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RESEARCH ARTICLE

Coronary blood flow influences tolerance to environmental extremes in fish

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ABSTRACT

Approximately half of all fishes have, in addition to the luminal venous O_2 supply, a coronary circulation supplying the heart with fully oxygenated blood. Yet, it is not fully understood how coronary O_2 delivery affects tolerance to environmental extremes such as warming and hypoxia. Hypoxia reduces arterial oxygenation, while warming increases overall tissue O_2 demand. Thus, as both stressors are associated with reduced venous O_2 supply to the heart, we hypothesised that coronary flow benefits hypoxia and warming tolerance. To test this hypothesis, we blocked coronary blood flow (via surgical coronary ligation) in rainbow trout (*Oncorhynchus mykiss*) and assessed how *in vivo* cardiorespiratory performance and whole-animal tolerance to acute hypoxia and warming was affected. While coronary ligation reduced routine stroke volume relative to trout with intact coronaries, cardiac output was maintained by an increase in heart rate. However, in hypoxia, coronary-ligated trout were unable to increase stroke volume to maintain cardiac output when bradycardia developed, which was associated with a slightly reduced hypoxia tolerance. Moreover, during acute warming, coronary ligation caused cardiac function to collapse at lower temperatures and reduced overall heat tolerance relative to trout with intact coronary arteries. We also found a positive relationship between individual hypoxia and heat tolerance across treatment groups, and tolerance to both environmental stressors was positively correlated with cardiac performance. Collectively, our findings show that coronary perfusion improves cardiac O_2 supply and therefore cardiovascular function at environmental extremes, which benefits tolerance to natural and anthropogenically induced environmental perturbations.

KEY WORDS: Cardiorespiratory performance, Coronary artery, Hypoxia, Warming

INTRODUCTION

Rapid changes in water temperature and O_2 levels are normal phenomena in aquatic ecosystems, but their incidence and magnitude are exacerbated from ongoing anthropogenic activities such as eutrophication, habitat degradation and climate change; trends that are projected to continue and worsen in the future (Breitburg et al., 2018; Diaz, 2001; Ummenhofer and Meehl, 2017; Woodward et al., 2010). To predict how fish and other aquatic organisms will be impacted, there is a need to understand the physiological mechanisms underlying tolerance to these environmental perturbations. Heat and hypoxia tolerance are often correlated in fish, indicating a functional relationship between the physiological processes that dictate these environmental boundaries (Anttila et al., 2013; Zhang et al., 2018).

The overall metabolism and thus O_2 demand increases in fish during warming, which is met by elevations in cardiovascular O_2 delivery and tissue O_2 extraction. This coincides with elevations in cardiac O_2 demand as cardiac output and cardiac workload (a product of cardiac output and blood pressure) increases along with a direct stimulatory effect of warming on cardiac cellular metabolism (Ekström et al., 2016; Eliason and Anttila, 2017). Similarly, cardiac work appears to increase within a certain hypoxia range in some fish species, e.g. rainbow trout (*Oncorhynchus mykiss*), as cardiac output is either maintained or elevated while ventral aortic blood pressure increases (Holeton and Randall, 1967; Perry and Desforges, 2006; Steffensen and Farrell, 1998; Wood and Shelton, 1980), whereas other species reduce cardiac work (see table in Stecyk, 2017). Even so, both warming and hypoxia may constrain the supply of O_2 to the heart as arterial blood oxygenation at the gills is impaired, tissue O_2 extraction increases and the partial pressure of O_2 (P_{O_2}) in the venous blood returning to the cardiac lumen decreases (Clark et al., 2008b; Ekström et al., 2016; Steffensen and Farrell, 1998; Thomas et al., 1994). Reductions in P_{O_2} are well known to impair the contractility of cardiac muscle strips *in vitro* (Roberts and Syme, 2018), as well as perfused heart preparations (Davie and Farrell, 1991; Davie et al., 1992; Petersen and Gamperl, 2010), suggesting a linkage between cardiac O_2 supply, cardiac performance and environmental tolerance limits.

The hearts of many fishes are composed exclusively of spongy myocardium, which generally relies entirely on the luminal venous blood for O_2 supply. Other fishes (e.g. salmonids) have a coronary circulation, which delivers oxygenated arterial blood and is generally, although not exclusively, associated with myocardial compaction (Axelsson, 1995; Farrell et al., 2012; Farrell and Smith, 2017). The primordial fish heart was hypothesized to be fully vascularized and composed of a mix of spongy and compact myocardium. Yet, the adaptive trade-offs of this cardiac arrangement remain unclear because more than half of all extant fish species lack coronary arteries (Durán et al., 2015; Farrell et al., 2012). Still, hypoxia and cardiac work are thought to be the main evolutionary drivers for coronary circulations (Farrell et al., 2012), highlighting the possibility that exposure to physiological and environmental extremes that induce increased myocardial work and reduced cardiac luminal O_2 supply, have been important selection pressures in the evolutionary history of the coronary circulation. Consequently, coronary perfusion capacity can be hypothesized to play an important role in determining the ability of fishes to withstand environmental challenges.

Salmonid fishes have a coronary circulation that perfuses the compact myocardium of the ventricle, which comprises ~20–50% of the total ventricular mass (Brijs et al., 2017; Ekström et al., 2017,

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2019; Farrell et al., 2009; Farrell and Smith, 2017). Yet, chronic surgical ligation of the coronary artery has been shown to either have little influence on routine cardiac function (heart rate, stroke volume and cardiac output; Gamperl et al., 1994a) or slightly elevate heart rate. This latter response is likely a compensation for compromised cardiac contractility and stroke volume to maintain cardiac output and arterial blood pressure, but there is currently no information available on absolute cardiac output after coronary ligation to substantiate this (Ekström et al., 2018, 2017, 2019; Steffensen and Farrell, 1998). However, consistent with the idea that the adaptive benefits of the coronary circulation are predominantly evident when fish are exposed to environmental and physiological challenges, coronary blood flow in salmonids increases markedly during acute hypoxia (Axelsson and Farrell, 1993; Gamperl et al., 1994b, 1995) and warming (Ekström et al., 2017), as well as during exercise (Gamperl et al., 1995). The functional significance of this response is evident after coronary artery ligation, which reduces relative stroke volume and cardiac output in rainbow trout exposed to acute warming (Ekström et al., 2017; Ekström et al., 2019), and impairs ventral aortic blood pressure generation in rainbow trout swimming in hypoxia (Steffensen and Farrell, 1998). Moreover, coronary ligation reduced the critical thermal maximum (CT_{max}; Ekström et al., 2017; Ekström et al., 2019) and compromised cardiac and aerobic metabolic capacity in rainbow trout in normoxia (Ekström et al., 2018), as well as the maximum sustained swimming speed in chinook salmon (Farrell and Steffensen, 1987). However, there is currently no information detailing the influence of coronary flow on absolute cardiac output responses during hypoxia or warming in salmonids, or in governing tolerance to hypoxia in fish in general.

The specific mechanisms dictating hypoxia tolerance are multi-faceted and can be broadly related to various combinations of a fish's capacity to: (i) maintain aerobic metabolism during hypoxia, (ii) depress overall metabolism and/or (iii) sustain anaerobic metabolism (for review, see Mandic and Regan, 2018; Richards, 2011). Hypoxia tolerance is often quantified by the critical Pw_{O_2} (P_{crit}), defined as the Pw_{O_2} below which the fish cannot sustain the O_2 consumption rate ($\dot{M}_{O_2}$; a proxy for aerobic metabolic rate) needed for aerobic standard metabolic rate (SMR) in hypoxia (Negrete and Esbaugh, 2019; Ultsch and Regan, 2019). When the animal cannot sustain SMR below P_{crit}, a mismatch between tissue ATP supply and demand develops and ultimately leads to loss of equilibrium at a certain Pw_{O_2} (i.e. P_{LOE}; Claireaux and Chabot, 2016; Negrete and Esbaugh, 2019; Rogers et al., 2016). There are several cardiovascular adjustments thought to promote O_2 extraction, transport and delivery to tissues in hypoxia (Gamperl and Driedzic, 2009; Sandblom and Axelsson, 2011; Stecyk, 2017). Below a certain Pw_{O_2}, obligate water-breathing fishes typically develop a vagal reflex inhibition of heart rate (i.e. hypoxic bradycardia; Farrell, 2007; Sandblom and Axelsson, 2005; Sandblom et al., 2009; Stecyk, 2017), which is initiated by stimulation of O_2-sensitive chemoreceptors in the gills (see Milsom, 2012). This bradycardia has been suggested to benefit cardiac performance in hypoxia by, for example, decreasing cardiac work and O_2 demand (Speers-Roesch et al., 2010), as well as improving the O_2 delivery to the myocardium by increasing end-diastolic volume and the residence time of blood in the heart lumen, which promotes O_2 diffusion into the spongy myocardium (for review, see Farrell, 2007; Stecyk, 2017). The prolongation of diastole with bradycardia also elevates stroke volume through increased diastolic filling and contractile force (i.e. Frank–Starling mechanism and the negative force–frequency relationship; for reviews, see Sandblom and Axelsson, 2007; Shiels et al., 2002). This allows cardiac output to be maintained, or even increase in hypoxia (Stecyk, 2017; Wood and Shelton, 1980). Interestingly, coronary blood flow may play an important role in this response, as coronary perfusion improves the contractility of isolated heart preparations during hypoxic conditions (Davie and Farrell, 1991; Davie et al., 1992). Furthermore, the hypoxic bradycardia likely enhances coronary blood flow by extending the diastolic period when coronary blood flow peaks (Axelsson and Farrell, 1993; Farrell, 2007). Taken together, these observations strongly indicate an important role of the coronary arteries for promoting cardiac performance in hypoxia, which likely extends to governing hypoxia tolerance in fish.

