EGG GLUCOCORTICOIDS AND MATERNAL STRESSOR EXPOSURE AS MODULATORS OF OFFSPRING QUALITY IN PACIFIC SALMON

by

NATALIE MARIE SOPINKA

B.Sc., McMaster University, 2009

M.Sc., McMaster University, 2010

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Forestry)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

February 2015

Abstract

Maternal experiences can have profound effects on offspring phenotype. In oviparous animals, prolonged maternal exposure to stressors can elevate circulating cortisol and is thought to also elevate egg cortisol. Experimental increases in egg cortisol are known to affect offspring performance. In fishes, past research has focused on how chronic maternal exposure to stressors affects egg size and embryonic survival, but changes to egg hormones and progeny phenotype beyond early development are not well understood. The aim of this thesis was to test the hypothesis that maternal exposure to a stressor alters behavioural and physiological attributes of offspring, and that those alterations are mediated by increases in egg cortisol. The research focused on Pacific salmon; animals that, as adults, encounter diverse stressors during their once-in-a-lifetime migration to spawning areas. I found that experimentally elevating egg cortisol, mimicking the presumed outcome of maternal stress, modified offspring morphology, swimming performance, and behavioural responses to conspecific intruders and simulated predator attacks. However, when I chronically exposed females to a daily chase stressor during sexual maturation, egg cortisol at spawning was not affected. Despite the absence of differences in egg hormone content, maternal stressor exposure did have latent effects on offspring swimming performance and physiological stress responses. Collectively, the evidence presented in this thesis suggests that, in Pacific salmon, experimentally manipulating egg cortisol elicits changes in offspring, but maturing females may have the capacity to buffer eggs from increases in cortisol. Gametic properties other than the concentration of cortisol are likely affected by maternal stress and responsible for the observed changes to offspring performance. The multidirectional

effects of maternal stress and egg cortisol treatment on offspring traits I report in this thesis support an emerging notion that the intergenerational effects of stress are highly-context dependent, as are interpretations of the adaptive *versus* maladaptive nature of changes to offspring. I conclude that the complexities of maternal stress and egg hormone deposition are not always captured when examining a single offspring trait at one life stage, without consideration of all components of the intergenerational process.

Preface

All of the work presented henceforth was conducted in the Pacific Salmon Ecology and Conservation Laboratory at the University of British Columbia, Vancouver campus (chapters 2-5) and at the University of Ottawa (chapter 5). All projects and associated methods were approved by the University of British Columbia Committee on Animal Care (#A11 0215) and met the Canadian Council for Animal Care guidelines.

Chapter 2: I was the lead investigator, responsible for concept development and design, data collection and analysis, and manuscript composition. Scott Hinch and David Patterson contributed to concept refinement and manuscript edits. Stephen Healy and Graham Raby assisted with data collection and provided manuscript edits.

Chapter 3: I was the lead investigator, responsible for concept development and design, data collection and analysis, and manuscript composition. Stephen Healy, Scott Hinch and David Patterson contributed to concept refinement and manuscript edits. Stephen Healy also assisted with data collection.

Chapter 4: A version of chapter 4 has been published [Sopinka N.M., Hinch S.G., Middleton C.T., Hills J.A., Patterson D.A. *Oecologia* 175: 493-500]. I was the lead investigator, responsible for concept development and design, data collection and analysis, and manuscript composition. Scott Hinch and David Patterson contributed to concept refinement and manuscript edits. Jayme Hills and Collin Middleton assisted with data collection.

Chapter 5: I was the lead investigator, responsible for data collection and analysis as well as manuscript composition. Jennifer Jeffrey and Katie Gilmour contributed to concept development and design. Katie Gilmour, Scott Hinch, Jennifer Jeffrey and David Patterson contributed to concept refinement and manuscript edits. Nicholas Burnett assisted with data collection.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ºC	degree Celsius
17α,20β-P	17α,20β-dihydroxy-4-prengnen-3-one
ACTH	adrenocorticotropic hormone
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BKA	bacteria killing ability
bp	base pairs
cAMP	cyclic adenosine monophosphate
CBG	corticosteroid binding globulin
cDNA	complementary deoxyribonucleic acid
cm	centimeter
CRF	corticotropin-releasing factor
DFO	Fisheries and Oceans Canada
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
EPOC	excess post-exercise oxygen consumption
EST	expressed sequence tags
g	gram
g	gravity
GC	glucocorticoid
GH	growth hormone
GSH	reduced glutathione
GSSG	oxidized reduced glutathione/glutathione disulfide
HIREC	human-induced rapidly changing environments
HPA	hypothalamic-pituitary-adrenal
HPI	hypothalamic-pituitary-interrenal
hpf	hours post-fertilization
HPG	hypothalamic-pituitary-gonadal
HSD11β2	type 2, 11β-hydroxysteroid dehydrogenase
HSD	honest significant difference
HSI	hepatosomatic index
IGF-1	insulin-like growth factor 1
kg	kilogram
km	kilometer
μL	microlitre
L	litre
m	metre
min	minute
MC2R	melanocortin 2 receptor
mL	milliliter
mm	millimeter
mRNA	messenger ribonucleic acid
MS222	tricaine methanesulfonate

n	sample size
ng	nanogram
ORAC	oxygen radical absorbance capacity
P450scc	cytochrome P450 side chain cleavage enzyme
PAR	Predictive Adaptive Responses
PC	principal component
PCA	Principal Components Analysis
PCR	polymerase chain reaction
PKA	protein kinase A
POA	preoptic area
PVC	polyvinyl chloride
PVN	paraventricular nucleus
RT-PCR	reverse transcription polymerase chain reaction
RU-486	mifepristone
s	second
SE	standard error
StAR	steroidogenic acute regulatory protein
UBC	University of British Columbia

Acknowledgements

Many thanks to: my ever patient and supportive supervisor (Scott Hinch), insightful and encouraging supervisory committee (Colin Brauner, Steven Cooke, Kathy Martin, David Patterson), lab mates (Mandy Banet, Arthur Bass, Marie-Ève Bélair-Bambrick, Nolan Bett, Nicholas Burnett, Jenn Burt, Matt Casselman, Alison Castle, Sylvia Chow, Mike Donaldson, Matt Drenner, Erika Eliason, Nathan Furey, Marika Gale, Steve Healy, Ken Jeffries, Sarah Larocque, Michael Lawrence, Melissa Lesko, Andrew Lotto, Eduardo Martins, Collin Middleton, Vanessa Minke-Martin, Vivian Nguyen, Graham Raby, Jessica Taylor, Catherine Thompson, Johan Spens, Sam Wilson, Charlotte Whitney), wonderful collaborators (Katie Gilmour, Jennifer Jeffrey), DFO Environmental Watch (Antoine Faure, Jayme Hills, Lisa Thompson, D'Arcy McKay, Taylor Nettles), Fraser River Pacific salmon, DFO Cultus Lake Salmon Research Laboratory, DFO Stock Assessment, DFO Chilliwack River Hatchery, Pacific Salmon Foundation, Chehalis First Nation, DFO Pacific Biological Station Molecular Genetics Lab, EWOS Canada Ltd., a multitude of dedicated undergraduate volunteers and research assistants, lovely friends (Trisha Atwood, Jordan and Cody Burke, Alison Collins, Laurence Dionne-Wilson, Lindsay Dores, Tara MacDonald (and Charles, Ella & Erhet Hatry), Julie Marentette, Julia Redfern, Susan Marsh-Rollo, Tonya Ramey, the Kendra-bo-bendra Robinson, Nicole Staresina, Monica Tse), dedicated supporters of graduate studies (Jackie Brinkman, Gayle Gosh, Christine Mutia, Rosemarie Cheng), dedicated supporters of mental health (Anne Massar, Karen Flood), thoughtful providers of monies (American Fisheries Society [Washington-British Columbia Chapter, Western Division, Canadian Aquatic Resources Section], Natural Sciences and Engineering Council of Canada, Ocean

Tracking Network, Fisheries Society of the British Isles, Society for Experimental Biology, University of British Columbia), and my family (Alicia Sopinka, Michael Sopinka (II & III), William Morrison, Halina Szczawinski).

Chapter 1: Introduction

1.1 Stress: origins and outcomes

1.1.1 *The stress response*

The stress response involves a suite of physiological and behavioural changes, and is thought to be adaptive by enabling animals to endure and overcome an acute stressor (i.e., short-term challenges to their fitness; Sapolsky et al. 2000). The conventional view of stress (activation of a stress response – the "general adaptation syndrome", Selye 1950; emergency life history stage, Wingfield et al. 1998) is that while the resultant changes to behaviour and physiology enhance fitness, a sustained response during chronic, long-term stressor exposure usually results in restricted realized animal performance and the potential for harmful pathologies (i.e., allostatic overload; McEwen and Wingfield 2003; Schreck 2010). The neuroendocrine system that regulates the response to stressors in vertebrates is the hypothalamic-pituitary-adrenal (HPA, mammals/rodents [Reeder & Kramer 2005], birds [Siegel 1980]) or hypothalamic-pituitary-interrenal (HPI, herpetofauna [Guillette et al. 1995], fishes, [Wendelaar Bonga 1997; Mommsen et al. 1999; Barton 2002]) axis. The HPA/-I axis is a complex signaling pathway that originates at the paraventricular nucleus (PVN) in the preoptic area (POA) of the hypothalamus (Herman & Cullinan 1997). Activation of the PVN is accomplished by sensory perception of immediate and forthcoming stressors by cerebral structures in ancient and advanced areas of the brain. Immediate stressors (e.g., imminent predator attack) may be processed by areas in the highly conserved brainstem, such as the medulla oblongata that is associated with respiration and blood pressure regulation (Loeschcke 1973). Forthcoming stressors (e.g., upcoming thesis defense) may be processed by areas in the forebrain, such as the

amygdala that is associated with emotional memory formation (Balleine & Killcross 2006). Instantaneously upon perception (or anticipation) of the stressor, catecholamine hormones (i.e., norepinephrine and epinephrine) are released by the adrenal medulla in mammals (Reeder & Kramer 2005) and birds (Siegel 1980), and in fishes (Wendelaar Bonga 1997; Barton 2002) and herpetofauna (Guillete et al. 1995), by interrenal chromaffin cells, *via* outputs of the sympathetic nervous system from the PVN that innervate these tissues. The actions of catecholamines are often associated with Walton Cannon's traditional "fight or flight reaction" that ensures an animal has adequate energy to either endure or escape the stressor (Cannon 1929). Catecholamines increase heart rate and ventilation rate, enhance blood oxygen transport, and promote the breakdown of fat (i.e., lipolysis), and of glycogen stores in the liver, to increase circulating glucose (i.e., glycogenolysis) (Wendelaar Bonga 1997; Reeder & Kramer 2005).

Simultaneous to the activation of catecholamine release, the PVN also releases a corticotropin-releasing factor (CRF) which, upon reaching the pituitary gland, stimulates the secretion of adrenocorticotropic hormone (ACTH) by corticotropes (Flik et al. 2006). ACTH secretion and responses thereafter are delayed relative to catecholamine release. ACTH travels through the bloodstream to the site of glucocorticoid production (adrenal cortex in mammals and birds, interrenal tissue in herpetofauna and fishes) and binds to melanocortin 2 receptors (MC2R) on steroidogenic cells of the target tissue. That binding results in production of cyclic adenosine monophosphate (cAMP) (Grantz & Fong 2003) and phosphorylation of protein kinase A (PKA). PKA then activates cholesterol ester hydroxylase to convert cholesterol esters to free cholesterol (Jefcoate et al. 1992). The transport protein, steroidogenic acute regulatory protein (StAR), shuttles cholesterol into the inner mitochondrial membrane of the interrenal cell (Stocco 2001). Within the mitochondrion, cholesterol is cleaved to pregnenolone by cytochrome P450 side

chain cleavage enzyme (P450scc, Payne & Hales 2004). Movement of cholesterol into the inner mitochondrial membrane and its subsequent cleaving are the rate-limiting steps of glucocorticoid synthesis. Following cleaving, a series of additional enzymes (e.g., 11β-hydroxylase) facilitate conversion of cholesterol to the taxon-specific primary glucocorticoid (cortisol: humans, fishes; corticosterone: rodents, birds, herpetofauna, Bury & Sturm 2007). The most well-studied class of hormone in the stress response system, glucocorticoids bind to receptors on target tissues (e.g., the brain, heart, adrenal glands/interrenal tissue, liver) to activate secondary and tertiary processes (see Sections 1.1.2, 1.1.3). Negative feedback of the HPA/-I axis occurs in the brain and adrenal glands/interrenal tissue (Wendelaar Bonga 1997). Cerebral binding of glucocorticoids occurs in hypothalamic and pituitary regions and results in downregulation of CRF and ACTH synthesis. At the adrenal glands/interrenal tissue, the binding of glucocorticoids directly inhibits further production of glucocorticoids.

Given the slower release of glucocorticoids (GCs herein) in comparison to catecholamines, and the variety of tissues GCs can be extracted from (e.g., plasma, saliva, hair/fur, feathers, feces; Sheriff et al. 2011), the concentration of GCs is the predominant metric (rather than catecholamines) measured to establish if an animal is stressed (i.e., has elicited a stress response; Cooke & O'Connor 2010, Dantzer et al. 2014). The effects of stressor-induced elevations in GCs have been well-studied across taxa, primarily within a generation.

1.1.2 *Effects of transiently elevated glucocorticoids*

In response to an acute stressor (e.g., seconds to minutes in duration, predator attack), GCs enhance catecholamine-induced increases in cardiovascular output, promote glycogenolysis (breakdown of glycogen stores in liver), move immune cells to infected/inflamed tissue and

promote the formation of memories (Sapolsky et al. 2000; McEwen & Wingfield 2003). There are also short-term suppressive effects of GCs during the response to acute stressors, including restrictions on non-essential physiological processes (e.g., gonad development, Wingfield & Sapolsky 2003; growth *via* changes to food intake and metabolism, Wendelaar Bonga 1997) and behaviours (e.g., courtship, foraging, Schreck et al. 1997). Exposure to an acute stressor that elevates circulating GCs also elevates plasma glucose (*via* glycogenolysis) and lysozyme activity (indicative of enhanced immunity, Demers & Bayne 1997), and reduces concentrations of plasma sex steroid hormones (Kubokawa et al. 1999), growth hormone (Pickering et al. 1991), and the appetite stimulant ghrelin (Pankurst et al. 2008). Experiments that impose rapid exogenous increases in GCs (e.g., *via* direct injection) find immediate reductions of non-essential behaviours (e.g., courtship, Burmeister et al. 2001; foraging, Santema et al. 2013), suggestive of a direct link between GCs and behaviour. Sex (Kubokawa et al. 1999), sexual maturity (Cook et al. 2011), age class (e.g., hyporesponsive period early in development, Sapolsky & Meaney 1986), parental care (Jeffrey et al. 2014a), and domestication (Woodward & Strange 1987; Lepage et al. 2000) can all modify the strength of the GC response to an acute stressor and the resulting secondary and tertiary effects. Arguably the most potent modifier of stressor-induced GC production and regulation is the chronicity of the stressor.

1.1.3 *Effects of chronically elevated glucocorticoids*

Chronic exposure to a stressor (e.g., hours to weeks or more in duration) is typically associated with chronically elevated circulating GCs *via* continuous stimulation of the HPA/-I axis or allostatic overload (McEwen 1998; McEwen & Wingfield 2003 but see Cyr & Romero 2009 regarding habituation). Subordinate social status (Sapolsky et al. 1997; Sloman et al.

2001a), long-term confinement or crowding (Pickering & Pottinger 1989; Campbell et al. 1994), inhabiting urbanized habitats (Zhang et al. 2011), chemical pollution (Hopkins et al. 1997), low food availability (Kitaysky et al. 2001) and repeated exposure to an acute stressor (Stratholt et al. 1997) have been identified as causes of chronically elevated GCs. In contrast to the adaptive outcomes of transient GC elevation, chronic GC elevation impairs metabolic efficiency and growth (DiBattista et al. 2006) and reproduction (e.g., persistently suppressed sex steroid hormones, Moore et al. 1991). Immune function is also compromised by chronically elevated GCs (Barcellos et al. 2004) increasing susceptibility to fatal pathologies (Woo et al. 1987). Other physiological effects of chronic stress can include neuronal cell death (Bachis et al. 2008), reduced metabolic scope (Lankford et al. 2005), increased fat deposition (Rebuffe-Scrive et al. 1992), and oxidative stress (Lucca et al. 2009). Behaviourally, chronic stress can lead to disrupted parental care (in conjunction with reduced prolactin levels in birds, Angelier & Chastel 2009), and reduced foraging, aggression and activity (as observed in subordinate individuals, Gilmour et al. 2005). Isolating the effects of stressor-induced chronically elevated GCs without stressor application is possible with the advent of synthetic GCs (Gamperl et al. 1994).

Exogenous manipulations of circulating GCs are used to mimic chronic HPA/-I axis activation and investigate the lethal and sub-lethal effects of chronic GC elevation. Intra-coelomic injection of GCs (suspended in carriers such as cocoa butter for slow, continuous release) elevates circulating GCs and reduces energetic reserves in largemouth bass (*Micropterus salmoides*, O'Connor et al. 2013), and suppresses plasma estradiol in female salmonids (Carragher et al. 1989). Belding's ground squirrels (*Urocitellus beldingi)* fed a GC-laced diet had increased serum GCs and fecal GC metabolites, and reduced bacterial killing ability (BKA) following immune challenge (Brooks & Mateo 2013). In birds, surgically-inserted silastic GC

implants increased plasma GCs and reduced feather growth (Romero et al. 2005), territorial behaviour (Wingfield & Silverin 1986) and parental care (Schultner et al. 2013). A meta-analysis by Costantini et al. (2011) found prolonged experimental elevation of GCs increased tissue-specific oxidative damage across vertebrate taxa. Dermal application of GCs dissolved in sesame oil increases circulating GC levels and the number of dead neonates in lizards (Meylan et al. 2010) and snakes (Robert et al. 2009). Like endogenously elevated GCs, exogenously elevated GCs are also implicated in individual survival (Pickering & Pottinger 1989).

The effects of chronic stress on reproduction are intimately linked to animal fitness. GC-mediated reductions in gamete and progeny quality can reduce parental fitness. Such effects are especially consequential for semelparous species (e.g., Pacific salmon, *Oncorhynchus* spp.) that reproduce only once in their lifetimes. GC-mediated modification of the hypothalamic-pituitary-gonadal (HPG) axis is multilevel (Rivier & Rivest 1991; Wingfield & Sapolsky 2003). For females, stressor exposure/elevated GCs can dampen production of sex steroid hormones (e.g., estradiol) by acting on hypothalamic and pituitary precursors (Wingfield & Sapolsky 2003). For oviparous species (i.e., lay eggs), estradiol is the key stimulator of hepatic production of the egg yolk protein vitellogenin (Wallace 1985), a component of eggs necessary for proper embryo development. Indeed, chronically elevated GCs can diminish plasma vitellogenin levels (Carragher et al. 1989; Campbell et al. 1994; Salvante & Williams 2003). Further, in oviparous species, increased maternal GCs are correlated with increased egg GCs (Hayward & Wingfield 2004), which can have notable effects on offspring phenotype (see Section 1.3). Maternal stressor exposure during sexual maturation/gestation may also have the potential to alter gene expression of developing eggs/embryos (Gheorghe et al. 2010). Embryos with sufficiently abnormal genetics may ultimately be aborted (Wilmut et al. 1986). It is therefore unsurprising,

given the relatively large investment by females into eggs/embryos, that research efforts have focused mostly on the effects of maternal (*versus* paternal) stress on offspring.

1.2 Maternal stress and glucocorticoids: intergenerational responses

The effects of maternal stress on offspring phenotype have been thoroughly examined in biomedicine using traditional rodent and human models, with the overwhelmingly accepted view that maternal stress and elevated GCs are harmful to offspring (Weinstock 2005; 2008). Experiments using non-rodent or human subjects generally corroborate this view; maternal stress, and proxies thereof, in wild and domesticated animals can have maladaptive effects on offspring phenotype. In birds, females chronically exposed to a stressor produce smaller offspring (Coslovsky & Richner 2011) with impaired learning (Lindqvist et al. 2007) and reduced foraging in competitive scenarios (Janczak et al. 2007). Exogenously manipulating circulating GCs to mimic maternal stress is also a potent modulator of offspring condition reducing immunocompetence and growth (Love et al. 2005), and amplifying HPA reactivity (Schultner et al. 2013), which can be associated with lower probability of survival (Blas et al. 2007). Maternal stress in farmed and captive mammals results in progeny with a range of impaired behaviours (e.g., social, exploratory/play, sexual) and reduced reproductive success (Braastad 1998). GC-treated female lizards birth lower condition offspring (Meylan & Clobert 2005). In fishes, chronically stressed females produce smaller offspring (McCormick 2006; 2009) with reduced survival (Campbell et al. 1992; 1994), and impaired learning (Roche et al. 2012) and antipredator behaviour (McGhee et al. 2012). When maternal GCs are experimentally increased, offspring have lower survival and growth (Eriksen et al. 2006). Accordingly, it would

seem appropriate to predict that chronic maternal stressor exposure resulting in sustained elevations in plasma GCs would be detrimental to offspring.

There is however a body of evidence for adaptive offspring outcomes following maternal stressor exposure and/or increases in maternal GCs. Experimental increases in maternal GCs of the lizard *Bassiana duperreyi* increased progeny growth (Warner et al. 2009). Elevations in maternal red squirrel (*Tamiasciurus hudsonicus*) GCs *via* resource competition also increased progeny growth (Dantzer et al. 2013). In fishes, threepined stickleback (*Gasterosteus aculeatus*) progeny reared from predator exposed females that reliably elevated plasma GCs in response to the stressor, Bell et al. 2007), demonstrated tighter schooling, an antipredator tactic (Giesing et al. 2011). The later example highlights the need to interpret the intergenerational effects of stress in an ecological context; are the outcomes of maternal stress matched or mismatched to the environment progeny will encounter (i.e., maternal match/mismatch; Breuner 2008; Love et al. 2013; Sheriff & Love 2013)? In a maternal match scenario, enhanced antipredator strategies would be beneficial for offspring facing a high predation environment similar to their mothers. However, if the offspring environment differs from the maternal environment (i.e., low predation) heightened vigilance may be hyper-responsive and impose an energetic cost. Seemingly negative traits may prove to be adaptive when maternal match/mismatch is considered (Sheriff & Love 2013). Fledgling European starling (*Sturnus vulgaris*) exposed to signals of poor maternal condition (i.e., increased yolk GCs) had reduced growth rates (Love et al. 2005). However, a stressed female in poor condition may not be able to provide sufficient resources for offspring with high growth rates. Thus, offspring with lower growth may outperform offspring with higher growth when reared by a stressed female (Love & Williams 2008a). Despite a general consensus that maternal stress has negative effects on offspring in

domesticated species, the variable, and potentially adaptive, offspring effects in wild animals has bolstered a continued interest in this field of study. Questions still remain regarding the physiological pathways by which maternal stress programs offspring phenotype. An obvious candidate pathway is that of maternal stress hormones (GCs, Groothuis et al. 2005; Weinstock 2005; Meylan et al. 2012) which, in oviparous animals, can accumulate in eggs (e.g., Okuliarová et al. 2010) and have direct consequences for offspring development.

1.3 Oviparity: a model for maternal effects of stress

In oviparous species, females transfer hormones, including GCs, to developing eggs (birds, Groothuis & Schwabl 2008; reptiles, Radder 2007; fishes, Brooks et al. 1997) without a placental buffer (Painter et al. 2002). The mechanism of hormone transfer is still a topic of debate (Groothuis & Schwabl 2008; Moore & Johnston 2008), and relationships between resting maternal hormones and egg hormones are broadly inconsistent (Groothuis & Schwabl 2008). In birds, hormone concentrations vary by egg order which could be suggestive of deliberate maternal investment (Groothuis & Schwabl 2008). Due to the lipophilic nature of hormones, passive uptake from maternal circulation is thought to occur in fish eggs (Mommer 2013), enabling the use of hormone baths as a method for experimentally-manipulating egg hormones (Auperin & Geslin 2008; Sloman 2010; Burton et al. 2011). Whether actively regulated or passively transferred (Groothuis & Schwabl 2008; Moore & Johnston 2008), egg hormones can have important effects on offspring.

Direct (*via* egg exposure) or indirect (*via* maternal state) experimental manipulation of egg hormone content (e.g., androgens, GCs, thyroid hormones) can modify progeny development, as can natural variation in egg hormones. Natural variation in egg hormone content

in birds is related to laying order and contributes to progeny immune function (Groothuis et al. 2005). In fishes, egg hormone content can vary depending on position within the ovary and spawning order, and influence embryo viability (Suter 2002). For herpetofauna, the duration of oviduct residence can affect the extent of hormone accumulation in eggs (Moore & Johnston 2008). Maternal nutrition (Warner et al. 2007), social experience (McCormick 2006; 2009), and predator (Pitk et al. 2012) and contaminant (Verboven et al. 2008) exposure can all influence egg hormone levels in oviparous taxa. When egg GC concentration is directly adjusted *via* hormone injection (birds, reptiles, and fishes) or immersion (fishes), progeny survival and growth, HPA/-I axis activity, cardiovascular function, aggression, metabolism and learning are affected (e.g., Love & Williams 2008a,b; Warner et al. 2009; Sloman 2010; Burton et al. 2011; Nesan & Vijayan 2012). The range of methods available to measure and manipulate egg hormones greatly facilitates the use of oviparous animals as models for the study of the intergenerational effects of maternal stress.

Among oviparous taxa, fishes are a useful but underused model for maternal effects. There are a number of reasons to use fishes for studies on the maternal effects of stress. Previous research has shown that when female fish are chronically exposed to ecological (McCormick 2006; 2009) and experimental (Stratholt et al. 1997) stressors, or injected with cortisol to mimic stressor-induced cortisol elevation (Eriksen et al. 2006), cortisol becomes elevated in plasma and eggs. Unfertilized or fertilized eggs can also be bathed directly in hormone-laden ovarian fluid or water, respectively, to achieve increased cortisol concentrations (Sloman 2010; Burton et al. 2011). Methods for *in vitro* egg fertilization and embryo rearing are well-established in fishes because they have been employed in aquaculture for decades. Offspring can be reared from gametes collected from wild-caught females exposed to natural stressors or females reared in

captivity and experimentally exposed to stressors. The survival and behaviour of the resulting offspring can then be monitored in the wild or laboratory settings. Other egg metrics that are affected by maternal stress (e.g., size, Campbell 1992; 1994) can be easily measured and predictive of offspring survival. Importantly, fishes are exposed to numerous anthropogenic stressors (e.g., chemical and noise pollution, warming water temperature, physical habitat destruction, eutrophication, fisheries capture and release) many of which are increasing in frequency and intensity. These stressors, which individuals cannot often escape from, can be attributed to declines in fish populations (Kappal 2005; Dudgeon et al. 2006). The intergenerational effects of maternal stress may further contribute to decreased fish productivity, but this hypothesis has yet to be investigated as there remains little basic knowledge on the effects of maternal stress in fishes. An integral objective of this thesis was to contribute to this knowledge deficit.

1.4 North American Pacific salmon: perishing parents?

Pacific salmon have a remarkable life cycle (see Groot & Margolis [1991] and Quinn [2005] for detailed species- and population-specific descriptions). Females deposit eggs in gravel in freshwater rivers which are simultaneously fertilized with sperm from males. Following spawning, both females and males die. Fertilized eggs incubate overwinter, embryos hatch and hatchlings/alevins absorb the yolk sac, converting the lipids and proteins into developing tissue. In spring, timed with freshwater algal blooms, fully formed fry emerge from the gravel and migrate to nearby freshwater rearing areas, estuaries or immediately to sea depending on the species. Young sockeye (*O. nerka*), coho (*O. kisutch*) and Chinook (*O. tshawytscha*) salmon fry residing in freshwater rearing lakes and streams stay for 1-2 years feeding and growing prior to

undergoing physiological (e.g., osmoregulation) and behavioural (e.g., schooling) metamorphoses that prepare them for entry into seawater (i.e., smoltification). As smolts, juvenile salmon migrate downstream to the Pacific Ocean where feeding and growth occur for 1-4 years depending on reproductive strategy (e.g., precocious male maturation or jacking) and species (e.g., pink salmon [*O. gorbuscha*] have a 2 year life cycle whereas sockeye salmon have a 4 year life cycle). Pink and chum (*O. keta*) salmon fry migrate downstream as underyearlings without a freshwater rearing phase. Adult salmon then undergo physiological (e.g., gonadal growth) and behavioural (e.g., cessation of feeding) changes that prepare individuals for reproduction and entry back into freshwater. Adults migrate from ocean feeding areas to their natal freshwater rivers, spawn and die. As semelparous fishes (only one lifetime opportunity to reproduce), any compromise to offspring quality can significantly affect fitness.

The productivity of Pacific salmon populations in British Columbia, Canada has declined since the early 1990s; for each spawning female, the number of offspring returning to spawn has decreased (Noakes et al. 2000). However, declines vary by species and population. In the Fraser River system, pink salmon productivity has generally been stable since the 1980s (Grant & MacDonald 2012). In contrast, coho salmon productivity has declined since the 1980's (Decker & Irvine 2013). Similarly, total sockeye salmon productivity has declined, but some populations remain stable or are increasing in productivity (Grant & MacDonald 2012). These trends are of considerable importance given the role of Pacific salmon in British Columbia's culture (e.g., aboriginal ceremonies), ecology (e.g., nutrient cycling, Schindler et al. 2003) and economy (e.g., commercial and recreational fisheries generate $1 billion annually, DFO 2008). To date, research has focused on how exposure to a variety of ecological and anthropogenic stressors affects adult salmon survival (fisheries capture, Raby et al. 2014; water temperature, Martins et al. 2012;

disease, Miller et al. 2014; flow regimes, Nadeau et al. 2010; hydroelectric dam passage, Burnett et al. 2014). Exposure of adults to environmental challenges during spawning migration generally reduces migration success and survival, and reductions are most pronounced in females (Martins et al. 2012). The number of fish arriving on spawning grounds is inherently critical in sustaining population stability. However, variability in the condition of fish that do spawn could also affect population productivity *via* variation in offspring quality. Neither parental spawner condition nor the influence of migratory stressors on offspring quality are normally considered when fisheries managers assess a population's productive capacity, nor when they use stock-recruit models to forecast the numbers of adults that will return to spawn. A consideration of intergenerational effects in population forecasting was recently identified by a federal judicial inquiry into declining salmon stocks as an important consideration (Cohen 2012).

Parental identity influences Pacific salmon progeny size and survival (Nadeau et al. 2009), response to thermal stress (Burt et al. 2012a) and swimming performance (Patterson et al. 2004a; Burt et al. 2012b). Parental condition has resonating effects as well. Tierney et al. (2009) found that moribund female sockeye salmon (characterized as lethargic and not actively swimming) with characteristically elevated plasma cortisol levels (Hruska et al. 2010) produced offspring that did not school as readily and fatigued sooner when compared to offspring reared from vigorous, spawn-ready females. These differences could be related to egg quality, which was not explicitly assessed. It is not known whether egg cortisol concentration differs between spawn-ready and moribund females. The eggs retained in moribund females may be 'overripe', which can result in altered lipid and protein content (Craik & Harvey 1984) and reduced offspring survival (Barnes et al. 2000). In the context of maternal stress, female sockeye salmon exposed to experimentally enhanced flow regimes did not alter reproductive investment or

offspring quality (Hinch et al. unpublished data), but regular exercise while in captivity does improve progeny survival (Patterson et al. 2004b). However, neither Hinch et al. (unpublished data) nor Patterson et al. (2004b) measured egg hormones or found differences in maternal plasma cortisol. Following chronic exposure to an experimental chase stressor, female coho salmon produced eggs with higher egg cortisol content than did undisturbed females, but offspring survival and size were not assessed (Stratholt et al. 1997). Parental treatment effects on offspring responses can be observed in the absence of survival or size differences (Rubolini et al. 2005). Thus, it is important to consider that the maternal effects of stress (and proxies thereof) may not manifest until later in offspring development and/or when offspring are tested under ecologically-relevant conditions.

For young Pacific salmon offspring, maternal stressor exposure and elevated egg cortisol could affect an array of life history and survival traits. For example, reduced egg and offspring size can jeopardize survival *via* insufficient yolk resources to survive through to spring, or through increased predation and/or reduced food intake (Brooks et al. 1997). Reductions in offspring swimming performance could compromise the success of fry migration to freshwater rearing areas, feeding efficiency, or predator evasion. While rearing in freshwater, prior to migration to sea as smolts, certain species of Pacific salmon (e.g., coho salmon, see chapter 3) establish and defend feeding territories (Groot & Margolis 1991; Quinn 2005). Decreased aggression and competitive ability could have effects on juvenile territory quality, and reproductive success as adults, if correlated with adult aggression/competitive ability (e.g., Francis 1990). Impaired antipredator behaviours influence survival directly by increasing predation risk. Finally, given the links between responsivity to acute stressors and survival (Blas et al. 2007; Cook et al. 2014), changes to offspring HPI function could also have fitness

consequences. Therefore, assessment of the maternal effects of stress and egg cortisol on offspring can be best achieved by examining a range of physiological and behavioural traits to provide a more holistic understanding of the latent effects of maternal experience.

Substantial effort has been invested into understanding how adult exposure to stressors affects adult survival but little is known about how sub-lethal effects of stressor exposure (e.g., increases in egg cortisol) are influencing the next generation of this significant natural resource (Cohen 2012). With limited replication of the relationships between stressor exposure, egg GCs and offspring quality in fishes, it cannot be ruled out that these relationships could be contributing to current trends in Pacific salmon productivity. Needed is a concerted effort to understand not only stressor-induced effects on maternal survival but also that of her progeny.

1.5 Thesis objectives and organization

This chapter has summarized the literature, knowledge gaps and rationale for investigating how egg glucocorticoids and maternal stressor exposure modulate offspring quality in Pacific salmon. The aims of the remainder of this thesis were to 1) determine the effects of experimentally elevated egg cortisol, a predicted outcome of maternal stress, on Pacific salmon offspring, and 2) substantiate experimental egg manipulations by examining the intergenerational effects of maternal stress in wild-caught, stressor-exposed salmon. Given the evolving understanding that in non-rodent systems maternal stressor exposure and egg GCs can result in offspring with traits that can be interpreted as a adaptive or maladaptive, specific predictions were not employed. The overarching hypothesis was that experimental modifications to eggs (chapters 2 and 3) and spawning females (chapters 4 and 5) would alter offspring performance. Chapters 2 and 3 explored the impacts of exogenously elevated egg cortisol on fry performance

and behaviour. Chapters 2 and 3 also served as a proof of concept that egg hormone baths can elevate egg cortisol concentrations and alter offspring. Chapter 2 focused on how elevated egg cortisol affects fry morphology and swimming capacity under benign conditions (i.e., within a swim tunnel). The egg cortisol effects were examined between populations (river *versus* channel-spawning) and between species of Pacific salmon (chum and sockeye salmon) to determine if offspring responses to elevated egg cortisol vary intra- and inter-specifically. Chapter 3 determined the effects of elevated egg cortisol on progeny behavioural responses under ecologically-relevant conditions; territory intrusion and threat of predation. Chapter 3's study design also aimed to reveal consistent correlations among behaviours (i.e., behavioural syndromes) and consistent inter-individual differences in behaviour (i.e., personality; Sih et al. 2004). This chapter used coho salmon as other species of salmon readily school, do not defend feeding territories, and exhibit low levels of aggression. Chapters 4 and 5 assessed the outcomes of chronic maternal exposure to a stressor on egg cortisol levels and offspring performance. To corroborate findings of chapter 2, chapter 4 described how repeated maternal exposure to an acute stressor alters egg cortisol allocation and fry swimming capacity in sockeye salmon. Chapter 5 then identified effects of maternal exposure to a stressor on offspring physiology by assessing the hormonal and genomic responses of juvenile sockeye salmon offspring to an acute stressor. Chapter 6 discusses thesis limitations, provides specific and general research directions regarding maternal effects of stress in fishes and draws conclusions on the roles of gametic properties and maternal stressor exposure in shaping offspring phenotype.

Chapter 2: Effects of experimentally elevated egg cortisol on juvenile Pacific salmon morphology and swimming performance

2.1 Synopsis

The objective of this chapter was to determine if increases in egg cortisol concentration, which can occur under conditions of maternal stress, affect offspring development and performance traits in juvenile salmon. Specifically, I investigated whether experimentally elevated egg cortisol (*via* hormone egg baths) affected juvenile morphology (body size and depth, fin size, eye size) and burst swimming performance in two wild populations of chum salmon (*O. keta*; spawn in natural river, spawn in man-made channel), and one wild population of sockeye salmon (spawn in natural river). Egg hormone baths significantly elevated egg cortisol concentrations in each of the populations and species examined. Sockeye salmon fry reared from cortisol-treated eggs had smaller fins, more robust bodies and smaller eyes compared to untreated conspecifics. In contrast, channel chum salmon fry reared from cortisol-treated eggs were larger, with larger fins, streamlined bodies and larger eyes compared to untreated channel fry. River chum salmon fry morphology was not altered by egg cortisol treatment. Fry burst swimming rate, but not duration, was reduced in egg cortisol-treated river sockeye and chum salmon, whereas no swimming traits were affected by egg cortisol in channel chum salmon. My results demonstrate that closely related species and populations can respond differently to elevated egg cortisol. Future predictions of offspring performance based on maternal stress and egg composition need to consider the potential for divergent responses among species and populations.

