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The adaptive capacity of thermal tolerance in chinook salmon

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Biology

THE ADAPTIVE CAPACITY OF THERMAL TOLERANCE IN CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*)

(Thesis format: Integrated Article)

by

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Abstract

With global temperatures projected to surpass the limits of thermal tolerance for many species, evaluating the capacity for evolutionary and phenotypically plastic changes in thermal tolerance is key to our understanding of the biological consequences of climate change. Within quantitative genetic breeding designs and multiple rearing environments, I measured the thermal performance of cardiac function among families of two populations of chinook salmon (*Oncorhynchus tshawytscha*). I found significant indirect genetic, plastic, and additive genetic effects contributing to cardiac performance and thermal tolerance, representing a variety of adaptive mechanisms available to salmon populations faced with climate change. These results enhance our understanding of the mechanisms of thermal adaptation in fish and suggest a resiliency to rising temperatures among these ecologically, economically, and culturally important fish.

Keywords

Acclimation, adaptation, aerobic capacity, climate change, egg size, evolutionary potential, heart rate, maternal effects, phenotypic plasticity, temperature

Co-Authorship Statement

A version of Chapter 2 has been accepted for publication in *Proceedings of the Royal Society B* with Katja Anttila, Zhong Chen, John Heath, Anthony Farrell, and Bryan Neff as co-authors. This publication also includes parts of Chapter 1. **Nicolas Muñoz** contributed to study design, conducted the heart rate and critical thermal maximum experiments, analyzed data, and drafted the manuscript, **Dr. Neff** contributed to study design and provided input regarding the manuscript, **Dr. Farrell** collaborated in performing the heart rate experiment and provided input regarding the experimental design and manuscript, **Dr. Heath** collaborated in collecting and rearing fish, and provided input regarding the experimental design, and **Dr. Anttila and Zhong Chen** collaborated in conducting the critical thermal maximum experiment.

A version of Chapter 3 is currently being prepared for submission as a manuscript to *Nature Climate Change* with John Heath, Anthony Farrell, and Bryan Neff as co-authors. **Nicolas Muñoz** contributed to study design, conducted the heart rate experiment, and analyzed data, **Dr. Neff** contributed to study design, **Dr. Farrell** collaborated in performing the heart rate experiment and provided input regarding the experimental design, and **Dr. Heath** collaborated in collecting and rearing fish, and provided input regarding the experimental design.

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List of Abbreviations

CT_{max}	critical thermal maximum
h^2	narrow-sense heritability
IPCC	Intergovernmental Panel on Climate Change
f_H	heart rate
f_{Hmax}	maximum heart rate
f_{Hpeak}	highest f_{Hmax} reached following temperature increase
G × E	genotype-by-environment interaction
T_{opt}	optimum temperature at which aerobic scope is maximal
T_{crit}	upper critical temperature at which aerobic scope is zero
T_{AB}	Arrhenius break temperature of maximum heart rate
T_{arr}	cardiac arrhythmia temperature
T_{win}	thermal window between T_{AB} and T_{arr}
T_{peakfH}	temperature at which f_{Hpeak} occurs
Q_{10}	thermal sensitivity of biological rate process
YIAL	Yellow Island Aquaculture Ltd.

Chapter 1

1 General introduction

Temperature plays a fundamental role in shaping the form and function of species. Central to the limits of organisms' environmental tolerance, temperature drives local adaptation and may serve as a basis for ecological speciation (Keller and Seehausen 2012). Indeed, for ectothermic organisms, temperature has been described as the "ecological master factor" (Brett 1971) because all aspects of their physiology are directly dependent on their thermal environment. Consequently, species are adapted to their thermal environment at scales both local (e.g. Weber et al. 2012) and global (e.g. Thomas et al. 2012), such that breadths of thermal tolerance and optima for performance generally correspond to the thermal conditions in which species evolved (Angilletta 2009). Understanding the nature of such thermal adaptation is an increasingly important endeavor for biologists in the current era of climate change; the average global air temperature is projected to increase by 1.7-4.8°C by 2100 (IPCC 2013). This projected rate of increase exceeds any found in the fossil record (Allan et al. 2005) and could have deleterious effects on species that are unable to adjust their thermal window for performance through physiological or evolutionary means.

Responses to climate change will be modulated by the adaptive capacity of organisms, which is defined as the intrinsic ability of individuals or populations to adapt to environmental change (Williams et al. 2008). Adaptive capacity may involve (1) evolutionary adaptation or (2) phenotypic plasticity. Evolutionary adaptation involves a change in the genetic makeup of a population following natural selection for favourable genotypes. Traits conferring a selective advantage must be heritable for such adaptation to occur. Conversely, phenotypic plasticity is a non-genetic change in the physiology, phenology, morphology, or behaviour of an individual elicited by changes in environmental conditions. Although there is evidence that both evolutionary and plastic responses have occurred in response to climate change (e.g. Bradshaw and Holzapfel 2006; Crozier and Hutchings 2014), the relative importance of each response remains an important question for climate change research (Gienapp et al. 2008; Visser 2008).

A trait can respond to natural selection when there is genetic variation underlying the trait and when this variation is heritable from one generation to the next (Blows and Hoffmann 2005). This evolutionary potential is often described as narrow-sense heritability (h^2), which is the ratio between additive genetic variance and total phenotypic variance in the trait. Additive genetic effects are the effects of alleles that influence offspring phenotype irrespective of any other alleles that are similarly inherited. Conversely, non-additive genetic effects occur due to the interaction of alleles within an individual, either at the same locus (i.e. dominance) or at different loci (i.e. epistasis). These interactions are not reliably passed down from parent to offspring and are consequently not heritable between generations. As such, non-additive genetic variation cannot respond to selection and thus does not contribute to the evolutionary potential of traits (reviewed in Neff and Pitcher 2005).

A powerful means of describing the genetic variation underlying traits is using quantitative genetic breeding designs such as the North Carolina II to partition phenotypic variation among offspring into additive genetic, non-additive genetic, and environmental maternal effects (Lynch and Walsh 1998). Such designs involve the crossing of adult males and females, measuring a phenotype of interest among offspring, and partitioning the variation in offspring phenotype into different sources based on the differences in reproductive investment between males and females. Because males (i.e. sires) often provide only genes to their offspring, the variation in offspring phenotype that is attributed to paternal ID is indicative of additive genetic variance. Similarly, because non-additive genetic effects occur due to the interaction of alleles within an individual, the variation in offspring phenotype that occurs due to the interaction of paternal and maternal ID is indicative of non-additive genetic variance. Also, because females (i.e. dams) often provide both genes and environmental effects such as egg nutrients to their offspring, the maternal effects contributing to offspring phenotype can be calculated as the difference between the variation in offspring phenotype due to maternal ID and that due to paternal ID. Such designs have been principally utilized in agricultural and plant sciences, but have more recently been applied to study the genetics of fitness-related traits in animals (e.g. Evans et al. 2007; Pitcher and Neff 2007) and to evaluate the potential for adaptation to environmental change (e.g. Sunday et al. 2011).

In aquatic ectotherms such as fish, the limits of thermal tolerance are attributed to the loss of aerobic scope (i.e. the difference between minimum and maximum oxygen consumption rates) (Pörtner 2001; Pörtner and Farrell 2008). As temperature rises above an animal's optimal temperature for aerobic scope (T_{opt}), the maximum capacity of the cardiorespiratory system to deliver oxygen to tissues cannot keep pace with increased oxygen demands, primarily due to limitations on the ability to increase heart rate beyond a maximum level (Farrell 2009). Aerobic scope is thereby reduced until an upper critical temperature (T_{crit}) is reached, above which an animal's capacity for aerobic activity cannot exceed routine rates. Because of the lack of oxygen available for aerobic metabolism above routine needs, such loss of scope reduces the capacity for growth, reproduction, and aerobic swimming, which can lead to reduced survival (Pörtner and Knust 2007; Farrell et al. 2008). Indeed, this oxygen- and capacity-limited thermal tolerance framework is now being broadly used to evaluate the susceptibility of aquatic ectotherms to climate change (Pörtner and Farrell 2008; Pörtner 2010; Somero 2010; Munday et al. 2012).

Because cardiac function is the primary mechanism supporting oxygen delivery and, subsequently, aerobic scope (Farrell 2009), the temperatures over which heart rate (f_H) becomes limited can be used as rapid estimates of oxygen-limited thermal tolerance (Casselman et al. 2012). Indeed, differences in the thermal performance of cardiac function explain broad patterns of biogeography in intertidal invertebrates (Logan et al. 2012), and a high degree of thermal plasticity in cardiac function can allow fish to inhabit environments that fluctuate widely in temperature (Jayasundara and Somero 2013). These measurements generate two transition temperatures – the Arrhenius break temperature (T_{AB}) and the arrhythmic temperature (T_{arr}) of maximum f_H (f_{Hmax}) – that provide functional indications of corresponding transition temperatures associated with a limitation in aerobic scope at and above T_{opt} (Fig. 1.1). Increasing routine f_H until f_{Hmax} is reached is the primary way in which fish supply the increased oxygen demands that occur during acute warming (Fry 1947; Farrell 2009). Thus, when increases in f_{Hmax} with increasing temperature start to become limited (i.e. at T_{AB}), there should be a corresponding limitation in aerobic scope that ultimately sets T_{opt}. Similarly, the temperature at which f_{Hmax} becomes arrhythmic should signal an approaching T_{crit}, as

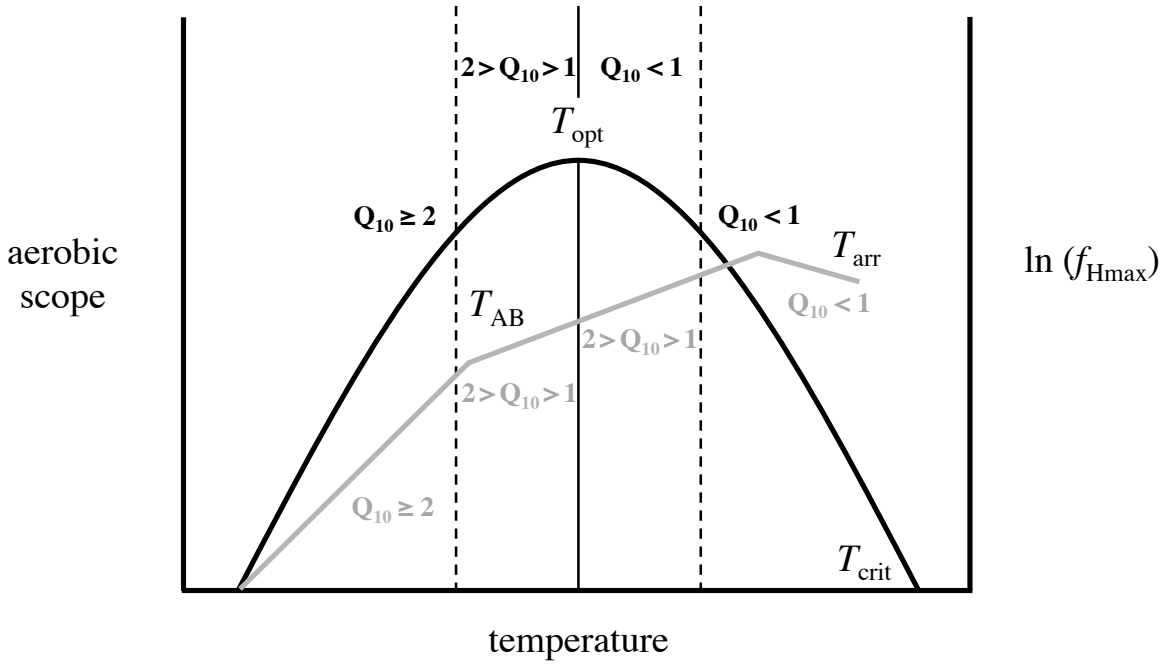

Fig. 1.1. The relationship between aerobic scope (black line), maximum heart rate (f_{Hmax}; grey line), and temperature in Pacific salmon (*Oncorhynchus* spp.). Shown are the optimum temperature (T_{opt}) and upper critical temperature (T_{crit}) for aerobic scope, as well as the Arrhenius break temperature (T_{AB}) and arrhythmic temperature (T_{arr}) of f_{Hmax}. The solid vertical line represents T_{opt}, and the dashed lines indicate the optimum temperature window in which aerobic scope is ≥ 90% of that at T_{opt}. Also shown are the temperature sensitivities (Q_{10}) of aerobic scope (black) and f_{Hmax} (grey). A $Q_{10} \geq 2$ represents an exponential increase with temperature. As described by Casselman et al. (2012) and Anttila et al. (2013a), when f_{Hmax} becomes limited with increasing temperature (i.e. at T_{AB}), there is a corresponding limitation in aerobic scope that ultimately sets T_{opt}. Similarly, when high temperatures induce cardiac arrhythmia (T_{arr}), the capacity for aerobic activity is highly reduced, thus corresponding with the upper critical temperature for aerobic scope (T_{crit}). This mechanistic relationship between f_{Hmax} and aerobic scope allows the use of T_{AB} and T_{arr} as comparative estimates of T_{opt} and T_{crit}, respectively.

aerobic capacity above this temperature would be highly reduced with an arrhythmic heartbeat. Indeed, in all the studies performed to date, T_{AB} and T_{arr} have been found to be within 1-2°C of T_{opt} and T_{crit}, respectively (Casselman et al. 2012; Anttila et al. 2013a).

