The effect of cortisol administration on hepatic and plasma estradiol-binding capacity in immature female rainbow trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. **80:** 264-273.

Quinn, T.P. 1988. Estimated swim speeds of migrating adult sockeye salmon. Can. J. Zool. **66**: 2169-2163.

Quinn, T.P., and Adams, D.J. 1996. Environmental changes affecting the migratory timing of American shad and sockeye salmon. Ecology, **77**: 1151-1162.

Quinn, T.P., Hendry, A.P., and Wetzel, L.A. 1995. The influence of life history trade-offs and the size of incubation gravels on egg variation in sockeye salmon (*Oncorhynchus nerka*). Oikos, **74**: 425-438.

Rand, P.S., and Hinch, S.G. 1998. Swim speeds and energy use of upriver-migrating sockeye salmon (Oncorhynchus nerka): simulating metabolic power and assessing risk of energy depletion. Can. J. Fish. Aquat. Sci. **55**: 1832-1841.

Robertson, O.H., and Wexler, B. 1957. Pituitary degeneration and adrenal tissue hyperplasia in spawning Pacific salmon. Science, **125**: 1295-1296.

Roff, D.A. 1992. The evolution of life histories: theory and analysis. Chapman Hall, London.

Roff, D.A. 1998. The detection and measurement of maternal effects. *In* Maternal Effects as Adaptations. *Edited by* T.A. Mousseau and C.W. Fox. Oxford University Press, Oxford. pp. 83-96.

Rossiter, M.C. 1996. Incidence and consequences of inherited environmental effects. Annu. Rev. Ecol. Syst. **27**: 451-476.

Schreck, C.B., Contreras-Sanchez, W. and Fitzpatrick, M.S. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture, **197**: 3-24.

Schubert, N.D., and Fanos, B.P. 1997. Estimation of the 1994 late run sockeye (*Oncorhynchus nerka*) escapement to the Stuart River system. Can. Manuscr. Rep. Fish. Aquat. Sci. **2431**.

Scott, A.P., and S.M. Baynes. 1980. A review of the biology, handling and storage of salmonid spermatozoa. J. Fish. Biol. **17**: 707-740

Scrivener, J.C. 1988. Two devices to assess incubation survival and emergence of salmonid fry in an estuary streambed. N. Am.. J. Fish. Manage. **8**: 248-258.

Servizi, J.A., and Jensen, J.O.T. 1977. Resistance of adult sockeye salmon to acute thermal shock. Int. Pac. Salmon Fish. Comm. Prog. Rep. **34**.

Smith, C.C., and Fretwell, S.D. 1974. The optimal balance between size and number of offspring. Am. Nat. **108**: 499-506.

Smoker, W.W., Gharrett, A.J., Stekoll, M.S., and Taylor, S.G. 2000. Genetic variation of fecundity and egg size in anadromous pink salmon *Oncorhynchus gorbuscha* Walbaum. Alaska Fishery Res. Bull. **7**: 44-50.

Sokal, R.R. and Rohlf, F.J. 2000. Biometry, 3rd edition. W.H. Freeman and Company. New York.

Springate, J.R.C., Bromage, N.R., Elliott, J.A.K., and Hudson. D.L. 1984. The timing of ovulation and stripping and their effects on the rates of fertilization and survival to eyeing, hatch and swim-up in the rainbow trout (*Salmo gairdneri R.*). Aquaculture, **43**: 313-322.

Srivastava, R.K., and Brown, J.A. 1991. The biochemical characteristics and hatching performance of cultured and wild Atlantic salmon (*Salmo salar*) eggs. Can. J. Zool. **69**: 2436-2441.

Su, G., Liljedahl, L., and Gall, G.A.E. 1997. Genetic and environmental variation of female reproductive traits in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, **154**: 115-124.

Sumpter, J.P., Carragher, J.F., Pottinger, T.G., and Pickering, A.D. 1987. Interaction of stress and reproduction in trout. *In* Proceedings of the 3rd International Symposium on Reproductive Physiology of Fish. *Edited by* D.R. Idler, L.W. Crim, and J.M. Walsh. St. John's, Newfoundland, pp. 299-302.

