

TRANSGENERATIONAL EFFECTS OF STRESS IN SOCKEYE SALMON  
(*ONCORHYNCHUS* NERKA) ON OFFSPRING FERTILIZATION SUCCESS AND  
EMBRYONIC SURVIVAL

By

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## ABSTRACT

Fish cope with stress by mounting a primary endocrine response via the sustained release of glucocorticoid steroid hormones – particularly cortisol. Exposure of fish to chronic stress and the sustained release of cortisol is known to have a wide range of effects on physiological processes, although less is known about its effects on reproduction. I utilized wild sockeye salmon from the Fraser River, British Columbia to examine some of the earliest effects of parental exposure to chronic stress and egg exposure to cortisol on viable offspring production. Fraser River sockeye salmon have declined precipitously in numbers and productivity in recent years. Increasingly stressful spawning migrations and the subsequent detrimental effects increased parental stress has on offspring has been put forth as a hypothesis to help explain these recent declines. I simulated chronic stress by periodically chasing female sockeye salmon for 6 weeks prior to ovulation. Egg survival to hatch remained high in both wild (90%) and control (80%) treatments but was significantly reduced as a result of chronic maternal stress (63%; Kruskal-Wallis test,  $H = 12.64$ ,  $P = 0.002$ ). To simulate the effect of circulating levels of maternal cortisol, eggs from wild caught females were fertilized in water dosed with control (0 ng/mL), low (300 ng/mL), and high (1000 ng/mL) levels of cortisol. Egg fertilization success remained high in the control group (90%), but was significantly reduced by exposure to both low and high levels of cortisol (78% and 79% respectively; Kruskal-Wallis test,  $H = 13.27$ ,  $P = 0.001$ ). Regardless of stress or cortisol exposure, embryos were highly likely to survive to emergence if they hatched. Results from this study indicate that transgenerational effects of stressful migrations are real and this has ramifications to the productivity of populations and spawner fitness. Transgenerational effects of stress may be one explanatory mechanism contributing to declines in Fraser River sockeye salmon.

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## **Introduction**

### *Stress, the stress response and reproduction*

Stress is omnipresent in the lives of all vertebrates and can stem from stimuli related to changes in an organism's physical, chemical, or environmental living conditions (Romero 2004).

Individuals cope with stress by eliciting a suite of integrated physiological responses when a threat to their homeostasis is perceived (Moberg 2000). This reaction is termed a stress response, and is similar across vertebrates (Romero 2004; Haussman *et al.* 2011). Indeed, it has been demonstrated that the cumulative effects of a prolonged stress response can ultimately lead to a reduction in survival of individuals (McEwan 1998; Wingfield *et al.* 1998; Sapolsky *et al.* 2000; Haussman *et al.* 2011); however, the primary function of this mechanism is to encourage survival during stressful events.

The primary endocrine response of vertebrates mounting a stress response is the release of glucocorticoid steroid hormones – cortisol and corticosterone (Sapolsky *et al.* 2000). Fish and mammals primarily release cortisol, whereas reptiles, amphibians, birds, and many rodents release corticosterone (Romero 2004). During a stress response, the release of these hormones brings about different physiological changes to promote survival (Sapolsky 2000). Across vertebrates, glucocorticoids act to mobilize energy stores for immediate utilization in working muscle, stimulate immune function, inhibit reproduction, decrease appetite and feeding, and increase alertness (Wingfield *et al.* 1998; Moberg 2000; Sapolsky *et al.* 2000; Haussman *et al.* 2011). When homeostasis is restored, glucocorticoids quickly return to their baseline levels; however, exposure to repeated or prolonged stressors can give rise to chronic stress and the sustained release of these steroid hormones (Haussman *et al.* 2011). This prolonged stress response can have a wide range of effects on physiological processes in vertebrates, including reproduction (Sapolsky *et al.* 2000).

It is the sustained release of corticosteroids acting at the level of the hypothalamus-pituitary-adrenal (HPA; or HPI – hypothalamus-pituitary-interrenal in fish) axis and the gonads in vertebrates that are responsible for the effects of stress on reproductive endocrinology (Barton 2002; Wingfield and Sapolsky 2003; Denver 2009). Much is known about the subsequent effects of stress-induced inhibition of reproductive functions, and both acute and chronic stress is shown to adversely affect a range of reproductive indices in vertebrates (Rivier and Rivest 1991; Tilbrook *et al.* 2000). For example, prolonged stress can result in a suppressive effect on

reproductive behaviour, impaired gonadal development, and a reduction in the size, number, and quality of gametes produced across taxa (e.g., in humans, Charmandari *et al.* 2005; other mammals, Wingfield *et al.* 1997; birds, O'Reilly and Wingfield 2001; amphibians and reptiles, Moore and Jessop 2003; and fish, Schreck *et al.* 2001). It is possible that observations like the former and measuring concentrations of glucocorticoids in adults can be used to assess the inhibitory influence of stress on reproduction. However, the most significant metric to assess any animal's full reproductive capability is the successful production of viable offspring (Campbell *et al.* 1994). Across vertebrate taxa, it is the effect of stress on this ultimate reproductive parameter that is of greatest importance (Campbell *et al.* 1994; Painter *et al.* 2005; Sheriff *et al.* 2009); the extent to which offspring are affected by parental exposure to stress has received less attention.

### *Transgenerational effects of stress*

There is a great deal of variation in the effects of a stressed parent on a number of offspring traits across vertebrate taxa (Table 1). Yet, the quantification of these effects is often based only on the immediate survival of offspring following fertilization, and/or on egg/progeny size, with few studies examining fertilization success or embryonic development, especially in fish (Schreck 2001). Examining how stress affects the fertilization success of eggs and survival through the subsequent stages of embryogenesis in fish can provide a more precise estimate of survival in progeny of by parents that may be predisposed to stressor events prior to or during reproduction.

**Table 1. Transgenerational effects of stress on offspring physiology and behaviour.**

<b>Species</b>	<b>Parental stressor</b>	<b>Effect on offspring</b>	<b>Citation</b>
Rat ( <i>Rattus norvegicus</i> )	Injected with glucocorticoids	Reduced birth weights	Drake <i>et al.</i> 2004
Snowshoe hares ( <i>Lepus americanus</i> )	Exposed to the presence of a predator	Reduced birth weights	Sherrif <i>et al.</i> 2009
Mouse ( <i>Mus musculus</i> )	Indirectly exposed to disease	Increased immune response when exposed to the same disease and less aggression in social groups	Curno <i>et al.</i> 2009



Great tit ( <i>Parus major</i> )	Exposed to ectoparasites	Faster growth	Buechler <i>et al.</i> 2002
Zebra finch ( <i>Taeniopygia guttata</i> )	Crowding stress from increased brood sizes	Reduction in body size and shorter wing lengths in $F_1$ and $F_2$ generations	Naguib <i>et al.</i> 2005
White leghorn chickens ( <i>Gallus gallus domesticus</i> )	Exposed unpredictable light-dark rhythms	Reduced learning, increased competitiveness, and faster growth	Lindqvist <i>et al.</i> 2007
Eastern narrow-mouthed toads ( <i>Gastrophryne carolinensis</i> )	Exposed to a contaminated environment	Reduced hatching success, abnormal swimming, and significant craniofacial abnormalities	Hopkins <i>et al.</i> 2006
Scincid lizard ( <i>Pseudemoia pagenstecher</i> )	Exposed to the scent of a predator	Increase in chemosensory behaviour and increased body weight	Shine and Downes 1999
Striped bass ( <i>Morone saxatilis</i> )	Eggs developed in polluted estuary	Reduction in body length and volume, but an increase in liver size	Ostrach <i>et al.</i> 2008
Atlantic salmon ( <i>Salmo salar</i> )	Females given intraperitoneal cortisol implants	Increase offspring mortality and mass, diminished yolk sac volume and utilization, and more aggression in dominant individuals	Eriksen <i>et al.</i> 2006; 2011
Rainbow ( <i>Oncorhynchus mykiss</i> ) and brown trout ( <i>Salmo trutta</i> )	Chronic chasing and confinement	Reduction in survival	Campbell <i>et al.</i> 1992; 1994

### *Study species*

Sockeye salmon are an excellent species in which to examine the parental effects of stress on offspring as a great deal is known about their distribution, migratory life history, and spawning physiology (Groot & Margolis 1991; Quinn 2005; Hinch *et al.* 2006). The oceanic range of sockeye salmon covers the entire North Pacific Ocean, Bering Sea, and Sea of Okhotsk, with spawning and rearing grounds extending from tributaries of the Columbia River to western Alaska along coastal North America, and throughout the entire Kamchatka Peninsula in Russia (Groot and Margolis 1991).

