INFLUENCES OF PARENTAL IDENTITY AND ELEVATED INCUBATION TEMPERATURE ON THE SURVIVAL, DEVELOPMENT AND EARLY LIFE HISTORY TRAITS IN SOCKEYE SALMON

by

Jennifer Mary Burt

B.Sc., McGill University, 2005

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

The Faculty of Graduate Studies

(Forestry)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

June 2011

ABSTRACT

Pacific salmon experience intense selection pressures during their early development, and offspring survivorship and fitness traits are influenced by both parental and environmental influences. Given that elevated water temperature can critically impact early development, this thesis focuses on how individual spawners within a population influence the variation in offspring responses to thermal stress.

The importance of parentage in assessing temperature effects on fish early life history was first examined in a comprehensive literature review. Only 20% of search-identified studies relating to incubation temperature assessed parental influences, but over 90% of those studies reported significant parentally mediated thermal responses. Research gaps in this area included a paucity of studies on offspring physiological traits (11% of studies), performance traits (2%), and on offspring responses beyond endogenous feeding stages (21%), providing impetus for future experiments. A review of the research on intergenerational temperature effects from adult thermal holding studies was also examined.

Sockeye salmon were used in a cross-fertilization experiment to test the hypothesis that significant variation in offspring responses to embryonic temperature stress could be explained by parental identity. Using gametes from Weaver Creek spawners, 10 offspring families were replicated and incubated at 12, 14, and 16ºC from fertilization to hatch. Offspring families had substantially different survival responses across the thermal gradient (crossing reaction norms), and post-treatment mortality and offspring size reflected persistent temperature and parental influences. Within temperature treatments, substantial variation in embryonic survival, alevin mass, time-to-hatch, and hatch duration was attributable to family identity, and most traits exhibited significant temperature-family interactions.

The same families were reared for three weeks after emergence then subjected to a second experiment assessing swim performance. Swim performance was reduced in

fish exposed to the elevated incubation treatments and offspring parentage was found to have a significant effect on fry burst swim time; findings previously undocumented in salmon. Significant temperature-by-family interactions provide further evidence that parental and temperature influences cannot be examined in isolation. In the context of climate change, these findings collectively highlight the importance of family-level variation in influencing future selection within salmonid populations exposed to elevated thermal regimes.

PREFACE

This research is part of a multi-disciplinary research program investigating the impacts of climate warming on Pacific salmon. I held primary responsibility for the design and initiation of experiments, the collection and analysis of data, as well as the writing and submission of manuscripts. During this process, I received considerable guidance, input and support from my two supervisors, Dr. Scott Hinch and David Patterson, who are subsequently listed as co-authors on the manuscripts developed from Chapters 2-4. All experimental procedures were approved by the University of British Columbia Animal Care Committee (#A08-0388) and conducted in accordance with guidelines set by the Canadian Council on Animal Care.

Chapter 2: The importance of parentage in assessing temperature effects on fish early life history: a review of the experimental literature

Published: Burt, J.M., Hinch S.G., and D.A. Patterson. In press. The importance of parentage in assessing temperature effects on fish early life history: a review of the experimental literature. Reviews in Fish Biology and Fisheries. DOI: 10.1007/s11160-010-9179-1
Acceptance: Accepted November 2, 2010, available on-line.
Comments: This review was conducted and written by JMB under the supervision of SGH and DAP who helped conceptualize the study and contributed to the preparation of the manuscript.

Chapter 3: Parental identity influences progeny responses to incubation thermal stress in sockeye salmon (*Oncorhynchus nerka*)

Authors: Jenn M. Burt, Scott G. Hinch, and David A. Patterson
Acceptance: Submitted March 1, 2011
Comments: This study was conducted and written by JMB under the supervision of SGH and DAP who helped conceptualize the experiment and contributed to the preparation of the manuscript.

Chapter 4: Developmental temperature stress and parental identity shape offspring burst swimming performance in sockeye salmon (*Oncorhynchus nerka*)

Authors: Jenn M. Burt, Scott G. Hinch, and David A. Patterson
Acceptance: In the process of submission, June 2011
Comments: Same comments as for Chapter 3

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

It's been a long road, but here I am at the end,
but there are so many people to whom thanks I extend!

First and foremost, is a well-deserved thanks to Prof Hinch[1],
Without your support and encouragement, I'd be in a real pinch!
Great mentorship, so approachable, good advice is your game,
You even sent me to a wonderful salmon conference in Spain!

David Patterson is next, the fert experiment gu-ru,
I'm not sure that this project would've been possible without you! (and E-watch)
You taught me the art of gamete collection and experimental design,
You've been an excellent editor and given me so much of your time!

Dr. John Richardson completes my committee of three,
A big thanks for your input, careful edits, and smile so cheer-y!

And I could never forgot all the helpers - SO MANY!
But the king of them all is most surely field Andy[2],
Mr. Lotto you're a genius, a wet-lab master-mind,
A more knowledgeable, reliable, positive worker one could not find!

There are so many great people who assisted in lab-fishy fun,
Jayme[3], Lisa[4], Merran[5], Jessica[6], Vanessa[7], D'Arcy[8] to name some!
Lucas[9] taught me to swim sockeye, with Lindsay[10] we swam 2000+ fry,
All Hinch members helped with ferts, picking morts, counting 'eyed.'

[1] Dr. Scott Hinch, [2] Andrew Lotto, [3] Jayme Hills, [4] Lisa Thompson, [5] Merran Hague, [6] Jessica Carter, [7] Vanessa Ives, [8] D'Arcy McKay, [9] Lucas Pon, [10] Lindsay Neilsen

But who are these "Hinch members"? This fantabulous crew!
What made these years great was working alongside of you!
Marika[11], Mike[12], Erika[13], Kim[14], Eduardo[15], Tim[16], and Roscoe[17],
Matt[18], Al[19], Kendra[20], Charlotte[21], Brian[22], who could say no?
And there's Andrea[23], and Eric[24], and even dear Glen[25],
And last (just because), is cheery ol' Ken[26]!

Now life as a student, isn't all milk and honey,
And I'm very thankful to the sources that have supported me financially!
A Faculty of Forestry Recruitment Award allowed me to initiate my work,
Followed by the Mary and David Macaree Fellowship and a one-year NSERC.

But the support I have leaned on, quite possibly the most,
Is the kind that all grad students most certainly boast,
It comes from my friends, loved ones, and close famil-y,
Who have kept me a-float when times were hard or craz-y.

To mom, dad, Will, and Sam, you have been so supreme,
You have nurtured my learning, supported my dreams!
And at last to the sockeye, at the heart of this endeavor,
Here's hoping your populations will flourish, for ever and EVER!

---

[11] Marika Gale, [12] Michael Donaldson, [13] Erika Eliason, [14] Kim Hruska, [15] Eduardo Martins, [16] Timothy Clark, [17] David Roscoe, [18] Matt Drenner, [19] Alison Collins, [20] Kendra Robinson, [21] Charlotte Whitney, [22] Brian Ma, [23] Andrea Haas, [24] Erik Vogt, [25] Glenn Crossin, [26] Ken Jeffries

CHAPTER 1: Introduction

Background to thesis topic

In most animal species, and particularly in marine and freshwater fish, offspring survival and fitness in the early life history stages is a critical determinant of population dynamics and successful regeneration. The interaction between organism and environment is central to evolutionary theory, and it is a well-known biological phenomenon that variation in offspring phenotype is a function of their parentally mediated genetic and non-genetic contributions, as well as the external environment that influences phenotypic expression. Thus, gathering information on how both parental influences and early life environmental conditions affect the offspring of particular species will provide a greater understanding of the factors affecting within population reproductive success, resilience and adaptability. This thesis focuses on sockeye salmon (*Oncorhynchus nerka*) and examines the role of parental influences and elevated water temperatures in shaping within-population offspring survival and fitness traits during early development.

Traditionally, spawning populations of fish are assessed as a whole without regard to variation between individuals. In doing so, the differences in reproductive output or offspring fitness between individual females or males are overlooked (Green et al. 2008). With increasing management-driven interest in understanding the factors that influence recruitment and resilience, mounting evidence from research (particularly in marine fishes) is suggesting that spawning stocks should not be viewed as a single entity, but rather as composed of individuals that range in size, age, condition and hence, reproductive potential (Marshall et al. 1998, Trippel et al. 1997). Fish that reproduce in freshwater are also characterized by extensive within-population phenotypic diversity. For example, spawning populations of sockeye salmon contain individuals that vary widely in size, age, physiological condition and energetic status (Patterson, 2004; Hruska, 2010). In addition to phenotypic diversity, the underlying importance of genetic diversity ('genetic infrastructure') within populations is acknowledged as a foundational

component to the adaptability and conservation of wild salmon populations (Gharrett and Smoker, 1993).

Parental influences

The variation among spawning individuals in a population can translate into important variation in the offspring generation. This is because the survival and phenotype of offspring are shaped by the genetic and non-genetic contributions of their individual parents. Genetic influences are comprised of two components: the independent effects of parental genotypes (additive genetic effects) and the interaction between parental genotypes (non-additive genetic effects). Numerous studies using salmonid fish provide evidence of these genetic effects on offspring survivorship and fitness traits (reviewed in Garcia de Leaniz et al., 2007 and Carlson and Seamons, 2008). The non-genetic influences of individual parents on their offspring are referred to as "maternal, paternal or parental *effects*." Parental effects encompass the phenotypic variation in offspring that is attributable specifically to the parental phenotype, sometimes called an inherited environmental effect (Roff, 1998; Wolf and Wade, 2009). In this thesis, the terms "maternal, paternal or parental *influence"* will be used. These terms do not imply a separation of genetic and non-genetic effects, and have been used in many other studies to examine parentally mediated offspring variation in fish (Benoit and Pepin, 1999; Nagler et al., 2000; Green and McCormick, 2005; Green, 2008; Nadeau et al., 2009).

In general, parental influences have been more extensively studied in organisms whose reproductive cycles are easy to manipulate, and which consist of large numbers of offspring with rapid regeneration times, such as invertebrates or plants. However, parental, and especially maternal influences, are increasingly being studied in order to explore mechanisms of offspring variation and adaptation in a larger range of reproductively complex animals (Mousseau and Fox, 1998). Interestingly, parental influences in fish – a hugely diverse group of vertebrates with enormous variation in reproductive strategies – have historically received less recognition despite being remarkably common. This research area has become increasingly popular (see reviews by

Reznick, 1991; Marshall et al., 2008; and Green, 2008) due to the interest in how within-population variation influences fish recruitment, population dynamics, and adaptation.

Parental influences on offspring can be either of maternal or paternal origin. Examination of non-genetic effects tends to focus on maternal effects, as mothers often choose where eggs are deposited and provide offspring with their initial nutritional requirements. For example, female size is known to influence offspring size and quality in a variety of taxa, including plants (Roach and Wuff, 1987), amphibians (Kaplan, 1998) and fish (Heath and Blouw, 1998). For fishes, this may be an important relationship as differences in egg sizes can affect survival to hatch, survival under starvation, hatchling size, and growth rate (reviewed in Reznick, 1991). Many other ecologically significant examples of variation in fish offspring traits have been attributed to morphological (*e.g.,* size at maturity), physiological (*e.g.,* age, nutrition, toxin load) and behavioral (*e.g.,* mate choice, site selection) maternal traits (reviewed in Green, 2008).

The non-genetic influence of individual males (sires) on their offspring has received less attention in the scientific literature because their contribution is primarily genetic. However, studies on paternal effects in many taxa are increasing as growing evidence supports that male sperm characteristics and reproductive behaviors can influence offspring phenotype. In fish species, studies have demonstrated that male spawners can influence hatching success (Rideout et al., 2004), sperm motility can influence larval size (Evans and Geffen, 1998), paternal nest care can influence larval growth rate (Green and McCormick, 2005), and reproductive mating strategy can affect juvenile metabolic capacity and muscle development (Morasse et al., 2008). In some cases, sire effects become more apparent slightly later in development, near the onset of exogenous feeding, and may be due to diminishing maternal effects (Heath et al., 1999). Overall, it is important to consider how both parents affect offspring survival and phenotype as the relative magnitude of maternal and paternal influence may shift during ontogeny (Heath et al., 1999; Kamler, 2005).

Environmental influences

In addition to endogenous parental influences, offspring phenotypic expression is moderated by exogenous environmental conditions. There are two central pathways by which environmental conditions and parentage act together to influence offspring traits. Firstly, the parental environment can affect offspring indirectly, by acting on the physiology or condition of adults during gametogenesis and reproductive maturation (Figure 1.1a). If females are exposed to a toxic environment, then toxic effects may be transferred to the offspring and affect developmental success (reviewed in Kime, 1995). Similarly, environments that are thermally stressful may interfere with gamete maturation processes that affect egg or sperm quality and subsequent progeny viability (Flett et al., 1996; Pankhurst and King, 2010). In the second pathway (Figure 1.1b), the external environment during early development can directly influence the expression or selection of parentally mediated traits among offspring. In a variety of taxa, the consequences of maternal effects on egg size can depend on environmental quality (insects - Plaistow et al., 2006; amphibians - Parichy and Kaplan, 1992; Fish - Einum and Fleming, 1999). In aquatic systems, water quality factors such as salinity, oxygen, temperature, and xenobiotic pollutant levels each exerts a substantial influence on the physiology and phenotypic expression of developing fish (reviewed for salmonids in Finn, 2007). For example, many early life history traits in salmonids are influenced by genotype-temperature interactions, whereby the relative fitness of offspring families depends on the thermal environment they are exposed to during initial incubation (Hutchings, 2011).

For ecothermic organisms, water temperature is a critical environmental factor due to its direct influences on physiological and developmental processes. Of particular interest to population dynamics and evolutionary responses, is the concept of thermal stress, whereby increases in temperature cause a change in biological functions leading to decreased survival or impaired fitness (McCullough et al., 2001). In migrating adult salmon, high temperatures can cause the collapse of aerobic scope (Farrell et al., 2008) and higher levels of parasitic infections (Crossin et al., 2008). Supraoptimal water temperatures also have critical influences on sedentary eggs, which unlike adult fish, have zero capacity to behaviorally thermoregulate. Compared to adults, fish eggs and

larvae are generally more stenothermal, with critical limits for survival and fitness within a narrower temperature range (Blaxter, 1992). In the early stages of life, water temperatures above thermal optima can significantly decrease survival, induce teratogenic effects, or shift phenotypic sex ratio (Finn, 2007). In addition, thermal stress in early development can affect biological functions that may persist beyond the incubation period and influence survival and fitness on longer time scales (Finn, 2007).

Understanding the effects and consequences of changing thermal regimes is of importance in a wide array of fish taxa. However, in the North East Pacific, there is a critical need to understand the impacts of elevated water temperatures on native salmonids. Pacific salmon (*Oncorhynchus* spp.) are an iconic group of species, highly valued economically, culturally, and ecologically. Commercial and recreational salmon fisheries are worth millions of dollars to the coastal economies of both the United States and Canada (Kristianson and Strongitharm, 2006; BCMOE, 2007). First Nations groups depend on Pacific salmon as an integral component of their culture, nutrition and economy (Garner and Parfitt, 2006). Ecologically, Pacific salmon are an important source of marine derived nutrients and contribute greatly to the productivity of freshwater and riparian communities (Naiman et al., 2002). In particular, sockeye salmon (*O. nerka*) are considered a 'keystone' species on which over 190 species of plants and animals rely (Cederholm et al., 2000). The importance of understanding temperature-related impacts on salmon is reflected in the scientific literature, with multiple review articles recently devoted specifically to this topic (Mote et al., 2003; Crozier et al., 2008; Bryant, 2009; Jonsson and Jonsson, 2009; Elliott and Elliott, 2010; McDaniels et al., 2010; Healey, 2011).

Reproduction and early development in Pacific salmon

Pacific salmon are semelparous and migrate into river systems, up to thousands of kilometers, to invest everything into one pre-terminal reproductive event. For each spawner, successful migration, gamete production and progeny development is critical, because failure in one of these steps results in zero lifetime fitness. The thermal

environment is also important in each of these steps, and salmon have evolved specific adaptations to ensure migration and reproductive success. For example, sockeye salmon demonstrate significant genetic control over the date of river entry and subsequent spawning, such that spatially separated populations have different entry timings that correspond to the encountered thermal regimes of their natal rivers during egg incubation (Brannon, 1987). Populations of sockeye salmon in the same river system also demonstrate physiological adaptations that correspond to their historical thermal conditions during migration (Eliason et al., 2011).

During their spawning migrations, salmon are undergoing reproductive development. Females undergo six major stages in egg production - oogenesis, primary oocyte growth, cortical aveolis stage, vitellogenesis, maturation, and ovulation (Tyler and Sumpter, 1996) - the latter half of vitellogenesis and subsequent events occurring primarily after freshwater entry. Males go through a process of spermatogenesis in which spermatozoa are formed, stored and then released through the sperm duct (spermiation). These events are under hormonal control, and during migration there is a general increase in plasma levels of several reproductive hormones (*e.g.,*17β-estradiol, testosterone, and 11-ketotestosterone) (Truscott et al., 1986). Although the reproductive cycle in salmonids is primarily driven by photoperiod (Billard, 1985), water temperature has an important modifying role, particularly in the timing of gamete maturation and spawning (Pankhurst and King, 2010). Studies primarily conducted on Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) show that exposure to elevated temperatures during gametogenesis can impair gonadal steroid synthesis, reduce maternal investment into oocytes and subsequently decrease gamete viability (reviewed in Pankhurst and King, 2010).

In the months that follow spawning, fertilized eggs incubate and develop within gravel redds. As described by Velson (1980), embryogenesis begins with early cleavage (initial cell divisions), followed by gastrulation and epiboly (cellular differentiation), and the beginning of eye pigmentation. The final phases of embryo development involve organogenesis, yolk vascularization and the completed pigmentation of the eyes (referred

to as ‘eyed’ stage). The ‘eyed’ eggs continue developing until hatching stage, when a hatching enzyme is produced to digest the egg capsule and release the active embryo. Hatched alevins remain in the gravel and feed endogenously on their yolk reserves until they are depleted, at which point they emerge from the gravel, begin foraging for food, and in the case of sockeye salmon, embark on a migration to their nursery lake. The average egg-to-fry survival for wild Pacific salmon is generally low, with estimates ranging from 7 to 9 % for pink (*O. gorbuscha*), chum (*O. keta*) and sockeye (*O. nerka*) (Bradford, 1995).

During this long period in the gravel, developing eggs are passive recipients of their external environment and water temperature is the fundamental determinant of physiological processes and development rate (Quinn, 2005). Most fish have an optimal thermal range for embryonic development, and for salmonids this range falls between 6 - 10 ºC (McCullough et al., 2001). Although temperature is the primary determinant, development rate in salmonids also has a genetic component, such that hatch timing and emergence can vary among different progeny within a population (Beacham, 1988). Significant variation among salmon offspring families exists for other characteristics such as egg size (egg weight variation can be greater than 30% within populations - Brannon, 1987), metabolic rates (Pakkasmaa and Jones, 2002), and muscle physiology (Johnston and McLay, 1997). The selective advantage of these genetic or phenotypic differences is not well understood (one exception may be the “bigger is better” hypothesis, Sogard 1997), however, these intrinsic family-based differences may become important determinants of relative fitness under conditions of thermal stress (Hoffmann and Parsons, 1991).

Climate and human induced thermal stress on Pacific salmon populations

Increased river temperatures resulting from climate change and anthropogenic sources are a major concern to the recruitment, health and distributions of many salmon populations in the North East Pacific (Rand et al., 2006; Crozier et al., 2008; Taylor, 2008). An improved understanding of the influence of stressful thermal regimes on

Pacific salmon reproductive success is becoming important, as rivers not only show evidence of warming, but an increase in the frequency of 'extreme temperature events' (Pike et al., 2008; Mantua et al., 2010; Hague et al., 2011). One of the largest salmon producing rivers in the world, the Fraser River, has documented a 1.5°C increase in peak summer temperatures since the 1940's (Patterson et al., 2007) with climate models predicting a 0.12°C increase in water temperature each decade from 2000 to 2100 (Ferrari et al., 2007). In the event that hot summer temperatures extend into September and October, or 'extreme temperature events' occur during these months, it is possible that water temperatures could surpass the thresholds for optimal spawning and initial incubation. Furthermore, since 1996, and for unknown reasons, certain stocks of late-run sockeye have been initiating their upriver spawning migration several weeks earlier than normal, when river temperatures are significantly increased (Cooke et al., 2004). To date, this has not altered their historical spawning time, but successful spawning and initial incubation could be substantially impacted if this were to change.

Increased water temperatures in salmonid freshwater habitat can also be the result of anthropogenic perturbations such as flow modifications (dams) and riparian deforestation. On four major rivers in Washington and Oregon, large dams have been identified as the central cause of lower water temperatures in the summer and higher water temperatures in the fall, subsequently affecting Chinook spawning success and fry emergence (Angilletta et al., 2008). Below the Hells Canyon Dam on the Snake River, fall Chinook salmon have been documented initiating spawning at temperatures well above the US State water quality standards, resulting in negative effects on their survival and emergence timing (Geist et al., 2006). Increases in stream temperatures resulting from logging or development-associated riparian removal have also directly impacted salmon incubation, with resulting warmer temperatures causing early emergence timing and altered growth patterns (Hartman and Scrivener, 1990).

Study population

This research is focused on sockeye salmon from the Fraser River. Sockeye salmon are the most commercially valuable and second most abundant salmon species in the Fraser. However, declining population trends in the last two decades prompted the launch in November 2009 of a federal commission of inquiry into the Fraser River sockeye decline (www.cohencommission.ca). It is becoming clear that climate change and the effects of projected increases in freshwater thermal regimes are critical issues affecting Fraser River sockeye. This work will provide fundamental information that will assist in understanding temperature tolerance and adaptability in the early developmental stages within sockeye populations.

The wealth of existing knowledge on sockeye salmon fertilization, incubation and development also provides a clear rationale for the study of this species. Detailed information on sockeye salmon embryonic development (Velson, 1980) and a predictive temperature-specific development timing model is available (Jensen and Jensen, 1999). Initial work by Patterson (2004) and subsequent studies (Patterson et al., 2004a; Nadeau, 2007; Nadeau et al., 2009) has resulted in well-established protocols for fertilization and incubation of sockeye salmon. Under optimal thermal conditions, these laboratory studies revealed high overall survivorship through early developmental stages, yet also showed significant differences in sockeye progeny survival based on parental gamete origin. Work by Beacham and Murray (1989) demonstrated that survival differences in sockeye offspring families were greatest when incubated at extreme high or low incubation temperatures. However, to date, no studies have examined among-family differences in sockeye offspring across a gradient of supraoptimal temperatures, or the persistence of thermal/parental effects beyond endogenous development stages. This may be particularly important for sockeye salmon given they have been identified among the poorest adapted of the Pacific salmon species to surviving high incubation temperatures (Beacham and Murray, 1990).

The experiments conducted herein use sockeye salmon from the Weaver Creek population. This is a relatively large coastal population, spawning in both Weaver Creek

(a tributary to the Harrison River located 2 km downstream from Harrison Lake) and in an adjacent artificial spawning channel (refer to Chapter 3, Fig. 3.1). Previous incubation and fry swimming studies have used this same population, providing evidence of parental influence on offspring traits (Beacham and Murray, 1989; Patterson et al., 2004a; Pon et al., 2007; Tierney et al., 2009; Nadeau et al., 2009) and good comparative data. An inter-population study on Fraser River sockeye also suggested that the Weaver Creek adult population may be less physiologically adapted to dealing with high temperature stress compared to other populations (Eliason et al., 2011).

Thesis goal and objectives

The overall goal of this research was to determine how individual parentage and elevated water temperatures influence within-population offspring survival and fitness traits during early development in salmon. There were three main objectives. The first objective was to review the scientific literature and provide a synthesis of the importance of parentage in assessing temperature effects on fish early life history. The second objective was to experimentally investigate how individual parentage can affect progeny responses to early developmental temperature stress in sockeye salmon. The third objective was to determine whether the effects of developmental temperature stress and/or parental influences in sockeye salmon were detectable (*i.e.,* persistent) beyond emergence. Each of these objectives has been addressed in a separate chapter of this thesis as outlined below:

Chapter 2 is a quantitative literature review that provides context for the thesis research and seeks to identify knowledge gaps, study limitations and to characterize the existing experimental studies in fish that address two important questions: 1) How might temperature experienced by the parent generation during reproductive development affect offspring through influencing adults' ability to spawn or gamete quality? and 2) How might incubation temperature during embryogenesis influence parentally mediated offspring traits and development? The review synthesizes information on the taxa

studied, the origins of parental stocks used, the types and persistence of offspring responses examined, and research study motivations.

Chapter 3 reports the findings from a cross-fertilization experiment in which multiple offspring families of Weaver Creek sockeye were subjected to three levels of temperature stress during early incubation. Differences in progeny survival, hatch timing, and size were examined across the thermal gradient (generating a reaction norm) and also within each temperature treatment. Collectively these findings highlight the role of parental influences in shaping selection processes in salmonid populations exposed to elevated thermal regimes.

Chapter 4 provides the results of a swim performance experiment that was conducted using the same fish from Chapter 3, but at a later development stage (three weeks post emergence). This study tested the hypotheses that both incubation temperature treatments and family identity would significantly influence fry burst swim capacity. The results showed that both incubation thermal stress and parental identity can have persistent effects on fry performance traits, which in turn may influence patterns of selection and population structure.

Chapter 5 provides a synthesis and conclusions and suggests possible areas for future investigation.

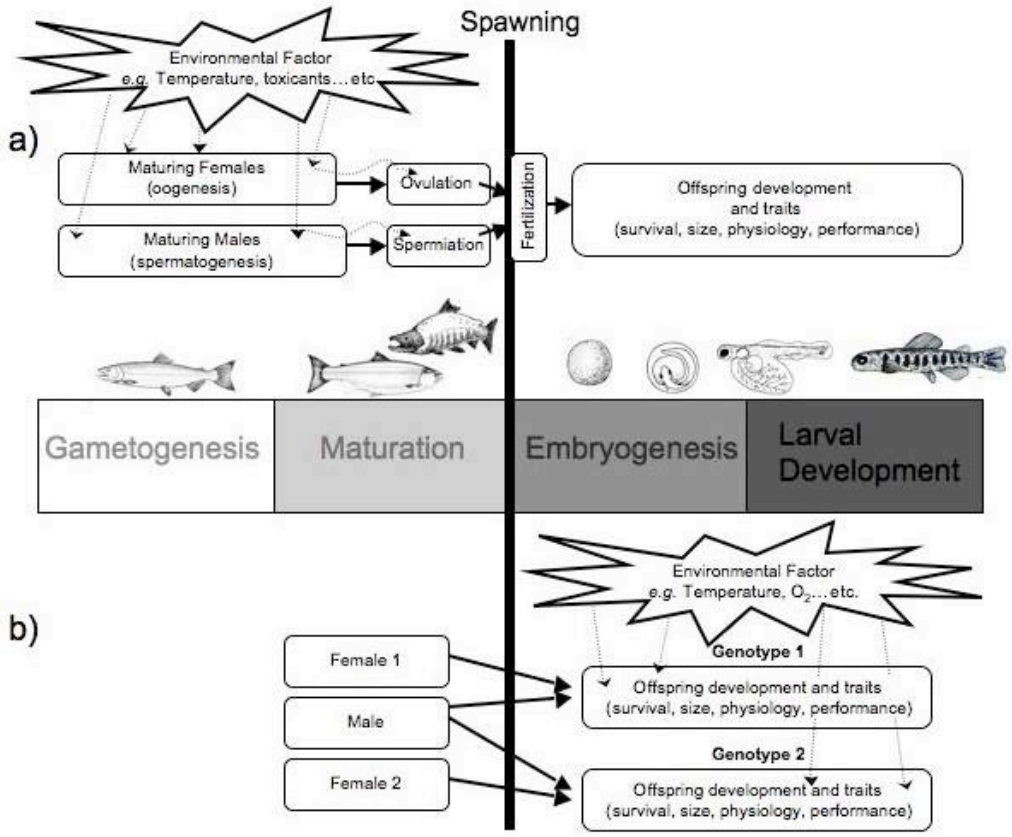

Figure 1.1. Diagram showing the two central pathways that parentally mediated environmental effects can affect offspring development and phenotypic traits. Pathway a) shows an environmental factor acting on the parents during reproductive development (gametogenesis and maturation processes) which may affect gamete production, fertilization capacity, and subsequently offspring traits. Pathway b) shows environmental influences acting directly on developing offspring. The developmental environment may interact with parental influences to differentially shape offspring responses and trait expression among offspring families (genotypes). Fry illustrations obtained with permission from Kamler 1992. Adult fish images obtained from on-line Creative Commons sources.

CHAPTER 2: The importance of parentage in assessing temperature effects on fish early life history: a review of the experimental literature[1]

Introduction

In most marine and freshwater fish species, early life history is characterized by high mortality and strong selection pressures (Kamler, 1992; Houde, 1997). In the majority of freshwater and marine fish species, eggs and sperm are either broadcast into the pelagic environment, released on a substrate or deposited in prepared areas or nests. During development, the fertilized embryos are influenced by an interplay between the external environment as well as the intrinsic embryo properties endowed to them by their parents (Kamler, 2005). For fish, water temperature is a critical factor that can influence early life history via the thermal regimes experienced by parents, as well as the thermal regimes experienced by developing progeny. In both pathways, genetic and non-genetic linkages between parents and progeny can influence how temperature effects will manifest during early development and shape larval and juvenile fitness.

In the context of climate change and expanding human alterations to aquatic thermal regimes, scientists are increasingly being called upon to predict how temperature changes may affect fish reproduction and development. Understanding population responses to temperature requires studying thermal effects across a generational boundary; linking parents to progeny. This knowledge is required by the aquaculture industry and hatchery operations whose objectives to optimize reproductive output are reliant on information about how parental or early development rearing environments can affect offspring production and quality. From a fisheries management perspective, it would be beneficial to know if extreme thermal events experienced by fish in the wild have intergenerational consequences, or if certain fish populations have the capacity to

---

[1] A version of this chapter has been accepted for publication. Burt, J.M., S.G. Hinch, and D.A. Patterson. In press. The importance of parentage in assessing temperature effects on fish early life history: a review of the experimental literature. Reviews in Fish Biology and Fisheries. DOI: 10.1007/s11160-010-9179-1

adapt to thermal change. In regards to early life history stages, understanding how parentally mediated variation (*e.g.,* additive genetic or maternal effects) can affect offspring fitness traits in adverse environmental conditions is an important component to assessing within-population adaptability and resilience (Charmantier and Garant, 2005). This knowledge is particularly applicable to the conservation and management of endangered stocks with small breeding numbers, and to fish populations affected by anthropogenically induced temperature shifts (Angilletta et al., 2008). In considering offspring responses to environmental temperature in the context of linking parent generations to their progeny, this review seeks to consolidate and characterize the peer-reviewed literature that addresses two important questions. Firstly, how might temperature experienced by the parent generation affect offspring through influencing adults' ability to spawn or the quality of their gametes? Secondly, how might incubation temperature during embryogenesis influence parentally mediated offspring traits and development?

The effects of parental temperature exposure on offspring

The environment experienced by adults during reproductive development can alter their physiological condition and gametogenesis processes, providing a means for non-genetic "parental effects" to be transferred to their offspring (Rossiter, 1996; Lacey, 1998). In ectothermic fish, temperature is a highly influential environmental factor affecting many processes involved in reproduction (Lam, 1983; Van der Kraak and Pankhurst, 1997). In addition to its controlling influence on metabolism and growth, temperature can act as a reproductive cue (Hilder and Pankhurst, 2003) and influence physiological pathways associated with gamete development (Brooks et al., 1997; Pankhurst and King, 2010). Water temperature experienced by females during oogenesis can affect egg sizes (Chambers, 1997) and egg biochemical composition (Buckley et al., 1990; Jobling et al., 1995; Atse et al., 2002), which could result in changes in offspring ontogeny and fitness (Kamler, 1992). In males, temperature can influence spermatogenesis and sperm properties, which may influence offspring production. High temperatures have been shown to inhibit spermiation (Manning and Kime, 1985) or affect

sperm motility (Alavi and Cosson, 2005), which are important influences on fertilizing capacity.

Alterations to offspring production and quality are likely mediated via temperature's influence on various reproductive endocrine pathways (Van der Kraak and Pankhurst, 1997). Elevated temperatures can alter the circulating levels of reproductive hormones, which can have downstream effects on ovarian and testicular steroid production (Manning and Kime, 1985; King et al., 2007). Disrupted steroid synthesis and subsequent alterations in hepatic vitellogenesis can result in reduced maternal investment and gamete viability (Tveiten and Johnsen, 1999; King et al., 2003). Temperature can also act directly at the level of the oocyte. High temperatures have been shown to disrupt processes involved in final oocyte maturation (FOM) and inhibit ovulation in many individuals (Pankhurst et al., 1996; Webb et al., 2001). Failure of adults to spawn as a result of experiencing suboptimal temperatures could alter the conspecific density of the surviving offspring. This could affect changes in early growth patterns, behavior, and the overall population structure, through known patterns of density dependence (Einum et al., 2008). Similarly, alterations in the proportions of genotypes represented in the offspring generation may reduce phenotypic variation and affect overall fitness.

The physiological pathways evoked by stress responses are highly interconnected with the endocrine processes associated with maturation and spawning (Schreck et al., 2001). Exposure of mature rainbow trout (*Oncorhynchus mykiss*) to repeated acute stress during gametogenesis caused ovulation delay, reduced egg sizes, lower sperm counts and lower survival rates of progeny (Campbell et al., 1992). Maternal transfers of the stress induced hormone, cortisol, to eggs has been demonstrated in some species (Stratholt et al., 1997; McCormick, 1998). However, variable effects of oocyte cortisol on development have been reported (Stratholt et al., 1997; McCormick, 1998; Schreck et al., 2001; Auperin and Geslin, 2008). If temperature induces a stress response in maturing adults, sufficient to alter levels of reproductive hormones (King et al., 2003), this may provide another means by which offspring outcomes could be affected.

The effects of temperature and parentage during offspring early development

Compared to adult fish, developing embryos and larvae have limited to no capacity for behavioral modification and are passive recipients of their thermal environment. As such, the early development stages are more stenothermal and can be profoundly affected by even minute temperature changes (Blaxter, 1992). Accordingly, thermal optima and lethal incubation limits are well established for many species and the effects of temperature exposures during initial development stages have been extensively studied (Pepin, 1991; Kamler, 1992; Blaxter, 1992; Kamler, 2008). For example, the direct relationship that incubation temperature has on development time has been mathematically determined for a wide array of marine and freshwater species (Pauly and Pullin, 1988; Teletchea et al., 2009). The thermal regime during early ontogeny has also been shown to influence larval size (Blaxter, 1992), meristic counts (Fowler, 1970), yolk utilization efficiency (Kamler, 2008), muscle physiology (Johnston et al., 1996), larval deformities (Angelopoulou et al., 2007), and progeny sex ratios in certain species (Baroiller et al., 1999).