The aim of this study was to determine the role of coronary O_2 supply to the heart during exposure to acute hypoxia and warming, and to study the mechanisms dictating cardiac and whole-animal tolerance to these environmental extremes. We determined the effects of coronary ligation on cardiac (absolute cardiac output, heart rate and stroke volume) and respiratory performance ($\dot{M}_{O_2}$) in rainbow trout subjected to progressive acute hypoxia to determine P_{crit} and P_{LOE}, as well as a standardized thermal challenge protocol to determine CT_{max}. Specifically, we hypothesized that coronary ligation would impair cardiac performance and the ability to sustain aerobic metabolism in hypoxia and during warming, which would compromise tolerance to hypoxia (i.e. elevate P_{crit} and/or P_{LOE}) and heat (i.e. reduce CT_{max}). We also analysed the relative contribution of key cardiorespiratory traits underlying intra-specific variation in environmental tolerance limits, as well as the relationship between hypoxia and heat tolerance across experimental treatment groups to unravel potential common underlying cardiorespiratory mechanisms.

MATERIALS AND METHODS

Fish and holding conditions

Rainbow trout (*Oncorhynchus mykiss* Walbaum 1792; see Table 1 for morphological details) of mixed sex where obtained from a commercial fish farm (Vänneåns Fiskodling AB, Halland, Sweden). The fish were acclimated to laboratory conditions for a minimum of 4 weeks and were held in 1000 litre tanks continuously supplied with aerated recirculating freshwater at 10±0.5°C under a 12 h:12 h light:dark photoperiod. The fish were fed pellets (7 mm, Protec Trout pellets, Skretting, Norway) twice a week until the start of the experiments, and were fasted for 3 days before the day of the surgical procedure to ensure they were in a post-absorptive state throughout the protocol. All experimental procedures were covered by ethical permit 165-2015, approved by the regional ethical committee in Gothenburg.

Surgery and instrumentation

Individual fish were anesthetized prior to surgery in freshwater containing 150 mg l^{-1} MS-222 (Tricaine methanesulfonate, Scan Aqua AS, Årnes, Norway) buffered with 300 mg l^{-1} $NaHCO_3$. Once ventilatory movements ceased, indicating a surgical plane of anaesthesia, a 300 µl blood sample was drawn from the caudal vessel for analysis of haematological variables (haematocrit and [haemoglobin]). Fork length and body mass (M_b) were recorded, and the fish was then placed on its left side on a surgery table covered with wet foam. During the surgery, the gills were perfused with a continuous flow of aerated freshwater (10°C) containing 75 mg l^{-1} MS-222 and 150 mg l^{-1} $NaHCO_3$. A small incision was made in the isthmus and blunt dissection was used to expose the ventral aorta and the coronary artery. Care was taken not to damage surrounding vessels and nerves and to ensure that the pericardium remained intact. One group of fish had their coronary artery ligated with a 6-0 silk suture (coronary-ligated group), downstream from where the coronary artery branches from the hypobranchial artery. Another group of fish

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Table 1. Morphological and haematological variables in sham-operated and coronary-ligated rainbow trout (*Oncorhynchus mykiss*)

Measured variables	Sham-operated	Temperature effect	Coronary-ligated	Temperature effect
Body mass (g)	1179.9±96.1		1217.4±73.5	
Fork length (mm)	454.6±10.0		471.2±13.0	
Condition factor	1.17±0.06		1.17±0.03	
Relative spleen mass (%)	0.12±0.03		0.15±0.01	
Relative ventricular mass (%)	0.08±0.01		0.08±0.01	
Compact myocardium (%)	34.5±1.9		34.1±1.4	
Haematocrit (%)				
Before surgery	32.3±1.5	$F_{1,21}$=28.151, P<0.001	31.9±1.3	$F_{1,21}$=11.991, P=0.002
After thermal challenge	41.4±1.8		38.4±1.6	
[Haemoglobin] (g l^{-1})				
Before surgery	82.4±3.0	$F_{1,21}$=8.999, P=0.007	79.7±2.7	$F_{1,21}$=3.354, P=0.081
After thermal challenge	95.7±3.3		86.8±2.9	
Mean corpuscular [haemoglobin] (g l^{-1})				
Before surgery	257.8±6.5	$F_{1,22}$=2.371, P=0.138	250.9±6.8	$F_{1,20}$=7.543, P=0.012
After thermal challenge	242.0±6.8		226.8±5.7	

Haematological variables were obtained from the caudal vessels and analysed before surgery and after the thermal challenge. All values are means±s.e.m. (sham-operated, n=10 and coronary-ligated, n=13). There were no significant differences between treatment groups. Statistical significance was accepted at P<0.05.

underwent an identical surgical protocol with the exception that the coronary artery was not ligated and left intact (sham-operated group). A 2.5 mm Transonic transit-time blood flow probe (L type; Transonic Systems, Ithaca, NY,USA) was then placed around the ventral aorta to allow recordings of ventral aortic blood flow (cardiac output). The probe was secured with 3-0 silk sutures inside the opercular cavity and in the skin just outside of the opercular cavity and a final (2-0) suture in front of the dorsal fin. Following instrumentation, the fish were placed individually in 10 litres Perspex respirometers submerged in a ∼120 liters experimental tank filled with aerated recirculating freshwater at 10.5±0.05°C. The fish were allowed to recover from surgery for ∼20 h, throughout which $\dot{M}_{O_2}$ was continuously recorded (see below for details). The experiments were run in pairs daily with one sham-operated and one coronary-ligated fish in each run. All fish were then subjected to a hypoxia challenge followed by a temperature challenge as described below.

Hypoxia challenge

Measurements of cardiorespiratory variables in normoxia commenced in the morning and were recorded for a minimum of 2 h before the start of the hypoxia challenge. Once steady state heart rate values had been obtained during the initial recording period, the hypoxia challenge commenced and was performed using closed circuit respirometry following previously described protocols (Negrete and Esbaugh, 2019; Reemeyer and Rees, 2019). Briefly, by turning off the inflow of normoxic water ($P\mathrm{w}_{O_2}$ ∼21 kPa at 100% air saturation) into the respirometers, the O_2 consumption of the fish caused a gradual decrease in $P\mathrm{w}_{O_2}$ to hypoxic levels until the fish reached P_{LOE} (Nilsson and Östlund-Nilsson, 2004), which was defined as the $P\mathrm{w}_{O_2}$ where the fish lost equilibrium for longer than 10 s. Cardiac measurements were recorded at P_{LOE} for one more minute, after which the individual respirometer was reperfused with aerated water to bring the $P\mathrm{w}_{O_2}$ inside the respirometer back to normoxic conditions. The duration of the hypoxia challenge averaged 61 min and ranged between 25 to 109 min. All fish recovered once normoxic conditions were restored.