Sockeye salmon are anadromous, that is, they migrate from the ocean to freshwater to spawn and typically exhibit 6 general stages of their migratory life cycle (Hinch *et al.* 2006). Upon successful spawning during the fall months, fertilized eggs incubate and develop over winter within the gravel or cobble substrates common in most streams and lakes (Quinn 2005; Hinch *et al.* 2006). In the spring, embryos hatch into alevins where they remain amongst the safety of the substrate and absorb nutrients from their yolk sacs while developing into fry (Quinn 2005; Hinch *et al.* 2006). Upon absorbing the yolk sac, fry emerge from the substrate and migrate to a nursery lake where they feed and grow for 1-2 years (Quinn 2005; Hinch *et al.* 2006). In the spring months, after rearing for 1-2 years, juveniles migrate downstream toward the ocean while undergoing smoltification, the process whereby osmoregulatory physiology changes to prepare for entry into saltwater (Quinn 2005; Hinch *et al.* 2006). While in the ocean, sockeye salmon feed and grow for 1-4 years, covering thousands of kilometers, before sexual maturation is initiated, cuing a directed migration back to the coast and natal rivers (Quinn 2005; Hinch *et al.* 2006). In the final stages of life from late spring to early fall, sockeye salmon re-enter freshwater and migrate upriver to their natal stream to spawn and eventually die (Quinn 2005; Hinch *et al.* 2006). It is during this final life stage that sockeye salmon undergo the astonishing physical transformations for which they are best known. Both sexes exhibit drastic changes in colour from their bright silvery marine hues to sexually mature adults characterized by bright green heads and brilliant red bodies, with males developing a distinct large hump on their backs (Groot and Margolis 1991). These unique changes in shape and colour are paralleled by distinct changes in physiology during this final stage of life.

Sockeye salmon undergo constant physiological changes throughout the various stages of their migratory life history (for review see Hinch *et al.* 2006). However, those that are most

challenging and pertinent to successful reproduction are the changes associated with entry into freshwater and upriver spawning migrations (Cooke *et al.* 2006). During migrations, levels of reproductive and steroid hormones regulating energy use, sexual, and gonadal development change continually up to and following spawning (Hinch *et al.* 2006). Circulating levels of cortisol increase dramatically as upriver migration progresses (Schmidt and Idler 1962), with increases particularly pronounced in females (Hane and Roberston 1959; Schmidt and Idler 1962; McBride 1986). For example, in sockeye salmon from the Fraser River in British Columbia, levels of cortisol in females can reach 800 ng ml<sup>-1</sup> during upriver migrations (Hinch *et al.* 2006), and fall to levels of 350 ng ml<sup>-1</sup> upon arrival at spawning grounds, before increasing again to 1200 ng ml<sup>-1</sup> post spawning immediately before death (Hruska *et al.* 2010). Given the correlations of increased maternal and egg cortisol in salmonids (Stratholt *et al.* 1997), an effect of parental stress on reproduction under these conditions is likely to play a vital role in the subsequent physiological and behavioural development of sockeye salmon offspring.

In salmonids, there is a fine line between too much and too little exposure to cortisol (Hinch *et al.* 2006; Sloman 2010). The adaptive role of this hormone during adult reproductive migrations and in response to stress is context specific, but the role of cortisol in juvenile development remains unclear (Mommensen *et al.* 1999). Increased levels of cortisol could serve as a maternally mediated cue that alters offspring phenotypes and behaviour in preparation for a stressful environment (Groothuis *et al.* 2005; Rubolini *et al.* 2005; Haussman *et al.* 2011), or as a mechanism that reduces the overall survival of progeny (Campbell *et al.* 1992, 1994; McEwan 1998; Wingfield *et al.* 1998; Sapolsky *et al.* 2000; Eriksen *et al.* 2006; 2007; Haussman *et al.* 2011). These observations in combination with the known effects of increased stress and the role of cortisol on reproductive, survival, and behavioural traits documented in salmonids that are important to aquaculture, present a unique opportunity to experimentally examine the effects of parental exposure to stress on wild sockeye salmon progeny from the Fraser River, British Columbia.

The use of Fraser River sockeye salmon in this study is of particular interest given the significant economic, ecologic, and cultural importance of this species in the province of British Columbia. The Fraser River is the largest salmon producing system in British Columbia, and sockeye salmon are its most commercially valuable and second most abundant species (Hinch and Martins 2011). Sockeye salmon are essential to the prosperity of the people of British

Columbia. The commercial fishery for this species has in the past been the most economically valuable salmon fishery in Canada (Williams 2007; Jacob *et al.* 2007), with the current recreational fishery helping support a multi-billion dollar industry and a number of jobs throughout communities in British Columbia (Kristianson and Strongitharm 2006; British Columbia 2010; Hinch and Martins 2011). Sockeye salmon are essential to marine and freshwater food webs, and decaying adult sockeye salmon carcasses are primary sources of nutrients in coastal watersheds, contributing significantly to ecosystem productivity and the energy available to aquatic and terrestrial organisms (Cederholm *et al.* 1999; Helfield and Naiman 2001; Hinch and Martins 2011). For thousands of years, many First Nations along the Fraser River have depended on sockeye salmon runs for food, trade, and ceremonial purposes (Jacob *et al.* 2010). However, since the early 1990s, Fraser River sockeye salmon have declined precipitously in productivity and abundance, ultimately leading to the designation of some populations as ‘endangered’ by the IUCN and the Committee on the Status of Endangered Wildlife in Canada (IUCN-SSG 2009; COSEWIC 2010; Peterman *et al.* 2010; Hinch and Martins 2011).

Evidence suggests that the recent declines in Fraser River sockeye salmon can be attributed to unfavourable ocean conditions, increasing river temperatures, earlier than usual entry into freshwater, and high en route mortality. However, no single cause of decline can be pointed to (Peterman *et al.* 2010). Rather, it is suggested that all mechanisms operate simultaneously with additive or multiplicative effects on migrating fish (Peterman *et al.* 2010; Hinch and Martins 2011). A number of hypotheses have been proposed to further explain these mechanisms and investigate the decline of Fraser River sockeye salmon returns well below historic levels, including those examining the transgenerational effects of stress (Peterman *et al.* 2010; Hinch and Martins 2011).

