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In situ cardiac function in Atlantic cod (*Gadus morhua*): effects of acute and chronic hypoxia

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SUMMARY

Recent *in vivo* experiments on Atlantic cod (*Gadus morhua*) acclimated to chronic hypoxia (6–12 weeks at 10°C; $P_{W_{O_2}}$ ~8–9 kPa) revealed a considerable decrease in the pumping capacity of the heart. To examine whether this diminished cardiac performance was due to the direct effects of chronic moderate hypoxia on the myocardium (as opposed to alterations in neural and/or hormonal control), we measured the resting and maximum *in situ* function of hearts from normoxia- and hypoxia-acclimated cod: (1) when initially perfused with oxygenated saline; (2) at the end of a 15 min exposure to severe hypoxia (P_{O_2} ~0.6 kPa); and (3) 30 min after the hearts had been reperfused with oxygenated saline. Acclimation to hypoxia did not influence resting (basal) *in situ* cardiac performance during oxygenated or hypoxic conditions. However, it caused a decrease in maximum cardiac output ($\dot{Q}_{max}$) under oxygenated conditions (from 49.5 to 40.3 ml min^{-1} kg^{-1}; by 19%), that was due to diminished values for maximum stroke volume (V_S) and scope for V_S. Severe hypoxia reduced $\dot{Q}_{max}$ in both groups to ~20 ml min^{-1} kg^{-1}, yet, the hearts of hypoxia-acclimated fish were better able to sustain this level of $\dot{Q}$ under hypoxia, and the recovery of $\dot{Q}_{max}$ (as compared with initial values under oxygenated conditions) was significantly improved (94% *vs* 83%). These data show that acclimation to hypoxia has a direct effect on cod myocardial function and/or physiology, and suggest that the cod heart shows some adaptations to prolonged hypoxia.

Key words: chronic hypoxia, cod, heart, cardiac performance, recovery.

INTRODUCTION

Hypoxia is encountered by many fish species, and exposure to low oxygen environments results in complex behavioural and physiological responses (Pihl et al., 1991; Val et al., 1995; Van Ginneken et al., 1995; Dalla Via et al., 1998). Of these, changes in cardiovascular function have been of particular interest, and a large amount of information currently exists on the *in vivo* cardiovascular responses of teleosts to short-term (acute) hypoxia (Wood and Shelton, 1980; Farrell, 1982; Bushnell et al., 1984; Glass et al., 1990; Glass et al., 1991; Perry et al., 1999; Sandblom and Axelsson, 2005). In contrast, only one study has investigated the effect of chronic (weeks of) hypoxia on fish *in vivo* cardiovascular function (Petersen and Gamperl, 2010). This study showed that cod acclimated to hypoxia ($P_{W_{O_2}}$ 8–9 kPa, 40% air saturation) had significantly lower values for resting and maximum stroke volume and cardiac output, and a significantly lower scope for stroke volume when swimming in hypoxic water as compared with normoxia-acclimated fish. While this work has provided novel insights into how fish cardiorespiratory physiology is impacted by prolonged exposure to hypoxia, the reason(s) for the diminished cardiac function in hypoxia-acclimated cod is not clear. For example, stroke volume in fishes is controlled by aneural and neural factors such as cardiac filling/venous pressure, blood oxygen levels and chemistry, myocardial contractility, circulating hormones, and by alterations in cholinergic and adrenergic nervous activity (Kiceniuk and Jones, 1977; Farrell, 1984; Axelsson, 1988; Axelsson and Nilsson, 1986; Satchell, 1991; Farrell, 1991; Zhang et al., 1998; Sandblom and Axelsson, 2005; Sandblom and Axelsson, 2006; Hanson et al., 2006; Hanson and Farrell, 2007). Further, while 3 weeks of acclimation to a water oxygen partial pressure ($P_{W_{O_2}}$) of 5 kPa (25% air saturation) induced myocardial degeneration and subsequent fibrosis in the flounder (*Platichthys flesus*) heart (Lennard and Huddart, 1992), Driedzic et al. (Driedzic et al., 1985) showed that myocardial strips from eelpout (*Zoarces vivparous*) acclimated to a $P_{W_{O_2}}$ of 6 kPa (~30% air saturation) for 4–6 weeks were better able to sustain peak tension development during anoxia in the presence of elevated Ca^{2+} levels.

In situ heart preparations, first developed for fish by Farrell et al. (Farrell et al., 1982), are devoid of any nervous and hormonal input, are extremely robust and tractable, and perform at maximum levels typical of those measured *in vivo* (Farrell et al., 1985; Farrell et al., 1989; Hanson et al., 2006; Hanson and Farrell, 2007). Furthermore, they have proved to be a very valuable tool for understanding teleost heart function during periods of oxygen deprivation (Farrell et al., 1989; Arthur et al., 1992; Hanson and Farrell, 2007), the importance of circulating catecholamines in supporting cardiac function under conditions experienced during severe exercise [e.g. hypoxemia, hyperkalemia and acidosis (Hanson et al., 2006; Hanson and Farrell, 2007)], and for elucidating several aspects of preconditioning in the teleost heart (Gamperl et al., 2001; Gamperl et al., 2004; Faust et al., 2004; Overgaard et al., 2004b). Thus, the present study used *in situ* heart preparations to investigate the effects of acclimation to hypoxia on the normoxic and hypoxic performance of the Atlantic cod heart, and ultimately to determine whether the reduced *in vivo* cardiac performance observed in hypoxia-acclimated cod (Petersen and Gamperl, 2010) was a direct result of a decrease in the pumping capacity of the heart.

MATERIALS AND METHODS

These studies were conducted in accordance with the guidelines of the Canadian Council on Animal Care, and approved by the

Institutional Animal Care Committee of Memorial University of Newfoundland (Protocol #05-03-KG).

Experimental animals

Experiments were performed on adult (0.53±0.04 kg; range 0.30–0.86 kg) Atlantic cod (*Gadus morhua* L.) at the Ocean Sciences Centre (OSC; Memorial University, St John's, Newfoundland, Canada). Cod were obtained from stocks hatched at the Aquaculture Research and Development Facility (ARDF) and held in sea-cages at Hermitage Bay (Newfoundland, Canada) for approximately 18 months before being transported back to the OSC. At the OSC, the fish were held in a 12,000 l tank supplied with aerated seawater at 10°C for at least 2 months prior to being moved to the acclimation tanks. The fish were fed a commercial cod diet three times a week, and maintained on ambient photoperiod.

Experimental conditions and surgery

Prior to the experiments, 20 fish from the holding tank were acclimated at a P_{O_2} of 19.4±0.1 kPa or 8.6±0.1 kPa in ~1300 l tanks for 6–12 weeks at 10±1°C as described in Petersen and Gamperl (Petersen and Gamperl, 2010). The normoxic fish were fed three times a week with commercial pellets at a ration equal to that consumed by the hypoxic group (1.4% body mass day^{-1}).

The fish were netted and anaesthetized in seawater containing tricaine methane sulphonate (MS-222; 0.1 g l^{-1}) until ventilatory movements ceased. The fish were then weighed and measured, before being transferred to an operating table where chilled (4°C), oxygenated seawater, containing a lower dose of MS-222 (0.05 g l^{-1}), was continuously irrigated over their gills. On the operating table the fish were placed on a wetted sponge in a supine position, injected with 1.0 ml of heparin (50 i.u. ml^{-1} of saline solution; Sigma Chemical Co., St Louis, MO, USA) *via* the caudal vein, and an *in situ* heart preparation was obtained as described by Farrell et al. (Farrell et al., 1986; Farrell et al., 1989) with some minor modifications. Briefly, the peritoneal cavity was exposed through a midline incision and by cutting through the abdominal muscle in a ventral–dorsal direction just posterior to the pectoral fins. Blood flow to the stomach, intestines and other abdominal organs was stopped by tying off the gastrointestinal tract, inferior to the liver, with umbilical tape. Then, the abdominal and digestive organs were carefully removed to permit proper placement of the input cannula whilst keeping the liver intact. A hepatic vein was selected for cannulation, and after the other one was tied off with 3-0 silk suture, a small cut was made in the hepatic vein and a steel cannula (0.9 mm outer diameter; o.d.) was inserted and tied in place. At this point, perfusion of the heart with ice-cold (4°C) oxygenated saline was begun, and the first and second gill arches were cut on each side of the fish to prevent excessive pressure development by the heart; the level of saline in the perfusion bottle set at the same height as the heart (i.e. 0 kPa input pressure) in order to obtain basal cardiac output and prevent cardiac stretch.