Often neglected in the investigation of temperature effects during early development is a consideration of parentally mediated offspring variation. First and foremost, offspring are under the genetic influences of their parents. For example, paternally-derived offspring variation is used in quantitative genetics approaches to represent the additive genetic variation (linked to estimates of heritabiliy) for a trait within a population. Another source of offspring variation however, comes from the influence of non-genetic maternal or paternal effects; a source of phenotypic variation that is specifically attributable to the phenotypic traits of the female or male parents, respectively (Bernardo, 1996; Roff, 1998). In fish populations, maternal effects are increasingly being recognized as significant drivers of offspring variation (Heath and Blouw, 1998; Marshall et al., 2008; Green, 2008). Although the most commonly reported maternal effect in fish populations is the positive correlation between maternal size and egg/offspring size (Chambers, 1997), variation between female spawners has also been shown to affect embryonic survival (Nagler et al., 2000; Trippel et al., 2005; Nadeau et al., 2009), yolk size and composition (Kamler, 2005), growth rate (Green and

McCormick, 2005), metabolic physiology (Patterson et al., 2004a; Pakkasmaa et al., 2006), stress response (Heath et al., 1993), and swim ability (Garenc et al., 1998). Despite the predominant role of females in the determination of fecundity, site selection and yolk provisioning, there is increasing evidence that male spawners can exert significant paternal effects on offspring traits (Evans and Geffen, 1998; Rideout et al., 2004; Probst et al., 2006; Morasse et al., 2008). In many cases, if genetic and non-genetic effects are not distinguishable, the term 'parental influence' refers more generally to any detectable offspring variation attributable to maternal or paternal identity (Green, 2008).

Investigations of incubation temperature in fish more commonly assess whole populations without regard to how offspring responses may vary as a result of their individual parentage (within population variation). Similarly, despite the increasing popularity of quantitative genetic approaches for examining the heritability of offspring traits (*e.g.*, in salmonids, reviewed by Carlson and Seamons, 2008), parentally mediated genetic effects are rarely quantified under different thermal regimes (Beacham, 1988; Heath et al., 1993; Heath et al., 1994; Hebert et al., 1998; Muller-Belecke et al., 2002; Saillant et al., 2006; Jensen et al., 2008). In fish early development, when mortality and selection pressures are high, an increased understanding of the individual variability in offspring response patterns is useful for predicting how populations may respond or adapt to environmental change (Hutchings, 2004). Failure to consider parental influences when assessing offspring responses to an environmental factor means that parentally attributable variation is included in the estimate of the treatment effects. Some authors suggest that this offers incomplete explanations of juvenile ecology and a less than accurate interpretation of treatment influences (Bernardo, 1996).

Bridging the gap – connecting parents to progeny

While thermal effects are frequently investigated in fish during distinct life history stages, intergenerational temperature effects that connect parental influences to progeny outcomes remain poorly understood. There is enormous diversity in fish reproductive strategies and subsequently in the developmental attributes and trajectories

during early life stages, suggesting the need to examine parent-progeny temperature effects over a broad range of taxa, aquatic habitats, and types of offspring responses. In order to gauge the persistence of parentally mediated thermal effects, it is necessary to investigate the developmental duration over which offspring responses are observed. Also, examining the motivations for the study of parent-progeny temperature effects, which can span diverse areas of fish research, may provide useful insights into the choice of experimental designs and how experimental findings may be applied.

This review seeks to explore the scope of experimental research related to parentally mediated temperature effects on progeny. We conducted a systematic literature search to consolidate laboratory based research that examines how temperature treatments applied to adults prior to fertilization, or to progeny just after fertilization, can affect the number of offspring produced and early offspring traits during development. Through systematically characterizing each study by aspects of their design, our principal objective was to evaluate the strengths, weaknesses and gaps in the experimental literature and assess our overall understanding of parent-progeny temperature effects in fish. Specifically, our goal was to examine trends in the taxa studied, the origins of parental stocks used, the types and persistence of offspring responses investigated, and the general motivations for conducting this type of research. We have discussed our results using examples from particular studies and identified research gaps that will help to guide future investigations.

Methods

We conducted a systematic, keyword based, literature search for peer-reviewed articles examining the effects of controlled temperature exposure on finfish during adult reproduction and offspring development. For the following two reasons, we chose only to focus on studies that used controlled laboratory approaches to investigate temperature effects: 1) observational or correlative studies are often confounded by numerous interacting environmental factors, resulting in difficulty in isolating temperature effects,

and 2) applying controlled thermal treatments in an experimental manner can allow researchers to examine cause-and-effect mechanisms which are valuable to understanding temperature effects at an individual level. Although the findings from such studies are published in many government and technical reports, as well as in books, our search was limited to peer-reviewed articles in order to represent the scientific literature that is most generally available to researchers.

A keyword search was conducted using two widely used commercial academic search engines, ISI Web of Knowledge (WOK) and Aquatic Sciences and Fisheries Abstracts (ASFA). The search included articles as early as was covered by the databases, 1965 to July 2009. Due to the vast number of papers that use common keywords such as 'temperature', 'thermal', 'offspring' or 'development', our search was conducted in a specific manner to acquire articles that contained multiple keywords (one from each of 4 groupings), which helped to identify them as appropriate for review. Many other keywords and search combinations were tested, but the following combination was chosen as it yielded the greatest number of relevant publications: (temperature* or thermal*) AND (held or hold* or expos* or experienc* or rear* or incubat* or male* or female* or maternal* or paternal* or parental or sire* or dam*) AND (egg* or propagule* or oocyte* or sperm or embryo* or larva* or hatch* or offspring or progeny) AND (surviv* or mortality or fitness or viability or size* or growth or development or quality or sex or muscle). Finally, it was also necessary to include the keywords (fish* or teleost*) as qualifiers in our WOK search.

To reduce the large number of publications identified by our combined search, we applied specific review criteria. Articles were included if they 1) experimentally examined how temperature exposures to the parent generation prior to fertilization in some way affected offspring through influencing gamete production or quality, or 2) examined the effects of incubation temperature applied during embryo and larval development using a controlled breeding design to account for parental influences and parent-by-temperature interactions. In the first category of adult thermal studies, it was required that investigators applied controlled temperature treatments and measured some

sort of response in the output or quality of gametes or offspring (Table 2.1). In the second category, our goal was to isolate studies that examined both the effects of early temperature exposure (thermal treatments applied to developing embryos or recent hatchlings) and parental influence on offspring development and phenotype (Table 2.2). This meant studies had to have a controlled fertilization design, or utilize offspring parental assignment techniques, enabling the examination of maternal, paternal (often reported as additive genetic variation) or family influences on offspring responses in addition to temperature effects. With the desire to focus more on the potential effects of chronic temperature exposures, studies examining the effects of acute cold/heat shocks, fluctuating temperatures, and temperature-mediated gene expression were not considered within the scope of this review (such topics essentially require a different set of search keywords). Following the identification of relevant studies through our primary keyword search, the references from all criteria-meeting articles were scanned for cited publications that were relevant to the review. This was done in order to capture relevant publications that may not have had electronic abstracts and keywords or had been excluded by our initial search specifications. These additional references were then added to our database if they met review criteria.

All articles within the two databases were classified according to the fish taxa studied (order), reproductive habitat type (saltwater or freshwater), motivation for scientific inquiry, and the types of offspring responses examined (Tables 2.1 and 2.2). Articles were also classified by origin of parental stock used (lab reared, hatchery population, farmed/domesticated source, or wild). Fish reared in a facility for one or more years were considered of lab origin. In a few cases where specific details of fish origin were not given but the terms ‘strain’ or ‘broodstock’ were used, parents were assumed to be from a farmed or domesticated stock. Experimental duration was assessed in order to understand the degree to which the persistence of temperature effects had been measured in offspring. Accordingly, studies were classified with a number from 1-5 based on the latest life stage at which an offspring response was examined, as follows: 1) experiment terminated before fertilization of gametes, 2) offspring measures were taken at any point between fertilization and eyed stage, 3) offspring measures were recorded up to and

including when eggs hatched, 4) offspring measures were recorded up to and including yolk absorption or metamorphosis, 5) offspring measures were recorded beyond endogenous feeding. Due to their uniqueness, we did not apply the 1-5 scale to classify two studies that used viviparous species (females give birth to live fry ready for exogenous feeding). Therefore these studies were excluded from our assessment of experiment duration. For the category of 'motivation for scientific inquiry', each publication was classed according to how authors framed the application of their research. This was done to gain insight into the motives that presently drive experimental investigations of parent-progeny temperature effects. Although it was quite clear when studies were conducted in the context of aquaculture needs, classifying studies into other motivation categories such as 'understanding species ecology', 'management applications in the wild' or purely, 'furthering knowledge on reproductive or developmental biology' was challenging and often not obvious. To eliminate possible subjectivity in our grouping, we chose only to classify study motives as either fish culture related or other (research conducted for biological, ecological or management objectives).

Results

Adult thermal holding studies

A total of 41 peer-reviewed articles were identified that experimentally examined the effects of controlled temperature exposures in reproducing adults on subsequent offspring production and development (Table 2.3). Freshwater reproducing species were the subject of the majority of articles (73%, n = 30); mostly driven by studies on fish from the order Salmoniformes. Overall, Salmoniformes was the most commonly studied fish order constituting nearly half (46%, n = 19) of all adult exposure studies. Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) were the most frequently examined species (nine and six articles, respectively) followed by Arctic char (*Salvelinus alpinus*) and Atlantic cod (*Gadus morhua*), each with three studies. After Salmoniformes, Perciformes (17%, n = 7) then Gadiformes (10%, n = 4) were the next most commonly

studied orders. Six other fish orders were represented, generally by a single publication on a particular species.

A large proportion of adult temperature exposure experiments (66%, n = 27) were classified as having study motivations related to aquaculture. Related to this observation was the finding that a majority of studies (83%, n = 34) used non-wild parental stocks of domesticated (n = 20), lab-reared (n = 13) or hatchery (n = 1) origins. Five out of the 41 studies (12%) used wild parental fish to examine the effects of adult temperature exposure on gametes or offspring. Two other studies used a combination of domesticated and wild fish.

The majority of adult thermal exposure experiments were terminated at relatively early offspring life stages. Eighty-three percent of studies (n = 34) did not examine offspring survival, quality or fitness beyond egg hatching: seven studies were terminated before fertilization, 16 were terminated between fertilization and eyed egg stages and 11 studies were terminated at hatching stage. In studies that followed offspring effects beyond hatch (n = 5), four were terminated when offspring reached yolk absorption or metamorphosis and one was terminated when offspring had reached 90 days post hatch.

To identify the different progeny effects examined in response to adult temperature exposure, publications were categorized according to the type of offspring responses they studied (descriptions Table 2.1, results Table 2.4). Reproductive output was the most commonly examined response measure and was most often reported in the context of the quantity of eggs produced or whether or not females would ovulate. Physiological measures in the gametes or offspring (*e.g.,* sperm properties, egg/larval composition or physiology) were the least examined progeny responses.

Incubation temperature and parental influence (ITPI) studies

Our initial keyword search revealed a large number of articles examining the effects of incubation temperature applied during the stages of embryonic and larval

development (n = 222). However, the majority of these studies (80%, n = 178), were not interested in the influence of individual spawners, and applied temperature treatments to offspring that were the products of pooled fertilizations or unknown parentage. In contrast, 44 studies (20%) met our criteria for review in that they employed controlled mating designs that enabled researchers to also report on how offspring responses were affected by parental influences (maternal, paternal, or family). By searching the cited references of these articles, we were able to identify an additional 12 publications for review. In total we reviewed and characterized 56 studies that examined both incubation temperature and parental influence (Table 2.5); these will hereafter be referred to as ITPI studies.

Seventy-five percent (n = 42) of ITPI studies addressed fish species that reproduce in a freshwater habitat while 25% (n = 14) of studies were conducted on marine species. Fish from the order Salmoniformes were investigated in 29 out of the 42 studies on freshwater-reproducing species, constituting 52% of all ITPI studies. Most of the Salmoniformes incubation experiments were conducted on Pacific salmon (*Oncorhynchus spp.*) species (83%, n = 24). Perciformes was the second most commonly studied order (25%, n = 14), and five other orders of fish were represented in the remaining studies (n = 14).

Parental broodfish used in ITPI studies were more balanced between wild (55%, n = 31) and non-wild origins (39%: domesticated strains n = 14, hatchery n = 4, lab n = 4) compared to the adult temperature experiments. Three studies used a combination of parental fish from wild and non-wild origins. Unlike the adult temperature exposure experiments, fewer ITPI studies appeared to be directly motivated by aquaculture applications (23%, n = 13). Overall, the focus of research within ITPI studies was very diverse in nature, addressing questions related to a variety of unique offspring developmental and fitness responses under potential thermal and parental influence.

ITPI studies were categorized according to the offspring responses they examined (descriptions Table 2.2, results Table 2.6). Measures of larval morphology and offspring

viability were the most common, while the influence of temperature and parentage on offspring performance or behavior was only examined in two studies. After survival and morphology, the offspring response that was most consistently investigated for both temperature and parental influence was progeny sex ratios. Seventeen studies were identified that explicitly set out to determine how temperature-influenced sex ratios could vary among the progeny of different individual spawners (seeking to understand genoype-by-environment interactions).

Offspring responses in ITPI studies were examined up to later development stages (longer experiment duration) compared to the offspring responses examined adult thermal holding studies. It is important to note that ITPI articles examining offspring sex ratio responses were excluded in the analysis of experimental duration. This was because their termination at later development stages was necessary to allow for sufficient gonad differentiation, and not with the intention to examine the persistence temperature or parental influences. Out of the remaining 39 ITPI studies, the majority of experiments examined offspring measures beyond hatch (77%, n = 30). This included a large number of studies which terminated at yolk absorption or metamorphosis stages (56%, n = 22), leaving eight studies (21%) that measured offspring responses beyond endogenous feeding stages.

From our total of 56 ITPI studies, only 4 did not detect an influence of parentage on offspring. Forty-two (75%) studies reported that offspring variation attributable to parentage (female, male or family/genotype) was statistically significant, and 29 of these studies reported a significant parent or family-by-temperature interaction. Ten other studies observed considerable family or female influences, but did not present findings statistically. The largest proportion of ITPI studies conducted their incubation temperature experiment using independent full-sib families of offspring (55%, n = 31). These studies reported parental influence in terms of family or genotypic effects. Maternal influences were either examined explicitly (18%, n = 10), or examined in combination with paternal influences by use of a factorial mating design (25%, n = 14). Only one incubation temperature study explicitly examined paternal influences.

Discussion

Common trends in parent-progeny temperature studies

A major focus on fish from the order Salmoniformes was clearly evident from our review of both adult thermal holding and incubation temperature parental influence (ITPI) studies. This is not surprising considering the high recreational and commercial value of salmonids and the fact that they are reared intensively in hatcheries and aquaculture facilities around the globe. Additional reasoning for the large amount of salmonid temperature studies could be the fact that salmonids mature and spawn in freshwater, which exposes them to a much more thermally variable environment (fluctuations in air temperatures, runoff, discharge, precipitation and land use patterns) than most marine species (Bryant, 2009). One benefit of the abundance of salmonid research is that multiple, similarly conducted studies exist on the same species, and allow for findings related to temperature exposure to be built upon and synthesized across experiments. For example, Pankhurst and King (2010) review the results from salmonid thermal holding studies and discuss in detail the physiological responses resulting from adult temperature exposure and the implications of climate change for aquaculture production. Referencing mostly studies on Atlantic salmon and rainbow trout, they provide details on some of the reproductive consequences of holding maturing female and male salmon at elevated temperatures: disrupted gametogenesis, impaired endocrine function, delayed or inhibited ovulation, inhibited spermiation, reductions in egg fertility and offspring viability. Because the paper is discussing rising temperature implications for aquaculture, Pankhurst and King (2010) also discuss various thermal stress mitigation options that could be used in the management of salmonid aquaculture broodstock.

A similar synthesis of findings from salmonid ITPI studies does not exist, however the importance of parentage in influencing thermal responses was apparent in the articles we identified. In all 29 salmonid ITPI studies, significant offspring responses to incubation temperature were reported, covering every category of offspring response we examined (viability, morphology, development rate, physiology, abnormalities, behavior, and sex ratio). Reviewing their findings, progeny temperature responses varied

significantly based on parentage in all but one study (Killeen et al., 1999; maternal migratory type did not influence offspring ontogeny). For example, in multiple studies on different Pacific salmon species, embryonic survival and size-related responses consistently showed a high degree of variation between individual families as incubation temperatures both increased and decreased from a thermal optima (Beacham 1988, 1990; Beacham et al., 1988; Beacham and Murray, 1985, 1986a,b, 1987, 1988, 1989; Murray and Beacham, 1986, 1987, 1989; Murray et al., 1990; Beacham and Varnavskaya, 1991). In another example, muscle cellularity and response to incubation temperature varied among families of Atlantic salmon, also revealing temperature-family interactions (Johnston and McLay, 1997). These authors attributed family thermal response differences to possible variation in egg sizes and quality, but concluded genetic and non-genetic influences from both parents likely contributed to the observed offspring variation. Using a quantitative analysis approach, Beacham (1988) found that increased environmental variance was the major component of the observed survival variation (little additive genetic variation) in pink (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*), but also that maternal effects accounted for up to 40% of the variation in temperatures where mortality was highest. In the same study, additive genetic variation (estimated as the paternal component of variance) played a greater role in influencing development time and size characteristics, however in both of these traits, maternal effects were still considerable and varied in their magnitude between incubation temperatures. These results, and similar findings from other salmonid ITPI studies (refer to Table 2.5) highlight the significant capacity of both female and male spawners to influence the magnitude of intra-population variability among progeny across an incubation temperature gradient. As such, the potential for selection to act on these family level differences may be an indication of adaptive phenotypic plasticity (Hutchings, 2004), and may be important to processes involved in local adaptation (Gharrett and Smoker, 1993; Garcia de Leaniz et al., 2007).

The focus on salmonids in experimental studies has been very informative, but would also suggest that our taxonomic scope for understanding parent-progeny temperature effects is limited. Finfish are diverse in their reproductive strategies and

geographic distributions, and it is known that environmental temperature can modulate reproduction in different ways for different species (Lam, 1983; Van der Kraak and Pankhurst, 1997). In the adult thermal holding studies we reviewed, non-salmonid species demonstrated a variety of offspring-impacting outcomes in response to elevated temperatures that were not observed in the salmonids. For example, the batch spawning Japanese whiting (*Sillago japonica*) showed a 2.7 fold increase in the number of eggs produced (coupled with a 14.7% decrease in egg volume) within three days of increasing holding temperatures from 22ºC to 28ºC (Hotta et al., 2001). Despite this drastic change in egg output, the hatching rates in this study were not statistically different between the two holding temperatures. The effects of warmer temperatures were observed to be cumulative in a three year study on Atlantic halibut (*Hippoglossus hippoglossus*) in which spawning time became progressively later, along with reduced absolute fecundity with each successive year of high temperature exposure (Brown et al., 2006). Above average holding temperatures of 18ºC caused females to undergo ovary regression or oocyte atresia in white sturgeon (*Acipenser transmontanus*; Webb et al., 1999) and impaired deposition of oocyte yolk granules in striped bass (*Morone saxatilis*; Clark et al., 2005). In contrast, for Atlantic cod (*Gadus morhua*), it was the low temperature holding treatment (only 3ºC below ambient) that resulted in females with the highest prevalence of atretic oocytes (Yoneda and Wright, 2005). Finally, Hilder and Pankhurst (2003) demonstrate that temperature can act as an important cue in the reproductive cycle of the tropical spiny damselfish (*Acanthochromis polyacanthus*). Their study showed that a 4ºC decrease in adult holding temperature was sufficient to significantly reduce the frequency of spawning events and offspring survivorship. Overall, these studies reveal that the manner in which reproduction and offspring output are affected by thermal exposure in the adult generation can vary widely depending on species' reproductive strategies and reproductive habitats.

Similar to adult thermal holding publications, the studies we found that investigated both parental and thermal responses during incubation stages were largely conducted on salmonids, with fewer studies on marine reproducing species and non-salmonid freshwater taxa. Salmonids possess a phylogenetically unique reproductive

strategy and developmental biology (Teletchea et al., 2009) which presents considerable challenges in extrapolating biological understandings of maternal, paternal and temperature effects to other marine or freshwater taxa. Although fewer in number, the experiments we found on marine fishes were some of the most explicit in their aims to examine female and male parental influences (Conover and Kynard, 1981; Conover and Heins, 1987; Hoie et al., 1999; Benoit and Pepin, 1999; Green and McCormick, 2005; Ojaveer, 2006). In an experiment designed to investigate both thermal and maternal effects (primarily egg size) on hatching characteristics in yellowtail flounder (*Pleuronectes ferrugineus*), Benoit and Pepin (1999) demonstrated that the variation in hatch size was significantly affected by a non-additive interaction between female and incubation temperature. In this study, the offspring from eggs produced by different females (albeit of similar sizes) varied in their maximum larval lengths when incubated at different temperatures, within the range of those experienced in the wild. Similarly, paternity along with male-by-female-by-temperature interactions were responsible for offspring variation in larval growth rate and performance in the tropical clownfish, (*Amphiprion melanopus*) (Green and McCormick, 2005). These authors both emphasize that marine fish populations are characterized by high larval mortality due to size and growth selective processes, and that identifying the parental and environmental sources of offspring variation is important to understanding recruitment dynamics. In another example, while attempting to better understand how changes in the thermal environment affect the sex-ratios of juvenile Atlantic silverside (*Menidia menidia*), Conover and Kynard (1981) followed by Conover and Heins (1987) were among the first studies to show that the progeny produced by different parental individuals varied significantly in their sensitivity to temperature sex-determination. Subsequently, interactions between parental genotype and incubation temperature have been identified in the determination of progeny sex ratio in other marine species (European sea bass, *Dicentrarchus labrax*) and non-salmonid freshwater taxa (primarily tilapia; *Oreochromis spp.*) (see references in Table 2.5 marked under 'offspring sex ratio'). Overall, the aforementioned studies add to the growing volume of knowledge that both temperature and parental factors (particularly maternal effects on egg size) exert considerable influence on the variability of thermally related life history traits as well as recruitment dynamics in marine species (see reviews

by Chambers, 1996, 1997; Marshall et al., 2008; Green, 2008). Despite this knowledge, more laboratory experiments akin to the plethora of salmonid ITPI studies that aim to quantify the combined influences of parentage and temperature would be beneficial.

Trends within the adult thermal holding studies

Within the adult thermal-holding experiments, scientific application was largely related to fish culture applications. This included examining temperature effects in relation to photothermal conditioning protocols for producing offspring out of season (Morrison and Smith, 1986; Johnston et al., 1992; Davies and Bromage, 2002; Clark et al., 2005; Van der Meeren and Ivannikov, 2006), concerns for broodstocks at facilities in geographical locations with elevated ambient thermal profiles (Pankhurst et al., 1996; Webb et al., 1999, 2001; Watts et al., 2004; Pornsoping et al., 2007) and strategies to mitigate temperature stress in broodstock (Dzikowski et al., 2001; King and Pankhurst, 2004b). Conversely, fewer studies were conducted to address temperature exposures in fish within an ecological or conservation context (Buckley et al., 1990; Bonner et al., 1998; St Mary et al., 2001; Ouellet et al., 2001; Hilder and Pankhurst, 2003; Ito et al., 2008). For example, although behavioral alterations, reduced spawning, and gonadal deformities have been observed in many populations inhabiting thermal effluent zones (Meffe, 1991; Sandstrom et al., 1997; Luksiene et al., 2000; Cooke et al., 2003), we identified only one experimental study that was loosely framed in the context of thermal discharge threats and wanting to understand the physiological mechanisms via which thermal effects on spawning are mediated (Hotta et al., 2001). Certainly, research related to fish culture is important for developing aquaculture management protocols and understanding how offspring production can be manipulated by thermal regimes (Pankhurst and King, 2010). However, the applications of these data in ecological contexts may be problematic given the highly manipulated rearing conditions (compressed photothermal cycles, prophylactic treatments, and hormone administration) or the use of domesticated broodstocks.

An overwhelming majority of adult thermal holding experiments used domesticated, lab reared or hatchery broodstocks. As previously stated, one likely reason for this is the desire to understand parentally mediated thermal effects in the context of aquaculture practices or fish husbandry. Alternatively, the use of domesticated and lab-reared broodfish may reflect issues of practicality. Experimental facilities with full environmental control and offspring rearing capacity are likely rare outside of aquaculture and hatchery contexts, where they have available broodstock and the infrastructure for experimentation. Although it did appear evident in the papers we identified, it is worth noting that use of domesticated or hatchery broodstocks can also reflect the fact that experimentation on wild populations may not be possible due to the populations of interest having endangered or threatened status. With little information from experimental studies using wild broodfish, the question remains as to how transferable the findings are from experiments on captive bred fish to temperature responses in natural populations? Domesticated broodstocks are often directly selected for higher growth rates and other attributes, which can consequently alter their genetic make-up, physiology and reproductive capabilities (Fleming et al., 2002; Tymchuck et al., 2006). In salmonids, there is evidence that the thermal tolerance of domesticated broodstocks can be both higher and lower than their wild counterparts. Carline and Manchung (2001), found that the critical thermal maxima (CTMax) of hatchery reared brown trout (*Salmo trutta*) was 0.5 - 1.6ºC lower then that of fish from comparable wild populations. In contrast, Molony et al. (2004) found the LT_{50}'s (the time required for 50% of thermally treated fish to perish) of a domesticated strain of rainbow trout to be approximately double that of a naturalized line taken from the wild. Regardless of the nature of the difference, the possibility for the physiology and/or thermal responses to differ between wild and domesticated fish presents a realistic concern in the transferability of findings from studies that use farmed, hatchery or lab-reared broodstocks to situations concerning wild fish. As such, the use of captive wild populations has been recommended as preferable for laboratory studies designed to understand the factors that affect fish egg and larval production (Lambert and Thorsen, 2003). Although it is acknowledged that not all species may be equally conducive to wild captive breeding, five studies from our search featuring Atlantic cod (Ouellet et al.,

2001), flagfish (*Jordanella floridae*) (St Mary et al., 2001), fountain darter (*Etheostoma fonticola*) (Bonner et al., 1998), Japanese whiting (Hotta et al., 2001), and winter flounder (*Pseudopleuronectes americanus*) (Buckley et al., 1990), in addition to other captive wild experiments using sockeye salmon (*Oncorhynchus nerka*) (Patterson et al., 2004b) and Atlantic haddock (*Melanogrammus aeglefinus*) (Trippel and Neil, 2004), demonstrate that using wild adults in adult temperature holding experiments is possible.

Our understanding of how fish progeny are impacted by temperature or their individual parents is based upon the traits that researchers choose to examine and the durational extent to which those traits are measured. In adult thermal holding experiments, measures of egg production (*e.g.,* ovulation timing, reproductive output, and initial survival) were examined much more frequently compared to other indicators of progeny fitness (*e.g.,* morphology, physiology, performance). Also, very few studies measured offspring effects beyond eggs or hatchlings. This is possibly because egg production data are straightforward to collect and of high relevance to fish culturists. As well, there are considerable logistical constraints associated with prolonged offspring rearing and it is often assumed that reporting survival to hatch is an adequate and ecologically relevant stage to assess progeny viability (Kamler, 2002). However, there is evidence to suggest that examining a greater diversity of progeny effects (beyond initial survival), or assessing impacts on progeny development at later stages may be worthwhile. For example, our review revealed three studies that demonstrated that adult thermal exposures can influence offspring egg matter composition or sperm quality (Buckley et al., 1990; Jobling et al., 1995; Atse et al., 2002). In a study on the effects of temperature on the reproductive development of Arctic charr (*Salvelinus alpinus*), Jobling et al. (1995) found that the exposure of maturing females to high temperatures resulted in oocytes with lower proportions of essential fatty acids. Although the authors did not correlate these biochemical changes with changes to offspring survival or fitness, they acknowledge that even small decreases in these essential fatty acids (necessary for developing brain and neural tissue) could place developing larvae at a disadvantage. In other early development studies, egg matter composition has been shown to be an important factor influencing larval survival (Kamler, 2005). In regards to the persistence

of biochemical alterations to offspring from thermally treated parents, Buckley et al. (1990) found that the content of protein and RNA in first feeding winter flounder larvae was still attributable to the thermal experiences of the maturing adults, in addition to the incubation thermal regime of offspring. Similarly, the potential importance of examining the persistence of intergenerational temperature effects was revealed in two studies that showed larval mortality patterns were dramatically different before and after hatch (Aegerter and Jalabert, 2004; Phelps et al., 2007). In both of these experiments, authors observed significant post-hatch mortality occurring in the progeny of parents held at elevated temperatures that would not have been detected if the studies had been terminated at hatch. Although much is known about how elevated holding temperatures affect hormonal pathways and gamete properties leading up to spawning (Van Der Kraak and Pankhurst, 1997; Pankhurst and King, 2010), many unresolved questions remain regarding the physiological or biochemical mechanisms that influence offspring viability and phenotype, and the potential for parentally mediated thermal effects to persist later into offspring development.

Trends within the incubation temperature and parental influence (ITPI) studies

We found that only a small percentage of publications examining incubation thermal effects during early ontogeny were designed to investigate the influence of parentage on offspring responses and traits. Of the 222 incubation temperature studies initially identified by our keyword search, 44 (20%) used a controlled fertilization design to assess the influence of individual spawners. Furthermore, we believe this 20% is likely an overestimate of the general prevalence of ITPI studies considering that our search was in fact directed to pull in publications with parentally linked keywords, thus likely omitting a plethora of other general incubation temperature studies (which would reduce the proportion of ITPI studies). Our search findings are consistent with the conclusions of other authors who claim that the individual spawner variation has traditionally been neglected as a source of variation in fish recruitment (Green, 2008) and that a general tendency exists among ecologists to homogenize among-family variation, thus ignoring parental influences (Bernardo, 1996).

Though only 20% percent of search-identified studies relating to incubation temperature looked at parental influences, over 90% of those studies detected a direct influence of parentage on offspring. In some cases, researchers were able to explain large proportions of the variability in progeny thermal responses. In two studies, genotype-by-temperature interactions were reported to account for between 5 and 60% of the variance in pink and chum salmon offspring developmental characters (Beacham 1988, 1990). Beacham (1988) reported that the proportion of phenotypic variation attributable to genotype-by-temperature interactions was greater then both additive and maternal variation, indicating how dependent the genetic control of trait expression was on the incubation thermal environment. In a third study, the proportion of variance in yellowtail flounder hatching length explained by female spawner identity varied significantly between incubation temperatures, however, explained up to 38% of the variance at intermediate temperatures (Benoit and Pepin, 1999). Lastly, paternity explained 52% of the variance in post-hatching clownfish growth rates, with an additional 30% explained by interactions between paternity, maternity and temperature (Green and McCormick, 2005). These substantial proportions of offspring variation attributable to parentage correspond to other larval development studies, where female identity was found to account for up to 74% of variation in initial survival (days 0 - 4) in Baltic cod (*Gadus morhua*) (Trippel et al*.,* 2005), and up to 23% of variation in embryonic survival in sockeye salmon (Nadeau et al*.,* 2009). Taken together, these studies indicate that progeny responses to temperature can vary substantially depending on the individual male and female spawner. Consideration of this parentage is important in experimental design if researchers seek to understand and explain the sources of variation among individual responses to temperature treatments. In the wild, progeny variability in thermal responses is crucial because in the face of environmental change, selective processes will occur at the individual level (Stearns, 1992). As climate change increases thermally related selective pressures on populations, the role of parentally linked variation may become increasingly important with respect to the conservation and management of populations with small breeding numbers (Liao and Reed, 2009) and those reliant on supportive breeding programs (Fraser, 2008).

Within ITPI studies, there was considerable variation in the offspring traits that researchers examined. The majority of the studies looked for parental and temperature effects on offspring survival and morphological traits, not surprisingly as Green (2008) suggested that these traits are the least expensive and easiest measures for examining parental influences. Also, these traits are important because variation in early life survival can be a critical determinant of population fluctuations (Houde, 1997), and size-based attributes are commonly linked to fitness (Pepin, 1991; Sogard, 1997; Einum and Fleming, 1999). Though less frequently examined, both temperature and parentage were found to have influences on offspring physiological traits such as larval muscle cellularity in Atlantic salmon (Johnston and McLay, 1997) and RNA/DNA ratios in herring (Hoie et al., 1999). In a study by Turner et al. (2007), the use of NMR metabolomics enabled the authors to document metabolic changes in steelhead (*Oncorhynchus mykiss*) embryo development, revealing that the behaviors of embryonic amino acids were being influenced by thermal stress and were expressed differently among the progeny of individual females. Offspring performance and behavioral measures were also rarely examined in ITPI studies, however the two studies found in this category demonstrated the potential to detect parental and thermal influences. For instance, the critical swimming ability (U-crit) of clownfish incubated at 28ºC was higher then for fish incubated at 25ºC, however there were marked differences in the swimming abilities of larvae from different parent females (Green and McCormick, 2005). In the other example, the incidence of jacking behavior in Chinook salmon (*Oncorhynchus tshawytscha*) offspring was higher when fish were incubated in a heated regime (2ºC higher then unheated group), in addition to being significantly influenced by paternity (male being a jack or a non-jack) (Heath et al., 1994). In this study, male-by-temperature interactions were also observed.

Our search for ITPI studies identified a large number of studies (n = 17) that investigated the offspring response of sex ratio. This is due to the fact that some species are known to have thermosensitive sex determiniation when temperature treatments are applied during the early incubation stages of development (reviewed in Baroiller et al.,

1999; Devlin and Nagahama, 2002). In addition, as evidenced by the findings in publications included in this review (see references in Table 2.5 marked under 'offspring sex ratio'), a high variability in thermosensitivity can exist among progenies of the same population or strain, linked to interactions between incubation temperature and individual parental genotype.

The implications of these parental and genotypic influences are of great importance in thermosensitive species that are of high aquaculture value (*e.g.,* talapia, *Oreochromis* spp.*)* because of the desire to rear more profitable monosex populations (Baroiller et al., 1995; Abucay et al., 1999; Tessema et al., 2006). In contrast, many other ITPI sex ratio studies were conducted under the premise of understanding the evolutionary complexity of temperature and genetically influenced sex determination mechanisms (Conover and Kynard, 1981; Sullivan and Schultz, 1986; Lagomarsino and Conover, 1993; Strussmann et al., 1996; Baroiller et al., 1996; Bezault et al., 2007). The mechanistic details and overall significance of how parentage and temperature determine progeny sex outcomes has been reviewed by several authors (Baroiller and D'Cotta, 2001; Piferrer et al., 2005; Baroiller et al., 2009), but in the context of this review, the aforementioned ITPI studies lend weight to the argument that there is a great diversity of offspring traits that are affected both by individual parentage and the early incubation thermal environment.

Because we found only a handful of ITPI studies that examined offspring beyond endogenous yolk stages, the persistence of parental and thermal influences on later offspring development remains largely unknown. This may be due in part to the logistic constraints of long-term rearing and evidence supporting that parental and temperature effects tend to diminish with ontogeny (Heath and Blouw, 1998; Heath et al., 1999). Nonetheless, significant offspring selection can occur at the transition stage between endogenous and exogenous feeding, or beyond (Kamler, 2002). Indeed, three ITPI studies found that post-emergence survival rates were influenced by interactions between parentage and temperature (De March, 1995; Muller-Belecke et al., 2002; Turner et al., 2007). Similarly, Heath et al. (1993) found that the physiological stress responses in

Chinook salmon juveniles were affected by sire and dam identity as well as genotype-by-incubation temperature interactions. In studies that separately address parental or temperature effects, findings have provided support for investigating post-emergence or post-metamorphic traits. The enzyme profiles of sockeye salmon fry (Patterson et al., 2004a) and swim performance of three-spine stickleback juveniles (*Gasterosteus aculeatus*) (Garenc et al. 1998) were shown to remain under detectable parental influence. Similarly, European sea bass progeny exposed to 15ºC during early development showed a significantly higher swimming capacity as juveniles then those incubated at 20ºC (Koumoundouros et al., 2009). Macqueen et al. (2008) showed that in Atlantic salmon, the effects of incubation temperature during a small window in embryogenesis can affect the myogenic phenotype of adults three years later. In the case of species with thermosensitive sex determination, the incubation temperature effects on offspring sex phenotype are permanent, thus playing a significant role in influencing population dynamics (Conover and Kynard, 1981; Baroillier et al., 2009).