2.2 Introduction

For oviparous taxa, repeated exposure to stressors that reliably elevate circulating levels of maternal GCs (e.g., predator exposure, Cockrem & Silverin 2002; competitive interactions, Sloman et al. 2001a) have the potential to influence the next generation *via* differential hormonal investment in eggs (e.g., McCormick 2006; 2009; Saino et al. 2005; Pitk et al. 2012). When elevated experimentally, egg GCs can have important effects on progeny physiology and behaviour. Generally, trade-offs are observed between egg GCs and offspring quality. Elevated egg GCs reduce offspring survival (Saino et al. 2005; Gagliano & McCormick 2009), growth (Hayward et al. 2006), immune function (Rubolini et al. 2005), cardiac function (Nesan & Vijayan 2012), aggression and social status (Burton et al. 2011). However, evidence is emerging for adaptive outcomes (e.g., increased vigilance in the lizard *Lacerta vivipara*, Uller & Olsson 2006; increased flight performance in European starlings (Chin et al. 2009) suggesting that offspring responses to egg GCs may vary within (see Sloman 2010 and Burton et al. 2011) and among species (Warner et al. 2009). Identifying intra- and inter-specific patterns in offspring responses to egg GCs could reveal useful insight into which animals are likely to be tolerant or vulnerable to stressor-induced increases in egg GCs. Of particular interest and concern are aquatic organisms, especially fishes, now chronically exposed to numerous stressors (e.g., temperature, Ryan 1995; contaminants, Pratap & Wendelaar Bonga 1990; fisheries capture, Marcalo et al. 2006) that can increase circulating cortisol, and thus potentially that of eggs. As important populations of fishes are declining in the face of multiple stressors (Hutchings & Reynolds 2004; Crain et al. 2008), investigating the role of egg cortisol in mediating intergenerational processes is of fundamental and applied interest.

Population (Dunlap & Wingfield 1995; Trompeter & Langkilde 2011; Dahl et al. 2012) and species (Møller & Mousseau 2007) differences in the effects of stressors on adult animals are observed across oviparous taxa, including commercially targeted salmonids (Donaldson et al. 2012; Jeffries et al. 2014). Intra- and inter-specific variation in response to changes in egg hormone composition has received little attention. The high spawning stream fidelity in many salmonids generates genetically distinct populations within species, resulting in divergent life history, physiological, and behavioural traits (Vander Zanden et al. 2000; Lahti et al. 2002; Rodnick et al. 2004; Eliason et al. 2011). In addition to striking intra-specific variation, inter-specific variation can also be observed within a breeding locale (Quinn 1999). Importantly, intra- (Woodward & Strange 1987) and inter-specific (Barton 2000; Donaldson et al. 2014) differences in stressor-induced cortisol production and regulation have also been detected in juvenile and adult salmonids, respectively. Such variation in adults suggests that gametic regulation of cortisol and the latent effects of such regulation on offspring performance could also demonstrate intra- and inter-specific variation. Examination of population- and species-specific effects on offspring traits are largely limited to early development when offspring are typically immobile (Essington et al. 2000; Whitney et al. 2013 but see Patterson et al. 2004a; Pon et al. 2007), and rarely considered in the context of maternally-derived cortisol.

In salmonids, offspring swimming performance is important to survival (Taylor & McPhail 1985a), varies intra- (Taylor & McPhail 1985a; Sopinka et al. 2013) and inter-specifically (Hawkins & Quinn 1996), and can be influenced by maternal effects (Burt et al. 2012b), including maternal cortisol (Tierney et al. 2009). At emergence (complete yolk sac absorption), Pacific salmon fry must quickly transition to exogenous feeding by migrating to rearing areas to feed before migrating to the ocean as smolts (Quinn 2005). During this period of

growth, young fish must successfully evade predators. It remains unclear how egg cortisol influences metrics related to predator avoidance (e.g., swimming capacity and hydrodynamic morphology), or if offspring responses to egg cortisol manipulation vary intra- or inter-specifically. Offspring burst swimming performance varies among populations of sockeye salmon and reflects the difficulty of adult reproductive migration more so than that of fry migration (Sopinka et al. 2013). Interspecific differences in offspring swimming capacity also exist. For example, chum salmon fry do not school as tightly as sockeye salmon fry (Salo 1991). These underlying intra- and inter-specific differences in offspring swimming performance, linked with intra- and inter-specific differences in offspring morphology (Taylor & McPhail 1985a; Hawkins & Quinn 1996), could potentially be masked, attenuated or unchanged by elevated egg cortisol.

The diverse life histories of Pacific salmon provide a useful model for understanding the intra- and inter-specific effects of elevated egg cortisol on offspring performance. Species- and population-level differences could be taken under consideration by conservation practitioners when trying to mitigate or predict animal sensitivity to stressors (Donaldson et al. 2012). Research that illustrates functional differentiation among closely related species and populations can help justify conservation actions aimed at protecting diversity, consistent with the stated principles of Pacific salmon management (in Canada; DFO 2005). Accordingly, I examined the hypothesis that experimental elevation of egg cortisol (*via* egg hormone bath, Auperin & Geslin 2008; Burton et al. 2011) would alter embryo survival, fry morphometrics and burst swimming performance, and that such alterations would vary between species (within a population) and between populations (within a species). Eggs were collected from two wild and geographically-distinct populations of chum salmon (river *versus* channel spawning) and one wild population of

sockeye salmon (river spawning). Emergent fry from the river spawning chum salmon population (Harrison River) swim downstream to reach estuaries, whereas fry from the channel spawning population of chum salmon (Weaver Creek) must swim upstream and then downstream to reach estuaries. Populations of salmon faced with this upstream migration appear to have enhanced swim performance (Patterson et al. 2004a; Pon et al. 2007 but see Sopinka et al. 2013). In the majority of sockeye salmon populations, emergent fry migrate to and reside in freshwater rearing areas for 1-2 year before migrating to the sea (Burgner 1991). In contrast, Harrison River sockeye salmon migrate downstream to estuaries as underyearlings (< 1 year old, Birtwell et al. 1987), a life history similar to that of chum salmon (Salo 1991). However, underyearling sockeye salmon will be considerably smaller than underyearling chum salmon and may experience differential predation pressure in estuarine environments. The life history differences among populations and species may drive intra- and inter-specific differences in how offspring respond to egg cortisol-treatment.

2.3 Materials and methods

2.3.1 *Egg cortisol treatment and offspring rearing*

Ripe fish were collected from two spawning areas in the Fraser River watershed in British Columbia, Canada. Following capture and euthanasia *via* cerebral percussion eggs and sperm were stripped from channel spawning chum salmon [Weaver Creek (49°19'22" N, 121°52'49" W)] and river spawning chum and sockeye salmon [Harrison River (49°17′5" N, 121°54′27" W)]. For further description of the Weaver Creek spawning channel see Raby et al. (2013). Wet egg mass was determined by averaging the mass (to the nearest 0.0001 g) of three replicates of ten unfertilized eggs from each female. Gametes were transported to (~2 hours) and

fertilized at the University of British Columbia (UBC) as follows in replicate: 5 full sibling crosses of channel chum salmon, 8 full sibling crosses of river chum salmon, and 7 full sibling crosses of river sockeye salmon. For each full sibling cross 15 g of eggs were mixed with 1 mL of milt and then 30 mL of water was added to activate sperm. After 2 min, 400 mL of water was added to the sperm-egg mixture and left to stand for 2 hours. One replicate received water with 1000 ng/mL cortisol (H4001, Sigma, www.sigmaaldrich.com, Auperin & Geslin 2008) that was initially dissolved in 95% ethanol (0.002 % final ethanol concentration). The other replicate received untreated water (0 ng/mL cortisol) with the same concentration of ethanol as the cortisol-treated eggs (0.002%). Stressor-induced (Cook et al. 2011; 2014) and resting (Hruska et al. 2010) plasma levels of maternal cortisol can approach and exceed 1000 ng/mL in maturing and moribund Pacific salmon. After 2 hours of incubation (following Burton et al. 2011), separated by replicate and family, fertilized eggs were rinsed thoroughly with fresh water and transferred to Heath stacks with mean (± SE) water temperature of 8.1 ± 0.4°C. Dead eggs and embryos were removed every other day from stacks to prevent decomposition and spread of fungus to healthy embryos. Dead eggs and embryos were stored in Stockard's solution (5% formaldehyde (40%), 4% glacial acetic acid, 6% glycerin, 85% water) to determine fertilization success, and survival to eyed, hatch and emergence. At emergence, families were pooled and fry were transferred to 1000 L flow-through troughs (~1500 fish per trough), separated by species, population and egg cortisol treatment. Photoperiod in the lab was adjusted to mimic the photoperiod at latitude 49°18´N. Water temperature ranged from 5-9°C due to natural fluctuations in the municipal water source used. Fry were fed powdered fishmeal (EWOS Canada Ltd., www.ewos.com) *ad libitum* twice daily until 24 hours prior to burst swimming trials.

2.3.2 *Egg cortisol assay*

To assess the efficacy of the cortisol treatment, egg cortisol concentrations were quantified with a commercially-available enzyme-linked immunosorbent assay (ELISA, Neogen Corporation, www.neogen.com, product #402710). Three unfertilized eggs from each female and three eggs from each replicate 2 and 24 hours post-fertilization (hpf) were frozen in liquid nitrogen and transferred to a -80°C freezer for storage. In the laboratory, eggs (~0.5 g) were homogenized in 1200 µL of assay buffer. The homogenate was vortexed with 3 mL of diethyl ether, centrifuged for 5 min at 10,000 *g*, and flash frozen at -80°C for 30 min. The liquid phase was poured off, evaporated under nitrogen and the residue was reconstituted in 1200 µL of assay buffer. Reconstituted samples were warmed for 10 min at 65°C, and a 50 µL subsample was removed for use on the ELISA plate. Egg cortisol samples were run in duplicate on three assay plates with intra- and inter- assay coefficients of variation of 4% and 9%, respectively.

2.3.3 *Fry burst swimming performance*

For detailed description of swim trial equipment and protocol, see Sopinka et al. (2013). Fry 2-3 months post-emergence from each species and population were randomly selected from a trough and placed in a sectioned area (30 cm length x 6.9 cm width x 4 cm depth) of a swim flume (230 cm length x 17 cm width x 4 cm depth) with fixed water speed of 39 and 34 cm/s for chum (mean ± SE fork length, 4.69 ± 0.03 cm) and sockeye salmon (3.80 ± 0.04 cm) fry, respectively (speed adjusted to achieve ~8-9 fork lengths/s, speeds maintained using anaerobic metabolism, Brett 1964). Each 1000 L flow-through trough held up to ~1500 fry pooled from all families within a species/treatment/population; random selection of fry reduced the likelihood of pseudoreplication within a single family. The front 20 cm of the sectioned area of the swim

flume was shaded and a light shone on the back 10 cm to encourage fish to swim forward. All trials were recorded with a digital camera (Canon EOS Rebel T3i; www.canon.com). Exhaustion, and the end of the swim trial, was defined as failure of a fish to move from the back of the sectioned area after 3 probes with a blunt instrument. Exhausted fish were removed from the flume and euthanized with an overdose of buffered tricaine methanesulfonate (MS222; 0.25 g/L). Body mass was recorded to the nearest 0.0001 g. A photograph was taken of each fish against graph paper (for scale) with a digital camera (Nikon D40, www.nikonusa.com) for morphological analyses (see Section 2.3.4).

Burst swimming duration and bursting rate were quantified from recorded videos. In total 271 fish were tested for swimming performance (79 river sockeye salmon [0 ng/mL, n=29; 1000 ng/mL, n=50]); 92 river chum salmon [0 ng/mL, n=42; 1000 ng/mL, n=50]; 100 channel chum salmon [0 ng/mL, n=50; 1000 ng/mL, n=50]). Fish swam one continuous bout until exhaustion or re-initiated swimming after falling to the back, un-shaded portion of the flume (more than one bout) prior to exhaustion. Burst swimming duration was calculated as the duration (in s) of the continuous bout and for fish that swam multiple bouts, the summed times of all bouts. Burst swimming rate was defined as the number of bouts completed per 10 s of swimming (i.e., the number of instances a fish fell to the back of the flume then re-initiated swimming and burst swam back to the front).

2.3.4 *Fry morphology*

Morphological traits (Figure 2.1) were measured from the digital images using ImageJ (rsbweb.nih.gov/ij/). Eleven measurements were taken in total; fork length (FL), fin size which included pectoral fin length (PEC, left fin only), caudal fin length (upper segment [CAUD1],

lower segment [CAUD 2]), caudal fin height (CAUD 3), and caudal fin area (CAUD 4), body depth which comprised three measures (distance between dorsal and pelvic fins [DORPEL], distance between adipose and anal fins [ADAN], and caudal peduncle height [PED]), and eye area which was calculated using the formula: π x [0.5 x EW] x [0.5 x EL] (Neff 2004). See Table 2.1 for mean ± SE values of all morphometric features measured.

2.3.5 *Statistical analyses*

All statistical tests were conducted using JMP 10.0.2 (SAS Institute Inc.). Data were assessed for normality and $\log_{10}$ (egg cortisol) or cube root (burst swimming duration) transformed to achieve normality and enable use of parametric tests. If data could not be transformed to achieve normality, non-parametric tests were used. Differences in unfertilized egg cortisol concentrations were assessed among groups (river sockeye salmon, river chum salmon, channel chum salmon) using analysis of variance (ANOVA). Effectiveness of the egg cortisol baths was examined separately for each group using two-way repeated measures ANOVAs with egg treatment level (0 ng/mL *versus* 1000 ng/mL cortisol dose) and time period (2 and 24 hpf) as fixed effects, and female ID nested within egg treatment as a random factor. Absolute change in egg cortisol elevation ([treated eggs] – [untreated eggs]) was assessed separately for each group using ANOVAs at 2 and 24 hpf. Temporal analysis ([2 hpf] – [24 hpf]) of cortisol levels was assessed separately for each group using ANOVAs for both untreated and cortisol-treated eggs. Pearson correlations were used to detect relationships between egg mass and unfertilized egg cortisol levels. Egg cortisol treatment effects on fertilization success and offspring survival were examined using Wilcoxon signed-rank tests. Morphological traits (Figure 2.1, excluding EW and EL), eye area and body mass were loaded into a Principal Components Analysis (PCA). PC1

explained 73% of the variation (eigenvalue = 8.0) and positively correlated with all contributed metrics (all positive eigenvectors, see Table 2.2). PC1 thus represented a general trend of body size and depth, eye size and fin size, and was used in subsequent analyses. Eleven fish (river sockeye salmon 1000 ng/mL, n=4; river chum salmon 0 ng/mL, n=1, 1000 ng/mL, n=2; channel chum salmon 0 ng/mL, n=1, 1000 ng/mL, n=3) were excluded from the PCA analyses as one of the metrics included in the PCA could not be obtained from the digital images. Effects of egg cortisol treatment on PC1 among fry groups (river sockeye salmon, river chum salmon, channel chum salmon) were analyzed using a factorial ANOVA. Separately for each fry group, Fisher's exact tests were used to determine the effects of egg cortisol treatment on swim failure. An analysis of covariance (ANCOVA) with PC1 as a covariate, and cortisol treatment as a fixed effect was used to establish egg treatment differences in sockeye salmon burst swimming duration. An ANCOVA with PC1 as a covariate, and cortisol treatment and population as fixed effects was used to establish egg treatment differences in chum salmon burst swimming duration. Cortisol treatment effects on burst swimming rate were quantified with a Wilcoxon signed-rank test separately for each fry group as bursting rates could not be normalized. Separated by egg cortisol treatment and fry group, relationships between PC1 and burst swimming rate were examined using Spearman's rank correlations and a Bonferroni correction for the 6 separate tests (P=0.008).

2.4 Results

2.4.1 *Fertilization success and embryo survival*

Across all fry groups, fertilization success, and cumulative survival to eyed, hatch and

emergence did not differ for progeny reared from untreated and cortisol-treated eggs (Wilcoxon signed-rank tests, all $P>0.05$).

2.4.2 *Egg cortisol levels*

Egg cortisol levels in unfertilized eggs were highest in river spawning sockeye salmon and lowest in river spawning chum salmon; levels were intermediate in channel spawning chum salmon (Table 2.3). Two-hour incubation in the cortisol-treated water (1000 ng/mL) significantly elevated egg cortisol levels for all salmon (Table 2.3). Egg cortisol levels were elevated to levels observed in previous egg treatment studies (Burton et al. 2011), mirrored levels detected in eggs of coho salmon stressed *via* net chasing twice daily for two weeks (Stratholt et al. 1997), and thus represented upper, but not pharmacological or pathological, hormone levels. Egg cortisol levels remained elevated 24 hpf in chum, but not sockeye, salmon (Table 2.3). Absolute change in cortisol elevation ([treated eggs] – [untreated eggs]) did not vary among groups at 2 (ANOVA, $F_{2,18}=0.44$, $P=0.65$) or 24 hpf ($F_{2,18}=0.40$, $P=0.68$). For chum salmon, cortisol-treated and untreated egg cortisol concentrations decreased from 2 to 24 hpf (Table 2.3). Cortisol concentrations significantly decreased from 2 to 24 hpf in cortisol-treated, but not untreated, sockeye salmon eggs (Table 2.3). The magnitude of cortisol reduction ([2 hpf] – [24 hpf]) did not vary among groups for either cortisol-treated ($F_{2,18}=2.11$, $P=0.15$) or untreated ($F_{2,18}=0.32$, $P=0.73$) eggs. No relationship was detected between egg mass and cortisol concentration in unfertilized eggs of chum (Pearson correlation, $r^2=0.001$, n=14, $P=0.93$) and sockeye ($r^2=0.14$, n=7, $P=0.41$) salmon.

2.4.3 *Fry morphology and swimming performance*

Effects of egg cortisol treatment on PC1 scores varied among species and populations. Compared to sockeye salmon fry reared from untreated eggs, fry reared from cortisol-treated eggs had lower PC1 scores indicating smaller body size, more robust body shape, and smaller fins and eyes (Factorial ANOVA, treatment x group, $F_{2,254}$=11.74, P<0.0001; Figure 2.2). In contrast channel chum salmon fry reared from cortisol-treated eggs had higher PC1 scores compared to fry from untreated eggs, indicating larger body size, more streamlined body shape and larger fins and eyes (Figure 2.2). River chum salmon morphology was not affected by egg cortisol treatment (Figure 2.2).

Egg cortisol treatment altered fry burst swimming performance depending on population and species (Table 2.4). Burst swimming duration was not affected by egg cortisol treatment (Table 2.4). Burst swimming duration did not differ between populations of chum salmon (Table 2.4). PC1 positively correlated with burst swimming duration for chum (Pearson correlation, r^2=0.22, n=155, P<0.0001) and sockeye salmon (r^2=0.42, n=70, P<0.0001). Interactions between PC1 and egg cortisol treatment were not detected indicating egg cortisol treatment did not alter relationships between fry morphology and burst swimming duration. River chum and sockeye salmon fry treated with cortisol as eggs had reduced burst swimming rates compared to fry from untreated eggs (Table 2.4). Burst swimming rates of channel chum salmon were not influenced by egg cortisol treatment (Table 2.4). No statistically significant relationships were detected between PC1 and burst swimming rate for any of the fry reared from untreated or cortisol-treated eggs (Table 2.5). Cortisol treatment of eggs did not affect the proportion of fish that failed to swim in the flume (Fisher's exact test, all P>0.05).

2.5 Discussion

I detected species-specific and population effects of elevated egg cortisol on morphology and swimming performance in wild salmon. Morphology of river sockeye salmon and channel, but not river, chum salmon was altered by egg cortisol treatment in opposite directions. Burst swimming duration was not affected by egg cortisol treatment. Egg cortisol treatment appeared to reduce burst swimming rate in sockeye and chum salmon fry from the river spawning population. Offspring survival was not affected by egg cortisol treatment. Cortisol-mediated effects on survival may be masked in this study as all embryos were reared under identical, controlled hatchery conditions. In Asian seabass (*Lates calcarifer*) survival under hyposaline conditions did not vary between untreated and cortisol-treated embryos, but under hypersaline cortisol-treated embryos had higher survival (Sampath-Kumar et al. 1993). Inter- and intra-specific variation in life history, sensitivity to stressors, and physiological buffering capacity could drive the differential influence of elevated egg cortisol levels on morphology and swimming performance.

Species-specific differences in response to elevated egg cortisol were evident morphologically but not with regard to swimming performance. It is unknown why morphology was altered by cortisol treatment in sockeye but not chum fry from Harrison River. Species-specific effects of egg GC treatment on juvenile growth are observed in lizards, though the species are not geographically sympatric (Warner et al. 2009). Early life history is similar between Harrison River sockeye salmon and chum salmon: both species migrate to estuaries as underyearlings (Birtwell et al. 1987, Salo 1991). Egg-fry/juvenile-adult survival rates are similar for chum and sockeye salmon (Bradford 1995). The adult migration is also similar: both enter the Fraser River late September, migrate ~100 km upstream to the Harrison River system, and hold

for approximately 6-12 weeks prior to spawning mid-November (D. Patterson, personal communication). Cortisol was higher in unfertilized sockeye salmon eggs compared to chum salmon from the same population; however the efficacy of egg cortisol treatment did not differ. The physiological processes hormones target that could drive these phenotypic changes have yet to be identified (Lema 2014). Burst swimming rate was reduced, albeit subtly, following egg cortisol treatment in river spawning sockeye and chum salmon fry. This response could compromise predator evasion by reducing the capacity to repeatedly swim away from an attacking predator. The physiological mechanism that led to reduced burst swimming performance cannot be determined with this dataset but may be related to cortisol-mediated changes in oxygen consumption (Sloman 2010), muscle or plasma lactate regulation (Tierney et al. 2009), and/or activity levels of metabolic enzymes such as citrate synthase and cytochrome *c* oxidase (see Patterson et al. 2004a).

The population-specific effects of egg cortisol treatment I observed were also trait specific. Fry swimming performance was altered by egg cortisol treatment in river but not channel spawning chum salmon, though the inverse was true of fry morphology. There was no evidence of intra-specific variation in egg cortisol uptake and/or eradication: egg cortisol concentration patterns did not differ between the two populations of chum salmon examined here. In the spring following emergence, both populations of chum salmon tested in this study migrate a similar distance to the Fraser River estuary prior to entering the Pacific Ocean to rear. Thus, early life history is not likely to be a driver of the differential responses observed between these two populations. With regard to adult life history, river spawning female chum salmon need maintain position while defending nests in water flows that are more turbulent compared to the slow laminar flows in a fishing-free spawning channel (Weaver Creek). I speculate that

swimming performance (*versus* body morphology) may be more responsive to egg cortisol in river spawning populations. In contrast, morphological traits may be more sensitive to egg cortisol in channel spawning chum salmon given that elevated egg cortisol can signal increased competition (McCormick 2006) and thus drive changes in phenotypic traits strongly associated with reproductive success during adulthood (i.e., morphology/size, Steen & Quinn 1999). The observed larger body fin and eye size in channel chum salmon reared from cortisol treated eggs can also be advantageous early in life during predator avoidance (Taylor & McPhail 1985b). A similar effect is observed in North American red squirrels; females exposed to natural and simulated high density conditions had elevated fecal GCs and produced offspring with accelerated growth rates (Dantzer et al. 2013).

Hormonal composition of eggs has ecologically important effects in highly fecund animals; however, these effects can be inconsistent and multifaceted when examining multiple traits, species, or populations. Such caveats should be considered when drawing evolutionary conclusions and forming theoretical predictions regarding the adaptive (or maladaptive) effects of egg hormones (Dufty et al. 2002; Love et al. 2013; Sheriff & Love 2013). Given that egg hormones are maternally derived, and the environment a breeding female experiences can alter the hormonal composition of an egg, similar considerations can be applied to interpretation of maternal effects. The use of wild populations (in conjunction with domesticated species) will guide our understanding of how maternally-mediated processes (i.e., gametic cortisol deposition and its effects on phenotype) occur in the context of environmental change in the wild. Egg cortisol baths can facilitate investigation into potential outcomes of maternally-meditated processes in the many species experiencing rapid human-mediated change.

Figure 2.1 Morphological traits of fry. Body size (FL), depth (DORPEL, ADAN, PED) and fin (PEC, CAUD 1-4) metrics measured for each fish. Eye area was calculated using the formula: π x [0.5 x eye width (EW)] x [0.5 x eye length (EL)]. See Section 2.3.4 for further details.

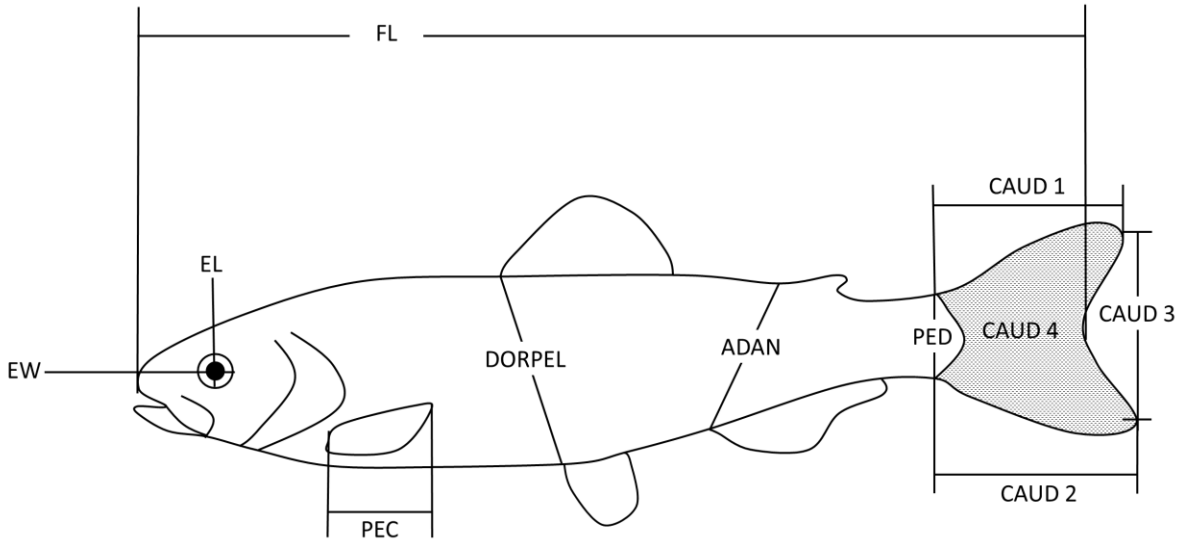


Figure 2.2 Comparison of principal component output (PC1, mean ± SE) among fry groups (river sockeye salmon, river chum salmon, channel chum salmon) and egg cortisol treatment (untreated, 0 ng/mL, open bars; cortisol-treated, 1000 ng/mL, filled bars). PC1 was generated using body size, eye size, 3 metrics of body depth and 5 measures of fin size. See Figure 2.1 and Section 2.3.4. Different letters denote significant differences among fry groups (P<0.05).

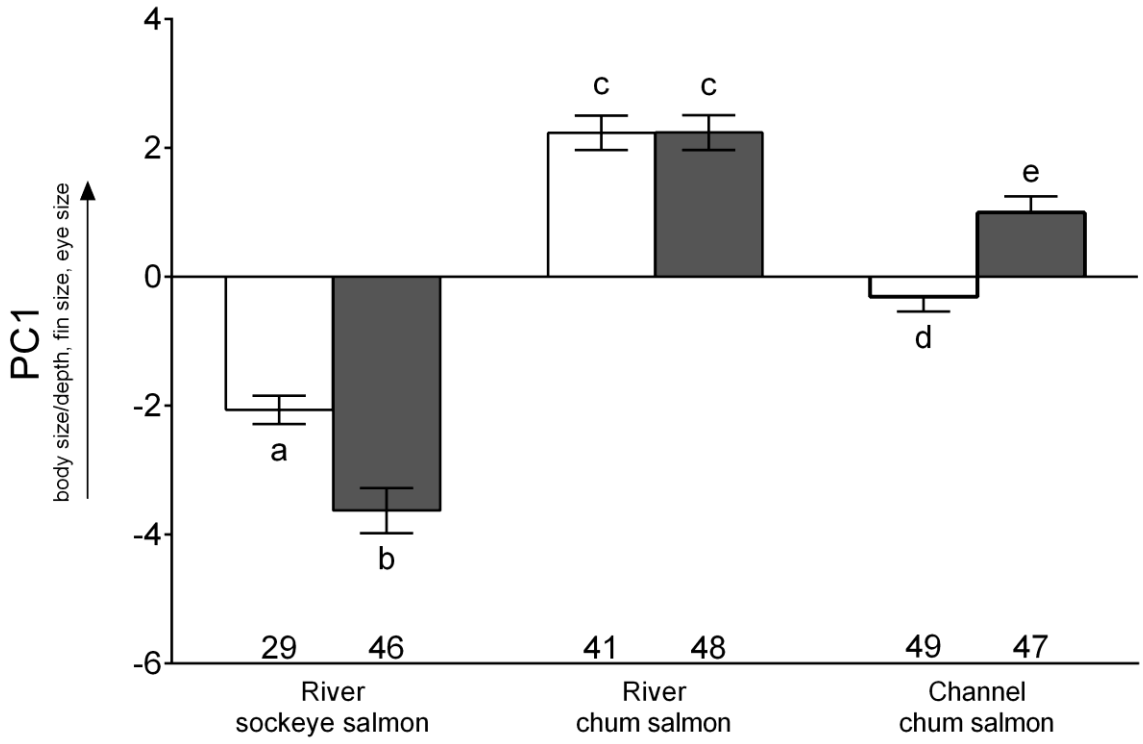


Table 2.1 Measures of fry morphological traits. Mean ± SE body mass, fork length (FL), eye area (π x [0.5 x EW] x [0.5 x EL]), and metrics for fin size (PEC, CAUD 1, CAUD 2, CAUD 3, CAUD 4) and body depth (DORPEL, ADAN, PED; see Figure 2.1) of salmon fry reared from untreated (0 ng/mL) and cortisol-treated (1000 ng/mL) eggs. Values are only presented for fish that were included in the principal components analysis (PCA, see Section 2.3.5).

Morphometric trait	River sockeye salmon		River chum salmon		Channel chum salmon	
	0 ng/mL (n=29)	1000 ng/mL (n=46)	0 ng/mL (n=41)	1000 ng/mL (n=48)	0 ng/mL (n=49)	1000 ng/mL (n=47)
Body mass (g)	0.523 ± 0.018	0.397 ± 0.019	0.858 ± 0.029	0.828 ± 0.022	0.579 ± 0.016	0.681 ± 0.021
FL (cm)	3.967 ± 0.042	3.711 ± 0.059	4.871 ± 0.052	4.879 ± 0.049	4.423 ± 0.037	4.660 ± 0.042
PEC (cm)	0.465 ± 0.007	0.444 ± 0.011	0.609 ± 0.018	0.530 ± 0.008	0.472 ± 0.007	0.544 ± 0.008
CAUD 1 (cm)	0.764 ± 0.010	0.725 ± 0.015	0.916 ± 0.009	0.908 ± 0.009	0.871 ± 0.009	0.909 ± 0.010
CAUD 2 (cm)	0.765 ± 0.009	0.736 ± 0.015	0.957 ± 0.012	1.019 ± 0.059	0.880 ± 0.009	0.944 ± 0.010
CAUD 3 (cm)	1.022 ± 0.017	0.938 ± 0.023	1.115 ± 0.019	1.249 ± 0.017	1.101 ± 0.014	1.139 ± 0.018
CAUD 4 (cm)	0.295 ± 0.008	0.265 ± 0.010	0.448 ± 0.011	0.469 ± 0.010	0.438 ± 0.008	0.478 ± 0.009
DORPEL (cm)	0.676 ± 0.010	0.604 ± 0.015	0.789 ± 0.011	0.778 ± 0.009	0.660 ± 0.008	0.714 ± 0.009
ADAN (cm)	0.592 ± 0.008	0.543 ± 0.011	0.711 ± 0.009	0.708 ± 0.007	0.621 ± 0.007	0.662 ± 0.010
PED (cm)	0.278 ± 0.004	0.262 ± 0.005	0.337 ± 0.004	0.331 ± 0.003	0.295 ± 0.003	0.308 ± 0.004
Eye area (mm^2)	0.638 ± 0.010	0.600 ± 0.014	0.910 ± 0.012	0.941 ± 0.010	0.908 ± 0.059	0.871 ± 0.008

Table 2.2 Results of the principal components analysis (PCA) on fry morphological traits. Body mass (g), fork length (FL, cm), eye area (π x [0.5 x EW] x [0.5 x EL], mm^2), and metrics for fin size (PEC, CAUD 1, CAUD 2, CAUD 3, CAUD 4, cm) and body depth (DORPEL, ADAN, PED, cm; see Figure 2.1) were loaded into the PCA (see Section 2.3.5). Only loadings with an eigenvalue > 1 are presented.

PCA

Morphometric variable	PC1 loading	Communality (h^2)
Eigenvalue	8.0	
% variance explained	73	
Body mass	0.34	0.95
FL	0.34	0.96
PEC	0.25	0.46
CAUD 1	0.32	0.84
CAUD 2	0.21	0.29
CAUD 3	0.28	0.63
CAUD 4	0.32	0.94
DORPEL	0.33	0.97
ADAN	0.34	0.91
PED	0.33	0.90
Eye area	0.22	0.36

Table 2.3 Repeated measures ANOVA results for cortisol concentration (ng/g) 2 and 24 hours post-fertilization (hpf) for untreated (0 ng/mL) and cortisol-treated (1000 ng/mL) eggs. Mean ± SE egg cortisol concentrations are presented with range in brackets; untransformed data are presented for ease of interpretation and statistical analyses used log_{10} transformed data. Different letters denote statistical significance for river sockeye salmon (A,B,C), river chum salmon (a,b,c) and channel chum salmon (X,Y,Z) following Tukey's HSD test ($P < 0.05$). Differences in egg cortisol concentrations among unfertilized eggs are denoted by x,y.

	F, *P*-value	Unfertilized	2 hpf		24 hpf	
			0 ng/mL	1000 ng/mL	0 ng/mL	1000 ng/mL
River sockeye salmon (n=7)	hpf: $F_{1,18}$=28.53, *P*<0.0001 Egg treatment: $F_{1,18}$=20.59, *P*=0.0003 hpf x Egg treatment: $F_{1,18}$=6.80, *P*=0.02	17.35 ± 4.71^{x} (7.63-36.10)	10.83 ± 4.44^{A}	33.71 ± 1.43^{B}	4.95 ± 0.75^{A}	8.12 ± 0.81^{A}
River chum salmon (n=9)	hpf: $F_{1,24}$=200.54, *P*<0.0001 Egg treatment: $F_{1,24}$=179.39, *P*<0.0001 hpf x Egg treatment: $F_{1,24}$=29.17, *P*<0.0001	5.82 ± 1.69^{y} (2.28-18.96)	5.48 ± 0.52^{a}	22.30 ± 1.43^{b}	2.89 ± 0.40^{c}	5.08 ± 0.38^{a}
Channel chum salmon (n=5)	hpf: $F_{1,12}$=46.43, *P*<0.0001 Egg treatment: $F_{1,12}$=53.60, *P*<0.0001 hpf x Egg treatment: $F_{1,12}$=3.79, *P*=0.08	7.17 ± 1.60^{xy} (4.22-12.63)	7.60 ± 2.20^{X}	24.19 ± 1.39^{Y}	3.54 ± 0.52^{Z}	7.29 ± 0.66^{X}

Table 2.4 Comparisons of burst swimming duration (ANCOVA) and burst swimming rate (Wilcoxon signed-rank tests) for fry reared from untreated (0 ng/mL) and cortisol-treated (1000 ng/mL) eggs. Mean ± SE burst swimming duration and burst swimming rate presented; untransformed data (burst swimming duration) are presented for ease of interpretation and statistical analyses used cubed root transformed values. Statistical significance (P<0.05) for burst swimming duration is denoted by different letters: sockeye salmon (A,B), chum salmon (a,b; populations pooled). Statistical significance (P<0.05) for burst swimming rates is denoted by different letters: sockeye salmon (A,B), river chum salmon (a,b), channel chum salmon (X,Y). n are presented below means.

