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Interactive effects of pre- and post-natal stressors on Chinook salmon performance and fitness

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INTERACTIVE EFFECTS OF PRE- AND POST-NATAL STRESSORS ON CHINOOK SALMON PERFORMANCE AND FITNESS

By

Pauline M. Capelle

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Interactive effects of pre- and post-natal stressors on Chinook salmon performance and fitness

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DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research, as follows: I am the sole author of Chapters 1 and 5, and am the primary author on all other chapters (Chapters 2–4). Chapters 2 through 4 are co-authored with my co-supervisors, Dr. Oliver Love and Dr. Christina Semeniuk, and collaborators Dr. Natalie Sopinka and Dr. John Heath. In all cases, the key ideas, primary contributions, experimental designs, data analysis and interpretation, were performed by the author, and the contribution of co-authors was primarily through help with experimental design, interpretation, editing, and providing funding and logistical support. Chapter 4 is also co-authored with Mr. Chris Harris, who contributed to data analysis and edited the manuscript.

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Thesis Chapter	Publication title/full citation	Publication status
Chapter 2	Pre-natal stress exposure generates higher early survival and smaller size without impacting developmental rate in a Pacific salmon	Will be resubmitted to *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*

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ABSTRACT

Assessing the intergenerational effects of stress is an important factor in determining how populations will respond to changing environments. Stressful maternal environments often lead to perceived negative effects on offspring phenotype (e.g., small size, slow growth, high fearfulness/anxiety), yet these changes may better equip offspring for stressful conditions (i.e., environmental matching), showing that intergenerational effects have the potential to dampen negative effects of environmental stressors. This thesis aims to test this theory by manipulating a maternal signal of stress and measuring offspring phenotype and performance in multiple environments. I treated Chinook salmon (*Oncorhynchus tshawytscha*) eggs with cortisol (low dose (LD), high dose (HD), control dose (CD)) and found that LD and HD groups had higher embryonic survival than the CD group and HD fish were smaller than CD fish at yolk-sac absorption. Following a 30-day post-natal stressor (low water depths in stream channels), juvenile fish from the LD group in low water conditions displayed optimal phenotypic/performance traits, based on measures of size, physiology, and behaviour. However, traits measured in the HD group were sub-optimal, indicating that fish were likely prepared for a more severely stressful environment. The CD group only displayed some indicators of being mismatched to the low water conditions, which may have been more apparent should conditions have been chronically stressful. Collectively, my results emphasize the potential for positive early-life effects of maternally-derived stress and support environmental matching theory while showing detrimental effects of unreliable cues. As environments change rapidly and also become more unpredictable, it is timely to further determine the outcomes of intergenerational stress in both matched and mismatched future environments.

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CHAPTER 1 – GENERAL INTRODUCTION

Environmental stress

Anthropogenic stressors are having increasingly negative impacts on wildlife due to a growing mismatch between the severity of stressors and the ability of species to respond to these disturbances (Butchart et al. 2010; Hoffmann et al. 2010). One way that populations can respond to disturbances is through evolutionary adaptation (Hoffmann and Sgro 2011), however, alterations in the environment may arise too quickly for evolutionary processes to respond (Visser 2008). Phenotypic plasticity, occurring when multiple phenotypes arise from one genotype in different environments, may therefore play a large role in the ability of organisms to respond to environmental change (Thibert-Plante and Hendry 2011). When the parental environment affects offspring phenotype, this form of transgenerational plasticity can further influence the offspring's ability to respond to changing conditions (Mousseau and Fox 1998). While multiple terms are used to describe the role of a parent's environment in shaping offspring phenotype (e.g., transgenerational plasticity: Shama et al. 2014; maternal or paternal effects: Mousseau and Fox 1998; Guillaume et al. 2016, parental effects: Burgess and Marshall 2014), herein I refer to this notion broadly as intergenerational effects. When discussing more specifically the effects of a stressful maternal environment during reproduction on offspring phenotype and performance, I refer to these effects as pre-natal/maternal stress.

Glucocorticoid hormones: signals of maternal stress

Hormones mediate interactions between an organism's internal physiological state and the environment, making them an ideal candidate for translating signals about

environmental conditions (Lessels 2008). Measuring levels of stress hormones (glucocorticoids (GCs)) can provide a rapid assessment of how individuals in a population respond to changes in their environment (Busch and Hayward 2009; Angelier and Wingfield 2013). GCs are energetic hormones with two major functions: regulating daily energy expenditure at baseline levels (Landys et al. 2006) and playing a critical role in the stress response (McEwen and Wingfield 2003). Elevation of GCs in response to an acute stressor mobilizes energy resources and leads to adaptive physiological and behavioural changes in response to an environmental challenge (Breuner et al. 2008). Chronic exposure to stressors can lead to prolonged and maladaptive elevations in baseline GCs resulting in suppressed growth, immunity, and reproduction (Sapolsky et al. 2000; Schreck et al. 2001). However, small elevations in baseline GCs can also be positively associated with reproductive success/fitness (Crossin et al. 2016), showing that GCs likely play a context-dependent role in the relationship between stress and reproduction, particularly when characterizing the intergenerational effects of stress.

Chronic exposure to ecological stressors such as predation threat (Sheriff et al. 2009, 2011), high conspecific densities (Bian et al. 2015), or low food availability (Kitaysky et al. 1999), can lead to reproductive females having elevated baseline levels of GCs. In addition, elevated GC levels have also been observed in populations exposed to anthropogenic stressors such as degraded habitat quality (Marra and Holberton 1998) or human disturbance (e.g. recreational activity: Thiel et al. 2008; hunting pressure: Bryan et al. 2015). Hormones (including GCs) are transferred from mother to offspring through the placenta in mammals and other viviparous species (Benediktsson et al. 1997) and directly to eggs in oviparous species (Groothius and Schwabl 2008; Sopinka et al. 2017),

where movement of lipophilic hormones into and out of eggs is a dynamic process (Moore and Johnston 2008). Increased deposition of GCs from mother to developing eggs or embryos during sexual maturation/gestation is therefore a mechanism by which a maternal stress signal can be passed from mother to offspring and lead to changes in offspring phenotype and performance (Love et al. 2013).

In fishes, cortisol is the primary GC and maternally-derived cortisol is present in developing eggs along with maternal mRNAs, lipids, proteins, and other hormones (Brooks et al. 1997). During ontogeny, embryos only begin endogenously producing cortisol around hatching (e.g., Feist and Schreck 2001); therefore, cortisol-mediated processes during early embryogenesis involve maternally-derived cortisol (Nesan and Vijayan 2016). Among fish species, including salmonids, stressor-exposed females with higher circulating cortisol levels also have eggs with higher concentrations of cortisol (Stratholt et al. 1997; Eriksen et al. 2006; but see Ghio et al. 2016). Elevated cortisol levels in fish eggs are known to influence embryonic development (cell division: Kleppe et al. 2013; muscle development and mesoderm formation: Nesan et al. 2012), modify offspring fitness-related traits early in development (survival: Li et al. 2010; size: McCormick 1998; Burton et al. 2011), and have later effects on phenotype and performance (growth: Li et al. 2010; stress response: Auperin and Geslin 2008; Colson et al. 2015; Sopinka et al. 2016a; aggression and boldness: Sloman 2010; Burton et al. 2011; Sopinka et al. 2015a; activity: Eriksen et al. 2011). There are a multitude of experimental approaches that can be used to elevate egg cortisol levels and examine the potential of cortisol as a maternal stress signal in fishes (Sopinka et al. 2015b). Maternal stress exposure can be manipulated by: i) applying a stressor to the female which is

known to elevate circulating cortisol (e.g., predation risk: McGhee et al. 2012; chasing: Stratholt et al. 1997; Mileva et al. 2011; Sopinka et al. 2014, 2016a; intraspecific competition: McCormick 1998, 2006), ii) directly manipulating circulating cortisol levels in the females (intraperitoneal injection: Eriksen et al. 2006; osmotic pump: Kleppe et al. 2013; soaked food pellets: Faught et al. 2016; Ghio et al. 2016), or iii) directly manipulating egg cortisol (immersion in a hormone bath: Auperin and Geslin 2008; Sloman 2010; Burton et al. 2011; Colson et al. 2015; Sopinka et al. 2015a, 2016b; Ghio et al. 2016; microinjection of eggs: Nesan and Vijayan 2012). The variety of methodologies available to directly and indirectly manipulate maternal stress signals make fishes an excellent study system to test the intergenerational effects of stress.

Maternal stress as a predictive signal

While research has focused on short-term negative impacts of maternal stress on offspring early survival and growth (e.g., Campbell et al. 1992; Saino et al. 2005), the potential adaptive nature of maternal stress signals has recently come into focus (Sheriff and Love 2013). From biomedical research, it has been shown that pregnant women experiencing stressful conditions, such as malnutrition, are more likely to deliver preterm babies with lower birth weights (Lobel et al. 1992; Wadhwa et al. 1993). While appearing to be a cost of maternal stress, the thrifty phenotype hypothesis (Hales and Barker 1992; Hales and Barker 2001) proposes that stress during pregnancy may provide a signal that the future environment is stressful, programming a small offspring phenotype with modified metabolism. This phenotype would be adaptive under predicted low resource conditions (i.e., decreased demand), but it can be more susceptible to disease, and hence

maladaptive, when resources are readily available (Bateson et al. 2002; Gluckman and Hanson 2004). Accordingly, when offspring of stressed mothers develop in an environment that is not stressful (i.e., mismatched from the maternal environment), they may display traits that are ultimately maladaptive such as a prolonged stress response, heightened anxiety and depression, and increased susceptibility to diseases such as coronary heart disease, type 2 diabetes, and obesity (Fernandez-Twinn and Ozanne 2006; Weinstock 2008).

Manipulating exposure to maternal stress (by applying a stressor to females or directly exposing eggs to GCs) results in changes to offspring morphology (e.g., reduced body size: McCormick 1998; Love et al. 2005), physiology (e.g., altered stress response: Hayward and Wingfield 2004; Sopinka et al. 2016a), and behaviour (e.g., impaired feeding and predator avoidance: Janczak et al. 2007; McGhee et al. 2012). While these maternally-mediated effects on offspring traits have conventionally been interpreted as negative, the environmental context of offspring in relation to maternal environment is rarely considered, and traits can often be interpreted as adaptive for offspring if faced with stressful conditions (e.g., Meylan and Clobert 2005; Chin et al. 2009; Coslovsky and Richner 2011; Dantzer et al. 2013; Bestion et al. 2014). Evolved responses to environmental cues whereby fitness advantages are not observed until later in life have been more broadly referred to as predictive adaptive responses (PARs: Gluckman et al. 2005). This evolutionary framework can be used to examine whether links exist between maternal environmental conditions during reproduction (and signals thereof) and offspring phenotype and performance in captive and wild animal populations. Sheriff and Love (2013) extended this framework to free-living systems with the maternal or

environmental matching hypothesis, stating that when mother and offspring environments overlap, maternal stress exposure can serve as a predictive signal to offspring about the quality of their future environment. For example, population densities cycle with predator-prey interactions in snowshoe hares (*Lepus americanus*), and predator-induced maternal stress leads to smaller, lighter offspring with a heightened stress response, traits that are advantageous in high predation environments (Sheriff et al. 2010, 2011). Similarly, high conspecific densities induce maternal stress in coral reef damselfish (*Pomacentrus amboinensis*) and lead to smaller offspring with large yolk sacs that are better able to disperse away from the stressful, high density environment (McCormick 1998, 2006; Gagliano et al. 2007). This work emphasizes the necessity of assessing offspring traits within a relevant environment context to predict outcomes for performance and fitness; however, studies rarely directly test offspring in the appropriate matched and mismatched environments.

Importance of environmental context

The existence of adaptive maternal stress signals requires the maternal environment to be predictive of the future conditions offspring will face, and reliability of stress signals can vary according to life history (Sheriff and Love 2013). For example, in species with fluctuating population cycles such as many small mammals, population densities are predictable from one generation to the next but unpredictable across longer time scales (e.g., Sheriff et al. 2009; Dantzer et al. 2013; Bian et al. 2015). In species that provide maternal care, mother and offspring environments overlap temporally and maternal quality during the period of post-natal care affects the reliability of the stress signal (e.g.,

Love et al. 2005; Love and Williams 2008). In species where there is no post-natal care given, mother and offspring environments can still overlap spatially, and maternal stress signals can serve as a predictive cue to offspring regarding future environmental quality (e.g., Gibbs and Van Dyck 2009). Considering the life history as well as the current environmental conditions of a species will therefore help determine whether there is relevant environmental matching between mother and offspring. However, the majority of current studies manipulate the maternal environment to create stressed and non-stressed maternal treatments, but only assess offspring for certain metrics in a single, benign environment (i.e., matched to the non-stressed maternal treatment, mismatched to stressed maternal treatment). While these results indicate that exposure to maternal stress modifies offspring traits, the results lack context to examine the adaptive potential of this modified phenotype in matched or mismatched environments. Indeed, recent work has emphasized that the pre- and post-natal environment should interact to influence offspring phenotype and therefore expected performance and fitness in future environments (Sheriff and Love 2013). Studies across taxa that manipulate both the pre- and post-natal environments and assess performance metrics have garnered some support for environment matching (e.g., Zimmer et al. 2013; Shama et al. 2014; Bian et al. 2015; Merrill and Grindstaff 2015), with groups in matched conditions outperforming groups in mismatched conditions. However, outcomes are dependent on many factors, and elements such as choice of offspring metrics and life stage in which traits are measured also affect outcomes of intergenerational stress.

Assessing offspring phenotype and performance

Choosing what offspring metrics to measure is an important component of studying intergenerational effects; early-life phenotypic and fitness-related traits such as size and survival are most commonly assessed, likely due to the logistical constraints of rearing offspring long-term (Sopinka et al. 2017). While these are important metrics of fitness, interpretations are contingent on the offspring's environment (e.g., small size can both increase and decrease susceptibility to predation: Lundvall et al. 1999). Including a measure of offspring performance such as growth (e.g., Li et al. 2010), physiology (e.g., Auperin and Geslin 2008), or behaviour (e.g., Sopinka et al. 2015a), improves the extension of interpretations to biologically-relevant situations, although performance is again specific to a certain environmental context. Outcomes of performance traits also vary across life-history stages; for example, activity levels in rainbow trout (*Oncorhynchus mykiss*) from eggs treated with cortisol were unchanged at 2 months post-fertilization (mpf), and increased at 5 mpf (Colson et al. 2015). Consequently, rearing offspring in multiple environmental contexts and using an integrated approach to move past phenotype and assess performance and fitness at different life-history stages will provide the most rigorous test of the adaptive nature of maternal stress signals.

Study system

Chinook salmon (*Oncorhynchus tshawytscha*) are a Pacific salmon species native to North America and their range extends from Central California to Northwest Alaska (Healey 1991). They are an ideal species to study interactive effects of pre- and post-natal stress because of spatial overlap in maternal and offspring environment during freshwater

life stages (i.e., spawning for females, juvenile parr stage for offspring) and fluctuating climate regimes that lead to environmental variability in freshwater streams (i.e., time scale of 2–7 years: Ware 1995; Petersen and Kitchell 2001). On a fixed energy budget due to cessation of feeding, mature Pacific salmon migrate upstream from the Pacific Ocean to their natal streams to spawn once before death (i.e., semelparous life history: Groot and Margolis 1991). Across species and populations, spawning migrations vary in difficulty (i.e., distance, duration, average flow rate, elevation gain) and exposure to stressors (Cooke et al. 2011). In particular, warming water temperatures due to climate change and interactions with fisheries capture are known to greatly reduce migration and spawning success (Farrell et al. 2008; Donaldson et al. 2012; Martins et al. 2012). In the stream environment, adult female salmon must endure potentially unfavourable water conditions (e.g., low water levels/drought), avoid predation, and compete for optimal redd sites to gain reproductive success (Groot and Margolis 1991). During their reproductive migration, plasma cortisol levels of spawning adults naturally increase, which leads to tissue degradation, suppression of immune function, and eventual senescence (Robertson and Wexler 1959; Maule et al. 1996; Maldonado et al. 2002). However, spawning adults can still physiologically respond to additional stressors during migration (i.e., elevate cortisol: Cook et al. 2011), and gravid females that are repeatedly exposed to stressors have higher circulating cortisol as well as higher egg cortisol (Stratholt et al. 1997).

Previous studies in salmonids have shown diverse effects of elevated egg cortisol as a proxy of maternal stress on offspring reared in benign environments. For instance, cortisol-implanted Atlantic salmon (*Salmo salar*) females had increased egg cortisol

levels and smaller offspring with lower early survival (to first feeding: Eriksen et al. 2006). It is noted that egg cortisol levels in this study were significantly higher than those observed in stressor-exposed salmon (~100 ng/g vs. ~25 ng/g in coho salmon, *O. kisutch*: Stratholt et al. 1997). Small elevations in egg cortisol levels via hormone bath (i.e., ~3 ng/g higher than untreated eggs) lowered juvenile stress responsiveness in rainbow trout (Auperin and Geslin 2008). Larger elevations in egg cortisol levels (both supraphysiological and within concentrations observed in unfertilized eggs) both increased (Sloman 2010) and decreased (Burton et al. 2011) conspecific aggression in two studies on brown trout (*Salmo trutta*). In addition, elevations in egg cortisol levels similar to those of stressor-induced salmon increased social dominance and boldness in juvenile coho salmon (*O. kisutch*: Sopinka et al. 2015a) and reduced structural size of juvenile sockeye salmon (*O. nerka*: Sopinka et al. 2016b). While these studies underscore the idea that when the offspring environment is mismatched to the maternal stress signal (i.e., individuals receiving maternal stress signal are reared in a benign environment), offspring phenotype is altered, the adaptive potential of these signals in stressful offspring environments is largely unknown.

In the wild, fertilized salmon eggs develop in stream gravel throughout the winter, hatching and absorbing their yolk-sac before emerging from stream gravel in spring to begin feeding exogenously (fry life stage). Egg-to-fry survival in the wild is generally low (Bradford 1995), making the fry stage critical for future success, and timing of emergence and fry size are both important factors determining survival rates (Jones et al. 2015). During the parr life stage, fish rear in the freshwater environment for months to years, depending on the species and type (e.g., ocean-type vs. stream type Chinook

salmon), until downstream migration to the ocean as smolts (Groot and Margolis 1991). During this critical life-history stage, juvenile salmon parr may be exposed to similar stressors their mothers were exposed to during spawning. Young salmon must evade aerial and aquatic predators, compete for optimal habitat among con- and heterospecifics, and withstand variable stream conditions (Groot and Margolis 1991). Survival and sufficient growth during freshwater rearing is necessary for successful migration of juveniles to the ocean as smolts (Groot and Margolis 1991), and novel stressors such as high stream temperatures and low flows have been linked with greater mortality rates in juvenile Chinook salmon populations (Crozier and Zabel 2006). Juvenile salmon must physiologically respond to these stressors (i.e., mount the stress response) to ensure survival, and water conditions (e.g., flow, temperature) in semi-natural stream channels have been linked to changes in resting cortisol levels (Flodmark et al. 2004; Kuehne et al. 2012). Behaviour of stream-dwelling salmon is known to be influenced by a trade-off between survival and growth; since predation risk is high in daytime but foraging is much more efficient, individuals preparing to migrate downstream are often diurnal (i.e., active in daytime, inactive at nighttime) to promote growth, but this strategy can come at the cost of survival (Metcalfe et al. 1998). Overall, juvenile salmon in stream environments are faced with many abiotic (e.g., water temperature, flow, depth) and biotic (e.g., competitive interactions, predators) factors that are similar to those faced by females during spawning migrations, and affect performance and ultimately determine survival.

Thesis objectives

My thesis tested predictions of environmental matching theory by examining short-and longer-term impacts of a pre-natal stress signal on offspring phenotype and performance in matched and mismatched post-natal conditions. Specifically, I used a manipulative approach at the egg stage (hormone bath) to simulate pre-natal (maternal) stress exposure, then lowered water levels (to partially mimic conditions during a drought) in semi-natural stream channels as a post-natal environmental stressor (Figure 1.1). I measured a suite of offspring metrics related to energetics (i.e., baseline cortisol, size, growth), coping with environmental stress (i.e., stress response, behaviour), and fitness (i.e., survival) across multiple important life stages (Figure 1.2). By measuring phenotype and performance within relevant environmental conditions (i.e., matched and mismatched), I tested the hypothesis that pre-natal stress acts as a predictive signal of environmental quality; therefore, individuals in matched pre- and post-natal stressful conditions should display phenotypic and fitness-related traits that indicate higher performance compared to mismatched individuals not primed for stressful conditions. My thesis emphasizes the importance of applying relevant post-natal environmental stressors and considering non-linear and trait-specific responses when investigating the intergenerational effects of stress.

In **Chapter 2**, I first assess whether multiple pre-natal stress signals (low cortisol dose, high cortisol dose, control dose) have differential, short-term effects on early survival, development, size, and morphology in Chinook salmon offspring at yolk-sac absorption (fry life stage). In **Chapter 3**, I examine the interactive longer-term effects of pre- and post-natal stress on energy demand (baseline cortisol), stress responsiveness

(post-stress cortisol), size, and survival of juvenile parr in fresh water, and growth and survival of smolts in salt water. By rearing offspring in freshwater semi-natural stream channels, I was able to apply a relevant, post-natal environmental stressor by lowering water levels in channels to simulate drought conditions. In **Chapter 4**, I characterize diel behaviour of individuals in semi-natural stream channels to explore effects of pre- and post-natal stress on shifts in behaviour and the relationship among behaviour, growth, and survival. Individual movement among microhabitats created within stream channels was tracked and used to quantify activity, exploration, and risk aversion behaviours, which provide insight into the trade-off between growth and survival under different levels of risk (i.e., increased perceived risk in low water). By assessing key offspring traits across multiple life stages (Figure 1.2), my thesis as a whole represents a novel examination of how stress experienced within and across generations shapes performance and fitness of an economically and ecologically important species.

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Figures

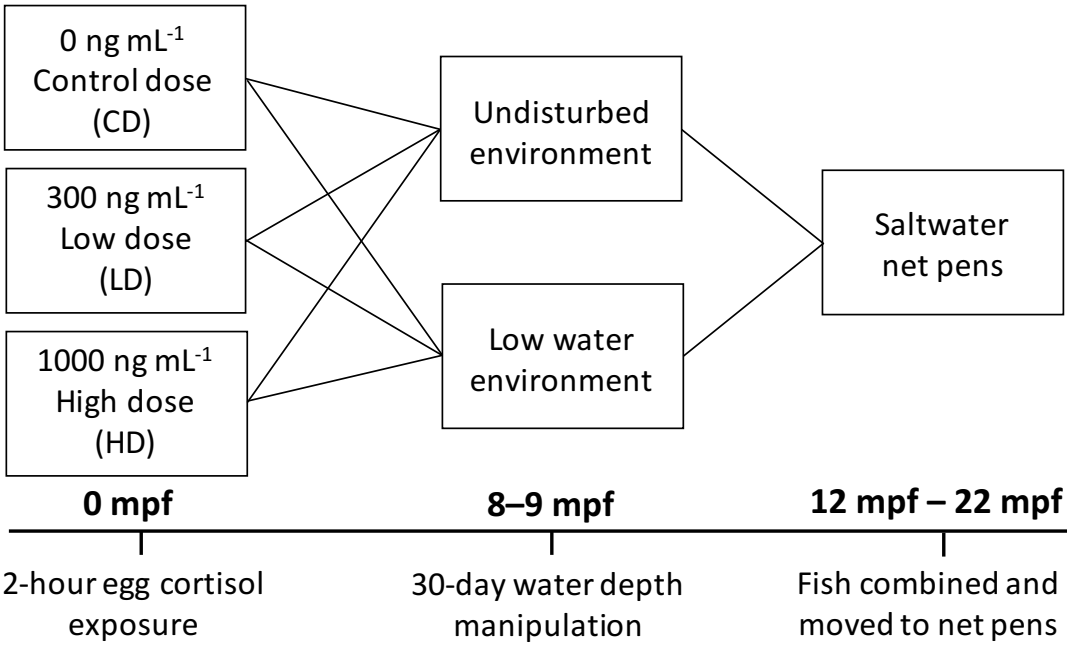

Figure 1.1 Outline of the thesis experimental design. Immediately post-fertilization, Chinook salmon eggs were treated with cortisol (control dose, low cortisol dose, or high cortisol dose) as a signal of pre-natal stress. Egg cortisol treatment groups were transferred to semi-natural stream channels and from 8–9 months post-fertilization (mpf), channels (n=2 replicates per treatment) were exposed to undisturbed or low water depth conditions for a 30-day period as a decrease in post-natal environmental quality (i.e., post-natal stress). All fish were then combined and transferred to 2 saltwater net pens and monitored from 12–22 mpf.

