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

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With global temperatures projected to surpass the limits of thermal tolerance for many species, evaluating the heritable variation underlying thermal tolerance is critical for understanding the potential for adaptation to climate change. We 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 and the critical thermal maximum (CT_{max}) of 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, cardiac arrhythmia temperature and CT_{max}. 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. Such indirect genetic effects could accelerate evolutionary responses to the selection imposed by rising temperatures and could contribute to the population-specific thermal tolerance that has recently been uncovered among Pacific salmon populations.

1. Introduction

Climate change is projected to have widespread impacts on biodiversity [1], with rising temperatures being of particular concern owing to the pervasive effects of temperature on organisms [2]. 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 [3,4]. Indeed, there is growing evidence that evolutionary adaptations to climate change will be key for the long-term viability of populations [5]. 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 [6]. A powerful means of describing evolutionary potential is using quantitative genetic breeding designs to partition phenotypic variation into additive (i.e. heritable) and non-additive genetic effects [7]. However, using only these direct estimates of heritability can underestimate evolutionary potential owing to the presence of indirect genetic effects [8]. 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 effects act indirectly and provide heritable variation on which selection can act [9]. Indirect genetic effects are largely attributed to heritable maternal effects [10,11], 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 among offspring, including larval survival [12],

stress response [13] and metabolic enzyme activity [14], 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 [15]. 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 [16]. In its place, the oxygen- and capacity-limited thermal tolerance framework offers a functional understanding of how temperature limits organisms in the wild. It attributes the limits of thermal tolerance to the loss of aerobic scope (i.e. the difference between minimum and maximum oxygen consumption rates) [17]. 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 owing to limitations on the ability to increase heart rate beyond a maximum level [18]. 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 [19,20].

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, whereas 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 in Pacific salmon populations at both the juvenile [21] and adult stage [22]. Indeed, a collapse of aerobic scope has been empirically linked to high mortality during spawning migrations of sockeye (*Oncorhynchus nerka*) salmon [20]. 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 [23,24]; however, the heritability of thermal tolerance and its evolutionary potential to respond to rising temperatures remain largely unknown.

The aim of this study was to evaluate the evolutionary potential of oxygen-limited thermal tolerance within a coastal population of chinook salmon (*Oncorhynchus tshawytscha*). To do so, we 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. Because the thermal tolerance of Pacific salmon populations appears to be adapted to local river temperatures [23,24], we predicted that we would detect additive genetic variation for thermal tolerance.

2. Material and methods

The study population consisted of wild chinook salmon from the Big Qualicum River, British Columbia, Canada. This population is augmented by a hatchery release programme. Such programmes can increase the standing genetic variation within populations that might have low genetic diversity owing to a large decline in abundance from historic levels [25]. On 8 October 2011, adult fish completing their spawning migration were collected using diversion channels located at the Fisheries and Oceans Canada salmon hatchery on the Big Qualicum River. 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 and transported on ice to Yellow Island Aquaculture Ltd on Quadra Island, BC, where mean egg diameter was measured using 30 eggs from each female (± 0.01 mm).

(a) Breeding design and offspring rearing

Gametes were crossed in a full-factorial breeding design (North Carolina II cross) [7] in which all possible crosses were conducted between five males and five females, producing 25 unique families. Fertilized eggs were incubated in a Heath stack, with all families exposed to the same thermal conditions throughout development (see the electronic supplementary material for more details). After entry into the exogenous feeding stage, hatched offspring were tagged using visible implant elastomers (Northwest Marine Technology, Shaw Island, WA, USA) and transported to 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 $9.3 \pm 0.7^{\circ}$C.

(b) Cardiac performance measurements

We used the response of maximum heart rate (f_H) to warming [26] to evaluate the genetic architecture underlying the thermal performance of cardiac function. 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} (figure 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 [18,27]. 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 aerobic capacity above this temperature would be highly reduced with an arrhythmic heartbeat.

At their acclimation temperature of 10°C, individuals were anaesthetized in MS-222 (Sigma-Aldrich, St Louis, MO, USA) and measured for their resting f_H in recirculating holding troughs (see [26] for apparatus details). f_{Hmax} was pharmacologically induced and measured at every $+1^{\circ}$C temperature increment until the heartbeat became arrhythmic (see the electronic supplementary material for detailed methodology). A total of 228 trials were conducted using 8–10 individuals from each of the 25 families. The pharmacological stimulation occasionally had incomplete or unexpected effects on individuals, such as cardiac arrhythmias occurring soon after stimulation. In such cases, individuals were removed from the study (32 fish were removed).