Thompson, W.F. 1945. Effect of the obstruction at Hells Gate on the sockeye salmon of the Fraser River. Int. Pac. Salmon Fish. Comm. Bull. **1**

Thorarensen, H., Gallaugher, P.E., Kiessling, A.K., and Farrell, A.P. (1993). Intestinal blood flow in swimming chinook salmon *Oncorhynchus tshawytscha* and the effects of hematocrit on blood flow distribution. J. Exp. Biol. **179**: 115-129.

Tyler, C.R., and Sumpter, J.P. 1996. Oocyte growth and development in teleosts. Rev. Fish Biol. Fish. **6**: 287-318.

Tyler, C.R., Sumpter, J.P., and Bromage, N.R. 1990. An *in-vitro* culture system to study vitellogenic growth of ovarian follicles of the rainbow trout, *Salmo gairdneri*. J. Exp. Zool. **248**: 199-206.

Tyler, C.R., Nagler, J.J., Pottinger, T.G., and Turner, M.A. 1994. Effects of unilateral ovariectomy on recruitment and growth of follicles in the rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. **13**: 309-316.

Tyler, C.R., Pottinger, T.G., Santos, E., Sumpter, J.P., Price, S-A., Brooks, S., and Nagler, J.J. 1996. Mechanisms controlling egg size and number in rainbow trout, *Oncorhynchus mykiss*. Biol. Reprod. **54**: 8-15.

van den Berghe, E.P. and Gross, M.R. 1989. Natural selection resulting from female breeding competition in a Pacific Salmon (Coho: *Oncorhynchus kisutch*). Evolution, **43**: 125-140.

Washburn, B.S., Frye, D.J., Hung, S.S.O., Doroshov, S.I. and Conte, F.S. 1990. Dietary effects on tissue composition, oogenesis and the reproductive performance of female rainbow trout (*Oncorhynchus mykiss*). Aquaculture **90**: 179-195.

Webb, P.W. 1995. Locomotion. *In* Physiological Ecology of Pacific Salmon. *Edited by* C. Groot, L. Margolis, and W.C. Clarke. UBC Press, Vancouver. pp. 69-99.

West, C.J., and Larkin, P.A. 1987. Evidence for size-selective mortality of juvenile sockeye salmon (*Oncorhynchus nerka*) in Babine Lake, British Columbia. Can. J. Fish. Aquat. Sci. **44**: 712-721.

Wiegand, M.D. 1996. Composition, accumulation, and utilization of yolk lipids in teleost fish. Rev. Fish Biol. Fish. **6**: 259-286.

Williams, I.V., Brett, J.R., Bell, G.R., Traxler, G.S., Bagshaw, J., McBride, J.R., Fagerlund, U.H.M., Dye, H.M., Sumpter, J.P., Donaldson, E.M., Bilinski, E., Tsuyuki, H., Peters, M.D., Choromanksi, E.M., Cheng, J.H.Y., and Coleridge, W.L. 1986. The 1983 early run Fraser and Thomspon River pink salmon; morphology, energetics, and fish health. Int. Pac. Salmon Fish. Comm. Bull. **23**

Wood, A. W., and Van Der Kraak, G.J. 2001. Apoptosis and ovarian function: novel perspectives from the teleosts. Biol. Reprod. **64**: 264-271.

Woody, C.A., Olsen, J., Reynolds, J., and Bentzen, P. 2000. Temporal variation in phenotypic and genotypic traits in two sockeye salmon populations, Tustumena Lake, Alaska. Trans. Amer. Fish. Soc. **129**: 1031-1043.

Wootton, R.J. 1998. Ecology of teleost fishes. Kluwer Academic Publishers, London.

Yanagimachi, R., Cherr. G.N., Pillai, M.C., and Baldwin, J.D. 1992. Factors controlling sperm entry into the micropyles of salmonid and herring eggs. Develop. Growth and Differ. **34**: 447-461.

Zohar, Y. 1980. Dorsal aorta catheterization in rainbow trout (*Salmo gairdneri*): I. Its validity in the study of blood gonadotropin patterns. Reprod. Nutr. Dev. **20**: 1811-1823.