	Burst swimming duration (s)			Burst swimming rate		
	F, *P*-value	0 ng/mL	1000 ng/mL	Z, *P*-value	0 ng/mL	1000 ng/mL
River sockeye salmon	PC1: $F_{1,67}$=40.78, P<0.0001 Egg treatment: $F_{1,67}$=2.20, P=0.14	75.99 ± 4.42^{A} (29)	71.11 ± 4.37^{A} (41)	Z=2.13, P=0.03	0.37 ± 0.04^{A} (29)	0.29 ± 0.03^{B} (41)
River chum salmon	PC1: $F_{1,151}$=25.89, P<0.0001 Egg treatment: $F_{1,151}$=2.74, P=0.10 Population: $F_{1,151}$=0.61, P=0.44	20.80 ± 4.65^{a} (28)	28.05 ± 3.83^{b} (41)	Z=6.29, P=0.01	2.08 ± 0.3^{a} (28)	1.44 ± 0.26^{b} (41)
Channel chum salmon		17.38 ± 3.39 (41)	22.34 ± 3.34 (45)	Z=1.05, P=0.30	3.50 ± 0.88^{X} (41)	2.94 ± 0.49^{X} (45)

Table 2.5 Spearman's rank correlations (r_s) between body morphology (PC1) and burst swimming rate for salmon reared from untreated (0 ng/mL) and cortisol-treated (1000 ng/mL) eggs. PC1 was generated using body size, eye size, 3 metrics of body depth and 5 measures of fin size (see Figure 2.1 and Section 2.3.4). Burst swimming rate was defined as the number of bouts completed per 10 s of swimming (i.e., the number of instances a fish fell to the back of the flume then re-initiated and burst swam back to the front). Statistical significance was set following Bonferroni correction at P=0.008.

	n	0 ng/mL	n	1000 ng/mL
River sockeye salmon	29	r_s=-0.10, P=0.60	41	r_s=-0.38, P=0.01
River chum salmon	28	r_s=-0.40, P=0.03	41	r_s=0.01, P=0.97
Channel chum salmon	41	r_s=0.04, P=0.82	45	r_s=-0.07, P=0.63

Chapter 3: Egg cortisol treatment affects the behavioural response of coho salmon to a conspecific intruder and threat of predation

3.1 Synopsis

To build on chapter 2 in which I observed effects of egg cortisol treatment on fry morphology and swim performance, this chapter reports on an experiment in which I assessed whether exogenously increased egg cortisol affected behavioural responses of juvenile coho salmon to a conspecific intruder and the threat of predation. When exposed to a conspecific intruder, coho salmon treated with cortisol *in ovo* increased activity, feeding and shelter monopolization, whereas coho salmon reared from untreated eggs reduced such behaviours. Egg hormone treatment did not affect aggression toward the conspecific intruder. Egg hormone effects on antipredator behaviour were only detected for coho salmon that had interacted with the conspecific intruder. Following exposure to a simulated predator attack, coho salmon reared from cortisol-treated eggs behaved in a bold manner by increasing activity and feeding, and reducing shelter use. In contrast, fish reared from untreated eggs reduced activity and feeding, and increased shelter use. For coho salmon that interacted with the conspecific intruder, egg hormone treatment generated evidence of personality; behaviour during the resource contest positively correlated with behaviour under threat of predation for coho salmon reared from cortisol-treated, but not untreated, eggs. The results reported here contribute to our understanding of intergenerational components of the hormone-behaviour nexus, and highlight the complex influence of egg cortisol on offspring behaviour, which can be modulated by individual experience.

3.2 Introduction

Investigation into how GCs correlate with tertiary processes like animal behaviour is extensive, from controlled studies in biomedical laboratories (Gregus et al. 2005) to opportunistic observation of free-ranging wildlife (Creel 2005). Within the framework of physiological stress, the effects of GCs on behaviour range from "suppressive" to "preparative", and have been well studied across taxa *via* exposure to a stressor or exogenous GC manipulation (see Sapolsky et al. 2000). Generally, elevations in GCs result in reductions in behaviour. For example, the experimental elevation of circulating GCs dampened aggression in rainbow trout *Oncorhynchus mykiss* (Øverli et al. 2002). In red-sided garter snakes (*Thamnophis sirtalis parietalis*) mating behaviours were reduced in males injected with GCs (Moore & Mason 2001). Black-legged kittiwakes (*Rissa tridactyla*) with silastic implants releasing GCs reduced nest attendance and deserted offspring (Schultner et al. 2013). In all cases, the GC-mediated changes to the observed behaviours are apt to reduce fitness. These studies, however, only recorded behaviour within a single context and reported averages behaviour of individuals. There is now an intensifying interest (Careau & Garland 2012) in the proximate mechanisms underlying consistency of between-individual differences in behaviour (i.e., personality, Sih et al. 2004; coping style, Koolhaus et al. 1999). Of particular interest is how the production and regulation of GCs contributes to behavioural variation in light of the notion that hormones can modulate "suites of correlated traits" (McGlothlin & Ketterson 2008). This "bottom-up" approach (*sensu* Bell [2007]) to exploring animal personality can reveal whether endocrine processes, with previously identified effects on individual behaviours, are also driving correlations among the behaviours (i.e., behavioural syndrome, Sih et al. 2004) and generating personality.

There is an evolving understanding of how circulating GCs relate to behavioural types (i.e., shy *versus* bold) in oviparous taxa. In rainbow trout endogenous GC synthesis in response to a stressor has an instrumental role in predicting an array of behaviours associated with behavioural types. Competitive ability, foraging and locomotion correlate with heritable, stressor-induced levels of GCs, suggesting that behavioural types can have genetic and hormonal underpinnings (Øverli et al. 2007). Individuals bred for low-responsiveness to an acute stressor tend to be more aggressive, become dominant during dyadic interactions and resume feeding faster following transfer from communal tanks to isolation (Øverli et al. 2007). Similar links between stressor-induced GCs and behavioural types are also identified in birds (Cockrem 2007). For oviparous species, circulating GCs are not only modifying behaviour but also are transferring to eggs (Hayward & Wingfield 2004). To complement the advances in our understanding of how circulating GCs associate with behavioural processes, needed is the study of how gametic GCs also influence behaviour of progeny.

In oviparous taxa, maternal GCs are thought to be passively absorbed into developing eggs (birds, Groothuis & Schwabl 2008; reptiles, Radder 2007; fishes, Mommer 2013 but see Moore & Johnston 2008 for discussion on the potential for maternal and embryonic control of yolk hormone deposition). When egg GCs are elevated, behavioural effects on offspring can be profound and variable. Yellow-legged gulls (*Larus michahellis*) reared from GC-treated eggs did not beg as loudly or as frequently (Rubolini et al. 2005). Swimming performance of the common lizard (*Lacerta vivipara*) was not affected by elevated egg GCs but the same offspring remained under protective cover significantly longer following a simulated predator attack, compared to offspring reared from untreated eggs (Uller & Olsson 2006). In brown trout (*Salmo trutta*), elevated egg cortisol concentration increased aggression displayed toward a mirror image

(Sloman 2010) and decreased aggression displayed toward a conspecific (Burton et al. 2011). Sloman (2010) also found elevated egg cortisol elicited differences in the cognitive capacity of juveniles. Experiments manipulating egg GCs predominantly use this methodology as a proxy for maternal stress. How elevated egg GC levels (*via* maternal stress and/or exogenous manipulation) modify progeny behaviour, and whether these effects are adaptive (Meylan et al. 2012) is an emerging topic of interest in behavioural and evolutionary ecology. Such GC-mediated effects may be especially impactful in species with minimal parental care (e.g., salmonids), and thus, limited opportunities for female-embryo interactions (see Love & Williams 2008a). It remains unclear how fluctuations in maternally derived egg GCs (and proxies of) may be influencing variation in progeny behavioural responses across environmental contexts (e.g., presence/absence of intruder/predator).

In fishes, aggression toward conspecifics and activity under threat of predation (or boldness) are positively correlated within individuals (Huntingford 1976; Bell & Stamps 2004). Accordingly, these two behavioural metrics are commonly examined when investigating animal personality from both ecological and evolutionary perspectives (Sih et al. 2004). Genomic (Aubin-Horth et al. 2012) and ecological (Bell & Sih 2007) drivers of aggressive-bold personalities continue to be revealed in fishes. Do maternally-inherited hormones have a role in programming animal personality as well? The hormonal composition of eggs/ovaries is known to vary among females (McCormick 1999), be influenced by maternal experience (Stratholt et al. 1997; McCormick 2006 but see chapter 4), and may predict average level aggression and predator avoidance of juvenile fish. For example, brown trout reared from cortisol-treated eggs were less aggressive toward conspecifics (Burton et al. 2011). Threespined stickleback females exposed to a predator produced offspring with impaired predator avoidance (McGhee et al.

2012), possibly a result of increased egg cortisol level (Giesing et al. 2011). Still, how inherent hormonal properties shape behavioural coupling and repeatability within individuals remains largely unknown.

I experimentally increased the concentration of cortisol in coho salmon eggs and examined how elevated egg cortisol influenced mean and individual level behavioural responses to a conspecific intruder and the threat of predation by an aerial predator. Following emergence from spawning ground gravel, juvenile coho salmon inhabit stream environments for 1-2 years prior to migrating out to the ocean as smolts (Sandercock 1991). During this rearing period the threat of predation by numerous avian (e.g., great blue heron, *Ardea herodias*, Clements et al. 2012) and piscivorous predators is significant (Sandercock 1991). While evading predators, juvenile coho salmon must acquire sufficient resources to fuel growth necessary for successful downstream migration to sea. In contrast to other juvenile Pacific salmon (e.g., sockeye salmon, chum salmon, pink salmon), juvenile coho salmon are more aggressive toward conspecifics (Hutchison & Iwata 1997), defend feeding territories in streams and do not readily school (Dill et al. 1981). The two ecological scenarios theoretically require an individual to be behaviourally plastic; increased activity and feeding can assert dominance in a competitive interaction (Abbott & Dill 1985) but such behaviours would increase predation risk in the presence of a predator (Lima & Dill 1990). Increased shelter occupancy is suitable for both competitive (Faria et al. 1998) and predatory (Lima & Dill 1990) scenarios. Increased occupancy during a dyadic interaction blocks the opponent's access to the resource. Increased occupancy under threat of predation reduces risk of capture and mortality. However, under competitive and predatory conditions, increased occupancy would limit opportunities to feed, potentially reducing growth.

The dynamic early life history of coho salmon provides an ideal opportunity to investigate how egg hormones affect behavioural responses to ecologically-relevant challenges.

I predicted that embryonic survival would vary between progeny reared from cortisol-treated and untreated eggs. At the treatment level I predicted that behaviour while interacting with a conspecific and following a simulated predator attack would differ between coho salmon reared from untreated and cortisol-treated eggs. At the individual level I predicted that the correlational strength between behavioural responses would differ between coho salmon reared from untreated and cortisol-treated eggs given behavioural correlations emerge following exposure to a stressor (Bell & Sih 2007), represented here as elevated GCs as a potential signal of maternal stress.

3.3 Materials and methods

3.3.1 *Egg cortisol treatment and offspring rearing*

Sperm and eggs were taken from 15 ripe male and 15 ripe female coho salmon that had migrated to the Fisheries and Oceans Canada (DFO) Chilliwack River Hatchery in Chilliwack, British Columbia, Canada and transported to the University of British Columbia (UBC, ~2 hours). Following chapter 2, in duplicate, 15 g of eggs from each female were fertilized with sperm from a male to create full sibling crosses (i.e., each female was paired once with a male). Facility water (30 mL) was added to the sperm-egg mixture to activate sperm motility. Approximately 400 mL of water treated with 1000 ng/mL cortisol (H4001, Sigma, www.sigmaaldrich.com) dissolved in 95% ethanol (0.002 % final concentration) was then added to one replicate, and 400 mL of control water (0 ng/mL cortisol) added to the other replicate with the same concentration of ethanol as hormone-treated eggs. Cortisol concentrations reflect

plasma cortisol concentrations detected in mature female Pacific salmon (Hruska et al. 2010) and were used previously (Auperin & Geslin 2008; chapter 2) to exogenously elevate salmonid egg cortisol. As in chapter 2, egg cortisol concentrations were determined using ELISA at 2 and 24 hours post-fertilization for each full sibling cross and each hormone treatment (0 and 1000 ng/mL). Intra- and inter plate variability were 5.3% and 8.6%, respectively. Egg cortisol concentrations were significantly higher after 2 hour immersion in cortisol-treated water (Paired *t*-test: t=7.20, n=15, $P<0.0001$, Figure 3.1), and levels were comparable to those detected in unfertilized eggs from adult coho salmon stressed in captivity *via* chronic exposure (2 weeks) to a chase stressor (Stratholt et al. 1997). Cortisol concentrations reduced by 24 hours post-fertilization in both egg treatments (0 ng/mL, t=-4.20, n=15, $P=0.001$; 1000 ng/mL, t=-12.93, n=15, $P<0.0001$) but concentrations in cortisol-treated eggs remained higher (t=2.50, n=15, $P=0.03$, Figure 3.1).

Fertilized eggs were moved to individual flow-through baskets after soaking for 2 hours in the cortisol-dosed or undosed water, and reared in Heath stacks until emergence (full yolk sac absorption). Water temperature was 7-8°C throughout incubation. Dead eggs and embryos were removed from heath stacks daily and stored in Stockard's solution to determine fertilization success, and survival to eyed, hatch and emergence. The 0 ng/mL full-sibling crossing for one female was accidently placed in Stockard's solution in its entirety and fertilization success and progeny survival could not be determined for this female. Emergent fish were transferred to 1000 L flow-through troughs (~1500 fish per trough), separated by egg hormone treatment and families pooled. Light cycle was adjusted throughout rearing to match photoperiod at latitude 49°18´N and water temperature in troughs ranged from 6-11°C due to natural fluctuations in

municipal water sources. Fish were fed fishmeal (EWOS Canada Ltd.; www.ewos.com) daily *ad libitum* up until the day of behavioural trials (~3 months post-emergence).

3.3.2 *Behavioural trials*

Fifty-eight behavioural trials were completed in a sectioned area (26 cm length x 24 cm width x 23 cm height of water) of an aquarium (50 cm length x 25 cm width x 31 height) filled with dechlorinated water. The area of the divided section of the aquarium was chosen based on territory size of juvenile coho salmon in the wild (Dill et al. 1981), and contained a 2 cm layer of 1 cm gravel, a 21 cm aquarium plant and a 34 cm black PVC tube with 0.25 cm randomly drilled holes (Figure 3.2). A perforated partition facilitated oxygen flow between the open area and the area containing an airstone (Figure 3.2).

Fish (~6 months post-fertilization) were lightly anesthetized with buffered tricaine methanesulfonate (MS222; 0.02 g/L) between 15:00 and 19:00, weighed to the nearest 0.0001 g, and fork length measured to the nearest 0.1cm. To identify resident and intruder fish, non-toxic acrylic paint was injected subcutaneously on the left and right side of the fish anterior to the dorsal fin. Pairs of fish were size-matched (average body mass difference = 0.04 g, range = 0.00 – 0.15 g; average fork length difference = 0.1 cm, range = 0-0.4 cm) and consisted of one individual reared from cortisol-treated eggs and one individual reared from untreated eggs which were assigned as the resident or the intruder. The resident was reared from cortisol-treated eggs for half the trials and reared from untreated eggs for the other half of the trials. Fish recovered in aerated water within an hour of injection and were placed in an aquarium; the resident fish was placed into the open sectioned area and the intruder fish placed into the PVC tube (Figure 3.2). Both fish were then fed ~0.04 g (~4% body mass) of fishmeal.

Eighteen-hours after placement in the aquarium, ~0.04 g of fishmeal was slowly strewn into a back corner of the open sectioned area. After 3 min the PVC tube was slowly lifted out of the aquarium revealing the intruder (resource contest). Three min after introduction of the intruder, the intruder was removed from the aquarium, euthanized with an overdose of buffered MS222, and body mass and fork length measured. Three hours later, ~0.04 g of fishmeal was again slowly strewn into a back corner of the open sectioned area. After 3 min, a model great blue heron (head to beak, 39 cm) was rapidly submerged into the open sectioned area 3 times (predator avoidance). Three min after the simulated predator attack the resident was removed the aquarium and euthanized as above. All behavioural trials were video recorded using a Canon EOS Rebel T3i digital camera (www.canon.com) at 60 frames per s.

Behaviour of resident and intruder coho salmon was obtained from the digital videos for four different 3 min intervals; before and after intruder introduction, and before and after the simulated predator attack. When applicable the following behaviours were tabulated: activity (total s focal fish swam more than 1 body length), feeding (number of times focal fish nibbled at a particle of food), shelter occupancy (total s focal fish spent behind the plant), chase (number of rapid and directed movements by focal fish toward opposing fish that caused the opposing fish to flee rapidly) and displacement (number of times focal fish slowly approached opposing fish causing the opposing fish to flee rapidly). Total aggression was calculated as the sum of chases and displacement. Average frequencies of resident coho salmon behaviours are presented in Table 3.1. Video observer was blind to the egg treatment of the focal fishes.

3.3.3 *Statistical analyses*

Egg cortisol treatment effects on fertilization success and offspring survival were examined using Wilcoxon signed-rank tests. Following Wilson & Godin (2009), change in candidate behaviours were loaded into a principal components analysis (PCA) separately for each behavioural context (resource contest, predator avoidance) to create composite behaviour scores for resident coho salmon. Changes in resident activity, feeding and shelter occupancy were calculated as: behaviour after intruder introduction/simulated predator attack – behaviour before intruder introduction/simulated predator attack. During the resource contest change in activity, feeding and shelter occupancy positively correlated with PC1 (Table 3.2). Following the simulated predator attack PC2 positively correlated with change in activity and feeding, and negatively correlated with shelter occupancy (Table 3.2). An additional PCA was performed for behaviour of intruder coho salmon during the resource contest; activity levels and feeding, and shelter occupancy positively and negatively correlated with PC3, respectively (Table 3.2). Wilcoxon signed-rank tests were then used to determine differences in behavioural responses (PC scores, aggression) of resident and intruder coho salmon reared from untreated and cortisol-treated eggs. Behavioural correlations were first detected using PCA output, and then statistically analyzed using Spearman's rank tests. Statistical analyses were performed using JMP 10 (SAS Institute Inc.; www.jmp.com).

3.4 Results

3.4.1 *Fertilization success and embryo survival*

Fertilization success, and cumulative survival to eyed, hatch and emergence did not differ between eggs fertilized in control (0 ng/mL) and cortisol-treated (1000 ng/mL) water (Wilcoxon signed-rank tests, all $P>0.05$).

3.4.2 *Behavioural response to a conspecific intruder*

Opposing fish did not interact in 15 out of 29 (52%) trials where the resident coho salmon was reared from untreated eggs, and in 10 out of 29 (34%) trials where the resident coho salmon was reared from cortisol-treated eggs (Chi-square test: $\chi 2=1.77$, n=58, $P=0.18$). Of pairs of fish that interacted, resident coho salmon reared from cortisol-treated eggs increased activity, feeding and shelter occupancy whereas resident coho salmon reared from untreated eggs decreased these behaviours (Wilcoxon signed-rank test: Z=3.08, n=33, $P=0.002$, Figure 3.3A). Egg cortisol treatment did not alter behavioural responses of resident coho salmon that did not interact with the intruder (Z=-1.22, n=25, $P=0.22$, Figure 3.3A). Regardless of whether intruder coho salmon interacted with the resident, intruder behaviour did not vary between egg hormone treatments (no interaction, Z=-1.69, n=25, $P=0.09$; interaction, Z=1.44, n=33, $P=0.15$). Total aggression (sum of chases and displacements) did not vary between egg cortisol-treated and untreated resident coho salmon (Z=0.11, n=58, $P=0.90$). Resident and intruder aggression did not vary from each other or between egg hormone treatment (all $P>0.05$). Total aggression positively correlated with PC1 for resident coho salmon reared from cortisol-treated, but not untreated, eggs; fish that were more aggressive had also increased activity levels, feeding and shelter occupancy following

exposure to the intruder (Spearman's ranks correlation: 0 ng/mL, r_s=-0.05, n=19, *P*=0.84; 1000 ng/mL, r_s=0.64, n=14, *P*=0.01, see Table 3.2 for PC scores).

3.4.3 *Behavioural response to a simulated predator attack*

Social interaction appeared to modify egg cortisol-mediated effects on antipredator behaviour. If resident coho salmon had not interacted with the intruder, juveniles reared from cortisol-treated and untreated eggs both did not change behaviour following the simulated predator attack (Z=0.69, n=25, *P*=0.49, Figure 3.3B). When opposing fish had interacted, resident coho salmon reared from cortisol-treated eggs acted in a bold manner following the simulated predator exposure increasing activity and feeding, and reducing shelter occupancy (Z=2.95, n=33, *P*=0.003, Figure 3.3B). Resident coho salmon reared from untreated eggs reduced activity and feeding, and increased shelter occupancy (Figure 3.3B).

3.4.4 *Consistency of behavioural responses*

Generally, following either the introduction of the intruder or simulated predator attack, resident coho salmon that increased activity also increased foraging (Table 3.2). However, when these relationships were explored between egg hormone treatments and in consideration of whether resident coho salmon had interacted with the conspecific, such behavioural correlations were not as apparent. Individual change in behaviour during the resource contest and following the simulated predator attack only correlated for resident coho salmon reared from cortisol-treated eggs that had interacted (Table 3.3). Coho salmon reared from cortisol-treated eggs that were more active, fed more and occupied the shelter for longer during the resource contest, also

were more active, fed more and did not seek shelter as often following the simulated predator attack.

3.5 Discussion

I found that egg cortisol treatment altered the behavioural responses of coho salmon in a context-dependent manner; egg hormone effects were evident for coho salmon that aggressively interacted with the conspecific intruder. Similar to chapter 2 findings, we did not detect any effect of exogenously elevated egg cortisol on offspring survival. Despite absence of cortisol-mediated effects on embryo survival in the present study, latent effects on juvenile coho salmon behaviour and personality were detected.

Increases in activity, feeding and shelter occupancy seen in cortisol-treated coho salmon likely imposed additional energetic costs during the agonistic interaction (e.g., increased metabolism/oxygen consumption, Ros et al. 2006). However, in fishes, bold/active individuals tend to become dominant (Sundström et al. 2004). Also, rainbow trout juveniles reared from cortisol-treated eggs have an attenuated plasma cortisol stress response (Auperin & Geslin 2008) and strains of rainbow trout with attenuated stress responses are more likely to be dominant (Øverli et al. 2007). In this study individual level aggression positively correlated with the PC score generated for the resource contest; however, stress response was not measured nor was dominance status assigned given the short (3 min) duration of the interaction. Thus, it is not clear from this study if bold, egg cortisol-treated resident coho salmon were more apt to achieve dominance over the intruder compared to untreated resident coho salmon. Extending observation periods and incorporating measurement of physiological parameters before (e.g., stress coping style, Øverli et al. 2007) and/or after (e.g., metabolic recovery/excess post-exercise oxygen

consumption [EPOC], Killen et al. 2014) the behavioural interaction can aid in disentangling the costs and benefits of egg cortisol mediated behavioural change.

In contrast to previous work (Sloman 2010; Burton et al. 2011), variation in aggression between egg hormone treatments was not detected. Sloman (2010) measured aggression toward a mirror image which is not always coupled with aggression toward a real competitor (Elwood et al. 2014). Burton et al. (2011) observed aggression over 2 days (3 min observation periods [n=12]) in triads of fish. Thus, methodology and time competitors interacted could account for lack of egg hormone treatment effects on aggression levels in this study. The absence of interaction in ~50% of trials herein could be influenced by seasonality. During winter young coho salmon move to highly sheltered areas in streams which can lead to "bottlenecks" (Nickelson et al. 1992), driving increased competition for limited space (Gregory & Griffith 1996). Offspring were tested during the summer when juvenile coho salmon do not exhibit strong habitat preference resulting in lower site-specific densities (Nickelson et al. 1992), and potentially reduced competitive pressure and motivation to compete. Interestingly, the proportion of fish not interacting did not vary between egg hormone treatments suggesting that egg cortisol levels do not necessarily alter juvenile motivation to interact but behaviour following a decision to interact. Also, resident coho salmon were exposed to olfactory cues of the conspecific before physical introduction, and exposure to such cues can reduce aggression (Giaquinto & Volpato 1997). The use of mesocosms and experimental streams, again over extended periods of interaction, would best mimic the environmental and social aspects of young coho salmon life and provide insight into how gametic factors affect progeny in the wild.

Egg cortisol exposure also modified behavioural responses when fish were under threat of predation, but only if individuals had previously interacted with a conspecific. This study was

designed with the objective of determining how egg cortisol treatment affected personality. A fixed-order experiment was executed with individuals exposed to the assay expected to generate the more lasting carryover effect last (i.e., simulated predator attack after conspecific intruder, Bell 2013). The significant lack of response to the conspecific intruder for both egg cortisol treatments was not expected. However, the two groups that emerged from the conspecific interaction (i.e., coho salmon that did and did not exhibit aggressive behaviours toward each other) were distinct enough to statistically address the study objective. Although a randomized design would be most ideal, Bell (2013) outlines logistical reasons why this design is not always feasible. Here, for example, sample size would need to be doubled to incorporate randomized ordering of the behavioural assays. However, this would double the study length at which point age effects may obscure the study objectives. The period between the behavioural assays could be extended to days *versus* hours to allow animals to recover regardless of whether or not they interacted with the conspecific intruder. Again, this would extend the study length potentially introducing age and, on longer timescales, seasonal effects. The carryover effect that did emerge is still interesting and warrants further discussion.

Within a type of situation (e.g., dyadic interaction, predator avoidance), previous experience influences future performance of individuals (Hsu et al. 2006; Lönnstedt et al. 2012). Díaz-Uriarte (1999) found that following an aggressive interaction, territorial lizards were more bold (emerging from shelter sooner) following a simulated predator attack. Díaz-Uriarte (1999) attributed this behaviour to individuals anticipating a future intruder (in comparison to individuals who did not interact with an intruder and hid in shelter longer). This behavioural trade-off may be occurring with egg cortisol-treated coho salmon but cannot be ascertained as all fish were exposed to an intruder, with only a subset of fish interacting (*versus* some fish not

exposed to an intruder). With regard to fish that interacted and demonstrated a response to the simulated predator attack, reductions in activity and feeding, and increases in shelter occupancy are the predicted, adaptive responses to a predator (Lima & Dill 1990). Indeed, coho salmon reared from untreated eggs, reduced activity levels, and feeding, and increased shelter occupancy. In contrast, coho salmon reared from cortisol-treated eggs increased activity and feeding and reduced shelter occupancy in response to the simulated predator attack, suggestive of impaired antipredator behaviour. In threespined stickleback, when females were exposed to a model predator, their offspring also demonstrated maladaptive antipredator behaviour not orienting to a live Northern pike (*Esox lucius*) predator as often and subsequently being captured by the pike more quickly (McGhee et al. 2012). The physiological mechanism for this unexpected behavioural response is thought to be *via* an apparent increase in egg cortisol following maternal exposure to a predator (see Giesing et al. 2011). These findings are consistent with my study whereby egg cortisol levels were associated with impaired antipredator behaviour. Considering competitive and antipredator behaviour at the mean level, it appears the adaptive potential of egg cortisol treatment on juvenile salmonids is context-dependent.

Consistency of boldness across contexts (i.e., personality) was linked to experimental elevation of egg cortisol; behaviour during the resource contest was positively correlated with antipredator behaviour in coho salmon reared from cortisol-treated, but not untreated, eggs (Table 3.3). Ecological and life history factors can affect behavioural consistency within and across contexts (e.g., age and population, Bell & Stamps 2004). Why elevated egg cortisol contributed to individual behavioural consistency is not known. The strength of behavioural consistency may depend on what elevated egg GCs signal within a species or taxon (e.g., challenging and/or fluctuating environment). Indeed, within a generation, exposure to predation

stress evokes personality in threespined stickleback (Bell & Sih 2007). Within a context, boldness in cortisol-treated coho salmon may be driven by egg cortisol-mediated effects on neuroendocrine (Mommer & Bell 2014) or cognitive function (Sloman 2010; Roche et al. 2012). As mentioned previously, egg cortisol treatment and corresponding boldness may be associated with dampened physiological stress. Both fishes (Auperin & Geslin 2008) and birds (Love & Williams 2008b) reared from GC-treated eggs have attenuated plasma GC stress responses. Linking behavioural consistency with measures of physiological effort (i.e., pace of life syndrome, Careau & Garland 2012) will continue to enhance our understanding of animal personality.

Here, I show that variation in egg hormones has the potential to regulate average behaviour and behavioural plasticity in a salmonid fish. Such information can be extended to both basic and applied future research. For example, high density conditions in the wild can increase frequency of aggressive interactions and ovarian cortisol concentrations resulting in smaller offspring (McCormick 2006). Such processes are highly relevant to aquaculture practices with fish often kept at high densities throughout sexual maturation; any subsequent egg-cortisol mediated effects on progeny personality have implications on the success of released fish in the wild (Sundström et al. 2004; Mittelbach et al. 2014). Continued investigation of egg hormone influences on offspring behaviour will be enhanced by tandem examination of physiological parameters, and extension to maternal effects of stress on egg hormones and progeny programming (Meylan et al. 2012). Importantly, the use of multiple environmental/ecological conditions can reveal context-dependent GC-mediated effects on behaviour and personality.

Figure 3.1 Coho salmon egg cortisol levels (mean ± SE) for eggs fertilized in control (0 ng/mL cortisol, open bars) and cortisol-treated (1000 ng/mL, filled bars) water at 2 and 24 hours post-fertilization (hpf). Asterisks and asterisks with bars denote statistical significance at $P<0.05$ between egg hormone treatment groups (0 *versus* 1000 ng/mL) and fertilization time points (2 *versus* 24 hpf), respectively. n presented at bottom of bars.

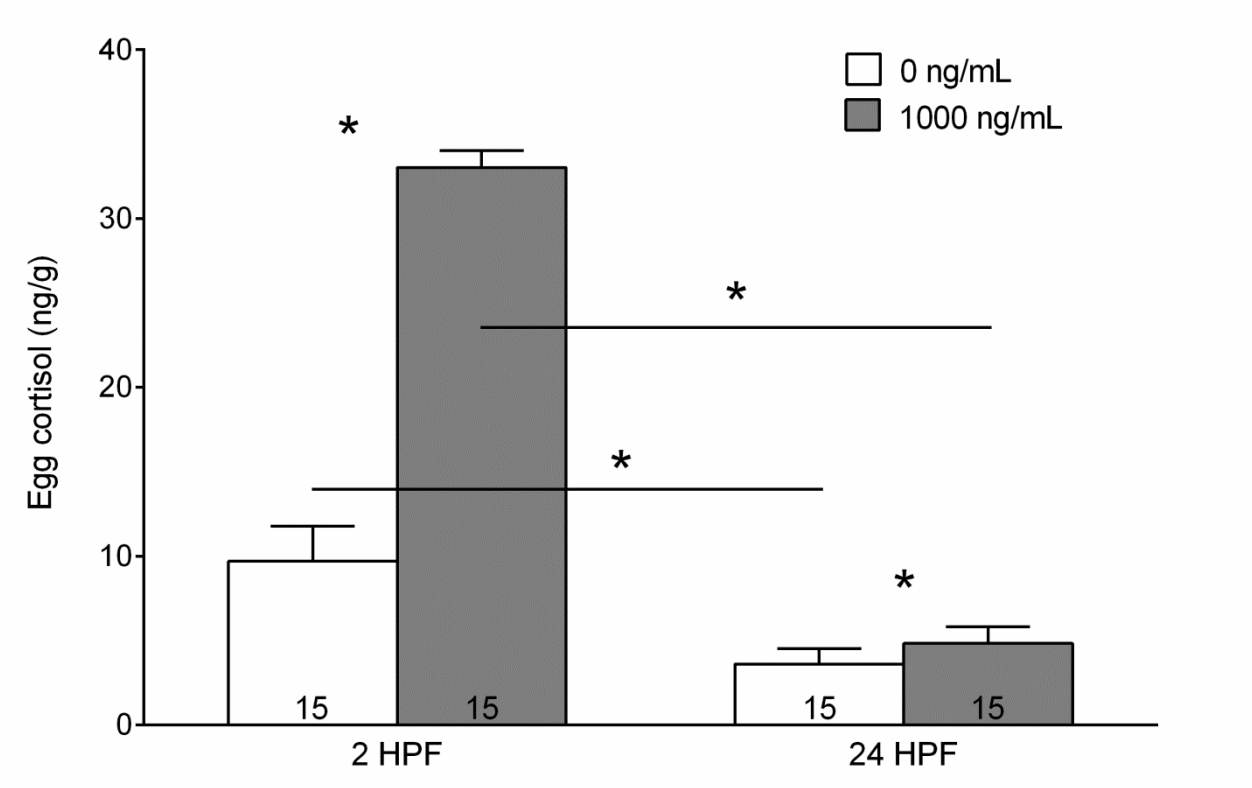


Figure 3.2 Initial experimental aquarium setup. Area of the section containing the resident coho salmon was chosen based on territory size of juvenile coho salmon in the wild (Dill et al. 1981), and contained a 2 cm layer of 1 cm gravel, a 21cm aquarium plant (for shelter) and a 34 cm black PVC tube with 0.25 cm randomly drilled holes The conspecific intruder was contained within the PVC tube for 18 hours before the resource contest. The resource contest was initiated when the PVC tube was slowly lifted out of the aquarium and the intruder was revealed. The intruder was removed from the aquarium 3 hours prior to the predator avoidance trial. Figure not to scale; see Section 3.3 for further details.

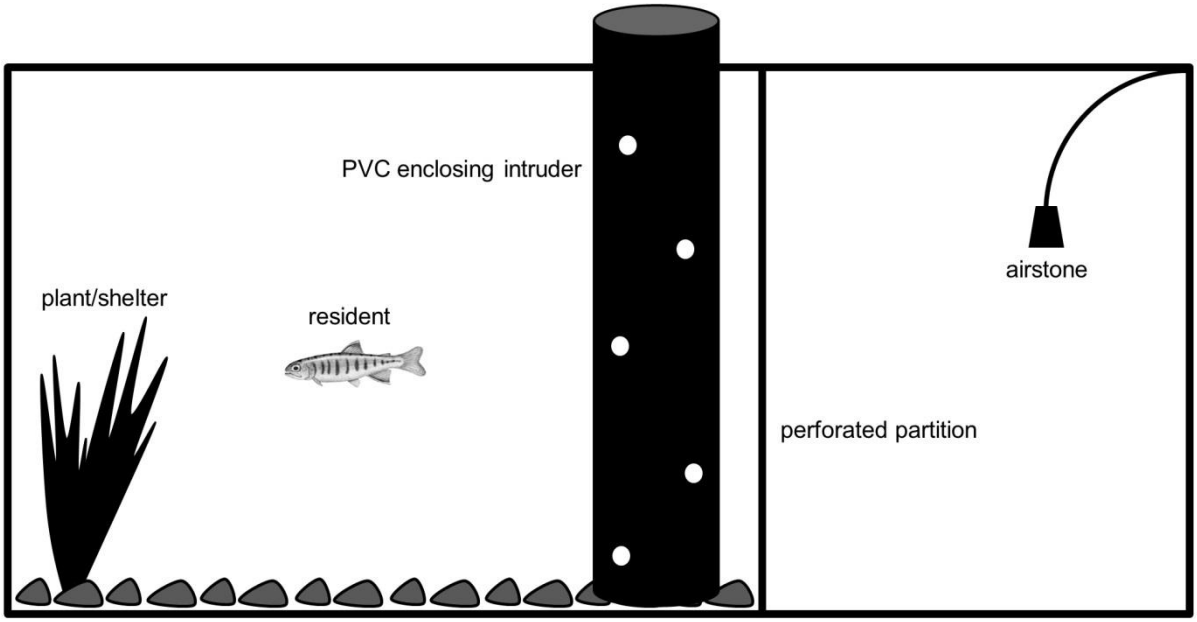


Figure 3.3 Comparison of mean (± SE) change in behaviour of resident coho salmon reared from untreated (0 ng/mL cortisol, open bars) and cortisol-treated (1000 ng/mL, filled bars) eggs, and exposed to an **(A)** intruder and **(B)** simulated predator attack. Change in behaviour was calculated as: behaviour after minus behaviour before the intruder/simulated predator attack, and assessed for resident coho salmon that did (Interaction) and did not (No Interaction) interact with the intruder during the resource contest. Behaviour encompassed activity levels, shelter occupancy and feeding, and were generated using Principal Components Analysis (PCA), see Section 3.3 and Table 3.2. Different letters denote statistical significance (P<0.05) between egg hormone treatments. n presented at bottom of bars **(B)**.

.

