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RESEARCH ARTICLE

In situ cardiac performance of Atlantic cod (*Gadus morhua*) at cold temperatures: long-term acclimation, acute thermal challenge and the role of adrenaline

Glenn J. Lurman[1,*], Lene H. Petersen[2,†] and A. Kurt Gamperl[2,‡]

[1]Alfred-Wegener-Institut für Polar- und Meeresforschung, PO Box 120161, 27515 Bremerhaven, Germany,
[2]Ocean Sciences Centre, Memorial University of Newfoundland, St John's, NL, Canada, A1C 5S7

*Present address: Institute of Anatomy, University of Bern, Baltzerstrasse 2, CH-3012, Bern, Switzerland
†Present address: Department of Biology, University of North Texas, 1155 Union Circle, Denton, TX 76203, USA
‡Author for correspondence (kgamperl@mun.ca)

SUMMARY

The resting and maximum *in situ* cardiac performance of Newfoundland Atlantic cod (*Gadus morhua*) acclimated to 10, 4 and 0°C were measured at their respective acclimation temperatures, and when acutely exposed to temperature changes: i.e. hearts from 10°C fish cooled to 4°C, and hearts from 4°C fish measured at 10 and 0°C. Intrinsic heart rate (f_H) decreased from 41 beats min^{-1} at 10°C to 33 beats min^{-1} at 4°C and 25 beats min^{-1} at 0°C. However, this degree of thermal dependency was not reflected in maximal cardiac output (Q_{max} values were ~44, ~37 and ~34 ml min^{-1} kg^{-1} at 10, 4 and 0°C, respectively). Further, cardiac scope showed a slight positive compensation between 4 and 0°C (Q_{10}=1.7), and full, if not a slight over compensation between 10 and 4°C (Q_{10}=0.9). The maximal performance of hearts exposed to an acute decrease in temperature (i.e. from 10 to 4°C and 4 to 0°C) was comparable to that measured for hearts from 4°C- and 0°C-acclimated fish, respectively. In contrast, 4°C-acclimated hearts significantly out-performed 10°C-acclimated hearts when tested at a common temperature of 10°C (in terms of both Q_{max} and power output). Only minimal differences in cardiac function were seen between hearts stimulated with basal (5 nmol l^{-1}) *versus* maximal (200 nmol l^{-1}) levels of adrenaline, the effects of which were not temperature dependent. These results: (1) show that maximum performance of the isolated cod heart is not compromised by exposure to cold temperatures; and (2) support data from other studies, which show that, in contrast to salmonids, cod cardiac performance/myocardial contractility is not dependent upon humoral adrenergic stimulation.

Key words: cod, heart, heart rate, cardiac output, temperature, adrenergic stimulation, catecholamines.

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INTRODUCTION

Temperature is a critical environmental factor that influences all life functions through changes in the rates of biochemical and physiological processes, and alterations in the stability of biological molecules. Consequently, the thermal tolerance range of aquatic organisms has been studied for decades (e.g. Brett, 1971; Fry, 1971; Beitinger et al., 2000; Pörtner, 2001), and there is accumulating evidence that: (1) the thermal tolerance of marine organisms (including fishes) is limited by blood oxygen transport and aerobic scope; and (2) at the limits of acclimation capacity, temperature-dependent constraints on these physiological processes translate into alterations in population dynamics and biogeography (Pörtner et al., 2001; Pörtner, 2002; Pörtner and Knust, 2007; Farrell, 2009; Pörtner, 2010; Eliason et al., 2011). Although it is difficult to determine the thermal limits of marine organisms under field conditions, studies on the influence of acclimation/acclimatization on physiological mechanisms/processes and thermal tolerance can be very insightful (Sokolova and Pörtner, 2003; Stillman, 2003; Seebacher et al., 2005; Franklin et al., 2007). For example, these latter authors reported that even archetypical stenothermal fish (e.g. the Antarctic fish *Pagothenia borchgrevinki*) display considerable plasticity in cardiovascular and metabolic control, and swimming performance, as a result of temperature acclimation.

Based on the above, it is clear that additional research is needed on how acclimation temperature and thermal history influence the temperature limits of various fish species, and on what physiological processes mediate thermal tolerance. Thus, in this study, we used an *in situ* heart preparation to examine how acclimation to 10, 4 and 0°C as well as acute temperature changes (10 to 4°C, 4 to 10°C and 4 to 0°C) influence maximum cardiac performance in Atlantic cod. In our experiments, we chose temperatures below 10°C to examine the relationship between cardiac function and temperature because cod that inhabit the continental shelf off Atlantic Canada typically face water temperatures between 0.7 and 8°C (Lear, 1984; Clark and Green, 1991), and these temperatures span those used by Claireaux et al. (Claireaux et al., 2000) to examine the influence of acclimation temperature on cod aerobic scope. Our research complements previous work as there are currently no data on cod cardiac performance below 5°C. In addition, to our knowledge, only one study [A. P. Farrell, J. Altimiras, C. E. Franklin and M. Axelsson, unpublished; data presented in Axelsson (Axelsson, 2005)] has measured maximum cardiac performance/cardiac scope in a non-Antarctic teleost at temperatures of, or approaching, 0°C.

This study also addresses the role that circulating catecholamines play in regulating temperature-dependent cardiac performance in cod. Specifically, data on 10°C-acclimated Atlantic cod (Axelsson, 1988) suggest that adrenaline is not required for basal or maximum

cardiac performance, whereas studies on several teleosts show that adrenergic sensitivity is a critical compensatory mechanism that enables the fish myocardium to maintain contractility during acute cold exposure (e.g. Franklin and Davie, 1992; Keen et al., 1993; Aho and Vornanen, 2001; Shiels et al., 2003; Galli et al., 2009), hypoxia and alterations in blood chemistry that are associated with intense exercise (Hanson et al., 2006). However, the apparent lack of myocardial responsiveness to adrenaline in cod may be due to the fact that only a single experimental temperature (10°C) has been examined to date. For example, research on other teleosts indicates that acclimation to 'warm' temperatures or those within a fish's optimal thermal range may reduce myocardial adrenergic sensitivity and/or adrenoreceptor density (Graham and Farrell, 1989; Keen et al., 1993; Shiels et al., 2003; Farrell et al., 2007). Thus, by examining the effects of adrenergic stimulation on maximum cardiac performance at several temperatures (0, 4 and 10°C), we were able to further evaluate what role circulating catecholamines play in supporting cod cardiac performance, and indeed, whether this species differs from other teleosts in this regard.