During their freshwater spawning migration, Fraser River sockeye salmon face a suite of increasing anthropogenic and environmental stressors related to fisheries, contaminated water, hetero/con-specific competition, increased flows, or less than optimal water temperatures that can lead to high en route mortality before arrival at spawning grounds (Hinch and Martins 2011). Still, many fish are able to successfully spawn, posing the question of what the effects of this increased stress are on the next generation. Hruska *et al.* (2010) have demonstrated that near fully mature Fraser River sockeye salmon females arriving on spawning grounds have higher

baseline stress levels than males at a similar level of maturation. So additional stress experienced during migration may be more detrimental to females and their offspring (Hinch and Martins 2011). Studies of hatchery and farmed raised salmonids suggest there are transgenerational effects of maternal stress related to survival and behaviour in progeny (Campbell *et al.* 1992, 1994; Eriksen *et al.* 2006, 2011), and both Macdonald *et al.* (2000) and Patterson (2004) provided evidence that Fraser River sockeye salmon encountering high flows and temperatures, and adverse river conditions can have low embryo survival. However, transgenerational effects in Fraser River sockeye salmon in terms of whether offspring fitness can be affected as a result of stressed maternal condition are not yet supported in the literature (reviewed in Peterman *et al.* 2010). Therefore, investigating the effects of maternal exposure to increased stress on viable offspring production and progeny development through the later stages in life is imperative as significant transgenerational effects could cause changes in recruit / spawner ratios and contribute to population declines (Peterman *et al.* 2010).

### *Thesis aims*

In an effort to elucidate more of the effects of parental exposure to stress on the progeny of wild Fraser River sockeye salmon, this thesis examined some of the earliest parameters essential to viable offspring production. The aim of my thesis was to determine the effects of maternal exposure to an exogenous stressor and gametic exposure to cortisol, on egg fertilization success and offspring survival through the early stages of embryogenesis. This study utilized gametes from individual females of a wild population of Fraser River sockeye salmon collected during their upriver spawning migration that were chronically exposed to an exogenous stress or whose eggs were exposed to ecologically relevant levels of cortisol during fertilization. I utilized offspring produced by stressed mothers or those from exposed eggs to address two hypotheses.

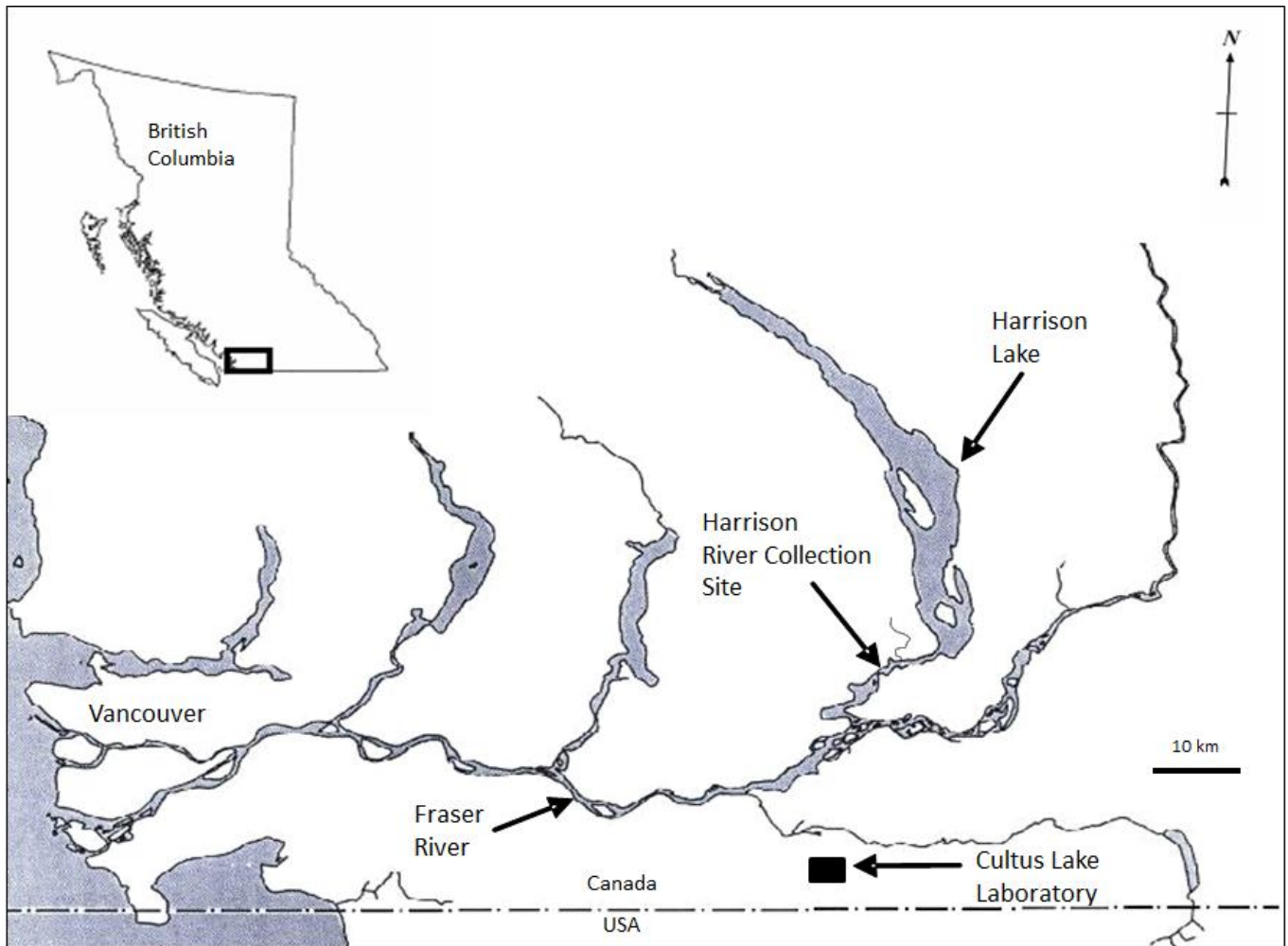
First, that chronic exposure of female adult sockeye salmon to a repeated exogenous stressor will adversely affect offspring fertilization success and early embryo survival. I predicted that when compared to unstressed or control fish captured on spawning grounds, offspring from stressed mothers would have significantly lower fertilization success and overall lower survival of eggs to the hatched stage. Secondly, that exposure of eggs from females captured during peak spawning to varying elevated levels of cortisol during fertilization would have negative effects on egg fertilization success and reduce offspring survival through early embryogenesis. I predicted that fertilization success and survival to hatch would be significantly

lower in eggs exposed to high levels of cortisol when compared to those of low exposure or controls.

## **Methods**

### *Fish collection and handling*

From September 28-30, 2011, male and female sockeye salmon ( $n = 300$ ) from the Harrison Rapids population were collected by beach seine from the Harrison River ( $49^{\circ}17'5$  N,  $121^{\circ}54'27$  W), a major tributary of the Fraser River in British Columbia (Figure 1). All collection occurred roughly 6 weeks prior to peak spawning while fish were en route to their natal spawning grounds approximately 130 km upstream from the Fraser River mouth below Harrison Lake on Chehalis First Nation's land. Temperatures of the Harrison River during the time of collection were approximately  $14^{\circ}\text{C}$ . All fish were transported live by vehicle for approximately 1 hour in aerated tanks (at densities no greater than  $25 \text{ kg fish/m}^3$  of water) continually monitored for dissolved oxygen levels to the Fisheries and Oceans Canada Cultus Lake Salmon Research Laboratory (CLL), British Columbia (Figure 1).