Limitations, knowledge gaps and future research

While extremely valuable, conducting experiments to examine parentally mediated temperature effects on fish offspring is inherently challenging. To examine progeny consequences resulting from thermal effects in adults, experimental facilities with full environmental control and offspring rearing capacity are needed. For ITPI studies, experimental difficulties arise in incubating and rearing independent offspring families. For example, many marine species have large numbers of dispersive larvae, species such as salmon have long incubation durations, and replication of families within and across treatments is limited by gamete quantities and rearing space. For these reasons, many of the ITPI studies we reviewed were restricted to comparisons between very few (< 5) families (Beacham and Murray, 1986a,b, 1987; Murray and Beacham, 1986; Heath et al., 1993; Benoit and Pepin, 1999; Ojaveer et al., 2006; Gagliano et al., 2007) or had little to no replication of experimental units within temperature treatments (Beacham and Murray, 1985; Heath et al., 1994; Yamamoto et al., 1996; Fujioka, 2001; Fox et al., 2003). Furthermore, measuring offspring traits for many individuals in each

replicate, family and temperature treatment may be time consuming (*e.g.,* for physiological or performance traits), or obscured if larvae are compared at slightly different development stages.

Our review revealed several gaps in our current knowledge of parentally mediated temperature effects in fish, which may guide future research. Considering that much of our experimental understandings come from salmonid species, future studies examining temperature responses in other taxa would be useful. In the context of global climate change, more experimentally based information is needed from non-salmonid species in order to form a broader mechanistic understanding of how thermal changes may have intergenerational impacts. At the same time however, our review suggests there are still important knowledge gaps within the salmonid parent-progeny literature. For example, not one of the salmonid adult thermal holidng studies was conducted using a wild parent population. Many wild Pacific salmon stocks are facing warming, often stressful, thermal regimes during reproductive migration and incubation (MacDonald, 2000; Mote et al., 2003; Angilletta et al., 2008; Strange, 2010). For many of these populations, it would be useful to simulate these extreme temperature experiences in a controlled experimental setting to explore the potential for gamete quality or offspring traits to be affected. While experimental approaches using these thermally threatened populations have revealed important physiological (Farrell et al., 2008) and fitness consequences (Crossin et al., 2008) related with migration at high temperatures, the intergenerational repercussions of thermal exposure in wild salmon populations remains to be explored.

Future parent-progeny-temperature experiments will likely benefit from a more in-depth examination of offspring physiological and performance-based fitness traits. In adult thermal holding studies, physiological sampling has greatly aided in elucidating the endocrine mechanisms responsible for temperature impacts on maturing spawners (Pankurst and King, 2010), however less is known about how adult temperature exposure may affect the biochemical properties of gametes, and how those properties might influence progeny development. In small larval organisms, such as those investigated in ITPI studies, methods of assessing phsysiological and performance traits are considerably

more challenging. Fortunately, the examination of physiological traits is becoming more feasible in early life history research due to technological and methodological advances (*e.g.,* egg manipulation, miniaturization techniques, metabolic fingerprinting, image analysis) (Burggren and Blank, 2009). A comprehensive review by Kamler (2008) documents the recent progress made in our understanding of the compositional dynamics (amino acids, proteins, fatty acids, lipids), onogenetic sequence, and metabolism of developing teleost larvae. Bearing in mind that offspring performance can also be an expression of physiological capacity, measures such as swimming ability (examined in only one ITPI study we found; Green and McCormick, 2005) may provide additional ways to consider how both temperature and parental influences can manifest and interact. Overall, the integration of physiological measures can strengthen basic approaches to larval biology and allow for further insight into developmental mechanisms and individual variability (Burggren and Blank, 2009).

In the publications we have reviewed, it is apparent that both the parental thermal environment and the developmental thermal environment can influence offspring early life histories. However, presumably due to the logistic complexity involved, rarely do experiments examine the interactions between adult thermal holding treatments and egg incubation temperature treatments. These studies have been done in other organisms (*e.g.,* insects), and show that intergenerational effects can indeed be context-dependent: the expression of environmentally-induced parental effects in offspring depend on the conditions in which the offspring develop (Stillwell and Fox, 2005; Plaistow et al., 2006). We identified one study by Buckley et al. (1990), who found that the offspring of winter flounder produced by adults held at low temperatures showed better growth at low incubation temperatures, and offspring from parents held at high temperatures were accordingly better suited for growth at higher incubation temperatures. These findings are relevant in the context of climate change, considering the likelihood that both adult thermal environments and offspring incubation environments would be affected. Overall, the context-dependency of intergenerational effects in fish remains largely unexplored (Donelson et al., 2009), and may be an interesting area of focus for future studies.

In this review, we have outlined the value in considering parentage in the assessment of temperature effects on fish early life history. More specifically, we have demonstrated the importance of addressing how thermal treatments to adult fish may affect subsequent offspring fitness, and the significance of considering parentally mediated variation when examining the direct impact of temperature on developing embryos. Experimental investigations that focus on these issues help illustrate cause-and-effect mechanisms and explain sources of variation, which will be useful in understanding population responses to natural or anthropogenically influenced thermal changes. Additionally, these experiments have been shown to be helpful in answering questions relating to aquaculture protocols and fish husbandry, fish management, and evolutionary research. Increased understanding of the parental and temperature sources of variation in spawning production or offspring fitness may furthermore be useful to incorporate into population models that estimate recruitment magnitude and fluctuations (Green, 2008). We found there to be sufficient literature to suggest that temperature during the adult reproductive stages can significantly affect progeny outcomes and fitness. However, this body of literature is predominantly informed by aquacultural studies on commercial species (mainly salmonids), with less focus on other taxa, wild broodfish, and post-hatch offspring responses. Similarly, we found that parentage can play an important role in how offspring respond to incubation temperature during embryogenesis, and that in some cases, these parental and thermal influences may persist into later development. We suggest that further research in this area is warranted as incubation temperature experiments with controlled breeding designs were relatively uncommon, spanned a wide range of designs and measured offspring responses, and had limited experimental durations. Climate change is altering thermal habitats for many freshwater and marine species at a rapid rate (Kaushal et al., 2010), and a greater understanding of the intergenerational and evolutionary consequences in temperature-impacted populations is needed (Crozier et al., 2008; Chown et al., 2010). Overall, we recommend that future studies should explore parent-progeny relationships, particularly focusing on interactions with temperature, as this information is an important step in understanding thermally related reproductive consequences, changes in early life fitness, and the adaptive capacity of vulnerable populations.

Table 2.1. Categorical description of the responses related to offspring production or development that were examined in adult thermal holding studies

Response category	Definition	Examples of measures from incubation temperature parental influence studies
Reproductive timing	Measures relating to timing aspects of egg / sperm production	- normal, delayed or inhibited spawning - synchronicity of ovulation or spermiation - gestation period, batch or brood intervals
Reproductive output	Measures reflecting the quantity or number of offspring produced	- fecundity, clutch size, 'egg production' - decreased egg output due to egg atresia, ovary regression, inhibited spawning - temperature induced adult mortality
Offspring viability	Measures of offspring survival to a distinct stage of development	- fertilization success - survival to eyed stage, hatch, emergence or beyond
Offspring morphology	Measures of size or weight of developing larvae	- length or weight (wet or dry mass) of embryos/larvae
Offspring abnormalities	Measures or reports of deviations in gamete or progeny morphology and development	- egg/larval deformities or abnormalities - observation or measurement of overripe eggs - abnormal deposition of yolk granules in egg - abnormal gonad or gamete development
Offspring physiology	Measures the compositional contents or microscopic properties of eggs, sperm, or larvae	- Egg/larval protein, lipid, energy content - Egg or larval RNA/DNA contents - Sperm concentration, glucose, or motility

Table 2.2. Categorical description of the responses examined in studies concerning the effects of incubation temperature and parental influence

Offspring responses category	**Definition**	**Examples of measures from adult thermal holding studies**
Offspring viability	Measures of offspring survival to a distinct stage of development	- fertilization success - survival to eyed stage, hatch, emergence or beyond
Offspring morphology	Measures of size or weight of developing larvae	- length and weight of embryos or larvae - morphometric characters - measurements of growth
Developmental rate	Measures the duration/rate of development progression	- time to hatching or emergence (days) - embryo development rate (ontogenetic progression)
Offspring abnormalities	Measures of deformities and abnormal morphology or development	- number of physically deformed individuals - asymmetry in meristic characters
Offspring physiology	Measures of the biochemical and biophysical attributes of gametes or offspring during development	- muscle cellularity - RNA and DNA content - yolk utilization - physiological stress response
Offspring sex ratio	Measures of sex-ratio outcomes of progeny	- sex-ratio
Offspring performance	Measures relating to physical performance or behavior	- swim ability - occurrence of jacking behavior

Table 2.3. Characterization of 41 adult thermal holding studies. Studies are grouped by fish order, then species. Each publication is categorized according to the aspects of the experimental design and offspring responses investigated (described in Table 2.1)

		Experimental design				Offspring responses investigated					
Species name	**Reference**	Reproductive Habitat	Scientific inquiry context	Parental Stock Source	Experiment Duration	Reproductive Timing	Reproductive Output	Offspring Viability	Offspring Morphology	Offspring Abnormalities	Offspring Physiology
Salmoniformes											
Oncorhynchus mykiss	Aegerter and Jalbert (2004)	fw	fc	d	4			*		*	*
Oncorhynchus mykiss	Davies and Bromage (2002)	fw	fc	d	2	*		*		*	
Oncorhynchus mykiss	King et al. (2007)	fw	fc	d	2	*	*	*	*		
Oncorhynchus mykiss	Morrison and Smith (1986)	fw	fc	d	2	*	*	*	*		
Oncorhynchus mykiss	Pankurst and Thomas (1998)	fw	fc	lr	2	*	*	*	*		
Oncorhynchus mykiss	Pankurst et al. (1996)	fw	fc	d	2	*	*	*	*		
Oncorhynchus mykiss	Pornsoping et al. (2007)	fw	fc	d	2		*	*		*	
Salmo salar	Johnston et al. (1992)	fw	fc	lr	3	*	*				
Salmo salar	King and Pankhurst (2004a)	fw	fc	d	2	*	*	*			
Salmo salar	King and Pankhurst (2004b)	fw	fc	d	2	*	*	*			
Salmo salar	King et al. (2003)	fw	fc	d	2	*		*	*	*	
Salmo salar	Taranger and Hansen (1993)	fw	fc	d	2	*	*	*			
Salmo salar	Taranger et al. (2003)	fw	fc	d	2	*	*	*			
Salmo salar	Vikingstad et al. (2008)	fw	fc	d	2		*	*			
Salmo salar	Watts et al. (2004)	fw	fc	d	2	*		*	*		
Salvelinus alpinus	Atse et al. (2002)	fw	fc	lr	4		*	*	*		*
Salvelinus alpinus	Gillet (1991)	fw	fc	lr	2	*	*	*		*	
Salvelinus alpinus	Jobling et al. (1995)	fw	o	d	1	*			*		*
Salvelinus fontinalis	Hokanson et al. (1973)	fw	o	h	3	*	*	*			*
Perciformes											
Acanthochromis polyacanthus	Hilder and Pankhurst (2003)	sw	o	lr	3	*	*	*			
Anarhichas lupus	Tveiten and Johnsen (1999)	sw	o	lr	2	*	*	*			
Anarhichas lupus	Tveiten et al. (2001)	sw	o	lr	3	*	*			*	*
Etheostoma fonticola	Bonner et al. (1998)	fw	o	w	3		*	*			
Macropodus opercularis	Haung and Cheng (2006)	fw	fc	lr	3		*	*			
Morone saxatilis	Clark et al. (2005)	fw	fc	d	1	*	*			*	
Sillago japonica	Hotta et al. (2001)	sw	o	w	3		*	*	*		
Gadiformes											
Gadus morhua	Ouellet et al. (2001)	sw	o	w	3		*	*	*		*
Gadus morhua	Van der Meeren & Ivannikov (2006)	sw	fc	lr	2		*	*		*	

Table 2.3. continued

Species name	Reference	Experimental design				Offspring responses investigated					
		Reproductive Habitat	Scientific inquiry context	Parental Stock Source	Experiment Duration	Reproductive Timing	Reproductive Output	Offspring Viability	Offspring Morphology	Offspring Abnormalities	Offspring Physiology
Gadus morhua	Yoneda et al. (2005)	sw	o	h/w	1		*				
Pollachius pollachius	Suquet et al. (2005)	sw	fc	lr	3	*	*	*		*	
Cyprinodontiformes											
Jordanella floridae	St. Mary et al. (2001)	fw	o	w	1		*				
Poecilia reticulata	Dzikowski et al. (2001)	fw	fc	d	!	*	*			*	
Poecilia reticulata	Karayucel et al. (2008)	fw	fc	d	!	*	*	*			
Acipenseriformes											
Acipenser transmontanus	Webb et al. (1999)	fw	fc	lr	3		*				
Acipenser transmontanus	Webb et al. (2001)	fw	fc	d	1	*	*	*	*		
Cypriniformes											
Pimephales promelas	Brungs et al. (1971)	fw	o	lr	5		*	*			
Tinca tinca	Kamler and Stachowiak (1992)	fw	o	d	1						*
Pleuronectiformes											
Hippoglossus hippoglossus	Brown et al. (2006)	sw	fc	lr	3	*	*	*	*		
Pseudopleuronectes americanus	Buckley et al. (1990)	sw	o	w	4			*	*		*
Atheriniformes											
Odontesthes bonariensis	Ito et al. (2008)	sw	o	d/w	1		*			*	
Siluriformes											
Ictatlurus spp.	Phelps et al. (2007)	fw	fc	d	4	*	*	*			

fw, reproduces in freshwater (includes anadromous species); sw, reproduces in saltwater; fc, fish culture; o, other; d, domesticated or farm cultured broodstock; h, hatchery broodstock; lr, lab reared broodstock; w, wild parents used; 1, experiment terminated before fertilization of gametes; 2, offspring measures were taken at any point between fertilization and eyed stage; 3, offspring measures were recorded up to and including when eggs hatched; 4, offspring measures were recorded up to and including yolk absorption or metamorphosis; 5, offspring measures were recorded beyond endogenous feeding; ! experimental duration not assigned to experiments that used viviparous species

Table 2.4. The number (and percent) of adult thermal holding studies from a total of 41 that examined a particular response influencing the offspring generation

Response Category	Number of studies
Reproductive output	35 (85%)
Offspring viability	32 (78%)
Reproductive timing	26 (63%)
Offspring morphology	13 (32%)
Offspring abnormalities	11 (27%)
Offspring physiology	8 (20%)

Table 2.5. Characterization of 56 incubation temperature parental influence (ITPI) studies. Studies are grouped by fish order, then species. Each publication is categorized according to the aspects of the experimental design and offspring responses investigated (described in Table 2.2).

		Experimental design				Offspring responses investigated						
Species name	**Reference**	Reproductive Habitat	Scientific inquiry context	Parental Stock Source	Experiment Duration	Offspring Viability	Offspring Morphology	Development Rate	Offspring Abnormalities	Offspring Physiology	Offspring Sex Ratio	Offspring Performance
Salmoniformes												
Oncorhynchus gorbuscha	Beacham and Murray (1986b)	fw	o	w	4	*	*					
Oncorhynchus gorbuscha	Beacham and Murray (1988)	fw	o	w	4	*	*	*				
Oncorhynchus gorbuscha	Beacham and Varnavskaya (1991)	fw	o	w	4	*		*				
Oncorhynchus gorbuscha	Hebert et al. (1998)	fw	o	w	3			*				
Oncorhynchus gorbuscha	Murray and Beacham (1986)	fw	o	w	3	*						
O. gorbuscha, O. keta	Beacham and Murray (1987)	fw	o	w	4	*	*					
O. gorbuscha, O. keta	Beacham (1988)	fw	o	w	4	*	*	*				
Oncorhynchus keta	Beacham (1990)	fw	o	w	5		*		*			
Oncorhynchus keta	Beacham and Murray (1985)	fw	o	w	4	*	*					
Oncorhynchus keta	Beacham and Murray (1986a)	fw	o	w	4	*	*					
Oncorhynchus keta	Beacham et al. (1988)	fw	o	w	4	*	*	*				
Oncorhynchus keta	Murray and Beacham (1989)	fw	o	w	5		*					
Oncorhynchus keta	Yamamoto et al. (1996)	fw	o	h	2				*			
Oncorhynchus kisutch	Konecki et al. (1995)	fw	o	w	3			*				
Oncorhynchus kisutch	Murray et al. (1990)	fw	o	w	4	*	*					
Oncorhynchus mykiss	Danzmann and Ferguson (1988)	fw	o	d	4	*						
Oncorhynchus mykiss	Turner et al. (2007)	fw	o	h	5	*	*		*	*		
Oncorhynchus nerka	Craig et al. (1996)	fw	o	w	5*						*	
Oncorhynchus nerka	Hendry et al. (1998)	fw	o	w	4	*	*	*				
O.tshawytscha	Heath et al. (1993)	fw	fc	d	5	*	*			*		
O.tshawytscha	Heath et al. (1994)	fw	o	d	5	*						*
O.tshawytscha	Rombough (1985)	fw	o	w	4			*				
O. tshawytscha, O. nerka	Beacham and Murray (1989)	fw	o	w	4	*	*					
O. tshawytscha, O. keta	Murray and Beacham (1987)	fw	o	w	3	*	*	*				
Sallvelinus alipinus	De March (1995)	fw	fc	d	5	*						
Salmo salar	Berg and Moen (1999)	fw	o	w/d	3			*				
Salmo trutta	Jensent et al. (2008)	fw	o	w	4		*	*				

Table 2.5. continued

Species name	Reference	Experimental design				Offspring responses investigated						
		Reproductive Habitat	Scientific inquiry context	Parental Stock Source	Experiment Duration	Offspring Viability	Offspring Morphology	Development Rate	Offspring Abnormalities	Offspring Physiology	Offspring Sex Ratio	Offspring Performance
Salmo salar	Johnston and McLay (1997)	fw	o	w	4					*		
Salmo trutta	Killeen *et al.* (1999)	fw	o	w	4			*				
Perciformes												
Amphiprion melanopus	Green and McCormick (2005)	sw	o	lr	4	*	*					*
Anarhichas lupus	Pavlov and Moksness (1995)	sw	fc	d	4	*			*			
Anarhichas minor	Hansen and Falk-Petersen (2001)	sw	fc	lr	4	*	*		*			
Dicentrarchus labrax	Saillant et al. (2002)	sw	fc	w	5*						*	
Dicentrarchus labrax	Saillant et al. (2006)	sw	fc	w/lr	5		*					
Oreochromis aureus	Desprez and Melard (1998)	fw	fc	d	5*						*	
Oreochromis niloticus	Abucay et al. (1990)	fw	fc	d	5*						*	
Oreochromis niloticus	Baras et al. (2001)	fw	fc	d	5*						*	
Oreochromis niloticus	Baroiller et al. (1995)	fw	o	d	5*						*	
Oreochromis niloticus	Baroiller et al. (1996)	fw	o	d	5*						*	
Oreochromis niloticus	Bezault et al. (2007)	fw	o	w	5*						*	
Oreochromis niloticus	Kwon et al. (2002)	fw	fc	d	5*						*	
Oreochromis niloticus	Tessema et al. (2006)	fw	fc	d	5*						*	
Pomacentrus ambionensis	Gagliano et al. (2007)	sw	o	w	4	*	*			*		
Atheriniformes												
Menidia menidia	Bengtson et al. (1987)	sw	o	w	3		*					
Menidia menidia	Conover and Heins (1987)	sw	o	w	5*						*	
Menidia menidia	Conover and Kynard (1981)	sw	o	w	5*						*	
Menidia menidia	Lagomarsino and Conover (1993)	sw	o	w	5*						*	
Odontesthes bonariensis	Strussmann et al. (1996)	fw	o	d	5*						*	
Cypriniformes												
Carassius langsdorfii	Muller-Belecke et al. (2002)	fw	fc	d	5	*	*			*		
Gnathopogon caerulescens	Fujioka (2001)	fw	o	lr	5*						*	
Gnathopogon caerulescens	Fujioka (2006)	fw	o	w/lr	5*						*	
Clupeiformes												
Clupea harengus	Hoie et al. (1999)	sw	o	w	3		*			*		
Clupea pallasi	Ojaveer (2006)	sw	o	w	4	*		*	*			

Table 2.5. continued

		Experimental design				Offspring responses investigated						
		Reproductive Habitat	Scientific inquiry context	Parental Stock Source	Experiment Duration	Offspring Viability	Offspring Morphology	Development Rate	Offspring Abnormalities	Offspring Physiology	Offspring Sex Ratio	Offspring Performance
Pleuronectiformes												
Pleuronectes ferrugineus	Benoit and Pepin (1999)	sw	o	lr	3	*	*	*				
Pleuronectes platessa	Fox *et al.* (2003)	sw	o	h	3			*				
Cyprinodontiformes												
Poeciliopsis lucida	Sullivan and Schultz (1986)	fw	o	d	5*						*	

fw, reproduces in freshwater (includes anadromous species); sw, reproduces in saltwater; fc, fish culture; o, other; d, domesticated or farm cultured broodstock; h, hatchery broodstock; lr, lab reared broodstock; w, wild parents used; 2, offspring measures were taken at any point between fertilization and eyed stage; 3, offspring measures were recorded up to and including when eggs hatched; 4, offspring measures were recorded up to and including yolk absorption or metamorphosis; 5, offspring measures were recorded beyond endogenous feeding; 5*, experiments that investigated sex-ratio and therefore necessitate a longer experimental duration

Table 2.6. The number (and percent) of studies from a total of 56, that examined the influence of both temperature and parentage on a particular response in developing offspring.

Response Category	Number of studies
Offspring viability	25 (45%)
Offspring morphology	24 (43%)
Offspring sex ratio	17 (30%)
Developmental rate	15 (27%)
Offspring abnormalities	6 (11%)
Offspring physiology	6 (11%)
Offspring performance	2 (4%)

CHAPTER 3: Parental identity influences progeny responses to incubation thermal stress in sockeye salmon (*Oncorhynchus nerka*)[2]

Introduction

Stressful temperatures can be a strong evolutionary force, and selection is expected to favor individuals that are better behaviorally or physiologically adapted. During development, the eggs of Pacific salmon (*Oncorhynchus* spp.) incubate for long durations in constructed gravel redds where, unable to move, they are passive recipients of their thermal environment. Optimal development conditions vary across species but generally range between 6ºC – 10ºC, with temperatures above that range affecting survival and phenotypic traits that relate to fitness (McCullough et al., 2001, and references within). In the context of climate change, predicted increases in mean temperatures and the frequency of extreme temperature events in salmonid freshwater habitat (Hague et al., 2011) may result in incubation environments surpassing thresholds for optimal development. Human development activities around aquatic environments (*e.g.,* power generation, forestry) also threaten to increase thermal regimes in salmonid spawning habitat (Angilletta et al., 2008). While exposure to elevated incubation temperature is known to detrimentally affect survival and fitness in early ontogeny (Combs, 1965; Murray and McPhail, 1988; Blaxter, 1992), little is known about the degree to which offspring responses from different spawning individuals varies within a population.

'Parental influences' are increasingly recognized as significant drivers of offspring variation in fish populations (reviewed in Green, 2008; Marshall et al., 2008), and show the potential for adaptive significance (Mousseau and Fox, 1998). Individual spawners can influence the survival and life history traits of their offspring through both genetic and non-genetic means. In salmonids, female spawner identity can significantly

---

[2] A version of this chapter has been submitted for publication. Burt, J.M., S.G. Hinch, and D.A. Patterson. In review. Parental identity influences progeny responses to incubation thermal stress in sockeye salmon (*Onchorhynchus nerka*)

affect embryonic survival (Nagler et al., 2000; Huuskonen et al., 2003), larval size (Heath et al., 1999), larval physiology (Johnston and McLay, 1997; Patterson et al., 2004a; Pakkasmaa et al., 2006), and emergence timing (Rombough, 1985; Angilletta et al., 2008). These differences are most commonly linked to maternal effects on egg size or egg composition (Kamler 2005). Variation in offspring growth rate, survival and other fitness-related traits have also been attributed to male spawner influence (Garant et al., 2002; Morasse et al., 2008; Eilertsen et al., 2009; Nadeau et al., 2009). However, the majority of these studies were examined under a single set of environmental conditions, and less is known about how the expression of maternal and paternal influences is modified by the environment. For example, despite its controlling effect on early developmental processes, only a small proportion of studies have examined family-level offspring variation in response to incubation temperature in fish (Chapter 2 of this thesis, Burt et al., In press).

There is evidence to suggest that parentally mediated variation can be more pronounced under harsh environmental conditions (Rossiter, 1998; Rasanen and Kruuk, 2007). In some cases, the impact of variation in egg sizes on offspring success is only observed if progeny are reared in a stressful, or resource-limited environment (Parichy and Kaplan, 1992; Fox and Mousseau, 1996). Although a large number of examples come from invertebrate and amphibian studies, Einum and Fleming (1999) showed that Brown trout (*Salmo trutta*) juveniles from large eggs experienced survival and growth advantages only when reared in a poor growth environment. Similarly, Donelson et al. (2009) demonstrated that the parental condition of the tropical damselfish (*Acanthochromis polyacanthus*) affected juvenile survival in low, but not high food, environments. Genetically based variation may also be enhanced in stressful environments (Hoffmann and Parsons, 1991). Genotypes can vary in their ability to buffer stress during development, which can result in the expression of variation that is not normally observed under optimal developmental conditions (Rutherford, 2000). In salmonids, several studies have shown that as incubation temperatures deviate from optimum, the variation in survivorship of different offspring families increases (Beacham and Murray, 1985, 1986a, 1989). The magnitude of this family variation is documented

to be highest at extreme or stressful temperatures (Beacham, 1988; Beacham and Murray, 1989; Murray et al., 1990; Beacham and Varnavskaya, 1991; Janhunen et al., 2010), however, studies that examine within-population variation at extreme thermal exposures are rare, with no studies that specifically focus on family-level variation across a gradient of supraoptimal temperatures.

The combination of environmental and parental influences on offspring survival and life-history traits is often complex and interactive in nature. Phenotypic plasticity refers to when a given genotype produces different phenotypes under different environmental conditions; such responses are represented graphically as a reaction norm (Schlichting and Pigliucci, 1998). Individual families may vary in their levels of response to the environment, resulting in crossing reaction norms, or genotype-by-environment interactions (Hutchings, 2004). In experiments with fish, genetic differences in thermal responses are more commonly documented among populations (Hendry et al., 1998; Haugen and Vollestad, 2000; Hutchings et al., 2007; Jensen et al., 2008), however, significant variation within populations has been demonstrated by different shaped, family-level, thermal reaction norms (Beacham and Murray, 1985, 1988, 1989), and by significant family/genotype-by-temperature interactions (Heath et al., 1993; Benoit and Pepin, 1999; Hoie et al., 1999; Purchase et al., 2010). This family-level variation in environmental sensitivity may be important for salmonids, considering the potential for selective processes to act on genetic differences in reaction norms when optimum phenotypic expression shifts in unpredictable environments (reviewed in Hutchings, 2011).

Beyond the thermal ranges for normal early development, it is possible eggs may experience both physiological and biochemical changes that could affect development and survival at later stages. In incubation temperature experiments where fish were reared and examined beyond the onset of exogenous feeding, juvenile traits such as survival (Gunnes, 1979), vertebrae deformities (Wargelius et al., 2005), muscle cellularity (Macqueen et al., 2008) and growth (Martell et al., 2005) were affected by early embryonic thermal exposures. Parental influences have also been demonstrated to affect

variation in post-emergence traits between offspring families (Heath et al., 1993; Patterson et al., 2004a; Nadeau et al., 2009), although it is far more common for parent-progeny studies to focus on egg and hatchling stages. For salmonids, factors affecting post-emergence survival or performance are likely critical, due to elevated selection pressure in association with increased competition for food, predator exposure and migration (Groot and Margolis, 1991). Whereas it is evident that both parentage and the external environment have controlling effects on early development processes, less is known about the persistence of incubation temperature effects and parental influences in later development stages (Chapter 2 of this thesis; Burt et al. In press).

The objective of the present study is to examine how individual parentage can affect progeny responses to early developmental temperature stress in sockeye salmon (*Oncorhynchus nerka*). To this end, independent fertilizations were performed using gametes from mature sockeye adults to create maternal and paternal half-sib families that were exposed to three levels of embryonic temperature stress and then reared until early fry stage. Our study tested the hypothesis that significant variation in offspring responses (survival, size, and hatch timing) to embryonic temperature stress could be explained by parental identity. We predicted (1) that parentally mediated variation in offspring survival and development traits would be greater under conditions of thermal stress, (2) that individual families would vary in their levels of response to the thermal stress (indicated by crossing survival reaction norms and a significant family-by-incubation temperature interaction), (3) that offspring survival and size would continue to be under detectable parental and temperature influence in post-emergence fry stages.

Methods

Fish collection and larval rearing

The study population consisted of sockeye salmon from Weaver Creek, one of the major stocks in the lower Fraser River of southern British Columbia, Canada (Fig. 3.1). On 15-18 September 2008, adult sockeye salmon were caught by beach seine in the

Harrison River, on Chehalis First Nations territory 117km from the mouth of the Fraser and 5km downstream from their spawning grounds. Upon capture, fish were immediately transported to the Fisheries and Oceans Canada Cultus Lake Laboratory where an adipose fin clip was taken to confirm stock identification (Beacham et al., 2005) and adults were randomly placed in holding tanks (~20 000 L) until the fish reached sexual maturity, confirmed by gentle abdominal pressure extruding either eggs or sperm.

Gametes were collected from six mature females and six mature males on October 10, 2008. Fish were sacrificed by cerebral concussion and eggs and milt were carefully extracted into individual dry plastic containers avoiding water and blood contamination. Upon terminal sampling, postorbital-hypural (POH) length and body mass (wet mass, ±10 g) was measured for each individual spawner. Three 10-egg sub-samples were also taken from each female to calculate egg wet mass (± 0.001 g) as well as egg dry mass (± 0.001 g), obtained after heating eggs in an oven for 36 h at 60ºC. Containers holding gametes were filled with 99.99% pure oxygen and transported in a cooler on ice (~ 4ºC) within two hours to Simon Fraser University Alcan Aquatic Facilities for fertilizations.

Fertilization protocol and incubation design

Ten offspring families were created by independent fertilizations. The eggs of five females (numbered 1-5) were crossed with the milt from a single male (male A) to create the first five families (labeled 1A, 2A, 3A, 4A, 5A). Next, the eggs of a single female (female 6) were crossed using the milt from different males (letters B-F) to create five more half-sib families (labeled 6B, 6C, 6D, 6E, 6F). A full factorial cross design was not possible due to limitations associated with gamete (egg) quantities and the desire to replicate families within temperature treatments. For each family cross (n = 10), nine replicates were created by separate fertilizations to provide three replicate families to be placed in each of the three incubation temperature treatments. A dry fertilization protocol was used for all crosses, a technique shown to be effective (≥ 94% embryonic survival) in other incubation studies using Weaver Creek sockeye (Patterson et al., 2004a). For each cross, approximately 10 g of eggs were combined with 0.15 ml of milt and activated with

15 ml of water. After two min, excess sperm was removed by adding 60 ml of fresh water. Fertilized eggs were kept in their jars for a minimum of 45 min to allow for water hardening, during which time they were transported to the University of British Columbia (< 1 h transport time) for incubation at the Forest Sciences Aquatic Laboratory.

Each replicate from each family cross was transferred into a separate netted cylindrical egg capsule (total n = 90 family replicates) and placed in a flow-through Heath stack fed by recirculating dechlorinated city water at one of three incubation temperatures (12, 14 or 16ºC) (Fig. 3.2). Each family was represented once in a single tray, in a single Heath stack (three Heath stacks per temperature treatment). Incubation temperatures were selected based on existing data for sockeye salmon (Beacham and Murray, 1990) to encompass a thermal range that included a treatment within the species' upper incubation optimum (12ºC), a thermally stressful temperature associated with an approximate 50% fertilization-to-hatch mortality (16ºC), and an intermediate treatment (14ºC). Additionally, data obtained from the Weaver Creek spawning channel indicated these temperatures were ecologically relevant; peak spawning tends to occur around 12ºC, but maximum temperatures during the entire spawning period can range up to 16ºC (Fig. 3.3).

Following fertilization, all egg capsules were initially incubated at 12ºC and elevated to treatment temperatures over three days by incremental adjustments to multiple small aquarium heaters placed in the bottom collection/intake basins. This was done to ensure all egg capsules had equal fertilization capacities, and to avoid high mortality rates associated with sub-optimal temperature exposure occurring before epiboly (Beacham and Murray, 1987). Once the desired incubation temperature was obtained, it was held constant until the families had completed hatching. Recorded actual treatment means (± S.D.) were 11.8ºC ± 0.4, 13.8ºC ± 0.4, 15.7ºC ± 0.4. Dissolved oxygen levels were maintained above 8.0 $mg \bullet mL^{-1}$, based on periodic checks using an Oxyguard© meter and stacks were covered to reduce light exposure. Water flow through Heath trays was held constant at ~10 L/min and temperature was recorded daily with

digital thermometers and hourly by Vemco© temperature loggers placed in the top and bottom of each stack.

Eggs were checked every 3-4 days until hatching began, after which they were checked daily. Mortalities were recorded and dead eggs were removed and cleared in Stockard's Solution (5% formaldehyde (40%), 4% glacial acetic acid, 6% glycerin, 85% water). Dead eggs that showed the presence of an eyed embryo were classified as surviving to 'eyed stage'. Within one day of each family capsule's 95% hatch date, five alevins were sampled to obtain both mass and length at hatch. Alevins were subjected to a lethal dose of tricaine methanesulfonate (MS222), blotted and weighed (± 0.001 g). Lengths (± 0.1 mm) were measured from photographs of each alevin taken against 1 mm graph paper.

Post-hatch incubation and rearing

After families in a temperature treatment reached 95% hatch, the water temperature was slowly lowered (1ºC per day) to the ambient temperature of the laboratory water supply (10ºC in early December and declining to 5ºC by February). All 90 family crosses therefore experienced identical, ambient post-hatch temperatures. Previous research has shown that increasing or decreasing temperature transitions during later development stages has little influence on alevin survival rates (Murray and Beacham, 1986, 1987), suggesting that any observed post-hatch mortality would be a result of persistent effects from pre-hatch treatment and not a result of the incremental lowering of temperatures. Alevins were checked every two days, and mortalities were recorded.

Upon reaching fry emergence stage, families were transferred from incubation capsules into 10 L netted rearing enclosures placed in 1,000 L flow-through troughs. At this stage, fish had no visible external yolk sac (entirely covered by chromatophores), and in the wild would be ready to emerge from their incubation gravel, migrate to their nursery lake, and begin exogenous feeding. The number of ATUs (accumulated thermal

units) obtained at fry transfer was confirmed to be close to the value that wild fry from Weaver Creek spawning channel emerge (~1000 ATUs; R. Stitt, Weaver Creek Spawning Channel, Department of Fisheries and Oceans, personal communication). Transferred fry were placed in enclosures at equal stocking densities (30 fish/10 L enclosure); however, rearing densities were lower (15-28 fry) for several 16ºC exposed families that had experienced high mortality during incubation. After a 24 h adjustment period, fry were fed powdered fishmeal (EWOS Canada Ltd.) in two daily installments of ~1% total body mass. Lighting conditions were adjusted regularly to reflect the natural photoperiod (49º18′ N). The experiment was concluded after families completed three weeks of exogenous feeding, at which point 10 fish were sampled from each rearing enclosure for fry mass and length.