Temperature challenge

Following >20 h of recovery from the hypoxia challenge, which was sufficient for all cardiorespiratory variables to return to routine values, baseline values were again recorded for a minimum of 2 h before the start of an acute heating challenge. The temperature was then increased from 10.5°C to 20°C in 5°C h^{-1} increments, with the temperature ramp lasting approximately 30 min and the target temperature being maintained for another 30 min. When 20°C was reached, the temperature was increased further by 1°C steps every 30 min (2°C h^{-1}), where the ramping lasted approximately 10 min and the target temperature was maintained stable for 20 min. CT_{max} was determined as the temperature at which the fish lost the ability to maintain an upright body position for more than 10 s. Before each temperature increment, two $\dot{M}_{O_2}$ slopes were recorded by turning off the flush pump and letting the % air saturation decrease by ∼10% before reinitiating flushing. Once CT_{max} was reached, the fish were removed from the respirometer and euthanized via blunt trauma to the head, immediately followed by drawing a blood sample from the caudal vessels for analyses of haematological variables. To ensure that the coronary artery had been successfully ligated, the position and integrity of the suture was verified post mortem. Finally, the wet mass of the spleen and heart ventricle (after careful removal of atrium, bulbus and any remaining blood in the lumen) were determined. The ventricle was preserved in 70% ethanol for further analyses (see below).

Data acquisition and calculations

The % air saturation inside the respirometer was continuously measured using an O_2 optode connected to a Firesting O_2 system (PyroScience, Aachen, Germany). $\dot{M}_{O_2}$ was determined using automated intermittent closed respirometry and calculated from the decline in % air saturation (i.e. slope) in the respirometer between flush cycles. $\dot{M}_{O_2}$ measurements at rest were typically performed in continuous 20 min cycles with the flush cycle set to 15 min. However, to ensure that water air saturation remained >85%, the cycles were reduced to 15 min cycles with 12 min flushing periods for some fish.

$\dot{M}_{O_2}$ was calculated as:

$$\dot{M}_{O_2} = \frac{(V_r - V_f) \times (\Delta\%\mathrm{Sat}/t) \times \alpha}{M_b}, \quad (1)$$

where V_r is the volume of the respirometer, V_f is the volume of the fish assuming that 1 g of fish equals 1 ml of water, $\Delta\%\mathrm{Sat}/t$ is the change in % O_2 saturation per unit time, α is the solubility coefficient of O_2 in freshwater and adjusted for the different experimental temperatures

and M_b is the body mass of the fish (Clark et al., 2013). SMR was calculated as the lowest 20th percentile of the $\dot{M}_{O_2}$ values (Chabot et al., 2016) obtained throughout the ∼20 h overnight post-surgery recovery period up until the start of the hypoxia challenge. Routine metabolic rate (RMR) throughout the hypoxia protocol was calculated from the average slope at 10% air saturation intervals until the air saturation reached 50%, and then at 5% intervals until P_{LOE} (i.e. starting at 90, 80, 70, 60, 50, 45, 40, 35, 30, 25, 20, 15 and 10% air saturation). SMR values were then plotted against Pw_{O_2}, and P_{crit} was determined by fitting a linear regression to RMR values during the hypoxia exposure that were below the normoxic SMR to identify the Pw_{O_2} where the regression line intercepted the SMR (P_{crit}) obtained in normoxia (see supplementary material, Fig. S1; McBryan et al., 2016). Prior to the temperature challenge, RMR at 10.5°C was obtained by calculating the average from four $\dot{M}_{O_2}$ slopes when the fish was in a steady state, as determined by a low and steady heart rate. During the temperature challenge, mean values were calculated from the two $\dot{M}_{O_2}$ slopes at each temperature step when the temperature had stabilized. Measurements of bacterial background respiration were performed following each trial after the removal of the animals from the respirometers and the acquired slopes were subtracted from the $\dot{M}_{O_2}$ slopes of the fish.

The Transonic flow probe was connected to a Transonic blood flow meter (model T206; Transonic Systems, Ithaca, NY) and the signals where recorded using a PowerLab system (ADInstruments, Castle Hill, Australia) at a sampling rate of 10 Hz using LabChart pro data acquisition software (v.7.3.2, AD Instruments, Castle Hill, Australia). To correct for any temperature effects on the Transonic flow probe readings, all probes were individually bench calibrated as specified by the user manual at 10.5, 15, 20, 22, 24, 26, 28 and 30.0°C. Briefly, the transonic flow probe was mounted on a calibration tubing submerged in a temperature controlled water bath. A flow of temperature-regulated water was pumped through the tubing using a peristaltic pump (Gilson 312 Minipuls 3, Villiers-Le-Bel, France) for 1 min. The outflowing water was collected and weighed to determine the flow gravimetrically. This process was repeated at increasing flow rates covering the flow range of the probe (1 to 100 ml min^{-1}). The values obtained from the transonic flow probe were plotted against the recorded gravimetric flow and the resulting regression equation was used to correct the obtained *in vivo* cardiac output values. The heart rate was calculated from the pulsating blood flow traces using the cyclic measurements module in LabChart Pro. Stroke volume was calculated as the quotient of cardiac output divided by heart rate.

The Pw_{O_2} at the onset of hypoxic bradycardia was determined via segmental linear regression analyses using GraphPad prism 8.3.0, according to established methods for identifying critical break points (Yeager and Ultsch, 1989). Briefly, this method fits two lines that intersect at the Pw_{O_2} where heart rate starts to decrease. The Pw_{O_2} and average value for peak stroke volume in hypoxia (peak $SV_{hypoxia}$), and cardiac output at peak $SV_{hypoxia}$ (CO at peak $SV_{hypoxia}$) were determined for each fish. We also determined the temperatures and average peak values for cardiac output, heart rate and MO_2 during warming (peak $CO_{warming}$, peak heart rate and peak $\dot{M}_{O_2}$, respectively), as well as the average stroke volume at peak $CO_{warming}$ (SV at peak $CO_{warming}$).

Haematocrit was determined by spinning the blood in capillary tubes in a microhaematocrit centrifuge for 5 min at 10,000 ***g*** and measuring the resulting fraction of red blood cells. [Haemoglobin] was measured using a handheld Hb 201+ analyser (Hemocue, Ängelholm, Sweden), and the values were recalculated for fish blood according to Clark et al. (Clark et al., 2008a). Mean corpuscular haemoglobin concentration (MCHC) was calculated as:

$$\text{MCHC} = [\text{haemoglobin}]/\text{haematocrit} \times 100. \quad (2)$$

The relative ventricular mass was calculated as wet mass of the ventricle/M_b×100. To determine the proportion of ventricle compact myocardium, the compact and spongy layers were separated and weighed according to Farrell et al. (2007). The percentage of compact myocardium was then calculated as the dry mass of compact myocardium/dry mass of ventricle×100. The relative spleen mass was calculated as wet mass of the spleen/M_b×100. The overall condition factor for individual fish was calculated as:

$$\text{Condition factor} = (M_b/\text{fork length}^3) \times 100. \quad (3)$$

Statistical analyses

Statistical analyses were performed using SPSS statistics 24 for Windows (IBM Corp., Armonk, NY, USA). Differences between groups (i.e. sham-operated versus coronary-ligated) in morphological variables, environmental tolerance indices (i.e. CT_{max}, P_{crit} and P_{LOE}), specific cardiorespiratory indices (e.g. peak responses, bradycardia) and mean routine cardiorespiratory values (at ≥18.9 kPa and 10.5°C) were analysed using independent-sample *t*-tests, Welch *t*-test (if variances were unequal) or Mann–Whitney *U*-tests (if data were not normally distributed). Differences in haematological variables where analysed using a linear mixed model with an unstructured repeated covariance structure, with fish ID as subject variable and sampling time (initial versus final), treatment (sham-operated versus coronary-ligated) and their interaction as fixed factors. Differences in cardiorespiratory variables during the hypoxia and temperature challenges were analysed using a linear mixed model with a within-subjects factor (Pw_{O_2} or temperature), treatment and the interaction between the within-subject factor and treatment as fixed factors, and fish ID as subject variable. Either a first-order autoregressive or a heterogeneous first-order autoregressive repeated covariance structure was used, which provided best fit to the models (as indicated by the lowest Akaike's information criterion, AIC). Pw_{O_2} values ranging from ≥18.9 to 2.1 kPa were included in the models assessing the effects of hypoxia and values at P_{LOE} were excluded. Models assessing the effects of temperature included temperatures ranging from 10.5 to 22°C, as this was the lowest temperature at which individual fish peaked across all variables. If significant interactions were found, these were further explored with between- and within-treatments pair-wise comparisons, where confidence intervals were adjusted for multiple testing using Bonferroni correction. For the statistical analysis of the hypoxia challenge, cardiac output, heart rate and $\dot{M}_{O_2}$, as well as $\dot{M}_{O_2}$ during the temperature challenge, were transformed to their natural logarithm to comply with the assumption of homoscedasticity of the residuals. P_{crit} was transformed to its natural logarithm to comply with the assumption of normality.