Despite its importance to the resilience of populations to climate change, the evolutionary potential of thermal tolerance has received little empirical study outside of *Drosophila* spp. (e.g. Chown et al. 2009; Santos et al. 2012). Still, heritable variation in thermal tolerance has been found in some fish species (Beacham and Withler 1991; Meffe et al. 1995; Perry et al. 2005; Robinson et al. 2008; Anttila et al. 2013b), as have quantitative trait loci for thermal tolerance in rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpinus*) (Perry et al. 2001; Quinn et al. 2011). Although these studies suggest a genetic basis for thermal tolerance that might respond to selection, they used time to mortality or the loss of equilibrium to measure thermal tolerance, which are measures that are unlikely to simulate how temperature limits organismal activity in the wild (Pörtner and Knust 2007). With the recent development of the oxygen- and capacity-limited thermal tolerance framework for understanding the ecological limits to thermal tolerance, studies of the heritability of oxygen-limited thermal tolerance would increase our understanding of how functional traits can respond to the selective pressures of temperature change.

Pacific salmon (*Oncorhynchus* spp.) provide an excellent system for understanding the effects of climate change on fishes; their anadromous life history exposes them to pressures found in both freshwater and marine environments, while their ecological, economic, and cultural value make their long-term viability a chief concern among stakeholders. Anomalously high river temperatures have recently been identified as a significant cause of mortality among Pacific salmon populations at both the juvenile (Crozier and Zabel 2006) and adult stage (Keefer et al. 2008). Indeed, a collapse of aerobic scope has been empirically linked to high mortality during spawning migrations of sockeye (*O. nerka*) salmon (Farrell et al. 2008). A clear, population-specific correspondence between adult T_{opt} and the modal temperature historically experienced during spawning migrations suggests that natural selection imposed by river conditions has shaped thermal adaptation in salmon (Lee et al. 2003 Eliason et al. 2011); however,

the heritability of thermal tolerance and its evolutionary potential to respond to rising temperatures remain largely unknown.

In the research presented here, I used two wild populations of chinook salmon (*O. tshawytscha*) to examine the genetic and environmental bases of juvenile thermal tolerance. I chose this life stage because juvenile survival has been identified as key for the recovery of threatened salmon populations (Kareiva et al. 2000; Zabel et al. 2006; Chittenden et al. 2010). Moreover, temperature-induced mortality of juveniles has been documented in chinook salmon populations (Crozier and Zabel 2006), with future climate warming projected to reduce juvenile survival and population viability in these populations (Crozier et al. 2008). Chinook salmon populations are highly philopatric, displaying a high degree of local adaptation to environmental conditions among populations (Waples et al. 2004; Heath et al. 2006). Coastal populations – such as those used in Chapters 2 and 3 – are characterized by short river migrations between natal and marine habitats and brief juvenile residencies (*ca*. 2-3 months) in fresh water (Groot and Margolis 1991). In these populations, adults generally complete their spawning migration from the ocean in the autumn. Fertilized eggs are deposited into the streambed, where the eggs remain over winter. The eggs eventually hatch into alevins, which feed endogenously and remain in the streambed until their yolk sac is completely used. At this time – usually in the late winter or early spring – the fish emerge from the gravel as free-swimming juveniles, feeding on small invertebrates. After a residency in fresh water, fish migrate to their marine feeding grounds, where they remain for several years before returning to their natal habitat to spawn and complete their life cycle.

The objective of this research was to evaluate the adaptive capacity of oxygen-limited thermal tolerance within populations of chinook salmon. To do so, I applied the mechanistic framework of the oxygen- and capacity-limited thermal tolerance hypothesis within a quantitative genetic framework and multiple rearing environments such that the heritability as well as the plasticity of thermal tolerance could be determined.

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Chapter 2

2 Indirect genetic effects underlie oxygen-limited thermal tolerance within a coastal population of chinook salmon

Evaluating the heritable variation underlying thermal tolerance is critical for understanding the potential for adaptation to climate change. I examined the evolutionary potential of thermal tolerance within a population of chinook salmon (*Oncorhynchus tshawytscha*) by conducting a full-factorial breeding design and measuring the thermal performance of cardiac function in offspring from each family. Additive genetic variation in offspring phenotype was mostly negligible, although these direct genetic effects explained 53% of the variation in resting heart rate (f_H). Conversely, maternal effects had a significant influence on resting f_H, scope for f_H, and cardiac arrhythmia temperature. These maternal effects were associated with egg size, as indicated by strong relationships between the mean egg diameter of mothers and offspring thermal tolerance. Because egg size can be highly heritable in chinook salmon, our finding indicates that the maternal effects of egg size constitute an indirect genetic effect contributing to thermal tolerance.

2.1 Introduction

Climate change is projected to have widespread impacts on biodiversity (Thomas et al. 2004), with rising temperatures being of particular concern due to the pervasive effects of temperature on organisms (Dillon et al. 2010). Macrophysiological studies have projected that, in the absence of adaptive responses, temperatures will surpass the limits of thermal tolerance for many species and consequently drive extinction or extirpation (Deutsch et al. 2008; Sinervo et al. 2010). Indeed, there is growing evidence that evolutionary adaptations to climate change will be key for the long-term viability of populations (Hoffmann and Sgrò 2011). The evolutionary potential of populations to adapt to change depends on the amount of existing genetic variation for environmental tolerance as well as the extent to which it is heritable and can thus respond to natural selection (Blows and Hoffmann 2005). A powerful means of describing evolutionary potential is using quantitative genetic breeding designs to partition phenotypic variation into additive (i.e. heritable) or non-additive genetic effects (Lynch and Walsh 1998; Neff and Pitcher 2005). However, using only these direct estimates of heritability can underestimate evolutionary potential due to the presence of indirect genetic effects (McAdam et al. 2002). Indirect genetic effects occur when a trait is influenced by heritable traits expressed in the environment (i.e. in an interacting individual). Because the genes influencing the focal trait are expressed in other individuals, these genetic effects act indirectly and provide heritable variation on which selection can act, outside of any direct genetic variation (Wolf et al. 1998). Indirect genetic effects are largely attributed to heritable maternal effects (Mousseau and Fox 1998; Räsänen and Kruuk 2007), which usually occur as a result of egg provisioning. In fishes, for example, maternal effects are known to contribute to a wide range of traits amongst offspring including larval survival (Evans et al. 2010), stress response (Heath et al. 1993), and metabolic enzyme activity (Patterson et al. 2004a), suggesting indirect genetic effects may be important to the evolutionary dynamics of populations.

In aquatic ectotherms such as fish, upper temperature tolerance has traditionally been measured using the critical thermal maximum (CT_{max}), defined as the temperature at which an individual loses equilibrium and a righting response (Lutterschmidt and

Hutchison 1997). While CT_{max} represents a functional collapse of the animal, its ecological relevance is questionable because organ systems key to fitness-promoting activities (e.g. predator avoidance, growth) likely decline before CT_{max} is reached (Anttila et al. 2013a). In its place, the oxygen- and capacity-limited thermal tolerance hypothesis offers a functional understanding of how temperature limits organisms in the wild and is now being broadly applied to evaluate the susceptibility of aquatic ectotherms to climate change (Pörtner and Farrell 2008; Pörtner 2010; Somero 2010; Munday et al. 2012).

A recent application of the oxygen- and capacity-limited thermal tolerance hypothesis by Eliason et al. (2011) found differences in aerobic scope among populations of sockeye salmon, with some populations being more vulnerable to rising temperatures than others. For example, the thermal window between T_{opt} and T_{crit} ranged from 6.3ºC to 12.6ºC in the six populations sampled. These differences were related to environmental conditions, with the T_{opt} of populations closely corresponding with the modal historic temperature of the population. Such correspondence could be due to local adaptation or to lasting phenotypic effects of developmental environments (i.e. developmental plasticity). I hypothesize that the correspondence between thermal tolerance and environmental conditions is a result of local adaptation. To test this hypothesis, I measured the thermal performance of juvenile cardiac function within a quantitative genetic breeding design, and partitioned the phenotypic variation into additive genetic, non-additive genetic, and maternal effects (see Muñoz et al. 2014). I predicted that I would detect additive genetic variation for thermal tolerance.

2.2 Methods

2.2.1 Fish and gamete collection

On October 8, 2011, adult chinook salmon completing their spawning migration were collected using diversion channels located at the Fisheries and Oceans Canada salmon hatchery on the Big Qualicum River, Vancouver Island, BC. Only unmarked, non-hatchery raised fish were selected for the study. Prior to gamete collection, each spawner was euthanized by cerebral concussion and measured for post-orbital hypural body length (± 0.1 cm). Egg and milt samples from five females and five males were collected, stored

in dry plastic containers, and transported on ice to Yellow Island Aquaculture Ltd. (YIAL) on Quadra Island, BC, where mean egg diameter was measured using 30 eggs from each female (± 0.01 mm).

2.2.2 Breeding design and offspring rearing

Gametes were crossed in a full-factorial breeding design (North Carolina II cross) (Lynch and Walsh 1998) in which all possible crosses were conducted between five males and five females, producing 25 different full-sib families. Each cross was replicated (n = 50 families total) using 60-155 eggs per replicate. Upon arrival at YIAL (< 4 h post-collection), eggs were fertilized in the order in which they were collected using a dry fertilization protocol (Patterson et al. 2004b). Each replicate was then randomly assigned to a cell within five trays of a single Heath incubation stack such that all families experienced the same temperature regime. The natural groundwater that fed this stack was pumped through an outdoor pipe system so that water temperature would fluctuate with the ambient temperature. Because both YIAL and the Big Qualicum River experience the mild, coastal climate of Vancouver Island's east coast, these ambient temperatures simulated the natural thermal conditions that the study population experiences. Water temperature was measured with a HOBO temperature logger (Onset, Bourne, MA, USA) deployed in the Heath stack; the mean ± S.D. water temperature throughout incubation was 5.6 ± 1.1°C, varying seasonally between 2.2 and 8.2°C and daily by 0.2–3.9°C.

Upon entry into the exogenous feeding stage (140 days post-fertilization), hatched offspring were moved into rearing barrels, with replicated families remaining separated. Fish were fed daily to satiation for the remainder of the experiment using organic fish pellets (Nelson & Sons, Tooele, UT, USA). From April 10–13, 2012, juvenile offspring were given family- and replicate-specific tags using Visible Implant Elastomers (Northwest Marine Technology, Shaw Island, WA, USA). On April 20, six fish from each replicate (n = 300 individuals) were placed in a 1000 l water tote and transported by truck and ferry to freshwater holding facilities located at the University of British Columbia in Vancouver. There, the fish were kept for the remainder of the experiment in a 1000 l tank that averaged 9.3 ± 0.7°C.

2.2.3 Cardiac performance measurements

I measured the response of maximum heart rate (f_H) to warming, developed by Casselman et al. (2012), in individuals from each family to evaluate the genetic architecture underlying the thermal performance of cardiac function. Following a starvation period of 24 h, two individuals were anesthetized in 75 mg l^{-1} MS-222 and 75 mg l^{-1} sodium bicarbonate solution, measured for body mass (± 0.1 g), and left anesthetized in a flume with recirculating water maintained at 10°C by a 3016D in-line chiller (Fisher Scientific, Ottawa, ON, Canada) (see Casselman et al. 2012 for apparatus details). After 30 min, resting f_H (in beats min^{-1}) was measured using non-invasive electrocardiogram techniques. Pharmacological stimulation was then used to induce f_{Hmax} using separate intraperitoneal injections of 2.4 mg kg^{-1} atropine sulphate (Sigma-Aldrich, St. Louis, MO, USA) and 8 μg kg^{-1} isoproterenol (Sigma-Aldrich) to block vagal tone and stimulate cardiac adrenergic β-receptors, respectively. At 10°C, the atropine injection significantly increased resting f_H by 9.3 ± 7.2 beats min^{-1} ($P < 0.001$), with a further increase of only 1.0 ± 3.2 beats min^{-1} ($P = 0.08$) after the isoproterenol injection. Once the rhythmic heartbeat had stabilized to its maximum rate (f_{Hmax}), temperature was progressively increased in 1°C increments at a rate of 10°C h^{-1}. For each 1°C interval, f_{Hmax} increased until it became stable and was measured ($n = 15$ heartbeats per measurement). Temperature was continuously increased beyond f_{Hmax} reaching its highest value (f_{Hpeak}) until an arrhythmic heartbeat signified the upper critical temperature for cardiac performance (i.e. T_{arr}). At this point, fish were removed from the apparatus and recovered for future experiments. Between May 3 and June 7, 2011, a total of 114 trials (two fish per trial, $n = 228$ fish total) were conducted using 8–10 individuals from each of the 25 full-sib families, in random order. The pharmacological injections occasionally had incomplete and/or unexpected effects on individuals, such as cardiac arrhythmias occurring soon after injection. In such cases, individuals were removed from the study (32 fish were removed). Unclear electrocardiogram readings prevented arrhythmias from being properly detected in another two individuals; these two individuals were thus removed from the calculations for the T_{arr}, thermal window between T_{AB} and T_{arr} (T_{win}; = $T_{arr} - T_{AB}$), f_{Hpeak}, and scope for f_H (= f_{Hpeak} − resting f_H) analyses, but remained in the resting f_H and T_{AB} analyses.

