Temperature-dependent plasticity mediates heart morphology and thermal performance of cardiac function in juvenile Atlantic salmon (*Salmo salar*)

Carlie A. Muir, Shawn R. Garner, Sashko Damjanovski, and Bryan D. Neff*

Department of Biology, Western University, London, ON, Canada

*Corresponding author: bneff@uwo.ca

Keywords: thermal tolerance, cardiac performance, salmonid, plasticity

Abstract

In many fishes, upper thermal tolerance is thought to be limited in part by the heart's ability to meet increased oxygen demands during periods of high temperature. Temperature-dependent plasticity within the cardiovascular system may help fishes cope with the thermal stress imposed by increasing water temperatures. In this study, we examined plasticity in heart morphology and function in juvenile Atlantic salmon (*Salmo salar*) reared under control (+0°C) or elevated (+4°C) temperatures. Using noninvasive Doppler echocardiography, we measured the effect of acute warming on maximum heart rate, stroke distance, and derived cardiac output. A 4°C increase in average developmental temperature resulted in a > 5°C increase in the Arrhenius breakpoint temperature for maximum heart rate and enabled the hearts of these fish to continue beating rhythmically to temperatures approximately 2°C higher than control fish. However, these differences in thermal performance were not associated with plasticity in maximum cardiovascular capacity, as peak measures of heart rate, stroke distance, and derived cardiac output did not differ between temperature treatments. Histological analysis of the heart revealed that while ventricular roundness and relative ventricle size did not differ between treatments, the proportion of compact myocardium in the ventricular wall was significantly greater in fish raised

at elevated temperatures. Our findings contribute to the growing understanding of how the thermal environment can affect phenotypes later in life and identifies a morphological strategy that may help fishes cope with acute thermal stress.

Introduction

Anthropogenic climate change is projected to have extensive impacts on biodiversity, as temperature highly constrains the physiological processes of ectothermic organisms (Dillon et al., 2010; Kappelle et al., 1999). As average global temperatures increase, it is also projected that the frequency and severity of transient heat waves will increase (IPCC, 2021). Organisms will therefore have to contend with warmer temperatures throughout their lives, as well as unprecedented acute temperature events. Over short timeframes, the ability of organisms to cope with these thermal challenges will depend predominantly on their current proximity to upper thermal limits and their capacity to respond to warmer temperatures through phenotypic plasticity (Crozier and Hutchings, 2014; Huey et al., 2012; Pörtner and Peck, 2010; Somero, 2010). Phenotypic plasticity in response to temperature can be partitioned into developmental plasticity and reversible thermal acclimation. Developmental plasticity refers to the shaping of phenotypic traits by early life environments, which can occur across developmental stages (Angilletta Jr and Angilletta, 2009). Developmental plasticity can have profound, and often permanent, effects on traits expressed throughout an organism's life. In contrast, thermal acclimation occurs within a single life stage, often in a reversible manner (Angilletta Jr and Angilletta, 2009). In the face of rapid climate warming, it is important understand the capacity for plasticity in ectotherms, as well as the proximate mechanisms through which plasticity in thermal tolerance is achieved.

Aerobic demands increase with temperature in ectotherms, and as such, cardiorespiratory traits are thought to play an important role in the response to warming. In fishes, increasing heart rate is one of the primary mechanisms for meeting increased tissue oxygen demands during acute warming (Eliason and Anttila, 2017; Farrell, 2009). Heart rate increases rapidly with temperature until an inflection point known as the Arrhenius breakpoint temperature (T_{AB}), after which temperature-dependent increases in maximum heart rate (f_{Hmax}) begin to slow (Anttila et al.,

2013a; Casselman et al., 2012). With further increases in temperature, the heart eventually becomes arrhythmic at the cardiac arrhythmia temperature (T_{Arr}), which represents a collapse of normal cardiac function (Anttila et al., 2013a; Casselman et al., 2012). T_{AB} has been proposed to serve as a measure of the optimal temperature for cardiac performance, and T_{Arr} as a measure of the upper limit for cardiac function (Anttila et al., 2013a; Casselman et al., 2012). In Pacific salmon, these cardiorespiratory measures have been used to predict temperature-related mortality in the wild and assess vulnerability to environmental change (Cooke et al., 2012; Farrell et al., 2008; Muñoz et al., 2015). Acute warming experiments have also demonstrated that cardiac output (the product of heart rate and stroke volume) peaks and subsequently declines at temperatures below the upper critical temperature in most fish species (Clark et al., 2008; Ekström et al., 2016; Eliason et al., 2013; Farrell et al., 1996; Gollock et al., 2006; Mendonça and Gamperl, 2010; Penney et al., 2014; Sandblom and Axelsson, 2007). Cardiac performance has therefore been implicated as an important contributor to upper thermal limits in fishes (Farrell, 2009; Pörtner and Knust, 2007).

Traits conferring increased cardiac capacity have been linked to improved upper thermal limits in many fishes. In Atlantic salmon (*Salmo salar*), Chinook salmon (*Oncorhynchus tshawytscha*), redband trout (*Oncorhynchus mykiss gairdneri*), and the common killifish (*Fundulus heteroclitus*), individuals with higher upper thermal limits were shown to have higher peak heart rates (Anttila et al., 2014b; Chen et al., 2018; Muñoz et al., 2015; Safi et al., 2019). Relative ventricle size, which correlates with stroke volume (Franklin and Davie, 1992), was positively correlated with upper thermal tolerance in both Atlantic salmon (Anttila et al., 2013b) and European sea bass (*Dicentrarchus* labrax; Ozolina et al., 2016). Oxygen supply to the heart itself is also thought to limit cardiac performance and thermal tolerance in fishes (Clark et al., 2008; Ekström et al., 2017; Farrell, 2009). In over half of all teleost species, the ventricular wall is comprised solely of a spongy myocardium that receives its oxygen supply from the oxygen that remains in the luminal venous blood after the oxygen demands of systemic tissues have been met (Davie and Farrell, 1991). In athletic teleosts, such as salmonids, the spongy myocardium is encased by a compact myocardium which receives an oxygen-rich coronary blood supply from the gills (Davie and Farrell, 1991). The relative thickness of the compact myocardium appears to vary with the metabolic requirements of a fish's life history, both interspecifically and intraspecifically (Davie and Farrell, 1991; Eliason et al., 2011; Graham and Farrell, 1992; Pombo

et al., 2012; Santer and Walker, 1980). In *Oncorhynchus mykiss*, Graham and Farrell (1992) found that anadromous fish had thicker compact myocardial layers compared to lake-dwelling conspecifics –presumably to help meet the greater aerobic demands associated with migration. Similarly, Eliason et al. (2011) demonstrated that sockeye salmon (*Oncorhynchus nerka*) with more metabolically challenging migration routes possessed larger ventricles with improved coronary blood supplies. The pyramidal shape of the salmonid ventricle is also pivotal to optimal cardiac performance, with ventricular roundness negatively correlating with cardiac output and critical swim speed (Claireaux et al., 2005). Altogether, there is a clear link between heart morphology, cardiac performance, and aerobic capacity in active fishes like salmonids.

In recent years, extreme heat events have had catastrophic impacts on migration success and survival across species of salmonids (Baisez et al., 2011; Farrell et al., 2008; Hinch et al., 2012; Martins et al., 2011; Martins et al., 2012; Munoz et al., 2020), which numerous studies have attributed to cardiorespiratory limitations at elevated temperatures (Crossin et al., 2008; Eliason et al., 2011; Farrell et al., 2008; Martins et al., 2012). While thermal tolerance in salmonids tends to be locally adapted to the historical conditions of ancestral rivers (Chen et al., 2015; Eliason et al., 2011; Gradil et al., 2016; Poletto et al., 2017; Verhille et al., 2016), experimental manipulations have demonstrated a significant role for phenotypic plasticity in determining the thermal performance of cardiac function. Acclimation to a common temperature can erase population differences in upper thermal tolerance (Anttila et al., 2014b), while developing under warmer thermal conditions can increase the optimal temperature for cardiac performance (Muñoz et al., 2015). In some fish species, it is thought that thermal acclimation during later life-stages may underestimate the organism's full plastic potential by excluding persistent effects of developmental plasticity (Crozier and Hutchings, 2014). As rates of climate warming threaten to outpace adaptation (Morgan et al., 2021; Muñoz et al., 2015), it is important to understand how the cardiorespiratory system of fishes can respond to a warmer environment via phenotypic plasticity, including both developmental plasticity and reversible acclimation effects.