Multiple regression models were used to determine the relative contribution of key variables on the variances in hypoxia (P_{LOE}) and temperature tolerance (CT_{max}). In these analyses, values for sham-operated and coronary-ligated treatments were pooled. Moreover, to determine potential relationships between temperature and hypoxia tolerance, additional regression analyses were carried out with CT_{max} as the dependent variable and P_{LOE}, P_{crit} and treatment (sham-operated versus coronary-ligated) as independent variables. The variables used in the multiple regression models were selected as they lacked multicollinearity and appeared to follow a linear relationship with the dependent variables (P_{LOE} and CT_{max}) when assessed by visual inspection of partial regression plots and fitted

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the assumptions of the model. Variables were subsequently gradually eliminated from the model using stepwise backward regression until only the variables that best explained the variability in the dependent variable remained. Only adjusted R^2 values are reported. Regression coefficients, standard errors and confidence intervals are included in Table S1. In addition, correlations between peak cardiac variables and cardiac morphological variables were analysed for each treatment using Pearson's correlations (P_{corr}). Statistical significance was accepted at $P<0.05$. All data are presented as means±s.e.m.

RESULTS

Morphological and haematological characteristics

There were no differences in overall body characteristics (M_b, fork length and condition factor), cardiac morphology (relative ventricular mass and % compact myocardium), haematological variables or relative spleen mass between treatment groups (Table 1).

Impacts of coronary blood flow on hypoxia tolerance and cardiorespiratory performance

In normoxia, there were no differences in SMR between sham-operated control and coronary-ligated trout (58.6±4.5 versus 60.8±2.8 mg O_2 kg^{-1} h^{-1}, respectively; Fig. S1). Similarly, there were no differences between control and coronary-ligated fish in routine cardiac output (13.4±1.2 versus 11.4±1.3 ml min^{-1} kg^{-1}, respectively; Fig. 1B) in normoxia. Even so, heart rate was significantly elevated after ligation (57.1±1.9 versus 47.6±3.9 beats min^{-1}; $t_{11.888}=-2.213$, $P=0.047$; Fig. 1C), which coincided with a significantly reduced stroke volume relative to control trout (0.20±0.03 versus 0.29±0.02 ml kg^{-1}; $t_{21}=2.461$, $P=0.012$; Fig. 1D).

Given the methodology used, body mass affected the rate of decline in Pw_{O_2} during the gradual hypoxia exposure. However, there was no linear relationship between the duration of the hypoxia protocol or body mass with P_{crit} or P_{LOE} (as indicated by visual inspection of scatterplots; data not shown). Throughout the hypoxia challenge, there were no significant differences in $\dot{M}_{O_2}$ between treatment groups (Fig. 1A). Moreover, P_{crit} occurred at similar Pw_{O_2} levels in control and coronary-ligated trout (Table 2). However, P_{LOE} was significantly higher in coronary-ligated trout ($t_{21}=-2.284$, $P=0.033$; Table 2). As hypoxia progressed, an increasingly pronounced bradycardia developed in both groups (Fig. 1C), but the onset of bradycardia occurred at a significantly higher Pw_{O_2} by 2.6 kPa in coronary-ligated fish ($t_{17}=-2.384$, $P=0.029$; Table 2). Moreover, while cardiac output was maintained in hypoxia via a

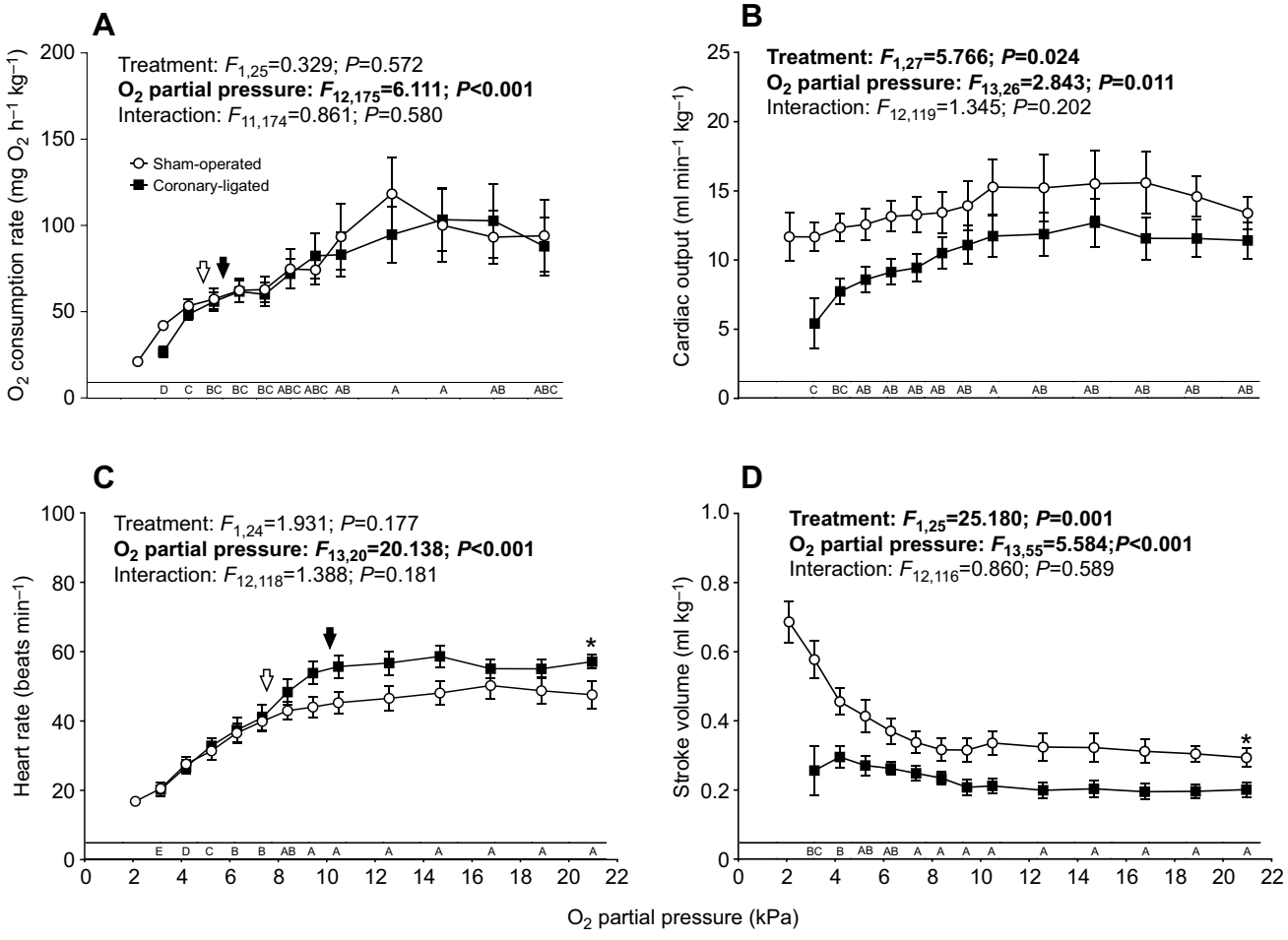

Fig. 1. Effect of coronary ligation on whole-animal O_2 consumption rate and cardiac responses in rainbow trout (*Oncorhynchus mykiss*) during acute hypoxia. (A) Whole-animal O_2 consumption rate ($\dot{M}_{O_2}$), (B) cardiac output, (C) heart rate and (D) stroke volume of sham-operated control (open circles, n=9–10) and coronary-ligated rainbow trout (filled squares; n=13). The results from the mixed model for the respective variables are presented in each panel. The arrows represent the critical O_2 tensions for $\dot{M}_{O_2}$ (i.e. P_{crit}) and heart rate (i.e. onset of bradycardia) for sham-operated (white arrow) and coronary-ligated (black arrow) rainbow trout. Mean routine cardiorespiratory values at ≥18.9 kPa were analysed using independent-sample t-tests (*P<0.05 between treatment groups). Different letters indicate a general statistically significant difference (P<0.05) at different P_WO_2. Values are means±s.e.m.