The lower jaw and operculum were then removed, the first and second gill arches cut away, and the third and fourth gill arches cut in half and clamped with cable ties (4″) to prevent leakage. Finally, the isthmus between the second and the third gill arches was cut to expose the ventral aorta in cross section, the ventral aorta was dissected free from the surrounding tissue, and a steel output cannula (0.8 mm o.d.) was inserted into the ventral aorta and tied in place with 3-0 silk suture.

After the output cannula was secured in place, the ducts of Cuvier were tied off by passing a large needle with attached silk suture (1-0) from the corner of the opercular cavity to the muscle of the abdominal wall, and then into the oesophagus and back into the buccal cavity. When this suture was subsequently pulled tight, it occluded the ducts of Cuvier and other veins entering the heart, and crushed the cardiac branches of the vagus nerve; crushing of the nerves was confirmed by a noticeable pectoral fin twitch and transitory cardiac arrest. This procedure ensured that any fluid entering the heart was from the input cannula, and that nervous stimulation of the heart was prevented during the experiment. Once surgery was completed, the fish was bisected just posterior to the pectoral fins, and placed in a water-jacketed saline-filled bath maintained at the fish's acclimation temperature.

Stabilization

After placing the *in situ* preparation in the experimental bath, the input cannula was attached to an adjustable constant-pressure head that was used to manipulate atrial filling pressure (input pressure; P_{in}), and the output cannula was connected to tubing, the height of which could be adjusted to control end-diastolic pressure (output pressure; P_{out}). The heart was then perfused with oxygenated physiological saline (see recipe below) from temperature controlled (10°C) water-jacketed bottles. P_{out} was maintained at 2 kPa during the first 10 min to let the heart recover from surgery, and to prevent excessive cardiac work while P_{in} was being set to a physiologically relevant resting cardiac output ($\dot{Q}$; 16 ml min^{-1} kg^{-1}) (Axelsson and Nilsson, 1986; Fritsche and Nilsson, 1989; Webber et al., 1998). After this initial period, P_{out} was increased to a physiological output pressure of 5 kPa (Axelsson and Nilsson, 1986) and the heart was allowed to stabilize for 15 min at this resting (basal) cardiac output.

Experimental protocol

A schematic diagram of the entire protocol can be seen in Fig. 1. After stabilization was complete, resting cardiac parameters (input pressure, P_{in}; heart rate, f_H; cardiac output, $\dot{Q}$; stroke volume, V_S; and power output, PO) were recorded, and then maximum cardiac output ($\dot{Q}_{max1}$) was determined by increasing P_{in} from the height required to achieve resting cardiac output (~0 to 0.05 kPa) to 0.4 kPa, and then in a stepwise fashion to 0.5, to 0.55 and finally to 0.6 kPa (Fig. 1). During the $\dot{Q}_{max1}$ test, P_{out} was maintained at 5 kPa, and each increase in P_{in} was maintained for approximately 30 s to allow enough time for cardiac functional parameters ($\dot{Q}_{max}$, $f_{H\,max}$, $V_{S\,max}$ and PO) to stabilize and be recorded, but short enough to avoid excessive cardiac stretch and/or myocardial damage. After determining $\dot{Q}_{max1}$, input pressure was again reduced to levels required to obtain resting cardiac output and the heart was allowed to recover for 10 min.

After 10 min of recovery under oxygenated conditions, the heart was exposed to 15 min of severe hypoxia (perfusate P_{O_2} of ~0.6 kPa), during which time P_{in} was not adjusted. This allowed for a determination of whether the heart's ability to maintain basal levels of performance during severe hypoxia differed between normoxia- and hypoxia-acclimated cod. This 15 min of hypoxia was followed by a second $\dot{Q}_{max}$ test ($\dot{Q}_{max2}$), after which the heart was perfused with oxygenated saline and allowed to recover at resting cardiac output (16 ml min^{-1} kg^{-1}) for 30 min. Most hearts survived this hypoxic exposure, but some stopped pumping either at the end of the 15 min of basal cardiac function or during the $\dot{Q}_{max2}$ test. After 30 min of recovery in oxygenated saline, a third $\dot{Q}_{max}$ test ($\dot{Q}_{max3}$) was performed, and this was followed by a maximum power output (PO_{max}) test. This final test was performed while the input pressure of the heart remained at 0.6 kPa (i.e. that used to obtain $\dot{Q}_{max}$), and involved decreasing P_{out} from 5 kPa to 3 kPa, and then increasing P_{out} in 1 kPa steps until the heart could no longer pump (or until an

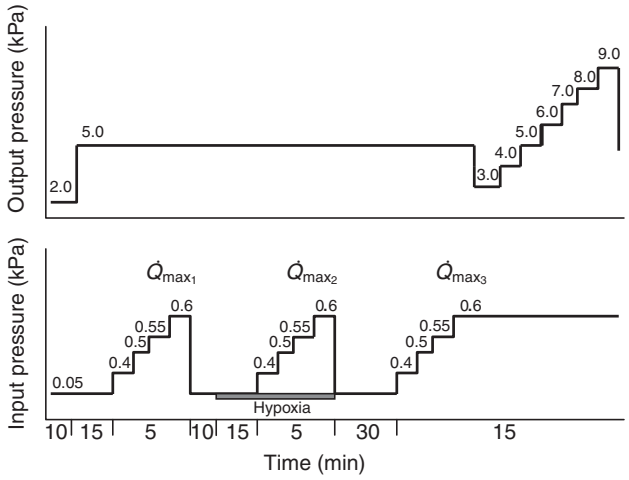

Fig. 1. Experimental protocol used to assess the resting and maximal cardiac performance on *in situ* hearts from normoxia- and hypoxia-acclimated Atlantic cod. Upper and lower panels show output and input pressures, respectively. P_{out} was normally set to a physiologically realistic value of 5 kPa; however, a sub-physiological level of P_{out} (2–3 kPa) was used for the first 10 min of the protocol to let the heart recover from surgery. Then, P_{out} was raised to 5 kPa and P_{in} continuously adjusted to give a cardiac output of 16–17 ml min^{-1} kg^{-1}. Resting conditions were then recorded for 15 min while the heart was perfused with oxygenated saline. The next set of steps indicates the first maximum cardiac output test ($\dot{Q}_{max1}$), where P_{in} was raised sequentially from 0.05 kPa to 0.6 kPa. The heart was then left to recover for 10 min and was then exposed to severe hypoxia (P_{O_2} 0.6 kPa, grey bar) for 15 min without adjusting P_{in}. This was followed by a second $\dot{Q}_{max}$ test, identical to the first one, but this time in hypoxic saline. Subsequently, the heart was allowed to recover at 0.05 P_{in} in oxygenated saline for 30 min before a final normoxic $\dot{Q}_{max}$ test was performed. Finally, while P_{in} remained at 0.60 kPa, output pressure was decreased from 5 kPa to 3 kPa and then raised in 1 kPa steps until the heart could no longer pump (~9 kPa).

output pressure of 9 kPa was reached). The time spent at each level of output pressure was just long enough to allow cardiac performance to stabilize, approximately 20–30 s.

After each experiment, the heart was tested to ensure that no leaks were present. This was done by clamping the input perfusate line with a pair of haemostats and ensuring cardiac output fell to zero, then raising the output tube to ~10 kPa and ensuring that no significant backflow occurred. Data from hearts that 'leaked' were excluded from analysis. The heart was then dissected from the fish and the cardiac chambers were separated, blotted dry, and weighed.