In Atlantic salmon, it remains unresolved whether exposure to warmer average temperatures starting in early development can maintain or improve cardiac performance when juvenile fish are exposed to short-term heat stress. The purpose of this study was to assess the capacity for phenotypic plasticity in the acute thermal tolerance of juvenile Atlantic salmon, while

investigating potential mechanistic underpinnings in heart morphology and function. To this end, we reared Atlantic salmon under control (+0°C) or elevated (+4°C) temperature conditions from fertilization. In juvenile salmon, we measured the response of f_{Hmax} to warming using a noninvasive Doppler echocardiograph system (Muir et al., 2021), in order to determine cardiorespiratory thresholds for thermal performance (T_{AB}, T_{Arr}) in the two temperature treatments. In addition to measures of f_{Hmax}, we simultaneously measured blood flow at the atrioventricular valve. Stroke distance, the distance travelled by the blood during a cardiac cycle, was used as a proxy for how stroke volume responds to acute warming (as volume is calculated by multiplying stroke distance by valve area). Measures of f_{Hmax} and stroke distance were also used in conjunction with estimates of AV valve area to calculate derived cardiac output (Q_D) as a function of temperature (Muir et al., 2021). Using histology, we examined morphological characteristics of the heart which were previously associated with improved aerobic performance in salmonids, comprising the proportion of compact myocardium in the ventricle, ventricular roundness, and relative ventricle size. We additionally examined relative ventricle size via measures of relative ventricular mass (RVM). If there is phenotypic plasticity in the thermal performance of heart function, we would expect fish raised at elevated temperatures to have higher upper thermal limits for cardiorespiratory performance. We predicted that plasticity in upper thermal limits would be associated with differences in maximum cardiovascular capacity. Furthermore, we predicted that plasticity in thermal performance would be associated with morphological changes in the heart that enhance aerobic capacity in salmon (e.g., larger, more pyramidal ventricles with higher proportions of compact myocardium). The overall goal was to elucidate how thermal conditions experienced from the start of development affect thermal performance phenotypes later in life and provide insight into the mechanistic drivers of improved thermal tolerance at the level of the heart.

Materials and Methods

Experimental animals

Atlantic salmon descended from the LaHave River population (Nova Scotia, Canada; 44.4°N, 64.5°W) were obtained as fertilized eggs from the OMNRF (Ontario Ministry of Natural

Resources and Forestry) Normandale Research Facility (Vittoria, Ontario) in the Fall of 2016 and 2017. This population has been reared in OMNRF hatcheries for 6 generations, and in a recent study continued to show local adaptation to the thermal conditions of the LaHave River (Gradil et al., 2016). Eggs were transported to a hatchery facility at the University of Western Ontario 24 hours after fertilization and housed in egg trays corresponding to one of two thermal treatments: control (+0°C) or elevated (+4°C). The rearing temperatures selected for early development were 7°C for the control treatment and 11°C for the elevated treatment, both of which fall within the natural range for the autumn spawning season in the LaHave River (Harding Gradil, 2015). We selected a 4°C temperature differential to follow a previous assessment of plasticity in thermal tolerance in juvenile Chinook salmon (Muñoz et al., 2015). OMNRF recommends that Atlantic salmon eggs are incubated between 7-10°C in aquaculture (OMNRF, 2010). We therefore selected 7°C as the egg incubation temperature for the control treatment, as this would allow for a 4°C temperature differential between treatments, while keeping the elevated temperature group close to the recommended incubation range (11°C).

Rearing temperatures were held constant within 0.5°C of the specified temperatures. Daily and seasonal fluctuations in water temperature were excluded in order to separate potential effects of seasonal temperature variation from the effects of average rearing temperature. Atlantic salmon develop in a temperature-dependent manner, such that fish raised in warmer temperatures complete endogenous feeding and emerge from the substrate earlier than those reared at lower temperatures (approximately 4 months post-fertilization for control fish and 2.5 months for elevated temperature fish). For this reason, experiments were staggered between temperature treatments based on the difference in time to exogenous feeding (Muñoz et al., 2015). Given this difference in developmental rate, allowing rearing temperature to fluctuate seasonally could have contributed to confounding effects on thermal tolerance based on when trials were performed. Instead, fry were transferred to 40 L tanks at the conclusion of endogenous feeding and the rearing temperatures were raised by 4°C to mimic the seasonal increase in temperature that cues the transition to exogenous feeding. This resulted in juvenile rearing temperatures of 11°C for the control treatment and 15°C for the elevated temperature treatment.

Juvenile fish were provided with pelleted feed *ad libitum* for the remainder of the rearing period (EWOS Commercial Feeds, Bergen, Norway), and experiments were performed in mixed-sex 1+ yearlings (~1 year post-fertilization for elevated temperature fish and ~1.5 years post-fertilization for control fish). All experiments were carried out according to Western University Animal Care protocol 2018-084.

Thermal performance of cardiac function

Blood flow at the atrioventricular valve was measured in juvenile Atlantic salmon using the Indus Doppler Flow Velocity (DFV) System, as previously described by Muir et al. (2021). Briefly, test fish were anaesthetized using 100 mg L^{-1} of MS-222 buffered with 200 mg L^{-1} sodium bicarbonate, and body mass was then measured. Anaesthetized fish were stabilized in a weighted sling (ventral side up) within a holding reservoir to ensure correct orientation for placement of the Doppler probe. Individuals were ram-ventilated and maintained at their respective rearing temperatures (11°C or 15°C) with recirculating water from a temperature-controlled water bath (VWR, Edmonton, AB, Canada) containing a maintenance dose of anesthetic (75 mg L^{-1} of MS-222 buffered with 150 mg L^{-1} sodium bicarbonate). Reservoir water temperature was monitored with a digital thermometer (Omega, St-Eustache, QC, Canada).

Following a 15-minute stabilization period in the holding reservoir, the 20 MHz transducer probe was held perpendicular to the ventral side of the fish, posterior to the gills, such that direction of the probe's ultrasound beam was parallel to the direction of blood flow through the atrioventricular valve of the heart. Signals were digitized and displayed as continuous, real-time grayscale DFV spectrograph waveforms in the Doppler Signal Processing Workstation software (Indus Instruments, Houston, TX, USA). Baseline spectrographs were visually examined to ensure normal heart function before starting the thermal performance trial. Fish were then pharmacologically stimulated to reach f_{Hmax} using intraperitoneal injections of 1.2 mg kg^{-1} atropine sulphate (Sigma-Aldrich, St. Louis, MO, USA) and 4 μg kg^{-1} isoproterenol (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.9% NaCl, following Casselman et al. (2012). Atropine sulphate was administered to block vagal tone, while isoproterenol was used to fully

stimulate adrenergic β-receptors. Each injection was followed by a 15-minute equilibration period, then a stepwise temperature increase was applied in 1°C increments every 6 minutes (Casselman et al., 2012). After each 1°C increase, we ensured that water in the holding reservoir had stabilized at the desired temperature for a few seconds before recording spectrograph waveforms of atrioventricular blood flow in 30 second intervals (10 per temperature). Once a shift from rhythmic to arrhythmic heart beats appeared in the spectrogram, the fish was removed from the apparatus and euthanized by lethal overdose of MS-222. In a subset of fish (N= 6 per treatment), the ventricle was excised, rinsed twice with PBS to remove excess blood, and weighed to determine the relative ventricular mass (RVM). Relative ventricular mass was calculated using the formula $RVM=(M_V/M_B)\times100\%$, where M_V is ventricle mass (g) and M_B is body mass (g). The remaining fish were prepared for histological sectioning, described in detail below.

Velocity spectrographs were later processed in Doppler Signal Processing Workstation to calculate f_{Hmax} and total stroke distance at each temperature, following Muir et al. (2021). Measures of f_{Hmax} and stroke distance were subsequently used to calculate derived cardiac output across temperature, in conjunction with estimates of AV valve area (described in detail below). From these data, we determined peak f_{Hmax}, stroke distance, and derived cardiac output for each fish, and identified the temperatures that these peak measures occurred at ($T_{peak\mathit{f}H}$, T_{peakSD}, and T_{peakQD}, respectively). Given the difference in growth rate between the temperature treatments, our initial sampling design resulted in the control fish being substantially smaller than the elevated temperature fish. To address a potential confound between body size and the experimental treatments, our analyses included only the subset of fish which fell within the overlapping range of body sizes between treatments (16-35 g; N= 13 for +0-treatment, N= 16 for +4-treatment). An additional 15 fish were removed because the atrioventricular flow signal was of poor quality, or the pharmacological injections induced an immediate cardiac arrythmia.

Measures of f_{Hmax} from Doppler spectrographs were also used to determine the Arrhenius breakpoint temperature (T_{AB}) of f_{Hmax} and arrhythmia temperature (T_{Arr}). T_{AB} was calculated in SigmaPlot 13.0 (Systat Software, San Jose, CA, USA) as described by Muñoz et al. (2015). Briefly, an Arrhenius plot was generated for each fish by plotting the natural logarithm of f_{Hmax} as a function of the inverse of temperature (K). A piecewise, two-segment linear equation was

applied to fit a biphasic line to the data using the software's 'Dynamic Fit Wizard' tool. The point at which the slope changed, representing the point at which temperature-induced increases in f_{Hmax} shift to a lower exponent, was identified as the Arrhenius breakpoint temperature, T_{AB} (Yeager and Ultsch, 1989). T_{Arr}, which signifies the upper thermal limit for cardiac performance, was identified as the temperature at which arrythmias appeared in the spectrograph waveforms.