**Figure 1. Map of the lower Fraser River, British Columbia, Canada, with the locations of the Harrison River collection site and the Fisheries and Oceans Canada Cultus Lake laboratory (CLL).**

Immediately upon arrival at CLL, fish were distributed among 10 tanks each of 10 000 L holding 30 fish (15 females and 5 males). Each tank was fed with fresh water circulating around the periphery at approximately 25 m/s so fish were able to orient themselves and maintain a constant swimming speed. Air bubblers were constantly in use creating dissolved oxygen levels above 90% at all times, with levels monitored daily. Water for the holding tanks was drawn directly from the Cultus Lake hypolimnion reflecting natural changes in temperature over the course of the study.

Of the ten tanks, 5 served as controls, and 5 as treatment. Treatment began on October 2, 2011 to allow fish time to recover from any stress that may have been caused by transport to the Cultus Lake facility. Over the course of this experiment, treatment fish were subjected to an exogenous stressor and chased around their tanks manually with a net for 3 minutes, twice daily

at random times between 0900 and 1700 for approximately 6 weeks. Control fish were left unchased for the duration of the experiment. After approximately 6 weeks, coinciding with the timing of peak spawning, fish from both treatment groups were dip-netted from their tanks and quickly examined for maturity to minimize air exposure. Fish were considered mature when milt or eggs were easily released from the vent by firmly squeezing along the lateral lines. Immature fish were returned to their tanks and mature fish were immediately sacrificed by cerebral concussion and sampled (see below). Fish were assessed for maturity until November 13<sup>th</sup>, after which all immature fish were euthanized and the experiment was ended.

Before gamete collection, all males had their vents wiped dry with a sterile paper towel to prevent contamination of milt with water or urine. Gametes from each individual were collected into clean, dry, and sterile Tupperware containers and given a dose of O<sub>2</sub> in preparation for transport to the Pacific Salmon Ecology and Conservation Lab at the University of British Columbia (UBC) in Vancouver. Between 1 – 4 ml of milt was collected from each male and approximately 100 g of eggs were collected from each female. Measurements of standard length, fork length, postorbital–hypural bone length, and postorbital-fork length were recorded to the nearest 0.1 cm. Fish were weighed for individual total mass and recorded to the nearest 0.01 kg. Gonad mass was measured and recorded to the nearest g. Female gonad mass was the summation of the initial amount of eggs stripped before dissection and that of the mass of any eggs remaining in the body cavity after dissection.

In addition to all the above collection and sampling of experimental fish, 20 male and 20 female sockeye salmon were collected from the same location on November 9, 2011 during peak spawning to serve as ‘wild’ control specimens. All wild fish were subjected to the same terminal sampling and gamete collection as described previously for fish sampled on the Harrison River.

#### *Fertilization, cortisol exposure, and incubation*

Gametes from 90 (45 males, 45 females) adult sockeye were used to for this study, allowing for up to 45 full sib crosses (15 unique families across the three treatment groups) to be created. Fertilizations took place from November 8 – 13, 2011 in the UBC lab, with only eggs from a single female crossed with the milt of a single male. Crossing designs included wild × wild, control × control, and control × stress pairings; sample sizes are summarized in Table 2.



**Table 2. Summary of crossing design between male and female Harrison Rapids sockeye salmon conducted at the Pacific Salmon Ecology and Conservation lab at the University of British Columbia, Vancouver.**

	<b>Control Male</b>	<b>Stress Male</b>	<b>Wild Male</b>
<b>Control Female</b>	<b>n = 14</b>	<b>n = 5</b>	
<b>Stress Female</b>	<b>n = 14</b>		
<b>Wild Female</b>			<b>n = 20</b>

All fertilizations were carried out following a modified method of that presented in Patterson *et al.* (2004). In a sterile, dry Mason jar, approximately 20 g of eggs were combined with 0.15 mL of milt prior to the addition of 30 mL of water to activate sperm; after 1 minute, an additional 30 mL of water was added. This same dry fertilization technique was applied to all crosses of experimental and ‘wild’ control fish – with the exception of varying doses of cortisol in the water used in ‘wild’ crosses. Fertilizations of ‘wild’ control crosses were each bathed in water containing levels of cortisol equivalent to those found in migrating Fraser River sockeye as described by Hinch *et al.* (2006) and Hruska *et al.* (2010). To simulate elevated levels of cortisol in eggs, water for these fertilizations was dosed with 0 ng/mL (control), 300 ng/mL (low), or 1000 ng/mL (high) of cortisol. After remaining undisturbed for approximately 1 hour for water hardening, each group of fertilized eggs was placed into an individual basket and distributed randomly among trays within Heath stacks for incubation. Each stack was supplied with a constant volume of de-chlorinated City of Vancouver water and monitored daily for dissolved oxygen and temperature levels. All Heath stacks were covered in black plastic to maintain darkness and monitored daily until fry emergence, when the yolk sac is fully absorbed or fry are determined “button-up”. Dead eggs were routinely picked from incubation baskets and placed into vials containing Stockard’s solution (5% formaldehyde (40%), 4% glacial acetic acid, 6% glycerin, 85% water) for preservation and future embryo analysis. Individual vials were labeled with the date of collection and the basket number from which dead eggs were picked to later aid in identification of families.

#### *Fertilization identification*

All dead eggs from different families preserved in vials of Stockard’s solution were examined for fertilization success. Using a dissecting microscope at 10x magnification, and tweezers, individual eggs were carefully examined for evidence of fertilization. Date picked from Heath

tray, basket number, total number of fertilized and unfertilized eggs were all recorded for each individual vial. Fertilization success was evaluated based on the presence of an early morula visible inside an egg and following the methods of Velsen (1980) who previously documented time to varying developmental stages in sockeye salmon eggs. Further identification aid was provided by a colour photographic index of embryonic development in Chinook salmon (*Oncorhynchus tshawytscha*) eggs presented by Boyd *et al.* (2010). Careful attention was paid to the stage of development of each egg with records kept of the number of fertilized, eyed, or hatched embryos contained in each vial.

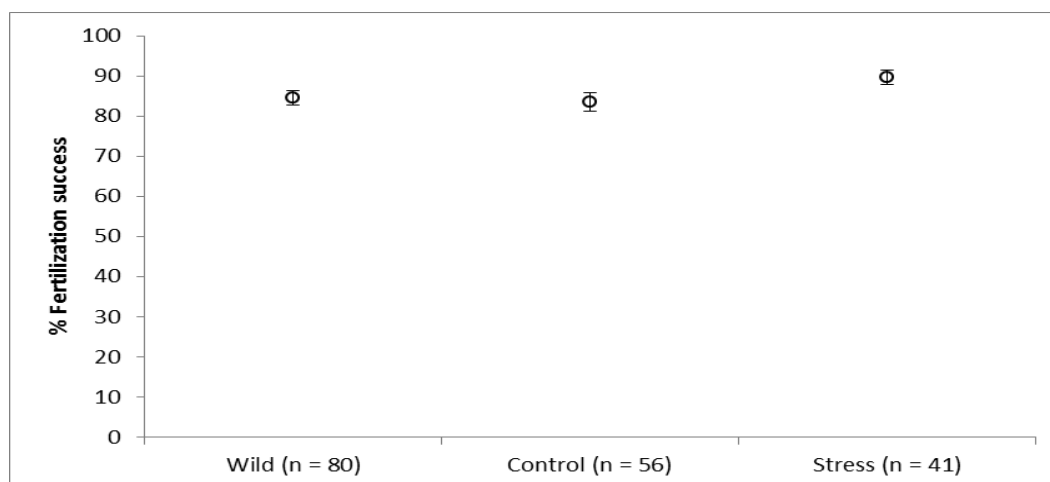
### *Statistical analysis*

All statistical analyses were performed with JMP, version 10 (SAS Institute Inc.; [www.sas.com](http://www.sas.com)). Data could not be transformed to meet the assumptions of normality and thus, non-parametric tests were used to examine the effects of parental stress and cortisol exposure on fertilization success and offspring survival at different stages of embryogenesis. A Kruskal-Wallis test was used to compare mean fertilization success and offspring survival to hatch and hatch to emergence across treatment groups. Post-hoc differences were identified using the Tukey HSD test. Standard error bars are presented on all figures and different letters on graphs denote significant differences between treatment groups measured at a  $P < 0.05$  level.

