



Effects of hypoxic acclimation on contractile properties of the spongy and compact ventricular myocardium of steelhead trout (*Oncorhynchus mykiss*)

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Abstract

The trout ventricle has an outer compact layer supplied with well-oxygenated arterial blood from the coronary circulation, and an inner spongy myocardium supplied with oxygen poor venous blood. It was hypothesized that: (1) the spongy myocardium of steelhead trout (*Oncorhynchus mykiss*), given its routine exposure to low partial pressures of oxygen (PO₂), would be better able to maintain contractile performance (work) when exposed to acute hypoxia (100 to 10% air saturation) relative to the compact myocardium, and would show little benefit from hypoxic acclimation; and (2) the compact myocardium from hypoxia-acclimated (40% air saturation) fish would be better able to maintain work during acute exposure to hypoxia relative to normoxia-acclimated individuals. Consistent with our expectations, when PO₂ was acutely lowered, net work from the compact myocardium of normoxia-acclimated fish declined more (by ~73%) than the spongy myocardium (~50%), and more than the compact myocardium of hypoxia-acclimated fish (~55%), and hypoxic acclimation did not benefit the spongy myocardium in the face of reduced PO₂. Further, while hypoxic acclimation resulted in a 25% (but not significant) decrease in net work of the spongy myocardium, the performance of the compact myocardium almost doubled. This research suggests that, in contrast to the spongy myocardium, performance of the compact myocardium is improved by hypoxic acclimation; and supports previous research suggesting that the decreased contractile performance of the myocardium upon exposure to lowered PO₂ may be adaptive and mediated by mechanisms within the muscle itself.

Keywords Trout · Ventricle · Muscle · Hypoxia · Acclimation · Power

Introduction

Environmental hypoxia [a reduction in the percent air saturation of water oxygen levels, as reflected in a reduced partial pressure of oxygen, PO₂] is a stressor that can occur acutely (i.e., over minutes–hours) or over extended periods (i.e., weeks–months) in freshwater and marine environments (Altieri and Gedan 2015; Breitburg et al. 2018; Diaz 2001; Diaz and Rozenberg 2008; Kangur et al. 2005; La and Cooke

2011; Mulholland et al. 2005), and for some fish species has important physiological implications (e.g., Farrell and Richards 2009; Rodnick and Gesser 2017; Stecyk 2017). Many fishes possess mechanisms that allow them to live over a wide range of PO₂ values as might occur regularly in their habitats (Farrell and Richards 2009), and inter-individual variability in hypoxia tolerance of fish has been shown to reflect sensitivity of the contractile response of ventricular muscle to hypoxia (Joyce et al. 2016a, b). With respect to cardiovascular function, many species maintain or elevate cardiac performance (cardiac output) during acute hypoxia, at least to an extent that allows adequate oxygen transfer to the tissues (Farrell and Jones 1992; Gamperl and Driedzic 2009). However, the heart itself is comprised of highly aerobic tissue, and so it might be expected that the contractile abilities of the myocardium would be negatively affected by hypoxia. For example, myocardial inotropy tends to decline following exposure to acute hypoxia (e.g., Driedzic and Gesser 1994), a response that has been proposed to be the

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result of cellular oxygen sensing mechanisms, and perhaps, not simply a failure of muscle performance (Carnevale et al. 2020; Gesser and Rodnick 2019; Syme et al. 2013).

In addition to expecting a depressive effect of hypoxia, it might also be predicted that hypoxia-intolerant fish would acclimate to the local oxygen environment, via changes in cardiac performance or its sensitivity to reduced oxygen availability. However, there is only evidence that acclimation to hypoxia is detrimental to cardiac/myocardial performance in species such as Atlantic cod (*Gadus morhua*) and steelhead trout (*Oncorhynchus mykiss*). For example, prolonged (6–12 weeks) acclimation to hypoxia (40% air saturation) decreased Atlantic cod in vivo cardiac performance [stroke volume and cardiac output] during exercise (swimming) in normoxia, and reduced normoxic, in situ maximum cardiac function when compared to normoxia-acclimated fish (Petersen and Gamperl 2010a, b). Cod in vivo stroke volume was lower under normoxic conditions, and hypoxia-acclimated individuals had a reduced capacity to elevate both cardiac output and stroke volume when exposed to acute hypoxia (Petersen and Gamperl 2011). Trout exposed to chronic hypoxia were unable to increase cardiac output and stroke volume to the same extent as normoxia-acclimated conspecifics when exposed to an incremental increase in water temperature (i.e., a CT_{Max} test), and this diminished cardiac performance was not related to changes in heart morphology (i.e., relative ventricular mass, the percentage of compact myocardium, maximum end-diastolic volume or gross ventricular anatomy) (Motyka et al 2017). Finally, Carnevale et al. (2020) recently showed that the maximum net work and power of strips from the spongy myocardium of hypoxia-acclimated steelhead trout were consistently lower (by ~35% and ~15%, respectively) as compared to normoxia-acclimated fish, and also that recovery of myocardial contractile capacity following acute hypoxia was poorer. This latter study suggests that the compromised cardiac function observed in hypoxia-acclimated fish in vivo is a direct result of the loss of cardiac inotropy.

The ventricle of salmonids has two types of myocardium, an outer compacta and inner spongiosa, connected via collagenous fibers to maintain a physical link between the layers during contraction. Icardo (2017) and Farrell and Smith (2017) review anatomical and functional differences between these layers. These include an outer compact myocardium, associated with athletic species, formed by multiple layers of myocardial cells that wrap around the ventricle in a fashion that facilitates ejection, particularly high pressure; this layer comprises ~30–40% of ventricular mass in most salmonids, and is vascularized and receives well-oxygenated blood from the coronary circulation. The inner spongy myocardium has a trabecular organization, forming a central lumen and smaller peripheral chambers that are presumed to distribute stress and aid with near complete

ejection during systole. The spongy layer is perfused with venous blood that circulates through the trabecular sheets, and is, thus, exposed to low blood PO_2 during both normoxia and environmental hypoxia (Gamperl et al. 1994; Thomas et al. 1994). This configuration creates two different oxygen microenvironments, which might influence the contractile responses of these two ventricular tissue types to hypoxic acclimation. Thus, it is important to compare the myocardial performance of these tissue layers in response to oxygen deprivation before conclusions are made with respect to how myocardial dysfunction contributes to the loss of in vivo cardiac function following hypoxic acclimation.

We measured the work output of spongy and compact myocardial strips from steelhead trout acclimated to hypoxia (40% air saturation, ~8.4 kPa PO_2) vs. normoxia (100% air saturation, 21 kPa PO_2), before, during and after exposure to acute hypoxia (i.e., PO_2 levels of 13.1, 5.1 and 2.0 kPa). We hypothesized that: (1) the contractile performance of spongy myocardium, given its routine exposure to low PO_2 even during normoxia, would be less impaired by acute hypoxia and less influenced by hypoxic acclimation relative to the compact myocardium; and (2) the compact myocardium, which routinely experiences relatively high PO_2 , would accrue greater protective benefits from hypoxic acclimation than the spongy myocardium, so that the decrease in work from compact myocardium upon exposure to acute hypoxia would be less in strips from hypoxia-acclimated vs normoxia-acclimated fish.