(c) Thermal tolerance measurements

We measured the CT_{max} of offspring from each family to determine the genetic and maternal effects underlying CT_{max} and to assess whether these effects are similar to those underlying cardiac performance. Fish were kept in a 50 l tank at 10°C for 1 h, and then temperature was continuously increased until an associated loss of directed locomotor capacity and equilibrium was observed (see the electronic supplementary material for detailed methodology). A total of 173 individuals were sampled, using 6–10 offspring from each full-sib family.

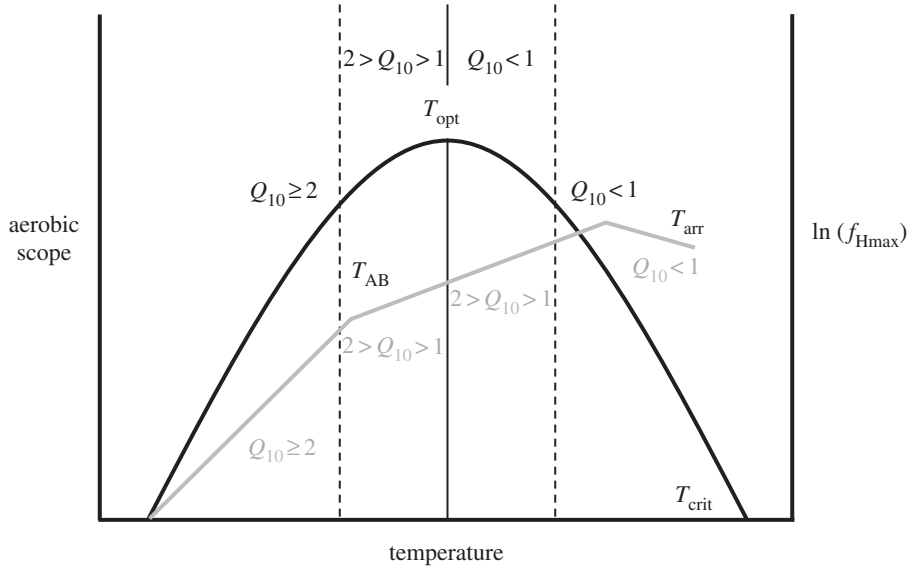

Figure 1. The relationship between aerobic scope (black line), maximum heart rate (f_{Hmax}; grey line), and temperature in Pacific salmon (*Oncorhynchus* spp.). Shown are the optimum temperature (T_{opt}) and upper critical temperature (T_{crit}) for aerobic scope, as well as the Arrhenius break temperature (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 range in which aerobic scope is $\geq$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. 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 T_{crit} [16,26].

(d) Statistical analyses

We calculated the T_{AB} of f_{Hmax} using the program presented in [28]. This program fits two-segmented straight lines, which allowed us to identify the point 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 lowest Arrhenius break point, T_{AB}. The temperature sensitivity (Q_{10}) of f_{Hmax} was also calculated between each temperature increment using the formula $(f_{Hmax\,n+1}/f_{Hmax\,n})\,\wedge\,(10/T_{n+1} - T_n)$, 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 (five levels) and cell location (16 levels) as fixed factors. We then partitioned the variation in offspring resting f_H, highest f_{Hmax} reached (f_{Hpeak}), scope for f_H (= f_{Hpeak} − resting f_H), T_{AB}, T_{arr}, thermal window between T_{AB} and T_{arr} (T_{win}), and CT_{max} into additive genetic, non-additive genetic and maternal effects using a two-way restricted maximum-likelihood-based ANOVA with *sire* and *dam* identity and their interaction as random factors. Additive genetic, non-additive genetic and maternal effects were calculated following Lynch & Walsh [7] (see the electronic supplementary material for calculations). 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 [29]. All statistical analyses were performed using SPSS 20 (IBM, Armonk, NY, USA). All means are reported $\pm$1 s.d.