2.2.4 CT_{max} measurements

We used a modified CT_{max} experiment (Cowles and Bogert 1944) to assess the genetic and environmental maternal effects underlying CT_{max} and whether they are similar to those underlying cardiac performance. These experiments were conducted with individuals that had already completed the f_H measurements, with a recovery time of at least five days allowed before experimentation. This recovery time was likely sufficient, as heat hardening effects on CT_{max} have not been documented in fishes to last for more than 24 h after initial exposure to acute thermal stress (Bilyk et al. 2012). Following a fasting period of 24 h, between 20 and 26 offspring at a time were placed in a 50 l insulated tank for 1 h prior to experiments. Water temperature was maintained at 10°C during this time by a 3016D in-line chiller (Fisher Scientific), which pumped recirculating, aerated water through the tank. Temperature was acutely increased at an initial rate of 0.3°C min^{-1}. When temperature reached 20°C, the heating rate was reduced to 0.1°C min^{-1} to allow more accurate assessments of thermal limits. CT_{max} was defined as the temperature at which individuals lost righting response (i.e. an associated loss of a directed locomotor capacity and an inability to escape from high temperatures). When this loss was observed, the individual was removed from the experimental tank, euthanized by cerebral concussion, and measured for body mass. A total of 173 individuals were sampled, using 6–10 offspring from each full-sib family.

2.2.5 Statistical analyses

We calculated the T_{AB} of f_{Hmax} using the program presented by Yeager and Ultsch (1989). On an Arrhenius plot (natural log of f_{Hmax} on the y-axis, inverse of temperature in Kelvin on the x-axis), this program fits two-segmented straight lines to the data to identify the temperature at which temperature-induced increases in f_{Hmax} shift to a lower exponent. When data could not be adequately fitted by the program to reflect this change, they were manually fitted with two lines using SigmaPlot (Systat Software, San Jose, CA, USA) by comparing the residuals of all possible groupings of f_{Hmax} at high versus low temperatures. The point of intersection was calculated for the two lines of best fit to estimate the Arrhenius break point, T_{AB} (see Appendix A and B for example calculation). The temperature sensitivity (Q_{10}) of f_{Hmax} was also calculated between each temperature

increment using the formula ($f_{Hmax\ n+1}$ / $f_{Hmax\ n}$) ^ (10 / T_{n+1} − T_n), whereby $f_{Hmax\ n}$ is the maximum heart rate at temperature step n and T_n is the temperature at step n.

We tested for potential rearing location effects on offspring cardiac performance and thermal tolerance by using a two-way ANOVA with tray position (5 levels) and cell location (16 levels) as fixed factors. We then partitioned the variation in offspring resting f_H, f_{Hpeak}, scope for f_H, T_{AB}, T_{arr}, T_{win}, and CT_{max} into additive genetic, non-additive genetic, and maternal effects using a two-way restricted maximum likelihood (REML)-based ANOVA with *Sire* and *Dam* identity and their interaction as random factors. Following Lynch & Walsh (1998), I estimated additive genetic variance by multiplying the *Sire* variance component by four. Non-additive genetic variance was calculated as four times the *Sire* × *Dam* variance component, while the maternal effect was calculated as four times the difference between the *Dam* and *Sire* components of variance. Because *Dam* effects include both additive genetic and maternal environmental effects whereas the *Sire* effect captures only additive genetic effects, maternal effects are calculated as the difference between the *Dam* and *Sire* components of variance. The proportional contributions of additive genetic, non-additive genetic, and maternal effects to the measured phenotypes were calculated by dividing their respective variance components with the total phenotypic variance (i.e. the sum of the *Sire*, *Dam*, *Sire* × *Dam* and residual variance components). Assumptions of the ANOVA were tested and, because each trait was negatively skewed, Box-Cox power transformations were performed to normalize the data (see Appendix C for power exponents used). Body mass was used as a covariate in each analysis and was removed from the model whenever non-significant. We also examined adult phenotypic correlates (female body length and mean egg diameter, male body length) of offspring performance by using linear regression with multiple *Y* values for every *X* value (Sokal and Rohlf 1995). The Box-Cox power transformations were performed in SAS 9.3 (SAS Institute, Cary, NC, USA), while all other statistical analyses were performed using SPSS 20 (IBM, Armonk, NY, USA). All means are reported ± 1 S.D.

2.3 Results

Upon entry into the juvenile stage of their life cycle, offspring survival across all families averaged 90 ± 15%. Offspring body mass averaged 0.59 ± 0.32 g at this time and increased to 3.6 ± 1.1 g during the measurements of cardiac performance and thermal tolerance. A two-way ANOVA revealed no significant effect of tray position or cell location on offspring cardiac performance and thermal tolerance ($0.858 \geq P \geq 0.078$ across all measures).

In general, f_H increased with temperature from a resting f_H of 71.1 ± 9.4 beats min^{-1} to the f_{Hpeak} of 163.3 ± 25.8 beats min^{-1} (Fig. 2.1), with f_{Hpeak} occurring at 21.2 ± 2.4°C. T_{AB} averaged 15.0 ± 1.1°C among all individuals, which corresponded to the incremental Q_{10} decreasing from 2.5 ± 0.2 at 10.0°C to 1.9 ± 0.3 at T_{AB} (Fig. 2.2). T_{arr} averaged 22.4 ± 2.5°C and was lower than CT_{max}, which averaged 26.5 ± 1.0°C. Thus, the f_{Hpeak} of the average fish occurred 1.2°C before T_{arr} and 5.3°C before the loss of their righting response.

Body mass was significantly and positively correlated with T_{arr} (Pearson's r = 0.225, P = 0.002), T_{win} (r = 0.253, $P < 0.001$), f_{Hpeak} (r = 0.177, P = 0.013), and scope for f_H (r = 0.147, P = 0.041), but not with either T_{AB} (r = 0.016, P = 0.822) or resting f_H (r = 0.090, P = 0.210). In the *Sire* and *Dam* ANOVA, body mass significantly covaried with resting f_H (P = 0.007), T_{arr} (P = 0.015), and T_{win} (P = 0.001), and was thus included in these models.

Residual, unexplained variation comprised most of the phenotypic variance for each trait; however, additive genetic, non-additive genetic, or maternal effects were detected in each of the traits measured (Table 2.1). *Dam* effects, which include both maternal additive genetic and environmental effects, significantly contributed to resting f_H, scope for f_H, T_{arr}, T_{win}, and CT_{max}. Conversely, *Sire* effects significantly contributed to only resting f_H. Using the *Sire* variance component, additive genetic variance for resting f_H was estimated to be 9.7×10^9 ($= 4 \times [2.4 \times 10^9]$), representing 53% ($= [9.7 \times 10^9] / [1.4 \times 10^{10}]$) of the total phenotypic variance in resting f_H; no non-additive genetic or maternal

effects were detected for this trait. The dam- and sire-based variation in each of the analyzed traits is shown in Fig. 2.3.

Regression analyses revealed further evidence of maternal influence on offspring phenotype, with mean egg diameter being strongly associated with scope for f_H (r^2 = 0.88, F = 20.3, df = 1, 3, P = 0.019) and CT_{max} (r^2 = 0.85, F = 19.2, df = 1, 3, P = 0.022), and marginally non-significantly with T_{AB} (r^2 = 0.76, F = 9.20, df = 1, 3, P = 0.054), f_{Hpeak} (r^2 = 0.70, F = 6.39, df = 1, 3, P = 0.082), T_{arr} (r^2 = 0.59, F = 4.37, df = 1, 3, P = 0.130), and T_{win} (r^2 = 0.51, F = 3.14, df = 1, 3, P = 0.177; Fig. 2.4). Using mass residuals of the cardiac performance traits resulted in these relationships being weaker yet still positive (data not shown), indicating that the effects of egg size were partially but not wholly mediated by offspring body size. No such relationships were found across all measures for both dam body length ($0.858 \geq P \geq 0.179$) and sire body length ($0.766 \geq P \geq 0.357$).

There were strong phenotypic correlations between T_{AB}, T_{arr}, and f_{Hpeak} (T_{arr} and T_{AB}: r = 0.511, df = 192, $P < 0.001$; T_{arr} and f_{Hpeak}: r = 0.789, df = 192, $P < 0.001$; T_{AB} and f_{Hpeak}: r = 0.569, df = 192, $P < 0.001$), while resting f_H was significantly correlated with f_{Hpeak} (r = 0.266, df = 192, $P < 0.001$) and T_{arr} (r = 0.147, df = 192, P = 0.040) but not T_{AB} (r = 0.082, df = 194, P = 0.250). Using Spearman's rank-order correlations (r_s) to correlate families' performance in the CT_{max} and f_H experiments (ranked from high to low for each trait), we found highly negative correlations between CT_{max} and T_{arr} (r_s = -0.541, df = 23, P = 0.005), T_{AB} (r_s = -0.622, df = 23, P = 0.001), f_{Hpeak} (r_s = -0.563, df = 23, P = 0.003), and scope for f_H (r_s = -0.643, df = 23, P = 0.001). Indeed, the offspring of Dam 1 reached the highest T_{arr}, yet the lowest CT_{max} values (Fig. 2.3d and 2.3e).

Fig. 2.1. The average change in heart rate (f_H) with temperature in Big Qualicum River chinook salmon (*Oncorhynchus tshawytscha*). Resting f_H was measured at their acclimation temperature, and their maximum f_H (black line) was stimulated and measured as a function of temperature until cardiac arrhythmia was observed. Shown are the averages for resting f_H, highest f_H (f_{Hpeak}), scope for f_H, Arrhenius break temperature (T_{AB}), arrhythmic temperature (T_{arr}), and thermal window (T_{win}) based on all fish (n = 196) used in the study. The grey line indicates the rise in f_H from resting f_H to f_{Hmax} following stimulation. Also shown is the average critical thermal maximum (CT_{max}), which was determined using alternative methods (see text).

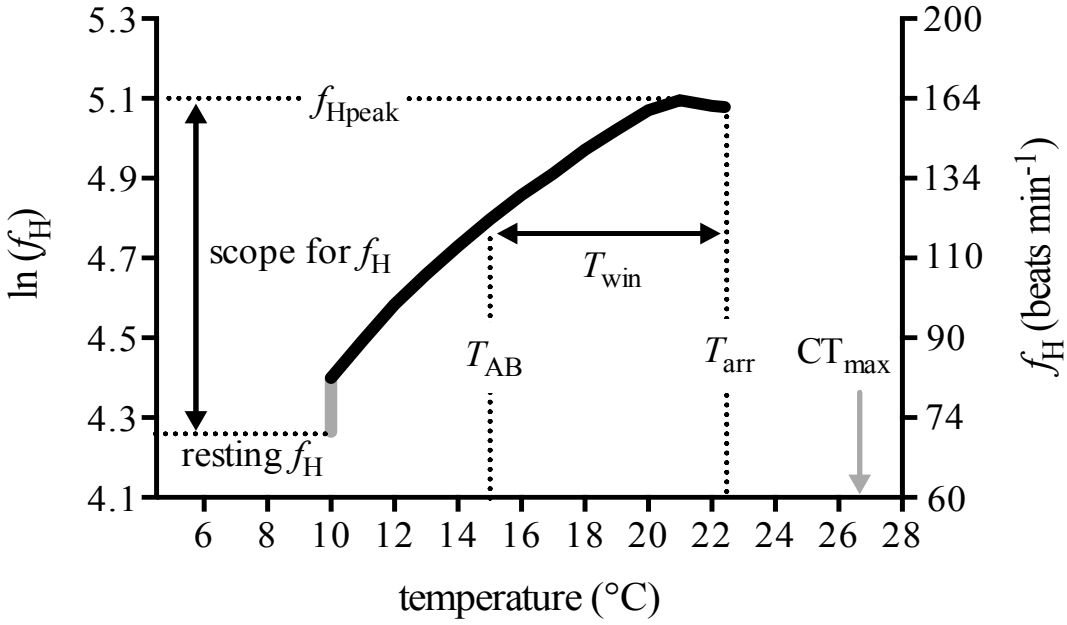


Fig. 2.2. Incremental Q_{10} values of maximum heart rate in Big Qualicum River chinook salmon (*Oncorhynchus tshawytscha*). The dashed lines indicate where maximum heart rate increases to an exponent of two (Q_{10} = 2) and does not change (Q_{10} = 1) with temperature. The asterisk denotes the mean Arrhenius break temperature of maximum heart rate (15.0°C). Values are mean ± 1 standard deviation.