Methods

Experimental animals and holding conditions

All applicable international, national, and institutional guidelines for the care and use of animals were followed. All animal care and handling procedures were approved by the animal care committees of both the University of Calgary (Protocol #AC14-0214) and Memorial University of Newfoundland (Protocol #15-89-kg), and followed guidelines of the Canadian Council on Animal Care. Juvenile, seawater-acclimated, steelhead trout were obtained from Cold Water Fisheries' (Coldwater, Ontario, Canada) cage-sites on the south coast of Newfoundland, Canada. The fish were initially housed for several months at the Dr. Joe Brown Aquaculture Research Building at the Ocean Science Centre of Memorial University of Newfoundland (St. John's, Newfoundland, Canada) at 14 °C, with salinity at 32 ppt and photoperiod at 12-h light: 12-h dark. After the initial holding period, eighty trout were divided equally between two ~1.2 m³ square tanks equipped with wooden lids to control for external stimuli (noise, human presence, etc.), that were supplied with 13–14 °C seawater

at 10 L min⁻¹ and had a water oxygen partial pressure of 19–20 kPa (i.e., ~95% air saturation). After a 2-week acclimation period, the water oxygen level in the tank that was to be made hypoxic was gradually lowered to the desired value (8.4 ± 0.1 kPa; 40% air saturation) over a period of 2 weeks. This was accomplished by: (1) slowly reducing the flow to the tank to 5 L min⁻¹ (i.e., allowing fish metabolism to partially reduce the water O₂ saturation); and (2) using a custom-designed system to control gas flow to the tank (Electronic Workshop, Memorial University of Newfoundland). This system monitored the oxygen saturation in the tank continuously by pumping tank water through an external circuit of tubing (Tygon Food, 6-419, Cole Parmer) that contained a galvanic oxygen electrode (CelIOx 325, WTW, Weilheim, Germany) placed in a D201 flow cell (WTW). This oxygen probe was connected to an oxygen meter (Oxi 340, WTW), which was interfaced with an electronic controller that set the open/closed position of two solenoid valves: one that bubbled pure N₂ gas into the tank when O₂ reached the upper limit of 9 kPa; the other bubbling air into the tank when oxygen saturation levels reached 8 kPa. Fish were then acclimated to either hypoxia or normoxia for a period of at least 8 weeks before experiments began. Fish in both tanks were fed daily with commercial trout pellets (Skretting 500 EP) at 1% of body mass. However, if the hypoxic fish failed to eat their full ration (satiation determined by the accumulation of a few pellets on the bottom of the tank), the normoxic fish were only given the same amount of food. Ammonia and nitrite levels were also monitored to ensure that these compounds did not exceed 0.02 and 0.5 mg L⁻¹, respectively.

Isolation of myocardial strips

For the experiments, fish (~500–650 g, Table 1) were netted from their respective acclimation tanks and euthanized using cerebral percussion followed by pithing of the brain and spinal cord. Immediately following euthanasia, the hearts were removed, the ventricle was separated from the atrium and bulbus arteriosus, cut in half, and placed in a dissection dish containing chilled (4 °C) physiological saline: in mM, NaCl 132; KCl 2.6; CaCl₂ 2.7; MgSO₄ 1.0; NaH₂PO₄ 1.0; glucose 10; HEPES buffer 10, pH = 7.8 (adapted from Altringham and Johnston 1990). From each ventricle, strips of compact and spongy myocardium (see Table 2) were dissected, with the cross-sectional area of the strips < 1 mm² to reduce/eliminate O₂ diffusion limitations in the tissues. For preparations of spongy myocardium, whole trabeculae were selected based on having a columnar shape and minimal branching of the muscle fibers. As muscle fiber alignment is not superficially apparent for compact myocardium, these preparations were selected based on the perceived alignment of fibres as observed during spontaneous contractions. Subsequent experiments were conducted on both spongy and compact strips from each fish simultaneously, using two identical systems, with the system used for spongy and compact strips alternated between each fish to avoid potential systematic biases.

Following dissection, lengths of 6–0 silk suture were secured to each end of the strips, and the strips were tied via these sutures to the arm of a servo motor (model 350, Cambridge Technology Inc., Bedford, MA, USA) and a force transducer (model 400a, Aurora Scientific, Aurora, ON, Canada). The muscle segments were then lowered into

Table 1 Body and cardiac morphometrics for the steelhead trout used in these experiments

Acclimation group	Fork length (cm)	Total length (cm)	Fish mass (g)	Relative ventricular mass	Ventricular mass (g)
Normoxia	31.5 ± 0.48	34.0 ± 0.54	600 ± 31	0.087 ± 0.0055	0.52 ± 0.036
Hypoxia	30.7 ± 0.46	33.1 ± 0.53	510 ± 34	0.090 ± 0.0076	0.46 ± 0.052

Data are means ± SEM, *N* = 19 normoxia, *N* = 18 hypoxia. Relative ventricular mass = ventricle mass/fish mass. None of the measurements were different between acclimation groups (two-sample *t* test, *P* > 0.05)

Table 2 Morphological parameters for the myocardial strips used to examine the effects of tissue type, hypoxic acclimation and acute hypoxic exposure on myocardial performance in steelhead trout

Ventricular tissue	Acclimation	<i>N</i>	Mass (mg)	Length (mm)	Cross-Sectional Area (mm ²)
Spongy	Normoxic	10	2.80 ± 0.46	6.10 ± 0.62	0.39 ± 0.068
	Hypoxic	9	2.36 ± 0.43	5.79 ± 0.26	0.40 ± 0.066
Compact	Normoxic	9	5.36 ± 1.1	7.02 ± 0.41	0.68 ± 0.081
	Hypoxic	9	4.96 ± 0.56	8.33 ± 0.44	0.60 ± 0.099

Data are means ± SEM. Values were not significantly different between acclimation groups within tissue type (*P* > 0.05), but strips taken from the compact myocardium had significantly greater values for mass, length and cross-sectional area as compared to those from the spongy myocardium (*P* < 0.05)

a chamber containing physiological saline that was maintained at 14 ± 0.2 °C using Peltier thermoelectric modules and a temperature controller (TC-24-12; TE Technology, Traverse City, MI).

Saline PO_2 was maintained at specific levels by bubbling a mixture of O_2 and N_2 gas delivered from a Wöstoff gas mixing pump (DIGAMIX 6KM301, Bochum, Germany) into a reservoir of saline that was then drained via stainless-steel tubing into the muscle chamber, circulated through the chamber and then back to the reservoir. Complete turnover of the saline in the 30-mL chamber occurred approximately once per min. The PO_2 in the muscle chamber was continuously monitored using a calibrated, fiber optic dipping oxygen probe (PSt3; PreSens, Regensburg, Germany) and oxygen meter (Fibox 3; PreSens).

Measurement of contractile performance

Custom software written using the LabView development system (National Instruments, Austin, TX) and a 12-bit analog/digital converter card (PCI MIO 16E 4; National Instruments) controlled the stimulator and servomotor (5 kHz D/A output), and collected muscle force, muscle length (servomotor arm position) and stimulus signals (1 kHz A/D input). The stimulator (Isostim A320, WPI, FL, USA) was connected to two platinum plates, one on each side of the submerged muscle preparation, and delivered a 1-ms stimulus pulse with voltage set 50% above that needed to obtain maximum force production.

Measurements of work were obtained from the spongy and compact myocardium of normoxia-acclimated fish ($N=10$ and 9 , respectively) and hypoxia-acclimated fish ($N=9$ and 9 , respectively) at $50 \text{ beats min}^{-1}$ (BPM) using the work loop method. This method measures the ability of the strips to do work during repeated cycles of lengthening and shortening (strain) that mimic those during contraction of a beating heart (Carnevale et al. 2020; Harwood et al. 1998; Syme et al. 2013; Syme and Josephson 1995). The resting length of each preparation was initially optimized to obtain maximum work output. To establish the optimum length, the strips were subjected to a series of 5 cycles of 10% strain at a frequency of 50 BPM, with the net work output from the final strain cycle used for comparison. The length of the preparation was increased in increments of 0.1 mm between series of work measurements until net work output no longer increased. This optimum length was then used for all subsequent measures of muscle performance. Fifty BPM was chosen as the contraction frequency as it approximates the in vivo heart rate for rainbow trout at temperatures similar to those used in the present study (Farrell and Smith 2017).

Two measures of isometric twitch kinetics were first recorded at 100% air saturation (normoxia). The measures

were: time taken for force to rise from 10 to 90% of maximal during contraction; and the time taken for force to fall from 90 to 10% during relaxation. These values were obtained from the last twitch in a series of 30 isometric twitches at a frequency of 50 BPM.

The effects of acute hypoxia exposure on isometric force and work output of spongy and compact strips, from the normoxia- and hypoxia-acclimated groups, were then measured. Work output and twitch force were measured at 100% air saturation ($PO_2 = 21$ kPa), 65% air saturation ($PO_2 = 13.1$ kPa), 25% air saturation ($PO_2 = 5.1$ kPa), and 10% air saturation ($PO_2 = 2.0$ kPa). These values of PO_2 were chosen specifically to mimic those in the spongy and compact myocardium during environmental normoxia and hypoxia; 65% and 25% air saturation are near the in vivo arterial (P_aO_2) and venous (P_vO_2) oxygen partial pressures, respectively, during environmental normoxia, while 10% air saturation is close to P_vO_2 during hypoxia (Gamperl et al. 1994). The PO_2 was briefly (for about 10 min) returned to 100% air saturation between the measures taken at 25% and 10% air saturation, allowing for a brief period of recovery from severe hypoxia to reduce the cumulative effects of repeated hypoxic exposure.