Experimental solutions

Hearts were perfused with physiological marine teleost saline during surgery and during the experimental period. This saline (pH 7.76 at 8°C) contained (in g l^{-1}): 10.5 NaCl; 0.49 $MgSO_4{\cdot}7H_2O$; 0.37 KCl; 0.33 $CaCl_2{\cdot}2H_2O$; 0.14 $NaH_2PO_4{\cdot}H_2O$; 1.84 sodium TES base ($C_6H_{14}NO_6SNa$); 0.59 TES acid ($C_6H_{15}NO_6S$); 1.0 glucose. The TES buffer system was used to simulate the buffering capacity of cod plasma and epinephrine (10 nmol l^{-1}) was added to the perfusate to ensure the long-term viability of the *in situ* heart (Graham and Farrell, 1989). These chemicals were obtained from Fisher Scientific (Fair Lawn, NJ, USA), with the exception of the TES salt and adrenaline bitartrate salt, which were purchased from Sigma Chemical Co. (St Louis, MO, USA). The saline was continuously gassed with oxygen during both surgery and when the heart was not being exposed to severe hypoxia during the experiment. To achieve severe hypoxia, the saline in the perfusion bottles was gassed with pure N_2 for at least 60 min before the hypoxic trial, and saline in the experimental bath was gassed with pure N_2 beginning approximately 5 min before the onset of the hypoxic experiment. The use of Masterflex® tubing, with low oxygen permeability (Tygon Food, ser. 6-419, Cole Parmer, Montreal, QC, Canada), to deliver saline to the *in situ* hearts further ensured that oxygen from external sources was minimized.

Data collection and analysis

Input and output pressures were measured using Gould (P23 ID, Oxnard, CA, USA) and Grass (PT300, Warwick, RI, USA) pressure transducers, respectively, and cardiac output was measured with a 2N in-line flow probe in conjunction with a T206 flow meter (Transonic Systems Inc., Ithaca, NY, USA). Input and output pressures were corrected to account for cannula resistance between the point of measurement and the heart [using predetermined calculations in Faust et al. (Faust et al., 2004)], and the pressure transducers were calibrated daily against a static column of water; with zero pressure equal to the level of saline in the bath. Pressure and flow signals were collected at a sample rate of 20 Hz, and filtered and amplified, using a Model MP100A-CE data acquisition system (BIOPAC Systems Inc., Santa Barbara, CA, USA), and the acquired signals were stored and analyzed using AcqKnowledge Software (BIOPAC Systems Inc.) installed on a 300 MHz Macintosh G3 computer.

Cardiac function was continuously recorded throughout the experiment by measuring P_{in}, P_{out}, $\dot{Q}$ (in ml min^{-1} kg^{-1}), f_H (in beats min^{-1}) and V_S (in ml kg^{-1}). Although data were continuously collected, cardiac function was only analyzed at specific intervals during each experiment. Resting cardiac parameters ($\dot{Q}$, f_H and V_S) were measured just prior to each of the $\dot{Q}_{max}$ tests. Maximum cardiac function was quantified by measuring $\dot{Q}_{max}$, $f_{H\,max}$, $V_{S\,max}$ and power output (PO_{max}). The first three parameters were measured at an input pressure of 0.6 kPa during the maximum cardiac output tests. Maximum power for each fish was calculated by fitting a second- or third-order regression to the power output *vs* P_{out} relationship. These data were then used to calculate the mean PO_{max} for each group. f_H was calculated by counting 20 systolic peaks, dividing by the measurement period (s), and multiplying by 60. Cardiac output (ml min^{-1} kg^{-1}) was calculated by dividing absolute flow (ml min^{-1}) by the mass of the fish (kg). V_S (in ml kg^{-1}) and power output (mW g^{-1} ventricle) were calculated as follows:

$$V_S = \dot{Q} / f_H , \quad (1)$$

$$PO = [\dot{Q} (P_{out} - P_{in})\, a] / M_v , \quad (2)$$

where P_{out} and P_{in} are output and input pressures (in cm H_2O), respectively, M_v is ventricle mass and a=0.098 (mW min ml^{-1} cm^{-1} H_2O) is a conversion factor to milliwatts (mW) (Farrell et al., 1996).

Statistical analyses

Statistical analyses were carried out using SPSS (v. 13.0; SPSS, Chicago, IL, USA). Paired *t*-tests and one-way ANOVAs were used to test for statistical differences within groups and between groups, respectively, for: (1) body and cardiac morphometrics (Table 1); (2) resting cardiac parameters (Table 2); (3) routine, maximum and scope of cardiac parameters (Table 3); and (4) all maximum cardiac parameters (Table 4). GLM repeated measures analyses were used to determine the effect of time and acclimation condition on cardiac parameters (f_H, V_S, $\dot{Q}$ and PO) when hearts were exposed to either oxygenated or hypoxic saline (see Fig. 2). This analysis was also

Table 1. Body and cardiac morphometrics for Atlantic cod acclimated to either normoxia (water oxygen partial pressure of 20 kPa) or hypoxia (8–9 kPa) for 6–12 weeks

	Body and cardiac morphometrics				
	Animal mass (kg)	Length (cm)	Heart mass (g)	Relative ventricular mass (RVM, %)	Condition factor (K)
Normoxia	0.49±0.05	39.3±1.2	0.35±0.03	0.071±0.003	0.80±0.03
Hypoxia	0.56±0.05	40.1±1.3	0.40±0.05	0.072±0.003	0.85±0.03

Values shown are means ± s.e.m. (N=8 for each group).

performed to determine the effect of: (1) P_{in} and acclimation condition on $\dot{Q}_{max1}$, $\dot{Q}_{max2}$, $\dot{Q}_{max3}$; and (2) P_{out} and acclimation condition on PO_{max}. These analyses were followed by Dunnett's *post-hoc* tests to determine when there was a significant change in a cardiac parameter from the resting value within each group. One-way ANOVAs were used to determine significant differences at each time point (Fig. 2) or each P_{in}/P_{out} (Figs 3 and 5) between the normoxic and hypoxic groups. One-way ANOVA was also performed to determine significant differences between $\dot{Q}_{max1}$ and $\dot{Q}_{max3}$ within each group (Fig. 4). Unless otherwise stated, a result was considered significant when P<0.05. All data presented in the text, figures and tables are means ± standard error of the mean (s.e.m.).

RESULTS

Cardiac morphometrics and resting (basal) performance

Acclimation to hypoxia did not affect the cod's mass, condition factor, ventricular mass or relative ventricular mass (RVM; Table 1), or basal *in situ* cardiac performance under oxygenated conditions (Table 2). In oxygenated saline, resting f_H was 61.9±2.3 beats min^{-1} and 60.4±3.0 beats min^{-1} in the normoxia- and hypoxia-acclimated groups, respectively. Furthermore, at this f_H, hearts from both groups required a slightly positive P_{in} (means 0.05 *vs* 0.08) and a V_S of ~0.28 ml kg^{-1} to achieve a cardiac output of 16–17 ml min^{-1} kg^{-1}.

During the 15 min of acute severe hypoxia (saline P_{O_2} ~0.6 kPa; during which time input pressure was not adjusted) changes in cardiac function were almost identical in the two groups. $\dot{Q}$ decreased gradually in both groups, this decrease in $\dot{Q}$ becoming significant after ~8 min of hypoxia, and $\dot{Q}$ after 15 min of severe hypoxia falling from ~16 to ~10 ml min kg^{-1} (i.e. by ~35%; Fig. 2, Table 3). This diminished $\dot{Q}$ was mirrored by changes in PO, and was the sole result of hypoxia-induced reductions in V_S. For example, in both groups, only slight (~2–3 beat min^{-1}) decreases in f_H were observed while V_S fell by 27% and 38% in normoxia- and hypoxia-acclimated fish, respectively.

Following 30 min of recovery in oxygenated saline, values for P_{in}, $\dot{Q}$, f_H and V_S were nearly identical between groups, and to those measured at the start of the experiment (i.e. prior to $\dot{Q}_{max1}$). The only exception was P_{in} which was 0.1 kPa higher in hearts from hypoxia-acclimated cod (P<0.10). However, this result was not surprising given that f_H was ~4 beats min^{-1} lower (but not significantly so) in the hearts from hypoxia-acclimated cod.