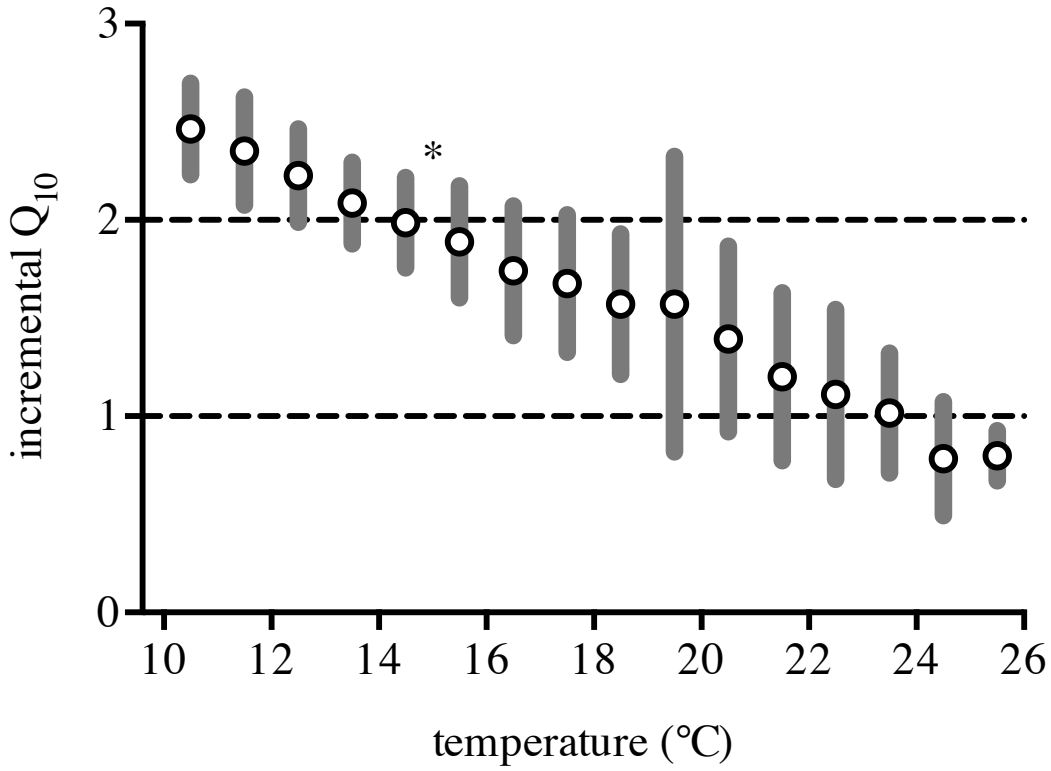


Table 2.1. The sire and dam effects contributing to cardiac performance and thermal tolerance in Big Qualicum River chinook salmon (*Oncorhynchus tshawytscha*). The results of the two-way ANOVA are summarized for resting heart rate (f_H), highest recorded f_H (f_{Hpeak}), scope for f_H, Arrhenius break temperature (T_{AB}), arrhythmic temperature (T_{arr}), thermal window (T_{win}), and critical thermal maximum (CT_{max}).

	DF	SS	*F*	*P*	σ^2	% phenotypic var	
Resting f_H							
Dam	4	3.2×10^{11}	12.9	**< 0.001**	1.8×10^{9}	Maternal	0
Sire	4	4.5×10^{11}	18.7	**< 0.001**	2.4×10^{9}	Additive	53
Sire × *Dam*	16	9.4×10^{10}	0.39	0.983	0.0	Nonadditive	0
Residual	170				1.4×10^{10}		
f_{Hpeak}							
Dam	4	1.2×10^{11}	2.17	0.118	4.0×10^{8}	Maternal	10
Sire	4	2.2×10^{10}	0.39	0.812	0.0	Additive	0
Sire × *Dam*	16	2.3×10^{11}	0.88	0.594	0.0	Nonadditive	0
Residual	169				1.6×10^{10}		
Scope for f_H							
Dam	4	1.5×10^{6}	3.13	**0.044**	6.7×10^{3}	Maternal	22
Sire	4	4.5×10^{5}	0.93	0.470	0.0	Additive	0
Sire × *Dam*	16	2.0×10^{6}	1.06	0.395	0.0	Nonadditive	0
Residual	169				1.2×10^{5}		
T_{AB}							
Dam	4	425.2	0.25	0.909	0.0	Maternal	0
Sire	4	4064.0	2.34	0.099	14.39	Additive	18
Sire × *Dam*	16	6969.2	1.44	0.129	5.54	Nonadditive	7
Residual	171				304.9		
T_{arr}							
Dam	4	4.1×10^{10}	4.32	**0.013**	2.2×10^{8}	Maternal	29
Sire	4	1.2×10^{10}	1.40	0.311	2.1×10^{7}	Additive	3
Sire × *Dam*	16	3.7×10^{10}	0.93	0.540	0.0	Nonadditive	0
Residual	168				2.5×10^{9}		
T_{win}							
Dam	4	468.9	6.28	**0.003**	2.69	Maternal	39
Sire	4	132.9	1.79	0.179	0.333	Additive	5
Sire × *Dam*	16	296.8	0.85	0.625	0.0	No-additive	0
Residual	168				21.49		
CT_{max}							
Dam	4	2.6×10^{77}	9.79	**< 0.001**	2.0×10^{75}	Maternal	77
Sire	4	4.4×10^{76}	1.65	0.203	0.0	Additive	0
Sire × *Dam*	16	1.0×10^{77}	0.60	0.880	0.0	Nonadditive	0
Residual	148				1.0×10^{76}		

N.B. The percent phenotypic variance explained by maternal, additive genetic, and non-additive genetic effects was calculated following Lynch and Walsh (1998).

Fig. 2.3. Variation in cardiac performance and thermal tolerance among families of Big Qualicum River chinook salmon (*Oncorhynchus tshawytscha*). Five males and five females were crossed in a full-factorial breeding design and offspring from each family were measured for their (*a*) resting heart rate (f_H); (*b*) highest f_H (f_{Hpeak}); (*c*) Arrhenius break temperature (T_{AB}); (*d*) arrhythmic temperature (T_{arr}); and (*e*) critical thermal maximum (CT_{max}). Vertical bars are ± 1 standard deviation from the family mean, while the dashed horizontal lines indicate the mean phenotype across all families.

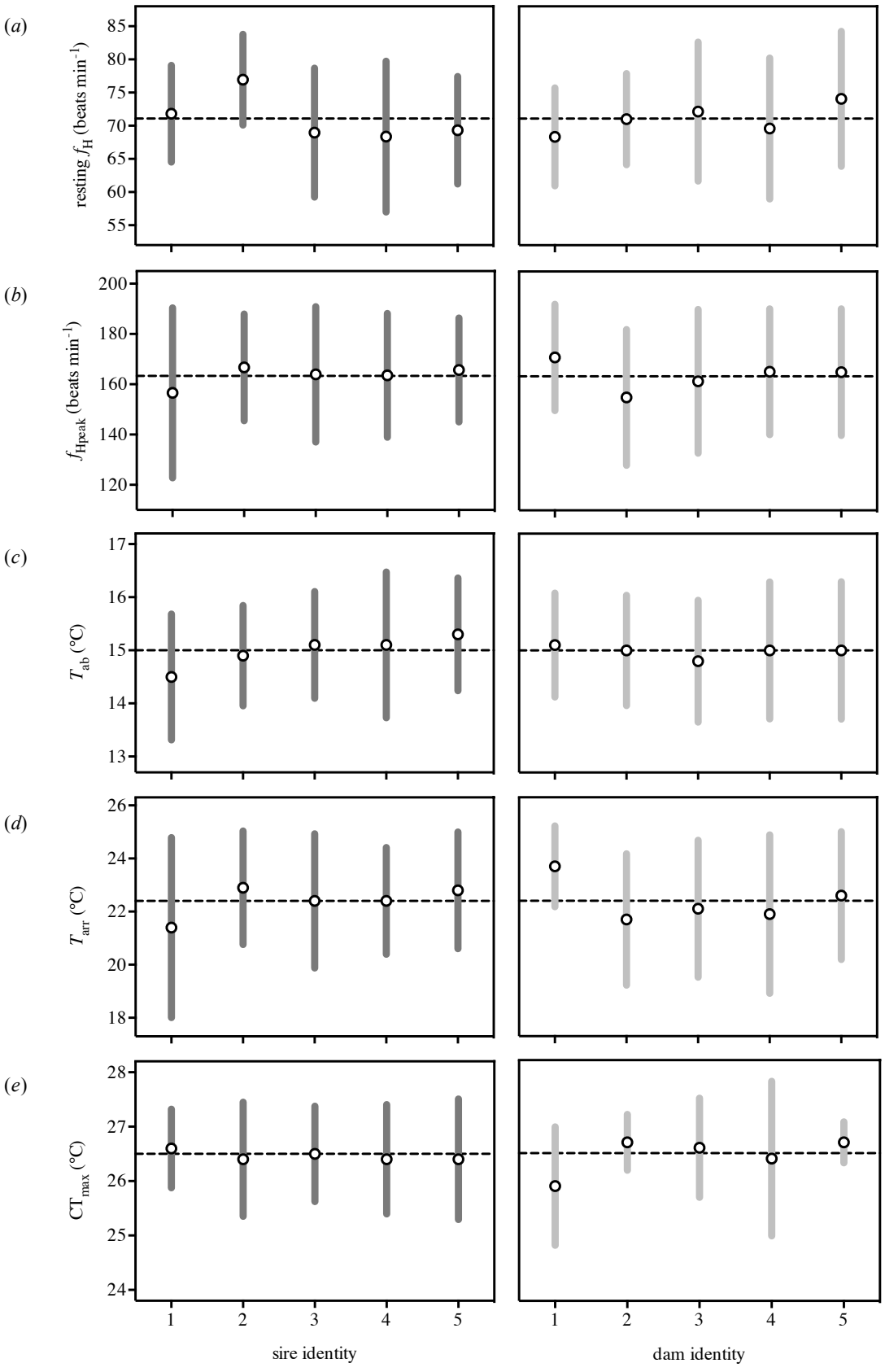
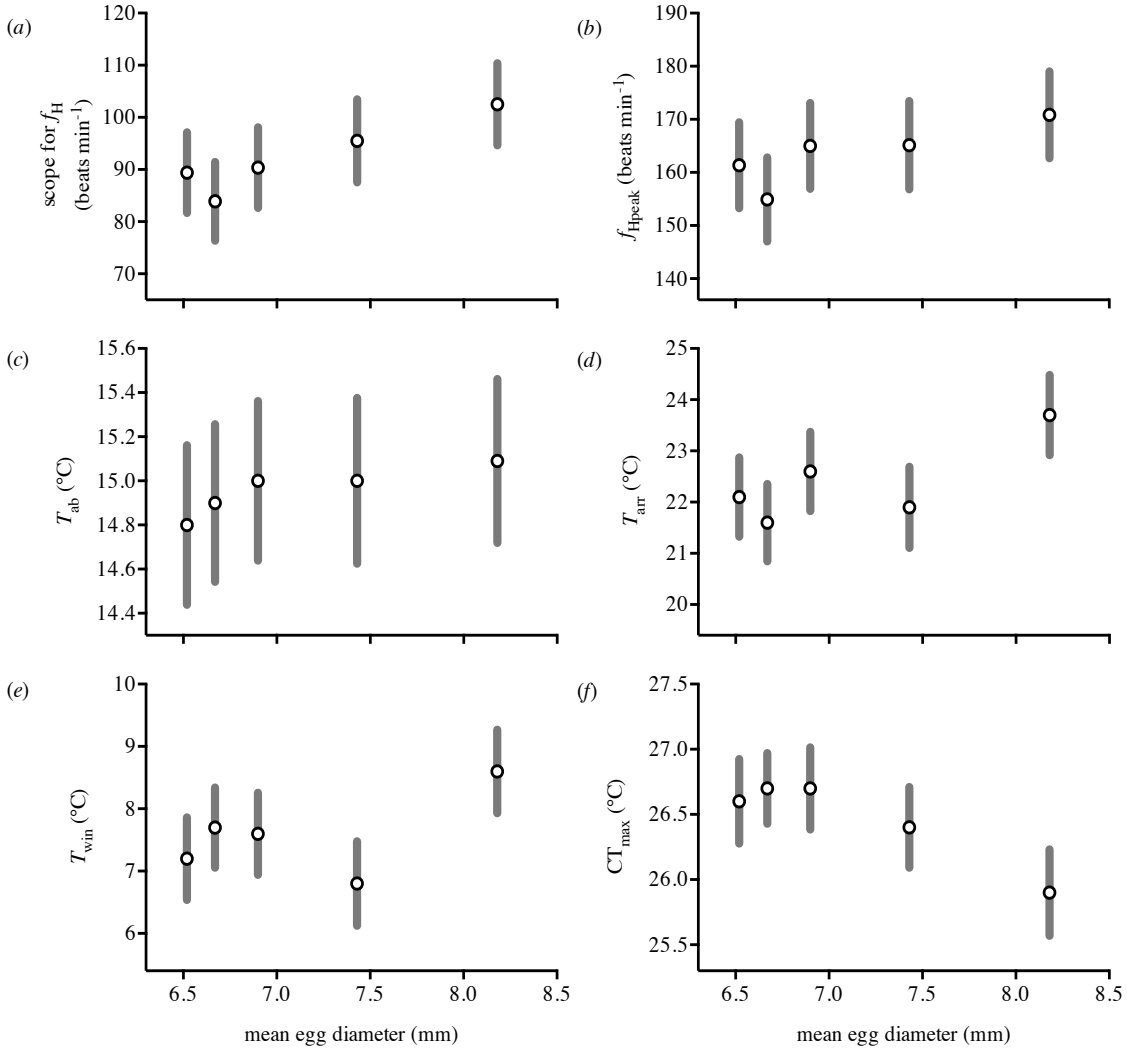

