VARIATION IN EMBRYONIC THERMAL TOLERANCE AMONG POPULATIONS
OF SOCKEYE SALMON: OFFSPRING SURVIVAL, GROWTH, AND HATCH TIMING
IN RESPONSE TO ELEVATED INCUBATION TEMPERATURE

by

Charlotte Kathryn Whitney

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Abstract

Populations of Pacific salmon are genetically and morphologically distinct across large watersheds, and these differences may reflect long-term adaptation to environmental factors such as temperature. While climate warming is predicted to affect sockeye salmon, it is likely that such impacts will happen differentially across life stages and populations. Given that selective pressures during early development plays an important role in lifetime fitness, and that elevated water temperatures can critically affect embryo success, this thesis focuses on inter-population differences in offspring response to supra-optimal temperatures during incubation.

Variation in embryonic thermal tolerance was explored among populations of sockeye salmon using a common garden approach. The gametes of 15-20 families per population (N = 9) were incubated at water temperatures of 10°C, 14°C, and 16°C. Survival from fertilization to hatch varied significantly by temperature and population, and crossing reaction norms showed an interaction of genotype (population) and environment (incubation temperature). Thermal tolerance within the study was related to historical temperatures during early development in nature. From this correlation it seems that population thermal adaptation may exist, and be driven by elevated spawning ground temperatures.

The same fertilization experiment was used to evaluate differences in egg size among populations, and to test the effect of temperature, population, and egg size on offspring size and hatching characteristics. Egg size varied among groups and was not related to hatch timing variation, but was tightly correlated to alevin size. Alevin length but not mass was significantly related to incubation temperature, perhaps due to a theoretical tradeoff between development rate and metabolic rate at high temperatures. Most embryos seemed to compensate for increased growth rates at high temperatures by requiring more thermal units to hatch than at lower temperatures.
Overall, I found that populations of sockeye salmon responded differently to thermal stress during embryo development, and populations responded best to temperatures that reflected their historical natal thermal regime. In the context of climate change, these results show that inter-population thermal tolerance may influence future selection among populations, and additionally, that this intraspecific thermal adaptation will be important in ensuring population, and therefore species, persistence.
Preface

This work follows in the footsteps of a large collaborative research program studying the effects of warming water temperatures on adult migrating and spawning sockeye salmon. I held primary responsibility for the experimental design, fieldwork planning, data analysis, and writing of chapters within this thesis. Co-authorship in both Chapters 2 and 3 acknowledges the expertise, advise, and reviews of Scott Hinch and David Patterson; and a large dataset provided by DFO-PSC by David Patterson was integral to Chapter 2. Field collections were conducted under the leadership and logistical support of David Patterson. All experimental procedures were carried out under the pre-approval of the University of British Columbia Animal Care Committee (#A08-0388) and in accordance with the Canadian Council of Animal Care guidelines.

Chapter 2: Differences in embryonic thermal reaction norms for survival among populations of sockeye salmon

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Chapter 3: Population and water temperature trump egg size in regulating early development timing and offspring traits in salmonids

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Chapter 1: Introduction

Background: Population variation and adaptation

Discreet groups of conspecific individuals within a species that do not interbreed create genetic variation within that species. These ‘populations’ may be genetically distinct from one another, thanks to a suite of evolutionary processes including genetic drift, mutation, and natural selection. Over time, genetic drift can result in reproductively isolated populations within a small or large geographic area. In addition, natural selection, acting upon traits that affect the individual’s lifetime fitness, can result in population divergence driven by different selective regimes within the populations’ environments (Conover et al. 2006). For most species, and particularly those whose habitat spans a wide range of geographical and ecological conditions, population differentiation allows species persistence through time, granting a buffer against environmental change as selection processes favor some genotypes over others. Correlations between environments and phenotypes can provide evidence for local adaptation among populations, and the role that this adaptation plays as a mechanism of selection can drive population fitness and persistence (Hutchings 2011). Within species, populations have typically evolved adaptive mechanisms and responses unique to their local environmental conditions (Taylor 1991). These adaptive differences suggest that different populations display unequal suitability to specific environmental states, and that they respond to an altered environment through phenotypic changes that result in a functional improvement in survival, reproduction, or growth (Stearns 1992, Beacham 1988).
Thermal adaptation and populations

Changing thermal conditions can drive adaption within and among species. For example, Charmantier et al. (2008) used a long-term population study on the great tit (Parus major) to show that shifting reproductive timing and behavioural response allowed the local population to adapt to changing thermal regimes. Despite significant changes in seasonal temperatures, the birds maintained a close relationship with hatch timing and food availability by changing their reproductive timing, suggesting that selection on those traits were adaptive and allowed the population to maintain fitness within their environment. As heritable variation in such phenotypic responses to thermal conditions is required in order for potential thermal adaptation to exist (Angilletta et al. 2002, Bryant 2008, reviewed in Carlson and Seamons 2008), exploring such variation among populations can show that some species, such as the great tit, may have the potential to adapt to changing environmental regimes (Jensen et al. 2008).

In fish, high offspring survival during embryogenesis and early development is critical for determining population success in the long term (Jensen et al. 2008). Year-class recruitment is largely based on a fitness response to environmental conditions during early development (Bradford 1995). Selection during early life history determines which genotypes will produce the next generation of offspring, and as such environmental effects that drive embryo selection can result in rapid population change (Hendry and Kinnison 1999). Moreover, the embryonic stages are a meaningful phase to assess population differences in direct response to thermal variation, as later organismal demands such as movement, feeding, and social structure are not a factor. Variation in offspring phenotypes is what allows certain individuals to persist through environmental change, as survival during the larval and early life stages and high mortality during this period is directly linked to environmental factors (Kamler 2008).
Pacific salmon life history and early life stage development

Pacific salmon (\textit{Oncorhynchus} spp.) are anadromous, spawning in freshwater and rearing there as juveniles before migrating to the ocean where they mature prior to returning to spawn in their natal areas (Groot and Margolis 1991, Quinn 2005). Pacific salmon are also semelparous, meaning that they have only one chance to migrate to natal spawning streams and successfully reproduce in order to secure lifetime fitness before senescence and death. During their spawning migration, Pacific salmon undergo significant physiological changes in preparation for reproduction. In females, the process of egg production requires significant energy reserves, so much so that parents must balance investment into eggs with energy needed to complete the migration and spawn (Kinnison \textit{et al.} 2001, Crossin \textit{et al.} 2004). Eggs begin development as oocytes through six steps prior to spawning: oogenesis, primary oocyte growth, the cortical alveolus stage, vitellogenesis, final maturation, and subsequent ovulation (Tyler and Sumpter 1996), all of which can be directly or indirectly affected by changes in environmental conditions (Brooks \textit{et al.} 1997, Kamler 2008). Males develop spermatozoa through the process of spermatogenesis, before release in the spawning event (spermiation). Both males and females also undergo the energetically costly development of secondary sexual characteristics in order to attract mates, including development of sexually dimorphic features in males including dorsal ‘humps’, jaw enlargement, and for both sexes, coloration changes (Groot and Margolis 1991, Quinn 2005). During this reproductive development stage, water temperature can modify gametogenesis, increase metabolic costs, and negatively affect egg development and subsequent gamete viability (reviewed in Pankhurst and King 2010, Pankhurst and Munday 2011). Even small increases in temperature beyond thermal optima can affect gametogenesis prior to spawning, embryo development during incubation, and survival to hatch (Rombough 1994,
Spawning and fertilization success may also be affected by physical disturbance or temperature (Jensen and Alderice 1993).

Following a successful spawning event, fertilized eggs incubate in gravel redds for several months prior to hatching as alevin. During early embryogenesis, preliminary cell division is followed by epiboly (cellular differentiation) and initial eye development. Subsequently, eggs undergo organogenesis, yolk development (vascularization) and reach the ‘eyed’ stage when completed eye pigmentation occurs. Hatching enzymes are finally produced, allowing the embryo to emerge from the egg capsule (as described by Velson 1980). At this point alevin subsist on their endogenous yolk reserve, which provides sufficient energy for maintenance metabolism and growth until they emerge from the gravel as fry, necessitating foraging for food.

These early life stages are characterized by high mortality (~ 91 - 95%, Bradford 1995, Kamler 2008). Throughout this process, the sessile embryos are exposed to a wide range of environmental conditions that may affect development. Water temperature, as the primary determinant of development for incubating embryos, affects incubation rate, hatch timing, musculature development, and ultimately survival (Quinn 2005). However, genetic differences among and within populations can also affect development, as a driver of phenology, or reproductive and development timing (Beacham and Murray 1989, 1990). Differences in maternal investment through egg size also interacts with incubation temperature, and it seems that larger eggs require more incubation time to hatch and to complete yolk absorption, although this pattern is not consistent across populations (Brannon 1987). In the limited amount of research on early development population differences, it is apparent that intra-species variation in hatch timing is complex, and that these differences often reflect local adaptation to thermal environments (Brannon 1987, Beacham and Murray 1987, 1989, Hendry et al. 1998, Kamler 2008, Miller et al. 2012).
The influence of temperature and climate change on Pacific salmon

In fishes, temperature, the ‘master’ abiotic factor for ectothermic fishes, directly affects all physiological processes (Brett 1971, Fry 1971, Bryant 2009, Farrell et al. 2009). These effects are amplified in developing embryos, as temperature regulates embryonic development, immune function, hatching, growth rates, and reproductive maturation (Warren and Davis 1967, Ojanguren and Brana 2000, Alcorn et al. 2002). Embryonic thermal conditions are of particular import for stenothermic fish such as salmonids, as adaptation during sensitive sessile embryonic life stages has cumulative effects for lifetime performance and fitness (Brannon 1987, Blaxter 1992, Rombough 1997, Janhunen et al. 2010). In addition, their anadromous lifecycle means they are exposed to both marine and freshwater ecosystems and any potential environmental changes therein (Bryant 2009, Martins et al. 2011).

Globally, the effects of climate change have now been observed to affect the behavior and distribution of species across all ecosystems, and such changes are only expected to accelerate in the future (Parmesan 2006, IPCC 2007). Species that inhabit a range of ecosystems and experience environmental variation throughout their life history may be especially at risk due to the additive effects of changing conditions over time and space. Evolutionary response to climate change is largely driven by reproductive timing, or phenology, and by reproductive success through adaption to environmental conditions (Bradshaw and Holzapfel 2008). As the freshwater portion of the salmonid life cycle is thus thought to more heavily influence lifetime fitness (Bradford 1995), understanding temperature effects during early development is critical. Within this period, salmonids are perhaps most susceptible to temperature changes during their immobile embryonic and alevin stages (Brannon 1987), when elevated temperatures can result in sub-lethal or lethal effects on developing eggs and larvae (Blaxter 1992). With small tolerance
limits around thermal optima (Rombough 1997), adaptation to thermal conditions during this stage is of great importance (Janhunen et al. 2010).

In the northeast Pacific, in-river temperatures are increasing, driven by broad-scale climate change as well as habitat loss and degradation. Indeed, summer temperatures in the Fraser River have increased by ~1.8°C since the 1950’s, with 13 of the past 20 summers exhibiting the warmest water temperatures on record (Patterson et al. 2007), and a similar increase has been observed in the Columbia River (Quinn and Adams 1996, Hodgson and Quinn 2002, Crozier et al. 2008). Climate models suggest that this warming trend will continue at a rate of at least 0.12°C per decade (Ferrari et al. 2007), and that temperatures will increase disproportionately more during winter months while eggs are incubating (Walker and Sydneysmith 2008). In addition, the frequency of extreme or abnormal temperature events is predicted to increase (Pike et al. 2008, Hague et al. 2011), which may push certain populations to or beyond their critical thermal maxima (Crozier et al. 2008, Reed et al. 2011). Although periods of abnormally high temperatures have been correlated with mortality and thermal stress for populations of Pacific salmon within the region (Macdonald et al. 2010, Martins et al. 2011), there has been limited research into the thermal tolerance or thermal adaptive potential of Pacific salmonids across life stages (e.g. Eliason et al. 2011, Reed et al. 2011; reviewed in Healey 2011).

Experimental research has found evidence of broad differences among sockeye salmon populations in juvenile thermal tolerance (Beacham and Murray 1989, Hendry et al. 1998). Other studies have suggested that egg and juvenile traits including size at hatch (Beacham and Murray 1989), yolk absorption efficiency (Brannon 1987), and egg development rate (Bams 1969) (summarized in Linley 1988, 1993) may respond differentially to temperature depending on natal origin. Taken together, these findings suggest that variation among populations in thermal
tolerance may reflect adaptations to historical incubation temperatures, and that such local adaptation may drive adaptive potential to climate change (i.e. Jensen et al. 2008). Though individual lifetime adaptation to temperature can occur through exposure driven acclimation (Angilletta et al. 2002), generations are required for selection to alter key thermal performance functions at a population level (Kingsolver and Huey 1998). The potential for populations to successfully adapt therefore depends on the temporal scale of thermal change, although thermal sensitivity may be one aspect of physiological performance that can respond quickly to environmental change (Angilletta et al. 2002). However, if climate change effects accelerate as predicted (IPCC 2007), adaptive potential may prove to be insufficient to maintain population or species viability.

**Focal species: British Columbia sockeye salmon**

Pacific salmon species are important economically, culturally, and ecologically throughout the northeast Pacific. Both commercial and recreational fisheries depend on the continued abundance of the five species of Pacific salmon, and are worth millions of dollars to local, regional, and national economies of both the United States and Canada (BCMOE 2007). First Nations groups consider salmonids as an important part of their culture and non-market based economy, for food as well as a connection with traditional ecological knowledge (Jacob et al. 2010). Ecologically, Pacific salmon provide an important source of nitrogen and other marine-derived nutrients to terrestrial systems, increasing productivity for nearby plants and trees, particularly in small stream systems (Helfield and Naiman 2001, Hocking and Reynolds 2011). Moreover, many animal species (i.e. coastal bear populations, Ursus spp.) depend on productive salmon populations as an integral portion of their annual energy budget (Schindler et al. 2003).
In British Columbia, hundreds of genetically distinct populations of sockeye salmon (*O. nerka*) spawn throughout August to November each year in reaches of the Fraser, Skeena, and Columbia rivers, as well as smaller coastal systems (Groot and Margolis 1991, Beacham *et al.* 2004a). These populations employ incredibly variable life history strategies and are thus exposed to a wide range of environmental conditions. Migration effort, elevation change, and distance varies between coastal-spawning stocks, with short migrations of ~100km, and interior-spawning stocks, some of which travel over 1200 km to reach their natal spawning streams (Groot and Margolis 1991). A strong homing tendency and low incidence of straying (returns to non-natal streams) has resulted in these distinct populations with measureable physiological and behavioral differences, linked to unique life history strategies employed by different groups to order to maximize fitness (Ricker 1972, Beacham and Murray 1989, 1993; Crossin *et al.* 2004, Quinn 2005, Bryant 2009, Eliason *et al.* 2011). Optimal thermal conditions vary within the watershed, but are thought to be in the range of 6 to 8°C during embryo development (Beacham and Murray 1989, McCullough *et al.* 2001); temperatures above that range during incubation may have deleterious consequences for survival and phenotypically plastic traits that relate to lifetime fitness.