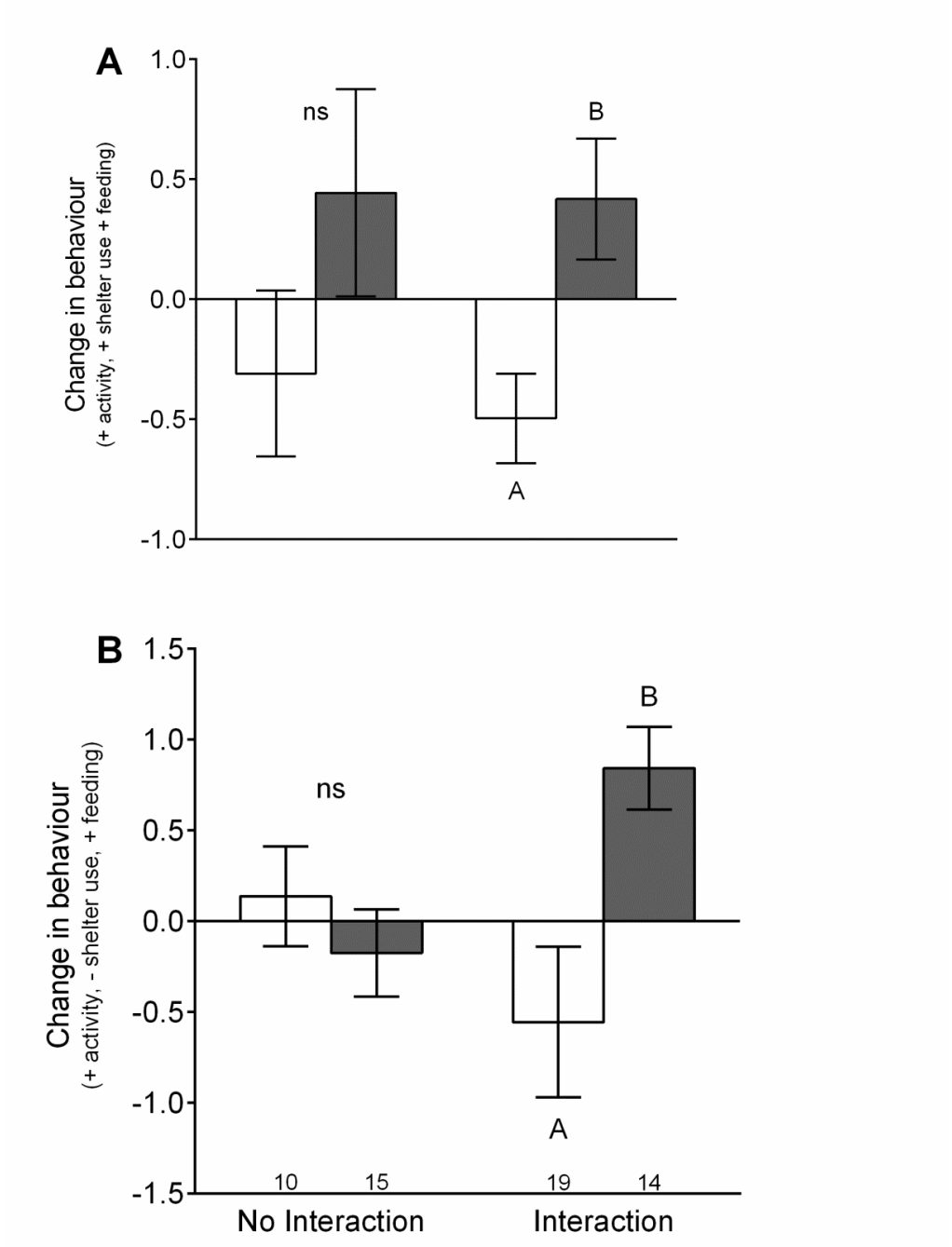


Table 3.1 Summary of resident coho salmon behaviours during the resource contest and predator avoidance observation periods. Means (± SE) are presented with ranges in brackets.

Behavioural Assay	Behaviour			
	Activity (s)	Feeding attempts (n)	Shelter occupancy (s)	Aggressive acts (n)
Resource contest	83 ± 11 (0-285)	3 ± 1 (0-43)	74 ± 16 (0-360)	3 ± 1 (0-27)
Predator avoidance	112 ± 15 (0-360)	5 ± 1 (0-40)	52 ± 15 (0-360)	-

Table 3.2 Principal components analysis (PCA) of resident and intruder coho salmon candidate behaviours during the resource contest and predator avoidance trials. Δ represents change (behaviour after minus behaviour before the intruder/simulated predator attack) in activity levels, shelter occupancy, and feeding. Only loadings with eigenvalues >1.0 are presented.

Behavioural context	Behaviour	PC1 Loading	% Variation explained
Resource contest	Δ Activity levels	0.46	49.4
(resident)	Δ Shelter occupancy	0.52	
	Δ Feeding	0.72	
Resource contest	Activity levels	0.64	58.1
(intruder)	Shelter occupancy	-0.51	
	Feeding	0.58	
Predator avoidance	Δ Activity levels	0.66	60.7
(resident)	Δ Shelter occupancy	-0.46	
	Δ Feeding	0.60	

Table 3.3 Spearman's ranks correlations (r_s) comparing change in behaviour of resident coho salmon (reared from untreated [0 ng/mL] and cortisol-treated [1000 ng/mL] eggs) during the resource contest (PC1, before and after introduction of conspecific) and predator avoidance (PC2, before and after simulated predator attack) trials. Behaviours (activity levels, shelter use and feeding) were transformed into composite scores using Principal Components Analysis (PCA) (see Section 3.3 and Table 3.2). Significant correlations (P<0.05) indicated in bold.

Egg hormone treatment	Conspecific interaction	r_s	*P*-value	n
0 ng/mL	Yes	0.20	0.40	19
	No	0.20	0.58	10
1000 ng/mL	Yes	0.63	**0.02**	14
	No	-0.39	0.15	15

Chapter 4: Effects of maternal exposure to a stressor on offspring performance at different life stages in a semelparous fish

4.1 Synopsis

Given that results from chapters 2 and 3 demonstrated that experimentally elevated egg cortisol can alter progeny morphology, swimming capacity and behavioural responses, the objective of this chapter was to evaluate whether repeated exposure of female salmon to a chase stressor elevated egg cortisol levels and affected gamete or offspring traits. Egg size and early offspring survival were not influenced by maternal exposure of sockeye salmon to the repeated acute stressor. However, when exposed to a swimming test later in development, fry reared from stressed females swam for shorter periods of time but possessed a superior capacity to re-initiate bouts of burst swimming. The mechanisms driving the observed effects do not appear to be related to elevations in egg cortisol concentration, as egg hormone concentrations did not vary between stressed and undisturbed females. Sockeye salmon appear to possess buffering strategies that protect offspring from deleterious effects of maternal stress that could otherwise compromise progeny during highly vulnerable stages of development. A gametic buffering system would be advantageous for semelparous species with only a single opportunity to reproduce before dying. Whether stressed sockeye salmon females endow offspring with traits that are matched or mismatched for survival in the unpredictable environment they encountered is discussed. This chapter highlights the importance of examining intergenerational effects across offspring early life history to fully assess the scope of impact of maternal stress.

4.2 Introduction

With a growing multitude and intensity of anthropogenic and environmental stressors, comes a need to understand interactions between ecological stressors, animal physiology, and behaviour. Existing knowledge on how stress modifies traits within and across generations is primarily derived from biomedical research on domesticated species (Romero 2004), and to date, key predictions (e.g., elevated GC levels) are only modestly corroborated by trends emerging from wild populations (Busch & Hayward 2009; Dickens & Romero 2013). Free-living populations exposed to ecological disturbances do demonstrate endocrine changes that can be implicated in the reprogramming of offspring performance (Dufty et al. 2002; Love et al. 2013; Sheriff & Love 2013). Such intergenerational effects are heavily attributed to altered maternal investment into offspring, as maternal provisioning (e.g., nutrients, hormones, parental care) typically exceeds the genetic investment of fathers (Mousseau & Fox 1998). Indeed, maternal stress influences various offspring parameters in vertebrates with multiple reproductive opportunities (e.g., birds, Henriksen et al. 2011), allowing adjustment of current reproductive investment when faced with unfavourable breeding conditions (Love & Williams 2008a). Theoretical and experimental evidence suggests that the resonating evolutionary consequences of transformed developmental trajectories can potentially shape how individuals will cope with future environmental change (Räsänen & Kruuk 2007; Badyaev & Uller 2009; Love et al. 2013). Currently lacking is how intergenerational effects of exogenous maternal stress manifest in semelparous species where there is no opportunity to postpone reproduction when under stressful conditions.

The striking contrasts between semelparous and iteroparous reproductive life histories are apt to influence maternal effects of stress (Heath & Blouw 1998). With only a single chance to maximize fitness, semelparous females must arm offspring with resources that will ensure adequate growth and survival, especially in the absence of or limited parental care. Arguably, due to immense selection pressure, semelparous species should be evolved to persist through ecological stressors without compromising reproduction (Wingfield & Sapolsky 2003). However, recent studies show that under simulated conditions of ecological stress senescing animals can mount a GC stress response (Cook et al. 2011; Donaldson et al. 2014) and reproduction can be impaired (e.g., incomplete egg release, McConnachie et al. 2012; depressed reproductive hormone concentrations, Jeffries et al. 2012a). Furthermore, Heath & Blouw (1998) showed that maternal effects (e.g., body size affecting egg size) are stronger in semelparous fish than they are in iteroparous species. This relationship, coupled with potential reproductive sensitivity to stressors, suggests that offspring of semelparous females may be particularly responsive to maternal effects of stress. Generally, it is accepted that maternal effects of stress are predominantly deleterious. Evidence is now growing that stressed females may confer adaptive information to their offspring (Love et al. 2013), and that maternal effects initially described as negative may actually increase fitness of both offspring and female when "matched" to stressful environmental conditions (Sheriff & Love 2013). Disentangling these contrasting (but not necessarily mutually exclusive, see Coslovsky & Richner 2011) types of maternal effects is especially pertinent given the recent declines of semelparous populations of Pacific sockeye salmon (Cohen 2012), a commercially, culturally, and ecologically significant species that is exposed to a gauntlet of ecological stressors during reproductive maturation.

In contrast to other vertebrate classes, the mechanisms, and specific outcomes of maternal effects of stress are largely unknown in wild fishes. Cortisol, the primary GC in fish, is the most widely accepted maternally-derived candidate driving observed 'downstream' effects of maternal stress (Schreck et al. 2001), yet the developmental role of egg cortisol is still unclear (Mommsen et al. 1999). Furthermore, beyond egg size and offspring survival data (Campbell et al. 1992; 1994), the effects of maternal exposure to a stressor on offspring performance traits represent an ecological black box (but see McGhee et al. 2012; Roche et al. 2012). Proxies for imposing maternal stress *via* exogenous cortisol injection (Eriksen et al. 2011) and immersion of eggs in cortisol at fertilization (Sloman et al. 2010; Burton et al. 2011) are logistically less challenging than chronically exposing adults to stressors. Such experimental designs have yielded intriguing behavioural carry over effects of maternal stress, though this and the aforementioned work remain limited to domesticated and/or iteroparous fish. Extension of those research findings to wild fish should be cautioned given the notable differences in behaviour and reproductive maturation between hatchery and wild fish (Johnsson 1997). Nevertheless, a foundation exists for exploring how adult exposure to stressors affects subsequent generations in wild populations of fish.

Semelparous populations of Pacific salmon that migrate through and spawn in the Fraser River watershed in British Columbia, Canada can be used to understand the latent consequences of maternal stress for offspring. After spending 1-3 years in the Pacific Ocean, adult salmon migrate to natal freshwater spawning grounds on a fixed energy budget due to cessation of feeding, in tandem with senescence and deterioration of major physiological processes (Groot & Margolis 1991). Returning salmon encounter a myriad of aquatic stressors: pollution, hydroelectric dams, warming water temperatures, predators and the fishing nets/hooks of

recreational, commercial and indigenous fishers. The cumulative effects of endogenous stress and exogenous stressors are thought to be driving the population declines of sockeye salmon (Cohen 2012), but there is minimal investigation into how intergenerational effects of maternal experience may be implicated in changes in population dynamics (but see Braun et al. 2013). With only a single breeding episode before death to produce the next generation of spawners, and brief parental care to mediate negative effects of maternally derived handicaps (e.g., elevated egg GC levels, Love & Williams 2008b), how maternal stress translates to offspring phenotypes could considerably influence a female's lifetime fitness. Specifically, if offspring performance traits, such as burst swimming ability, are compromised, this could have detrimental effects at various stages of development. Fry migrating to nursery areas where they will feed and grow for 1-2 years (Groot & Margolis 1991) need adequate burst swim performance to successfully avoid predators (Taylor & McPhail 1985b).

I sought to quantify the intergenerational effects of maternal stress in wild (i.e., lived from egg to adult in the wild and originate from parents produced in the wild) semelparous sockeye salmon. I predicted that egg size and hormonal composition, as well as embryo survival and fry burst swimming performance would differ between females repeatedly exposed to an acute chase stressor and those left undisturbed.

4.3 Materials and methods

4.3.1 *Adult exposure and offspring rearing*

Wild adult sockeye salmon were collected in fall, during their up-river migration along a main tributary of Fraser River (British Columbia, Canada), the Harrison River (49°17′5 N, 121°54′27 W), approximately 6 weeks prior to historical peak spawning (mid-November). Fish

were transported 60 km (~1 hour) to the DFO Cultus Lake Salmon Research Laboratory (Cultus Lake, British Columbia, Canada) and held in 10 large, circular tanks (3 m diameter, 2 m deep, 10 000 L capacity). Water temperature in tanks decreased from 12.6 to 7.6°C, reflective of changes in water temperature experienced in the Harrison River. Each tank held 26-30 fish (14-26 females and 4-12 males). Tanks were divided into two treatment groups; 1) control fish that were not disturbed throughout the duration of experimental holding (five tanks) and 2) stressed fish that were chased (five tanks), but not trapped, with a net for 3 min, two times a day to mimic the repetitive nature of encounters with physical stressors (e.g., fisheries net, predator) experienced by fish migrating in the wild. Similar methods whereby experimenters manually chase wild Pacific salmon for 3 min show that heart rate, plasma lactate, and plasma cortisol levels are significantly increased (Donaldson et al. 2010), indicating individuals mount a stress response when exposed to such a stressor. Fish were chased at randomized times between 9:00 and 17:00 to reduce habituation. Fish were terminally sampled after 37 (n=22 females; n=22 males) and 42 (n=13 females; n=13 males) days of chasing, as timing of ripeness was staggered in time. The proportion of ripe females was, however, equal among control (46%) and stressed (45%) tanks at the termination of study, and similar numbers of fish were removed and sampled from each tank. Sampled fish were sacrificed using cerebral concussion and total mass and fork length were measured to the nearest 0.01 kg and 0.01 cm, respectively. Gametes were extracted and total ovary mass was recorded to the nearest g for females. Mass per egg was assessed by weighing (to the nearest 0.0001g) three replicates of 10 eggs from each female wet and after being dried in an oven for 48 hours at 60ºC. Three unfertilized, wet eggs from all but one female (control) were also frozen in liquid nitrogen and transferred to -80°C for cortisol analysis.

Gametes were fertilized at UBC following methods described in chapter 2. Eggs from 18 control and 17 stressed females were fertilized with sperm from control males to create full sibling crosses. Control males were paired once with a control female and once with a stressed female (one male was paired only once, with a control female). Each full-sibling cross was replicated three times. Full-sibling crosses were used as the focus of the study was to determine differences at the level of maternal stressor treatment. Consequently, replication between but not within females does not exclude the influence of parental effects. Following fertilization, eggs still separated by family and replicate were incubated in Heath trays with mean (± SE) water temperature of 8.4 ± 0.6ºC. Developing embryos were monitored daily and dead eggs/embryos were removed from Heath trays and stored in Stockard's solution to evaluate fertilization success. When fry reached emergence (complete absorption of the yolk sac) approximately 5 months from fertilization, up to five fish from each replicate of each family were sacrificed with an overdose of buffered tricaine methanesulfonate (MS222), blotted dry and body mass (to the nearest 0.0001 g) and fork length (to the nearest 0.1 cm) were measured for each fish. Replicates with <35% survival were not sampled to ensure adequate numbers of fish for swim trials. Separated by maternal treatment (families pooled), emerged fry were then transferred to a single 1000 L flow-through rectangular pond until swim trials. Water supply to the ponds came from local municipal reservoirs and flowed continually with minimal flows. Photoperiod was adjusted throughout rearing to mimic natural photoperiod at latitude 49°18´N. Water temperature in ponds ranged from 6-11°C throughout rearing due to natural changes in municipal water. Fish were fed powdered fishmeal (EWOS Canada Ltd; www.ewos.com) *ad libitum* twice daily until 24 hours before burst swimming trials. Fish were monitored daily and all mortalities were recorded. These methods have been used previously to raise Pacific salmon in lab (Burt et al. 2012a).

4.3.2 *Egg cortisol assay*

Following methodologies described in chapters 2 and 3, cortisol concentration of unfertilized eggs were determined for each female used for fertilizations (n=34, eggs were not obtained from one control female). Samples were run in duplicate on three assay plates with intra- and inter- assay coefficients of variation 4.5% and 5.9%, respectively.

4.3.3 *Fry burst swimming performance*

At approximately 1 month post-emergence, control (n=100) and stressed (n=100) fry (6 weeks post-emergence) were swum in a fixed speed test to assess burst swim performance. Methods followed those described in chapter 2 and Sopinka et al. (2013). Water speed was kept constant at 25 cm/s (which equated to 9 fork lengths/s) throughout the duration of swim trials. Water temperature in the flume during swim trials was 8.0ºC. All swim trials were recorded at 60 frames per s using a digital camera (Canon EOS Rebel T3i; www.canon.com). On average fry swam for 62 s (range, 8-302 s). Upon exhaustion (failure to swim after three probes with a blunt instrument), fish were then removed from the flume and sacrificed with an overdose of buffered tricaine methanesulfonate (MS222), blotted dry and body (to the nearest 0.001 g) and fork length (to the nearest 0.1 cm) were measured.

Burst swimming duration and burst swimming rate were quantified from videos viewed in Quicktime Pro (www.apple.com). For fish that burst swam for one continuous bout, the length of that bout was defined as total burst swimming duration. For fish that reinitiated swimming after falling back out of the covered area, the length of all the bouts of burst swimming were summed to quantify total burst swimming duration. Burst swimming rate was calculated by

summing the number of times (per 10 s) fish re-entered the covered area at the front of the flume after falling back out of said covered area.

4.3.4 *Statistical analyses*

Statistical analyses were based on treatment level means. When data could not be transformed (log_{10}, logit) to meet assumptions of normality or equal variance, non-parametric tests were employed. Body condition was calculated using the formula: (body mass/fork length^3) x 100%. To assess differences between control and stressed females, Student's *t*-tests (female body condition, egg cortisol, offspring survival to 1 month post-emergence, offspring body condition at 1- month post-emergence), an ANCOVA with maternal treatment (control or stressed) as a fixed effect and female body mass as a covariate (female ovary mass, egg mass [wet, dry]), and a mixed effects ANOVA with maternal treatment as a fixed effect and Family ID as a random effect (fertilization success, offspring survival to emergence, offspring body condition at emergence) were used. Pearson correlations were used to determine relationships between egg cortisol and fertilization success, offspring survival to emergence and emergent offspring body condition. Chi-squared tests were used to evaluate differences in the proportion of non-swimming fry and fry that required stimulation to initiate swimming due to maternal exposure to a stressor. Body mass (Pearson correlation, r^2=0.21, n=169, P<0.0001) and fork length (r^2=0.13, n=169, P<0.0001) positively correlated with burst swimming duration. Differences in swimming duration between offspring from control and stressed females were thus examined using an ANCOVA, with fry body mass or length as a covariate and maternal treatment as a fixed effect. Maternal treatment differences in burst swimming rate were determined with a Wilcoxon signed-rank test. Non-significant interactions (P>0.05) were

removed from all models. *Post-hoc* differences ($P<0.05$) were determined using Tukey's HSD test.

4.4 Results

4.4.1 *Maternal condition and early offspring traits*

Maternal exposure to the repeated stressor did not affect female body condition, egg mass (dry and wet), egg cortisol or fertilization success (Table 4.1). Chased females did however have reduced ovary mass (Table 4.1). Across multiple stages of development, no differences in survival or body condition were detected between offspring from control and stressed females (Table 4.1). Offspring survival to emergence was however more variable for stressed females (Levene's test, F=4.69, P=0.04; coefficient of variation, control females=0.36, stressed females=0.58). Across females (control and stressed), egg cortisol levels did not correlate with fertilization success (Pearson correlation, adjusted r^2=-0.03, n=34, P=0.70) or offspring survival to emergence (adjusted r^2=-0.03, n=34, P=0.79). There was a tendency for emergent offspring body condition to decrease with increased egg cortisol (adjusted r^2=0.14, n=26 P=0.06).

4.4.2 *Fry swimming performance*

Similar proportions of fry from control (13%) and stressed (18%) females failed to burst swim in the flume (Chi-squared test, $\chi 2$=0.96, n=200, P=0.33). Burst swimming performance was related to maternal treatment. Controlling for body mass (ANCOVA, maternal treatment: $F_{1,166}$=32.53, P<0.0001; body mass: $F_{1,166}$=43.04, P<0.0001), and length (maternal treatment: $F_{1,166}$=24.88, P<0.0001; body length: $F_{1,166}$=17.34, P<0.0001), fry reared from stressed females burst swam for shorter periods of time compared to fry reared from control females (Figure

4.1A). When burst swimming rate was quantified, the relationship between maternal treatment and swim performance reversed; fry reared from stressed females had superior burst swimming rates compared to fry reared from control females (Wilcoxon signed-rank test, $Z=5.29$, $n=169$, $P<0.0001$, Figure 4.1B). Following Sopinka et al. (2013), as burst swimming rate increased, total burst swimming duration decreased (Spearman's rank correlation, control: $\rho=-0.52$, $n=87$, $P<0.0001$; stressed: $\rho=-0.50$, $n=82$, $P<0.0001$).

4.5 Discussion

I have shown for the first time in a wild semelparous vertebrate that females repeatedly exposed to a chase stressor do not incur impairment to reproductive and early offspring parameters (gametic composition, embryo survival). However, burst swimming performance of emergent fry was influenced by maternal exposure to the stressor. Gametic/offspring buffering (Schreck et al. 2001) and maternal match/mismatch (Breuner 2008) are discussed herein. Furthermore, a lack of variation in egg cortisol between stressed and control females prompts discussion of alternative mechanisms driving the observed behavioural differences in offspring. It is noted that relative ovary mass was lower in chased females but mass per egg did not differ between chased and undisturbed females. Calculating fecundity by dividing ovary mass by mass per egg suggests chased females produced fewer eggs. Egg number is thought to be fixed in salmonids by mid-vitellogenesis or 4 months before ovulation (Tyler et al. 1994), which would generally be prior to freshwater entry of Pacific salmon. Atresia may have occurred in females exposed to the repeated experimental chasing. Fathead minnow (*Pimephales promelas*) exposed to low pH/acidified water (McCormick et al. 1989) or dietary methylmercury (Drevnick et al. 2006), and red gurnard (*Chelidonichthys kumu*) confined for 72-96 hours (Clearwater &

Pankhurst 1997) exhibited increased egg atresia. However, as ovaries were only weighed at the end of the experimental treatment advancement of atresia and/or histological quantification of atretic oocytes was not possible.

Egg size (Brooks et al. 1997) and cortisol (Gagliano & McCormick 2009) can influence offspring survival and hence the capacity for females to effectively mediate these effects is advantageous. Such a system would be especially advantageous for sockeye salmon given that in the wild, typical egg-fry survival is already very low (7%, Bradford 1995). In this study, a "progeny-protecting system" (Schreck et al. 2001) may account for the observed resilience to increases in egg cortisol and reductions to egg size and offspring survival in sockeye salmon chronically exposed to an acute stressor. Though overall ovary mass was reduced in stressed females, absence of reduced egg size and increased egg cortisol could be the result of maternal modulation of egg lipo-protein (e.g., vitellogenin) and hormone content. It is unclear however, when this potential modulation would have occurred (onset of stressor treatment *versus* final stages of ovulation). In mammals, hormonal modulation of offspring is accomplished *via* the placental enzyme 11β-hydroxysteroid dehydrogenase (HSD11β2) which converts cortisol to its inactive form cortisone (Benediktsson et al. 1997). Rainbow trout ovarian follicles, ovulated eggs, and fertilized embryos show evidence of converting cortisol to cortisone, which suggests the presence of similar protective enzymes in fishes (Li et al. 2012). I speculate that in semelparous, senescing animals HSD11β2 levels may increase in tandem with GCs (Baker & Vynne 2014) due to degeneration of tissues that regulate the HPI axis (Maldonado et al. 2002). In combination with stress response attenuation as maturation progresses (Wingfield & Sapolsky 2003; Cook et al. 2011), it is possible that semelparous females possess superior mechanisms to reduce offspring exposure to elevated levels of GCs.

Maternal match/mismatch is receiving increasing attention (Breuner 2008; Love & Williams 2008b; Love et al. 2013; Sheriff & Love 2013). I hypothesize that chased females perceived the daily chase stressor as a chronic, unpredictable, large, predator (such as a surface attacking bear) and/or an enclosing seine/gill fishing net (both tangible threats to migrating Pacific salmon that can elicit a burst swimming response). The riverine environment shared by females and later by their offspring would be characterized as unpredictable and having a high predation risk. Accordingly, females produced offspring with phenotypes (increased burst swimming rates) that could be beneficial if fry also encountered a world with chronic, unpredictable stressors that required individuals to repeatedly swim away to ensure survival; consistent with maternal match predictions. Although fry may not encounter the same types of stressors as migrating adults, higher burst swimming rates can still be advantageous when escaping life-stage appropriate predators (e.g., great blue heron *Ardea herodias*; larger/older Pacific salmon). Furthermore, swim capacity traits manifesting in young fry can correlate with adult swim requirements in sockeye salmon (Sopinka et al. 2013). Females that complete a longer and more arduous migration produce offspring with higher burst swimming rates, suggesting that females may prime offspring with swimming abilities detectable early in development that also attribute to successful completion of a more challenging migration later in life (Sopinka et al. 2013). Alternatively, from a mismatch perspective increased burst swimming rates may be a hyperactive response and exhaust energy stores more rapidly. Reduced burst swimming durations could result in faster and/or inevitable capture by a predator/fishing net. Energetically and behaviourally inefficient predator escape would conclude that maternal stress conferred unfavorable traits to offspring. Interestingly, locomotory differences occurring in the

absence of differences in egg cortisol clearly indicate that intergenerational effects can become manifest *via* mechanisms other than cortisol.

Hormone and epigenetic-mediated maternal effects have received considerable attention across taxa. In fish, cortisol is the predominant hormone manipulated by researchers to mimic maternal stress and evoke differences in offspring performance. One of the primary objectives was to determine whether repeated exposure to an acute stressor would increase maternal deposition of cortisol into eggs. Comparable egg cortisol levels between stressed and control females did not support previous findings with hatchery coho salmon (Stratholt et al. 1997). Other egg hormones (sex and thyroid hormones) not measured may have varied and contributed to differences detected later in offspring development. Other egg components that could elicit latent behavioural effects are those related to the genetic contribution of females to their offspring. In non-migrating fish (that do not move through as many diverse environments throughout life) genetic influences may be under greater selection pressure and maternal-matching is apt to be a more reliable strategy to confer adaptive information to offspring as the environment of female and offspring is considerably more similar. In migrating sockeye salmon, a female's genetic makeup affects fry burst swim performance (Burt et al. 2012b; Sopinka et al. 2013) and cellular machinery supporting burst swimming varies at the family and population level (Garenc et al. 1998). It is possible that changes to maternal gene expression associated with maternal stress (e.g., Jeffries et al. 2012b; 2014) could interact with inherent maternal effects, consequently altering the phenotype of offspring that would emerge under undisturbed conditions. Exposure to a temperature stressor during embryonic development modifies parental effects on emergent sockeye salmon burst swimming performance (Burt et al. 2012b). Due to logistical constraints I was unable to comprehensively control for parental effects within

treatment groups. If the mechanisms driving stress-induced epigenetic changes are heritable, stressed females could reprogram offspring to express traits necessary to survive a similarly stressful environment (i.e., context-specific maternal effect, Badyaev & Uller 2009). Further research quantifying the heritability of performance traits between ecologically stressed and undisturbed Pacific salmon will help quantify and qualify the impact of maternal effects and enrich current population modelling.

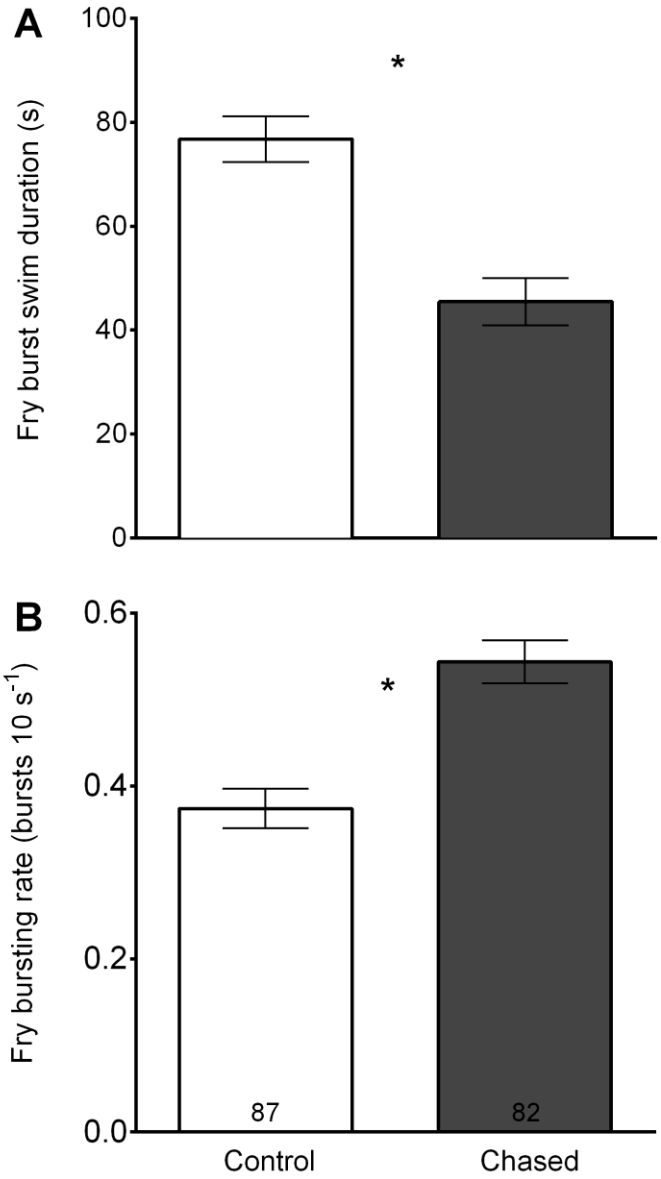
This study stresses the importance of examining different metrics of offspring performance at different life stages. Becoming more evident is that predicting the latent effects of ecological stressors in wild populations is not as straightforward as previously thought (Dickens & Romero 2013; Fefferman & Romero 2013). Free-living animals are now exposed to cumulative stressors, across all stages of life and stress-coping mechanisms vary with species-specific reproductive strategies. Approaching maternal effects in a holistic manner that encompasses the ecology, endocrinology and behaviour of a species throughout its life history will provide a more complete picture of how the environment affects more than one generation at a time (Sheriff & Love 2013).

Table 4.1 Effects of maternal exposure to the chase stressor on reproductive, gametic and offspring traits in sockeye salmon.

Trait	Maternal Treatment	n	Mean ± SE (range)	t/F, *P*-value
Body condition	Control	18	1.12 ± 0.02 (1.01 – 1.32)	t=-0.20 *P*=0.84
	Chased	17	1.11 ± 0.02 (0.98-1.31)	
Ovary mass (kg)	Control	18	0.554 ± 0.022 (0.387-0.719)	$F_{1,32}$=9.00 *P*=0.01
	Chased	17	0.503 ± 0.021 (0.295-0.620)	
Wet egg mass (g)	Control	18	0.155 ± 0.004 (0.119-0.190)	$F_{1,32}$=0.05 *P*=0.82
	Chased	17	0.154 ± 0.004 (0.130-0.194)	
Dry egg mass (g)	Control	18	0.063 ± 0.002 (0.050-0.072)	$F_{1,32}$=0.64 *P*=0.43
	Chased	17	0.060 ± 0.007 (0.051-0.075)	
Egg cortisol (ng/g)	Control	17	11.59 ± 1.74 (3.21-28.26)	t=0.55 *P*=0.58
	Chased	17	13.03 ± 1.99 (4.12-32.78)	
Fertilization success (%)	Control	18	82.30 ± 4.30 (28.44-98.62)	$F_{1,33}$=0.76 *P*=0.39
	Chased	17	86.94 ± 3.04 (56.05-98.30)	
Survival to emergence (%)	Control	18	76.19 ± 6.53 (8.74-97.50)	$F_{1,33}$=0.58 *P*=0.45
	Chased	17	67.52 ± 9.46 (1.60-98.73)	

Trait	Maternal Treatment	n	Mean ± SE (range)	t/F, *P*-value
Survival 1 month post-emergence (%)	Control	18	85.61 ± 4.65 (28.77-99.90)	t=-0.75 *P*=0.33
	Chased	17	92.53 ± 2.02 (69.05-99.90)	
Fry body condition (at emergence)	Control	15	8.14 ± 0.18 (6.96-9.64)	$F_{1,25}$=0.15 *P*=0.71
	Chased	12	8.24 ± 0.18 (7.57-9.26)	
Fry body condition (1 month post-emergence)	Control	100	6.81 ± 0.07 (5.34-8.64)	t=-0.53 *P*=0.60
	Chased	100	6.87 ± 0.08 (5.20-10.13)	

Figure 4.1 (A) Mean (± SE) length-adjusted burst swimming duration (in s) of sockeye salmon fry reared from control (open bars) and chased (filled bars) females. Untransformed data presented for illustrative purposes only. Statistical differences were generated using an ANCOVA and $\log_{10}$ transformed data. (**B)** Mean burst swimming rate (± SE) for fry from control and stressed females. Burst swimming rate was calculated by summing the number of times (per 10 s) fry re-entered the shaded area at the front of the flume after falling back out of the shaded area. Asterisks denote significant differences between fry groups (P<0.05).



Chapter 5: Maternal programming of offspring stress response in wild sockeye salmon

5.1 Synopsis

Chapter 4 revealed that maternal stressor exposure modified offspring swimming capacity. The present chapter sought to examine effects on physiological responses of offspring. Specifically, I examined whether repeated maternal exposure to a chase stressor altered offspring's plasma cortisol stress response or hypothalamic-pituitary-interrenal (HPI) activity in sockeye salmon. Resting plasma cortisol and preoptic area corticotropin-releasing factor (CRF) mRNA abundance did not vary between offspring reared from undisturbed females and those exposed to the stressor. However, resting mRNA abundance of head kidney melanocortin 2 receptor (MC2R), steroidogenic acute regulatory protein (StAR), and cytochrome P450 side-chain cleavage enzyme (P450scc) appeared to be elevated in offspring reared from stressor-exposed females. One hour after an acute chase stressor, juveniles reared from chased females had lower plasma cortisol levels than did juveniles reared from undisturbed females. Compared to resting mRNA abundance, head kidney mRNA abundance was reduced post-chase in offspring reared from chased females. Head kidney mRNA abundance did not change post-chase compared to baseline levels for offspring reared from control females. Together, the results suggest maternal programming of progeny with respect to baseline and stressor-induced mediators of HPI axis activity. Whether the intergenerational effects on HPI axis observed here are adaptive will require further investigation into the tissue-specific glucocorticoid receptors and enzymatic metabolism of cortisol, and more importantly, direct measures of fitness (e.g., predator avoidance).

5.2 Introduction

Intergenerational effects of maternal stressor exposure are taxonomically broad (e.g., fishes, McCormick 2009; birds, Saino et al. 2005; herpetofauna, Shine & Downes 1999; mammals, Dantzer et al. 2013), and have been examined for an array of traits using an array of experimental manipulations (e.g., predator exposure, competitive interactions). The majority of studies report effects of maternal stress on measures of offspring size and survival. Evidence that offspring behaviour is also altered by maternal stressor exposure is now accumulating (e.g., foraging, Janczak et al. 2007; predator avoidance, McGhee et al. 2012; dispersal, Bestion et al. 2014). However, how maternal stress influences physiological processes of offspring, that potentially drive variation in size, behaviour and survival, is a developing field. The hypothalamic-pituitary-adrenal [HPA] axis (in birds, mammals; HP-interrenal [HPI] axis in herpetofauna, fishes) of offspring is one such physiological response that could be affected by maternal stress. Maternally-mediated effects on offspring HPA/-I can be discerned by relatively straightforward measurement of plasma GCs following stressor exposure. Effects of maternal stress on the offspring GC stress response are of particular ecological and evolutionary importance. The degree of GC elevation post-stressor can be linked with different behaviours, including aggression (Øverli et al. 2004) and nest-site selection (Seltmann et al. 2014). Also, the stress response is hypothesized to be linked with fitness (Wingfield et al. 1998; Blas et al. 2007; Breuner et al. 2008; MacDougall-Shackleton et al. 2009; Cook et al. 2014). However, the production of GCs is the final outcome following a number of neuroendocrine and cellular processes (see below). Thus, investigating all levels of the offspring HPA/-I axis can provide a more comprehensive understanding of how maternal stressor exposure affects progeny stress physiology.