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Table 2. Hypoxia tolerance, peak cardiac responses and onset of bradycardia during exposure to progressive acute hypoxia in sham-operated and coronary-ligated rainbow trout (*O. mykiss*)

Measured variables	Sham-operated	Coronary-ligated
Duration of the hypoxia protocol (min)	61.5±8.5 (10)	60.5±5.9 (13)
P_{crit} (kPa)	4.8±0.7 (8)	5.6±0.7 (9)
P_{LOE} (kPa)	2.6±0.2 (10)	3.2±0.2 (13) *
Pw_{O_2} at onset of bradycardia (kPa)	7.5±0.6 (8)	10.1±0.8 (11) *
Peak $SV_{hypoxia}$ (ml kg^{-1})	0.58±0.05 (10)	0.36±0.03 (13)***
Pw_{O_2} at peak $SV_{hypoxia}$(kPa)	3.3±0.2 (10)	5.1±0.4 (13)**
CO at peak $SV_{hypoxia}$ (ml min^{-1} kg^{-1})	11.6±1.0 (10)	8.2±0.8 (13)**

P_{crit}, critical O_2 level; P_{LOE}, P_{O_2} at loss of equilibrium; $SV_{hypoxia}$, stroke volume in hypoxia; CO, cardiac output; Pw_{O_2}, water P_{O_2}. Values are means±s.e.m. and sample sizes are presented within brackets. *, ** and *** denote significant treatment effects with statistical significance accepted at P<0.05, P<0.01 and P<0.001, respectively.

significant increase in stroke volume in the sham-operated control fish (Fig. 2B and D), the stroke volume in coronary-ligated trout was significantly lower during the hypoxia exposure (Fig. 1D). Consequently, the peak $SV_{hypoxia}$ was significantly lower (t_{21}=4.747, P<0.001) and occurred at a higher Pw_{O_2} (U=102, P=0.021; Table 2), which coincided with a significantly lower cardiac output in coronary-ligated fish during the hypoxia exposure (Fig. 1B). Multiple regression analyses revealed that relative ventricular mass, percentage compact myocardium and peak $SV_{hypoxia}$ combined, significantly explained part of the variation in P_{LOE} ($F_{3,19}$=4.896, P=0.011, R^2=0.35).

Impacts of coronary blood flow on temperature tolerance and cardiorespiratory performance

There were no differences between control and coronary-ligated trout in normoxia at 10.5°C, following the recovery from the hypoxia exposure, with regards to routine $\dot{M}_{O_2}$ (53.0±4.6 versus 59.5±4.0 mg O_2 kg^{-1} h^{-1}, respectively; Fig. 2A) and cardiac output (11.2±1.2 versus 10.0±1.0 ml min^{-1} kg^{-1}, respectively; Fig. 2B). Moreover, although routine heart rate was not significantly different between groups (41.3±3.5 versus 50.8±3.0 beats min^{-1}, respectively), a trend towards a higher heart rate was observed in coronary-ligated trout (t_{21}=−2.003, P=0.058; Fig. 2C), which coincided with a significantly lower stroke volume (0.29±0.03 versus 0.20±0.02 ml kg^{-1}; t_{21}=2.248, P=0.035; Fig. 2D).

$\dot{M}_{O_2}$ did not differ between treatment groups and increased with warming at a similar rate across treatment groups (Fig. 2A). However, the overall temperature tolerance was significantly

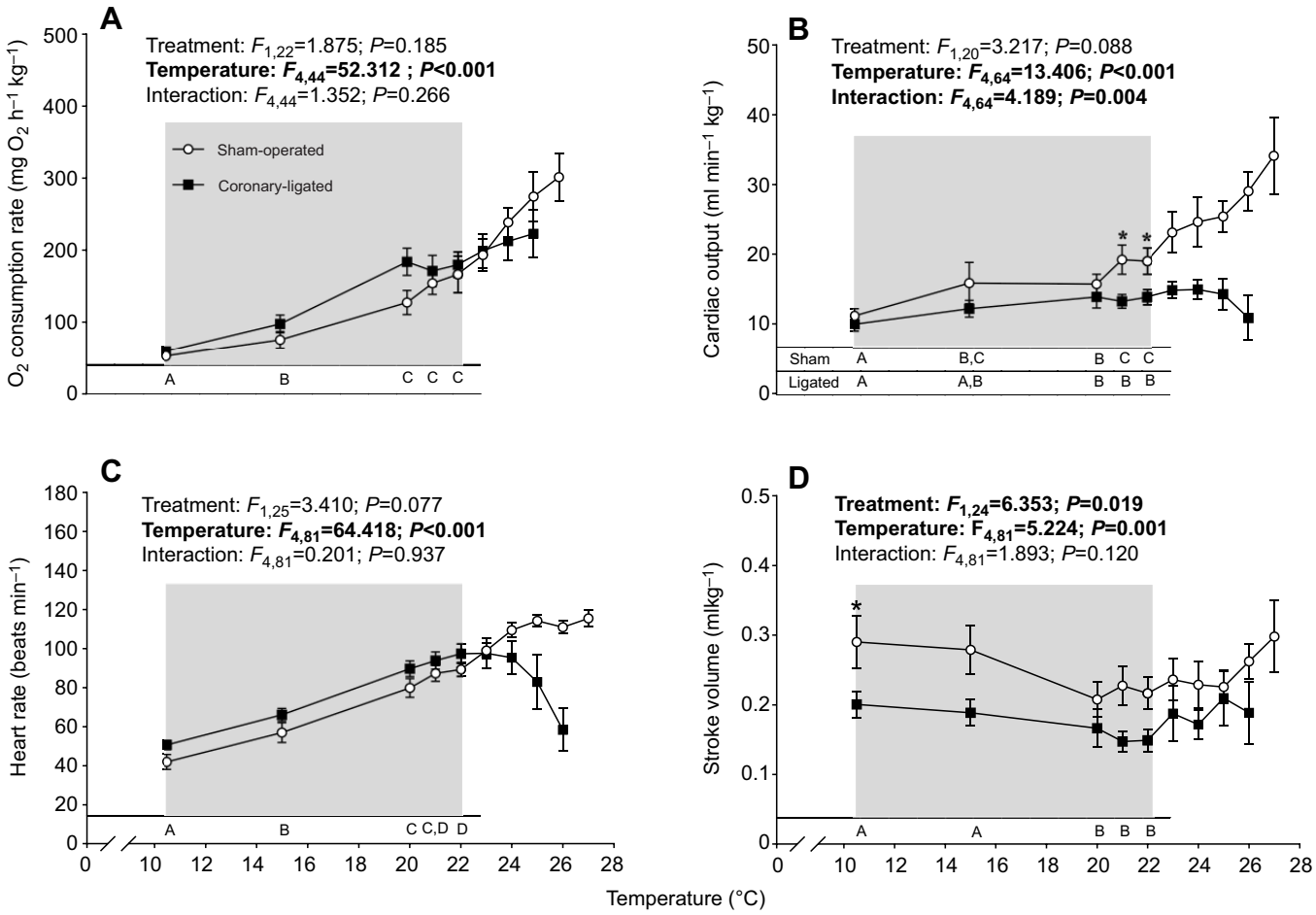

Fig. 2. Effect of coronary ligation on whole-animal O_2 consumption rate and cardiac responses in rainbow trout (*O. mykiss*) during acute warming. (A) Whole-animal O_2 consumption rate ($\dot{M}_{O_2}$), (B) cardiac output, (C) heart rate and (D) stroke volume of sham-operated (open circles, n=9–10) and coronary-ligated rainbow trout (filled squares; n=12–13). The results from the mixed model for the respective variables are presented in each panel. Only values between 10.5 and 22°C, the latter representing the lowest individual cardiorespiratory breakpoint temperature, were included in the mixed model (indicated by the shaded area). Mean routine cardiorespiratory values at 10.5°C were analysed using independent-sample t-tests (*P<0.05 between treatment groups). Different letters in A,C and D indicate a general statistically significant difference (P<0.05) between temperatures. In B, where a significant interaction effect was found between treatment and temperature the different letters indicate a significant difference within the two treatment groups. Values are means±s.e.m.

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reduced in coronary-ligated trout as indicated by a 1.3°C reduction in CT_{max} (U=20, P=0.004; Table 3). Consistent with the increase in $\dot{M}_{O_2}$, cardiac output also increased with temperature in both groups, although the increase was greater in control fish from 21°C onwards (temperature $effect_{sham}$: $F_{4,51}$=12.528, P<0.001; temperature $effect_{ligated}$: $F_{4,51}$=3.947, P=0.007; Fig. 2C). The increased cardiac output was governed by elevations in heart rate in both groups (Fig. 2B,C). Stroke volume remained significantly lower in coronary-ligated trout throughout the thermal challenge relative to the control group (Fig. 2D). Consequently, the peak $CO_{warming}$ was lower in coronary-ligated fish ($t_{12.275}$=3.696, P=0.003; Table 3), which was due to a decreased SV at peak $CO_{warming}$ ($t_{11.902}$=3.476, P=0.005; Table 3). Moreover, the temperatures at peak heart rate, peak $\dot{M}_{O_2}$ and peak $CO_{warming}$ were lower in the ligated compared with the sham-operated trout (U=16, P=0.002; U=23, P=0.047 and U=22, P=0.006, respectively; Table 3). Multiple regression analyses revealed that the peak $CO_{warming}$ significantly explained part of the variation in CT_{max} ($F_{1,21}$=9.685, P=0.005, R^2=0.28), such that CT_{max} was positively correlated with $CO_{warming}$ (P_{corr}=0.548).