For measures of isometric force, each preparation was subjected to 30 consecutive contractions at 50 BPM with the muscle held at a fixed length. For measures of work, preparations were subjected to stimuli during 30 strain cycles at a frequency of 50 BPM, and a strain amplitude of 10% applied in a sinusoidal trajectory. The proportion of the sine cycle that comprised shortening versus lengthening was adjusted for each preparation at each PO_2 so that net work output was maximized; the intent was to closely match the period of muscle shortening with the period of muscle contraction (i.e., active force generation), with the remainder of the cycle comprising muscle lengthening, similar to how a beating heart functions in vivo (Carnevale et al. 2020).

Following exposure to graded hypoxia, the PO_2 was returned to 100% air saturation for 20 min., and work output was measured again at 50 BPM using a set of 30 strain cycles. This final measure of work output was compared to the initial measure of work output at 100% air saturation to assess recovery of the strips from acute hypoxia.

Analysis

After the experiments were completed, the preparations were removed from the apparatus and viewed under a microscope, and tissue that was outside of the ties or clearly non-viable was dissected free from the strips. The strips were then blotted on filter paper to remove surface moisture, and weighed (Mettler-Toledo MT5, Highston NJ, USA). The optimal length, mass and cross-sectional area of the strips are presented in Table 2.

For the analysis of developed isometric twitch force (the difference between resting and maximum force during an isometric twitch), force was expressed relative to muscle cross-sectional area (kN m^{-2}) assuming a muscle density of 1.05 g/cm^3 . For measures of work, work was expressed relative to muscle mass (J kg^{-1}). The net work output from ventricular strips was calculated as the difference between the shortening work and lengthening work done during a complete cycle. Shortening work is produced by the muscle during shortening (contraction), and lengthening work is performed by the servomotor as it lengthens the preparations during relaxation. Work was measured from the last cycle in each series of 30. However, when net work differed by more than 10% between the last two cycles (29 and 30) of a series (i.e., when work tended to oscillate between successive cycles), that particular measure was excluded from analysis as such instability was not considered representative of normal function (i.e., the membrane action potential was likely becoming refractory). In the compact myocardium from normoxia-acclimated fish, this occurred once at 100% air saturation, once at 65% air saturation, twice at 25% air saturation, and three times at 10% air saturation, but did not occur in compact tissue from hypoxia-acclimated fish or any spongy myocardial strips.

Values of area-specific force from spongy and compact myocardium at all values of PO_2 studied, and mass-specific work measured at 100% air saturation, are provided in the results to allow for the assessment of differences between the two tissue types and the effects of acclimation on contractile performance. The pattern of differences in work between the tissue types and acclimation states was consistent across all values of PO_2 tested. Differences between tissue types not only may reflect a true difference in contractile capacity, but may also reflect experimental factors that could impact mass- and area-specific measures of force and work (see “Discussion”). Thus, to avoid complications in interpretation arising from this potential experimental variability, the data for assessing the impact of PO_2 on work was normalized to measurements made at 100% air saturation for each preparation. This allowed for an assessment of the relative effect of PO_2 on work in the two tissue types and acclimation states independent of the mass of the muscle strip. However, statistical analysis was performed on the non-normalized, mass-specific values, using repeated-measures analyses to account for variability of mass between preparations.

Analyses of contraction and relaxation twitch kinetics were conducted using two-way ANOVAs with the main effects of acclimation and tissue, and including an interaction term. The other contractile properties (developed isometric twitch force; net, lengthening and shortening work) were analyzed using a mixed-effects model with the fully crossed fixed-effects structure: $\text{PO}_2/\text{tissue type}/\text{acclimation}$. Because of variability between tissue types

that might be introduced into the analysis due to biases in dissection (preparation dimensions, amount of non-viable tissue), measures of muscle work were also analyzed separately within each tissue type, with the fully crossed fixed-effects structure: $\text{PO}_2/\text{acclimation}$. A random intercept term was included in both these models for individual preparations to control for variability in preparation-specific performance (i.e., due to the non-independence of repeated measures on tissues, and the size differences amongst myocardial strips). The proportion of net work measured when the PO_2 was returned to air saturation following hypoxia exposure, relative to the beginning of the experiment, was compared between tissues and acclimation groups using a mixed-effects model: $\text{pre/post hypoxia exposure} \times \text{tissue type} \times \text{acclimation}$, again with a random intercept estimated for each preparation. P-values of the fixed effects in mixed-effects models were estimated using Satterthwaite estimation of denominator degrees of freedom. Degrees of freedom using this method are estimated from one moment and two moment approximation (Fai and Cornelius 1996; Kuznetsova et al. 2013). All analyses were conducted using SAS v9.4 (SAS 2012). Significant differences were reported when $P < 0.05$, and Tukey’s correction factors were applied to post hoc contrasts.

Results

None of the measures of trout and ventricle size differed significantly between acclimation groups (Table 1). The strips of compact myocardium dissected from the ventricles and used for experiments had a greater mass, length and cross-sectional area than those from the spongy myocardium (Table 2). This was a result of their different morphology which necessitated different approaches to dissection. However, the size of the muscle strips of each tissue type was not different between acclimation groups.

Effects of acclimation and tissue type

Twitch kinetics

Isometric twitch contraction and relaxation kinetics did not differ significantly between the acclimation states within either tissue type (Fig. 1). However, both contraction and relaxation took significantly longer in the spongy myocardium as compared to the compact myocardium. The interaction between acclimation and tissue type was not significant in either measure of twitch kinetics (contraction $P = 0.59$, relaxation $P = 0.18$).

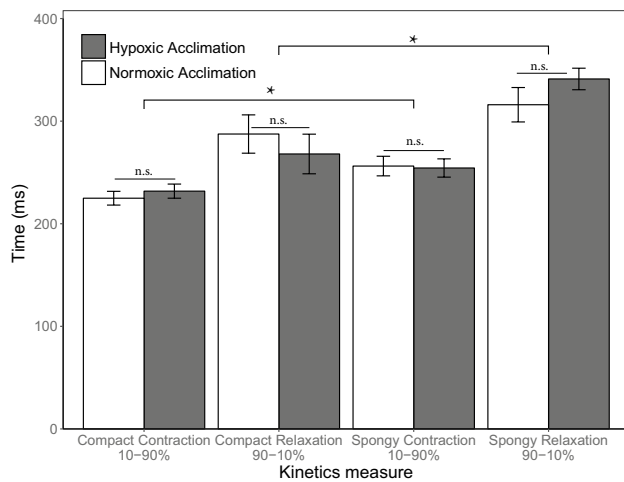


Fig. 1 Isometric twitch kinetics during contraction (time for force to rise from 10 to 90% of maximal) and relaxation (time for force to fall from 90 to 10% of maximal) of spongy and compact myocardium from steelhead trout acclimated to normoxia or hypoxia (40% air saturation). All measures were made in 100% air saturation. Twitches were measured at a contraction frequency of 50 BPM. Data are means \pm SEM. There were no significant differences between acclimation states for either tissue type (contraction $P=0.925$, relaxation $P=0.866$); horizontal bars with n.s. span groups that are not significantly different. Spongy myocardium had significantly longer durations of contraction and relaxation than the compact myocardium ($P=0.0022$ and $P=0.0046$, respectively); horizontal brackets with asterisks span groups that are significantly different. Spongy normoxia $N=10$, spongy hypoxia $N=9$, compact normoxia $N=9$, compact hypoxia $N=9$

Force and work

The spongy myocardium produced more force (by \sim three-fold), shortening work (by \sim 2.2-fold) and net work (by \sim 3.7-fold), but not lengthening work, than the compact myocardium on average across the two acclimation states (Fig. 2, Tables 3 and 4). Hypoxic acclimation did not affect isometric force produced by either the spongy or compact myocardium (Fig. 2). In spongy myocardium, while there was a trend toward reduced net and shortening work with hypoxic acclimation, this effect was not significant when measured at 100% air saturation (Table 3), either when the results from spongy and compact were analyzed together across all values of PO_2 tested (Table 4) or when the spongy myocardium was analyzed separately (Table 5). Conversely, for compact myocardium, hypoxic acclimation resulted in a substantial increase in net (\sim 200%) and shortening (\sim 25%) work, but not lengthening work, compared with normoxic acclimation; this effect was statistically significant when the data for compact myocardium was analyzed separately (Table 5), but not when results at only 100% air saturation were compared (Table 3) or when the results from the spongy and compact myocardium were analyzed together across all values of PO_2 tested (Table 4).