(a)
resting f_H (beats min^{-1})
85
80
75
70
65
60
55
(b)
f_{Hpeak} (beats min^{-1})
200
180
160
140
120
(c)
T_{ab} (°C)
17
16
15
14
13
(d)
T_{arr} (°C)
26
24
22
20
18
(e)
CT_{max} (°C)
28
27
26
25
24
1
2
3
4
5
sire identity
dam identity

Fig. 2.4. Relationships between dam mean egg diameter and offspring (*a*) scope for heart rate (f_H); (*b*) highest f_H (f_{Hpeak}); (*c*) Arrhenius break temperature (T_{AB}); (*d*) arrhythmic temperature (T_{arr}); (*e*) thermal window (T_{win}); and (*f*) critical thermal maximum (CT_{max}) in Big Qualicum River chinook salmon (*Oncorhynchus tshawytscha*). The error bars are ± 1 standard error of the family mean.



2.4 Discussion

Maternal effects have been found to be key determinants of phenotypic variation among offspring for a wide range of traits and taxa, and are thus increasingly recognized as having an important role in the evolutionary dynamics of populations (Mousseau and Fox 1998; Räsänen and Kruuk 2007). For example, a study of 17 life history and fitness-related traits among wild chinook salmon populations – including the study population used here – found that maternal effects contribute more to phenotypic divergence between populations than do additive genetic effects (Aykanat et al. 2012). In this study, I detected a maternal influence on offspring thermal tolerance that far exceeded the direct influence of parental genes, with females with larger eggs having more thermally tolerant offspring. When maternal effects are themselves heritable, populations can still respond to natural selection via indirect genetic effects (Wolf et al. 1998). Indeed, in a captive population of chinook salmon, a mother-daughter regression revealed egg mass to be highly heritable ($h^2 = 0.39$), indicative of a genetic basis for egg provisioning (Heath et al. 2003). Although heritability is population- and environment-specific, within-population variation in egg size is common in demersal egg-laying species like salmon (Einum and Fleming 2002) and has been found to be similarly heritable in other species of Pacific salmonids (Su et al. 1997; Gall and Neira 2004). If female salmon inherit the ability to provision eggs, these maternal effects would increase the "total heritability" of thermal tolerance and could accelerate any evolutionary response to the selection imposed by rising temperatures. Furthermore, this indirect genetic effect could contribute to the population-specific thermal tolerance uncovered across a number of Pacific salmon populations (Lee et al. 2003; Eliason et al. 2011; Chen et al. 2013). While the correspondence between thermal tolerance and environmental conditions suggests local adaptation brought about by selection on additive genetic effects (e.g. Barrett et al. 2011), our study suggests that an indirect genetic effect – mediated by egg size – could instead underlie the variation. Indeed, across many sockeye salmon populations, egg size is positively correlated with natural incubation temperature (Braun et al. 2013) and juvenile thermal tolerance (Chen et al. 2013), suggesting a mode of thermal adaptation.

Heart rate varies considerably both within and between fish species, with resting f_H being primarily determined by metabolic rate and hemodynamic requirements, and f_{Hmax} being limited by mechanistic constraints such as pacemaker potential, excitation-contraction properties, and myocardium structure (Lillywhite et al. 1999). Ultimately, resting f_H and f_{Hmax} are 'set' by a balance between these mechanistic constraints and the evolutionary pressures created by hemodynamic and oxygen requirements. We found that additive genetic effects account for a significant amount of intraspecific variation in resting f_H but not f_{Hmax} in juvenile chinook salmon. These differences could be due to stronger selective pressures on maximum rates of oxygen uptake than on resting rates. Indeed, the upper limit for f_H is about 120 beats min^{-1} across many species of adult ectothermic vertebrates (Farrell 1991), suggesting selection has increased maximum cardiac capacity as much as possible given common mechanistic constraints. We measured f_{Hmax} using pharmacological stimulation and acute increases in temperature, whereas resting f_H was measured in anesthetized fish at their acclimation temperature. Whether the high levels of genetic variation for resting f_H still exist at high temperatures – when aerobic scope is reduced – should be investigated; indeed, individuals that can maintain a greater scope for aerobic performance by having lower resting oxygen demands might be selected for as temperatures rise, thereby allowing evolutionary adjustments of thermal tolerance.

The loss of righting response used to estimate CT_{max} could be caused by any effect of temperature that impairs neuronal or skeletal muscle function. A switch from aerobic to anaerobic metabolism occurs in fish as temperatures approach their CT_{max} (Beers and Sidell 2011), typical of animals experiencing hypoxic conditions. Indeed, CT_{max} and hypoxia tolerance are positively correlated among families of Atlantic salmon (*Salmo salar*) (Anttila et al. 2013b), suggesting a genetic basis for anaerobic capacity. We found that juvenile chinook salmon reach their maximum f_H (i.e. f_{Hpeak}) 1.2°C cooler than their T_{arr} and 5.3°C cooler than their CT_{max}. Thus, the hearts of chinook salmon begin to collapse at cooler temperatures than the whole animal, meaning their ability to aerobically avoid predation, forage, or grow wanes well before CT_{max}. Such mismatch between tissue function and loss of righting response questions the functional and perhaps ecological utility of CT_{max} beyond that as a simple measure of relative thermal

tolerance among groups of organisms. Moreover, because f_{Hmax} is an aerobic trait due to the heart's inability to work anaerobically at maximal levels (Farrell and Stecyk 2007), the negative correlation between CT_{max} and cardiac capacity among families of chinook salmon suggests a tradeoff between aerobic and anaerobic capacity consistent with earlier suggestions (Reidy et al. 2000; Blake 2004).

Our evidence for maternally-mediated indirect genetic effects underlying thermal tolerance adds to the growing body of evidence for such 'heritable environmental' effects having important roles in the evolutionary potential of populations (Mousseau and Fox 1998; Räsänen and Kruuk 2007) and highlights the need for these effects to be quantified in studies of potential evolutionary responses to climate change. Indeed, with increasing evidence for parental influences on offspring environmental tolerance beyond those directly due to the genes inherited by offspring (e.g. Donelson et al. 2012), indirect genetic effects appear to be a promising source of evolutionary potential in natural populations challenged by a changing environment.

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Chapter 3

3 Genetic and plastic effects on oxygen-limited thermal tolerance within a population of chinook salmon

The capacity for evolutionary adaptation and phenotypic plasticity are key determinants of the susceptibility of organisms to environmental change. I examined the adaptive capacity of thermal tolerance in a population of chinook salmon (*Oncorhynchus tshawytscha*) by rearing families in current and future (+4°C) temperatures and measuring the response of maximum heart rate (f_{Hmax}) to warming. Plastic adjustments of cardiac performance were detected, with the Arrhenius break temperature of f_{Hmax} (T_{AB}), the highest recorded f_{Hmax} (f_{Hpeak}), and the temperature at which f_{Hpeak} occurs ($T_{peak/H}$) significantly increasing between the current and future groups. Additive genetic effects significantly influenced T_{AB} and scope for heart rate within both treatment groups. These data suggest that genetic and plastic effects on cardiac function can shape thermal adaptation among salmon populations and represent adaptive mechanisms by which populations might respond to climate change.

3.1 Introduction

As the earth's climate changes at unprecedented speeds, uncovering the adaptive capacity of organisms has become an increasingly central goal of organismal biologists (Williams et al. 2008). Adaptive capacity is the intrinsic ability of individuals or populations to respond to environmental change via evolutionary adaptation or phenotypic plasticity. Whereas evolutionary adaptation involves population-level changes in phenotype following natural selection on heritable genetic variation, phenotypic plasticity is the environmentally-induced expression of different phenotypes by an individual. A common form of plasticity is termed acclimation, which is defined as a phenotypic adjustment made in response to a chronic change in a single environmental variable (Prosser 1986). Developmental acclimation refers to all phenotypic, often permanent changes that occur across developmental stages, whereas reversible acclimation occurs within a developmental stage and often in a reversible manner (Angilletta 2009). While the factors influencing acclimatory capacity have been subject to theoretical and experimental study for several decades (e.g. Janzen 1967; Prosser 1986; Leroi et al. 1994), there have been enhanced research efforts in recent years in wake of anthropogenic climate change (e.g. Tewksbury et al. 2008); indeed, given the immediate, within-generational nature of acclimation, the capacity for such adjustment is a key determinant of how species can cope with rapid environmental change (Somero 2010).

Phenotypic plasticity can be depicted using reaction norms, which are graphical representations of trait values in two or more environments for a given genotype; the degree of plasticity is indicated by the extent to which trait values change in response to the change in environment (i.e. the slope of the line connecting trait values) (Schlichting and Pigliucci 1998). Use of reaction norms has elucidated the presence of genotype-by-environment (G × E) interactions, whereby certain genotypes are more plastic than others (Hutchings 2011). For example, a reciprocal transplant experiment revealed an effect of developmental environment on offspring survival and growth in two populations of chinook salmon (*Oncorhynchus tshawytscha*), with the magnitude of this effect differing among families (Evans et al. 2010). Such genetic variation in plasticity indicates that plasticity can itself respond to selection, such that it may evolve as an adaptation to

environmental variation (Via et al. 1995; Nussey et al. 2007). With environments becoming increasingly variable as a result of anthropogenic activity, selection for increased plasticity may be an important response to this change.

The oxygen- and capacity-limited thermal tolerance hypothesis (Pörtner 2001) provides a mechanistic framework for understanding the ecological limits of thermal tolerance in aquatic ectotherms with which we can address key questions in organismal biology regarding the nature of thermal adaptation and the potential for responses to rising temperatures. Recent research has highlighted the differences between eurythermal (i.e. thermal generalist) and stenothermal (i.e. thermal specialist) fish, further elucidating the mechanisms of thermal adaptation (e.g. Eliason et al. 2011; Healy and Schulte 2012; Anttila et al. 2013; Chen et al. 2013; Jayasundara and Somero 2013). For example, the optimum temperature for aerobic scope (T_{opt}) is tightly linked with the Arrhenius break temperature (T_{AB}) of maximum heart rate (f_{Hmax}) in juvenile rainbow trout (*O. mykiss*) and coho salmon (*O. kisutch*), with an increased dependence on anaerobic metabolism occurring in temperatures just beyond T_{opt} (Anttila et al. 2013). The thermal performance of cardiac function responds much less readily to thermal acclimation in the athletic species sockeye salmon (*O. nerka*) (Chen et al. 2013) than it does in the eurythermal, sedentary goby fish *Gillichthys mirabilis* (Jayasundara and Somero 2013), indicative of a tradeoff between capacity for aerobic performance and thermal breadths over which performance can be maintained. Indeed, previous findings suggest that warm acclimation provides little or no benefit to CT_{max} – another measure of the upper limit of thermal tolerance – in salmon (Brett 1956; Chen et al. 2013). Given the temperature-induced mortality that has been recently documented within salmon populations (Crozier and Zabel 2006; Keefer et al. 2008), a complete picture of how plastic and genetic effects combine to form the adaptive capacity of thermal tolerance is needed.

The thermal specialization observed among populations of Pacific salmon (Lee et al. 2003; Eliason et al. 2011) could be the result of local adaptation or phenotypic plasticity. I hypothesized that thermal conditions can elicit plastic responses that change the thermal tolerance of individuals. Furthermore, because G × E effects have been widely documented in salmonids (reviewed in Hutchings 2011), I hypothesized that

different genotypes differ in their capacity for thermal plasticity. Using a coastal population of chinook salmon from the Quinsam River, British Columbia, I measured the thermal performance of f_{Hmax} within a quantitative genetic breeding design and between two thermal environments. I predicted that I would detect phenotypic differences between individuals from the two thermal environments, and that the degree of these differences would be family-specific.