Data and statistical analysis

All replicates of every family (n = 90) were used in the analysis given that all fertilizations yielded viable offspring and no family replicates experienced 100% mortality during the experiment. Prior to analysis, proportional survival data (% of total eggs that survived) were arcsine square root transformed to meet the normality (Kolmogorlov-Smirnov test) and homoscedasticity assumptions of parametric tests. Three periods of survival were examined: survival-to-hatch (S_H), alevin survival (S_A: survival hatch-to-emergence), and fry survival (S_F: survival post-emergence). Family means were used as response variables for alevin mass and length at hatch (M_H and L_H), as well as fry mass and length (M_F and L_F). Daily information on the number of eggs hatching was used to calculate the median hatch date (H_{50}) and the hatching duration (*HD*; number of days between 5% and 95% hatch) for each incubation egg capsule. All analyses were done using mixed effect ANOVAs in SAS 9.1 (SAS Institute: www.sas.com), which compute F-tests (typeIII SS) to determine the statistical significance of both fixed and random variables.

To examine the variables of interest at a 'whole model' scale, the independent and interacting effects of temperature and parentage (family identity) on offspring response variables were analyzed using a nested, mixed model ANOVA:

$$y_{jkl} = \mu + T_j + P_k + F(P)_{l(k)} + T \times P_{jk} + T \times F(P)_{jl(k)} + \varepsilon_{jl(k)}$$

where y = offspring response variable (S_H, M_H, L_H, H_{50}, HD, S_A, S_F , M_F, or L_F), T_j = temperature for treatment j, P_k = parent spawner used to generate half-sibs (k = 'female' or 'male' parent spawner), F_l = family identity nested within male or female spawner (l = 1-5 within 'male' or 6-10 within 'female'), and ε is the random error term. All variable interaction terms are also included in the model. Heath stack effects were included in the original model, but were found to be non-significant in all analyses, and were subsequently removed from the current model. For all analyses, T and P were considered fixed effects while all other variables, including family identity, were considered to be random effects. Differences among temperature treatments were assessed using pairwise t-tests and a Bonferronic alpha correction (Whitlock and Schluter, 2009).

In order to examine among-family variation in offspring traits in isolation from interactions across treatment temperatures, a simplified nested mixed model ANOVA model was used separately within the 12, 14, and 16ºC treatment groups:

$$y_{kl} = \mu + P_k + F(P)_{l(k)} + \varepsilon_{l(k)}$$

where variables y, P, and F are defined the same as in the model above. Originally included in this model, the effect of Heath stack was removed after determining it to be non-significant in the analysis of each y variable, at each temperature. In order to compare female vs. male spawner influence on offspring variables (y), paired t-tests from the simplified ANOVA model were used to compare family means and assess whether differences were significant among paternal half-sib families, among maternal half-sib families, or both. Finally, Pearson's correlations using family means were used to examine the relationships between egg/alevin size and offspring survival, hatching characteristics and offspring survival, as well as egg size and alevin/fry size. When

correlations were significant, their respective coefficient, (r), is given. The significance level for all statistical procedures was set at $\alpha = 0.05$.

Results

Embryonic survival

At the end of the thermal treatment period, both incubation temperature and family identity showed significant effects on embryonic survival to hatch (ANOVA, $F_{2,16} = 33.2$, n = 90, $P < 0.0001$ and $F_{8,16} = 2.79$, n = 90, $P < 0.05$, respectively, Fig. 3.4a). Mean survival to hatch was high for the 12ºC incubation (95% ± 4.5 S.D.), but decreased significantly at 14ºC (84% ± 18.7 S.D.) and 16ºC (60% ± 23.2 S.D.), indicating the higher temperatures were indeed thermally stressful. The relative variation among family-specific means was also 8 times higher at 16ºC compared to 12ºC (c.v. 4.8% at 12ºC, 22.3% at 14ºC and 38.7% at 16ºC).

At 16ºC, variation in mean progeny survival was significant (ANOVA, $F_{8,20} = 5.12$, n = 30, $P < 0.001$) with family means ranging from 31.2 - 92.3%. Differences in progeny survival were significant among crosses varying in female spawner identity and among crosses varying in male spawner identity (paired t-tests, n = 30, various pairs with $P < 0.005$, Fig. 3.4a). For the 14ºC treatment, variation among families was not significant (ANOVA, n = 30, $P > 0.05$) due to a higher amount of variation among family replicates (see Fig. 3.4a). Within the 12ºC treatment, variation due to family identity was significant (ANOVA, $F_{8,20} = 2.58$, n = 30, $P > 0.05$); however, significant differences were only found among families of differing female parentage (paired t-tests, n = 30, various pairs with $P < 0.005$).

To visualize the among-family differences in survival reaction norms over the incubation thermal gradient, mean survivorship vs. temperature data were plotted (Fig. 3.5). Although the reaction norms ‘cross’ (indicating the presence of an interaction between temperature and survival), statistical analysis using the ‘whole model’ ANOVA

revealed the interaction between incubation temperature and family identity to be only marginally significant (ANOVA, $F_{16,60} = 1.63$, n = 90, $0.05 < P < 0.10$). However, when the analysis was run without nesting maternally-linked and paternally-linked families (*i.e.,* considering the 10 families as independent), the interaction term was significant (ANOVA, $F_{18,60} = 1.96$, n = 90, $P < 0.05$).

Hatching characteristics and offspring size at hatch

Development time to median hatch (H_{50}) was affected by incubation temperature (ANOVA, $F_{2,16} = 88.9$, n = 90, $P < 0.0001$), with hatching occurring first within the 16ºC families, followed by 14ºC then 12ºC families (Fig. 3.6a). The duration of the hatching (HD) was also affected by temperature (ANOVA, $F_{2,16} = 50.47$, n = 90, $P < 0.0001$), with the hatching process taking twice as long at 14ºC (mean = 7.2 days), and three times as long at 16ºC (mean = 9.1 days), compared to fairly synchronous hatching at 12ºC (mean = 2.9 days) (Fig. 3.6b). Temperature effects on hatching also included a three times higher incidence of precocious hatching (eggs hatching prior to the 5% hatch date) at 16ºC. A total of 48 eggs hatched precociously among all combined eggs incubated at 16ºC, compared to 11 and 15 precociously hatched eggs in the in 12º and 14ºC treatments, respectively.

H_{50} and HD were also influenced by family identity (ANOVAs, $F_{8,16} = 1.95$ and 2.96, respectively, n = 90, both $P < 0.05$) and a temperature-by-family interaction (ANOVAs, $F_{16,60} = 88.9$ and 50.5, respectively, n = 90, both $P < 0.05$). At 12ºC, variation in H_{50} and HD due to family identity was not significant (ANOVAs, n = 30, both $P > 0.05$); average hatch timing among families was similar, and most families hatched within 1 – 3.6 days. At 14ºC, both H_{50} and HD varied among families (ANOVAs, $F_{8,20} = 18.9$ and 8.05, respectively, n = 30, all $P < 0.05$); median hatch ranged from 46 – 52 days and hatch duration from 3 – 10 days. At 16ºC, H_{50} varied among families (ANOVA, $F_{8,20} = 3.99$, n = 30, $P < 0.001$) and family variation in HD was marginally significant (ANOVA, $F_{8,20} = 2.22$, n = 30, $0.05 < P < 0.1$). Median hatch at 16ºC took 43 - 51d and average hatch duration from 7.3 – 11 days (however, some egg baskets required up to14 days to complete hatching). Inter-family variation was significant among male half-sib

crosses and female half-sib crosses at 14ºC (paired t-tests, n = 30, various pairs with $P < 0.005$). The same was true at 16ºC, however differences among male and female half-sibs were only significant before Bonferroni corrections ($P < 0.05$).

Alevin mass at hatch was primarily influenced by maternal identity (Fig. 3.4b). Alevin mass varied only among half-sib families of differing maternity (paired t-tests, n = 30, various pairs with $P < 0.005$), and was explained by individual differences in egg dry mass (Pearson's correlations, n = 5, $P < 0.05$, $r = 0.98$ at 12ºC, $r = 0.93$ at 14ºC, $r = 0.91$ at 16ºC), although relationships were strongly driven by the particularly heavy family (4A) that came from large eggs. Conversely, temperature had a small, albeit significant, effect on alevin mass at hatch (ANOVA, $F_{2,16} = 16.8$, n = 90, $P < 0.001$), whereby average alevin mass was slightly lower at 12ºC compared to 14 and 16ºC (Fig. 3.4b). Length at hatch was influenced by incubation temperature (ANOVA, $F_{2,16} = 9.6$, n = 90, $P < 0.01$) but not by family identity (ANOVA, n = 90, $P > 0.05$). Variation in length at hatch was high among family replicates, possibly due to a less precise method used for length estimation. The family-by-temperature interaction term was not significant for either mass or length at hatch (ANOVAs, n = 90, both $P > 0.05$).

Neither egg dry mass nor hatching characteristics were correlated to offspring survival at hatch. Inter-family differences in survivorship observed at 12 and 16ºC (no differences at 14ºC) were not related to egg wet mass, dry mass or alevin mass (Pearson's correlations, n = 5, all $P > 0.10$). Similarly, embryonic survivorship at 16ºC was not related to H_{50} or HD (Pearson's correlations, n = 10, both $P > 0.20$). However, all of these correlations were subject to small sample sizes (using family means) leaving survival relationships hard to discern.

Persistent temperature effects and family influences

After each treatment group achieved hatching, temperatures were lowered to an identical ambient thermal profile. Post-hatch mortality of alevin and fry was significantly affected by pre-hatch temperature treatments (ANOVA, $F_{2,16} = 16.5$, n = 90, $P < 0.001$, Fig. 3.7). Alevin and fry mortality was significantly lower within the treatment groups

initially at 12ºC (alevin = 0.4% ± 0.68 S.D., fry = 0%) and 14ºC (alevin = 2.3% ± 2.7 S.D., fry = 0.1% ± 0.39 S.D.). Despite removal of the high temperature stress, mean alevin and fry mortality (13.7% ± 14.0 S.D. and 1.5% ± 1.9 S.D., respectively) were significantly higher in fish that had experienced the 16ºC exposure (all $P < 0.001$).

Within the 16ºC treatment group, alevin mortality (between hatching and emergence) showed significant variation among families (ANOVA, $F_{8,20} = 8.1$, n = 30, $P < 0.0001$, Fig. 3.7) but not post-emergence fry mortality ($P > 0.05$). In particular, four families (2A, 4A, 6D, 6E) experienced high post-hatch alevin mortality ranging between 15.0% and 42.4% (Fig. 3.7). Similar to embryonic survival, post-hatch survival of families was not related to any offspring size variables we measured (Pearson's correlations, n = 5, $P > 0.05$).

Temperature effects that had not been strongly present at hatch were apparent after three weeks of exogenous feeding for fry mass and length (ANOVAs, $F_{2,16} = 13.9$, n = 90, $P < 0.001$ and $F_{2,16} = 66.5$, n = 90 $P < 0.0001$, respectively). Fry initially incubated at 16ºC weighed less and were shorter than fry from the 12 and 14ºC treatments (Fig. 3.8). Family identity significantly influenced fry mass and length (ANOVAs, $F_{8,16} = 4.0$ and 5.1, respectively, n = 90, both $P < 0.05$), however temperature-by-family interactions were also significant (ANOVAs, $F_{16,60} = 2.4$ and 2.5, respectively, n = 90, both $P < 0.05$).

Within each temperature group, variation in fry mass and length was attributable to family identity (ANOVAs, 12ºC - $F_{8,20} = 7.2$ and 8.3, 14ºC - $F_{8,20} = 4.3$ and 6.7, 16ºC - $F_{8,20} = 3.3$ and 4.8, n = 30, all $P < 0.01$) and influenced by both female and male spawner identity (paired t-tests, n = 30, various pairs with $P < 0.005$). Unlike at hatch, inter-female variation in progeny mass at fry stage was no longer predicted by initial egg dry mass, although the two families coming from larger eggs tended to be heavier and longer. Interfamily variation in fry size variables was less pronounced in the 16ºC exposed group (Fig. 3.8), but this may be due to non-random sampling of "surviving" fish in a treatment that experienced high mortality.

Discussion

By examining temperature-induced shifts in survivorship among families of sockeye salmon, the present study highlights the substantial degree to which individual spawners can influence their progeny's response to high temperature exposure during early incubation. Based on an optimal range for sockeye salmon incubation of between 4 –12.5ºC (McCullough et al., 2001), the substantial decreases in survival at 14 and 16ºC is an indication these temperatures were thermally stressful. Consistent with our original prediction, among-family variation in survival to hatch was much higher at 16ºC compared to 12ºC. This is similar to other salmonid studies that show interfamily survival variation tends to increase as temperatures diverge from an incubation thermal optimum (Beacham and Murray, 1985, 1989). Along with among-family variation, within-family variation also increased in the higher incubation temperature treatments. When eggs were exposed to 14ºC, family-based differences in embryonic survival were obscured by high levels of variation among family replicates, as also reported by Janhunen et al. (2010) using Arctic charr (*Salvelinus alpinus*). Higher levels of 'developmental noise' are common when organisms develop in sub-optimal conditions. That is, genotypic replicates reared in the same conditions can have different outcomes due to higher rates of random perturbations in developmental pathways (Willmore and Hallgrimsson, 2005). Whereas a population effect (decreased overall survival) was observed at both 14 and 16ºC, large distinctions between families with overall high versus low survivorship only occurred in the 16ºC treatment. Accordingly, the threshold for observing a clear genetic basis for selection appears to be greater the higher the thermal exposure from the population norm.

The observed variation in offspring survivorship was not attributable to any parent-mediated phenotypic traits, such as initial egg size. This is consistent with results from some salmonid studies that found egg size did not affect embryonic viability (Hutchings, 1991; Pitcher and Neff, 2007; Nadeau et al., 2009), and in contrast to others that observed both positive (Heath et al., 1999) and negative (Beacham and Murray, 1985) relationships between egg size and survival. In some cases, incubation at higher temperatures has been shown to remove the significant survival advantage of larger eggs

observed at lower, more optimal incubation temperatures (Gagliano et al., 2007; Janhunen et al., 2010). Given the impact that physiological stress in adult spawners can have on gamete quality, this may also influence offspring viability (Campbell et al., 1992; Eriksen et al., 2006). Gagliano and McCormick (2009) observed that the embryonic and post-hatch survival of damselfish (*Pomacentrus amboinensis*) offspring was linked to their levels of the maternally derived hormone cortisol, presenting a mechanism by which female spawners can directly influence the survival potential of their progeny. However in the present study, further analysis revealed no correlation between the stress-related physiological status of the 10 parent spawners and the variation observed in progeny survival (see Appendix A, Table A.1 and A.2). Although other unmeasured maternal effects such as egg matter composition and maternal mRNA may have influenced (Morrison et al., 2002) progeny viability (Kamler, 2005), the lack of parentally mediated phenotypic traits that explain survival variability suggests that in conditions of high temperature stress, family survivorship may be more linked to the genetic influences of parent spawners (Rudolfsen et al., 2005). This is supported by our finding that incubation at 16ºC resulted in significant variation among half-sib families sired by different males. Differences in 'genetic quality' due to particular parental combinations has been observed to affect both early and late larval survival in whitefish (*Coregonus* spp.) (Wedekind et al., 2008) as well as survival and growth in Chinook salmon (*O. tshawytscha*) (Pitcher and Neff, 2007). Stressful incubation temperatures could also have resulted in the release of cryptic genetic variation, for example in genes linked to thermotolerance, that was previously underexpressed in environmentally optimal conditions (Rutherford and Lindquist, 1998).

Our data showed that individual families had different survival responses across the incubation thermal gradient, or interacting survival reaction norms, suggesting that there was no clear additive effect of temperature on family. Some families had a large magnitude of change in survival between 12º and 14ºC, others between 14ºC and 16ºC, while two families (1B, 4B) sustained relatively high mean survival to hatch in all three temperature environments. This is in agreement with a multitude of studies in developing teleosts which have found several complex phenotypic traits to be governed by

interactions between genotype and incubation temperature (Chapter 2 of this thesis, Burt et al., In press). Revealing family-level differences in phenotypic plasticity associated with fitness traits, or in this case survival itself, is important because it conveys information about the ability of individual genotypes, and therefore populations, to respond to environmental change (Hard, 1995; Hutchings et al., 2007). In a similar incubation temperature experiment, evidence of genetic variation in temperature tolerance was indicative that adaptive divergence had occurred among sockeye populations linked to their thermal environments within Lake Washington (Hendry et al., 1998).

Offspring families were reared into the exogenous feeding stage to test for the presence of latent temperature effects and persistent parental influences. Despite identical thermal conditions post-hatch, alevin and fry mortality were significantly higher in fish initially exposed to the 16ºC treatment, with almost no post-hatch mortality occurring in families from the initial 12ºC and 14ºC exposures. Latent temperature effects on alevin mortality have been observed when salmonid eggs are transferred from extreme cold to warm (and vice versa) temperatures during initial incubation (Murray and Beacham, 1986, 1987), however, this is the first study to our knowledge to demonstrate that pre-hatch exposure to supraoptimal temperatures can have latent effects on both alevin survival and post-emergence fry survival. While the mechanisms of this delayed mortality are unclear, they could be a result of thermal alterations to genetic or biochemical pathways during the critical embryogenesis stages that eventually affect later survival. Johnston (2006) proposed that thermally-induced changes in transcriptional networks, extracellular signaling molecules, and the expression of certain genes that control muscle differentiation during embryogenesis may be responsible for the 'imprint' that early temperature exposures can have on later-stage muscle phenotypes. Takle et al. (2004) demonstrated that a short (~ 4days) temperature elevation of only 4ºC during organogenesis led to altered expression of several 'stress sensitive' genes linked to heart function and metabolism in Atlantic salmon (*Salmo salar*).

Latent temperature effects on alevin survival were experienced in different magnitudes for different offspring families. For example, two of the families (4A, 6D) with the highest survival to hatch in the 16ºC treatment had the highest post-hatch mortality despite ambient temperature conditions. In general, parental influences are observed to fade with ontogeny (Mousseau and Fox, 1998), but our findings lend weight to those of other studies that demonstrate certain traits and responses in salmonid offspring can remain under significant parental influence beyond hatch and exogenous feeding (stress response – Heath et al., 1993; enzyme activity – Patterson et al,. 2004; fry survival – Nadeau et al., 2009). Very few studies exist examining stage-specific, family dependent, thermally-induced mortality (Murray and Beacham, 1986; Beacham and Murray, 1987), however, evidence of these complex mortality patterns emphasizes the importance of extending the duration of future incubation studies beyond treatment exposures or hatching stages.

In addition to survival, offspring size characteristics were influenced by both temperature and parentage. At hatch, alevin mass was most influenced by the maternal effects of egg size, consistent with other studies on sockeye salmon (Hendry et al., 1998; Patterson et al., 2004a), whereas temperature effects were surprisingly minimal. Although a decrease in larval mass at hatch at the higher temperatures would have been expected (Beacham and Murray, 1989), one explanation for the lack of temperature effects at hatching could be that treatment groups were at varying levels of development when families were sampled. For example, the fish at 14 and 16ºC had substantially longer hatching durations, and therefore may have had more opportunity to grow before being sampled for mass.

Interestingly, despite post-hatch rearing at a common temperature, the three treatment groups had significantly different average mass and lengths when measured as 3-week-old fry (12ºC > 14ºC > 16ºC, Fig. 3.8). In teleosts, a negative effect of increasing incubation temperature on larval body size has several explanatory hypotheses: temperature acts differentially on growth versus developmental rate, the efficiency of yolk utilization is reduced at higher temperatures, and vertebrae number is reduced

during development at high temperatures (see Kamler, 2008 and references within). However, these effects apply in the context of continuous high temperature exposure up to a sampling point, and less so in the context where persistent temperature effects on size or growth are observed after rearing in controlled post-hatch environments. The latter context is seldom documented, however, Martell *et al.* (2005) found that incubation temperatures (4, 6, and 8ºC) affected haddock (*Melanogrammus aeglefinus*) growth well beyond hatch (> 35 days), despite rearing at a common post-hatch temperature (6ºC), and attributed these delayed growth effects to alterations in muscle cellularity that persisted from early thermal exposures (Martell and Kieffer, 2007). Such could be the case in this experiment, however, we cannot rule out the possibility that temperature-related size differentials were present at hatch (albeit undetected) and became amplified during yolk absorption and exogenous feeding.

In this experiment, a paternal influence on the mass and length of 3-week-old fry was evident within all temperature treatments. Together with a less pronounced relationship between egg size and fry mass/length (a trend was still apparent), this suggests that there may be a greater influence of the offspring genome on size compared to maternal effects at this later development stage (Heath et al., 1999). However, as was observed for survivorship, size variables were characterized by family-temperature interactions, which suggest that a complex interplay between the two factors influenced post-hatch growth.

Models describing an inverse relationship between time-to-hatch and temperature are well developed for salmonids (*e.g.,* Beacham and Murray, 1990), but variation at the family level, and trends in hatch duration are seldom reported. In the current study, hatch timing and hatch duration were fairly synchronous among families at 12ºC, whereas the higher incubation temperatures resulted in substantial among-family variation in both traits (influenced by maternity and paternity). Although no studies exist on sockeye salmon, these results support the findings from other *Oncorhynchus* species*;* that time-to-hatch has genetic, maternal and temperature influenced components (Beacham, 1988; Hebert et al., 1998). Our results suggest the same may be true for hatching duration,

which showed family differences, and was increased two-fold at 14ºC and three-fold at 16ºC, compared to 12ºC. High temperatures can cause earlier secretion of hatching enzymes, combined with increased embryo mobility that causes alevins to hatch prematurely (Kamler, 2008). However, this does not explain why only some individuals in a family hatched earlier than others, and why hatching within families incubated at 14 and 16ºC was distributed over a greater number of days (up to 14 days in some families). To our knowledge, only one other study has examined hatching duration in salmonids, and their findings were contradictory whereby higher incubation temperatures promoted more synchronous hatching (Humpesch, 1985). Further studies are required to better understand the influence of temperature on hatching duration, and to find out if there are fitness consequences or benefits related to hatch timing within an asynchronous distribution.

In summary, the results of this study support a growing literature showing that offspring responses to thermal stress within salmonid populations are influenced by individual parentage (Beacham, 1988; Turner et al., 2007; Janhunen et al., 2010). Although quantitative partitioning of variation between additive genetic, maternal and environmental sources was not possible in our design, we were able to show that significant differences in offspring survival, size, hatching timing, and hatch duration are attributable to maternal and paternal identity, and that the expression of these parental influences was dependent on temperature. However, further research is needed to illuminate the relationship between individual spawner attributes (genetic and/or non-genetic) and offspring success in suboptimal environments. Work that focuses on unraveling the physiological/genetic basis of early development and survival, as well as experiments that use larger sample sizes of parent spawners (representing a wider distribution of phenotypes or genotypes) may increase our ability to explain offspring differences. Future studies may also wish to further investigate persistent temperature and parental influences on offspring traits linked to fitness (*e.g.,* size, swimming performance, physiology), as post emergence stages represent another critical bottleneck for survival in Pacific salmon life history (Groot & Margolis, 1991).

Climate change and human activities are expected to continue raising the thermal regimes in freshwater systems, placing pressure on salmonids that use this habitat to migrate, spawn and rear (Crozier et al., 2008). Further knowledge of how individual spawners influence early life history traits, how temperature can modulate these influences, and the persistence of parental and temperature effects, would be helpful in improving our ability to predict the immediate and evolutionary responses of sockeye populations to high temperature events. Our findings highlight the crucial role that parental influences on offspring fitness may have in shaping future selection within salmonid populations exposed to elevated thermal regimes, overall emphasizing the benefit of maintaining genetic diversity for future conservation.

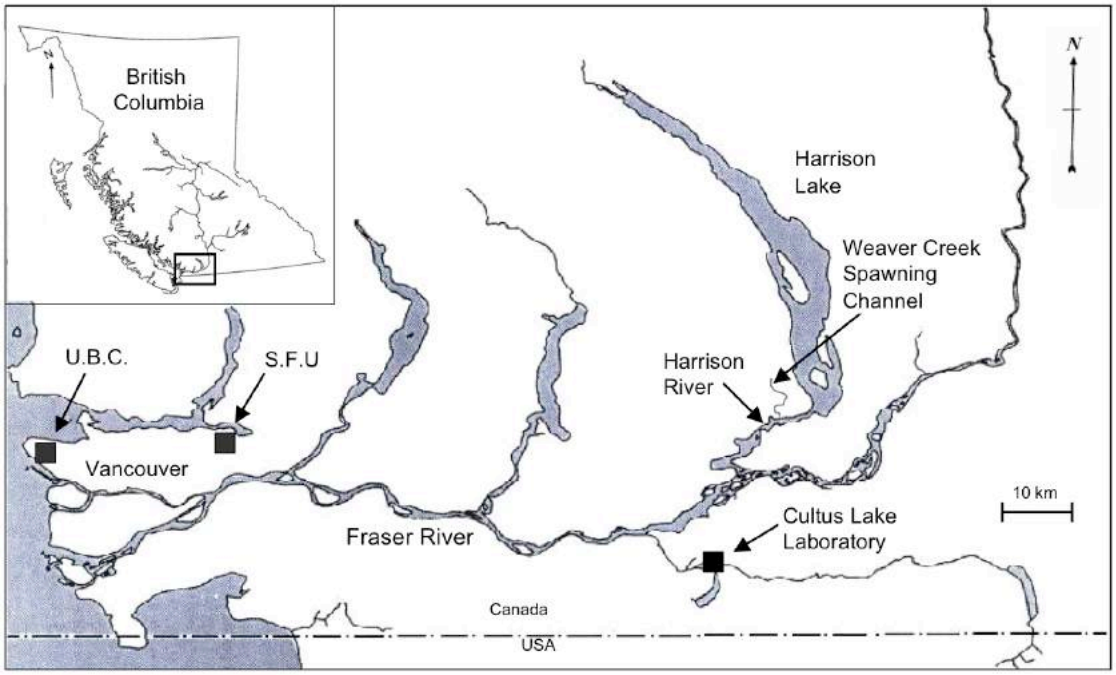

Figure 3.1. Map of the lower Fraser River, British Columbia, Canada. The collection site for Weaver Creek salmon is indicated by the arrow on the Harrison River. Laboratory locations are abbreviated: U.B.C., University of British Columbia; S.F.U., Simon Fraser University.

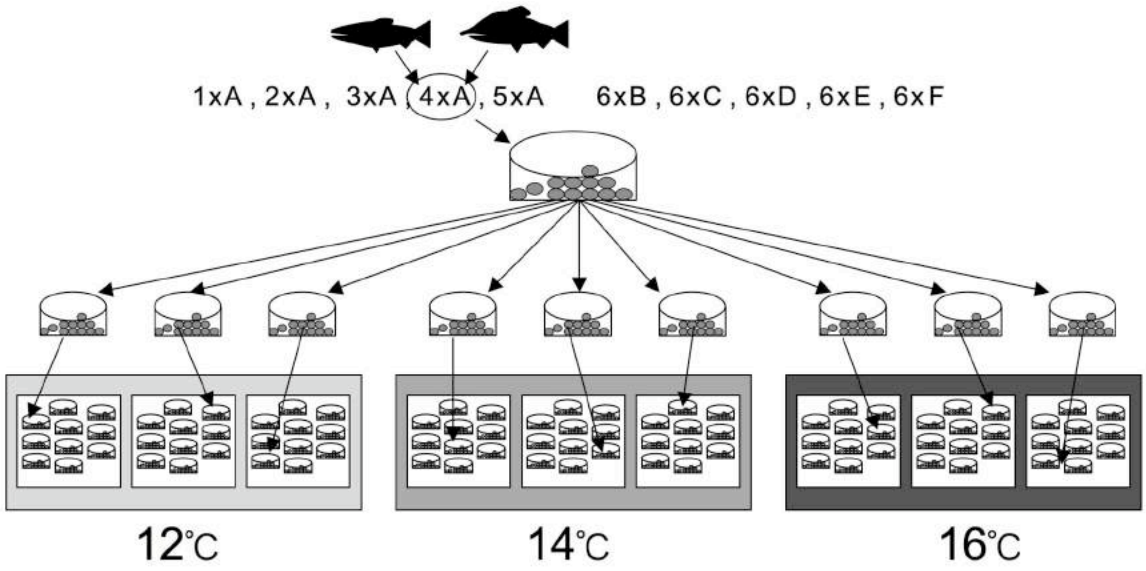

Figure 3.2. Diagram of incubation experimental design. Numbers represent female spawners and letters represent male spawners used for each family cross. Fertilized eggs were placed in cylindrical capsules and randomly arranged in separate Heath stacks (white squares) within three incubation temperature treatments.

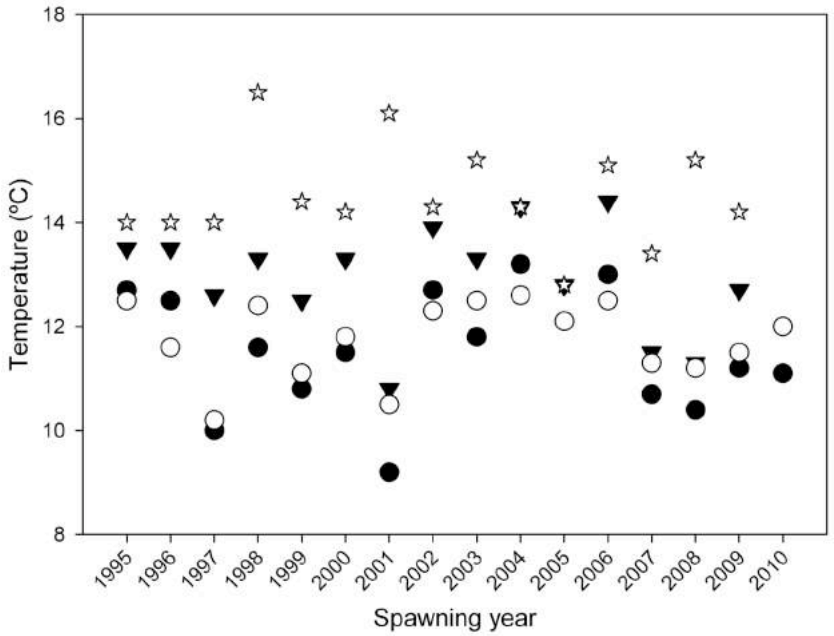

Figure 3.3. Water temperatures during sockeye salmon spawning in the Weaver Creek spawning channel from 1995 - 2010. Black symbols show the average (circle) and maximum (triangle) water temperatures recorded during the interval of peak spawning. White symbols show the average (circle) and maximum (star) water temperatures recorded over the entire spawning duration. Data obtained from R. Stitt, Department of Fisheries and Oceans.

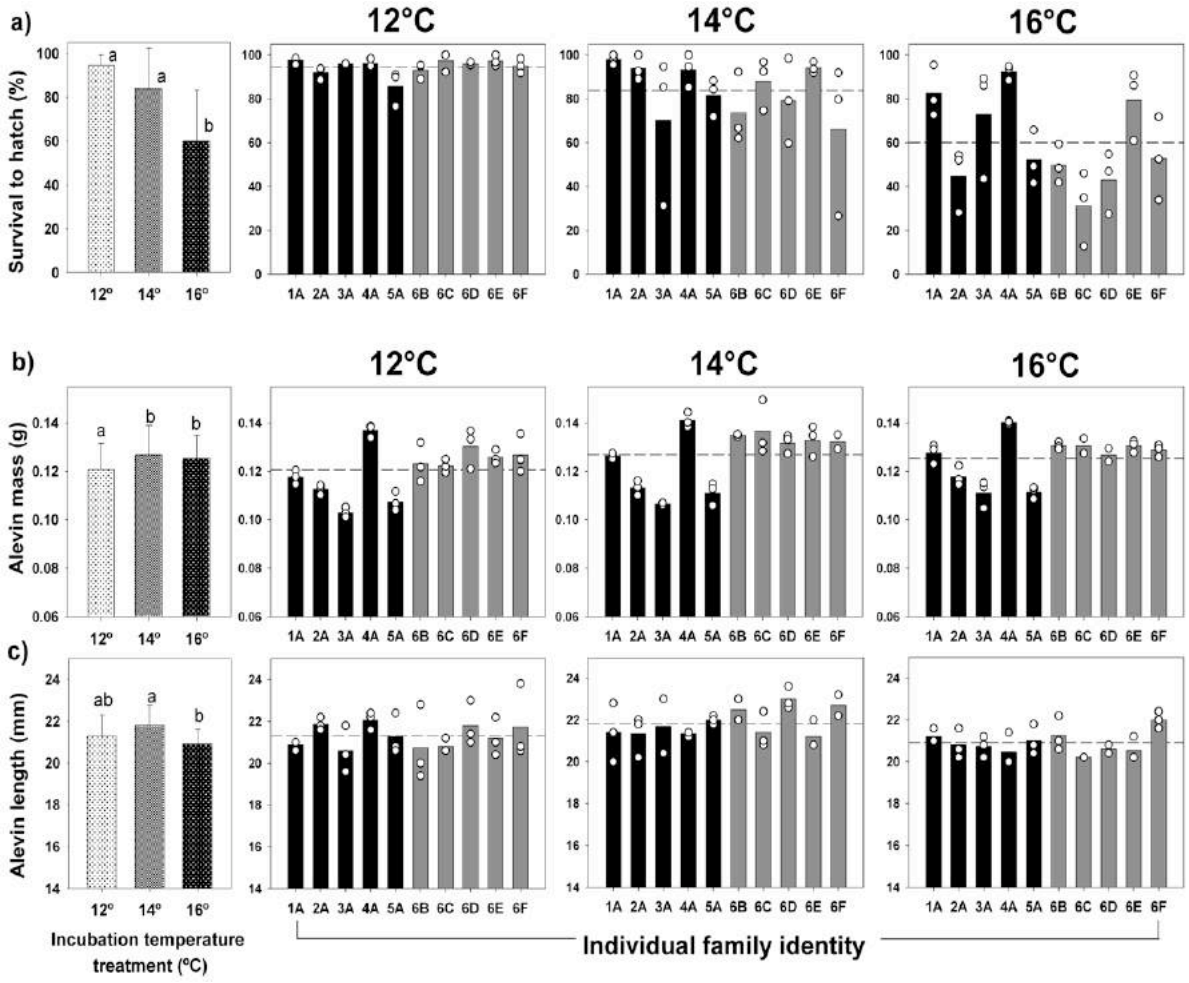

Figure 3.4. The effects of incubation temperature treatments and family identity on selected offspring traits at hatch: (a) survival, (b) alevin mass, (c) alevin length. Graphs on the left show incubation temperature treatment means (n = 30) +S.D. and letters indicate significant differences ($P < 0.01$). Graphs to the right show individual family responses. Bar heights represent the family mean and the white circles show the independent family replicate values. The dashed line shows the overall mean within a temperature treatment. Black bars show the influence of female spawner identity (females 1-5 crossed to male A) and the grey bars show the influence of male spawner identity (males B-F crossed to female 6).