## **Results**

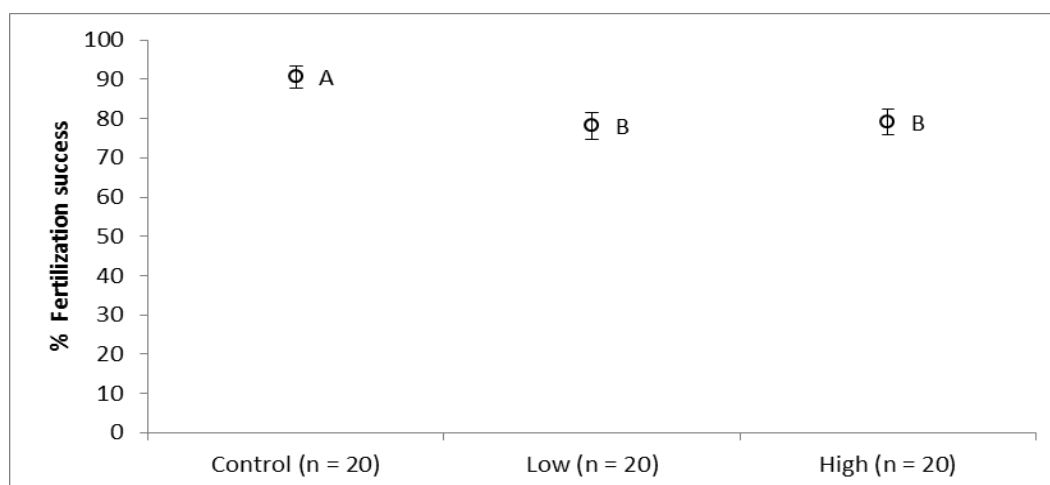
### *Fertilization success*

Fertilization success of eggs from mothers exposed to exogenous stress was high ( $> 83\%$ ), but did not vary among treatment groups (Kruskal-Wallis test,  $H = 12.64$ ,  $P = 0.13$ ; Figure 2).



**Figure 2. Fertilization success of Harrison Rapids *O. nerka* eggs from mothers of wild, control, and exogenous stress treatments.**

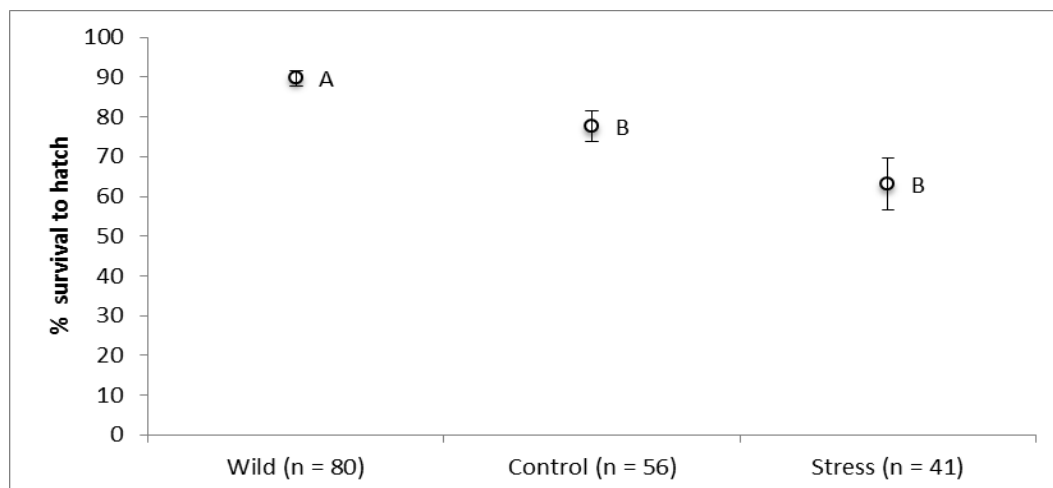
Eggs exposed to varying levels of cortisol exhibited significantly different levels of fertilization success among treatment groups (Kruskal-Wallis test,  $H = 13.27$ ,  $P = 0.001$ ; Figure 3). Eggs exposed to low and high doses of cortisol had similar rates of fertilization success (78% and 79% respectively), with highest success in eggs from the control group (90%) (Figure 3).



**Figure 3. Fertilization success of Harrison Rapids *O. nerka* eggs exposed to control (0 ng/ml), low (300 ng/ml), and high (1000 ng/ml) levels of cortisol during artificial spawning. Values not connected by same letter are significantly different.**

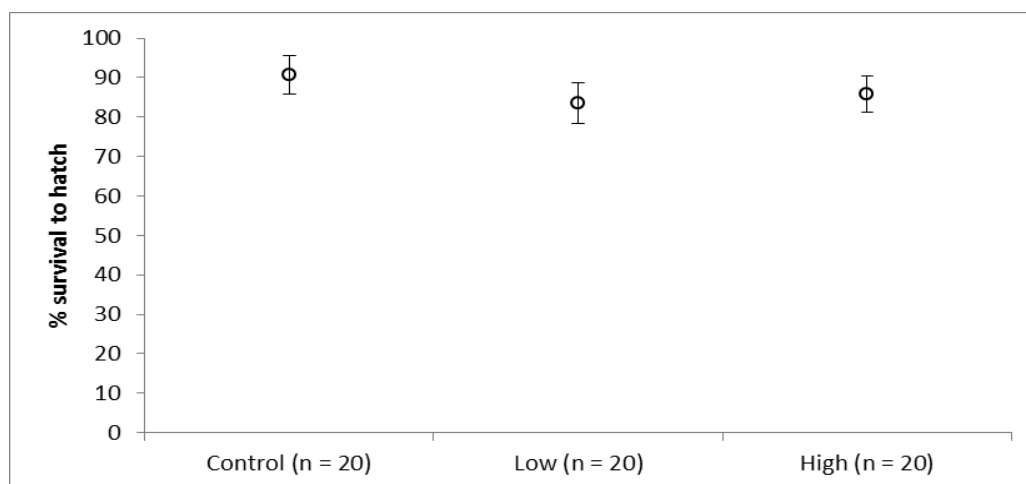
### *Survival to hatch*

In eggs from mothers exposed to exogenous stress, the percentage that survived to hatch differed significantly among treatment groups (Kruskal-Wallis test,  $H = 12.64$ ,  $P = 0.002$ ; Figure 4). Survival to hatch was lowest (63%) in eggs from stressed mothers, and highest for the wild treatment group (90%) with control levels being intermediate (Figure 4).



