LOCAL ADAPTATION TO COLD TEMPERATURES BY LARVAL COHO SALMON (*ONCORHYNCHUS KISUTCH*) FROM DIFFERENT POPULATIONS THROUGHOUT BRITISH COLUMBIA

by

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ABSTRACT

The influence of environmental variables on larval development of coho salmon (*Oncorhynchus kisutch*), with specific focus on the influence of near-freezing incubation temperatures, was examined across populations within British Columbia. A survey across the geographical distribution within British Columbia was conducted to determine the range and variability of incubation temperatures experience by incubating coho salmon. Temperatures throughout incubation differed significantly among locations, averaging approximately 1 °C in colder interior locations and approximately 5 °C in warmer coastal locations. Environmental variables influenced egg size, fecundity, female size and gonadal somatic index, such that higher latitude of spawning grounds increased, larger systems decreased, and increased temperatures experienced by a population increased the four life-history traits. Suggesting significant effects of latitude of spawning grounds, size of spawning system and temperatures experienced by a population on shaping patterns of reproductive investment. A laboratory incubation study revealed no difference in survival and performance between families from a southern and a northern population reared at near-freezing incubation temperatures. These findings suggest plasticity in developmental processes of coho salmon, as each population was successful across a wide range of temperatures, and in particular developed successfully with minimal fitness effects at the extreme ranges of near-freezing temperatures.

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PROLOGUE

Temperature profoundly affects how organisms function, and is particularly important for poikilotherms. Temperature not only influences the attributes within a habitat such as dissolved oxygen levels, conductivity and productivity, but also physiology and survival of poikilotherms such as fish. Consequently, temperature is one of the leading factors governing growth and development in fish (Fry 1971; Blaxter 1992). Effects of temperature on poikilotherms are cumulative, as it has a direct effect on metabolic rate and therefore their rate of development (Fry 1971; Brett 1995). Cold temperatures cause a decrease in metabolic rates and development rates that in turn cause a decrease in multiple measures of performance (Perry and Tufts 1998). Consequently, ectothermic animals have evolved to cope with specific temperature regimes and relatively small temperature changes can have measurable effects on community and population structure.

Adaptive evolution occurs when the genetic constitution of a population changes as a consequence of natural selection (Merilä and Hendry 2014). Plasticity occurs when a given genotype is unchanged, but adjust their phenotype due to the conditions experienced within the environment (Merilä and Hendry 2014). When investigating the influence of temperature on the physiology of poikilotherms such as metabolic rate and development, there are a number of measures commonly used. The cumulative effect of temperature over time is measured with the unit of accumulated thermal units which are calculated by the sum of the average daily temperature experienced. Additionally, the temperature coefficient (Q_{10}) represents the factor by which the rate of a reaction increases for every ten degree rise in temperature. It is useful as it may be used to infer mechanistic insight about the physiological processes under investigation. The temperature coefficient is defined as:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

where Q_{10} is the *factor* by which the reaction rate increases when the temperature is raised by ten degrees. Q_{10} is a unitless quantity. R_1 is the measured reaction rate at temperature T_1 (where $T_1 < T_2$). R_2 is the measured reaction rate at temperature T_2 (where $T_2 > T_1$).

For species with large geographic distributions, considerable differences in temperature may be experienced, which have been shown to translate into differences in thermal tolerance (Fangue et al. 2006). Such patterns, however, have not been adequately determined. Adaptive phenotypic change through evolution or phenotypic plasticity may be crucial for persistence of populations experiencing variable selective pressures. This is of particular interest if demographic rescue from neighbouring populations is unlikely, such as in salmonid fish, which have strong homing behaviour (Reed et al. 2011; Visser 2008). Changing climates can result in loss of biodiversity (Thomas et al. 2004), shifts in distribution (Brander 2003; Perry et al. 2005; Grebmeier et al. 2006), migration failure (Farrell et al. 2008), and altered species productivity (Pörtner and Peck 2010), and demonstrate the importance of temperature for animal function (Rosenzweig et al. 2008). An understanding of the interaction between evolutionary and ecological processes, therefore, is needed to determine the effect of anthropogenic disturbance and climate change on natural populations (Reed et al. 2011).

Coho salmon (*Oncorhynchus kisutch*) have a large geographic distribution and spawn around the Pacific Rim from California to Japan (Figure P.1; Shaul et al. 2007; Sandercock 1991). Moreover, throughout British Columbia (BC) coho salmon spawn in over 970 rivers and small streams (Sandercock 1991). With such a large geographic distribution and with

spawning occurring along the coast as well as through the interior of BC (Figure P.1), populations of coho salmon experience different ranges of temperatures during spawning and incubation, and are expected to tolerate a wide range in temperatures. Coho salmon return to their natal spawning grounds during the fall and spawn during fall and early winter, showing a very narrow spawning window in comparison to other species of Pacific salmon. Spawners migrate to small tributary streams, and even in coastal rivers coho salmon tend to spawn in the upper reaches of a watershed (Shaul et al. 2007). Fry emerge from gravel the following spring, approximately 4 to 7 months later (Murray and Beacham. 1988; Sandercock 1991; DFO 2002). Fry emergence corresponds with periods of high discharge, and fry colonise flooded habitats created by spring freshets (Sandercock 1991). Juveniles feed within freshwater for one year and then migrate to the ocean as smolts (DFO 2005; Murray and Beacham 1988). Studying early life-stage development is an important focus for conservation and protection because environmental influences directly affect the physiological function of developing coho salmon. Larval development is also of interest as it is generally the time of highest mortality in salmonids (Quinn 2005).

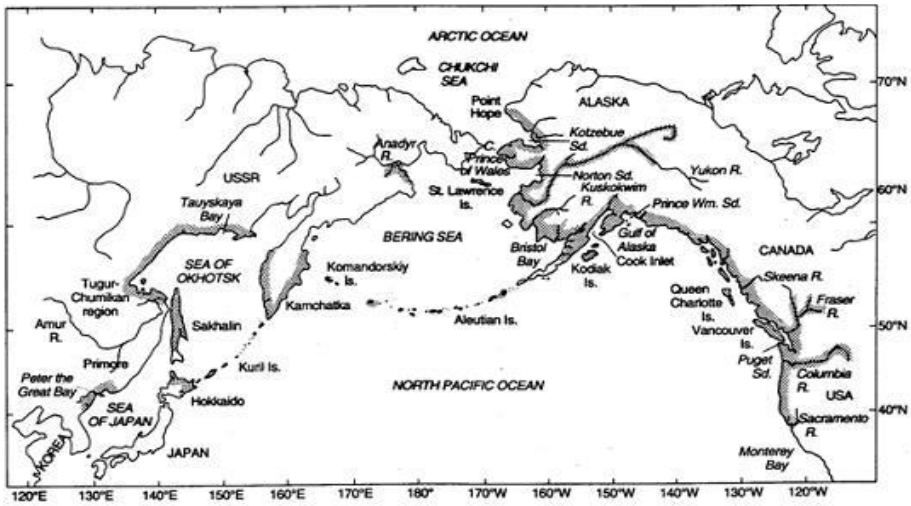


Figure P.1. The distribution of coho salmon (*Oncorhynchus kisutch)* throughout the Pacific Rim of North America and Asia (Sandercock 1991).

For my thesis I investigated the effect of temperature on a critical period of life history and physiology of coho salmon – spawning and early juvenile development. Chapter 1 summarizes a survey of the temperatures experienced throughout incubation across the geographical distribution of coho salmon within British Columbia. Chapter 2 investigated the different life-history traits, and the environmental variables that affect them, found in populations of coho salmon across their geographical distribution within BC. Specifically, I examined environmental variables that may influence egg size, fecundity, female size and reproductive investment (gonadal somatic index). From the environmental variables that I found to influence life-history traits of coho salmon, I further examined incubation temperature in Chapter 3. A controlled incubation study was conducted to test the effects of incubation temperatures on the development of coho salmon from two populations, one from northern BC and one from southern BC, with a specific focus on temperatures reaching near-freezing during incubation. This research will provide a better understanding of how temperature influences a species and how adaptive and plastic animals are to ranges in temperature currently experienced. The potential response of fish to future changes in climate are also of significant interest currently as temperatures are expected to change and be less consistent from year to year – a concern for salmon found throughout watersheds draining into the North Pacific Ocean.

CHAPTER 1

Variation in incubation temperature experienced by coho salmon (*Oncorhynchus kisutch*) across their geographic range in British Columbia

Abstract

Coho salmon (*Oncorhynchus kisutch*) have a large geographic distribution throughout British Columbia. It is expected that populations will experience highly variable temperatures throughout their range, especially at the extremes of their distribution, but the temperature regimes experienced by incubating coho salmon are not well understood. Currently, most data on the conditions experienced by coho salmon throughout incubation have been collected from surface waters, and not within the gravel environment of the redds. To gain a better understanding of the temperature regimes experienced by coho salmon throughout the incubation period, I surveyed both surface and intergravel temperatures within ten systems across British Columbia. I found that incubation temperatures varied significantly across British Columbia, which demonstrates the plasticity of this species as each population is successful across such a wide range of temperatures. Moreover, all the populations spawn at approximately the same time, despite the large geographic distances among them. Incubation temperature within the gravel differed significantly from surface temperatures. This is an important finding when attempting to extrapolate incubation temperature from surface water temperature as even small differences can affect incubation and development because the effects of temperature are cumulative. Considerable differences in accumulated thermal units, therefore, will result from small differences in temperature. Consequently, it is important to determine temperature regimes for each population, as future research needs to

be carried out on incubation temperatures within the redds themselves and how variation influences larval development.

Introduction

Coho salmon (*Oncorhynchus kisutch*) have a large geographic distribution throughout the north Pacific Rim. In North America, they can be found along the Pacific Coast from California to Alaska and are found to spawn in over 970 rivers and small streams throughout British Columbia (BC) (Sandercock 1991). With such a large geographic distribution, a wide range of temperatures throughout spawning and incubation must be experienced by different populations of coho salmon. Intergravel temperatures have been reported down to approximately 3 ºC for south coastal populations in BC (Shepherd et al. 1986). Coho salmon from an interior BC watershed, however, experience much lower temperatures during incubation, which can approach freezing at the coldest part of winter (McRae 2009).

Habitat use for spawning is dependent on physical characteristics of the stream and intergravel environment (McRae et al. 2012). An important factor for spawning site selection of coho salmon is intergravel temperature (McRae et al. 2012). Temperature is of interest for fisheries management as it has been found to be one of the leading factors in governing the growth and development of fish (Fry 1971; Blaxter 1992). Temperature effects in poikilotherms are cumulative as temperature has a direct effect on their metabolic rate, and therefore rate of development (Fry 1971; Brett 1995). Cold temperatures cause a decrease in metabolic and development rates that in turn cause a decrease in performance (Perry and Tufts 1998). Consequently, ectothermic animals have evolved to cope with specific temperature regimes, and relatively small temperature changes can have measurable effects

on community and population structure (Daufresne et al. 2009; Zuo et al. 2012; Paaijimans et al. 2013).

Intergravel temperature within a stream is dependent on two factors: the infiltration of stream water, and the upwelling of groundwater that together mix in the hyporheic zone. Intergravel water temperatures can differ considerably from surface water temperatures, and each system can vary based on the ratio of upwelling and downwelling (Freeze and Cherry 1979). Due to the relatively constant temperature of groundwater, intergravel temperatures are generally found to be warmer in winter and cooler in summer than surface water. Intergravel temperatures also show less response to diurnal heating than surface temperatures and will have a lag effect as intergravel temperatures are buffered by the thermal mass of the substrate and possible upwelling groundwater.

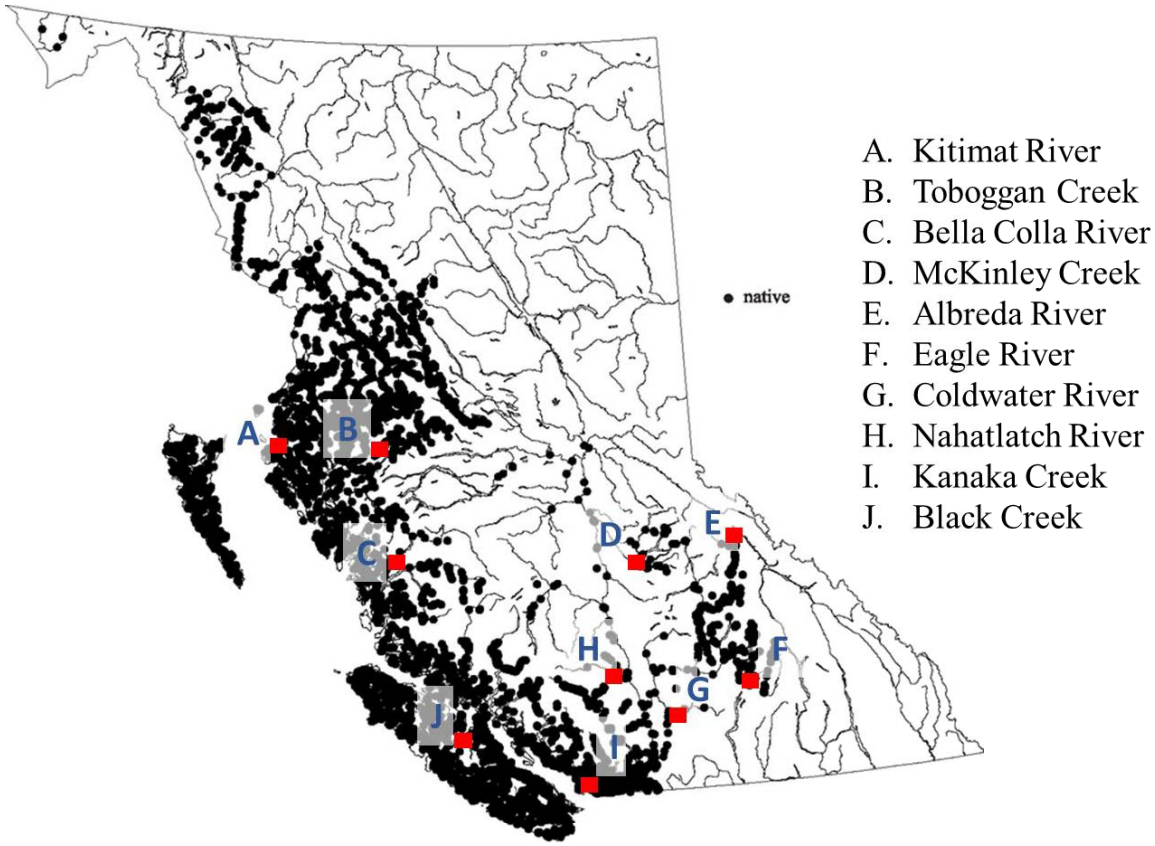
Little work has been done examining the range of temperatures that larval coho salmon experience during incubation. In an earlier study, Murray and Beacham (1988) found that survival rates of eggs and larvae from 13 populations of coho salmon from BC were highest at approximately 4 ºC; complete mortality occurred above 14 ºC. The study by Murray and Beacham (1988), however, may not represent the full range of temperatures that coho salmon experienced during incubation in BC. McRae (2009) found that coho salmon from an interior BC watershed experienced near-freezing temperatures of 0.3 ºC at the coldest part of winter during incubation. Studies need to be expanded, therefore, to elucidate the potential range of intergravel temperatures that coho salmon experience throughout their distribution. Additionally, surface temperature is often used as a proxy for incubation temperature, but the relationship between surface water temperature and redd temperature is poorly understood. Thus, the objectives of this research were to 1) determine the range of

temperatures experienced throughout the incubation period by populations of larval coho salmon across their distribution in BC, and 2) determine if surface temperature approximate the intergravel temperatures experienced by coho during incubation.

Methods

Stream Temperature across British Columbia

Ten systems across BC were chosen to represent the range of locations where coho salmon spawn (Figure 1.1). The systems were chosen based on their geographic position (latitude and longitude) and migration distance in an attempt to characterize as much of the distribution of coho salmon and potential physical environmental variability that exists throughout the province. Populations chosen were from four coastal regions with short migration distances but different latitudes (Kanaka Creek 49 ºN, Black Creek 50 ºN, Bella Coola River 52 ºN, and Kitimat River 54 ºN), from three regions with intermediate migration distances in central BC (Coldwater River 50 ºN, Nahatlach River 50 ºN, and Toboggan Creek 55 ºN) and from three interior regions with long-distance migrations (Eagle River 51 ºN, Albreda River 52 ºN, and McKinley Creek 52 ºN).



Map 44. The B.C. distribution of coho salmon, *Oncorhynchus kisutch*.

Figure 1.1: The distribution of coho salmon in British Columbia, represented as black circles and the ten study systems, represented as red squares: four coastal short migration distance populations (**I**-Kanaka Creek 49 ºN, **J**-Black Creek 50 ºN, **C**-Bella Coola River 52 ºN, and **A**-Kitimat River 54 ºN), three central BC populations with intermediate migration distances (**G**-Coldwater River 50 ºN, **H**-Nahatlach River 50 ºN, and **B**-Toboggan Creek 55 ºN), and three interior BC populations with long-migration distances (**F**-Eagle River 51 ºN, **E**-Albreda Creek 52 ºN and **D**-McKinley Creek 52 ºN).

For each river system, temperature loggers were placed in three different locations within the system where coho salmon were observed to spawn. Sites were selected based on historic knowledge of spawning locations provided by Fisheries and Oceans Canada and enhancement facility personnel, direct observation of the presence of spawning coho salmon at a location, or the presence of redds where coho salmon had previously spawned. For some

of the systems the different locations were within the main stem river, but different reaches. For other systems, the sample locations were located in different tributaries of the main stem river.

Nine to twelve HOBO U-22 temperature loggers (Onset Computer Corporation, Bourne MA) were deployed in each system in October and November prior to or during the spawning period. Three intergravel temperature loggers and one surface temperature logger were deployed at up to three sites within a system; sites were a minimum of three meters apart to capture some of the potential variation in temperatures from different redds within each system. Intergravel temperature loggers were buried approximately 25 cm upstream and to the side of redds to minimize disturbance to eggs; 25 cm is the average depth of coho salmon redds (Shephard et al. 1986; McRae et al. 2012). Surface temperature loggers were placed on top of the gravel in a pool close to the intergravel loggers. Loggers recorded temperature every hour for approximately six months throughout incubation from November 2012 to May 2013.

Statistical Analysis

Data collected from each logger were screened to remove data that indicated the logger was not in the gravel or was out of the water. This could be seen as periods when intergravel loggers had increased daily oscillations in temperature. Loggers that were out of the water also exhibited increased daily oscillations and an overall decrease in temperatures. Temperature regimes were analyzed using a repeated measure analysis of variance (ANOVA) to assess differences in means of incubation temperatures among and within the ten systems and their location in the system (gravel verses surface). Due to the requirement for equal number of samples per system, a sample size of 5 was used (number of loggers

placed in the Nahatlatch River system). For systems with data from more than five temperature loggers, five samples were chosen at random. Data from McRae (2009) was used to supplement two additional sites within the McKinlay Creek samples. A single surface temperature logger from each site within a system was paired with up to three intergravel loggers. Little difference in temperature has previously been observed for surface water temperature within a stream reach (McRae 2009; Williamson 2006). The assumption of sphericity was not met, thus the measure of Greenhouse-Geisser was used. Two time periods were used in the analysis; the temperature throughout the incubation period from November 22 to April 30, and the temperature during the coldest period of incubation – the month of January. Significant temperature differences from intergravel loggers and surface loggers between systems were also compared using post hoc Tukey test's. Statistical analysis was performed using SPSS statistical software (version IC 18; SPSS Inc., PASW Statistics, Chicago, IL).

