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Cardiovascular and haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature increase

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Summary

For fish to survive large acute temperature increases (i.e. >10.0°C) that may bring them close to their critical thermal maximum (CTM), oxygen uptake at the gills and distribution by the cardiovascular system must increase to match tissue oxygen demand. To examine the effects of an acute temperature increase (~1.7°C h^{-1} to CTM) on the cardiorespiratory physiology of Atlantic cod, we (1) carried out respirometry on 10.0°C acclimated fish, while simultaneously measuring *in vivo* cardiac parameters using Transonic® probes, and (2) constructed *in vitro* oxygen binding curves on whole blood from 7.0°C acclimated cod at a range of temperatures. Both cardiac output ($\dot{Q}$) and heart rate (f_H) increased until near the fish's CTM (22.2±0.2°C), and then declined rapidly. Q_{10} values for $\dot{Q}$ and f_H were 2.48 and 2.12, respectively, and increases in both parameters were tightly correlated with O_2 consumption. The haemoglobin (Hb)–oxygen binding curve at 24.0°C showed pronounced downward and rightward shifts compared to 20.0°C and 7.0°C, indicating that both binding capacity and affinity decreased. Further, Hb levels were lower at 24.0°C than at 20.0°C and 7.0°C. This was likely to be due to cell swelling, as electrophoresis of Hb samples did not suggest protein denaturation, and at 24.0°C Hb samples showed peak absorbance at the expected wavelength (540 nm). Our results show that cardiac function is unlikely to limit metabolic rate in Atlantic cod from Newfoundland until close to their CTM, and we suggest that decreased blood oxygen binding capacity may contribute to the plateau in oxygen consumption.

Key words: *Gadus morhua*, temperature, cardiac output, heart rate, stroke volume, metabolic rate, haemoglobin, blood oxygen concentration.

Introduction

Temperature is an important environmental factor influencing all life functions, and a large body of literature exists on the thermal tolerance limits of salmonids and other freshwater fish species (e.g. Cherry et al., 1976; Jobling, 1981; Beitinger et al., 2000). The Atlantic cod *Gadus morhua* is a marine fish species whose thermal biology has also received considerable attention (Saunders, 1963; Clark and Green, 1991; Jobling, 1988; Schurmann and Steffensen, 1997; Otterlei et al., 1999; Claireaux et al., 2000; Björnsson et al., 2001; Despatie et al., 2001; Peck et al., 2003; Petersen and Steffensen, 2003). Recently, it was shown that North Sea cod thermal tolerance is limited by the capacity of oxygen supply mechanisms (Sartoris et al., 2003; Lannig et al., 2004). Further, Lannig et al. suggested, based on data collected by magnetic resonance imaging (MRI), that there is a progressive mismatch between oxygen delivery and demand above 5.0°C because temperature dependent increases in heart rate (f_H) do not result in similar increases in blood flow (i.e. cardiac output) (Lannig et al., 2004). The conclusion that oxygen delivery limits thermal tolerance in this species is consistent with the current literature on marine ectotherms (e.g. Pörtner, 2002). However, the reported insensitivity of blood flow to temperature increases above 5.0°C is an interesting finding, which suggests that the cardiorespiratory system of North Sea cod may respond differently to an acute thermal challenge compared to other fish species. For example, Overgaard et al. (Overgaard et al., 2004) showed that as a result of increased f_H and the maintenance of stroke volume (V_S), maximum cardiac output ($\dot{Q}$) increased with temperature (Q_{10}=1.8) in the *in situ* rainbow trout heart. Furthermore, 8.0°C acclimated winter flounder (*Pleuronectes americanus*) exposed to acute elevations in temperature can increase $\dot{Q}$ until approx. 1–2°C before their critical thermal maximum (CTM) (25.0°C) (P. C. Mendonca and A.K.G., unpublished).

Although free swimming Atlantic cod held in thermally stratified water move to preferred temperatures (Claireaux et al., 1995), sea-caged fish are limited in their movement in the

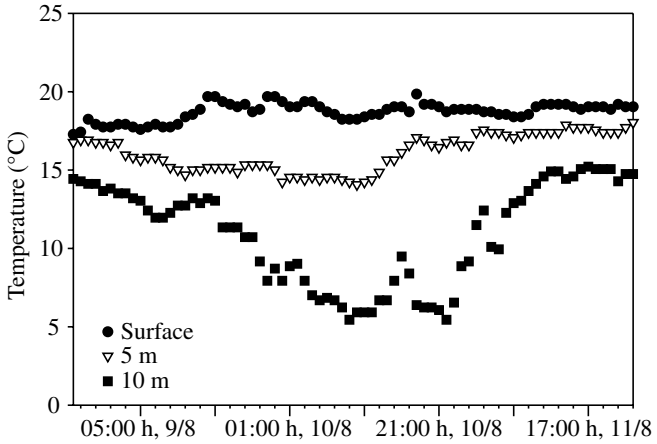

Fig. 1. Temperature profile at a cod cage-site aquaculture facility (Pool's Cove, Newfoundland) during the summer of 2003. This trace illustrates how quickly temperatures can change (especially at the depths where fish congregate at this time of the year; >5 m). Temperature was recorded at several depths; however, surface, 5 m and 10 m temperatures profiles only are shown to allow for clarity of data presentation.