Initiating in the hypothalamus, a first step of the HPA/-I axis is the hypothalamic release of corticotropin-releasing factor (CRF), which act stimulate synthesis and release of adrenocorticotropic hormone (ACTH) from corticotropic cells in the pituitary. Circulating ACTH binds to melanocortin 2 receptors (MC2R) on steroidogenic cells within the adrenal glands (birds, mammals) or interrenal tissues (herpetofauna, fishes). Following binding of ACTH, GCs are produced through a series of enzymatic processes (see below). To date, proxies of maternal stressor exposure are primarily used to investigate intergenerational effects of maternal stress on offspring HPA/-I (but see Mommer & Bell 2013). Females implanted with GCs *via* silastic tubing or, in oviparous species, eggs injected with GCs produce offspring with attenuated (Auperin & Geslin 2008; Love & Williams 2008b; Hayward et al. 2006 [female progeny]; Jeffrey 2014), hyperresponsive (Hayward & Wingfield 2004, Marasco et al. 2012; Schultner et al. 2013) or unaltered (Hayward et al. 2006 [male progeny]; Henriksen et al. 2013) HPA/-I activity (as measured by plasma/whole body GC concentrations). Recovery following progeny exposure to an acute stressor can also be modified by elevated egg GCs (Haussmann et al. 2011). With the exception of biomedical, rodent-based research (see Weinstock 2005), how the HPA/-I axis is affected in offspring reared from females directly exposed to a stressor is presently lacking.

If maternal exposure to a stressor can modulate the plasma GC response of offspring exposed to a stressor themselves, what mechanisms might generate such variation in GC synthesis and regulation? Again, measuring only post-stressor plasma GCs limits the scope of interpretation of HPA/-I axis function. In fishes, for example, ACTH binds to the G-coupled protein MC2R on the interrenal cells of the head kidney (Aluru & Vijayan 2008). This binding initiates a cAMP-signaling cascade leading to the shuttling of cholesterol to the inner

mitochondrial membrane *via* steroidogenic acute regulatory protein (StAR). There, cholesterol is cleaved by cytochrome P450 side chain cleavage enzyme (P450scc) to pregnenolone (Aluru & Vijayan 2006); subsequent enzymatic modification yields cortisol. Using mRNA abundance as a proxy for protein levels, CRF, MC2R, StAR and P450scc mRNA abundance are increased following exposure to a stressor (Geslin & Auperin 2004; Doyon et al. 2005; Fuzzen et al. 2010; Jeffrey 2014; Jeffrey et al. 2014b). Thus, the aforementioned maternal effects of stress on the final outcome of the offspring stress response (i.e., plasma GC concentration) may be driven by changes in the activity at higher levels of the HPA/-I axis. Fishes are an excellent group in which to study maternal effects of stress on the offspring stress response given knowledge of the HPI axis (Wendelaar Bonga 1997; Mommsen et al. 1999). Further, many species of fishes do not exhibit the extensive parental care typical of birds and rodents, which has the potential to further modify stress axis activity (Meaney 2001; Love & Williams 2008b). Also, our knowledge of stress-mediated maternal influences on offspring traits remains limited to metrics of egg size and embryo survival (Schreck 2001).

I explored how chronic maternal exposure of wild-caught adult sockeye salmon to an acute chase stressor affected hormonal and molecular components of the progeny HPI axis. Each year millions of adult salmon migrate to freshwater spawning areas in the Fraser River watershed in British Columbia, Canada encountering numerous ecological (e.g., predation) and anthropogenic (e.g., warming river temperatures, fisheries) stressors. Exposure to elevated water temperature (Jeffries et al. 2014) and fisheries capture and release (Donaldson et al. 2014) elevate circulating cortisol as well as genetic signatures of cellular stress in salmon. Maternal exposure to a repeated acute stressor alters the swim performance of their offspring (chapter 4), and can increase cortisol content of eggs (Stratholt et al. 1997 but see chapter 4) which can

dampen progeny HPI activity (Auperin & Geslin 2008). The implications on fitness of any potential physiological adjustment are significant for Pacific salmon given their semelparous life history. Populations of sockeye salmon are of particular concern owing to population-specific declines (Cohen 2012). I predicted that the plasma cortisol and glucose stress response of offspring reared from chased females would be different from that of offspring reared from control females (i.e., degree of elevation and rate of recovery would vary significantly). I also predicted that differences in the plasma cortisol response would be mirrored by differences in baseline and post-stressor exposure mRNA abundance of CRF, MC2R, StAR and P450scc. Also, given that exposure to an acute stressor initiates glycogenolysis in the liver (Wendelaar Bonga 1997), I predicted that liver size would be altered in offspring following stressor exposure.

5.3 Materials and methods

5.3.1 *Adult exposure and offspring rearing*

Adult holding and stressor exposure and offspring rearing were carried out as described in chapter 4. Fish were fed fishmeal (EWOS Ltd. http://www.ewos.com) *ad libitum* up to the day of transfer to buckets (see below).

5.3.2 *Offspring exposure to an acute stressor*

Offspring (~ 1 year post-fertilization) plasma cortisol and glucose concentration and tissue mRNA levels of CRF (brain preoptic area [POA]), MC2R, StAR and P450scc (head kidney) were examined before (baseline) and 0, 1, 4, 8 or 24 hours following a 5 min chase stressor. Offspring reared from control and stressed females were randomly selected from holding troughs and placed into 10 L flow-through buckets (n=6 fish per bucket) held in 1000 L

flow-through troughs. Buckets were covered with an opaque tarpaulin and left undisturbed for 24 hours. Buckets were randomly assigned to be sampled before and 0, 1, 4, 8 or 24 hours post-chase. The next day, fish in all buckets were simultaneously chased for 5 min with a net, except the bucket containing fish to be sampled before the stressor (baseline). The single, acute chase stressor was chosen to mimic a single, predation episode whereby offspring may be required to swim to exhaustion to escape an aerial or piscivorous predator. At the designated sampling time, fish were removed from buckets and euthanized by immersion in an overdose of buffered tricaine methanesulfonate (MS222). Body mass was measured to the nearest 0.001 g. Blood (~10 μL) was collected into heparinized microcapillary tubes *via* caudal severance and centrifuged at 6000 *g* for 5 min; the plasma layer was removed and stored at -80^{o}C until further analysis. The POA of the brain and a piece of head kidney were excised, placed in RNAlater® solution, stored at 6^{o}C for 24 hours and then transferred to -80^{o}C until further analysis. Liver tissue was also removed and placed in pre-weighed vials filled with RNAlater® solution to be used in future analyses not presented in this thesis. Vials containing liver tissue were weighed a second time to determine liver mass to the nearest 0.001 g. The entire experiment was replicated to have a final sample of 12 for each time point for offspring reared from both chased and control females.

5.3.3 *Plasma cortisol and glucose concentrations*

Plasma cortisol was analyzed using ELISA, previously validated for use in sockeye salmon (e.g., Jeffries et al. 2011). Values for intra- and inter-plate variation for cortisol analysis were 2.4% and 5.5%, respectively. Owing to plasma volume constraints, samples of plasma were pooled for analysis of glucose concentrations. Plasma from two individuals from the same maternal treatment, same time point post-chase and same experimental day was pooled to

generate one sample. Plasma glucose concentration was analyzed spectrophotometrically according to Bergmeyer (1983). Values for intra- and inter-plate variation for glucose analysis were 4.5% and 5.6%, respectively.

5.3.4 *Gene sequences*

Partial sequences for CRF, MC2R, StAR, P450scc, and 18S were generated as in Jeffrey et al. (2014a). Gene-specific primers (Table 5.1) were designed using Primer3 (Sim-Gene.com) based either on rainbow trout sequences (CRF, MC2R, and P450scc) or expressed sequence tags (EST) for sockeye salmon (StAR and 18S). For MC2R and 18S, a second set of primers was used to extend the sequence. These primer sets were designed such that the forward primer of the second set of primers was embedded within the sequence of the product of the first set of primers. Synthesized cDNA (see below) from the POA (for CRF) and head kidney (for MC2R, StAR, P450scc, and 18S) were used in 25 µl PCR reactions as follows: 2 µl cDNA, 0.25 µl Choice Taq DNA polymerase (Denville), 0.2 mM dNTPs (Life Technologies), and 0.2 µM primer. A Bio-Rad S100 Thermal Cycler (Bio-Rad) was used for all PCR reactions with the following cycling conditions; annealing temperature 55°C (30 s) and elongation temperature 72°C (30 s) for 38 cycles. Products were run on 1.5% agarose (Life Technologies) gels with ethidium bromide (Fisher Scientific) and extracted using QIAquick gel extraction kit (QIAGEN). Using QIAGEN PCR cloning kit (QIAGEN) and DH5α Competent Cells (Life Technologies), amplicons were ligated into a vector and cloned following the manufacturers' protocols; after which plasmids were extracted using QIAprep Spin Miniprep Kit (QIAGEN). Plasmids were sequenced by GenScript USA Inc., resulting in partial sequences for CRF (392 bp), MC2R (831

bp), StAR (643 bp), P450scc (695 bp), and 18S (655 bp) subsequently used to generate primers for semi-quantitative real-time RT-PCR.

5.3.5 *Tissue mRNA relative abundance*

Relative mRNA abundance was assessed using semi-quantitative real-time RT-PCR. Following the manufacturer's protocol, total RNA was extracted from 10 to 100 mg of POA or head kidney tissue using TRIzol reagent (Life Technologies). Tissues were homogenized in TRIzol using, sequentially, 18- and 23-gauge needles attached to a syringe. A NanoDrop ND-1000 UV–Vis Spectrophotometer was used to quantify RNA. The synthesis of cDNA was completed using QuantiTect Reverse Transcription Kit (QIAGEN) following the manufacturer's protocol with the exception that half reactions were used yielding a volume of 10 rather than 20 µL. Semi-quantitative real-time RT-PCR was then conducted using the Rotor-Gene SYBR Green RT-PCR kit (QIAGEN) and a Rotor-Gene Q system (QIAGEN) according to manufacturer's instructions with the exception that reaction volumes were scaled to 10 µL. Gene-specific primers were generated using Primer3 (Sim-Gene.com; Table 5.2) and amplicons were sequenced to verify the specificity of each primer set. Standard curves were also generated to optimize reaction compositions based on the efficiency of the reaction which were between 0.89 and 1.07. Primer concentrations were 10 µM for 18S, MC2R and StAR, and 0.4 µM for P450scc and CRF. The cDNA was diluted 10-fold for analysis of CRF, MC2R, P450scc and StAR in head kidney, and 2000- and 20 000-fold for18S in brain and head kidney, respectively. Cycling parameters were constant across reactions; 95°C (5 s) and 60°C (10 s) for 40 cycles. To rule out genomic DNA contamination, no reverse transcriptase (cDNA synthesis without reverse transcriptase) and no template (water replaces cDNA in real-time RT-PCR reactions) controls

were run on each plate. For each gene of interest, samples were run in duplicate on 3 plates with average intra- and inter-plate variation across genes of 0.5% and 1.3%, respectively. To compare the mRNA abundance of CRF, MC2R, StAR and P450scc between offspring reared from control and chased females, mRNA abundance of each gene was determined relative to baseline mRNA levels of offspring reared from control females (n=5-6) using the modified delta-delta Ct method with 18S as the normalizing gene (see Pfaffl 2001; Jeffrey et al. 2012). The mRNA abundance of 18S in brain and head kidney tissue did not vary across time points (ANOVAs, $P>0.05$) or between treatments (Student's *t*-tests, $P>0.05$).

5.3.6 *Statistical analyses*

Data were assessed for normality and equal variance (Bartlett's test) and transformed (log_{10}) when necessary to achieve normality. Two-way ANOVAs with maternal treatment (offspring reared from control *versus* chased females) and time post-chase as fixed effects were used to examine offspring plasma cortisol and glucose levels following exposure to the 5 min chase stressor. A two-way ANCOVA with maternal treatment and time post-chase as fixed effects, and offspring body mass as a covariate was used to examine changes in offspring liver size following the chase stressor. Non-significant interactions ($P>0.05$) were removed from models. Student's *t*-tests were used to determine differences in baseline relative mRNA levels between offspring groups. One-way ANOVAs were used to assess how relative mRNA abundance changed over time following the chase stressor, separately for each offspring group. Two outliers (CRF mRNA abundance for offspring reared from a control female, at baseline and 1 hour post-chase) were detected and excluded from analyses (Grubbs' test, $P<0.05$).

5.4 Results

5.4.1 *Plasma cortisol concentration*

Offspring reared from control and chased females both exhibited elevated plasma cortisol levels following the 5 min chase stressor that returned to baseline levels within 4-8 hours (two-way ANOVA, time x maternal treatment: $F_{5,132}$=9.21, P<0.0001, Figure 5.1A). Cortisol levels were highest for both offspring groups at 1 hour post-chase, but absolute levels were significantly lower in offspring reared from chased females (Figure 5.1A). Cortisol levels in offspring reared from chased females returned to baseline by 4 hours post-chase, whereas values for offspring reared from undisturbed females returned to baseline levels by 8 hours post-chase (Figure 5.1A). Baseline plasma cortisol (no chase) did not differ between offspring groups (Figure 5.1A).

5.4.2 *Plasma glucose concentration*

For offspring reared from undisturbed and chased females, plasma glucose levels were elevated 4 hours following exposure to the chase stressor, and remained elevated 24 hours post-chase (two-way ANOVA, time: $F_{5,62}$=10.79, P<0.0001; Figure 5.1B). There were no statistically significant differences in glucose levels between offspring groups (maternal treatment: $F_{1,62}$=2.19, P=0.14, Figure 5.1B). Given that plasma cortisol levels were lower in offspring reared from chased females, we expected glucose to also be lower. Further examination of maternal treatment effects using one-tailed Student's *t*-tests revealed that at 4 and 8 hours post-chase, glucose levels tended to be lower in offspring reared from chased females (one-tailed Student's *t*-test, 4 hours: t=-1.60, n=12, P=0.08; 8 hours: t=-2.24, n=12, P=0.02; Figure 5.1B).

5.4.3 *Relative mRNA abundance of genes of the HPI axis*

Baseline CRF relative mRNA levels did not differ between offspring groups (Student's t-test, t=0.70, n=12, *P*=0.500, Figure 5.2). Baseline relative mRNA levels of MC2R (t=3.38, n=12, *P*=0.01, Figure 5.3A) and StAR (t=2.57, n=12, *P*=0.04, Figure 5.3B) were higher in offspring reared from chased females compared to offspring reared from undisturbed females. There was a tendency for relative mRNA abundance of P450scc to also be higher in offspring reared from chased females (t=2.00, n=12, *P*=0.08, Figure 5.3C).

Significant changes in mRNA abundance following the chase stressor were not observed in offspring reared from control females for any gene. There was a trend for CRF relative mRNA abundance to increase post-chase (ANOVA, $F_{5,28}$=2.43, *P*=0.06, Figure 5.2); this pattern was not detected for relative mRNA abundance of MC2R ($F_{5,30}$=0.95, *P*=0.46, Figure 5.3A), StAR ($F_{5,30}$=1.59, *P*=0.19, Figure 5.3B) or P450scc ($F_{5,30}$=0.73, *P*=0.60, Figure 5.3C). Relative CRF mRNA abundance did not change over time following the chase stressor in offspring reared from chased females ($F_{5,30}$=0.81, *P*=0.55, Figure 5.2). Exposure to the chase stressor did appear to reduce relative mRNA abundance of MC2R ($F_{5,30}$=6.60, *P*=0.0003, Figure 5.3A), StAR ($F_{5,30}$=2.37, *P*=0.06, Figure 5.3B) and P450scc ($F_{5,30}$=2.46, *P*=0.05, Figure 5.3C) from baseline levels, however, mRNA levels did not change over time post-chase (Figure 5.3A-C).

5.4.4 *Liver mass*

Relative to baseline liver mass, at 4 hours post-chase liver mass of offspring reared from control and chased females was reduced (ANCOVA, time: $F_{5,136}$=3.07, *P*=0.01; body mass: $F_{1,136}$=100.78, *P*<0.0001, Figure 5.4). Offspring liver mass did not vary between maternal

treatment at any time point pre- or post-chase (maternal treatment: $F_{1,136}$=0.84, P=0.36, Figure 5.4).

5.5 Discussion

I found evidence that maternal stressor exposure altered the offspring stress response; plasma variables and molecular signatures of the HPI axis differed between offspring reared from chased and control females. Plasma GC, and to a lesser extent glucose, levels in response to an acute stressor were lower in progeny of chased females. Love & Williams (2008b) also observed this pattern in European starling fledglings reared from eggs with experimentally elevated GCs to mimic poor maternal condition. The authors suggested that if elevated egg GCs reliably signal to offspring a forthcoming environment that is unfavourable and unpredictable, then an attenuated stress response would be adaptive (e.g., "Predictive Adaptive Responses" (PARs); Gluckman et al. 2005), by preventing exaggerated activity of energetically costly GC-mediated physiological processes (e.g., gluconeogenesis, osmoregulation in salmon, Richman & Zaugg 1987) or behaviours (e.g., begging in birds, Kitaysky et al. 2001; mating vocalizations in amphibians, Leary et al. 2006; thermoregulation in reptiles, Belliure & Colbert 2004). In adult sockeye salmon migrating to freshwater spawning grounds, individuals with smaller changes between baseline and stressor-induced plasma GC levels had a greater likelihood of successfully passing hydro-dynamically challenging rapids (Cook et al. 2014). Enhanced survival is also linked with dampened GC reactivity in reptiles (Romero & Wikelski 2001) and birds (Blas et al. 2007). Future studies comparing offspring cortisol stress response and a direct fitness correlate (e.g., survival in presence of live predator) can advance our understanding of how maternal stress affect relationships between the acute stress response and fitness (Breuner et al. 2008).

Interestingly, chronic maternal exposure to the chase stressor did not elevate egg cortisol concentrations (chapter 4), yet effects of maternal stress still were observed in juveniles. Similarly, Jeffrey (2014) found that egg cortisol concentration was equal between dominant and subordinate zebrafish (*Danio rerio*) yet offspring reared from subordinate females had attenuated plasma cortisol levels following an acute stressor. Thus, a mechanism other than maternally-derived egg cortisol concentration appears to be modulating offspring HPI and potentially acting as a predictive cue to offspring of future environmental conditions. Given that baseline mRNA levels of HPI axis genes varied between offspring reared from chased and control females, epigenetic mechanisms (e.g., DNA methylation, histone modification) may contribute to changes in gene expression and the observed changes in offspring HPI axis function. Measuring stressor-induced changes in non-hormonal maternally derived components of eggs warrants future investigation.

Baseline relative mRNA abundance of head kidney MC2R, StAR and P450scc were elevated in offspring reared from chased females. Increased baseline mRNA levels observed in the present study may be another PAR if offspring are entering an environment similar to that which adult females experienced (i.e., a repeated exposure/threat of exposure to a stressor and repeated HPI axis stimulation). Having a larger pool of MC2R, StAR and P450scc mRNA may facilitate cortisol production without an immediate requirement to replace mRNA. Levels of head kidney mRNA fell post-chase suggesting translation of mRNA to protein, but mRNA did not subsequently increase suggesting no immediate replenishment of mRNA. If baseline mRNA levels of stress axis genes are higher in offspring reared from chased females, why were stress-induced plasma cortisol levels lower? First, mRNA abundance does not linearly correlate to protein expression; elevated baseline mRNA abundance of head kidney genes does not guarantee

that enzymatic activity, and thus cortisol production, is increased. Second, reduced activity of enzymes at later stages of the HPI axis (e.g., 11β-hydroxylase, converts 11-deoxycortisol to cortisol) could reduce post-chase cortisol production despite potential increases in activity of StAR and P450scc. Third, a higher proportion of cortisol may be bound to corticosteroid-binding globulins (CBG). However, the prevailing view is that fishes have significantly lower proportions of bound GCs, in contrast to other vertebrates (Desantis et al. 2013). Lastly, cortisol may be being converted at a higher rate to its inactive form, cortisone, *via* type 2 11β-hydroxysteroid dehydrogenase (HSD), which increases in response to an acute stressor, Alderman & Vijayan 2012). Significant differences in baseline CRF mRNA levels were not observed between the offspring types. Resting level differences in CRF mRNA also were not detected between strains of rainbow trout differing in their cortisol response to a standardized stressor (Backström et al. 2011). The present study found evidence for maternal programming of constitutive expression of HPI axis genes in the head kidney, but further work is needed to understand the dynamics of protein expression and enzymatic activity following exposure to a stressor.

The type and severity of the acute stressor to which sockeye salmon offspring were exposed could account for the lack of mRNA response over time post-stressor. When rainbow trout held in isolation for 22 days were chased for 5 min with a net, no changes in head kidney mRNA levels were observed (Geslin & Auperin 2004). In contrast, when rainbow trout held in groups (n=12) for 16 days in large tanks were captured, confined in a smaller tank (2 min), transferred to a tank with phenoxy-2 ethanol (anesthetic, 3 min), and finally returned to the original larger tanks, MC2R, StAR and P450scc mRNA levels were elevated (Geslin & Auperin 2004). Also, rainbow trout confined in a submerged net for 1 hour exhibited increased MC2R,

StAR and P450scc mRNA levels (Jeffrey et al. 2014b). Variability in post-stressor CRF and CRF binding protein (CRF-BP) mRNA levels is also influenced by stressor type (e.g., social interaction, hypoxia, confinement/netting) and duration (Doyon et al. 2005; Bernier et al. 2008; Huising et al. 2004). The solitary 5 min chase stressor employed in this study may not have been severe enough to require protein production of MC2R, StAR and P450scc beyond what was possible with constitutive mRNA, and thus synthesis of more mRNA was not required post-chase (see Huising et al. 2004). Although head kidney mRNA levels showed no obvious stressor-induced changes, a trend was observed for CRF to increase over time post-chase for offspring reared from undisturbed females. This pattern could suggest tissue-specific HPI axis regulation following exposure to an acute stressor, and that maternal stress influences such regulation in offspring. Investigation of how both acute and chronic stressors influence physiological (i.e., HPI activity) and behavioural (e.g., fleeing) aspects of the offspring stress response can help elucidate the interplay between maternal programming and exposure to ecological stressors.

Elevated cortisol during the stress response in fishes has numerous targets, including the liver. We found significant, albeit subtle, reductions in offspring liver mass following the acute chase stressor. Severity of the stressor may account for the slight changes in liver mass; chronic stressors can influence liver mass more dramatically (e.g., social status, Sloman et al. 2001b; Sopinka et al. 2009; toxicant exposure, Lowe-Jinde & Niimi 1984; disease, Perelberg et al. 2003). The small change in liver mass could be the result of activation of glycogenolysis following exposure to an acute stressor; glucose levels were elevated 4 hours post-chase. However, catecholamines (*versus* cortisol) primarily drive stressor-induced glycogenolysis *via* activation of $ß_2$ -adrenergic receptors (Fabbri et al. 1998), but were not measured in this study.

No treatment level differences in liver mass despite differences in the cortisol response between offspring reared from chased and undisturbed females is therefore not surprising.

Presently the fitness implications of the observed multi-level effects of maternal stress on activity of the HPI axis in juvenile sockeye salmon are not yet clear. Examination of GR abundance at target tissues (e.g., liver, brain, Alderman et al. 2012) could aid in determining whether the muted stressor-induced cortisol response in offspring of chased females is linked with responsiveness at the tissue level. There also remains a suite of peripheral regulators of HPI axis activity (e.g., arginine vasotocin, Gilchriest et al. 2000; neuropeptide Y, Doyon et al. 2003; serotonin, Winberg et al. 1997) that might also be modified in offspring reared from stressed females. Finally, tandem examination of offspring stress physiology and behavioural responses (e.g., Piato et al. 2011) can provide a holistic understanding of how maternal stress alters progeny HPI axis function.

Table 5.1 Oligonucleotide primer sets used for gene cloning in sockeye salmon.

Gene	Primer direction	Sequence (5'-3')	Accession number*
CRF	Forward	gct cat tgc ttt ctt acc gc	NM001124286.1
	Reverse	tgg gct tgt tgc tgt aac tg	
MC2R	Forward 1	tga tgt ctc tgc tct ccc ct	NM001124680
	Reverse 1	gaa tag cat ggt gag cgt ga	
	Forward 2	atc ggc tgc atc tgt agc tt	
	Reverse 2	gga gca gaa cag cat ctt cc	
StAR	Forward	tgc ctg caa ctt tca aac tg	EV384867.1
	Reverse	act ggg ccg cat cac tat ac	
P450scc	Forward	tgg tga ggt gga gtg tgt gt	EV377682.1
	Reverse	ctg acg cat cgt cct gta ga	
18S	Forward 1	gga ggt tcg aag acg atc ag	EV376582.1, EV383560.1
	Reverse 1	ctc taa gaa gtt gga cgc cg	
	Forward 2	cat ggc cgt tct tag ttg gt	
	Reverse 2	tcc att att cct agc tgc gg	

*Accession number of sequence used to design primer set(s)

Table 5.2 Oligonucleotide primers used for semi-quantitative real-time RT-PCR in sockeye salmon.

Gene	Primer direction	Sequence (5'-3')	Product size (bp)
CRF	Forward	agt ctc ttc ccc tcc tga cg	20
	Reverse	ggg tac atg cct tta ggt gc	20
MC2R	Forward	gag aac ctg ttg gtg gtg gt	20
	Reverse	gag gga gga gat ggt gtt ga	20
StAR	Forward	tgg gga agg tgt tta agc tg	20
	Reverse	agg gtt cca gtc tcc cat ct	20
P450scc	Forward	aca gga caa atg gac cac cg	20
	Reverse	caa cat cag gcc cag acg tt	20
18S	Forward	ggc ggc gtt att ccc atg acc	21
	Reverse	ggt ggt gcc ctt ccg tca att c	22

Figure 5.1 Baseline and 0, 1, 4, 8, and 24 hours post-chase plasma **(A)** cortisol and **(B)** glucose levels (mean ± SE) of sockeye salmon reared from control (open bars) and chased (filled bars) females. Different letters denote statistically significant differences at $P<0.05$ (maternal treatments pooled, glucose only). [1]one-tailed Student's *t*-test, P=0.08; [2]one-tailed Student's *t*-test, $P=0.02$. Plasma [cortisol], n=12; [glucose], n=4-6.

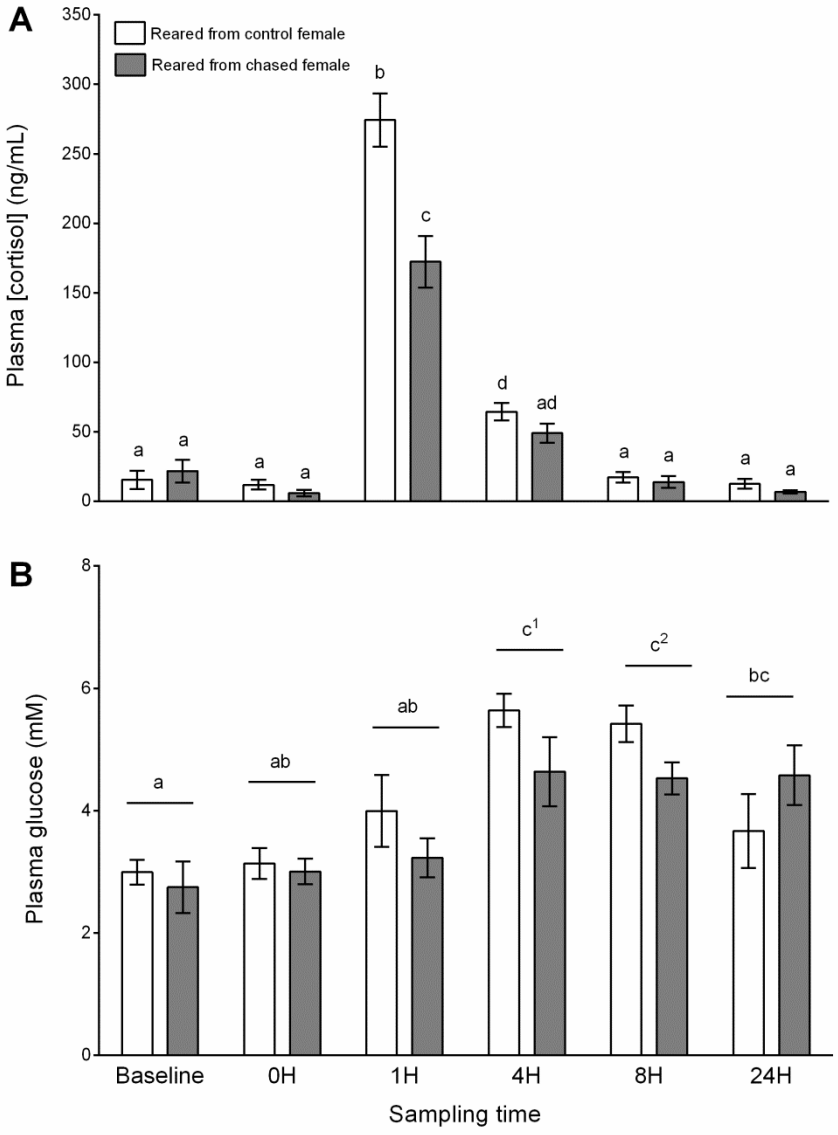


Figure 5.2 Baseline and 0, 1, 4, 8, and 24 hours post-chase relative mRNA abundance (mean ± SE) of corticotropin-releasing factor (CRF) in the preoptic area (POA) of sockeye salmon reared from control (open bars) and chased (filled bars) females. n=5-6.

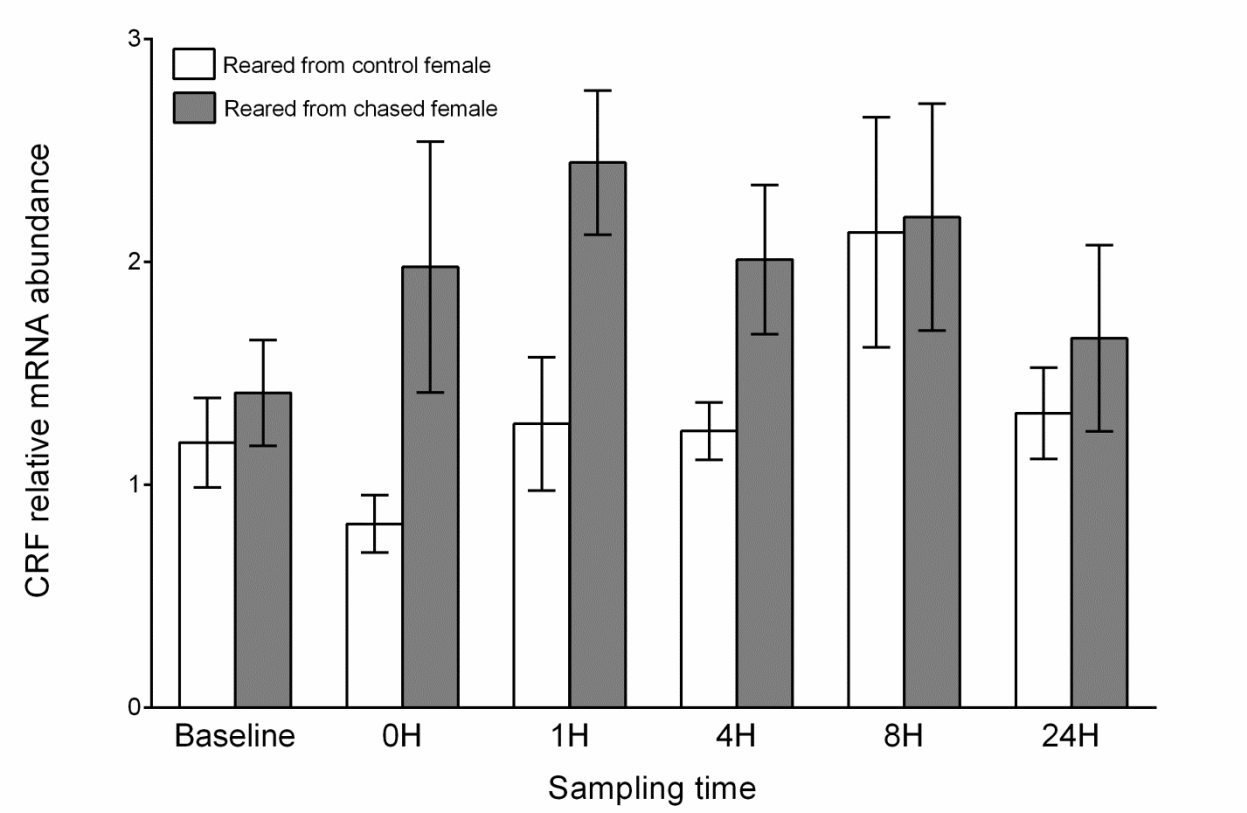


Figure 5.3 Baseline and 0, 1, 4, 8, and 24 hours post-chase relative mRNA abundance (mean ± SE) of **(A)** melanocortin 2 receptor (MC2R), **(B)** steroidogenic acute regulatory protein (StAR) and **(C)** cytochrome P450 side chain cleavage enzyme (P450scc) in head kidney of sockeye salmon reared from control (open bars) and chased (filled bars) females. Different letters denote statistically significant differences at $P<0.05$ across time points, within a maternal treatment. Asterisks denote statistically significant differences at $P<0.05$ between maternal treatments for baseline values. n=6.