MATERIALS AND METHODS

This research conformed to the guidelines published by the Canadian Council on Animal Care and was approved by Memorial University's Institutional Animal Care Committee (Protocol 04-01-KG).

Experimental animals

The mixed-gender, 2-year+ Atlantic cod *Gadus morhua* L. used in this study were transported from a sea-cage facility at Northwest Cove (Hermitage Bay, Newfoundland, Canada) to the Aquaculture Research and Development Facility (ARDF) at the Ocean Sciences Centre in St John's, Newfoundland, in March 2006. The fish were held in 3000 l tanks at the ARDF supplied with aerated seawater at 10°C for 3–4 months post-transfer, and then acclimated to 10, 4 and 0–1°C for at least 6 weeks prior to experimentation; water temperature was lowered by 1°C every 2–3 days until the desired temperature was reached. While at the ARDF, the cod were fed a commercial cod diet daily and maintained under ambient photoperiod.

Surgical procedures

In situ heart preparations were obtained for the cod with only minor modifications of the protocol of Farrell et al. (Farrell et al., 1982), as described in Mendonça et al. (Mendonça et al., 2007). Each fish was then bisected just posterior to the pectoral fins, placed in a water-jacketed saline-filled bath maintained at the fish's acclimation temperature, and the input and output cannulae were immediately connected to a tube delivering perfusate at constant pressure and to tubing whose height could be adjusted to control the end-diastolic pressure developed by the ventricle, respectively. The saline contained (in $g\,l^{-1}$): 10.5 NaCl; 0.49 $MgSO_4{\cdot}7H_2O$; 0.37 KCl; 0.34 $CaCl_2{\cdot}2H_2O$; 0.14 $NaH_2{\cdot}PO_4{\cdot}H_2O$; 1.84 sodium TES base; 0.59 TES acid; and 1.0 glucose, pH 7.67 at 20°C. Shortly before use, 250 µl of 0.1 $\mu mol\,l^{-1}$ adrenaline bitartrate salt (AD) dissolved in distilled H_2O was added to 500 ml of saline to give a final concentration of 5 $nmol\,l^{-1}$ AD, a concentration similar to resting plasma concentrations in Atlantic cod (Wahlqvist and Nilsson, 1980). Alternatively, 500 µl of 2 $\mu mol\,l^{-1}$ AD was added to obtain a final concentration of 200 $nmol\,l^{-1}$ AD; this concentration designed to mimic maximal *in vivo* concentrations measured in stressed fish (Wahlqvist and Nilsson, 1980). Adrenaline was added to fresh (0 $nmol\,l^{-1}$ adrenaline) perfusate bottles every 20 min to avoid photo-degradation, and thus loss of potency.

Cardiac performance tests

See Fig. 1 for a graphical representation of the complete protocol. After mounting the *in situ* preparation in the experimental bath, the heart was allowed to recover from surgery at the acclimation temperature for approximately 5–10 min at an output pressure (P_{out}) of 2 kPa and input pressure (P_{in}) was continuously adjusted to maintain a cardiac output (Q) of 16, 10 or 8 $ml\,min^{-1}\,kg^{-1}$ at 10, 4 and 0°C, respectively. These values were estimates of *in vivo* resting Q based on published values for cod at various temperatures (Webber et al., 1998). After this initial period, P_{out} was increased to a physiological level of 5 kPa (Pettersson and Nilsson, 1980; Axelsson and Nilsson, 1986), and the hearts were allowed to stabilise for a further 20 min at resting Q and P_{out}. Thereafter, the temperature was changed in those hearts subjected to an acute thermal challenge (i.e. from 10 to 4°C or from 4°C to 10 or 0°C) over 60 min, and they were allowed an additional 20 min to stabilise at their final test temperature before their maximum performance was assessed. An equivalent period under resting conditions was allowed for hearts tested at their acclimation temperature to control for any deteriorations in cardiac function with time.

For all hearts, a maximum cardiac output (Q_{max}) test was initially performed at 5 $nmol\,l^{-1}$ AD by increasing input pressure in steps from resting values to 0.4, 0.5, 0.55 and finally 0.6 kPa. Each increase in P_{in} was held for ~30 s, and output pressure was maintained at

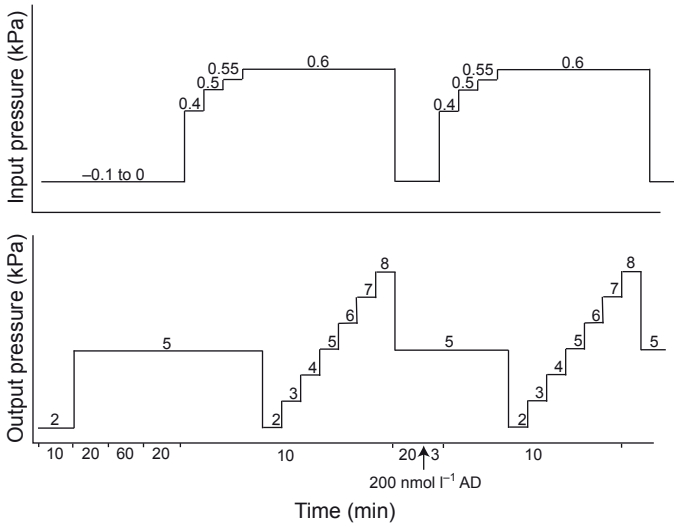

Fig. 1. Experimental protocol used to examine the effects of temperature acclimation and acute changes in temperature on Atlantic cod *in situ* resting and maximum cardiac function. After the *in situ* heart preparation was placed in the experimental bath at the fish's acclimation temperature [with 5 $nmol\,l^{-1}$ adrenaline (AD) in the perfusate] and allowed to recover at a sub-physiological output pressure (2 kPa) for 10 min, the output pressure head was increased to a physiological value of 5 kPa for another 20 min. A period of 1 h was then used to change the temperature for the acutely challenged hearts, and these hearts were allowed a further 20 min to stabilise at their test temperature before the maximum cardiac output test. This test was performed by increasing input pressure in four steps (0.4, 0.5, 0.55 and finally 0.6 kPa), and was followed immediately by a maximum power output test where the P_{in} was left at 0.6 kPa and P_{out} dropped to 2 kPa before being increased in 1 kPa steps to 8 kPa. After these initial tests, the hearts were allowed to recover for 20 min and cardiac output was set at the appropriate resting level by adjusting P_{in}. Thereafter, the AD level in the perfusate was increased to 200 $nmol\,l^{-1}$, resting parameters were recorded after 3–5 min at the new level of AD, and a second set of maximum cardiac output and maximum power output tests was performed. Note: the time-line for fish that were tested at their acclimation temperature was the same as described above.