Results

The maximum, average and minimum temperature regimes for all ten systems showed different trends over time (Figure 1.2). Most systems in my study were characterized by a gradual decline in temperature until the coldest months of the winter (January and February) and then a gradual increase in temperature towards the spring (Figure 1.2). The rate of increase and decrease in temperatures, however, varied among systems. The duration of the coldest temperatures also varied from a few weeks to more than a month (Figure 1.2).

Incubation temperatures for two of the systems were near-freezing throughout the coldest period. McKinley Creek was the coldest system that coho salmon incubated in with temperatures in all locations reaching 0.3 °C over the month of January. Toboggan Creek

reached a stable temperature of 0.7 °C early in incubation and maintained this temperature in all locations until a warming period in late March (Figure 1.2). Coldwater, Eagle, Nahatlatch and Bella Coola Rivers were found to be on average much warmer with coldest temperatures only reaching approximately 3.5 °C. There was considerable variation in temperatures among spawning sites within each of the warmest systems. Kanaka Creek was the warmest system for the longest period of time, with a gradual decrease in temperature until a very abrupt drop in temperature to 3 °C for a short period of time before an increase in temperature.

Within each watershed intergravel temperatures were significantly warmer than surface temperatures, for both the temperature throughout the entire incubation period ($F_{1, 4}$ = 22.01, P = 0.009) and the temperature throughout the coldest period of incubation ($F_{1, 4}$ = 28.96, P = 0.006) (Table 1.1; Figure 1.3). There was also a significant within-system effect for both the temperature throughout the entire incubation period ($F_{9, 44}$ = 24.353, P = 0.001) and the temperature throughout the coldest period of incubation ($F_{9, 44}$ = 10.650, P < 0.001) (Table 1.1). Additionally, there was a significant difference in incubation temperature among the systems, with the southern coastal (Kanaka Creek, Black Creek, Nahatlatch River, Eagle River and Bella Coola River) systems being significantly warmer than the northern coastal and interior systems (Kitimat River, Toboggan Creek and McKinley Creek) for temperature throughout the incubation period ($F_{9, 44}$ = 1463 P < 0.001) and during the coldest part of incubation ($F_{9, 44}$ = 98468 P < 0.001) (Tables 1.1 and 1.2; Figure 1.3).

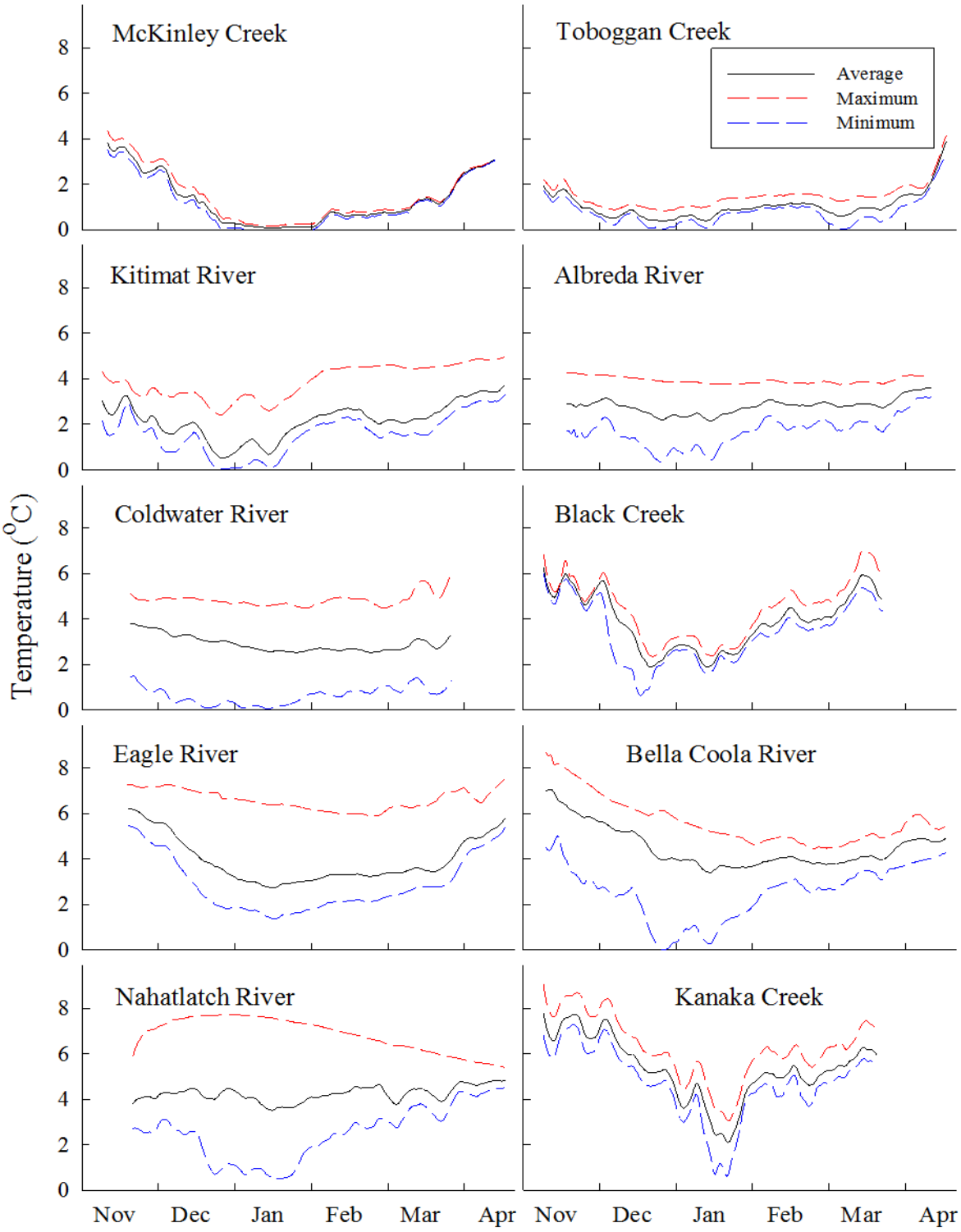


Figure 1.2: The running average (over 2 weeks) of the intergravel maximum, average, and minimum temperature regimes found across ten systems across BC where coho salmon are found to spawn. The systems are ordered based on latitude from the most northern on the top down to the most southern systems on the bottom.

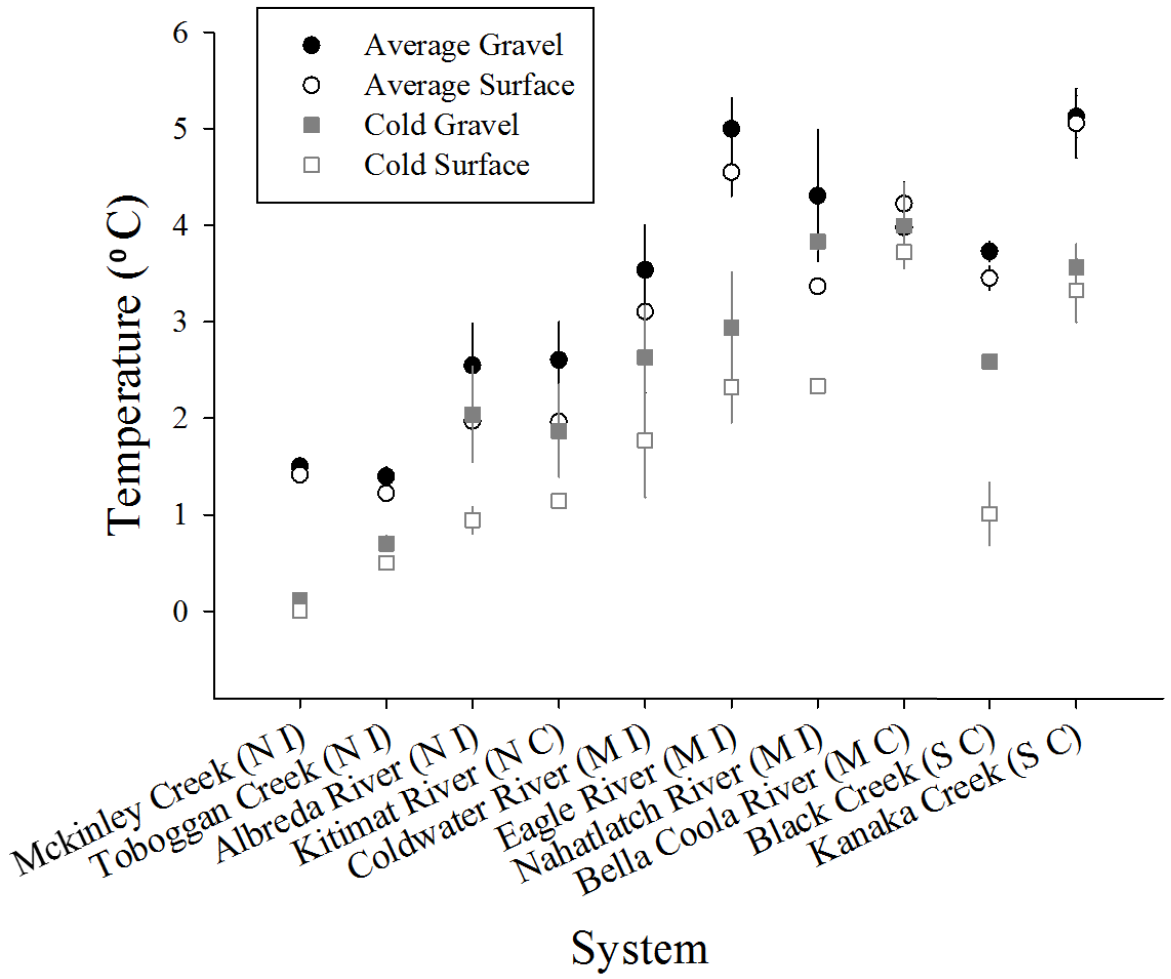


Figure 1.3: Average surface (open symbols) and intergravel (solid symbols) temperatures throughout the incubation period (2012-2013) for coho salmon across all ten systems measured in British Columbia. The average temperature throughout the incubation period of November 22, 2012 to April 30, 2013 is represented as circles and the coldest period throughout the incubation period (January) is represented as squares with standard error bars. The systems are ordered based on latitude from the most northern to the most southern systems (N = Northern latitude, Mid = Mid latitude, S = Southern latitude) and then ranked from interior to coastal within each of the latitudes (I = Interior, C = Coastal).

Table 1.1: Results from repeated measures analysis of variance testing whether average intergravel temperature during the incubation period (November 22, 2012 – April 30, 2013) and the coldest portion of incubation (January) differed among spawning locations of coho salmon in British Columbia. The variation is summarized based on the difference between systems across British Columbia and the logger location within each system (intergravel redd temperature and surface temperature), n = 5 pairs of surface and intergravel logger per system with a total n of 50.

Incubation Temperature		Source of Variation	SS	F	P
Average	Within-Subject Effect	System	201.98	24.54	0.001
		Logger location	3.31	22.01	0.009
		System x Logger location	2.307	0.983	0.414
	Between-Subject Effect		1032.36	1463.76	<0.001
Cold Period	Within-Subject Effect	System	117.49	10.65	<0.001
		Logger location	4.38	28.96	0.006
		System x Logger location	7.46	0.987	0.396
	Between-Subjects Effect		339.84	98468	<0.001

Table 1.2: Summary of statistics comparing study systems determined by post hoc Tukey's Test. Systems that differed significantly are represented with an "X" for the average intergravel temperature throughout incubation above the diagonal (November to April) and a "#" for the coldest period of incubation below the diagonal (January). Populations chosen are from four coastal regions with short migration distances but different latitudes (Kanaka Creek (Kan) 49 ºN, Black Creek (Blk) 50 ºN, Bella Coola River (Bel) 52 ºN, and Kitimat River (Kit) 54 ºN), from three regions with intermediate migration distances in central BC (Coldwater River (Col) 50 ºN, Nahatlach River (Nah) 50 ºN, and Toboggan Creek (Tob) 55 ºN) and from three regions with long distance migrations within the interior of BC (Eagle River (Eag) 51 ºN, Albreda River (Alb) and McKinley Creek (McK) 52 ºN). See Figure 1 for locations.

System	McK	Tob	Alb	Eag	Nah	Col	Kit	Bel	Bla	Kan
McK				X	X			X	X	X
Tob				X	X	X		X	X	X
Alb				X				X		X
Eag	#	#				X	X			
Nah	#	#								
Col										
Kit								X		
Bel	#	#								X
Bla										X
Kan	#	#								

Discussion

This study showed that incubation temperature experienced by coho salmon in BC differed significantly across populations (Table 1.1 and 1.2, Figure 1.3). Such differences demonstrate the plasticity of this species, as all the populations spawn at approximately the same time of year, despite the large geographic distance in locations and wide range of temperatures. Temperatures in the hyporheic environment where the northern interior populations spawn were near-freezing throughout the coldest period of incubation, January, demonstrating an impressive physiological capacity of these fish (Figure 1.2 and 1.3). Emergence time of fry is thought to differ across each of these systems as incubation periods and growth rates of salmonids are dependent on the temperatures experienced within each redd, specifically the number of accumulated thermal units (ATU) (Beacham and Murray 1990). Salmonid eggs incubating in warmer temperatures develop faster than those incubating at cold temperatures as it takes longer to reach the required number of ATUs to emerge from the redds. It is believed that, in general, populations of most species of salmon spawn at specific times of the year to increase the chances of fry emergence coinciding with the increased spring productivity (Burger et al. 1985). Populations spawning in colder systems, therefore, will do so earlier to increase the length of incubation so development will be completed for emergence at optimal spring productivity. Coho salmon, however, have a relatively narrow spawning window compared to other salmonid species, with all populations within BC spawning from October to December (Groot and Margolis 1991). Thus, populations of coho salmon appear atypical compared to other species, as interior populations are found to spawn when stream temperatures are already very cold, but at a similar time of the year to southern populations. In contrast, other species of Pacific salmon

have a wider spawning period and interior populations often spawn earlier in the fall when stream temperatures are warmer (Groot and Margolis 1991).

Intergravel water temperatures within each redd differed significantly from river surface temperature, and were consistently warmer than surface temperatures throughout all ten systems (Table 1.1, Figure 1.3). The effect of temperature on development of salmonids is cumulative, resulting in considerable differences in ATU for very small differences in water temperature. Thus, hatching and emergence dates could vary significantly with only a small difference in temperature, especially for systems at near-freezing temperature where developing embryos are likely to be highly sensitive to the temperature differences. For example, 250 ATU are required to reach hatching, which takes 21 days at 0.5 °C, whereas approximately 52 days are required at 0.2 °C. Consequently, surface water temperatures are not likely to be a useful estimate of intergravel temperatures when analyzing incubation conditions. Additionally, the temperature regimes found within the hyperheic zone are more stable in comparison to the river surface temperatures, which have significant diurnal variation as a result of several processes. The major factor influencing both air and stream temperature is incoming solar radiation. Daily variation in stream temperature is related to the amount of cover from riparian zones (Johnson and Jones 2000). Increased shading of a stream is found to significantly decrease the maximum stream temperature by minimizing the exposure of direct sunlight (Johnson 2004). Wind speed, relative humidity, subsurface saturation of the bedrock and substrate all can influence stream temperature (Johnson 2003). Stream temperatures can also be influenced by the amount of snowpack insulating the system as well as anchor ice throughout the winter, which can freeze incubation sites (Cunjak and Power 1986; Cunjak 1996). However, the hyperheic zone is found to be much more stable in

comparison as it is dependent on two factors: the infiltration of stream water and the upwelling of groundwater that mix in the hyporheic zone. Intergravel water temperatures will also differ considerably from surface water temperatures based on the ratio of upwelling and downwelling water (Shepherd et al. 1986). Based on these patterns, Shephard et al. (1986) also believed that intergravel temperatures would differ from surface temperatures, affecting the precision of ATU and development rate predictions when relying on surface conditions. Thus, it should not be assumed that intergravel redd environments can be characterized from surface water variables – but knowledge of the hyporheic zone should be considered for fish enhancement and habitat management projects.

Temperature within a system differed significantly (Figure 1.2, Table 1.1), suggesting that the used spawning habitat selected within a system was not always consistent within a population. A number of intergravel loggers in redds of coho salmon, however, were directly above upwelling ground water, resulting in consistently warmer temperature (5 to 7 °C) throughout the entire incubation period in comparison to other intergravel loggers deployed in redds within the same system. Salmonids will often spawn in areas with discharging ground water (Cunjak and Power 1986; Garrett et al. 1998), specifically when winter incubation conditions are severe (Baxter and McPhail 1999). Offspring of salmonids found spawning in locations with groundwater discharge have improved survival as redds influenced by groundwater are not only warmer, but more stable than locations without groundwater (Baxter and McPhail 1999; McRae et al. 2012). Groundwater, however, has lower levels of dissolved oxygen (McRae et al. 2012), which is found to slow development (Davis 1975). This suggests that enhanced development achieved by selecting warmer temperatures may be offset by lower dissolved oxygen, which limits growth.

McRae et al. (2012), found a significant difference between used and unused spawning habitat, suggesting that the small variation among sites within my study may indicate selection of sites for spawning. Site selection would contribute to more uniform offspring development, survival among individuals, and random survival within a population. These factors are important for maintaining large effective population sizes (Waples 1990a), which contributes to resiliency of a population to stochastic events that may decrease numbers of fish. Waples (1990b) showed that low-frequency alleles are subject to rapid extinction in Pacific salmon where effective population size is small and can lead to inbreeding depression. Thus, populations with small effective sizes are at a much greater risk for extinction (Newman and Pilson 1997), which may occur with little warning (Frankham 1995). Shrimpton and Heath (2003) demonstrated that available spawning habitat is positively correlated with effective population size, suggesting that more area available for spawning enhances population resilience. The relative effect on redd success is not known from my study, but may lead to population-level effects on the effective size of breeding stock. Temperature and site selection of individuals, therefore, can play a large role in effective population size and should be considered in management practices.