water column. For example, in Newfoundland waters, cod can be exposed to temperatures of up to 20.0°C (even at depths of 6 m) and short-term (daily/weekly) temperature fluctuations of as much as 10.0°C during the summer months (Fig. 1). Since Atlantic cod are generally considered to have a preferred temperature of 8–15°C (Despatie et al., 2001; Petersen and Steffensen, 2003), it is important to determine whether high temperatures negatively impact oxygen delivery in Newfoundland Atlantic cod, as has been suggested for the North Sea populations (Lannig et al., 2004). This is particularly pertinent, as there is evidence to suggest that physiological responses to imposed challenges differ between cod populations (Nelson et al., 1994).

In this study, we fitted 10.0°C acclimated adult cod of Newfoundland origin with Transonic® flow probes, and measured cardiac variables and oxygen consumption ($\dot{M}_{O_2}$) as water temperature was increased to the cod's CTM. Further, we constructed *in vitro* haemoglobin-oxygen dissociation curves at 7.0, 20.0 and 24.0°C to investigate the effects of an acute increase in temperature on blood oxygen-binding capacity.

Materials and methods

Experimental animals

The Atlantic cod *Gadus morhua* L. used to examine the effect of an acute temperature change on *in vivo* metabolism and cardiac function (body mass 1.125±0.048 kg; range=0.861–1.335 kg) were transported from a sea-cage facility at Northwest Cove (Hermitage Bay, Newfoundland, Canada) to the Aquaculture Research Development Facility (ARDF) at the Ocean Sciences Centre in St John's, Newfoundland. These fish were maintained in a ~3000 litre tank in the ARDF supplied with aerated seawater at 10.0–11.0°C for at least 2 months prior to experimentation. The fish were fed a commercial cod diet daily, and photoperiod was maintained at 8 h:16 h light:dark.

To perform the *in vitro* studies of haemoglobin-oxygen binding we used 7.0–8.0°C acclimated cod (0.383±0.016 kg; range=0.206–0.483 kg) reared at the ARDF. Prior to experimentation these animals were maintained in a 17 500 litre tank at the Ocean Science Centre. These fish were also fed commercial cod pellets daily, and maintained on an ambient photoperiod.

Surgical procedures

Fish were netted and placed in seawater containing tricaine methanesulfonate (MS-222, Finquel; 0.15 g l^{-1}) until ventilatory movements ceased. The fish were then weighed and measured, before being transferred to a surgery table, where oxygenated seawater containing MS-222 (0.055 g l^{-1}) continuously irrigated their gills. The procedure used to implant the flow probe was modified from that described (Thorarensen et al., 1996) for the rainbow trout. Briefly, the cod was placed on its right side on a wetted sponge, and the operculum on the left side was lifted and secured in place to allow access to the gill arches. Then, a surgical thread was placed around the gill arches and tied to allow access to the ventral aorta. A small (approx. 7–10 mm) incision was made in the tissue just below the junction of the second and third gill arches with a scalpel, and the ventral aorta was located by carefully cutting away connective tissue. Without disrupting the pericardium, the vessel was then freed from the surrounding tissue using blunt dissection, and a 2.5S Transonic® blood flow probe (Transonic Systems, Ithaca, NY, USA) was placed around the aorta. The cable of the flow probe was then secured to the animal with 3 skin sutures (1-0 silk thread, American Cyanamid Company, Pearl River, NY, USA); one close to the incision, one just ventral to the pectoral fin, and finally, one close to the dorsal fin.

Respirometry and cardiac output measurements

Once surgery had been completed, fish were transferred to a 142-litre custom-designed swim tunnel with the water speed set at 0.2 body lengths per second (*BL* s^{-1}). This current velocity allowed the fish to hold position, without having to swim actively. All fish commenced ventilation almost immediately after being transferred to the swim tunnel, and were given at least 18 h to recover from surgery. Acute temperature challenges were carried out the day after surgery, by increasing temperature from baseline (10–11°C) by ~1.7°C h^{-1} until the fish lost equilibrium; the temperature at which the fish lost equilibrium was recorded as the animal's CTM. $\dot{M}_{O_2}$ measurements were made during the last 12 min at each temperature, as we had previously determined that temperature had reached a steady state by this time. From preliminary experiments we estimated that the CTM was approx. 22.0°C, and therefore oxygen measurements were taken every 30 min, starting 5.0°C before the fish's expected