Although sockeye salmon in the Fraser River are the second most abundant salmonid species in the watershed, several populations have been in precipitous decline in recent decades, and one (Cultus Lake) is designated as endangered under the Committee for the Status of Endangered Wildlife in Canada (COSEWIC 2003, Beacham *et al.* 2004b). Indeed, this phenomenon prompted a federal judicial inquiry into the state of Fraser River sockeye salmon (the Cohen Commission; [www.cohencommission.ca](http://www.cohencommission.ca)), and one of its main goals is to better understand the effects of climate change on this species (Hinch and Martins 2011).
Despite previous research comparing divergence and differentiation among multiple populations of salmonids in various locales, and evidence of variable thermal tolerance among populations of adult sockeye salmon in British Columbia, embryonic thermal tolerance has not been thoroughly investigated within this region, nor linked with historical thermal experience. These experiments used sockeye salmon from eight geographically distinct populations that spawn in the Fraser River watershed, and one that spawns in the upper reaches of the Columbia River. These populations were chosen to represent a range of migration distance, elevation, spawning time, and thermal regime. In order to provide better information for the sustainable management of sockeye salmon, it is increasingly evident that a population specific understanding of physiological response, developmental characteristics, phenotypic plasticity, and heritable thermal tolerance at all life stages is required (e.g. Reed et al. 2011).

**Thesis goal and objectives**

The overall goal of this thesis was to investigate differences in early developmental response to temperature in survivorship and growth among populations of sockeye salmon within British Columbia. There were two main objectives. The first objective was to test embryonic thermal tolerance among many distinct populations of sockeye salmon, and to explore how those populations respond differently to early development thermal stress. The second objective was to determine whether that same temperature stress affected growth and development characteristics of the offspring, and whether those responses varied by among populations. Both of these objectives were addressed and are outlined in separate chapters herein:
Chapter 2 describes the results from a laboratory-based incubation study in which families representing nine populations of sockeye salmon were raised under three temperature treatments during early development. Differences in offspring survival among populations were compared across the gradient of thermal treatment and reactions norms for survival were created, showing genetic and environmental influence on thermal tolerance. Furthermore, historical thermal history was correlated to this experimental response, showing the potential role of adaptation in driving selective processes among populations. Collectively these results show the importance of inter- and intra-population variation in thermal tolerance in ensuring species viability under warming conditions.

Chapter 3 reports the additional results from analyses of the effect of temperature on offspring size and hatching characteristics among populations. Differences in egg size, hatching characteristics, and progeny size were examined across populations, as well as the influence of incubation temperature. These findings showed that both thermal regime and population shape growth and timing characteristics, which can affect phenology and competitive selection for offspring, in turn driving population structure.

Chapter 4 is a synthesis of the above and conclusions. Within this chapter I also suggest areas for future research to order to elucidate the thermal adaptive potential and heritable aspects of the thermal response among populations.
Chapter 2: Provenance matters for embryonic thermal tolerance: differences in survival among populations of sockeye salmon

Introduction

There is an increasing need to understand how various taxa, species, and populations will respond to shifts in their thermal environment (Baumann and Conover 2011) with both the continued threat of anthropogenic climate change to natural systems (IPCC 2007), and the poorly understood fitness consequences of those regime changes (Crozier et al. 2008). Adaptive differences in response to current environmental conditions among fish populations (Eliason et al. 2011) suggest that different populations will display unequal suitability to future environmental states. A response to an altered thermal environment through phenotypic changes could result in a functional improvement in survival, reproduction, or growth (Stearns 1992, Beacham 1988). Exploring variation in thermal tolerance among populations can be used to highlight the potential for different populations to adapt to climate change (Jensen et al. 2008).

The cumulative impacts of climatic shifts will likely affect all taxa, but aquatic species that exist within freshwater ecosystems are particularly at risk due to changes in both hydrology (e.g. frequency of stochastic flood events) and thermal regimes (e.g. shifting cyclical thermal patterns) (Pike et al. 2008). The life history diversity, distinct spawning population structure, and prior research connecting survival to temperature effects make sockeye salmon (*Oncorhynchus nerka*) a good species to study population level variation in thermal tolerance. Sockeye salmon are an anadromous poikilothermic fish that remain immobile during incubation in a variety of freshwater streams, rivers and lakes for 4 to 10 months, making developing embryos especially vulnerable to increasing freshwater temperatures (Bryant 2009; Healey 2011; Martins et al. 2012). Within British Columbia, Canada, millions of sockeye salmon representing hundreds of genetically and geographically distinct populations spawn in the Fraser, Skeena, and Columbia
watersheds, as well as smaller coastal systems, from June to December each year (Groot and Margolis 1991, Beacham et al. 2004a). The unique environmental conditions experience by each population have already been linked to distinct life history strategies employed by different groups in order to maximize fitness (Ricker 1972, Quinn 2005, Eliason et al. 2011). Most prior research on embryo development and temperature has focused on population differences in parentally mediated traits, such as egg size and development timing. These may be due in part to local adaptation to thermal environments (Berg and Moen 1999), as growth rate, encompassing a suite of developmental and physiological processes, is highly sensitive to water temperature (Angilletta et al. 2002; see Chapter 3 of this thesis). Predicted increases in incubation temperatures within the region (Walker and Sydneysmith 2008) could differentially impact fish survival across populations of sockeye salmon if the current traits associated with thermal acclimation vary in response to future temperature change (Bradshaw and Holzapfel 2008, Healey 2011).

While morphological differences among populations are well documented, there has been limited experimental research on broad differences in population-level adaptive thermal tolerance whether by genetic differences or phenotypically plastic responses to environmental variation (reviewed in Burt et al. 2011). This variable response among populations can be represented by reaction norms (see Schlichting and Pigliucci 1998), where phenotypic variation in response to the environment, or a genotype by environment interaction, can be visually depicted by crossed reaction norms. Reaction norms can represent a suite of behavioural, morphological, meristic, life history, or physiological traits, but in terms of lifetime fitness, using a reaction norm approach for survival is one of the best means of assessing adaptive potential of populations (Hutchings 2004, 2011). Several studies have used these methods to assess thermal tolerance in salmonids via survival under varying conditions in a common garden trial (e.g.
Hendry et al. 1998, Jensen et al. 2008, reviewed in Hutchings 2011), a powerful way to explore phenotypic plasticity in reaction to environmental variation. The design of a common garden experiment assumes that environmental effects are minimized or eliminated, so that a differential response in the trait(s) of interest thus reflects additive genetic variation.

There are several key empirical examples of examining thermal tolerance in embryo development among salmonid populations using a common-garden environment. Hendry et al. (1998) found significant differences in survival to hatching among four recently differentiated sockeye salmon populations from Lake Washington, although the sample sizes were small and the divergent populations did not represent a wide range of environmental conditions. Within British Columbia, Beacham and Murray (1989) looked at only two populations under cold temperatures and found that an interior-spawning population of Fraser River sockeye salmon (Adams River) had better survival during embryo development at lower incubation temperatures than a coastal-spawning population (Weaver Creek). They proposed that this was attributable to probable adaptation to lower historical incubation temperatures in the interior of the province, but did not suggest how populations might respond to elevated temperatures as would be expected with climate change. Further afield, Jensen et al. (2008) found local adaptation in early developmental traits by common garden trials among populations of brown trout (Salmo trutta) in Norway, and although they used only moderately elevated experimental temperature treatments, suggested potential differences in adaptive variation to climate change. Thus, thermal tolerance may be found to differ within a species through evidence of phenotypic plasticity (Gienapp et al. 2008) whereby evolutionary selection upon thermal tolerance will drive selective pressures on populations and species.

However, a variable response to environmental conditions may only be indicative of phenotypic plasticity, not necessarily adaptive potential, unless the heritability of that variation is
known. To date most observed shifts in species survival due to climate change have likely been due to plastic responses within a genotype, rather than by evolutionary selection (Gienapp et al. 2008). Adaptation to environmental factors such as temperatures are likely driven more by the extreme events, rather than long-term average trends or steady shifts in climatic conditions (Jentsch et al. 2007). The potential for populations to successfully adapt therefore depends on the temporal scale of thermal change. If climate change occurs at too rapid a rate, then thermal adaptive potential could be insufficient to maintain population viability. There is some evidence that salmonids may be able to quickly adjust to environmental change (Angilletta et al. 2002) and adapt to new regimes within 10 to 20 generations (Hendry et al. 1998; Hendry and Kinnison 1999).

In sockeye salmon there has been a large focus on the impact of high migration temperatures during adult migration, more specifically population specific thermal tolerance (e.g. Martins et al. 2012) and the predictive ability to adapt to future climate change (Eliason et al. 2011; Hague et al., 2011, Reed et al. 2011). This work has suggested that evolutionary response to climate change will likely be driven by a combination of behavioral changes in reproductive timing (e.g. migration timing, Reed et al. 2011), and by physiological adaptation to environmental conditions (e.g. thermal tolerance, Eliason et al. 2011). However, it is not known whether populations that currently have a high thermal tolerance during the adult stage, will also tolerate higher temperatures during early life stages. This would imply the physiological basis for thermal tolerance exhibited by adults may extend to include the non-motile embryo stages.

Early life stage thermal sensitivity was explored in nine populations of sockeye salmon from British Columbia, Canada through a common garden incubation study, using full-sibling crosses across all groups. As thermal tolerance among populations can reflect local adaptation by natural selection (Haugen and Vollestad 2000), the following predictions were made: (1)
populations would differ in their embryonic survival response to increased temperature, and that (2) thermal tolerance would reflect historical incubation temperatures from natal spawning grounds. Two additional expectations were as follows: that within population variation in thermal tolerance would vary, indicative of within-population plasticity to temperature changes and future potential to adapt; and that upstream migration temperatures would also be positively related to embryo thermal tolerance, suggesting that thermal tolerance can cross life-history stages.

**Materials and methods**

*Fish Capture and Gamete Collection*

I examined populations of sockeye salmon from the Fraser (n = 8) and Columbia (n = 1) Rivers (Figure 2.1). The Fraser River populations are geographically distinct within the watershed, reflect a range of spawning ground and adult migration thermal conditions, and exhibit a range of life history characteristics (Table 2.1). Okanagan River sockeye from the Columbia River system were included in the study because their spawning adults are some of the most thermally tolerant of all sockeye salmon (Hyatt *et al.* 2003)

Reproductively mature adult sockeye salmon (n = 15-20 unique pairs) were captured from the spawning grounds of natal subwatersheds during peak spawning. All adults used in this study were sacrificed immediately via concussion to the head. All procedures were in accordance with those recommended by the Canadian Council on Animal Care and approved by the University of British Columbia (UBC) Animal Care Committee (#A08-0388). Eggs and milt were hand expelled and collected in clean, dry plastic containers that were immediately oxygenated and placed on ice for transport to laboratory facilities at UBC. As some populations were geographically isolated and transport to UBC took much longer than others (Figure 2.1), all
groups were fertilized at a standardized 24 hours post-collection. Previous work has showed that transporting unfertilized gametes results in higher survival than transporting activated, developing eggs (Jensen and Alderice 1983), and that salmonid eggs and sperm will remain viable with a 95 -100% fertilization potential for up to five days as long as temperatures are low and sufficient oxygen levels are maintained within the containers (Jensen and Alderice 1984).

**Fertilization Protocol and Incubation Methods**

Gametes were crossed by a randomized mating design wherein each male was paired once with each female, resulting in 15-20 unique families per population. For each cross, three replicates of 15g of eggs (~80-140 eggs) were fertilized with 0.3 mL of milt and activated with 30 mL of water at either 10°, 14°, or 16° C, according to their planned incubation temperature. After 1 minute to allow fertilization, an additional 100 mL of water was added to each jar to increase dissolved oxygen levels, and the jars were left undisturbed for 45 minutes (Jensen 1988) to allow the eggs to fully harden. This dry fertilization method that has been used in various studies on Fraser River sockeye and been shown to result in >90 - 95% survival during embryonic development at ideal temperatures (e.g. Patterson *et al.* 2004). Family baskets were then randomly distributed in vertical stack incubators, with single replicates of each family at 10°, 14°, or 16° C (Figure 2.2). Parentally mediated effects are certainly a significant aspect of early developmental plasticity (Janhunen *et al.* 2010, Burt *et al.* 2012a, b), but as the focus of this study was on inter-population differences, the logistical constraints of my study design did not allow us to fully investigate parental effects by including replication at the family level. I did conduct a concurrent experiment to test the effects of fertilization at high temperatures. To do this, I created an additional single set of crosses (n = 15-20 per population) for each of four populations (Adams River, Chilko River, Scotch Creek, and Harrison River) at 10°C. Following
the same fertilization protocol as the main experiment, I incubated them at 10°C until post-
epiboly (~12 days, estimated according to Jensen 1988), and slowly transferred them to 14°C,
and finally to 16°C. I found no differences in fertilization success in these four populations
between the transferred embryos and the embryos fertilized at 10°, 14°, or 16°C (Table 2.3).

Temperatures were chosen to characterize extreme high, elevated, and mean natural
incubation temperatures during spawning among the chosen populations (D. Patterson Fisheries
and Oceans, unpublished data). Additionally, long-term data on the thermal range for the species
suggested 10°C as approximating the thermal optima for incubation, 16°C a stressful temperature
likely to result in ~50% mortality from fertilization to hatch, and 14°C an appropriate
intermediate temperature (Beacham and Murray 1990, McCullough et al. 2001). Populations
were selected to represent a range of geographic location and spawning timing, and thereby
capture a range of spawning and incubation grounds thermal conditions (Table 2.1).

Fertilized eggs were left undisturb in the incubation stacks for the first 24-48 hours,
and thereafter were checked every 48 hours for mortality (observed by translucent eggs) and
hatching. Stacks were covered to reduce light exposure, except while completing egg checks (< 5
minutes/day/family). Dissolved oxygen concentrations were held constant above 8.0 mg•ml^{-1},
water flow was maintained at ~10 l min^{-1} through all Heath trays, and temperature was held
constant (± 0.3°C) using a system of small aquarium heaters, chillers, and temperature control
computers. All dead eggs were promptly removed and cleared in Stockard’s Solution (5%
formaldehyde (40%), 4% glacial acetic acid, 6% glycerin, 85% water) (Rugh 1952) for minimum
24 hours and visually assessed for fertilization. When groups had reached 430-450 accumulated
thermal units (ATUs), successfully eyed eggs (estimated according to Jensen 1988) were counted
and any surviving uneyed eggs discarded. Alevin mortality and survival were recorded during
daily observations for each group at each temperature. Alevin were maintained within Heath
stacks until emergence and subsequent husbandry in the laboratory (for additional analyses, see Chapter 3 of this thesis).