Maximum cardiac output and power output tests

Although prolonged exposure to hypoxia (Pw_{O_2} ~8–9 kPa; 40% air saturation) did not influence resting (basal) *in situ* cardiac performance during oxygenated or hypoxic conditions, there were several important differences in maximum cardiac performance between the two groups. First, acclimation to hypoxia caused a decrease in maximum cardiac output (from 49.5 to 40.3 ml min^{-1} kg^{-1}; by 19%) and the scope for $\dot{Q}$ (from 32.8 to 23.8 ml min^{-1} kg^{-1}; by 28%) during the initial oxygenated $\dot{Q}_{max}$ test ($\dot{Q}_{max1}$). This difference was again due to diminished values for maximum V_S and scope for V_S in hearts from hypoxia-acclimated cod; the decrease in f_H due to myocardial stretch (~7 beats min^{-1}) was similar in the two groups (Fig. 3A; Table 3). Second, although exposure to 15 min of severe hypoxia reduced maximum $\dot{Q}$ and V_S to similar levels in both groups ($\dot{Q}$ to 18 and 23 ml min^{-1} kg^{-1} and V_S to 0.34 and 0.443 ml kg^{-1}), (1) the decrease in maximum $\dot{Q}$ between $\dot{Q}_{max1}$ and $\dot{Q}_{max2}$ was much greater in normoxia- *vs* hypoxia-acclimated cod (~31.5 *vs* 17.2 ml min^{-1} kg^{-1}; Table 4), and (2) $\dot{Q}$ remained constant in the hypoxia-acclimated cod hearts as P_{in} was increased from 0.4 to 0.6 kPa, yet fell significantly from ~26.5 to 18 ml min^{-1} kg^{-1} in the normoxia-acclimated group (Fig. 3B). Finally, although maximum $\dot{Q}$ and V_S values following 30 min of recovery from hypoxia were similar to initial values (i.e. during $\dot{Q}_{max1}$) in the hypoxia-acclimated group, both these parameters were lower ($\dot{Q}$ significantly) in normoxia-acclimated fish during $\dot{Q}_{max3}$ (Fig. 4; Table 4). This resulted in percentage recovery values of only ~83% for maximum $\dot{Q}$ and V_S in normoxia-acclimated cod, whereas values for hypoxia-acclimated cod were 93.5 and 90.1%, respectively. Collectively, these results show that whereas hearts from hypoxia-acclimated cod have a reduced maximum pumping capacity as compared with those from normoxia-acclimated individuals, they can maintain maximum cardiac function better when faced with acute hypoxia, and their recovery is enhanced following an acute hypoxic insult.

Power output increased slightly in both groups as P_{out} was raised from 3 to 5 kPa. However, it decreased rapidly thereafter, with PO

Table 2. Resting (basal) cardiac parameters for *in situ* hearts from normoxia- and hypoxia-acclimated cod under a variety of test conditions

	Oxygenated saline			Hypoxia			Recovery (oxygenated saline)		
	P_{in} (kPa)	Stroke volume (ml kg^{-1})	Heart rate (beats min^{-1})	P_{in} (kPa)	Stroke volume (ml kg^{-1})	Heart rate (beats min^{-1})	P_{in} (kPa)	Stroke volume (ml kg^{-1})	Heart rate (beats min^{-1})
Normoxia	0.05±0.03	0.27[a]±0.01	61.9±2.3	0.02±0.03	0.19±0.02	59.9±2.3	0.03[+]±0.04	0.26±0.02	64.0±3.7
Chronic hypoxia	0.08±0.02	0.28[a]±0.01	60.4±3.0	0.08±0.03	0.18±0.03	57.6±2.4	0.13±0.06	0.28±0.02	59.7±3.5

Values shown are means ± s.e.m. (N=8 for each group). [a]Indicates a significant difference (P<0.05) between parameters measured under oxygenated and hypoxic conditions within each acclimation condition. [+]Indicates a significant (P<0.10) difference between groups (normoxia- *vs* hypoxia-acclimation) within a particular test.

Input pressure (P_{in}) was that required by each heart to maintain a cardiac output of 16–17 ml min^{-1} kg^{-1} under oxygenated conditions, or at the onset of the hypoxic exposure.

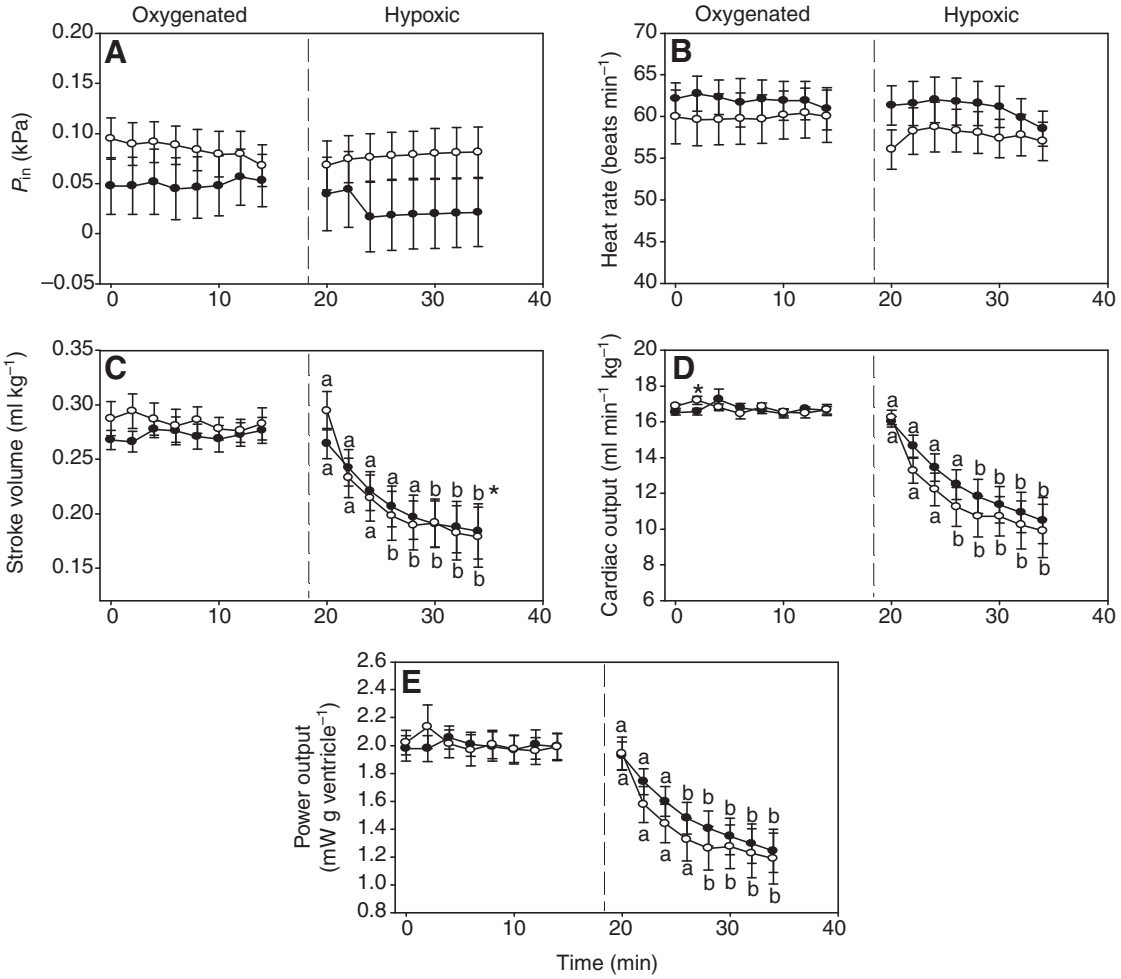

Fig. 2. Effect of acute hypoxia on (A) input pressure, P_{in}; (B) heart rate; (C) stroke volume; (D) cardiac output and (E) power output of *in situ* hearts from normoxia- (black circles; Pw_{O_2} of 20 kPa) and hypoxia-acclimated (white circles, Pw_{O_2} of 8–9 kPa) cod. The isolated hearts were first exposed to oxygenated saline during which cardiac output was maintained at 16–17 ml min^{-1} kg^{-1} by adjusting P_{in} (left hand side of each figure). Then, the hearts were exposed to 15 min of severe hypoxia (saline P_{O_2} of 0.6 kPa) during which P_{in} was held constant (right hand side of each figure). Values are means ± s.e.m. (N=8 for each group). *Value significantly (P<0.05) different in the normoxia- and hypoxia-acclimated groups. Dissimilar letters indicate values that were significantly different from those recorded at time 0 during the acute hypoxic exposure.

at 8 kPa falling to <0.05 mW g^{-1} ventricle (Fig. 5). Although *PO* was somewhat lower in hearts from hypoxia-acclimated cod at output pressures from 3–5 kPa, there was no significant difference in maximum power output between the two groups (normoxia-acclimated 4.7±0.5 mW ventricle^{-1}; hypoxia-acclimated 4.0± 0.8 mW ventricle^{-1}).