Table 1. Morphometric data for all groups used to evaluate the effect of temperature and adrenaline concentration on Atlantic cod *in situ* cardiac function

Acclimation temperature (°C)	10		4			0
Test temperature (°C)	10	4	10	4	0	0
Mass (kg)	0.60±0.03	0.54±0.03	0.54±0.03	0.59±0.02	0.49±0.10	0.56±0.17
Length (cm)	41.6±0.6	40.5±0.8	40.3±0.5	40.8±0.4	40.8±1.7	39.9±1.2
Ventricle mass (g)	0.49±0.02	0.46±0.03	0.49±0.04	0.47±0.02	0.40±0.04	0.48±0.03
Atrial mass (g)	0.154±0.011	0.123±0.008	0.133±0.008	0.133±0.008	0.123±0.017	0.117±0.006
RVM (%)	0.083±0.014	0.080±0.012	0.091±0.012	0.083±0.010	0.081±0.009	0.089±0.013
RAM (%)	0.026±0.003	0.023±0.004	0.025±0.003	0.023±0.002	0.025±0.006	0.022±0.004

Relative ventricular (RVM) and atrial mass (RAM) are presented as a percentage of body mass. All values are means ± s.e.m. No differences were identified between the groups for any parameter. N=7–10 except hearts from 4°C-acclimated fish tested at 0°C, where N=4.

5 kPa. Then, P_{in} was left at 0.6 kPa and a maximum power output (PO_{max}) test was performed by decreasing P_{out} to 2 kPa and increasing it in 1 kPa steps until 8 kPa. Following these initial Q_{max} and PO_{max} tests, the hearts were allowed to recover under resting conditions for 20 min. After this, the perfusate AD concentration was changed from 5 to 200 nmol l^{-1}, and after 3 min, resting parameters were recorded and the Q_{max} and PO_{max} tests were repeated as described above. Finally, the hearts were removed from all fish so that ventricular mass and relative ventricular mass (RVM) and relative atrial mass (RAM) could be determined.

Data collection and analysis

Cardiac output was measured using a model T206 small animal blood flow meter in conjunction with a pre-calibrated in-line flow probe (2N, Transonic Systems, Ithaca, NY, USA). A Gould Statham pressure transducer (Model P23 ID, Oxnard, CA, USA) was used to measure P_{out}, and P_{in} was measured using a Grass pressure transducer (Model PT300, Warwick, RI, USA). Before the start of each experiment, the pressure transducers were calibrated against a static column of water, where zero pressure (0 cm H_2O) was set at the saline level in the experimental bath (note: 1 cm H_2O=0.098 kPa). Pressure and flow signals were collected at 20 Hz, and amplified and filtered using a Model MP100A-CE data acquisition system (BIOPAC Systems, Santa Barbara, CA, USA). The acquired signals were then analysed and stored using Acknowledge 3.7 software (BIOPAC Systems). Analysis included using pre-determined calibrations (Faust et al., 2004) to adjust P_{in} and P_{out} to account for the resistance in the tubing between the points of pressure measurement and the heart.

Cardiovascular performance was continuously measured throughout the experiment by measuring Q, P_{in} and P_{out}. P_{in} was measured before each Q_{max} test in order to determine the input pressure required to obtain resting Q. Cardiac output, heart rate (f_H), stroke volume, (V_S) and P_{in} were also measured/calculated at each step of the Q_{max} and PO_{max} tests. Heart rate was calculated by measuring the number of systolic peaks during a 30 s interval. Cardiac output (ml min^{-1} kg^{-1}) and stroke volume (ml kg^{-1}) were calculated as:

$$Q = Q / M \quad (1)$$

and

$$V_S = (Q / f_H) / M, \quad (2)$$

where M is fish mass (kg), and myocardial power output (PO; mW g^{-1} ventricle) was calculated as:

$$PO = [Q \times (P_{OUT} - P_{IN}) \times a] / M_V. \quad (3)$$

where P_{out} and P_{in} are output and input pressures (cm H_2O), respectively, a (0.0016 mW min ml^{-1} cm^{-1} H_2O) is a conversion factor to mW, and M_V is ventricular mass.

Statistical analysis was performed using SigmaStat 3.5 (Systat Software, Chicago, IL, USA). Two-way repeated-measures ANOVAs were used to test for the effects of temperature and adrenaline concentration, and Holm–Sidak *post hoc* tests were then used to examine differences between groups when main effects were significant (P<0.05). Values in the text, and those presented in figures and tables, are means ± 1 s.e.m.

RESULTS

Acclimation temperature had no significant effect on cod ventricular or atrial mass, or RVM or RAM (RVM=0.080–0.091% and RAM=0.023–0.026%; Table 1).

Effects of temperature

Cardiac performance at rest

Under resting conditions, with 5 nmol l^{-1} AD, 10°C hearts (when acclimated to this temperature or acutely exposed to it) required a slightly positive P_{in} (0.004±0.020 kPa) to maintain the required resting Q of ~16 ml min^{-1} kg^{-1} (Table 2). This value was ~0.03 to 0.08 kPa higher than the negative input pressures required by 4°C hearts (again when acclimated or acutely challenged) to maintain resting Q or of the 0°C-acclimated hearts tested at this temperature (these hearts requiring the lowest input pressure, –0.11 kPa). Both Q and PO were significantly higher at 10°C than at 4 or 0°C, and at 4°C *versus* 0°C due to our manipulation of Q to reflect *in vivo* values at these different temperatures. These differences in Q were mirrored primarily by resting values for f_H (41.4 at 10°C, 33.2 at 4°C and 24.9 at 0°C), as V_S was not significantly different between the groups (range 0.33–0.4 ml kg^{-1}).

Maximum cardiac performance

Acclimation temperature also had a significant effect on heart rate during the Q_{max} test, with heart rate falling from ~40.5 to 29.1 beats min^{-1} between 10 and 0°C (Fig. 2A). Q_{max} decreased significantly with acclimation temperature (from approximately 44 to 34 ml min^{-1} kg^{-1}). However, the Q_{10} values for changes in Q_{max} with temperature were quite low (1.40, 1.25 and 1.34 for 10–4°C, 4–0°C and 10–0°C, respectively). This was because V_{Smax} increased slightly (i.e. from 1.15 and 1.30 ml kg^{-1}), although not significantly (P=0.30), between 10 and 0°C (Fig. 2B). Maximum power output of 10°C-acclimated hearts was 6.7 mW g^{-1} (Fig. 3). Although PO_{max} was lower at both 4 (6.1 mW g^{-1}) and 0°C (4.6 mW g^{-1}), none of the values for PO_{max} were significantly different (P=0.12).