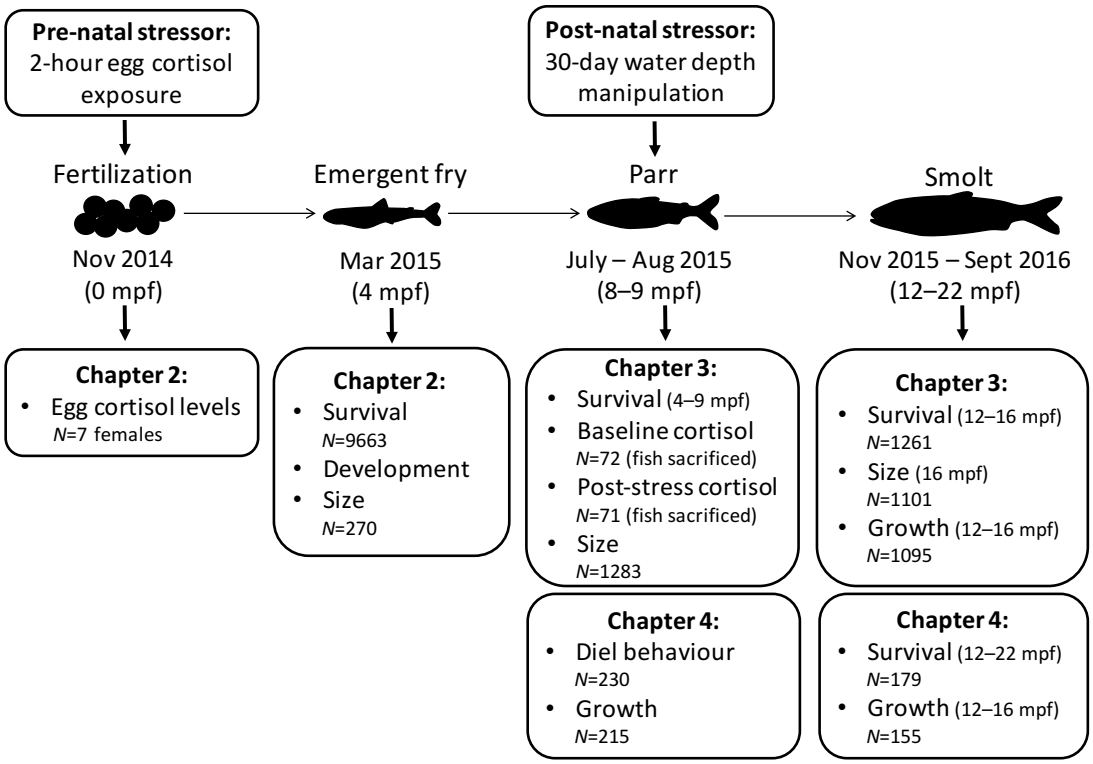

Figure 1.2 Timeline of Chinook salmon life stages outlining when experimental measures were taken for each thesis chapter and indicating phenotypic and performance traits measured with associated sample sizes.

CHAPTER 2 – PRE-NATAL STRESS EXPOSURE GENERATES HIGHER EARLY SURVIVAL AND SMALLER SIZE WITHOUT IMPACTING DEVELOPMENTAL RATE IN A PACIFIC SALMON[1]

Introduction

Maternally-derived hormones play a major role in the early development of vertebrates (Groothius and Schwabl 2008; Nesan and Vijayan 2013), and determining how they shape offspring phenotype as well as how this phenotype interacts with environmental variation to affect fitness is a rapidly emerging field of research (Meylan et al. 2012). In particular, glucocorticoid (GC) hormones manage energy balance at baseline levels (Landys et al. 2006) and facilitate homeostatic return in response to acute stressors (Sapolsky et al. 2000; Barton 2002). Circulating levels of GCs in reproductive females depend on individual condition and environment (Love et al. 2005; McCormick 2006; Sheriff et al. 2009, 2011), thereby providing a link among maternal state, reproduction, and quality of progeny (Schreck et al. 2001; Love et al. 2005). Indeed, stressful environments due to natural or anthropogenic perturbations have the potential to shape offspring phenotype through hormonal mechanisms (Leatherland et al. 2010; Meylan et al. 2012; Love et al. 2013), such as maternal transfer of elevated levels of GCs to offspring (Saino et al. 2005; McCormick 2006; Dantzer et al. 2013).

Exposure to elevated maternal GCs is known to impact offspring phenotypic traits (e.g., smaller size, slower growth, morphological changes) and outcomes are typically deemed a maladaptive outcome for offspring (Saino et al. 2005; Eriksen et al. 2006). However, less work has examined how and why this phenotypic variation impacts fitness

[1]This chapter is a result of joint research with C. Semeniuk, N. Sopinka, J. Heath, and O. Love, and is being resubmitted to *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*

metrics such as early survival and performance (Sheriff and Love 2013). It has been suggested that phenotypic traits such as small size and slower growth may actually be adaptive responses in preparation for expected lowered resources in the future environment (i.e., predictive adaptive responses (PARs): Gluckman et al. 2005). Evolutionary ecologists have extended the PAR framework from laboratory mammals and humans to free-living systems (i.e., maternal or environmental matching: Love et al. 2005; Love and Williams 2008) to test the adaptive potential of maternal stress (Sheriff and Love 2013). For example, European starlings (*Sturnus vulgaris*) exposed to elevated embryonic GCs (a pre-natal stress signal) were smaller at independence (Love and Williams 2008), but had improved flight performance, presumably in response to expected increased predation risk (Chin et al. 2009). In addition, Dantzer and colleagues (2013) found that wild red squirrels (*Tamiasciurus hudsonicus*) with experimentally elevated GCs produced faster growing offspring, which is positively associated with fitness in this population when high densities occur. Despite these well-rooted hypotheses, further research needs to be conducted at more specific life-history stages and in species where mothers and offspring share a common environment to appreciate how complex phenotypic responses interact with environmental variation to impact fitness (Sheriff and Love 2013).

Pacific salmon are an ideal group of species to explore impacts of stressors across generations, as adults undergo arduous migrations from the ocean to freshwater natal spawning grounds and encounter multiple anthropogenic stressors (e.g., fisheries capture, warming water temperatures) that can reduce migration success (Donaldson et al. 2012; Martins et al. 2012). While baseline levels of circulating cortisol (the primary GC in fish)

are known to increase in mature female salmon during migration to spawning areas (Baker and Vynne 2014), individuals remain responsive to acute stressors in the freshwater enviroment, and levels of circulating cortisol are linked to survival on spawning grounds (Cook et al. 2011; McConnachie et al. 2012). Furthermore, maternally-derived cortisol is present in salmon eggs and can increase following maternal exposure to a repeated stressor (Stratholt et al. 1997). Pacific salmon have a semelparous life-history, undergoing a single breeding attempt, and provide little parental care (Groot and Margolis 1991); therefore, egg cortisol-mediated effects on offspring phenotype are expected to have significant fitness consequences for mothers, suggesting that maternal GC transfer should be under strong selection (Love et al. 2009, 2013). Moreover, studies in salmonid species that have experimentally elevated egg cortisol levels via egg immersion have shown that cortisol can be an important regulator of offspring phenotype (Auperin and Geslin 2008; Li et al. 2010; Sloman et al. 2010; Burton et al. 2011; Colson et al. 2015; Sopinka et al. 2015, 2016).

In this study, we explore the effects of biologically-relevant egg cortisol exposures (low dose, high dose, control) on three important early life-history traits in Chinook salmon (*Oncorhynchus tshawytscha*): offspring survival, developmental rate, and morphology. Survival was monitored to the embryonic stage (eyed egg stage) as well as to first-feeding (fry stage), and rate of development (based on degree of yolk sac absorption) and morphology were measured at the fry stage. We focused on the egg-to-fry stage since these three early life-history traits all contribute to determining the success of juvenile salmon in fresh water (Einum and Fleming 2000; Jones et al. 2015). We hypothesized that a signal of pre-natal stress (elevated egg cortisol) would lead to an

accelerated developmental trajectory based on other fish studies showing higher heart rates (McCormick and Nechaev 2002), as well as earlier hatching (Gagliano and McCormick 2009) and faster yolk-sac absorption (Mathiyalagan et al. 1996) in cortisol-dosed embryos. We also predicted that elevated egg cortisol would lead to smaller-sized fry (McCormick 1999; Burton et al. 2011), and that these developmental differences could also impact egg-to-fry survival. Finally, low and high cortisol exposure doses were chosen with the prediction that they could represent optimal and supra-optimal doses, respectively, since effects of stress are often non-linear (Schreck 2010). Therefore, we predicted that there would be phenotypic differences in offspring from the low and high cortisol dose groups, with the low dose showing positive effects and the high dose showing negative effects compared to controls (Li et al. 2010).

Methods

Fish origin

This study was completed at Yellow Island Aquaculture Ltd. (YIAL), a small Chinook salmon farm on Quadra Island in British Columbia, Canada. YIAL's domestic stock originated in 1985 from gametes taken from Robertson Creek and Big Qualicum hatcheries on Vancouver Island (see Lehnert et al. 2014 for further details). The females whose eggs were used in the current study were the progeny of a self-crossed fish that was bred in Autumn 2009 (Komsa 2012). This breeding design allowed us to minimize the influence of genetic maternal effects on early survival and size (Burt et al. 2012) by using a more homogeneous pool of eggs.

Egg cortisol treatment

In Autumn 2014, eggs were collected from 7 females which were euthanized by cerebral percussion. Three unfertilized eggs were taken from each female and frozen at –80ºC for cortisol analysis to compare natural levels of pre-fertilization egg cortisol among females. For each female, we measured total body mass (mean ± SE: 3.41 ± 0.30 kg, range: 2.09–4.49 kg), fork length (62.2 ± 1.6 cm, 55.4–67.7 cm), and total ovary mass (849.65 ± 88.41 g, 476.49–1159.16 g). Individual egg mass was measured by weighing 3 sets of 10 eggs, dividing the average weight by 10, and taking the average of the 3 sets. Mean ± SE individual egg mass was 0.29 ± 0.01 g and ranged from 0.27–0.32 g. Eggs from all 7 females were pooled prior to fertilization to reduce female-specific maternal effects among egg cortisol treatment groups. Pooled eggs were split into 18 containers of 180 g of eggs (~600 eggs), with 6 replicate containers assigned to each of the 3 egg cortisol treatment groups. Milt was collected from 5 males by applying pressure to the abdomen and then pooled. Containers of eggs were fertilized with ~1 mL of pooled sperm, and egg-milt mixtures were left for 2 minutes before adding hatchery water (Shrimpton et al. 2012). After 2 minutes (at which time sperm would no longer be motile: Hoysak and Liley 2001), egg-milt mixtures were immersed in water containing either a 1) low cortisol dose (300 ng ml^{-1}), 2) high cortisol dose (1000 ng ml^{-1}), or 3) control dose (0 ng mL^{-1}) for a 2-hour treatment period (Sopinka et al. 2015, 2016). The low and high cortisol doses contained cortisol powder (H4001, Sigma) dissolved in 90% ethanol (HPLC grade, Sigma; 7.5×10^{-6} low dose final concentration, 2.5×10^{-5} high dose final concentration) and diluted in hatchery water, while the control solution contained water with the same concentration of ethanol as the low dose. Low and high cortisol dose concentrations were

chosen based on previous work that used similar concentrations in salmonid species and showed that dosages result in biologically-relevant elevations in egg cortisol content (Auperin and Geslin 2008; Sopinka et al. 2015, 2016) and can lead to biphasic effects on offspring phenotype (Li et al. 2010).

Following the exposure period, eggs were washed thoroughly with hatchery water and placed in a flow-through, vertical-stack incubator. Eggs from each replicate container were split into 3 replicate cells (18 total replicates per treatment group) and randomly placed among 4 trays (each tray divided into 16 cells) within the stack. Three eggs were collected from each replicate cell (54 total replicate cells across all egg treatment groups) 2- and 24-hours post-fertilization (hpf) and frozen at –80ºC to determine the level and duration of cortisol elevation following the cortisol treatment. At the eyed stage, embryos were counted and dead embryos were removed from each replicate cell. Survival was then monitored every 3 days until the fry stage by removing and recording any dead embryos. Mean ± SE water temperature during incubation was 7.4 ± 0.2 ºC, and ranged from 5.5–8.9 ºC. Mean ± SE dissolved oxygen concentration was 10.2 ± 0.2 mg L^{-1} and ranged from 9.1–11.3 mg L^{-1}.

Developmental rate and morphology

At 4 months post-fertilization (fry life stage), we randomly sampled 5 fry from each replicate cell (n=90 fry per egg cortisol treatment group), recorded their body mass (to the nearest 0.01 g), and took a photograph (Canon EOS Rebel XT) for future morphometric analysis. Digital photographs were analyzed using ImageJ (http://imagej.nih.gov/ij/). Measurements taken included fork length (FL), gape (GAPE),

3 measures of body depth (depth from dorsal fin perpendicular to fish length (BD1), depth to widest point of yolk sac protrusion (BD2), depth from dorsal fin to anal fin (BD3)), caudal peduncle width (PED), and caudal fin width (CAUD) (Figure 2.1; Table 2.1). Additional body-depth measurements were taken to account for variation among individual fry in depth based on amount of residual yolk sac. From this variation in residual yolk sac, a measure of developmental rate was assessed from the digital photographs by assigning a 0–3 ranking for each fry based on protrusion of the yolk sac (Figure 2.1). A rank of 0 indicated minimal yolk sac protrusion (higher degree of yolk sac absorption) and a rank of 3 indicated maximal yolk sac protrusion (lower degree of yolk sac absorption).

Egg cortisol assays

We determined egg cortisol levels with enzyme immunoassays (EIA - Cayman Chemicals, Ann Arbor, USA) using methods modified from Sopinka et al. (2015). Briefly, 3 eggs per container replicate (or 3 eggs per female for unfertilized eggs) were homogenized with 1200 uL of assay buffer, and 3 mL diethyl ether was added. Samples were vortexed for 30 seconds and then centrifuged for 5 minutes at 4000 rpm. Samples were allowed to settle for 30 minutes, flash frozen at –80 ºC for 30 minutes, and then the liquid layer was decanted and evaporated overnight in a fume hood. Dried samples were reconstituted with 1200 uL of assay buffer. To check for parallelism across multiple dilutions and determine an optimal dilution factor, a pooled sample was extracted as described above and a serial dilution performed using assay buffer (1:10, 1:20, 1:40, 1:57, 1:81, 1:116). Samples from the serial dilution were run on a single Cayman cortisol plate.

Following this optimization, all samples were diluted using a 1:57 dilution factor and run in triplicate wells following kit insert instructions. Three plates were run and read with a plate reader at 412 nM wavelength. Intra- and inter-assay coefficients of variation were 4.5% and 10.0%, respectively.

Statistical analyses

Statistical analyses were completed using R version 3.2.4 (R Core Team 2016). Model assumptions were assessed by graphical inspection: residuals versus fitted values were plotted to verify homogeneity, and quantile-quantile plots and histograms of the residuals were plotted to verify normality. A two-way ANOVA with time (2 hpf, 24 hpf), egg cortisol treatment (control, low dose, high dose), and a time × egg cortisol treatment interaction was used to analyze differences in egg cortisol levels. Following a significant interaction term, post hoc slice tests were used to test for differences among groups for both fixed effects at the level of the other fixed effect using the lsmeans package (Lenth 2016). Survival to eyed and fry stages was totaled per replicate cell (number dead and number alive) and data were converted to binary form with 0 representing a dead embryo and 1 representing an alive embryo (buildbinary function in the fullfact package; Houde and Pitcher 2016). To examine survival differences to the eyed and fry stages, generalized linear mixed models for binary data were fit with the logit link function (lme4 package; Bates et al. 2015). Replicate, treatment container, and incubation tray position were included in the models as random effects. Likelihood ratio tests were used to compare model fit and test significance of the fixed effect (egg cortisol treatment). To examine differences in fry morphology, a Principal Component Analysis (PCA) was

used. Morphological traits (mass, FL, GAPE, BD1, BD2, BD3, PED, CAUD; Figure 2.1; Table 2.1) were loaded into a PCA to reduce redundancies and find trends in fry body size and shape based on multiple measurements. Two components were extracted based on the Kaiser criterion (eigenvalue >1) and visual inspection of variance plots (Table 2.2). PC1 (eigenvalue 3.2) explained 40% of the variation and represented structural size (PC1 positively correlated with mass, FL, CAUD, and BD1, BD2, and BD3). PC2 (eigenvalue 1.6) explained an additional 21% of the variation and represented developmental rate (PC2 positively correlated with FL, GAPE, and PED, and negatively correlated with BD1 and BD2). Effects of egg cortisol treatment on PC1 and PC2 were assessed using linear mixed models (lme4 package; Bates et al. 2015). Categorical yolk sac rankings were compared among egg cortisol treatments using a cumulative link mixed model for ordinal data (ordinal package; Christensen 2015). All models included replicate, treatment container, and incubation tray position as random effects. Significance of the fixed effect was again tested using likelihood ratio tests fit with maximum likelihood, and then final models were fit with restricted maximum likelihood estimation. For significant models (P<0.05), pairwise differences between treatment groups were assessed using Tukey post hoc tests (lsmeans package; Lenth 2016).

Results

Egg cortisol levels

Egg cortisol treatment interacted with time to affect levels of egg cortisol ($F_{2,30}$=5.07, P=0.01; Figure 2.2). Eggs that were treated with the high cortisol dose had higher cortisol levels immediately following the exposure period (2 hpf) compared to control dose eggs

(P=0.03) and tended to have higher levels than the low dose eggs (P=0.052). Cortisol levels in the high dose eggs were elevated within one standard deviation of the cortisol levels measured in non-manipulated, unfertilized eggs (mean: 14.07 ng g^{-1}, SD: 7.45 ng g^{-1}, range: 5.02–27.21 ng g^{-1}), confirming that the high dose treatment represented a biologically-relevant elevation in egg cortisol. We found no difference in egg cortisol levels between the low dose eggs and control eggs (P=0.95) immediately following the exposure period. Egg cortisol levels no longer differed among the three treatment groups twenty-four hours after the exposure period; however, the high dose treatment showed a significant decrease in egg cortisol levels from 2 to 24 hpf (P=0.002; Figure 2.2), while levels in the control dose and low dose groups did not change from 2 to 24 hpf (control dose: P=0.31; low dose: P=0.19).

Early survival

There was a significant effect of egg cortisol treatment on survival to the eyed stage (χ^2=22.82, df=2, P<0.0001; Figure 2.3a), with all three treatment groups being significantly different from each other (high versus low: P=0.007, high versus control: P=0.0005, low versus control: P<0.0001). Survival to the eyed stage was highest for low cortisol dose eggs (mean ± SE: 77.5 ± 1.5 %), intermediate for high cortisol dose eggs (63.7 ± 2.6 %) and lowest for control dose eggs (43.4 ± 2.4 %).

There was also a significant effect of egg cortisol treatment on survival to the fry stage (χ^2=22.55, df=2, P<0.0001; Figure 2.3b). Survival to the fry stage of low and high cortisol-treated eggs was significantly higher compared to survival of control dose eggs (both P<0.0001). However, survival of low and high cortisol dose eggs did not differ

from one another (P=0.07). Survival to the fry stage was 65.2 ± 1.5 % for low cortisol dose eggs, 52.2 ± 2.9 % for high cortisol dose eggs and 28.0 ± 2.6 % for control dose eggs.

Developmental rate and morphology

Egg cortisol treatment had a significant effect on structural size (PC1 scores: χ^2=6.99, df=2, P=0.03; Figure 2.4a), with fry from high cortisol dose eggs having a tendency to be structurally smaller compared to fry from control dose eggs (P=0.056). However, fry from low cortisol dose eggs were not different in structural size compared to fry from high cortisol dose eggs (P=0.85) or fry from control dose eggs (P=0.15). Egg cortisol treatment did not have a significant effect on rate of development (PC2 scores: χ^2=5.02, df=2, P=0.08; Figure 2.4b). In addition, when assessed categorically based on yolk sac protrusion (see Figure 2.1), developmental rate did not differ among egg cortisol treatments (χ^2=2.90, df=2, P=0.23).

Discussion

Importance of early survival for future success

Compared to offspring reared from control eggs, we found 37% and 24% higher survival of fry reared from eggs exposed to a low and high cortisol dose, respectively. While elevated egg cortisol levels have been reported to negatively affect embryonic survival (Eriksen et al. 2006; Li et al. 2010), offspring survival is often not affected when egg cortisol levels are elevated within a physiologically relevant range (Auperin and Geslin 2008; Sloman 2010; Burton et al. 2011; Colson et al. 2015; Sopinka et al. 2015, 2016).

Our results are the first to our knowledge to demonstrate survival benefits following exposure to elevated cortisol immediately post-fertilization, particularly relatively small increases (i.e., low dose here); however, the mechanisms by which exogenous cortisol applied post-fertilization functions to mimic those observed in eggs with endogenously elevated maternally derived cortisol warrants further study. In the wild, egg-to-fry survival of Pacific salmon ranges from ~7–20% (Bradford 1995), meaning that the fry stage is a critical period which determines future success of juveniles migrating to the ocean (Groot and Margolis 1991). As such, our results suggest that variation in egg cortisol among female salmon contributes to early survival differences, yet little is known about the extent of egg cortisol variation within and between individuals in a population, or the ecological/evolutionary underpinnings for such variation (Sopinka et al. 2017). Moreover, these findings highlight the complex nature of hormonally-mediated effects and that impacts of increasing doses are not consistently linear (Gagliano and McCormick 2009; Li et al. 2010), reaffirming the importance of using multiple exposure doses to properly represent the spectrum of phenotypic effects. Although our breeding design (i.e., self-crossed strain of females) was specifically used to reduce maternal effects, the design may be responsible for the low (28%) survival of control dose offspring. Pure crosses using this strain led to reduced survival and growth at 7, 11, and 17 months post-fertilization, possibly indicative of inbreeding depression, although gamete quality was not assessed (Komsa 2012). Hybrid crosses performed between the self-crossed female strain and YIAL males in Autumn 2015 (i.e., same breeding design as the current study) showed that egg-to-fry survival for untreated eggs (i.e., not exposed to

cortisol) was again low (38%), indicating a repeatable degree of survival (Capelle, unpublished data).