DISCUSSION

This *in situ* study confirms earlier *in vivo* measurements (Petersen and Gamperl, 2010) showing that stroke volume and cardiac output during well-oxygenated conditions are significantly reduced in hypoxia-acclimated (>6 weeks at Pw_{O_2}=8 kPa) cod. Thus, this research strongly suggests that *in vivo* cardiac function in hypoxia-acclimated cod was not lower because of alterations in nervous and/or humoral control or venous vascular tone, but because of the direct effects of prolonged hypoxia on the myocardium. The present study also showed that hearts from hypoxia-acclimated cod could maintain maximium performance longer when faced with severe hypoxia (P_{O_2} ~0.6 kPa), and recovered better than hearts from normoxia-acclimated fish following an acute severe hypoxic insult. These latter results suggest that although their normoxic performance may be reduced, they may be better able to perform under conditions of limited O_2 supply.

Resting (basal) cardiac performance

The resting (basal) V_S recorded for *in situ* hearts from normoxia-acclimated cod (0.27±0.01 ml kg^{-1}) corresponds well with previous data for most normoxic *in situ* cod heart preparations (~0.3 ml kg^{-1}) (Mendonca et al., 2007) (A.K.G. and A. G. Genge, unpublished data), but is lower than the *in vivo* V_S recorded in normoxia-acclimated Newfoundland cod (0.60–0.73 ml kg^{-1}) (Gollock et al., 2006) (Petersen and Gamperl, 2010) and North Sea cod (0.39–0.51 ml kg^{-1}) (Axelsson and Nilsson, 1986; Axelsson, 1988; Fritsche and Nilsson, 1989). The lower V_S *in situ*, as compared with *in vivo*, results because cholinergic nervous tone on the cod heart (Axelsson and Nilsson, 1986) is eliminated during the surgical procedures used to obtain the *in situ* heart preparation, and a lower V_S is thus required to achieve resting $\dot{Q}$ at the intrinsic rate of the heart. For example resting f_H *in vivo* is ~25–40 beats min^{-1} at 10°C (Jones et al., 1974; Pettersson and Nilsson, 1980; Wahlqvist and Nilsson, 1980; Smith et al., 1985;

Table 3. Routine, maximum and scope for cardiac parameters measured using *in situ* cod hearts from normoxia- and hypoxia-acclimated (water P_{O_2} ~8–9 kPa) individuals

	Normoxia-acclimated						Hypoxia-acclimated					
	Oxygenated saline			Hypoxic saline			Oxygenated saline			Hypoxic saline		
	f_H (beats min^{-1})	V_S (ml kg^{-1})	$\dot{Q}$ (ml min^{-1} kg^{-1})	f_H (beats min^{-1})	V_S (ml kg^{-1})	$\dot{Q}$ (ml min^{-1} kg^{-1})	f_H (beats min^{-1})	V_S (ml kg^{-1})	$\dot{Q}$ (ml min^{-1} kg^{-1})	f_H (beats min^{-1})	V_S (ml kg^{-1})	$\dot{Q}$ (ml min^{-1} kg^{-1})
Routine	61.9±2.3	0.27[a]±0.01	16.7[a]±0.1	59.9±2.3	0.19±0.02	10.9±1.1	60.4±3.0	0.28[a]±0.01	16.5[a]±0.3	57.8±2.7	0.18±0.03	10.3±1.4
Max	54.1±1.9	0.92[a]*±0.04	49.6[a]*±2.3	55.2±2.3	0.34±0.07	18.0±3.2	53.0±2.4	0.77[a]±0.08	40.3[a]±4.0	53.0±2.3	0.44±0.03	23.1±1.7
Scope	7.8±0.9	0.65[a]*±0.04	32.8[a]*±2.4	4.8±1.4	0.20±0.04	9.8±1.5	7.4±1.5	0.49[a]±0.08	23.8[a]±3.9	3.8±1.2	0.20±0.04	10.1±2.1

Values shown are means ± s.e.m. (N=8 for each group). [a]Indicates a significant difference between hearts perfused with oxygenated and hypoxic saline within each acclimation condition. *Indicates a significant (P<0.10) difference between groups (normoxia *vs* hypoxia acclimation) within a particular test condition.
Maximum cardiac output tests (see Fig. 1) were initially performed while hearts pumped oxygenated saline, and then after 15 minutes of exposure to hypoxic saline (P_{O_2} ~0.6 kPa).

Table 4. Maximum cardiac parameters for *in situ* hearts from cod acclimated to normoxia or hypoxia (water P_{O_2} ~8–9 kPa) for 6–12 weeks

	$\dot{Q}_{max}$				$V_{S\,max}$				$f_{H\,max}$			
	max1	max2	max3	% rec	max1	max2	max3	% rec	max1	max2	max3	% rec
Normoxia	49.6*±2.3	18.0[a]±3.2	40.9[a]±2.6	83.2*±5.7	0.92*±0.04	0.34[a]±0.07	0.75±0.06	82.26±5.81	54.1±1.9	55.2±2.3	55.0±2.2	101.7±2.2
Hypoxia	40.3±4.0	23.1[a]±1.7	38.2±3.1	93.6±4.1	0.77±0.08	0.43[a]±0.03	0.73±0.06	90.11±4.55	53.0±2.4	53.0±2.3	53.0±3.0	102.7±2.7

Values are means ± s.e.m. (N=8 for each group). % rec, % recovery: [(max3/max1)/max1]×100 within each acclimation condition. [a]Significant difference (P<0.05) between max1, and max2 or max3, within each acclimation condition. *Significant difference (P<0.10) between groups (normoxia *vs* hypoxia acclimation) within a particular test condition.
Each heart was given three maximum cardiac output ($\dot{Q}_{max}$) tests. An initial $\dot{Q}_{max}$ test in oxygenated saline ($\dot{Q}_{max1}$); $\dot{Q}_{max2}$, a test performed after hearts were exposed to severe hypoxia (saline P_{O_2} ~0.6 kPa) for 15 minutes; and a final $\dot{Q}_{max}$ test ($\dot{Q}_{max3}$), performed 30 minutes after the hearts were allowed to recover from the severe hypoxic exposure.

Axelsson and Nilsson, 1986; Axelsson, 1988; Butler et al., 1989; Fritsche and Nilsson, 1989; Webber et al., 1998; Gollock et al., 2006) whereas *in situ* heart rates are generally in the range of 50–60 beats min^{-1} (Mendonca et al., 2007) (A.K.G. and A. G. Genge, unpublished data; the present study).

In situ f_H in the two groups was comparable, and not affected by severe hypoxia (e.g. see Fig. 2). These results are contrary to findings *in vivo*. For example, Petersen and Gamperl (Petersen and Gamperl, 2010) showed that cod acclimated to chronic hypoxia have significantly higher heart rates than normoxia-acclimated animals, and L.H.P. and A.K.G. (unpublished data) report that f_H decreases from 32 to 18 beats min^{-1} at the point at which cod lose equilibrium (water P_{O_2} ~2.7 kPa). These data confirm the importance of alterations in adrenergic and cholinergic nervous function in the regulation of teleost f_H during hypoxia (Wood and Shelton, 1980; Fritsche and Nilsson, 1989; Fritsche, 1990) and suggests they are also critical for cardiovascular adjustments to chronic hypoxia. Cardiac output and power output decreased gradually in both groups during the 15 min of severe hypoxia (saline P_{O_2} ~0.6 kPa), and $\dot{Q}$ and V_S at the end of the hypoxic period were only ~60–70% of initial values. The magnitude and rate of decrease in these variables (myocardial performance) is very similar to that measured for rainbow trout (*Oncorhynchus mykiss*) and cod myocardial strips (Hartmund and Gesser, 1996) and for the *in situ* trout heart (Gamperl et al., 2001; Faust et al., 2004; Gamperl et al., 2004; Overgaard et al., 2004a).