An acute drop in temperature from 4 to 0°C had no effect on any of the measured maximum cardiovascular parameters, and only f_H changed significantly (decreasing by ~25%) when 10°C-acclimated hearts were tested at 4°C compared with 10°C (Figs 2, 3). In contrast,

Table 2. Input pressure (P_{in}), heart rate (f_H), stroke volume (V_S), cardiac output (Q) and power output (PO) under resting conditions at 5 or 200 nmol l^{-1} adrenaline (AD)

Acclimation temperature (°C)	10				4				0			
Test temperature (°C)	10		4		10		4		0		0	
AD (nmol l^{-1})	5	200	5	200	5	200	5	200	5	200	5	200
P_{in}	0.004±0.020	−0.008±0.018	−0.036±0.022	−0.039±0.025	0.004±0.027	0.002±0.044	−0.084±0.039	−0.073±0.033	−0.007±0.030	−0.001±0.033	−0.112±0.049	−0.090±0.045
f_H (beats min^{-1})	41.4±1.0^{a}	42.6±1.5^{a}	34.3±1.5^{b}	34.9±4.1^{b}	45.2±1.3^{a}	43.6±2.6^{a}	33.2±1.5^{b}	32.3±1.2^{b,c}	27.0±2.9^{c}	26.6±1.8^{c}	24.9±0.5^{c}	24.7±1.0^{c}
V_S (ml kg^{-1})	0.40±0.02	0.42±0.02	0.32±0.02*	0.37±0.02	0.34±0.02*	0.41±0.02	0.31±0.02*	0.39±0.02	0.34±0.03	0.35±0.03	0.33±0.01	0.36±0.03
Q (ml min^{-1} kg^{-1})	16.3±0.1^{a}	18.0±0.7^{a}	10.8±0.6^{b,*}	12.9±0.7^{b}	15.6±2.2^{a,*}	17.5±3.6^{a}	10.2±0.1^{b,*}	12.5±0.9^{b}	8.8±0.1^{b}	9.1±0.7^{c}	8.3±0.1^{b}	8.9±0.9^{c}
PO (mW g^{-1} ventricle)	2.75±0.13^{a}	3.02±0.13^{a}	2.08±0.11^{a,b,*}	2.48±0.11^{a,b}	2.88±0.14^{a,*}	3.35±0.14^{a}	1.83±0.13^{b,c,*}	2.21±0.13^{b,c}	1.51±0.18^{b,c}	1.57±0.18^{b,c}	1.19±0.14^{c}	1.25±0.14^{c}

Q_{10} values for heart rate were 1.44 between 10 and 4°C, 2.05 between 4 and 0°C and 1.66 between 10 and 0°C for hearts tested at their acclimation temperature and with 5 nmol l^{-1} AD. Values are means ± 1 s.e.m. Values with dissimilar letters are significantly different (P<0.05) within a particular adrenaline concentration. An asterisk (*) indicates a significant difference between the 5 and 200 nmol l^{-1} AD doses within each group. N=7–10 except hearts from 4°C-acclimated fish tested at 0°C, where N=4.

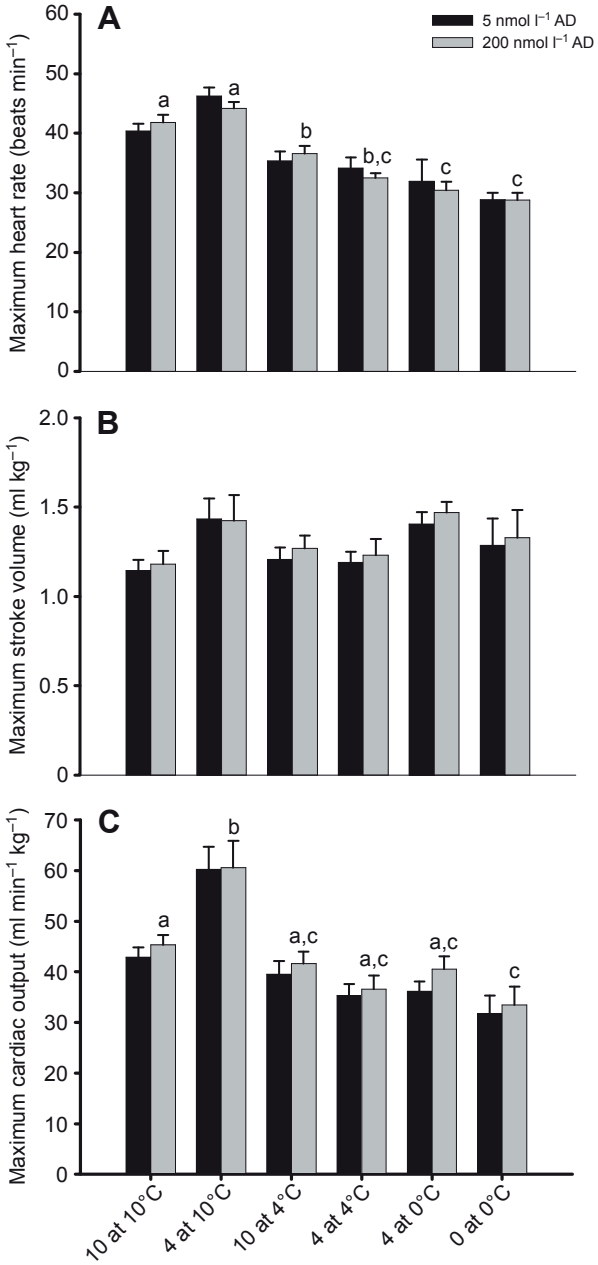

Fig. 2. Maximum values for heart rate (A), stroke volume (B) and cardiac output (C) for Atlantic cod hearts tested at their acclimation temperature (0, 4 and 10°C) and after an acute decrease or increase in temperature. Black bars indicate 5 nmol l^{-1} AD, while grey bars indicate 200 nmol l^{-1} AD in the perfusate. Groups without a letter in common are significantly different (P<0.05). Increasing the perfusate AD concentration did not significantly influence cardiac function in any group, although 200 nmol l^{-1} AD had a slight, but significant, overall positive effect on cardiac output and stroke volume. Bars indicate +1 s.e.m. N=7–10 except hearts from 4°C-acclimated fish tested at 0°C, where N=4.