**Data Analysis**

All statistical analyses were based on population means (*i.e.* population averages of the values for each family) at each temperature treatment to evaluate the population response as determined by family replicates. Survival to hatch from fertilization ($S_{HI}$) was used rather than total egg numbers in order to separate the effects of incubation treatment from the potential effects of temperature on fertilization or other preexisting issues that could affect egg viability and therefore fertilization success (Brooks *et al.* 1997, Vladic and Jarvi 1997).

Proportional survival data (% of fertilized eggs) were logit transformed to meet the normality and homoscedasticity assumptions of parametric tests (Warton and Hui 2011). Temperature and population differences were tested using mixed-effect ANOVAs (Type III SS) to determine the statistical significance of fixed effects, and subsequent Tukey’s HSD post-hoc multiple comparisons tests for survival and hatch timing analysis. All analyses were conducted using R (R Foundation; [www.r-project.org](http://www.r-project.org)).

The survival response was assessed using a linear mixed model with population and temperature treatment as the fixed effects, and family as the random effect. Population variation was assessed using a base model with the form:

$$y_{jk} = \mu + T_j * P + F(P) + \varepsilon_{jk}$$

where $y$ is the response variable ($S_{HI}$), $T$ represents the temperature treatment ($j$), $P$ is the population, and $F$ is the family identity (full-sib families, $k$). The effect of distinct Heath stacks within the incubation design as well as egg size were evaluated as a metric for maternal investment, but these were found to be non-significant and were removed from the current
model. Differences among fixed effects (temperature treatments and population) were tested using Tukey’s HSD tests.

For each population, embryos experience the warmest water temperatures during the initial egg deposition and early development period, as all populations spawn on the descending limb of thermograph and fry emerge during cool spring temperatures. Therefore, I surmised that the initial spawning period would represent the most critical thermal stress period for developing embryos. Historical water temperatures recorded during adult migration and peak spawn periods for each natal subwatershed (D. Patterson, Fisheries and Oceans Canada, unpublished data) were used to calculate mean temperatures during the spawning season for each Fraser River population, and peak spawning temperatures were selected from observational data for ‘peak’ spawning activity at each location. Using these estimates of spawning duration with the temperature data, I calculated mean, maximum and minimum temperatures during peak spawning over the last two decades (1990-2010) for each population. For the Columbia River population (Okanagan R.), average migration temperatures were taken from analyses of Columbia River spawning temperatures (Hodgson and Quinn 2002), and spawning temperatures from Hyatt et al. (2003), with assistance from K. Hyatt (pers. comm.) as well as the Okanagan Nation Alliance (S. Folks and R. Bussanich, pers. comm.). While I included the Columbia River population in the overall model, it is more difficult to predict the link between temperatures and salmon adaptation in this highly manipulated (with numerous hydro power dams) river system (Quinn et al. 1997), and as such the Fraser River populations were analyzed separately.

Using historical temperature as a proxy for population within an adjusted version of the same base model, I tested for the relationship between historical thermal regime and performance within the incubation study. A mixed-model ANOVA was used overall to test for the interaction of incubation temperature and historical spawning and migration temperatures:
\[ y_{jk} = \mu + T_j * H_k + P_k + F(P_{kl}) + \epsilon_{jk} \]

where \( y \) is offspring survival to hatch, \( H \) represents historical temperature during peak spawn (mean, maximum or minimum) or adult migration, and \( T, P, \) and \( F \) are defined the same as within the original model. Within this adjusted model, temperature and historical temperature were considered fixed effects, while population and family were nested random effects. Again, the effects of heath stack and egg weight were not significant and were subsequently removed from this final model. A simplified version of this model was used separately within each temperature treatment.

Additionally, Pearson correlations were calculated to assess the relationship between population level offspring survival and historical spawning and adult migration temperatures for each population, and the requisite \( r \)-values are stated. The significance level for all tests was set at \( \alpha = 0.05 \).

**Results**

**Embryonic Survival**

Survival varied significantly by the interaction of population and temperature treatment (ANOVA, \( F_{16,422} = 2.17, P = 0.0057 \); Figure 2.3). Mean survival to hatch for fertilized eggs was highest across all populations within the 10°C treatment (93% ± 16 SD), declined at 14°C (85% ± 20 SD), and decreased significantly at 16°C (55% ± 27 SD; a 41% decline compared to 10°C) (Figure 2.4). The relative variation among mean survival estimates increased with temperature, and was 3 times higher among stocks at 16°C than 10°C (c.v. 17.1% at 10°C, 23.3% at 14°C, and 48.9% at 16°C). While maternal effects (egg size) were different among groups (see Chapter 3 of this thesis for more details), this did not significantly affect population mean offspring survival to hatch, and was therefore not included in the base model.
The post-hoc Tukey HSD comparisons on the model estimates showed that there was no difference among population-level survival at the coolest temperature (10°C). At the intermediate treatment (14°C), population response to temperature differed significantly (ANOVA; \( F_{8,94} = 4.08, P = 0.0003 \)). Specifically, post-hoc Tukey HSD tests showed that Scotch Creek offspring survived better than those from the Okanagan \((P = 0.0159)\), Harrison River \((P = 0.0171)\), and Chilko River populations \((P < 0.01)\). Chilko Lake alevin survived significantly poorer than those from Weaver Creek population \((P < 0.01)\); indeed, Chilko fish performed the worst at 14°C compared to all other populations. At the highest temperature (16°C), population differences in survival were again strongly evident (ANOVA; \( F_{8,92} = 5.35, P < 0.0001 \)), and the Scotch Creek families again outperformed all others, especially the late run Harrison River \((P < 0.01)\), Chilko Lake \((P < 0.01)\), and Gates Creek \((P = 0.024)\) populations. Chilko Lake survival was lower than the other summer-run populations (Horsefly River; \(P < 0.01\)) and even a late-run Adams River population \((P < 0.01)\). Similarly, the latest timed group, the Harrison River eggs also responded poorly to warm incubation temperatures, with lower survival than the other late timed Adams \((P = 0.0128)\) population.

Within-population variation increased with increasing temperature, but the magnitude of this shift was dependent on population (Figure 2.5). The relative variation among family means within a population was up to 50.8 times higher at 16°C than at 10°C \((i.e.\) Weaver Creek alevin, coefficient of variation (c.v.): 1% at 10°C, 18% at 14°C, 56.7% at 16°C). Relative variation within populations ranged from 0.4x times higher to 50.8 times higher at 16°C than 10°C. Interestingly, those populations with the highest survivorship at 16°C (high ‘thermal tolerance’) were more often to have the lowest within-population variation, while the populations with an ‘average’ thermal tolerance had high plasticity among families (especially Okanagan, Weaver, and Stellako).
**Connection with Historical Temperatures**

In general, populations with higher historical spawning temperatures had higher embryonic survival to hatch at warmer incubation temperatures. Mean peak spawning temperatures for all populations represented population differences as related to offspring survival in the overall model (ANOVA; interaction of the effects of incubation treatment and mean historical spawning temperatures, all populations; $F_{2,308} = 13.36, P < 0.0001$), as did maximum peak spawning temperatures ($F_{2,308} = 13.43, P < 0.0001$). This relationship was much stronger at the high temperature treatment (mean peak spawning temperature correlated with survival at $16^\circ$C ($r = 0.32, P < 0.0001$; maximum peak spawning temperature $r = 0.29, P = 0.0001$), especially when this relationship was tested among only Fraser River populations (mean spawning temperature, $r = 0.393, P < 0.0001$; Figure 2.6). The correlation was not significant for either all populations or just Fraser River populations at either $14^\circ$C ($r = 0.05, P = 0.47, r = 0.09, P = 0.29$, respectively), or $10^\circ$C ($r = 0.06, P = 0.43, r = 0.05, P = 0.59$, respectively). All three metrics of spawning temperatures examined, maximum, mean, and minimum, provided similar results for Fraser River stocks. Interestingly, there was no relationship between adult migration temperatures and thermal embryo tolerance for both Fraser River and Columbia River populations ($r = -0.04, P = 0.34$).

**Discussion**

Exposure to high temperatures through embryo development until hatching resulted in a decreased but differential survival across nine genetically distinct populations of sockeye salmon in a common garden trial. The magnitude of thermal effects varied among populations in a manner that suggested adaptation to long-term environmental conditions. Population
differentiation through local adaptation means that native populations have a higher fitness within their natal environment than other populations of the same species. Additionally, intra-population phenotypic plasticity in survival increased with increasing temperatures, as previously shown at the family level (Beacham et al. 1988, Burt et al. 2012b). Considering both inter- and intra-population variability in thermal tolerance suggests that forming predictions for future population adaptation to warming waters is not as simple as identifying current tolerance limits; it will be important to consider population-specific thermal scope for activity, and to understand the large-scale ecological drivers of thermal tolerance (Sunday et al. 2012).

**Population Specific Responses to High Temperatures**

The observed differences in embryonic survival among populations were substantial, and the interaction of genotype and environment observed through crossing survival reaction norms suggested there was no clear additive effect of temperature across populations. Genotypes responded differentially, with some populations exhibiting a greater magnitude of survival response between 10°C and 14°C, while for other populations the increase in temperature between 14°C and 16°C resulted in significant mortality. The fact that several populations had high survival at 14°C, and even to a lesser extent at the high temperature (16°C) treatment, suggests that plasticity to temperature within *O. nerka* may be relatively high in this early life stage (Hutchings et al. 2007, Hutchings 2011). Certainly, the overall response of all populations to high temperatures supports a concern for sockeye salmon with warming waters, but the differential response by population suggests that there is the potential for some populations to maintain embryonic viability with increasing temperature regimes.

Considering the adaptive implications of observing differences in survival response, a long-term temperature database was used to assess historical thermal regimes at each of the
populations and compare these norms with the results that were observed in the common garden study. This study was able to link thermal tolerance to the embryonic response of developing eggs within an incubation experiment, and thus suggests that thermal tolerance as exhibited within this study is reflected in historical adaptation in nature. Indeed, it seems that populations that have historically experienced cool spawning temperatures (i.e. Gates Creek, Chilko River, Harrison River) were less able to cope with increased temperature within this common garden trial, whereas populations that spawn during warmer conditions respond better to higher incubation temperatures (i.e. Scotch Creek, Adams River; especially clear at 16°C). More specifically, the populations with the lowest embryonic survival in this study were all populations that spawn in cool- and cold-acclimated spawning regions. Chilko River flows from a glacially fed lake, and the water consequently remains cold throughout most of the year. Harrison River *O. nerka* may be cold adapted as this population spawns in the late fall, when river temperatures have notably decreased. While Gates Creek is an ‘early summer’ run population, water temperatures are cold in comparison to the other populations within this run timing group, as the inflow to the spawning area derives from high mountain streams. Conversely, the ‘best’ survivors were those derived from populations that historically have had higher probability of experiencing near 16 °C water temperatures during spawning, and therefore may be better adapted to warm incubation temperatures. An exception to this trend was the upper Columbia River population, the Okanagan River sockeye, which had only moderate thermal tolerance within this study, yet experience the highest migration and spawning temperatures (Stockwell *et al.* 2001, Hodgson and Quinn 2002, Hyatt *et al.* 2003). However, this thermal regime is a product of intensive management of the river for hydroelectric power production and has led to warming of the river only in recent decades (Quinn and Adams 1996, Quinn *et al.* 1997). As the thermal consequences of dams are likely to affect late spawning populations more
than early spawners, these impacts may affect the speed of development and growth more than thermal tolerance, and certainly will take time to change thermal adaptation (Quinn et al. 1997, Angilletta et al. 2008).

Populations that respond more favorably to higher temperatures may already be at or near their thermal capacity, implying that further warming could have considerable negative consequences for those groups (Stillman 2003). While variation in among-population survival response implies a breadth of thermal tolerance for the species, within-population variation suggests phenotypic plasticity within a genotype. Consistent to the original prediction, within-family variation in survival to hatch increased with temperature, especially when comparing the 16°C and 10°C treatments. This trend has been observed in other studies of salmonids, which noted that variation among families increases as thermal conditions diverge from environmental norms, or thermal optima (Beacham and Murray 1985, 1989; Hendry et al. 1998, Burt et al. 2012b). Adaptive differences suggest that different populations display unequal suitability to specific environmental states, and that they respond to an altered environment through phenotypic changes that result in a functional improvement in survival, reproduction, or growth (Stearns 1992, Beacham 1988). Phenotypic plasticity allows populations and species to accommodate environmental variation; greater plasticity will allow population survival in the short term without necessitating selection for genetic change. However, within-population plasticity for thermal tolerance does not necessarily pair with mean group thermal tolerance; the data herein shows that those populations with the highest thermal tolerance were also those with lower within-population variation in this response. This may indicate less plasticity for thermal scope in those groups, although without elevating temperatures further this is merely a suggestion. Certainly, current tolerance for elevated temperatures does not necessarily reflect the potential to withstand additional warming; for populations that already experience warm
temperatures, the species-level critical thermal maxima indicates that such populations will soon become compromised (Crozier and Zabel 2006). Therefore, it is important to understand the climate change at a regional scale that is relevant to the spatial structure of the salmon population under investigation, and not just provide broad predictions.

Previous experimental research has found evidence of broad differences in population-level adaptive thermal tolerance using survival (e.g. Beacham and Murray 1989, Hendry et al. 1998, Hutchings et al. 2007, Jensen et al. 2008; reviewed in Hutchings 2011). In general, analyses of thermal tolerance during embryonic development have found that populations tend to be best suited to temperatures that most closely resemble the historical thermal regime of their natal habitat (Taylor 1991). Many studies have showed that when looking at traits linked to fitness, populations respond best to temperatures that approximate their thermal optima in the wild (Hutchings 2011). However, increasing winter temperatures (Walker and Sydneysmith 2008) may push certain groups to or beyond their thermal range (Hague et al. 2011, Reed et al. 2011). Indeed, this concept may suggest that those populations with the greatest thermal tolerance at present may not be those populations that will best manage increasing water temperatures. Work on thermal tolerance in crabs (Stillman 2003) has surmised that current thermal tolerance is not necessarily indicative of future thermal tolerance, as those populations may already be at or near the limit of their thermal scope. This is important at a species level, as variation amongst populations will drive species-level resistance, resilience, and subsequent selective response to climate change (Hutchings et al. 2007, Lyon et al. 2008, Bradshaw and Hopzapfel 2008).

An interesting aspect of this study is that, and contrary to the original expectation, thermal tolerance varies greatly by life stage (Beacham and Murray 1987, reviewed in Healey 2011), suggesting it will be important to avoid generalizing from studies on other life stages,
such as those on adults (e.g. Eliason et al. 2011) or fry (e.g. Beacham and Murray 1989). For example, while Chilko Lake adults have been identified as thermally tolerant during their up-river migration (Eliason et al. 2011), Chilko embryos were the least tolerant of warming temperatures within this study. Similarly, adult Okanagan sockeye salmon experience the warmest upstream migration conditions (Hyatt et al. 2003), but do not have the highest thermal tolerance as embryos. Indeed, adult migration temperatures were not correlated with offspring survival in this study, as opposed to the positive correlation found with spawning ground temperatures. Moreover, results from this study should be viewed with caution, as they consider only the sessile embryonic development stage during incubation, the portion of the life history where increasing spawning ground water temperatures may have a direct effect in the near future (Healey 2011), and does not consider the latent effects of supraoptimal temperature regimes. Carry-over effects of elevated temperature during incubation has been shown to affect later life stages, even if high temperature is removed (Beacham and Murray 1987, Burt et al. 2012a, b). For this reason, it is important to consider these differences in population viability under thermal stress with additional precaution, considering that the effects of temperature on developmental pathways may significantly affect future competitive behaviors, including swimming ability, as well as lifetime fitness though survival.