Following 30 min of recovery in oxygenated saline, values for P_{in}, $\dot{Q}$, f_H and V_S were almost identical between groups, and also to those measured at the start of the experiment. The only exception being P_{in}, which was 0.1 kPa higher in hypoxia-acclimated as compared with normoxia-acclimated hearts; an increase that is probably due to heart rate in the hypoxia-acclimated group being 4 beats min^{-1} lower. It is quite surprising that the cod heart did not require a substantially higher P_{in} to maintain a resting $\dot{Q}$ of 16–17 ml min^{-1} kg^{-1} after recovering from severe hypoxia as this has been routinely reported for the rainbow trout heart (Gamperl et al., 2001; Faust et al., 2004; Gamperl et al., 2004; Overgaard et al., 2004a). This difference may be due to the presence of only spongy myocardia in cod, as compared with both compact and spongy myocardium in the trout. However, a definitive answer as to why the cod heart retains its sensitivity to filling pressure following exposure to acute severe hypoxia and reperfusion awaits further study.

Maximum cardiac function: normoxia

Maximum $\dot{Q}$ in oxygenated saline was 49.6±2.3 ml min^{-1} kg^{-1}, and maximum V_S and f_H at maximum $\dot{Q}$ were 0.92±0.04 ml kg^{-1} and 54.1±1.9 beats min^{-1}, respectively, in the normoxia-acclimated group. These values for $\dot{Q}$ and V_S are very similar to *in vivo* values reported for 10°C-acclimated cod that were exercised to exhaustion [(Petersen and Gamperl, 2010) ~45 ml min^{-1} kg^{-1} and 0.99 ml kg^{-1}, respectively] and comparable to those recorded in cod exposed to a temperature increase up to their critical thermal maximum, when the elevation in f_H is taken into account [(Gollock et al., 2006) ~52.6 ml min^{-1} kg^{-1} and 0.78 ml kg^{-1})]. Furthermore, the reported values are in line with other studies using *in situ* cod hearts although there is some variation between studies [$\dot{Q}$, 48–58 ml min^{-1} kg^{-1}; V_S, 0.95–1.2 ml kg^{-1} (Mendonca et al., 2007) (A.K.G., G. Lurman, L.H.P. and H. O. Portner, unpublished data; A.K.G. and A. G. Genge, unpublished data)].

A very significant finding was that maximum $\dot{Q}$ and V_S were 19 and 28% lower, respectively, under oxygenated conditions in hearts

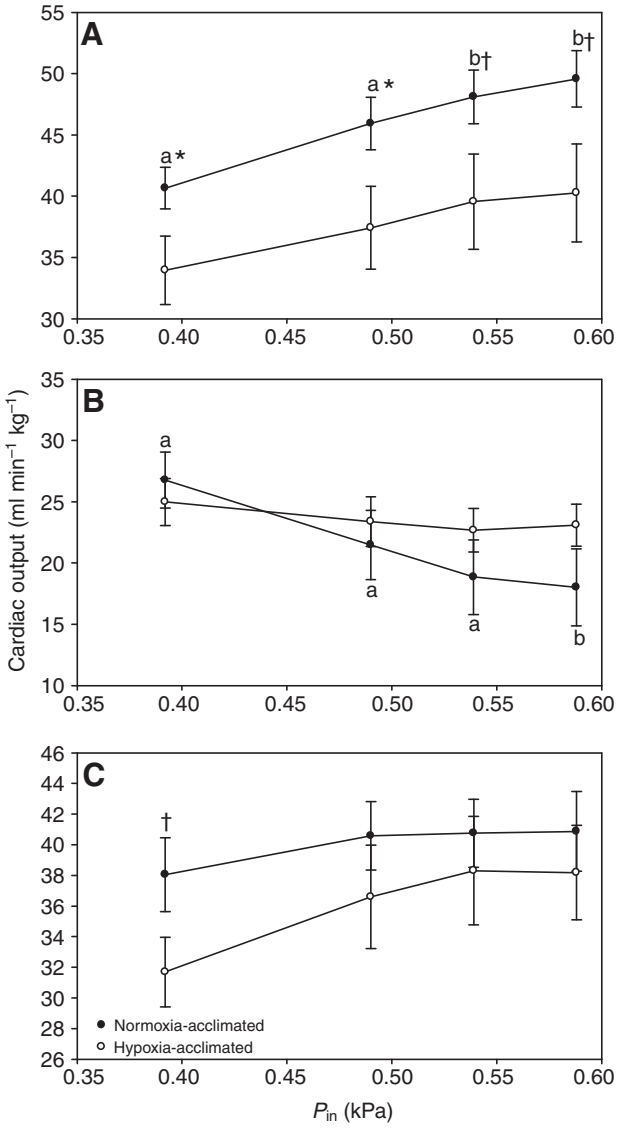

Fig. 3. The relationship between input pressure (P_{in}) and cardiac output for cod *in situ* hearts during three maximum cardiac output ($\dot{Q}_{max}$) tests. (A) Initial maximum cardiac output test ($\dot{Q}_{max1}$) in oxygenated saline; (B) $\dot{Q}_{max2}$ test performed after hearts were exposed to severe hypoxia (saline P_{O_2} ~0.6 kPa) for 15 min; (C) the final $\dot{Q}_{max}$ test ($\dot{Q}_{max3}$), performed 30 min after the hearts were allowed to recover from the severe hypoxic exposure (black circles; normoxia-acclimated cod; white circles; hypoxia-acclimated cod). Values are means ± s.e.m. (N=8 for each group). *Value significantly different (P<0.05) in normoxia- and hypoxia-acclimated groups. †Value significantly different (P<0.10) in normoxia- and hypoxia-acclimated groups at 0.55 kPa (P=0.075) and 0.60 kPa (P=0.064) in A and P=0.080 at 0.4 kPa in C. Dissimilar letters indicate values within the normoxia-acclimated group that were significantly different from those recorded at 0.40 kPa.

from hypoxia-acclimated cod, and that these reductions mirrored those seen *in vivo* (~25%) when normoxia- and hypoxia-acclimated cod were given a U_{crit} test under normoxic conditions (Petersen and Gamperl, 2010). These data strongly suggest that the reduced *in vivo* capacity of hearts from hypoxia-acclimated cod to pump during exhaustive exercise was not the result of alterations in the ability of nervous or hormonal mechanisms to stimulate cardiac function, or of the fish to modulate venous (filling) pressure (e.g. Sandblom and Axelsson, 2005; Sandblom and Axelsson, 2006), but a direct effect of chronic hypoxia on the myocardium.

There has been very little research on the effects of chronic hypoxia on fish cardiac physiology and morphology. However, there are at least three potential explanations for the poor pumping capacity of *in situ* hearts from hypoxia-acclimated fish under oxygenated conditions. First, it is possible that the cod myocardium was damaged by constant exposure to low oxygen conditions. Such a conclusion would be consistent with the findings of Lennard and Huddart (Lennard and Huddart, 1992) who showed that cardiomyocytes in flounder (*Platichthys flesus*) subjected to 3 weeks of hypoxia (water P_{O_2} ~5 kPa) showed striking changes in mitochondrial morphology (decreased size, budding and necrosis) and evidence of myofibril degeneration. However, the level of hypoxia in this study (8–9 kPa) was not nearly as severe as that used by Lennard and Huddart (Lennard and Huddart, 1992), and several studies have shown that, at least in the trout heart, acute (<30 min) exposure to severe anoxia (perfusate P_{O_2} ≤1 kPa) does not result in myocardial necrosis or a disruption in myocardial energetic and enzymatic status (Faust et al., 2004; Overgaard et al., 2004a; Overgaard et al., 2004b). These data thus raise the question of whether myocardial damage and/or necrosis was experienced by our hypoxia-acclimated cod. Second, the hearts of hypoxia-acclimated cod could have been 'stunned' [i.e. experiencing mechanical dysfunction that persists after reoxygenation and/or reperfusion despite the absence of irreversible damage; see Bolli and Marban (Bolli and Marban, 1999)] when initially tested under oxygenated conditions, and thus that the decreased pumping capacity of hearts from hypoxia-acclimated cod represented a reduced functional capacity that was not related to major morphological or structural alterations of the myocardium. This conclusion would be consistent with the findings of a number of authors who showed that the trout heart is stunned when acutely exposed to severe hypoxia (Faust et al., 2004; Overgaard et al., 2004a; Overgaard et al., 2004b). However, we feel it is unlikely that the decrease in performance during the initial test under oxygenated conditions was the result of stunning. This is because the hearts of hypoxia-acclimated cod showed no deficit in cardiac performance after exposure to severe hypoxia and reperfusion (see below). Finally, it is possible that hypoxia-induced myocardial remodelling reduced the maximum V_S of the heart. Although the similar RVM in hypoxia- and normoxia-acclimated cod (0.071 and 0.072%, respectively) provides some evidence against extensive cardiac remodelling in the chronically hypoxic cod, Marques et al. (Marques et al., 2008) showed that acclimation of zebrafish (*Danio rerio*) and the cichlid (*Haplochromis piceatus*) to a P_{WO_2} of 2 kPa (10% air saturation) for 21 days increased cardiac myocyte density and that this resulted in a smaller ventricular outflow tract and reductions in the size of the central ventricular cavity and lacunae. Such a decrease in the capacity of the ventricle to fill with blood would certainly explain why maximum *in situ* and *in vivo* V_S were reduced by 28 and 25%, respectively, in the hypoxia-acclimated cod. We feel this is the most likely explanation, and we will be initiating experiments shortly to investigate whether this phenomenon also occurs in cod when exposed to chronic hypoxia.