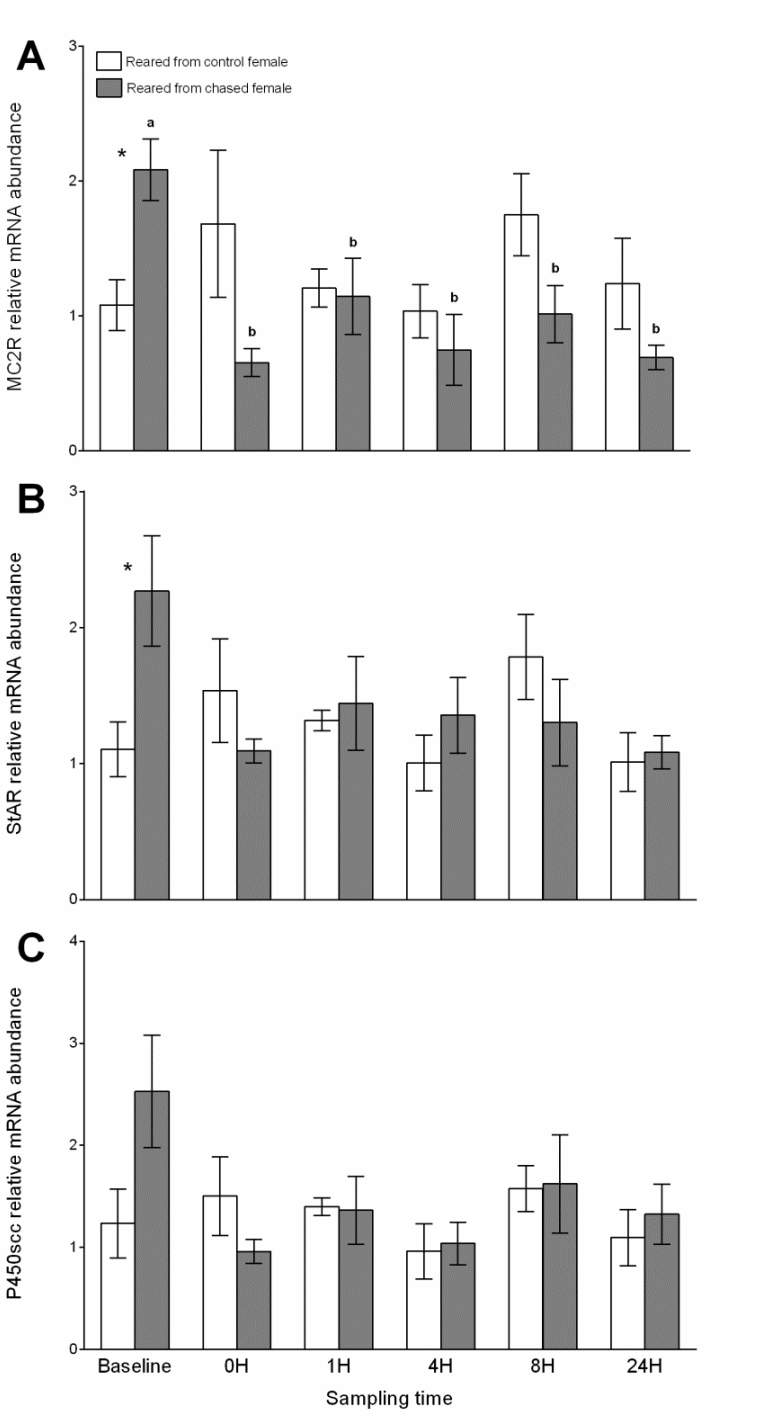
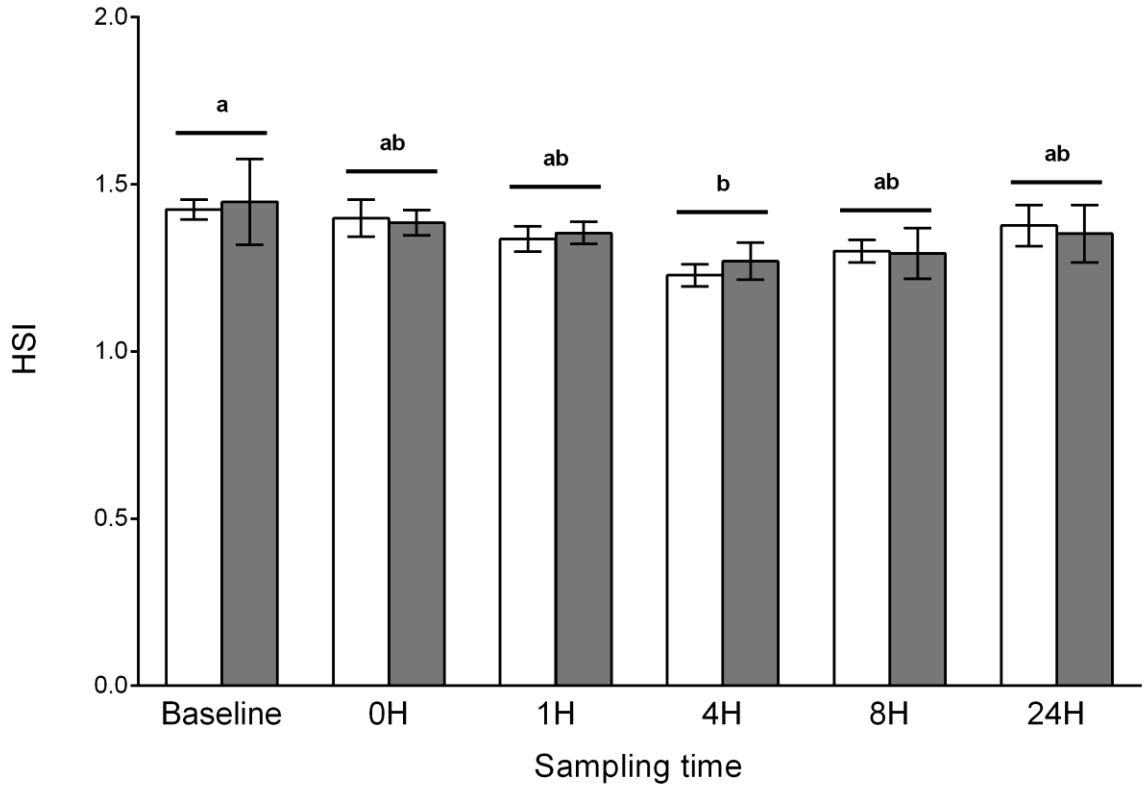


Figure 5.4 Baseline and 0, 1, 4, 8, and 24 hours post-chase liver size (mean ± SE, g) of sockeye salmon reared from control (open bars) and chased (filled bars) females. Hepatosomatic index (HSI) values are presented for illustrative purposes only and calculated as: [liver mass (g)/ body mass (g) – liver mass (g)] x 100%. Statistical differences were generated using an ANCOVA, see Section 5.3.6. Different letters denote statistically significant differences at $P<0.05$ across time points, maternal treatments pooled. n=12.



Chapter 6: Conclusions

This thesis sought to elucidate the effects of maternal stressor exposure on offspring performance and determine if observed effects were associated with differences in egg cortisol levels. It was evident in chapters 2 and 3 that offspring morphology and behaviour could be altered by exogenously elevating egg cortisol concentrations within 4 standard deviations (or ~10 ng/g) of concentrations measured in eggs collected from chronically stressed Pacific salmon (Stratholt et al. 1997). With egg cortisol content as a plausible hormonal driver of offspring performance (chapters 2 and 3; Sloman 2010; Burton et al. 2011), and there being evidence for stressor-induced increases in egg cortisol (Stratholt et al. 1997; McCormick 2006; 2009) and reductions in offspring quality (e.g., size, McCormick 2006; 2009; survival, Campbell et al. 1994), I hypothesized that exposing female salmon experimentally to a stressor would alter gametic traits and egg cortisol levels. Chapter 4 did not support this hypothesis as egg size and cortisol, and embryo survival were not affected by maternal stressor exposure. Still, in the absence of elevated egg cortisol, chapters 4 and 5 did reveal latent effects of maternal stressor exposure; emergent offspring swimming capacity and juvenile (~1 year post-fertilization) plasma cortisol stress response differed between offspring reared from stressor-exposed and control females.

6.1 Glucocorticoids and maternal stress: the good and the bad

As outlined in chapter 1, when an animal's fitness is threatened the stress response and release of GCs is adaptive. GCs stimulate physiological and behavioural changes to ensure an animal successfully escapes from or endures an acute stressor. However, under conditions of chronically elevated GCs *via* chronic stressor exposure, pathological effects can manifest.

Schreck (2010) proposed relating the dichotomous outcomes of low *versus* high levels of stress to the pharmacological/toxicological concept of hormesis. As defined by Mattson (2008), hormesis is a "biphasic dose response to an environmental agent characterized by a low dose stimulation or beneficial effect and a high dose inhibitory or toxic effect". Schreck (2000) discusses how acute exposure to certain stressors (e.g., a handling stressor that is minutes in duration) can facilitate quicker recovery following subsequent exposure to the same stressor *via* "stress hardening". Such priming degrades when individuals are chronically exposed to severe stressors whereby individuals cannot recover from allostatic overload (Schreck 2000). With a focus on GCs and reproduction, Milla et al. (2009) reviewed how depending on the timing of elevation, reproduction can be stimulated or impaired by GCs. For example, cortisol is critical for the final stages of egg development (e.g., hydration) in fishes and resting levels increase naturally throughout sexual maturation in salmonids (Milla et al. 2009). However, prior to ovulation during vitellogenesis, when GC levels are chronically elevated due to stressor exposure, HPG function is compromised and deleterious effects are observed (e.g., reduced sex steroid hormone levels, smaller eggs; Sections 1.13 and 1.3). Sheriff & Love (2013) and Love et al. (2013) have recently extended this interpretation across generations by highlighting studies when maternal stress/GCs endowed offspring with potentially beneficial traits (summarized in Section 1.2). Together, it is necessary that the full spectrum of influence of GCs is appreciated when studying effects of stressor exposure and GCs within and across generations.

The variable results from my thesis may be driven by underlying hormetic processes. Variable offspring effects following egg cortisol treatment observed in chapter 2 may be the result of intra- and inter-specific hormetic thresholds. An egg cortisol dose of 1000 ng/mL may be detrimental for sockeye salmon but not high enough to elicit change in chum salmon (Figure

2.2). The use of multiple doses as suggested in Study 1 (Section 6.1) can help elucidate whether variation in morphological and behavioural outcomes are driven by hormetic thresholds to egg cortisol treatment. Auperin & Geslin (2008) and Li et al. (201) detected dose-specific responses of egg cortisol treatment on rainbow trout offspring. Variable offspring effects following maternal stressor exposure observed in chapters 4 and 5 may suggest that the daily acute stressor treatment applied to females may not have been severe enough to harm offspring. The stressor exposure may have acted as an informative signal of maternal environment that resulted in offspring exhibiting traits suitable for entry into the pre-natal environment (e.g., increased burst swimming ability, Figure 4.1). In contrast, stressors used in studies that have found detrimental effects may be more severe (e.g., daily air exposure for 9 months, Campbell et al. [1992]) and/or applied during a period of maturation when gametes are more sensitive to maternal stress (e.g., Stratholt et al. 1997 but see Contreras-Sánchez et al. 1998). Offspring fitness was not explicitly measured in this thesis, and so whether the variable magnitude and direction of offspring responses are adaptive is not yet clear. Maternal match/mismatch and hormesis inherently intersect and when exploring maternal effects of stress and should be considered together.

6.2 Maternal buffering in fishes

My thesis contributes to a body of literature on the interplay between maternal stressor exposure, egg cortisol content and offspring performance in wild fishes (Table 6.1). However, with equivocal patterns related to the questions of whether maternal stressor-exposure elevates egg cortisol levels and whether offspring changes are dependent on elevations in egg cortisol (Table 6.1), the results of my thesis caution that there are limitations to using experimental elevations in egg cortisol as a proxy for chronic maternal stress, unless such a relationship is

established for the study species (e.g., McCormick 2006; 2009). Experimental elevations within naturally-occurring levels (e.g., 1-2 S.D. of population mean, fishes, Burton et al. 2011) are still useful to furthering our understanding of how natural variation in maternally-derived egg hormones shape offspring phenotypes. Specifically for salmonids, with so few studies assessing whether chronic maternal stress affects egg cortisol concentration (Stratholt et al. 1997; Contreras-Sánchez 1996; chapter 4) concrete hypotheses cannot be drawn regarding the existence of maternal buffering.

6.2.1 *Potential buffering mechanisms*

In fishes, Schreck (2001) proposed a maternal buffering program based on a number of lines of evidence. Maternal plasma cortisol levels are higher than that of eggs and ovarian fluid (Contreras-Sánchez 1996; Stratholt et al. 1997), suggesting at least some degree of buffering between circulating and gametic levels. The maternal buffering mechanisms could include corticosteroid-binding globulins (CBG) in plasma and the activity of type 2 11β-hydroxysteroid dehydrogenase (HSD11β2) (Schreck 2001). As discussed in chapter 5, the role CBGs have in the stress physiology of fishes is unclear, compared with other vertebrates (Mommsen et al. 1999; Desantis et al. 2013). Barry et al. (2001) did find that semelparous Chinook salmon had higher total cortisol levels and lower bound cortisol levels at spawning compared to iteroparous Chinook salmon. Based on the review by DeDantis et al. (2013), it does not appear that new work measuring bound and unbound cortisol in salmonids has occurred in over two decades. A priority for determining how CBGs contribute to maternal buffering in salmonids would be to determine if maternal stressor exposure changes the percentage of bound *versus* unbound circulating cortisol.

We now know that mRNA abundance of HSD11β2 in rainbow trout is widely distributed across tissues including the liver, head kidney, muscle, heart, testes and ovaries (Kusakabe et al. 2003). Evidence of conversion of cortisol to cortisone is detected in rainbow trout ovarian follicles and ovulated eggs but not ovarian fluid (Li et al. 2012), with transcripts of HSD11β2 increasing in abundance in follicles throughout late vitellogenesis to ovulation (Kusakabe et al. 2003). It remains unclear whether ovarian HSD11β2 increases under conditions of maternal stress in fishes. Activity of HSD11β2 in the zebrafish brain is increased in response to exposure to an acute stressor (as indicated by increased conversion of radiolabeled cortisol to cortisone and reduced cerebral mRNA abundance, Alderman & Vijayan 2012). In rodents, acute stress can increase placental HSD11β2 activity but chronic stress can decrease placental HSD11β2 activity (Welberg et al. 2005). Thus, the severity and duration of a stressor may influence the extent to which ovarian HSD11β2 can buffer gametes from exposure to maternal cortisol. However, as proposed in chapter 4, life history strategy may also play a role in the degree of enzymatic-mediated buffering. Throughout their adult migration to spawning grounds, senescencing, semelparous Pacific salmon plasma cortisol levels increase (Baker & Vynne 2014) which could be driven by degradation of HPI tissues (Maldonado et al. 2002). Chapter 4 hypothesized that regulation of constitutive HSD11β2 is also disrupted resulting in chronically elevating levels. As smolts, when circulating cortisol levels are also chronically increasing (Barton et al. 1985), gill mRNA abundance of HSD11β2 increases as well (Kiilerich et al. 2007). Given that elevations in cortisol levels are an evolved component of salmon migratory physiology, *versus* chronic activation of the HPI axis, one might assume there is also an evolved maternal buffering mechanism. Barry et al. (2010) did find that coho salmon kidney treated with 17α,20β-dihydroxy-4-prengnen-3-one (17α,20β-P), which regulates final gamete maturation (Nagahama

1994) and naturally increases during senescence (Scott et al. 1983), produced more cortisone suggesting cortisol metabolism increases as salmon mature. However, the authors propose that conversion of cortisol to cortisone in fact contributes to cortisol excess; cortisone inhibits the enzyme renal sulfotransferase (SULT) from metabolizing cortisol to cortisol-21-sulfate, ultimately leading to death (Barry et al. 2010). To determine how HSD11β2 contributes to maternal buffering under benign and noxious conditions, HSD11β2 expression in plasma and ovarian tissue of unripe, ripe, moribund, chronically stressed, acutely stressed and unstressed female salmonids is needed.

Active embryonic buffering (*sensu* Moore & Johnston 2008) once eggs are released from the female (with fixed hormonal content) and fertilized may also be occurring. In birds, studies injecting radiolabeled hormones into the yolk have found evidence of embryonic metabolism of maternally derived hormones (von Engelhardt et al. 2009; Paitz et al. 2011; Vassallo et al. 2014). Throughout incubation the levels of free yolk corticosterone in developing Japanese quail (*Coturnix japonica*, Vassallo et al. 2014) and European starling (Paitz et al. 2011) eggs reduced and the levels of conjugated forms of the GC increased (Vassallo et al. 2014). Incubating tissues in media with radiolabelled cortisol, Li et al. (2012) detected conversion of cortisol to cortisone in early rainbow trout embryos, in addition to pre-fertilized eggs, providing support for a dynamic hormone transfer model in fishes that includes both maternal, gametic, and offspring buffering. Fish eggs can also be microinjected (Nesan & Vijayan 2012). Microinjection of radiolabeled cortisol into fish eggs would facilitate comparison of embryonic GC metabolism between birds and fishes. It may be possible that cortisol is actively shuttled out of the egg (*via* a membrane pump) before and/or after fertilization. Measuring the amount of radiolabelled cortisol and cortisol metabolites in the water surrounding fish embryos is likely as important as

measuring the amount of those compounds in the embryo itself. Measurement of hormones and hormone metabolites in water is presently a method employed to non-invasively quantity the cortisol stress response (Scott et al. 2008). Continued examination of embryonic metabolism of maternally derived GCs is needed not only in fishes, but across oviparous taxa. A basic understanding of existent embryonic buffering mechanisms is needed before determining how maternal and/or embryonic exposure to a stressor alters such mechanisms.

6.2.2 *Time course of egg cortisol deposition*

A key, yet undefined, relationship in fishes is when a female increases hormone deposition following stressor exposure. Great tit (*Parus major*, Pitk et al. 2012) and barn swallow (*Hirundo rustica*, Saino et al. 2005) eggs laid the morning following a single 60 min or 2 h exposure to a model predator, respectively, had elevated egg GC levels. Tyler & Sumpter (1996) proposed that uptake of hormones (e.g., cortisol), vitamins, and metals in fishes may occur during vitellogenesis *via* attachment to vitellogenin/other yolk proteins or *via* hormone-specific receptors/transporters on the egg surface at stages in oogenesis that have yet to be determined. At least in the case of vitamins/carotenoids, this mode of uptake appears to be true (Lubzens et al. 2003). There are a number of factors to consider when examining hormone uptake during stressor exposure. For example, secretagogues of hepatic vitellogenin (e.g., gonadotropins, estradiol) can be dampened in concentration by stressor-induced GCs (see chapter 1) and thus timing of vitellogenesis may be delayed in stressed *versus* undisturbed females.

Even under undisturbed conditions, reproductive cycles vary considerably in length among fishes (e.g., days in zebrafish, months in salmonids, years in sturgeon) and thus when and for how long a stressor is applied may affect potential increases in egg cortisol levels. In zebrafish, a single stressor exposure on the order of hours (e.g., dyadic interaction to establish social rank) could be severe enough to chronically elevate maternal cortisol levels, influence egg cortisol deposition days later during vitellogenesis and sustain elevated egg concentrations through to ovulation (e.g., Figure 6.1a, solid line). In contrast, a single stressor exposure prior to or early in vitellogenesis on the order of hours may elevate circulating GCs only briefly in salmonids and marginally in their eggs. A repeated stressor exposure throughout sexual maturation and active vitellogenesis that continuously activates the HPI axis and chronically elevates circulating cortisol could be predicted to steadily increase salmonid egg cortisol (Figure 6.1b, solid line). However, no differences in egg cortisol (or ovarian fluid) were found among rainbow trout females stressed repeatedly during early (9-6 months before spawning), late (3 months before spawning) or throughout sexual maturation (9 months before spawning, Contreras-Sánchez et al. 1996; 1998). Similarly, sockeye salmon stressed during late sexual maturation (~6 weeks before spawning) did not have increased egg cortisol (chapter 4). On the other hand, coho salmon stressed during late sexual maturation but only two weeks before spawning did produce eggs with elevated cortisol (Stratholt et al. 1997). I can only speculate that 1) among salmonids, species-specific and life history differences and/or timing of stressor exposure during sexual maturation affect stressor-induced increases in egg cortisol (e.g., stressing females two *versus* six weeks before spawning, semelparous *versus* iteroparous).

Developing eggs may possess the capacity to metabolize steroids, converting cortisol to cortisone (Figure 6.1, dashed/dotted lines). Egg-mediated buffering may be occurring in iteroparous trout (Contreras-Sánchez et al. 1996; 1998) and sockeye salmon (chapter 4). It cannot be ruled out however, that rainbow trout and sockeye salmon females stressed early in maturation had elevated egg cortisol that was then metabolized by the time of egg sampling (and thus, undetected; Figure 6.1b, dashed line). Similarly, if egg hormone metabolism occurs immediately and continuously during chronic maternal stress (Figure 6.1b, dotted line), it is plausible that elevations in egg cortisol would be dampened throughout the treatment.

The use of species with shorter reproductive cycles is logistically less challenging for research on intergenerational effects, as stressor exposure can be applied at various stages leading up to ovulation and ovarian tissue continuously sampled (e.g., in birds see Okuliarová et al. 2010). In fishes, the only published work attempting to disentangle this issue has been done in a salmonid (Contreras-Sánchez et al. 1998). An additional layer of complexity to bear in mind is that offspring phenotype has been reported to vary depending on egg position within the ovary (Burton et al. 2013) and this may be related to variation in egg cortisol content (Suter 2002), perhaps mediated by proximity to blood vessels.

6.3 Maternal stress and non-glucocorticoid components of eggs

The candidate factor mediating maternal effects of stress examined in this thesis was cortisol given the well-established relationship between stressors, GCs and animal performance (i.e., tertiary effects of stress). However, there are a multitude of egg constituents I did not examine that could be affected by maternal stress and account for the differences in offspring performance I observed; sex and thyroid hormones, vitamins/carotenoids (Palace & Werner

2006), antioxidants (Taylor 2014), immune factors, mRNAs and genetic information (Lam 1994; Tyler & Sumpter 1996; Brooks et al. 1997; Bobe & Labbé 2010).

6.3.1 *Sex and thyroid hormones, vitamins and carotenoids, immune factors*

The function of sex steroids in developing eggs is not clear, though they are probably not related to sex-determination (Devlin & Nagahama 2002). Maternal stress reduces circulating levels of sex steroid hormones (see chapter 1), but it is not known if hormone reductions are mirrored in eggs. In birds, chronic maternal stress reduced (~60 day exposure, Okuliarová et al. 2010) and increased (~30 day exposure, Guibert et al. 2011) yolk testosterone content. Similarly, the role of thyroid hormones in fish eggs is not fully understood. Elevated egg thyroid hormone levels *via* maternal injection did (Ayson & Lam 1993) and did not (Mylonas et al. 1994) affect embryo survival or progeny size. Thyroid hormones are certainly linked to the HPI axis but connections between stressor exposure and thyroid hormone production are highly complex (Peter 2011). Vitamins and carotenoids in eggs largely reflect maternal diet and nutrition and effects on offspring survival and size are mixed (Palace & Werner 2006). If maternal stress impairs foraging or the stressor itself is food deprivation then egg vitamin and carotenoid content could be reduced. Vitamins and carotenoids are well known for their antioxidant properties in fishes (Palace & Werner 2006). Brown et al. (2014) and Taylor (2014) predicted that maternal antioxidant damage (e.g., acrolein, ratio of reduced to oxidized glutathione [GSH/GSSG]) and/or antioxidant capacity (using the oxygen radical absorbance capacity [ORAC] assay) would be reflected in fish eggs and embryos but did not find evidence to support their predictions. Carotenoids are also linked to immunocompetence. In birds, immunocompromised females deposited less carotenoid into eggs (Saino et al. 2002). A trade-off is also observed with regard

to yolk carotenoids and immunoglobins; eggs with increased carotenoids have lower levels of immunoglobins (Blount et al. 2002). Female-egg relationships are poorly understood in fishes and (as mentioned above) would be dependent on stressor type, timing, and duration. Building on relationships detected in avian species can help prioritize the next egg components to target as mediators of intergenerational effects of stress in fishes, especially in an applied context (e.g., aquaculture and maternal dietary/carotenoid status).

6.3.2 *mRNA and epigenetic programming*

The variety of tools available for measuring genetic expression facilitates examination of non-hormonal maternal effects of stress on eggs and offspring (Ho & Burggren 2010). With regard to maternal mRNAs in fish eggs, Jeffrey (2014) found gene-specific effects of maternal subordinate social status on zebrafish offspring mRNA abundance before hatch, when maternally-mediated changes to mRNA abundance would be especially impactful to progeny relying on maternal resources and not endogenously producing hormones and enzymes. Following hatch, when progeny are synthesizing hormones and enzymes *de novo*, there were gene- and age-specific effects of maternal social status on mRNA abundance (Jeffrey 2014). Maternal photoperiod and hormonal induction of ovulation also alter egg mRNA abundance (Bonnet et al. 2007). Epigenetic programming (e.g., DNA methylation) of offspring is well studied in rodents in relation to maternal care and progeny HPA activity (e.g., Weaver et al. 2004). With regard to maternal stress, Oberlander et al. (2008) found that pregnant human females experiencing a depressed/anxious mood produced offspring with higher post-stress cortisol levels and increased methylation of a gene coding for a GC receptor. Epigenetics in fishes is an emerging field which, if overlaid with maternal effects, would likely add

considerably to our understanding of the intergenerational effects of stress. For example, *via* maternal stress, modified expression of genes related to cardiovascular development or lactate metabolism could explain the modified offspring swimming performance observed in chapter 4. Similarly, modified expression of HPI genes could account for the differences in offspring plasma cortisol response observed in chapter 5. Such genetic programming would be equally relevant to male gametes given that paternal investment in progeny pre-fertilization is solely genetic.

6.4 A multi-trait and multi-life stage approach to maternal effects

This thesis examined multiple offspring traits beyond embryo survival (e.g., swimming performance, aggression, predator avoidance, stress response) at varying life history stages (e.g., embryo, fry, juvenile) revealing latent effects of maternal and egg hormone experimental manipulation. This approach provided a comprehensive assessment that produced some patterns that were relatively simple to interpret and others more complex. Following offspring throughout development and measuring a variety of traits ensured that effects responsive and unresponsive to maternal stressor exposure/elevated egg cortisol were identified (including trade-offs between traits). Importantly, conclusions were not skewed based on examination of a single trait at a single time point. Recent discussions on the adaptive potential of maternal stress, "predictive adaptive responses" and maternal match/mismatch (Gluckman et al. 2005; Breuner 2008; Love et al. 2013; Sheriff & Love 2013) further the argument for measuring multiple traits at multiple time points and under ecologically-relevant conditions. These two concepts are interconnected and to date are generally *post-hoc* interpretations given the predominant assumption that maternal stress results in poor quality offspring. Biomedical research may be driving

unidirectional predictions but maternal stressors used in rodent dominant studies are not always ecologically-relevant, nor are models of human disease (e.g., addiction). There is much for the field of ecology to obtain from biomedical research (Romero 2004), but future studies of maternal stress should focus on how maternal environment/state programs offspring, based on the environment and challenges offspring will encounter.

I propose a framework for incorporating both laboratory and field-based examination of maternal effects of stress in wild fishes (Figure 6.2). There are benefits and drawbacks associated with each approach. At the level of the adult female, stressor exposure (acute and chronic) can occur in the lab and field (A,D). However, in the field, repeated exposure over days may be a logistical challenge without animal tagging, especially for migratory species (e.g., adult Pacific salmon). Gametes can be obtained from adults held in captivity to maturation and from adults caught in the wild during peak spawning. Fertilized embryos can be reared in the environment gametes were stripped (black solid arrows) and/or in the opposite environment (black dashed arrows). Offspring rearing in the laboratory ensures higher survival but is an artificial environment where nuances of photoperiod (i.e., sunrise and sunset), water temperature (i.e., diurnal fluctuations) and substrate can deviate from nature (Section 6.1, B *versus* E). For offspring, testing can also be conducted in the environment embryos were reared (grey solid arrows) and/or in the opposite environment (grey dashed arrows). Laboratories provide controlled settings and a wider array of physiological and behavioural assays are feasible (C). The ecological relevance of responses measured in the laboratory is strengthened if responses are captured under conditions with increased ecological relevance (e.g., swimming performance under threat of predation) and linked to offspring fitness (i.e., survival in presence of live predator). Ultimately, responses could be artefacts of laboratory conditions and, when possible,

monitoring offspring in the wild *via* mesocosms or telemetry (F) will more accurately reflect naturally-occurring processes. Not all laboratory equipment is appropriate for the field but the range of responses measurable in the field continues to expand as technology (e.g., telemetry, biologging) and statistical approaches are developed. For example, movement/activity levels, acceleration, personality (e.g., Taylor & Cooke 2014), heart rate, oxygen consumption and foraging (in combination with stable isotope analyses, Cunjak et al. 2005) can all be determined for individuals from remotely amassed data (Cooke et al. 2004), and/or biogenetics models based on calibration of tagging technology (e.g., Wilson et al. 2013). Embryo incubation in the laboratory and field (B and E) can establish how interactions between maternal stress and early rearing environment shape offspring phenotypes (e.g., Giorando et al. 2014). Observing offspring in the laboratory and field (C and F) can establish whether laboratory behaviour correlates with behaviour in the wild (e.g., Höjesjö et al. 2002).

6.5 Specific future directions of this dissertation

In addition to study-specific future directions mentioned in each chapter, there remain limitations of this dissertation that can be addressed in future research. For all chapters, offspring rearing occurred under controlled, laboratory conditions ensuring high survival of embryos and adequate sample sizes for experimental testing. However, such conditions (e.g., uniform water temperature, fiberglass tanks devoid of substrate, abrupt changes in photoperiod, *ad libitum* access to food) hardly reflected conditions experienced by young salmon in the wild. The implementation of outdoor rearing (see Section 6.4), variable feeding regimes and enriched tanks could minimize doubt that differences are simply artefacts of the laboratory environment, and that offspring reared in the laboratory are behaving similar to offspring reared in the wild.

Another limitation present in all chapters is the inability to examine interactions between paternal and maternal effects and treatment level effects. A non-reciprocal cross design was chosen as the focus of this thesis was how egg and maternal level treatments affected offspring performance. Also, rearing all crosses in a full-reciprocal design to stages beyond fry were not logistically feasible in the facilities available. Space limitations in university wet lab facilities also resulted in offspring from varying experimental treatments being housed in single tanks raising concerns of tank effects. I do acknowledge that maternal and paternal effects are important mediators of offspring performance in Pacific salmon (e.g., Burt et al. 2012a,b) and future studies should aim to incorporate inherent maternal and paternal effects. Hatcheries may have sufficient space to house more Heath stacks and rearing tanks in comparison to the university facilities used in this thesis.

To presently address the hypothesis that maternal exposure to a stressor alters offspring quality *via* changes to egg cortisol levels I would conduct the following studies:

Study 1) Establish the natural timing of egg cortisol deposition in a single species and population of Pacific salmon. In the laboratory, females collected from coastal marine areas can be housed until sexual maturation. Eggs can be collected at regular intervals from a subset of females and egg cortisol levels quantified. In the field, eggs can be collected and assayed for cortisol from females caught in the coastal marine area, estuaries and at various locations along a known migration route for a particular population. Egg development at river entry varies across populations(Crossin et al. 2004) and GC deposition may too vary across populations in relation to migration distance required to reach spawning grounds. Though logistically challenging, collection of

animals from a single population would be best. Aim to assess egg cortisol deposition for at least 25 females.

Study 2) Establish the naturally occurring, inter-female variation in egg cortisol concentrations (at spawning) in a single species and population of Pacific salmon across 4-5 consecutive years. Aim to assess egg cortisol for at least 25 females.

Study 3) During the last 4-6 weeks of sexual maturation, chronically expose adult, female salmon in holding to a stressor (ideally ecologically relevant; e.g., fisheries capture and release). Once ripe, sample eggs from stressed and control females and determine egg cortisol concentrations. Aim to assess egg cortisol for at least 25 females. To assess both treatment and maternal level effects, fertilize eggs from stressed and control females with sperm of control males creating full reciprocal crosses. Rear offspring to yolk sac absorption/emergence at which time a fitness trait is assessed (e.g., survival in presence of predator) for at least 25 offspring from each cross. Determine if egg cortisol levels are within those detected in Study 1. Replicate this study for (at least) a second consecutive year to validate findings.

Study 4) If Study 3 found that maternal stressor exposure increased egg cortisol, further experiments investing intergenerational effects of stress could use egg hormone baths to experimentally elevate egg cortisol levels. Determine the appropriate doses to utilize in the egg hormone baths that achieves levels of egg cortisol detected in chronically, stressed females and that reflect the upper range of egg cortisol concentrations detected in Study 2. The use of two doses can explore potential thresholds at which elevations in egg cortisol generate effects. To facilitate comparison between methodologies (egg

hormone bath *versus* maternal stressor exposure), replicate full reciprocal crossing design and offspring rearing and testing as in Study 3.

6.6 Animal stress in the wild remains a complex story

Do resonating effects of maternal stress on progeny have the capacity to influence population level changes? Meylan et al. (2012) argue yes, and for the existence of a hormonally-mediated mechanism. However, still in debate is whether elevated GCs are synonymous with a chronically "stressed" animal (Dickens & Romero 2013 but see Dantzer et al. 2014) Evidence for distinct and heritable stress coping styles (Pottinger & Carrick 1999) is an additional complexity to studying maternal stress. There is much to be discerned and appreciated regarding maternal stressor exposure and offspring performance in fishes, including the dynamics between maternal and egg cortisol transfer, and how best to track changes in offspring traits. Given that aquatic environments are undergoing relatively rapid flux as a result of anthropogenic stressors, characterizing variation in maternal responsivity to relevant stressors is a priority (e.g., cortisol response, epigenetic markers). The range and degree of responsivity can indicate the proportions of high *versus* low responding individuals within a population, and could also indicate the potential magnitude of intergenerational effects.

My thesis explored what I thought to be a transparent story in nature – chronic, maternal stressor exposure reduces offspring quality. However, the ending of my story is complex and may find its place in the early chapters of future research theses on maternal effects of stress in fishes. I conclude from my thesis that the hormonal profile of an egg and exposure of females to a stressor can mold an offspring's phenotype, but relationships and mechanisms among maternal stressor exposure, egg hormones and offspring potential are not always as anticipated.

mother's potent mark
hidden by my thought and tool
revealed, now evolve

Table 6.1 Summary of gamete/offspring outcomes following maternal stressor-exposure in fishes. - indicates trait was not measured, ND indicates no differences detected in a trait between stressed and control treatments, ⬆ indicates an increase in the trait, ⬇ indicates a decrease in the trait

Species	Stressor	Duration	Outcome			Reference
			Plasma/egg cortisol	Egg or offspring size	Offspring performance	
Rainbow trout (*Oncorhynchus mykiss*)	3 min air exposure *via* tank draining	9 months, 1-10 times per week	⬆[1]/-	⬇	⬇ survival	Campbell et al. 1992
	chronic tank confinement	2 weeks	⬆/-	⬇	⬇ survival	Campbell et al. 1994
	randomized among 15 min crowding *via* reducing water levels, 5 min air exposure *via* tank draining, 5 min noise disturbance *via* pipe banging on tank, 5 min net chase	daily, 5 days/week, 45 days (early maturation)	ND/ND	⬇	ND in survival, growth, disease resistance	Contreras-Sánchez 1996; et al. 1998
		daily, 5 days/week, 45 days (late maturation)	ND/ND	ND	ND in survival, growth, disease resistance	Contreras-Sánchez 1996; et al. 1998
		daily, 5 days/week, 90 days (early and late maturation)	ND/ND	ND	ND in survival, growth, disease resistance	Contreras-Sánchez 1996; et al. 1998
Brown trout (*Salmo trutta*)	chronic tank confinement	2 weeks	⬆/-	⬇	⬇ survival	Campbell et al. 1994
Coho salmon (*Oncorhynchus kisutch*)	60 s net chase	twice daily, 2 weeks	⬆/⬆	-	-	Stratholt et al. 1997

Species	Stressor	Duration	Outcome			Reference
			Plasma/egg cortisol	Egg or offspring size	Offspring performance	
Damselfish (*Pomacentrus amboinensis*)	increased female density resulting in increased frequency of aggressive interactions	3 months	-/⬆	⬇	-	McCormick 2006
	increased heterospecific density resulting in increased frequency of aggressive interactions		-/⬆	⬇	-	McCormick 2009
African cichlid (*Neolamprologus pulcher*)	net chase then confinement in submerged net	net chase for 2-5 min, twice daily (net chase) for 50 days, followed by net confinement once daily for 30 days	-/ND	⬇	-	Mileva et al. 2010
Zebrafish (*Danio rerio*)	dyadic interaction	48 hours	-/ND	ND	ND in survival, altered mRNA abundance and stress response	Jeffrey 2014
Sockeye salmon (*Oncorhynchus nerka*)	3 min net chase	twice daily, 37 or 42 days	-/ND	ND	ND in survival, altered swimming performance and stress response	chapters 4, 5

[1]sampled after 5 months of stressor application

Figure 6.1 Proposed time course of egg hormone deposition in **(A)** a species with a relatively short reproductive cycle (days, e.g., zebrafish) following exposure to an acute stressor, and **(B)** a species with a relatively long reproductive cycle (months, e.g., salmon) during chronic exposure to a stressor. Following exposure to an acute stressor **(A)** egg cortisol levels increase during vitellogenesis and peak (solid line) upon ovulation when further uptake of vitellogenin (and hormones bound to the protein) is not predicted as eggs are less permeable (Tyler & Sumpter 1996). Alternatively, cortisol levels may initially rise during vitellogenesis but began to fall (dashed line) as developing follicles metabolize cortisol (*via* of type 2 11β-hydroxysteroid dehydrogenase [HSD11β2]) converting the hormone to its inactive form cortisone. Following chronic exposure to a stressor throughout vitellogenesis **(B)** egg cortisol levels increase and peak (solid line) or increase and fall (dashed line) as described for **(A)**. Cortisol metabolism may be occurring more regularly (dotted line) in species with longer periods of vitellogenesis. See Section 6.2.2 for further details.