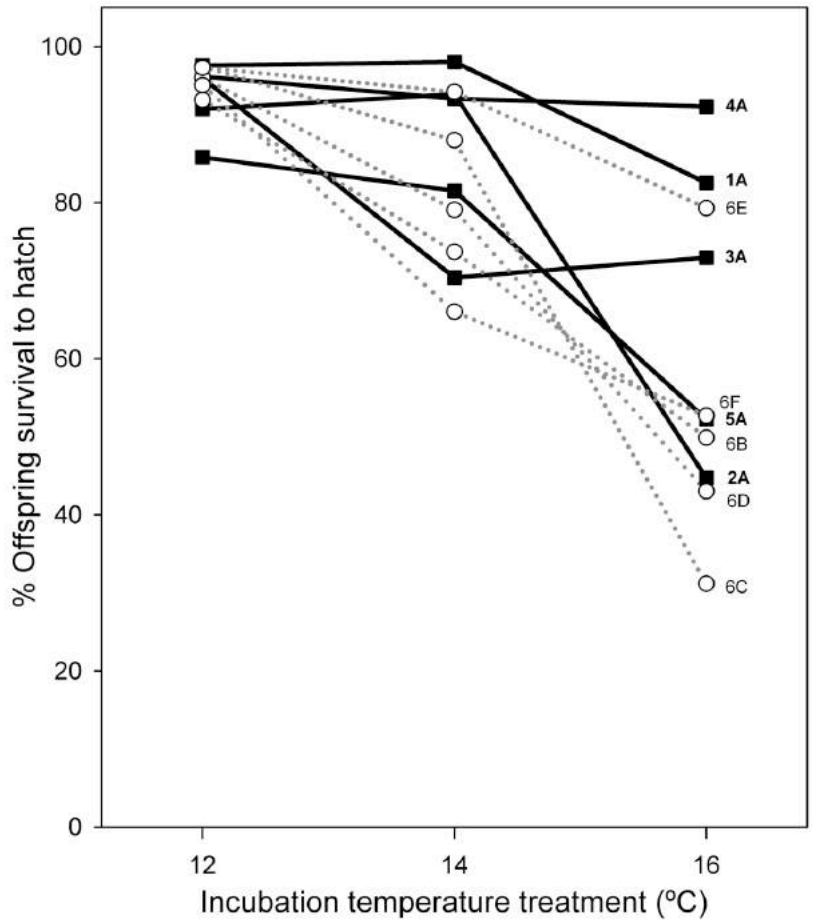

Figure 3.5. Reaction norm showing mean family survival to hatch at incubation temperatures 12º, 14º, and 16ºC. Black lines with square symbols show half-sib families of different female parentage (1A, 2A, 3A, 4A, 5A) and grey dotted lines with circles show half-sib families of different male parentage (6B, 6C, 6D, 6E, 6F).

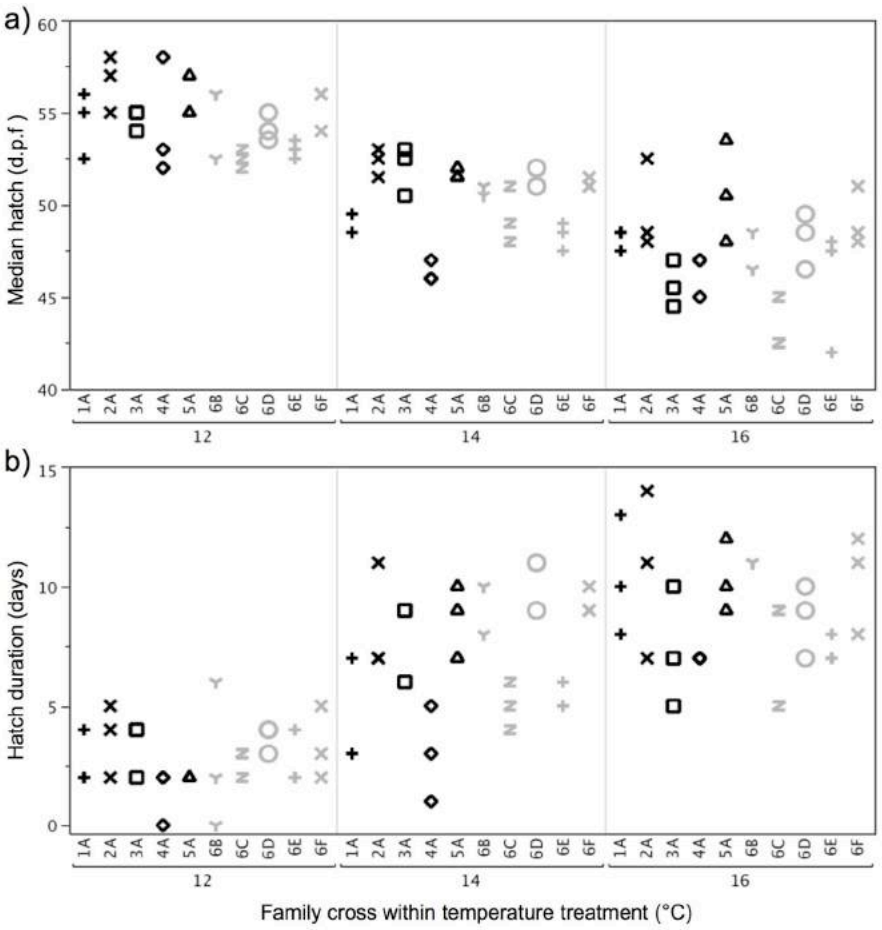

Figure 3.6. Hatch timing characteristics for family crosses within temperature treatments: a) median hatch (days post fertilization) and b) hatch duration. Black symbols show the influence of female spawner identity (females 1-5 crossed to male A) and grey symbols show the influence of male spawner identity (males B-F crossed to female 6). Note: a hatching duration of '0' indicates that all eggs hatched within 1day. Overlapping symbols appear as just one symbol.

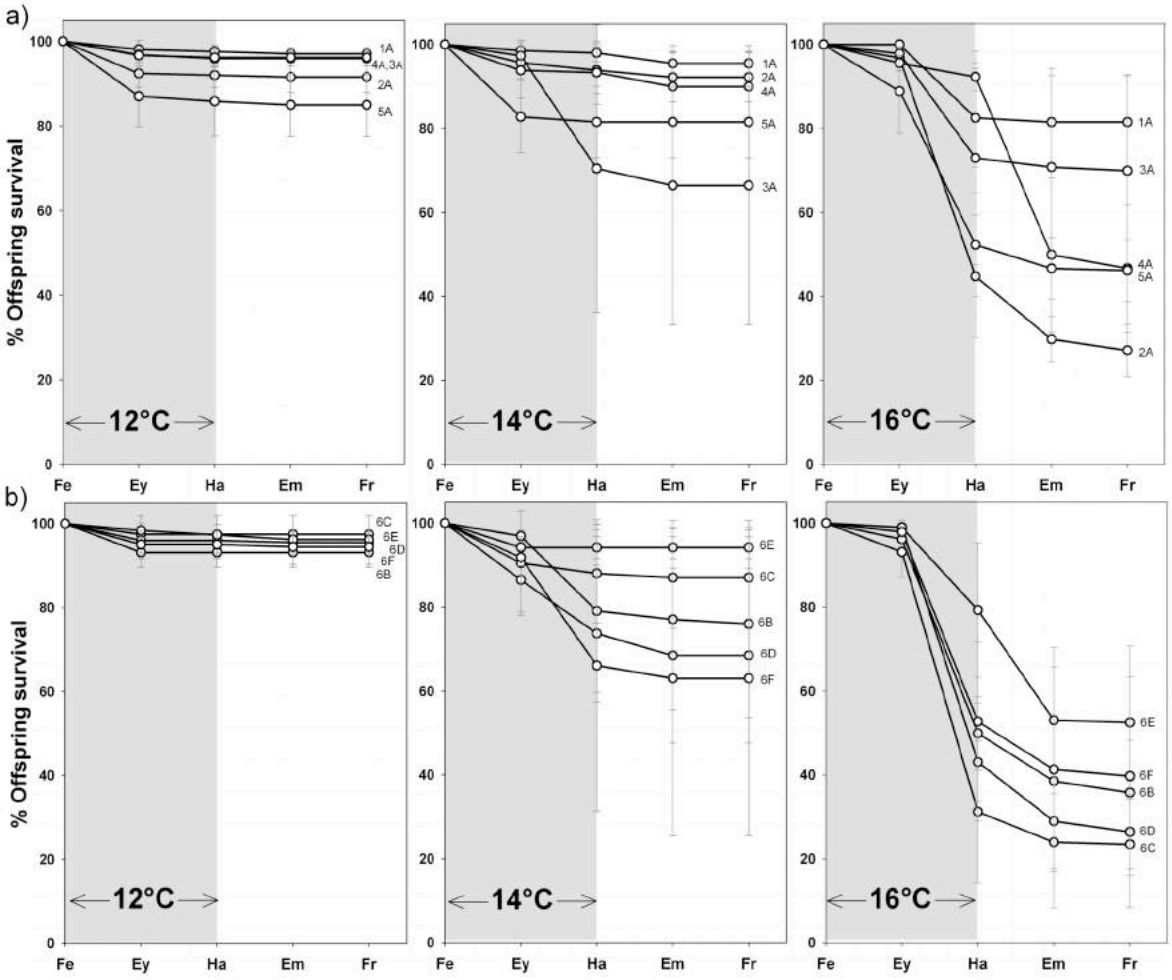

Figure 3.7. Mean survival (± S.D.) for offspring crosses within temperature treatments at different life history stages. a) shows the influence of female spawner identity (females 1-5 crossed to the single male spawner 'A'). b) shows the influence of male spawner identity (males B-F crossed to the single female spawner '6'). The x-axis depicts discrete development stages: Fertilization (Fe), Eyed embryo (Ey), Hatch (Ha), Emergence (Em), 3-week-old fry (Fr). The shaded area shows the duration that eggs were exposed to incubation temperature treatments, after which all offspring experienced identical low ambient temperatures.

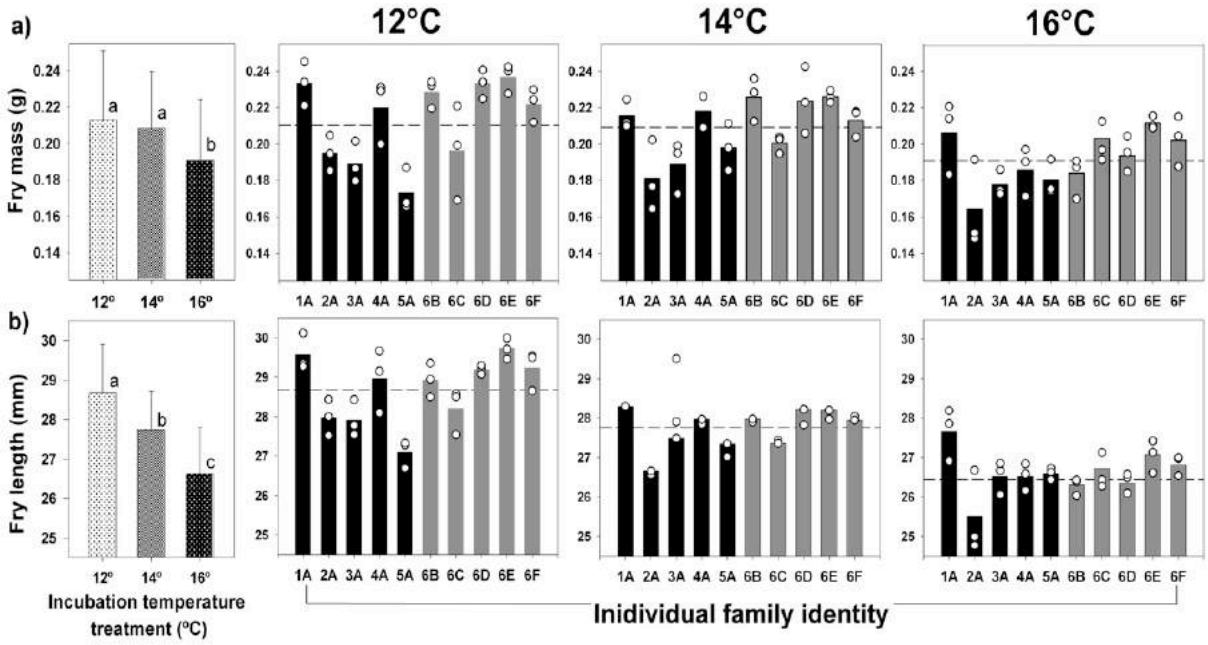

Figure 3.8. The effects of incubation temperature treatments and family identity on fry mass and length after three weeks of exogenous feeding. Graphs on the left show incubation temperature treatment means (n = 30) +S.D. and letters indicate significant differences ($P < 0.01$). Graphs on right show individual family responses. Bar heights represent the family mean and the white circles show the independent family replicate values. The dashed line shows the overall mean within a temperature treatment. Black bars show the influence of female spawner identity (females 1-5 crossed to male A) and the grey bars show the influence of male spawner identity (males B-F crossed to female 6).

CHAPTER 4: Developmental temperature stress and parental identity shape offspring burst swimming performance in sockeye salmon (*Oncorhynchus nerka*)[3]

Introduction

In Pacific salmon (*Oncorhynchus* spp.), the period directly following fry emergence is critical to survival and has an important influence on population dynamics (Elliott, 1989). During this transition from endogenous to exogenous feeding in sockeye salmon (*O. nerka*), performance-linked traits such as size and swimming ability are highly important as individual salmon migrate to natal lakes, forage for food, and interact with competitors and predators (Burgner, 1991). Fry-to-smolt survival rates are relatively low (Bradford, 1995) and initial selection during this period will depend on individuals' performance capacity and behavior (Eggers, 1978). Intrinsically, variation in size or performance at this post-larval emergence stage is derived from two sources: environmental and parental influences during development. Whereas many studies have examined the influence of these factors on embryonic and larval phenotypes, few have investigated the persistence of both the embryonic environment and parental influences on early juvenile performance-linked traits (Beacham, 1990; Heath et al., 1993; Green and McCormick, 2005).

Water temperature is a critical regulator of physiological processes in aquatic poikilotherms, and is an especially poignant factor affecting the developmental rates and survival of eggs (Blaxter, 1992). The thermal regime during embryogenesis can also influence the development and expression of important phenotypic traits (*e.g.,* size - Atkinson, 1996; meristics - Fowler, 1970; muscle cellularity - Johnston & McLay 1997). Concurrently, there is increasing evidence that thermally-induced phenotypic plasticity in

[3] A version of this chapter is in the process of submission for publication. Burt, J.M., S.G. Hinch, and D.A. Patterson. Developmental temperature stress and parental identity shape offspring burst swimming performance in sockeye salmon (*Oncorhynchus nerka*)

early ontogeny can affect subsequent performance at later life stages (Elphick and Shine, 1998; Pechenik et al., 1998; Watkins, 2000; Albokhadaim et al., 2007). In fish populations, an increase of 2ºC in the water temperature during development is enough to alter the muscle phenotype of post-hatch larvae (Johnston, 2006; Martell and Kieffer, 2007); an effect that can remain imprinted in later juvenile stages and result in decreased swim performance (Koumoundouros *et al.*, 2009). At developmental temperatures that approach upper tolerance limits, thermal stress can result in the disturbance of cellular/genetic pathways with potential consequences for offspring morphology, physiology and behavior (Takle et al., 2004; Wargelius et al., 2005; Turner et al., 2007). Whereas thermal stress during development is shown to have latent effects on locomotor performance in herpetofaunal studies (Brana and Ji, 2000), similar embryonic thermal stress experiments have not been conducted within fish populations.

Along with environmental effects, individual metabolic and performance capacities are determined by genetic and non-genetic parental influences. In many species of fish, embryonic and larval characteristics such as size, meristics, metabolism, muscle cellularity and growth are shown to vary among offspring families as a function of either female or male parental identity (Beacham, 1990; Chambers and Leggett, 1996; Heath et al., 1999; Trippel et al., 2005; Pakkasmaa et al., 2006; Morasse et al., 2008; Rossignol et al., 2010). Whereas the majority of these studies are limited to assessing phenotypes during egg and hatchling stages, there is increasing evidence to suggest that parental influences can show considerable temporal persistence. In experiments that investigated offspring beyond the onset of exogenous feeding, substantial parentally mediated variation has been detected in offspring size (Nadeau et al., 2009), muscle fibres and growth (Macqueen et al., 2008), stress response (Heath et al., 1993), and swim performance (Garenc et al., 1998; Tierney et al., 2009).

Experimental tests of swimming capacity have been used in many species of fish as a quantitative assessment of performance linked to fitness (Beamish, 1979). In studies specific to salmonid fry and juveniles, differences in swim performance have been assessed between species (Hawkins and Quinn, 1996; McDonald et al., 1998; Hale,

1999), and between populations (Taylor and McPhail, 1985a, b; Taylor and Foote, 1991; Pon et al., 2007), but rarely between offspring families or as a function of their developmental environment. Despite evidence that exposure to incubation thermal stress can affect survival, morphology and physiology in salmonid fry (Chapter 3 of this thesis; Finn, 2007; Turner et al., 2007), the potential for elevated developmental temperatures to have latent effects on fry swim performance has not been examined. Similarly, although several studies have shown considerable among-family variation in the enzymatic correlates of locomotor activity (Garenc et al., 1998; Patterson et al., 2004a; Rossignol et al., 2010), we found only one study that has investigated parental identity as a potential source of variation in salmonid swimming capacity (Nadeau et al., 2009).

In the context of climate change, predicted increases in mean temperatures and the frequency of extreme temperature events in salmonid freshwater habitat (Hague et al., 2011) may result in incubation environments surpassing thresholds for optimal development. As such, understanding the persistence of parental and temperature influences will be essential in gaining a more comprehensive view of how environmental change may influence early life history selection processes and survival. The objective of this experiment was to determine whether exposure to developmental high-temperature stress results in latent effects on fry swim performance, and whether swim performance differs among offspring families. To do this, we tested the burst swim endurance of individual, 3-week-old fry from 10 offspring families that had been exposed to three levels of temperature stress between fertilization and hatch. We predicted that (1) burst swim performance would be lower in the fish that experienced higher thermal stress during incubation, and (2) differences in fry burst swim endurance within temperature treatments would be attributable, at least in part, to offspring parental identity. While investigating these predictions, we sought to determine whether any variation in fry swimming performance (among temperature treatments, among families, and at the individual level) could be explained by differences in fry size attributes or selective processes resulting from exposure to high temperatures during development.

Methods

Fish collection and incubation design

The collection of gametes used for this study was conducted as described in Chapter 3. Briefly, sockeye salmon from the Weaver Creek population were captured in the Harrison River in September 2008 and transported to the Cultus Lake Laboratory (Department of Fisheries and Oceans Canada) where they were held until reaching sexual maturity. Eggs and milt were collected from six mature females and six mature males, then transferred on ice to the Alcan Aquatic Facilities (Simon Fraser University) where a dry fertilization procedure was used to create 10 offspring families. The eggs of five females (numbered 1-5) were crossed with the milt of a single male (male A) to create the first five families (labeled 1A, 2A, 3A, 4A, 5A). Next, the eggs of a single female (female 6) were crossed using the milt from different males (letters B-F) to create five more half-sib families (labeled 6B, 6C, 6D, 6E, 6F). Nine individual fertilizations were performed for each of the 10 gamete combinations, so that three replicates of each family cross could be incubated within three separate temperature treatments (total n = 90 family replicates).

Incubation took place in the Forest Sciences Aquatic Laboratory at the University of British Columbia. Nine flow-through Heath stacks were divided into three water temperature treatments: 12ºC - low thermal stress (within optimal range), 14ºC - intermediate thermal stress, 16ºC - high thermal stress (see Chapter 3 for details). Incubating in separate netted egg capsules, one replicate of each family cross was represented once in a single tray of every Heath stack (three replicate crosses in each temperature treatment). All egg capsules were initially incubated at 12ºC and incrementally elevated to treatment temperatures over three days. Once at treatment temperatures, recorded actual treatment means (± S.D.) were 11.8ºC ± 0.4, 13.8ºC ± 0.4, 15.7ºC ± 0.4. Egg capsules were checked regularly until hatch, and daily thereafter, and dead eggs were recorded and removed. Mortality between fertilization and hatch was significantly higher within families exposed to the 16ºC incubation treatment (39.9% ± 23.23 S.D.), moderate for families incubated at 14ºC (16.2% ± 18.7 S.D.), and low for

families incubated at 12ºC (5.4% ± 4.6 S.D.). Statistical details and analysis of survivorship between treatments and families is provided in Chapter 3.

Post-hatch incubation and rearing

After families in a temperature treatment reached 95% hatch, the water temperature was slowly lowered (1ºC per day) to the ambient temperature of the laboratory water supply (10ºC in early December and declining to 5ºC by February). All 90 family crosses therefore experienced identical ambient post-hatch temperatures up to, and during, fry swim trials. Alevins were checked every two days, and mortalities were recorded. Alevin mortality in fish from the 12 and 14ºC treatments were low (0.4% and 2.3%, respectively), however significant latent mortality occurred for alevins that had been exposed to 16ºC (13.7% ± 14.0 S.D, Chapter 3).

Families were determined to have reached "emergence" stage by visual inspection, when the yolk sacs of the fish were entirely covered by chromatophores ("buttoned up"). At this stage in the wild, fish would emerge from their incubation gravel, migrate to their nursery lake, and begin exogenous feeding. Upon observing that families from the three temperature treatments reached emergence stage in the same time period, all family replicates were transferred over 9 consecutive days from their Heath stacks into individual netted 10 L rearing enclosures (n = 90). Although we initially expected emergence timing to be staggered, we observed compensatory development of alevins from different thermal regimes, as observed in other experiments (Beacham and Murray, 1985). This is possibly explained by the fact that the 12ºC families required a lower number of accumulated thermal units (~1086 ATUs) to "button up" compared to the 16ºC families (~1230 ATUs) which is a known mechanism of stabilizing emergence timing within salmon populations in the wild (Brannon, 1987). For assurance, we obtained confirmation that the total number of ATUs attained by our 12ºC emergent fry was close to the value attained by fry upon emergence from the Weaver Creek spawning channel (~1000 ATUs; R. Stitt, Weaver Creek Spawning Channel, Department of Fisheries and Oceans, personal communication).

Transferred fry were placed in rearing enclosures at equal stocking densities (30 fish/10 L enclosure) and enclosures were randomly positioned within multiple 1,000 L flow-through troughs. Rearing densities were slightly lower (15-28 fry) for several 16ºC exposed families that experienced high mortality during incubation. Fry were fed powdered fishmeal (EWOS Canada Ltd.) in two daily installments of ~1% total body mass for a period of just over three weeks, at which point swim trials began following 24 h of fasting (see below). During rearing, lighting conditions were adjusted regularly to reflect the natural photoperiod (49º18′N). Mortality during this exogenous feeding stage was generally low (0% in 12ºC group, 0.1% in 14ºC group, and 1.5% in 16ºC group, Chapter 3).

Burst swimming protocol and measurements

A fixed-velocity test of burst swimming was performed on individual fish to assess endurance capacity as a component of overall fitness. Burst swimming is defined here as a maximal swimming effort that can be sustained for only a short period (usually ≤ 20s; Beamish, 1979), but for a longer duration than a startle response (usually < 1s) for which the term burst swimming has also been used (Taylor and McPhail, 1985b). Burst swim trials have successfully been used to evaluate intra- and inter-specific differences in anaerobic capacity in juvenile salmonids (McDonald et al., 1998; Pon et al., 2007; Nadeau et al., 2009) and are advantageous in comparison to other measures of prolonged swimming (*i.e.,* U_{crit}) in that they do not take long, and many individuals can be tested in a short period of time.

Burst swim trials were conducted in an open-top rectangular flume (230 cm length x 17 cm width, described in detail by Pon et al., 2007) within a sectioned off swimming channel measuring 30 cm long and 6.9 cm wide (water depth = 1.8 cm). To maintain laminar flow, fresh flowing water passed through two honeycomb flow straighteners before entering the swim channel which was gated with wire mesh at either end. The swim channel was illuminated with incandescent light from above, apart from a

dark shaded region at the head of the channel provided to encourage fry to remain actively swimming and avoid falling back. Encountered flow velocity was calculated from discharge/cross-sectional area. Flow velocity was maintained constant at 23.0 $cm \cdot s^{-1}$ (± 0.7 $cm \cdot s^{-1}$) throughout duration of swim tests, equating to an average fish swimming speed of 8.0 - 8.6 $BL \cdot s^{-1}$ (dependent on fish length). Preliminary trials established that fry could maintain position swimming at maximal effort against this current speed for approximately 30 s before fatiguing and falling back to the rear gate of the swim area. The water temperature for all trials was 5.8°C (± 0.2°C).

A maximum of ten fry from each independently reared family replicate (n = 90 family replicates) were subjected to a burst endurance swim trial between February12-18, 2009. Families were swum in corresponding order to the day they were transferred to rearing enclosures, thus ensuring all fish had experienced a similar number of feeding days (22 or 23) prior to the test. To avoid any effects of feeding or digestion on swim performance, families were not fed for 24 h prior to their swim trial ensuring complete food evacuation.

To begin each trial, one fry was randomly scooped from its rearing enclosure (fry scooped and transported in water to minimize exertion and stress) and introduced directly into the flowing current of the swim channel. Most fry would immediately initiate burst swimming, holding position under the shaded area at the head of the swim channel until fatigued. Fish that did not initially burst were stimulated using a blunt probe, which in most cases promoted immediate bursting. If fish were not able to initiate bursting or swim against the current for > 5 s, these fish were recorded as "non-swimmers" and sampled for length and weight. In the event of a "non-swimmer", another fish was randomly selected from the same rearing enclosure to perform the trial with the aim of obtaining 10 valid swim trials for each family replicate.

Burst swimming time was calculated as the interval between the initiation of burst swimming (T_0) and the point when fatigued fish fell back beyond the line marked by the shaded region (T_F). Upon fall back, fish were immediately stimulated with a blunt probe

to confirm complete fatigue. If fish re-initiated burst swimming, this swimming duration and successive bursting episodes were incorporated into the calculation of cumulative burst swim time (*BST*):

$$BST = \sum_{i=1}^{n} (T_F - T_0)$$

where n = the number of bursting episodes. Individual burst swim times were obtained by video analysis of swimming trails recorded by two wide-angle lens video cameras (Panasonic WV-BP312; 4.5 mm focal length) connected to a time lapse VCR (Panasonic AG-6124). Upon trial completion, fish were removed from the channel, sacrificed by overexposure to MS-222, blotted dry and measured for length (± 0.1 mm) and mass (± 0.001 g). Video data from one of the three 14ºC family replicates (family 4B) was corrupted and subsequently excluded.

Data and statistical analysis

Data from 858 individual burst swim trials were analyzed using SAS 9.1 (SAS Institute: www.sas.com). Since body size can influence salmonid swim performance (Taylor and McPhail, 1985a, b; McDonald et al., 1998), analyses were carried out to examine the influence of size on burst swim time (*BST*). We confirmed that both fry mass and length had small, but significant, influences on individual fry *BST* by calculating regressions using a logarithmic transformation (Fig. 4.1). Subsequently, we generated both mass-independent and length-independent burst swim times according to the methods described by Packard and Boardman (1988). *BST* values for individual fry were adjusted to the overall average fry mass or fry length and scaled using the coefficients obtained from the logarithmic regressions. No significant differences were detected in the slopes of the 12, 14, and 16ºC fry mass-by-*BST* logarithmic regressions (ANCOVA, $F_{2,852} = 2.28$, n = 858, $P > 0.05$), or fry length-by-*BST* logarithmic regressions (ANCOVA, $F_{2, 852} = 1.63$, n = 858, $P > 0.05$), so single scaling coefficients from the slope of the common regression line were used (Fig. 4.1). Mass-adjusted burst swim times (BST_M) and length-adjusted burst swim times (BST_L) were obtained by the following equations:

$$BST_M = \frac{BST}{(M / \overline{M})^{b1}} \quad \text{or} \quad BST_L = \frac{BST}{(L / \overline{L})^{b2}}$$

where *BST* is the unadjusted burst swim time of an individual fry, $\overline{M}$ is the grand mean for fry mass, $\overline{L}$ is the grand mean for fry length, *b1* is the slope of the mass versus *BST* regression (*b1* = 0.932), and *b2* is the slope of the length versus *BST* regression (*b2* = 2.77).

To examine the first hypothesis and test for a difference in swimming performance among temperature treatments, in addition to the interaction between temperature and parentage (family identity), a nested mixed model ANOVA was used:

$$y_{jkl} = \mu + T_j + P_k + F(P)_{l(k)} + T \times P_{jk} + T \times F(P)_{jl(k)} + \varepsilon_{jl(k)}$$

where y = burst swim time (*BST*, BST_M or BST_L), T_j = temperature for treatment j, P_k = parent spawner used to generate half-sibs (k = 'female' or 'male' parent spawner), F_l = family identity nested within male or female spawner (l = 1-5 within 'male' or 6-10 within 'female'), and ε is the random error term. Heath stack effects (family replicates) were included in the original model, but were found to be non-significant, and were subsequently removed from the current model. For all analyses, *T* and *P* were considered fixed effects while all other variables, including family identity, were considered to be random effects. Differences among temperature treatments were assessed using pairwise t-tests and a Bonferronic alpha correction (Whitlock and Schluter, 2009). In all analyses, burst swim times were square-root transformed to meet the assumptions of normality (Kolmogorlov-Smirnov test) and homoscedasticity required for parametric tests.

To examine our second hypothesis and test for differences in swimming performance among families, a simplified mixed model ANOVA model was used separately within the 12, 14, and 16ºC treatment groups:

$$y_{kl} = \mu + P_k + F(P)_{l(k)} + \varepsilon_{l(k)}$$

where y = burst swim time (*BST*, BST_M or BST_L), and the variables *P* and *F* are defined the same as in the model above. Again, the effect of Heath stack (family replicate) was not significant in the analysis of burst swim time within each temperature treatment and

was subsequently removed from the model. In order to compare female vs. male spawner influence on burst swim time (using BST_M), we sub-divided the data within temperature treatments into maternal half-sib crosses and paternal half-sib crosses. One-way ANOVAs were used to test the significance of the variation among families differing in maternal identity or paternal identity.

We conducted contingency analyses on several behavioral observations recorded for each swim trial (n = 858 fry). Pearson's chi-square tests were performed to compare the proportions of fry among temperature treatments with regard to whether they, 1) needed physical stimulation to initiate swimming (1 = yes, 0 = no), 2) were able to complete swim trials in one continuous burst (1 = yes, 0 = no), and 3) fatigued frequently during swim trial (proportion of fry demonstrating ≥ 3 or ≥ 5 fall-backs). The proportion of "non-swimmers" (unable to burst swim and hold against current for > 5 s) from each temperature treatment was also compared using a Pearson's chi-square test (total n = 922, "non-swimmer" fry added to the number of fry with valid swim times).

Finally, due to the high mortality experienced in the families exposed to the 16ºC treatment, we used a linear regression to examine whether mean family survivorship (% offspring survived) was predictive of mean fry swimming performance (BST_M).

Results

Developmental temperature effects

The range (2 S.D.) in length of fry that were swim tested was 24.9 – 30.5 mm, while the range in mass was 0.134 – 0.276 g. Significant differences were found among temperature treatments for mean fry length (12ºC > 14ºC > 16ºC) and mass (12ºC ≈ 14ºC > 16ºC, Fig. 4.2). Overall, fry mass and length explained a relatively small amount of the variation in individual fry burst swim time. Individual fry mass explained slightly more of the variation (15.4%) compared to fry length (10.6%)(Fig. 4.1).

Without adjustment for size, the burst swim time of 3-week-old fry was significantly affected by developmental temperature treatment (ANOVA, $F_{2,16} = 6.4$, n = 858, $P < 0.01$, Fig. 4.3a). Specifically, fry exposed to the 12ºC incubation were able to maintain burst swimming for a longer time (37.4 s ± 13.9 S.D.) than fry exposed to 14ºC (31.9 s ± 11.9 S.D.) and 16ºC (32.1 s ± 14.7 S.D.) pre-hatch temperatures (Fig. 4.3a). Among-treatment differences in burst swim time were partly attributable to temperature effects on fry size. When individual burst swim times were scaled to account for differences in fry mass, only the 12 and 14ºC treatment means remain significantly different (Fig. 4.3b). Analysis using length-adjusted burst swim times revealed no differences in swim performance between temperature groups (Fig. 4.3c).

Observations involving physical stimulation or fatiguing fry during swim trials showed that fish incubated at 16ºC performed more poorly (Table 4.1). A larger proportion of individuals from the 16ºC treatment needed stimulation to promote initial burst swimming ($X^2_2 = 83.2$, n = 858, $P < 0.0001$). Fish from the 16ºC group were less able to burst for a single continuous duration ($X^2_2 = 24.6$, n = 858, $P < 0.0001$), showing a higher occurrence of swim trials during which fry fell back ≥3 times ($X^2_2 = 25.3$, n = 858, $P < 0.0001$) or fell back ≥5 times ($X^2_2 = 29.3$, n = 858, $P < 0.0001$). Finally, a greater number of fish from the 16ºC treatment were classified as “non swimmers” ($X^2_2 = 78.5$, n = 922, $P < 0.0001$), unable to burst swim and hold against the current.

Family effects

Fry burst swim time was affected by a significant interaction between incubation temperature and family (ANOVA, $F_{16,828} = 2.95$, n = 858, $P < 0.0001$), indicating the influence of family identity on fry burst swimming time was not consistent between treatments (Fig. 4.4). This interaction remained significant when burst swim times were analyzed independent of fry mass and length (ANOVA, $F_{16,828} = 3.23$ and 3.30, respectively, n = 858, both $P < 0.0001$). Fry mass and length were also influenced by significant temperature-by-family interactions (ANOVA, $F_{16,828} = 3.59$ and 4.79, respectively, n = 858, both $P < 0.0001$).

Within incubation temperature treatments, mean burst swim times varied significantly among offspring families (ANOVAs, 12ºC: $F_{8,290} = 5.95$, n = 300, $P < 0.001$, 14ºC: $F_{8,280} = 3.10$, n = 290, $P < 0.01$, and 16ºC: $F_{8,258} = 2.50$, n = 268, $P < 0.05$). When individual burst swim times were adjusted for differences in fry mass, the variation among families remained significant (ANOVAs, 12ºC: $P < 0.0001$, 14ºC: $P < 0.01$, and 16ºC: $P < 0.0001$, Fig. 4.4). Results were similar when length-adjusted values were used. Overall, the analysis using mass-independent or length-independent burst swim values only resulted in some minor changes in the relative ranking of families compared to using the un-adjusted burst swim values (Table 4.2).

Maternal and paternal influences on fry burst swim performance varied between temperature treatments (Fig. 4.4). Within the 12ºC treatment, variation in mean family burst swim time was attributable to maternal identity (ANOVA, $F_{4,145} = 8.20$, n = 150, $P < 0.0001$) and paternal identity (ANOVA, $F_{4,145} = 7.32$, n = 150, $P < 0.0001$). Within the 14ºC treatment, mean burst swim time only varied among crosses differing in paternal identity (ANOVA, $F_{4,145} = 4.85$, n = 150, $P < 0.01$). Within the 16ºC, mean burst swim time only varied among crosses differing in maternal identity (ANOVA, $F_{4,139} = 9.11$, n = 144, $P < 0.0001$).

Within the 16ºC incubation treatment group, mean family swim performance was related to mean family survivorship ($R^2 = 0.65$, n = 10, $P = 0.005$, Fig. 4.5). Specifically, families that had a higher percent of offspring survive from fertilization to swim test day were characterized by a lower mean burst swim time, whereas families that had a low percent of offspring survive were capable of higher mean burst swim times.

Discussion

This study provides the first evidence in sockeye salmon that both developmental temperature and individual parentage act together to influence fry swim performance. Exposure to high temperature stress during embryogenesis resulted in reduced endurance

capacity and impaired swimming behavior in later fry stages. As the post-hatch rearing environment for all temperature treatments was controlled at a cool ambient level, we attributed these effects to temperature exposure during embryonic development. In addition to temperature, and independent of offspring size, both maternal and paternal identity was shown to influence offspring swim performance. However, a strong temperature-by-family interaction indicated that parentally mediated influences are temperature dependent.