Maximum cardiac function: hypoxia and reperfusion

When hearts from normoxia-acclimated fish were exposed to severe hypoxia (P_{O_2} ≤1 kPa) at 10°C, maximum $\dot{Q}$ was reduced to approximately one-third of initial values under oxygenated conditions (Tables 3 and 4). This decrease in $\dot{Q}_{max}$ was attributed to a decrease in V_S as f_H did not change, and its magnitude is

consistent with earlier studies on the effect of hypoxia on the cardiac performance of isolated trout and dogfish hearts. For example, the $\dot{Q}_{max}$ of 10°C *in situ* trout hearts decreased by 25–50% when perfusate P_{O_2} was lowered to ~3.3 kPa (Farrell et al., 1989; Hanson et al., 2006), and Davie and Farrell (Davie and Farrell, 1991) showed that $\dot{Q}_{max}$ in an isolated 15°C dogfish heart preparation was decreased by 59% at 1 kPa. However, it is much greater than the <20% reduction in $\dot{Q}_{max}$ experienced by 15°C eel hearts exposed to a P_{O_2} of 1.6 kPa (Davie et al., 1992), although these hearts were only exposed to 8 min of hypoxia before $\dot{Q}_{max}$ was assessed.

Exposure of hearts from normoxia-acclimated cod to hypoxia, followed by a $\dot{Q}_{max}$ test at the end of the 15 min hypoxic period, resulted in a ~17% reduction in $\dot{Q}_{max}$. Furthermore, although we did not measure PO_{max} at the beginning of the experiment, a comparison of our PO_{max} data with other studies on cod hearts where PO_{max} was only evaluated under oxygenated conditions (Fig. 6) indicates that exposure to hypoxia reduced PO_{max} by ~30% (4.7 *vs* ~7 mW g^{-1} ventricle, respectively) and shifted the P_{out} where PO_{max} occurred from ~6-7 to 4 kPa. These hypoxia-induced reductions in $\dot{Q}_{max}$ and PO_{max} are very similar to values reported for 10°C-acclimated trout (Faust et al., 2004; Overgaard et al., 2004a; Overgaard et al., 2004b) and cod (A.K.G. and A. G. Genge, unpublished data), and were probably associated with stunning of the myocardium and not myocardial damage or disruptions in energy metabolism. This conclusion is based on the fact that several studies on trout have failed to demonstrate myocardial necrosis after periods of anoxia or severe hypoxia as long as 30 min (Gamperl et al., 2001; Faust et al., 2004; Overgaard et al., 2004a; Overgaard et al., 2004b), and Overgaard et al. (Overgaard et al. 2004a) reported that the energetic state of trout hearts exposed to 20 min of anoxia and 30 min of reperfusion was similar to that measured in hearts constantly perfused with oxygenated saline.

The hearts of hypoxia-acclimated cod were better able to maintain $\dot{Q}_{max}$ during severe hypoxia and showed significantly enhanced recovery following 30 min of reperfusion with oxygenated saline (Figs 3 and 4, Table 4). These results suggest that acclimation to chronic hypoxia increases myocardial hypoxia tolerance, and are consistent with the substantial body of research that has been conducted on chronically hypoxic mammals (for a review, see Ostadal and Kolar, 2007). Furthermore, they are in agreement with those of Driedzic et al. (Driedzic et al., 1985) who showed that hearts from hypoxia-acclimated (4–6 weeks; P_{O_2}, 4–4.7 kPa) eelpout were better able to sustain peak tension development during anoxia in the presence of elevated levels of Ca^{2+} in the bathing media.

In fish, as in mammals, the loss of myocardial function during severe hypoxia or anoxia is primarily due to the inability of the heart to maintain the rate of ATP production through anaerobic metabolism (Farrell et al., 1985; Overgaard and Gesser, 2004), and changes in the intracellular environment of the myocyte that result (Godt and Nosek, 1989). For example, anaerobic metabolism leads to the production of lactate and a corresponding build up of hydrogen ions that diminishes hypoxic performance (Gesser and Poupa, 1974). In addition, cellular high energy phosphate levels decline during hypoxia and this leads to an accumulation of intracellular phosphates (Hartmund and Gesser, 1996; Arthur et al., 1992), which further impairs contractility by reducing the calcium sensitivity of troponin C (Gesser and Jorgensen, 1982; Nosek et al., 1987). There are several strategies that can be utilized to balance cellular ATP demand and supply during hypoxia, including upregulating glycolytic energy production, and reducing energy demand to levels that can be supported by the reduced ATP availability. Measurements were not taken in this study to elucidate the mechanisms whereby hearts from hypoxia-acclimated cod were better able to sustain maximum cardiac performance under severe hypoxia. However, data from the fish and mammalian literature points to several mechanisms that might have been important for balancing ATP supply and demand. First, it is possible that enhanced glucose uptake and glycolytic capacity were primary factors mediating the enhanced cardiac function of hearts from hypoxia-acclimated cod. Lennard and Huddart (Lennard and Huddart, 1992) report that endogenous glycogen stores are increased in flounder myocytes after 3 weeks at a Pw_{O_2} of 5 kPa. Several studies have reported the reliance of hypoxic and anoxic teleost cardiac performance on extracellular glucose (Driedzic et al., 1985; Bailey

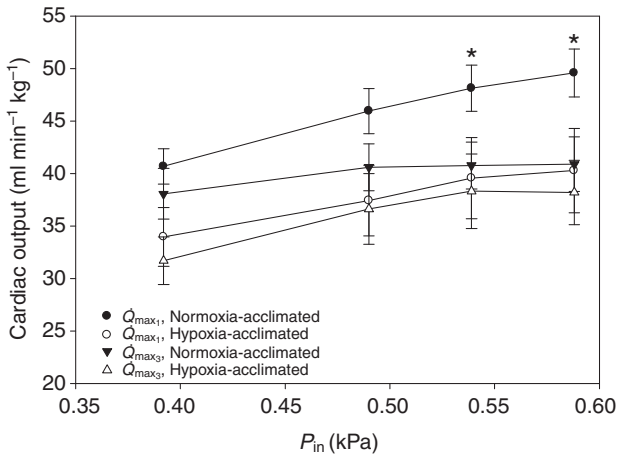

Fig. 4. The relationship between input pressure (P_{in}) and cardiac output for cod *in situ* hearts during the $\dot{Q}_{max}$ tests performed in oxygenated saline. $\dot{Q}_{max1}$ is the initial $\dot{Q}_{max}$ test and $\dot{Q}_{max3}$ is the third $\dot{Q}_{max}$ test, performed 30 min after the hearts were allowed to recover from severe hypoxia (see legend of Fig. 1). Values are means ± s.e.m. (N=8 for each group). *Value significantly different (P<0.05) between $\dot{Q}_{max1}$ and $\dot{Q}_{max3}$ for the normoxia-acclimated group.