Temperature is one of the most influential abiotic features affecting fish throughout their life cycle. Stream temperatures have become a major issue and are at the centre of policy debate, because elevated temperatures can negatively impact cold-water fish species, such as threatened or endangered salmonids (Johnson 2003). Salmonids have evolved temperature-specific life-history strategies to ensure that spawning occurs at a time of year that will maximize incubation and emergence survival of their offspring (Quinn 2005). Thus, an understanding of the full range of temperatures experienced by coho salmon throughout

incubation, and how populations have adapted and evolved throughout their distribution, specifically for northern interior populations, is of interest both for management and conservation initiatives. Future research should include long-term monitoring of intergravel incubation temperatures experienced by coho salmon throughout a wider range of systems, as my study only examined a single year and only 10 river systems. Stream temperatures can vary from year to year, so a long-term monitoring study would increase our understanding of temperature influences on development and survival of coho salmon. Also, quantifying incubation temperatures within spawning sites and other apparently suitable, but unused, sites within each system would provide a better understanding of whether coho salmon are using spawning sites with specific temperature conditions to increase survival and development throughout incubation, as suggested by McRae et al. (2012). Selecting for specific spawning temperatures within a system could result in optimal emergence times in relation to the spring freshet.

CHAPTER 2

Spatial and temporal variation in life-history strategies of coho salmon (*Oncorhynchus kisutch*) populations throughout British Columbia

Abstract

Coho salmon (*Oncorhynchus kisutch*) have a narrow spawning window, from late October to early December, throughout British Columbia. However, they have a very large geographic distribution across British Columbia, and different environmental conditions are experienced by populations throughout their range. Escapement for most populations is low in number compared to other species of Pacific salmon, and so a better understanding of the environmental variables influencing individual populations is needed to properly manage and conserve this species. Here, I examined the effect of several environmental variables on egg size, fecundity, female size and reproductive investment of populations of coho salmon from across British Columbia. Egg size increased with higher latitude, warmer incubation temperature, longer migration distance, more years of hatchery enhancement programs, and smaller size of the spawning system. Both female size and fecundity increased with latitude, warmer incubation temperature, and longer migration distance, but decreased with larger size of spawning system. Gonadal somatic index decreased with higher latitude, warmer incubation temperature and larger size of spawning system. Taken together, these results reveal that latitude of spawning grounds, size of spawning system and temperatures experienced by a population have a significant effect on shaping patterns of reproductive investment. Thus, these three environmental variables should be considered when conserving and developing management strategies for individual populations of coho salmon.

Introduction

Species with wide geographic distributions experience a range of physical factors, but arguably temperature is the dominant factor for poikilotherms. I found that temperature differed significantly throughout the geographical range within British Columbia that coho salmon are known to spawn (Chapter 1). Understanding how individual organisms successfully exploit habitats within their range is important to fully understand how to manage the species not only at the population level, but throughout their geographic range. This is especially important when working with and managing species of concern because conservation efforts would benefit from a fuller understanding of the species' physiology, ecology and life history. A species able to exploit a range of habitats throughout their distribution may use different life-history traits and display different trade-offs to maximize fitness (Lappalainen 2008). Specific differences in life-history traits among populations during the spawning and incubation period are of particular interest in Pacific salmon because the highest rates of mortality occur during the incubation and alevin stages (Groot and Margolis 1991; Quinn 2005).

Svardson (1949) suggested that allocation of resources to egg production, defined as the product of egg number and egg size, will be optimized but may vary due to local selection pressures. There is, however, a trade-off between the size and number of eggs produced, which precludes investing maximally in both traits simultaneously (Svardson 1949). The number of eggs, therefore, will vary in response to selective pressures both on egg size and total investment in egg production (Fleming and Gross 1990). Previous work has shown that fecundity of fish generally increases with latitude and increases with female size (Leggett and Carscadden 1978; Beacham 1982; Fleming and Gross 1990; Beacham and

Morley 1985). Populations of coho salmon in Alaska have significantly higher fecundity than lower latitude populations in British Columbia (BC) and California (Beacham 1982). Fleming and Gross (1990) also found that total fecundity and egg size of Pacific salmon increased with higher latitude and was independent of other influences such as competition and migration distance.

Despite widespread distribution of coho salmon, most studies have focussed on populations from the United States, the southern mainland of BC, and Vancouver Island. While some studies have been conducted in Alaska, surprisingly these data were not presented in several investigations (Beacham 1982; Fleming and Gross 1990; Beacham and Murray 1993). Thus, data from northern and long-distance migrating interior populations of coho salmon are needed to fully understand the variation in life-histories of coho salmon and potential differences among populations.

Variation in life-history strategies among populations of coho salmon have been linked to both spatial and temporal effects, but rarely have studies incorporated other environmental factors that may influence life-history traits. Such variables include migration distance, which is energetically costly for an individual, the temperature of the system, and the size and type of the headwater system that feeds each system, as it will have an influence on the temperature and productivity of each system. My objective was to understand how life-history traits differ for populations of coho salmon, both spatially and temporally, throughout British Columbia, and how these life-history traits relate to environmental conditions found within the incubation habitat. To characterize habitat features that may influence egg size, fecundity, female size, and gonadal somatic index, six environmental variables were measured for locations throughout BC where coho salmon spawn. Spawning

systems were chosen based on available historic data which coincided with the systems in Chapter 1. This research will provide a better understanding of how environmental variables influence coho salmon populations across their distribution in BC, but also investigate how environmental variables interact, which has not been addressed in previous studies.

Methods

Stocks used in the analysis and data collection

Life-history traits were compared for eight populations of coho salmon selected from different regions across BC that represented a range of migration distances and potential incubation conditions experienced by this species (Figure 2.1). Data were obtained from Fisheries and Oceans Canada (FOC) or published sources (Beacham 1982; Fleming and Gross 1900), and included female size (post orbital length), fecundity, and mean egg size. Archived data were obtained from the Kitimat River Hatchery for the Kitimat population, Snootli Creek Hatchery for the Bella Coola population, Spius Creek Hatchery for the Eagle River and Coldwater River populations, Toboggan Creek Hatchery for the Toboggan Creek population, Kanaka Creek Hatchery for the Kanaka Creek population, Quesnel River Hatchery for the McKinley Creek population, and Big Qualicum Hatchery for Black Creek population. Each population was assessed over multiple years (Table 2.1). Additional life-history data on mean fecundity and female size were also obtained from Beacham (1982) for populations on Vancouver Island that were used in addition to the data from the Black Creek (Table 2.1).

Data on egg size were available either as weight or volume measurements. I used egg weight in my calculations and for consistency I transformed volume to weight using Bonham's (1976) equation:

$$Weight\frac{g}{egg} = volume\frac{ml}{egg}\ x\ 1.076$$

Gonadal somatic index (GSI), a ratio of gonad weight to somatic weight, was calculated by:

$$GSI = \frac{Weight\frac{g}{egg}\ x\ Fecundity}{Total\ Female\ Weight}$$

Female weight, however, was not collected for each population, but instead data on length were collected. Based on a condition factor of 1.06 g · cm^{-3} (CF; 100 W · L^{-3}), determined from Kitimat River population females (Chapter 3), length was used to calculate an approximate total weight. A CF of 1.06 for mature female coho salmon falls within the range reported by Scholz et al. (2011) for spawning female coho salmon in the Puget Sound Lowlands.

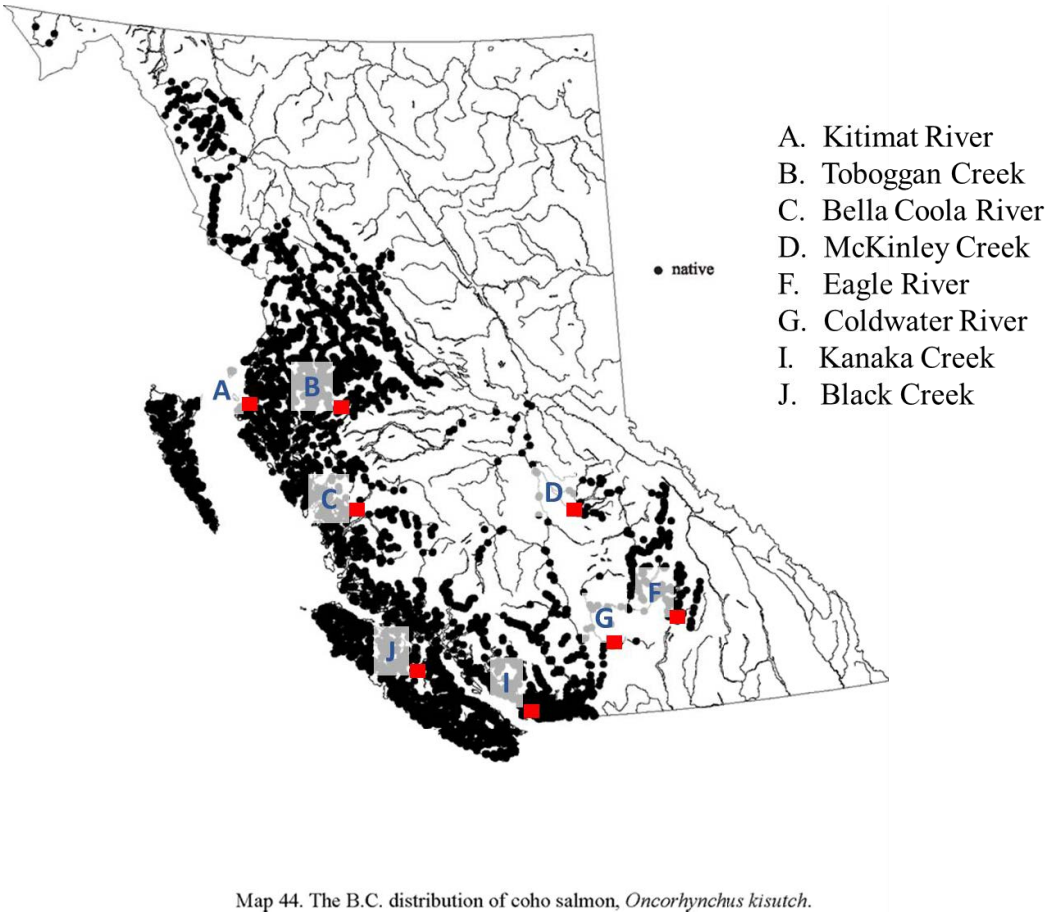


Figure 2.1. Locations where data were collected for environmental variables and life-history traits of coho salmon across British Columbia for this study. Location are ladled based on the systems used throughout Chapter 1. The missing systems are due to lack of available life-history data.

Table 2.1: Summary of the available data collected from each system for each life-history trait (egg size, fecundity, female size at maturity, gonadal somatic index [GSI]). The range of sample years and total number of years collected for each life-history trait.

System		Life-history Trait (n)			
	Years	Egg Size	Fecundity	Female Size	GSI
McKinley	1983-1990	8	1	N/A	N/A
Toboggan	1989-2008	20	20	20	20
Kitimat	1984-2013	15	30	13	4
Black	1959-1976; 2008-2014	N/A	15	15	N/A
Coldwater	2000-2013	14	14	14	14
Eagle	1998-2013	8	8	8	8
Bella Coola	1999-2013	8	4	N/A	N/A
Kanaka	2011-2014	4	4	4	4

Model Development

The effects of environmental variables on life-history traits were analyzed using truncated regression models and negative binomial regression to determine variations across each system. Truncated regression models were used to examine variation in egg size, female size and gonadal somatic index as they are continuous data with the exclusion of values below zero. Negative binomial regressions were used to explain the variation in fecundity as the data were over-distributed count data. Variance inflation factors (VIF) were examined for each independent variable to determine if they resulted in multicollinearity within models. Variables with a VIF greater than 10 were considered to have a high degree of multicollinearity and not used in the model with other highly collinear variables. Categorical variables were adjusted to create a design matrix to correct for linear dependencies in each model. System was used in each model as a random factor to account for non-independence of data for each system.

Model Parameters

Candidate models were developed from a set of predictor variables recognized as important for explaining variation in egg size, fecundity, female size, and gonadal somatic index of coho salmon. A total of seven predictor variables were used: system temperature, which was the average temperature (°C) throughout the entire incubation period; average gravel temperature (°C) throughout the coldest period of incubation (January); average temperature (°C) throughout the peak of spawning; latitude; migration distance from the ocean (km); number of years of hatchery operation; system size (ranked as large or small based on the size of the system where coho salmon are found to spawn); and type of headwater influence (Table 2.2).

Table 2.2: List of environmental variables used in analyses of variation in life-history traits of coho salmon.

Model Parameter	Description	Categories	Reference
Temperature	January Temperature – average gravel temperature within each system throughout the coldest period of incubation (average throughout January) determined in Chapter 1.	Continuous	Murray and Beacham 1988 Shepherd et al. 1986 Clarke and Johnston 1999 McRae et al. 2012
	System Average – average gravel temperature for each system throughout the duration of incubation from November - May determined in Chapter 1.	Continuous	Murray and Beacham 1988 Clarke and Johnston 1999 McRae et al. 2012
	Spawning Temperature – average river surface temperature for each system throughout the peak spawning period of November determined in Chapter 1.	Continuous	Murray and Beacham 1988 McCullough 1999
Latitude	The average latitude measured across the three study sites within each system studied in Chapter 1 were used as the latitudinal coordinate of each system. Average latitude was calculated from GPS coordinates taken at each site with an accuracy of ± 3 meters.	Continuous	Leggett and Carscadden 1978 Beacham 1982 Fleming and Gross 1990 Chavaries et al. 2010
Migration Distance	The average migration distance in kilometers traveled from the ocean to the spawning site in each system.	Continuous	Groot and Margolis 1991 Brett 1995
Stream Size	The size of stream where coho were found to spawn based on study sites used for the temperature data collection in Chapter 1.	Small (1) Big (2)	Rasenfeld et al. 2000
Headwater Type	The source of headwaters that feeds into each system.	Snow melt (1) Lake (2)	

Model Selection

An information theoretic approach was used to evaluate competing models for explaining the influence of environmental variables on life-history traits. Akaike's Information Criterion, corrected for small sample sizes (AICc), was used to rank models within candidate model sets (Burnham and Anderson 2002). The model with the lowest AICc in a candidate model set was considered the most parsimonious model, but those models within 2 AICc units from the best approximating model were considered competitive (Burnham and Anderson 2002). Akaike weights (ωi), the relative likelihood that the candidate model is the best model given the data and the model set (Burnham and Anderson 2002), are reported for each model. I interpreted parameter estimates from a single top model within the candidate model set when ω_i of the top model exceeded 0.90; in cases where there was model selection uncertainty (i.e., $\omega_i < 0.90$), I calculated model-averaged estimates of parameter estimates using all models within the candidate model set with $\Delta AICc < 2$ (Burnham and Anderson 2002). Model-averaging minimizes the effect of uninformative parameters (confidence intervals that overlap 0) with little impact on the bias and precision of the more useful parameter estimates (Arnold 2010). Statistical analysis was performed using STATA statistical software (version IC 12; StataCorp, College Station, TX).

Results

Egg Size

The best approximating model for egg size variation suggested that latitude, January incubation temperature, migration distance, number of years that hatchery enhancement programs were run, and size of system were influential (Table 2.3). The second ranked model was similar except that it did not include the number of years that hatchery

enhancement programs were run. The null model for egg size and all other life-history traits were found to do very poorly. Model-averaged estimates suggested that egg size increased with higher latitude (β = 12.16, SE = 4.22, 95% CI [3.84 to 20.37]), longer migration distance (β = 0.19, SE = 0.03, 95% CI [0.11 to 0.26]), and with decreasing size of a system (β = -55.17, SE = 10.81, 95% CI [-76.37 to -33.98]). Warmer January temperatures (β = 4.82, SE = 5.91, 95% CI [-6.77 to 16.41]) and the number of years of hatchery enhancement to the system (β = 0.56, SE = 0.33, 95% CI [-0.09 to 1.22]), however, were uninformative as the confidence intervals overlapped zero.

Fecundity

Environmental variables in the best approximating model explaining variation in fecundity included latitude and January incubation temperature. The second ranked model was similar, but also included size of system. The third competitive model included size of system and migration distance in addition to latitude and January temperature (Table 2.4). Model-averaged estimates indicated that latitude (β = 0.06, SE = 0.02, 95% CI [0.02 to 0.09]), January incubation temperature (β = 0.085, SE = 0.03, 95% CI [0.02 to 0.14]) had a positive effect on fecundity. Migration distance (β = 0.001, SE = 0.0002, 95% CI [-0.0003 to 0.0006]) and size of system (β = -0.01, SE = 0.01, 95% CI [-0.028 to 0.007]), however, were uninformative as the confidence intervals overlapped zero.

Female Size

For size of female coho salmon at maturity, the best approximating model included latitude, spawning temperature throughout November, and size of system (Table 2.5). A second competitive model was similar, but also included migration distance. Model-averaging indicated that size of females increased with higher latitude (β = 10.44, SE = 0.51,

95% CI [9.38 to 11.38]) and spawning temperature (β = 7.33, SE = 7.33, 95% CI [6.08 to 8.57]). Migration distance (β = -0.002, SE = 0.002, 95% CI [-0.005 to 0.001]) and the size of the river system (β = -18.10, SE = 0.96, 95% CI [-19.97 to -16.22]), however, were uninformative as the confidence intervals overlapped zero.

Gonadal Somatic Index

The best approximating model for GSI included January incubation temperature and size of system. The second competitive model was similar, but also included latitude (Table 2.6). Model-averaging suggested that all three variables were negatively related to GSI. Gonadal somatic index was lower with warmer incubation temperatures (β = -5.93, SE = 1.15, 95% CI [-7.99 to -3.50]), and in larger systems (β = -4.48, SE = 0.83, 95% CI [-5.86 to -2.61]). Latitude was uninformative as the confidences intervals overlapped zero (β = 0.08, SE = 0.41, 95% CI [-0.87 to 0.72]).

Table 2.3: Summary of AICc ranking of candidate models for environmental variables influencing egg size of coho salmon. Lat = Latitude, JanG = average temperature found in each system throughout the coldest period of incubation (January), SysAvg = average temperature found in each system throughout the incubation period from November to April, YOH = total number of years an enhancement hatchery for coho salmon has been in operation within each system, Mig = migration distance to each system from the ocean, Size = size (using ranks) of each river, HeadW = type of headwater that feeds each system.

Model Parameters	K	AICc	ΔAICc	ωi
Lat + JanG + Mig + YOH + Size	6	893.3	0.0	0.486
Lat + JanG + Mig + Size	5	893.7	0.3	0.413
Lat + JanG + Mig + YOH + HeadW	6	898.1	4.8	0.045
Lat + HeadW	3	900.2	6.8	0.016
Lat + JanG + Mig + HeadW	5	901.7	8.3	0.008
Lat	2	902.1	8.8	0.006
Lat + JanG + HeadW	4	902.2	8.8	0.006
Lat + Size	3	902.2	8.8	0.006
Lat + SysAvg	3	903.7	10.4	0.003
Lat + YOH	3	903.8	10.4	0.003
Lat + JanG	3	904.1	10.8	0.002
Lat + Mig	3	904.2	10.8	0.002
Lat + JanG + Size	4	904.3	11.0	0.002
Lat + JanG + YOH	4	905.0	11.6	0.001
Lat + JanG + Mig	4	905.9	12.5	0.001

Table 2.4: Summary of AICc ranking of candidate models for environmental variables influencing fecundity of coho salmon. Lat = Latitude, JanG = average temperature found in each system throughout the coldest period of incubation (January), SysAvg = average temperature found in each system throughout the incubation period from November to April, YOH = total number of years an enhancement hatchery for coho salmon has been in operation within each system, Mig = migration distance to each system from the ocean, Size = size (using ranks) of each river, HeadW = type of headwater that feeds each system.