Less than 10 studies have examined the effects of developmental temperature on later-stage swimming ability in fish, and these have been limited to marine cold-water species (Table 4.3). In general, these studies have found that differences in the swimming performance between fish from different incubation thermal regimes were primarily the result of temperature effects on muscle physiology and development. Koumoundouros et al. (2009) found that European sea bass (*Dicentrarchus labrax*) juveniles initially reared at 15ºC exhibited higher maximum aerobic swimming capacities than those initially reared at 20ºC. These authors attributed the increased swimming capacity of the cold-water reared fish to having a greater relative red muscle area and increased number of red myofibres and mitochondria than the warmer reared fish. In contrast, an experiment with Atlantic herring (*Clupea harengus*) demonstrated that fish incubated in a colder temperature regime had reduced maximum velocities during fast-starts than warmer-incubated fish due to their reduced development of swimming musculature, neural activity and fins (Johnston et al., 2001). In another study on larval herring, Batty et al. (1993) showed rearing temperature had no observable physiological effect on swimming performance, except through its influence on larval size.

Variation in juvenile salmonid swim performance has been shown to relate to body length and mass (Bams, 1967; Taylor and McPhail, 1985a, b; McDonald et al., 1998). However, in studies using sockeye salmon fry, there is evidence of both a strong relationship (Bams 1967) and no existing relationship (Pon et al., 2007; Nadeau et al., 2009) between body size and individual swim performance. In the present study, differences in the average fry burst swim time between thermal treatment groups were

partly explained by the effects of incubation temperature on fry size (Chapter 3 of this thesis, Beacham and Murray, 1990, Kamler 2008). Consistent with findings from Bams (1967), significant differences in average fry length (12ºC > 14ºC > 16ºC) explained treatment-level differences in average swimming endurance. Nevertheless, at the individual level, fry length did not explain a large amount of variation in burst swim time (10%), and fry mass was only slightly better (15%). When mass-adjusted burst swim times were analyzed, the 12ºC treated fish still swam better on average than the 14ºC group, suggesting that other inherent treatment-level effects on swim performance may be present. Overall, these results suggest that temperature plays an important role in determining average fry size and subsequent average swimming performance, however the considerable individual variation in fry swim performance is only marginally related to individual differences in size.

Perhaps more significant than differences in total burst swim time, is the observation that 16ºC treated fish were less willing to initiate swimming and fatigued more frequently during their bursting episodes. These findings correspond with those from a study on hatchling lizards (*Podarcis muralis*) in which individuals exposed to a stressful incubation temperature showed a more disjointed running pattern (high frequency of stops) during locomotor trials (Brana and Ji, 2000). The observed differences in 16ºC exposed fish would not appear to be advantageous considering that swimming stamina and initial burst responses have been emphasized as critical swimming characteristics for predator avoidance in emergent sockeye salmon (Bams, 1967). Similarly, Tierney et al. (2009) suggested that a reduced stimulus response and higher rate of fatiguing in sockeye parr were associated with poor schooling behavior and could lead to decreased survivorship. All together, the reduced mass, length, burst swim time, and swimming motivation of individuals from the high temperature exposure suggest that ecologically relevant shifts in fry fitness may result from deviations in incubation temperature above optimum.

Given that eggs in this study were incubated at temperatures approaching the upper limit of viability for embryonic development (McCullough et al., 2001), it is

possible that temperature-related influences on fry swim performance were affected by selective mortality. Overall, between fertilization and the swim test date, fish exposed to the 16ºC treatment experienced just over 50% mortality (largely during embryogenesis) whereas fish incubated at 14 and 12ºC lost only 19% and 6%, respectively. With the possibility that surviving fish incubated at 14 and 16ºC were physiologically different to the ones that perished, conducting swim trails on these 'survivors' may have reduced our capacity to find even greater differences in swim performance between temperature treatments.

Along with incubation temperature, offspring parentage had a strong effect on fry swimming ability. Within all three temperature treatments, the family with the highest mean burst swim time was able to swim almost twice as long (1.7 times) as the family with the lowest mean swim time. Family level differences in salmonid swim performance have only been detected in two previous studies, neither of which examined a measure of sprint/burst swimming capacity. Rossignol et al. (2010) found that maternal and paternal identity influenced spontaneous activity (transiting from one location to another) in Atlantic salmon (*Salmo salar*), whereas Tierney et al. (2009) demonstrated sockeye parr from moribund females had reduced schooling behavior, startle responses and fatigued more easily than offspring from spawn-ready females. Parental influences on swim performance have been detected in a limited number of studies on non-salmoid fish (Garenc et al., 1998; Green and McCormick, 2005), but overall the capacity for parentage to shape offspring performance traits is not commonly assessed (Chapter 2 of this thesis; Burt et al., In press).

Our finding that offspring families vary in their burst swim performance corresponds well with previous work on emergent and juvenile sockeye salmon. Examining the same population of Weaver Creek sockeye used for this study, Patterson et al. (2004a) found significant among-family differences in the mass-specific and protein-specific levels of several enzymes, including lactate dehydrogenase (LDH), in unfed emergent fry. LDH is a critical component in the glycolytic pathways involved in sprint or burst swimming and is a frequently examined biochemical correlate of burst

swim performance (Kolok, 1992; Garenc et al., 1998; Gibb and Dickson, 2002). Interestingly, Nadeau et al. (2009) were unable to detect maternal influences on the burst swim endurance of 4-month-old sockeye fry from the Weaver Creek population. Together with our findings, this supports the notion that parentally mediated variation in swimming capacity is considerable following emergence but fades with further feeding and growth (Garenc et al., 1998).

Our results demonstrate that incubation temperature interacts with family identity to influence fry swim performance. The dependence of parentally mediated variation on incubation temperature has been observed for a number of other life history traits (*e.g.,* size, development rate, metabolic fingerprints, sex ratios, stress response) that are characterized by significant temperature-by-family interactions (Beacham, 1988; Heath et al., 1993; Benoit and Pepin, 1999; Turner et al., 2007). Of particular interest is the variation observed at both 12ºC and 16ºC. Incubation at a constant 12ºC is arguably warmer than what eggs would experience in the wild, however, this treatment is within the optimal range for development and was characterized in this experiment by very high survival. Our finding that both female and male parental identity contributed to similar levels of variation in burst swim time within this treatment, independent of size effects, suggests that genetic factors may be important determinants of offspring performance under regular incubation conditions in the wild.

Interestingly, within the 16ºC treatment there was a negative relationship between the thermotolerance of families (based on survivorship) and their average burst swimming capacity. One possible explanation for this relationship may be that selection occurring within families during incubation favored individuals that were physiologically superior (better burst swimmers), which resulted in higher average swim times for the families that experienced higher mortality. This has been shown in studies on birds and insects where directional selection imposed by stressful conditions resulted in a shift in the mean value of certain morphological traits (Brown and Brown, 1998; Hoffmann and Hercus, 2000). An alternative explanation is that the negative relationship between family survivorship and burst swim time could reflect a trade-off between physiological or

genotypic traits that confer stress resistance and those that affect performance (Hoffmann and Parsons, 1991). Selection experiments in plants and invertebrates have shown that genotypes selected for stress resistance exhibit trade-offs with other fitness-related traits (*e.g.,* growth rate, metabolic rate - Hoffman and Parsons, 1989, 1991). In this study, a potential trade-off between surviving thermal stress and endurance capacity remains unclear, and will require further research on the physiological mechanisms associated with thermotolerance in developing fish.

Collectively, our findings demonstrate for the first time, that both developmental temperature stress and parental identity can shape the burst swimming performance of sockeye salmon offspring. Burst swimming performance has shown to be related to predator evasion in salmonids (Bams 1967, Taylor and McPhail 1985b) and therefore may be an important determinant of survival and fitness in sockeye salmon fry during the lake-rearing stages of their life history. This study provided evidence that exposure to high temperatures in early salmon development can result in persistent, parentally mediated effects on performance. As such, scientists and managers should have an increased awareness of the possibility that thermal stress events may have population effects in life stages beyond when the stress is experienced. The exposure of developing eggs to temperature stress will become an increasingly important concern in the context of climate change and human developments that impact salmonid incubation thermal regimes. However, it is important to consider that this research focuses on only one performance component among a myriad of early life history factors (*e.g.,* population density; Einum et al., 2008) that likely influence salmonid population dynamics. In order to build upon our findings, future studies may benefit from taking a quantitative genetics approach. This would enable researchers to partition swim performance variation into environmental, genetic, and non-genetic effects, as well as demonstrate whether the expression of genetic variation shifts under favorable versus unfavorable incubation conditions.

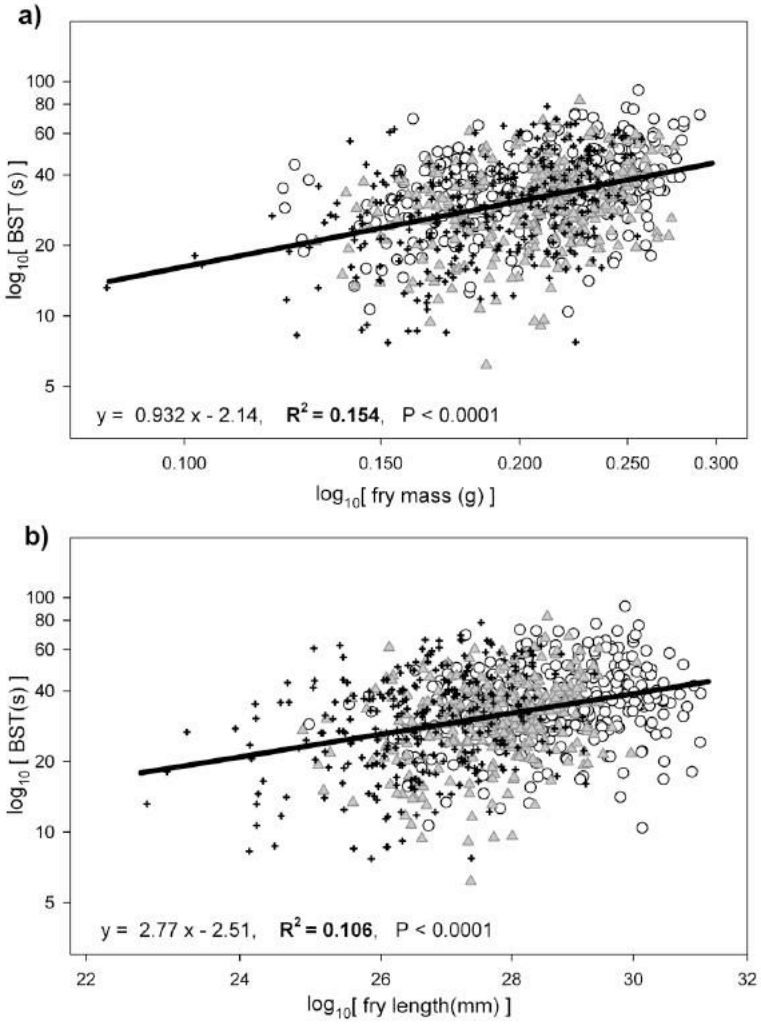

Figure 4.1. Linear regressions using logarithmic transformations of individual fry burst swim times (*BST*) as a function of a) fry mass, and b) fry length. Data points from each temperature treatment are indicated by the different symbols: ○ = 12ºC, ▲ = 14ºC, ✚ = 16ºC. The common regression line for all individuals (n = 868 fry) is shown along with the corresponding equation, R^2, and P-value. Axes show logarithmic scaling.

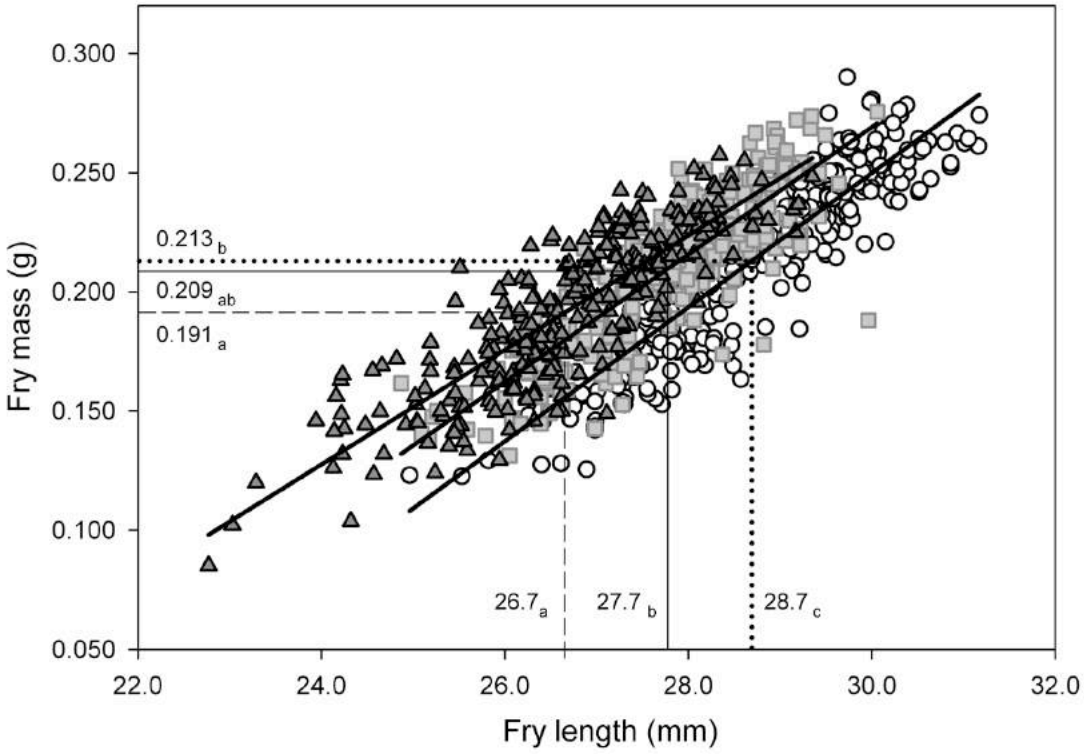

Figure 4.2. The relationship between length and mass of fry for the three incubation temperature treatments applied between fertilization and hatch (○ = 12ºC, ■ = 14ºC, ▲= 16ºC). Temperature-specific regression lines are shown and the average values for fry length and mass within each temperature treatment are listed next to the dotted (12ºC), solid (14ºC) and dashed (16ºC) lines. Letters indicate significant differences in average mass or length between treatments ($P < 0.01$).

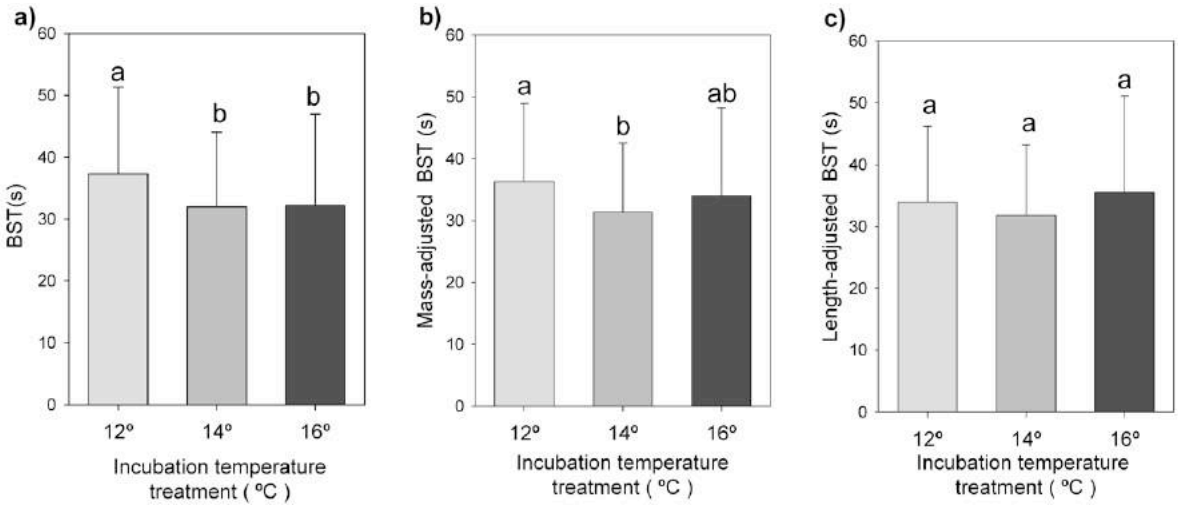

Figure 4.3. The effects of incubation temperature treatment experienced between fertilization and hatch on the mean burst swim time (*BST*) of 3-week-old fry: a) *BST* is not size adjusted, b) mass-adjusted *BST*, c) length-adjusted *BST*. The white bars in panel a) indicate the mean treatment values if "non swimmers" are included. Error bars show ± S.D. ($n \approx 300$ fry per treatment) and letters indicate significant differences between the three treatments ($P < 0.01$).

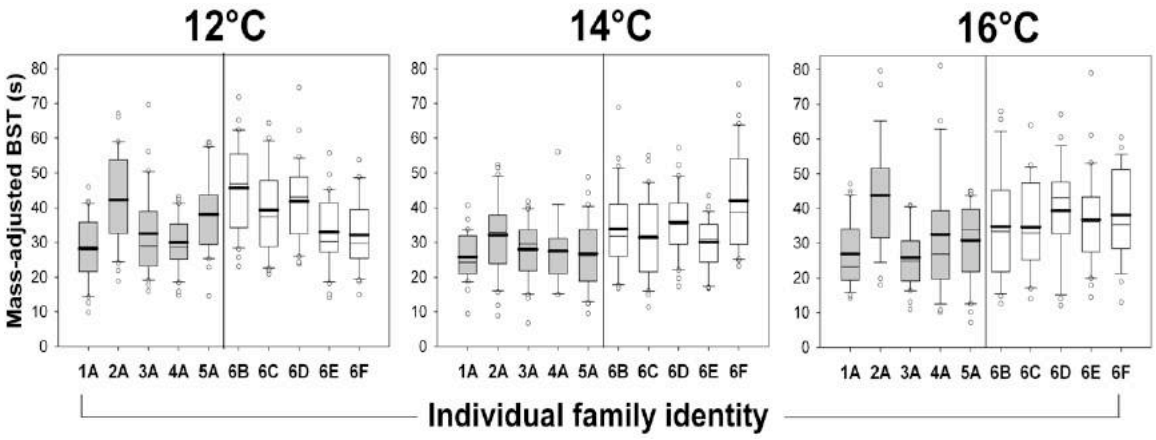

Figure 4.4. The effects of family identity on the mass-adjusted burst swim times (*BST*) of 3-week-old fry within pre-hatch incubation temperature treatments. Each box plot represents the distribution of individual fry swim times within a family cross ($n \approx 30$). Grey boxes show the influence of female spawner identity (females 1-5 crossed to male A) and the white boxes show the influence of male spawner identity (males B-F crossed to female 6). The box displays the interquartile range, the median and mean (bold), and the 10^{th} and 90^{th} percentiles (error bars). The white circles represent individual fish swim times that fall outside of the 10^{th} and 90^{th} percentiles.

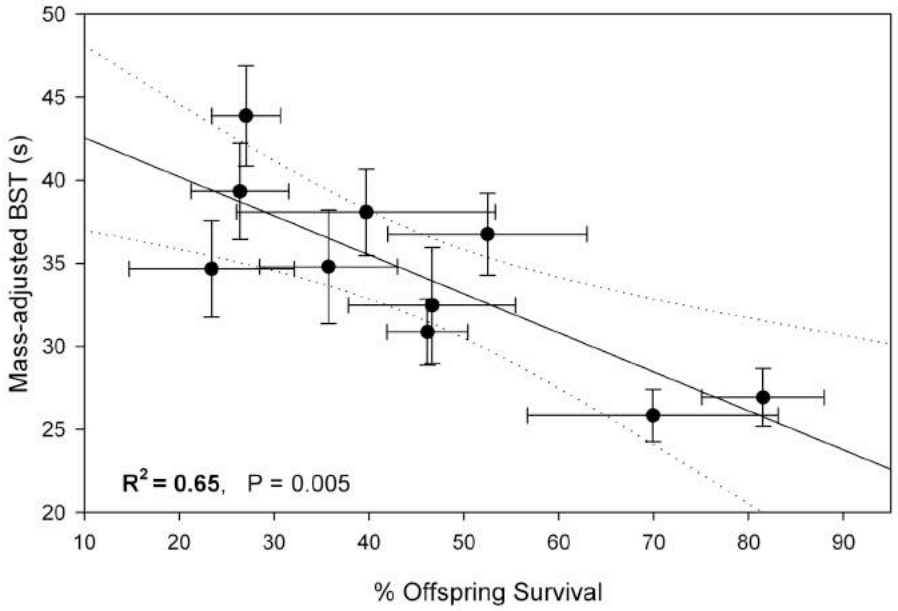

Figure 4.5. Linear regression showing only families exposed to the 16ºC incubation temperature treatment. Mean fry burst swim time (mass-adjusted *BST* ± S.E.) is shown as a function of mean family survivorship (± S.E.) Survivorship was measured as the % of offspring surviving from fertilization to 3-week-old fry. The 95% confidence intervals (dotted lines), R^2, and p-value are also given.

Table 4.1. Proportion of within-temperature treatment fry which (1) needed physical stimulation to initiate swimming, (2) were able to complete swim trials in one continuous burst, or (3) fatigued frequently during swim trial (≥ 3 or ≥ 5 fall-backs). The number of fry from each treatment that were classified as "non-swimmers" (not included in total *n*) are also indicated (4).

Burst swim characteristic	**12ºC** (*n* = 300)	**14ºC** (*n* = 290)	**16ºC** (*n* = 270)
(1) Stimulation	0.20	0.23	0.47
(2) One continuous burst	0.40	0.42	0.24
(3) Fatigue during swim trial			
≥ 3 fall backs	0.27	0.26	0.44
≥ 5 fall backs	0.030	0.0070	0.10
(4) Non-swimmers	3	6	55

Table 4.2. Temperature-specific family means for the mass, length, and burst swim time (*BST*) of 3-week-old fry. The left columns show the relative ranking of families for *BST*, mass-adjusted BST_M, and length-adjusted BST_L.

T (ºC)	Family cross	Mass (g)	Length (mm)	BST (s)	Rank (*BST*)	Rank (BST_M)	Rank (BST_L)
	1A	0.233	29.6	31.6 ± 10.5	9	10	10
12	2A	0.195	28.0	40.9 ± 15.5	3	2	3
	3A	0.189	27.9	29.5 ± 10.1	10	7	8
	4A	0.220	29.0	31.8 ± 8.5	8	9	9
	5A	0.173	27.1	32.2 ± 8.9	7	5	5
	6B	0.229	28.9	50.3 ± 15.1	1	1	1
	6C	0.196	28.2	37.9 ± 15.5	4	4	4
	6D	0.233	29.2	47.3 ± 15.4	2	3	2
	6E	0.237	29.7	37.6 ± 11.2	5	6	6
	6F	0.222	29.2	34.3 ± 10.1	6	8	7
	1A	0.215	28.3	27.1 ± 7.3	8	10	10
14	2A	0.181	26.7	28.4 ± 10.1	7	4	5
	3A	0.189	27.5	26.1 ± 9.4	9	7	9
	4A	0.218	28.0	28.8 ± 9.2	6	8	7
	5A	0.198	27.3	25.9 ± 9.4	10	9	8
	6B	0.226	28.0	36.2 ± 11.3	3	3	3
	6C	0.200	27.4	30.7 ± 12.2	5	5	4
	6D	0.224	28.2	38.7 ± 10.8	2	2	2
	6E	0.226	28.2	32.8 ± 8.3	4	6	6
	6F	0.213	27.9	43.6 ± 15.7	1	1	1
	1A	0.206	27.7	27.1 ± 10.6	9	9	9
16	2A	0.164	25.5	36.2 ± 15.7	4	1	1
	3A	0.178	26.5	22.5 ± 7.8	10	10	10
	4A	0.187	26.6	30.7 ± 18.5	7	7	7
	5A	0.180	26.6	27.3 ± 10.4	8	8	8
	6B	0.184	26.3	31.6 ± 16.4	6	5	6
	6C	0.203	26.7	35.1 ± 15.9	5	6	5
	6D	0.194	26.4	37.9 ± 16.3	2	2	2
	6E	0.212	27.1	37.3 ± 12.4	3	4	4
	6F	0.204	26.9	38.2 ± 14.1	1	3	3

Table 4.3. Review of fish studies that have examined the effects of developmental temperature on swimming performance

Species	Common name	Significant effect of developmental temperature	Authors
Dicentrarchus labrax	European sea bass	• juvenile critical swimming speed (Ucrit)	Koumoundourous et al. (2009)
Gadus morhua, Myoxocephalus scorpius	Atlantic cod, shorthorn sculpin	• larval critical swimming speed (Ucrit)	Guan et al. (2008)
Ampiprion melanopus	Tropical clownfish	• larval critical swimming speed (Ucrit)	Green and McCormick (2005)
		• larval critical swimming speed (Ucrit)	Green and Fisher (2004)
Clupea harengus	Atlantic herring	• maximum velocity (Ucrit) during fast-starts	Johnston et al. (2001)
Hippoglossoides platessoides	American plaice	• larval maximum swimming speed, swimming distance and escape speed	Shepherd et al. (2000)
Clupea harengus	Herring	• (no effect) larval burst-swimming speed during C-start escape	Batty et al. (1993)

CHAPTER 5: Synthesis and conclusions

The purpose of this thesis was to determine how individual parentage and elevated water temperatures influence within-population offspring survival and fitness traits during early development in salmon. The approaches taken in this research were a quantitative literature review and two laboratory experiments examining the existence and persistence of parentally mediated temperature effects in Weaver Creek sockeye salmon.

In the first objective, to summarize the evidence from the current experimental literature (Chapter 2), I found that parental influences play an important role in assessing temperature effects on fish early life history. In particular, experiments that examined the post-spawning incubation environment provided a useful framework for the experimental work in this thesis. One significant finding was that only 20% of the studies (defined by the literature search parameters, n = 222) relating to incubation temperature effects in offspring, assessed parental influences. Hence, many of the parentally mediated thermal effects I observed in Weaver Creek sockeye can be considered important contributions to the growing literature on parental influences in fish. Building on a large compendium of incubation studies on Pacific salmon by the authors Beacham and Murray (see publications between 1985 - 1991), Chapter 3 provides the first evidence in sockeye salmon of survivorship reaction norms over a gradient of supraoptimal temperatures, differences in post-stress mortality among families exposed to a 16ºC developmental temperature treatment, as well as temperature and parental influences on hatching duration. The swimming performance experiment in Chapter 4 demonstrated that fry burst swimming ability was significantly influenced by offspring parentage and an interaction between family and temperature, two findings previously undocumented in salmon. These results correspond well with the gaps identified by the literature review; given that few incubation-temperature-parental-influence studies were found to assess performance traits (2 % of studies) or offspring traits beyond endogenous feeding stages (21 %).

The literature review also included studies investigating how controlled temperature exposures during adult reproduction can affect offspring production and fitness. Collectively, these experiments indicated that high temperatures during the reproductive development stages in fish can significantly affect successful spawning and gamete quality. However, this body of literature is predominantly informed by aquacultural studies focused on reproductive output, revealing a lack of information on the effects in wild fish and post-hatch intergenerational responses. My Chapters 3 and 4 focused on incubation temperature effects in sockeye salmon, however, understanding the early life history of this species will also require information about how offspring are impacted by the temperature regimes experienced by their parents. For example, in the past 15 years, Weaver Creek sockeye and other late run stocks have been migrating back through the Fraser River during periods of abnormally high temperatures (Cooke et al., 2004). Scientists have hypothesized that this is linked to considerable en route and pre-spawn mortality (Hruska, 2010; Mathes et al., 2010), but we have no knowledge of possible sub-lethal intergenerational effects on subsequent offspring generations. Based on the findings from my Chapter 2 review (Burt et al., In press) and in light of conservation concerns for Fraser River sockeye, this issue of 'intergenerational effects' has been officially noted in the federal Cohen Commission as a "priority research area" (Hinch and Martins, 2011, p. 109).

Consistent with previous studies on sockeye salmon, my results showed that extended periods of water temperature above 12ºC during intra-gravel egg development are thermally stressful. Whereas a population effect (decreased overall survival) was observed at both 14 and 16ºC, major distinctions in the survivorship among families only occurred in the 'extreme' 16ºC treatment. At this temperature, I did not find that offspring thermotolerance was related to egg size (although a positive trend was apparent, n = 5) or the physiological status of parents. It is possible that survivorship under thermal stress is related to an offspring phenotypic trait that was not measured (*e.g.,* metabolic rate - Hoffman and Parsons, 1991), but perhaps more likely that family-level differences are related to the differential ability of genotypes to buffer against cellular perturbations

associated with thermal stress (Dworkin, 2005). Interestingly, I also found that families selected for higher thermotolerance had lower swim performance (Chapter 4), meaning there could be potential trade-offs between stress resistance and performance. Further research to confirm these speculations will have to take a more "mechanistic approach" to understanding the function of genes, proteins and biochemical networks linked to thermotolerance (Dalziel et al., 2009). Potential cellular, molecular and genetic mechanisms linked to thermosensitivity in fish are being explored (Portner et al., 2006), but this area of research is in its infancy (Dalziel et al., 2009).

The capacity for individuals and populations to respond to environmental fluctuation is paramount to species persistence. Therefore, my research has broader implications for the adaptation of sockeye populations to climate change. If the optimal expression of a given trait were rigid, requiring specific thermal conditions, individual fitness would decline with shifts in environmental temperature. However, in my experiments, almost all of the traits that were examined were characterized by significant family-by-temperature interactions (crossing reaction norms) (Fig. 5.1). This implies that there are genetic differences in how phenotypes respond to thermal change, and this phenotypic plasticity may be what allows successful early development to occur, and genetic variability to be maintained, in spite of unpredictable inter- and intra-annual temperatures on spawning sites. In a recent review article focused on salmonids, Hutchings (2011) emphasizes the importance of reaction norms in "refining our capacity to predict how populations respond to natural and anthropogenic environmental variability." Under current climate change predictions for further warming of salmonid freshwater environments (Hague et al., 2010), the observed plasticity in phenotypic responses to temperature may help ensure population persistence or even facilitate adaptive evolutionary change.

An important caveat to the above assumption is that the rate of future climate-induced warming is currently unknown, yet this will drastically affect the capacity for populations to adaptively respond. Another important limitation, specific to my experiment, was that the design did not permit me to quantitatively partition the observed

variation into its environmental, genetic, and non-genetic (maternal) components. Quantitative genetic studies in salmonids conducted under different incubation thermal environments are relatively few (Beacham, 1988; Heath et al., 1993, 1994; Hebert et al., 1998, Jensen et al., 2008; Janhunen et al., 2010), and no such studies exist in sockeye salmon. In juvenile Chinook, within-population heritability for heat tolerance has been demonstrated (Beacham and Withler, 1991), however further studies are needed to understand how additive genetic variation within salmon populations may change under favorable and unfavorable thermal conditions in order to better predict evolutionary potential (Charmantier and Garant, 2005).

Based on my results, it is interesting to speculate that if developing eggs in the wild are exposed to a prolonged period of 16ºC, non-random embryonic survival based on parental gamete origin could result in alterations to the intergenerational gene flow and genetic architecture within populations. This may be of particular concern for small populations with fewer breeding numbers in which stress-induced selection may result in a loss of genetic variation and corresponding decline in adaptive capacity (Hoffman and Miriam, 2000). Stress-induced losses may act in concert with other process such as genetic drift and inbreeding depression, further threatening the persistence of small populations on the edge of extinction (Liao and Reed, 2009). In the Fraser River, several small sockeye populations are considered threatened (*e.g.,* Cultus lake, Gates Creek, and Early Stuart stocks - Rand, 2008) and events of extreme developmental temperature stress could pose a particular conservation concern.

Of particular importance is my experiment showing that the effects of thermal stress experienced during the early development of sockeye salmon could persist into subsequent development stages. Despite a reduction from the elevated temperature treatments after hatch completion, latent mortality, reduced size and poorer swimming performance were observed in alevin and fry initially exposed to 16ºC. This should be especially relevant to scientists and managers because it infers that population effects resulting from altered phenotypes could occur in later life history stages beyond an initial exposure to ‘extreme temperature’. Nevertheless, it remains to be established what length

of exposure is necessary to induce such latent effects (the exposure in my study was considerable), or whether critical windows of sensitivity to thermal stress exist during embryogenesis (Beacham and Murray, 1987). Some salmonid studies found that exposure duration as little as 24 hours at 12ºC (up from 6ºC) was sufficient to induce permanent spinal deformities (Wargelius et al., 2005). Other studies that exposed eggs to variable temperature treatments found no significant phenotypic effects (Bjornevik et al., 2003, Geist et al., 2005). Given that salmon eggs in the wild incubate under a declining and variable thermal regime, experiments aiming to maximize ecological relevance should attempt to mimic these thermal profiles under treatment regimes with different baselines (Geist et al., 2005).

It is evident that there are several limitations to my experiments that should be highlighted. This study was conducted only on a single population of Fraser River sockeye from Weaver Creek, and it is likely that responses may differ across geographically isolated stocks (Beacham and Murray, 1989). This study also focused exclusively on temperature, but there are many other important environmental factors that interact to influence development and trait expression (*e.g.,* oxygen, water flow, environmental pollutants, density). Finally, the early life stages cannot be considered in isolation of the salmon life cycle. Climate change is likely to produce conflicting selection pressures in different salmon life stages (Crozier et al., 2008), and a considerable challenge for managers, ecologists and theorists will be to predict the overall patterns of salmon productivity and evolutionary change given a mosaic of interacting factors.

In conclusion, the findings in this thesis demonstrate that within a population of sockeye salmon, temperature and parental influences interact in a complex manner to shape survival and fitness traits. Specifically, offspring traits that include survival, hatch timing, size, and swimming performance are sensitive to embryonic temperature stress, but responses vary considerably depending on offspring parentage (linked to both maternal and paternal spawner identity). My observation of considerable family-level variation in temperature responses reflects positively on the ability of sockeye

populations to adapt to elevated temperature regimes or periods of thermal stress, however this will surely vary across populations and is dependent on the rates and magnitude of water temperature change. Overall, I recommend that future studies expand on this exploration of parent-progeny-temperature relationships, with particular attention paid to intergenerational temperature effects, the cellular/genetic mechanisms of thermotolerance, and the differences among sockeye populations in thermal reaction norms. Although much remains to be learned about the environmental, genetic and non-genetic influences in salmon early development, an evolutionary approach to salmon conservation is needed, aimed at maintaining genetic diversity and the conditions for natural selection to operate.