Rapidly changing thermal regimes will certainly affect species distribution of both terrestrial and aquatic species, shifts particularly driven by the increasing frequency of extreme climatic events (Jentsch et al. 2007). In birds, reproductive timing is shifting as temperatures increase, implying that sufficient phenotypic plasticity in reproductive traits will be critical for population persistence (Lyon et al. 2008). In terms of climate change adaptation, it’s seasonal timing shifts that may affect species persistence more than any other response, as populations have evolved synchronous events within their life history in response to environmental norms in
their natal habitat (Brannon 1987, Bradshaw and Holzapfel 2008, Charmantier et al. 2008). With shifting thermal regimes, potential changes in the timing of reproductive events has the capacity to severely impact species like *O. nerka*, which have clearly evolved differential capacity for their environment (Eliason et al. 2011; Hague et al. 2011). In addition, while increasing water temperatures will have detrimental effects for developing offspring, they will also affect reproductive maturation and ovulation of their parents (Pankhurst et al. 1996, King et al. 2007), and in some cases could be related to spawning failure (Crozier et al. 2008). Prior to the spawning event itself, warming river temperatures may negatively affect the ability of some returning adults to successfully migrate to spawning areas (Eliason et al. 2011), and the survival cost of maturation and reproduction will affect overall population success (Kuparinen et al. 2012). If they survive to spawning grounds, intergenerational temperature effects may persist from early development and deleteriously affect lifetime success of their offspring (Burt et al. 2012a, b; Salinas and Munch 2012). As shown here, a ‘successful’ spawning event does not assume offspring survival, especially considering elevated incubation temperatures (Pankhurst and Munday 2011). Life stage specific thermal tolerance may affect specific survival of developing eggs, alevin, or fry, and the cumulative effects of warming waters across life history stages will likely have severe deleterious consequences for lifetime fitness.
Figure 2.1. Map of the study populations within the Fraser River watershed and the upper Columbia River, within British Columbia, Canada. Embryos and adult samples from each group were transported back to laboratory facilities at the University of British Columbia in Vancouver, British Columbia, for fertilization, incubation and husbandry throughout early development.
Table 2.1: Environmental characteristics and ecology for nine populations of sockeye salmon, eight from the Fraser River and one from the Columbia River (C.R.). Peak spawning date, mean, maximum and minimum spawning temperatures for Fraser River populations are reported from a ten day period encompassing the estimated mean peak spawning date during the years 1990-2010. Temperatures are reported ± SD. Peak river entry and mean migration temperatures are reported for historical averages, but for Adams, Weaver, and Harrison, where recent shifting run timings to earlier river entry are reported in brackets. Source: Fraser River populations, PSC-DFO; Okanagan River, Hodgson and Quinn 2001, Hyatt et al. 2003, K. Hyatt, Fisheries and Oceans Canada, Pacific Biological Station, and S. Folks & R. Bussanich, Okanagan Nation Alliance, pers.comm.

<table>
<thead>
<tr>
<th>Population</th>
<th>Gates Creek</th>
<th>Scotch Creek</th>
<th>Chilko River</th>
<th>Horsefly River</th>
<th>Stellako River</th>
<th>Okanagan Lake (C.R.)</th>
<th>Adams River</th>
<th>Weaver Creek</th>
<th>Harrison River</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run Timing Group</td>
<td>Early Summer</td>
<td>Early Summer</td>
<td>Summer</td>
<td>Summer</td>
<td>Summer</td>
<td>Columbia River</td>
<td>Late</td>
<td>Late</td>
<td>Late</td>
</tr>
<tr>
<td>Migration Distance from River Entry</td>
<td>400</td>
<td>484</td>
<td>642</td>
<td>796</td>
<td>1100</td>
<td>1200</td>
<td>484</td>
<td>161</td>
<td>121</td>
</tr>
<tr>
<td>Elevation (m)</td>
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<td>366</td>
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<td>716</td>
<td>276</td>
<td>366</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Unique families/pop</td>
<td>18</td>
<td>20</td>
<td>12</td>
<td>14</td>
<td>15</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Mean Spawning Temperature</td>
<td>9.59 ± 0.76</td>
<td>10.84 ± 2.75</td>
<td>9.95 ± 1.39</td>
<td>12.72 ± 1.92</td>
<td>10.95 ± 2.33</td>
<td>12.1 ± 0.82</td>
<td>11.81 ± 1.96</td>
<td>10.43 ± 1.41</td>
<td>8.50 ± 1.13</td>
</tr>
<tr>
<td>Max. Spawning Temperature</td>
<td>10.22 ± 0.90</td>
<td>11.92 ± 2.79</td>
<td>10.83 ± 1.16</td>
<td>14.06 ± 1.98</td>
<td>11.97 ± 2.44</td>
<td>14.2 ± 2.91</td>
<td>13.07 ± 2.01</td>
<td>11.57 ± 1.48</td>
<td>9.09 ± 1.29</td>
</tr>
<tr>
<td>Min. Spawning Temperature</td>
<td>8.90 ± 0.70</td>
<td>9.46 ± 3.02</td>
<td>8.45 ± 2.75</td>
<td>11.21 ± 1.91</td>
<td>9.81 ± 2.27</td>
<td>11.1 ± 1.23</td>
<td>9.95 ± 2.66</td>
<td>9.42 ± 1.52</td>
<td>7.73 ± 1.40</td>
</tr>
<tr>
<td>Migration Temperature (Median)</td>
<td>17.6</td>
<td>17.6</td>
<td>16.6</td>
<td>16.6</td>
<td>16.2</td>
<td>17.5</td>
<td>14.2 (16.9)</td>
<td>14.9 (17.4)</td>
<td>14.8 (17.4)</td>
</tr>
</tbody>
</table>
**Figure 2.2.** Diagram of the experimental design for the incubation study, showing incubation temperatures and family crosses for each population. Numbers represent individual females and letters represent individual males used for each unique cross, creating up to 20 distinct families per population. Fertilized eggs were placed in cylindrical incubation baskets and randomly placed in separate Heath stacks (squares) within the three temperatures. Adapted with permission from Burt 2011.
Figure 2.3. Reaction norms showing mean population survival to hatch at incubation temperatures of 10°C, 14°C, and 16°C. Reaction norms show the mean response per group at a given incubation temperature, representing different phenotypes expressed by genotypes across the environmental gradient.
Figure 2.4. The effect of temperature on embryo survival across incubation temperatures of 10°, 14°, and 16°C, showing averages across all populations. Dark lines show median, boxes show 25th and 75th quartiles, whiskers show range of data, with extremes as open circles.
Figure 2.5. The effect of temperature on populations, across incubation temperatures of 10°, 14°, and 16°C. Populations are ranked in all temperatures by their survival at 16°C. Dark horizontal lines show median values per population, with data contained within the upper 75th and lower 25th quartile contained within open boxes, and data to 95th quartile shown by whiskers, and extreme data shown in open circles.
Figure 2.6. Correlation between offspring survival at 16°C and mean historical spawning temperatures during peak spawn for Fraser River populations (Pearson’s $r = 0.39$, $P < 0.0001$).
Table 2.2. Mean proportional population survival from fertilization to hatching, ± SE, across incubation temperatures of 10°C, 14°C, and 16°C. Populations are in order of their river entry timing (Early Summer to Summer to Late Run stocks).

<table>
<thead>
<tr>
<th>Population</th>
<th>10°C</th>
<th>14°C</th>
<th>16°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gates Creek</td>
<td>0.906 ± 0.011</td>
<td>0.864 ± 0.031</td>
<td>0.435 ± 0.046</td>
</tr>
<tr>
<td>Scotch Creek</td>
<td>0.909 ± 0.044</td>
<td>0.944 ± 0.023</td>
<td>0.734 ± 0.051</td>
</tr>
<tr>
<td>Chilko River</td>
<td>0.802 ± 0.099</td>
<td>0.688 ± 0.072</td>
<td>0.321 ± 0.068</td>
</tr>
<tr>
<td>Horsefly River</td>
<td>0.924 ± 0.047</td>
<td>0.870 ± 0.055</td>
<td>0.663 ± 0.052</td>
</tr>
<tr>
<td>Okanagan River</td>
<td>0.953 ± 0.023</td>
<td>0.824 ± 0.041</td>
<td>0.519 ± 0.050</td>
</tr>
<tr>
<td>Adams River</td>
<td>0.944 ± 0.010</td>
<td>0.844 ± 0.052</td>
<td>0.692 ± 0.039</td>
</tr>
<tr>
<td>Stellako River</td>
<td>0.968 ± 0.011</td>
<td>0.818 ± 0.057</td>
<td>0.567 ± 0.074</td>
</tr>
<tr>
<td>Weaver Creek</td>
<td>0.989 ± 0.002</td>
<td>0.904 ± 0.037</td>
<td>0.544 ± 0.069</td>
</tr>
<tr>
<td>Harrison River</td>
<td>0.937 ± 0.024</td>
<td>0.810 ± 0.033</td>
<td>0.381 ± 0.057</td>
</tr>
</tbody>
</table>

Table 2.3. Fertilization success for 10°C, 14°C, and 16°C fertilizations of four populations compared with a ‘transfer’ protocol where eggs were fertilized at 10°C, transferred just after epiboly over 24 hours to 14°C, held for two days, and subsequently transferred over 24 hours to 16°C for the duration of incubation. See Table 2.1 for sample sizes. Proportion fertilization ± SD.

<table>
<thead>
<tr>
<th>Population</th>
<th>10°C</th>
<th>14°C</th>
<th>16°C</th>
<th>Transfer protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scotch Creek</td>
<td>0.808 ± 0.06</td>
<td>0.772 ± 0.09</td>
<td>0.773 ± 0.12</td>
<td>0.799 ± 0.28</td>
</tr>
<tr>
<td>Chilko River</td>
<td>0.390 ± 0.32</td>
<td>0.484 ± 0.31</td>
<td>0.380 ± 0.29</td>
<td>0.593 ± 0.31</td>
</tr>
<tr>
<td>Adams River</td>
<td>0.726 ± 0.10</td>
<td>0.667 ± 0.19</td>
<td>0.712 ± 0.11</td>
<td>0.750 ± 0.11</td>
</tr>
<tr>
<td>Harrison River</td>
<td>0.494 ± 0.34</td>
<td>0.481 ± 0.32</td>
<td>0.366 ± 0.31</td>
<td>0.445 ± 0.34</td>
</tr>
</tbody>
</table>
Chapter 3: Population and water temperature trump egg size in regulating early development timing and offspring traits in salmonids

Introduction

Within-species, genetically distinct populations are known to differ in parentally mediated developmental qualities such as egg size and alevin morphology that are commonly related to local adaptation (Beacham and Murray 1989, Crossin et al. 2004, Jensen et al. 2008). Resource limitations impose developmental constraints, often on reproductive processes, and a finite energy budget for breeding individuals implies life history consequences that may differ among groups with different environmental conditions (Stearns 1992, Hendry 1998). Individuals which experience long and difficult migrations to spawn have been selected over evolutionary time for swimming efficiency, and therefore have less maternal investment by producing either smaller or fewer eggs per female (Brannon 1987, Linley 1988, Beacham and Murray 1989, Hendry 1998, Crossin et al. 2004, Rollinson and Hutchings 2011). Egg size plays an important role in development of fishes; larger eggs, resulting in larger alevin and fry, may take longer to develop and survive longer without post-emergent feeding (Bagenal 1969). Egg size and quality thus affects offspring size, morphology, and fitness (Brooks et al. 1997), which can significantly affect lifetime survival.

Temperature is a regulatory factor that affects all reproductive processes in fish. Elevated temperature can elevate basal metabolism and development rates, affecting embryogenesis, offspring size, growth, and survival (Pankhurst and Munday 2011). Stenothermic fish such as salmonids are perhaps most susceptible to temperature changes during their sessile embryonic and alevin stages (Brannon 1987), since as passive recipients of their surrounding environment, any significant shifts in temperatures can result in sub-lethal or lethal effects on developing eggs.
and larvae (Blaxter 1992). With small tolerance limits around thermal optima (Rombough 1997), adaptation to thermal conditions during this stage is of critical importance (Janhunen et al. 2010).

In British Columbia, hundreds of genetically distinct populations of sockeye salmon spawn throughout July to December each year in a variety of small streams, lakes, and rivers of the Fraser, Skeena, and Columbia watersheds (Groot and Margolis 1991, Beacham et al. 2004a). These populations, referred to as stocks, employ incredibly variable life history strategies and are thus exposed to a wide range of environmental conditions. Migration effort, elevation change, and distance varies among coastal-spawning stocks, with short migrations of ~20 km, and interior-spawning stocks, some of which travel over 1200 km to reach their natal spawning streams (Groot and Margolis 1991). A strong homing tendency and low incidence of straying (returns to non-natal streams) has resulted in these distinct populations with measureable physiological and behavioral differences, linked to unique life history strategies employed by different groups to order to maximize fitness (Ricker 1972, Beacham and Murray 1989, 1993; Bryant 2009, Eliason et al. 2011).

Offspring size and viability is affected by maternal influence through egg size, thermal history, and population adaptation to environmental conditions. Indeed, Beacham and Murray (1989) observed that under the same incubation treatment, an interior-spawning stock produced longer and heavier alevin and fry than coastal-spawning stocks, relative to egg size. This was largely due to differences in yolk conversion efficiency between eggs from different populations (Beacham and Murray 1987, 1989). Direct and indirect parental effects clearly play an important role in offspring survival, viability, and competitiveness (Burt et al. 2012b). Environmental factors such as elevated water temperature during maturation can also affect parental condition and reproductive development. For instance, elevated temperatures can affect hormonal changes and reproductive systems regulated by the hypothalamo-pituitary-gonadal (HPG) axis. Early
gamete development requires minimum temperatures for the stimulation of the endocrine system, but thermal inhibition of reproductive pathways occurs at high temperatures, above which suppression of reproductive development occurs (~18-24°C for cold temperate and temperate fish species; Pankhurst and Munday 2011). While the trade-off between maternal investment in egg size and number in a species with extremely low parental care may suggest that females should increase egg numbers rather than egg size, the post-emergent competitive benefits of large eggs (larger alevin and fry, improved offspring condition, lower immediate exogenous energy requirements, improved competitiveness) may be more important when considering the additive negative consequences of elevated incubation temperatures on offspring viability (Kinnison et al. 2001).