Mechanisms underlying variation in survival

While only the high cortisol dose (1000 ng mL^{-1}) successfully elevated egg cortisol within a biologically-relevant range following the 2-hour exposure period, we argue that the low cortisol dose (300 ng mL^{-1}) may have cleared from the eggs rapidly but still provided a pre-natal stress signal to the developing embryo, given the early survival differences. While initial research assumed that steroid hormones in eggs of oviparous species moved passively due to their lipophilic nature, recent work has shown that the process can instead be highly dynamic (Moore and Johnston 2008). In fishes, ovarian follicles and fertilized eggs/embryos metabolize cortisol to cortisone and cortisol- and cortisone sulfates (Tagawa et al. 2000; Li et al. 2012), and cortisol metabolites were detectable *in vitro* within 4 hours in ovarian follicles co-incubated with radio-labelled cortisol (Li et al. 2014). In zebrafish (*Danio rerio*), *in vitro* cortisol treatment of ovarian follicles for 4 hours increased expression of 11beta-hydroxysteroid dehydrogenase 2 (11β-HSD2), an enzyme that rapidly metabolizes cortisol to its inactive form, cortisone (Faught et al. 2016). Cortisol may also be actively transported out of the egg via ATP-binding cassette (ABC) transporters that are known to regulate cortisol uptake within 6 hours in threespined stickleback (*Gasterosteus aculeatus*) eggs immersed in a cortisol solution (Paitz et al. 2016). We found decreases in egg cortisol from 2 to 24 hpf in the high cortisol dose, which is generally observed in fish eggs post-fertilization (e.g., Sopinka et al. 2016). We did not find decreases in control or low dose groups, which is

also reported in some egg cortisol exposure studies (Auperin and Geslin 2008), but could have been due to low sample sizes and pooling of eggs across females.

Our survival results are novel in showing a positive effect on early survival (both to the eyed and fry stages) following a biologically-relevant and temporally short (i.e., <24 hours) exposure to exogenous cortisol. We propose that increased cortisol can adaptively program early development (and therefore survival to eyed/fry stages) through glucocorticoid receptor (GR) signaling in eggs. In zebrafish, maternally-derived GR mRNA transcripts are abundant in unfertilized eggs and early-stage embryos (Pikulkaew et al. 2010). Maternal GR deficiency early in embryogenesis is associated with the up- and down-regulation of hundreds of other mRNA transcripts and leads to deformities and compromised embryo viability (Pikulkaew et al. 2011). Moreover, experiments elevating embryonic cortisol and/or performing GR knockdowns have shown the essential role of cortisol-mediated GR signaling in cytogenesis, mesoderm formation, and muscle development (Hillegass et al. 2008; Nesan et al. 2012; Kleppe et al. 2013). While the negative developmental implications of over-exposure to cortisol (outside of the normal physiological range) are clear, less is known about how biologically-relevant elevations in egg cortisol can impact early survival. We propose that increased levels of cortisol bound to maternal GR led to changes in the regulation of other maternal mRNA transcripts during early embryogenesis (Pikulkaew et al. 2011), ultimately promoting embryo viability.

Effects of elevated cortisol on offspring developmental rate and size

We found that fry reared from eggs exposed to a high cortisol dose were structurally smaller than fry reared from control dose eggs, which is in agreement with previous work in oviparous taxa (McCormick 1999; Meylan and Clobert 2005; Saino et al. 2005; Burton et al. 2011). Other studies have demonstrated that effects of elevated egg GCs on body size are not consistently linear (Li et al. 2010), occur at different life stages (Burton et al. 2011), and can be species-specific (Sopinka et al. 2016). For example, nestlings exposed to elevated pre-natal corticosterone were lighter at hatching but heavier at fledging due to compensatory growth (European starling: Love et al. 2005, 2008; House wren (*Troglodytes aedon*): Strange et al. 2016). Our results can be interpreted as a result of cortisol-mediated GR signaling early in embryogenesis, as GR knockdown in zebrafish leads to delayed hatching and embryos with shorter body length (Nesan et al. 2012; Wilson et al. 2016). As predicted, we found that egg cortisol treatment affected size but had no impact on developmental rate. This suggests that timing of yolk sac absorption should have been similar across treatment groups in the wild and fry would have been in competition for resources, with size therefore being a determinant of survival. Body size is an important fitness metric and fry length can be positively correlated with survival (Einum and Fleming 2000); this suggests fry reared from high dose eggs would have been at a disadvantage by being smaller. However, we cannot make conclusions about the outcome of a small body size without appreciating the future interactive role of environmental effects (Love and Williams 2008; Sheriff and Love 2013), since investing in offspring with a small phenotype may increase offspring fitness in stressful environments (e.g., high predation risk environment; Chin et al. 2009; Sheriff et al. 2009,

2011). For example, juvenile salmon that rear in freshwater streams with high predation may benefit from being small if they are less likely to be detected by predators, but a small phenotype may make juvenile salmon more susceptible to predation if they display lower swimming performance and are slower to escape predators (Lundvall et al. 1999). As such, offspring phenotypic responses to elevated GCs during egg/embryo development and their expected fitness outcomes are complex and depend on environmental context (Sheriff and Love 2013).

Conclusions

We show that a brief exposure of Chinook salmon eggs to biologically-relevant concentrations of cortisol immediately post-fertilization can lead to increased early survival and smaller body size, without changing developmental rate. Overall, we provide evidence for a biphasic effect of pre-natal stress exposure, with positive effects observed following a low cortisol dose (increased early survival), and a positive impact coupled with an apparently negative effect following a high cortisol dose (increased early survival and smaller size). If egg cortisol levels function as a general representation of a female's stress load prior to reproduction, a short-term increase in egg cortisol may represent a female encountering acute stressors (Stratholt et al. 1997). The low dose may have been an optimal pre-natal stress signal that programmed an early survival benefit, while the high dose was supra-optimal by resulting in a small body-size phenotype (Schreck 2010). Alternatively, the high dose may have signaled to developing embryos that future stream conditions were stressful, and the early survival benefit and small body-size phenotype could have been adaptive under challenging environmental conditions (Sheriff and Love

2013). Future work should tease apart these possibilities by following individuals across life-history stages to assess performance in different environments that match and do not match the maternal environment. Advancing this field of reproductive ecology will ultimately help quantify the transgenerational fitness implications of hormonally-mediated changes in offspring phenotype.

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Tables

Table 2.1 Morphological traits (mean ± SE) of Chinook salmon at first-feeding (fry) originating from eggs treated with a control dose, low cortisol dose, or high cortisol dose.

Trait	Control (*n*=90)	Low dose (*n*=90)	High dose (*n*=90)
Body mass (g)	0.44 ± 0.003	0.43 ± 0.004	0.42 ± 0.004
Fork length (mm)	36.13 ± 0.09	35.91 ± 0.07	35.69 ± 0.06
Gape (mm)	7.46 ± 0.02	7.43 ± 0.03	7.38 ± 0.02
Body depth 1 (mm)	6.53 ± 0.05	6.27 ± 0.05	6.30 ± 0.04
Body depth 2 (mm)	8.10 ± 0.05	7.80 ± 0.05	7.86 ± 0.04
Body depth 3 (mm)	6.46 ± 0.04	6.31 ± 0.03	6.28 ± 0.04
Caudal peduncle width (mm)	1.91 ± 0.02	1.96 ± 0.01	1.94 ± 0.01
Caudal fin width (mm)	7.04 ± 0.04	6.96 ± 0.04	6.90 ± 0.04

Table 2.2 PCA loadings (PC1 – structural size; PC2 – developmental rate) for morphological traits of Chinook salmon at first-feeding (fry) where bolded values (>0.3) indicate loadings that contribute significantly to the PC scores.

Trait	PC1 loading	PC2 loading
Eigenvalue	3.2	1.6
% variance explained	40	21
Body mass	**0.42**	−0.02
Fork length	**0.37**	**0.39**
Gape	0.28	**0.52**
Caudal fin width	**0.36**	0.13
Body depth 1	**0.42**	**−0.43**
Body depth 2	**0.36**	**−0.48**
Body depth 3	**0.40**	−0.05
Caudal peduncle width	0.12	**0.38**

Figures

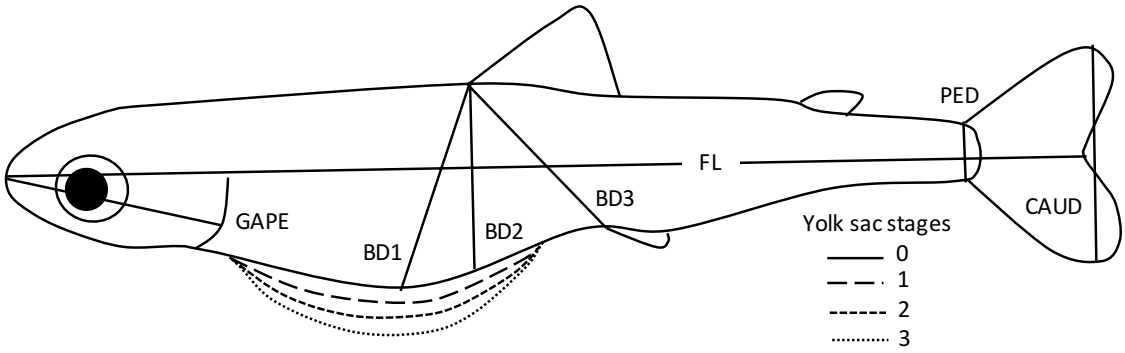

Figure 2.1 Morphological measurements taken from digital photographs of Chinook salmon at first-feeding (fry). Measurements include fork length (FL), gape (GAPE), 3 measures of body depth (BD1, BD2, BD3), caudal peduncle width (PED), and caudal fin width (CAUD). Rate of development was assessed by assigning a 0–3 ranking for each fry based on protrusion of the yolk sac, where a rank of 0 indicated minimal yolk sac protrusion (higher degree of yolk sac absorption) and a rank of 3 indicated maximal yolk sac protrusion (lower degree of yolk sac absorption).

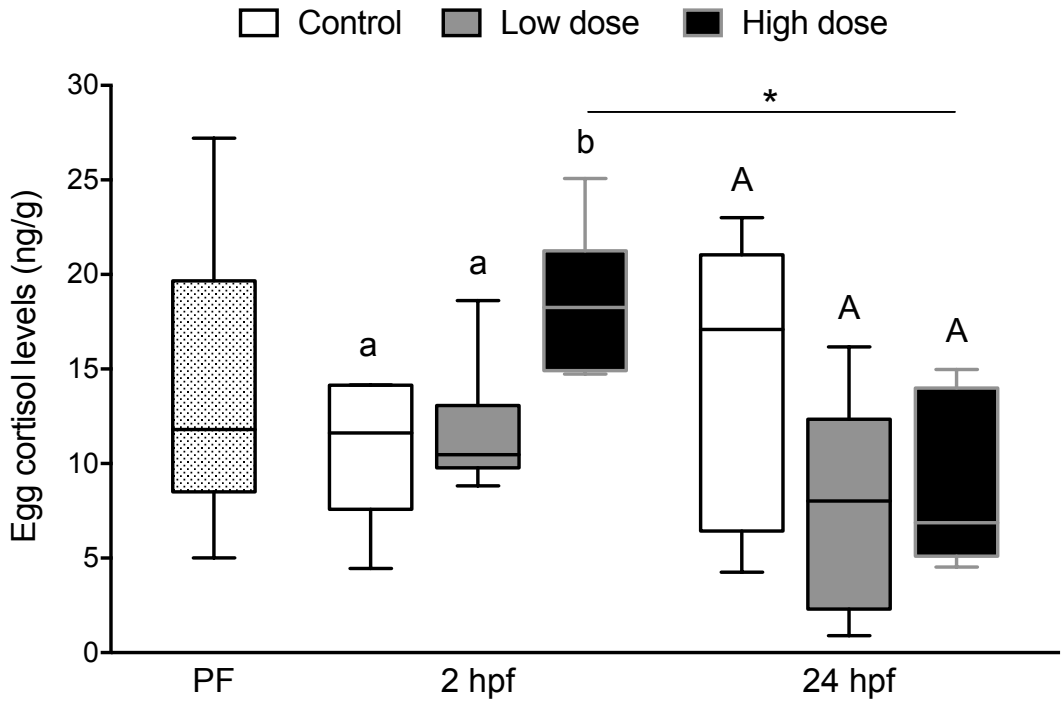

Figure 2.2 Egg cortisol concentrations in pre-fertilization (PF) eggs (*n*=7 females), and eggs treated with a control dose (0 ng ml^{-1}; *n*=6 pooled egg samples), low cortisol dose (300 ng ml^{-1}; *n*=6 pooled egg samples), or high cortisol dose (1000 ng ml^{-1}; *n*=6 pooled egg samples) immediately following the exposure period (2 hours post-fertilization (hpf)) and 24 hpf. The middle line represents the median egg cortisol concentration, the boxes represent the first and third quantiles, and the whiskers represent the maximum and minimum values. Different lower-case letters represent a significant difference (control dose-high dose; $P<0.05$) and marginally significant difference (low dose-high dose; $P=0.052$) between egg cortisol groups at 2 hpf based on slice tests following a significant time by egg cortisol treatment interaction. The asterisk represents a significant difference ($P<0.05$) between egg cortisol levels 2 and 24 hpf in the high dose group, based on a slice test following the significant time by egg cortisol treatment interaction.

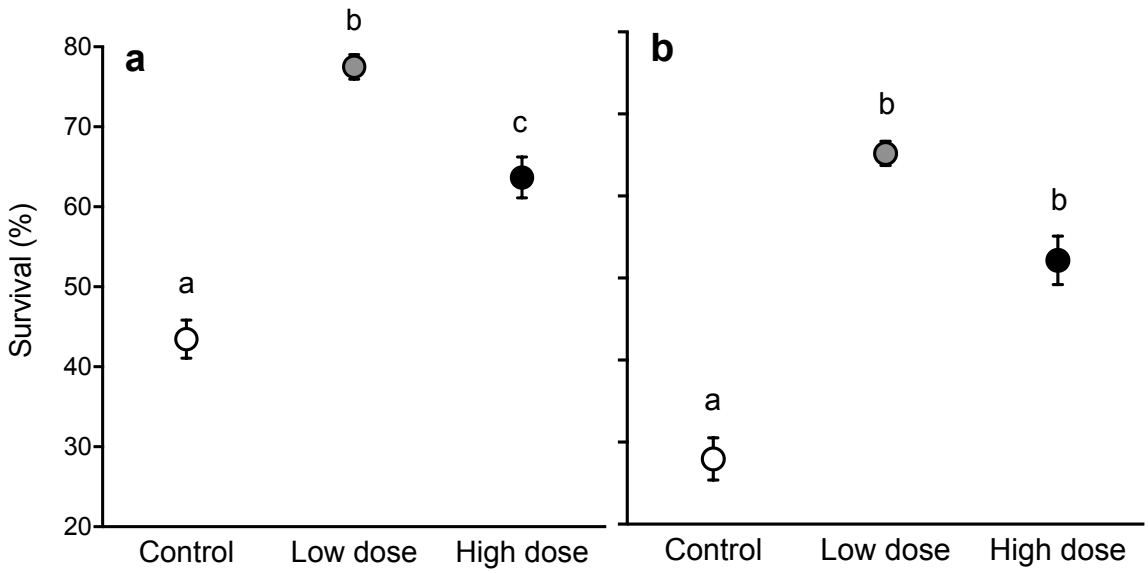

Figure 2.3 Percent survival (mean ± SE) to (a) embryonic (eyed) stage and (b) first-feeding (fry) stage for Chinook salmon embryos/fry originating from eggs treated with a control dose, low cortisol dose, and high cortisol dose (n=18 replicates per treatment). Data presented based on % average survival per replicate cell while statistical analyses were performed on binary response data (0, 1) per individual. Different letters represent significant differences based on Tukey post hoc tests (P<0.05).

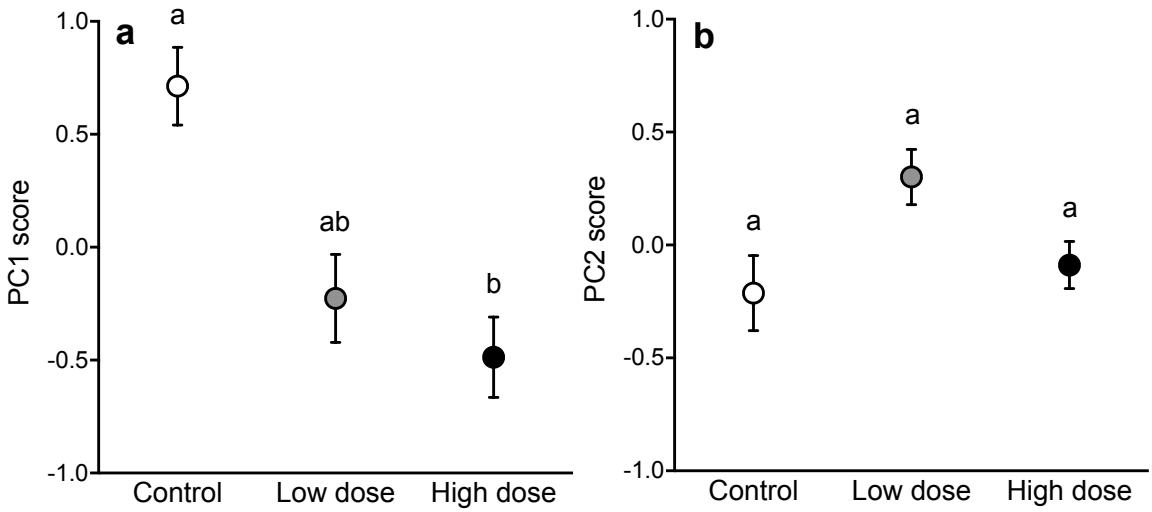

Figure 2.4 Mean ± SE values for (a) PC1 scores as a measure of structural size and (b) PC2 scores as a measure of developmental rate in Chinook salmon at first-feeding (fry) from eggs treated with a control dose, low cortisol dose, and high cortisol dose (n=90 per treatment group). Different letters represent a marginally significant difference in PC1 scores based on a Tukey post hoc test (control dose-high dose; P=0.056).

CHAPTER 3 – DEGREE OF PRE-NATAL STRESS DIFFERENTIALLY IMPACTS PERFORMANCE AND SURVIVAL FOLLOWING EXPOSURE TO A STRESSFUL POST-NATAL ENVIRONMENT[2]

Introduction

Environmental stress (e.g., reduced access to resources, increased predation pressure) has diverse impacts on performance and fitness that are observed both within and across generations (Mousseau and Fox 1998; Dufty et al. 2002), and physiological tools can help characterize the mechanisms underlying these impacts (Wikelski and Cooke 2006). Glucocorticoid (GC) hormones are known for their role in enabling individuals to adaptively respond to acute and chronic environmental perturbations (Busch and Hayward 2009; Angelier and Wingfield 2013). Baseline GCs regulate energy balance (Landys et al. 2006) and fluctuate according to life-history stage and environmental conditions (Crespi et al. 2013; Wingfield 2013), meaning they can serve as a context-dependent signal of individual state (Bonier et al. 2009; Madliger and Love 2014). Given their integration with energetic demand and therefore tight regulation (Landys et al. 2006), variation in baseline GCs has a central role in the interaction between environmental stressors and reproductive decisions or outcomes (Schreck et al. 2001; Wingfield and Sapolsky 2003; Crossin et al. 2016). Since offspring may be indirectly exposed to environmental stressors via maternally-derived GCs (Meylan et al. 2012), intergenerational stress is thought to play an especially important, and increasingly studied, role in linking environmental variation with offspring performance and fitness.

Exposure to maternally-derived GCs is known to influence multiple components of offspring phenotype (body size and growth: Love et al. 2005; Burton et al. 2011;

[2]This chapter is a result of joint research with C. Semeniuk, N. Sopinka, J. Heath, and O. Love.

physiology: Hayward and Wingfield 2004; Auperin and Geslin 2008; Haussmann et al. 2012; behaviour: Janczak et al. 2006; Zimmer et al. 2013; Colson et al. 2015). Moreover, recent work has emphasized that these altered phenotypes interact with future environmental effects to ultimately impact performance and fitness (Sheriff and Love 2013; Benowitz-Fredericks et al. 2015). As such, while short-term effects on offspring phenotype can often be interpreted as 'negative' (i.e., smaller size and slower growth: Saino et al. 2005; Burton et al. 2011), they may in fact positively influence performance and fitness if the interaction with the future environment is fully considered (Chin et al. 2009; Dantzer et al. 2013). Exposure to maternally-derived GCs in response to environmental stressors has been proposed as a means by which offspring can phenotypically prepare for an expected stressful future environment (i.e., predictive adaptive responses (PARs): Gluckman et al. 2005; maternal/environmental matching: Sheriff and Love 2013; Figure 3.1a), in contrast with the traditional view that pre-natal stress leads to net future costs regardless of the offspring's environment (Figure 3.1b). Indeed, a handful of recent studies from invertebrate to vertebrate taxa have suggested that individuals exposed to both pre- and post-natal stressors can perform better than expected in stressful (i.e., environmentally matched) conditions, provided that maternal and offspring environment overlap temporally and/or spatially (e.g., Saastamoinen et al. 2013; Zimmer et al. 2013; Shama et al. 2014; Bian et al. 2015; Merrill and Grindstaff 2015). Still, little research to date has manipulated pre- and post-natal environmental contexts and examined how changes in offspring phenotype impact performance and fitness metrics.

In this study, we examine how the interaction between a pre-natal hormonal signal of environmental stress and a post-natal decrease in environmental quality shape offspring phenotype, performance, and survival in Chinook salmon (*Oncorhynchus tshawytscha*). Chinook salmon are an ecologically and economically important Pacific salmon species that migrate from freshwater streams to the ocean as juveniles and return to fresh water to spawn once before death (Groot and Margolis 1991). Since females only have one breeding attempt (semelparous life history) and provide little post-natal care, there should be strong selection for intergenerational effects in Pacific salmon (Love et al. 2009). In addition, their migratory life cycle makes Pacific salmon especially susceptible to anthropogenic stressors such as increasing stream temperatures and seasonal periods of low flow leading to drought conditions, which have been proposed as a contributing factor for significant population declines across their North American range (Crozier et al. 2006, 2008; Mantua et al. 2010). On spawning grounds, circulating cortisol (the primary GC in fishes) levels of mature females are linked to their survival (McConnachie et al. 2012), and maternally-derived cortisol is found in eggs (Stratholt et al. 1997). While studies have explored the effects of elevated egg cortisol on salmonid offspring traits (survival: Li et al. 2010; Chapter 2; size: Burton et al. 2011; physiology: Auperin and Geslin 2008; Colson et al. 2015; behaviour: Sloman 2010; Burton et al. 2011; Sopinka et al. 2015), phenotypic responses have generally only been tested in benign post-natal environments.