Model Parameter	K	AICc	ΔAICc	ωi
Lat + JanG	3	1589.3	0.0	0.275
Lat + JanG + Size	4	1590.7	1.4	0.137
Lat + JanG + Mig + Size	5	1590.8	1.5	0.130
Lat + JanG + YOH	4	1591.3	2.0	0.101
Lat + JanG + Mig + YOH + Size	6	1591.3	2.1	0.098
Lat + JanG + Mig	4	1591.5	2.2	0.093
Lat + JanG +HeadW	4	1591.5	2.2	0.093
Lat + JanG + Mig + YOH	5	1593.	4.1	0.035
Lat + JanG + Mig + YOH + Size + HeadW	7	1593.7	4.4	0.031
Lat + JanG + Mig + HeadW	5	1593.7	4.4	0.031
Lat + JanG + Mig + YOH + HeadW	6	1595.4	6.1	0.013
Lat + SysAvg	3	1596.4	7.1	0.008
Lat + YOH	3	1607.4	18.1	0.000
Lat + Mig	3	1614.3	25.0	0.000
Lat	2	1615.2	26.0	0.000

Table 2.5: Summary of AICc ranking of candidate models for environmental variables influencing female size of coho salmon. Lat = Latitude, SpTemp = average temperature found in each system throughout the peak spawning period in November, SysAvg = average temperature found in each system throughout the incubation period from November to April, YOH = total number of years an enhancement hatchery for coho salmon has been in operation within each system, Mig = migration distance to each system from the ocean, Size = size (using ranks) of each river, HeadW = type of headwater that feeds each system.

Model Parameters	K	AICc	ΔAICc	ωi
Lat + SpTemp + Size	4	685.2	0.0	0.689
Lat + SpTemp + Mig + Size	5	687.4	2.2	0.230
Lat + Size	3	690.1	4.9	0.059
Lat + SpTemp + Mig	4	692.3	7.1	0.020
Lat + Mig	3	702.5	17.3	0.001
SpTemp + Size	3	702.9	17.7	0.000
Lat + SpTemp + YOH	4	704.3	19.1	0.000
Size	2	704.6	19.4	0.000
Lat + HeadW	3	708.1	22.9	0.000
Lat + SpTemp + HeadW	4	709.4	24.2	0.000
Lat + JanG	3	712.1	26.9	0.000
Lat + YOH	3	713.6	28.4	0.000
Lat	2	724.0	38.8	0.000
Lat + SysAvg	3	726.1	40.9	0.000
SpTemp + Mig	3	736.2	51.0	0.000

Table 2.6: Summary of AICc ranking of candidate models for environmental variables influencing gonadal somatic index of coho salmon. Lat = Latitude, JanG = average temperature found in each system throughout the coldest period of incubation (January), SysAvg = average temperature found in each system throughout the incubation period from November to April, YOH = total number of years an enhancement hatchery for coho salmon has been in operation within each system, Mig = Mig distance to each system from the ocean, Size = size (using ranks) of each river, HeadW = type of headwater that feeds each system.

Model Parameters	K	AICc	ΔAICc	ωi
JanG + Size	3	348.8	0.0	0.711
Lat + JanG + Size	4	351.1	2.2	0.230
Lat + Size	3	356.4	7.5	0.016
Lat + YOH	3	356.6	7.7	0.015
JanG + YOH	3	357.2	8.4	0.011
Lat + JanG + YOH	4	357.5	8.7	0.009
JanG + Mig	3	359.1	10.2	0.004
Lat + JanG + Mig	4	361.1	12.3	0.002
JanG + HeadW	3	362.0	13.5	0.001
JanG	2	362.7	13.8	0.001
Lat + JanG + HeadW	4	364.7	15.8	0.000
Lat + JanG	3	364.8	15.9	0.000
Lat + Mig	3	366.2	17.3	0.000

Discussion

This study represents the first examination of how environmental factors affect life-history variation in a wide-ranging species which incorporated not only physical variables that fish experience during incubation across a range of latitudes, but also migration distance. A number of previous studies have examined life-history variation among populations at different latitudes, but the populations have been limited to coastal watersheds or short migration distance into interior river systems (Fleming and Gross 1990; Beacham and Murray 1993; Braun et al. 2013). In my study, data were collected from populations of coho salmon that migrate approximately 20 km to stocks that spawn more than 750 km from the ocean. Coho salmon are a good model organism for this study due to this wide geographic distribution, but also their relatively narrow spawning window from late October to early December. A general pattern observed was that females from populations in northern systems were larger and more fecund with larger eggs compared to female spawners from southern systems. Coho salmon from northern interior populations that migrate longer distances were also found to have larger eggs than southern coastal stocks and are, therefore, investing considerably more energy into not only somatic growth, but also reproductive potential. Such a finding contrasts with the theory of reproductive investment trade-offs presented in earlier literature for anadromous salmon – that with an increase in latitude number of eggs increases, but egg size and total biomass of eggs produced decreases (Beacham 1982; Fleming and Gross 1990; Beacham and Murray 1993). The variables that affect the life-history traits examined in this study and potential reasons for the apparent disparity from earlier work are explored below.

Egg Size

Egg size of coho salmon was found to increase in more northern populations, but also with greater migration distance – the opposite trend presented in the studies by Fleming and Gross (1990) and Beacham and Murray (1993). The geographic distribution in the study by Fleming and Gross (1990) was relatively narrow and did not extend into the northern distribution of coho salmon within BC compared to the geographical distribution that was covered within my study. Beacham and Murray (1993) examined latitudes from 47° to 55°, but did not incorporate migration distance in their analysis. My study examined latitudes from 49° to 55° with migrations distances up to 700 km into the northern interior of British Columbia. The earlier studies suggested egg size decreased with increased latitude as a trade-off for an increase in the number of eggs produced allowing reproductive investment to be similar across latitudes but increase offspring fitness. With fewer juvenile competitors, increased spring productivity, and size selective gradients in higher latitudes it was suggested that selection for larger egg size was reduced.

Fleming and Gross (1990) also suggest that the decrease in egg size at higher latitudes is due to the decreased incubation temperatures, and the efficiency of conversion of yolk to body tissues and offspring size relationships found by Johnston and McLay (1997). Within my model egg size increased with an increase in temperature experienced throughout the coldest period of incubation, but the 95% confidence intervals overlapped zero, indicating incubation temperature throughout January on its own was not influential. It is surprising that temperature did not influence egg size due to the direct effect of temperature on metabolic rate and yolk conversion efficiency (Stickland et al. 1988; Johnston and McLay 1997; Kileen et al. 1999). Hyperstatic growth of white muscle fibers was found to decrease

with increased incubation temperatures, thus decreasing the efficiency of conversion of yolk to body tissues (Stickland et al. 1988; Johnston and McLay 1997; Kileen et al. 1999). With the decrease in conversion efficiency in warmer temperatures, salmon will produce smaller alevin and fry for a given egg size at higher temperatures, thus larger eggs in warmer incubation temperature will maximize overall growth (Stickland et al. 1988; Johnston and McLay 1997). Murray and Beacham (1988) also found that northern and mainland stocks incubated at low temperatures (1.5 to 2.0 °C) were found to have larger, heavier alevin and fry than southern and coastal populations. My findings that temperature was not influential may be due to how cold incubation conditions were for some of the populations of fish examined. When temperatures reach near-freezing these trends may reverse as fish are found to develop at rates faster than expected; less ATU are required to reach the same development stage (Williamson 2006; Murray and Beacham 1988; Chapter 3). Metabolic rates of fish incubating at near-freezing temperatures are not well understood. Development at such cold temperatures, however, must be metabolically costly.

The fact that years of hatchery operation was in the top model suggest that this variable is important, however, it also proved to be uninformative. The number of years of hatchery enhancement within a system was previously shown to have a positive effect on egg size. Fleming and Gross (1990) and Quinn et al. (2004) found coho salmon eggs were larger for hatchery populations than wild populations. The data of Fleming and Gross (1990), however, was later reanalyzed by Murray and Beacham (1993); addition of a regional variable resulted in a lack of difference among populations. In contrast, Heath et al. (2003) and Haring et al. (2016) found that enhancement programs relaxed selective pressure for

larger eggs in Chinook salmon, driving selection for decreased gonadal investment, increased fecundity and smaller eggs.

My findings also suggest that females spawning in smaller systems have larger eggs – opposite to previous studies. It was suggested that smaller systems will experience less bedload movement resulting in smaller substrate as the lower flow will not wash out fine sediments from among the larger gravel and boulders (Knighton 1998). Smaller eggs would be selected for in systems with smaller gravel size, which have reduced oxygen level within the gravel as they have reduced oxygen demand and more efficient surface-to-volume ratio in comparison to larger eggs (Quinn et al. 1995; Rollinson and Hutchings 2011). Smaller systems within this study were found to be warmer coastal systems with relativity good spawning substrate, suggesting that the temperature influence and available nutrition on emergence may have a bigger influence on egg size. Larger systems such as the Nahatlatch River, Eagle River and Coldwater River had smaller gravel in areas where redds were located, thus these finding may be correlated with the effects of gravel size on egg size. Stream size, therefore, is a variable that is difficult to relate to life-history traits.

Fecundity

Fecundity increased with latitude and migration distance consistent with a number of previous studies. The increase in fecundity with latitude has been suggested to be a reproductive trade-off to produce more, smaller eggs rather than few, large eggs (Beacham 1982; Fleming and Gross 1990; Beacham and Murray 1993). This reproductive trade-off, however, was not found among the coho salmon populations in my study, which have an overall increase in reproductive investment, as the northern populations are found to have increased fecundity and egg size with increased latitude – although latitude was included in

the model for GSI, surprisingly the effect was not found to be influential (see below). Fecundity of sockeye salmon (*Oncorhynchus nerka*) has also been found to increase with increased female size (Braun et al. 2013), consistent with my findings as large females were found to spawn farther north and at longer migration distances.

Temperature had a positive influence on fecundity such that females whose offspring had warmer incubation temperatures were more fecund. Systems with higher temperatures were found along the coast in warmer, wetter climates, with longer growth seasons. Longer growing seasons with higher productivity may support more individuals with less competition for resources. The model also suggested that females spawning within smaller systems had higher fecundity. Smaller rivers in my study were mostly found within the coastal warmer systems. Conversely, systems with longer migration distances and colder temperatures have longer and harsher winters resulting in shorter growth periods with lower productivity. Lower fecundity may be offset by fewer, larger eggs to lower competition within lower productivity systems and increase available energy reserves for initial development. Although size of system and migration distance from the ocean have the potential to affect fecundity, these two variables were found to be uninformative in my model.

Female size

Female size and morphology have previously been shown to be influenced by total reproductive investment (higher in larger females), migration distance (thinner, smaller fish with longer caudle peduncle in streams with longer migration distance), and competition for redd location on spawning grounds (more pronounced kype, larger body size, and brighter colouration with increased competition) (Fleming and Gross 1989). Braun et al. (2013) found

that larger female sockeye salmon produced more large eggs than smaller females – an overall increase in reproductive investment. Informative variables in my model were that female size increased with latitude, increased with spawning temperature, and decreased with size of system. The previous models for egg size and fecundity showed an increase in fecundity and larger egg size with higher latitude, demonstrating increased reproductive investment of larger females at higher latitudes. Smaller females also spawned within larger systems, a pattern consistent with the previous models that less reproductive investment was allocated by spawners in larger systems as both fecundity and egg size decreased. Van Den Berghe and Gross (1989) found female size contributed to overall fitness in three ways, increase initial biomass of egg production, the ability to acquire a high-quality territory for redds, and success in nest defense. Thus, in smaller systems that may have limited spawning habitat and higher competition, larger females may be more successful. The type of head water influence to a system may also influence the productivity and temperature of a system suggesting that females are larger in lake fed systems (Rasenfeld et al. 2000) – however, system type was not in any of my top models.

My study used length of female as a measure of size due to data availability. Although my model suggested that length of female decreased with increased migration distance, the large confidence intervals suggest this variable was uninformative. Smaller females have been found from stocks that migrate longer distances; Fleming and Gross (1989) reported that both body depth and length of female coho salmon decreased with increased migration distances. Taylor and McPhail (1985) found that interior Fraser juvenile coho salmon have a more fusiform (streamlined) body shape, which may persist throughout life into adult migration, in comparison to costal populations. Adults in my study from

interior populations with more energetically demanding migrations also may have distinctive fusiform morphology as it is superior for sustained swimming compared to costal populations having more robust bodies (Taylor and McPhail 1985; Fleming and Gross 1989). To better understand the effect of environmental variables on female size condition factor and overall morphology should be considered in the future where data are available.

Gonadal Somatic Index

Gonadal somatic index decreased for fish spawning in systems that were larger and had warmer incubation temperatures. Potentially females from larger systems were allocating a smaller proportion of their energy budget to reproduction and defending somatic size. Larger systems could have higher discharge resulting in more difficult migration for an individual. Larger systems may also have larger substrate due to increased bedload movement with higher discharge. Thus, more somatic energy may be needed to ensure the females are large enough to migrate and spawn in larger systems. Colder incubation temperatures were also found for the northern interior systems where not only did the eggs incubate at colder temperature, but temperature was colder for spawning and most likely for migration. Braun et al. (2013) found that gonadal mass decreased with increased migration difficulties for sockeye salmon – a finding consistent with my study as migration is more difficult for adult salmon at warmer temperatures (Farrell et al. 2008). Additionally, less energy is required for metabolic processes at colder temperature, thus there may be limited benefit of producing large eggs, allowing females to allocate energy to other processes such as producing more eggs and somatic growth (Stickland et al. 1988; Johnston and McLay 1997). Latitude was found to be uninformative within the top models for GSI, however, it is a significant variable for all other life-history traits examined. With latitude significantly

influencing egg size, fecundity and female size, one would think it would also be a significant environmental driver of GSI. Unfortunately, limited data were available for GSI. An increased data set with increased latitudinal distribution may result in a significant influence of latitude being detected.

Conclusion

Care must be taken when interpreting data as a few limitations do arise when working with large data sets compiled from enhancement programs and previous studies. The availability of life-history data was inconsistent across the populations of interest, resulting in uneven representations of populations across each of the life-history models. Dependent on the data collected by hatcheries in the past and the year sampling occurred within a hatchery, data for specific life-history traits were not always available for each population or for consecutive years, resulting in variation in sample sizes. However, a minimum of ten years for each population was used whenever possible to create a robust data set. One area of interest for further investigation would be to include the age of spawners in addition to the life-history traits incorporated in the present study, as this may also vary within and among populations due to environmental influences.

With these considerations in mind, the data suggests that based on geographic distribution, three environmental variables have the most influential on reproductive investment of coho salmon. The models reveal that latitude of spawning grounds, size of spawning system and temperatures experienced by a population have a significant effect on shaping patterns of reproductive investment throughout British Columbia. Thus, these three environmental variables should be considered at a population level when conserving and developing management strategies for individual populations.

CHAPTER 3

The effects of near-freezing incubation temperatures on development, metabolic rate and yolk utilization in coho salmon (*Oncorhynchus kisutch*)

Abstract

Populations of coho salmon experience incubation temperatures that differ across their distribution in British Columbia; from near-freezing for some populations to above 6 °C for others. Incubation at near-freezing temperatures demonstrates an impressive physiological capacity, and may represent local adaptation to increase survival. To investigate potential adaptation to cold temperatures, an incubation study was conducted on a northern and a southern population of coho salmon with pure and hybrid families incubated at three different temperature regimes (cold reaching 0.5 °C, mid at 4.5 °C, and warm at 9 °C). The near-freezing incubation temperatures resulted in less accumulated thermal units to reach each development stage, lower condition factors, lower resting metabolic rate and more yolk available for somatic growth than the warmer incubation temperatures. There was little difference between the two populations, however, suggesting little local adaptation for development at cold temperatures. Larval development, however, was strongly influenced by maternal origin, which was associated with egg size; smaller eggs from the southern population produced smaller offspring that developed faster than offspring from the northern population with larger eggs. Coho salmon exhibit exceptional phenotypic plasticity as little difference was found between families from the northern and southern populations.

Introduction

Temperature is one of the most influential variables governing reproductive investment. In Chapter 2, I showed the importance of temperature for fecundity, female size and gonadal somatic index, but temperature also governs growth, development and survival of salmon at other stages of development. Even small changes have profound effects on the development rate of fish as the effect of temperature is cumulative. This is particularly evident for larval fish; intergravel temperature throughout early development of coho salmon (*Oncorhynchus kisutch*) was found to be important for spawning site selection and development (McRae et al. 2012; Chapter 2). For a species such as coho salmon with large geographic distributions, considerable differences in temperature are experienced, yet the effect of such differences are not clear. In the killifish (*Fundulus herteroclitus*), latitudinal differences in populations have been shown to translate into differences in thermal tolerance (Fangue et al. 2006). Adaptive phenotypic change or phenotypic plasticity may be crucial for population persistence where selection pressures are altered. Use of spawning sites by salmonids has been examined in two interior BC systems, McKinley Creek and Davis River. These studies found that surface waters and even intergravel temperatures within redds of coho salmon and bull trout (*Salvelinus confluentus*) were close to freezing for long periods (McRae 2009; Williamson 2006). The development of embryos at such extreme temperatures represents an impressive physiological capacity within a species and is not yet fully understood. Fish that are adapted to cold temperatures have higher metabolic rates (Johnston et al. 2000) and proportionally faster rates of development at a given temperature (Williamson 2006). This increase in metabolism required for survival at extreme temperatures near-freezing may come at a cost to other measures of performance.

Temperature tolerance of larval Pacific salmon has been examined previously (Murray and Beachem 1988; Beacham and Murray 1990; Jonsson and Jonsson 2011; Whitney at al. 2014). Murray and Beachem (1988) suggested that Pacific salmon appear to adapt to specific spawning temperatures to maximize survival and size of fry (Murray and Beachem 1988). Their study on coho salmon used 13 populations from BC, reared at five different temperatures from 1.5 to 15 ºC, and showed survival was highest between 4 and 5 ºC and complete mortality occurred above 14 ºC. The study by Murray and Beachem (1988), however, may not represent the full range of temperatures that coho salmon experience during incubation in British Columbia. For example, McRae (2009) found that coho salmon from an interior BC watershed experience near-freezing temperatures that averaged 0.3 ºC at the coldest part of winter during incubation. Additionally, I showed that temperature regimes experienced throughout incubation by coho salmon across BC differed significantly, ranging from 0.1 to 5 ºC on average throughout the coldest period of incubation (Chapter 1).