Haematocrit was significantly increased following the temperature challenge in both control and coronary-ligated fish (Table 1), whereas [Haemoglobin] only increased significantly in the control group (Table 1). There were no significant differences in MCHC between treatment groups at the end of the temperature challenge (treatment $effect_{afterCTmax}$: $F_{1,20}$=2.964, P=0.101; Table 1). However, MCHC was significantly reduced compared with pre-surgery values in coronary-ligated trout indicating pronounced red blood cell swelling, but this was not observed in sham-operated fish (Table 1).

Linkages between hypoxia and temperature tolerance

A multiple regression was run to predict CT_{max} from P_{crit}, P_{LOE} and treatment. The model revealed that treatment ($F_{1,21}$=12.352, P=0.002, R^2=0.34), P_{LOE} ($F_{1,21}$=8.485, P=0.008, R^2=0.25) and treatment in combination with P_{LOE} (Fig. 3) significantly predicted CT_{max}. This indicates that individuals with lower P_{LOE} (and thus greater hypoxia tolerance) were also the most temperature tolerant, which strengthens the above findings that coronary ligation reduces tolerance to both environmental extremes.

A significant negative correlation was found in the coronary-ligated fish between the percentage compact myocardium and peak $SV_{hypoxia}$ (P_{corr}=−0.698; P=0.008; Fig. 4A), and consequently CO at peak $SV_{hypoxia}$ (P_{corr}=−0.609; P=0.027). Similarly, peak $CO_{warming}$ (P_{corr}=−0.655; P=0.015; Fig. 4B) and SV at peak $CO_{warming}$ (P_{corr}=−0.629; P=0.021) were also negatively correlated with percentage compact myocardium. Thus, the acute coronary ligation in individuals with a large proportion of compact myocardium (which was presumably devoid of coronary blood supply) resulted in lower peak stroke volume and cardiac output during exposure to both hypoxia and warming. These significant correlations were not observed in the sham-operated control group (Fig. 2A,B).

Table 3. Temperature tolerance and peak cardiorespiratory responses during acute warming in sham-operated and coronary-ligated rainbow trout (*O. mykiss*)

Measured variables	Sham-operated	Coronary-ligated
CT_{max} (°C)	26.7±0.3 (10)	25.4±0.3 (13)**
Peak heart rate (beats min^{-1})	117.3±3.2 (10)	109.1±5.1 (13)
Temperature at peak heart rate (°C)	25.3±0.2 (10)	23.4±0.3 (12)**
Peak $CO_{warming}$ (ml min^{-1} kg^{-1})	29.9±3.4 (10)	16.4±1.4 (13)**
Temperature at peak $CO_{warming}$ (°C)	24.8±0.3 (10)	23.0±0.5 (13)**
SV at peak $CO_{warming}$ (ml kg^{-1})	0.27±0.03 (10)	0.15±0.01 (12)**
Peak $\dot{M}_{O_2}$ (O_2 kg^{-1} h^{-1})	290.9±28.9 (8)	256.9±20.7 (12)
Temperature at peak $\dot{M}_{O_2}$ (°C)	24.9±0.3 (8)	23.3±0.5 (12)*

CT_{max}, upper critical thermal maximum; $CO_{warming}$, cardiac output; $\dot{M}_{O_2}$, whole-animal O_2 consumption rate during warming and stroke volume (SV). Values are means±s.e.m.; sample sizes are presented within brackets. *P<0.05 and **P<0.01 significant treatment effects.

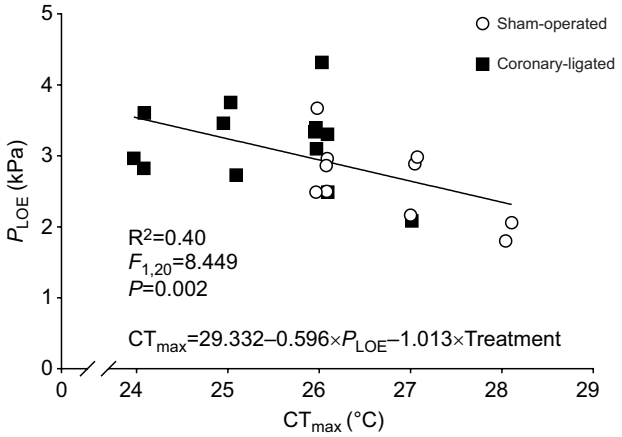

Fig. 3. Relationship between the O_2 tension for loss of equilibrium (P_{LOE}) and upper critical thermal maximum (CT_{max}) in sham-operated and coronary-ligated rainbow trout (*O. mykiss*). Linear regression between P_{LOE} and CT_{max} in sham-operated (open circles, n=10) and coronary-ligated rainbow trout (filled squares; n=13). The output of the multiple linear regression model with CT_{max} as dependent variable and P_{LOE} and treatment as independent variables is shown in the figure. The regression equation for CT_{max} as a function of treatment and P_{LOE} is displayed. The P-value indicates whether treatment groups and P_{LOE} added statistically significantly to the prediction (P<0.05) of CT_{max}.

DISCUSSION

Coronary blood flow promotes cardiac stroke volume in trout at rest

Similarly to previous studies, coronary ligation resulted in either a trend towards an elevated routine heart rate (prior to the heating protocol) or a significantly elevated routine heart rate (prior to hypoxia protocol) in rainbow trout (Ekström et al., 2018, 2017, 2019; Steffensen and Farrell, 1998). Here, we can show for the first time that this response serves to maintain O_2 consumption rate and cardiac output by compensating for a ∼44% decrease in stroke volume following the coronary ligation. The elevations in heart rate are most likely mediated via a reduced cholinergic (i.e. vagal) tone on the heart, presumably by a reduction in ventral aortic blood pressure inducing a barostatic reflex (see Sandblom and Axelsson, 2011). Indeed, Ekström et al. (2019) showed that treatment with the muscarinic antagonist atropine, which normally would abolish the cholinergic tone on the heart thus leading to an elevated heart rate, had no effect on heart rate in coronary-ligated rainbow trout suggesting that the ligation per se had already caused a release of cholinergic tone.

Restrictions of coronary blood flow impairs cardiac performance in hypoxia and affect whole-animal hypoxia tolerance

In both treatment groups, the bradycardic responses to hypoxia occurred at a Pw_{O_2} (7.5–10.1 kPa), which is within the range of previously reported values for this species (see table in Stecyk,

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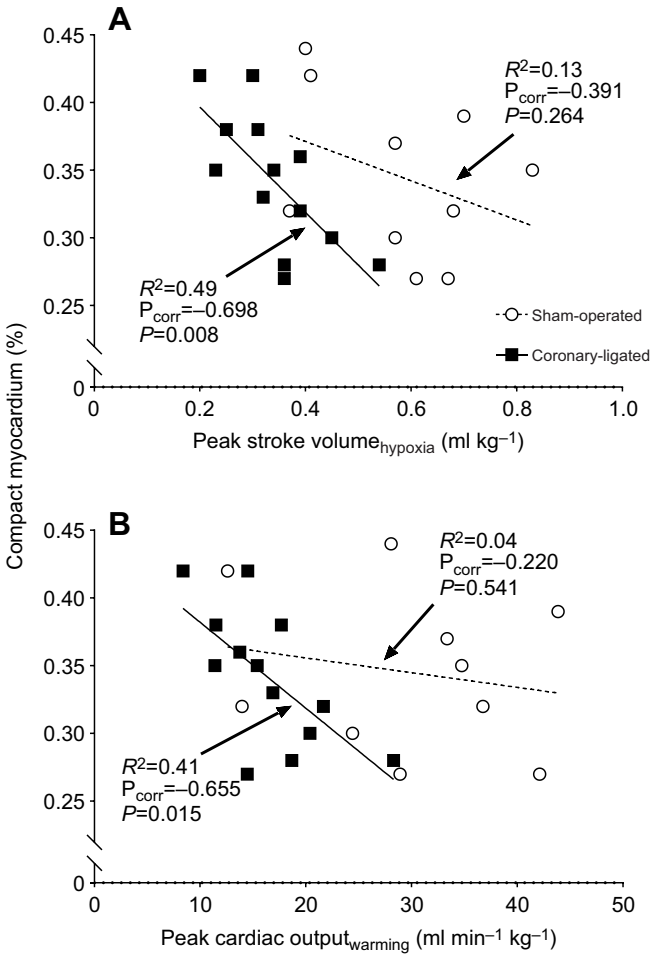

Fig. 4. Relationship between percentage compact myocardium and peak cardiac responses in sham-operated and coronary-ligated rainbow trout (*O. mykiss*). Linear regression between percentage compact myocardium and (A) peak stroke volume during hypoxia and (B) peak cardiac output during warming in sham-operated (hatched lines and open circles, n=10) and coronary-ligated trout (solid lines and filled squares; n=13). Pearson's correlations (P_{corr}) indicates the strength and direction of the association between the two variables. The P-value indicates statistically significant correlations (P<0.05) between treatments.