We mimicked a maternally-derived signal of environmental stress (i.e., pre-natal stress) by treating eggs with exogenous cortisol (low and high dose) immediately post-fertilization (e.g., Sopinka et al. 2015, 2016), then decreased offspring's environmental

quality (i.e., post-natal stress) by lowering water levels in semi-natural streams to simulate drought conditions (i.e., riskier environment with decreased amount of cover, increased predation risk: Lonzarich and Quinn 1995; Piccolo et al. 2007; Gibson and Erkinaro 2009) at the juvenile parr stage. We measured energetic demand (via baseline cortisol), stress responsiveness (via post-stress cortisol), size, and survival in juvenile parr, and then explored longer-term effects by measuring size, growth, and survival when fish transitioned to the saltwater smolt stage. Within the framework of environmental matching, we predicted that pre-natally stressed individuals (low and high cortisol dose) in a stressful post-natal environment (low water) would exhibit traits (e.g., energy demand, stress responsiveness, body size) that enable the individual to perform (e.g., grow, survive) better or no worse than individuals that do not receive the pre-natal stress signal (i.e., these individuals would be better matched to their future stressful environments: Sheriff and Love 2013; Figure 3.1a). We were also able to test whether exposure to pre-natal stress leads to detectable costs when the post-natal environment is benign and individuals are mismatched (Figure 3.1a), or leads to unavoidable costs to offspring regardless of the post-natal environment (Figure 3.1b). Furthermore, it was expected that any interactive effects of pre- and post-natal stress would persist and influence growth and survival of smolts in the saltwater stage.

Methods

Pre-natal manipulation of egg cortisol levels and fish rearing

Experiments were conducted at Yellow Island Aquaculture Ltd. (YIAL), a small Chinook salmon aquaculture facility located on Quadra Island, British Columbia, Canada. In

Autumn 2014, pooled eggs from 7 Chinook salmon females were fertilized with milt pooled from 5 Chinook salmon males (Sloman 2010; Colson et al. 2015). Following fertilization, egg-milt mixtures underwent a 2-hour cortisol treatment via immersion in water dosed with either a 1) low cortisol dose (LD; 300 ng mL^{-1}) 2) high cortisol dose (HD; 1000 ng mL^{-1}) or 3) control (CD; 0 ng mL^{-1}) (see Chapter 2). Doses were based on previous studies with the goal of elevating egg cortisol within a physiologically relevant range (Auperin and Geslin 2008; Sopinka et al. 2015, 2016) and representing cortisol levels in eggs of chronically-stressed females (Stratholt et al. 1997). Following the immersion, eggs were washed with hatchery water and placed in a flow-through, vertical-stack incubator. Beginning at the eyed stage, dead eggs were removed and mortalities recorded until full yolk sac absorption. We found that the LD and HD groups had higher embryonic survival to yolk sac absorption compared to the CD and the HD fish were structurally smaller than CD fish (Chapter 2).

Upon yolk sac absorption (~4 months post-fertilization (mpf)), fish were transferred to 12 outdoor stream channels (L × W: 3 m × 1 m) made from galvanized steel culverts and enclosed by ¼ inch dark green mesh netting. Stream channels were installed within 2 large naturalized channels (15 m × 3.5 m) with a flow-through water system (i.e., first channel drained into second channel) and netting around the sides to reduce predation (Madison et al. 2013). Fish were kept separate by egg cortisol treatment and replicated twice in each of the larger naturalized channels (n=150 fish per stream channel). Each stream channel contained a layer of medium-sized gravel and upright 4-inch PVC tubing for refuge and behavioural enrichment. Fish were manually fed evenly throughout the stream channels three times a day to satiation (Starter Feed, Taplow). Lids

made of fine mesh were kept on top of the stream channels from 4–8 mpf (until the post-natal manipulation) to further limit any possibility of avian predation risk and reduce risk of mortalities occurring before the post-natal manipulation period.

Post-natal manipulation of water depth

For a 30-day period at the parr stage (8–9 mpf), water levels were lowered in the second of the two larger channels (included 2 of the 4 replicate stream channels per egg cortisol treatment) by modifying the outflow to simulate drought conditions (i.e., a decrease in environmental quality). In stream channels where the water level was not changed (undisturbed channels), mean water depth (±SE) was 64 ± 1 cm (range 61–66 cm), whereas in low water stream channels the water level was lowered to 33 ± 1 cm (range 30–38 cm) (Table 3.1). The manipulated water depth was chosen to represent the lower end of preferred stream depth microhabitat for juvenile Chinook salmon, while the undisturbed depth was chosen to represent the mid-range of preferred stream depth microhabitat for juvenile Chinook salmon (Hillman et al. 1987; Allen 2000). During the 30-day manipulation of water depth, water temperature (Vernier Software and Technology) and dissolved oxygen (OxyGuard) were measured in the stream channels every morning and afternoon to assess water quality differences due to the depth manipulation (summarized in Table 3.1). Irrespective of depth treatment, there was no detectable water flow within the individual stream channels (using a flowmeter; Vernier Software and Technology).

Baseline and post-stress cortisol

During the final 6 days of the water depth manipulation, blood samples were collected from parr in 2 stream channels per day between 09:00 and 11:00 to control for diel variation in cortisol levels. We seined 12 fish from each channel and blood sampled 6 fish within 3 minutes to ensure baseline cortisol levels were captured (Romero and Reed 2005). The remaining 6 fish were held out of water for 1 minute, then transferred to an opaque 53 L tote with water and an air bubbler and held for 1 hour before being blood sampled for peak stress-induced cortisol levels (Barton and Iwama 1991). Fish were placed in a lethal dose of clove oil (50 ppm; Sigma) and then blood was collected via the caudal vein of the fish using 70 µl heparinized microcapillary tubes. Blood samples were centrifuged at 10000 rpm for 10 minutes within 1 hour of collection to separate out plasma, which was stored at -20°C until being transported to the laboratory in a dry nitrogen shipper and stored at -80°C until cortisol analysis.

Levels of plasma cortisol were determined using a cortisol enzyme immunoassay (EIA - Cayman Chemicals, Ann Arbor, USA) following optimization of the assay for the species (Chapter 2). Non-extracted plasma samples were run in triplicate following kit insert instructions. Baseline samples were run using a 1:20 dilution factor and post-stress samples were run using a 1:160 dilution factor. Assay plates were read with a plate reader at 412 nM wavelength and intra- and inter-assay coefficients of variation were 2.7% and 18.1%, respectively.

Freshwater morphology and survival

Following the completion of blood sampling for a stream channel, remaining fish were caught via seining, lightly anesthetized with clove oil (20 ppm; Sigma), and a 12 mm passive integrated transponder (PIT) tag (Biomark, Boise, Idaho) was inserted into the abdominal cavity. Fish were then weighed and a digital photograph was taken with a ruler for scale (Canon EOS Rebel XT). Five morphological traits were measured from digital photographs using ImageJ (http://imagej.nih.gov/ij/): fork length (FL), eye width (EW), gape (GAPE), body depth (BD), and caudal peduncle height (CP) (Figure 3.2). Freshwater survival (4–9 mpf) was calculated for each stream channel by recording a 1 for each fish found alive and a 0 for each fish not found and presumed dead ($n_{4\ mpf}$=150 total per stream channel; see statistical analyses section). Following PIT tagging, all fish were combined (n=1298) and split into 2 outdoor troughs (2000 L) for a 2-week recovery period.

Saltwater growth and survival

Following the recovery period, fish were moved into 2 saltwater net pens (L × W × D: 3 m × 3 m × 3 m) in the Pacific Ocean (50°7′N and 125°19′W) with ~650 fish in each net pen. Each net pen was fed 1% net pen biomass/day across 3 feedings per day (Organic Grower Feed, Taplow). At 12 mpf (n=1261) and 16 mpf (n=1102), fish from both net pens were seined and each fish was PIT tag scanned and weighed under light anesthesia with clove oil (20 ppm; Sigma). Specific growth rate (SGR) in salt water (smolt stage, 12 –16 mpf) was calculated using the formula $SGR=100[(\ln W1 - \ln W0)\ t^{-1}]$ where W_1 represented final body mass at 16 mpf, W_0 represented initial body mass at 12 mpf, and t

represented number of days between the initial and final masses (123 days). Saltwater survival (12–16 mpf) was calculated by coding 1 for fish that were alive at both sampling dates, and 0 for fish that were alive at 12 mpf but no longer found at 16 mpf (see statistical analyses section).

Statistical analyses

All statistical analyses were completed using R version 3.2.4 (R Core Team 2016). Model assumptions were verified by visual inspection of residual versus fitted and quantile-quantile plots. Baseline cortisol levels were $\log_{10}$ transformed to improve normality and homogeneity of variances. Morphological traits at the freshwater stage (Table 3.2) were loaded into a Principal Component Analysis (PCA) to reduce redundancies in correlated traits (n=15 fish were excluded due to inability to obtain one or more of the measurements from the photograph; total n=1283). One component was extracted from the analysis based on eigenvalue >1 and represented a positive correlation in all traits (representation of structural size; Table 3.3). At the freshwater stage, $\log_{10}$ baseline cortisol, post-stress cortisol, and PC1 (structural size) were analyzed using linear mixed models (lme4 package; Bates et al. 2015). Individual fish length and mass had non-significant effects on baseline or post-stress cortisol values, and were therefore not included in the final cortisol models. Freshwater survival was converted to binary response data (0=dead individual, 1=alive individual) (buildbinary function in the fullfact package; Houde et al. 2016) and analyzed using a generalized linear mixed model (GLMM) for binary data with the logit link function. Specific growth rate in salt water was analyzed using a linear mixed model, controlling for initial mass by including it as a

random effect in the model. Saltwater survival was analyzed using a GLMM, and since there were only 37 mortalities (out of n=1298) between PIT tagging (9 mpf) and the first saltwater time point (12 mpf), this time interval was excluded from analyses as the few mortalities were likely due to the PIT tagging process. All freshwater and saltwater models included pre-natal environment (egg cortisol), post-natal environment (water depth), and their interaction as fixed effects, and either semi-natural channel identity or sea pen identity as a random effect for freshwater or saltwater analyses, respectively. Significance of fixed effects was assessed using likelihood ratio tests fit with maximum likelihood (ML). The interaction was tested first and if a significant interaction was present (P<0.05), the full model was kept and refitted with restricted maximum likelihood estimation (REML). Following significant interactions, post hoc comparisons were run for the fixed effects at each level of the other fixed effect (i.e., slice tests) using the lsmeans package (Lenth 2016). If the interaction was non-significant (p>0.05), main effects were tested by sequentially removing each from the model and comparing the models using likelihood ratio tests, and significant main effects were assessed with pairwise Tukey post hoc tests when necessary.

Results

Baseline and post-stress cortisol

There was a significant pre- × post-natal environment interaction on baseline cortisol (χ^2 =15.13, df=2, P=0.0005; Figure 3.3a). Comparing the undisturbed environment to the low water environment, baseline cortisol decreased in the LD group (P=0.03), increased in the HD group (P=0.03), while levels remained consistent across environments in the

CD group (P=0.66) (Figure 3.3a). There was also a significant pre- × post-natal environment interaction on post-stress cortisol (χ^2 =8.18, df=2, P=0.02; Figure 3.3b). While stress-induced cortisol in the CD and HD groups decreased between the undisturbed and low water environments (CD: P=0.04; HD: P=0.01), the LD group showed similar post-stress cortisol between environments (P=0.97) (Figure 3.3b).

Size and growth

At the freshwater parr stage (9 mpf), there was a marginally significant pre- × post-natal environment interaction on structural size (PC1: χ^2 =5.32, df =2, P=0.07; Figure 3.4a). Individuals in the HD group were smaller in the low water compared to the undisturbed water treatment (P=0.01), and individuals in the CD group tended to be smaller (P=0.06), whereas the LD group did not differ in size between environments (P=0.36) (Figure 3.4a). There was no pre- × post-natal environment interaction (χ^2 =0.0001, df=2, P=0.99) on saltwater growth, but there was a main effect of pre-natal environment (χ^2 =10.04, df=2, P=0.007; Figure 3.4b); individuals in the CD and LD groups displayed higher growth than the HD group (CD-HD: P=0.02; LD-HD: P=0.04). There was also a main effect of post-natal environment (χ^2 =4.48, df=1, P=0.03); individuals from the low water group had higher saltwater growth than those from the undisturbed environment group (Figure 3.4b). This variability in growth rates across treatments resulted in individuals in the HD group having a lower body mass compared to the CD group (main effect of pre-natal environment: χ^2 =7.69, df=2, P=0.02; CD-HD: P=0.02) by the second saltwater smolt time-point (16 mpf) (Figure 3.4c).

Survival

There was an effect of post-natal environment (manipulated from 8–9 mpf) on freshwater survival from 4–9 mpf (χ^2 =4.90, df=1, *P*=0.03; Figure 3.5a); individuals in the undisturbed environment had higher survival compared to the low water environment. However, there was no effect of pre-natal environment (χ^2 =0.74, df=2, *P*=0.69) or interaction between pre- and post-natal environment (χ^2 =1.38, df=2, *P*=0.50). In salt water, there was no detectable effect of pre-natal environment (χ^2 =2.91, df=2, *P*=0.23), post-natal environment (χ^2 =0.06, df=1, *P*=0.81), or their interaction (χ^2 =0.28, df=2, *P*=0.87) on smolt survival (12–16 mpf; Figure 3.5b). Raw values for all metrics are presented in Table 3.4.

Discussion

We show that pre-natal stress signals (egg cortisol treatment) interact with post-natal environmental quality (manipulation of water depth) to influence Chinook salmon phenotype and performance at freshwater and saltwater stages. Impacts on energetic demand (baseline cortisol), stress responsiveness (post-stress cortisol), body size, and growth provided support for our predictions based on the environmental matching hypothesis (Sheriff and Love 2013; Figure 3.1a); fish from the LD group displayed the most indicators of high performance in the matched low water environment (i.e., low energetic demand, maintenance of stress responsiveness, coupled with large size in fresh water and high growth in salt water). Our results also indicate that increasing levels of pre-natal stress do not consistently provide benefits to offspring in stressful environments, given that the HD group in low water displayed high energetic demand,

decreased stress responsiveness, and small size in fresh water followed by low growth in salt water. The HD group may have been mismatched to the low water conditions and suffered costs of the HD egg cortisol exposure (i.e., as predicted in Figure 3.1b), or was primed for a more severely stressful environment (i.e., as predicted in Figure 3.1a) that we did not test. The CD (control) group, predicted to perform poorly in low water, only displayed some indicators of low performance (dampened stress response, smaller size in fresh water), indicating that the low water stressor may not have been severe enough (i.e., presence of actual rather than simply simulated increases in predation risk) to lead to long-term negative effects. All pre-natal stress groups in low water had lower freshwater survival compared to the undisturbed environment, but there were no carry-over effects on saltwater survival, possibly due to saltwater conditions being relatively benign (i.e., ad libitum food, lack of predators).

Interactive effects of pre- and post-natal stressors on energetic demand

In response to low water conditions, we expected shifts in energetic demand (i.e., baseline cortisol) since lowered depths can impede feeding ability/efficiency (Piccolo et al. 2007) and decrease cover (Gibson and Erkinaro 2009). We found no difference in energetic demand between environments in the CD group, while the LD group displayed lowered energetic demand in low water. This may indicate that LD fish in low water were feeding effectively and able to allocate energy to other processes (Busch and Hayward 2009), providing support for environmental matching. Lowering baseline cortisol may also have been an adaptive response to expected nutritional deficits in low water, in an attempt to conserve energy (Kitaysky et al. 2005). Energetic demand in the HD group

increased in low water, indicating that HD fish may not have been feeding as effectively in low water. The HD pre-natal signal appears to be mismatched to the low water conditions, but could have been better matched to a more severely stressful post-natal environment (e.g., food restriction, chronic predation risk: Scheuerlein et al. 2001). Alternatively, the HD signal could have represented a maladaptive signal outside of the relevant ecological range (Figure 3.1b) and led to offspring that were metabolically inefficient, since maternally-derived hormones are known to affect metabolic rate (Sloman 2010; Nilsson et al. 2011). However, the high cortisol dosage resulted in egg cortisol levels well within the normal physiological range (Chapter 2), therefore it seems likely that the HD group was prepared for a more severely stressful environment.

Interactive effects of pre- and post-natal stressors on stress responsiveness

The LD group maintained high responsiveness in low water, which is proposed to be associated with a better ability to cope with environmental change (Cockrem 2013), which further supports environmental matching in the LD group given the increase in environmental stress expected in a low water environment. In contrast, the CD and HD groups both had lower responsiveness in low water, indicating a decreased ability to cope with further stressors (Barton 2002; Romero 2004; Busch and Hayward 2009). Both the pre- and post-natal environment can affect expression of glucocorticoid receptors and impact negative feedback efficiency of the stress axis (Zimmer and Spencer 2014); sustained activation of the stress axis and enhanced negative feedback efficiency could have led to lower responsiveness in the CD and HD groups. In multiple fish species, other environmental stressors such as degraded water quality and the presence of pollutants

lead to attenuated post-stress cortisol levels due to continual activation of the stress axis (Hontela et al. 1992; Norris et al. 1999). In contrast, low stress responsiveness is proposed to serve as an adaptive response to conserve energy in chronically stressful environments; San Marcos salamanders (*Eurycea nana*) had lower stress responsiveness when exposed to a frequently encountered predator compared to a rarely encountered predator (Davis and Gabor 2015). However, in response to challenging abiotic conditions such as shifts in water depth, maintaining responsiveness to other important stressors such as predators would likely be beneficial, supporting the notion that the CD and HD groups appear to be mismatched to the low water environment.

Implications for size, growth, and survival

Variation in parr size in fresh water provides some additional support for environmental matching, as the mismatched CD group tended to show decreased size while the matched LD group maintained size following the low water manipulation. In addition, HD fish were significantly smaller in low water, potentially due to being metabolically inefficient or due to lower foraging ability, as proposed above. A 21-day exposure to low water depth reduced growth via lower food intake in juvenile brown trout (*Salmo trutta*), which was attributed to lack of space to feed (Flodmark et al. 2004). In this study, parr exposed to the 30-day low water environment during freshwater residence had lower survival compared to the undisturbed environment. We were not able to recover mortalities from the stream channels from 4–8 mpf (prior to the water depth manipulation) and therefore cannot be certain that survival differences occurred during the period of low water. However, water quality in the two large, naturalized channels (containing all 12 stream

channels) was similar from 4–8 mpf (dissolved oxygen, top channel: 11.5 ± 0.3, bottom channel: 11.3 ± 0.2; water temperature, top channel: 10.2 ± 0.5, bottom channel: 10.2 ± 0.7) and fresh mortalities were observed at 9 mpf following the 30-day manipulation (Capelle, personal observation), providing evidence that survival differences were likely due to the period of low water. Since the stream channels were mostly protected from predation (i.e., coverage by netting), mortalities in low water may have been due to increased competition for feeding opportunities, as lowered water depth is known to change dominance hierarchies in brown trout (Sloman et al. 2001). While we previously found that elevated egg cortisol benefits early survival (Chapter 2), there were no carry-over effects on freshwater parr survival (although our fish were protected from actual predation), suggesting that the post-natal environment likely plays a larger role in determining survival when individuals are exposed to challenging post-natal conditions (Uller et al. 2013).

Growth rates in salt water were similar for CD and LD groups, showing that the LD group incurred no future growth cost of the pre-natal signal and providing indirect support for environmental matching (i.e., performing as well as CD group). The HD group displayed lower growth than the CD and LD groups independent of environmental treatment and lower body mass at the final saltwater time-point, showing long-term effects of pre-natal stress treatment on growth that possibly supports the conventional maternal stress model (Figure 3.1b). Alternatively, it is again possible that the HD group may have been prepped for a more stressful environment where small size or slower growth would be beneficial if traded off with higher survival. Interestingly, individuals from the low water environment had higher growth compared to the undisturbed

environment independent of pre-natal environment, showing evidence for compensatory growth in salt water (Won and Borksi 2013). This rapid growth could be beneficial for fish from the low water treatment as larger body size in the saltwater phase is important for survival (Duffy and Beauchamp 2011). Vindas et al. (2016) applied an unpredictable, chronic stressor to Atlantic salmon (*Salmo salar*) parr at 10 mpf and found that fish in the stress treatment exhibited compensatory growth during the transfer to salt water, which was interpreted as an ability to cope better with future stressful conditions. However, compensatory growth is also associated with costs such as lower burst swimming performance (Metcalfe and Monaghan 2001; Álvarez and Metcalfe 2007), which could increase susceptibility to predation in the wild. While individuals were able to compensate for the decrease in environmental quality and lower survival was not observed in captive conditions, in the wild, the period of rapid growth may have led to costs that ultimately affected marine survival. Overall, there were no detectable carry-over effects of pre- or post-natal environment on saltwater survival, which may have been because individuals surviving to that point were those who survived both pre- and post-natal stressors and might have displayed certain phenotypic and performance traits that led to success across life stages and environmental contexts.

Conclusions

We provide strong evidence for environmental matching based on multiple phenotypic and performance traits (i.e., LD group), and also show costs of a potential environmental mismatch (i.e., HD group and CD group for some traits), while highlighting that offspring responses are trait- and context-specific. This study reinforces other work

showing that effects of pre-natal stress are not consistently linear (Auperin and Geslin 2008; Gagliano and McCormick 2009), and low doses may lead to positive effects while higher doses show detrimental effects (Li et al. 2010). Overall, this work provides important insight on the impacts of stressors both within and across generations, and emphasizes the potential for hormonally-mediated stress signals to play a role in the ability of populations to cope with changing environments. Future work should continue to explore the adaptive potential of stress signals by setting up matched and mismatched environments, with a focus on incorporating multiple relevant and competing stressors in light of rapid environmental change.

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Tables

Table 3.1 Water quality metrics (mean ± SE with ranges in brackets) during the 30-day manipulation of water depth where water temperature and dissolved oxygen were measured in the morning and afternoon in each stream channel.

Water quality metric	Time of day	Undisturbed environment	*N*	Low water environment	*N*
Depth (cm)	NA	64 ± 1 (60-66)	6	33 ± 1 (30-38)	6
Temperature (°C)	Morning	13.6 ± 0.08 (12.4–14.8)	103	13.7 ± 0.08 (12.4–14.9)	106
	Afternoon	14.4 ± 0.09 (13.2–16.2)	102	14.7 ± 0.09 (13.3–16.4)	104
Dissolved oxygen (mg L^{-1})	Morning	9.8 ± 0.03 (9.0–10.6)	97	9.4 ± 0.04 (8.2–10.6)	101
	Afternoon	10.1 ± 0.04 (9.1–11.1)	103	9.7 ± 0.04 (8.6–10.5)	104

Table 3.2 Morphological traits (mean ± SE) of juvenile Chinook salmon reared from control dose eggs (CD; 0 ng mL^{-1}), low cortisol dose eggs (LD; 300 ng mL^{-1}), or high cortisol dose eggs (HD; 1000 ng mL^{-1}) and exposed to undisturbed or low water environmental conditions.

Trait	Post-natal environment	CD	*N*	LD	*N*	HD	*N*
Body mass (g)	Undisturbed	11.57 ± 0.15	215	10.93 ± 0.13	231	12.43 ± 0.14	257
	Low water	9.78 ± 0.11	219	9.89 ± 0.12	195	9.84 ± 0.15	181
Fork length (mm)	Undisturbed	100.37 ± 0.43	212	98.51 ± 0.35	226	100.85 ± 0.36	256
	Low water	95.09 ± 0.35	219	95.50 ± 0.39	192	94.95 ± 0.47	178
Eye width (mm)	Undisturbed	6.29 ± 0.01	212	6.20 ± 0.01	226	6.18 ± 0.01	256
	Low water	6.18 ± 0.01	219	6.15 ± 0.01	192	6.03 ± 0.02	178
Gape (mm)	Undisturbed	22.66 ± 0.07	212	22.31 ± 0.07	226	22.34 ± 0.07	256
	Low water	22.02 ± 0.07	219	21.96 ± 0.08	192	21.67 ± 0.08	178
Body depth (mm)	Undisturbed	21.98 ± 0.13	212	21.44 ± 0.10	226	22.31 ± 0.11	256
	Low water	20.54 ± 0.10	219	20.51 ± 0.10	192	20.12 ± 0.13	178
Caudal peduncle width (mm)	Undisturbed	7.62 ± 0.04	212	7.49 ± 0.03	226	7.84 ± 0.03	256
	Low water	7.60 ± 0.04	219	7.70 ± 0.04	192	7.23 ± 0.04	178

Table 3.3 PCA loadings for morphological traits of juvenile Chinook salmon where PC1 was extracted from the PCA based on eigenvalue >l and all traits contributed significantly to the PC1 loading.