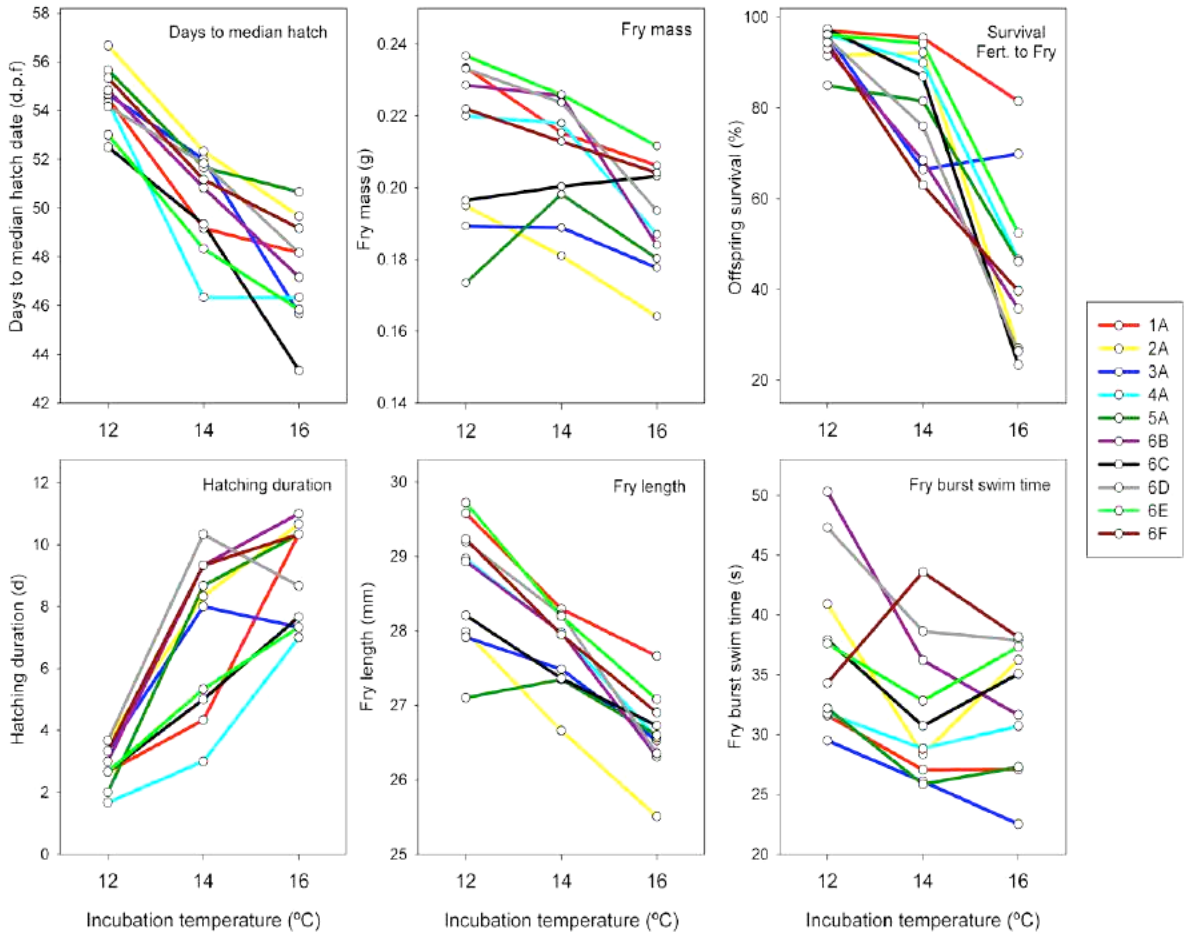

Figure 5.1. Reaction norms for sockeye salmon early life history traits across a gradient of incubation temperature treatments applied from fertilization to hatch (post-hatch temperatures were reduced and identical). Each point on a single line represents the average response (n = 3 family replicates) for a family cross. The legend (right) shows family crosses; females 1-5 crossed to male A, males B-F crossed to female 6. In the top left panel, d.p.f = days post fertilization. In the top right panel, % offspring survivorship is from fertilization to 3-week-old fry.

REFERENCES:

Abucay, J. S., G. C. Mair, D. O. F. Skibinski, and J. A. Beardmore. 1999. Environmental sex determination: the effect of temperature and salinity on sex ratio in *Oreochromis niloticus* L. Aquaculture 173(1-4):219-234.

Aegerter, S., and B. Jalabert. 2004. Effects of post-ovulatory oocyte ageing and temperature on egg quality and on the occurrence of triploid fry in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 231(1-4):59-71.

Alavi, S. M. H., and J. Cosson. 2005. Sperm motility in fishes. I. Effects of temperature and pH: a review. Cell Biology International 29(2):101-110.

Albokhadaim, I., C. L. Hammond, C. Ashton, B. H. Simbi, S. Bayol, S. Farrington, and N. Stickland. 2007. Larval programming of post-hatch muscle growth and activity in Atlantic salmon (*Salmo salar*). Journal of Experimental Biology 210(10):1735-1741.

Angelopoulou, A., P. Kaspiris, P. Divanach, and G. Koumoundouros. 2007. Temperature effects on cranial deformities in European sea bass, *Dicentrarchus labrax* (L.). Journal of Applied Ichthyology 23(1):99-103.

Angilletta, M. J., E. A. Steel, K. K. Bartz, J. G. Kingsolver, M. D. Scheuerell, B. R. Beckman, and L. G. Crozier. 2008. Big dams and salmon evolution: changes in thermal regimes and their potential evolutionary consequences. Evolutionary Applications 1(2):286-299.

Atkinson, D. 1996. Ecotherm life-history responses to developmental temperature. Pages 183-204 *in* I. A. Johnston, and A. F. Bennett, editors. Animals and Temperature; Phenotypic and Evolutionary Adaptation. Cambridge University Press, Cambridge.

Atse, C. B., C. Audet, and J. de la Noue. 2002. Effects of temperature and salinity on the reproductive success of Arctic charr, *Salvelinus alpinus* (L.): egg composition, milt characteristics and fry survival. Aquaculture Research 33(4):299-309.

Auperin, B., and M. Geslin. 2008. Plasma cortisol response to stress in juvenile rainbow trout is influenced by their life history during early development and by egg cortisol content. General and Comparative Endocrinology 158(3):234-239.

Bams, R. A. 1967. Differences in performance of naturally and artificially propagated sockeye salmon migrant fry as measured with swimming and predation tests. Journal of the Fisheries Research Board of Canada 24(5):1117-1153.

Baras, E., B. Jacobs, and C. Melard. 2001. Effect of water temperature on survival, growth and phenotypic sex of mixed (XX-XY) progenies of Nile tilapia *Oreochromis niloticus*. Aquaculture 192(2-4):187-199.

Baroiller, J. F., D. Chourrout, A. Fostier, and B. Jalabert. 1995. Temperature and sex chromosomes govern sex ratios of the mouthbrooding cichlid fish *Oreochromis niloticus*. Journal of Experimental Zoology 273(3):216-223.

Baroiller, J. F., and H. D'Cotta. 2001. Environment and sex determination in farmed fish. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 130(4):399-409.

Baroiller, J. F., H. D'Cotta, E. Bezault, S. Wessels, and G. Hoerstgen-Schwark. 2009. Tilapia sex determination: Where temperature and genetics meet. Comparative Biochemistry and Physiology Part A 153(1):30-38.

Baroiller, J. F., Y. Guiguen, and A. Fostier. 1999. Endocrine and environmental aspects of sex differentiation in fish. Cellular and Molecular Life Sciences 55(6-7):910-931.

Baroiller, J. F., I. Nakayama, F. Foresti, and D. Chourrout. 1996. Sex determination studies in two species of teleost fish, *Oreochromis niloticus* and *Leporinus elongatus*. Zoological Studies 35(4):279-285.

Batty, R. S., J. H. S. Blaxter, and K. Fretwell. 1993. Effect of temperature on the escape response of herring larvae. Marine Biology 115(4):523-528.

BCMOE. 2007. British Columbia's Fisheries and Aquaculture Sector, Oceans and Marine Fisheries Branch Reports and Publications. BC Ministry of the Environment. http://www.env.gov.bc.ca/omfd/reports/BC-Fisheries-Aquaculture-Sector-2007.pdf (Accessed May 11, 2011).

Beacham, T. D. 1988. A genetic analysis of early development in pink (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*) at three different temperatures. Genome 30(1):89-96.

Beacham, T. D. 1990. A genetic-analysis of meristic and morphometric variation in chum salmon (*Oncorhynchus keta*) at three different temperatures. Canadian Journal of Zoology 68(2):225-229.

Beacham, T. D., J. R. Candy, B. McIntosh, C. MacConnachie, A. Tabata, K. Kaukinen, L. T. Deng, K. M. Miller, R. E. Withler, and N. Varnavskaya. 2005. Estimation of stock composition and individual identification of sockeye salmon on a Pacific Rim basis using microsatellite and major histocompatibility complex variation. Transactions of the American Fisheries Society 134(5):1124-1146.

Beacham, T. D., and C. B. Murray. 1985. Effect of female size, egg size, and water temperature on developmental biology of chum salmon (*Oncorhynchus keta*) from the Nitinat River, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 42(11):1755-1765.

Beacham, T. D., and C. B. Murray. 1986a. Comparative developmental biology of chum salmon (*Oncorhynchus keta*) from the Fraser River, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 43(2):252-262.

Beacham, T. D., and C. B. Murray. 1986b. Comparative developmental biology of pink salmon, *Oncorhynchus gorbuscha*, in Southern British Columbia. Journal of Fish Biology 28(2):233-246.

Beacham, T. D., and C. B. Murray. 1987. Effects of transferring pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon embryos at different developmental stages to a low incubation temperature. Canadian Journal of Zoology 65(1):96-105.

Beacham, T. D., and C. B. Murray. 1988. Variation in developmental biology of pink salmon (*Oncorhynchus gorbuscha*) in British Columbia. Canadian Journal of Zoology 66(12):2634-2648.

Beacham, T. D., and C. B. Murray. 1989. Variation in developmental biology of sockeye salmon (*Oncorhynchus nerka*) and Chinook salmon (*Oncorhynchus tshawytscha*) in British Columbia. Canadian Journal of Zoology 67(9):2081-2089.

Beacham, T. D., and C. B. Murray. 1990. Temperature, egg size, and development of embryos and alevins of five species of Pacific salmon: A comparative analysis. Transactions of the American Fisheries Society 119(6):927-944.

Beacham, T. D., and N. V. Varnavskaya. 1991. Effect of parental heterozygosity on pink salmon (*Oncorhynchus gorbuscha*) embryonic and alevin survival and development at extreme temperatures. Canadian Journal of Zoology 69(9):2485-2489.

Beacham, T. D., R. E. Withler, C. B. Murray, and L. W. Barner. 1988. Variation in body size, morphology, egg size, and biochemical genetics of pink salmon in British Columbia. Transactions of the American Fisheries Society 117(2):109-126.

Beamish, R. J. 1979. Swimming capacity. Pages 101-187 *in* W. S. Hoar, and D. J. Randall, editors. Fish Physiology, volume 7 - Locomtion. Academic Press Inc., New York.

Bengtson, D. A., R. C. Barkman, and W. J. Berry. 1987. Relationships between maternal size, egg diameter, time of spawning season, temperature, and length at hatch of Atlantic silverside, *Menidia menidia*. Journal of Fish Biology 31(5):697-704.

Benoit, H. P., and P. Pepin. 1999. Interaction of rearing temperature and maternal influence on egg development rates and larval size at hatch in yellowtail flounder (*Pleuronectes ferrugineus*). Canadian Journal of Fisheries and Aquatic Sciences 56(5):785-794.

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

Bernardo, J. 1996. Maternal effects in animal ecology. American Zoologist 36(2):83-105.

Bezault, E., F. Clota, M. Derivaz, B. Chevassus, and J. F. Baroiller. 2007. Sex determination and temperature-induced sex differentiation in three natural populations of Nile tilapia (*Oreochromis niloticus*) adapted to extreme temperature conditions. Aquaculture 272:S3-S16.

Billard, R. 1985. Environmental factors in salmonid culture and the control of reproduction. Conference Procedings. R. N. Iwamoto, and S. Sower, editors. Salmonid Reproduction - An international symposium, Bellevue, Washington (Oct. 31-Nov.2, 1983).

Blaxter, J. H. 1992. The effect of temperature on larval fishes. Netherlands Journal of Zoology 42(2-3):336-357.

Bonner, T. H., T. M. Brandt, J. N. Fries, and B. G. Whiteside. 1998. Effects of temperature on egg production and early life stages of the fountain darter. Transactions of the American Fisheries Society 127(6):971-978.

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

Brana, F., and X. Ji. 2000. Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). Journal of Experimental Zoology 286(4):422-433.

Brannon. 1987. Mechanism stabilizing salmonid fry emergence timing. L. Smith, L. Margolis, and C. C. Wood, editors. Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Canadian Special Publications of Fisheries and Aquatic Sciences, No. 96, pp.120-124.

Brooks, S., C. R. Tyler, and J. P. Sumpter. 1997. Egg quality in fish: What makes a good egg? Reviews in Fish Biology and Fisheries 7(4):387-416.

Brown, C., and B. B. Brown. 1998. Intense natural selection on body size and wing and tail asymmetry in cliff swallows during severe weather. Evolution 52(5):1461-1475.

Brown, N. P., R. J. Shields, and N. R. Bromage. 2006. The influence of water temperature on spawning patterns and egg quality in the Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture 261(3):993-1002.

Brungs, W. A. 1971. Chronic effects of constant elevated temperature on the fathead minnow (*Pimephales promelas Rafinesque*). Transactions of the American Fisheries Society 100(4):659-664.

Bryant, M. D. 2009. Global climate change and potential effects on Pacific salmonids in freshwater ecosystems of Southeast Alaska. Climatic Change 95(1-2):169-193.

Buckley, L. J., A. S. Smigielski, T. A. Halavik, and G. C. Laurence. 1990. Effects of water temperature on size and biochemical composition of winter flounder *Pseudopleuronectes americanus* at hatching and feeding initiation. Fishery Bulletin 88(3):419-428.

Burggren, W., and T. Blank. 2009. Physiological study of larval fishes: challenges and opportunities. Scientia Marina 73:99-110.

Burgner, R. L. 1991. Life history of sockeye salmon (*Oncorhynchus nerka*). Pages 3-117 *in* C. Groot, and L. Margolis, editors. Pacific salmon life histories. UBC Press, Vancouver.

Burt, J. B., S. Hinch, and D. A. Patterson. In press. The importance of parentage in assessing temperature effects on fish early life history: a review of the experimental literature. Reviews in Fish Biology and Fisheries: DOI:10.1007/s11160-010-9179-1.

Campbell, P. M., T. G. Pottinger, and J. P. Sumpter. 1992. Stress reduces the quality of gametes produced by rainbow trout. Biology of Reproduction 47(6):1140-1150.

Carline, R. F., and J. F. Machung. 2001. Critical thermal maxima of wild and domestic strains of trout. Transactions of the American Fisheries Society 130(6):1211-1216.

Carlson, S. M., and T. R. Seamons. 2008. A review of quantitative genetic components of fitness in salmonids: implications for adaptation to future change. Evolutionary Applications 1(2):222-238.

Cederholm, C. J., D. H. Johnson, R. E. Bilby, L. G. Dominguez, A. M. Garrett, W. H. Graeber, E. L. Greda, M. D. Kunze, B. G. Marcot, J. F. Palmisano, R. W. Plotnikoff, W. G. Pearcy, C. A. Simenstad, and P. C. Trotter. 2000. Pacific salmon and wildlife - ecological contexts, relationships, and implications for management. , Special Edition Technical Report, Prepared for D. H. Johnson and T. A. O'Neil (Managing directors), Wildlife-Habitat Relationships in Oregon and Washington. Olympia, Washington.

Chambers, R. C. 1997. Environmental influences on egg and propagule sizes in marine fishes. Pages 63-102 *in* R. C. Chambers, and E. A. Trippel, editors. Early life history and recruitment in fish populations, volume Fish and Fisheries. Chapman & Hall, London.

Chambers, R. C., and W. C. Leggett. 1996. Maternal influences on variation in egg sizes in temperate marine fishes. American Zoologist 36(2):180-196.

Charmantier, A., and D. Garant. 2005. Environmental quality and evolutionary potential: lessons from wild populations. Proceedings of the Royal Society B-Biological Sciences 272(1571):1415-1425.

Chown, S. L., A. A. Hoffmann, T. N. Kristensen, M. J. Angilletta, N. C. Stenseth, and C. Pertoldi. 2010. Adapting to climate change: a perspective from evolutionary physiology. Climate Research 43(1-2):3-15.

Clark, R. W., A. Henderson-Arzapalo, and C. V. Sullivan. 2005. Disparate effects of constant and annually cycling day length and water temperature on reproductive maturation of striped bass (*Morone saxatilis*). Aquaculture 249(1-4):497-513.

Combs, B. D. 1965. Effect of temperature on the development of salmon eggs. Progressive Fish Culturist 27:134-137.

Conover, D. O., and S. W. Heins. 1987. The environmental and genetic components of sex ratio in *Menidia menidia* (Pisces, Atherinidae). Copeia (3):732-743.

Conover, D. O., and B. E. Kynard. 1981. Environmental sex determination - Interaction of temperature and genotype in a fish. Science 213(4507):577-579.

Cooke, S. J., S. G. Hinch, A. P. Farrell, M. F. Lapointe, S. R. M. Jones, J. S. MacDonald, D. A. Patterson, M. C. Healey, and G. Van Der Kraak. 2004. Abnormal migration timing and high en route mortality of sockeye salmon in the Fraser River, British Columbia. Fisheries 29:22-33.

Cooke, S. J., J. Schreer, D. P. Philipp, and P. J. Weatherhead. 2003. Nesting activity, parental care behavior, and reproductive success of smallmouth bass, *Micropterus dolomieu*, in an unstable thermal environment. Journal of Thermal Biology 28(6-7):445-456.

Craig, J. K., C. J. Foote, and C. C. Wood. 1996. Evidence for temperature-dependent sex determination in sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences 53(1):141-147.

Crossin, G. T., S. G. Hinch, S. J. Cooke, D. A. Welch, D. A. Patterson, S. R. M. Jones, A. G. Lotto, R. A. Leggatt, M. T. Mathes, J. M. Shrimpton, G. Van Der Kraak, and A. P. Farrell. 2008. Exposure to high temperature influences the behaviour, physiogy, and survival of sockeye salmon during spawning migration. Canadian Journal of Zoology 86:127-140.

Crozier, L. G., A. P. Hendry, P. W. Lawson, T. P. Quinn, N. J. Mantua, J. Battin, R. G. Shaw, and R. B. Huey. 2008. Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon. Evolutionary Applications 1(2):252-270.

Dalziel, A. C., S. M. Rogers, and P. M. Schulte. 2009. Linking genotypes to phenotypes and fitness: how mechanistic biology can inform molecular ecology. Molecular Ecology 18(24):4997-5017.

Danzmann, R. G., and M. M. Ferguson. 1988. Temperature-dependent genotypic selection and embryonic survival of rainbow trout. Biochemical Genetics 26(1-2):69-82.

Davies, B., and N. Bromage. 2002. The effects of fluctuating seasonal and constant water temperatures on the photoperiodic advancement of reproduction in female rainbow trout *Oncorhynchus mykiss*. Aquaculture 205(1-2):183-200.

De March, B. G. E. 1995. Effects of incubation temperature on the hatching success of Arctic char eggs. Progressive Fish Culturist 57:132-136.

Desprez, D., and C. Melard. 1998. Effect of ambient water temperature on sex determinism in the blue tilapia *Oreochromis aureus*. Aquaculture 162(1-2):79-84.

Devlin, R. H., and Y. Nagahama. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture 208(3-4):191-364.

Donelson, J. M., P. L. Munday, and M. I. McCormick. 2009. Parental effects on offspring life histories: when are they important? Biology Letters 5(2):262-265.

Dzikowski, R., G. Hulata, I. Karplus, and S. Harpaz. 2001. Effect of temperature and dietary l-carnitine supplementation on reproductive performance of female guppy (*Poecilia reticulata*). Aquaculture 199(3-4):323-332.

Eggers, D. M. 1978. Limnetic feeding behavior of juvenile sockeye salmon in Lake Washington and predator avoidance. Limnology and Oceanography 23(6):1114-1125.

Eilertsen, E. M., B. J. Bardsen, S. Liljedal, G. Rudolfsen, and I. Folstad. 2009. Experimental evidence for paternal effects on offspring growth rate in Arctic charr (*Salvelinus alpinus*). Proceedings of the Royal Society B-Biological Sciences 276(1654):129-136.

Einum, S., and I. A. Fleming. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. Proceedings of the Royal Society of London Series B-Biological Sciences 266(1433):2095-2100.

Einum, S., G. Robertsen, and I. A. Fleming. 2008. Adaptive landscapes and density-dependent selection in declining salmonid populations: going beyond numerical responses to human disturbance. Evolutionary Applications 1(2):239-251.

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

Elliott, J. M. 1989. Mechanisms responsible for population regulation in young migratory trout, *Salmo trutta* I., the critical time for survival. Journal of Animal Ecology 58(3):987-1001.

Elliott, J. M., and J. A. Elliott. 2010. Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus* : predicting the effects of climate change Journal of Fish Biology 77(8):1793-1817.

Elphick, M. J., and R. Shine. 1998. Longterm effects of incubation temperatures on the morphology and locomoter performance of hatchling lizards (*Bassiana duperreyi,* Scincidae). Biological Journal of the Linnean Society 63:429-447.

Eriksen, M. S., M. Bakken, A. Espmark, B. O. Braastad, and R. Salte. 2006. Prespawning stress in farmed Atlantic salmon *Salmo salar*: maternal cortisol exposure and hyperthermia during embryonic development affect offspring survival, growth and incidence of malformations. Journal of Fish Biology 69(1):114-129.

Evans, J. P., and A. J. Geffen. 1998. Male characteristics, sperm traits, and reproductive success in winter-spawning Celtic Sea Atlantic herring, *Clupea harengus*. Marine Biology 132(2):179-186.

Farrell, A. P., J. Gallaugher, J. Fraser, D. Pike, P. Bowering, A. K. M. Hadwin, W. Parkhouse, and R. Routledge. 2001. Successful recovery of the physiological status of coho salmon on board a commercial gillnet vessel by means of a newly designed revival box. Canadian Journal of Fisheries and Aquatic Sciences 58:1932-1946.

Farrell, A. P., S. G. Hinch, S. J. Cooke, D. A. Patterson, G. T. Crossin, M. Lapointe, and M. T. Mathes. 2008. Pacific salmon in hot water: Applying aerobic scope models and biotelemetry to predict the success of spawning migrations. Physiological and Biochemical Zoology 81(6):697-708.

Ferrari, M. R., J. R. Miller, and G. L. Russell. 2007. Modeling changes in summer temperature of the Fraser River during the next century. Journal of Hydrology 342:336-346.

Finn, R. N. 2007. The physiology and toxicology of salmonid eggs and larvae in relation to water quality criteria. Aquatic Toxicology 81(4):337-354.

Fleming, I. A., T. Agustsson, B. Finstad, J. I. Johnsson, and B. B. Bjornsson. 2002. Effects of domestication on growth physiology and endocrinology of Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 59:1323-1330.

Flett, P. A., K. R. Munkittrick, G. Van Der Kraak, and J. F. Leatherland. 1996. Overripening as the cause of low survival to hatch in Lake Erie coho salmon (*Oncorhynchus kisutch*) embryos. Canadian Journal of Zoology 74:851-857.

Fowler, J. A. 1970. Control of vertebral number in teleosts - an embryological problem. Quarterly Review of Biology 45(2):148-167.

Fox, C. J., A. J. Geffen, R. Blyth, and R. D. M. Nash. 2003. Temperature-dependent development rates of plaice (*Pleuronectes platessa* L.) eggs from the Irish Sea. Journal of Plankton Research 25(11):1319-1329.

Fox, C. W., and T. A. Mousseau. 1996. Larval host plant affects fitness consequences of egg size variation in the seed beetle *Stator limbatus*. Oecologia 107(4):541-548.

Fraser, D. J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. Evolutionary Applications 1(4):535-586.

Fujioka, Y. 2001. Thermolabile sex determination in honmoroko. Journal of Fish Biology 59(4):851-861.

Fujioka, Y. 2006. Patterns of sex ratio response to water temperature during sex determination in honmoroko *Gnathopogon caerulescens*. Fisheries Science 72(5):1034-1041.

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

Gagliano, M., M. I. McCormick, and M. G. Meekan. 2007. Temperature-induced shifts in selective pressure at a critical developmental transition. Oecologia 152(2):219-225.

Garant, D., P. M. Fontaine, S. P. Good, J. J. Dodson, and L. Bernatchez. 2002. The influence of male parental identity on growth and survival of offspring in Atlantic salmon (*Salmo salar*). Evolutionary Ecology Research 4(4):537-549.

Garcia de Leaniz, C., I. A. Fleming, S. Einum, E. Verspoor, W. C. Jordan, S. Consuegra, N. Aubin-Horth, D. Lajus, B. H. Letcher, A. F. Youngson, J. H. Webb, L. A. Vollestad, B. Villanueva, A. Ferguson, and T. P. Quinn. 2007. A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biological Reviews 82(2):173-211.

Garenc, C., F. G. Silversides, and H. Guderley. 1998. Burst swimming and its enzymatic correlates in the threespine stickleback (*Gasterosteus aculeatus*): full-sib heritabilities. Canadian Journal of Zoology 76(4):680-688.

Garner, K., and B. Parfitt. 2006. First Nations, salmon fisheries and the rising importance of conservation, Report to the Pacific Fisheries Resources Conservation Council.

http://www.fish.bc.ca/files/First%20Nations%20Salmon%20Fisheries.pdf (Accessed May 11, 2011).

Geist, D. R., C. S. Abernethy, K. D. Hand, V. I. Cullinan, J. A. Chandler, and P. A. Groves. 2006. Survival, development, and growth of fall Chinook salmon embryos, alevins, and fry exposed to variable thermal and dissolved oxygen regimes. Transactions of the American Fisheries Society 135(6):1462-1477.

Gharrett, A. J., and W. W. Smoker. 1993. A perspective on the adaptive importance of genetic infrastructure in salmon populations to ocean ranching in Alaska. Fisheries Research 18(1-2):45-58.

Gibb, A. C., and K. A. Dickson. 2002. Functional morphology and biochemical indices of performance: is there a correlation between metabolic enzyme activity and swimming performance? Integrative and Comparative Biology 42:199-207.

Gillet, C. 1991. Egg production in an Arctic charr (*Salvelinus alpinus* L.) brood stock: Effects of temperature on the timing of spawning and the quality of eggs. Aquatic Living Resources 4(2):109-116.

Green, B. S. 2008. Maternal Effects in Fish Populations. Pages 1-105 *in* D. W. Sims, editor. Advances in Marine Biology, volume 54. Academic Press, San Diego.

Green, B. S., and R. Fisher. 2004. Temperature influences swimming speed, growth and larval duration in coral reef fish larvae. Journal of Experimental Marine Biology and Ecology 299(1):115-132.

Green, B. S., and M. I. McCormick. 2005. Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish. Marine Ecology-Progress Series 289:263-272.

Groot, C., and L. Margolis. 1991. Pacific Salmon Life Histories. UBC Press, Vanvouver.

Guan, L., P. V. R. Snelgrove, and A. K. Gamperl. 2008. Ontogenetic changes in the critical swimming speed of *Gadus morhua* (Atlantic cod) and *Myoxocephalus scorpius* (shorthorn sculpin) larvae and the role of temperature. Journal of Experimental Marine Biology and Ecology 360(1):31-38.

Gunnes, K. 1979. Survival and development of Atlantic salmon eggs and fry at 3 different temperatures. Aquaculture 16(3):211-218.

Hague, M. J., M. R. Ferrari, J. R. Miller, D. A. Patterson, G. L. Russell, A. P. Farrell, and S. Hinch. 2011. Modelling the future hydroclimatology of the lower Fraser River and its impacts on the spawning migration survival of sockeye salmon. Global Change Biology 17:87-98.

Hale, M. E. 1999. Locomotor mechanics during early life history: Effects of size and ontogeny on fast-start performance of salmonid fishes. Journal of Experimental Biology 202(11):1465-1479.

Hansen, T. K., and I. B. Falk-Petersen. 2001. The influence of rearing temperature on early development and growth of spotted wolffish *Anarhichas minor* (Olafsen). Aquaculture Research 32(5):369-378.

Hard, J. J. 1995. A quantitative genetic perspective on the conservation of intraspecific diversity. Pages 304-326 *in* J. L. Nielsen, editor. Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation, volume 17. American Fisheries Society, Bethesda.

Hartman, G. F., and J. C. Scrivener. 1990. Impacts of forestry practices on a coastal stream ecosystem Carnation Creek British Columbia Canada. Canadian Bulletin of Fisheries and Aquatic Sciences (223):1-148.

Haugen, T. O., and L. A. Vollestad. 2000. Population differences in early life-history traits in grayling. Journal of Evolutionary Biology 13(6):897-905.

Haung, W. B., and F. L. Cheng. 2006. Effects of temperature and floating materials on breeding by the paradise fish (*Macropodus opercularis*) in the non-reproductive season. Zoological Studies 45(4):475-482.

Hawkins, D. K., and T. P. Quinn. 1996. Critical swimming velocity and associated morphology of juvenile coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead trout (*Oncorhynchus mykiss*), and their hybrids. Canadian Journal of Fisheries and Aquatic Sciences 53(7):1487-1496.

Healey, M. 2011. The cumulative impacts of climate change on Fraser River sockeye salmon (*Oncorhynchus nerka*) and implications for management. Canadian Journal of Fisheries and Aquatic Sciences 68:718-737.

Heath, D. D., N. J. Bernier, J. W. Heath, and G. K. Iwama. 1993. Genetic, environmental, and interaction effects on growth and stress response of Chinook salmon (*Oncorhynchus tshawytscha*) fry. Canadian Journal of Fisheries and Aquatic Sciences 50(2):435-442.

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

Heath, D. D., R. H. Devlin, J. W. Heath, and G. K. Iwama. 1994. Genetic, environmental and interaction effects on the incidence of jacking in *Oncorhynchus tshawytscha* (Chinook salmon). Heredity 72:146-154.

Heath, D. D., C. W. Fox, and J. W. Heath. 1999. Maternal effects on offspring size: Variation through early development of Chinook salmon. Evolution 53(5):1605-1611.

Hebert, K. P., P. L. Goddard, W. W. Smoker, and A. J. Gharrett. 1998. Quantitative genetic variation and genotype by environment interaction of embryo development rate in pink salmon (*Oncorhynchus gorbuscha*). Canadian Journal of Fisheries and Aquatic Sciences 55(9):2048-2057.

Hendry, A. P., J. E. Hensleigh, and R. R. Reisenbichler. 1998. Incubation temperature, developmental biology, and the divergence of sockeye salmon (*Oncorhynchus nerka*) within Lake Washington. Canadian Journal of Fisheries and Aquatic Sciences 55(6):1387-1394.

Hilder, M. L., and N. W. Pankhurst. 2003. Evidence that temperature change cues reproductive development in the spiny damselfish, *Acanthochromis polyacanthus*. Environmental Biology of Fishes 66(2):187-196.

Hinch, S. G., and E. G. Martins. 2011. A review of potential climate change effects on survival of Fraser River sockeye salmon and an analysis of interannual trends in en route loss and pre-spawn mortality, Tech Report 9, The Cohen Commission of Inquirey into the Decline of Sockeye Salmon in the Fraser River. Vancouver, B.C. www.cohencommission.ca (Accessed May 10, 2011).

Hoffmann, A. A., and M. J. Hercus. 2000. Environmental stress as an evoluationary force. Bioscience 50(3):217-226.

Hoffmann, A. A., and P. A. Parsons. 1989. An integrated approach to environmental stress tolerance and life-history variation: desiccation tolerance in *Drosophia* Biological Journal of the Linnean Society 37:117-136.

Hoffmann, A. A., and P. A. Parsons. 1991. Evolutionary Genetics and Environmental Stress. Oxford University Press, Oxford.

Hoie, H., A. Folkvord, and A. Johannessen. 1999. The influence of different parental combinations and incubation temperature on the RNA and DNA content of herring larvae at hatching: A pilot study. Journal of Fish Biology 55(sA):110-118.

Hokanson, K. E., McCormic.Jh, B. R. Jones, and J. H. Tucker. 1973. Thermal requirements for maturation, spawning, and embryo survival of brook trout, *Salvelinus fontinalis*. Journal of the Fisheries Research Board of Canada 30(7):975-984.

Hotta, K., M. Tamura, T. Watanabe, Y. Nakamura, S. Adachi, and K. Yamauchi. 2001. Changes in spawning characteristics of Japanese whiting *Sillago japonica* under control of temperature. Fisheries Science 67(6):1111-1118.

Houde, E. D. 1997. Patterns and consequences of selective processes in teleost early life histories. Pages 172-196 *in* R. C. Chambers, and E. A. Trippel, editors. Early Life History and Recruitment in Fish Populations. Chapman and Hall, London.

Hruska, K. A. 2010. Factors affecting egg retention and longevity in spawning female sockeye salmon (*Oncorhynchus nerka*). PhD Thesis, University of British Columbia, Canada. p. 1-136.

Humpesch, U. H. 1985. Inter- and intra-specific variation in hatching success and embryonic development of five species of salmonids and *Thymallus thymallus*. Arch. Hydrobiol 104(1):129-144.

Hutchings, J. A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. Evolution 45(5):1162-1168.

Hutchings, J. A. 2004. Norms of reaction and phenotypic plasticity in salmonid life history. Pages 154-175 *in* A. P. Hendry, editor. Evolution Illuminated: Salmon and their Relatives. Oxford University Press, New York.

Hutchings, J. A. 2011. Old wine in new bottles: reaction norms in salmonid fishes. Heredity 106:421-437.

Hutchings, J. A., D. P. Swain, S. Rowe, J. D. Eddington, V. Puvanendran, and J. A. Brown. 2007. Genetic variation in life-history reaction norms in a marine fish. Proceedings of the Royal Society B-Biological Sciences 274(1619):1693-1699.

Huuskonen, H., O. Penttinen, and J. Piironen. 2003. Effects of temperature and parental background on the embryonic survival and metabolic rate of newly hatched Arctic charr. Pages 35-44 *in* The big fish bang: Proceedings of the 26th Annual Larval Fish Conference. Institute of Marine Research (Norway).

Ito, L. S., A. M. Cornejo, M. Yamashita, and C. A. Strussmann. 2008. Thermal threshold and histological process of heat-induced sterility in adult pejerrey (*Odontesthes bonariensis*): A comparative analysis of laboratory and wild specimens. Physiological and Biochemical Zoology 81(6):775-784.

Janhunen, M., J. Piironen, and N. Peuhkuri. 2010. Parental effects on embryonic viability and growth in Arctic charr *Salvelinus alpinus* at two incubation temperatures. Journal of Fish Biology 76:2558-2570.

Jensen, J. O. T., and M. E. Jensen. 1999. IncubWin: A windows 95/98/NT computer program for predicting embryonic stages in Pacific salmon and steelhead trout, http://www.pac.dfo-mpo.gc.ca/science/aquaculture/sirp/incubwin-eng.htm (Accessed April 22, 2011).

Jensen, L. F., M. M. Hansen, C. Pertoldi, G. Holdensgaard, K. L. D. Mensberg, and V. Loeschcke. 2008. Local adaptation in brown trout early life-history traits:

implications for climate change adaptability. Proceedings of the Royal Society B-Biological Sciences 275(1653):2859-2868.

Jobling, M., H. K. Johnsen, G. W. Pettersen, and R. J. Henderson. 1995. Effect of temperature on reproductive development in Arctic charr, *Salvelinus Alpinus* (L.). Journal of Thermal Biology 20(1-2):157-165.

Johnston, C. E., M. J. Hambrook, R. W. Gray, and K. G. Davidson. 1992. Manipulation of reproductive function in Atlantic salmon (*Salmo salar*) kelts with controlled photoperiod and temperature. Canadian Journal of Fisheries and Aquatic Sciences 49(10):2055-2061.

Johnston, I. A. 2006. Environment and plasticity of myogenesis in teleost fish. Journal of Experimental Biology 209(12):2249-2264.

Johnston, I. A., and H. A. McLay. 1997. Temperature and family effects on muscle cellularity at hatch and first feeding in Atlantic salmon (*Salmo salar* L). Canadian Journal of Zoology 75(1):64-74.

Johnston, I. A., V. L. A. Vieira, and J. Hill. 1996. Temperature and ontogeny in ectotherms: muscle phenotype in fish. Pages 153-181 *in* I. A. Johnston, and A. F. Bennett, editors. Animals and Temperature; Phenotypic and Evolutionary Adaptation. Cambridge University Press, Cambridge.

Johnston, I. A., V. L. A. Vieira, and G. K. Temple. 2001. Functional consequences and population differences in the developmental plasticity of muscle to temperature in Atlantic herring *Clupea harengus*. Marine Ecology Progress Series 213:285-300.