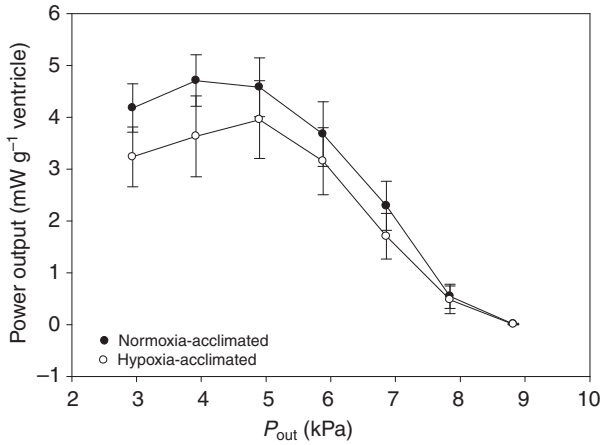

Fig. 5. The effect of increased output pressure (P_{out}) on the myocardial power output of *in situ* Atlantic cod hearts from normoxia- or hypoxia-acclimated individuals. These measurements were taken while input pressure was left at the level (0.6 kPa) used to obtain maximum cardiac output. Values are means ± s.e.m. (N=8 for each group). There was no statistically significant difference in maximum power output (see Table 3) between the two groups even though maximum power output was routinely lower in the hypoxia-acclimated group.

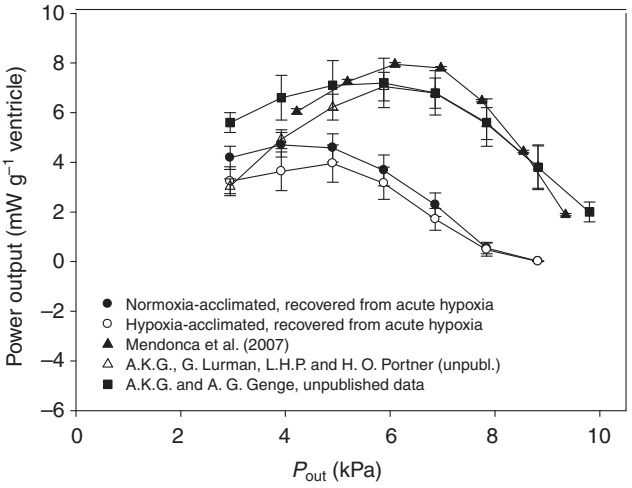

Fig. 6. Comparison of the relationship between power output and output pressure (P_{out}) for hearts in the current study (i.e. after recovery from 15 min of severe hypoxia) with that obtained in several other studies on Atlantic cod *in situ* cardiac performance where the hearts did not experience a hypoxic insult prior to the maximum power output test. In these other studies, all fish were acclimated to normoxic conditions.

et al., 2000; Gamperl et al., 2001; Clow et al., 2004), and Clow et al. (Clow et al., 2004) showed that glucose uptake was increased threefold in the cod heart during hypoxia (~P_{O_2} 5 kPa). Finally, Martinez et al. (Martinez et al., 2006) reported increases in myocardial hexokinase, pyruvate kinase and triose phosphate isomerase activity ranging from 18–28% in Gulf killifish (*Fundulus grandis*) exposed to a Pw_{O_2} of 3 kPa for 4 weeks, and Marques et al. (Marques et al., 2008) showed increased expression of pyruvate kinase (by 2.7-fold) and aldolase b (by 4.3-fold) mRNA after zebrafish were exposed to a Pw_{O_2} of 2 kPa for 21 days. However, we feel this is unlikely as Hall et al. (Hall et al., 2009) showed that increases in the expression of genes related to glucose transport, uptake and metabolism are short-lived when cod are acclimated to hypoxia (Pw_{O_2} ~8.0–9.0 kPa; i.e. hypoxia-acclimated cod do not appear to have an enhanced capacity for anaerobic metabolism). Second, a caveat with these experiments is that the heart cannot be made completely anoxic (i.e. P_{O_2} was ~0.6 kPa), and a small amount of aerobic metabolism can go a long way in supplying cellular ATP demands, given the large amount of ATP generated per molecule of glucose (36 *vs* 2 through anaerobic metabolism). Given that myoglobin plays an important role in oxygen metabolism in fish hearts at low extracellular P_{O_2}s by facilitating oxygen diffusion from the extracellular space to the mitochondria (e.g. see Legate et al., 1998), one might expect to see an increase in myocardial myoglobin levels in hearts of chronically hypoxic individuals. However, this is unlikely as myoglobin levels or mRNA expression were unchanged in both eelpout (Driedzic et al., 1985) and cod (Hall et al., 2009) following acclimation to hypoxia. Third, Na^+/K^+-ATPase activity constitutes 20–40% of cellular energy expenditure in excitable tissues (Rolfe and Brown, 1997), and Paajanen and Vornanen (Paajanen and Vornanen, 2003) showed that acclimation to hypoxia reduces the Na^+/K^+-ATPase activity of crucian carp (*Carassius carassius*) cardiac homogenates by 33%. This overall ~10% saving in cellular energy expenditure may be an important component to the enhanced performance exhibited by hypoxia-acclimated cod. Finally, there are several other mechanisms that have been reported to confer hypoxia tolerance on the mammalian heart during periods of prolonged oxygen deprivation. Amongst these are ATP-sensitive potassium (K_{ATP}) channels [both sarcolemmal (sK_{ATP}) and mitochondrial (mK_{ATP})], nitric oxide (NO), HIF-1α, and various protein kinases (including PKC) (Kolar and Ostadal, 2004; Ostadal and Kolar, 2007). However, research on the importance of these mechanisms in conferring cardioprotection in fishes is still in its infancy, and is often contradictory (see MacCormack and Driedzic, 2002; Chen et al., 2005; Rissanen et al., 2006; Marques et al., 2008).

With regards to the ability of fish hearts to recover maximum cardiac function following a period of oxygenated perfusion, Overgaard et al. (Overgaard et al., 2004a) showed that functional impairment of trout hearts following anoxic exposure occurs even though energetic and biochemical status of the myocardium is not compromised (altered); and they suggested that increased levels of oxygen radicals were responsible for the stunning of trout hearts following recovery from severe hypoxia and anoxia. Based on this information, it could be hypothesized that the improved functional recovery shown by hearts of hypoxia-acclimated cod following 15 min of severe hypoxia was due to an increased ability to protect the myocardium against the negative effects of reactive oxygen species (ROS). Indeed, this is a plausible explanation, as Marques et al. (Marques et al., 2008) showed that the expression of six genes important for protection against ROS were upregulated (by 2.1- to 6.5-fold) in zebrafish hearts following 21 days of acclimation to a Pw_{O_2} of 2 kPa.

Perspectives and future research

In summary, this study shows that acclimation to a moderate level of hypoxia (Pw_{O_2} ~8 kPa) has a direct negative impact on the maximum performance of the cod heart, but enhances its ability to maintain maximum pumping capacity under conditions of oxygen shortage, and to recover better following a period of oxygenated reperfusion. While these results may appear contradictory, and we presently have few mechanistic explanations for how these differences in functional capacity might be mediated in fishes, we hypothesize, based on the literature, that they reflect two different types of myocardial adaptation: (1) a remodelling of the heart (probably involving significant hyperplasia) that limits ventricular volume (stroke volume) but diminishes the workload of individual cardiomyocytes and prevents severely hypoxic/anoxic cores in the cardiomyocytes, and thus limits apoptosis and/or myocardial necrosis (see Des Tombe et al., 2002; Laarse et al., 2005; Marques et al., 2008); and (2) a reprogramming of cellular metabolism that ameliorates the potentially negative effects of long-term hypoxia on the capacity for aerobic metabolism, and attenuates mitochondrial ROS production (Papandreou et al., 2006; Kim et al., 2006; Kelly, 2008). Whether these hypotheses are correct, and apply to fishes in general, awaits a significant amount of research effort. However, we expect this effort will reveal novel insights into myocardial plasticity and adaptation in fishes (vertebrates), and the molecular and biochemical pathways that protect the heart from environmental insults that might normally lead to cardiac dysfunction, myocardial damage, and eventually mortality.

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