the maximum performance of hearts from 4°C-acclimated cod increased dramatically when tested at 10°C. Heart rate was 40% higher, Q_{max} and PO_{max} increased by 65% (to 61 ml min^{-1} kg^{-1}) and 80% (to 10.9 mW g^{-1}), respectively, and this substantial enhancement in pumping capacity resulted in both of these latter parameters being significantly higher (by 37 and 50%, respectively) compared with hearts from 10°C-acclimated fish when tested at 10°C. Interestingly, these increases in maximum pumping capacity were not associated

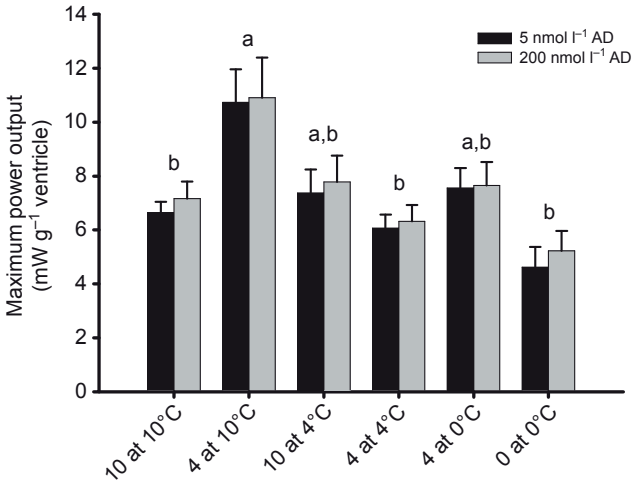

Fig. 3. Maximum power output (PO_{max}) for Atlantic cod hearts tested at their acclimation temperature (0, 4 and 10°C) and after an acute decrease or increase in temperature (e.g. 4 at 10°C indicates hearts from 4°C-acclimated fish that were tested at 10°C). Black bars indicate 5 nmol l^{-1} AD, while grey bars indicate 200 nmol l^{-1} AD in the perfusate. Groups without a letter in common are significantly different (P<0.05). Increasing the perfusate AD concentration did not significantly influence cardiac function in any group. Bars indicate +1 s.e.m. N=7–10 except hearts from 4°C-acclimated fish tested at 0°C, where N=4.

with statistically significant changes in V_S (although mean V_S was 25% higher in 4°C-acclimated fish; see Fig. 2B), or in the shape or position of the relationships between PO or Q and P_{out} (Fig. 4). Maximum values for power output were recorded between 5 and 6 kPa of diastolic pressure, and Q values for hearts from 4°C-acclimated cod tested at 10°C were substantially higher compared with all other groups at all values of P_{out}.

Adrenergic effects

Increasing the adrenaline concentration from 5 to 200 nmol l^{-1} generally had a positive inotropic effect on hearts tested under 'resting' levels of performance at 10 and 4°C, with Q, V_S and PO significantly or noticeably increased by ~10–20% at the higher concentration. However, no such stimulatory effect of the high adrenaline dose was observed in hearts tested at 0°C (Table 2), and f_H was unaffected by the 200 nmol l^{-1} AD dose at any temperature. A marginally positive effect of 200 nmol l^{-1} AD on heart function was also observed when comparing maximum cardiac performance (Q_{max}, V_{Smax} and PO_{max}) using the overall model (P=0.009, 0.03 and 0.05, respectively; see Figs 2, 3). However, *post hoc* Holm–Sidak t-tests did not reveal any significant effects of increased (200 *versus* 5 nmol l^{-1}) adrenaline on maximum cardiac parameters between groups at any of the acclimation/test-temperature combinations.

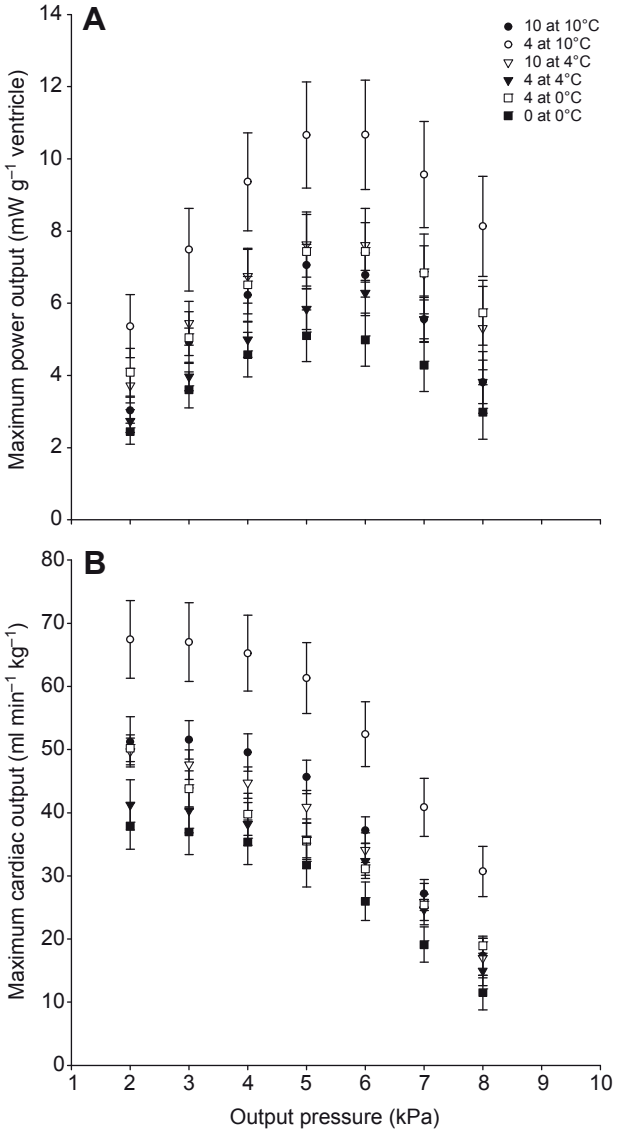

Fig. 4. Power (A) and cardiac (B) output for Atlantic cod hearts during the maximum power output test. Hearts were tested at their acclimation temperature (0, 4 and 10°C) and after an acute decrease or increase in temperature (e.g. 4 at 10°C indicates hearts from 4°C-acclimated fish that were tested at 10°C). In the maximum power output test, input pressure was maintained at 6 kPa, and diastolic output pressure was increased from 2 to 8 kPa. These data were obtained using 5 nmol l^{-1} AD. Note: Increasing the perfusate AD concentration to 200 nmol l^{-1} had no significant effect on either parameter, or on the shapes of the curves. At all P_{out} values, cardiac and power output for the 4°C-acclimated fish tested at 10°C were significantly higher than all other groups. Bars indicate ±1 s.e.m. N=7–10 except hearts from 4°C-acclimated fish tested at 0°C, where N=4.