Trait	PC1 loading
Eigenvalue	4.9
% variance explained	81.3
Body mass	0.43
Eye width	0.33
Gape	0.41
Fork length	0.44
Body depth	0.43
Caudal peduncle	0.40

Table 3.4 Phenotypic and performance traits (mean ± SE) of Chinook salmon reared from control dose eggs (CD; 0 ng mL^{-1}), low cortisol dose eggs (LD; 300 ng mL^{-1}), or high cortisol dose eggs (HD; 1000 ng mL^{-1}) and exposed to undisturbed or low water environmental conditions (8–9 mpf). Data from 4–9 mpf and 12–16 mpf collected from freshwater and saltwater stages, respectively.

Trait	Time point	Post-natal environment	CD	*N*	LD	*N*	HD	*N*
Baseline cortisol (ng ml^{-1})	9 mpf	Undisturbed	14.9 ± 3.5	12	21.8 ± 3.7	12	9.3 ± 2.1	12
		Low water	18.4 ± 5.0	12	6.6 ± 1.5	12	34.7 ± 9.6	12
Post-stress cortisol (ng ml^{-1})	9 mpf	Undisturbed	142.7 ± 16.8	12	148.2 ± 14.3	12	182.6 ± 8.3	12
		Low water	86.0 ± 11.0	12	147.8 ± 15.4	11	105.6 ± 12.9	12
PC1 (structural size)	9 mpf	Undisturbed	0.87 ± 0.15	212	0.15 ± 0.13	226	1.03 ± 0.14	256
		Low water	−0.61 ± 0.13	219	−0.56 ± 0.14	192	−1.35 ± 0.16	178
Survival (%)	4–9 mpf	Undisturbed	86.0 ± 2.0	300	85.0 ± 2.1	300	93.7 ± 1.4	300
		Low water	81.0 ± 2.3	300	73.0 ± 2.6	300	68.3 ± 2.7	300
Specific growth rate (% body mass gain/day)	12–16 mpf	Undisturbed	0.36 ± 0.01	189	0.36 ± 0.01	192	0.32 ± 0.01	213
		Low water	0.39 ± 0.01	188	0.39 ± 0.01	161	0.37 ± 0.01	152
Body mass (g)	16 mpf	Undisturbed	56.7 ± 0.8	189	55.8 ± 0.8	194	55.6 ± 0.7	215
		Low water	56.7 ± 0.7	188	55.9 ± 0.8	163	53.3 ± 0.8	152
Survival (%)	12–16 mpf	Undisturbed	83.2 ± 2.6	209	78.8 ± 2.7	226	76.6 ± 2.7	248
		Low water	80.7 ± 2.7	212	74.9 ± 3.1	191	78.9 ± 3.1	175

Figures

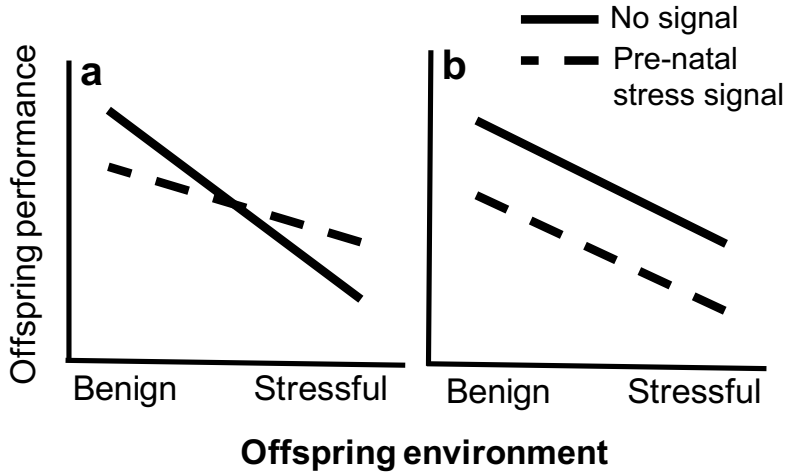

Figure 3.1 Schematic representing theoretical predictions of how performance is influenced by both the pre- and post-natal environment. Environmental matching (a) proposes that while individuals not receiving a pre-natal signal (control) have higher performance than those receiving a pre-natal stress signal in benign environments, individuals receiving a pre-natal stress signal have higher performance than control individuals in stressful environments (i.e., pre- and post-natal environments are matched). In the traditional maternal stress model (b), a signal of pre-natal stress leads to lower performance in both post-natal environments, with no interaction occurring between pre- and post-natal environments.

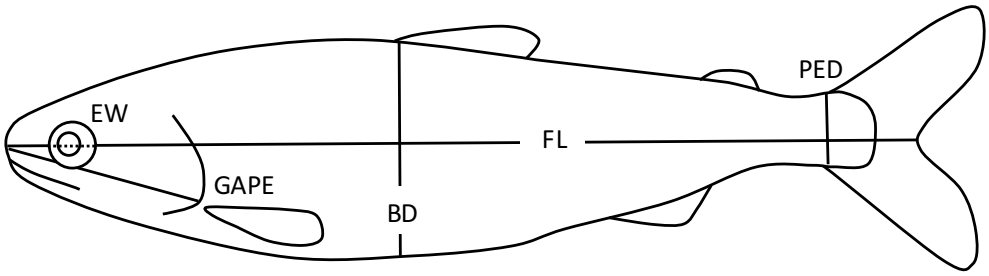

Figure 3.2 Morphological measurements taken from digital photographs of juvenile Chinook salmon (9 mpf). Measurements include eye width (EW), gape (GAPE), body depth (BD), fork length (FL) and caudal peduncle width (PED).

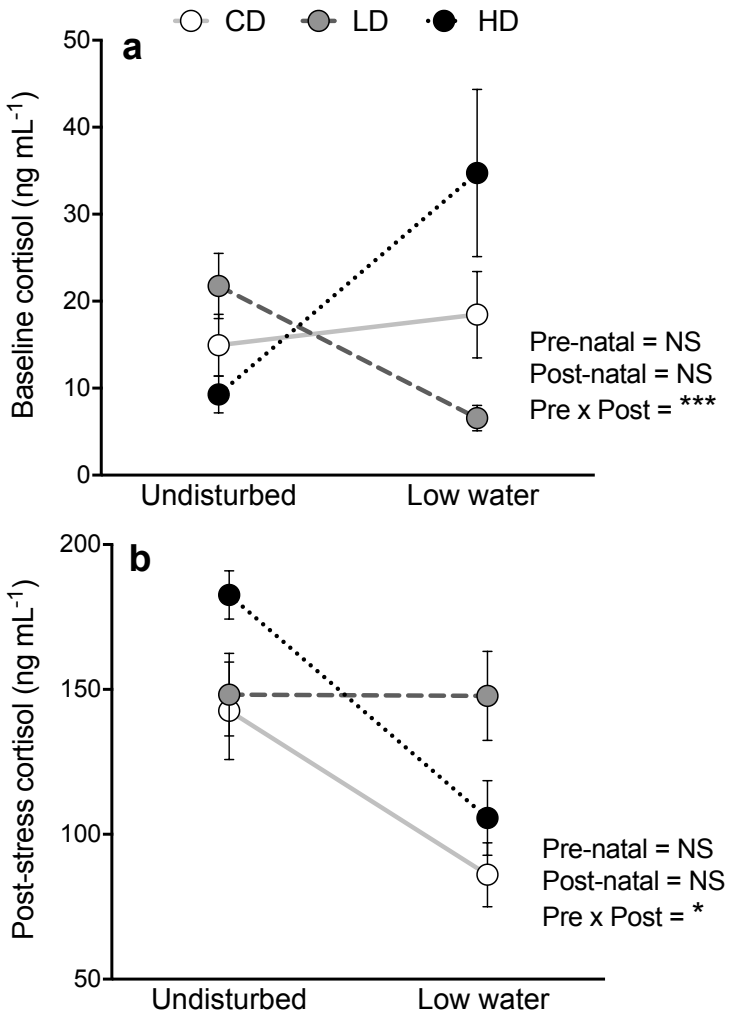

Figure 3.3 Baseline plasma cortisol (a) and post-stress plasma cortisol (b) of juvenile Chinook salmon parr (9 mpf) reared from eggs treated with a control dose (CD; 0 ng mL^{-1}), low cortisol dose (LD; 300 ng mL^{-1}), or high cortisol dose (HD; 1000 ng mL^{-1}) as a pre-natal manipulation and exposed to an undisturbed or low water environment from 8–9 mpf. Values presented as means ± SE and n=12 for all treatment groups except n=11 for the LD-low water group in (b). Values presented as means ± SE. NS, *, **, and *** represent P-values that were >0.05, <0.05, <0.01, and <0.001, respectively.

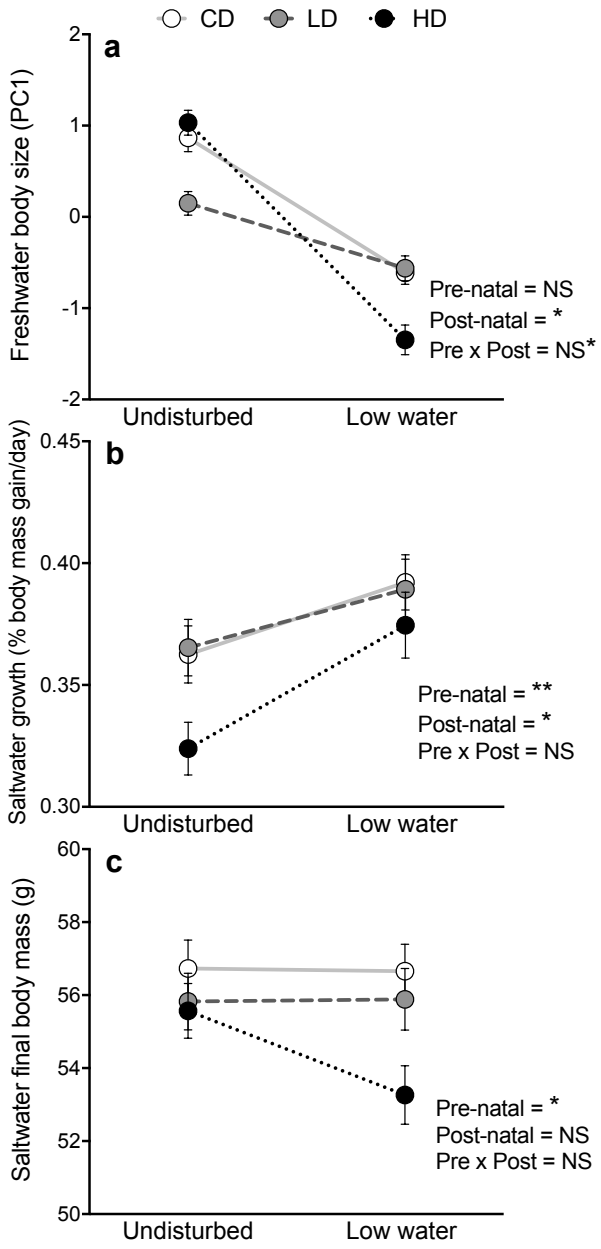

Figure 3.4 Freshwater structural size at 9 mpf (a), specific growth rate in salt water (12–16 mpf) (b), and final body mass in salt water (16 mpf) (c) of Chinook salmon reared from eggs treated with a control dose (CD; 0 ng mL^{-1}), low cortisol dose (LD; 300 ng mL^{-1}), or high cortisol dose (HD; 1000 ng mL^{-1}) and exposed to undisturbed or low water environments (8–9 mpf). Values presented as means ± SE. NS, *, **, and *** represent *P*-values that were >0.05, <0.05, <0.01, and <0.001, respectively, and NS* indicates a marginally significant interaction (*P*=0.07*)*. Sample sizes are indicated in Table 3.4.

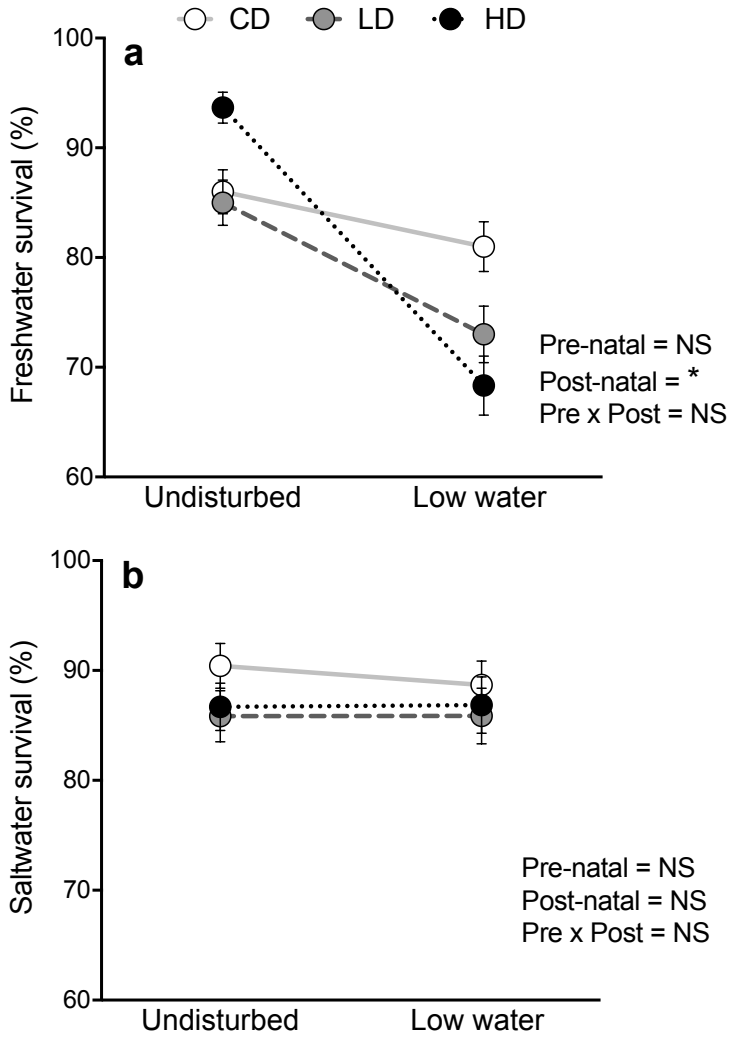

Figure 3.5 Survival in (a) fresh water (4–9 mpf) and (b) salt water (12–16 mpf) for Chinook salmon juveniles reared from eggs treated with a control dose (CD; 0 ng mL^{-1}), low cortisol dose (LD; 300 ng mL^{-1}), or high cortisol dose (HD; 1000 ng mL^{-1}) and exposed to an undisturbed or low water environment from 8–9 mpf in fresh water. Values presented as means ± SE and NS, *, **, and *** represent *P*-values that were >0.05, <0.05, <0.01, and <0.001, respectively. Sample sizes are indicated in Table 3.4.

CHAPTER 4 – DROUGHT CONDITIONS MODULATE DIEL BEHAVIOUR OF JUVENILE SALMON EXPOSED TO A PRE-NATAL STRESS SIGNAL[3]

Introduction

Variation in environmental quality is an important evolutionary driver, and rapid environmental change has intensified the need to study how animal populations respond to different conditions (Visser 2008). Stressors can be defined as environmental perturbations having direct implications for individual performance and fitness (Schulte 2014), and stressors experienced during reproduction can equally have indirect impacts on offspring phenotype via intergenerational effects mediated via the mother (Mousseau and Fox 1998). Poor maternal environmental conditions often lead to lowered offspring survival and/or other negatively perceived traits when phenotypes are examined within benign post-natal conditions (e.g., small size, slow growth, increased fearfulness/anxiety; Campbell et al. 1992, 1994; Saino et al. 2005; McCormick 2006; Janczak et al. 2006). While not always immediately apparent and infrequently tested, poor maternal/early developmental environments may produce phenotypes capable of buffering offspring from future environmental stressors (i.e., predictive adaptive responses (PARs): Gluckman et al. 2005). The theory of PARs has been extended to free-living vertebrates with maternal/environmental matching, which predicts that exposure to maternal stress can increase relative offspring fitness when maternal and future offspring environments are matched (i.e., equally poor: Sheriff and Love 2013). For instance, in multiple fish species, exposing parents to warmer water temperatures (predicted under climate change) led to offspring having better thermal tolerance and higher growth under the same

[3] This chapter is a result of joint research with O. Love, N. Sopinka, C. Harris, J. Heath, and C. Semeniuk

warmer temperatures (Donelson et al. 2012; Salinas and Munch 2012; Shama et al. 2014), providing support for environmental matching and highlighting important implications for population dynamics and conservation (Meylan et al. 2012).

Behaviour often serves as an individual's first response to altered environments, making it a key mechanism that can facilitate or hinder acclimation or adaptation to environmental change, and hence affect the persistence of a population (Wong and Candolin 2015). Nevertheless, the ability of animals to cope with stressors in their environment is dependent not only on their underlying genotype-phenotype, but can be further modified by intergenerational effects. For example, parental exposure to high CO_2 levels improves offspring escape performance under similar high CO_2 environmental conditions (Allan et al. 2014). As such, intergenerational effects can alleviate negative effects of environmental stressors by promoting adaptive behavioural responses, and these responses are often mediated by maternally-derived hormones (Groothius and Schwabl 2008; Nesan and Vijayan 2013). In oviparous species, increased levels of maternally-transferred glucocorticoids (GCs) in eggs influence behavioural traits such as activity (Epsmark et al. 2008; Eriksen et al. 2011), exploration (Zimmer et al. 2013), as well as aggression and dominance (Sloman 2010; Burton et al. 2011; Ahmed et al. 2014; Sopinka et al. 2015) that are important for coping with environmental stressors. However, only testing offspring in a benign post-natal environment limits the ability to explore and determine how changes in behaviour impact performance across multiple environmental contexts. Moreover, the balance between individual consistency in behaviour (i.e., personality: Réale et al. 2007) and behavioural flexibility is thought to be an important determinant of adaptive or maladaptive responses to environmental change (Briffa et al.

2008; Dingemanse et al. 2010). Although maternal stress signals may therefore modulate behavioural consistency and/or flexibility to benefit offspring in a matched stressful environment, this has largely been unexplored.

Pacific salmon are particularly susceptible to the effects of climate change due to their migratory life-history and reliance on freshwater streams, which are now experiencing rapid changes in temperature and flow rates (Crozier et al. 2008). Mature adult salmon that return to natal freshwater streams to spawn only have one chance to reproduce (i.e., semelparous life-history), and are increasingly facing prevalent stressors such as warming stream temperatures and fluctuating water levels that are known to reduce survival to spawning grounds (Martins et al. 2012). Survival of female salmon on spawning grounds is linked to circulating cortisol levels, the primary GC in fishes (McConnachie et al. 2012), and cortisol is transferred from maternal circulation to developing eggs (Stratholt et al. 1997). Juvenile salmon offspring rear in the same freshwater streams as their mothers for months to years before making their downstream migration to the ocean as smolts, and as such face similar stressors (e.g., high stream temperatures and flows) that also negatively affect survival at a juvenile life stage (Connor et al. 2003; Crozier and Zabel 2006). Attaining a large body size in the face of strong intra- and interspecific competition while avoiding predation is key to success and influences behavioural decisions of stream-dwelling juvenile salmon (Groot and Margolis 1991). Diel changes in behaviour (i.e., over a 24-hour period) are influenced by a trade-off between growth and survival; diurnal activity patterns are generally favoured in summertime since daytime foraging is more efficient for vision-dependent foragers, although risk of predation is higher (Metcalfe et al. 1999). Diel behaviour is also

influenced by environmental factors (Orpwood et al. 2010), individual state (Metcalfe et al. 1998), and personality (Watts et al. 2015), therefore, examining diel behaviour in different environmental conditions can provide insight into juvenile salmon performance. For instance, low water depths in streams are known to decrease the amount of protective cover (Gibson and Erkinaro 2009), change dominance structures (Sloman et al. 2001), and impair feeding ability (Piccolo et al. 2007) of juvenile salmon. Exploring the potential for maternally-derived hormones to mediate and potentially dampen negative effects of stressors through behavioural responses (or lack thereof) in stressful environments will help establish whether organisms will be able to cope with their rapidly changing environment.

To determine how a pre-natal hormonal signal modulates diel behaviour in stressful and non-stressful post-natal environments, and how behavioural patterns subsequently influence growth and survival, we reared juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from eggs with manipulated cortisol levels (pre-natal stress) and exposed them to undisturbed or low water depths (post-natal stress) in semi-natural streams. We chose to simulate low-water (drought) conditions as a stressful environment because these conditions are both occurring more frequently due to climate change (Crozier et al. 2008) and known to affect behaviour and performance of juvenile salmon (Lonzarich and Quinn 1995; Sloman et al. 2001). We measured behavioural traits under hormonal influence (Archard et al. 2012) that represent a measure of energetic expenditure (activity), use of a novel, risky habitat (exploration), and responses to perceived predation risk (refuge use). We expected all groups to respond to stressful low water conditions by increasing refuge use during the day and being more

active/exploratory at night to avoid perceived risky, daytime foraging (Metcalfe et al. 1998, 1999). In addition, we also expected that pre-natal stress groups may display the most diel flexibility in activity (largest changes in behaviour from daytime to nighttime) and be overall the most risk-averse in the matched, stressful conditions. In the undisturbed environment, we expected the control group in matched conditions to be the most active and exploratory in daytime and have low refuge use, while pre-natal stress groups in mismatched conditions may exhibit more risk averse (i.e., less optimal) behaviour. Finally, we anticipated that measures of behaviour should contribute to explaining variation in growth during the water depth manipulation, but may not carry-over to affect growth and survival in salt water, a common environment and different life-history stage (Smith and Blumstein 2008).

Methods

Egg cortisol treatment

The captive Chinook salmon used in these experiments were from Yellow Island Aquaculture Ltd., an aquaculture facility on Quadra Island, British Columbia, Canada. In Autumn 2014, eggs were pooled among 7 Chinook salmon females and fertilized with a pool of milt from 5 Chinook salmon males. For a 2-hour period immediately post-fertilization, eggs were immersed in water dosed with either a 1) low cortisol dose (LD; 300 ng mL^{-1}) 2) high cortisol dose (HD; 1000 ng mL^{-1}) or 3) control (CD; 0 ng mL^{-1}) as described in Chapter 2, with doses chosen to result in biologically-relevant elevations in egg cortisol (Auperin and Geslin 2008; Sopinka et al. 2015) and mimic transfer of cortisol from a chronically-stressed female to eggs (Stratholt et al. 1997). Eggs were then

incubated in flow-through, vertical-stack incubation trays until emergence (full yolk-sac absorption). The LD and HD doses led to higher survival to emergence compared to the CD group and fry from the HD group were smaller than the CD fish, showing early positive fitness and phenotypic effects of pre-natal stress signals (see Chapter 2).