2017). The onset of bradycardia occurred at a Pw_{O_2} that was 2.6 kPa higher in the ligated group compared with the control group. It is possible that this was because the input from the branchial O_2-sensitive chemoreceptors in hypoxia overrode the barostatic response that may initially have kept heart rate elevated in the coronary-ligated fish. Even so, below a Pw_{O_2} of ∼8 kPa the heart rate was similar between treatment groups, suggesting that the hypoxic bradycardia response dominated. However, in contrast to the control trout where stroke volume increased substantially as the hypoxic bradycardia developed, the stroke volume was only marginally elevated in trout with ligated coronary arteries, which meant that cardiac output collapsed and was halved immediately prior to P_{LOE} relative to the cardiac output recorded in normoxia. Thus, the abolished O_2 delivery to the compact myocardium hampered the capacity to elevate stroke volume and maintain cardiac output at lower Pw_{O_2} levels, in contrast to trout with an intact coronary flow, which were far better able to maintain cardiac output at reduced ambient Pw_{O_2}. Another possible contributing factor that requires further testing is that along with an insufficient myocardial oxygenation, ligation of the coronary circulation also prevents catecholamines from reaching and effecting the compact myocardium. These are normally released during acute hypoxia (Perry and Reid, 1994, 1992) and protect myocardial function from hypoxia, acidosis and hyperkalaemia (see Driedzic and Gesser, 1994; Hanson et al., 2006; Roberts and Syme, 2018).

Despite a cardiac output that was twice as high in the control relative to coronary-ligated trout immediately prior to P_{LOE}, coronary-ligated trout were only marginally less tolerant to acute hypoxia as their P_{LOE} was only elevated by 0.6 kPa (∼3% air saturation). Moreover, the P_{crit} for both treatment groups were within the range of previously reported values for rainbow trout (Williams et al., 2019; Wood, 2018), and while P_{crit} was numerically slightly higher in the ligated fish (by 0.8 kPa), this was not statistically significant. Thus, it seems likely that the coronary-ligated fish initiated some response in hypoxia that compensated for the severely reduced capacity for circulatory O_2 delivery to the tissues, which in turn minimized the negative impacts of the coronary obstruction on overall hypoxia tolerance. Indeed, both treatment groups maintained a similar $\dot{M}_{O_2}$ throughout most of the hypoxia protocol, suggesting a significantly increased tissue O_2 extraction in the coronary-ligated trout when cardiac output collapsed in hypoxia. Given that Steffensen and Farrell (1998) found no difference in venous P_{O_2} between sham-operated and coronary-ligated rainbow trout, and since blood O_2 carrying capacity (as indicated by a similar haematocrit) did not differ between treatments, one possibility is that there was a right (Bohr effect) and possibly downward shift (Root effect) in the haemoglobin O_2 dissociation curve in the coronary-ligated trout, thus augmenting O_2 unloading at the tissues (Harter and Brauner, 2017; Rummer and Brauner, 2015; Rummer et al., 2013). This shift could have been caused by an exacerbated acidosis in coronary-ligated fish in hypoxia, but unfortunately neither blood pH nor blood O_2 content was measured in the current or earlier studies to confirm this. Although the overall effects of acute coronary ligation on hypoxia tolerance where relatively mild, the effects of a sustained impaired coronary O_2 delivery over the long-term on organismal hypoxia tolerance remains to be explored.

Coronary blood flow restrictions during warming are linked to a compromised stroke volume, early onset of heart rate collapse and reduction in upper thermal tolerance

Our study is the first to demonstrate the role of the coronary circulation in maintaining cardiac output and stroke volume along with respiratory performance during warming. Cardiac output is mainly elevated via heart rate increases in response to warming (Eliason and Anttila, 2017). Similarly to previous observations (Ekström et al., 2017; Ekström et al., 2019), the heart rate peaked at a ∼1.6°C lower temperature in coronary-ligated trout, although the peak heart rate was similar for both treatment groups. It is possible that the lower temperature for peak heart rate during warming was simply an effect of the slightly higher routine heart rate often observed in coronary-ligated trout (Ekström et al., 2018, 2017, 2019; Steffensen and Farrell, 1998). The peak $CO_{warming}$ was driven by the peak heart rate in both control and ligated fish, which meant that the peak $CO_{warming}$ occurred at 1.8°C lower temperature in trout with ligated coronary arteries. However, despite the similar peak heart rate response, the peak $CO_{warming}$ was reduced by 45% in ligated fish. Similarly to the situation in hypoxia, this impaired capacity to elevate cardiac output with warming was explained by a severely constrained stroke volume (reduced by ∼44%) at peak $CO_{warming}$ in coronary-ligated trout. This is consistent with the ∼50% reduction in peak relative cardiac output (recorded using

Doppler flow probes) in coronary-ligated trout observed by Ekström et al. (2019). Despite the reduced cardiac output during warming, peak $\dot{M}_{O_2}$ was not statistically different between groups; again, however, the peak occurred at a lower temperature in ligated fish. Nevertheless, the earlier collapse of cardiorespiratory function in the coronary-ligated trout was associated with a lower CT_{max}, indicating a loss of heat tolerance (Ekström et al., 2017; Ekström et al., 2019).

Following thermal ramping, haematocrit and [haemoglobin] increased in the control fish, indicating splenic contraction (Perry and Kinkead, 1989), whereas haematocrit increased and MCHC was reduced in the coronary-ligated group, indicating erythrocyte swelling (Nikinmaa, 1982; Templeman et al., 2014). Both responses are consistent with an increase in circulating catecholamines, which commonly occurs following acute warming and increases blood O_2 carrying capacity (Templeman et al., 2014) and haemoglobin O_2 affinity by elevating erythrocytic intracellular pH (Nikinmaa, 1982). However, as the reduction in MCHC was more pronounced in the coronary-ligated trout, it is possible that this reflects a more pronounced increase of circulating catecholamines in this group in an attempt to mitigate the effect of blood acidosis on haemoglobin O_2 affinity and to increase blood O_2 carrying capacity in face of hypoxemia at elevated temperatures (Boutilier et al., 1986; Perry et al., 1989).

Myocardial oxygenation and cardiac performance constitute a common underlying mechanism influencing hypoxia and heat tolerance

Although acute tolerance limits to both hypoxia and warming have been previously linked to cardiac function, it is less clear if there are common underlying mechanisms that confer tolerance to both environmental extremes. For example, intra-specific differences in whole-animal hypoxia tolerance in European seabass (*Dicentrarchus labrax*) were positively correlated with contractile force production of ventricular strip preparations under both normoxia and hypoxia (Joyce et al., 2016). Moreover, studies in various teleost species indicate that intra-specific warming tolerance is positively correlated with cardiac morphological traits such as ventricle size (Anttila et al., 2013; Ozolina et al., 2016) and cardiac myoglobin levels (Anttila et al., 2013). Anttila et al. (2013) also found a positive association between hypoxia and thermal tolerance, indicating that a better O_2 supply to the heart (i.e. due to increased myoglobin levels) and a capacity for maintaining a higher cardiac output (given the positive association between cardiac output and ventricle size; Franklin and Davie, 1992) could be a common trait associated with tolerance to both environmental drivers. In the current study, 33% of the variation in P_{LOE} could be explained by the combined variation in relative ventricular mass, percentage compact myocardium and peak $SV_{hypoxia}$, whereas peak $CO_{warming}$ had the most pronounced influence on temperature tolerance and accounted for 26% of the individual variability in CT_{max}. This highlights not only the importance of cardiac function in determining overall hypoxia and temperature tolerance but also some of the underlying mechanisms that allow for such enhanced performance. Indeed, a relatively larger ventricle can generate a larger stroke volume for a given size fish (Franklin and Davie, 1992) and a larger percentage compact myocardium may allow for a greater pressure generating capacity, especially as hearts become larger and require a greater ventricular wall tension production (Laplace's law; Brijs et al., 2017; Farrell, 1991; Farrell et al., 2009). Similarly, ventricle size and percentage compact myocardium has been shown to positively correlate with other fitness traits such as migratory capacity in sockeye salmon (*Oncorhynchus nerka*), highlighting the relationship between these cardiac morphological traits and the ability of the fish to overcome the challenges they face in their upstream spawning migrations (Eliason et al., 2011). Our data also indicate that the impaired cardiac performance in hypoxia and during warming after coronary ligation was specifically related to an ischaemic compact myocardium, because there was a negative relationship between percentage compact myocardium and the peak $SV_{hypoxia}$ and CO at peak $SV_{hypoxia}$, as well as between percentage compact myocardium and peak $CO_{warming}$ and SV at peak $CO_{warming}$. We interpret this finding as individuals with a larger proportion of O_2-deprived compact myocardium suffered greater reductions in ventricular contraction force as luminal O_2 became increasingly limiting in hypoxic and warming conditions.