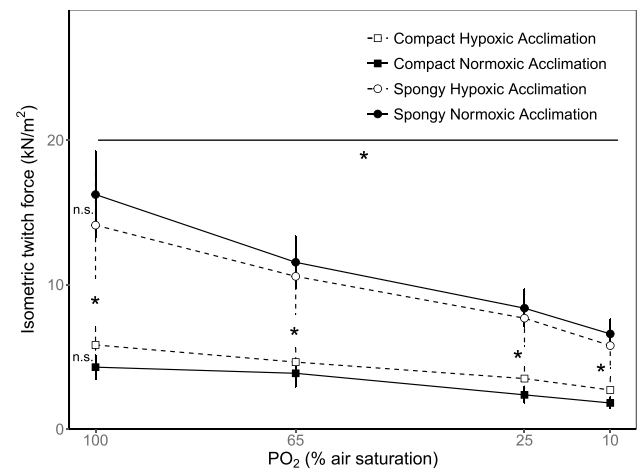


Fig. 2 Developed isometric twitch force of spongy and compact myocardium from steelhead trout acclimated to normoxia or hypoxia, when acutely exposed to different PO_2 levels. Data are means \pm SEM. Force declined with reduced PO_2 in both tissue types ($P<0.001$); horizontal bar with asterisk spans groups that are significantly different across PO_2 . There was no effect of hypoxic acclimation on twitch force in either tissue type ($P=0.591$, n.s. indicates no significant difference between acclimation states), and no significant interactions (PO_2 /tissue type, $P=0.320$; PO_2 /acclimation, $P=0.11$; tissue type/acclimation, $P=0.52$; PO_2 /tissue type/acclimation, $P=0.80$). The spongy myocardium produced higher force than the compact ($P=0.002$); asterisks indicate significant difference between spongy vs compact groups. Spongy normoxia $N=10$, spongy hypoxia $N=9$, compact normoxia $N=9$, compact hypoxia $N=9$

Effects of acute hypoxia on myocardial performance

Developed isometric twitch force declined as PO_2 was lowered in both tissue types (Fig. 2). No significant interactions were found between PO_2 /tissue type, PO_2 /acclimation, tissue type/acclimation, or PO_2 /tissue type/acclimation, suggesting that both tissue types from normoxia- and hypoxia-acclimated fish responded similarly to reduced PO_2 .

Net work also declined with reduced PO_2 in both tissue types and acclimation states (Fig. 3, Tables 4 and 5). A significant interaction between tissue type and PO_2 (Table 4), a near-significant ($P=0.059$) interaction between tissue type and acclimation state (Table 4), and a significant acclimation effect for compact myocardium (Table 5), collectively indicate that with reduced PO_2 there was a significantly larger decline in net work output in compact myocardial strips from normoxia-acclimated fish (\sim 73%) as compared to those from hypoxia-acclimated fish and as compared to the spongy myocardium (\sim 55% for both layers) (Fig. 3). None of the other interactions were significant (Tables 4 and 5).

Similar to net work, shortening work declined significantly with reduced PO_2 in both tissue types (Fig. 4, Tables 4 and 5). A significant tissue type/ PO_2 interaction (Table 4), a near-significant ($P=0.076$) tissue type/acclimation interaction (Table 4), and an acclimation effect

Table 3 Mass-specific work performed by the compact and spongy myocardium from fish acclimated to normoxia and hypoxia, all measured at the start of the experiment at 100% air saturation

Ventricular tissue type	Acclimation	Shortening work (J kg ⁻¹)	Lengthening work (J kg ⁻¹)	Net work (J kg ⁻¹)
Spongy	Normoxic	1.30 ^a ± 0.104	0.305 ^a ± 0.040	1.00 ^a ± 0.0792
	Hypoxic	1.08 ^{ab} ± 0.181	0.311 ^a ± 0.080	0.768 ^a ± 0.119
Compact	Normoxic	0.478 ^b ± 0.104	0.319 ^a ± 0.091	0.157 ^b ± 0.0729
	Hypoxic	0.602 ^b ± 0.210	0.280 ^a ± 0.0940	0.321 ^b ± 0.137

Data are means ± SEM, *N* = 9 except spongy normoxia *N* = 10. Values with superscripted letters in common are statistically different from other acclimation state and/or tissue type (*P* < 0.05)

Table 4 *F*- and *P*-values for the effects of tissue, oxygen level, acclimation state and their interactions on net, shortening and lengthening work (mass specific) produced by the spongy and compact ventricular strips

Effect	Net work		Shortening work		Lengthening work	
	<i>F</i> value	<i>P</i>	<i>F</i> value	<i>P</i>	<i>F</i> value	<i>P</i>
Main effects						
Tissue	21.09	<0.001	10.92	0.003	0.34	0.564
PO ₂	37.64	<0.001	33.45	<0.001	4.71	0.004
Acclimation	0.66	0.425	1.44	0.241	0.26	0.613
Interactions						
Tissue*PO ₂	12.84	<0.001	7.25	<0.001	0.038	0.990
Tissue*acclimation	3.92	0.059	3.40	0.076	0.47	0.499
PO ₂ *acclimation	0.12	0.948	0.24	0.869	0.64	0.592
Tissue* PO ₂ *acclimation	0.46	0.709	0.12	0.947	1.11	0.358

Table 5 *F*- and *P*-values for the effects of oxygen level, acclimation state and their interactions on the net, shortening and lengthening work (mass specific) produced by the ventricular strips when analyzed within each tissue type

Effect	Net work		Shortening work		Lengthening work	
	<i>F</i> value	<i>P</i>	<i>F</i> value	<i>P</i>	<i>F</i> value	<i>P</i>
Compact myocardium						
Main effects						
PO ₂	10.72	<0.001	5.80	0.002	0.82	0.491
Acclimation	4.86	0.042	3.59	0.076	0.93	0.351
Interaction						
PO ₂ *acclimation	1.03	0.386	0.19	0.902	0.19	0.899
Spongy myocardium						
Main effects						
PO ₂	31.8	<0.001	34.36	<0.001	8.28	<0.001
Acclimation	1.34	0.263	0.70	0.414	0.01	0.913
Interaction						
PO ₂ *acclimation	0.16	0.922	0.19	0.902	0.84	0.480

for compact myocardial strips (Table 5) indicate a significantly smaller effect of reduced PO₂ on shortening work from hypoxia- vs normoxia-acclimated compact myocardium (Fig. 4). None of the other interactions were significant (Tables 4 and 5).

Reduced PO₂ resulted in a statistically significant reduction in lengthening work, but only in the spongy myocardium (Fig. 5, Tables 4 and 5). However, the extent of the decline was quite modest, ranging from essentially no decline even at the lowest PO₂ tested in spongy and compact myocardial strips from fish acclimated to hypoxia, to about

20% in muscle acclimated to normoxia. Again, none of the interactions were significant (Fig. 5).