3. Results

Upon entry into the juvenile stage of their life cycle, offspring survival across all families averaged 90 $\pm$ 15%. Offspring body mass averaged 0.59 $\pm$ 0.32 g at this time and increased to 3.6 $\pm$ 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 $\pm$ 9.4 beats min^{-1} to the f_{Hpeak} of 163.3 $\pm$ 25.8 beats min^{-1}, with f_{Hpeak} occurring at 21.2 $\pm$ 2.4°C (electronic supplementary material, figure S1). T_{AB} averaged 15.0 $\pm$ 1.1°C among all individuals, which corresponded with the incremental Q_{10} decreasing from 2.5 $\pm$ 0.2 at 10.0°C to 1.9 $\pm$ 0.3 at T_{AB} (electronic supplementary material, figure S2). T_{arr} averaged 22.4 $\pm$ 2.5°C and was lower than CT_{max}, which averaged 26.5 $\pm$ 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.

Larger offspring generally had enhanced cardiac capacity, with body mass being 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 ($r = 0.147$, $P = 0.041$). 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 1). *Dam* 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. The dam- and sire-based variation in each of the analysed traits is shown in table 1 and the electronic supplementary material, figure S3.

Regression analyses revealed further evidence of maternal influence on offspring phenotype, with mean egg diameter

Table 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 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}). Shown are the variance components of each source (σ^2), as well as the contributions to phenotypic variance (% phenotypic var) of maternal, additive genetic, and non-additive genetic effects. Significant values ($p < 0.05$) are italicized.

	d.f.	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	non-additive	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	non-additive	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	non-additive	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	non-additive	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	non-additive	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	non-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	non-additive	0
residual	148				1.0×10^{76}		

being strongly associated with scope for f_H ($r^2 = 0.88$, $F_{1,3} = 20.3$, $p = 0.019$) and CT_{max} ($r^2 = 0.85$, $F_{1,3} = 19.2$, $p = 0.022$), and marginally non-significantly with T_{AB} ($r^2 = 0.76$, $F_{1,3} = 9.20$, $p = 0.054$), f_{Hpeak} ($r^2 = 0.70$, $F_{1,3} = 6.39$, $p = 0.082$), T_{arr} ($r^2 = 0.59$, $F_{1,3} = 4.37$, $P = 0.130$) and T_{win} ($r^2 = 0.51$, $F_{1,3} = 3.14$, $p = 0.177$; figure 2). 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. Conversely, no significant 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}: $r = 0.511$, d.f. = 192, $p < 0.001$; T_{arr} and f_{Hpeak}: $r = 0.789$, d.f. = 192, $p < 0.001$; T_{AB} and f_{Hpeak}: $r = 0.569$, d.f. = 192, $p < 0.001$), whereas resting f_H was significantly correlated with f_{Hpeak} ($r = 0.266$, d.f. = 192,