**Figure 4. Percentage of Harrison Rapids *O. nerka* eggs that survived to hatch from mothers of wild, control, and exogenous stress treatments. Values not connected by same letter are significantly different.**

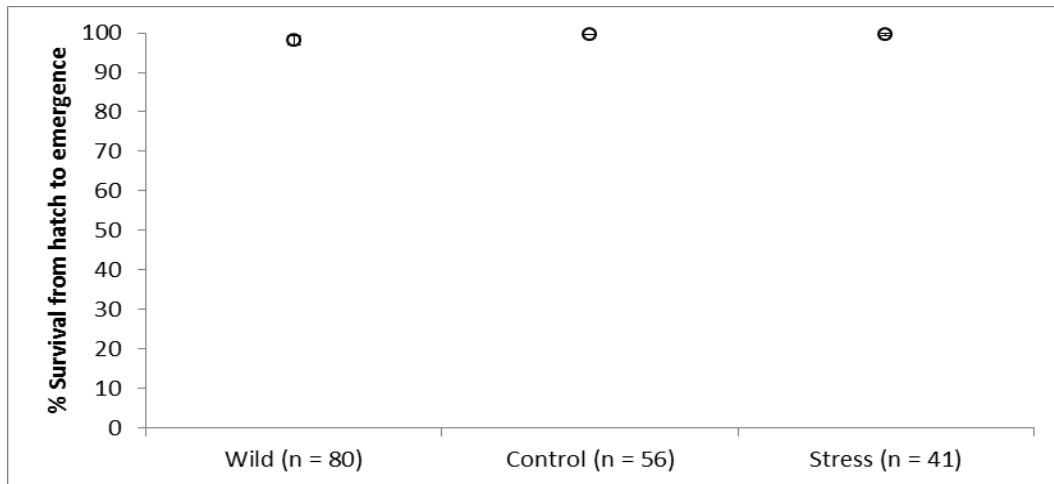
In eggs exposed to different levels of cortisol during artificial spawning, overall levels of survival to hatch were high (>84%) and did not differ across treatments (Kruskal-Wallis test,  $H = 4.35$ ,  $P = 0.11$ ; Figure 5).



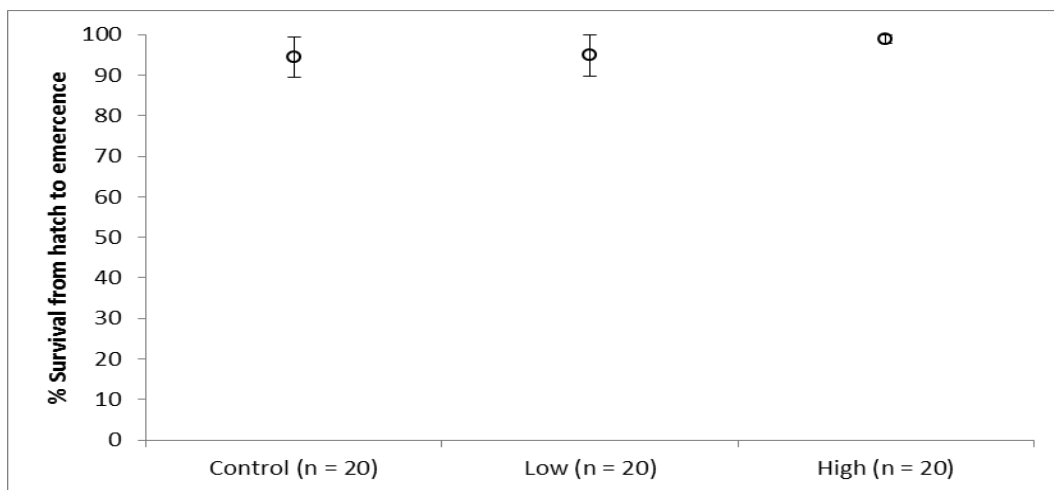
**Figure 5. Percentage of Harrison Rapids *O. nerka* eggs exposed to control (0 ng/ml), low (300 ng/ml) and high (1000 ng/ml) levels of cortisol during artificial spawning that survived to hatch.**

### *Survival from hatch to emergence*

Overall survival of eggs from hatch to emergence was very high (>95%). Eggs from any treatment group that survived to hatch were likely to survive to emergence regardless of exposure to exogenous stress or cortisol (Kruskal-Wallis test,  $H = 2.30$ ,  $P = 0.32$ ; Figure 6; Kruskal-Wallis test,  $H = 2.32$ ,  $P = 0.31$ ; Figure 7).



**Figure 6.** Survival from hatch to emergence of Harrison Rapids *O. nerka* eggs from mothers of wild, control, and exogenous stress treatments.



**Figure 7.** Survival from hatch to emergence of Harrison Rapids *O. nerka* eggs exposed to control (0 ng/ml), low (300 ng/ml) and high (1000 ng/ml) levels of cortisol during artificial spawning.

## Discussion

I examined some of the earliest effects of transgenerational stress in wild sockeye salmon life history. By subjecting wild caught mothers to chronic exogenous stress during the final stages of maturation and ovulation, or exposing eggs to varying levels of cortisol during fertilization, I was able to examine some of the effects of stress on immediate fertilization success and survival through the early stages of embryonic development. Maternal exposure to exogenous stress did not play a role in fertilization success as no differences were detected among treatments. In contrast, I found exposure of eggs to varying levels of cortisol during fertilization resulted in notable differences in fertilization success among treatments. Overall, fertilization success remained high irrespective of stress treatment, suggesting this is not the critical parameter

influencing the effects of maternal stress on viable offspring production. A significant reduction in survival to hatch in eggs from stressed mothers indicates survival through the subsequent stages of embryonic development is influenced by maternal stress. However, egg survival to hatch was not affected by direct exposure to cortisol. Furthermore, regardless of maternal stress or cortisol exposure, if eggs survived to hatch they were likely to survive to emergence as survival remained above 94% between experiments and across all treatment groups. These results are similar to those of Burt *et al.* (2012) who found post hatch alevin mortality was low in Fraser River sockeye salmon juveniles fertilized and incubated in identical but thermally stressful conditions. Preliminary results from the present study suggest the effects of parental stress on viable offspring production are first realized during the early stages of embryonic development following fertilization and prior to emergence. The following discussion will 1) highlight some of the differences and similarities between this and other studies of stressed salmonids, and 2) explore some of the mechanisms and possible causes or explanations for the differences in fertilization success and survival through development observed in sockeye salmon progeny from stressed mothers or gametes exposed to cortisol during fertilization.

#### *Fertilization success*

Few studies have specifically examined the fertilization success of eggs from wild but artificially stressed salmonids. Using the same dry fertilization technique as was used in this study, Patterson *et al.* (2004) and Galbraith *et al.* (2006) found fertilization success in wild caught Fraser River sockeye salmon varied between 86-98% in ideal laboratory settings where fertilization could be maximized, thus providing a standard from which to measure the potential effect of stress on fertilization in this study. Furthermore, Campbell *et al.* (1992) observed rainbow trout eggs from both control and parents subjected to prolonged acute environmental stress maintained fertilization success over 90% regardless of treatment.

Contrary to what was predicted, but within the range of values from the previously mentioned studies, egg fertilization success in the present study was not affected by parental exposure to exogenous stress and overall remained quite high between 83-89% across wild, control, and stressed treatments. These results, supported by those of Campbell *et al.* (1992), suggest there is little detrimental effect of parental exposure to exogenous stress on offspring fertilization success. Given only control males were used for fertilization in this study, any effect of stress on sperm motility or viability can be ruled out; suggesting female sockeye salmon have the

physiological capacity to buffer some aspects of their reproductive systems against the effects of stress, particularly to do with achieving high fertilization success.