Net work measured at 100% air saturation following recovery from the series of acute reductions in PO₂ was significantly lower than prior to reducing PO₂, in both tissue types and acclimation states (*P* = 0.006 for all comparisons), although the magnitude of the reduction was relatively small: post/pre ratio = 0.821 ± 0.174 SEM for compact myocardium from normoxia-acclimated fish, 0.915 ± 0.077 for compact myocardium from hypoxia-acclimated fish, 0.955 ± 0.087 for spongy myocardium

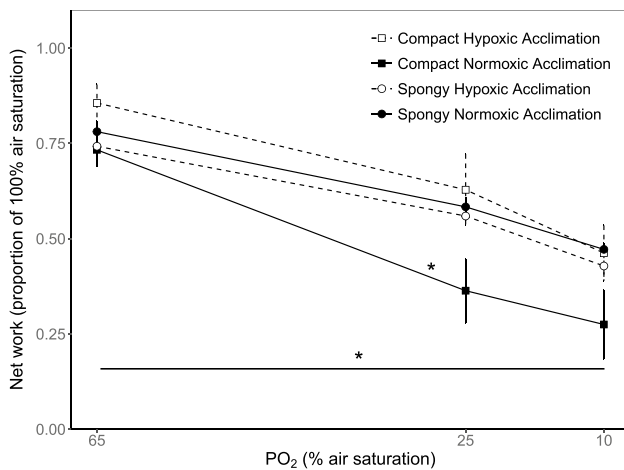


Fig. 3 Net work from the spongy and compact myocardium from steelhead trout acclimated to normoxia or hypoxia, when acutely exposed to different PO₂ levels. Values are expressed as a percentage of that measured initially at 100% air saturation. Data are means \pm SEM. Horizontal bar with asterisk spans groups that are significantly different across PO₂. Asterisk indicates significant effect of normoxic acclimation on compact myocardium vs hypoxic and spongy in either acclimation state. Spongy normoxia $N=10$, spongy hypoxia $N=9$, compact normoxia $N=9$, compact hypoxia $N=9$

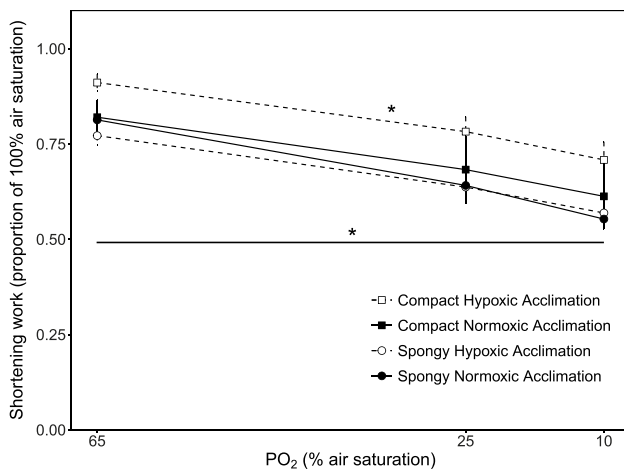


Fig. 4 Shortening work from the spongy and compact myocardium from steelhead trout acclimated to normoxia or hypoxia, when acutely exposed to different PO₂ levels. Values are expressed as a percentage of that measured initially at 100% air saturation. Data are means \pm SEM. Horizontal bar with asterisk spans groups that are significantly different across PO₂. Asterisk indicates significant effect of hypoxic acclimation on compact myocardium vs normoxic and spongy in either acclimation state. Spongy normoxia $N=10$, spongy hypoxia $N=9$, compact normoxia $N=9$, compact hypoxia $N=9$

from normoxia-acclimated fish, and 0.818 ± 0.063 for spongy myocardium from hypoxia-acclimated fish. Further, the proportion of net work recovered (post/pre) was not significantly different between tissue types ($P=0.856$) or acclimation states ($P=0.84$).

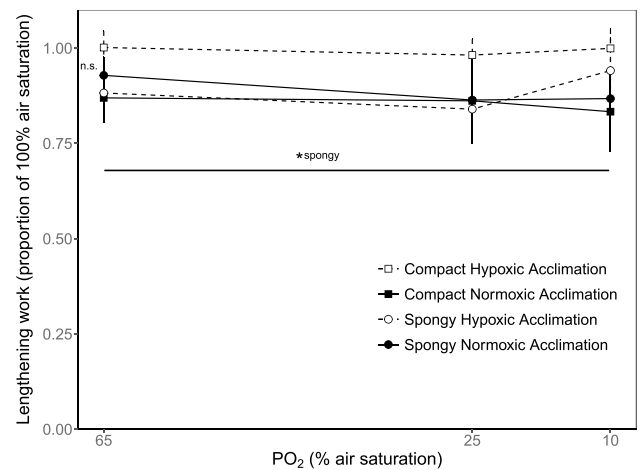


Fig. 5 Lengthening work from the spongy and compact myocardium from steelhead trout acclimated to normoxia or hypoxia, when acutely exposed to different PO₂ levels. Values are expressed as a percentage of that measured initially at 100% air saturation. Data are means \pm SEM. Horizontal bar with asterisk spans spongy myocardial groups that are significantly different across PO₂; PO₂ did not affect lengthening work of compact myocardium. n.s. indicates no significant effect of acclimation state within spongy or compact tissue. Spongy normoxia $N=10$, spongy hypoxia $N=9$, compact normoxia $N=9$, compact hypoxia $N=9$

Discussion

Differences in work and force between tissue types

The spongy myocardium produced more isometric force, and mass-specific shortening and net work, as compared to the compact myocardium (Fig. 2, Table 3). Because the trout heart is mostly composed of spongy myocardium (i.e., > 60%; Farrell et al. 2009; Farrell and Smith 2017), this may be of great functional significance to cardiac output, also when considering the decrease in work capacity in the spongy myocardium vs. the increase in compact myocardium following hypoxic acclimation. However, understanding the physiological implications of this difference would require a detailed analysis of ventricular anatomy and mechanics in the trout heart. There are also other factors that could have influenced these results. While attempts were made to keep both preparations similar in size, muscle strips isolated from the compact myocardium were larger than those from the spongy layer due to differences in the physical characteristics of the tissue in the different layers (Table 2). Preparation size should not impact mass- and area-specific measures of muscle contractile capacity, or responses to acclimation and PO₂. The larger size of the compact myocardial preparations could result in greater oxygen diffusion limitations compared with spongy, but most of the differences in preparation size were associated with differences in length, while differences in cross-sectional area were modest. Furthermore, it

is unlikely that small differences in diffusion distance could account for the spongy myocardium outperforming compact across the full spectrum of PO_2 s tested; i.e., to the extent that performance of the compact myocardium at the highest PO_2 remained less than the spongy myocardium at the lowest PO_2 tested (Fig. 2).

Tissue differences in muscle fiber orientation and water content could also have contributed to the reported differences in mass-specific work. Fiber orientation was more evident and could more readily be accounted for during dissection of spongy myocardial preparations. This likely resulted in more of the muscle fibers in spongy myocardial strips being oriented parallel to the long-axis of the preparation, and more of the strip being comprised of intact, viable muscle. In addition, the spongy myocardium could have potentially lost more water when blotted before weighing due to its lower fiber density (packing), leading to higher values of mass-specific work and area-specific force. Assessment of the effects of acclimation would be less impacted by such experimental factors, as the physical characteristics of strips from normoxia- and hypoxia-acclimated fish were similar within a tissue type (Table 2), and such differences would have little impact on the effects of reduced PO_2 .

Effects of hypoxic acclimation

Previous studies report that hypoxic acclimation affords improved performance in the hearts of some fish when exposed to subsequent acute hypoxia (Driedzic et al. 1985; Faust et al. 2004; Joyce et al. 2016a, b; Petersen and Gamperl 2010a), but there is mounting evidence that it may impair aspects of myocardial contractile capacity in some fishes, including trout (e.g., see Carnevale et al. 2020). The present study provides evidence of both effects and, notably, different responses in the spongy vs. compact myocardium. Mass-specific shortening and net work output of the spongy myocardium were approximately 25% lower (although not significantly) in hypoxia- vs. normoxia-acclimated trout when measured at 100% air saturation (Tables 3, 4 and 5). This is consistent with the approximately 35% lower mass-specific net work from the spongy myocardium of hypoxia- vs normoxia-acclimated steelhead trout when stimulated at 50 BPM (Carnevale et al. 2020). Little or no change in the work capacity of the spongy myocardium following hypoxic acclimation is also consistent with the hypothesis that the spongy myocardium would be relatively insensitive to hypoxic acclimation due to it normally being exposed to relatively low PO_2 levels even during environmental normoxia, and that it experiences only a relatively small drop in PO_2 during environmental hypoxia (Gamperl et al. 1994; Thomas et al. 1994). A decrease in work capacity following hypoxic acclimation could be interpreted as an impairment due to limited oxygen availability, perhaps as a result of

cardiac remodeling or stunning (Bolli and Marban 1999; Carnevale et al. 2020), or a regulated response (discussed below).