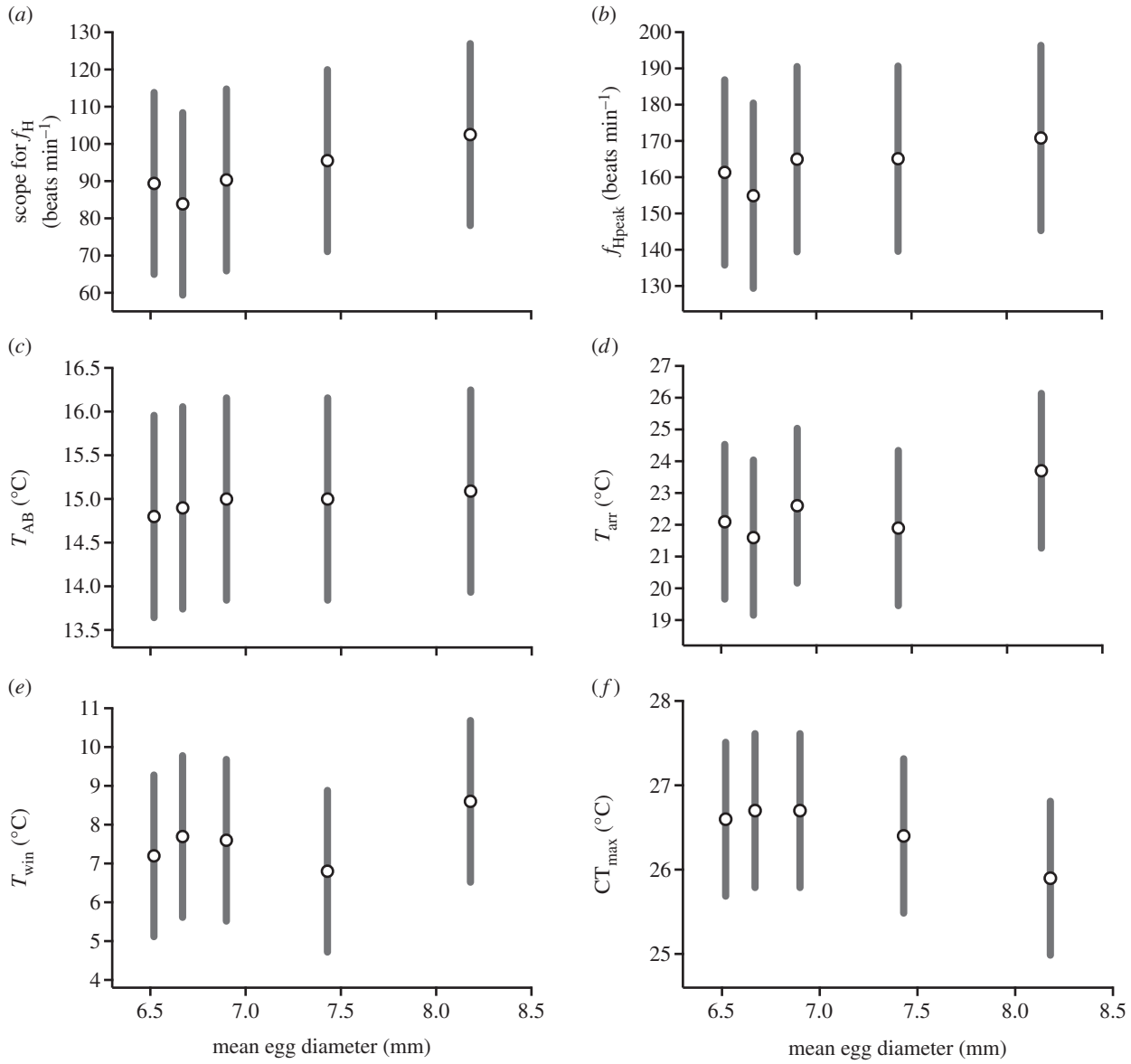

Figure 2. Relationships between dam mean egg diameter and offspring (*a*) scope for heart rate (f_H); (*b*) highest f_H (f_{Hpeak}); (*c*) Arrhenius break temperature (T_{AB}); (*d*) arrhythmic temperature (T_{arr}); (*e*) thermal window (T_{win}); and (*f*) critical thermal maximum (CT_{max}) in Big Qualicum River chinook salmon (*Oncorhynchus tshawytscha*). The error bars are ± 1 s.d. of the family mean.

$p < 0.001$) and T_{arr} ($r = 0.147$, d.f. $= 192$, $p = 0.040$). 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} ($r_s = -0.541$, d.f. $= 23$, $p = 0.005$), T_{AB} ($r_s = -0.622$, d.f. $= 23$, $p = 0.001$), f_{Hpeak} ($r_s = -0.563$, d.f. $= 23$, $p = 0.003$) and scope for f_H ($r_s = -0.643$, d.f. $= 23$, $p = 0.001$). Indeed, the offspring of dam 1 reached the highest T_{arr} yet the lowest CT_{max} value (electronic supplementary material, figure S2*d*,*e*).

4. Discussion

Describing the genetic and environmental underpinnings of thermal tolerance is key to our understanding of how populations might respond to climate change [30]. The findings presented here comprise one of the first quantitative estimates of the genetic variation underlying thermal tolerance in a wild fish population, and the first to do so using direct measures of oxygen-limited thermal tolerance. Within a coastal population of chinook salmon, we found strong maternal effects underlying thermal tolerance and cardiac performance. These results help elucidate the adaptive mechanisms available to fish populations that are faced with rising temperatures.

Maternal effects have been found to be key determinants of phenotypic variation among offspring in a wide range of traits and taxa, and are thus increasingly recognized as having an important role in the evolutionary dynamics of populations [10,11]. For example, a study of 17 life-history and fitness-related traits among wild chinook salmon populations found that maternal effects contribute more to phenotypic divergence between populations than do additive genetic effects [31]. In our study, we 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 [9]. Indeed, in a captive population of chinook salmon, a mother–daughter regression revealed egg mass to be highly

heritable, indicative of a genetic basis for egg provisioning [32]. Although heritability is population- and environment-specific, within-population variation in egg size is common in demersal egg-laying species such as salmon [33] and has been found to be similarly heritable in other species of Pacific salmonids [34,35]. If female salmon inherit the ability to provision eggs, then 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 [23,24,36]. While the correspondence between thermal tolerance and environmental conditions suggests local adaptation brought about by selection on additive genetic effects, 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 population-specific and positively correlated with natural incubation temperatures [37] and juvenile thermal tolerance [36].