It is possible that the physiological effects of parental exposure to exogenous stress are less important than effects of stress on parental behaviour. Stressed males and females arriving on spawning grounds could elicit behaviours that compromise their abilities to successfully spawn. For example, physiologically stressed females can prematurely release eggs, leading to water hardening before fertilization, or may choose inferior locations to excavate their redds. These behaviours could in turn give way to egg superimposition, less than adequate dissolved oxygen levels leading to egg suffocation, or higher amounts of suspended sediment impairing the ability of eggs to be fertilized (Galbraith *et al.* 2006). Stressed males could display courtship behaviours that deter mates or result in premature milt release. Indeed, timing is widely regarded as the critical factor in salmonid fertilization success (Liley *et al.* 2002) as eggs are fertilized less than 10 s after gamete release, and viability is reduced 20 s after release into the water (Hoysak and Liley 2001). Therefore, any effects of stress that might affect behaviour, particularly to do with spawning timing should be considered. The lack of differences observed in my results might be masked by laboratory procedures that eliminated behavioural variability and maximized fertilization success. Further studies utilizing natural spawning environments to examine the effects of parental stress and behaviour on spawning and fertilization warrant consideration. Regardless, my results suggest strong selective pressure for sockeye salmon eggs to be fertilized irrespective of female exposure to chronic stress; likely a result of the anadromous and semelparous nature of sockeye placing heavy emphasis on reproduction no matter the conditions during spawning. Greater detrimental effects on fertilization success could result from fertilization in stressful abiotic environments.

Consistent with my predictions, exposure of sockeye salmon eggs to varying levels of cortisol resulted in significantly reduced fertilization success rates that fell to below 80% as a result of cortisol exposure. However, a lack of significant difference in fertilization success between dosage levels suggests exposure to cortisol alone is enough to influence fertilization. If cortisol dosed water is representative of increasingly stressful migratory conditions, this suggests stressful abiotic environments play a role in influencing production of viable offspring. My results are contrary to those of Li *et al.* (2010) who immersed rainbow trout eggs in 100 and 1000 ng/mL doses of cortisol and ovarian fluid prior to fertilization and observed no difference

in fertilization success. These differences could be attributed to experimental design. A lack of ovarian fluid and the addition of cortisol-dosed water to initiate fertilization in my experiment likely allowed for the effects of cortisol to act simultaneously on both eggs and sperm and to not be influenced by ovarian fluid. Ovarian fluid is a maternal mechanism suggested to protect against the deleterious effects of hypercortisolism in eggs (Shrek *et al.* 2001). Contreras-Sanchez (1995) demonstrated that rainbow trout could contain 17 times less cortisol in their ovarian fluid than found in circulation, likely protecting oocytes from this hormone during development. By excluding ovarian fluid from eggs before fertilization and removing the cortisol buffering capacity of this substance in my study, I likely observed the unimpeded effects on fertilization success of elevated levels of this hormone.

Stressful abiotic incubation environments as reflected by cortisol could have the capacity to change structural characteristics of sockeye salmon eggs such that fertilization success is reduced simply by direct exposure to this hormone. Khan and Weis (1993) found that exposure of Mummichog (*Fundulus heteroclitus*) eggs to environmental pollutants results in artificial activation, blockage and swelling of the egg micropyle and lip, and a reduction in diameter and overall fertilization success. Similar effects could have taken place in this study, though I did not examine these parameters; future studies may look to examine the structural effects of cortisol on sockeye salmon eggs.

In the present study, milt was added to eggs prior to fertilization initiation with cortisol-dosed water. As a result, the effects of cortisol exposure on sperm motility cannot be excluded as a factor affecting interpretation. Sperm motility is a prerequisite for fertilization and correlates strongly with fertilization success (Rurangwa *et al.* 2004). In rainbow trout, Moccia and Munkittrick (1987) concluded that the number of motile sperm was the critical factor for fertilization. These conclusions, and results from this study, suggest cortisol exposure highly influences a reduction of sperm motility and contributes largely to the significant decline in fertilization success observed in the present study. Although direct exposure of sockeye salmon eggs to cortisol as applied here is unlikely to be replicated in the wild, other stressful environments resulting from increased contamination of Fraser River waters from development and land use activities like forestry and agriculture are likely to be encountered. Pollution and its effects on sperm motility are cause for concern as Kahn and Weis (1987) observed significantly reduced sperm motility in Mummichog after exposure to aquatic pollutants. It is likely that



sockeye salmon sperm experience the same fate in similarly contaminated environments, leading to a reduction in fertilization success and early embryo survival.

Stress appears to play a role in some aspects of fertilization, although the maintenance of successful fertilization was above 78% in this study regardless of treatment suggesting a great deal of capacity for sockeye salmon females to buffer against the effects of stress on one of the earliest parameters of viable offspring reproduction. Subsequent observations of the later stages of embryonic development suggest a significantly greater effect of stress post-fertilization and prior to embryo hatch.

#### *Embryonic survival to hatch*

Significant reductions in egg survival post-fertilization imply that transgenerational effects of maternal exposure to chronic exogenous stress exist. Consistent with my predictions, embryonic survival to hatch was significantly reduced in eggs from both control and stressed mothers, falling to as little as 63% in the stress treatment group. No significant differences existed between control and stress treatments, though survival to hatch was slightly higher in the control group, implying a possible effect of capture and captivity on stress. Beach seining is meant to minimize invasiveness; however, it is prone to greater delays in transfer, struggling, and air exposure (Nadeau 2007). The effects of captivity on stress from confinement and increased proximity of fish to pathogens combined with effects of the sampling process should not be ruled out. These effects could have created the slight differences in embryonic survival observed between stress and control treatments relative to wild fish. However, Crossin *et al.* (2008) found no physiological differences in stress measures between captive control and wild Fraser River sockeye salmon at the end of a study applying similar thermal stress, implying that treatment in this study was indeed stressful.

A significant reduction in survival to hatch after parental and embryonic exposure to stress was observed in previous studies conducted on salmonids. Using Fraser River sockeye salmon and thermal stress, Burt *et al.* (2012) observed egg survival to hatch was high in eggs incubated at optimal water temperatures of 12° C, but decreased significantly to less than 60% using thermally stressful water temperatures during incubation. The present study did not account for the effects of thermal stress; however, my results and those of Burt *et al.* (2012) could have additive effects and severely reduce sockeye salmon embryonic survival if stressors in the Fraser River continue to escalate (Patterson *et al.* 2007). Similar to my stress treatment,

Campbell *et al.* (1994) observed offspring from both brown and rainbow trout exposed to chronic exogenous stress before spawning exhibited significantly reduced survival in embryos to hatch. Our results, and those of the two previously mentioned studies, suggest salmonid eggs are particularly susceptible to the effects of parental stress post-fertilization.

A number of mechanisms may be responsible for the observed reductions in survival following stress. Eggs from stressed parents were smaller in size than controls (unpublished data). Though this was not likely a contributing factor to the observed reduction in survival as egg size is not a good indicator of egg quality in many teleosts (Bromage *et al.* 1992; Brooks *et al.* 1997). Rather, decreases in egg survival from stressed mothers could be a result of a reduction in egg quality that is related more to function and content. Reductions in egg enzymes and lipid reserves are a result of the stress response in female fish (Schreck *et al.* 2001; Leatherland *et al.* 2010). The reduction in embryonic survival I observed could be attributed to a lack of sustenance required for embryonic development. Enzymes are present in all teleost eggs and act to catalyze a number of metabolic processes vital for production of viable offspring (Brooks *et al.* 1997). Of particular importance are cathepsin enzymes that mediate the degradation of stored yolk proteins in free amino acids used by developing embryos (Sire *et al.* 1994; Brooks *et al.* 1997). Chronic parental stress may influence the production of these enzymes in eggs and could contribute to the reduction in survival observed in eggs from stressed mothers, particularly if enzymes are important to nutrient absorption in developing embryos. Maternal transfer of lipids during egg development is essential to quality egg and viable offspring production (Brooks *et al.* 1997). However, this process can be interrupted by the stress response in fish (Schreck *et al.* 2001) and plays a role in subsequent offspring development. A reduction in the transfer of lipids to eggs could contribute to the reduction of embryonic survival observed in this study. When confounded with a reduction in egg enzymes, the cumulative effects of parental stress on egg nutrient composition is likely enough to elicit the reduction in survival observed in this experiment.