3.2 Methods

3.2.1 Fish and gamete collection

On October 23, 2012, unmarked adult fish completing their spawning migration were collected at the Fisheries and Oceans Canada salmon hatchery on the Quinsam River using diversion channels. Eight females and eight males were euthanized by cerebral concussion, measured for post-orbital hypural body length (± 0.1 cm), and stripped of their gametes. These gamete samples were stored in dry, plastic containers and transported on ice to Yellow Island Aquaculture Ltd. (YIAL) on Quadra Island, BC.

3.2.2 Breeding design and temperature treatment

Gametes were crossed in four 2 × 2 full-factorial crosses (North Carolina II design) (Lynch and Walsh 1998), producing 16 different full-sib families. Each cross was replicated four times, using 31-83 eggs per replicate. These replicates were split between four trays of a single Heath incubation stack. The water flow into this stack was manipulated such that two separate recirculating systems fed the stack, with the recirculating water feeding two of the trays being separate from that of the other two trays. With a TR20 Aquarium Chiller (Teco, Ravenna, Italy) controlling the water temperature of each system, two replicates from each family were reared in one of two temperature treatments: *current* and *future*. The current group was maintained in temperatures reflecting current thermal conditions, whereas the future group was reared in elevated temperatures that reflect possible future conditions (~4°C higher than current conditions). Water temperature was measured every two hours with two HOBO temperature loggers (Onset) deployed in the Heath stack; the mean ± S.D. water

temperature throughout incubation was 5.6 ± 1.7°C and 10.0 ± 1.5°C in the current and future groups, respectively, varying seasonally between 3.6-10.1°C and 7.9-12.3°C.

Entry into the exogenous feeding stage occurred ~90 days and ~140 days post-fertilization in the future and current groups, respectively. At this time, hatched offspring were moved into rearing barrels, with replicated families remaining separated. Twenty individuals from each replicate were transported by truck and ferry to freshwater holding facilities at the University of British Columbia. Prior to transportation, offspring were given family- and replicate-specific tags using Visible Implant Elastomers (Northwest Marine Technology). The fish were kept at the University of British Columbia for the remainder of the experiment, with the future group kept at 12.4 ± 0.3°C and the current group at 8.0 ± 0.8°C.

3.2.3 Cardiac performance measurements

The response of f_{Hmax} to warming (Casselman et al. 2012) was measured in offspring from each family and temperature treatment to evaluate the adaptive capacity of the thermal performance of cardiac function. Using the methods described in Chapter 2, temperature was incrementally increased from the acclimation temperature of the fish (8°C for the current group, 12°C for the future group), and f_{Hmax} was measured at each 1°C increment. Because fish in the future temperature treatment entered the juvenile stage approximately two months earlier than those in the current treatment, they were measured before the current group (future group measured from April 11 to June 22, current group from June 24 to September 2). A total of 360 trials were conducted (future group: n = 178; current group: n = 182), with at least 15 individuals from each paternal family being measured. As in the study described in Chapter 2, the pharmacological injections often had incomplete and/or unexpected effects on individuals, and 37 and 43 individuals had to be removed from the future and current groups, respectively.

3.2.4 Statistical analyses

The T_{AB} and Q_{10} of f_{Hmax} were calculated using the methods described in Chapter 2. To examine the genetic and plastic effects on offspring resting f_H, f_{Hpeak}, scope for f_H, T_{AB}, T_{arr}, and temperature at which f_{Hpeak} occurs ($T_{peak/H}$), we used ANOVA models with

Treatment and *Block* as fixed factors and *Sire*, *Dam*, *Sire* × *Dam*, *Sire* × *Treatment, Dam* × *Treatment,* and *Treatment* × *Sire* × *Dam* as random factors. Because the parents were crossed in four 2 × 2 full-factorial blocks, the *Sire* and *Dam* effects were nested within *Block*. Also, because the *Sire* effect on offspring phenotype is indicative of additive genetic effects, the *Sire* × *Treatment* effect allowed me to assess the presence of G × E interaction effects. To control for differences in body mass between the two treatments, body mass was used as a covariate in each model. All statistical analyses were performed using SPSS 20 (IBM). All means are reported ± 1 S.D.

3.3 Results

Survival to the juvenile stage did not differ between the current and future treatments, averaging 87 ± 11% and 90 ± 9% in the two groups, respectively (P = 0.781). In these two groups, offspring body mass averaged 0.56 ± 0.06 g and 0.58 ± 0.06 g at this time, respectively, and increased to 5.4 ± 1.4 g and 4.0 ± 1.2 g during the cardiac performance measurements. A two-way ANOVA revealed no significant effect of tray position or cell location on offspring cardiac performance ($0.807 \geq P \geq 0.077$ across all measures).

In the current group, f_H increased with temperature from a resting f_H of 65.1 ± 6.6 beats min^{-1} to the f_{Hpeak} of 152.5 ± 18.2 beats min^{-1}, with $T_{peak,H}$ occurring at 20.8 ± 2.3°C (Fig. 3.1). T_{AB} averaged 14.0 ± 1.1°C, while T_{arr} averaged 24.2 ± 1.6°C. Conversely, the T_{AB} of fish in the future group averaged 16.1 ± 0.9°C, with $T_{peak,H}$ and T_{arr} occurring at 22.8 ± 1.9°C and 24.5 ± 2.2°C, respectively. Thus, the average T_{arr} increased only 0.3°C after developmental acclimation to future temperatures, whereas both T_{AB} and $T_{peak,H}$ increased ~2°C (Fig. 3.1). The thermal sensitivity of resting f_H – measured at the two acclimation temperatures of 8°C and 12°C – followed the normal, exponential effect of temperature on physiological rates (Q_{10} = 2.43), indicating that no compensatory adjustments of resting f_H were made. Indeed, the f_{Hmax} of the two groups did not significantly differ from one another until the 16°C temperature interval (t = 3.01, df = 279, P = 0.003).

The present-day stream temperatures during the juvenile residency of this population average 11.1 ± 3.7°C, with a maximum environmental temperature of 20.1°C

(Fig. 3.1). This maximum temperature is just below the average $T_{peak,fH}$ of the current group (20.8°C), indicating that the maintenance of maximum cardiac function corresponds with maximum environmental temperatures.

Sire effects significantly contributed to f_{Hpeak}, scope for f_H, and T_{AB}, whereas *Dam* effects significantly influenced T_{arr} (Table 3.1). Considerable plasticity of cardiac performance was detected, with temperature treatment having a significant effect on resting f_H, f_{Hpeak}, scope for f_H, T_{AB}, and $T_{peak,fH}$; only T_{arr} was not significantly different between treatment groups. No significant *Sire* × *Treatment* effects were found, indicating no G × E interaction effects (Fig. 3.2).

Fig. 3.1. Mean increase in maximum heart rate (f_{Hmax}) among all offspring from the current (black line) and future (grey line) treatment groups. Offspring were reared in two temperature treatments – reflecting current and future conditions – and the response of their f_{Hmax} to warming was measured from their acclimation temperature. Shown are the Arrhenius break temperatures of f_{Hmax} (T_{AB}), the temperatures at which the maximum f_{Hmax} occurs (T_{peakfH}), and the temperatures at which f_{Hmax} becomes arrhythmic (T_{arr}). Also shown are the stream temperatures during the juvenile residency of this population, from the mean spring temperature of 11.1°C to the maximum temperature of 20.1°C, highlighted in grey. These temperature data were collected from 2000 to 2011 and were provided by the Department of Fisheries and Oceans Canada.

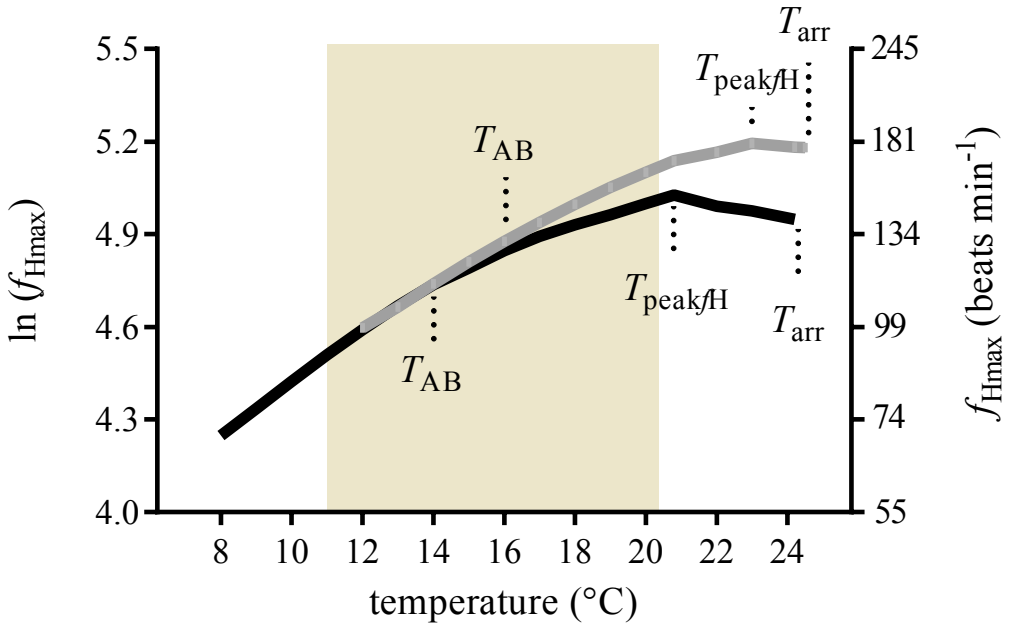


Table 3.1. The plastic and genetic effects contributing to offspring resting heart rate (f_H), highest recorded f_H (f_{Hpeak}), scope for f_H, Arrhenius break temperature (T_{AB}), arrhythmic temperature (T_{arr}), and the temperature at which f_{Hpeak} occurs (T_{peakfH}) in Quinsam River chinook salmon (*Oncorhynchus tshawytscha*). Families were reared in two temperature treatments, reflecting current and future temperatures.

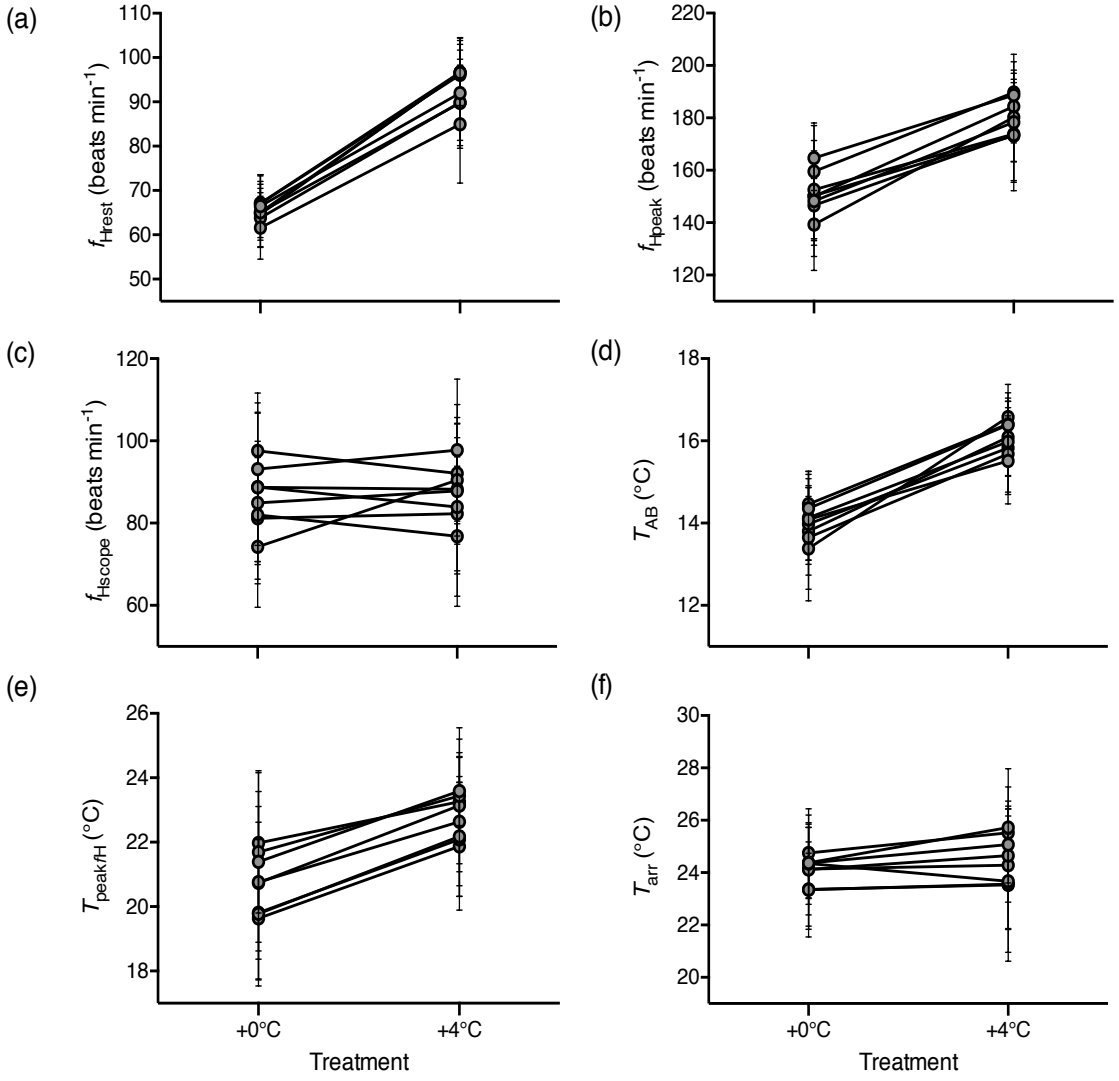
	DF	SS	*F*	*P*	Effect
Resting f_H					
Treatment	1	43629	753	**< 0.001**	**Plastic**
Dam	4	313	1.35	0.252	
Sire	4	467	2.02	0.092	
Sire × *Dam*	4	441	1.90	0.110	
Treatment × *Dam*	4	165	0.71	0.583	
Treatment × *Sire*	4	284	1.23	0.300	
Residual	260	15055			
f_{Hpeak}					
Treatment	1	30636	119	**< 0.001**	**Plastic**
Dam	4	938	0.91	0.457	
Sire	4	4458	4.34	**0.002**	**Additive**
Sire × *Dam*	4	1786	1.74	0.142	
Treatment × *Dam*	4	847	0.82	0.511	
Treatment × *Sire*	4	647	0.63	0.642	
Residual	260	66813			
Scope for f_H					
Treatment	1	1145	4.52	**0.034**	**Plastic**
Dam	4	1732	1.71	0.148	
Sire	4	4907	4.84	**0.001**	**Additive**
Sire × *Dam*	4	883	0.87	0.482	
Treatment × *Dam*	4	1321	1.30	0.269	
Treatment × *Sire*	4	1343	1.32	0.261	
Residual	260	65885			
T_{AB}					
Treatment	1	235	267	**< 0.001**	**Plastic**
Dam	4	3.04	0.87	0.485	
Sire	4	12.3	3.49	**0.009**	**Additive**
Sire × *Dam*	4	3.99	1.14	0.340	
Treatment × *Dam*	4	7.26	2.07	0.086	
Treatment × *Sire*	4	8.19	2.33	0.057	
Residual	260	228			