Jonsson, B., and N. Jonsson. 2009. A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, with particular reference to water temperature and flow. Journal of Fish Biology 75:2381-2447.

Kamler, E. 1992. Early Life History of Fish: An Energetics Approach. Chapman & Hall, London.

Kamler, E. 2002. Ontogeny of yolk-feeding fish: an ecological perspective. Reviews in Fish Biology and Fisheries 12(1):79-103.

Kamler, E. 2005. Parent-egg-progeny relationships in teleost fishes: an energetics perspecitve. Reviews in Fish Biology and Fisheries 15:399-421.

Kamler, E. 2008. Resource allocation in yolk-feeding fish. Reviews in Fish Biology and Fisheries 18(2):143-200.

Kamler, E., and J. Stachowiak. 1992. Egg size and reproductive effort in tench (*Tinca tinca* (L.)) females from heated waters. Polish Archives of Hydrobiology 39(1):101-107.

Kaplan, R. H. 1998. Maternal effects, developmental plasticity, and life history evoluation: An amphibian model. Pages 244-260 *in* T. A. Mousseau, and C. W. Fox, editors. Maternal Effects as Adaptations. Oxford University Press, New York.

Karayucel, I., A. K. Orhan, and S. Karayucel. 2008. Effect of temperature on some reproductive parameters of gravid females and growth of newly hatched fry in guppy, *Poecilia reticulta* (Peters, 1860). Journal of Animal and Veterinary Advances 7(10):1261-1266.

Kaushal, S. S., G. E. Likens, N. A. Jaworski, M. L. Pace, A. M. Sides, D. Seekell, K. T. Belt, D. H. Secor, and R. L. Wingate. 2010. Rising stream and river temperatures in the United States. Frontiers in Ecology and the Environment 8(9):461-466.

Killeen, J., H. A. McLay, and I. A. Johnston. 1999. Development in *Salmo trutta* at different temperatures, with a quantitative scoring method for intraspecific comparisons. Journal of Fish Biology 55(2):382-404.

Kime, D. E. 1995. The effects of pollution on reproduction in fish. Reviews in Fish Biology and Fisheries 5(1):52-95.

King, H. R., and N. W. Pankhurst. 2004a. Effect of maintenance at elevated temperatures on ovulation and luteinizing hormone releasing hormone analogue responsiveness of female Atlantic salmon (*Salmo salar*) in Tasmania. Aquaculture 233(1-4):583-597.

King, H. R., and N. W. Pankhurst. 2004b. Effect of short-term temperature reduction on ovulation and LHRHa responsiveness in female Atlantic salmon (*Salmo salar*) maintained at elevated water temperatures. Aquaculture 238(1-4):421-436.

King, H. R., N. W. Pankhurst, and M. Watts. 2007. Reproductive sensitivity to elevated water temperatures in female Atlantic salmon is heightened at certain stages of vitellogenesis. Journal of Fish Biology 70(1):190-205.

King, H. R., N. W. Pankhurst, M. Watts, and P. M. Pankhurst. 2003. Effect of elevated summer temperatures on gonadal steroid production, vitellogenesis and egg quality in female Atlantic salmon. Journal of Fish Biology 63(1):153-167.

Kolok, A. S. 1992. Morphological and physiological correlates with swimming performance in juvenile largemouth bass. American Journal of Physiology 263:1042-1048.

Konecki, J. T., C. A. Woody, and T. P. Quinn. 1995. Influence of temperature on incubation rates of coho salmon (*Oncorhynchus kisutch*) from ten Washington populations. Northwest Science 69(2):126-132.

Koumoundouros, G., C. Ashton, D. G. Sfakianakis, P. Divanach, M. Kentouri, N. Anthwal, and N. C. Stickland. 2009. Thermally induced phenotypic plasticity of

swimming performance in European sea bass *Dicentrarchus labrax* juveniles. Journal of Fish Biology 74(6):1309-1322.

Kristianson, G., and D. Strongitharm. 2006. The Evolution of Recreational Salmon Fisheries in British Columbia, Report to the Pacific Fisheries Resource Conservation Council. http://www.fish.bc.ca/files/R-39_EvolutionSalmonFisheries.pdf (Accessed May 11, 2011).

Kwon, J. Y., B. J. McAndrew, and D. J. Penman. 2002. Treatment with an aromatase inhibitor suppresses high-temperature feminization of genetic male (YY) *Nile tilapia*. Journal of Fish Biology 60(3):625-636.

Lacey, E. P. 1998. What is an adaptive environmentally induced parental effect? Pages 55-66 *in* T. A. Mousseau, and C. W. Fox, editors. Maternal Effects as Adaptations. Oxford University Press, New York.

Lagomarsino, I. V., and D. O. Conover. 1993. Variation in environmental and genotypic sex-determining mechanisms across a latitudinal gradient in the fish, *Menidia menidia*. Evolution 47(2):487-494.

Lam, T. J. 1983. Environmental influences on gonadal activity in fish. Pages 65-116 *in* W. S. Hoar, D. J. Randall, and M. E. Donaldson, editors. Fish Physiology, volume IX. Academic Press, New York.

Lambert, Y., and A. Thorsen. 2003. Integration of captive and wild studies to estimate egg and larval production of fish stocks. Journal of Northwest Atlantic Fisheries Science 33:71-79.

Liao, W., and D. H. Reed. 2009. Inbreeding-environment interactions increase extinction risk. Animal Conservation 12(1):54-61.

Luksiene, D., O. Sandstrom, L. Lounasheimo, and J. Andersson. 2000. The effects of thermal effluent exposure on the gametogenesis of female fish. Journal of Fish Biology 56(1):37-50.

MacDonald, J. S. 2000. Mortality during the migration of Fraser River sockeye salmon (*Oncorhynchus nerka*): A study of the effect of ocean and river environmental conditions in 1997. Canadian Technical Report on Fisheries and Aquatic Sciences 2315:118.

Macqueen, D. J., D. H. F. Robb, T. Olsen, L. Melstveit, C. G. M. Paxton, and I. A. Johnston. 2008. Temperature until the 'eyed stage' of embryogenesis programmes the growth trajectory and muscle phenotype of adult Atlantic salmon. Biology Letters 4(3):294-298.

Manning, N. J., and D. E. Kime. 1985. The effect of temperature on testicular steroid production in the rainbow trout, *Salmo gairdneri*, in vivo and in vitro. General and Comparative Endocrinology 57(3):377-382.

Mantua, N., I. Tohver, and A. Hamlet. 2010. Climate change impacts on streamflow extremes and summertime stream temperature and their possible consequences for freshwater salmon habitat in Washington State. Climatic Change 102(1-2):187-223.

Marshall, D. J., R. M. Allen, and A. J. Crean. 2008. The ecological and evolutionary importance of maternal effects in the sea. Pages 203-250 *in* R. N. Gibson, R. J. A. Atkinson, and J. D. M. Gordon, editors. Oceanography and Marine Biology: an Annual Review, volume 46. CRC Press - Taylor & Francis Group, Boca Raton.

Martell, D. J., and J. D. Kieffer. 2007. Persistent effects of incubation temperature on muscle development in larval haddock (*Melanogrammus aeglefinus* L.). Journal of Experimental Biology 210(7):1170-1182.

Martell, D. J., J. D. Kieffer, and E. A. Trippel. 2005. Effects of temperature during early life history on embryonic and larval development and growth in haddock. Journal of Fish Biology 66(6):1558-1575.

Mathes, M. T., S. G. Hinch, S. J. Cooke, G. T. Crossin, D. A. Patterson, A. Lotto, and A. P. Farrell. 2010. Effect of water temperature, timing, physiological condition, and lake thermal refugia on migrating adult Weaver Creek sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences 67:70-84.

McCormick, M. I. 1998. Behaviorally induced maternal stress in a fish influences progeny quality by a hormonal mechanism. Ecology 79(6):1873-1883.

McCullough, D., S. Spalding, D. Sturdevant, and M. Hicks. 2001. Summary of technical literature examining the physiological effects of temperature on salmonids. EPA Issue Paper 5 - EPA-910-D-01-005:1-113.

McDaniels, T., S. Wilmot, M. Healey, and S. Hinch. 2010. Vulnerability of Fraser River sockeye salmon to climate change: A life cycle perspective using expert judgments. Journal of Environmental Management 91(12):2771-2780.

McDonald, D. G., W. J. McFarlane, and C. L. Milligan. 1998. Anaerobic capacity and swim performance of juvenile salmonids. Canadian Journal of Fisheries and Aquatic Sciences 55(5):1198-1207.

Meffe, G. K. 1991. Life history changes in eastern mosquitofish (*Gambusia holbrooki*) induced by thermal elevation. Canadian Journal of Fisheries and Aquatic Sciences 48(1):60-66.

Molony, B. W., A. R. Church, and G. B. Maguire. 2004. A comparison of the heat tolerance and growth of a selected and non-selected line of rainbow trout, *Oncorhynchus mykiss*, in Western Australia. Aquaculture 241(1-4):655-665.

Morasse, S., H. Guderley, and J. J. Dodson. 2008. Paternal reproductive strategy influences metabolic capacities and muscle development of Atlantic salmon (*Salmo salar* L.) embryos. Physiological and Biochemical Zoology 81(4):402-413.

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

Morrison, J. K., and C. E. Smith. 1986. Altering the spawning cycle of rainbow trout by manipulating water temperature. Progressive Fish Culturist 48(1):52-54.

Mote, P. W., E. Parson, A. F. Hamlet, W. S. Keeton, D. Lettenmaier, N. Mantua, E. L. Miles, D. Peterson, D. L. Peterson, R. Slaughter, and A. K. Snover. 2003. Preparing for climatic change: The water, salmon, and forests of the Pacific Northwest. Climatic Change 61(1-2):45-88.

Mousseau, T. A., and C. W. Fox, editors. 1998. Maternal Effects as Adaptations. Oxford University Press, New York.

Muller-Belecke, A., K. Ohara, W. Koedprang, G. Horstgen-Schwark, and N. Taniguchi. 2002. Environmental influence on quantitative traits of naturally isogenic lines of ginbuna (*Carassius langsdorfii*). Aquaculture 203(3-4):251-262.

Murray, C. B., and T. D. Beacham. 1986. Effect of varying temperature regimes on the development of pink salmon (*Oncorhynchus gorbuscha*) eggs and alevins. Canadian Journal of Zoology 64(3):670-676.

Murray, C. B., and T. D. Beacham. 1987. The development of Chinook (*Oncorhynchus tshawytscha*) and chum salmon (*Onchorhynchus keta*) embryos and alevins under varying temperature regimes. Canadian Journal of Zoology 65(11):2672-2681.

Murray, C. B., and T. D. Beacham. 1989. Responses of meristic characters in chum salmon (*Oncorhynchus keta*) to temperature changes during development. Canadian Journal of Zoology 67(3):596-600.

Murray, C. B., T. D. Beacham, and J. D. McPhail. 1990. Influence of parental stock and incubation temperature on the early development of coho salmon (*Oncorhynchus kisutch*) in British Columbia. Canadian Journal of Zoology 68(2):347-358.

Murray, C. B., and J. D. McPhail. 1988. Effect of incubation temperature on the development of five species of Pacific salmon (*Oncorhynchus*) embryos and alevins. Canadian Journal of Zoology 66:266-273.

Nadeau, P. S. 2007. Parental contributions to the early life history traits of juvenile sockeye salmon (*Onchorhynchus nerka*): The roles of spawner identity and migratory experience. Masters Thesis, University of British Columbia, Canada. p.1-112.

Nadeau, P. S., S. G. Hinch, L. B. Pon, and D. A. Patterson. 2009. Persistent parental effects on the survival and size, but not burst swimming performance of juvenile sockeye salmon (*Oncorhynchus nerka*). Journal of Fish Biology 75(3):538-551.

Nagler, J. J., J. E. Parsons, and J. G. Cloud. 2000. Single pair mating indicates maternal effects on embryo survival in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 184(1-2):177-183.

Naiman, R. J., R. E. Bilby, D. E. Schindler, and J. M. Helfield. 2002. Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. Ecosystems 5(4):399-417.

Ojaveer, E. 2006. On the external and parental effects in early development of herring (*Clupea pallasi*) at the NE Kamchatka. Fisheries Research 81(1):1-8.

Ouellet, P., Y. Lambert, and I. Berube. 2001. Cod egg characteristics and viability in relation to low temperature and maternal nutritional condition. Ices Journal of Marine Science 58(3):672-686.

Packard, G. C., and T. J. Boardman. 1988. The misuse of ratios, indexes, and percentages in ecophysiological research. Physiological Zoology 61(1):1-9.

Pakkasmaa, S., and M. Jones. 2002. Individual-level analysis of early life history traits in hatchery-reared lake trout. Journal of Fish Biology 60(1):218-225.

Pakkasmaa, S., O. P. Penttinen, and J. Piironen. 2006. Metabolic rate of Arctic charr eggs depends on their parentage. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 176(5):387-391.

Pankhurst, N. W., and H. R. King. 2010. Temperature and salmonid reproduction: implications for aquaculture. Journal of Fish Biology 76(1):69-85.

Pankhurst, N. W., G. J. Purser, G. Van Der Kraak, P. M. Thomas, and G. N. R. Forteath. 1996. Effect of holding temperature on ovulation, egg fertility, plasma levels of reproductive hormones and in vitro ovarian steroidogenesis in the rainbow trout *Oncorhynchus mykiss*. Aquaculture 146(3-4):277-290.

Pankhurst, N. W., and P. M. Thomas. 1998. Maintenance at elevated temperature delays the steroidogenic and ovulatory responsiveness of rainbow trout *Oncorhynchus mykiss* to luteinizing hormone releasing hormone analogue. Aquaculture 166(1-2):163-177.

Parichy, D. M., and R. H. Kaplan. 1992. Maternal effects on offspring growth and development depend on environmental quality in the frog *Bombina orientalis*. Oecologia 91(4):579-586.

Patterson, D. A. 2004. Relating the sockeye salmon (*Oncorhynchus nerka*) spawning migration experience with offspring fitness: A study of intergenerational effects. Masters Thesis, Simon Fraser University, Canada. p. 1-135.

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

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

Patterson, D. A., J. S. Macdonald, K. M. Skibo, D. P. Barnes, I. Guthrie, and J. Hills. 2007. Reconstructing the summer thermal history for the lower Fraser River, 1941 to 2006, and implications for adult sockeye salmon (*Oncorhynchus nerka*) spawning migration Canadian Technical Report on Fisheries and Aquatic Sciences 2724:1-43.

Pauly, D., and R. S. V. Pullin. 1988. Hatching time in spherical, pelagic, marine fish eggs in response to temperature and egg size. Environmental Biology of Fishes 22(4):261-271.

Pavlov, D. A., and E. Moksness. 1995. Development of wolffish eggs at different temperature regimes. Aquaculture International 3(4):315-335.

Pechenik, J. A., D. E. Wendt, and J. N. Jarrett. 1998. Metamorphosis is not a new beginning. Bioscience 48(11):901-910.

Pepin, P. 1991. Effect of temperature and size on development, mortality, and survival rates of the pelagic early life-history stages of marine fish. Canadian Journal of Fisheries and Aquatic Sciences 48(3):503-518.

Phelps, R. P., R. Hastey, A. Pendetar, L. Linley, N. Papanikos, and R. A. Dunham. 2007. Effects of temperature on the induced spawning of channel catfish and the production of channel x blue catfish hybrid fry. Aquaculture 273(1):80-86.

Piferrer, F., M. Blazquez, L. Navarro, and A. Gonzalez. 2005. Genetic, endocrine, and environmental components of sex determination and differentiation in the European sea bass (*Dicentrarchus labrax* L.). General and Comparative Endocrinology 142(1-2):102-110.

Pike, R. G., D. L. Spittlehouse, K. E. Bennett, V. N. Eggington, P. J. Tschaplinski, T. Q. Murdock, and A. T. Werner. 2008. Climate change and watershed hydrology: Part 1 – Recent and projected changes in British Columbia. Streamline Watershed Management Bulletin 11(2):1-8.

Pitcher, T. E., and B. D. Neff. 2007. Genetic quality and offspring performance in Chinook salmon: implications for supportive breeding. Conservation Genetics 8(3):607-616.

Plaistow, S. J., C. T. Lapsley, and T. G. Benton. 2006. Context-dependent intergenerational effects: The interaction between past and present environments and its effect on population dynamics. American Naturalist 167(2):206-215.

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

Pornsoping, P., G. Unsrisong, T. Vearasilp, S. Wessels, and G. Horstgen-Schwark. 2007. Reproductive performance of female rainbow trout *Oncorhynchus mykiss* (Walbaum) kept under water temperatures and photoperiods of 13 degree and 51 degree N latitude. Aquaculture Research 38(12):1265-1273.

Portner, H. O., A. F. Bennett, F. Bozinovic, A. Clarke, M. A. Lardies, M. Lucassen, B. Pelster, F. Schiemer, and J. H. Stillman. 2006. Trade-offs in thermal adaptation: The need for a molecular to ecological integration. Physiological and Biochemical Zoology 79(2):295-313.

Probst, W. N., G. Kraus, R. M. Rideout, and E. A. Trippel. 2006. Parental effects on early life history traits of haddock *Melanogrammus aeglefinus*. Ices Journal of Marine Science 63(2):224-234.

Purchase, C. F., I. A. E. Butts, A. Alfonso-Fernandez, and E. A. Trippel. 2010. Thermal reaction norms in sperm performance of Atlantic cod (*Gadus morhua)*. Canadian Journal of Fisheries and Aquatic Sciences 67:498-510.

Quinn, T. P. 2005. The Behavior and Ecology of Pacific Salmon and Trout. University of Washington Press, Bethesda, Maryland.

Rand, P. S. 2008. *Oncorhynchus nerka*. In IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4. http://www.iucnredlist.org/apps/redlist/details/135301/0 (Accessed May 10, 2011).

Rand, P. S., S. G. Hinch, J. Morrison, M. G. G. Foreman, M. J. MacNutt, J. S. MacDonald, M. C. Healey, A. P. Farrell, and D. A. Higgs. 2006. Effects of river discharge, temperature, and future climates on energetics and mortality of adult migrating Fraser River sockeye slamon. Transactions of the American Fisheries Society 135:655-667.

Rasanen, K., and L. E. B. Kruuk. 2007. Maternal effects and evolution at ecological time-scales. Functional Ecology 21(3):408-421.

Reznick, D. N. 1991. Maternal effects in fish life histories. Pages 780-793 *in* E. C. Dudley, editor. The Unity of Evolutionary Biology. Discorides Press, Portland.

Rideout, R. M., E. A. Trippel, and M. K. Litvak. 2004. Paternal effects on haddock early life history traits. Journal of Fish Biology 64:695-701.

Roach, D. A., and R. D. Wuff. 1987. Maternal effects in plants. Annual Review of Ecology and Systematics 18:209-235.

Roff, D. A. 1998. The detection and measurement of maternal effects. Pages 83-96 *in* T. A. Mousseau, and C. W. Fox, editors. Maternal Effects as Adaptations. Oxford University Press, New York.

Rombough, P. J. 1985. Initial egg weight, time to maximum alevin wet weight, and optimal ponding times for Chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences 42(2):287-291.

Rossignol, O., J. J. Dodson, C. Marquilly, and H. Guderley. 2010. Do local adaptation and the reproductive tactic of Atlantic salmon (*Salmo salar* L.) affect offspring metabolic capacities? Physiological and Biochemical Zoology 83(3):424-434.

Rossiter, M. C. 1996. Incidence and consequences of inherited environmental effects. Annual Review of Ecology and Systematics 27:451-476.

Rossiter, M. C. 1998. The role of environmental variation in parental effects expression. Pages 112-134 *in* T. A. Mousseau, and C. J. Fox, editors. Maternal Effects as Adaptations. Oxford University Press, New York.

Rudolfsen, G., L. Figenschou, I. Folstad, J. T. Nordeide, and E. Soreng. 2005. Potential fitness benefits from mate selection in the Atlantic cod (*Gadus morhua*). Journal of Evolutionary Biology 18(1):172-179.

Rutherford, S. L. 2000. From genotype to phenotype: buffering mechanisms and the storage of genetic information. Bioessays 22(12):1095-1105.

Rutherford, S. L., and S. Lindquist. 1998. Hsp90 as a capacitor for morphological evolution. Nature 396(6709):336-342.

Saillant, E., M. Dupont-Nivet, P. Haffray, and B. Chatain. 2006. Estimates of heritability and genotype-environment interactions for body weight in sea bass (*Dicentrarchus labrax L.*) raised under communal rearing conditions. Aquaculture 254(1-4):139-147.

Saillant, E., A. Fostier, P. Haffray, B. Menu, J. Thimonier, and B. Chatain. 2002. Temperature effects and genotype-temperature interactions on sex determination in the European sea bass (*Dicentrarchus labrax* L.). Journal of Experimental Zoology 292(5):494-505.

Sandstrom, O., I. Abrahamsson, J. Andersson, and M. Vetemaa. 1997. Temperature effects on spawning and egg development in Eurasian perch. Journal of Fish Biology 51(5):1015-1024.

Schlichting, C. D., and M. Pigliucci. 1998. Phenotypic Evolution: A Reaction Norm Perspective. Sinauer Associates Inc., Sunderland, MA.

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

Shepherd, T. D., K. E. Costain, and M. K. Litvak. 2000. Effect of development rate on the swimming, escape responses, and morphology of yolk-sac stage larval American plaice, *Hippoglossoides platessoides*. Marine Biology 137(4):737-745.

Sogard, S. M. 1997. Size-selective mortality in the juvenile stage of teleost fishes: A review. Bulletin of Marine Science 60(3):1129-1157.

St Mary, C. M., C. G. Noureddine, and K. Lindstrom. 2001. Environmental effects on male reproductive success and parental care in the Florida flagfish *Jordanella floridae*. Ethology 107(11):1035-1052.

Stearns, S. C. 1992. The Evolution of Life Histories. Oxford University Press, Oxford, UK.

Stillwell, C. R., and C. W. Fox. 2005. Complex patterns of phenotypic plasticity: Interactive effects of temperature during rearing and oviposition. Ecology 86(4):924-934.

Strange, J. S. 2010. Upper thermal limits to migration in adult Chinook salmon: Evidence from the Klamath River basin. Transactions of the American Fisheries Society 139(4):1091-1108.

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

Strussmann, C. A., S. Moriyama, E. F. Hanke, J. C. C. Cota, and F. Takashima. 1996. Evidence of thermolabile sex determination in pejerrey. Journal of Fish Biology 48(4):643-651.

Sullivan, J. A., and R. J. Schultz. 1986. Genetic and environmental basis of variable sex ratios in laboratory strains of *Poeciliopsis lucida*. Evolution 40(1):152-158.

Suquet, M., Y. Normant, J. L. Gaignon, L. Quemener, and C. Fauvel. 2005. Effect of water temperature on individual reproductive activity of pollock (*Pollachius pollachius*). Aquaculture 243(1-4):113-120.

Takle, H., G. Baeverfjord, and O. Andersen. 2004. Identification of stress sensitive genes in hyperthermic Atlantic salmon (*Salmo salar*) embryos by RAP-PCR. Fish Physiology and Biochemistry 30(3-4):275-281.

Taranger, G., E. Vikingstad, U. Klenke, I. Mayer, S. Stefansson, B. Norberg, T. Hansen, Y. Zohar, and E. Andersson. 2003. Effects of photoperiod, temperature and GnRHa treatment on the reproductive physiology of Atlantic salmon (*Salmo salar* L.) broodstock. Fish Physiology and Biochemistry 28(1-4):403-406.

Taranger, G. L., and T. Hansen. 1993. Ovulation and egg survival following exposure of Atlantic salmon, *Salmo salar* L., broodstock to different water temperatures. Aquaculture and Fisheries Management 24:151-156.

Taylor, E. B., and C. J. Foote. 1991. Critical swimming velocities of juvenile sockeye salmon and kokanee, the anadromous and non-anadromous forms of *Oncorhynchus nerka* (Walbaum). Journal of Fish Biology 38(3):407-419.

Taylor, E. B., and J. D. McPhail. 1985a. Burst swimming and size-related predation of newly emerged coho salmon *Oncorhynchus kisutch*. Transactions of the American Fisheries Society 114(4):546-551.

Taylor, E. B., and J. D. McPhail. 1985b. Variation in burst and prolonged swimming performance among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. Canadian Journal of Fisheries and Aquatic Sciences 42(12):2029-2033.

Taylor, S. G. 2008. Climate warming causes phenological shift in Pink Salmon, *Oncorhynchus gorbuscha*, behavior at Auke Creek, Alaska. Global Change Biology 14(2):229-235.

Teletchea, F., A. Fostier, E. Kamler, J. N. Gardeur, P. Y. Le Bail, B. Jalabert, and P. Fontaine. 2009. Comparative analysis of reproductive traits in 65 freshwater fish species: application to the domestication of new fish species. Reviews in Fish Biology and Fisheries 19(4):403-430.

Tessema, M., A. Muller-Belecke, and G. Horstgen-Schwark. 2006. Effect of rearing temperatures on the sex ratios of *Oreochromis niloticus* populations. Aquaculture 258(1-4):270-277.

Tierney, K. B., D. A. Patterson, and C. J. Kennedy. 2009. The influence of maternal condition on offspring performance in sockeye salmon *Oncorhynchus nerka*. Journal of Fish Biology 75(6):1244-1257.

Trippel, E. A., G. Kraus, and F. Koster. 2005. Maternal and paternal influences on early life history traits and processes of Baltic cod *Gadus morhua*. Marine Ecology Progress Series 303:259-267.

Trippel, E. A., and S. R. E. Neil. 2004. Maternal and seasonal differences in egg sizes and spawning activity of northwest Atlantic haddock (*Melanogrammus*

aeglefinus) in relation to body size and condition. Canadian Journal of Fisheries and Aquatic Sciences 61(11):2097-2110.

Truscott, B., D. R. Idler, Y. P. So, and J. M. Walsh. 1986. Maturational steroids and gonadotropin in upstream migratory sockeye salmon. General and Comparative Endocrinology 62:99-110.

Turner, M. A., M. R. Viant, S. J. Teh, and M. L. Johnson. 2007. Developmental rates, structural asymmetry, and metabolic fingerprints of steelhead trout (*Oncorhynchus mykiss*) eggs incubated at two temperatures. Fish Physiology and Biochemistry 33(1):59-72.

Tveiten, H., and H. K. Johnsen. 1999. Temperature experienced during vitellogenesis influences ovarian maturation and the timing of ovulation in common wolffish. Journal of Fish Biology 55(4):809-819.

Tveiten, H., S. E. Solevaag, and H. K. Johnsen. 2001. Holding temperature during the breeding season influences final maturation and egg quality in common wolffish. Journal of Fish Biology 58(2):374-385.

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

Tymchuck, W., R. Devlin, and R. Withler. 2006. The role of genotype and environment in phenotypic differentiation among wild and cultured salmonids. Canadian Technical Report of Fisheries and Aquatic Sciences 2450 IV.

Van der Kraak, G., and N. W. Pankhurst. 1997. Temperature effects on the reproductive performance of fish. Pages 159-177 *in* C. M. Wood, and D. G. McDonald, editors. Global Warming: Implications for freshwater and marine fish. Cambridge University Press, Cambridge.

Van der Meeren, T., and V. P. Ivannikov. 2006. Seasonal shift in spawning of Atlantic cod (*Gadus morhua* L.) by photoperiod manipulation: egg quality in relation to temperature and intensive larval rearing. Aquaculture Research 37(9):898-913.

Velson. 1980. Embryonic development in eggs of sockeye salmon, *Oncorhynchus nerka.* Canadian Special Publication of Fisheries and Aquatic Science No. 49: 1-19.

Vikingstad, E., E. Andersson, B. Norberg, I. Mayer, U. Klenke, Y. Zohar, S. O. Stefansson, and G. L. Taranger. 2008. The combined effects of temperature and GnRHa treatment on the final stages of sexual maturation in Atlantic salmon (*Salmo salar* L.) females. Fish Physiology and Biochemistry 34(3):289-298.

Wargelius, A., P. G. Fjelldal, and T. Hansen. 2005. Heat shock during early somitogenesis induces caudal vertebral column defects in Atlantic salmon (*Salmo salar*). Development Genes and Evolution 215(7):350-357.

Watkins, T. B. 2000. The effects of acute and developmental temperature on burst swimming speed and myofibrillar ATPase activity in tadpoles of the pacific tree frog, *Hyla regilla*. Physiological and Biochemical Zoology 73(3):356-364.

Watts, M., N. W. Pankhurst, and H. R. King. 2004. Maintenance of Atlantic salmon (*Salmo salar*) at elevated temperature inhibits cytochrome P450 aromatase activity in isolated ovarian follicles. General and Comparative Endocrinology 135(3):381-390.

Webb, M. A. H., J. P. Van Eenennaam, S. I. Doroshov, and G. P. Moberg. 1999. Preliminary observations on the effects of holding temperature on reproductive performance of female white sturgeon, *Acipenser transmontanus* Richardson. Aquaculture 176(3-4):315-329.

Webb, M. A. H., J. P. Van Eenennaam, G. W. Feist, J. Linares-Casenave, M. S. Fitzpatrick, C. B. Schreck, and S. I. Doroshov. 2001. Effects of thermal regime on ovarian maturation and plasma sex steroids in farmed white sturgeon, *Acipenser transmontanus*. Aquaculture 201(1-2):137-151.

Wedekind, C., G. Evanno, D. Urbach, A. Jacob, and R. Muller. 2008. 'Good-genes' and 'compatible-genes' effects in an Alpine whitefish and the information content of breeding tubercles over the course of the spawning season. Genetica 134(1):21-30.

Whitlock, M. C., and D. Schluter. 2009. The Analysis of Biological Data. Roberts & Co. Publishers, Greenwood Village, CO.

Willmore, K. E., and B. Hallgrimsson. 2005. Within individual varation: Developmental noise versus developmental stability. Pages 191-218 *in* B. Hallgrimsson, and B. K. Hall, editors. Variation: A central concept in biology. Elsevier Academic Press, London.

Wolf, J. B., and M. J. Wade. 2009. What are maternal effects (and what are they not)? Philosophical Transactions of the Royal Society B-Biological Sciences 364(1520):1107-1115.

Yamamoto, T. S., W. Kobayashi, and T. Kuramoto. 1996. Twin malformation induced in chum salmon eggs by elevated water temperature, with a suggestion as to its mechanism. Canadian Journal of Zoology 74(3):485-491.

Yoneda, M., and P. J. Wright. 2005. Effects of varying temperature and food availability on growth and reproduction in first-time spawning female Atlantic cod. Journal of Fish Biology 67(5):1225-1241.

APPENDIX A: Physiological status of Weaver Creek sockeye parent spawners

Table A.1. Physiological variables sampled from females at the time of gamete collection and correlations with offspring survival. Individual values and means are given for several plasma ions and hormones from each female used in the 2008 fertilization experiment (refer to Chapter 3). Correlations use average values for offspring survival (S-ha and S-fry), and are conducted only among half-sib families (females 1 - 5 crossed to male A, total n = 5). The asterisk indicates a significant correlation with $P < 0.05$ and the correlation coefficient (r) is given. The bracketed signs indicate a positive (+) or negative (-) trend. For information on protocols for plasma ion and hormone sampling, refer to Farrell et al. (2001).

FEMALE ID		Mass (kg)	Standard length (cm)	10-egg wet mass (g)	10-egg dry mass (g)	Na+ ($mmol \cdot L^{-1}$)	K+ ($mmol \cdot L^{-1}$)	Cl- ($mmol \cdot L^{-1}$)	Osmolality (mosmol)	Glucose ($mmol \cdot kg^{-1}$)	Lactate ($mmol \cdot L^{-1}$)	Cortisol ($ng \cdot ml^{-1}$)	Estradiol ($ng \cdot ml^{-1}$)	Testoterone ($ng \cdot ml^{-1}$)
1		2.526	53.5	1.422	0.459	141	1.6	107.2	281	7.67	5.45	416.1	0.249	53.5
2		2.514	53.2	1.187	0.454	154	4.8	121.9	296	7.15	1.84	269.6	0.334	51.9
3		2.787	54.8	1.127	0.41	142	3.9	112.2	285	8.41	3.09	345.0	0.086	8.6
4		4.025	61.0	1.489	0.576	148	3.7	118.7	295	7.39	5.81	306.5	0.185	11.2
5		2.793	54.0	1.187	0.448	134	4.9	96.0	277	1.75	15.40	476.7	0.117	5.7
6		3.559	56.0	1.383	0.516	151	2.8	114.6	299	10.40	3.85	310.7	0.270	55.6
Mean		**3.304**	**55.4**	**1.299**	**.477**	**145**	**3.6**	**111.7**	**289**	**7.13**	**5.91**	**354.1**	**0.207**	**31.1**
Correlation matrix														
12ºC	S-ha[+]	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	(-)	P > 0.05	P > 0.05	***r = 0.89**	P > 0.05	P > 0.05	P > 0.05	P > 0.05
	S-fry[†]	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	(-)	P > 0.05	P > 0.05	***r = 0.90**	P > 0.05	P > 0.05	P > 0.05	P > 0.05
14ºC	S-ha[+]	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	(+)	P > 0.05
	S-fry[†]	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	(+)	P > 0.05
16ºC	S-ha[+]	P > 0.05	P > 0.05	P > 0.05	(+)	P > 0.05	(-)	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
	S-fry[†]	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	(-)	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05

[+] Offspring survival (%) from fertilization to hatch

[†] Offspring survival (%) from fertilization to 3-week-old fry

Table A.2. Physiological variables sampled from males at the time of gamete collection and correlations with offspring survival. Individual values and means are given for several plasma ions and hormones from each male used in the 2008 fertilization experiment (refer to Chapter 3). Correlations use average values for offspring survival (S-ha and S-fry), and are conducted only among half-sib families (males B - F crossed to female 6, total n = 5). No correlations were found to be significant ($P < 0.05$), indicated by 'NS' or 'not significant'. For information on protocols for plasma ion and hormone sampling, refer to Farrell et al. (2001).

MALE ID		Mass (kg)	Standard length (cm)	Na+ ($mmol \cdot L^{-1}$)	K+ ($mmol \cdot L^{-1}$)	Cl- ($mmol \cdot L^{-1}$)	Osmolality ($mOsm \cdot kg^{-1}$)	Glucose ($mmol \cdot L^{-1}$)	Lactate ($mmol \cdot L^{-1}$)	Cortisol ($ng \cdot ml^{-1}$)	Estradiol ($ng \cdot ml^{-1}$)	Testoterone ($ng \cdot ml^{-1}$)
A		3.989	61.0	139	5.3	112.8	287	7.70	10.30	454.5	0.066	1.6
B		4.107	55.1	151	3.1	111.3	300	7.40	3.43	193.5	0.105	19.0
C		2.728	56.5	133	6.4	95.1	279	4.23	16.40	480.3	0.049	2.0
D		3.095	59.8	146	2.7	109.6	285	8.86	5.81	432.8	0.082	26.0
E		2.209	53.8	143	3.5	107.5	335	9.77	2.93	491.0	0.068	18.6
F		2.879	57.7	130	3.1	96.4	262	9.76	4.99	565.9	0.028	1.9
Mean		**3.167**	**57.3**	**140**	**4.0**	**105.4**	**291**	**7.95**	**7.31**	**436.3**	**0.067**	**11.5**
Correlation matrix												
12ºC	S-ha[+]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	S-fry[†]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
14ºC	S-ha[+]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	S-fry[†]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
16ºC	S-ha[+]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	S-fry[†]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

+ Offspring survival (%) from fertilization to hatch

† Offspring survival (%) from fertilization to 3-week-old-fry