Heart rate varies considerably both within and between fish species, with resting f_H being primarily determined by metabolic rate and haemodynamic requirements, and f_{Hmax} being limited by mechanistic constraints such as pacemaker potential, excitation–contraction properties and myocardium structure [38]. Ultimately, resting f_H and f_{Hmax} are 'set' by a balance between these mechanistic constraints and the evolutionary pressures created by haemodynamic 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 owing 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 [39], 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 anaesthetized fish at their acclimation temperature. Whether the high levels of genetic variation for resting f_H still exist in 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} [16], typical of animals experiencing hypoxic conditions. Indeed, CT_{max} and hypoxia tolerance are positively correlated among families of Atlantic salmon (*Salmo salar*) [40], suggesting a genetic basis for anaerobic capacity. We found that juvenile chinook salmon reach their maximum heart rate (i.e. f_{Hpeak}) 1.2°C cooler than their arrhythmic temperature (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 [15]. Moreover, because f_{Hmax} is an aerobic trait owing to the heart's inability to work anaerobically at maximal levels [41], the negative correlation between CT_{max} and cardiac capacity among families suggests a trade-off between aerobic and anaerobic capacity consistent with earlier suggestions [42,43].

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 [10,11] 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 owing to the genes inherited by offspring [44], indirect genetic effects appear to be a promising source of evolutionary potential in natural populations challenged by a changing environment.

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Data accessibility. The heart rate and CT_{max} measures from all individuals used in this study: dryad doi:10.5061/dryad.682ns.

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Supplementary Materials

Breeding Design and Offspring Rearing

Gametes were crossed in a full-factorial breeding design (North Carolina II cross) [7] in which all possible crosses were conducted between five males and five females, producing 25 unique families. Each cross was replicated (n = 50 families total) using 60-155 eggs per replicate. Upon arrival at YIAL (< 3 h post-collection), eggs were fertilized in the order in which they were collected using a dry fertilization protocol [45]. Each replicate was then randomly assigned to a cell within five trays of a single Heath incubation stack. 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.

Cardiac Performance Measurements

Following a fasting period of 24 h, two individuals at a time were anesthetized in 75 mg l^{-1} MS-222 (Sigma-Aldrich, St. Louis, MO, USA) and 75 mg l^{-1} sodium bicarbonate solution, measured for body mass (± 0.1 g), and left anesthetized in the experimental apparatus with recirculating water maintained at 10°C by a 3016D in-line chiller (Fisher Scientific, Ottawa, ON, Canada) (see [26] 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 intraperitoneal injections of 2.4 mg kg^{-1} atropine sulphate (Sigma-Aldrich) 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 228 trials were conducted using 8–10 individuals from each of the 25 families. The pharmacological injections occasionally had incomplete 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 (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.

Thermal Tolerance Measurements

CT_{max} was measured using 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 [46]. 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 lowered 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.

Statistical Analyses

We partitioned the variation in offspring phenotype into additive genetic, non-additive genetic, and maternal effects using a two-way restricted maximum likelihood-based ANOVA with *Sire* and *Dam* identity and their interaction as random factors. Following Lynch & Walsh [7], we 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. Body mass was used as a covariate in each analysis and was removed from the model whenever non-significant.

Fig. S1. 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. Also shown is the average critical thermal maximum (CT_{max}), which was determined using alternative methods (see text).