DISCUSSION

The f_H of *in situ* hearts from 10°C-acclimated cod was 41 beats min^{-1} at 10°C, and maximum Q and V_S were 44 ml min^{-1} kg^{-1} and 1.14 ml kg^{-1}, respectively. This heart rate is substantially higher than that measured *in vivo* at 10°C after extended (1 week) recovery from surgery [28 beats min^{-1} (Webber et al., 1998)]. However, this difference was not unexpected given that the cholinergic tonus (37%) on the cod heart at 10°C is greater than the adrenergic tonus (21%) (Axelsson et al., 1998), and all nervous tone is eliminated in the *in situ* heart preparation. The reported Q_{max} and V_{Smax} also correspond well with *in vivo* data for this species. Petersen and Gamperl (Petersen and Gamperl, 2010) reported values for Q_{max} and V_{Smax} of 44.5 ml min^{-1} kg^{-1} and 0.99 ml kg^{-1} in cod swimming at their critical swimming speed (1.50 body lengths s^{-1}) at 10°C, while cod swimming at 0.7 m s^{-1} had a Q of 35 ml min^{-1} kg^{-1} (Webber et al., 1998). The close association between *in situ* and *in vivo* cardiac function in this species, is very similar to that for the rainbow trout (Claireaux et al., 2005), and further validates this preparation for studies of cardiac function in fishes.

Temperature effects on cardiac function

Cardiac hypertrophy is often associated with cold-acclimation/adaptation (Driedzic et al., 1996; Farrell, 1996; Axelsson et al., 1998; Aho and Vornanen, 2001), and has been previously reported to occur in Atlantic cod. Foster et al. (Foster et al., 1993) showed that the RVM of juvenile cod acclimated to 5°C for 43 days was 24% greater than for 15°C-acclimated fish. This latter result differs from this study where no difference in RVM was found between cod acclimated to temperatures between 0 and 10°C (Table 1). This discrepancy may be due to the age of the fish used in the two studies (juvenile *versus* adult), or the range of acclimation temperatures utilised (0–10°C *versus* 5 and 15°C). Nonetheless, this is not the first study to report that RVM does not increase with cold acclimation. Heart size did not change in the white bass (*Morone americana*) or yellow perch (*Perca flavescens*) when exposed to cold temperatures (Sephton and Driedzic, 1991). Although many studies report that RVM increases in rainbow trout at cold temperatures (e.g. Farrell et al., 1988; Graham and Farrell, 1989), Sephton and Driedzic (Sephton and Driedzic, 1995) did not observe any increase in heart size when trout were acclimated to 5°C for 4 weeks.

A clear effect of acclimation temperature was seen on intrinsic heart rate at rest, with Q_{10} values of 1.44, 2.05 and 1.66 between 10 and 4°C, 4 and 0°C, and 10 and 0°C, respectively. These results suggest that there was partial compensation of f_H between 10 and 4°C, but not as temperature fell further. The effect of acclimation temperature on f_H of the *in situ* cod heart is consistent with data for the rainbow trout and sea raven (*Hemitripterus americanus*). The Q_{10} value for trout hearts acclimated to 15 and 5°C and perfused with 5 nmol l^{-1} AD was 1.32 (Graham and Farrell, 1989), whereas sea raven hearts tested at 12–14°C *versus* 2–3°C had a Q_{10} for f_H of ~1.81 (Graham and Farrell, 1985). Collectively, these results suggest that the acclimation/acclimatization capacity of pacemaker cells and/or mechanisms that determine the kinetics of myocardial contraction are limited in temperate teleosts at temperatures near their lower thermal limit.

While acclimation temperature had a substantial effect on resting f_H, its influence on Q_{max} and PO_{max} was not as great. Q_{max} was not different between 10 and 4°C and only fell by 8.5% between 4 and 0°C (Q_{10}=1.25), and acclimation temperature had no significant effect on PO_{max} (Figs 2, 3). Further, cardiac scope showed a slight positive compensation between 4 and 0°C (Q_{10}=1.7), and a full, if not slight over compensation between 10 and 4°C (Q_{10}=0.9; Fig. 5). The ability of the Atlantic cod heart to largely compensate for the effects of temperature on Q_{max} and PO_{max} was due to the heart's ability to maintain, or slightly increase, V_{Smax} at the two colder acclimation temperatures (Fig. 2B). These results suggest that acclimation to cold temperatures does not have a negative impact on the intrinsic mechanical properties of the cod myocardium. This finding is in contrast to studies on most other teleosts. Sea raven acclimated to 2–3°C had a maximum stroke volume 20% lower than measured in fish acclimated to 12–14°C (Graham and Farrell, 1985). When the increase in ventricular mass in 5°C- *versus* 15°C-acclimated rainbow trout is taken into account, V_{Smax} (ml g^{-1} ventricle) was ~50% lower in fish acclimated to the lower temperature (Graham and Farrell, 1989). Finally, the pumping capacity of hearts from 5°C-acclimated carp (*Carassius carassius*) was only approximately one-third of that observed at 15°C (Matikainen and Vornanen, 1992).