The evolution of developmental phenology, e.g. the cycle of reproductive timing throughout the year, is a critical factor that will affect species response to climate change and potential adaptation to shifting environmental conditions (Bradshaw and Holzapfel 2008, Jensen et al. 2008). Development timing can vary among stocks, as reflected by local spawning conditions and thermal patterns (Beacham and Murray 1987, 1989, Brannon 1987) and related to subsequent fry migration timing to the ocean (Beacham 1988). This may be due in part to local thermal adaptation (Berg and Moen 1999), since growth rate, as mentioned previously, is highly sensitive to water temperature (Brannon 1987, Angilletta et al. 2002). In addition, incubation timing may have been selected over evolutionary time to increase fitness and improve the chances of population-wide survival considering natural predation in rearing lakes (Tallman 1986). Environmental conditions at hatch and emergence over evolutionary time tends to result in a well-timed development sequence that is likely to be affected by temperature shifts (Blaxter 1992, Pankhurst and Munday 2011), and potentially result in a mismatch of juvenile fish emergence with ecosystem processes and food availability (Bradshaw and Holzapfel 2008).
For juvenile fish, accumulated thermal units (ATUs), or degree-days, as a sum of temperature exposure since fertilization can be used as a means to predict developmental events in early life history. Under fixed conditions, such as within a common garden study, degree days to certain developmental stages can be a useful way to compare differences in development patterns among groups of individuals. Results are mixed among the breadth of previous research on development timing and incubation duration. In general, studies that tested differences in time requirements to hatching have suggested that there is little variation in hatch timing among populations (Beacham and Murray 1986, Berg and Moen 1999). However, there has been evidence of variation in incubation time among highly distinct populations, suggesting adaptive differentiation in response to varying environmental conditions (chum salmon, Tallman 1986; coho salmon, Konecki et al. 1995; sockeye salmon, Hendry et al. 1998). Asynchronous hatching may offer a competitive advantage and minimize density dependent growth for individuals within a population. In addition, incubation requirements may vary among families within populations, forming a significant portion of intra-population variation (Burt et al. 2012b). Developmental asynchrony within populations may increase under suboptimal conditions, but little is known of this relationship as this aspect of development timing is rarely explored in salmonids (Kamler 2002, Burt et al. 2012b). It is possible that asynchrony in development timing within populations may also be an adaptive response to resource competition, especially in years of high abundance as a means of lowering competition for scarce resources within a population (Godin 1982). While the link with fitness and hatch timing is less clear than for emergence timing, this trait could have consequences for survival of alevin within gravel redds, including challenges for oxygen availability, gravel ice in spawning habitat, or waste removal for developing fish (Rombough 1997). Hatch timing is closely associated with emergence timing (Crisp 1988), and as such is an interesting metric for the study of developmental phenology.
Experimentally, incubation temperature is likely to affect thermally adapted populations differentially depending on historical thermal regime. Previous research suggests that at a common temperature, late spawning groups may develop faster than early spawning populations, a response that may reduce thermally mediated hatching and emergence asynchrony among groups (Brannon 1987, Beacham and Murray 1990). Increased development rates can cause premature hatching, and at extremely high temperatures the additive effect of premature secretion of hatching enzymes can expedite development so that embryos develop too quickly (Kamler 2002). High temperatures during incubation are likely to result in smaller alevin at hatch due to reduced yolk utilization efficiency. Thermally elevated basal metabolic rates require a greater proportion of endogenous energy reserves, leaving less for growth and development (Beacham and Murray 1985, Kamler 2008, Jonsson and Jonsson 2011). Alternatively, Blaxter (1992) suggested that decreased larval size with increased incubation temperatures is due to the disproportionate increase in development rate versus growth rate, so that cell differentiation occurs before tissue develops sufficiently.

A common garden experiment was used to assess population-level differences in offspring development rates and alevin size at hatch using three incubation temperature treatments (10, 14, 16°C). These temperatures were chosen to span thermally optimal, average, and high incubation temperatures for the species (McCullough et al. 2001). I predicted that (1) within a common temperature, development timing would vary by population, and specifically that embryos from cool-experienced populations would develop faster than warm-experienced populations. Thus, while temperature should cause faster development overall, population differences in hatching timing will emerge, especially at the more ‘stressful’ high temperatures. Additionally, I predicted that (2) alevin size would be negatively related to time to hatch, as yolk conversion efficiency would be reduced at high temperatures, so that those fish that developed
faster in elevated temperatures would hatch as smaller alevi. In addition, I looked to confirm previous work on maternal investment within the study system that showed egg size to have an inverse relationship with adult migratory distance (Crossin et al. 2004) with a much larger sample size across many populations. Overall, this information was used to make predictions regarding future offspring response in a range of developmental traits to climate change among populations.

**Materials and methods**

*Offspring Collection*

This study used nine populations of sockeye salmon from the Fraser (8) and Columbia (1) Rivers. These Fraser River populations are all abundant stocks that are geographically distinct within the watershed, that represent a variety of migration and spawning conditions, and that display a range of life history characteristics (See Chapter 2 for details; Figure 2.1 and Table 2.1).

Gametes were collected from 15-20 pairs of reproductively mature male and female sockeye salmon collected from the spawning grounds of each population. Adults were sacrificed by cerebral concussion, and eggs and milt were hand expelled and collected in clean, dry plastic containers. All containers were immediately oxygenated and chilled to 0 - 4°C for immediate transportation to laboratory facilities at the University of British Columbia. As some populations were geographically isolated and transport to UBC took much longer than others, all groups were fertilized at a standardized 24 hours post-collection. Previous work has showed that transporting unfertilized gametes results in higher survival than transporting activated, developing eggs (Jensen and Alderice 1983), and that salmonid eggs and sperm will remain viable with a 95 -
100% fertilization potential for up to five days as long as temperatures are low and sufficient oxygen levels are maintained within the containers (Jensen and Alderice 1984).

**Fertilization Protocol and Incubation Methods**

Gametes were crossed by a randomized mating design where each male was paired once with each female (n = 15-20 families/population) (for fertilization details see Chapter 2 of this thesis). Family baskets were then randomly distributed in vertical stack incubators, with single replicates of each family at 10°, 14°, or 16° C.

Fertilized eggs were left undisturbed in the incubation stacks for the first 24-48 hours, and thereafter were checked every 48 hours for mortality and hatching. Stacks were covered to reduce light exposure, except while completing egg checks (< 5 minutes/day/family). Dissolved oxygen concentrations were held constant above 8.0 mg•ml⁻¹, water flow was maintained at ~10 l min⁻¹ through all Heath trays, and temperature was held constant (± 0.3° C) using a system of small aquarium heaters, chillers, and temperature control computers. All dead eggs were promptly removed and cleared in Stockard’s Solution (5% formaldehyde (40%), 4% glacial acetic acid, 6% glycerin, 85% water) (Rugh 1952) for minimum 24 hours and visually assessed for fertilization. When groups had reached 430-450 accumulated thermal units (ATUs), successfully eyed eggs (estimated according to Jensen 1988) were counted and any surviving uneyed eggs discarded. Mortality between fertilization and hatching was significantly higher for all populations at the 16°C treatment (45% ± 27 SD across all groups), versus 15% ± 15 SD at 14°C, and only 7% ± 16 SD at 10°C (details and analysis in Chapter 2 of this thesis).

Once hatching was observed for any family in a population at a given temperature, newly hatched alevin were recorded daily during daily observations, and hatching rate and duration were recorded. Within one day of each population’s 90-95% hatch point, 5 alevin (when
available) from each family were measured for length and weight at hatch. Alevin were euthanized in dilute MS-222 (tricaine methanesulfonate), blotted to remove excess moisture, weighed (±0.01 mg,) and measured for standard body length (L_S; ±0.5 mm). In cases of low survivorship within a family (see Chapter 2 of this thesis), as many alevin as were available were measured (<5) as among family means were used for population values, and those families with no remaining alevin were excluded from the analyses. All remaining alevin were maintained within Heath stacks until emergence and subsequent husbandry in the laboratory.

**Data Analysis**

All statistical analyses were based on population means (i.e. averages of family values for the offspring of each mating pair). Daily counts of egg hatching were used to describe accumulated thermal units prior to first hatch (H_o), 5% hatch (H_5), median hatch date (H_50) and hatching duration (H_D; number of days between 5 and 95% hatch per family). Dry egg mass (M_E), alevin mass (M_H) and length (L_H) at hatch were also measured.

I assessed alevin size response using a linear mixed model with population and temperature treatment as the fixed effects, and family as the random effect. Temperature and population differences were tested using mixed-effect three-way ANOVAs (Type III SS) using population, temperature, and family to determine the statistical significance of the fixed effects of population and temperature, and subsequent Tukey’s HSD post-hoc multiple comparisons tests for alevin size and hatch timing analysis. All analyses were conducted using R (R Foundation; [www.r-project.org](http://www.r-project.org)).
Population variation was assessed using a base model with the form:

$$y_{jk} = \mu + T_j \ast P + F(P_k) + \varepsilon_{jk}$$

where $y$ is the response variable ($H_o$, $H_s$, $H_{50}$, $H_{D}$, $M_{H}$, or $L_{H}$), $T$ represents the temperature treatment (j), $P$ is the population, and $F$ is the family identity (full-sib families, k). For all analyses, temperature and population were considered fixed effects, varying by the random effect of family. I evaluated the effect of distinct Heath stacks within the incubation design, but this was found to be non-significant and were removed them from the current model. Differences among fixed effects (temperature treatments and population) were tested using Tukey’s HSD tests.

Additionally, Pearson correlations were calculated to assess the relationship between egg mass, alevin size, and hatch timing. Where correlations were found to be significant, their $r$ value is stated. Regression analyses were used to compare egg size with migration distance, and offspring size ($M_{H}$, $L_{H}$) with adult female fork length ($F_{L}$). The significance level for all tests was set at $\alpha = 0.05$.

**Results**

*Hatching Characteristics*

Temperature, population, and the interaction of temperature and population (ANOVA, $F_{16,399} = 5.30, P < 0.0001$) significantly affected time to hatch ($H_5$) among populations (Table 3.1). All populations developed faster as incubation temperatures increased, but the magnitude of this difference varied by population (Tukey HSD among incubation temperatures, both $14^\circ$ and $16^\circ$C faster than $10^\circ$C, $16^\circ$C faster than $14^\circ$C, $P < 0.0001$). Alevin from the Chilko River required the longest incubation time of all populations at $10^\circ$C ($P < 0.01$), and longer than the Adams, Horsefly, and Scotch Creek stocks ($P < 0.01$) and late-run Harrison and Weaver stocks.
at 14°C ($P < 0.05$). At 16°C, Okanagan alevin incubated the longest (44 ± 2.03 days) among all populations ($P < 0.001$), while Harrison River fish hatched the fastest (38.1 ± 1.76 days; n.s.). Considering accumulated thermal units (ATUs) rather than days, all populations required more ATUs to reach hatching at higher temperatures, except for Harrison River embryos, which hatched sooner at 16°C than 10°C ($P = 0.0506$; Figure 3.1). In general, the among-population differences reflected the same pattern as for the metric of days to 5% hatch, whereby Chilko River alevin required longer incubation to hatch at both 10°C and 14°C, but Okanagan River stocks were the slowest to hatch at 16°C (Table 3.1). Egg mass was not correlated with either days ($r = -0.039$, $P = 0.41$) or ATUs to first hatch ($r = -0.07$, $P = 0.1375$) across temperatures. At 10°C, egg size was somewhat related to $H_5$, in that larger eggs took longer to hatch ($r = 0.187$, $P = 0.02$), while at 16°C the inverse was true ($r = -0.26$, $P = 0.0017$), so that larger eggs hatched faster. However, this is likely reflected in the compensatory hatch rate by population, as the largest eggs (Harrison River) also hatched faster at higher temperatures.

Development time to median hatch ($H_{50}$) was affected by incubation temperature (ANOVA, $F_{2,399} = 52.1$, $P < 0.0001$) in the same way, with hatching occurring earlier with higher temperatures (Figure 3.2). Both population and the interaction of population and temperature also significantly affected time to median hatch ($P < 0.0001$, Table 3.1). Alevin from the Chilko Lake stock took significantly more ATUs (672 ± 24.85) to reach median hatch at 10°C than those from all other populations ($P < 0.001$), while those from the Adams River required less (605.5 ± 19.32) than most other cool-adapted groups (Chilko, Gates, Harrison, Okanagan; $P < 0.001$), but the same as other warm-adapted populations ($P > 0.10$) (Table 2.1; Chapter 2 of this thesis). At 14°C, most populations required more ATUs to reach 50% hatch (average 661.5 ± 37.7, $P < 0.001$). In the high temperature treatment (16°C), Adams River alevin still required the most thermal units compared to all other populations (696.8 ± 37.6; $P < 0.001$), and in general
the ‘heat sum’ requirement for all populations was more than as under the intermediate treatment (average 694.2 ± 40.38; \( P < 0.0001 \)).

Hatch duration (5-95% hatch within families), also varied significantly by population, temperature, and the interaction of temperature and population (Figure 3.3, Table 3.1). Post-hoc analysis of temperature treatment showed hatching duration at the 10°C treatment (mean = 5.24 days ± 3.1 SD) took significantly less time than at the 16°C treatment (7.2 days ± 1.9 SD) across all populations (\( P < 0.0001 \)). Among populations, Gates Creek alevin took significantly longer to reach 95% hatch (9.2 days ± 2.8) than those from most other river systems (\( P < 0.001 \); mean of all populations 5.24 ± 3.12). At 14°C and 16°C, Horsefly River alevin exhibited greater hatch synchrony among families than other groups (than Adams, Okanagan and Stellako; \( P < 0.001 \) at 14°C).

Correlation analysis suggested that hatch timing was related to offspring survival, as \( H_5 \), \( H_{50} \) and \( H_D \) were correlated with offspring survival within the incubation experiment described in Chapter 2. Incubation duration to \( H_5 \) (at 16°C; \( r = -0.33, P < 0.0001 \)) and \( H_{50} \) (\( r = -0.24, P = 0.004 \)) were negatively related to offspring success at high temperatures, while longer hatch durations were negatively correlated with hatching success (\( r = -0.25, P < 0.0001 \)) over all temperatures but not significantly at 16°C (\( r = 0.11, P = 0.18 \)).