Energy during larval development is limited by yolk supply, which must be partitioned among metabolism, growth, development and activity (Callow 1985; Rombough 2006). Yolk protein serves two main functions: amino acids for tissue growth and energy for catabolic processes (Heming and Buddington 1988). Energetic trade-offs experienced by larval fish may arise when yolk reserves necessary for growth and development are used for activity associated with movement and maintaining metabolic rates (Brett and Groves 1979; Callow 1985). It is also important to understand how energy is allocated between somatic growth and metabolic processes, as the rates of absorption of yolk are important determinants of development, growth, and survival in larval fish (Heming and Buddington 1988). Transforming yolk to body tissue as efficiently as possible should have fitness consequences

because larger larvae are expected to be stronger swimmers and less susceptible to predation (Heming and Buddington, 1988). At colder temperatures, less energy is needed to maintain a stable metabolic rate, resulting in more available energy to be allocated to somatic growth. Thus, species at higher latitudes in colder environmental conditions will theoretically be able to grow larger as there is more energy available for growth.

To gain a better understanding of physiological adaptations that may occur at extremely cold temperatures experienced throughout incubation and early development, a controlled incubation study was conducted. I examined the effect of incubation temperature on the number of accumulated thermal units required for development, resting metabolic rate, yolk availability for somatic growth, size of fish, and survival. To test whether local adaptation had an effect on these variables, two populations were examined; one from southern and one from northern BC. Further, both pure strain and reciprocal hybrid families were created to assess maternal and paternal influences from both populations.

Methods

Gamete collection and breeding design

This study was conducted from November 2013 to June 2014. Two populations of coho salmon with short migration distances (approximately 50 km), but from different latitudes (a southern BC population, Kanaka Creek 49 ºN, and a northern BC population, Kitimat River 54 ºN), were used to minimize the influence of migration distance on development. Gametes from three males and three females were collected from both the Kanaka Creek Hatchery and the Kitimat River Hatchery and transferred to the Aquatic Animal Holding Facility at the University of Northern BC on November 22, 2013. Once at the Aquatic Animal Holding Facility, eggs were fertilized to create a replicated two by two

design with pure and hybrid families to determine treatment effect on each family. One male and one female from each population were crossed to create six pure and six hybrid families (Appendix 3.1). Each family was divided into six groups, one for each temperature regime and replicated twice within each temperature treatment.

Experimental treatments

Three incubation temperature regimes were used; a "cold" treatment which simulated the coldest temperatures experienced by coho salmon throughout BC (McKinley Creek), a "mid" treatment that was similar to the warmest temperatures (Kanaka Creek and Bella Coola River; see Chapter 1), and a "warm" treatment which was increased at the same rate that the cold treatment was decreased, until it reached close to the upper temperature tolerance range for larval coho salmon (9 °C). Incubation commenced at the same temperature (4.5 °C) for all three treatments to minimize stress on eggs when first transferred to the incubators. Temperature was then gradually altered to reach the target temperatures for each incubator (approximately 0.5 °C for the cold, 4.5 °C for the mid and 9 °C for the warm). Temperatures were increased in the spring for the cold treatment to mimic the natural warming of temperature regime of McKinley Creek (Figure 3.1).

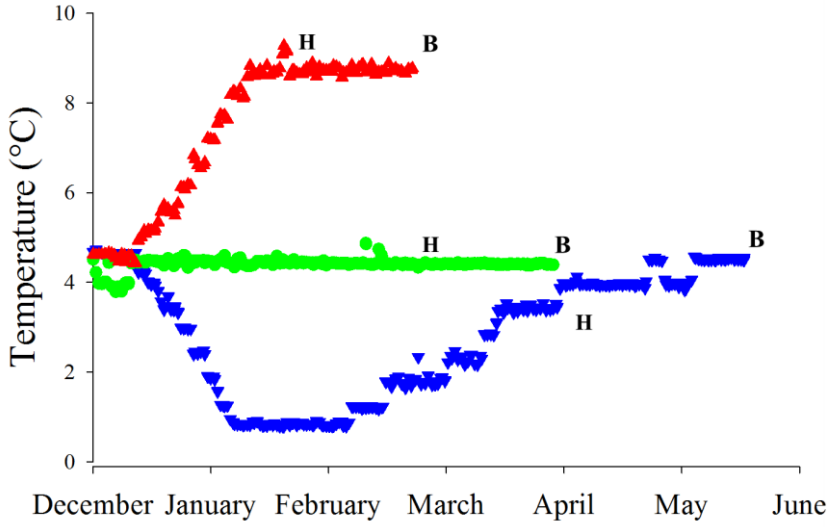


Figure 3.1: Temperature regimes followed throughout the incubation period for coho salmon until button-up (red = warm, green = mid, and blue = cold treatment). The approximate date of hatch (H) and button-up (B) are shown for each temperature regime.

Three Heath-style incubators were used, one for each incubation temperature regime, each with a large plastic barrel (200 L) as a headwater reservoir containing a D1-33 or D1-100 refrigeration unit (Frigid Units Inc., Toledo OH) to control temperature. Prince George municipal water, treated with aquarium water conditioner to remove chlorine, was used in each reservoir and introduced to the Heath stacks from the reservoir barrel by gravity, passed through the stack and pumped back into the reservoir by an aquarium pump, providing continuous circulation of water through the system. Each incubator contained four Heath trays, the top one to catch the outflow from the reservoir before flowing down over the eggs, the middle two were divided into 12 sections each holding different families incubated at the same temperature, and the bottom tray passed the water into a second reservoir where it was passed through a filter and then pumped back into the initial reservoir.

Heath stacks were covered in black plastic to block ambient light from the eggs and larvae. Temperature, dissolved oxygen, pH and conductivity were measured daily to assess water quality. Onset U-22 data loggers were placed in each reservoir and within each Heath

tray to measure temperature hourly throughout the study to detect any potential positional effects on the temperature experienced by the fish. Water was changed every 1-2 days (40 L per stack, total volume of each system was 280 L) to maintain water quality.

Size, Condition Factor and Survival

Samples were collected from each of the families at three development stages: one week after fertilization, hatch, and button-up. The hatching date was determined when 50% of the replicate was hatched; the period from hatch of the first alevin to complete hatch for the replicate did not differ among families within the same treatment, but it did vary among treatments as eggs in the cold treatment were much slower to hatch. Button-up was determined when the fish in each family had completely absorbed the yolk sack. Accumulated thermal units (ATU) were calculated for each stage of development as the number of days each family took to reach a particular development stage multiplied by the mean daily temperature. Once the eggs had eyed, mortalities were removed daily to determine survival rates for each stage of development. For development measurements, two fertilized eggs were sampled from each family, while four to six samples (based on population sizes of each replicate, as mortality rates were high for a few replicates) were taken from each replicate in each treatment for the last two development stages. Length (if possible) and weight was measured for each development stage, and a condition factor was calculated for hatch and button-up as $100(W \cdot L^{-3})$.

Oxygen Consumption

Oxygen consumption rates ($\mu g\ O_2 \cdot h^{-1}$) were measured on three fish from each replicate (six fish per family per treatment) at hatch and button-up for the middle and cold treatments using intermittent flow respirometry. The respirometry equipment was not fully

set-up and running in time to collect measurements for the warm treatment groups. An eight-chamber respirometer was used to measure six fish simultaneously, while two were left empty for each trial and used as a control. Horizontal mini glass chambers (length 35mm, ID 9mm, CH10645, Loligo Systems, Tjele, Denmark) were fitted with mini fibre optic O_2 sensor spots (5 mm, X11050, Loligo System), which do not consume oxygen.

At the beginning of each set of trials, fish were placed into individual glass chambers which were covered with black plastic to keep the chambers dark, and left to acclimatize and reach a resting state for an hour before oxygen consumptions rates were measured. The temperature bath and chambers were filled with water directly from the treatment incubator with recirculating pumps to maintain the temperature as close as possible to the rearing temperatures. The systems were filled prior to adding any fish to ensure the O_2 spots were completely wet before measurement. Two peristaltic pumps (PU10650, Loligo Systems) were used to flush the chambers with fresh oxygenated water from the temperature bath and two others used to create closed circuits with 1 mm (ID) tubing (TU11350, Loligo Systems). The pumps were left running for an hour prior to the first measurement to allow the fish to acclimatize to the chambers and reach resting metabolic rate. The flushing pumps were then shut off and oxygen consumption measured until oxygen levels within the chambers reached 70% of saturation, at which time the flushing pumps were turned back on to exchange the water within the chamber with 100% saturated water for 45 min. For each trial, fish were measured until a minimum of two runs were recorded with a linear decrease in oxygen in each chamber. Temperature and oxygen levels were recorded every 2 seconds using the Witrox 4 oxygen analysers (OX11875, Loligo Systems) and fibre cables (OX11150, Loligo Systems) which read the O_2 sensor spots. Measurements were converted with the DAQ-M

instrument (AR12500, Loligo Systems) and AutoResp respirometry software (AR12600, Loligo System). The length of time each test was run was dependent on the rate of oxygen consumption within the chamber, and thus varied for each trial and treatment. Resting metabolic rates were calculated as the decrease in oxygen in the chamber based on the chamber volume and divided by the mass of the fish within the chamber. The temperature coefficient (Q_{10}) which represents the factor by which the rate of a reaction increases for every ten degree rise in temperature was measured for each family between each treatment. The temperature coefficient is defined as:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

where Q_{10} is the *factor* by which the reaction rate increases when the temperature is raised by ten degrees. Q_{10} is a unitless quantity. R_1 is the measured reaction rate at temperature T_1 (where $T_1 < T_2$). R_2 is the measured reaction rate at temperature T_2 (where $T_2 > T_1$).

Yolk Area Analysis

To determine yolk area at hatch, images were taken of four to six fish from each population within each treatment stages using a MEIJI microscope and Motic Image Plus software. All images were taken at 1.4x magnification and the head was adjusted and maintained at the highest point possible on the microscope to ensure consistent results. Total length of fish, yolk area and yolk perimeter were measured in millimeters using the Motic Image Plus software. Similar methods have used imaging software to measure yolk cross-sectional area (Wells and Pinder 1996; Marty et al. 1997; Boucher 2012). Relative yolk area was measured by dividing the yolk area at hatch by the average egg area of the population.

Statistical Analysis

The data for weight, length, condition factor, yolk area, resting metabolic rate, and survival were collected using a partial factorial design with repeated measurements of each population nested within each temperature treatment. I used a nested three-way analysis of variance (ANOVA), with origin of maternal gamete and paternal gamete nested within treatment, for all measurements except resting metabolic rate at hatch for which I used a nested two-way ANOVA comparing the pure families. The two replicates for each family within a temperature treatment were combined because there was no difference in the results with all replicates analysed individually. Family groups were then used as replicates to test for effects of maternal and paternal origin. Maternal and paternal effects were analysed for each female and male cross however, families did not differ from one another, therefore for all subsequent analysis maternal origin and paternal origin were analysed by population. All assumptions for analysing each data set with a three-way ANOVA, (normal distribution, homoscedasticity and independence of observations) were assessed before analysis (Gotelli and Ellison 2004). For each measurement, the data met all the assumptions except variances were heteroscedastic. Transformations of the data (log +1, ln, and square root) did not achieve homoscedasticity. ANOVA, however, is robust to unequal variance when the sample size is large (Zar 1996). Tukey post hoc tests and pairwise comparison with an adjustment for unbalanced samples sizes were run to determine the specific significant differences present in each model. Statistical analysis was preformed using STATA statistical software (version IC 12; StataCorp, College Station, TX).

Results

Development Rate

All progeny in the mid and warm treatments required significantly more ATU to reach hatch than the cold treatment ($F_{2, 35} = 3438$, $P < 0.001$; Figure 3.2a). There was a significant maternal ($F_{1, 35} = 576$, $P < 0.001$) and paternal ($F_{1, 35} = 7.48$, $P = 0.012$) origin effect, and a maternal by paternal interaction ($F_{1, 35} = 231$, $P < 0.005$) effect on the number of ATU to reach hatch. Additionally, there was a significant interaction effect between all three variables (treatment, maternal origin and paternal origin) on the number of ATU required to reach hatch ($F_{2, 35} = 4.95$, $P = 0.01$). The progeny of the pure northern origin fish required significantly more ATU to reach hatch than the progeny from the southern maternal origin within all three treatments. Summary tables for all 3-way ANOVA results are presented in Appendix 3.2.

The number of ATU required to reach button-up was significantly greater with increased temperature treatment ($F_{2, 35} = 14020$, $P < 0.001$; Figure 3.2b). At button-up, however, there was no longer a significant interaction between all three variables ($F_{2, 35} = 1.31$, $P = 0.28$), but there was a significant interaction between treatment and maternal origin ($F_{2, 35} = 50.91$, $P < 0.001$), as well as treatment and paternal origin ($F_{2, 35} = 4.18$, $P = 0.03$). The progeny of the northern maternal origin required significantly more ATU to reach hatch than the progeny from the southern maternal origin within the cold and mid treatments (Figure 3.2b).

Size and Condition Factor

The weight of fish at hatch was strongly dependent on maternal origin ($F_{1, 364} = 222.35$, $P < 0.0001$) and treatment ($F_{2, 364} = 7.04$, $P < 0.001$). The progeny of the northern

females were significantly heavier than the progeny of the southern females within all three temperatures (Figure 3.3a). Families reared in the mid temperature regime were significantly larger than families reared in cold and warm temperature regimes (Figure 3.3a). The effect of maternal origin by treatment was significant for length ($F_{2, 364} = 10.79$, $P < 0.0001$) and condition factor ($F_{2, 364} = 10.00$, $P < 0.0001$). Progeny of southern females reared in the cold temperature regime were significantly shorter than fish reared at the mid and warm temperature regimes (Figure 3.3b). There was less difference for progeny of northern females, however, the fish reared at the mid temperature regime were significantly longer than cold and warm treatments. Condition factor also differed with treatment; the significantly shorter progeny from northern females reared at cold and warm temperatures resulted in significantly higher condition factor for these groups (Figure 3.3c).

The relationships found at button-up, however, differed from the findings at hatch. The interaction between all three variables (treatment, maternal origin and paternal origin) for weight at button-up, however, was not significant ($F_{2, 666} = 2.96$, $P = 0.053$). There was a significant interaction between maternal and paternal origin for weight ($F_{1,666} = 16.47$, $P < 0.0001$), such that the progeny of females of the northern origin were heavier than from females of the southern origin, and a paternal effect with hybrid families for both north and south origins were lighter than the pure families (Figure 3.4a). The effect of treatment was also significant for weight at button-up ($F_{2, 666} = 26.76$, $P < 0.0001$). Families reared in the warm temperature regime were larger than families reared in cold temperature regime and the families reared in the mid temperature regime were intermediate (Figure 3.4a). At button-up there was a significant interaction between maternal and paternal origin for length ($F_{1, 666} = 7.95$, $P = 0.005$) and also a significant interaction between treatment and maternal origin

for length ($F_{2, 666} = 8.01$, $P < 0.0001$); progeny of northern females reared at the coldest temperature regime were the longest (Figure 3.4b). The paternal effect was due to the pure strain families being heavier and longer than the hybrid families (Figure 3.4). For condition factor, there was also a significant treatment by maternal effect ($F_{2, 666} = 6.80$, $P < 0.002$). At button-up families incubated within the cold treatment were lighter but longer than families incubated within the mid and warm treatments which resulted in the lowest condition factors (Figure 3.4). Although, families from warm treatment groups at button-up were heaviest and exhibited the highest condition factor, fish within the cold treatment were lighter, but longer and exhibited the lowest condition factor at button-up; opposite to the findings at hatch (Figure 3.3c and 3.4c).

Oxygen Consumption

Resting metabolic rate was measured at hatch for the cold and mid temperature treatment groups. Temperature for the cold group was approximately 0.5 ºC and resting metabolic rate was significantly lower than the mid temperature treatment at 4.5 ºC ($F_{1, 279} = 186.43$, $P < 0.0001$; Figure 3.5a). The Q_{10} values at hatch between the mid (4.5 ºC) and the cold (0.5 ºC) treatments was 8.4 for the Kitimat Pure families and 8.2 for the Kanaka Pure families. There was no maternal or paternal origin effect on resting metabolic rate at hatch. Interestingly, at button-up there was a significant three-way interaction between treatment, female, and male origin ($F_{1, 326} = 16.51$, $P < 0.0001$, Figure 3.5b). The northern hybrid family within the mid treatment had a significantly higher metabolic rate and was the only family found to be significantly different than all other families within both treatments. Despite the different thermal histories experienced by the two groups, there was minimal treatment effect

on the resting metabolic rate as oxygen consumption was measured at similar temperatures for each treatment (Figure 3.5b).

Yolk Area

The yolk area at hatch was found to differ significantly between treatments ($F_{2, 315} = 19.72$, $P < 0.0001$), being significantly smaller in the warm treatment than the cold and mid treatments (Figure 3.6a). Yolk area was also strongly dependent on the maternal origin ($F_{1, 315} = 328.74$, $P < 0.0001$) as the progeny of females from the northern stock, both pure and hybrid families, had significantly larger yolks than the individuals with a southern maternal gamete. Standardizing yolk area based on initial egg size, however, the maternal effect was no longer significant. There was still a significant treatment effect ($F_{2, 315} = 19.11$, $P < 0.00001$) with the warm treatments having significantly smaller yolk area than the cold and mid (Figure 3.6b).

Survival

Temperature had no effect on overall survival. Survival for all families in all three treatments was over 50%. There was a significant maternal by paternal interaction on total percent survival ($F_{1, 71} = 7.90$, $P < 0.006$; Figure 3.7), such that the progeny of the southern paternal origin had significantly lower overall survival than the progeny of the northern paternal origin. Across all three treatments the northern hybrid families had the lowest overall percent survival. Both the southern pure and hybrid families had very high variation in survival among replicates (Figure 3.7). Timing of mortalities, however, differed across the treatments, with the most mortalities occurring between hatch and button-up within the warm treatments for all four families. Most mortalities in the cold temperature treatments occurred prior to hatch for all families.