Heart histology

To ensure that hearts were in diastole prior to fixation, an intraperitoneal injection of 100 mg kg^{-1} KCl was administered immediately prior to euthanasia. Euthanized fish were then fixed in 10% neutral buffered formalin (VWR, Radnor, PA, USA). After 48 hours, the midsection of the fish was trimmed (anterior to the eyes and posterior to the pectoral fins) and returned to 10% neutral buffered formalin for an additional 72 hours. Following serial dehydration, the dissected specimen was stored in 70% ethanol and sent to the Robarts Molecular Pathology Facility (London, ON) for embedding and histological sectioning. Serial sections (10 µm thickness) of the whole fish were taken through the sagittal plane, traversing the heart, and stained with Hematoxylin and Eosin (H&E). Two individuals were excluded from the analyses due to poor slide quality.

Sections were imaged using an Olympus SZX9 dissecting microscope equipped with an OPTIKA C-B5 camera and measurements were taken in OPTIKA PROView (OPTIKA Srl, Ponteranica, BG, Italy). Diameter of the atrioventricular (AV) valve opening was measured in serial sections to determine the maximum diameter (Supplemental Materials). To ensure that measures of ventricular morphology were taken at a consistent location within the heart across individuals, only sections that passed through both the atrioventricular channel and the bulbus arteriosus were considered for measures of ventricular area, ventricular roundness, and thickness of the compact myocardium, and only fish that fell within an overlapping size range between treatments were used for morphological comparisons (N= 5 per treatment). From the subset of slides passing through the atrioventricular channel and the bulbus arteriosus, subsequent measures of ventricle morphology were taken for each fish on the slide where AV valve diameter was maximized. Area of the ventricle was measured by tracing the outer edges of the ventricle

chamber using the 'Polygon' setting in OPTIKA PROView. Ventricular roundness was measured as the length to width (L:W) ratio of the ventricle. Ventricle length was measured from the dorsal edge of the bulbus arteriosus to the tip of the ventricular apex. Ventricular width was measured at the midpoint of ventricular length using a perpendicular line in the 'Vertical > Four Points' setting in OPTIKA PROView. The length to width (L:W) ratio of the ventricle was calculated as ventricle length divided by ventricle width. Thickness of the compact myocardium was measured at the outer edges of this ventricular midpoint line, and converted to a percentage of total ventricular width. Morphological measurements were adapted from Anttila et al., 2015, Claireaux et al., 2005 and Johnson et al., 2014. Representative sections showing morphological measurements are shown in Supplemental Figure 1.

Derived cardiac output

To calculate derived cardiac output (Q_D), we first estimated the radius of the AV valve for each fish using a linear regression of the relationship between body mass and observed AV radius (R^2= 0.59, p < 0.001) measured in histological sections for a subset of fish (N= 18; Supplemental Figure 2). Rearing temperature was not included as a predictor in this analysis, as its inclusion did not improve model fit. The area of the AV valve was then estimated assuming a circular shape using the formula πr^2, where r represents valve radius. Q_D (mL min^{-1} kg^{-1}) was calculated by multiplying stroke distance (cm) by AV valve area (cm^2) and f_{Hmax} (beats min^{-1}) at a given temperature, and divided by body mass (kg).

Statistical analysis

Body mass was compared between treatments using an unpaired t-test with Welch's correction, and RVM was compared using an unpaired t-test. Measures of thermal performance (T_{AB}, T_{Arr}, T_{peakfH}, T_{peakSD}, and T_{peakQD}), peak cardiac function (peak f_{Hmax}, peak stroke distance, and peak Q_D) and histological measures of heart morphology (ventricle size, ventricular L:W ratio, percentage compact myocardium) were examined using one-way ANCOVAs that included rearing treatment (+0, +4) as a fixed factor and body mass as a covariate. To examine how

cardiac function parameters responded to increasing temperature –and test if these responses differed between rearing treatments– f_{Hmax}, stroke distance, and Q_D were analyzed across temperature using a linear mixed model with AR (1) as the covariance structure, measurement temperature as the repeated measure, body mass as a covariate, and rearing treatment, measurement temperature, and their interaction as fixed factors. Statistical analyses were performed in Sigmaplot 13.0, GraphPad Prism 6 (GraphPad Software, La Jolla California USA), or SPSS v. 27 (IBM, Chicago, IL).

Results

Body size

Only fish within an overlapping size range across treatments were included in comparisons of thermal performance, cardiac function, and heart morphology (16-35 g). Within this subset, there was no significant difference in body mass between fish in the +4-treatment (26.2 ± 5.4 g) and fish in the + 0-treatment (22.3 ± 5.3 g; $t_{27} = 2.0$, $p = 0.054$). Body mass was included as a covariate in all of our analyses. Body mass was not a significant covariate for peak f_{Hmax} ($F_{1,26} = 2.2$, $p = 0.15$), but was associated with the temperature at which peak f_{Hmax} occurred (T_{peakfH}; $F_{1,26} = 9.0$, $p = 0.01$). Body mass was not a significant covariate for the other thermal performance metrics associated with f_{Hmax}: T_{AB} ($F_{1,26} = 1.4$, $p = 0.24$) and T_{Arr} ($F_{1,26} = 4.1$, $p = 0.05$). Body mass was not a significant covariate for peak stroke distance ($F_{1,26} = 1.6$, $p = 0.21$), nor the temperature at which peak stroke distance occurred (T_{peakSD}; $F_{1,26} = 0.07$, $p = 0.79$). Body mass significantly affected peak Q_D ($F_{1,26} = 7.9$, $p < 0.01$), but did not affect the temperature at which peak Q_D occurred (T_{peakQD}; $F_{1,26} = 3.0$, $p = 0.10$).

Body mass did not statistically differ between treatments within the subset of fish used for histological analysis of the heart ($t_8 = 1.4$, p = 0.20; Table 3). Across this size range, ventricle area varied with body mass ($F_{1,7} = 6.2$, $p = 0.04$), but did not vary with ventricular L:W ratio ($F_{1,7} = 0.96$, $p = 0.36$) and percentage compact myocardium ($F_{1,7} = 0.01$, $p = 0.94$).

Thermal performance of cardiac function

To examine the thermal sensitivity of cardiac performance in the two temperature treatments, we examined the responses of f_{Hmax}, stroke distance, and derived cardiac output (Q_D) to warming using linear mixed models. Q_D increased with warming in both groups, before reaching a peak and subsequently declining (Temperature effect: $F_{18,285} = 3.2$, $p < 0.001$; Fig. 1A). Increases in Q_D during warming were mediated by increases in f_{Hmax} (Temperature effect: $F_{18,278} = 29.4$, $p < 0.001$; Fig. 1B), while stroke distance tended to decline with warming in both groups (Temperature effect: $F_{18,284} = 2.1$, $p = 0.008$; Fig. 1C). We did not detect a significant treatment effect on Q_D (Treatment: $F_{1,39} = 0.18$; $p = 0.7$; Fig. 1A), f_{Hmax} (Treatment: $F_{1,32} = 2.3$; $p = 0.14$; Fig. 1B), nor stroke distance (Treatment: $F_{1,42} = 0.07$; $p = 0.8$; Fig. 1C); nor was there a significant interaction between the effects of rearing treatment and acute warming on Q_D (Temperature × Treatment effect: $F_{11,280} = 0.96$, $p = 0.47$; Fig. 1A), f_{Hmax} (Temperature × Treatment effect: $F_{11,274} = 0.35$, $p = 0.97$; Fig. 1B), or stroke distance (Temperature × Treatment effect: $F_{11,277} = 0.56$, $p = 0.86$; Fig. 1C).

We further examined the thermal performance of cardiac function by comparing key temperatures for f_{Hmax}, stroke distance, and Q_D between temperature treatments. The Arrhenius breakpoint temperature for f_{Hmax} (T_{AB}) was significantly higher for the +4-fish than the +0-fish ($F_{1,26} = 26.7$, $p < 0.001$; Fig. 2A; Table 1). Similarly, +4-fish attained their peak f_{Hmax} (T_{peakfH}) at higher temperatures than +0 fish ($F_{1,26} = 7.5$, $p = 0.01$; Table 1) and maintained cardiac function to higher temperatures before arrythmias occurred (T_{Arr}; $F_{1,26} = 12.2$, $p < 0.01$; Fig. 2A/B; Table 1). Peak stroke distance also occurred at significantly higher temperatures in +4-fish ($F_{1,26} = 26.3$, $p < 0.001$; Table 1). Finally, Q_D tended to peak at higher temperatures in the +4-group, but T_{peakQD} did not differ significantly between treatments ($F_{1,26} = 3.6$, $p = 0.07$; Table 1).

Cardiac function metrics

Despite differences in the thermal performance of cardiac function, we did not detect significant differences in peak cardiovascular capacity between temperature treatments (Table 2). While fish from the +4-treatment maintained rhythmic heart function to higher temperatures on average,

peak f_{Hmax} did not differ significantly between temperature treatments ($F_{1,26}$ = 0.7, p = 0.40), nor did peak measures of stroke distance ($F_{1,26}$ = 1.5, p = 0.23) and Q_D ($F_{1,26}$ = 0.1, p = 0.78).