Fish rearing and tagging

As described in Chapter 3, fry were moved to outdoor stream channels (L × W: 3 m × 1 m) at 4 months post-fertilization (mpf), and maintained within two large naturalized channels (15 m × 3.5 m) with flow-through water (Madison et al. 2013). Individual stream channels were made of galvanized steel culverts, enclosed by ¼ inch dark green mesh netting, and had medium-sized gravel and 4-inch PVC tubing for shelter. Fry from each egg cortisol treatment were ponded into individual stream channels (n=150 fish per channel), and replicated in each of the naturalized channels. The area surrounding the stream channels was closed off using mesh to mostly eliminate risk of aerial and terrestrial predation. From 4–8 mpf, fish in all 12 stream channels experienced similar conditions and were fed uniformly throughout the stream channels 3 times a day (Starter Feed, Taplow). At 8 mpf, each stream channel was divided into 3 microhabitats of 1m in length consisting of: i) a novel, downstream shallow microhabitat using two inverted 37.9 L plastic rectangular totes covered with a layer of gravel, ii) a middle area containing the upright 4-inch PVC which continued to serve as a refuge area, and iii) an upstream deep area which was left unmodified. (Figure 4.1). Following the creation of the microhabitats at 8 mpf, a random subset of fish (n=20) from each stream channel was caught via seining to ensure capture of all behavioural types (Biro 2013). Fish were lightly

anesthetized (20 ppm clove oil), their body mass recorded to the nearest 0.01 g, and they were then individually tagged via insertion of a passive integrated transponder (PIT) tag (12.5 mm × 2.1 mm, 134.2 kHz, Biomark Inc., Boise, Idaho) into the peritoneal cavity using pre-loaded single use injectors. PIT tag numbers were scanned using a Biomark 601 Reader and recorded manually. Fish were then returned to their original stream channels and given 5 days to recover.

Environmental manipulation of water depth

Water depth was lowered for a 30-day period (8–9 mpf) in 6 out of the 12 stream channels (each egg cortisol treatment, replicated) by modifying the outflow in the naturalized stream channel to simulate a decrease in post-natal environmental quality. We lowered water depth as a relevant post-natal environmental stressor since small decreases in depth (i.e., 10–20 cm) can negatively impact foraging ability (Piccolo et al. 2007) and increase use of shelter in juvenile salmon (Gibson and Erkinaro 2009), and depth affects other water quality metrics (e.g., temperature, dissolved oxygen: Lonzarich and Quinn 1995; Crozier and Zabel 2006). Water depth in the deep area of the undisturbed channels was (mean ± SE) 64 ± 1 cm (range: 61–66 cm), representing the mid-range of depth preference for juvenile Chinook salmon (Lister and Genoe 1970; Hillman et al. 1987; Allen 2000), while water depth was 30 ± 1 cm (range: 25–33 cm) in the shallow microhabitat, representing a riskier area (i.e., less cover from depth: Gibson and Erkinaro 2009; Figure 4.1). In the low water channels, the greatest water depth was 33 ± 1 cm (range: 30–38 cm), representing the lower range of depth preference for juvenile Chinook salmon and meaning that overall, the low water channels provided a lower surface

area/volume of water (i.e., one factor occurring in a drought situation: Magoulick and Kobza 2003). Water depth in the shallow area was further reduced to 7 ± 0.5 cm (range: 5–8 cm), representing a risky but still useable habitat as juvenile salmon reside in shallow riffles in some stream environments (Everest and Chapman 1972; Figure 4.1). There was no measurable rate of flow inside the individual stream channels regardless of overall water depth (using a flowmeter; Vernier Software and Technology). Water temperature (Vernier Software and Technology) and dissolved oxygen (OxyGuard) were measured in the deep and shallow microhabitats of each stream channel in morning and afternoon for the duration of the water depth manipulation. Dissolved oxygen levels were lower in low water channels, while water temperature was fairly consistent across depth treatments (Table 4.1).

Fish tracking using PIT tag array

Movement of PIT tagged fish within stream channels was monitored for a continuous 24-hour period using a PIT-tag monitoring array: 4 antennas and a IS1001 Multiplexing Transceiver System (MTS) consisting of 1 master controller and 4 IS1001 readers (Biomark Inc., Boise, Idaho). Four rectangular antennas (L × W × H: 72 cm × 84 cm × 8 cm) were set up in pairs inside a channel (Armstrong et al. 1997). To facilitate the tracking of movement direction between microhabitats, antennas were placed in pairs at the transition zones between the shallow, refuge, and deep microhabitats of the channel (Figure 4.2). Antennas were custom designed and encased in aluminum to reduce signal interference within antenna pairs (5 cm spacing between antennas, 20 cm total length including width of both antennas) and between pairs (~87 cm spacing), while maintaining

signal strength in the interior of the antennas. Equipment was tested on-site prior to the start of trials and a PIT tag was detected in all interior sections of each antenna including the middle where signal strength should be the weakest (antenna exciter voltage: 12-20 V DC, electronically adjustable). The first antenna and then the second consistently detected a PIT tag manually moved through both antennas of a pair.

Beginning on day 5 of the water depth manipulation (8 mpf + 5 days), the PIT tag array was deployed for the 24-hour trial in each of the 12 channels. To minimize the acclimation time of fish to the introduction of the PIT tag array to their habitat, replicate mock antennas made out of grey, 2-inch PVC pipe were deployed in the channel twenty-four hours prior to the addition of the antennas to a channel. Antennas were moved among channels each day between 15:00 and 20:00; times were staggered to account for the time needed to move the antennas between channels. Following the disturbance of adding the antennas to the channel, fish were given a 30-minute acclimation period and then the MTS was turned on and each antenna was automatically tuned in sequence. Antennas fired every 40 ms and each pair was fired in sequence. Each time a PIT-tagged fish was detected by one of the antennas, the date, time, antenna ID, and tag ID was recorded and data was written to a removable USB drive and stored in internal memory. Tag interference (two PIT tagged fish in read range of antenna) is a known limitation of this system and we aimed to reduce this issue by only tagging a small subset of the total fish in a channel (n=20 out of 150) and by using custom-built shielded antennas to reduce read range length-wise in the channel.

From the raw PIT tag tracking data, microhabitat-specific behavioural data were calculated for each individual using a VBA macro (Microsoft Excel 2013) which used the

current and previous antenna reads from a fish to position a given fish within a given microhabitat. Values calculated included percent time spent in each habitat, number of visits to a habitat, mean length of stay in a habitat, as well as an overall measure of habitat switches throughout the time period. Calculations were also included for time spent in transition areas between habitats (i.e., between the individual antennas of a pair) as well as time where the fish could not be placed in a specific location (e.g., fish moved straight from antenna 1 to 3 without being registered by antenna 2; represented <5% on average of the total time trial). These data were excluded from analyses as these values represented noise that we could not assign to a specific habitat. As we were interested in behaviour across the full 24-hour period as well as diel changes in behaviour, variables were calculated for the whole 24-hours as well as for daytime and nighttime. Data for each channel were split into day and night periods based on the sunrise and sunset times for the date that the tracking took place (latitude 50° 7′ N). Approximately 1 hour of data in the morning was excluded from the daylight totals and 24-hour totals due to a separate behavioural experiment conducted during this time interval.

Measures of survival and growth

Following the 30-day manipulation of water depth, all fish were caught via seining and removed from channels. The tagged subset of fish in each channel were identified and body mass was recorded again to the nearest 0.01 g. Specific growth rate (SGR) in fresh water (8–9 mpf) was calculated using the formula $SGR = 100[(\ln W1 - \ln W0)\, t^{-1}]$ where W_1 represented final body mass at 9 mpf, W_0 represented initial body mass at 8 mpf, and t represented number of days between the initial and final masses (30 days). The

remaining fish were then individually PIT tagged, and all fish were combined among treatments in two 2000 L troughs for a two-week recovery period, then transferred to 2 saltwater net pens (L × W × D: 3 m × 3 m × 3 m) in the Pacific Ocean (50°7′N and 125°19′W) and fed 1% net pen biomass/day across 3 feedings a day (Organic Grower Feed, Taplow). At 12 and 16 mpf, fish in each net pen were seined and then individually anesthetized so that their PIT tag could be read and body mass recorded (to the nearest 0.5 g). Survival was recorded as either alive or not found at each handling point and SGR was calculated as above but for the saltwater period (12–16 mpf; 123 days). From 16 to 22 mpf, survival was monitored weekly via recovery of mortalities on bottom of net pens and removal of PIT tag to verify fish identity.

Statistical analyses

Effect of pre-natal stress on diel behaviour of juveniles

All statistical analyses were completed using R version 3.2.4 (R Core Team 2016). Model assumptions of normality and homogeneity of variances were tested by visual inspection of residual versus fitted and quantile-quantile plots. To explore diel changes in behaviour among pre-natal stress groups during the water depth manipulation (8–9 mpf), a stacked dataset with day and night behavioural variables (percent time in a habitat; number of stays in a habitat; mean stay length in a habitat; number of total zone changes) for each individual was used. For all raw behavioural variables except for "percent time spent in deep habitat", a constant of 1 was added and then variables were $\log_{10}$ transformed to improve normality. Behavioural variables were expected to be correlated and therefore loaded into a Principal Component Analysis (PCA); while PCA does not have an

underlying assumption of normality, it can aid in better extracting components (Clary et al. 2014). There were three components that had an eigenvalue > 1 and therefore the PCA was followed by a varimax orthogonal rotation for three components. Component 1 represented a measure of activity (positively correlated with zone changes, number of stays in shallow, refuge, and deep habitats) and explained 36% of the variation; component 2 represented a measure of exploratory behaviour (Careau et al. 2009) (positively correlated with percent time spent in novel shallow habitat and mean stay length in shallow habitat, negatively correlated with percent time spent in deep habitat and mean stay length in deep habitat) and explained an additional 27% variation; and component 3 represented a measure of refuge use (positively correlated with percent time spent in refuge habitat and stay length in refuge habitat, negatively correlated with percent time spent in deep habitat and mean stay length in deep habitat) and explained an additional 23% variation (Table 4.2). Since individuals were represented twice in the dataset (daytime value and nighttime value) which violates the assumption of independence, separate PCAs were run on split day and night datasets and the same components were obtained, verifying the validity of using the stacked dataset (Dingemanse et al. 2007; Adriaenssens and Johnsson 2010). Linear mixed models (lmer package; Bates et al. 2015) were used to assess differences in the three behavioural components across treatments; the full model included period (day, night), egg cortisol treatment (control, low, high), and a period × egg cortisol interaction as fixed effects and channel identity and individual ID as random effects. Since body mass can influence behaviour (Brown et al. 2007), body mass prior to the water-depth manipulation was initially included in the models and then removed since the term was non-significant in

all models (P>0.05). Data were split by depth treatment (undisturbed, low water) and run as separate models since large differences in growth were expected during the manipulation, showing differential responses in the two conditions. Following methods in Zuur et al. (2009), likelihood ratio tests fit with maximum likelihood (ML) were used to first determine significance of the interaction by comparing the full model to a model with no interaction term. If a significant interaction was present (P<0.05), the full model was kept, refitted with restricted maximum likelihood estimation (REML), and post hoc tests were run for the fixed effects at each level of the other fixed effect (i.e., slice tests) using the lsmeans package (Lenth 2016). If the interaction was non-significant (P>0.05), main effects were tested by removing each from the model and comparing the models using likelihood ratio tests. When applicable, significant main effects were assessed with pairwise Tukey post hoc tests.

Effect of pre-natal stress and juvenile behaviour on growth rate and survival

Linear mixed models were used to explore differences in specific growth rate during the manipulation of water depth (8–9 mpf) attributable to egg cortisol treatment, depth treatment, and the egg cortisol × depth interaction, which were included as fixed effects, and stream channel identity which was included as a random effect. Survival of PIT tagged individuals following the water depth manipulation was 80% or greater in all treatment groups (i.e., 16 fish or greater out of 20 fish successfully recovered from each stream channel); as mortalities for the subset of tagged individuals could have been due to handling and tagging process rather than the manipulation, freshwater survival was not used in analyses. To examine whether behaviour during the water depth manipulation

related to current growth or future growth and survival, the full 24-hour behavioural dataset was used, as it was anticipated that how an individual behaves across a full day would influence growth and survival. Log_{10} transformed behavioural variables (except for percent time spent in the deep habitat) were again loaded into a Principal Component Analysis (PCA) with a varimax orthogonal rotation based on 3 components with an eigenvalue >1. Similar to the day and night stacked dataset, the first component from the 24-hour dataset represented activity (explained 42% variation), the second component represented refuge use (explained 23% variation), and the third represented exploratory behaviour (explained 22% variation) (Table 4.3). Linear mixed models were used to examine how each behaviour (activity, refuge use, or exploration), egg cortisol treatment, and a behaviour × egg cortisol treatment interaction influenced SGR in fresh water with channel identity included as a random effect, and SGR in salt water with net pen identity as a random effect. In addition, generalized linear mixed models (GLMMs) for binary data with a logit link function were used to assess relationship between the same fixed effects and saltwater survival (12–22 mpf), with net pen identity as a random effect. Models were again split by water depth treatment since large differences in growth from the freshwater manipulation were expected to have impacts on saltwater growth and survival, potentially swamping out other covariate effects.

Results

Effect of pre-natal stress on diel behaviour

In undisturbed channels, significant egg cortisol × period interactions were found for all 3 behaviours (activity: χ^2=26.2, df=2, P<0.0001, Figure 4.3a; exploration: χ^2=11.0, df=2,

P=0.004, Figure 4.3c; refuge use: χ^2=7.0, df=2, P=0.03, Figure 4.3e). Individuals in the CD group were less active (P<0.0001) yet more exploratory (P<0.0001) at night, and did not change refuge use (P=0.26). Individuals in the LD group did not change their activity from day to night (P=0.12), increased their exploratory behaviour at night, but to the lowest degree (P=0.03), and increased refuge use at night (P=0.0006). In the HD group, individuals were more active (P=0.01) and more exploratory (P<0.0001) at night, and showed no difference in refuge use (P=0.90).

In low water channels, a significant egg cortisol × period interaction was found for activity (χ^2=6.10, df=2, P=0.048, Figure 4.3b); while all egg cortisol treatment groups increased activity during the night, the CD group did so most (CD: P<0.0001; LD: P=0.0001; HD: P=0.0002;). Regardless of cortisol treatment, fish in low water increased exploratory behaviour (main effect of period: χ^2=52.8, df=2, P<0.0001, Figure 4.3d) at night. In contrast, there was no difference in refuge use between day and night (χ^2=0.16, df=2, P=0.69, Figure 4.3f) in the low water channels; however, there was an effect of egg cortisol treatment (χ^2=6.72, df=2, P=0.035, Figure 4.3f) on refuge use, driven by the HD group having lower refuge use.

Effect of pre-natal stress and behaviour on current growth, future growth, and survival

Initial mass at 8 mpf (prior to post-natal manipulation) did not differ among egg cortisol treatments (χ^2=1.03, df=1, P=0.60). Following the post-natal manipulation (8–9 mpf), fish in the low water treatment had lower growth than fish in the undisturbed treatment (main effect of depth: χ^2=18.96, df=1, P<0.0001; Figure 4.4). With day and night combined, higher exploratory behaviour of individuals in the undisturbed environment

was linked to higher growth (χ^2=5.48, df=2, P=0.02; Table 4.4). In low water, there was a marginally significant interactive effect of refuge use and egg cortisol treatment on growth (χ^2=5.79, df=2, P=0.055; Table 4.4); individuals in the LD and CD groups that had higher refuge use tended to have lower growth, while there was no such relationship in the HD group.

Egg cortisol treatment affected saltwater growth in fish from the undisturbed environment, with fish from the HD group having the slowest saltwater growth (Table 4.5). There was a marginal effect (P=0.050) of refuge use behaviour on saltwater growth in fish from the low water environment (Table 4.5); fish with higher refuge use in fresh water tended to have higher saltwater growth rates. There were no carry-over influences of the three behaviours or egg cortisol treatment on survival in salt water (Table 4.6).

Discussion

We characterized activity, exploration, and refuge use to determine flexibility in diel behaviour in response to low water (drought) and therefore high-risk conditions, and the potential for pre-natal hormonal signals to modulate behavioural flexibility at the group-level in matched and mismatched environments. In the low risk (undisturbed) environment, more exploratory individuals had higher growth. Moreover, the CD group displayed behaviour most likely to promote growth (diurnal activity, low refuge use), followed by the LD group (active throughout day and night, higher refuge use at night), while the HD group displayed risk-averse behaviours (nocturnal and high refuge use). Finally, the HD group also had lower growth following transfer to salt water, showing potential negative carry-over effects of sub-optimal behaviour in fresh water. In the

higher risk (low water) offspring environment, individuals in all groups displayed high diel flexibility in activity and exploration (nocturnal behaviour), but the HD group fish were the most risk-taking (low refuge use) and potentially better suited to a chronically stressful environment (under the risk allocation hypothesis: Lima and Bednekoff 1999). Increased refuge use led to lower growth in CD and LD groups but not in the HD group, reaffirming that CD and LD groups were favouring survival in the risky environment over growth, while the HD group was continuing to take risks to maintain growth. In addition, increased refuge use led to higher growth upon transfer to salt water, showing that more risk-averse behaviour may have led to positive carry-over effects in the common saltwater stage. We did not detect any carry-over effects of freshwater behaviour on survival into the saltwater phase; this was not unexpected, as fish experienced a common, less stressful environment in salt water and effects of behaviour on fitness are life stage specific (Smith and Blumstein 2008).

Changes in diel behaviour among pre-natal stress groups in undisturbed conditions

The optimal behaviours displayed by the CD group in the matched, undisturbed environment – more active in daytime yet more exploratory at night, and unaltered use of the refuge from day to night – are in agreement with previous work showing that juvenile salmon preparing to migrate should invest in high growth by displaying diurnal activity that leads to more efficient foraging (since efficiency declines with low light levels: Fraser and Metcalfe 1997; Metcalfe et al. 1998). The LD group displayed the second most optimal behaviour, suggesting that the LD treatment was somewhat mismatched to the benign environment. Since no growth differences were detected among pre-natal

stress groups during the 30-day period, maintaining high activity levels at night did not negatively affect growth in the LD group. However, since actual predation in our study was minimized with enclosed netting, we cannot make conclusive deductions about potential predation costs of having increased activity and exploration. Switching from a riskier strategy during the day (i.e., high activity, low refuge use) to a more risk-averse strategy at night may have allowed LD fish to monopolize feeding opportunities during the day by making use of the shallow habitat. However, since fish were only fed during the day, being active at night would not have led to higher food acquisition unless natural food sources in the outdoor stream channels were being utilized. While other work has also found that elevated pre-natal GCs can increase exploratory and risk-taking behaviour (Zimmer et al. 2013), our results are novel in showing effects of a hormonal signal on shifts in diel exploration and refuge-seeking behaviours.

As predicted, the HD group displayed the least optimal behaviours in the mismatched undisturbed environment; HD fish had a more risk-averse strategy by being nocturnal (more active at night), a behavioural pattern normally observed in wintertime under lower temperatures when metabolic rates are lower (Fraser and Metcalfe 1997). The HD group also had the steepest increase in exploration from day to night, and high overall risk-averse behaviour that did not change from day to night. Since HD fish had similar growth to the CD and LD groups during the 30-day period, lower levels of activity and exploration in daytime were sufficient to acquire food and maintain energetic balance. Studies under different food availability have shown that brief bouts of daytime foraging can have a large, positive impact on growth (Metcalfe et al. 1999). However, had HD fish not been predictably fed multiple times during the day (e.g., as would not

have occurred in the wild), they may not have been able to maintain a similar growth trajectory. Indeed, the HD group had lower growth following transfer to salt water, indicating that exhibiting risk-averse behavioural patterns in fresh water may have had negative carry-over effects. Taken together, these results indicate that variation in behaviours related to the trade-off between growth and survival are modulated by exposure to pre-natal stress signals. As the matched CD group displayed optimal behaviour while the potentially mismatched LD group, and in particular the HD group, showed less optimal behaviours in a relatively benign environment, this supports environmental matching theory (Sheriff and Love 2013) and the idea that exposure to stress signals will result in costs if the signal is unreliable. As environmental change is not only leading to more severely stressful conditions, but also more variable and unpredictable conditions, it is relevant to characterize the implications of experiencing mismatched conditions.

Changes in diel behaviour among pre-natal stress groups in low water conditions

We found that individuals in all pre-natal stress groups showed a high level of diel flexibility in response to low water conditions; activity and exploration were low in the daytime and showed a large increase from day to night (i.e., nocturnal behaviour). Low daytime activity and exploration was likely indicative of lower foraging behaviour, since all groups in low water had lower growth rates compared to fish in the undisturbed environment. Lower growth was likely incurred to promote survival in the stressful environment; fish may have adopted nocturnal behavior in response to less cover (Gibson and Erkinaro 2009) to limit time spent exposed to perceived risk of diurnal avian

predators (Valdimarsson and Metcalfe 1998) and visual piscivores (Metcalfe et al. 1999). Indeed, Railsback et al. (2005) used simulation modeling to show that low-flow conditions (reduced water area) are predicted to change activity patterns by increasing nocturnal feeding and reducing fish growth. The authors concluded that patterns of activity are modified by many factors including habitat conditions that change feeding ability and amount of cover (Railsback et al. 2005). While pre-natal stress groups displayed behavioural flexibility in response to low water, indicative of predation-sensitive foraging (reduced foraging activity in the presence of a predatory threat), we did find modulations in behaviour explained by pre-natal stress exposure, with CD and LD groups displaying more optimal behaviour compared to the HD group. The CD group had a steeper increase in activity from day to night compared to the LD and HD groups, displaying extremely low daytime activity and a very risk-averse behavioural phenotype. In food-restricted conditions, displaying such low daytime activity may ultimately lead to low feeding in the CD growth and consequences for growth (not observed in this study under a predictable feeding regime).

In contrast, the HD group had lower overall refuge use compared to the CD and LD groups, showing that the HD group took the most risks in the low water environment, a strategy that may have been better suited to more severely stressful conditions. Under the risk allocation hypothesis, individuals experiencing periods of risk should increase foraging during less risky periods, while individuals experiencing chronically risky situations have little choice but to forage under risk to survive (Lima and Bednekoff 1999). This framework supports our results showing that increased refuge use related to lower growth in the CD and LD groups during the low water manipulation, but not in the

HD group. We hypothesize that CD and LD groups traded-off growth to benefit survival by increasing refuge use, while the same trade-off was not observed in the HD group that had overall lower refuge use. Freshwater survival was not assessed statistically due to low mortality of tagged fish, and therefore we cannot make firm conclusions about the outcome of the growth-survival behavioural trade-offs in low water. In addition, our stream channels were protected from most predation, meaning that we were not able to assess consequences of decisions made under the perception of risk in low water. In a more severe high-risk environment, we predict that the HD group may have displayed appropriate behaviours (e.g., high anti-predator behaviour: Giesing et al. 2011) while the CD group may have exhibited sub-optimal behaviours, and future work could introduce different levels of a stressor (e.g., predation risk, realized predation) to tease apart these predictions.