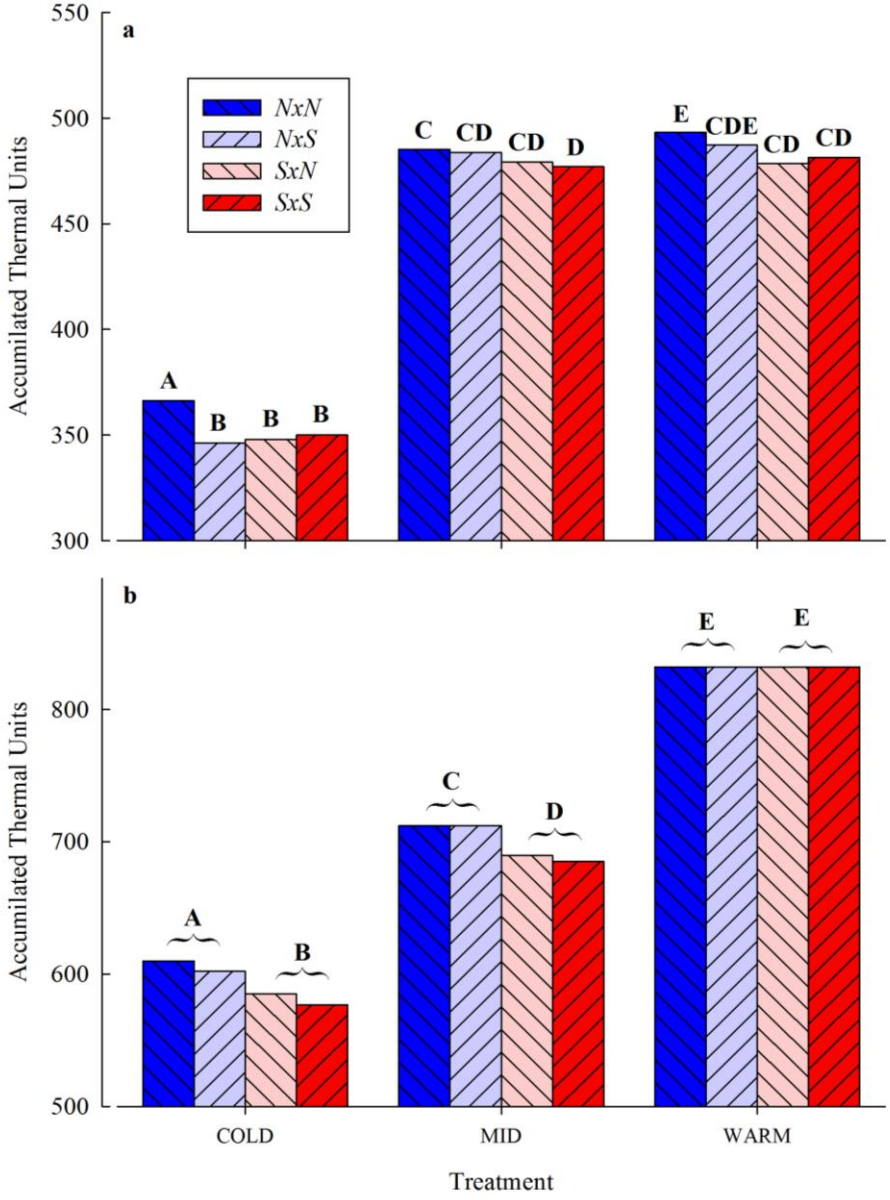


Figure 3.2: The required accumulated thermal units to reach hatch (a) and button-up (b) for the different pure and hybrid families of coho salmon reared at cold, mid and warm temperature regimes (see Figure 3.1). Families are represented as female X male pairs from a northern population (N: Kitimat River) or a southern population (S: Kanaka Creek). The upper-case letters above each bar in figure a) represent the significant difference between the three way interactions of treatment, maternal and paternal origin. The letters above each bar in figure b) represent the significant differences determined by the two-way interaction of treatment and maternal origin and the treatment and paternal origin.

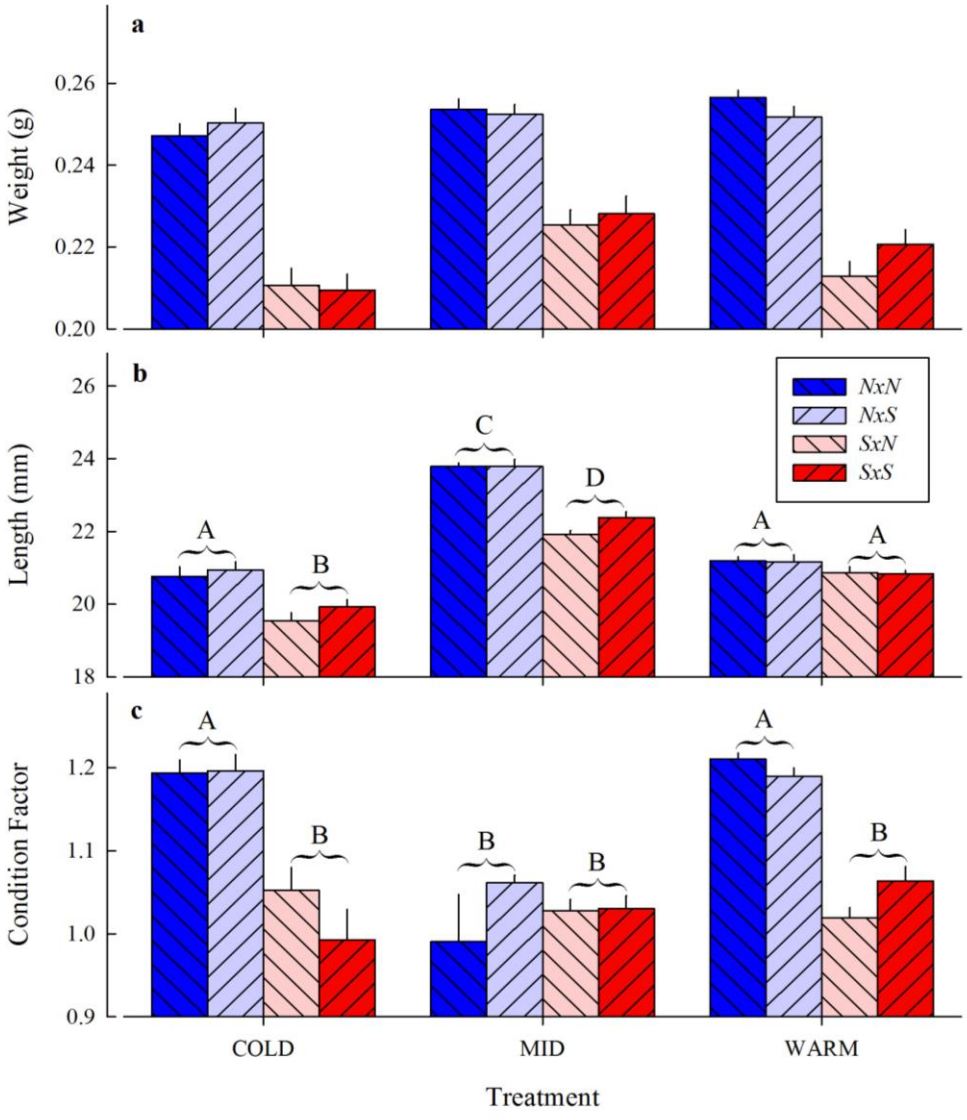


Figure 3.3: Weight (a), length (b) and condition factor (c) at hatch for the different pure and hybrid families of coho salmon reared at cold, mid and warm temperature regimes (see Figure 3.1). Families are represented as female X male pairs from a northern population (N: Kitimat River) or a southern population (S: Kanaka Creek). The upper-case letters above each bar represent the significant treatment and maternal origin interactions. Data are shown as means + standard error.

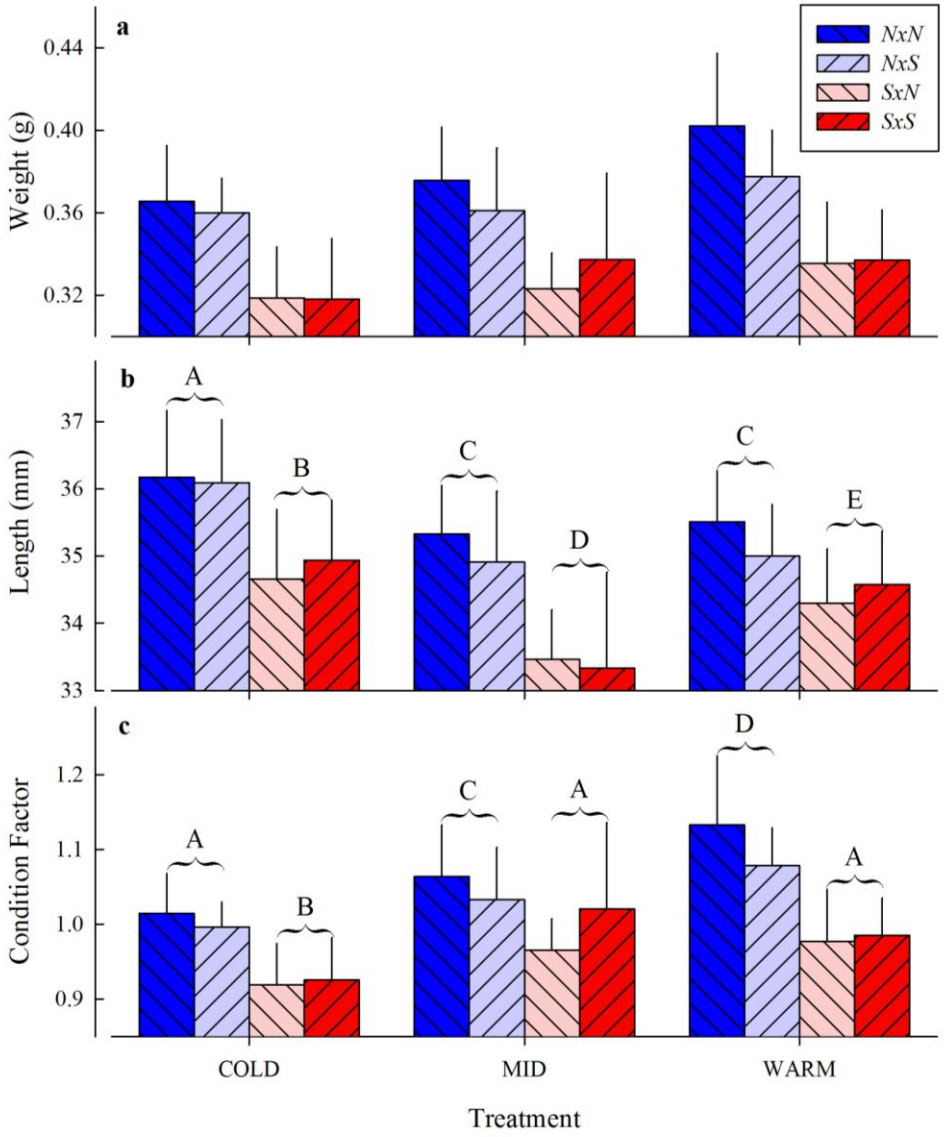


Figure 3.4: Weight (a), length (b), and condition factor (c) at button-up for the different pure and hybrid families reared at cold, mid and warm temperature regimes (Figure 3.1). Refer to Figure 3.3 for detailed description of the legend. The upper-case letter above each bar represent the interaction effect of treatment and maternal origin and the interaction effect of maternal and paternal origin. Figure a) has a three-way interaction effect of treatment, maternal origin and paternal origin and is described within the text. Data are shown as means + standard error.

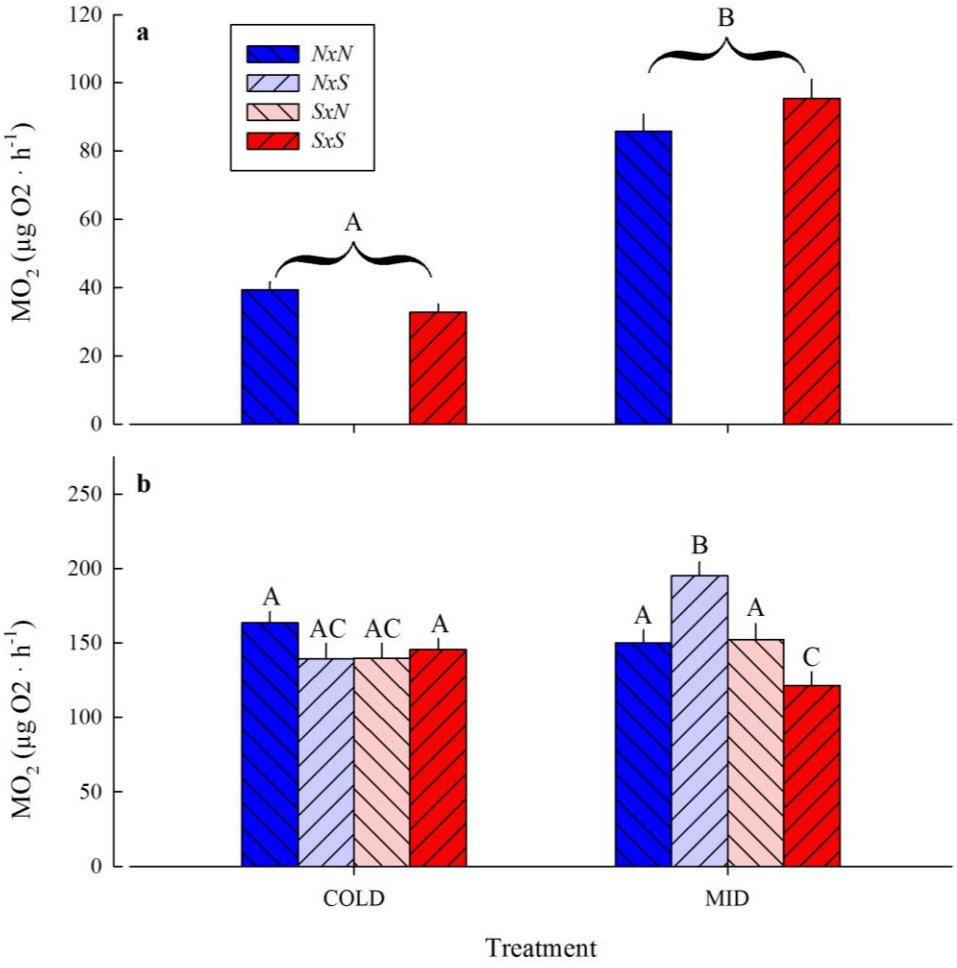


Figure 3.5: Resting metabolic rate at hatch (a) and button-up (b) for the different pure and hybrid families reared at cold (0.5 ºC at hatch, 4.5 ºC at button-up) and mid (4.5 ºC at hatch and button-up) temperature regimes (Figure 3.1). Refer to Figure 3.3 for detailed description of the legend. The upper-case letters above each bar represent a) the significant difference in treatment and b) the three-way interaction effect of treatment, maternal origin and paternal origin. Data are shown as means + standard error.

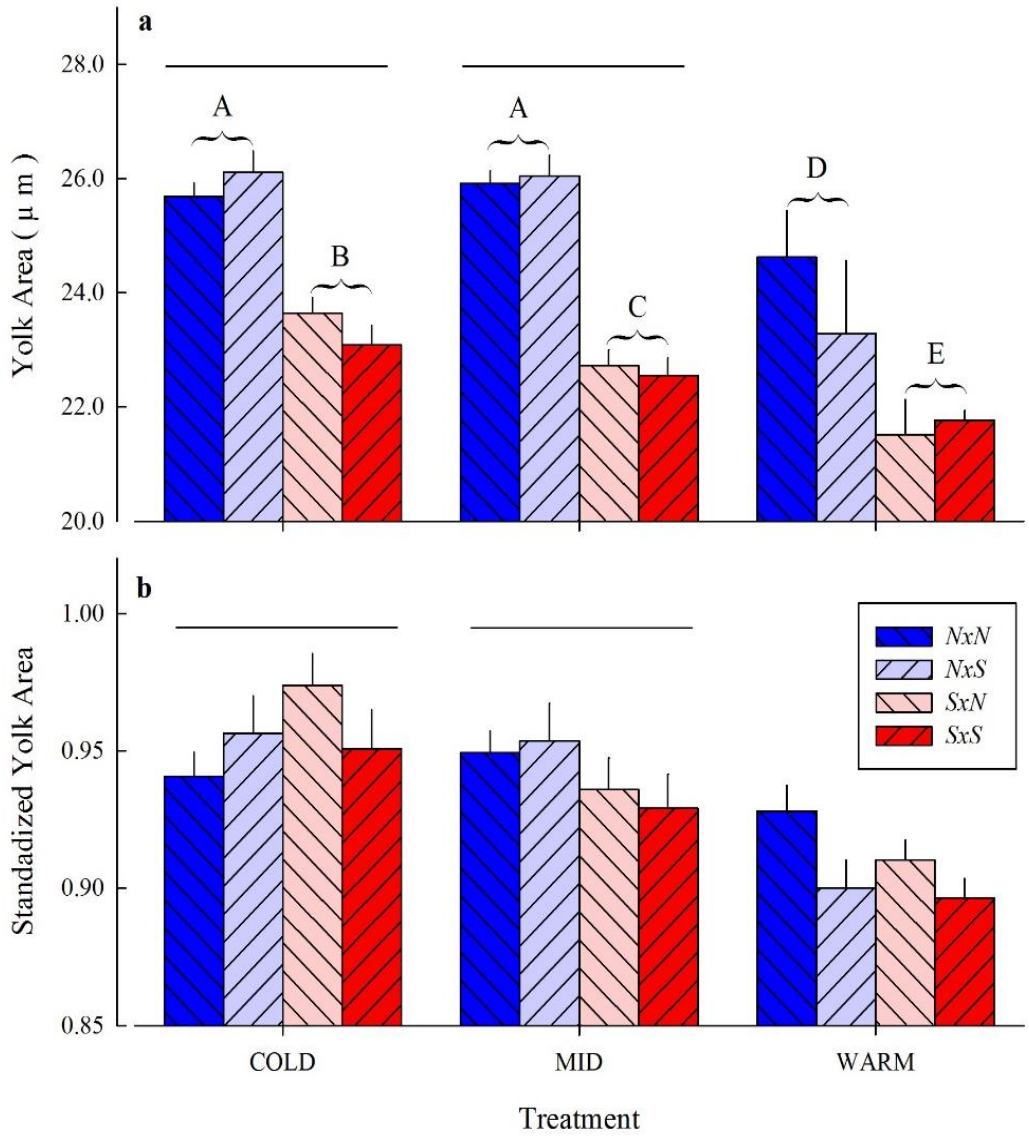


Figure 3.6: The yolk area (a) and standardized yolk area (b) at hatch for the different pure and hybrid families reared at cold, mid and warm temperature regimes (Figure 3.1). Refer to Figure 3.3 for detailed description of the legend. The solid line above each treatment represents the significant difference from the treatments without a solid line. The upper-case letters above each bar represent the significant difference between maternal origins. Data are shown as means + standard error.