Table 3.1 (continued)

	DF	SS	*F*	*P*	Effect
$\boldsymbol{T_{arr}}$					
Treatment	1	2.30	0.68	0.411	
Dam	4	35.2	2.59	**0.037**	**Maternal**
Sire	4	24.1	1.77	0.134	
Sire × *Dam*	4	6.70	0.49	0.740	
Treatment × *Dam*	4	1.47	0.11	0.980	
Treatment × *Sire*	4	9.66	0.71	0.585	
Residual	260	882			
$\boldsymbol{T_{peakfH}}$					
Treatment	1	101.6	26.9	**< 0.001**	**Plastic**
Dam	4	30.0	1.98	0.098	
Sire	4	23.4	1.54	0.191	
Sire × *Dam*	4	9.37	0.62	0.650	
Treatment × *Dam*	4	7.24	0.48	0.752	
Treatment × *Sire*	4	5.83	0.38	0.819	
Residual	260	985			

N. B. Shown are the source of variation, degrees of freedom (DF), sum of squares (SS), *F* statistic, *P*-value, and the presence of plastic, maternal, or additive genetic effects. Significant values ($P < 0.05$) are bolded.

Fig. 3.2. Norms of reaction among paternal half-sib families of Quinsam River chinook salmon (*Oncorhynchus tshawytscha*). Offspring were reared in current and future temperature conditions and measured for their (*a*) resting f_H (f_{Hrest}), (*b*) highest recorded f_H (f_{Hpeak}), (*c*) scope for f_H (f_{Hscope}), (*d*) Arrhenius break temperature (T_{AB}), (*e*) the temperature at which f_{Hpeak} occurs ($T_{peak/H}$), and (*f*) the arrhythmic temperature (T_{arr}).



3.4 Discussion

The cardiorespiratory system is key to the maintenance of thermal tolerance, making the thermal plasticity of cardiac function a crucial determinant of the ability of fish species to cope with thermal heterogeneity (Farrell 2009). Indeed, in the eurythermal goby fish *Gillichthys mirabilis*, significant shifts in f_{Hpeak} and $T_{peak f_H}$ among individuals acclimated to 9°C, 19°C, and 26°C suggest that a high degree of plasticity in cardiac performance allows this species to live in a wide range of temperatures (Jayasundara and Somero 2013). Here, I found that juvenile chinook salmon can significantly increase their T_{AB}, $T_{peak f_H}$, and f_{Hpeak} via acclimation to warm temperatures. By increasing the maximum capacity of their hearts as well as the temperatures at which this capacity begins to decline, these plastic adjustments enhance the ability of individuals to deliver oxygen to tissue in warm temperatures, thereby providing a greater breadth of temperatures over which aerobic performance can be maintained. Interestingly, T_{arr} did not exhibit such plasticity, non-significantly increasing from 24.2°C to 24.5°C in the current and future groups, respectively. While these results suggest that upper thermal limits (i.e. T_{arr}, T_{crit}, CT_{max}) may not be readily adjusted via phenotypic plasticity, the realized thermal niche of active fish species such as salmon is likely lower than that bounded by these upper thermal limits due to the need for an aerobic scope large enough to support the aerobic demands of life (Pörtner and Knust 2007). The plastic increases in T_{AB}, $T_{peak f_H}$, and f_{Hpeak} described here would likely change the shape of the aerobic scope-temperature relationship (Fig. 1.1) such that the T_{opt} and T_{opt} window would be shifted higher and closer to T_{crit} (i.e. a rightward skew of the curve). Such adjustment would maintain capacity for aerobic performance in the temperatures preceding T_{crit} and could thus contribute to the ability of salmon to cope with rising temperatures.

Several mechanisms can be adjusted via thermal acclimation that change the temperature dependency of f_{Hmax} and thus might have contributed to the plastic changes that I observed in T_{AB}, $T_{peak f_H}$, and f_{Hpeak}. These mechanisms include excitation-contraction properties, myocardium structure, and sensitivity to sympathetic modulation (Lillywhite et al. 1999). Two distinct types of acclimation responses are developmental and reversible acclimation (Angilletta 2009), with developmental acclimation occurring

across developmental stages and reversible acclimation occurring within a single developmental stage and usually in a reversible manner. The phenotypic adjustments that I detected were made in individuals that were exposed to one of two thermal regimes across the embryonic, alevin, and juvenile stages of their life cycle, with the response of f_{Hmax} to warming being measured from the acclimation temperatures of the two groups. The high degree of plasticity that was detected here was not found in a study by Chen et al. (2013) in which four populations of sockeye salmon were incubated at either 10°C or 14°C throughout embryonic development and then reared in common temperatures as alevin and juveniles. The plasticity of cardiac function was inconsistent among these four populations, with T_{AB} and f_{Hpeak} decreasing from the 10°C to the 14°C treatment group in one of the populations, increasing in another population, and remaining unchanged in the other two populations. Although this study by Chen et al. (2013) provides evidence for some lasting developmental effects on cardiac function, the roles of developmental and reversible (i.e. within developmental stage) acclimation remain unclear. Studies that measure the thermal performance of cardiac function within and among developmental stages are needed to clarify how thermal environments have immediate, lasting, or transgenerational (e.g. Donelson et al. 2012) effects on thermal tolerance, while the differences in plastic capacity among populations of Pacific salmon warrant a proximate or evolutionary explanation.

Present-day temperatures that this population can experience during juvenile residency in streams range widely from 3.3°C to 20.1°C and average 11.1°C. This maximum environmental temperature corresponds with the average $T_{peak,fH}$ of the current group (20.8 ± 2.3°C). This match may be due to a functional importance of $T_{peak,fH}$; aerobic scope would decline rapidly above this temperature due to the decline in maximum cardiac capacity, perhaps making $T_{peak,fH}$ a limit of thermal tolerance. In the future group, $T_{peak,fH}$ increased 2°C to 22.8°C ± 1.9°C. Although this change would increase the temperatures over which aerobic capacity can be maintained, it does not completely track the 4°C increase the fish were exposed to. Salmon populations may thus have to rely on other processes – such as evolutionary adaptation – to keep pace with a high level of warming.

Quantitative trait loci have been associated with CT_{max} in rainbow trout and Arctic charr (*Salvelinus alpinus*) (Perry et al. 2001; Quinn et al. 2011), suggesting a genetic basis for thermal tolerance that may be key for evolutionary responses to climate change. In this study, I found significant additive genetic effects underlying f_{Hpeak}, scope for f_H, and T_{AB}. Fish that have a greater capacity for cardiac function should be more 'equipped' to cope with exposure to high temperatures, meaning this heritable variation in cardiac capacity could allow responses to the selection imposed by deleteriously warm temperatures. Furthermore, because T_{AB} is mechanistically linked with T_{opt}, the additive genetic effects underlying T_{AB} suggest that the close correspondence between T_{opt} and local thermal conditions among Pacific salmon populations (Lee et al. 2003; Eliason et al. 2011) might be an adaptation brought about by selection on these genetic effects.

The findings presented here represent the first direct examination of heritable and plastic effects contributing to oxygen-limited thermal tolerance in a wild fish population. The results support my hypothesis that thermal conditions can elicit plastic changes in thermal tolerance. These changes might contribute to the population-specific thermal tolerance of Pacific salmon populations. However, the additive genetic variation in cardiac performance that I detected suggests that evolutionary adaptation could also contribute to this thermal specialization among populations. Despite not detecting any G × E effects contributing to thermal tolerance, these results indicate an intrinsic ability of salmon populations to respond to changing temperature conditions.

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Chapter 4

4 General discussion

Describing the genetic and environmental underpinnings of thermal tolerance is key to our understanding of how populations might respond to climate change (Somero 2010). The findings presented here comprise one of the first quantitative estimates of the genetic variation underlying thermal tolerance in wild populations of fish, and the first to do so using direct measures of oxygen-limited thermal tolerance. Within two coastal populations of chinook salmon, I found significant maternal, additive genetic, and plastic effects contributing to thermal tolerance. These results help elucidate the adaptive potential of fish populations that are faced with rising temperatures.

4.1 Genetic responses to climate change

Novel selection pressures are projected to be widespread as a result of climate change, affecting natural systems in a rapid and global fashion. Given the ubiquity of evolutionary adaptation to the environment, genetic changes within populations are expected (Hoffmann and Sgrò 2011). Many of the genetic responses to climate change that have been documented thus far involve phenological changes in response to earlier springs and longer growing seasons (Bradshaw and Holzapfel 2008). Indeed, evolutionary adjustments of migration timing may allow sockeye salmon populations to avoid harmful temperatures during future migrations (Reed et al. 2011). However, such phenological adaptation to climate change depends on concurrent changes in the physical and biological requirements of populations (e.g. growth requirements for migration, interactions with predators and prey), requirements that might limit the degree of such adaptation. For species that already live close to their geographic or physiological limits such as the Pacific salmon, the capacity for genetic changes in physiological tolerance will likely be important (Hoffmann and Sgrò 2011).

Although little is known about the heritability of thermal tolerance in fishes, the high degree of thermal adaptation of performance breadths observed locally (e.g. Jensen et al. 2008; Eliason et al. 2011) and across latitudinal clines (e.g. Fangue et al. 2006; Peck

et al. 2014) suggests an evolutionary potential of thermal tolerance. Indeed, the cold tolerance of threespine sticklebacks (*Gasterosteus aculeatus*) lowered 2.5°C as a result of evolutionary adaptation to cold temperature after only three generations of exposure (Barrett et al. 2011). In Chapter 3, I describe standing genetic variation for f_{Hpeak}, scope for f_H, and T_{AB} that may serve as the basis for genetic adjustments of thermal tolerance in chinook salmon. Conversely, no significant additive genetic effects on these traits were detected in Chapter 2. The study populations used in the two studies are genetically similar (Heath et al. 2006) and inhabit similar freshwater environments along the east coast of Vancouver Island, BC. With no clear difference in relevant selection pressures on aerobic performance between the two populations, a likely cause of the discrepancy in observed heritability is the number of genetic sources used in the two breeding designs: five males and five females were crossed in a full-factorial design in Chapter 2, whereas a total of eight males and eight females were used in the breeding design in Chapter 3, increasing the likelihood of detecting additive genetic variation. In fact, the sire effect contributing to T_{AB} in Chapter 2 was approaching significance (P = 0.099), potentially explaining up to 18% of the phenotypic variation. Certainly it would be valuable for future studies to incorporate more individuals, as well as to compare between populations, to more fully understand genetic variation for thermal tolerance within and among salmon populations.