Heart morphology

In addition to measures of cardiac function, we investigated heart morphology as a potential mechanistic driver of improved thermal performance in the +4-fish. Relative ventricle mass did not differ between treatment groups, with a mean RVM of 0.13 ± 0.02% in +0-fish and 0.12 ± 0.03% in +4-fish (t_{10} = 0.8, p = 0.44). Similarly, ventricle area did not differ significantly between rearing treatments when controlling for body mass ($F_{1,7}$ = 0.3, p = 0.59; Table 3). Ventricle shape was also similar between treatments, with no significant difference in ventricular L:W ratios ($F_{1,7}$ = 2.8, p = 0.14; Table 3). However, fish raised in the +4-treatment displayed significantly higher percentages of compact myocardium within their ventricles than fish raised in the +0-treatment ($F_{1,7}$ = 12.9, p = 0.01; Table 3).

Discussion

In fishes, the heart is typically one of the first organs to fail during thermal stress and has therefore been positioned as an 'ecological thermometer' (Iftikar and Hickey, 2013). In the present study, we found a remarkable degree of plasticity in the thermal performance of cardiac function in juvenile Atlantic salmon. Fish reared under warmer temperatures (+4°C) displayed a 5.3°C increase in the mean optimal temperature for cardiac performance (T_{AB}) and maintained rhythmic heart function (T_{Arr}) to temperatures 1.9°C higher than control fish. However, contrary to our prediction, shifts in the thermal limits for cardiorespiratory performance were not accompanied by treatment-level differences in peak cardiac function. As we discuss below, plasticity may be acting on traits which preserve cardiac function under high temperature conditions, rather than traits relating to maximum cardiovascular capacity.

As aerobic demands increase with temperature, heart rate and stroke volume represent the fundamental components of cardiac output that can be altered to increase oxygen delivery to tissues. In the present study, increases in derived cardiac output during acute warming were driven by increases in maximum heart rate –a seemingly universal finding in fishes (Eliason and Anttila, 2017; Farrell, 2009). Concomitantly with increases in heart rate, we observed declines in stroke distance —a proxy for stroke volume— as temperatures increased. This finding agrees with numerous studies in fishes that documented an inverse relationship between maximum heart rate and stroke volume during warming (Eliason et al., 2013; Farrell et al., 1996; Keen and Farrell, 1994; Morgenroth et al., 2021). As the frequency of heart contractions increases, diastolic filling between contractions and the systolic force of the contractions are reduced (Driedzic and Gesser, 1988; Farrell et al., 1996; Shiels et al., 2002). Oxygen limitation is also known to reduce cardiac contractility at high temperatures, further constraining stroke volume during acute warming (Ekström et al., 2016; Ekström et al., 2017). Because we measured blood flow at the atrioventricular valve, decreases in ventricular inflow may therefore reflect a reduction in cardiac filling time or an increase in the end-systolic volume remaining from preceding contractions (i.e., a decreased ejection fraction resulting from reduced contractility). Though not statistically significant, stroke distance tended to decline more rapidly with warming in +0-fish than +4-fish (Fig. 1C). Indeed, stroke distance peaked at much lower temperatures in the +0-treatment (Table 1). A preservation of stroke volume during rapid increases in heart rate could allow fish to increase cardiac output more rapidly during warming, though we did not detect a significant interaction between measurement temperature and temperature treatment in these fish.

Indicators of maximum cardiovascular capacity, such as peak heart rate and relative heart size, have previously been linked to increased upper thermal tolerance in fishes. However, estimates of peak Q_D did not differ between our rearing treatments. Peak stroke distance, used here as a proxy for stroke volume, was also unaffected by rearing temperature. This finding is perhaps unsurprising given that fish from the two treatments did not display differences in relative ventricle size nor ventricular shape. While fish raised at elevated temperatures displayed higher thermal limits for cardiac function and reached peak f_{Hmax} at significantly higher temperatures, peak f_{Hmax} did not differ between treatment groups. Although increases in maximum heart rate

are often observed with warm acclimation in juvenile and adult fish (Anttila et al., 2014b; Safi et al., 2019), exposure to elevated temperatures starting in early development can have varied effects on heart rate. In a Chinook salmon study, peak f_{Hmax} was significantly higher in fish reared under elevated temperatures from fertilization, but there was a lack of plasticity in T_{Arr} (Muñoz et al., 2015). In a sockeye salmon study, a 4°C increase in egg incubation temperature resulted in higher peak heart rates in one population, lower peak heart rates in another population, and had no effect on peak heart rate in the remaining two populations (Chen et al., 2013). Increased egg incubation temperature also had mixed effects on juvenile thermal tolerance across populations in this study. These patterns demonstrate the variation in plastic capacity that exists across fish species, populations, and life stages, as well as the diversity in proximate mechanisms through which thermal plasticity is achieved.

In many fish species, chronic changes in temperature elicit shifts in the relative proportion of spongy to compact myocardium within the ventricle (Keen et al., 2017). Here, we observed considerable plasticity in ventricular myoarchitecture, as the percentage of compact myocardium was 70% greater in fish reared at elevated temperatures. Similar plasticity in the cardiac phenotype has been observed following warm acclimation in a number of salmonids, including rainbow trout (Keen et al., 2017; Klaiman et al., 2011), Arctic char, and Atlantic salmon (Anttila et al., 2015). In parallel with the present findings, Anttila et al. (2014a) observed a greater proportion of compact myocardium in Atlantic salmon parr with higher optimal temperatures for cardiac function (T_{AB}) and better swimming performance. These trends toward higher percentages of compact myocardium among more thermally tolerant salmonids may imply an increased importance of coronary circulation under high-temperature conditions. While the compact myocardium's coronary blood supply is not required for maintaining routine cardiac output, it is thought to increase in importance as the heart's luminal oxygen supply becomes constrained during exercise, warming, or hypoxia (Davie and Farrell, 1991; Morgenroth et al., 2021). Indeed, previous studies in fish have demonstrated that acute thermal tolerance is reduced when coronary blood flow is blocked via surgical ligation (Ekström et al., 2017; Ekström et al., 2019; Morgenroth et al., 2021). Similar to the present findings in +0-fish, reduced thermal performance following coronary ligation was characterized by a failure to maintain stroke volume with warming and an early onset of heart rate collapse (Morgenroth et al., 2021). Our findings lend support to the notion that acute thermal tolerance in fishes may be limited by

oxygenation of the heart itself, whereby function of the oxygen-deprived myocardium declines at high temperatures and compromises oxygen delivery to systemic tissues (Clark et al., 2008; Ekström et al., 2017; Farrell, 2009). Further investigations into the degree of capillarization in the compact myocardium of these fish will be fruitful.

As the climate warms, one of the most noticeable impacts on salmonids will be acute thermal stress in freshwater environments (Crozier et al., 2008). Streams and rivers are less thermally stable than marine environments, making salmon particularly vulnerable to high water temperatures during freshwater stages. In juvenile salmonids, high river temperatures have been hypothesized to create aerobically constrained environments and limit survival during this critical life stage (Crozier and Zabel, 2006; Gradil et al., 2016; Muñoz et al., 2015). Acute cardiorespiratory responses to elevated temperature are therefore ecologically relevant in juvenile salmonids, and will only increase in importance as climate change increases the frequency and severity of extreme heat events (IPCC, 2021). In a previous study by Gradil et al. (2016), juveniles from this hatchery-bred population were found to be narrowly adapted to the summer temperature conditions of their native environment. In the Gradil study, upper thermal limits for cardiac performance (T_{Arr}) showed close alignment with peak summer temperatures in the LaHave River, suggesting a vulnerability to extreme heat events in this population. In the present study, we demonstrate a significant capacity for plasticity in juveniles from this population, such that exposure to chronically warmer temperatures starting during early development can increase acute thermal tolerance at the level of the heart. Thus, increases in average temperature may help buffer juvenile salmon against the thermal stress imposed by summer heat events in freshwater habitats.

In this study, Atlantic salmon were reared in one of two thermal regimes across the embryonic, alevin, and juvenile stages of their life cycle before thermal performance of cardiac function was examined. This study design has been used previously to examine phenotypic plasticity in thermal performance in Chinook salmon (Muñoz et al., 2015). However, since fish from the two temperature treatments were not returned to a common rearing temperature prior to experiments, this study design limits our ability to distinguish between lasting developmental effects and reversible thermal acclimation within the juvenile stage. To distinguish between these two processes would require an experimental design that includes an additional two treatments in

which individuals from each temperature are switched to the opposite temperature following development. Interestingly, developmental plasticity and reversible thermal acclimation are not mutually exclusive –as early life environments can not only have lasting effects on an animal's thermal performance, but also on their future acclimation capacity (Beaman et al., 2016). In zebrafish, exposure to elevated temperatures during embryonic development was shown to increase thermal acclimation capacity in later life and improve thermal hardiness (Scott and Johnston, 2012). In *Drosophila melanogaster*, heat tolerance is mediated by an interaction between current acclimation temperature and embryonic temperature (Willot et al., 2021). Thus, the phenotypic changes observed in this study are likely the result of exposure to elevated temperatures across multiple life stages.