In contrast to the spongy myocardium, there was a 20–25% increase in mass-specific shortening and net work in compact myocardial strips following hypoxic acclimation (Tables 3, 4 and 5). An increase in the work output of the compact myocardium from hypoxia-acclimated trout, and the fact that no compact myocardial strips from hypoxia-acclimated fish became refractory to stimulation whereas several from normoxia-acclimated fish did (see “Methods”), is also consistent with the hypothesis that acclimation to hypoxia would improve the resistance of this tissue to subsequent hypoxia exposure, given that it would experience a notable drop in PO_2 during hypoxic acclimation. However, a substantial increase in performance following hypoxic acclimation might seem surprising. If hypoxia limits energy availability, it might be expected that performance would be constrained, or maintained, but not enhanced. The higher PO_2 of blood perfusing the compact myocardium, even during hypoxia (Gamperl et al. 1994; Thomas et al. 1994) may support an enhanced capacity for work in compact myocardium following hypoxic acclimation, and only a reduced or maintained capacity in spongy myocardium. The cellular basis for such changes is not known. Differences in pH sensitivity and buffering capacity between the tissue layers may be important, as pH has a pronounced effect on contractile capacity of ventricular myocardium (e.g. Driedzic and Gesser 1994; Shiels et al. 2010). The compact layer may be better able to resist acidosis in hypoxia, resulting in maintained or improved performance compared to spongy. An increase in the performance of the compact myocardium following hypoxic acclimation also suggests that it might be a regulated response (see below).

In contrast to work, there was no effect of hypoxic acclimation on isometric twitch force in spongy and compact myocardium (Fig. 2). Isometric force would be most representative of myocardial contractile capacity during the isovolumetric portion of the cardiac cycle rather than ejection (Syme 1993), and there is evidence that isometric force is not always a reliable indicator of the work capacity of fish myocardium (Syme et al. 2013). Hence, this lack of an effect of acclimation on force, in contrast to work, may not be unusual. Twitch kinetics (Fig. 1) were also not significantly affected by hypoxic acclimation in either tissue layer, and Carnevale et al. (2020) reported only small effects (less than 10%) of hypoxic acclimation on the duration of trout spongy myocardial contraction and relaxation, perhaps suggesting little effect of acclimation on cardiac chronotropy. However, the enhanced ability of compact myocardial strips from hypoxic- vs. normoxia-acclimated fish to avoid becoming refractory to stimulation, particularly when exposed to low PO_2 , suggests that chronic hypoxia may increase the

maximum contraction rate (heart rate) at which this tissue can maintain contractility.

Effects of acute hypoxia

The > 40% decline in myocardial net work following acute reductions in PO₂, similar to what the myocardium might experience during environmental hypoxia (Fig. 3, Tables 4 and 5) is comparable to that measured in strips from normoxia-acclimated steelhead trout over a similar decrease in PO₂ (Carnevale et al. 2020), to the 20–25% drop in net work produced by the compact and spongy myocardium of steelhead trout when saline PO₂ was reduced from what the tissues experience in environmental normoxia vs. 60% air saturation (Roberts and Syme 2018), and to the ~55% drop in net work produced by isolated spongy myocardial strips from normoxia-acclimated Atlantic cod following a PO₂ change from ~21 kPa to 5.1 kPa (Syme et al. 2013). A drop in contractile force with reduced PO₂ is also commonly reported (Driedzic and Gesser 1994; Gamperl and Driedzic 2009; Rodnick and Gesser 2017). Interestingly, force and the work capacity of fish ventricular myocardium change with acute changes in PO₂ under conditions that are physiologically hypoxic through hyperoxic (Fig. 2; Gesser and Rodnick 2019). These responses speak to a potential role for oxygen sensing in the myocardium, rather than simply a failure of muscle contractility when PO₂ is reduced.

The response of compact myocardium to acute reductions in PO₂ showed a significant acclimation effect, with a greater decline in net work in normoxia- vs hypoxia-acclimated fish, and smaller reductions in shortening work in hypoxia- vs normoxia-acclimated fish (Figs. 3 and 4). Furthermore, there was no effect of hypoxic acclimation on the response to reduced PO₂ in the spongy myocardium (Figs. 3 and 4). Hypoxic acclimation may thus protect the contractile capacity of the compact but not spongy myocardium against the effects of acute hypoxia. In addition, Carnevale et al. (2020) noted that the effect of acclimation on the reduction in net work with acute hypoxia in spongy myocardial strips of steelhead trout was modest at 30 and 50 bpm, but more pronounced at 70 and 90 BPM. Our experiments were only conducted at one contraction frequency (50 BPM), and thus, the results may be different at higher contraction frequencies. In addition, the effects of adrenergic agonists were not examined in the present study, but may afford additional mechanisms to adjust myocardial contractility. Effects of adrenergic tone on fish myocardial performance in vivo are often modest, but under conditions of stress, such as reduced PO₂ and following hypoxic acclimation, they appear to be more substantial (e.g., Blank et al. 2002; Farrell 1984). While little is known about the effects of adrenergic tone on the capacity of isolated myocardium to produced power, independent of its effects in vivo, it appears to support these

in vivo observations. Hanson et al. (2006) noted that maximal adrenergic stimulation (500 nmol l⁻¹), but not routine (5 nmol l⁻¹), fully preserved maximal cardiac output and power in in situ rainbow trout hearts down to 2.0 kPa PO₂ (i.e. near venous PO₂ during intense activity), including in the presence of hyperkalemic and acidotic conditions that would accompany such exercise, but that cardiac output fell when PO₂ was reduced further. Likewise, the effects of adrenergic stimulation on work capacity of isolated trout myocardium are small in normoxia, but substantial in hypoxia, and different in the different tissue layers (Roberts and Syme 2018). Hence, adrenergic stimulation likely has an important role in protecting cardiac contractile capacity during hypoxia.

The differential effects of hypoxic acclimation on spongy vs compact myocardium resulted in similar relative changes in net work with reduced PO₂ in the two tissue layers, but only in the hypoxia-acclimated state. Spongy myocardium working at 2.1 kPa PO₂ (similar to venous PO₂ during environmental hypoxia) produced about 80% of the net work compared to working at 5.1 kPa PO₂ (the venous PO₂ during environmental normoxia), regardless of acclimation state (Fig. 3). And while the net work of the compact myocardium at 5.1 kPa PO₂ (an estimate of arterial PO₂ during environmental hypoxia) was only 50% of that at 13.1 kPa PO₂ (arterial PO₂ during environmental normoxia) if normoxia acclimated, it was about 85% if hypoxia acclimated, similar to results for the spongy myocardium (Fig. 3). Hence, when normoxia acclimated, the compact myocardium fails disproportionately during acute hypoxia as compared to the spongy myocardium, but when hypoxia acclimated, both tissue types are able to maintain at least 80% of their normoxia-acclimated performance during environmental hypoxia, which likely has important consequences for the working capacity of the intact heart.

The decrease in net work output at lower PO₂ levels in cod, rainbow trout, and steelhead trout spongy myocardium has been associated almost entirely with a decrease in shortening work (inotropy), and not to a change in lengthening work (lusitropy) (Carnevale et al. 2020; Roberts and Syme 2018; Syme et al. 2013). In the present study on steelhead trout, low PO₂ resulted not only in a large decline in shortening work (Fig. 4), but also in a small decrease in lengthening work in the spongy myocardium (Fig. 5, Table 5), although the changes in lengthening work were quite modest (10–20%) compared to the changes in shortening work (~40% or greater). Hence, changes in inotropy appear to dominate with regards to the effects of changes in PO₂ on net work and power output. Yet, even a small decline in lengthening work would effect a decreased amount of work required to extend the myocardium during acute hypoxia exposure, and this may benefit diastolic ventricular filling. When hypoxic bradycardia occurs, there is an accompanying

increase in stroke volume (Gamperl et al. 1994). The decline in lengthening work during acute hypoxia would promote ventricular filling and might contribute to an increase in stroke volume during hypoxia, without requiring changes to atrial contractility or vascular resistance. Nonetheless, these results are in contrast to those reported by others (Carnevale et al. 2020; Roberts and Syme 2018; Syme et al. 2013), and thus, the importance of changes in lengthening work to cardiac function during hypoxia in fishes remains unclear.