Further evidence that the cod heart is well adapted to, and has considerable capacity for thermal compensation at, cold temperatures comes from the acute temperature change experiments: (1) both groups of hearts that experienced drops in temperature (from 10 to 4°C and 4 to 0°C) had values of Q_{max} and PO_{max} that were equivalent to or slightly higher than measured in hearts acclimated to the lower temperature; and (2) hearts acclimated at 4°C but tested at 10°C had values for these two variables that far exceeded (by 37 and 50%, respectively) those measured in hearts from 10°C-acclimated fish (Figs 2, 3). Indeed, both these results are remarkable based on data for the majority of teleost species examined under similar experimental conditions. For example, *in situ* Q_{max} and PO_{max} were ~40–45% lower when hearts from summer-acclimated (12–14°C) sea raven were tested at 3.3°C, and 20 and 35% lower, respectively, in winter-acclimated (2–3°C) than summer-acclimated fish when tested at 13°C (Graham and Farrell, 1985). The maximum contractile force developed by the hearts of 4°C-acclimated rainbow trout was reduced by 60% when fish were acutely exposed to 10°C without AD, and 20% lower even when 100 nmol l^{-1} AD was added (Aho and Vornanen, 2001). The maximum isometric tension and pumping capacity of the burbot (*Lota lota*, another member of the family Gadidae) heart are maximum when acclimated to 1°C, but decrease precipitously when exposed to an acute increase in temperature (Tiitu and Vornanen, 2002). Finally, even in winter-active freshwater species (e.g. yellow perch and smallmouth bass, *Micropterus dolomieui*), where positive thermal compensation in contractile force has been reported, this effect is only seen at low contraction/pacing frequencies (<30 min^{-1}) (Bailey and Driedzic, 1990). In the present study, hearts from 4°C-acclimated cod were able to maintain or increase V_{Smax} in contrast to 10°C-acclimated fish, even though their intrinsic f_H at 10°C exceeded 45 beats min^{-1} (see Fig. 2B). It is unlikely, however, that the cod heart's ability to pump at or near maximal levels when chronically or acutely exposed to temperatures at the lower end of its thermal range is unique amongst temperate marine teleosts. Axelsson et al. (A. P. Farrell, J. Altimiras, C. E. Franklin and M. Axelsson, unpublished) [see fig. 6.4 in Axelsson (Axelsson, 2005)] showed that cardiac scope of the eurythermal sculpin (*Myoxocephalus scorpuis*) exposed to an acute temperature increase from 1 to 10°C is 1.6-fold that measured in fish held at 1°C.

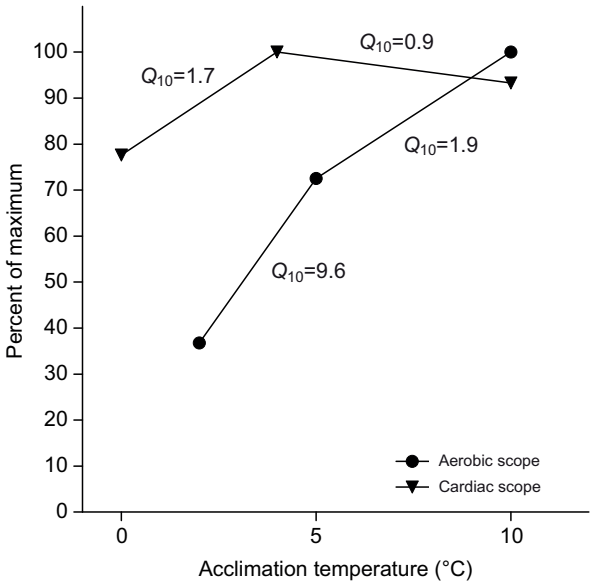

Fig. 5. Atlantic cod aerobic and cardiac scope as a function of acclimation temperature. Aerobic scope (net aerobic scope) calculated from Claireaux et al. (Claireaux et al., 2000). All data are normalised to the maximum value that was reported over the presented temperature range.

The ability of the cod heart to largely compensate for chronic and acute decreases in temperature, and to elevate performance when exposed to an acute increase in temperature from 4 to 10°C, must be predicated on aspects of cardiac and myocardial physiology. In this study, we did not investigate how cellular and molecular mechanisms important in myocardial plasticity and performance were affected by the imposed temperature regimes. However, we speculate that considerable remodelling of both the mechanical and electrical properties of the cod heart is probably involved in its superior cold performance. These alterations could include a prolonged action potential (Haverinen and Vornanen, 2008; Galli et al., 2009) and an enhancement of sarcolemmal Na^+ current (I_{Na}) that augments Ca^{2+} influx through Na^+/Ca^{2+} exchange (Haverinen and Vornanen, 2004). Further, the cod heart is rare amongst teleosts in that the force–frequency relationship is flat or positive over the range of f_H measured in this study, and this phenomenon can be eliminated by ryanodine [see fig. 7D in Driedzic and Gesser (Driedzic and Gesser, 1988)]. These latter data suggest that aspects of sarcoplasmic reticulum (SR) function may play a major role in enabling the *in situ* cod heart to maintain performance over a range of temperatures and to elevate performance when exposed to an acute increase in temperature. Indeed, this hypothesis has significant support in the literature. Several studies have shown that SR function is augmented in cold-acclimated or -resident species, and that SR Ca^{2+} cycling offers a mechanism for thermal plasticity in fish hearts (Aho and Vornanen, 1998; Aho and Vornanen, 1999; Shiels et al., 2006; Shiels et al., 2011). Tiitu and Vornanen (Tiitu and Vornanen, 2002) reported that excitation coupling of burbot hearts was more dependent on SR function after an acute temperature increase to 7°C than it was at the 1°C acclimation temperature.

Our data for cod *in situ* cardiac function suggest that this species should be able to maintain its performance capacity at cold temperatures, and when it encounters marked seasonal or diel temperature variations (e.g. D'Amours, 1993; Godø and Michalsen, 2000; Righton et al., 2010). However, this conclusion is in contrast to that reported for the effects of temperature on cod swimming and metabolic performance. Sylvestre et al. (Sylvestre et al., 2007) reported that cod swimming performance and metabolic capacity were reduced significantly (by 20–25%) following a short-term (over 2 days) drop in temperature from 7 to 3°C. Lurman et al. (Lurman et al., 2009) found that acclimation temperature (4 *versus* 10°C) had no or minimal effects on the active (maximum) metabolic rate and critical swimming speed of cod when tested at either temperature, but that these variables were significantly (10–20%) lower in both groups when tested at the other temperature. Finally, although cardiac scope changed little (i.e. by <20%) when our cod were acclimated to temperatures of 0, 4 and 10°C (present study), Claireaux et al. (Claireaux et al., 2000) showed that metabolic scope of the Atlantic cod decreased by 28% between 10 and 5°C and a further 48% between 5 and 2°C (Fig. 5). The large discrepancy between the effects of temperature on cod cardiac *versus* aerobic scope is an unexpected finding given the excellent relationship between cardiac output and oxygen consumption reported for Atlantic cod (e.g. Webber et al., 1998; Gollock et al., 2006), and suggests that there may be situations, particularly large acute changes in temperature and cold temperatures, where the relationship between cardiac function and oxygen consumption breaks down and the capacity of fishes to utilise oxygen becomes the limiting factor. However, these results will need to be confirmed *in vivo* as the influence of temperature changes on extrinsic mechanisms that control/influence fish cardiac function are precluded when using *in situ* heart preparations.