While direct genetic effects on thermal tolerance were found in Chapter 3, evidence for indirect genetic effects influencing thermal tolerance – acting via the maternal effects of egg size – was found in Chapter 2. Evidence for such 'heritable environmental' effects having important roles in the evolutionary trajectories of populations is accumulating (Mousseau and Fox 1998; Räsänen and Kruuk 2007). For example, in a long-term study of a wild population of red squirrels (*Tamiasciurus hudsonicus*), maternal effects had a large influence on juvenile body mass and were found to be highly heritable, such that the evolutionary potential of juvenile body mass increased more than three-fold when both direct and indirect genetic effects were considered instead of only direct effects (McAdam et al. 2002). Mothers can non-genetically affect the phenotype of their offspring through the partitioning of nutrients or hormones, but the ability to do so can show additive genetic variance that responds to

selection (Okuliarova et al. 2011). With increasing evidence for parental effects on thermal tolerance beyond those directly due to genes (see Section 4.2 below), indirect genetic effects appear to be a promising source of evolutionary potential in natural populations challenged with a warming climate. Because indirect genetic effects change the expected effect of selection on both genetic and phenotypic values (Wolf et al. 1998), studies of evolutionary potential should be designed such that the effect of indirect genetic variation can be quantified; indeed, had I not measured adult traits such as egg size in Chapter 2, I would not have found evidence for indirect genetic effects and their contribution to the adaptive capacity of thermal tolerance.

The adaptive significance and genetic architecture of phenotypic plasticity have been the subject of much debate (e.g. Via et al. 1995) and theoretical study (e.g. Reed et al. 2010), however explicit, non-correlative tests of the heritability and fitness benefits of phenotypic plasticity are rare (Kingsolver and Huey 1998; Angilletta 2009). A notable exception is a study by Nussey et al. (2005), in which the fitness consequences of individual plasticity in reproductive timing was examined within a population of great tits (*Parus major*) using a 32-year data set. The plasticity in reproductive timing was heritable, and females that were more able to adjust their laying date in response to warming temperatures achieved greater reproductive success than those that maintained a constant laying date. Comparable studies of physiological responses to temperature are lacking, perhaps due to the relative challenges posed by studying physiological traits in natural settings versus morphological or phenological characters. In Chapter 3, I found no evidence of G × E effects contributing to the thermal tolerance of Quinsam River chinook salmon, indicating that the evolutionary potential of thermal plasticity may be limited. Still, G × E effects on life-history traits have been widely documented in salmonids (Hutchings 2011) and have been detected across current and future temperature treatments in recent studies of body size in marine sticklebacks (Shama et al. 2014) and hatching success in the sea urchin *Heliocidaris erythrogramma* (Lymbery and Evans 2013), suggesting that the evolution of plasticity could contribute to the adaptation of populations to warmer and more variable environments.

4.2 Non-genetic responses to climate change

Phenotypic plasticity is fundamental to the ability of organisms to cope with environmental change. Indeed, most of the phenotypic responses to climate change that have been documented among fish species are attributable to plasticity (Crozier and Hutchings 2014), likely because it is the first response to change in populations that cannot disperse. Macrophysiological research has suggested that temperate species have an enhanced capacity for plasticity relative to tropical and polar species as a result of adaptation to climatic variability (Janzen 1967, Tewksbury et al. 2008; Peck et al. 2014). Still, Pacific salmon populations appear to have specialized their performance with respect to temperature (Brett 1956; Farrell 2009; Eliason et al. 2011), and such thermal specialization may tradeoff with plasticity (Angilletta 2009). In Chapter 3, I detected strong adjustments of T_{AB}, f_{Hpeak}, and $T_{peak}f_H$ in offspring that were reared in high temperatures, adjustments that are likely to enhance the capacity for aerobic performance in the temperatures preceding T_{crit}. However, these changes in cardiac performance did not completely track the extent of the warming that the fish were reared in, suggesting thermal plasticity alone may not keep pace with a high level of temperature increase. The degree of plasticity demonstrated here could be used in evolutionary models that combine demographic information with the heritability and plasticity of traits key to environmental tolerance to predict the maximum rate of environmental change that can be tolerated without local extinction (e.g. Chevin et al. 2010). This plasticity could also be considered in models of future mortality risk due to exposure to deleteriously high temperatures (e.g. Hague et al. 2011; Martins et al. 2011). Indeed, development in high temperatures might have lasting effects that could contribute to the variation in thermal tolerance observed among populations (Eliason et al. 2011; Chen et al. 2013).

Recent research has elucidated transgenerational plasticity as a powerful mechanism by which fish can cope with stressors such as high temperature (Donelson et al. 2012; Salinas and Munch 2012; Shama et al. 2014), hypoxia (Ho and Burggren 2012), and acidification (Miller et al. 2012). Transgenerational plasticity occurs when offspring inherit non-genetic effects from their parents that are dependent on the environment that their parents were exposed to. For example, the tropical damselfish *Acanthochromis*

polyacanthus can make transgenerational adjustments of thermal tolerance such that when both parents and their offspring are reared in high temperatures, offspring avoid the temperature-induced loss of aerobic scope that occurs when only offspring are reared in high temperatures (Donelson et al. 2012). This 'preparing' of offspring likely occurs through changes in offspring epigenetic state or through maternal partitioning of hormones. Indeed, marine stickleback offspring benefit from maternal transgenerational effects on body size, with offspring being largest when reared in the same acclimation temperature as their mother (Shama et al. 2014). In both Chapter 2 and 3, I found strong maternal effects on offspring thermal tolerance, with the effects in Chapter 2 seemingly acting through egg size. Adaptive phenotypic plasticity of egg size can occur when mothers adjust their reproductive investment in response to an environmental cue that is a reliable indicator of future environments (Fox et al. 1997). In salmonids, increased egg size can buffer the deleterious effects of high temperature on offspring growth and survival (Beacham and Murray 1985; Ojanguren and Braña 2003; Finstad and Jonsson 2012), and is associated with increased incubation temperatures (Braun et al. 2013) and thermal tolerance (Chen et al. 2013) at the population level. Future studies should evaluate the capacity for maternal adjustments of egg size in response to high temperatures, as well as the costs and tradeoffs inherent in such transgenerational responses.

4.3 Concluding remarks

While my findings of plastic, additive genetic, and indirect genetic effects underlying aerobic capacity help elucidate how salmon populations might respond to climate change, it's clear that other considerations must be taken to understand the full scope of salmon resilience to climate change. First, how the genetic and environmental contributions to thermal tolerance change throughout ontogeny should be studied, as spawning migrations are known to be a major source of temperature-induced mortality (Farrell et al. 2008). Also, changes in migration timing might allow populations to avoid high temperatures (Reed et al. 2011), thereby providing another target for selection. In addition to the directions for future research described in this chapter, a remaining question – and one of the most pressing in climate change research as a whole (Gilman et

al. 2010) – is the extent to which trophic interactions and thus community stability are disrupted with warming. Nevertheless, a crucial first step towards understanding species' vulnerability to climate warming is an understanding of their thermal physiology. As anthropogenic activity continues to have an increasingly profound influence on natural systems, continued research that undertakes the challenging task of integrating physiological, quantitative genetic, and ecological data will fill crucial gaps in our understanding of the scope for biological resilience to rapid environmental change.

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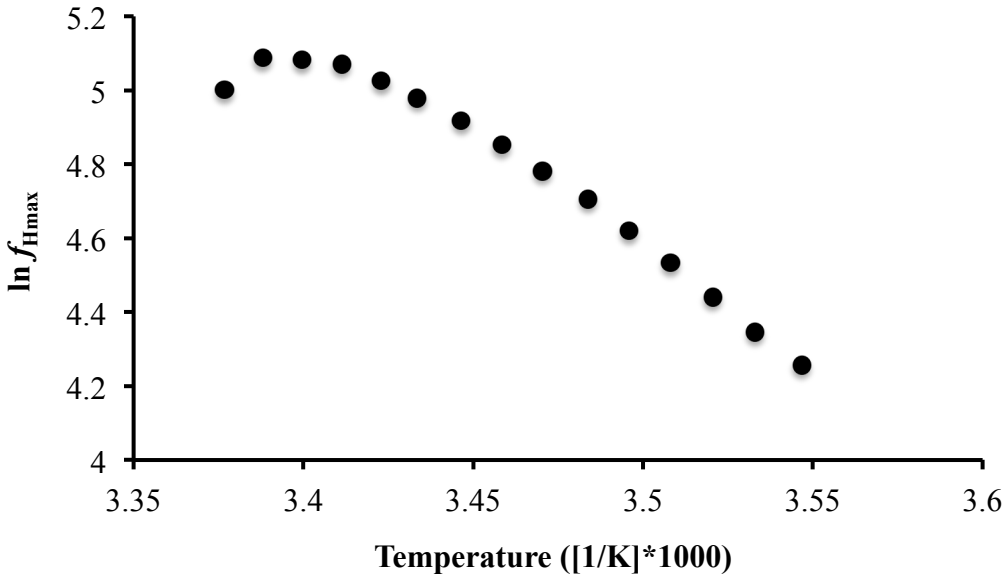
5 Appendices

Appendix A. Example response of maximum heart rate (f_{Hmax}) to temperature and the corresponding Arrhenius plot.

Test temperature		Maximum heart rate		Q_{10}
°C	(1/K)*1000	beats min^{-1}	ln	
7.8	3.5594	70.64	4.2576	2.22
8.9	3.5455	77.12	4.3454	2.57
9.9	3.5329	84.75	4.4397	2.58
10.9	3.5205	93.17	4.5344	2.37
11.9	3.5082	101.58	4.6208	2.31
12.9	3.4959	110.43	4.7044	2.00
14	3.4825	119.21	4.7808	2.04
15	3.4704	128.02	4.8522	1.94
16	3.4584	136.78	4.9184	1.74
17.1	3.4453	145.40	4.9795	1.67
18	3.4347	152.28	5.0257	1.57
19	3.4229	159.29	5.0707	1.13
20	3.4112	161.29	5.0832	1.06
21	3.3996	162.16	5.0886	0.42
22	3.3881	148.51	5.0007	



Appendix B. Calculation of T_{AB} based on example response of maximum heart rate (f_{Hmax}) to temperature in Appendix A.

Data split into two based on the two lines that best fit the data:

Test temperature		Maximum heart rate		Q_{10}
°C	(1/K)*1000	beats min^{-1}	ln	
7.8	3.5594	70.64	4.2576	2.22
8.9	3.5455	77.12	4.3454	2.57
9.9	3.5329	84.75	4.4397	2.58
10.9	3.5205	93.17	4.5344	2.37
11.9	3.5082	101.58	4.6208	2.31
12.9	3.4959	110.43	4.7044	2.00
14	3.4825	119.21	4.7808	2.04
15	3.4704	128.02	4.8522	1.94

Equation:
$y = -6.8x + 28.47$

Test temperature		Maximum heart rate		Q_{10}
°C	(1/K)*1000	beats min^{-1}	ln	
16	3.4584	136.78	4.9184	1.74
17.1	3.4453	145.40	4.9795	1.67
18	3.4347	152.28	5.0257	1.57
19	3.4229	159.29	5.0707	1.13
20*	3.4112	161.29	5.0832	1.06
21*	3.3996	162.16	5.0886	0.42
22*	3.3881	148.51	5.0007	

* Temperature steps removed when fitting data due to f_{Hmax} plateauing at these steps and thus skewing the temperature-f_{Hmax} relationship (see Arrhenius plot in Appendix A).

Equation:
$y = -4.3x + 19.79$

Solving for T_{AB}, the point of intersection of these two lines:

$$-6.8x + 28.47 = -4.3x + 19.79$$
$$2.5x = 8.68$$
$$x = 3.472$$

$$3.472 \left(\frac{1000}{\mathrm{K}}\right) = 14.9°\mathrm{C}$$

Thus, T_{AB} = 14.9°C

Appendix C. Power exponents used to transform resting heart rate (f_H), highest recorded f_H (f_{Hpeak}), scope for f_H, Arrhenius break temperature (T_{AB}), arrhythmic temperature (T_{arr}), thermal window (T_{win}), and critical thermal maximum (CT_{max}) data from Chapter 2. Box-Cox analyses were performed to find optimal power exponents to restore normality.

Trait	Exponent
resting f_H	3
f_{Hpeak}	2.5
scope for f_H	1.5
T_{AB}	1.8
T_{arr}	3.8
T_{win}	1.3
CT_{max}	26.9

Appendix D: Ethics statement.

All experiments followed ethical guidelines from the Canadian Council on Animal Care as reviewed and approved by the Animal Use Subcommittees at the University of Western Ontario (protocol # 2010-214) and the University of British Columbia (protocol #810-022).

6 Curriculum Vitae

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