Behaviour as a predictor of future growth and survival

We found no detectable carry-over effects of freshwater behaviour on growth during the early saltwater phase in fish from the low-risk, undisturbed environment; the shift in life-history stage from a freshwater stream to the saltwater ocean phase is dramatic and it is likely that selective pressures have led to different optimal suites of behaviour for each environment (Smith and Blumstein 2008). However, higher refuge use in fresh water led to higher saltwater growth in fish from the low water environment, suggesting that behaviours in response to risky conditions may have more of an influence on future performance traits. This shows the benefits of adopting optimal strategies across environmental contexts; seeking refuge in risky freshwater environments, as did the CD

and LD groups, was associated with faster growth in a food-rich, less risky saltwater environment. Interestingly however, behaviour measured in fresh water did not influence future survival of smolts in salt water. Fish in salt water were exposed to a relatively benign environment in net pens with regular feeding, and so we may not have detected the same survival differences that could have occurred in the wild. Alternatively, since juvenile stream behaviour is flexible and affected by many factors such as internal state (Metcalfe et al. 1998) and personality (Watts et al. 2015), overall daily behavioural patterns observed may not directly relate to future survival since the relationship between behaviour and fitness is context- and life-stage specific (Biro and Stamps 2008; Smith and Blumstein 2008).

Conclusions

Pacific salmon are experiencing drastic changes in stream conditions such as increased occurrences of droughts (Crozier et al. 2008), emphasizing the importance of characterizing the level of phenotypic and transgenerational plasticity in behavioural responses to stressful environments. Our results are novel in showing that pre-natal hormonal signals lead to shifts in behaviours representing a trade-off between growth and predator avoidance. The LD (low dose) group and CD (control) group exhibited optimal behavioural patterns in low water, and we predict that the low water conditions may not have been risky enough to lead to costs in the CD group. We found evidence that the HD (high dose) group was phenotypically prepared for a more chronically stressful environment, and further studies incorporating high-risk environments (e.g., predation risk) could confirm this idea. This work highlights the importance of not only assessing

the potential benefits of reliable intergenerational signals of stress, but also the costs of unreliable cues under conditions of rapid environmental change.

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Tables

Table 4.1 Water quality metrics (mean ± SE with ranges in brackets) in shallow and deep microhabitats of stream channels in morning and afternoon during 30-day manipulation of water depth.

Water quality metric	Time of day	Habitat	Undisturbed environment	*N*	Low water environment	*N*
Depth (cm)	NA	Shallow	30 ± 1 (25–33)	6	7 ± 0.5 (5–8)	6
	NA	Deep	64 ± 1 (60–66)	6	33 ± 1 (30–38)	6
Temperature (°C)	Morning	Shallow	13.6 ± 0.1 (12.4–14.8)	103	13.7 ± 0.1 (12.5–15.1)	106
		Deep	13.6 ± 0.1 (12.4–14.8)	103	13.7 ± 0.1 (12.4–14.9)	106
	Afternoon	Shallow	14.5 ± 0.1 (13.2–16.2)	103	14.7 ± 0.1 (13.3–16.5)	104
		Deep	14.4 ± 0.1 (13.2–16.2)	102	14.7 ± 0.1 (13.3–16.4)	104
Dissolved oxygen (mg L^{-1})	Morning	Shallow	9.8 ± 0.03 (9.0–10.6)	97	9.2±0.05 (7.8–10.4)	101
		Deep	9.8±0.03 (9.0–10.6)	97	9.4±0.04 (8.2–10.6)	101
	Afternoon	Shallow	10.2 ± 0.04 (9.1–11.2)	103	9.6±0.04 (8.3–10.7)	104
		Deep	10.1±0.04 (9.1–11.1)	103	9.7±0.04 (8.6–10.5)	104

Table 4.2 PCA loadings, eigenvalues, and percent variance explained for diel behavioural traits of juvenile Chinook salmon where 3 components were extracted based on eigenvalue >l and bolded values indicate loadings >0.3.

Dataset	**Day and night stacked**		
Component	1: Activity	2: Exploration	3: Refuge use
Eigenvalue	4.8	2.4	1.4
% variance explained	35.9	26.8	23.3
Percent time in shallow	0.26	**0.90**	−0.02
Percent time in refuge	0.30	0.08	**0.91**
Percent time in deep	0.17	**−0.63**	**−0.65**
Habitat switches	**0.94**	0.27	0.05
Number of visits to shallow	**0.74**	**0.53**	0.11
Number of visits to refuge	**0.94**	0.29	0.12
Number of visits to deep	**0.94**	0.01	−0.10
Mean stay length in shallow	0.30	**0.83**	−0.07
Mean stay length in refuge	−0.16	−0.13	**0.90**
Mean stay length in deep	−0.26	**−0.57**	**−0.48**

Table 4.3 PCA loadings, eigenvalues, and percent variance explained for 24-hour behavioural traits of juvenile Chinook salmon where 3 components were extracted based on eigenvalue >l and bolded values indicate loadings >0.3.

Dataset	**24-hour**		
Component	1: Activity	2: Refuge use	3: Exploration
Eigenvalue	4.9	2.4	1.4
% variance explained	42.4	23.0	21.6
Percent time in shallow	0.27	0.00	**0.92**
Percent time in refuge	**0.32**	**0.90**	−0.02
Percent time in deep	0.01	**−0.76**	**−0.45**
Habitat switches	**0.97**	−0.05	0.17
Number of visits to shallow	**0.77**	0.09	0.41
Number of visits to refuge	**0.97**	−0.02	0.19
Number of visits to deep	**0.91**	−0.19	0.01
Mean stay length in shallow	0.19	0.08	**0.89**
Mean stay length in refuge	**−0.34**	**0.88**	−0.11
Mean stay length in deep	**−0.79**	−0.28	−0.27

Table 4.4 Summary of linear mixed models examining the effects of egg cortisol treatment and behaviour on freshwater growth of juvenile Chinook salmon with stream channel identity included as a random effect. Significance was assessed using likelihood ratio tests where terms were sequentially removed (significant *P*-values indicated in bold).

Freshwater growth rate							
Environment	Model	Variable	Estimate	SE	χ^2	df	*P*
Undisturbed	1	Intercept	1.61	0.15	–	–	–
		Egg cortisol [LD]	−0.37	0.22	2.15	2	0.34
		Egg cortisol [HD]	−0.05	0.20			
		Activity	−0.06	0.11	1.84	1	0.17
		Egg cortisol [LD] × activity	0.31	0.16	3.82	2	0.15
		Egg cortisol [HD] × activity	0.10	0.13			
	2	Intercept	1.63	0.13	–	–	–
		Egg cortisol [LD]	−0.21	0.17	1.53	2	0.46
		Egg cortisol [HD]	−0.01	0.18			
		Refuge use	0.08	0.09	0.05	1	0.81
		Egg cortisol [LD] × refuge use	0.03	0.13	4.75	2	0.093
		Egg cortisol [HD] × refuge use	−0.14	0.12			
	3	Intercept	1.58	0.14	–	–	–
		Egg cortisol [LD]	−0.19	0.20	2.13	2	0.34
		Egg cortisol [HD]	−0.02	0.20			
		Exploration	0.10	0.07	5.14	1	**0.023**
		Egg cortisol [LD] × exploration	−0.04	0.09	0.21	2	0.90
		Egg cortisol [HD] × exploration	−0.02	0.09			
Low water	1	Intercept	0.81	0.18	–	–	–
		Egg cortisol [LD]	−0.11	0.25	1.70	2	0.43
		Egg cortisol [HD]	0.13	0.25			
		Activity	0.01	0.05	3.39	1	0.066
		Egg cortisol [LD] × activity	0.12	0.08	2.10	2	0.35
		Egg cortisol [HD] × activity	0.05	0.08			
	2	Intercept	0.81	0.20	–	–	–
		Egg cortisol [LD]	−0.07	0.29	1.30	2	0.52

	Egg cortisol [HD]	0.18	0.29			
	Refuge use	−0.04	0.06	0.01	1	0.93
	Egg cortisol [LD] × refuge use	−0.07	0.10			**0.055**
	Egg cortisol [HD] × refuge use	0.18	0.09	5.79	2	
3	Intercept	0.79	0.16	–	–	–
	Egg cortisol [LD]	−0.09	0.23	1.30	2	0.52
	Egg cortisol [HD]	0.08	0.23			
	Exploration	0.09	0.06	0.21	1	0.64
	Egg cortisol [LD] × exploration	−0.07	0.09	3.68	2	0.16
	Egg cortisol [HD] × exploration	−0.16	0.09			

Table 4.5 Summary of linear mixed models examining the effects of behaviour (measured in fresh water) and egg cortisol treatment on saltwater growth rate of Chinook salmon smolts with net pen included as a random effect. Significance was assessed using likelihood ratio tests where terms were sequentially removed (significant *P*-values indicated in bold).

Saltwater growth rate							
Environment	Model	Variable	Estimate	SE	χ^2	df	*P*
Undisturbed	1	Intercept	0.39	0.05	–	–	–
		Egg cortisol [LD]	−0.08	0.07	7.03	2	**0.030**
		Egg cortisol [HD]	−0.09	0.04			
		Activity	0.03	0.06	2.15	1	0.14
		Egg cortisol [LD] × activity	0.07	0.09	1.38	2	0.50
		Egg cortisol [HD] × activity	−0.01	0.06			
	2	Intercept	0.42	0.05	–	–	–
		Egg cortisol [LD]	−0.02	0.05	7.62	2	**0.022**
		Egg cortisol [HD]	−0.10	0.04			
		Refuge use	0.01	0.04	0.09	1	0.76
		Egg cortisol [LD] × refuge use	0.04	0.07	0.86	2	0.65
		Egg cortisol [HD] × refuge use	−0.02	0.05			
	3	Intercept	0.41	0.04	–	–	–
		Egg cortisol [LD]	−0.001	0.04	8.35	2	**0.015**
		Egg cortisol [HD]	−0.10	0.04			
		Exploration	0.01	0.03	0.01	1	0.94
		Egg cortisol [LD] × exploration	−0.07	0.05	4.14	2	0.13
		Egg cortisol [HD] × exploration	0.02	0.04			
Low water	1	Intercept	0.40	0.04	–	–	–
		Egg cortisol [LD]	−0.06	0.04	2.84	2	0.24
		Egg cortisol [HD]	−0.02	0.05			
		Activity	0.02	0.02	0.08	1	0.77
		Egg cortisol [LD] × activity	−0.001	0.03	3.84	2	0.15
		Egg cortisol [HD] × activity	−0.06	0.03			
	2	Intercept	0.38	0.04	–	–	–
		Egg cortisol [LD]	−0.05	0.04	5.22	2	0.074

		Egg cortisol [HD]	0.04	0.05			
		Refuge use	0.04	0.02	3.82	1	**0.050**
		Egg cortisol [LD] × refuge use	−0.01	0.04	0.36	2	0.84
		Egg cortisol [HD] × refuge use	−0.03	0.04			
	3	Intercept	0.39	0.03	–	–	–
		Egg cortisol [LD]	−0.05	0.04	3.07	2	0.21
		Egg cortisol [HD]	0.04	0.04			
		Exploration	0.04	0.03	2.12	1	0.14
		Egg cortisol [LD] × exploration	−0.06	0.04	4.79	2	0.091
		Egg cortisol [HD] × exploration	0.03	0.04			

Table 4.6 Results from generalized linear mixed models examining the effects of behaviour (measured in fresh water) and egg cortisol treatment on survival of Chinook salmon smolts with net pen included as a random effect. Significance was assessed using likelihood ratio tests where terms were sequentially removed.

Saltwater survival							
Environment	Model	Variable	Estimate	SE	df	χ^2	*P*
Undisturbed	1	Intercept	0.52	0.55	–	–	–
		Egg cortisol [LD]	−1.06	0.86	2	4.08	0.13
		Egg cortisol [HD]	0.00	0.67			
		Activity	0.39	0.89	1	1.98	0.16
		Egg cortisol [LD] × activity	0.15	1.17	2	0.02	0.99
		Egg cortisol [HD] × activity	0.13	1.02			
	2	Intercept	0.90	0.55	–	–	–
		Egg cortisol [LD]	−1.10	0.69	2	3.53	0.17
		Egg cortisol [HD]	0.06	0.73			
		Refuge use	0.40	0.67	1	1.60	0.21
		Egg cortisol [LD] × refuge use	−1.12	0.97	2	1.78	0.41
		Egg cortisol [HD] × refuge use	−0.91	0.78			
	3	Intercept	0.69	0.41	–	–	–
		Egg cortisol [LD]	−0.67	0.60	2	1.87	0.39
		Egg cortisol [HD]	0.11	0.58			
		Exploration	0.24	0.53	1	3.03	0.081
		Egg cortisol [LD] × exploration	−0.62	0.66	2	2.91	0.23
		Egg cortisol [HD] × exploration	−1.12	0.69			
Low water	1	Intercept	1.23	0.53	–	–	–
		Egg cortisol [LD]	−1.28	0.63	2	0.09	0.77
		Egg cortisol [HD]	−0.74	0.66			
		Activity	0.53	0.39	1	2.91	0.23
		Egg cortisol [LD] × activity	−0.94	0.55	2	3.88	0.14
		Egg cortisol [HD] × activity	−0.81	0.52			
	2	Intercept	0.80	0.42	–	–	–
		Egg cortisol [LD]	−0.70	0.57	2	3.07	0.22
		Egg cortisol [HD]	−0.43	0.61			

		Refuge use	0.12	0.37	1	0.87	0.35
		Egg cortisol [LD] × refuge use	−0.53	0.54	2	1.23	0.54
		Egg cortisol [HD] × refuge use	−0.53	0.58			
	3	Intercept	0.86	0.40	–	–	–
		Egg cortisol [LD]	−0.89	0.54	2	3.12	0.21
		Egg cortisol [HD]	0.11	0.65			
		Exploration	−0.10	0.39	1	0.53	0.47
		Egg cortisol [LD] × exploration	0.25	0.57	2	1.42	0.49
		Egg cortisol [HD] × exploration	0.76	0.65			

Figures

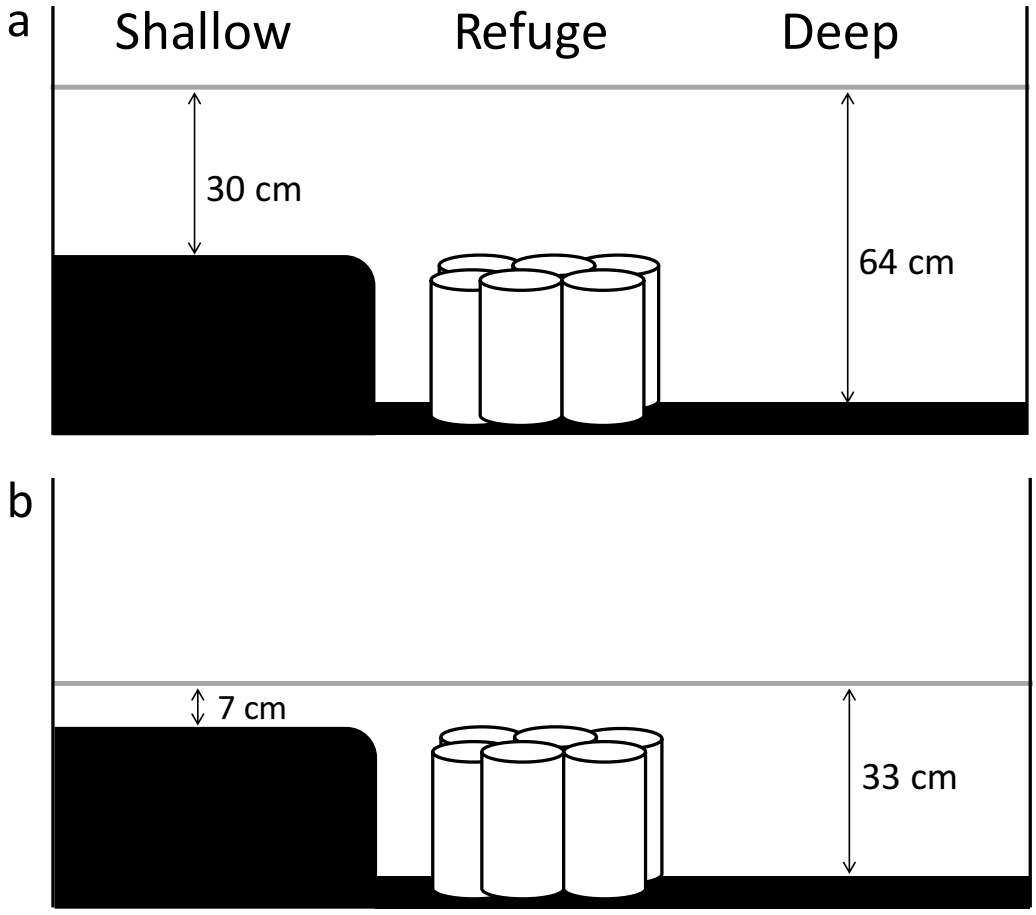

Figure 4.1 Side-view schematics of the (a) undisturbed stream channels and (b) low water depth stream channels containing three different microhabitats: a shallow downstream area, middle refuge area, and upstream deep area.

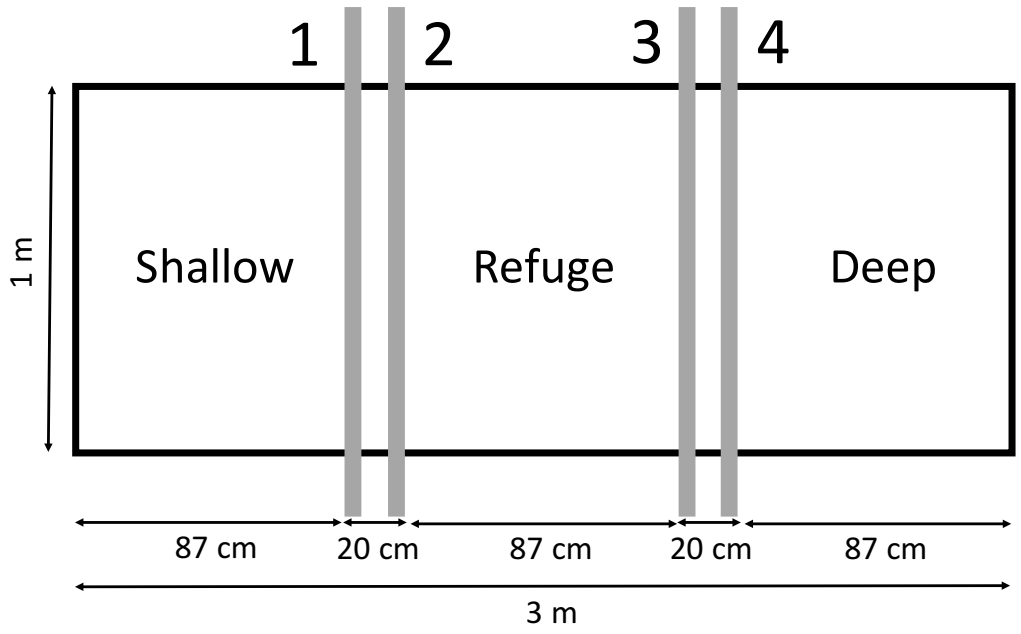

Figure 4.2 Top-down schematic showing placement of antenna pairs within a semi-natural stream channel, splitting each channel intro three microhabitats: downstream shallow area, middle refuge area, and upstream deep area. For more details regarding detection ranges and signal interference, see methods section.

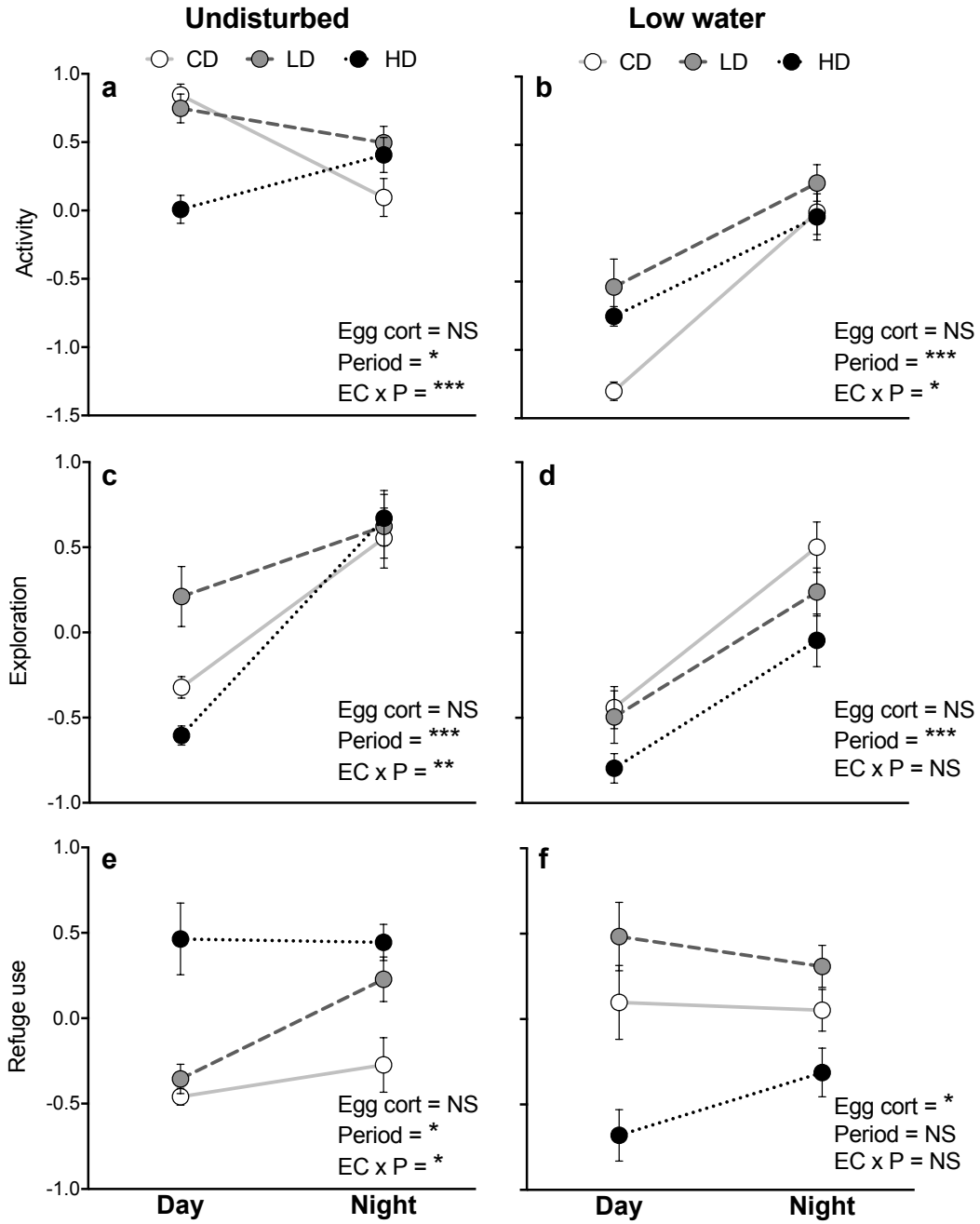

Figure 4.3 Diel behaviours (activity, exploration, refuge use) of Chinook salmon juveniles reared from eggs exposed to a control dose (CD; 0 ng mL^{-1}), low cortisol dose (LD; 300 ng mL^{-1}), or high cortisol dose (HD; 1000 ng mL^{-1}) and exposed to undisturbed (a, c, e) or low water (b, d, f) environments from 8–9 mpf in fresh water. Values presented as means ± SE and NS, *, **, and *** represent P-values that were >0.5, <0.05, <0.01, and <0.001 respectively.