Cardiac function and adrenergic stimulation

In this study, we showed that high levels of AD had minimal effects on the resting or maximum *in situ* performance of the Atlantic cod heart (e.g. see Figs 2, 3). This result was not because the basal level of AD (5 nmol l^{-1}) resulted in near-maximal cardiac stimulation or that the maximum level chosen (200 nmol l^{-1}) was not physiological. A pilot study conducted with three cod hearts at 10°C compared the effects of no AD with both 5 and 200 nmol l^{-1} AD, and showed little difference in performance between them (data not shown), and Gamperl and Genge (A.K.G. and A. G. Genge, unpublished data) found no observable differences in resting or maximum f_H, Q or V_S in cod hearts treated with 7 nmol l^{-1} AD *versus* those perfused with AD-free saline. Further, *in vivo* maximum post-stress plasma AD concentrations in the range of 100–300 nmol l^{-1} have been reported for this species (Wahlqvist and Nilsson, 1980; Alzaid, 2012). Instead, in agreement with Axelsson (Axelsson, 1988), our results indicate that even maximum circulating catecholamine levels have little inotropic or chronotropic effect on the cod heart. These data are in sharp contrast to data on many teleosts, including salmonids, the eel (*Anguilla dieffenbachia*) and tunas, where this hormone causes large increases in the heart's pumping capacity and in myocardial force development (Graham and Farrell, 1989; Franklin and Davie, 1992; Gamperl et al., 1994; Shiels et al., 2003; Galli et al., 2009). However, the cod does not appear to be unique in having a very limited capacity to elevate cardiac performance in response to increases in circulating catecholamines. For example, Mendonça and Gamperl (Mendonça and Gamperl, 2009) showed that the winter flounder heart is not dependent upon adrenergic stimulation at rest, and only reported increases of 6% in V_S and 10% in Q following the simultaneous injection of 0.2 and 0.4 μg kg^{-1} of AD and noradrenaline at 8°C, respectively. Maximum adrenergic stimulation (up to 500 nmol l^{-1}) has no effect on either heart rate or maximum Q, and only a modest (10–15%) positive inotropic effect on power output of the *in situ* sea bass (*Dicentrarchus labrax*) heart at 18 and 22°C (Farrell et al., 2007). Finally, Lague et al. (Lague et al., 2012) report that AD and noradrenaline concentrations as high as 5×10^{-6} mol l^{-1} have no effect on function of the 22°C *in situ* tilapia (*Oreochromis* hybrid) heart under conditions of normoxia, hypoxia or acidosis.

While this study did not investigate the mechanistic basis (or bases) behind the diminished sensitivity of cod heart function to adrenergic stimulation, there are several potential explanations. First, B_3-adrenoreceptors exist in the fish heart (Nikinmaa, 2003; Nickerson et al., 2003; Imbrogno et al., 2006) and may play a 'protective role' in some fish hearts (including the cod) by preventing excessive β_1/β_2-stimulation of the myocardium (Gauthier et al., 2007; Angelone et al., 2008). Second, catecholamine-induced sarcolemma Ca^{2+} influx varies between species, and may be somewhat independent of sarcolemma Ca^{2+} channel density. For example, Vornanen (Vornanen, 1998) showed that isoproterenol increased the basal Ca^{2+} current (I_{Ca}) by ~2.3-fold in trout myocytes but only by 1.4-fold in crucian carp (*Carassius carassius*) cardiac cells, despite the fact that there is a higher density of myocardial Ca^{2+} channels in the latter species. Alternatively, Shiels et al. (Shiels et al., 2006) suggest that Na^+/Ca^{2+} exchange may be the primary pathway for sarcolemma Ca^{2+} influx in the cold stenothermal burbot. If a similar phenomenon operates at cold temperatures in the cod heart, this would largely preclude an inotropic response to adrenergic stimulation. Third, changes in cod cardiac function are much more dependent on alterations in cholinergic than adrenergic tonus (Axelsson, 1988; Altimiras et al., 1997), and several authors (Laurent et al., 1983; Axelsson and Nilsson, 1986; Fritsche and Nilsson, 1990; Altimiras et al., 1997) suggest that the teleost heart

is also controlled by a non-adrenergic non-cholinergic (NANC) tonus, which could be more important in the cod heart than in other teleosts. For instance, although nitric oxide generally results in negative chronotropy and inotropy, it has also been identified as an important NANC regulator of cardiac performance in teleosts (Imbrogno et al., 2001; Tota et al., 2005). This raises the possibility that the cod heart has a diminished adrenergic sensitivity to catecholamines because other systems play a predominant role in controlling cardiac function.

Although our results show that AD has very limited direct effects on the cod heart, this does not preclude this hormone from having a significant role in supporting cardiac function. In rainbow trout, AD increases venous tone through an α-adrenergic dependent mechanism and decreases venous compliance (Sandblom and Axelsson, 2006; Zhang et al., 1998), and Sandblom et al. (Sandblom et al., 2005) showed that increases in mean circulatory filling pressure, central venous pressure and Q were abolished in swimming sea bass after α-adrenoceptor blockade. These results suggest that α-adrenergic stimulation of the cod's venous vasculature could mobilize venous blood, and increase cardiac preload and/or V_S.

Conclusions

In this study, we show that the isolated adult cod heart can maintain its pumping capacity when challenged with acute temperature decreases, and that maximum cardiac function is only reduced slightly when this species is acclimated to temperatures as low as 0°C. This degree of thermal independence when faced with decreasing temperatures has not been reported previously for fish cardiac function, and is likely to be of considerable benefit to this fish, which can be exposed to subzero winter temperatures and to significant temperature changes during diel vertical migrations (Righton et al., 2010). What mechanisms mediate this plasticity in cardiac function are not known, but it is evident that: (1) alterations in adrenergic sensitivity and heart size are not involved; and (2) the capacity for modifications in myocardial excitability and contractility with temperature acclimation must be considerable given the large increases in Q_{max} and PO_{max} exhibited by hearts from 4°C-acclimated fish when tested at 10°C.

LIST OF SYMBOLS AND ABBREVIATIONS

AD	adrenaline
ARDF	Aquaculture Research Development Facility
f_H	heart rate
I_{Ca}	calcium ion current
I_{Na}	sodium ion current
NANC	non-adrenergic non-cholinergic
P_{in}	input pressure
PO	power output
PO_{max}	maximum power output
P_{out}	output pressure
Q	cardiac output
Q_{10}	temperature quotient
Q_{max}	maximum cardiac output
RAM	relative atrial mass
RVM	relative ventricular mass
SR	sarcoplasmic reticulum
V_S	stroke volume
V_{Smax}	maximum stroke volume

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