The mechanisms responsible for the decline in work output with reduced PO_2 are not known. Myocardial stunning as a result of reduced PO_2 is a possibility (see Carnevale et al. 2020 for a discussion). However, the rapidity of the response in both tissues, the finding that the magnitude of the response was blunted in the compact myocardium following hypoxic acclimation, and observations that there is a PO_2 effect on inotropy across a very wide range of PO_2 values that encompasses hyperoxia through to severe hypoxia (e.g., the present study; Carnevale et al. 2020; Gesser and Rodnick 2019; Roberts and Syme 2018; Syme et al. 2013) are less consistent with stunning, and suggest that these changes are a result of an oxygen sensing mechanism at the cellular level. This could perhaps be an energy sparing mechanism upon exposure to reduced PO_2 , rather than just a reduction of work output through failure of the myocardium to contract (Carnevale et al. 2020; Gesser and Rodnick 2019; Syme et al. 2013). Several rapidly acting mechanisms could be involved in cellular oxygen sensing by the myocardium (Cserne Szappanos et al. 2017; Fago et al. 2012; Hool 2015; Scaringi et al. 2013; and see Gesser and Rodnick 2019 and Carnevale et al. 2020 for further discussion). The reduced sensitivity of the compact myocardium to reduced PO_2 following hypoxic acclimation is a very interesting/novel finding, and is consistent with changes in mass-specific work capacity of ventricular myocardium following hypoxic acclimation as noted in the present study and by Carnevale et al. (2020). These changes may involve the resetting of mechanisms that impart oxygen sensitivity, such that the muscle does less work at a given PO_2 following hypoxic acclimation. While the functional basis and mechanisms responsible for cellular regulation of muscle contraction associated with PO_2 are currently unknown in fish, the existence of rapid, reversible, and broad scale regulation of work output in response to PO_2 is supported by the present study.

The extent of the recovery of net work on returning the myocardium to 100% air saturation following exposure to reduced PO_2 was similar between the two acclimation states and tissue types, and was quite high (82–95%; see “Results”). This degree of recovery is similar to other studies that have acutely exposed fish myocardium to reduced PO_2 (Carnevale et al. 2020; Gamperl et al. 2001; Joyce et al. 2016a, b; Peterson and Gamperl 2010a; Roberts and Syme 2018; Syme et al. 2013). While Carnevale et al.

(2020) reported that the spongy myocardium of hypoxia-acclimated steelhead trout did not recover as well as that from normoxia-acclimated individuals following exposure to graded hypoxia down to 10% air saturation, those strips were exposed to a longer acute hypoxic protocol (i.e., more steps in PO_2) and not allowed to recover (i.e., exposed to 100% air saturation) between levels of hypoxia. This effect on recovery suggests that the capacity of hypoxia-acclimated myocardium to recover from hypoxia depends on the severity and/or duration of the hypoxic challenge. Collectively, these data suggest that the fish myocardium is able to rapidly restore a high proportion of its initial work output on return to high PO_2 , a finding that again supports the existence of an active sensing mechanism rather than simply fatigue or other type of failure of the tissue.

Conclusions and perspectives

Acute changes in PO_2 , even in physiological hyperoxia, result in rapid changes in the contractile capacity (inotropy) of both the spongy and compact myocardium of rainbow trout, steelhead trout and Atlantic cod (this study; Carnevale et al. 2020; Gesser and Rodnick 2019; Roberts and Syme 2018; Syme et al. 2013), and these changes appear to be rapidly reversible following the restoration of saline PO_2 . These results suggest that declines in contractile capacity during acute hypoxia are adaptive rather than a failure of the myocardium, and may serve to sustain adequate but not excess pumping capacity, and thus protect the heart during periods of limited oxygen availability. These observations also support the hypothesis that the response may be mediated by cellular oxygen sensing mechanisms. The differences in response of the compact and spongy myocardium to acute and chronic hypoxia may be related, at least in part, to the different PO_2 s and changes in PO_2 that they experience during environmental normoxia and hypoxia, and the nature and sensitivity of their sensing mechanisms.

Evidence suggests that chronic hypoxic acclimation has a depressive effect on mass-specific work output of the spongy myocardium, but little or no effect on this tissue’s response to acute hypoxia (e.g., the present study; Carnevale et al. 2020). Conversely, hypoxic acclimation had a small positive effect on work performed by the compact myocardium, and conferred increased resistance to the depressive effects of acute hypoxia. Given the anticipated changes in arterial and venous PO_2 during environmental hypoxia, the extent of the reduction in work capacity would be similar in both tissue types in hypoxia-acclimated fish, and perhaps limited to about 20%. The slightly increased work capacity of compact myocardium following hypoxic acclimation, and the slightly reduced or maintained work capacity of spongy myocardium, may reflect the PO_2 and magnitude of the PO_2 changes experienced by these tissues during environmental

hypoxia. The compact myocardium experiences a large drop in PO_2 during environmental hypoxia but is still perfused with blood at a relatively high PO_2 , which may allow it to increase its relative contributions to cardiac pumping. The spongy myocardium, experiencing only a small drop in PO_2 during environmental hypoxia but routinely being perfused with blood at a relatively low PO_2 , responds less to chronic hypoxia and is not situated to do more than maintain its contribution, at best.

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Author contributions AKG and DAS contributed to the study conception and design. Material preparation, data collection and analysis were performed by AKG, DAS, CC and JCR. The first draft of the manuscript was written by JCR and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval All applicable international, national, and institutional guidelines for the care and use of animals were followed. All animal care and handling procedures were approved by the animal care committees of both the University of Calgary (Protocol #AC14-0214) and Memorial University of Newfoundland (Protocol #15-89-KG), and followed guidelines of the Canadian Council on Animal Care.

Availability of data and material All authors attest that all data and materials as well as software application or custom code support their published claims and comply with field standards. All data generated or analysed during this study are included in this published article.