**Egg and Offspring Size**

Egg mass (dry) differed among populations, and for most populations this was related to migration distance (overall relationship, \( R^2 = 0.39, P < 0.0001 \)). The effect of egg mass was included in the original model for each hatch timing characteristic, but was found to be non-significant and was thus removed from the final analyses. However, days to median hatch were
not correlated with egg weight at either 14°C ($P = 0.907$) or 16°C ($P = 0.178$), but were slightly related at 10°C ($r = 0.168$, n = 152, $P = 0.0369$; Pearson’s correlations). Female standard length described some of the variation in egg mass across all populations ($R^2 = 0.13$, $P < 0.0001$; Figure 3.8). Egg mass explained a great deal of the variation in alevin mass, irrespective of thermal treatment ($R^2 = 0.59$, $P < 0.0001$, Figure 3.6), but not in alevin length ($R^2 = 0.05$, $P < 0.0001$, Figure 3.7), where incubation temperature and population played more of a role. Indeed, the interaction of temperature and population was significantly related to differences in alevin length (ANOVA; $F_{16,400} = 7.92$, $P < 0.0001$), but not mass (ANOVA; $F_{16,399} = 0.76$, $P = 0.7282$). Across all populations, alevin were shorter at hatch when incubated at higher temperatures than those that incubated at 10°C ($P < 0.0001$), while alevin mass did not vary across incubation temperatures (Figure 3.4). Across all temperatures, Chilko and Harrison alevin were much longer at hatch than all other populations ($P < 0.001$, Figure 3.5). While alevin mass did not vary significantly across incubation temperatures, alevin did weigh less at hatch when incubated at either 14°C or 16°C, compared to 10°C.

**Discussion**

Exposure of *O. nerka* to elevated temperatures (14° and 16° compared to 10°C) during incubation caused substantial differences both within and among-populations in hatch timing, embryonic development rates, and offspring size. Gametes were found to vary significantly in terms of egg size, a maternal trait, among populations, but this did not strongly affect most development traits. Considering the fitness implications of hatch timing and offspring size, these results suggest that sockeye salmon populations may respond differentially to the effects of climate warming during incubation.
**Hatching characteristics: timing and duration**

As predicted, incubation requirements varied by population, where the embryos of cool-experienced groups developed faster than did those of warm-experienced populations. This is consistent with other studies on hatching and emergence that suggests this may increase synchrony of future development events in later life stages, such as fry development in rearing lakes, smolt migration to ocean systems, and adult migration at sea (Godin 1982, Tallman 1986, Beacham and Murray 1987, 1988; Brannon 1987). This trend is not always distinct; Hendry *et al.* (1998) observed an adaptation to faster development in a late spawning population in Lake Washington compared to early-season spawning groups, but did not see this as a clear pattern among early spawners versus mid-season spawners. While most research on development timing has focused on emergence timing rather than hatch timing as discussed here, alevin hatch timing has a close relationship with emergence timing (Godin 1982, Crisp 1988) so these comparisons remain ecologically relevant.

It has been postulated that incubation time is often related to egg size as larger eggs may require longer incubation to reach hatching, perhaps because smaller eggs require an exogenous food supply sooner than larger eggs that enjoy greater maternal investment (Bagenal 1969). However, I suggest based on my results that incubation time depends more on other reproductive strategies than strictly on egg size, a pattern that has been seen in previous work on some of the same populations (Brannon 1987). Indeed, the population that hatched the fastest at high temperatures (Harrison River) was also that with the largest eggs, and the relationship between egg size and hatching time was variable within the other groups. Moreover, the effect of egg size did not significantly affect any of time to first hatch, 5% hatch, or 50% hatch.

Temperature could be a selective force driving development rates; research on rainbow trout (*O. mykiss*) suggests that parallel adaptation for rapid early development may be linked to
similar thermal regimes among geographically distinct populations (Miller et al. 2012). In that study, populations adapted to cool temperatures in early life both had very rapid development rates; in this study, Harrison River *O. nerka* are a cold-experienced late spawning stock (see Chapter 2 of this thesis), and similarly, did exhibit rapid embryonic development rates.

Development rate was also linked to timing in subsequent life stages. For example, it has been shown that growth timing during early life history can affect eventual adult size (in Atlantic salmon, Jonsson et al. 1996; in white-spotted charr, Morita et al. 1999). Development timing traits have likely been selected for within and among populations, as plasticity in timing within groups allows for environmental variation at a population level, while this variation among groups minimizes intra-population competition for food and habitat resources (Quinn 2005, Hutchings 2009). Post-hatching and emergence, juvenile salmonids experience high levels of mortality in the wild, and selection acts at this life stage to optimize timing cues to maximize food selection and growth, avoid predation, and successfully migrate to rearing streams and lakes. In this way, adult spawn timing, while related to adult thermal history, maturation, and migratory experience (Eliason et al. 2011), is also strongly linked to the thermal requirements and food availability of offspring during the subsequent spring (Beacham and Murray 1987, Brannon 1987, Konecki et al. 1995).

In poikilotherms, development rates are directly related to temperature (Brett 1971), but exposure to supraoptimal temperatures can result in a compensatory response in incubation rate, where the amount of ATUs required to reach a certain development stage, such as hatching, increases at temperatures beyond a certain threshold (Brannon 1987). If development rates were to increase proportionately to rising temperatures, certain aspects of development may not proceed as required, resulting in premature hatching. This disassociation between metabolic and growth rates is generally seen as temperatures exceed an upper thermal limit (Rombough 1994).
Other factors related to basal maintenance, such as oxygen consumption, are also elevated with temperature and may drive early hatching, especially considering that dissolved oxygen content of water decreases with increasing temperature (Jaworski and Kamler 2002).

This relationship between metabolic and growth rates is thought to vary by population, and to be linked with historical emergence date (Konecki et al. 1995). Evidence of a thermally driven compensatory response at hatch was observed, where most populations within the study required more ATUs to reach hatching at the 14°C and 16°C treatment than the cold treatment (10°C; Chilko), while one population, Harrison River, required less ATUs as incubation temperatures increased. While these data did suggest a compensatory response, this relationship could also be confounded by the increased mortality rates that occurred at high temperatures (see Chapter 2 of this thesis), as I did find a relationship between survival in that study and hatch timing, whereby increasing time to hatch was negatively related to survival. This was especially evident at both the moderate (14°C) and high (16°C) incubation temperature. This was confounded by the effect of temperature on survival, but the parallels between hatch timing and success should be investigated further.

Hatching duration also varied by temperature treatment, where higher temperatures were related to protracted hatching within a population. This is an effect that is rarely investigated, but this tendency towards less synchronous hatching was also observed in a common garden study on Weaver Creek *O. nerka* (Burt et al. 2012b). In reviewing the incubation literature, Kamler (2005) noted increased hatching asynchrony at elevated incubation temperatures in those studies that monitored this trait. Godin (1982) suggested that synchrony in fry emergence timing should allow populations to ‘swamp’ predators, whereby the abundance of prey (fry) is so concentrated that a sufficient proportion of them survive predation. While the direct fitness implications of hatch timing are less clear, the association between hatch and emergence timing is such that one
can speculate as to the consequences of asynchronous timing sequences throughout early
development.

**Offspring size and growth**

Consistent with earlier work on several populations of sockeye salmon, egg size differed
significantly among populations, and this variation in size occurred along a gradient of migration
distance from river entry, where longer migrations were associated with smaller eggs (Crossin *et
al.* 2004). While previous work has shown that egg size can be negatively or positively related to
survival (Beacham and Murray 1985, Heath *et al.* 1999, respectively), it is often unrelated to
offspring viability or embryonic response (Hutchings 1991, Nadeau *et al.* 2009, Burt *et al.*
2012a). Here, elevated temperatures increased development rates, resulting in earlier hatching
compared to those embryos incubated at cool temperatures, but egg size did not affect
developmental timing characteristics. However, the direct connection of egg size and offspring
size did hold true (Bagenal 1969), especially in regards to alevin mass (Beacham and Murray
1987). The fitness implications of egg size and subsequent offspring size (Kinnison *et al.* 2001),
in conjunction with the effects of incubation temperature on those offspring size characteristics,
suggest concern for the competitiveness and survivorship of small fry.

Across all populations, alevin hatched from high temperatures were shorter in body
length and lower in mass than their siblings raised under cool temperatures (10°C). This pattern
has been shown previously in sockeye salmon (Hendry *et al.* 1998) and other salmonids (pink
salmon, Murray and Beacham 1986; brown trout, Ojanguren and Brana 2003; Arctic charr,
Janhunen *et al.* 2010). In addition, while offspring size was affected by provenance. Alevin
mass was not significantly affected by temperature treatment, but length was, which is consistent
with the suggestion from previous research that suggests lower temperatures allow alevin to
more efficiently use a greater proportion of their yolk sac for growth prior to hatching and emergence, rather than for basal metabolism under increased temperatures (Beacham and Murray 1989). If alevin hatch faster under warmer temperatures, they use more of their endogenous yolk reserve for routine metabolism rather than growth, and are therefore smaller at hatch (Rombough 1994, Kamler 2008). Previous work has shown that if temperatures are high enough during incubation to affect the efficiency of yolk conversion to body tissue, alevin length may be affected more than alevin mass (Beacham and Murray 1985). In general, several incubation studies suggest that development rates reflect adaptation to local conditions (Taylor et al. 1991, Hendry et al. 1998). For instance, in a study on populations of brown trout (Salmo trutta), Jensen et al. (2008) found that growth rate, specifically for alevin length, was most efficient in temperatures proportionate to conditions in natal spawning rivers.

Regardless, suboptimal temperatures during incubation are likely to have deleterious consequences for developing embryos. In addition to affecting size, a concern as smaller alevin are at a competitive disadvantage in nature, high incubation temperatures can cause spinal deformities, changes in meristic characteristics such as the number of vertebrae, change muscular structure, and cause heart malformations (Jonsson and Jonsson 2011). Climate change and anthropogenic changes to rearing systems are likely to continue to cause increasing freshwater temperatures, and these changes are expected to affect populations of salmonids who depend on these environments (Crozier et al. 2008, Healey 2011). While warm incubation temperatures reduce alevin size at hatch, there is some evidence that for salmonids, selection will act on size to favour smaller individuals, as they may be more tolerant of elevated temperatures than larger fish (Clark et al. 2012). Further investigation into how populations vary in early life history traits, and how these genetic differences interact with environmental conditions such as temperature, will be necessary in order to allow us to predict the evolutionary response of sockeye salmon to
climate change and peak temperature events. This work highlights the importance of population variation in thermal adaptation, and is a reminder that maintaining genetic diversity within species should be a priority for conservation and management.
Figure 3.1. Required heat sum as measured by accumulated thermal units (ATUs, or summed degrees per day for the duration of incubation) to reach 5% hatching, across all populations at three incubation temperatures (10°, 14°, and 16°C). All populations except the cool-experienced Harrison population required more ATUs to reach hatch at higher temperatures.
Figure 3.2. The influence of incubation temperature among populations on time to median hatching ($H_{50}$) from fertilization (d.p.f. = days post fertilization), at three incubation temperatures ($10^\circ$, $14^\circ$, $16^\circ$C). Response for all treatments are ranked by the order of populations in the $10^\circ$C treatment. Black lines show the median response for a population, the boxes contain the data between the 25th and 75th quartile, and the range of data for each population are shown within the whiskers, with extreme values shown as open circles.
Figure 3.3. Hatch duration reaction norms by population, under incubation temperatures of 10°, 14°, and 16°C. Overall hatch synchrony across populations increased with temperature, while hatch synchrony within populations decreased. Hatch duration measured between 5 and 95% hatch for each family, shown as averages per population.
**Figure 3.4.** The effect of temperature on alevin length and alevin mass across all populations under three incubation temperatures. Black lines show median values, boxes contain all data within the 25th and 75th quartile, and dashed lines show the maximum and minimum range of the data. Only length varied significantly among temperatures ($P < 0.0001$).
Figure 3.5. The effect of incubation temperature (10°C, 14°C, 16°C) and population on alevin length (mm), showing median response (black lines) within populations, all data within the 25th and 75th quartile within open boxes, and the range of data for each population by the whiskers. Extreme data points are shown with open circles.
Figure 3.6. Relationship between dry egg mass and alevin mass across all temperatures, including all populations ($R^2 = 0.59, P < 0.0001$).
Figure 3.7. The relationship between dry egg mass and alevin length across all incubation temperatures, showing data from all populations ($R^2 = 0.05$, $P < 0.0001$).
Figure 3.8. Egg mass (g) as related to female standard length (cm) among all populations. Female length seems to fall along a gradient of migration distance, as does egg mass (see Figure 2.1 for a map of all populations with natal spawning sites), where long distance migrators have smaller body size and produce lighter eggs.
Table 3.1. Summary statistics for mixed-model ANOVA with family as random effects, temperature, population as fixed effects. Egg mass was significantly different among populations ($P < 0.01$) Results for each of six early life history traits: Time to first hatch ($H_0$) (both days and accumulated thermal units, or ATUs), hatch duration ($H_D$, days, 5-95% hatch), time to 50% hatch ($H_{50}$, days), alevin length ($L_H$), and mass ($M_H$). F values shown with degrees of freedom in parentheses, asterisks represent significance (*$P < 0.01$, **$P < 0.001$, n.s., not significant).

<table>
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<th>$H_0$, days</th>
<th>$H_0$, ATU</th>
<th>Hatch duration</th>
<th>$H_{50}$, days</th>
<th>Alevin length</th>
<th>Alevin mass</th>
</tr>
</thead>
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<td>temperature</td>
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<td>5.10 (2)*</td>
<td>37.84 (2)**</td>
<td>52.10 (2)**</td>
<td>13.49 (2) n.s</td>
<td>1.53 (2) n.s</td>
</tr>
<tr>
<td>population</td>
<td>17.29 (8)**</td>
<td>8.99 (8)**</td>
<td>15.86 (8)**</td>
<td>7.49 (8)**</td>
<td>12.95 (8)**</td>
<td>56.17 (8)**</td>
</tr>
<tr>
<td>temp x pop</td>
<td>6.57(16)**</td>
<td>5.05 (16)**</td>
<td>8.58 (16)**</td>
<td>3.39 (16)**</td>
<td>6.25 (16)**</td>
<td>1.11 (16)</td>
</tr>
</tbody>
</table>
Chapter 4: Conclusions

The aim of this thesis was to explore the response of juvenile sockeye salmon to increased temperature, and to assess inter-population differences in offspring survival, growth, and size in response to such elevated incubation temperatures. Using a common garden design, I focused on maintaining consistent environmental conditions in order to allow genotypic and phenotypic differences among and within populations to emerge. Specifically, in Chapter 2 I investigated the potential thermal tolerance of developing embryos from many populations of sockeye salmon, and compared these results with historical thermal history from natal subwatersheds to the potential thermal adaptation of those populations. In Chapter 3, I explored additional differences among populations, namely embryo size, growth characteristics in response to supraoptimal temperatures, and offspring size upon hatch.

Consistent with the significant amount of previous research on salmonid early development requirements (e.g. Beacham and Murray 1985-1991; reviewed in Burt et al. 2011), the results of Chapter 2 showed that water temperatures above 10°C have deleterious consequences for developing sockeye salmon embryos. Survival was significantly lower at both 14°C and 16°C than at the control temperature of 10°C, and intra-population variation in survival response increased with temperature for all populations. Except in some instances, offspring thermal tolerance was not affected by egg size or the physiological status of parents (Appendix A). I was able to show that embryonic thermal tolerance varies by population, as does the magnitude of mortality response to extreme elevated temperatures. Using reaction norms for survival, the interaction of genotype (population) and environment (temperature) showed the potential for differential and potentially adaptive response to thermal change among groups. Overall, the importance of inter-population variation in early life history traits was evident, and
the extent to which population-level phenotypic plasticity in thermal tolerance seems to vary by life stage was a surprise.