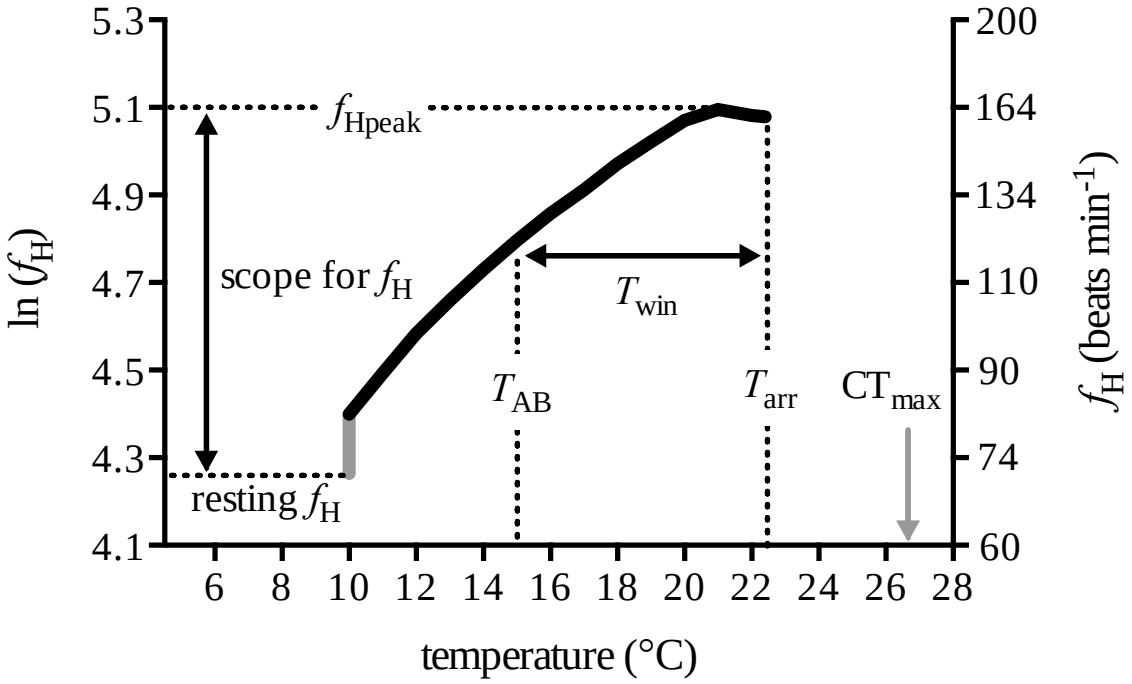


Fig. S2. Incremental Q_{10} values of maximum heart rate in Big Qualicum River chinook salmon (*Oncorhynchus tshawytscha*). The dashed lines indicate where maximum heart rate increases exponentially (Q_{10} = 2) and does not change (Q_{10} = 1) with temperature. The asterisk denotes the mean Arrhenius break temperature of maximum heart rate (15.0°C). Values are mean ± 1 standard deviation.