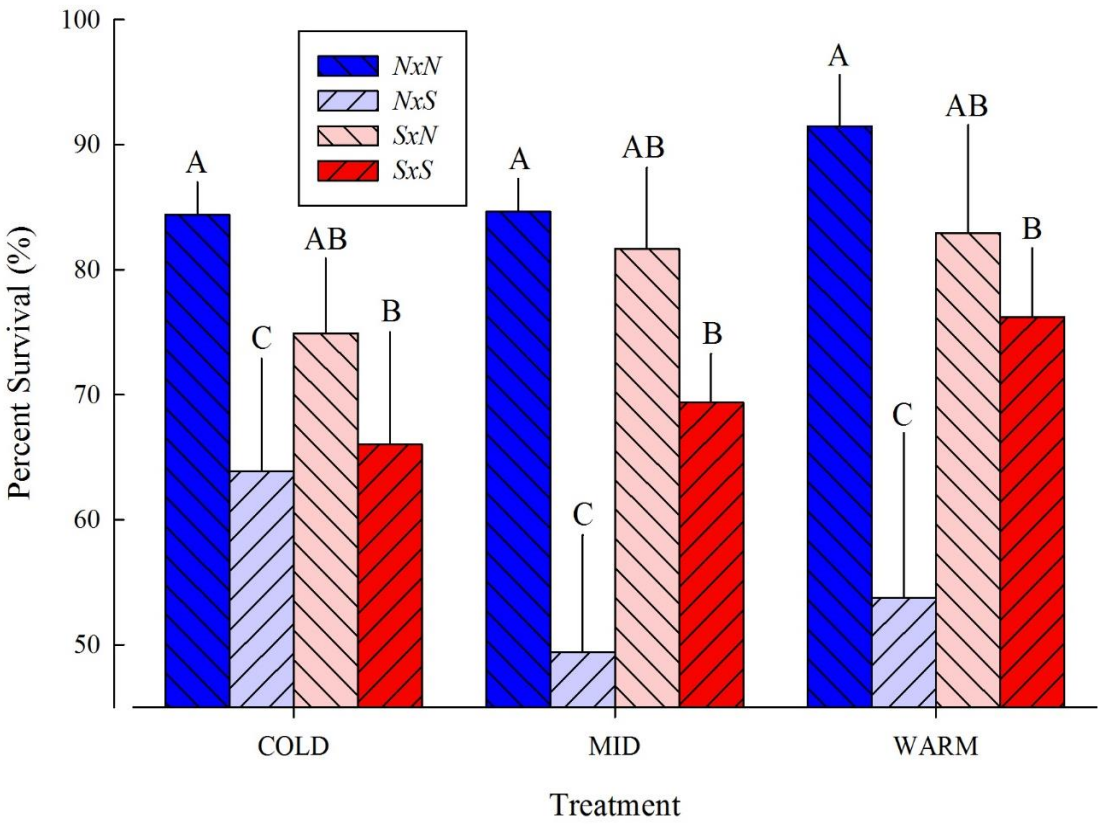


Figure 3.7: The total percent survival for the different pure and hybrid families reared at cold, mid and warm temperature regimes (Figure 3.1) from fertilization to button-up. Refer to Figure 3.3 for detailed description of the legend. The upper-case letters above each bar represent the interaction effect of maternal and paternal origin. Data are shown as means + standard error.

Discussion

This study is one of the first to examine the effects of near-freezing temperatures during incubation on the development of coho salmon. A number of previous studies have examined the effect of temperature on the development of Pacific salmon including coho salmon, but temperatures below 1.5 ºC have previously not been achieved (Heming 1982; Murray and Beacham 1988; Beacham and Murray 1989; Beacham and Murray 1990; Johnston et al. 2000; Whitney et al. 2014). In my study, incubation temperature regimes near-freezing were found to have a more profound effect on the development of coho salmon at hatch than the warmer incubation temperature regimes. Development, resting metabolic rate and yolk absorption of fish incubating in the cold treatment at hatch differed significantly from the warmer treatments. However, these differences decreased as the fish reached button-up and the cold treatment temperature regimes were increased gradually to 5 ºC. Additionally, survival was similar and above 50% across all three treatments, demonstrating the plasticity of this species and the ability to develop and mature successfully despite the thermal history experienced by the fish.

Larval development was also found to be strongly influenced by the maternal origin but not as strongly by paternal origin. Maternal effects on early development are associated with the maternal investment in egg size such that smaller eggs will produce smaller offspring that develop faster than offspring from larger eggs (Heming 1982; Beacham 1988; Murray and McPhail 1988; Heath et al. 1999).

Development Rate

Eggs in the cold treatment required two months longer to reach hatch and three months longer to reach button-up than in the warm treatment (Figure 3.2). The eggs in the cold

treatment, however, required significantly less ATU (approximately half) to reach hatch and button-up in comparison to the mid and warm treatments (Figure 3.2). More rapid morphological development at warmer temperatures, however, required a greater number of ATU, which is well documented in the literature (Heming 1982; Beacham and Murray 1985; Murray and Beacham 1986; Murray and McPhail 1988; Whitney et al. 2014). With colder systems typically having a longer winter period with decreased temperatures and later spring freshet, productivity and food availability would consequently not be available until later in the spring/summer compared to warmer coastal systems. Thus, a longer development period and later emergence would align with these natural processes and increase the survival prospects of juveniles. The date of hatch and fry emergence was found to be dependent on the environment during incubation (Beacham 1988) such that fry from smaller eggs emerge sooner than those from larger eggs (Beacham and Murray 1985; Heath et al. 1999). This trend was also present within my study as the families with the southern maternal origin and smaller eggs reached button-up earlier than the families with the northern maternal origin and larger eggs in all three treatments.

The rate of development and efficiency of converting yolk into somatic growth may not have been consistent throughout the development period for the cold treatment. Individuals at hatch were found to be significantly smaller than the individuals in the warm and mid treatment. However, size differences among the temperature treatments at button-up were reduced or the opposite trends were detected. Length at button-up within the cold treatment was greater than the warm treatment, which may be because alevin use their yolk sacs less for growth and more for basal metabolism (Rombough 1994; Kamler 2008; Whitney et al. 2014). This increase in size of fish within the cold treatment may also suggest

that as temperatures increase throughout the spring, development may accelerate resulting in a compensation for slow growth in the cold treatment and ultimately less difference in size among the treatment temperatures

Size and Condition Factor

Size at hatch was strongly influenced by an interaction between treatment and maternal origin, while at button-up the interaction between treatment and maternal origin was present, a significant interaction between maternal origin and paternal origin was also found (Figure 3.3 and 3.4). Within the literature incubation temperatures have been found to have a significant effect on the size of Pacific salmon throughout early development (Peterson 1977; Beacham 1985; Murray and McPhail 1988; Murray et al. 1990), a finding consistent with my study. Lower incubation temperatures produce larger alevin and fry (Peterson 1977; Beacham 1985; Murray and McPhail 1988; Murray et al. 1990). For coho salmon, alevin and fry were largest at 2 ºC (Murray and McPhail 1988), while Murray et al. (1990) found fry to be largest at mid temperatures ranging from 4-8 ºC. I found that fish were longer and slightly heavier at hatch in the mid treatment at 4.5 ºC (Figure 3.3). At button-up, however, this pattern changed with fish in the cold treatment being the longest and fish in the warm treatment being the heaviest (Figure 3.4).

Maternal effects on progeny body size and early development are well documented in the literature (Heming 1982; Beacham 1988; Murray and McPhail 1988; Heath et al. 1999). For Chinook salmon (*Oncorhynchus tshawytscha*), however, this maternal effect is only present during early development prior to emergence, and decreases post-hatch, becoming negative at the beginning of emergence when paternal effects become dominant (Heath et al. 1999). Beacham (1988) also found that size of fry of pink (*O. gorbuscha*) and chum (*O. keta*)

salmon was less influenced by maternal egg size than alevin size characters, and the hatch time was dependent on the environmental conditions during incubation. In my study, maternal origin had a significant effect on progeny size at hatch, but at button-up a significant interaction between the paternal effect and the maternal effect was present. This transition between a maternal effect throughout early development and the importance of egg size, to a paternal effect later in development also occurs in coho salmon populations. For Chinook salmon, Heath et al. (1999) suggested that the negative maternal effect observed at emergence is a result of different size eggs having different hatch dates and growth rates, such that progeny from smaller eggs hatch earlier and grew faster than progeny from larger eggs. Two mechanisms were suggested to explain these patterns: differential feeding behavior, such that smaller progeny from smaller eggs will attempt to compensate for their small size and feed more aggressively, and/or progeny from small eggs emerge earlier than those from large eggs and have longer exogenous feeding time. Both mechanisms would lead to increased risk of predation for progeny from small eggs, but these would overall gain a growth advantage (Heath et al. 1999). My fish were not fed exogenously, but a paternal effect was found for *NxN* and *NxS* families; it is possible that if I had continued the experiment longer a greater paternal effect would have been observed.

Condition factor can be used to determine fitness of fish. At hatch fish from the cold and warm treatments had higher condition factors compared to the mid treatment, and there was a significant maternal effect as progeny of northern maternal origin had higher condition factor at hatch and button-up (Figure 3.3 and 3.4). The maternal effect was likely due to the larger initial size of eggs of the northern female. Condition factor at button-up was more

consistent across all three temperatures, although fish from the cold treatment had significantly lower condition factor than the other treatments.

Oxygen Consumption

Temperature has a doubling effect on metabolic rate when body mass is taken into account; metabolic rate increases with increased temperature in teleost species (Clarke and Johnston 1999) including Pacific salmon such that metabolic rate increased 2-3 (Q_{10}) times with every 10 ºC increase in temperature (Brett 1971; Brett 1995; Perry and Tufts 1998). Resting metabolic rate at hatch within the cold treatment was significantly lower than within the mid treatment as expected (Figure 3.5). At colder temperatures there is a decrease in utilisation of energy towards maintaining a stable metabolic rate, resulting in more available energy to be allocated for somatic growth (Heming 1982). The Q_{10} value was found to be three times more the expected trend in the literature with a Q_{10} value of approximately 8 for all families. Such a finding would suggest that the expected increase in reaction of 2-3 with every 10 ºC increase is lost when the individuals are incubated at the extreme ranges in temperature.

At button-up, the pattern of differences in metabolic rate among families within the cold and mid treatment groups was quite complex (Figure 3.5). For the most part, however, resting metabolic rate did not differ between families or the cold and mid temperature treatments. The temperature regimes at button-up for the cold treatment had reached 5 ºC following the natural spring warming. Consequently, resting metabolic rate was not influenced by the thermal history experienced by an individual, just the temperature at the time of measurement. Resting metabolic rates have been found to increase significantly between hatch and emergence when temperatures remained consistent throughout incubation

(Rombough 2011), which was also found in this study as metabolic rate increased at button-up in the mid treatment which had a constant temperature throughout incubation. Although prior thermal history did not have an effect on metabolic rate, differences in condition factor and size of fish at hatch and button-up from the different temperature treatments might reflect prior rates of oxygen consumption. Weight of fish was lowest from the coldest temperature treatment, suggesting that less maternal energy stores were used for somatic growth in this group. Development at temperatures close to freezing potentially was less efficient metabolically, reducing the number of ATU required for growth.

Yolk Absorption

Yolk area at hatch was smallest for all four family groups reared in the warm treatment indicating that at warmer temperatures yolk was absorbed at a faster rate than at colder temperatures (Figure 3.6). Beacham and Murray (1989) found that yolk weight at hatch increased with decreasing temperatures. Heming (1983) found that the duration of yolk absorption and energy available for somatic growth was reduced at higher incubation temperatures, consistent with the metabolic rate measurements conducted at hatch in my experiment. This finding suggests that more energy was used to maintain higher metabolic rates in the warm treatment than the cold treatment. Although size at hatch did not differ in my study, smaller yolk area for the individuals within the warm treatment suggests that less of the yolk used was allocated to somatic growth and more used for routine basal metabolism (Beacham and Murray 1989; Rombough 1994; Kamler 2008).

The amount of yolk present is determined ultimately by ovum size (Heming 1982). Yolk area in my study was affected significantly by the maternal stock as the individuals with northern maternal gametes had significantly more yolk present at hatch than progeny

from southern females (Figure 3.6). After relative yolk area was calculated whereby yolk area was scaled to the initial egg size of each female, the maternal effect was no longer present. Therefore, maternal effects are likely due to differences in initial investment in egg size, and the rate of yolk absorption may be similar among families within the same treatment. This trend was also found by Heming (1982) as efficiency and rate of yolk absorption was dependent largely on rearing temperature after measurements were scaled to initial egg size.

Survival

Despite all of the differences found in development across the three treatments, there were no significant differences in overall survival among the treatments, demonstrating the plasticity and resiliency of this species to extreme temperatures (Figure 3.7). Additionally, survival was greater than 60% for all families within the cold treatment, also demonstrating the physiological capacity of coho salmon to survive a wide range of incubation temperatures, including near-freezing temperatures. Survival of most Pacific salmon are found to decrease as temperatures reach 3 ºC or less and increase with increased temperature until an upper thermal limit of approximately 12 ºC (Murray and McPhail 1998; Beacham 1990), but for coho salmon survival was above 80% at temperatures below 2 ºC (Beacham 1988; Murray and McPhail 1988). The paternal origin of progeny had a significant effect on survival as the progeny with the southern paternal origin had significantly lower survival across each treatment (Figure 3.7), suggesting that the genetic contributions from southern male were weaker despite the maternal origin or temperature treatment. Paternal origin was found to influence survival post emergence (Heath et al. 1999) and influence stress response and resistance to pathogens (Nadeau et al. 2009). Interestingly, an influence of paternal effect

has been linked to behaviour; the offspring of less aggressive males have been found to have higher survival (Peterson and Järvi 2007). Additionally, cryptic female choice has been found in salmonid populations, such that eggs differentially influence the fertilization success of sperm from different males (Rosengrave et al. 2016). My results demonstrate that this may be occurring as the southern males had lower fertilization success and lower overall survival – but this was particularly dramatic for the pairings with northern females.

Other than the difference in egg size that resulted in significant maternal effects in my study, there was remarkably little difference between families from the northern or southern populations in the variables measured. Paternal effects were seen in the sizes of some of the families at button-up, but these were subtle. Interestingly, the progeny from the northern populations did not show significantly improved performance in the cold temperature regime. Such a finding would suggest little evidence of local adaptation – rather exceptional phenotypic plasticity within this species is occurring.

APPENDIX 3.1

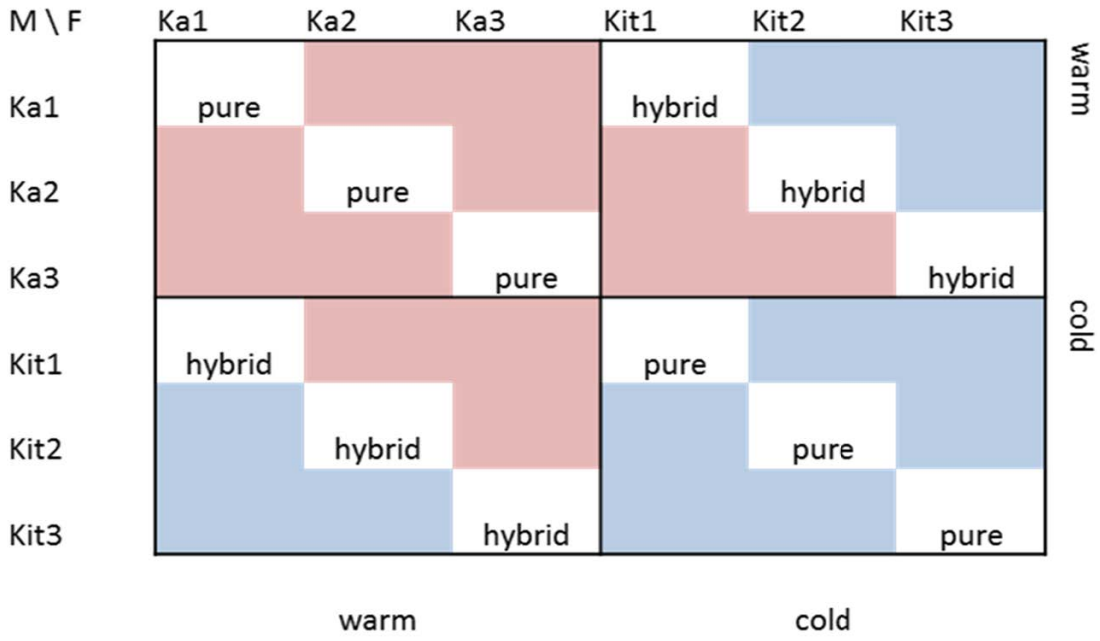


Figure A.1: Layout of the breeding design that was used to study the effect of temperature on the development of larval coho salmon. Three males and three females were collected from two populations with short migration distances to spawning locations but from different latitudes. One population was from a southern system (Kanaka) of BC with warm incubation conditions, represented by the red highlighted blocks, and one population was from a northern system (Kitimat) with cold incubation conditions, represented with the blue highlighted blocks. Six pure and six hybrid families were created by fertilizing one female by one male from one or both populations. Families created are shown with the white blocks.

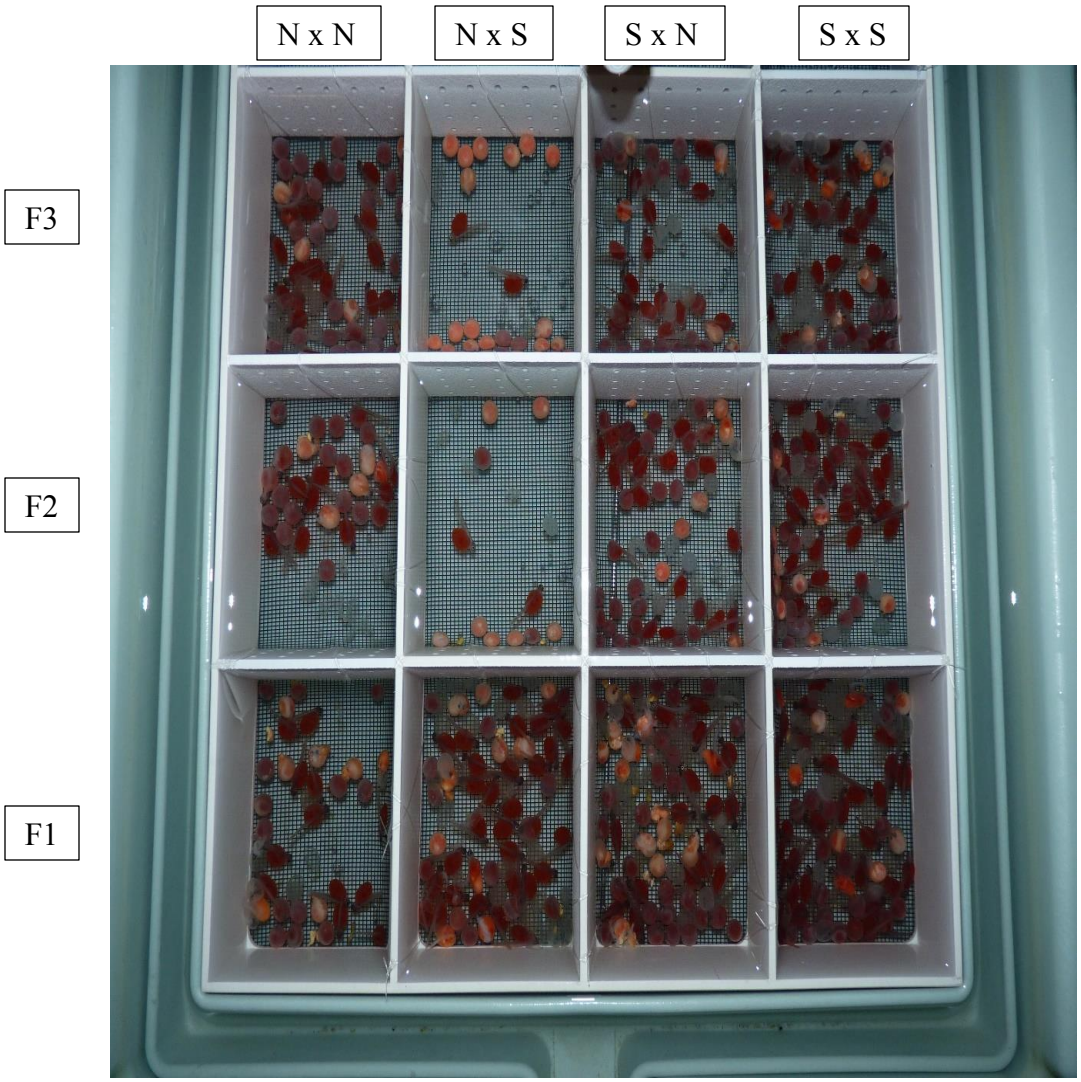


Figure A.2: Layout of the top Heath tray with the first replicate of each population of coho salmon in each of the three incubators at Hatch. F1, F2, and F3 refer to the different families; placement for families in the Heath trays differed to limit any positional effect on the variables measured. N (northern) and S (southern) represent the population of each female x male crossed within each cell.