Furthermore, I used historical temperature data for each natal subwatershed and noted that embryonic survival, especially at extremely high incubation temperatures, was correlated with historical average and maximum spawning ground temperatures. This was a similar trend that has been noted in other studies on salmonids (e.g. Hendry et al. 1998, Jensen et al. 2008) that suggest individuals respond best, in either growth, performance, or survival, to environmental conditions that best reflect their natural conditions. Previous research suggests that such an observation, especially when corroborated with significant genotype by environment interactions (as shown by crossing reaction norms for survival, Figure 4.1), is good evidence for adaptive genetic variation among groups (reviewed in Hutchings 2011). However, while this suggests local adaptation among populations, without quantitatively exploring the additive genetic variation and non-genetic components of this variation these trends are merely speculative.

In addition to the differential survival response among populations, Chapter 3 showed that developmental characteristics also differ among groups. Similar to earlier work on a smaller number of Fraser River populations (Crossin et al. 2004), I found that maternal investment through egg size differs vastly among populations, and that these differences can reflect a reproductive tradeoff between the maternal energy budget dedicated to swimming ability and to reproductive investment into eggs (e.g. Hendry 1998, Kinnison et al. 2001). Egg size is thought to affect incubation requirements, where large eggs may take longer to hatch (Kamler 2008). However, this trend was not evident in my study, and hatch timing differences were more related to population differences driven by phenology (e.g. Beacham et al. 1985, Brannon 1987, Hendry et al. 1998, Berg and Moen 1999). Differences in development characteristics and hatching rates
among populations of the same species suggest variability within the species and potential adaptive plasticity in response to thermal changes. Other metrics of hatch characteristics were found to be related to both genetic (population) differences and the overarching influence of thermal stress, rather than to egg size. Large alevin and fry size doubtless has a fitness advantage (e.g. Einum and Fleming 2000, Green and McCormick 2006). While I did not test whether larger alevin or fry had higher fitness in this thesis, analyses of burst swimming ability and endurance swimming ability across these populations are currently under investigation (N. Sopinka et al. in prep., E. Eliason et al. in prep).

Temperature is a commonly studied factor that is known to affect survival and development of Pacific salmon throughout their life cycle. However, this is the first study of such scale that investigates the thermal tolerance of wild Pacific salmon across many populations of the same species, and links this thermal response to the potential for historical adaptation. This analysis supports recent work on population-level physiological differences in this species at the adult life stage (Martins et al. 2011, Eliason et al. 2011), and augments the preceding work on sockeye salmon that focused on the thermal tolerance of two populations during early development (Beacham and Murray 1989). The populations studied differ in their ability to develop under alternative thermal regimes, suggesting phenotypic plasticity and potentially genetic heritability for thermal tolerance among those populations. Early developmental response to environmental change can have serious repercussions for subsequent life stages and lifetime fitness (Murray and Beacham 1986, 1987, Burt et al. 2012b), so plasticity in juvenile response to environmental regimes will be imperative when considering these shifts. The results imply that some sockeye salmon populations may have a greater ability to survive and, potentially, adapt to warming climate regimes than others. These differences in survival rates reflect adaptation to local environmental conditions at natal rivers, so by using historical knowledge of thermal
history in nature, current and future scope for survival may be predicted under various temperature regimes.

While thermal tolerance may suggest adaptive potential, it is likely not enough; sufficient heritable variation in phenotypic responses to thermal conditions is required in order for potential adaptation to exist (Angilletta et al. 2002, Bryant 2009). The ability of a species to thrive and persist under natural and anthropogenic change depends on variation within and among populations, as without adaptive phenotypic plasticity, fitness is likely to decline with changing environmental states (Crozier et al. 2008, Hutchings 2011). My research implies that population differences will play an important role in the potential for sockeye salmon to respond to climate change. Each of the traits examined in this thesis exhibited an interaction of genotype-by-environment by crossing reaction norms (Figure 5.1). These trends provide a representation of how genotypes may respond to environmental differences by producing different phenotypes. Reaction norms are a powerful means of suggesting adaptive variation, as there is a further assumption that part of the observed genetic variation is additive genetic variance, and can therefore be reflective of heritable adaptive ability (Hutchings 2011). Indeed, reaction norms for survival (Chapter 2) may be the best means of representing adaptive potential among groups due to the high association of survival with fitness (Hutchings et al. 2007, Hutchings 2011).

Considering the inter- and intra-population variation observed, I suggest that current thermal tolerance does not necessarily reflect future thermal tolerance. There is great uncertainty regarding the rate and extent of climate change effects on salmonids, and there are also many other additive changes, both anthropogenic and natural, that may interact with thermal changes to influence survival and development of these populations (Crozier et al. 2008, Healey 2011). The adaptive potential of populations in response to environmental differences has been demonstrated in other studies on a variety of taxa (Etterson and Shaw 2001, Charmantier and
Garant 2005, Janhunen et al. 2010), and suggests that the interaction of genotype and environment may result in differential effects of climate change on individual and population fitness.

To confidently predict the capacity for populations to respond to a changing environment, one must be able to accurately assess both the rate of environmental change as well as the potential rate of adaptation. A constraint of this experiment was that the study design did not allow me to delineate the observed variation into environmental, non-genetic (maternal) and genetic components (Aykanat et al. 2012). While this study was an important and previously unexplored step to greater understanding of the thermal adaptation of sockeye salmon, further research is certainly needed to understand the heritability of such thermal tolerance to be able to predict adaptive potential under climate change (e.g. Charmantier and Garant 2005, Reed et al. 2011). Considering that I observed differential survival within this study, and that survival represents a highly heritable trait that responds to selection in a variety of species (Bradford 1969, Hutchings 2011), it is likely that these differences in survival have a heritable component.

Since selection decreases genetic variation over time by favoring certain alleles over others (Roff 1997), life history traits subject to strong directional selection are likely to have lower heritability estimates than those subject to weaker or more variable selection (reviewed in Carlson and Seamons 2008). Nevertheless, there is some evidence that high heritability for fitness related life history traits can remain high over time, perhaps maintained by diversifying selection among heterogeneous environments and populations (Mousseau and Roff 1987).

Estimates of thermal optima for *O. nerka* certainly suggest that such high temperatures will negatively affect survival, development success, and subsequent lifetime fitness (McCullough et al. 2001). Return migration and spawning temperature regimes for these populations are historically cool, but have been increasing in recent years due to run timing shifts.
and warming freshwater temperatures (Patterson et al. 2007). While the effects of rising temperatures on migrating adults are well known (Eliason et al. 2011, Martins et al. 2011, 2012), the consequences for developing gametes, incubating gametes, and newly hatched alevin are much less defined (Healey 2011). This thesis shows that increasing water temperatures will differentially affect offspring characteristics among populations, and that these results may suggest consequences for lifetime fitness of those groups (e.g. Martins et al. 2011, Healey 2011).

Considering the likelihood of warming water temperatures in the near future due to climate change (Walker and Sydneysmith 2008), utmost caution should be taken in the management of in-stream flow and critical habitat in order to attempt to maintain historical temperatures on natal subwatersheds (e.g. Healey 2011, Macdonald et al. 2012), especially during this critical and sensitive sessile incubation life stage.

Overall, I recommend that future studies investigate the genetic component of thermal tolerance to estimate the adaptive potential of these populations to environmental change. In addition, a more intensive study looking into the eco-physiology of thermal stress as it affects intergenerational reproductive success would benefit an across life-history approach to determine climate impacts. In terms of management, it is important to consider the thermal and habitat needs of sockeye salmon differentially among life stages, and of course among populations. Differential variation in offspring survivorship is a serious conservation concern, and that fisheries management could benefit from this knowledge when setting population-specific spawning escapement targets (Holt and Peterman 2006, Macdonald et al. 2010). In order to maintain population viability in the future, it will be necessary to preserve the natural variation that is evident within this important species through population-specific management goals.
Figure 4.1. Summary of reaction norms: differences offspring traits for sockeye salmon early life history traits across a gradient of incubation temperatures applied from fertilization to hatch. Traits are described in detail in Chapter 3, except offspring survival, which are described in detail as associated with local adaptation in Chapter 2. Included here as a summary for the thesis as a whole.
References


### Appendix A: Physiological status of sockeye parent spawners from all populations

**Table A.1.** Physiological variables for males sampled at the time of gamete collection for each population, compared to correlations with offspring survival. Mean values (± SD) are given per population (n=15-20 per population, see Table 1) as used in the thermal tolerance fertilization experiment (Chapter 2). Correlations use average values for offspring survival (S_H) per population. Asterisks indicate significant correlations (P < 0.05) and the correlation coefficient (r) is given. Positive or negative trends are indicated with (+) or (-). See Farrell et al. 2001 for details on plasma ion and hormone analysis techniques.

<table>
<thead>
<tr>
<th>Population</th>
<th>Mass (kg)</th>
<th>Standard length (cm)</th>
<th>Condition factor</th>
<th>Na+ (mmol*L⁻¹)</th>
<th>Cl⁻ (mmol*L⁻¹)</th>
<th>K+ (mmol*L⁻¹)</th>
<th>Osmolality (mOsm*L⁻¹)</th>
<th>Glucose (mmol*L⁻¹)</th>
<th>Lactate (mmol*L⁻¹)</th>
<th>Testosterone (ng*ml⁻¹)</th>
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<td>Adams</td>
<td>3.10 ± 0.44</td>
<td>57.3 ± 2.0</td>
<td>1.2 ± 0.3</td>
<td>146 ± 9</td>
<td>120 ± 9</td>
<td>2.9 ± 0.7</td>
<td>309 ± 13</td>
<td>7.4 ± 3.0</td>
<td>9.0 ± 2.4</td>
<td>11.1 ± 4.9</td>
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<td>1.2 ± 0.1</td>
<td>130 ± 11</td>
<td>113 ± 16</td>
<td>3.6 ± 1.4</td>
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<td>125 ± 3</td>
<td>2.4 ± 1.5</td>
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<td>6.2 ± 1.0</td>
<td>3.5 ± 2.7</td>
<td>16.8 ± 8.7</td>
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<td>ND</td>
<td>ND</td>
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<td>134 ± 4</td>
<td>3.2 ± 0.7</td>
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<td>5.3 ± 2.7</td>
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<td>108 ±15</td>
<td>3.8 ± 1.3</td>
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<td>117 ± 5</td>
<td>3.2 ± 1.1</td>
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<td>7.2 ± 1.0</td>
<td>5.1 ± 1.5</td>
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<td>Scotch</td>
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<td>ND</td>
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<td>1.8 ± 0.7</td>
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<td>1.2 ± 0.1</td>
<td>138 ±10</td>
<td>117 ±14</td>
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<td>3.6 ± 0.9</td>
<td>297 ± 14</td>
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**Correlation matrix**

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<th>14C</th>
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<td>16C</td>
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* Asterisks indicate significant correlations (P < 0.05) and the correlation coefficient (r) is given. Positive or negative trends are indicated with (+) or (-).
Table A.2 Physiological variables for females sampled at the time of gamete collection for each population, compared to correlations with offspring survival. Mean values (± SD) are given per population (n=15-20 per population, see Table 1) as used in the thermal tolerance fertilization experiment (Chapter 2). Correlations use average values for offspring survival ($S_H$) per population. Asterisks indicate significant correlations ($P < 0.05$) and the correlation coefficient ($r$) is given. Positive or negative trends are indicated with (+) or (-). See Farrell et al. 2001 for details on plasma ion and hormone analysis techniques.

<table>
<thead>
<tr>
<th>Population</th>
<th>Mass (kg)</th>
<th>Standard length (cm)</th>
<th>Condition factor</th>
<th>GSI</th>
<th>10-egg dry mass (g)</th>
<th>Estradiol (ng*ml$^{-1}$)</th>
<th>Testosterone (ng*ml$^{-1}$)</th>
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<td>Chilko</td>
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<td>52.1 ± 2.1</td>
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<td>14.72±3.37</td>
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<td>0.44±0.12</td>
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<td>Horsefly</td>
<td>1.86 ± 0.93</td>
<td>51.4 ± 2.3</td>
<td>1.1 ± 0.05</td>
<td>12.00±3.53</td>
<td>0.36±0.04</td>
<td>0.50±0.19</td>
<td>34.12±24.5</td>
</tr>
<tr>
<td>Okanagan</td>
<td>1.16 ± 0.26</td>
<td>42.9 ± 2.5</td>
<td>0.98 ± 0.23</td>
<td>25.01±3.34</td>
<td>0.38±0.04</td>
<td>0.55±0.11</td>
<td>18.27±11.18</td>
</tr>
<tr>
<td>Scotch</td>
<td>2.02 ± 0.26</td>
<td>52.7 ± 1.6</td>
<td>1.1 ± 0.08</td>
<td>13.52±3.84</td>
<td>0.35±0.03</td>
<td>0.45±0.12</td>
<td>12.38±9.77</td>
</tr>
<tr>
<td>Stellako</td>
<td>1.93 ± 0.19</td>
<td>50.8 ± 1.7</td>
<td>1.1 ± 0.07</td>
<td>12.97±4.11</td>
<td>0.34±0.03</td>
<td>0.42±0.11</td>
<td>17.35±10.67</td>
</tr>
<tr>
<td>Weaver</td>
<td>2.53 ± 0.36</td>
<td>54.1 ± 2.7</td>
<td>1.2 ± 0.09</td>
<td>23.39±15.76</td>
<td>0.52±0.04</td>
<td>0.68±0.18</td>
<td>55.60±21.21</td>
</tr>
</tbody>
</table>

Correlation matrix

<table>
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<tr>
<th></th>
<th>10C</th>
<th>14C</th>
<th>16C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r = 0.18$</td>
<td>P &gt; 0.05</td>
<td>(+)</td>
<td></td>
</tr>
<tr>
<td>P &gt; 0.05</td>
<td>(+)</td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>(+)</td>
</tr>
<tr>
<td>P &gt; 0.05</td>
<td>*$r = -0.26$</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
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</tbody>
</table>
**Table A.3** Physiological variables for females sampled at the time of gamete collection for each population, compared to correlations with offspring survival. Mean values (± SD) are given per population (n = 15-20 per population, see Table 1) as used in the thermal tolerance fertilization experiment (Chapter 2). Correlations use average values for offspring survival ($S_H$) per population. Asterisks indicate significant correlations ($P < 0.05$) and the correlation coefficient ($r$) is given. Positive or negative trends are indicated with (+) or (-). See Farrell *et al.* 2001 for details on plasma ion and hormone analysis techniques.