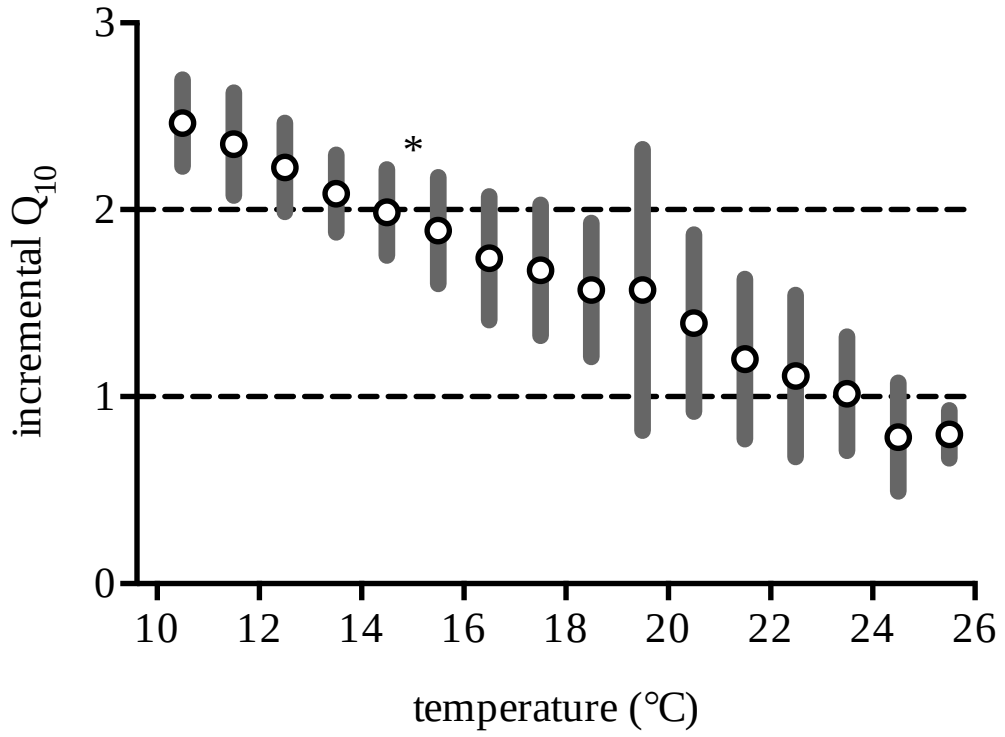
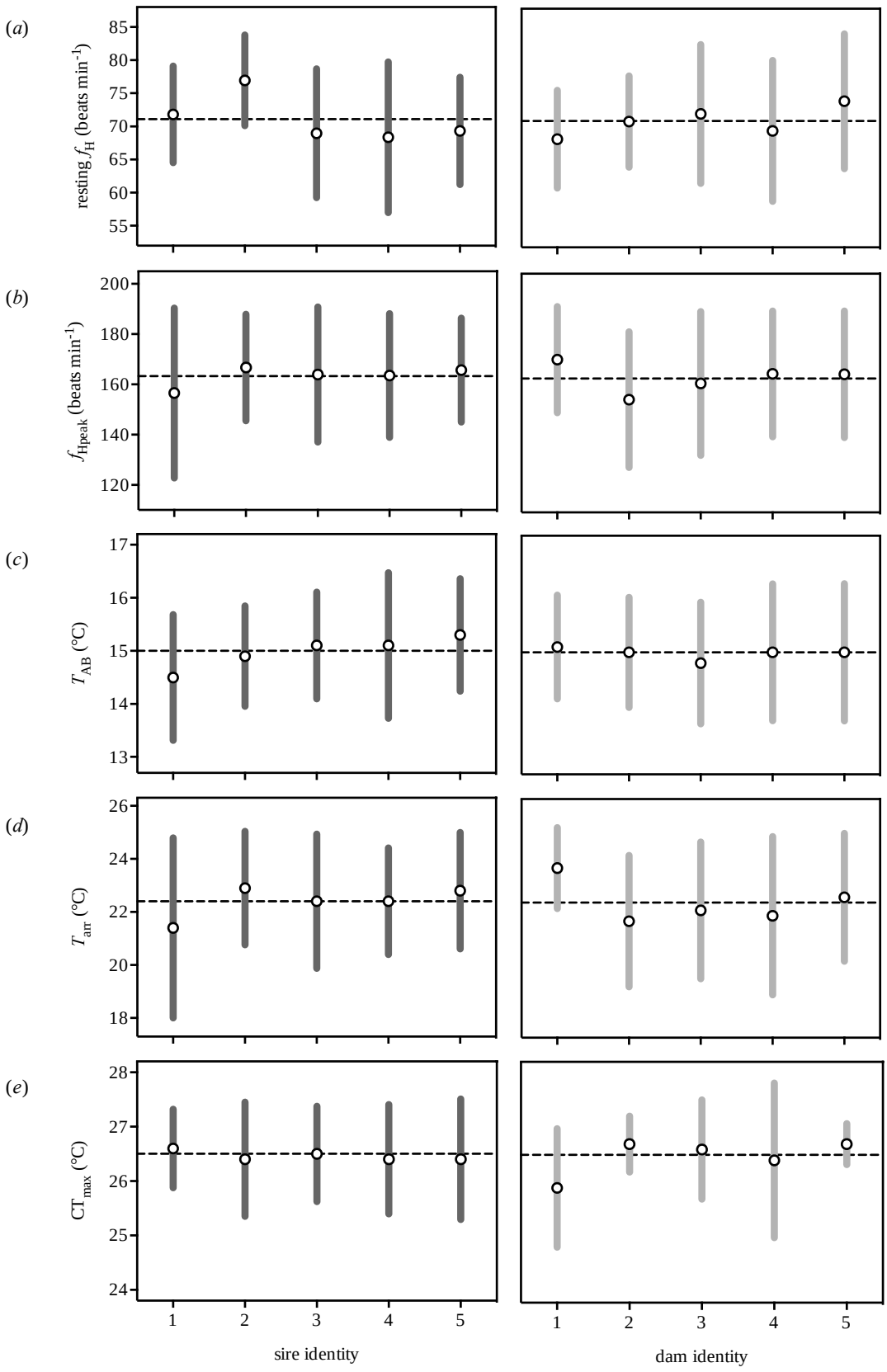


Fig. S3. Variation in cardiac performance and thermal tolerance among families of Big Qualicum River chinook salmon (*Oncorhynchus tshawytscha*). Five males and five females were crossed in a full-factorial breeding design and offspring from each family were measured for their (*a*) resting heart rate (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
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