Table 1. *Oxygen consumption and cardiac parameters measured in Atlantic cod exposed to a 1.7°C h^{-1} temperature increase from 10–11°C (resting) until they reached critical thermal maximum*

	Temperature (°C)		
	Resting	Maximum	CTM
Oxygen consumption (mg kg^{-1} h^{-1})	82.2±3.7[a]	210.8±7.2[b]	
Cardiac output (ml min^{-1} kg^{-1})	21.5±0.8[a]	52.6±2.8[b]	28.4±3.5[a]
Heart rate (beats min^{-1})	36.3±1.7[a]	71.8±3.6[b]	37.4±4.9[a]
Stroke volume (ml kg^{-1})	0.60±0.04[a]	0.76±0.05[a]	0.80±0.08[b]

Maximum refers to the maximum value recorded for each fish prior to critical thermal maximum (CTM), while cardiac parameters reported at CTM were measured very shortly (within 1 min) after the fish lost equilibrium.

Values are means ± s.e.m. (N=6–9). Dissimilar letters within each row indicate values that are significantly different (P<0.05).

CTM. $\dot{M}_{O_2}$ measurements were not taken after the fish lost equilibrium, as water temperature was rapidly reduced after brief (<1 min; see Table 1) cardiovascular measurements were made. This procedure was performed in an effort to recover the animals, but only one fish survived.

Oxygen concentration (mg O_2 l^{-1}) in the swim tunnel was continuously measured by pumping water through an external circuit using a peristaltic pump (Masterflex, Cole Palmer; Anjou, QC, Canada). The circuit was constructed of tubing with an extremely low gas permeability (Tygon Food, ser. 6-419, Cole Parmer; Anjou, QC, Canada), and contained a D201 flow cell (WTW; Weilheim, Germany) that housed a galvanic oxygen electrode (model CellOx 325, WTW). This oxygen probe was connected to an oxygen meter (model 330, WTW) with automatic temperature compensation so that water oxygen levels (mg l^{-1}) could be obtained. Dissolved oxygen in the water never dropped below 6.5 mg l^{-1} (~85% saturation at ~22.0°C) during the study, and $\dot{M}_{O_2}$ of the fish (in mg O_2 kg^{-1} h^{-1}) was calculated as:

$$\dot{M}_{O_2} = \Delta O_2 v / M_b t ,$$

where: ΔO_2 is the change in water oxygen content (mg l^{-1}), v is volume of the respirometer and external circuit (142 l), M_b is mass of the fish (kg) and t is time required to make the $\dot{M}_{O_2}$ measurement (h).

$\dot{Q}$ was directly measured by connecting the flow probe lead to a blood flow meter (model T206 Transonic Systems, Ithaca, NY, USA), which was interfaced with a MP100A-CE data acquisition system (Biopac Systems Inc., Santa Barbara, CA, USA) and a laptop PC running AcqKnowledge software (Biopac Systems Inc.). Data was recorded at a frequency of 10 Hz, and records of $\dot{Q}$ were obtained during each $\dot{M}_{O_2}$ measurement. Brief (<5 min) recordings of $\dot{Q}$ were also made after the fish reached its CTM. $\dot{Q}$ (ml min^{-1} kg^{-1}) was calculated in AcqKnowledge by dividing the raw data (ml min^{-1}) by the mass of the fish (kg). f_H (beats min^{-1}) was calculated by counting the systolic peaks during a 15–30 s measurement period, and V_S (ml kg^{-1}) was calculated from $\dot{Q}/f_H$. These measurements were repeated three times per temperature, and the mean value used for data and statistical analyses.

In vitro blood oxygen binding curves

Oxygen binding curves were constructed for Atlantic cod blood incubated at 7.0, 20.0 and 24.0°C. These temperatures were selected because they represented baseline levels, the temperature at which fish in the *in vivo* study started to show signs of sublethal stress (levelling off of fH and an increase in VS), and a temperature slightly above the highest CTM reached (23.2°C), respectively.