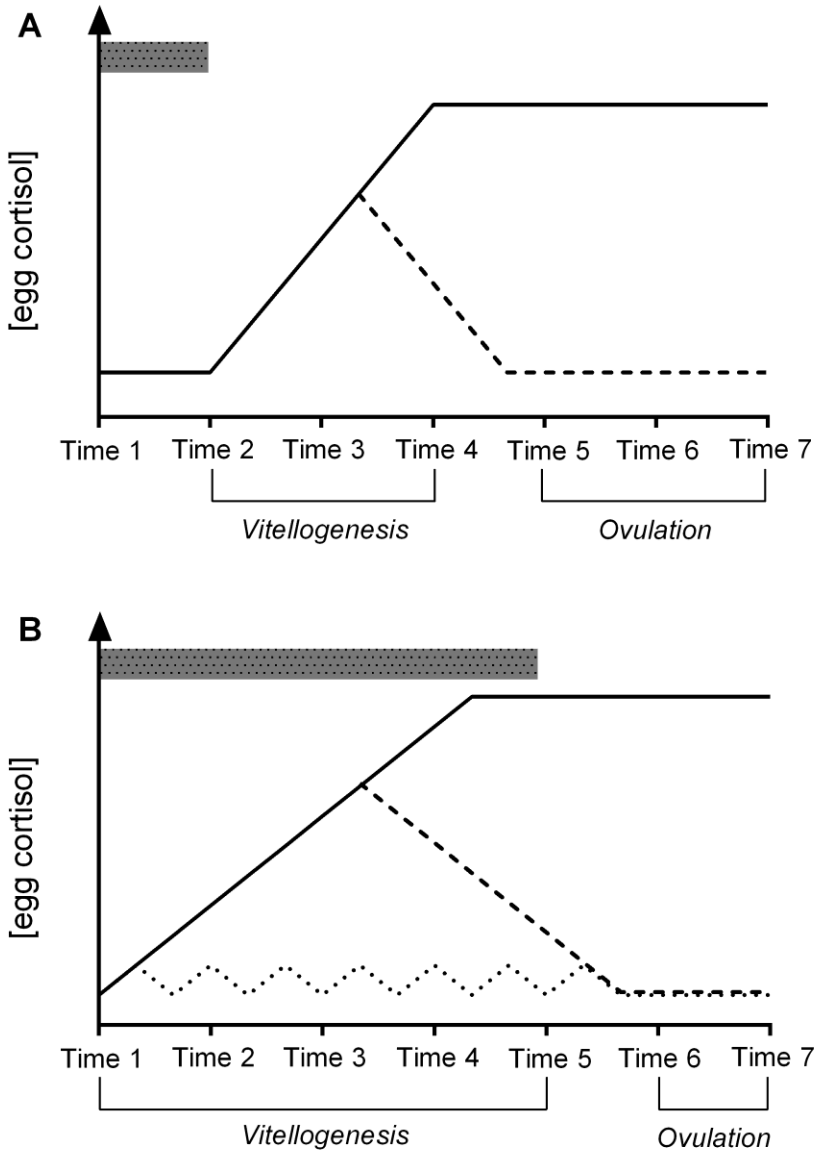
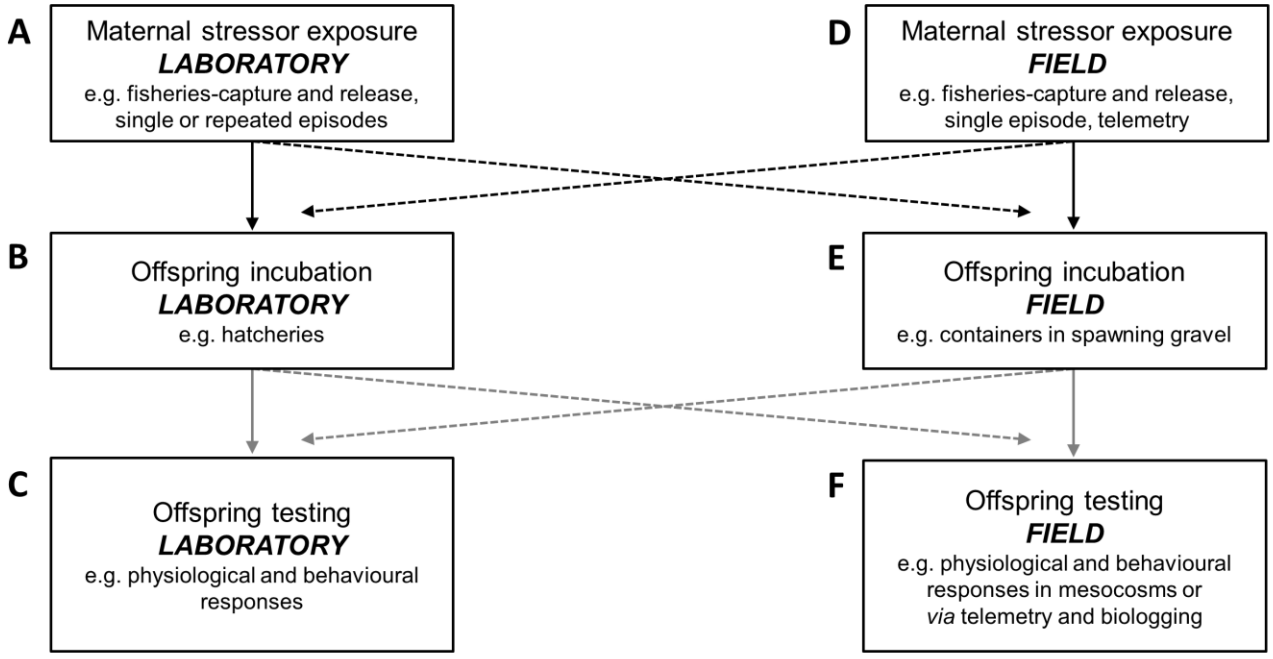


Figure 6.2 Framework for examining maternal effects of stress in fishes. Adult stressor exposure **(A,D)**, offspring rearing **(B,E)** and offspring testing **(C,F)** can occur in the laboratory and/or field. Combinations of laboratory and field rearing /testing (solid and dashed arrows) can be used to investigate interactive effects of maternal stressor exposure and rearing environment on offspring responses, and consistency of responses between laboratory and field settings. See Section 6.4 for further details.



References

Abbott, J.C. & Dill, L.M. 1985. Patterns of aggressive attack in juvenile steelhead trout (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences 42: 1702-1706.

Alderman, S.L. & Vijayan M.M. 2012. 11β-hydroxysteroid dehydrogenase type 2 in zebrafish brain: a functional role in hypothalamus-pituitary-interrenal axis regulation. Journal of Endocrinology 215: 393-402.

Alderman, S.L., McGuire, A., Bernier, N.J. & Vijayan, M.M. 2012. Central and peripheral glucocorticoid receptors are involved in the plasma cortisol response to an acute stressor in rainbow trout. General and Comparative Endocrinology 176: 79-85.

Aluru, N. & Vijayan, M.M. 2006. Aryl hydrocarbon receptor activation impairs cortisol response to stress in rainbow trout by disrupting the rate-limiting steps in steroidogenesis. Endocrinology 147: 1895-1903.

Aluru, N. & Vijayan, M.M. 2008. Molecular characterization, tissue specific expression and regulation of melanocortin 2 receptor in rainbow trout. Endocrinology 149: 4577-4588.

Angelier, F. & Chastel, O. 2009. Stress, prolactin and parental investment in birds: a review. General and Comparative Endocrinology 163: 142-148.

Aubin-Horth, N., Deschênes, M. & Cloutier, S. 2012. Natural variation in the molecular stress network correlates with a behavioural syndrome. Hormones and Behavior 61: 140-146.

Auperin. B. & Geslin, M. 2008. Plasma cortisol response to stress in juvenile rainbow trout is influenced by their life history during early development and by egg cortisol content. General and Comparative Endocrinology 158: 234-239.

Ayson, F.G. & Lam, T.J. 1993. Thyroxine injection of female rabbitfish (*Siganus guttatus*) broodstock: changes in thyroid hormone levels in plasma, eggs, and yolk-sac larvae, and its effects on larval growth and survival. Aquaculture 109: 83-93.

Bachis, A., Cruz, M.I., Nosheny, R.L. & Mocchetti, I. 2008. Chronic unpredictable stress promotes neuronal apoptosis in the cerebral cortex. Neuroscience Letters 442: 104-108.

Backström, T., Schjolden, J., Øverli, O., Thömqvist, P-O. & Winberg, S. 2011. Stress effects on AVT and CRF systems in two strains of rainbow trout (*Oncorhynchus mykiss*) divergent in stress responsiveness. Hormones and Behavior 59: 180-186.

Badyaev, A.V. & Uller, T. 2009. Parental effects in ecology and evolution: mechanisms, processes and implications. Philosophical Transactions of the Royal Society B: Biological Sciences 364: 1169-1177.

Baker, M.R. & Vynne, C.H. 2014. Cortisol profiles in sockeye salmon: sample bias and baseline values at migration, maturation, spawning, and senescence. Fisheries Research 154: 38-43.

Balleine, B.W. & Killcross, S. 2006. Parallel incentive processing: an integrated view of amygdala function. Trends in Neurosciences 29: 272-279.

Barcellos, L.J.G., Kreutz, L.C., de Souza, C., Rodrigues, L.B., Fioreze, I., Quevedo, R.M., Cericato, L., Soso, A.B., Fagundes, M., Conrad, J., de Almeida Lacerda, L. & Terra S. 2004. Hematological changes in jundiá (*Rhamdia quelen* Quoy and Gaimard *Pimelodidae*) after acute and chronic stress caused by usual aquacultural management, with emphasis on immunosuppressive effects. Aquaculture 237: 229-236.

Barnes, M.E., Hanten, R.P., Sayler, W.A. & Cordes, R.J. 2000. Viability of inland fall Chinook salmon spawn containing overripe eggs and the reliability of egg viability estimates. North American Journal of Aquaculture 62: 237-239.

Barry, T.P., Unwin, M.J., Malison, J.A. & Quinn, T.P. 2001. Free and total cortisol levels in semelparous and iteroparous chinook salmon. Journal of Fish Biology 59: 1673-1676.

Barry, T.P., Ashok, M. & Nunez, S. 2010. Inhibition of cortisol metabolism by 17α,20β-P: mechanism mediating semelparity in salmon? General and Comparative Endocrinology 175: 53-59.

Barton, B.A. 2000. Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. North American Journal of Aquaculture 62: 12-18.

Barton, B.A. 2002. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42: 517-525.

Barton, B.A., Schreck, C.B., Ewing, R.D., Hemmingsen, A.R. & Patiño, R. 1985. Changes in plasma cortisol during stress and smoltification in Coho Salmon, *Oncorhynchus kisutch*. General and Comparative Endocrinology 59: 468-471.

Bell, A. M., Backström, T., Huntingford, F. A., Pottinger, T. G. & Winberg, S. 2007. Variable neuroendocrine responses to ecologically-relevant challenges in sticklebacks. Physiology & Behavior 91:15-25.

Bell, A.M. 2007. Future directions in behavioural syndromes research. Proceedings of the Royal Society B: Biological Sciences 274: 755-761.

Bell, A.M. & Sih, A. 2007. Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). Ecology Letters 10: 828-834.

Bell, A.M. & Stamps, J.A. 2004. Development of behavioural differences between individuals and populations of sticklebacks, *Gasterosteus aculeatus*. Animal Behaviour 68: 1339-1348.

Bell, A.M. 2013. Randomized or fixed order for studies of behavioral syndromes? Behavioral Ecology 24: 16-20.

Belliure, J. & Clobert, J. 2004. Behavioral sensitivity to corticosterone in juveniles of the wall lizard, *Podarcis muralis*. Physiology & Behavior 81: 121-127.

Benediktsson, R., Calder, A.A., Edwards, C.R.W. & Seckl, J.R. 1997. Placental 11β-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. Clinical Endocrinology 46: 161-166.

Bergmeyer, H.U. 1983. Methods of Enzymatic Analysis. Academic Press, New York.

Bernier, N.H., Alderman, S.L. & Bristow, E.N. 2008. Heads or tails? Stressor-specific expression of corticotropin-releasing factor and urotensin I in the preoptic area and caudal neurosecretory systems of rainbow trout. Journal of Endocrinology 196: 637-648.

Bestion, E., Teyssier, A., Aubret, F., Clobert, J. & Cote, J. 2014. Maternal exposure to predator scents: offspring phenotypic adjustment and dispersal. Proceedings of the Royal Society B: Biological Sciences 281: 20140701.

Birtwell, I.K., Nassichuk, M.D. & Beune, H. 1987. Underyearling sockeye salmon (*Oncorhynchus nerka*) in the estuary of the Fraser River. Canadian Special Publication of Fisheries and Aquatic Sciences 9: a25–a35.

Blas, J., Bortolotti, G.R., Tella, J.L., Baos, R. & Marchant, T.A. 2007. Stress response during development predicts fitness in a wild, long lived vertebrate. Proceedings of the National Academy of Sciences 104: 8880-8884.

Blount, J.D., Surai, P.F., Nager, R.G., Houston, D.C., Møller, A.P., Trewby, M.L. & Kennedy, M.W. 2002. Carotenoid and egg quality in the lesser black-backed gull *Larus fuscus*: a supplemental feeding study of maternal effects. Proceedings of the Royal Society B: Biological Sciences 269: 29-36.

Bobe, J. & Labbé, C. 2010. Egg and sperm quality in fish. General and Comparative Endocrinology. 165: 535-548.

Bonnet, E., Fostier, A. & Bobe, J. 2007. Microarray-based analysis of fish egg quality after natural or controlled ovulation. BMC Genomics 8: 55.

Braastad, B.O. 1998. Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals. Applied Animal Behaviour Science 61: 159-180.

Bradford, M.J. 1995. Comparative review of Pacific salmon survival rates. Canadian Journal of Fisheries and Aquatic Sciences 52: 1327-1338.

Braun, D.C., Patterson, D.A. & Reynolds, J.D. 2013. Maternal and environmental influences on egg size and juvenile life-history traits in Pacific salmon. Ecology and Evolution 3: 1727-1740.

Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. Journal of the Fisheries Research Board of Canada 21: 1183-1226.

Breuner, C. 2008. Maternal stress, glucocorticoids, and the maternal/fetal match hypothesis. Hormones and Behavior 54: 485-487.

Breuner, C.W., Patterson, S.H. & Hahn, T.P. 2008. In search of relationships between the acute adrenocortical response and fitness. General and Comparative Endocrinology 157: 288-295.

Brooks, K.C. & Mateo, J.M. 2013. Chronically raised glucocorticoids reduce innate immune function in Belding's ground squirrels (*Urocitellus beldingi*) after an immune challenge. General and Comparative Endocrinology 193: 149-157.

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

Brown, A.C., Leonard, H.M., McGraw, K.J. & Clotfelter, E.D. 2014. Maternal effects of carotenoid supplementation in an ornamented cichlid fish. Functional Ecology 28: 612-620.

Burgner, R.L. 1991. Life history of sockeye salmon (*Oncorhynchus nerka*). In: Groot, C. & Margolis, L. (Eds) Pacific Salmon Life Histories. UBC Press, Vancouver, pp. 1-117.

Burmeister, S., Somes, C. & Wilczynski, W. 2001. Behavioral and hormonal effects of exogenous vasotocin and corticosterone in the green treefrog. General and Comparative Endocrinology 122: 189-197.

Burnett, N.J., Hinch, S.G., Braun, D.C., Casselman, M.T., Middleton, C.T., Wilson, S.M. & Cooke, S.J. 2014. Burst swimming in areas of high flow: delayed consequences of anaerobiosis in wild adult sockeye salmon. Physiological and Biochemical Zoology 87:587-598.

Burt, J.M., Hinch, S.G. & Patterson, D.A. 2012a. Parental identity influences progeny responses to incubation thermal stress in sockeye salmon *Oncorhynchus nerka*. Journal of Fish Biology 80: 444-462.

Burt, J.M., Hinch, S.G. & Patterson, D.A. 2012b. Developmental temperature stress and parental identity shape offspring burst swimming performance in sockeye salmon (*Oncorhynchus nerka*). Ecology of Freshwater Fish 21: 176-188.

Burton, T., Hoogenboom, M.O., Armstrong, J.D., Groothuis, T.G.G. & Metcalfe, N.B. 2011. Egg hormones in a highly fecund vertebrate: do they influence offspring social structure in competitive conditions? Functional Ecology 25: 1379-1388.

Burton, T., Hoogenboom, M.O., Beevers, N.D., Armstrong, J.D. & Metcalfe, N.B. 2013. Among-sibling differences in the phenotypes of juvenile fish depend on their location within the egg mass and maternal dominance rank. Proceedings of the Royal Society B: Biological Sciences 280: 20122441.

Bury, N.R. & Sturm, A. 2007. Evolution of the corticosteroid receptor signaling pathway in fish. General and Comparative Endocrinology 153: 47-56.

Busch, D.S. & Hayward, L.S. 2009. Stress in a conservation context: a discussion of glucocorticoids and how levels change with conservation-relevant variables. Biological Conservation 142: 2844-2853.

Campbell, P.M., Pottinger, T.G. & Sumpter, J.P. 1992. Stress reduces the quality of gametes produced by rainbow trout. Biology of Reproduction 47: 1140-1150.

Campbell, P.M., Pottinger, T.G. & 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.

Cannon, W.B. 1929. Organization for physiological homeostasis. Physiological Reviews 9: 399-431.

Careau, V., V & Garland, T. 2012. Personality, and energetics: correlation, causation, and mechanism. Physiological and Biochemical Zoology 85: 543-571.

Carragher, J.F., Sumpter, J.P., Pottinger, T.G. & Pickering, A.D. 1989. The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta* L. and *Salmo gairdneri* Richardson. General and Comparative Endocrinology 76: 310-321.

Chin, E.H., Love, O.P., Verspoor, J.J., Williams, T.D., Rowley, K. & Burness, G. 2009. Juveniles exposed to embryonic corticosterone have enhanced flight performance. Proceedings of the Royal Society B: Biological Sciences 276: 499–505.

Clearwater, S.J. & Pankhurst, N.W. 1997. The response to capture and confinement stress of plasma cortisol, plasma sex steroids and vitellogenic oocytes in the marine teleost, red gurnard. Journal of Fish Biology 50: 429-441.

Clements, S., Stahl, T. & Schreck, C.B. 2012. A comparison of the behavior and survival of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*O. mykiss*) in a small estuary system. Aquaculture 362-363: 148-157.

Cockrem, J.F. 2007. Stress, corticosterone responses and avian personalities. Journal of Ornithology 148: S169-S178.

Cockrem, J.F. & Silverin, B. 2002. Sight of a predator can stimulate a corticosterone response in the great tit (*Parus major*). General and Comparative Endocrinology 125: 248-255.

Cohen, B.I. 2012. Commission of Inquiry into the Decline of Sockeye Salmon in the Fraser River. Public Works and Government Services Canada, Ottawa. Cat. No.: CP32-93/2012E-1 (v.1).

Contreras-Sánchez, W.M. 1996. Effects of Stress on the Reproductive Performance and Physiology of Rainbow Trout (*Oncorhynchus mykiss*). PhD dissertation, Oregon State University, Corvallis, Oregon, USA.

Contreras-Sánchez, W.M., Schreck, C.B., Fitzpatrick, M.S. & Pereira, C.B. 1998. Effects of stress on the reproductive performance of rainbow trout (*Oncorhynchus mykiss*). Biology of Reproduction 58: 439-447.

Cook, K.V., McConnachie, S.H., Gilmour, K.M., Hinch, S.G. & Cooke, S.J. 2011. Fitness and behavioral correlates of pre-stress and stress-induced plasma cortisol titers in pink salmon (*Oncorhynchus gorbuscha*) upon arrival at spawning grounds. Hormones and Behavior 60: 489-497.

Cook, K.V., Crossin, G.T., Patterson, D.A., Hinch, S.G., Gilmour, K.M. & Cooke, S.J. 2014. The stress response predicts migration failure but not migration rate in a semelparous fish. General and Comparative Endocrinology 202: 44-49.

Cooke, S.J. & O'Connor, C.M. 2010. Making conservation physiology relevant to policy makers and conservation practitioners. Conservation Letters 3: 159-166.

Cooke, S.J., Hinch, S.G., Wikelski, M., Andrews, R.D., Kuchel, L.J., Wolcott, T.J. & Butler, P.J. 2004. Biotelemetry: a mechanistic approach to ecology. Trends in Ecology & Evolution 19: 334-343.

Coslovsky, M. & Richner, H. 2011. Predation risk affects offspring growth via maternal effects. Functional Ecology 25: 878-888.

Costantini, D., Marasco, V. & Møller, A.P. 2011. A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. Journal of Comparative Physiology B: Biochemical, Systems, and Environmental Physiology 181: 447-456.

Craik, J.C.A. & 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.

Crain, C.M., Kroeker, K. & Halpern, B.S. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecology Letters 11: 1304-1315.

Creel, S. Dominance, aggression, and glucocorticoids levels in social carnivores. 2005. Journal of Mammalogy 86: 255-264.

Cunjack, R.A, Roussel, J-M., Gray, M.A., Dietrich, J.P., Cartwright, D.F., Munkittrick, K.R. & Jardine, T.D. 2005. Using stable isotope analysis with telemetry or mark-recapture data to identify fish movement and foraging. Oecologia 144: 636-646.

Cyr, N.E. & Romero, L.M. 2009. Identifying hormonal habituation in field studies of stress. General and Comparative Endocrinology 161: 295-303.

Dahl, E., Orizaola, G., Winberg, S. & Laurila, A. 2012. Geographic variation in corticosterone response to chronic predator stress in tadpoles. Journal of Evolutionary Biology 254: 1066-1076.

Dantzer, B., Newman, A.E.M., Boonstra, R., Palme, R., Boutin, S., Humphries, M.M. & McAdam, A.G. 2013. Density triggers maternal hormones that increase adaptive offspring growth in a wild mammal. Science 340: 1215-1217.

Dantzer, B., Fletcher, Q.E., Boonstra, R. & Sheriff, M.J. 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? Conservation Physiology 2: cou023.

Decker, A.S. & Irvine, J.R. 2013. Pre-COSEWIC Assessment of Interior Fraser Coho Salmon (*Oncorhynchus kisutch*). Fisheries and Oceans Canada Canadian Science Advisory Secretariat Research Document 2013/121.

Demers, N.E. & Bayne, C.J. 1997. The immediate effects of stress on hormones and plasma lysozyme in rainbow trout. Developmental & Comparative Immunology 21: 363-373.

Desantis, L.M., Delehanty, B., Weir, J.T. & Boonstra, R. 2013. Mediating free glucocorticoid levels in the blood of vertebrates: are corticosteroid-binding proteins always necessary? Functional Ecology 27: 107-119.

Devlin, R.H. & Nagahama, Y. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture 208:191-364.

Díaz-Uriarte, R. 1999. Anti-predator behaviour changes following an aggressive encounter in the lizard *Tropidurus hispidus*. Proceedings of the Royal Society B: Biological Sciences 266: 2457-2464.

DiBattista, J.D., Levesque, H.M., Moon, T.W. & Gilmour, K.M. 2006. Growth depression in socially subordinate rainbow trout *Oncorhynchus mykiss*: more than a fasting effect. Physiological and Biochemical Zoology 79: 675-687.

Dickens, M.J. & Romero, L.M. 2013. A consensus endocrine profile for chronically stressed wild animals does not exist. General and Comparative Endocrinology 191: 177-189.

Dill, L.M., Ydenberg, R.C. & Fraser, A.H.G. 1981. Food abundance and territory size in juvenile coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Zoology 59: 1801-1809.

DFO (Fisheries and Oceans Canada). 2005. Canada's policy for conservation of wild Pacific salmon.

DFO (Fisheries and Oceans Canada). 2008. Canadian Fisheries Statistics 2006, DFO/2008-1518.

Donaldson, M.R., Clark, T.D., Hinch, S.G., Cooke, S.J., Patterson, D.A., Gale, M.K., Frappell, P.B. & Farrell, A.P. 2010. Physiological responses of free-swimming adult coho salmon to simulated predator and fisheries encounters. Physiological and Biochemical Zoology 83, 973-983.

Donaldson, M.R., Hinch, S.G., Raby, G.D., Patterson, D.A., Farrell, A.P. & Cooke, S.J. 2012. Population-specific consequences of fisheries-related stressors on adult sockeye salmon. Physiological and Biochemical Zoology 85: 729-739.

Donaldson, M.R., Hinch, S.G., Jeffries, K.M., Patterson, D.A., Cooke, S.J., Farrell, A.P. & Miller, K.M. 2014. Species-and sex-specific responses and recovery of wild, mature Pacific salmon to an exhaustive exercise and air exposure stressor. Comparative Biochemistry and Physiology A: Molecular & Integrative Physiology 173: 7-16.

Doyon, C., Gilmour, K.M., Trudeau, V.L. & Moon, T.W. 2003. Corticotropin-releasing factor and neuropeptide Y mRNA levels are elevated in the preoptic area of socially subordinate rainbow trout. General and Comparative Endocrinology 133: 260-271.

Doyon, C., Trudeau, V.L. & Moon, T.W. 2005. Stress elevates corticotropin-releasing factor (CRF) and CRF-binding protein mRNA levels in rainbow trout (*Oncorhynchus mykiss*). Journal of Endocrinology 186: 123-130.

Drenner, S.M., Hinch, S.G., Martins, E.G., Robichaud, D., Clark, T.D., Thompson, L.A., Patterson, D.A., Cooke, S.J. & Thomson, R.E. 2014. Variable thermal experience and diel thermal patterns of homing sockeye salmon in coastal marine waters. Marine Ecology Progress Series 496: 109-124.

Drevnick, P.E., Sandheinrich, M.B. & Oris, J.T. 2006. Increased ovarian follicular apoptosis in fathead minnows (*Pimephales promelas*) exposed to dietary methylmercury. Aquatic Toxicology 79: 49-54.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z-I., Knowler, D.J., Leveque, C., Naiman, R.J., Prieur-Richard, A-H., Stiassny, M.L.J. & Sullivan. C.A. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81: 163-182.

Dufty, A.M., Clobert, J. & Møller, A.P. 2002. Hormones, developmental plasticity and adaptation. Trends in Ecology & Evolution 17: 190-196.

Dunlap, K.D. & Wingfield, J.C. 1995. External and internal influences on indices of physiological stress. I. Seasonal and population variation in adrenocortical secretion of free-living lizards, *Sceloporus occidentalis.* Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 271: 36-46.

Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K.M., Gale, M.K., Patterson, D.A., Hinch, S.G. & Farrell, A.P. 2011. Differences in thermal tolerance among sockeye salmon populations. Science 332: 109-112.

Elwood, R.W., Stoilova, V., McDonnell, A., Earley, R.L. & Arnott, G. 2014. Do mirrors reflect reality in agonistic encounters? A test of mutual cooperation in displays. Animal Behaviour 97: 63-67.

Eriksen, M.S., Bakken, M., Espmark, Å., Braastad, B.O. & Salte, R. 2006. Prespawning stress in farmed Atlantic salmon *Salmo salar*: maternal cortisol exposure and hyperthermia during embryonic development affect offspring survival, growth and incidence of malformations. Journal of Fish Biology 69: 114-129.

Eriksen, M.S., Færevik, G., Kittilsen, S., McCormick, M.I., Damsgård, B., Braithwaite, V.A., Braastad, B.O., Bakken, M. 2011. Stressed mothers- trouble offspring: a study of behavioural maternal effects in farmed *Salmo salar*. Journal of Fish Biology 79: 575-586.

Essington, T.E., Quinn, T.P. & Ewert, V.E. 2000. Intra-and inter-specific competition and the reproductive success of sympatric Pacific salmon. Canadian Journal of Fisheries and Aquatic Sciences 57: 205-213.

Fabbri, E., Capuzzo, A. & Moon, T.W. 1998. The role of circulating catecholamines in the regulation of metabolism: an overview. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 120: 177-192.

Faria, C., Almada, V. & do Carmo Dunes, M. 1998. Patterns of agonistic behaviour, shelter occupation and habitat preference in juvenile *Lipophrys pholis*, *Coryphoblennius galerita* and *Gobius cobitis*. Journal of Fish Biology 53: 1263-1273.

Fefferman, N.H. & Romero, L.M. 2013. Can physiological stress alter population persistence? A model with conservation implications. Conservation Physiology 1: cot012.

Flik, G., Klaren, P.H.M., Van den Burg, E.H., Metz, J.R. & Huising, M.O. 2006. CRF and stress in fish. General and Comparative Endocrinology 146: 36-44.

Francis, R.C. 1990. Temperament in a fish: a longitudinal study of the development of individual differences in aggression and social rank in the Midas cichlid. Ethology 86: 311-325.

Fuzzen, M.L.M., Van Der Kraak, G. & Bernier, N.J. 2010. Stirring up new ideas about the regulation of the hypothalamic-pituitary-interrenal axis in zebrafish (*Danio rerio*). Zebrafish 7: 349-358.

Gagliano, M. & McCormick, M.I. 2009. Hormonally mediated maternal effects shape offspring survival potential in stressful environments. Oecologia 160: 657-665.

Gamperl, A.K., Vijayan, M.M. & Boutlier, R.G. 1994. Experimental control of stress hormone levels in fishes: techniques and applications. Reviews in Fish Biology and Fisheries 4: 215-255.

Garenc, C., Silversides, F.G. & Guderley, H. 1998. Burst swimming and its enzymatic correlates in threespine stickleback (*Gasterosteus aculeatus*): full-sib heritabilities. Canadian Journal of Zoology 76: 680-688.

Geslin, M. & Auperin, B. 2004. Relationship between changes in mRNAs of the genes encoding steroidogenic acute regulatory protein and P450 cholesterol side chain cleavage in head kidney and plasma levels of cortisol in response to different kinds of acute stress in the rainbow trout (*Oncorhynchus mykiss*). General and Comparative Endocrinology 145: 70-80.

Gheorghe, C.P., Goyal, R., Mittal, A. & Longo, L.D. 2010. Gene expression in the placenta: maternal stress and epigenetic responses. The International Journal of Developmental Biology 54: 507-523.

Giaquinto, P.C. & Volpato, G.L. 1997. Chemical communication, aggression, and conspecific recognition in the fish nile tilapia. Physiology and Behavior 62: 1333-1338.

Giesing, E.R., Suski, C.D., Warner, R.E. & Bell, A.M. 2011. Female sticklebacks transfer information via eggs: effects of maternal experience with predators on offspring. Proceedings of the Royal Society B: Biological Sciences 278: 1753-1759.

Gilchriest, B.J., Tipping, D.R., Hake, L., Levy, A. & Baker, B.I. 2000. The effects of acute and chronic stresses on vasotocin gene transcripts in the brain of the rainbow trout (*Oncorhynchus mykiss*). Journal of Neuroendocrinology 12: 795-801.

Gilmour, K.M., DiBattista, J.D. & Thomas, J.B. 2005. Physiological causes and consequences of social status in salmonid fish. Integrative and Comparative Biology 45: 263-273.

Giordano, M., Groothuis, T.G.G. & Tschirren, B. 2014. Interactions between prenatal maternal effects and posthatching conditions in a wild bird population. Behavioral Ecology 25: 1459-1466.

Gluckman, P.D., Hanson, M.A. & Spencer, H.G. 2005. Predictive adaptive responses and human evolution. Trends in Ecology & Evolution 20: 527-533.

Grant, S.C.H. & MacDonald, B.L. 2013. Pre-season run size forecasts for Fraser River Sockeye (*Oncorhynchus nerka*) and Pink (*O. gorbuscha*) salmon in 2013. Fisheries and Oceans Canada Canadian Science Advisory Secretariat Research Document 2012/145.

Grantz, I. & Fong, T.M. 2003. The melanocortin system. American Journal of Physiology – Endocrinology and Metabolism 284: E468-E474.

Gregory, J.S. & Griffith, J.S. 1996. Aggressive behaviour of underyearling rainbow trout in simulated winter concealment habitat. Journal of Fish Biology 49: 237-245.

Gregus, A., Wintink, A.J., Davis, A.C. & Kalynchuk, L.E. 2005. Effect of repeated corticosterone injections and restraint on anxiety and depression-like behavior in male rats. Behavioural Brain Research 156: 105-114.

Groot, C. & Margolis, L. 1991. Pacific Salmon Life Histories. UBC Press, Vancouver.

Groothuis, T.G.G., Müller, W., von Engelhardt, N., Carere, C. & Eising, C. 2005. Maternal hormones as tools to adjust offspring phenotype in avian species. Neuroscience and Biobehavioral Reviews 29: 329-352.

Groothuis, T.G.G. & Schwabl, H. 2008. Hormone-mediated maternal effects in birds: *mechanisms* matter but what do we know of them? Philosophical Transactions of the Royal Society B: Biological Sciences 363: 1647-1661.

Guibert, F., Richard-Yris, M-A., Lumineau, S., Kotrschal, K., Bertin, A., Petton, C., Möstl, E. & Houdelier, C. 2011. Unpredictable mild stressors on laying females influence the composition of Japanese quail eggs and offspring's phenotype. Applied Animal Behaviour Science 132: 51-60.

Guillette, L.J., Cree, A. & Rooney, A.A. 1995. Biology of stress: interactions with reproduction, immunology and intermediary metabolism. In: Warwick, C., Frye, F.L. & Murphy, J.B. (Eds) Health and Welfare of Captive Reptiles. Chapman and Hall, London, pp. 32-81.

Haussmann, M.F., Longenecker, A.S., Marchetto, N.M., Juliano, S.A. & Bowden, R.M. 2011. Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. Proceedings of the Royal Society B: Biological Sciences 279: 1447-1456.

Hawkins, D.K. & Quinn, T.P. 1996. Critical swimming velocity and associated morphology of juvenile coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead trout (*Oncorhynchus mykiss*), and their hybrids. Canadian Journal of Fisheries and Aquatic Sciences 53: 1487-1496.

Hayward, L.S. & Wingfield, J.C. 2004. Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. General and Comparative Endocrinology 135: 365-371.

Hayward, L.S., Richardson, J.B., Grogan, M.N. & Wingfield, J.C. 2006. Sex differences in the organizational effects of corticosterone in the egg yolk of quail. General and Comparative Endocrinology 146: 144-148.

Heath, D.D. & Blouw, D.M. 1998. Are maternal effects in fish adaptive or merely physiological side effects? In: Mousseau, T.A. & Fox, C.W. (Eds) Maternal Effects as Adaptations. Oxford University Press, New York, pp. 178-201.

Henriksen, R., Rettenbacher, S. & Groothuis, T.G.G. 2011. Prenatal stress in birds: Pathways, effects, function and perspectives. Neuroscience & Biobehavioral Reviews 35: 1484-1501.

Henriksen, R., Rettenbacher, S. & Groothuis, T. G.G. 2013. Maternal corticosterone elevation during egg formation in chickens (*Gallus gallus domesticus*) influences offspring traits, partly via prenatal undernutrition. General and Comparative Endocrinology 191: 83-91.

Herman, J.P. & Cullinan, W.E. 1997. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends in Neurosciences 20: 78-84.

Ho, D.H. & Burggren, W.W. 2010. Epigenetics and transgenerational transfer: a physiological perspective. The Journal of Experimental Biology 213: 3-16.

Höjesjö, J., Johnsson, J.I. & Bohlin, T. 2002. Can laboratory studies on dominance predict fitness of young brown trout in the wild? Behavioral Ecology and Sociobiology 52: 102-108.

Hopkins, W.A., Mendonça, M.T. & Congdon, J.D. 1997. Increasing circulating levels of testosterone and corticosterone in southern toads, *Bufo terrestris*, exposed to coal combustion waste. General and Comparative Endocrinology 108: 237-246.

Hruska, K.A., Hinch, S.G., Healey, M.C., Patterson, D.A., Larsson, S. & Farrell, A.P. 2010. Influences of sex and activity level on physiological changes in individual adult sockeye salmon during rapid senescence. Physiological and Biochemistry Zoology 83: 663-676.

Hsu, Y., Earley, R.L. & Wolf, L.L. 2006. Modulation of aggressive behavior by fighting experience: mechanisms and outcomes. Biological Reviews 81: 33-74.

Huising, M.O., Metz, J.R., van Schooten, C., Taverne-Thiele, A.J., Hermsen, T., Verburg-van Kemenade, B.M.L. & Flik, G. 2004. Structural characterisation of a cyprinid (*Cyprinus carpio* L.) CRH, CRH-BP and CRH-R1, and the role of these proteins in the acute stress response. Journal of Molecular Endorcinology 32: 627-648.

Huntingford, F.A. 1976. The relationship between anti-predator behaviour and aggression among conspecifics in the three-spined stickleback, *Gasterosteus aculeatus*. Animal Behaviour 24: 245-260.

Hutchings, J.A. & Reynolds, J.D. 2004. Marine fish population collapses: consequences for recovery and extinction risk. BioScience 54: 297-309.

Hutchison, M.J. & Iwata, M. 1997. A comparative analysis of aggression in migratory and non-migratory salmonids. Environmental Biology of Fishes 50: 209-215.

Janczak, A.M., Torgesen, P., Palme, R. & Bakken, M. 2007. Effects of stress in hens on the behaviour of their offspring. Applied Animal Behaviour Science 107: 66-77.

Jefcoate, C.R., McNamara, B.C., Artemenko, I. & Yamazaki, T. 1992. Regulation of cholesterol movement to mitochondrial cytochrome P450scc in steroid hormone synthesis. The Journal of Steroid Biochemistry and Molecular Biology 43: 751-767.

Jeffrey, J.D., Esbaugh, A.J., Vijayan, M.M. & Gilmour, K.M. 2012. Modulation of hypothalamic–pituitary–interrenal axis function by social status in rainbow trout. General and Comparative Endocrinology 176: 201-210.

Jeffrey, J.D. 2014. The role of social status, maternal stress and parental investment in mediation of hypothalamic-pituitary-interrenal axis function in teleosts. PhD dissertation. University of Ottawa, Ottawa, Ontario, Canada.

Jeffrey, J.D., Cooke, S.J. & Gilmour, K.M. 2014a. Regulation of hypothalamic-pituitary-interrenal axis function in male smallmouth bass (*Micropterus dolomieu*) during parental care. General and Comparative Endocrinology 204: 195-202.

Jeffrey, J.D., Gollock, M.J. & Gilmour, K.M. 2014b. Social stress modulates the cortisol response to an acute stressor in rainbow trout (*Oncorhynchus mykiss*). General and Comparative Endocrinology 196: 8-16.