<table>
<thead>
<tr>
<th>Population</th>
<th>Na+ (mmol*L⁻¹)</th>
<th>Cl⁻ (mmol*L⁻¹)</th>
<th>K⁺ (mmol*L⁻¹)</th>
<th>Osmolality (mOsm*L⁻¹)</th>
<th>Glucose (mmol*L⁻¹)</th>
<th>Lactate (mmol*L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adams</td>
<td>135 ±20</td>
<td>108 ±23</td>
<td>2.3 ± 0.8</td>
<td>292 ± 31</td>
<td>10.8 ± 4.9</td>
<td>10.1 ± 4.5</td>
</tr>
<tr>
<td>Chilko</td>
<td>129 ±12</td>
<td>115 ±17</td>
<td>3.8 ± 1.2</td>
<td>289 ± 14</td>
<td>10.4 ± 5.1</td>
<td>4.4 ± 4.0</td>
</tr>
<tr>
<td>Gates</td>
<td>127 ±18</td>
<td>108 ±21</td>
<td>2.0 ± 1.2</td>
<td>277 ± 23</td>
<td>9.2 ± 2.6</td>
<td>5.3 ± 3.3</td>
</tr>
<tr>
<td>Harrison</td>
<td>150 ± 4</td>
<td>136 ± 5</td>
<td>3.0 ± 0.6</td>
<td>320 ± 10</td>
<td>5.1 ± 0.8</td>
<td>5.0 ± 2.2</td>
</tr>
<tr>
<td>Horsefly</td>
<td>136 ± 13</td>
<td>114 ± 15</td>
<td>1.9 ± 1.5</td>
<td>306 ± 18</td>
<td>11.1 ± 4.1</td>
<td>7.2 ± 2.7</td>
</tr>
<tr>
<td>Okanagan</td>
<td>145 ± 9</td>
<td>114 ± 7</td>
<td>1.8 ± 1.3</td>
<td>290 ± 11</td>
<td>7.8 ± 1.9</td>
<td>5.6 ± 2.0</td>
</tr>
<tr>
<td>Scotch</td>
<td>122 ± 16</td>
<td>90 ± 18</td>
<td>1.8 ± 1.0</td>
<td>277 ± 22</td>
<td>16.6 ± 6.3</td>
<td>7.4 ± 2.7</td>
</tr>
<tr>
<td>Stellako</td>
<td>138 ± 11</td>
<td>123 ± 14</td>
<td>2.0 ± 1.1</td>
<td>310 ± 17</td>
<td>6.4 ± 2.2</td>
<td>7.8 ± 2.9</td>
</tr>
<tr>
<td>Weaver</td>
<td>138 ± 8</td>
<td>121 ± 13</td>
<td>4.2 ± 1.3</td>
<td>300 ± 15</td>
<td>6.6 ± 2.1</td>
<td>2.3 ± 1.6</td>
</tr>
</tbody>
</table>

**Correlation matrix**

<table>
<thead>
<tr>
<th></th>
<th>10C</th>
<th>14C</th>
<th>16C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P &gt; 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*r = 0.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P &gt; 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*r = 0.17</td>
<td></td>
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</tr>
<tr>
<td>P &gt; 0.05</td>
<td></td>
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</tr>
<tr>
<td>*r = 0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P &gt; 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*r = 0.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P &gt; 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix B: Population and incubation temperature affect hatch characteristics and offspring size

Table B.1. Summary data of hatch timing characteristics across populations and incubation temperatures. Collection date and number of families to survive to hatch shown by incubation temperature. \( H_0 \) represents first hatch within a population, \( H_5 \) and \( H_{50} \) the 5 and 95% hatch, respectively, and \( H_{50} \) is the median hatch point. Populations are ranked in order of collection date. C.R. = Columbia River.

<table>
<thead>
<tr>
<th></th>
<th>Gates Creek</th>
<th>Scotch Creek</th>
<th>Chilko River</th>
<th>Horsefly River</th>
<th>Stellako River</th>
<th>Okanagan Lake (C.R.)</th>
<th>Adams River</th>
<th>Weaver Creek</th>
<th>Harrison River</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Families/pop</strong> 10°/14°/16°C</td>
<td>18/18/15</td>
<td>20</td>
<td>10/11/7</td>
<td>14</td>
<td>15</td>
<td>20</td>
<td>20</td>
<td>20/19/19</td>
<td>16/16/9</td>
</tr>
<tr>
<td>( H_{0}, 10^\circ ) C</td>
<td>56.7 ± 2.7</td>
<td>59.85 ± 2.15</td>
<td>63.5 ± 1.51</td>
<td>59.0 ± 2.94</td>
<td>59.7 ± 1.4</td>
<td>62.6 ± 1.8</td>
<td>59.55 ± 1.5</td>
<td>59.85 ± 1.35</td>
<td>61.4 ± 1.15</td>
</tr>
<tr>
<td>14C</td>
<td>44.9 ± 2.1</td>
<td>43.7 ± 1.13</td>
<td>47.8 ± 1.84</td>
<td>42.1 ± 2.13</td>
<td>42.2 ± 1.7</td>
<td>45.2 ± 1.7</td>
<td>43.5 ± 0.9</td>
<td>44.1 ± 1.4</td>
<td>44 ± 1.2</td>
</tr>
<tr>
<td>16C</td>
<td>39.3 ± 2.8</td>
<td>37.65 ± 2.6</td>
<td>41.7 ± 1.8</td>
<td>36.8 ± 1.7</td>
<td>37.8 ± 2.5</td>
<td>41.65 ± 3.5</td>
<td>38.9 ± 0.9</td>
<td>38.9 ± 1.85</td>
<td>36.7 ± 2.6</td>
</tr>
<tr>
<td>5% ( H_{0}, 10^\circ ) C</td>
<td>58.8 ± 3.1</td>
<td>60.3 ± 1.4</td>
<td>65.5 ± 2.9</td>
<td>60.6 ± 2.1</td>
<td>60.2 ± 1.1</td>
<td>62.55 ± 1.2</td>
<td>60.1 ± 1.1</td>
<td>60.3 ± 1.3</td>
<td>62.75 ± 1.0</td>
</tr>
<tr>
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<td>45.4 ± 1.9</td>
<td>44.2 ± 1.0</td>
<td>47.5 ± 1.2</td>
<td>44.2 ± 1.3</td>
<td>45.1 ± 5.3</td>
<td>45.9 ± 1.6</td>
<td>44.25 ± 1.2</td>
<td>44.8 ± 1.1</td>
<td>44.7 ± 1.2</td>
</tr>
<tr>
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<td>40.5 ± 2.3</td>
<td>39.9 ± 1.3</td>
<td>42.85 ± 1.9</td>
<td>39.5 ± 1.0</td>
<td>39.9 ± 1.8</td>
<td>44.15 ± 2.0</td>
<td>40.1 ± 1.3</td>
<td>40.1 ± 1.6</td>
<td>38.1 ± 1.8</td>
</tr>
<tr>
<td>( H_{5}, 10^\circ ) C</td>
<td>9.2 ± 2.8</td>
<td>4.2 ± 1.5</td>
<td>5.2 ± 2.6</td>
<td>3.6 ± 1.0</td>
<td>2.9 ± 1.0</td>
<td>7.6 ± 3.5</td>
<td>2.8 ± 0.9</td>
<td>5.3 ± 2.7</td>
<td>5.75 ± 3.3</td>
</tr>
<tr>
<td>14C</td>
<td>6.0 ± 2.6</td>
<td>5.35 ± 2.4</td>
<td>6.1 ± 1.6</td>
<td>4.1 ± 1.3</td>
<td>8.9 ± 4.8</td>
<td>7.95 ± 2.6</td>
<td>8.3 ± 2.9</td>
<td>6.4 ± 1.8</td>
<td>6.1 ± 1.9</td>
</tr>
<tr>
<td>16C</td>
<td>8.1 ± 1.9</td>
<td>7.0 ± 1.8</td>
<td>7.1 ± 1.7</td>
<td>6.4 ± 1.7</td>
<td>7.8 ± 2.4</td>
<td>6.6 ± 1.9</td>
<td>8.2 ± 1.6</td>
<td>6.6 ± 1.6</td>
<td>6.9 ± 1.7</td>
</tr>
<tr>
<td>( H_{50}, 10^\circ ) C</td>
<td>63.3 ± 1.6</td>
<td>61.9 ± 1.2</td>
<td>67.2 ± 2.5</td>
<td>62.1 ± 1.6</td>
<td>60.9 ± 0.9</td>
<td>54.3 ± 2.6</td>
<td>60.6 ± 1.9</td>
<td>61.75 ± 1.2</td>
<td>64.4 ± 1.5</td>
</tr>
<tr>
<td>14C</td>
<td>47.05 ± 2.2</td>
<td>45.75 ± 1.5</td>
<td>50.4 ± 3.0</td>
<td>45.2 ± 1.3</td>
<td>46.5 ± 2.6</td>
<td>49.5 ± 2.8</td>
<td>47.0 ± 2.9</td>
<td>47.8 ± 2.1</td>
<td>46.5 ± 2.0</td>
</tr>
<tr>
<td>16C</td>
<td>43.7 ± 2.2</td>
<td>42.4 ± 1.7</td>
<td>45.6 ± 2.6</td>
<td>41.85 ± 1.5</td>
<td>42.4 ± 2.0</td>
<td>46.4 ± 2.4</td>
<td>43.6 ± 2.4</td>
<td>43.0 ± 1.9</td>
<td>41.2 ± 1.3</td>
</tr>
<tr>
<td>( ATUs, H_{0}, 10^\circ ) C</td>
<td>633.3 ± 16</td>
<td>619 ± 11</td>
<td>672 ± 25</td>
<td>621 ± 16</td>
<td>608.6 ± 9</td>
<td>642.5 ± 25</td>
<td>605.5 ± 19</td>
<td>617.5 ± 12</td>
<td>644 ± 15</td>
</tr>
<tr>
<td>14C</td>
<td>658.7 ± 31</td>
<td>640.5 ± 21</td>
<td>705 ± 42</td>
<td>633 ± 18</td>
<td>650.5 ± 36</td>
<td>692 ± 39</td>
<td>658.7 ± 41</td>
<td>670 ± 29</td>
<td>651 ± 28</td>
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<tr>
<td>16C</td>
<td>698.6 ± 35</td>
<td>678.4 ± 28</td>
<td>729 ± 41</td>
<td>669.7 ± 24</td>
<td>678.4 ± 31.8</td>
<td>741.6 ± 41</td>
<td>696.8 ± 38</td>
<td>688 ± 31</td>
<td>659 ± 21</td>
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</table>
Table B.2. Measurements for alevin mass ($M_H$) and length ($L_H$) at hatch, and fry mass ($M_E$) and length ($L_E$) at emergence across populations under the influence of three incubation temperatures ($10^\circ$, $14^\circ$, $16^\circ$C), ± SD. Raw data was ± 0.01 mg, ± 0.25 mm.

<table>
<thead>
<tr>
<th></th>
<th>Gates Creek</th>
<th>Scotch Creek</th>
<th>Chilko River</th>
<th>Horsefly River</th>
<th>Stellako River</th>
<th>Okanagan Lake (C.R.)</th>
<th>Adams River</th>
<th>Weaver Creek</th>
<th>Harrison River</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metric</td>
<td>temp.</td>
<td>H (mg)</td>
<td>L H (mg)</td>
<td>M E (mg)</td>
<td>M E (mg)</td>
<td>M E (mg)</td>
<td>M E (mg)</td>
<td>M E (mg)</td>
<td>M E (mg)</td>
</tr>
<tr>
<td></td>
<td>10°</td>
<td>115.3 ± 12.5</td>
<td>91.6 ± 7.9</td>
<td>110.6 ± 7.7</td>
<td>96.4 ± 16.0</td>
<td>91.1 ± 8.1</td>
<td>99.5 ± 11.7</td>
<td>105.4 ± 75.0</td>
<td>128.8 ± 12.9</td>
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<tr>
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<td>14°</td>
<td>104.6 ± 7.9</td>
<td>97.0 ± 64.9</td>
<td>111.1 ± 8.9</td>
<td>93.6 ± 9.2</td>
<td>91.6 ± 16.4</td>
<td>99.4 ± 15.2</td>
<td>99.1 ± 10.8</td>
<td>123.4 ± 13.8</td>
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<td>107.8 ± 17.9</td>
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<td>94.9 ± 10.5</td>
<td>90.9 ± 7.9</td>
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<td>97.9 ± 8.9</td>
<td>127.4 ± 13.0</td>
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<td>19.9 ± 1.6</td>
<td>19.4 ± 0.9</td>
<td>21.9 ± 1.0</td>
<td>19.7 ± 1.4</td>
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<td>20.2 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>14°</td>
<td>16.9 ± 1.4</td>
<td>17.9 ± 1.0</td>
<td>20.1 ± 0.9</td>
<td>18.6 ± 0.9</td>
<td>19.5 ± 1.3</td>
<td>18.4 ± 1.1</td>
<td>19.2 ± 1.3</td>
<td>18.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>16°</td>
<td>17.4 ± 1.1</td>
<td>17.7 ± 1.05</td>
<td>19.7 ± 1.1</td>
<td>18.3 ± 1.1</td>
<td>17.6 ± 1.3</td>
<td>17.9 ± 1.0</td>
<td>17.8 ± 0.9</td>
<td>18.6 ± 1.0</td>
</tr>
<tr>
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<td>141.3 ± 11.5</td>
<td>108.9 ± 14.2</td>
<td>138.9 ± 11.5</td>
<td>118.3 ± 10.4</td>
<td>110.7 ± 13.5</td>
<td>116.9 ± 20.0</td>
<td>118.6 ± 14.1</td>
<td>159.3 ± 20.2</td>
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<td>14°</td>
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<td>81.08 ± 10.2</td>
<td>103.9 ± 15.1</td>
<td>90.2 ± 11.4</td>
<td>99.8 ± 78.3</td>
<td>97.5 ± 14.1</td>
<td>96.3 ± 16.8</td>
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<td>81.1 ± 17.5</td>
<td>79.8 ± 15.5</td>
<td>89.6 ± 20.0</td>
<td>85.6 ± 20.2</td>
<td>119.9 ± 27.9</td>
</tr>
<tr>
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<td>26.12 ± 0.8</td>
<td>25.15 ± 0.8</td>
<td>26.69 ± 0.6</td>
<td>25.56 ± 0.7</td>
<td>24.9 ± 0.8</td>
<td>25.0 ± 0.9</td>
<td>24.8 ± 0.8</td>
<td>26.9 ± 1.1</td>
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<td>14°</td>
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<td>23.59 ± 0.7</td>
<td>24.28 ± 0.9</td>
<td>23.26 ± 1.0</td>
<td>22.9 ± 0.8</td>
<td>22.8 ± 0.9</td>
<td>23.1 ± 1.2</td>
<td>24.4 ± 1.3</td>
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<tr>
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<td>16°</td>
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<td>21.87 ± 1.1</td>
<td>22.58 ± 0.6</td>
<td>22.0 ± 1.4</td>
<td>21.2 ± 2.5</td>
<td>22.0 ± 1.0</td>
<td>21.9 ± 1.2</td>
<td>23.4 ± 1.1</td>
</tr>
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</table>