References

- Altieri AH, Gedan KB (2015) Climate change and dead zones. *Glob Change Biol* 21:1395–1406
- Altringham JD, Johnston IA (1990) Scaling effects on muscle function: power output of isolated fish muscle fibres performing oscillatory work. *J Exp Biol* 151:453–467
- Blank JM, Morrisette JM, Davie PS, Block BA (2002) Effects of temperature, epinephrine and Ca^{2+} on the hearts of yellowfin tuna (*Thunnus albacares*). *J Exp Biol* 205(Pt 13):1881–1888
- Bolli R, Marbán E (1999) Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev* 79:609–634
- Breitburg D, Levin LA, Oschlies A, Grégoire M, Chavez FP, Conley DJ, Garçon V, Gilbert D, Gutiérrez D, Isensee K, Jacinto GS, Limburg KE, Montes I, Naqvi SWA, Pitcher GC, Rabalais NN, Roman MR, Rose KA, Seibel BA, Telszewski M, Yasuhara M, Zhang J (2018) Declining oxygen in the global ocean and coastal waters. *Science* 5:359
- Carnevale C, Roberts JC, Syme DA, Gamperl AK (2020) Hypoxic acclimation negatively impacts the contractility of steelhead trout (*Oncorhynchus mykiss*) spongy myocardium. *Am J Physiol Regul Integr Comp Physiol* 318:214–226
- Cserne Szappanos H, Viola H, Hool LC (2017) L-type calcium channel: clarifying the “oxygen sensing hypothesis”. *Int J Biochem Cell Biol* 86:32–36
- Diaz RJ (2001) Overview of hypoxia around the world. *J Environ Qual* 30:275–281
- Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. *Science* 321:926–929
- Driedzic WR, Gesser H (1994) Energy metabolism and contractility in ectothermic vertebrate hearts: hypoxia acidosis, and low temperature. *Physiol Rev* 74:221–258
- Driedzic WR, Gesser H, Johansen K (1985) Effects of hypoxic adaptation on myocardial performance and metabolism of *Zoarces viviparus*. *Can J Zool* 63:821–823
- Fago A, Jensen FB, Tota B, Feelisch M, Olson KR, Helbo S, Lefevre S, Mancardi D, Palumbo A, Sandvik GK, Skovgaard N (2012) Integrating nitric oxide, nitrite and hydrogen sulfide signaling in the physiological adaptations to hypoxia: a comparative approach. *Comp Biochem Physiol A Mol Integr Physiol* 162:1–6
- Fai AH, Cornelius PL (1996) Approximate F-tests of multiple degree of freedom hypotheses in generalized least squares analyses of unbalanced split-plot experiments. *J Stat Comp Sim* 54(4):363–378
- Farrell AP (1984) A review of cardiac performance in the teleost heart: intrinsic and humoral regulation. *Can J Zool* 62:523–536
- Farrell AP, Jones DR (1992) The Heart. In: Hoar WS, Randall DJ, Farrell AP (eds) *Fish physiology 12a: the cardiovascular system*. Academic Press Inc., San Diego, pp 26–115
- Farrell AP, Richards JG (2009) Defining hypoxia: an integrative synthesis of the responses of fish to hypoxia. In: Richards JG, Farrell AP, Brauner CJ (eds) *Fish physiology: Hypoxia*, 1st edn. Elsevier, San Diego, pp 487–503
- Farrell AP, Smith F (2017) Cardiac form, function and physiology. In: Gamperl AK, Gillis TE, Farrell AP, Brauner CJ (eds) *Fish physiology 36a: the cardiovascular system: morphology, control and function*. Elsevier, San Diego, pp 155–264
- Farrell AP, Eliason EJ, Sandblom E, Clark TD (2009) Fish cardiorespiratory physiology in an era of climate change. *Can J Zool* 87(10):835–851
- Faust HA, Gamperl AK, Rodnick KJ (2004) All rainbow trout (*Oncorhynchus mykiss*) are not created equal: intra-specific variation in cardiac hypoxia tolerance. *J Exp Biol* 207:1005–1015
- Gamperl AK, Driedzic WR (2009) Cardiovascular function and cardiac metabolism. In: Richards JG, Farrell AP, Brauner CJ (eds) *Fish physiology: Hypoxia*, 1st edn. Elsevier, San Diego, pp 301–360
- Gamperl A, Pinder A, Grant R, Boutillier R (1994) Influence of hypoxia and adrenaline administration on coronary blood flow and cardiac performance in seawater rainbow trout (*Oncorhynchus mykiss*). *J Exp Biol* 193:209–232
- Gamperl AK, Todgham AE, Parkhouse WS, Dill R, Farrell AP (2001) Recovery of trout myocardial function following anoxia: preconditioning in a non-mammalian model. *Am J Physiol Regul Integr Comp Physiol* 281:1755–1763
- Gesser G, Rodnick KJ (2019) Is the teleost heart oxygen limited? Insights using ‘hyperoxic’ incubations of contracting cardiac tissue from rainbow trout. *Comp Biochem Physiol* 231A:124–130
- Hanson LM, Obradovich S, Mouniargi J, Farrell AP (2006) The role of adrenergic stimulation in maintaining maximum

- cardiac performance in rainbow trout (*Oncorhynchus mykiss*) during hypoxia, hyperkalemia and acidosis at 10 °C. *J Exp Biol* 209:2442–2451
- Harwood C, Young I, Altringham J (1998) Influence of cycle frequency, muscle strain and muscle length on work and power production of rainbow trout (*Oncorhynchus mykiss*) ventricular muscle. *J Exp Biol* 201:2723–2733
- Hool LC (2015) How does the heart sense changes in oxygen tension: a role for ion channels? *Antioxid Redox Signal* 22:522–536
- Icardo JM (2017) Heart morphology and anatomy. In: Gamperl AK, Gillis TE, Farrell AP, Brauner CJ (eds) *Fish physiology 36a: the cardiovascular system: morphology, control and function*. Elsevier, San Diego, pp 1–54
- Joyce W, Ozolina K, Mauduit F, Ollivier H, Claireaux G, Shiels HA (2016a) Individual variation in whole-animal hypoxia tolerance is associated with cardiac hypoxia tolerance in a marine teleost. *Biol Lett* 12:20150708. <https://doi.org/10.1098/rsbl.2015.0708>
- Joyce W, Simonsen M, Gesser H, Wang T (2016b) The effects of hypoxic bradycardia and extracellular HCO₃⁻/CO₂ on hypoxic performance in the eel heart. *J Exp Biol* 219:302–305
- Kangur K, Kangur A, Kangur P, Laugaste R (2005) Fish kill in Lake Peipsi in summer 2002 as a synergistic effect of cyanobacterial bloom, high temperature and low water level. *Proc Est Acad Sci Biol Ecol* 54:67–80
- Kuznetsova A, Brockhoff PB, Christensen RHB (2013). lmerTest Package: Tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package). R package 2.0–11
- La VT, Cooke SJ (2011) Advancing the science and practice of fish kill investigations. *Rev Fish Sci* 19:21–33
- Motyka R, Norin T, Petersen LH, Huggett DB, Gamperl AK (2017) Long-term hypoxic exposure alters the cardiorespiratory physiology of steelhead trout (*Oncorhynchus mykiss*), but does not affect their upper thermal tolerance. *J Therm Biol* 68B:149–161
- Mulholland PJ, Houser JN, Maloney KO (2005) Stream diurnal dissolved oxygen profiles as indicators of in-stream metabolism and disturbance effects: Fort Benning as a case study. *Ecol Indic* 5:243–252
- Petersen LH, Gamperl AK (2010a) In situ cardiac function in Atlantic cod (*Gadus morhua*): effects of acute and chronic hypoxia. *J Exp Biol* 213:820–830
- Petersen LH, Gamperl AK (2010b) Effect of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Atlantic cod (*Gadus morhua*). *J Exp Biol* 213:808–819
- Petersen LH, Gamperl AK (2011) Cod (*Gadus morhua*) cardiorespiratory physiology and hypoxia tolerance following acclimation to low-oxygen conditions. *Physiol Biochem Zool* 84:18–31
- Roberts JC, Syme DA (2018) Effects of epinephrine exposure on contractile performance of compact and spongy myocardium from rainbow trout (*Oncorhynchus mykiss*) during hypoxia. *Fish Physiol Biochem* 44:49–62
- Rodnick KJ, Gesser H (2017) Cardiac energy metabolism. In: Gamperl AK, Gillis TE, Farrell AP, Brauner CJ (eds) *Fish Physiology 36a: the cardiovascular system: morphology, control and function*. Elsevier, San Diego, pp 317–367
- SAS Institute Inc. (2012) *SAS user's guide: statistics*. SAS for windows, version 9.4. SAS Institute Inc. Cary
- Scaringi JA, Rosa AO, Morad M, Cleemann L (2013) A new method to detect rapid oxygen changes around cells: how quickly do calcium channels sense oxygen in cardiomyocytes? *J Appl Physiol* 115:1855–1861
- Shiels HA, Santiago DA, Galli GLJ (2010) Hypercapnic acidosis reduces contractile function in the ventricle of the Armored catfish *Pterygoplichthys pardalis*. *Physiol Biochem Zool* 83(2):366–375
- Stecyk JAW (2017) Cardiovascular function under limiting oxygen conditions. In: Gamperl AK, Gillis TE, Farrell AP, Brauner CJ (eds) *Fish Physiology 36b: the cardiovascular system: development, plasticity and physiological responses*. Elsevier, San Diego, pp 299–371
- Syme DA (1993) Influence of extent of muscle shortening on work from frog heart trabeculae and heart rate. *Am J Physiol* 265:R310–319
- Syme DA, Josephson RK (1995) Influence of muscle length on work from trabecular muscle of frog atrium and ventricle. *J Exp Biol* 198:2221–2227
- Syme DA, Gamperl AK, Nash GW, Rodnick KJ (2013) Increased ventricular stiffness and decreased cardiac function in Atlantic cod (*Gadus morhua*) at high temperatures. *Am J Physiol Regul Integr Comp Physiol* 305:864–876
- Thomas S, Fritsche R, Perry SF (1994) Pre- and post-branchial blood respiratory status during acute hypercapnia or hypoxia in rainbow trout, *Oncorhynchus mykiss*. *J Comp Physiol B* 164:451–458

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