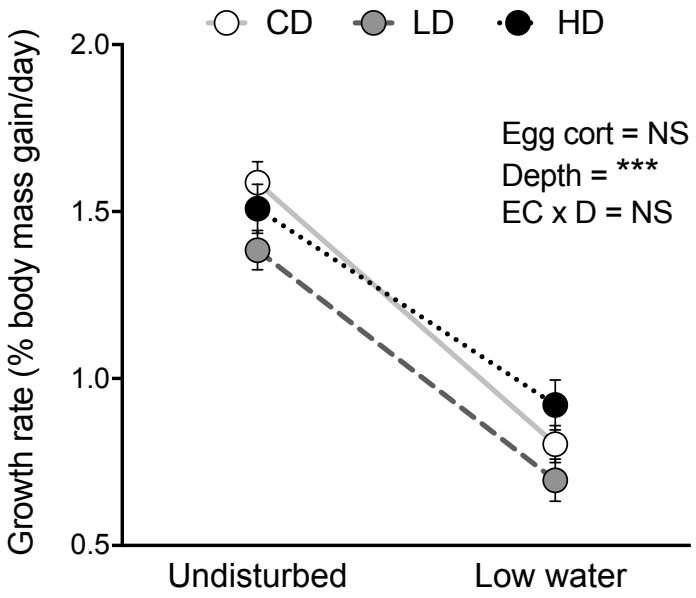

Figure 4.4 Specific growth rate in fresh water (8–9 mpf) of Chinook salmon juveniles reared from eggs treated with a control dose (CD; 0 ng mL^{-1}), low cortisol dose (LD; 300 ng mL^{-1}), or high cortisol dose (HD; 1000 ng mL^{-1}) and exposed to an undisturbed or low water environment from 8–9 mpf in fresh water. Values presented as means ± SE and NS and *** represent *P*-values >0.5 and <0.001, respectively.

CHAPTER 5 – GENERAL DISCUSSION

Introduction: the importance of intergenerational effects in coping with environmental change

Individuals can respond to changing environments through various means, one of which is phenotypic plasticity, whereby a certain genotype leads to different phenotypes in altered environments (Thibert-Plante and Hendry 2011). When a parent's environment influences offspring phenotype, this form of plasticity is referred to as transgenerational plasticity or intergenerational effects (Mousseau and Fox 1998). Since intergenerational effects have evolved in response to predictable shifts in the environment, they have the potential to mitigate negative effects of climate change-induced conditions (Allan et al. 2014; Shama et al. 2014). However, this is dependent on the parental environment continuing to serve as a reliable cue of future offspring environmental quality (i.e., PARs/environmental matching theory: Gluckman et al. 2005; Sheriff and Love 2013). Studies examining hormonally-mediated maternal stress (i.e., signals transmitted from mother to offspring via elevated glucocorticoids: Love et al. 2013) observe potential adaptive shifts in offspring phenotype in response to elevated maternally-derived hormones (e.g., Chin et al. 2009; Dantzer et al. 2013), yet offspring traits and fitness metrics measured in one environment or in a non-realistic context limit the interpretability of results. Only when measuring a suite of offspring traits and fitness-metrics in response to a maternal hormonal signal in both matched and mismatched environmental conditions can interpretations be extended to real-world situations (Sheriff and Love 2013). Consequently, quantifying intergenerational effects in the context of environmental change by setting up multiple offspring environments was the overall goal

of my thesis, as an important next step for characterizing organismal responses to climate change.

Taken together, the results from my thesis support predictions of environmental matching theory while emphasizing the potential costs of a signal being mismatched to future environmental conditions. Importantly, my results highlight the necessity of setting up realistic environmental contexts to test intergenerational effects and measuring multiple offspring traits across life-history stages. More specifically, I found that pre-natal hormonal signals of stress (low cortisol dose (LD) and high cortisol dose (HD) signals) benefitted early survival of Chinook salmon fry compared to the control dose (CD; Chapter 2), while post-natal environment (i.e., water depth) was a more important determinant of parr survival, with a risky, low water environment decreasing survival (Chapter 3). Interestingly, combining survival metrics to quantify cumulative survival indicated that while carry-over effects of pre-natal stress on longer-term survival may not be observed, cumulative effects of early offspring survival may still have large implications for overall maternal fitness (Figure 5.1). Low water conditions in semi-natural streams led to optimal responses in Chinook salmon parr from the LD group, indicated by low energetic demand, maintenance of stress responsiveness, and large size (Chapter 3), as well as nocturnal and risk-averse behaviour (Chapter 4), providing support for environmental matching. In contrast, the HD group displayed sub-optimal responses in low water such as increased energy demand, dampened stress response, small size, and lower growth (Chapter 3), as well as risky behavioural patterns (Chapter 4), and was likely primed for a more severely stressful environment (e.g., chronic predation risk). The CD group was predicted to be mismatched to stressful conditions but

only showed some indicators of sub-optimal performance (dampened stress response, small size; Chapter 3), meaning that the low water environment may not have been a severe enough stressor to lead to measurable costs across all traits in the CD group.

Why are context-dependent intergenerational effects observed and when are they adaptive?

In this section, I use the results of my thesis as a case study to discuss four ways that intergenerational effects are context dependent, and how consideration of these concepts can aid in interpretability of results and the end goal of concluding whether offspring responses to maternal stress are adaptive in the context of the offspring's environment. First, I discuss how manipulation of parental environment, in these instances a maternal stressor either imposed on the female directly or indirectly through elevation of GCs in the female or in her eggs, affects offspring responses (discussion of paternal effects is important but beyond the scope of this thesis; see Guillaume et al. 2016). In relation to this, I then discuss how choice of offspring rearing environment, ranging in degree from a benign, laboratory environment to chronically stressful conditions, modifies offspring responses and the level of matching/mismatching between parental and offspring conditions. Next, the conditions in which offspring are experimentally tested will shape the outcome of traits measured and are therefore an important consideration. Finally, I discuss how life-history traits ultimately determine the adaptability of intergenerational effects in a given study species, and how alternate mechanisms (e.g., bet hedging: Crean and Marshall 2009) may be more likely in some instances.

Manipulation of maternal stress

Within the realm of work examining hormonally-mediated maternal stress in oviparous taxa, there is a vast range of techniques to either apply a maternal stressor or mimic the transfer of elevated GCs to eggs (Sopinka et al. 2015a). Applying a maternal stressor (e.g., chasing, predation risk, competition) is the most realistic method of experimentally inducing maternal stress and leads to ease of matching maternal and offspring environments (i.e., can apply identical stressor to mother and offspring), but the stressor itself is likely causing a plethora of physiological and behavioural changes in the females and eggs, meaning that it is difficult to tease apart the mechanism inducing phenotypic effects. For example, exposure of female sticklebacks to predation risk leads to eggs with increased cortisol content (Giesing et al. 2011) but also leads to embryos with differential expression of genes involved in epigenetic modification (Mommer and Bell 2014), highlighting that offspring phenotypic effects may have been due to hormonal mechanisms, epigenetic mechanisms, or likely a combination of both (see Figure 1, Li and Leatherland 2013). For this reason, researchers can either expose females to GCs (e.g., Eriksen et al. 2006) or directly expose eggs to GCs (e.g., Sopinka et al. 2015b) to determine whether effects on offspring are GC-mediated responses. These studies provide more control over egg-GC levels and the potential to induce multiple signals of different strengths (i.e., low and high dose signals), which are known to have non-linear effects on offspring phenotype (Gagliano and McCormick 2009; Li et al. 2010). Indeed, my results support the existence of biphasic effects and propose that a low dose signal can lead to optimal early-life effects, while a higher dose may be supra-optimal (Chapter 2). For example, in the context of the offspring stream (i.e., rearing) environment, the low dose

signal again appeared to be optimal while the high dose signal was not, which was likely due to a mismatch of the high dose hormonal cues with the low water environment (Chapters 3 and 4). My thesis emphasizes the importance of using hormone doses that lead to levels within the physiological range and not supra-physiological levels (e.g., Sloman 2010; Colson et al. 2015), as this ensures that females could realistically spawn eggs containing that level of hormone and serving as an environmental cue. While it is difficult to estimate how different levels of GCs in eggs may serve as cues for different levels of environmental stress, using multiple doses aids in interpreting the potential level of matching/mismatching. Additionally, studies that apply an egg GC exposure in tandem with a maternal stressor exposure and quantify the same offspring traits in both treatments can help tease apart the mechanistic role of GCs in transmitting signals of maternal stress (e.g., Ghio et al. 2016).

Manipulating offspring rearing environment

Moving beyond the simple relationship between maternal stress and offspring phenotypic change, researchers have more recently begun to appreciate the interaction between pre- and post-natal environmental conditions, emphasizing the importance of measuring offspring responses to intergenerational stress in relevant stressful conditions in addition to more benign conditions (Sheriff and Love 2013). Examining phenotypes within both expected matched and mismatched environments is increasingly relevant since studies have shown that offspring often respond to maternal stress exposure by displaying traits that could be beneficial in stressful conditions or serve as a way to avoid/disperse from stressful environments (better flight performance: Chin et al. 2009; higher growth:

Dantzer et al. 2013; greater anti-predator behaviour and higher dispersal: Bestion et al. 2014). A few studies have exposed parents and offspring to identical stressful conditions and found evidence of environmental matching (i.e., matched offspring in stressful environments outperform offspring in mismatched environments: Donelson et al. 2012; Salinas and Munch 2012; Shama et al. 2014), yet results are often not this simple. When hormone exposures are used as a proxy of maternal stress, it is difficult to estimate what offspring environment is matched or mismatched to the signal, and equally convoluted to determine the life stage and length of time to apply the stressor, particularly in species with complex life-histories and different selective pressures occurring at different life stages. To characterize how different GC levels in eggs represent different environmental signals, more work is needed, particularly in fishes, to determine the connection between the maternal environment and egg GC content. In addition, my thesis emphasizes that even when a relevant offspring environmental stressor is chosen, imposing conditions in semi-natural environments may mean that outcomes of trade-offs normally observed in the wild are not seen in captivity. For example, I simulated a risky environment with less protective cover by lowering water depths, but was not able to fully measure the outcome of behavioural decisions without realized risk of predation (Chapter 4). While including multiple offspring environments is the first step towards properly interpreting the effects of maternal stress signals, researchers should also consider the severity of stressor imposed and relevance to conditions experienced in the wild.

Considering offspring experimental conditions

In addition to context-dependent effects of maternal stress observed due to benign versus stressful offspring rearing conditions, the experimental conditions in which offspring are tested can also lead to conflicting results. For example, two separate studies on brown trout (*Salmo trutta*) found both increased (Sloman 2010) and decreased (Burton et al. 2011) levels of aggression in offspring previously exposed to elevated egg GCs. These inconsistencies may be explained by differences in experimental design; one study tested offspring in isolation while the other study tested offspring in a group. Similar contradictory results were found in threespine stickleback (*Gasterosteus aculeatus*), where two studies found both lowered (McGhee et al. 2012) and elevated (Giesing et al. 2011) anti-predator behaviour in offspring whose mothers were exposed to predation risk. Again, inconsistent results can be attributed to differences in testing conditions; elevated anti-predator behaviour measured as tighter shoaling was assessed in groups, while lower anti-predator behaviour measured as survival in the face of a real predator was concluded following tests in isolation. My thesis aimed to minimize this issue by rearing offspring in semi-natural stream channels and conducting behavioural tests on individuals inside semi-natural stream channels (Chapter 4) without needing to remove individuals and test them in an unfamiliar environment or in isolation.

Considering study species life history

A final consideration for studies regarding intergenerational effects is to design and interpret experiments in light of a study species' life-history, since the likelihood of adaptive stress signals existing can be predicted based on key life-history traits. For

example, species that exhibit natal philopatry may rely more on intergenerational effects compared to species with high dispersal, since reliable signals depend on overlapping environments (Sheriff and Love 2013). My thesis made use of the stream-dwelling phase where maternal and offspring environment overlap spatially in Pacific salmon, albeit during different seasons, and it was likely that cues reflecting stream conditions could be relatively predictable. Climate fluctuations in the Northeast Pacific Ocean have historically occurred over different time-scales, with the shortest time-scale between 2–7 years (Ware 1995), and these fluctuations affect inland climate and stream conditions (Petersen and Kitchell 2001). However, manipulating the offspring environment earlier in development (e.g., during incubation period or immediately after emergence) may have helped better ensure higher predictability of conditions between generations, as Winter and Spring seasonal climates are the most correlated (Ware 1995). Knowing that the adaptive nature of intergenerational effects is based on the assumption that environmental conditions are both variable and provide reliable cues for predicting future conditions, few studies can actually quantify environmental predictability (Burgess and Marshall 2014), which is especially difficult in longer-lived species with complex life-histories. If environmental conditions underwent periods that were extremely variable and unpredictable, a diversifying bet hedging strategy may have been selected for, under the premise that investing in offspring with diverse phenotypes in an unpredictable environment will lead to a proportion of offspring with an optimal phenotype and benefit maternal fitness (Crean and Marshall 2009). Shama (2015) created a stressful maternal environmental treatment (i.e., elevated water temperature) as well as an unpredictable maternal environmental treatment (i.e., fluctuations in water temperature), and found

evidence for both adaptive intergenerational effects and bet hedging in coral reef damselfish. While there has been little work linking variability in egg hormones with potential bet-hedging strategies, Love et al. (2009) noted intra-clutch variability of yolk hormones in birds as a potential adaptive maternal effect if diversity in offspring traits (e.g., begging behaviour) reduces competition in the nest. While my thesis does not directly test predictions of bet-hedging theory by creating an unpredictable maternal environment, it is interesting to note the variability in phenotypic traits observed in offspring reared from different egg cortisol groups, knowing that all groups had physiologically-relevant egg cortisol levels. Assuming that some degree of intra-clutch variation in egg cortisol exists in fishes (e.g., based on ovary position; Suter 2002), mothers that invest in offspring broods comprised of a variety of phenotypes could have a selective advantage and be of particular relevance as climate change leads to increased unpredictability.

Future directions

Based on the results of my thesis and the above considerations of context-dependent intergenerational effects, future studies should focus broadly on 1) better characterizing egg hormones as a link between maternal environment and offspring performance, as well as 2) further examining matching and mismatching of real-world environmental contexts between generations. Firstly, my thesis indicates that changes in egg cortisol within the physiological range have diverse effects on offspring that are often non-linear and trait-specific; future studies should therefore focus on quantifying natural variation in egg cortisol levels across females, with the overall goal of characterizing the evolutionary

significance of such variation (Sopinka et al. 2017). For example, my results show that a physiologically-relevant boost in egg cortisol can have positive early effects on survival (Chapter 2), and it would be pertinent to first assess inter-female variation in egg cortisol, and then assess whether similar positive effects on survival are observed in offspring reared from eggs that had higher levels of cortisol, and whether this trades-off with any future costs of having higher egg cortisol. In addition to quantifying the implications of natural variation in egg hormones, more work is needed to determine the connection between female state and egg cortisol; while a link between lower female body condition and eggs with higher egg GCs has been well established in birds (Love et al. 2005), some studies in fishes report a positive link between a stressful maternal environment and egg cortisol levels (e.g., Stratholt et al. 1997; McCormick 2006; Sierra-Flores et al. 2015), while others find no such relationship (e.g., Mileva et al. 2010; Sopinka et al. 2014; Jeffrey and Gilmour 2016; Ghio et al. 2016). Future work should continue to explore the link between a stressful maternal environment and levels of egg cortisol, coupled with consideration of other mechanisms explaining how eggs may be buffered from increased levels of cortisol (e.g., metabolism via 11beta- hydroxysteroid dehydrogenase 2 (11β-HSD2): Faught et al. 2016, active transport out of the egg via ATP-binding cassette (ABC) transporters: Paitz et al. 2016). Furthering our knowledge of both the natural variability in egg GCs and the connection between maternal environment and egg GCs will help integrate mechanistic work on the relationship between GCs and embryonic development with studies of intergenerational effects.

I propose that future studies testing environmental matching theory could benefit from taking additional considerations when setting up offspring environments that are

matched and mismatched to the maternal stress signal. In studies manipulating egg GCs, it is difficult to quantify the level of matching between a maternal stress signal (i.e., elevated egg GCs) and the offspring environment. This could be improved in future work by releasing tagged parr into streams with quantifiable differences in environmental quality and tracking survival, or by setting up a gradient of environmental conditions to better capture different possible conditions. For example, including both an environment with a single stressor (e.g., high temperature) and an environment with multiple stressors (e.g., high temperature and predation risk), which are known to have cumulative effects on juvenile salmon (Kuehne et al. 2012), could help tease apart effects of different egg hormone dosages. This approach is increasingly relevant as organisms are more frequently exposed to a combination of stressors (Sih et al. 2011). Choosing relevant stressors as well as the timing and length of the applied stressors are important considerations, and choices should be made according to a species' life history. In addition to testing predictions of environmental matching, future work should also consider the potential influence of bet hedging strategies and aim to measure intra-brood variability in offspring responses as well as overall changes in mean responses (Crean and Marshall 2009). An integrative approach that considers different potential evolved responses to variability in the environment and sets up relevant offspring conditions with attention to a species' life-history will be the most useful in furthering our knowledge of the intergenerational effects of stress.

Relevance for Pacific salmon conservation

As a migratory group of species with complex life-histories, Pacific salmon face many ecological stressors (e.g., predation, competition, nutritional stress; Groot and Margolis 1991), and are now facing a myriad of novel stressors (e.g., warming water temperatures: Martins et al. 2012; fisheries capture: Donaldson et al. 2012, fluctuations in water flow: Crozier and Zabel 2006), demonstrating the importance of characterizing individual and population responses to changing conditions. Perhaps the most sensitive and selective life-history stages with regards to impacts of climate change are the juvenile parr and mature adult (i.e., spawning migration) stages, since the stream environment is susceptible to changes in water flow, temperature, and depth (Crozier and Zabel 2006; Mantua et al. 2010). Results from my thesis demonstrate that variation in egg cortisol levels among female Pacific salmon may act as predictive cues to offspring, producing phenotypes that are best suited to certain stream environments matched to the hormonal signal. This has implications for aspects of Pacific salmon conservation such as improving egg quality (i.e., ensuring hormone concentrations necessary for proper development) and egg-to-fry survival, as well as the success of juveniles in the stream environment. A brief increase in egg cortisol levels close to fertilization led to markedly increased egg-to-fry survival, reinforcing the potential for hormone levels to affect egg quality (Brooks et al. 1997; Bobe and Labbé 2010), although responses to exogenous increases in hormone may not reflect the same outcomes as naturally elevated levels of egg hormone in response to increased maternal circulating levels. Nonetheless, my thesis supports the notion that maternally-derived hormones in eggs are an important aspect of overall egg quality and further quantifying levels of egg hormones and the subsequent

effects of certain hormone levels on developing embryos is relevant to conservation initiatives such as stock enhancement.

My results indicate that egg cortisol levels could serve as an important modulator of offspring traits that dictate how offspring will perform in certain environmental conditions. While my thesis focused on offspring performance in low water depth conditions as a simulated risky environment, future work could further assess the potential for intergenerational effects to mitigate detrimental effects of climate change by manipulating water temperature in both maternal and offspring environments, creating an elevated temperature treatment predicted under the conditions of climate change. Other work in fishes has shown that experiencing matched water temperature environments between generations can improve performance (Donelson et al. 2012; Salinas and Munch 2012; Shama et al. 2014), showing the potential for populations to perform better than expected in response to warming water temperatures if reliable cues exist between generations. In addition, hatchery augmentation programs that rear salmon and release juveniles into streams to increase population numbers face issues such as balancing the maintenance of genetic diversity and fitness in the wild (Fraser 2008), and it could also be important to consider carryover effects from the maternal environment on offspring fitness in a specific environment (O'Connor and Cooke 2015). For artificially reared salmon, it may therefore be important to consider whether juveniles are being released into streams that are matched or mismatched to the conditions experienced in the maternal environment (i.e., leading to a certain hormonal signal in eggs of females), as this could affect the performance of released juvenile offspring. Some applied research has begun to consider intergenerational effects as an important mediator of offspring

fitness; Evans et al. (2014) found that Atlantic salmon (*Salmo salar*) offspring of parents exposed to a natural river environment (i.e., wild exposure) had increased survivorship compared to the offspring of captive parents. Overall, future research is needed in Pacific salmon to solidify the relationship among the maternal environment, hormone levels in eggs, and fitness outcomes in offspring, with the goal of characterizing how intergenerational effects of stress could be incorporated into conservation strategies.

Conclusions

My thesis provides a rigorous test of the adaptive nature of maternal stress signals, showing evidence for improved offspring performance when a stress signal is matched with risky offspring conditions, as well as costs of being in a mismatched environment. I have drawn attention to the context-dependent nature of intergenerational effects and methodological differences (e.g., choice of parental/offspring environments, egg hormone dosages, testing conditions) that lead to difficulties in interpreting findings. Ultimately, this work has implications for improving knowledge of the intergenerational effects of stress and their role in organismal responses to changing environments, with the potential for intergenerational effects to mitigate detrimental impacts of climate change.

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Figures

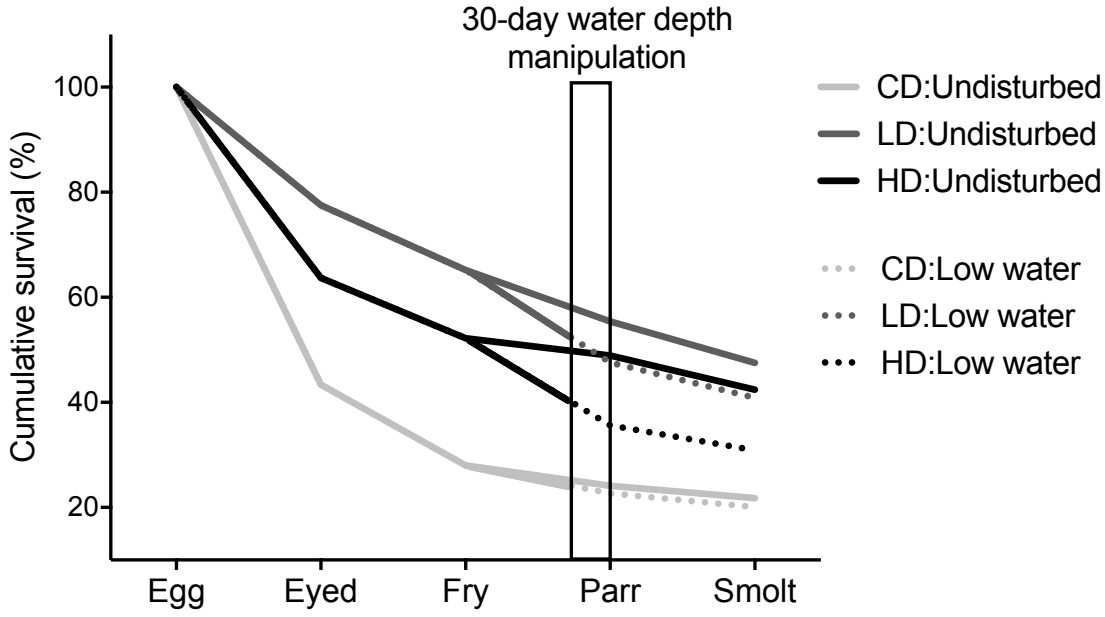

Figure 5.1 Cumulative survival (to 16 mpf) of Chinook salmon exposed to a control dose (CD; 0 ng mL^{-1}), low cortisol dose (LD; 300 ng mL^{-1}), or high cortisol dose (HD; 1000 ng mL^{-1}) at the egg stage, and then exposed to an undisturbed or low water environment for a 30-day period at the parr stage.

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