It is well understood that chronic stress results in increased maternal cortisol and is directly correlated with increases in egg cortisol in salmonids (Stratholt *et al.* 1997). Still, the significance of this hormone in eggs and whether it affects egg quality is less understood (Brooks *et al.* 1997). Mingist *et al.* (2007) demonstrated a significant negative relationship between egg survival to the eyed stage and egg cortisol content in masu salmon (*O. masou*), and Eriksen *et al.*

(2006) have shown that developing embryos from cortisol-stressed Atlantic salmon mothers exhibit increased mortality. This leads to the inference that maternal cortisol has a negative effect on salmonid eggs, and greatly enhances chances of progeny mortality post-fertilization. The effects of stress and presumed increases in maternal and egg cortisol is probably a significant contributing factor to the reduction in embryo survival prior to hatch observed in this study. Future analyses of egg and whole body cortisol content of juveniles used in this study are needed to examine this hypothesis.

Contrary to my predictions, artificially exposing eggs to elevated levels of cortisol did not influence egg survival to hatch, suggesting other maternal and egg aspects affected by stress aside from cortisol levels were equally as important to embryo survival post-fertilization. My findings contradict those of Li *et al.* (2010) who suggest the function of cortisol on egg survival is dose dependent. However, their results are likely influenced by the cortisol buffering capacity of ovarian fluid maintained in their experiment. Nevertheless, their results support the idea that there is a threshold level of cortisol (Hinch *et al.* 2006; Sloman 2010) that could affect salmonid offspring survival. A lack of difference in survival to hatch among treatments in my study could be a result of the applied dosage levels being too similar to naturally circulating levels of cortisol found in migrating female sockeye salmon. Perhaps if the applied dosage was higher and better reflected significantly high stress, differences in survival could have been observed. Given these dosages failed to produce results, wild female sockeye and their eggs may possess a capacity to buffer against relatively high levels of cortisol. Female sockeye salmon and their eggs have likely evolved a mechanism to ensure offspring survival during routine spawning. However, increased stressors beyond those encountered naturally during spawning migrations could compromise the natural capacity of females to buffer eggs from cortisol. Still, protection against increased maternal cortisol may be inherent in sockeye salmon eggs.

Another possible explanation for the differences in egg survival to hatch among treatments observed in this study is the ability of eggs to rapidly metabolize or excrete cortisol. Studies of egg cortisol content in salmonid eggs suggest this hormone clears rapidly from developing embryos by the late-eyed stage and may not be a factor in later development (Stratholt *et al.* 2007; Li *et al.* 2010). Li *et al.* (2010) noted a rapid decline in total embryo cortisol content immediately following fertilization of rainbow trout eggs bathed in elevated levels of cortisol. Stratholt *et al.* (1997) observed significantly elevated levels of cortisol in eggs

from stressed female coho salmon was followed by a rapid reduction in cortisol to levels no different from control egg levels only 8 days post-fertilization. Sockeye salmon eggs used in this study likely exhibited the same processes; no differences in survival to hatch across cortisol treatments could be explained by this phenomenon. These patterns suggest sockeye salmon eggs are likely adapted to increases in circulating maternal cortisol and are able to withstand certain threshold levels of this hormone before it becomes deleterious and impedes the very early stages of viable offspring production. This suggests stress and the resulting cortisol in females and eggs is not the ultimate factor determining embryonic survival, rather, offspring survival may be more influenced by the effects of stress on gene expression in eggs and how this relates to embryonic growth after fertilization (Barton 1991; Li *et al.* 2010). Or perhaps the real effects of stress and cortisol on viable offspring production are realized as post-emergence fry (Eriksen *et al.* 2006; 2007; 2011).

Subsequent juvenile development in the later stages of life may be compromised by the female stress response. Offspring may be more prone to behaviours that reduce survival (Eriksen *et al.* 2006), or transgenerational maternal stress may compromise juvenile stress responses (Wendelaar Bonga 1997) that ultimately affect progeny survival in the later stages of life. For example, a compromise in offspring stress response has been observed in the progeny of domestic chickens exposed to elevated levels of maternal cortisol (Haussman *et al.* 2011). The stress response of chicks shifted to that of more oxidative stress and a reduction in telomere length, which in many species of vertebrates ultimately leads to reduced offspring survival (Haussman *et al.* 2011). This has not been examined in sockeye salmon; however, a reduction in offspring stress response following maternal stress with similar consequences could compromise future viable offspring production. Future studies examining parental, egg, and offspring gene expression related to growth, behaviour, oxidative stress and telomere length in Fraser River sockeye salmon may be necessary to gain a more precise understanding of the stress response and role of cortisol in offspring development and how this hormone may ultimately affect survival.

### *Conclusions and Future Directions*

My study suggests increasingly-stressed maturing Fraser River sockeye salmon females have a great capacity to buffer against the effects of elevated physiological stress during migrations on fertilization success. However, observations that subsequent offspring survival can be

significantly decreased when female spawners encounter stressful migration conditions (Macdonald *et al.* 2000; Patterson 2004; Burt *et al.* 2011; 2012), and the significant reductions in embryonic survival observed in this study suggest transgenerational effects of maternal stress in Fraser River sockeye salmon exist. This has large ramifications for the productivity of populations and spawner fitness.

A number of different hypotheses have been put forth to explain the recent declines in Fraser River sockeye salmon abundance and productivity (Peterman *et al.* 2010; Hinch and Martins 2011), including transgenerational effects that reduce the overall fitness of subsequent generations. However, due to a lack of directed research and support from empirical analysis of the population data, transgenerational effects are considered very unlikely to have contributed to the long-term downward trend in productivity of Fraser sockeye salmon (Peterman *et al.* 2010). This study shows that detrimental transgenerational effects on production of viable future generations are real and should be considered as a potential contributing factor in the precipitous decline of Fraser River sockeye salmon.

Climate change, increased flows, and warming water temperatures are widely regarded as significant contributing factors to recent declines in Fraser River sockeye salmon (Peterman *et al.* 2010; Hinch and Martins 2011). Predictions of further river warming and increasingly difficult migrations will only increase en-route mortality and exacerbate the effects of stress on adults that survive to spawn (Patterson *et al.* 2007; Hinch and Martins 2011). It is therefore expected that transgenerational effects of stress will become increasingly prevalent and could lead to additional declines in sockeye salmon abundance and productivity in the Fraser River. Accordingly, further research is needed to examine subsequent aspects of transgenerational stress that could affect the overall fitness of future generations of Fraser River sockeye salmon.

A number of additional experiments should be conducted to build on the results from this study and enhance the current understanding of the effects of transgenerational stress in Fraser River sockeye salmon. Juvenile survival through the later stages of life must be monitored completely. Evaluating the swimming capability of juveniles from stressed mothers will aid in assessing how well offspring can successfully reach their nursery lakes. Behavioural studies of feeding and aggression in juveniles that ultimately affect survival in the wild should be conducted. Examination of the compromising capability of parental stress on the juvenile stress response is essential to determine how offspring will cope with increasingly stressful

environmental conditions. Effects on osmoregulation and smoltification should be examined to monitor how offspring from stressed parents cope with the transition to seawater. Additionally, sampling for contaminants and disease should include both parents and offspring (Peterman *et al.* 2010). To more fully comprehend transgenerational effects of stress in Fraser River sockeye salmon, all studies should be paired with gene expression to investigate the parameter of interest (Peterman *et al.* 2010).

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