A common trade-off associated with developing at higher temperatures is an accelerated developmental rate, but smaller juvenile body size. However, this pattern was not observed in the present study. On the contrary, +4-fish tended to be larger than +0-fish, likely because they began feeding approximately 6 weeks earlier, due to their accelerated rate of development. Additionally, temperature differences between treatments were maintained after development, and temperature generally shows a positive association with post-development growth rate in Atlantic salmon (Handeland et al., 2008). Interestingly, egg incubation temperature has also been shown to affect later growth performance in Atlantic salmon (Finstad and Jonsson, 2012), such that fish exposed to elevated temperatures during embryogenesis displayed faster growth rates as juveniles (particularly at warmer temperatures). Given the observed association between temperature and growth, we used a size-matching approach to ensure that there was no significant difference in body size between treatments for the fish used in our analyses. This approach adds a potential confound in that fish included from the +4-treatment were either younger or relatively slower growing on average, while fish included from the +0-treatment were either older or relatively faster growing on average. However, these size and growth differences are unlikely to have had a major influence on our conclusions, as we observed only weak associations between body size and cardiorespiratory measures, and the overall trends were similar when we included the smallest +0 fish and largest +4 fish. The thermal plasticity observed in this study appears to be adaptive, but future investigations into possible performance trade-offs associated with developmental temperature would be fruitful.

In summary, we demonstrated that exposure to higher average water temperatures starting early in development significantly increased the cardiorespiratory thresholds for thermal performance in a hatchery-bred population of Atlantic salmon. Contrary to our prediction, this plasticity in thermal tolerance was not accompanied by plasticity in maximum cardiovascular capacity, as peak f_{Hmax} and relative ventricular volume did not differ between treatments. Rather, fish from the warm treatment displayed higher proportions of compact myocardium in their ventricular walls, a trait commonly associated with increased aerobic demands. As the compact myocardium represents the portion of the ventricle receiving a coronary blood supply, this change in myoarchitecture may improve oxygen supply the heart itself under high temperature conditions (Anttila et al., 2013b; Ekström et al., 2017). In conclusion, this study contributes to our understanding of how average thermal conditions can affect the response to acute warming and identifies a morphological strategy which may improve the cardiac performance of fishes during thermal stress.

Competing Interests

No competing interests declared.

Acknowledgements

The results in this paper are reproduced from the PhD thesis of Carlie Muir (Western University, 2022).

Funding

This work was supported by the Natural Sciences and Engineering Research Council [RGPIN-2017-06045 and 494220-2016 STPGP to BDN, RGPIN-2018-06665 to SD, CGS-D scholarship to CAM].

References

Angilletta Jr, M. J. and Angilletta, M. J. (2009). *Thermal adaptation: a theoretical and empirical synthesis*. New York: Oxford University Press.

Anttila, K., Casselman, M. T., Schulte, P. M. and Farrell, A. P. (2013a). Optimum temperature in juvenile salmonids: Connecting subcellular indicators to tissue function and whole-organism thermal optimum. *Physiol. Biochem. Zool.* **86**, 245–256.

Anttila, K., Dhillon, R. S., Boulding, E. G., Farrell, A. P., Glebe, B. D., Elliott, J. A. K., Wolters, W. R. and Schulte, P. M. (2013b). Variation in temperature tolerance among families of Atlantic salmon (Salmo salar) is associated with hypoxia tolerance, ventricle size and myoglobin level. *J. Exp. Biol.* **216**, 1183–1190.

Anttila, K., Jørgensen, S. M., Casselman, M. T., Timmerhaus, G., Farrell, A. P. and Takle, H. (2014a). Association between swimming performance, cardiorespiratory morphometry, and thermal tolerance in Atlantic salmon (Salmo salar L.). *Front. Mar. Sci.* **1**, 1–10.

Anttila, K., Couturier, C. S., Øverli, Ø., Johnsen, A., Marthinsen, G., Nilsson, G. E. and Farrell, A. P. (2014b). Atlantic salmon show capability for cardiac acclimation to warm temperatures. *Nat. Commun.* **5**, 4252.

Anttila, K., Lewis, M., Prokkola, J. M., Kanerva, M., Seppanen, E., Kolari, I. and Nikinmaa, M. (2015). Warm acclimation and oxygen depletion induce species-specific responses in salmonids. *J. Exp. Biol.* **218**, 1471–1477.

Beaman, J. E., White, C. R. and Seebacher, F. (2016). Evolution of Plasticity: Mechanistic Link between Development and Reversible Acclimation. *Trends Ecol. Evol.* **31**, 237–249.

Casselman, M. T., Anttila, K. and Farrell, A. P. (2012). Using maximum heart rate as a rapid screening tool to determine optimum temperature for aerobic scope in Pacific salmon Oncorhynchus spp. *J. Fish Biol.* **80**, 358–377.

Chen, Z., Anttila, K., Wu, J., Whitney, C. K., Hinch, S. G. and Farrell, A. P. (2013). Optimum and maximum temperatures of sockeye salmon (Oncorhynchus nerka) populations hatched at different temperatures. *Can. J. Zool.* **91**, 265–274.

Chen, Z., Snow, M., Lawrence, C. S., Church, A. R., Narum, S. R., Devlin, R. H. and Farrell, A. P. (2015). Selection for upper thermal tolerance in rainbow trout (Oncorhynchus mykiss Walbaum). *J. Exp. Biol.* **218**, 803–812.

Chen, Z., Farrell, A. P., Matala, A. and Narum, S. R. (2018). Mechanisms of thermal adaptation and evolutionary potential of conspecific populations to changing environments. *Mol. Ecol.* **27**, 659–674.

Claireaux, G., McKenzie, D. J., Genge, A. G., Chatelier, A., Aubin, J. and Farrell, A. P. (2005). Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow trout. *J. Exp. Biol.* **208**, 1775–1784.

Clark, T. D., Sandblom, E., Cox, G. K., Hinch, S. G. and Farrell, A. P. (2008). Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (Oncorhynchus tshawytscha). *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* **295**, 1631–1639.

Cooke, S. J., Hinch, S. G., Donaldson, M. R., Clark, T. D., Eliason, E. J., Crossin, G. T., Raby, G. D., Jeffries, K. M., Lapointe, M., Miller, K., et al. (2012). Conservation physiology in practice: How physiological knowledge has improved our ability to sustainably manage Pacific salmon during up-river migration. *Philos. Trans. R. Soc. B Biol. Sci.* **367**, 1757–1769.

Crozier, L. G. and Hutchings, J. A. (2014). Plastic and evolutionary responses to climate change in fish. *Evol. Appl.* **7**, 68–87.

Crozier, L. G. and Zabel, R. W. (2006). Climate impacts at multiple scales: Evidence for differential population responses in juvenile Chinook salmon. *J. Anim. Ecol.* **75**, 1100–1109.

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

Davie, P. S. and Farrell, A. P. (1991). The coronary and luminal circulations of the myocardium of fishes. *Can. J. Zool.* **69**, 1993–2001.

Dillon, M. E., Wang, G. and Huey, R. B. (2010). Global metabolic impacts of recent climate warming. *Nature* **467**, 704–706.

Driedzic, B. Y. W. R. and Gesser, H. (1988). Differences in Force-Frequency Relationships and Calcium Dependency Between Elasmobranch and Teleost Hearts. *J. Exp. Biol.* **140**, 227–241.

Ekström, A., Brijs, J., Clark, T. D., Gräns, A., Jutfelt, F. and Sandblom, E. (2016). Cardiac oxygen limitation during an acute thermal challenge in the European perch: Effects of chronic environmental warming and experimental hyperoxia. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* **311**, 440–449.

Ekström, A., Axelsson, M., Gräns, A., Brijs, J. and Sandblom, E. (2017). Influence of the coronary circulation on thermal tolerance and cardiac performance during warming in rainbow trout. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* **312**, 549–558.

Ekström, A., Gräns, A. and Sandblom, E. (2019). Can´t beat the heat? Importance of cardiac control and coronary perfusion for heat tolerance in rainbow trout. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **189**, 757–769.

Eliason, E. J. and Anttila, K. (2017). Temperature and the Cardiovascular System. In *Fish Physiology, The Cardiovascular System: Development, Plasticity, and Physiological Responses* (ed. Gamperl, A. K., Gillis, T. E., Farrell, A. P., and Brauner, C. J.), pp. 235–297. Elsevier Inc.

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

Eliason, E. J., Clark, T. D., Hinch, S. G. and Farrell, A. P. (2013). Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon. *Conserv. Physiol.* **1**, 1–19.

Farrell, A. P. (2009). Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. *J. Exp. Biol.* **212**, 3771–3780.

Farrell, A. P., Gamperl, A. K., Hicks, J. M. T., Shiels, H. A. and Jain, K. E. (1996). Maximum cardiac performance of rainbow trout (Oncorhynchus mykiss) at temperatures approaching their upper lethal limit. *J. Exp. Biol.* **199**, 663–672.