Conclusions

Although coronary arteries appear to have been present in the hearts of evolutionarily ancient fishes, they have been lost multiple times throughout their evolutionary history (Durán et al., 2015; Farrell et al., 2012), raising questions regarding the adaptive significance of their presence or absence (Farrell et al., 2012). Our results highlight that while coronary blood flow is not essential for the maintenance of routine cardiac output, its importance increases when fish are exposed to environmental extremes such as severe hypoxia and high temperatures. This allows fishes with high aerobic capacity like salmonids to increase cardiac work even during conditions and physiological states when cardiac luminal O_2 supply is severely constrained. As a corollary, our findings also highlight that pathological restrictions in coronary blood flow (e.g. coronary arteriosclerosis), which are known to be highly prevalent in both farmed and wild salmonids (Brijs et al., 2020; Farrell, 2002), may severely impact on cardiac capacity and animal performance traits. The coronary O_2 delivery is likely of particular adaptive benefit during episodes of elevated physical activity and high cardiac workloads (e.g. swimming during challenging spawning migrations), specifically when combined with environmental conditions that constrain luminal O_2 supply such as extreme temperatures and hypoxia as examined here.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: E.S., A.E.; Validation: D.M., A.E.; Formal analysis: D.M., T.M., A.G.; Investigation: D.M., T.M., A.E.; Resources: M.A., E.S.; Data curation: D.M.; Writing - original draft: D.M.; Writing - review & editing: D.M., T.M., A.G., M.A., E.S., A.E.; Visualization: D.M.; Supervision: E.S., A.E.; Project administration: E.S., A.E.; Funding acquisition: E.S., A.E.

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Data availability

Data are available from the Dryad digital repository (Morgenroth, 2021): dryad.kprr4xh4g

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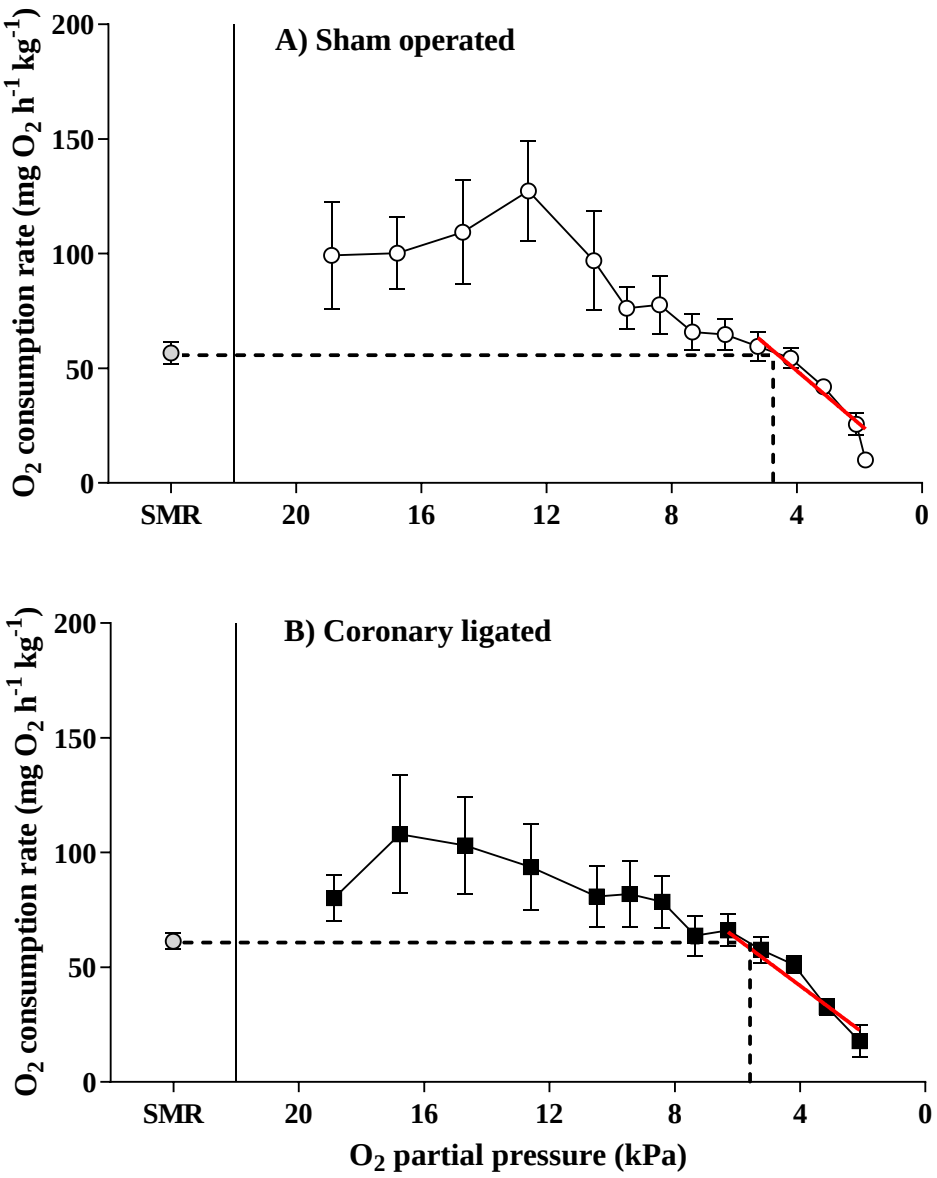

Figure S1. Method of determining the critical O_2 tension for oxygen consumption rate (P_{crit}) in rainbow trout. O_2 consumption of sham operated and coronary ligated rainbow trout during hypoxia. Whole-animal oxygen consumption (MO_2) in A) sham operated trout (open circles, n = 8) and B) coronary ligated trout (filled squares; n = 9). The grey data point on each graph represents standard metabolic rate (SMR). This plot includes only the MO_2 traces down to P_{LOE} of those individuals where P_{crit} could be estimated. Red lines represents the regression line including MO_2 values below and one data point above the interception point of SMR with the MO_2 traces. Values are means ± SEM.

Table S1. Summary of the coefficients of the multiple regression model for cardiovascular variables and environmental tolerances in sham operated and coronary ligated rainbow trout (*Oncorhynchus mykiss*).

		B	*Std. Error B*	*β*	*p*	95.0 % Confidence interval for *B*	
						Lower bound	Upper bound
P_{LOE}	Intercept	6.883	1.226		0.000	4.316	9.450
	Relative ventricular mass	-18.713	10.647	-0.303	0.095	-40.997	3.571
	Percentage compact myocardium	-4.410	2.125	-0.378	0.052	-8.857	0.038
	Peak $SV_{hypoxia}$	-2.230	0.666	-0.610	0.003	-3.625	-0.835
CT_{max}	Intercept	24.647	0.474		0.000	23.661	25.634
	Peak $CO_{warming}$	0.060	0.019	0.562	0.005	0.020	0.101
CT_{max}	Intercept	29.332	0.914		0.000	27.427	31.238
	P_{LOE}	-0.596	0.332	-0.331	0.087	-1.288	0.095
	Treatment	-1.013	0.404	-0.461	0.021	-1.856	-0.170

The output of the multiple linear regression model displaying the relative contribution of different variables in explaining the variability in temperature and hypoxia tolerance. The abbreviations are: unstandardized regression coefficient (*B*), standard error of *B* (*Std. Error B*) and standardized coefficient (*β*), PO_2 at loss of equilibrium (P_{LOE}), stroke volume in hypoxia ($SV_{hypoxia}$), upper critical thermal maximum (CT_{max}) and cardiac output during warming ($CO_{warming}$).