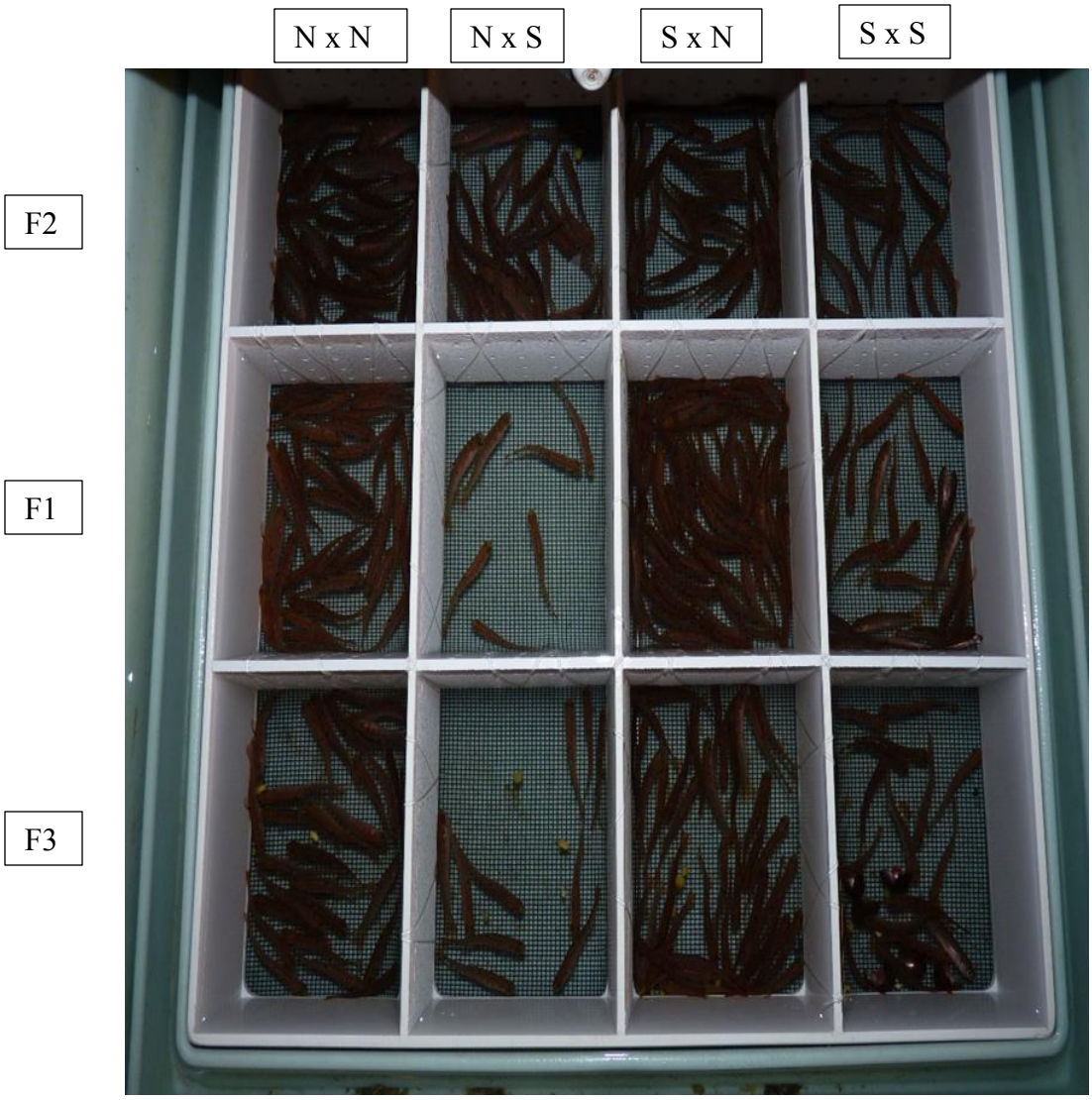


Figure A.3: Layout of the bottom Heath tray with the second replicate of each population of coho slamon in each of the three incubators at Button-up. F1, F2, and F3 refer to the different families; placement for families in the Heath trays differed to limit any positional effect on the variables measured. N (northern) and S (southern) represent the population of each female x male crossed within each cell.

APPENDIX 3.2

Development Rate

Table A3.1: Summary statistics for accumulated thermal units required to reach hatch.

ATU	SS	df	MS	F	Sig
Model	137866.4	11	12533.31	613.01	0.00001
Treatment	136583.1	2	68291.57	3438.25	0.00001
Maternal	575.66	1	575.666	28.98	0.00001
Paternal	148.602	1	148.602	7.48	0.0115
Treat*Mat	24.958	2	12.479	0.63	0.5421
Treat*Pat	106.177	2	53.088	2.67	0.0895
Mat*Pat	231.335	1	231.335	11.65	0.0023
Treat*Mat*Pat	196.589	2	98.294	4.95	0.0159
Residual	476.69	24	19.862		
Total	138343.166	35	3952.66		

Table A3.2: Summary statistics for accumulated thermal units required to reach button-up.

ATU	SS	df	MS	F	Sig
Model	347080.35	11	31552.75	2578.78	0.00001
Treatment	343120.89	2	171560.45	14021.49	0.00001
Maternal	2490.91	1	2490.91	203.58	0.00001
Paternal	105.21	1	105.22	8.60	0.0073
Treat*Mat	12445.70	2	622.85	50.91	0.00001
Treat*Pat	102.33	2	51.17	4.18	0.0277
Mat*Pat	5.877	1	5.87	0.48	0.4949
Treat*Mat*Pat	9.40	2	4.71	0.38	0.6851
Residual	293.65	24	12.23		
Total	347374.01	35	9924.97		

Size and Condition Factor

Table A3.3: Summary statistics for the weight at hatch.

Weight	SS	df	MS	F	Sig
Model	0.1223	36	0.0111	26.43	0.00001
Treatment	0.05921	2	0.00296	7.04	0.0010
Maternal	0.09354	1	0.09354	222.35	0.00001
Paternal	0.000096	1	0.000096	0.23	0.6328
Treat*Mat	0.0023	2	0.00119	2.83	0.0604
Treat*Pat	9.25-06	2	4.69-06	0.01	0.9891
Mat*Pat	0.000336	1	0.000336	0.80	0.3721
Treat*Mat*Pat	0.00099	2	0.000495	1.18	0.3090
Residual	0.14809	352	0.00042		
Total	0.27043	363	0.000744		

Table A3.4: Summary statistics for the length at hatch.

Length	SS	df	MS	F	Sig
Model	587.0475	11	53.368	50.57	0.0001
Treatment	396.36	2	198.1848	187.78	0.0001
Maternal	84.851	1	84.851	80.40	0.0001
Paternal	2.1444	1	2.1444	2.03	0.1549
Treat*Mat	22.731	2	11.3565	10.76	0.0001
Treat*Pat	1.570	2	0.7851	0.74	0.4760
Mat*Pat	0.9432	1	0.9432	0.89	0.3451
Treat*Mat*Pat	0.7027	2	0.3513	0.33	0.7171
Residual	372.5625	353	1.0554		
Total	959.612	364	2.6362		

Table A3.5: Summary statistics for the condition factor at hatch.

Length	SS	df	MS	F	Sig
Model	2.285	11	0.207794	8.54	0.0001
Treatment	0.5238	2	0.2619	10.77	0.0001
Maternal	0.9629	1	0.962921	39.59	0.0001
Paternal	0.03712	1	0.00371	0.15	0.6962
Treat*Mat	0.4865	2	0.2432	10.00	0.0001
Treat*Pat	0.05744	2	0.0287	1.18	0.3084
Mat*Pat	0.0094	1	0.0094	0.39	0.5326
Treat*Mat*Pat	0.7717	2	0.3858	1.59	0.2061
Residual	8.5848	353	0.02431		
Total	10.870	364	0.2986		

Table A3.6: Summary statistics for the weight at Button-up.

Length	SS	df	MS	F	Sig
Model	0.43871	11	0.03988	50.55	0.0001
Treatment	0.04223	2	0.02111	26.76	0.0001
Maternal	0.2676	1	0.2676	339.19	0.0001
Paternal	0.0032	1	0.00328	4.316	0.0418
Treat*Mat	0.00438	2	0.00219	2.78	0.0629
Treat*Pat	0.0023	2	0.00119	1.52	0.2205
Mat*Pat	0.1299	1	0.01299	16.47	0.0001
Treat*Mat*Pat	0.00467	2	0.00233	2.96	0.0525
Residual	0.5168	655	0.00078		
Total	0.9555	666	0.0014		

Table A3.7: Summary statistic for the length at button-up

Length	SS	df	MS	F	Sig
Model	418.293	11	38.0266	41.59	0.00001
Treatment	159.25	2	79.628	87.09	0.00001
Maternal	212.15	1	212.15	232.03	0.00001
Paternal	1.1567	1	1.156	1.27	0.2611
Treat*Mat	14.656	2	7.328	8.01	0.0004
Treat*Pat	3.9477	2	1.973	2.16	0.1163
Mat*Pat	7.2667	1	7.266	7.95	0.0050
Treat*Mat*Pat	1.3016	2	0.6508	0.71	0.4912
Residual	579.68	634	0.9143		
Total	997.97	645	1.5472		

Table A3.8: Summary statistics for condition factor at Button-up.

Length	SS	df	MS	F	Sig
Model	2.4208	11	0.2200	29.93	0.0001
Treatment	0.8592	2	0.4296	58.43	0.0001
Maternal	0.8254	1	0.8254	112.27	0.0001
Paternal	0.0062	1	0.0062	0.85	0.3562
Treat*Mat	0.0999	2	0.0499	6.80	0.0012
Treat*Pat	0.2562	2	0.1281	1.74	0.1759
Mat*Pat	0.119	1	0.1193	16.23	0.0001
Treat*Mat*Pat	0.0182	2	0.0091	1.24	0.2898
Residual	4.661	634	0.0073		
Total	7.0825	645	0.1098		

Oxygen Consumption

Table A3.9: Summary statistics of the two-way ANOVA for the metabolic rate at Hatch.

Length	SS	df	MS	F	Sig
Model	148796.26	3	49598.75	55.09	0.0001
Treatment	144355.78	1	144355.78	160.32	0.0001
Population	14.05	1	14.05	0.02	0.9007
Treat*Pop	1747.26	1	1747.26	1.94	0.1652
Residual	175578.25	195			
Total	324374.52	198			

Table A3.10: Summary statistics of the three-way ANOVA for the metabolic rate at Button-up.

Length	SS	df	MS	F	Sig
Model	134333	7	19190	5.93	0.00001
Treatment	4508.09	1	4508	1.39	0.22388
Maternal	37437	1	37437	11.57	0.0008
Paternal	81.56	1	81.56	0.03	0.8740
Treat*Mat	13680	1	13680	4.23	0.0406
Treat*Pat	5204.23	1	5204	1.61	0.2057
Mat*Pat	9864.88	1	9864	3.05	0.0818
Treat*Mat*Pat	53429	1	53429	16.51	0.0001
Residual	1032508	319	32326.7		
Total	1166841	326	3579.23		

Yolk Area

Table A3.11: Summary statistic for the yolk area at hatch.

Yolk	SS	df	MS	F	Sig
Model	735.017	11	68.455	36.01	0.00001
Treatment	74.966	2	37.483	19.72	0.00001
Maternal	624.97	1	624.97	328.74	0.00001
Paternal	3.186	1	3.1863	1.68	0.1964
Treat*Mat	7.135	2	3.5679	1.88	0.1549
Treat*Pat	4.376	2	2.1880	1.15	0.3177
Mat*Pat	1.393	1	1.393	0.73	0.3927
Treat*Mat*Pat	6.047	2	3.023	1.59	0.2055
Residual	577.938914	304	1.9011		
Total	1330.95	315	4.2252		

Table A3.12: Summary statistic for the standardized yolk area at hatch.

Yolk	SS	df	MS	F	Sig
Model	0.1730	11	0.0157	5.23	0.00001
Treatment	0.1149	2	0.0574	19.11	0.00001
Maternal	0.0020	1	0.0020	0.68	0.4111
Paternal	0.0052	1	0.0052	1.73	0.1893
Treat*Mat	0.0126	2	0.0063	2.10	0.1248
Treat*Pat	0.0058	2	0.0029	0.98	0.3772
Mat*Pat	0.0024	1	0.0024	0.83	0.3627
Treat*Mat*Pat	0.0084	2	0.0042	1.40	0.2489
Residual	0.9142	304	0.0030		
Total	1.0873	315	0.0034		

Survival

Table A3.13: Summary statistics for the total percent survival.

Survival	SS	df	MS	F	Sig
Model	11189	11	1017.21	3.74	0.0004
Treatment	310.50	2	155.25	0.57	0.5679
Maternal	278.40	1	278.40	1.02	0.3156
Paternal	7360.32	1	7360.32	27.08	0.00001
Treat*Mat	527.52	2	263.76	0.97	0.3848
Treat*Pat	280.34	2	140.17	0.52	0.5997
Mat*Pat	2147.98	1	2147.98	7.90	0.0067
Treat*Mat*Pat	284.32	2	142.16	0.52	0.5954
Residual	16310	60	271.84		
Total	27499	71	387.32		

EPILOGUE

This work expands the current knowledge of the importance of temperature on development of larval coho salmon and the environmental variables they experience throughout the incubation period. Coho salmon are of significant importance within BC, both economically and culturally. Interior Fraser coho salmon are currently listed as endangered by the Committee on the Status of Endangered Wildlife in Canada, but not currently listed by Species at Risk Act. As incubation is a critical stage in development for Pacific salmon, I would expect strong selective pressure at this life-history stage. The wide range of temperatures experienced by larval coho salmon across BC during the incubation period, some very cold and near freezing, would suggest that temperature is an important constraint on performance. Population genetics also suggests that local adaptation is likely to maximize performance for fish at the extreme limit of their range.

The importance of temperature on the early life history and development of coho salmon was confirmed in my studies, but I also showed that other variables influence life-history variation – latitude of spawning grounds, size of spawning system, and migration distance (Chapter 2). A number of previous studies have examined life-history variation among populations that differ in latitude, but the populations have been limited to coastal watersheds or those with short migration distances into interior river systems (Fleming and Gross 1990; Beacham and Murray 1993; Braun et al. 2013), and additionally they did not incorporate incubation temperatures and other environmental variables experienced. Thus, by creating models with a number of variables that influence early life-history traits I have helped focus management on the most significant variables.

With temperature being so important for early life history, governing the growth and development in fish (Chapter 2; Fry 1971; Blaxter 1992), and having direct effect on metabolic rate and therefore rate of development (Chapter 3; Fry 1971; Brett 1995), determining the range in temperatures experienced by coho salmon is of great interest. Chapter 1 demonstrated how temperatures experienced within the redd and river systems vary significantly throughout British Columbia. Additionally Chapter 3 demonstrated that small differences in temperature can have significant effects on early development. Temperature regimes ranged from 1 to 5 °C on average throughout incubation and from 0.1 to 4 °C during the coldest period of incubation, confirming the capacity of coho salmon to tolerate a wide range of temperatures. My findings demonstrate the importance of understanding the conditions present within the redds themselves throughout incubation at a population level and not just surface water conditions. The effect of temperature is cumulative with even very small differences in temperature having profound effect on the ATU required, resulting in considerable differences in the development of salmonids. Thus, hatching and emergence dates could vary significantly with only a small difference in temperature, especially for systems at near-freezing temperature where developing embryos are likely to be highly sensitive to the temperature differences.

To better understand the importance of temperature on early development of coho salmon and the adaptations that enhance their survival at near-freezing incubation temperatures, a controlled incubation experiment measuring early development differences between pure and hybrid families of two populations of coastal coho salmon was conducted. The results demonstrated that rate of development, growth, yolk utilization and metabolic rates were all significantly affected by temperature, specifically when temperatures approach

freezing. Near-freezing incubation temperatures resulted in less accumulated thermal units to reach each development stage, lower condition factors, lower resting metabolic rates and more yolk available for somatic growth than the warmer incubation temperatures. Additionally, the incubation experiment also demonstrated that temperature has more of an influence on early development of coho salmon compared to maternal and paternal genetic effects. Maternal and paternal origin did, however, have a significant effect on the variables measured, but compared to temperature these genetic influences were subtle. Maternal effects were related to the differences in egg size between the northern or southern populations. Paternal effects were more subtle and only seen for some families at button-up. The progeny from the northern populations also did not show significant improvement in performance in the cold treatments regime as predicted. Thus, the results from this experiment suggest little local adaptation on development at cold temperatures – rather exceptional phenotypic plasticity within this species is occurring.

To properly conserve and manage coho salmon, a better understanding of the temperature regimes experienced by different populations is needed, particularly at a finer scale within streams and at the extreme ranges in distribution. Future research could expand my work and continue to measure metabolic rates and development throughout later life stages such as parr and smolting to gain a better understanding of how populations adapt to different temperature regimes and how it affects overall fitness of these later life stages. Incubating at near-freezing temperatures, or cold-adapted populations incubating within warm temperature regimes, may not have a significant effect on early development but may reduce the overall fitness and success throughout smolting and maturation. Additionally, conducting more frequent sampling of yolk and metabolic rate at a larger range of

temperatures will allow a better understanding of how energy is being allocated and yolk is being used by larval fish from these populations within these near-freezing temperatures, and the effects on overall fitness. Previous studies have examined these processes (Heming 1982; Beacham and Murray 1989; Rombough 1994; Kamler 2008), but not at the near-freezing temperatures experienced by coho salmon at the limits of their range. My expectation was that the northern population, which is routinely exposed to cold temperatures during larval development, should have performed better than the southern population. Although there was a strong maternal effect, the lack of a paternal effect would argue that adaptation to rearing at cold temperatures was not strongly developed in the northern population. In contrast, my results show tremendous phenotypic plasticity exists within a single species that enable it to thrive under a wide range of temperatures during larval development.

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