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

Finstad, A. G. and Jonsson, B. (2012). Effect of incubation temperature on growth performance in Atlantic salmon. *Mar. Ecol. Prog. Ser.* **454**, 75–82.

Franklin, C. E. and Davie, P. S. (1992). Sexual Maturity Can Double Heart Mass and Cardiac Power Output in Male Rainbow Trout. *J. Exp. Biol.* **171**, 139–148.

Gollock, M. J., Currie, S., Petersen, L. H. and Gamperl, A. K. (2006). Cardiovascular and haematological responses of Atlantic cod (Gadus morhua) to acute temperature increase. *J. Exp. Biol.* **209**, 2961–2970.

Gradil, K. J., Garner, S. R., Wilson, C. C., Farrell, A. P. and Neff, B. D. (2016). Relationship between cardiac performance and environment across populations of Atlantic salmon (Salmo salar): a common garden experiment implicates local adaptation. *Evol. Ecol.* **30**, 877–886.

Graham, M. S. and Farrell, A. P. (1992). Environmental influences on cardiovascular variables in rainbow trout, Oncorhynchus mykiss (Walbaum). *J. Fish Biol.* **41**, 851–858.

Handeland, S. O., Imsland, A. K. and Stefansson, S. O. (2008). The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. *Aquaculture.* **283**, 36–42.

Harding Gradil, K. J. (2015). Thermal performance covaries with environmental temperature across populations of Atlantic salmon (Salmo salar).

Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M. and Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Philos. Trans. R. Soc. B Biol. Sci.* **367**, 1665–1679.

Iftikar, F. I. and Hickey, A. J. R. (2013). Do mitochondria limit hot fish hearts? Understanding the role of mitochondrial function with heat stress in Notolabrus celidotus. *PLoS One* **8**, E64120.

IPCC (2021). *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. (ed. Masson-Delmotte, V., Zhai, P., Pirani, S. L., Connors, C., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M. I., et al.). Cambridge University Press.

Johnson, A. C., Turko, A. J., Klaiman, J. M., Johnston, E. F. and Gillis, T. E. (2014). Cold acclimation alters the connective tissue content of the zebrafish (Danio rerio) heart. *J. Exp. Biol.* **217**, 1868–1875.

Kappelle, M., Van Vuuren, M. M. I. and Baas, P. (1999). Effects of climate change on biodiversity: a review and identification of key research issues. *Biodivers. Conserv.* **8**, 1383–1397.

Keen, J. E. and Farrell, A. P. (1994). Maximum prolonged swimming speed and maximum cardiac performance of rainbow trout, Oncorhynchus mykiss, acclimated to two different water temperatures. *Comp. Biochem. Physiol. -- Part A Physiol.* **108**, 287–295.

Keen, A. N., Klaiman, J. M., Shiels, H. A. and Gillis, T. E. (2017). Temperature-induced cardiac remodelling in fish. *J. Exp. Biol.* **220**, 147–160.

Klaiman, J. M., Fenna, A. J., Shiels, H. A., Macri, J. and Gillis, T. E. (2011). Cardiac Remodeling in Fish: Strategies to Maintain Heart Function during Temperature Change. *PLoS One* **6**, e24464.

Mendonça, P. C. and Gamperl, A. K. (2010). The effects of acute changes in temperature and oxygen availability on cardiac performance in winter flounder (Pseudopleuronectes americanus). *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* **155**, 245–252.

Morgan, R., Finnøen, M. H., Jensen, H., Pélabon, C. and Jutfelt, F. (2021). Low potential for evolutionary rescue from climate change in a tropical fish. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 33365–33372.

Morgenroth, D., McArley, T., Gräns, A., Axelsson, M., Sandblom, E. and Ekström, A. (2021). Coronary blood flow influences tolerance to environmental extremes in fish. *J. Exp. Biol.* **224**, jeb.239970.

Muir, C. A., Neff, B. D. and Damjanovski, S. (2021). Adaptation of a mouse Doppler echocardiograph system for assessing cardiac function and thermal performance in a juvenile salmonid. *Conserv. Physiol.* **9**, 1–11.

Muñoz, N. J., Farrell, A. P., Heath, J. W. and Neff, B. D. (2015). Adaptive potential of a Pacific salmon challenged by climate change. *Nat. Clim. Chang.* **5**, 163–166.

OMNRF (2010). *Lake Ontario Fish Communities and Fisheries: 2009 Annual Report of the Lake Ontario Management Unit*. Picton, ON.

Ozolina, K., Shiels, H. A., Ollivier, H. and Claireaux, G. (2016). Intraspecific individual variation of temperature tolerance associated with oxygen demand in the European sea bass (Dicentrarchus labrax). *Conserv. Physiol.* **4**, cov060.

Penney, C. M., Nash, G. W. and Kurt Gamperl, A. (2014). Cardiorespiratory responses of seawater-acclimated adult Arctic char (Salvelinus alpinus) and Atlantic salmon (Salmo salar) to an acute temperature increase. *Can. J. Fish. Aquat. Sci.* **71**, 1096–1105.

Poletto, J. B., Cocherell, D. E., Baird, S. E., Nguyen, T. X., Cabrera-Stagno, V., Farrell, A. P. and Fangue, N. A. (2017). Unusual aerobic performance at high temperatures in juvenile Chinook salmon, Oncorhynchus tshawytscha. *Conserv. Physiol.* 13.

Pombo, A., Blasco, M. and Climent, V. (2012). The status of farmed fish hearts: An alert to improve health and production in three Mediterranean species. *Rev. Fish Biol. Fish.* **22**, 779–789.

Pörtner, H. O. and Knust, R. (2007). Climate Change Affects Marine Fishes Through the Oxygen Limitation of Thermal Tolerance. *Science.* **315**, 95–97.

Pörtner, H. O. and Peck, M. A. (2010). Climate change effects on fishes and fisheries: Towards a cause-and-effect understanding. *J. Fish Biol.* **77**, 1745–1779.

Safi, H., Zhang, Y., Schulte, P. M. and Farrell, A. P. (2019). The effect of acute warming and thermal acclimation on maximum heart rate of the common killifish Fundulus heteroclitus. *J. Fish Biol.* **95**, 1441–1446.

Sandblom, E. and Axelsson, M. (2007). Venous hemodynamic responses to acute temperature increase in the rainbow trout (Oncorhynchus mykiss). *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* **292**, 2292–2298.

Santer, R. M. and Walker, M. (1980). Morphological studies on the ventricle of teleost and elasmobranch hearts. *J. Zool.* **190**, 259–272.

Scott, G. R. and Johnston, I. A. (2012). Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. *PNAS* **109**, 14247–14252.

Shiels, H. A., Vornanen, M. and Farrell, A. P. (2002). The force-frequency relationship in fish hearts - A review. *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* **132**, 811–826.

Somero, G. N. (2010). The physiology of climate change: How potentials for acclimatization and genetic adaptation will determine "winners" and "losers." *J. Exp. Biol.* **213**, 912–920.

Verhille, C. E., English, K. K., Cocherell, D. E., Farrell, A. P. and Fangue, N. A. (2016). High thermal tolerance of a rainbow trout population near its southern range limit suggests local thermal adjustment. *Conserv. Physiol.* **4**, 1–12.

Willot, Q., Loos, B. and Terblanche, J. S. (2021). Interactions between developmental and adult acclimation have distinct consequences for heat tolerance and heat stress recovery. *J. Exp. Biol.* **224**, 1–8.

Yeager, D. P. and Ultsch, G. R. (1989). Physiological Regulation and Conformation: A BASIC Program for the Determination of Critical Points. *Physiol. Zool.* **62**, 888–907.