Jeffries, K.M., Hinch, S.G., Donaldson, M.R., Gale, M.K., Burt, J.M., Thompson, L.A., Farrell, A.P, Patterson, D.A. & Miller, K.M. 2011. Temporal changes in blood variables during final maturation and senescence in male sockeye salmon *Oncorhynchus nerka*: reduced osmoregulatory ability can predict mortality. Journal of Fish Biology 79: 449-465.

Jeffries, K.M., Hiinch, S.G., Martins, E.G., Clark, T.D., Lotto, A.G., Patterson, D.A., Cooke, S.J., Farrell, A.P. & Miller, K.M. 2012a. Sex and proximity to reproductive maturity influence the survival, final maturation, and blood physiology of Pacific salmon when exposed to high temperature during a simulated migration. Physiological and Biochemical Zoology 85: 62-73.

Jeffries, K.M., Hinch, S.G., Sierocinski, T., Clark, T.D., Eliason, E.J., Donaldson, M.R., Li, S., Pavlidis, P. & Miller, K.M. 2012b. Consequences of high temperatures and premature mortality on the transcriptome and blood physiology of wild adult sockeye salmon (*Oncorhynchus nerka*). Ecology and Evolution 2: 1747-1764.

Jeffries, K.M., Hinch, S.G., Sierocinski, T., Pavlidis, P. & Killer, K.M. 2014. Transcriptomic responses to high water temperature in two species of Pacific salmon. Evolutionary Applications **7**: 286-300.

Johnsson, B. 1997. A review of ecological and behavioural interactions between cultured and wild Atlantic salmon. ICES Journal of Marine Science 54: 1031-1039.

Kappal, C.V. 2005. Losing pieces of the puzzle: threats to marine, estuarine, and diadromous species. Frontiers in Ecology and the Environment 3: 275-282.

Kiilerich, P., Kristiansn, K. & Madsen, S.S. 2007. Hormone receptors in gills of smolting Atlantic salmon, *Salmo salar*: expression of growth hormone, prolactin, mineralcorticoid and glucocorticoid receptors and 11β-hydroxysteroid dehydrogenase type 2. General and Comparative Endocrinology 152: 295-303.

Killen, S.S., Mitchell, M.D., Rummer, J.L., Chivers, D.P., Ferrari, M.C.O., Meekan, M.G. & McCormick, M.I. 2014. Aerobic scope predicts dominance during early life in a tropical damself fish. Functional Ecology 28: 1367-1376.

Kitaysky A., Wingfield, J.C. & Piatt, J.F. 2001. Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. Behavioral Ecology 12: 619-625.

Koolhaus, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W. & Blokhuis, H.J. 1999. Coping styles in animals: current status in behavior and stress-physiology. Neuroscience and Biobheavioral Reviews 23: 925-935.

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

Kusakabe, M., Nakamura, I. & Young, G. 2003. 11β-hydroxysteroid dehydrogenase complementary deoxyribonucleic acid in rainbow trout: cloning, sites of expression, and seasonal changes in gonads. Endocrinology 144: 2534-2545.

Lahti, K., Huuskonen, H., Laurila, A. & Piironen, J. 2002. Metabolic rate and aggressiveness between brown trout populations. Functional Ecology 16: 167-174.

Lam, T.J. 1994. Hormones and egg/larval quality in fish. Journal of the World Aquaculture Society 25: 2-12.

Lankford, S.E., Adams, T.E., Miller, R.A. & Cech, J.J. 2005. The cost of chronic stress: impacts of a nonhabituating stress response on metabolic variables and swimming performance in sturgeon. Physiological and Biochemical Zoology 78: 599-609.

Leary, C.J., Garcia, A.M. & Knapp, R. 2006. Elevated corticosterone levels eclicit non-calling mating tactics in male toads independeltly of changes in circulating androgens. Hormones and Behavior 49: 425-432.

Lema, S.C. 2014. Hormones and phenotypic plasticity in an ecological context: linking physiological mechanisms to evolutionary processes. Integrative and Comparative Biology 54: 850-863.

Lepage, O., Øverli, Ø., Petersson, E., Järvi, T. & Winberg, S. 2000. Differential stress coping in wild and domesticated sea trout. Brain, Behavior and Evolution 56: 259-268

Li, M., Christie, H.L. & Leatherland, J.F. 2012. The in vitro metabolism of cortisol by ovarian follicles of rainbow trout (*Oncorhynchus mykiss*): comparisons with ovulated oocytes and pre-hatch embryos. Reproduction 144: 713-722.

Lima, S.L. & Dill, L.M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68: 619-640.

Lindqvist, C., Janczak, A.M., Nätt, D., Baranowska, I., Lindqvist, N., Wichman, A., Lundeberg, J., Lindberg, J., Torjesen, P.A. & Jensen, P. 2007. Transmission of stress-induced learning impairment and associated brain gene expression from parents to offspring in chickens. PLoS ONE 2: e364.

Loeschcke, H.H. 1973. Respiratory chemosensitivity in the medulla oblongata. Acta Neurobiologiae Experimentalis 33: 97-112.

Love, O.P., Chin, E.H., Wynne-Edwards, K.E. & Williams, T.D. 2005. Stress hormones: a link between maternal condition and sex-biased reproductive investment. The American Naturalist 166: 751-766.

Love, O.P. & Williams, T.D. 2008a. The adaptive value of stress-induced phenotypes: effects of maternally derived corticosterone on sex-biased investment, cost of reproduction, and maternal fitness. The American Naturalist 172: E135-149.

Love, O.P. & Williams, T.D. 2008b. Plasticity in the adrenocortical response of a free-living vertebrate: The role of pre- and post-natal developmental stress. Hormones and Behavior 54: 496-505.

Love, O.P., McGowan, P.O. & Sheriff, M.J. 2013. Maternal adversity and ecological stressors in natural populations: the role of stress axis programming in individuals, with implications for populations and communities. Functional Ecology 27: 81-92.

Lowe-Jinde, L. & Niimi, A.J. 1984. Short-term and long-term effects of cadmium on glycogen reserves and liver size in rainbow trout (*Salmo gairdneri* Richardson). Archives of Environmental Contamination and Toxicology 13: 759-764.

Lubzens, E., Lissauer, L., Levavi-Sivan, B., Avarre, J. C. & Sammar, M. 2003. Carotenoid and retinoid transport to fish oocytes and eggs: what is the role of retinol binding protein? Molecular Aspects of Medicine 24: 441-457.

Lucca, G., Comim, C.M., Valvassori, S.S., Réus, G.Z., Vuolo, F., Petronilho, F., Dal-Pizzol, F., Gavioli, E.C. & Quevedo, J. 2009. Effects of chronic mild stress on the oxidative parameters in the rat brain. Neurochemistry International 54: 358-362,

MacDougall-Shackleton, S.A., Dindia, L., Newman, A.E., Potvin, D.A., Stewart, K.A. & MacDougall-Shackleton, E.A. 2009. Stress, song and survival in sparrows. Biology Letters 5: 746-748.

Maldonado, T.A., Jones, R.E. & Norris, D.O. 2002. Timing of neurodegeneration of beta-amyloid (Aβ) peptide deposition in the brain of aging kokanee salmon. Developmental Neurobiology 53: 21-35.

Marasco, V., Robinson, J., Herzyk, P. & Spencer, K.A., 2012. Pre- and post-natal stress in context: effects on the stress physiology in a precocial bird. Journal of Experimental Biology 215: 3955-3964

Marcalo, A., Mateus, L., Correia, J., Fryer, R. & Stratoudakis, Y. 2006. Sardine (*Sardina pilchardus*) stress reactions to purse seine fishing. Marine Biology 149: 1509-1518.

Martins, E.G., Hinch, S.G., Patterson, D.A., Hague, M.J., Cooke, S.J., Miller, K.M., Robichaud, D., English, K.K. & Farrell, A.P. 2012. High river temperature reduces survival of sockeye salmon approaching spawning grounds and exacerbates female mortality. Canadian Journal of Fisheries and Aquatic Sciences 69: 330-342.

Mattson, M. P. 2008. Hormesis defined. Ageing Research Reviews 7: 1-7.

McConnachie, S.H., Cook, K.V., Patterson, D.A., Gilmour, K.M., Hinch, S.G., Farrell, A.P. & Cooke, S.J. 2012. Consequences of acute stress and cortisol manipulation on the physiology, behavior, and reproductive outcome of female Pacific salmon on spawning grounds. Hormones and Behavior 62: 67-76.

McCormick, J.H., Stokes, G.N. & Hermanutz, R.O. 1989. Oocyte atresia and reproductive success in fathead minnows (*Pimephales promelas*) exposed to acidified hardwater environments. Archives of Environmental Contamination and Toxicology 18: 207-214.

McCormick, M.I. 1999. Experimental test of the effect of maternal hormones on larval quality of a coral reef fish. Oecologia 118: 412-422.

McCormick, M.I. 2006. Mothers matter: crowding leads to stressed mothers and small offspring in marine fish. Ecology 87: 1104-1109.

McCormick, M.I. 2009. Indirect effects of heterospecific interactions on progeny size through maternal stress. Oikos 118: 744-752.

McEwen, B.S. 1998. Seminars in medicine of the Beth Israel Deaconess Medical Center: protective and damaging effects of stress mediators. New England Journal of Medicine 338: 171-179.

McEwen, B.S. & Wingfield, J.C. 2003. The concept of allostasis in biology and biomedicine. Hormones and Behavior 43: 2-15.

McGhee, K.E., Pintor, L.M., Suhr, E.L. & Bell, A.M. 2012. Maternal exposure to predation risk decreases offspring antipredator behaviour and survival in threespined stickleback. Functional Ecology 26: 932-940.

McGlothlin, J.W. & Ketterson, E.D. 2008. Hormone-mediated suites as adaptations and evolutionary constraints. Philosophical Transactions of the Royal Society B: Biological Sciences 363: 1611-1620.

Meaney, M.J. 2001. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annual Reivew of Neuroscience 24: 1161-1192.

Meylan, S. & Clobert, J. 2005. Is corticosterone-mediated phenotype development adaptive? Maternal corticosterone treatment enhances survival in male lizards. Hormones and Behavior 48: 44-52.

Meylan, S., Haussy, C. & Voituron, Y. 2010. General and Comparative Endocrinology 169: 158-166.

Meylan, S., Miles, D.B. & Clobert, J. 2012. Hormonally mediated maternal effects, individual strategy and global change. Philosophical Transactions of the Royal Society B: Biological Sciences 367: 1647-1664.

Milla, S., Wang, N., Mandiki, S. N. M. & Kestemont, P. 2009. Corticosteroids: Friends or foes of teleost fish reproduction?. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 153: 242-251.

Miller, K.M., Teffer, A., Tucker, S., Li, S., Schulze, A.D., Trudel, M., Juanes, F., Tabata, A., Kaukinen, K.H., Ginther, N.G., Ming, T.J., Cooke, S.J., Hipfner, J.M., Patterson, D.A. & Hinch, S.G. 2014. Infectious disease, shifting climates, and opportunistic predators: cumulative factors potentially impacting wild salmon declines. Evolutionary Applications 7: 812-855.

Mittelbach, G.G., Ballew, N.G. & Kjelvik M.K. 2014. Fish behavioral types and their ecological consequences. Canadian Journal of Fisheries and Aquatic Sciences 71: 927-944.

Møller A.P. & Mousseau T.A. 2007. Determinants of interspecific variation in population declines of birds after exposure to radiation at Chernobyl. Journal of Applied Ecology 44: 909-919.

Mommer, B. 2013. The neuroendocrine and molecular mechanisms underlying stress-induced maternal effects in the threespine stickleback. PhD dissertation, University of Illinois at Urbana-Champaign, Champaign, Illinois, USA.

Mommer, B. C. & Bell, A.M. 2013. A test of maternal programming of offspring stress response to predation risk in threespine sticklebacks. Physiology & Behavior 122: 222-227.

Mommer, B. C. & Bell, A.M. 2014. Maternal experience with predation risk influences genome-wide embryonic gene expression in threespined sticklebacks (*Gasterosteus aculeatus*). PloS ONE 9: e98564.

Mommsen, T.P., Vijayan, M.M. & Moon, T.W. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries 9: 211-268.

Moore, I.T. & Mason, R.T. 2001. Behavioral and hormonal responses to corticosterone in the male red-sided garter snake, *Thamnophis sirtalis parietalis*. Physiology & Behavior 72: 669-674.

Moore, M.C., Thompson, C.W. & Marler, C.A. 1991. Reciprocal changes in corticosterone and testosterone levels following acute and chronic handling stress in the tree lizard, *Urosaurus ornatus*. General and Comparative Endocrinology 81: 217-226.

Moore, M.C. & Johnston, G.I.H. 2008. Toward a dynamic model of deposition and utilization of yolk steroids. Integrative and Comparative Biology 48: 411-418.

Mousseau, T.A. & Fox, C.W. 1998. The adaptive significance of maternal effects. Trends in Ecology & Evolution 13: 403-407.

Mylonas, C.C., Sullivan, C.V. & Hinshaw, J.M. 1994. Thyroid hormones in brown trout (*Salmo trutta*) reproduction and early development. Fish Physiology and Biochemistry 13: 485-493.

Nadeau, P.S., Hinch, S.G., Pon, L.B. & Patterson, D.A. 2009. Persistent parental effects on the survival and size, but not burst swimming performance of juvenile sockeye salmon *Oncorhynchus nerka*. Journal of Fish Biology 75: 538-551.

Nadeau, P.S., Hinch, S.G., Hruska, K.A., Pon, L.B. & Patterson, D.A. 2010. The effects of experimental energy depletion on the physiological condition and survival of adult sockeye salmon (*Oncorhynchus nerka*) during spawning migration. Environmental Biology of Fish 88: 241-251.

Nagahama, Y. 1994. Endocrine regulation of gametogenesis in fish. International Journal of Developmental Biology 38: 217-229.

Neff, B.D. 2004. Increased performance of offspring sired by parasitic males in bluegill sunfish. Behavioral Ecology 15: 327-331.

Nesan, D. & Vijayan, M.M. 2012. Embryo exposure to elevated cortisol level leads to cardiac performance dysfunction in zebrafish. Molecular and Cellular Endocrinology 363: 85-91.

Nickelson, T.E., Rodgers, J.D., Johnson, S.L. & Solazzi, M.F. 1992. Seasonal changes in habitat use by juvenile coho salmon (*Oncorhynchus kisutch*) in Oregon coastal streams. Canadian Journal of Fisheries and Aquatic Sciences 49: 783-789.

Noakes, D.J., Beamish, R.J. & Kent, M.L. 2000. On the decline of Pacific salmon and speculative links to salmon farming in British Columbia. Aquaculture 183: 363-386.

Oberlander, T.F., Weinber, J., Papsdork, M., Grunau, R., Misri, S. & Devlin, A.M. 2008. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (*NR3C1*) and infant cortisol stress responses. Epigenetics 3: 97-106.

O'Connor, C.M., Nannini, M., Wahl, D.H., Wilson, S.M., Gilmour, K.M. & Cooke, S.J. 2013. Sex-specific consequences of experimental cortisol elevation in pre-reproductive wild largemouth bass. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 319: 23-31.

Okuliarová, M., Šárniková, B., Rettenbacher, S., Škrobánek, P. & Zeman, M. 2010. Yolk testosterone and corticosterone in hierarchical follicles and laid eggs of Japanese quail exposed to long-term restraint stress. General and Comparative Endocrinology 165: 91-96.

Øverli, O., Kotzian & Winberg, S. 2002. Effects of cortisol on aggression and locomotor activity in rainbow trout. Hormones and Behavior 42: 53-61.

Øverli, O., Korzan, W.J., Höglund, E., Winberg, S., Bollig, H., Watt, M., Forster, G.L., Barton, B.A., Øverli, E., Renner, K.J. & Summers, C.H. 2004. Stress coping style predicts aggression and social dominance in rainbow trout. Hormones and Behavior 45: 235-241.

Øverli, O., Sørensen, C., Pulman, K.G.T., Pottinger, T.G., Korzan, W., Summers, C.H. & Nilsson, G.E. 2007. Evolutionary background for stress-coping styles: relationships between physiological, behavioral and cognitive traits non-mammalian vertebrates. Neuroscience and Biobehavioral Reviews 31: 396-412.

Painter, D., Jennings, D.H. & Moore, M.C. 2002. Placental buffering of maternal steroid hormone effects on fetal and yolk hormone levels: a comparative study of a viviparous lizard, *Sceloporus jarrovi*, and an oviparous lizard, *Sceloporus graciosus*. General and Comparative Endocrinology 127: 105-116.

Paitz, R.T., Bowden, R.M. & Casto, J.M. 2011. Embryonic modulation of maternal stress in European starlings *(Sturnus vulgaris*). Proceedings of the Royal Society B: Biological Sciences 278: 99–106.

Palace, V.O. & Werner, J. 2006. Vitamins A and E in the maternal diet influence egg quality and early life stage development in fish: a review. Scientia Marina 70: 41-57.

Pankhurst, N.W., King, H.R. & Ludke, S.L. 2008. Relationship between stress, feeding and plasma ghrelin levels in rainbow trout, *Oncorhynchus mykiss*. Marine and Freshwater Behaviour and Physiology 41: 53-64.

Patterson, D.A., Guderley, H., Bouchard, P., Macdonald, J.S. & Farrell, A.P. 2004a. Maternal influence and population differences in activities of mitochondrial and glycolytic enzymes in emergent sockeye salmon (*Oncorhynchus nerka*) fry. Canadian Journal of Fisheries and Aquatic Sciences 61: 1225-1234.

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

Payne, A.H. & Hales, D.B. 2004. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocrine Review 25: 947-970.

Penhoat, A., Naville, D. & Begeot, M. 2001. The adrenocorticotropic hormone receptor. Current Opinion in Endocrinology & Diabetes 8: 112-117.

Piato, Â.L., Capiotti, K.M., Tamborski, A.R., Oses, J.P., Barcellos, L.J.G., Bogo, M.R., Lara, D.R., Vianna, M.R. & Bonan, C.D. 2011. Unpredictable chronic stress model in zebrafish (*Danio rerio*): Behavioral and physiological responses. Progress in Neuro-Psychopharmacology & Biological Psychiatry 35: 561-567.

Pickering, A.D. & Pottinger, T.G. 1989. Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. Fish Physiology and Biochemistry 7: 253-258.

Pickering, A.D., Pottinger, T.G., Sumpter, J.P., Carragher, J.F. & Le Bail, P.Y. 1991. Effects of acute and chronic stress on the levels of circulating growth hormone in the rainbow trout, *Oncorhynchus mykiss*. General and Comparative Endocrinology 83: 86-93.

Pitk, M., Tilgar, V., Kilgas, P & Mänd, R. 2012. Acute stress affects the corticosterone level in bird eggs: a case study with great tits (*Parus major*). Hormones and Behavior 62: 475-479.

Perelberg, A., Smirnov, M., Hutoran, M., Diamant, A., Bejerano, Y. & Kotler, M. 2003. Epidemiological description of a new viral disease afflicting cultured *Cyprinus carpio* in Israel. Israeli Journal of Aquaculture 55: 5-12.

Peter, M.C. S. 2011. The role of thyrid hormones in stress response of fish. General and Comparative Endocrinology 172: 198-210.

Pfaffl, M.W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research 29: e45.

Pon, L.B., Hinch, S.G., Wagner, G.N., Lotto, A.G. & Cooke, S.J. 2007. Swimming performance and morphology of juvenile sockeye salmon, *Oncorhynchus nerka*: comparison of inlet and outlet fry populations. Environmental Biology of Fishes 78: 257-269.

Pottinger, T.G. & Carrick, T.R. 1999. Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. General and Comparative Endocrinology 116: 122-132.

Pratap, H.B. & Wendelaar Bonga, S.E. 1990. Effects of water-borne cadmium on plasma cortisol and glucose in the cichlid fish *Oreochromis mossambicus.* Comparative Biochemistry and Physiology Part C: Comparative Pharmacology 95: 313-317.

Quinn, T.P. 1999. Variation in Pacific salmon reproductive behaviour associated with species, sex and levels of competition. Behaviour 136: 179-204.

Quinn, T.P. 2005. The behaviour and ecology of Pacific salmon and trout. University of Washington Press, Seattle.

Raby, G. D., Cooke, S. J., Cook, K. V., McConnachie, S. H., Donaldson, M. R., Hinch, S. G., Whitney, C.K., Drenner, S.M., Patterson, D.A., Clark, T.D. & Farrell, A. P. 2013. Resilience of pink salmon and chum salmon to simulated fisheries capture stress incurred upon arrival at spawning grounds. Transactions of the American Fisheries Society 142: 524-539.

Raby, G.D., Donaldson, M.R., Nguyen, V.M., Taylor, M.K., Sopinka, N.M., Cook, K.V., Patterson, D.A., Robichaud, D., Hinch, S.G. & Cooke, S.J. 2014. Bycatch mortality of endangered coho salmon: impacts, solutions and aboriginal perspectives. Ecological Applications 24: 1803-1819.

Radder, R.S. 2007. Maternally derived egg yolk steroid hormones and sex determination: review of a paradox in reptiles. Journal of Biosciences 32: 1213-1220.

Räsänen, K. & Kruuk, L.E.B. 2007. Maternal effects and evolution at ecological time-scales. Functional Ecology 21: 408-421.

Rebuffé-Scrive, M., Walsh, U.A., McEwen, B. & Rodin, J. 1992. Effect of chronic stress and exogenous glucocorticoids on regional fat distribution and metabolism. Physiology & Behavior 52: 583-590.

Reeder, D.M. & Kramer, K.M. 2005. Stress in free-ranging mammals: integrating physiology, ecology, and natural history. Journal of Mammalogy 86: 225-235.

Richman, N.H. & Zaugg, W.S. 1987. Effects of cortisol and growth hormone on osmoregulation in pre- and desmoltified coho salmon (*Oncorhynchus kisutch*). General and Comparative Endocrinology 65: 189-198.

Rivier, C. & Rivest, S. 1991. Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: peripheral and central mechanisms. Biology of Reproduction 45: 523-532.

Robert, K.A., Vleck, C. & Bronikowski, A.M. 2009. The effects of maternal corticosterone levels on offspring behavior in fast- and slow-growth garter snakes (*Thamnophis elegans*). Hormones and Behavior 55: 24-32.

Roche, D.P., McGhee, K.E. & Bell, A.M. 2012. Maternal predator-exposure has lifelong consequences for offspring learning in threespined sticklebacks. Biology Letters 8: 932-935.

Rodnick, K.J., Gamperl, A.K., Lizars, K.R., Bennett, M.T., Rausch, R.N. & Keeley, E.R. 2004. Thermal tolerance and metabolic physiology among redband trout populations in south-eastern Oregon. Journal of Fish Biology 64: 310-335.

Romero, L.M. & Wikelski, M. 2001. Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Nino events. Proceedings of the National Academy of Sciences 98: 7366-7370.

Romero, L.M. 2004. Physiological stress in ecology: lessons from biomedical research. Trends in Ecology & Evolution 19: 249-255.

Romero, L.M., Strochlic, D. & Wingfield, J.C. 2005. Corticosterone inhibits feather growth: potential mechanism explaining seasonal down regulation of corticosterone during molt. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 142: 65-73.

Ros, A.F.H., Becker, K. & Oliveira, R.F. 2006. Aggressive behaviour and energy metabolism in a cichlid fish, *Oreochromis mossambicus*. Physiology & Behavior 89: 164-170.

Rubolini, D., Romano, M., Boncoraglio, G., Ferrari, R.P., Martinelli, R., Galeotti, P., Fasola, M. & Saino. N. 2005. Effects of elevated egg corticosterone levels on behavior, growth, and immunity of yellow-legged gull (*Larus michahellis*) chicks. Hormones and Behavior 47: 595-605.

Ryan, S.N. 1995. The effect of chronic heat stress on cortisol levels in the Antarctic fish *Pagothenia borchgrevinki*. Experientia 51: 768-774.

Saino, N., Suffritti, C., Martinelli, R., Rubolini, D. & Møller, A.P. 2002. Immune response covaries with corticosterone plasma levels under experimentally stressful conditions in nestling barn swallows (*Hrundo rustica*). Behavioral Ecology 14: 318-325.

Saino, N., Romano, M., Ferrari, R.P., Martinelli, R. & Møller, A.P. 2005. Stressed mothers lay eggs with high corticosterone levels which produce low-quality offspring. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 303A: 998-1006.

Salo, E.O. 1991. Life history of chum salmon (*Oncorhynchus keta*). In: Groot, C. & Margolis, L. (Eds) Pacific Salmon Life Histories. UBC Press, Vancouver, pp. 231-310.

Salvante, K.G. & Williams, T.D. 2003. Effects of corticosterone on the proportion of breeding females, reproductive output and yolk precursor levels. General and Comparative Endocrinology 130: 205-214.

Sampath-Kumar, R., Munro, A.D., Lee, J. & Lam, T.J. 1993. Exogenous cortisol promotes survival of Asian seabass (*Lates calcarifer*) hatchlings exposed to hypersalinty but not hyposalinty shock. Aquaculture 116: 247-255.

Sandercock, F.K. 1991. Life history of coho salmon (*Oncorhynchus kisutch*). In: Groot, C. & Margolis, L. (Eds) Pacific Salmon Life Histories. UBC Press, Vancouver, pp. 395-445.

Santema, P., Teitel, Z., Manser, M., Gennett, N. & Clutton-Brock, T. 2013. Effects of cortisol administration on cooperative behavior in meerkat helpers. Behavioural Ecology 24: 1122-1127.

Sapolsky, R.M. & Meaney, M.J. 1986. Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Research Reviews 11: 65-76.

Sapolsky, R.M., Alberts, S.C. & Altmann, J. 1997. Hypercortisolism associated with social subordinate or social isolation among wild baboons. Archives of General Psychiatry 54: 1137-1143.

Sapolsky, R.M., Romero, L.M. & Munck, A.U. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21: 55-89.

Schindler, D.E., Scheuerell, M.D., Moore, J.W., Gende, S.M., Francis, T.B. & Palen, W.J. 2003. Pacific salmon and the ecology of coastal ecosystems. Frontiers in Ecology and the Environment 1: 31-37.

Schreck, C.B., Olla, B.L. & Davis, M.W. 1997. Behavioral responses to stress. In: Iwama, G.K., Pickering, A.D., Sumpter, J.P. & Schreck, C.B. (Eds) Fish stress and health in aquaculture. Cambridge University Press, Cambridge, pp. 145-170.

Schreck, C.B. 2000. Accumulation and long-term effects of stress in fish. In: Moberg, G.P. & Mench, J.A. (Eds) The Biology of Animal Stress. CAB International, Wallingford, pp. 147–158.

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

Schreck, C.B. 2010. Stress and fish reproduction: the roles of allostasis and hormesis. General and Comparative Endocrinology 165: 549-556.

Schultner, J., Kitaysky, A.S., Gabrielsen, G.W., Hatch, S.A. & Bech, C. 2013. Differential reproductive responses to stress reveal the role of life-history strategies within a species. Proceedings of the Royal Society B: Biological Sciences 280: 20132090.

Scott, A.P., Sumpter, J.P. & Hardiman, P.A. 1983. Hormone changes during ovulation in the rainbow trout (*Salmo gairdneri* Richardson). General and Comparative Endocrinology 49: 128-134.

Scott, A.P., Hirschenhauser, K., Bender, N., Oliveria, R., Earley, R.L., Sebire, M., Ellis, T., Pavlidis, M., Hubbard, P.C., Huertas, M. & Canario, A. 2008. Non-invasive measurement of steroids in fish-holding water: important consideration when applying the procedure to behaviour studies. Behaviour 145: 1307-1328.

Seltmann, M.W., Jaatinen, K., Steele, B.B. & Öst, M. 2014. Boldness and stress responsiveness as drivers of nest-site selection in a ground-nesting bird. Ethology 120: 77-89.

Selye, H. 1950. Stress and the general adaptation syndrome. British Medical Journal 4667: 1383-1392.

Sheriff, M.J., Dantzer, B., Delehanty, B., Palme, R. & Boonstra, R. 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia 166: 869-887.

Sheriff, M.J. & Love, O.P. 2013. Determining the adaptive potential of maternal stress. Ecology Letters 16: 271-280.

Shine, R. & Downes, S.J. 1999. Can pregnant lizards adjust their offspring phenotypes to environmental conditions? Oecologia 119: 1-8.
Siegel, H.S. 1980. Physiological stress in birds. BioScience 30: 529-534.

Sih, A., Bell, A. & Johnson, J.C. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology & Evolution 19: 372-378.
Sloman, K.A., Metcalfe, N.B., Taylor, A.C. & Gilmour, K.M. 2001a. Plasma cortisol concentrations before and after social stress in rainbow trout and brown trout. Physiological and Biochemical Zoology 74: 383-389.

Sloman, K.A., Taylor, A.C., Metcalfe, T.N. & Gilmour, K.M. 2001b. Effects of an environmental perturbation on the social behaviour and physiological function of brown trout. Animal Behaviour 61: 325-333.

Sloman, K.A. 2010. Exposure of ova to cortisol pre-fertilisation affects subsequent behaviour and physiology of brown trout. Hormones and Behavior 58: 433-439.

Sopinka, N.M., Fitzpatrick, J.L., Desjardins, J.K., Stiver, K.A., Marsh-Rollo, S.E. & Balshine, S. 2009. Liver size reveals social status in the African cichlid *Neolamprologus pulcher*. Journal of Fish Biology 75: 1-16.

Sopinka, N.M., Hinch, S.G., Lotto, A.G., Whitney, C.K. & Patterson, D.A. 2013. Does among-population variation in burst swim performance of sockeye salmon *Oncorhynchus nerka* fry reflect early life migrations? Journal of Fish Biology 83: 1416-1424.

Steen, R.P. & Quinn, T.P. 1999. Egg burial depth by sockeye salmon (*Oncorhynchus nerka*): implications for survival of embryos and natural selection on female body size. Canadian Journal of Zoology 77: 836-841.

Stocco, D.M. 2001. StAR protein and the regulation of steroid hormone biosynthesis. Annual Review of Physiology 63: 192-213.

Stratholt, M.L., Donaldson, E.M. & Liley, N.R. 1997. Stress induced elevation of plasma cortisol in adult female coho salmon (*Oncorhynchus kisutch*), is reflected in egg cortisol content, but does not appear to affect early development. Aquaculture 158: 141-153.

Sundström, L.F., Petersson, E., Höjesjö, J., Johnsson, J. & Järvi, T. 2004. Hatchery selection promotes boldness in newly hatched brown trout (*Salmo trutta*): implications for dominance. Behavioral Ecology 15: 192-198.

Suter, H.C. 2002. The effects of maternal steroids on individual variation in juvenile salmonids. PhD dissertation, University of Glasgow, Glasgow, Scotland, UK.

Taylor, E.B. & McPhail, J.D. 1985a. Variation in burst and prolonged swimming performance among British Columbia populations of coho salmon *Oncorhynchus kisutch*. Canadian Journal of Fisheries and Aquatic Sciences 42: 2029-2033.

Taylor, E.B. & McPhail, J.D. 1985b. Burst swimming and size-related predation of newly emerged coho salmon *Oncorhynchus kisutch*. Transactions of the American Fisheries Society 114: 546-551.

Taylor, J.J. 2014. Effects of maternal oxidative stress and gametic cortisol exposure on Fraser River sockeye salmon (*Oncorhynchus nerka*) offspring. MSc thesis, Carleton University, Ottawa, Ontario, Canada.

Taylor, M.K. & Cooke, S.J. 2014. Repeatability of movement behaviour in a wild salmonid revealted by telemetry. Journal of Fish Biology 84: 1240-1246.

Tierney, K.B., Patterson, D.A. & Kennedy, C.J. 2009. The influence of maternal condition on offspring performance in sockeye salmon *Oncorhynchus nerka*. Journal of Fish Biology 75: 1244-1257.

Trompeter, W.P. & Langkilde, T. 2011. Invader danger: lizards faced with novel predators exhibit an altered behavioral response to stress. Hormones and Behavior 60: 152-158.

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

Tyler, C.R. & Sumpter, J.P. 1996. Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries 6: 287-318.

Uller, T. & Olsson, M. 2006. Direct exposure to corticosterone during embryonic development influences behaviour in an ovoviviparous lizard. Ethology 112: 390-397.

Vander Zanden, M.J., Shuter, B.J., Lester, N.P. & Rasmussen, J.B. 2000. Within- and among-population variation in the trophic position of a pelagic predator, lake trout (*Salvelinus namaycush*). Canadian Journal of Fisheries and Aquatic Sciences 57: 725-731.

Vassallo, B.G., Paitz, R.T., Fasanello, V.J. & Haussmann, M.F. 2014. Glucocorticoid metabolism *in ovo* environment modulates exposure to maternal corticosterone in Japanese quail embryos (*Coturnix japonica*). Biology Letters 10: 20140502

Verboven, N., Verreault, J., Letcher, R.J., Barielsen, G.W. & Evans, N.P. 2008. Maternally derived tesosterone and 17β-estradiol in the eggs of Arctic-breeding glaucous gulls in relation to persistent organic pollutants. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 148: 143-151.

von Engelhardt, N., Henriksen, R. & Groothuis, T.G.G. 2009. Steroids in chicken egg yolk: metabolism and uptake during early embryonic development. General and Comparative Endocrinology 163: 175-183.

Wallace, R.A. 1985. Vitellogenesis and Oocyte Growth in Nonmammalian Vertebrates. In: Browder, L. W. (Ed) Developmental Biology: A Comprehensive Synthesis, Volume 1 Oogeneis. Plenum Press, New York, pp.127-177.

Warner, D.A., Lovern, M.B. & Shine, R. 2007. Maternal nutrition effects reproductive output and sex allocation in a lizard with environmental sex determination. Proceedings of the Royal Society B: Biological Sciences 274: 883-890.

Warner, D.A., Radder, R. & Shine, R. 2009. Corticosterone exposure during embryonic development affects offspring growth and sex ratios in opposing directions in two lizard species with environmental sex determination. Physiological and Biochemical Zoology 82: 363-371.

Weaver, I.C.G., Cerboni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M. & Meaney, M.J. 2004. Epigenetic programming by maternal behavior. Nature Neuroscience 7: 847-854.

Weinstock, M. 2005. The potental influence of maternal stress hormones on development and mental health of the offspring. Brain, Behavior, and Immunity 19: 296-308.

Weinstock, M. 2008. The long-term behavioural consequences of prenatal stress. Neuroscience & Biobehavioral Reviews 32: 1073-1086.

Welberg, L.A.M., Thrivikraman, K.V. & Plotsky, P.M. 2005. Chronic maternal stress inhibits the capacity to up-regulate placental 11β-hydroxysteroid dehydrogenase type 2 activity. Journal of Endocrinology 186: R7-R12.

Wendelaar Bonga, S.E. 1997. The stress response in fish. Physiological Reviews 77: 591-625.

Whitney, C.K., Hinch, S.G. & Patterson, D.A. 2013. Provenance matters: thermal reaction norms for embryo survival among sockeye salmon *Oncorhynchus nerka* populations. Journal of Fish Biology 82: 1159-1176.

Wilmut, I., Sales, D.I. & Ashworth C.J. 1986. Maternal and embryonic factors associated with prenatal loss in mammals. Journal of Reproduction and Fertility 76: 851-864.

Wilson, S.W., Hinch, S.G., Eliason, E.J., Farrell, A.P. & Cooke, S.J. 2013. Calibrating acoustic acceleration transmitters for estimating energy use by wild adult Pacific salmon. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 164: 491-498.

Wilson, A.D.M. & Godin, J-G.J. 2009. Boldness and behavioral syndromes in the bluegill sunfish, *Lepomis macrochirus*. Behavioral Ecology 20: 231-237.

Winberg, S., Nilsson, A., Hylland, P., Söderstöm, V. & Nilsson, G. 1997. Serotonin as a regulator of hypothalamic-pituitary-interreanl activity in teleost fish. Neuroscience Letters 230: 113-116.

Wingfield, J.C. & Sapolsky, R.M. 2003. Reproduction and resistance to stress: when and how. Journal of Neuroendocrinology 15: 711-724.

Wingfield, J.C. & Silverin, B. 1986. Effects of corticosterone on territorial behavior of free-living male song sparrows *Melospiza melodia*. Hormones and Behavior 20: 405-417.

Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M. & Richardson, R.D. 1998. Ecological bases of hormone-behavior interactions: the "emergency life history stage". American Zoologist 38: 191-206.

Woo, P.T.K., Leatherland, J.F. & Lee, M.S. 1987. *Cryptobia salmositica*: cortisol increases the susceptibility of *Salmo gairdneri* Richardson to experimental cryptobiosis. Journal of Fish Diseases 10: 75-83.

Woodward, C. & Strange, R.J. 1987. Physiological stress responses in wild and hatchery-reared rainbow trout. Transactions of the American Fisheries Society 116: 574-579.

Zhang, S., Lei, F., Liu, S., Li, D., Chen, C. & Wang, P. 2011. Variation in baseline corticosterone levels of Tree Sparrow (*Passer montanus*) populations along an urban gradient in Beijing, China. Journal of Ornithology 152: 801-806.