Figures and Tables

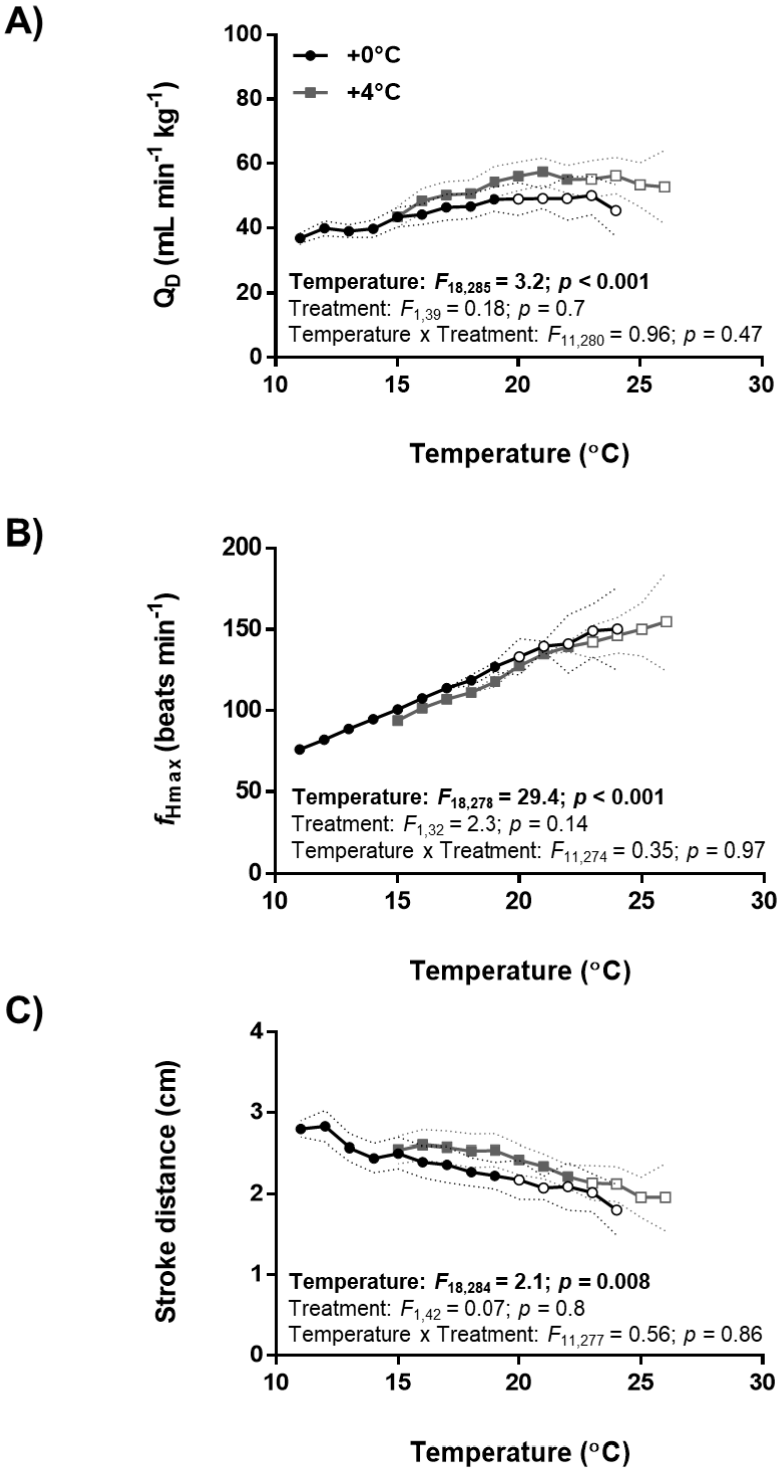

Figure 1. Effect of acute warming on cardiac performance in juvenile Atlantic salmon (*Salmo salar*) raised in two thermal regimes. Data are presented as means ± s.e.m. (dashed lines) for A) maximum heart rate (f_{Hmax}), B) stroke distance, and C) derived cardiac output (Q_D) of fish raised in the +0 (black, N= 13) and +4 (grey, N= 16) treatments. Open circles indicate temperatures at which some individuals were removed from analyses following the onset of arrythmias. Results of the mixed model are presented in each panel for the respective variable.

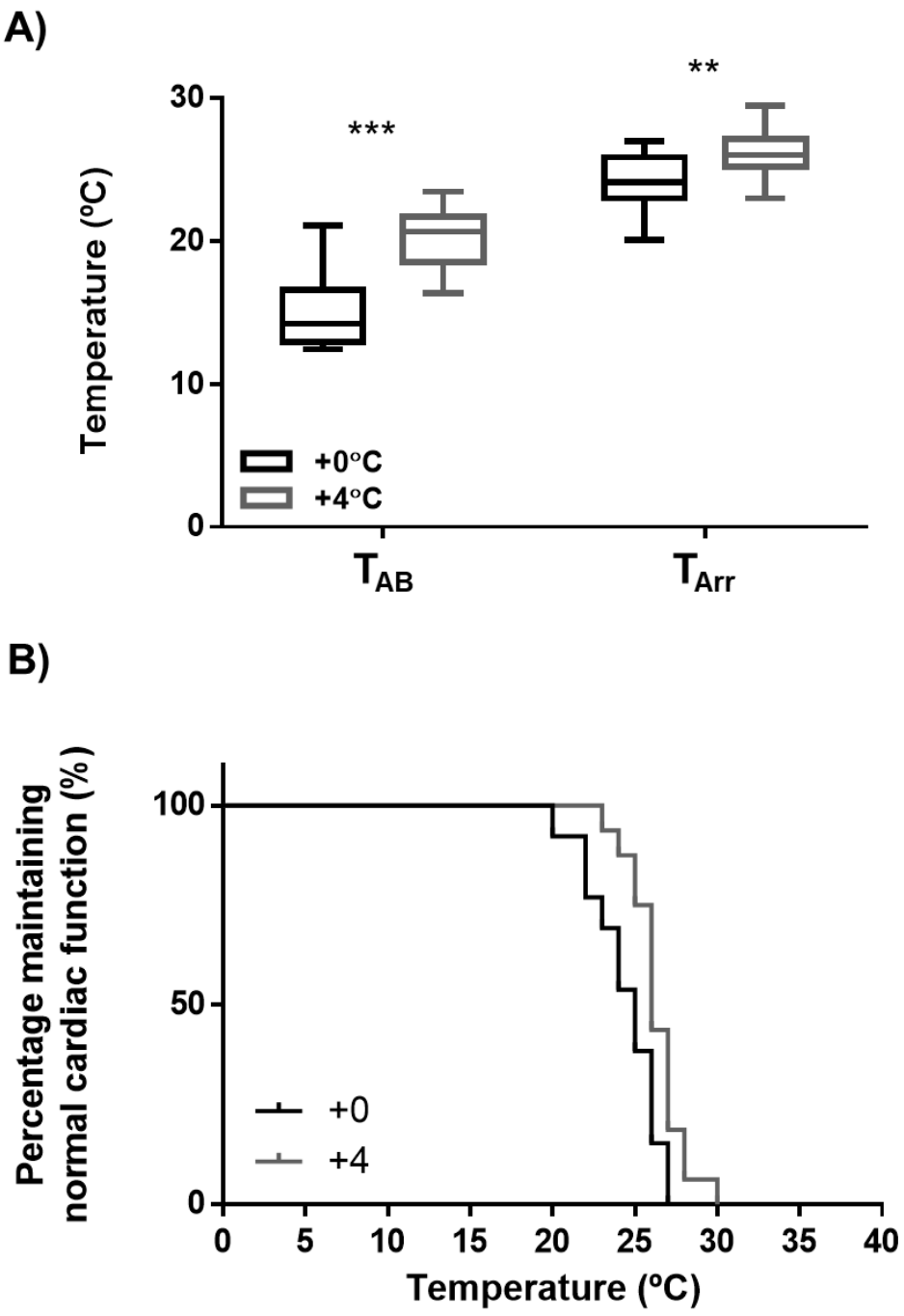

Figure 2. Effect of rearing temperature on thermal performance in juvenile Atlantic salmon (*Salmo salar*) raised in two thermal regimes. A) Data are presented as boxplots for the Arrhenius breakpoint temperature (T_{AB}) of maximum heart rate (f_{Hmax}) and the temperature at which cardiac arrythmias occurred (T_{Arr}) for fish raised under control (+0, black; N= 13) or elevated (+4, grey; N= 16) temperature conditions. The box plots display the median, 25th and 75th percentiles, with whiskers indicating the 10th and 90th percentiles and individual points used to show data outside this interval. T_{AB} and T_{Arr} were compared between treatment groups using one-way ANCOVAs with body mass as a covariate. Statistical significance was accepted at $p < 0.05$. *** $p < 0.001$; ** $p < 0.01$. B) Predicted survival during acute warming is plotted for each rearing treatment as the percentage of individuals that maintained cardiac function to that temperature without displaying cardiac arrythmias.

Table 1. Thermal performance measures for juvenile Atlantic salmon (*Salmo salar*) raised in thermal regimes reflecting either control (+0) or elevated (+4) temperatures.

Measure	+0 (°C)	+4 (°C)	F	df	*p*
T_{AB}	14.9 ± 2.6	20.2 ± 2.2	26.7	1,26	< 0.001
T_{Arr}	24.3 ± 2.1	26.2 ± 1.6	12.2	1,26	< 0.01
T_{peakfH}	23.2 ± 2.0	24.4 ± 2.3	7.5	1,26	0.01
T_{peakSD}	12.0 ± 1.3	18.6 ± 4.2	26.3	1,26	< 0.001
T_{peakQD}	20.6 ± 3.5	22.3 ± 3.2	3.6	1,26	0.07

Note: T_{AB}, Arrhenius breakpoint temperature; T_{Arr}, temperature at onset of cardiac arrythmias; T_{peakfH}, temperature for peak f_{Hmax}; T_{peakSD}, temperature for peak stroke distance; T_{peakQD}, temperature for peak derived cardiac output. Data presented as means ± s.d.; N= 13 for +0, N= 16 for +4. Thermal performance metrics were compared between treatment groups using one-way ANCOVAs with body mass as a covariate. Statistical significance was accepted at $p < 0.05$.

Table 2. Peak cardiac performance measures for juvenile Atlantic salmon (*Salmo salar*) raised in thermal regimes reflecting either control (+0) or elevated (+4) temperatures.

Measure	+0	+4	F	df	*p*
Peak $f_{H_{max}}$ (beats min^{-1})	149.8 ± 15.9	151.8 ± 15.8	0.7	1,26	0.40
Peak stroke distance (cm)	3.1 ± 0.6	2.9 ± 0.8	1.5	1,26	0.23
Peak Q_D (mL min^{-1} kg^{-1})	60.3 ± 10.0	64.4 ± 18.8	0.1	1,26	0.78

Note: f_{Hmax}, maximum heart rate; Data presented as means ± s.d.; N= 13 for +0, N= 16 for +4. Cardiac performance metrics were compared between treatment groups using one-way ANCOVAs with body mass as a covariate. Statistical significance was accepted at $p < 0.05$.

Table 3. Morphological measures for juvenile Atlantic salmon (*Salmo salar*) raised in thermal regimes reflecting either control (+0) or elevated (+4) temperatures.

Measure	+0	+4	Test statistic	df	*p*
Body mass (g)	21.3 ± 5.4	26.1 ± 5.5	1.4	8	0.20
Ventricle area (cm^2)	$5.9 \times 10^{-2} \pm 0.5 \times 10^{-2}$	$6.3 \times 10^{-2} \pm 2.0 \times 10^{-2}$	0.3	1,7	0.59
Ventricular L:W ratio	2.0 ± 0.3	2.3 ± 0.4	2.8	1,7	0.14
Compact myocardium (%)	8.9 ± 1.1	15.4 ± 3.2	12.9	1,7	0.01

Note: L:W ratio, length to width ratio. Data presented as means ± s.d.; N= 5 per treatment. Body mass was compared between treatments using an unpaired t-test for fish used in histological analysis of heart morphology. Remaining morphological variables were compared between treatments using one-way ANCOVAs with body mass as a covariate. Statistical significance was accepted at $p < 0.05$.

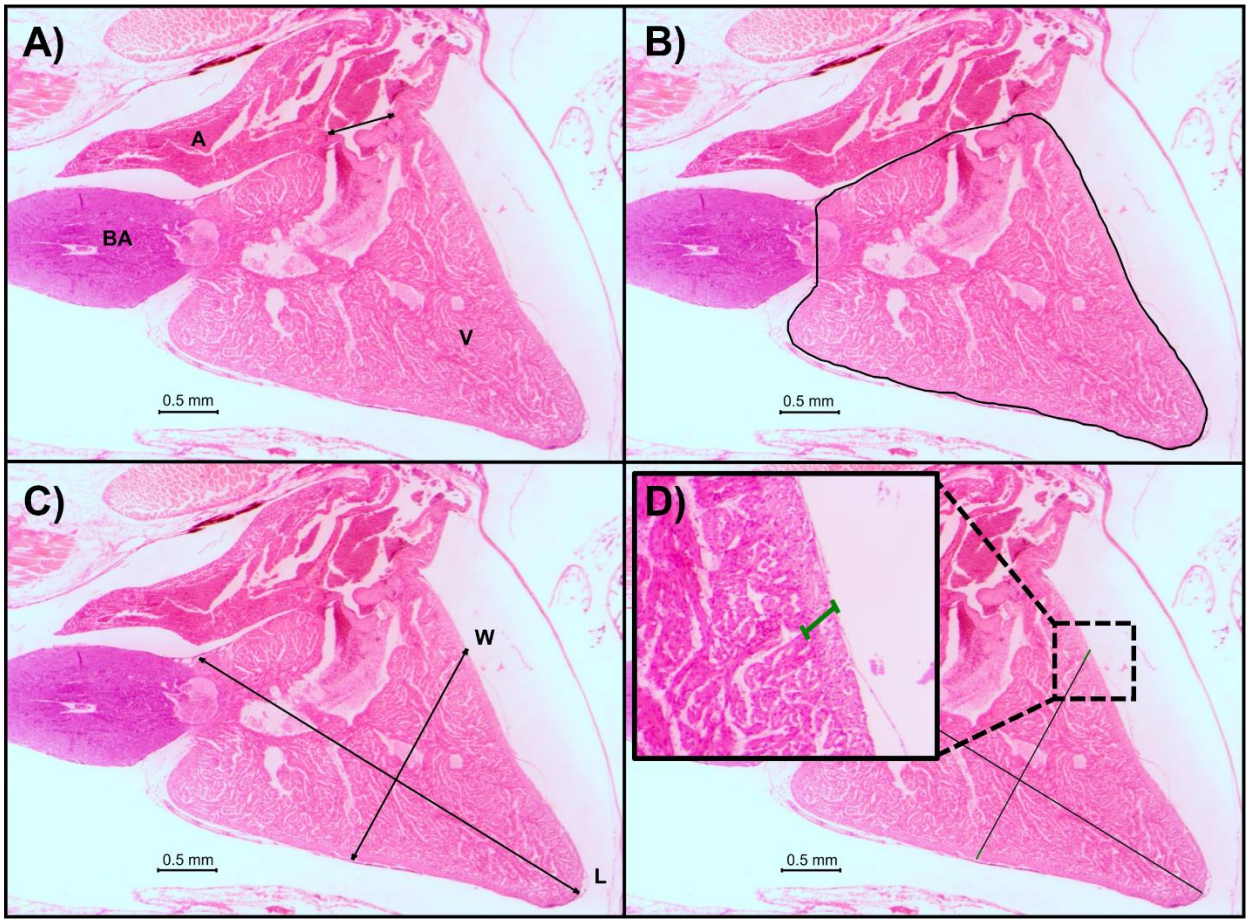

Fig. S1. Histological measurements of heart morphology. Haematoxylin and Eosin (H&E) stained sagittal section (thickness = 10 μM) of an Atlantic salmon (*Salmo salar*) yearling raised in the +0-treatment. Measurements were taken in OPTIKA PROView (OPTIKA Srl, Ponteranica, BG, Italy) for A) diameter of the atrioventricular (AV) valve opening (black line), B) ventricular area (black outline), C) ventricular length and width (black lines), and D) thickness of the compact myocardium (green line) relative to ventricle width (black line). Label abbreviations: A = atrium; BA = *bulbus arteriosus*; L = length; V = ventricle; W = width.

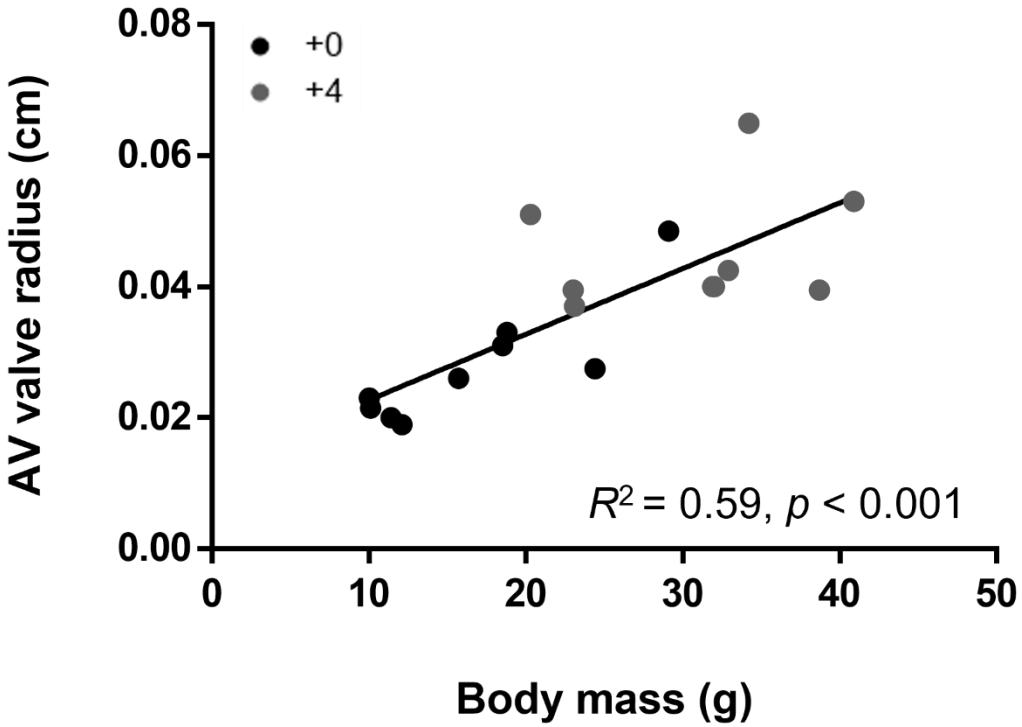

Fig. S2. Relationship between body mass and atrioventricular (AV) valve radius measured via histology in Atlantic salmon (*Salmo* salar) yearlings. A single linear regression analysis was used to assess the relationship between body mass and observed AV radius for the pooled subset of fish included in the histological analyses, as adding rearing temperature as a predictor did not improve model fit (N= 18). The resulting equation was used to calculate AV radius for each fish included in our cardiac performance analyses: *AV radius (cm) = 0.0130949 + 0.0009805*Body Mass (g).*