Fish were anaesthetized in seawater containing MS-222 (0.055 g l^{-1}) and 3 ml of blood was quickly withdrawn using caudal puncture and heparinized syringes. Haematocrit (Hct) was then determined in duplicate by centrifugation of blood in micro-haematocrit tubes at 10 000 ***g*** for 3–5 min, and the remaining blood sample adjusted to 20% haematocrit using marine teleost saline (Driedzic et al., 1985). After adjusting Hct, blood samples were placed in heparinized round-bottom flasks in a 7.0°C shaking water bath, and initially gassed with a humidified mix of 100% air/0.2% CO_2 (blood P_{O_2} 16–20 kPa). For experiments conducted at 7.0°C, these experimental conditions were maintained for 1 h. In contrast, blood used in the 20.0 and 24.0°C experiments was gradually warmed to the desired temperature for 1 h. After this initial 1 h equilibration period, eight different O_2 tensions ranging from 20 to 1.2 kPa were achieved by adjusting the relative percentages of N_2 and air (CO_2 remaining constant at 0.2%) using flow meters and a Wösthoff gas-mixing pump (H. Wösthoff Co., Bochum, Germany). Blood was allowed to equilibrate at each P_{O_2} level for approx. 30 min prior to sampling using gas-tight Hamilton syringes. Blood P_{O_2} was determined by injecting blood into a small thermostatted chamber containing a Clark-type oxygen electrode (Cameron Instrument Co., Port Aransas, TX, USA) set to the experimental temperature (7.0, 20.0 or 24.0°C), while blood oxygen content (Hb-O_2) was measured on 30 μl blood samples using Tucker's methods (Tucker, 1967) and a custom designed thermostatted Tucker chamber (volume=1.66 ml) maintained at 32.0°C. The oxygen electrodes were connected to an OM 200 oxygen meter (Cameron Instrument Co.) and a desktop PC running AcqKnowledge software. At each temperature blood from six individuals was used to generate the haemoglobin-oxygen binding curve.

At each P_{O_2} level, 50 μl blood samples were taken for the measurement of haemoglobin concentration ([Hb]), and immediately frozen in liquid nitrogen. [Hb] was subsequently measured in duplicate on 10 μl blood samples. These assays were performed using a commercially available haemoglobin assay kit (Sigma Chemical Co., St Louis, MO, USA) and a spectrophotometer (Beckman Coulter, Mississauga, ON, USA; model DU 640) set to a wavelength of 540 nm. Hct was determined at the end of the experiments (as above), and mean

corpuscular haemoglobin content (MCHC) (g 100 ml^{-1}) calculated as [Hb]/Hct×100.

To ensure that the lower values of [Hb] measured at 24.0°C were not due to haemoglobin degradation, or alterations in the nature of the chemical interaction between the Drabkin's reagent (used in the Hb assay) and the Hb protein at this high temperature, we performed a brief experiment on two cod. In this experiment, we placed cod blood with a Hct of 20% in heparinized round bottom flasks, warmed the blood by approx. 2.0°C every 30 min, and collected blood samples at 7.0, 16.0, 18.0, 22.0, 24.0 and 26.0°C. Then we conducted two sets of subsequent analyses. First, we performed wavelength scans (400–600 nm) on blood that was being analyzed for [Hb] to see if there was a change in the optimum wavelength (usually 540 nm) or the shape of the spectra. Secondly, we performed agar gel electrophoresis on 50 μl blood samples using the protocol described by Petersen and Steffensen (Petersen and Steffensen, 2003).

Data and statistical analyses

Haemoglobin oxygen binding curves were constructed for individual fish at 7.0, 20.0 and 24.0°C (Tucker, 1967) by plotting Hb-O_2 as a function of P_{O_2}, and fitting a 4-parameter sigmoidal curve to the data using Sigmaplot 2001 (SPSS, Chicago, IL, USA). The P_{50} value and Hill Coefficient (n) for each fish were derived from Hill plots (log[satHbO_2/(1–sat HbO_2] *vs* logP_{O_2}).

Statistical analyses were carried out using SPSS (v.11.0; SPSS, Chicago, IL, USA). $\dot{M}_{O_2}$, $\dot{Q}$, f_H and V_S measurements taken at each temperature were compared to both baseline levels and maximum levels prior to CTM using analysis of variance (ANOVA) and Dunnett's *post-hoc* tests. One-way ANOVAs followed by Tukey's *post-hoc* tests were used to examine whether resting, maximum and post-CTM cardiac parameters and $\dot{M}_{O_2}$ were different, and to evaluate the effect of incubation temperature on *in vitro* haematological parameters. Pearson's correlation analysis was carried out to define the strength of the relationship between $\dot{M}_{O_2}$ and both $\dot{Q}$ and f_H. Finally, a 2-way ANOVA with Tukey's *post-hoc* analysis was used to determine the effect of temperature and P_{O_2} on *in vitro* haemoglobin levels. A result was considered significant when $P<0.05$. All data presented in the text, figures and tables are means ± standard error (s.e.m.).

Results

Cardiac function and metabolism

At 10°C, cod $\dot{M}_{O_2}$ was 82.2±3.7 mgO_2 kg^{-1} h^{-1}, and values for $\dot{Q}$, f_H and V_S were 21.5±0.8 ml min^{-1} kg^{-1}, 36.3±1.7 beats min^{-1} and 0.6±0.04 ml kg^{-1}, respectively. $\dot{M}_{O_2}$

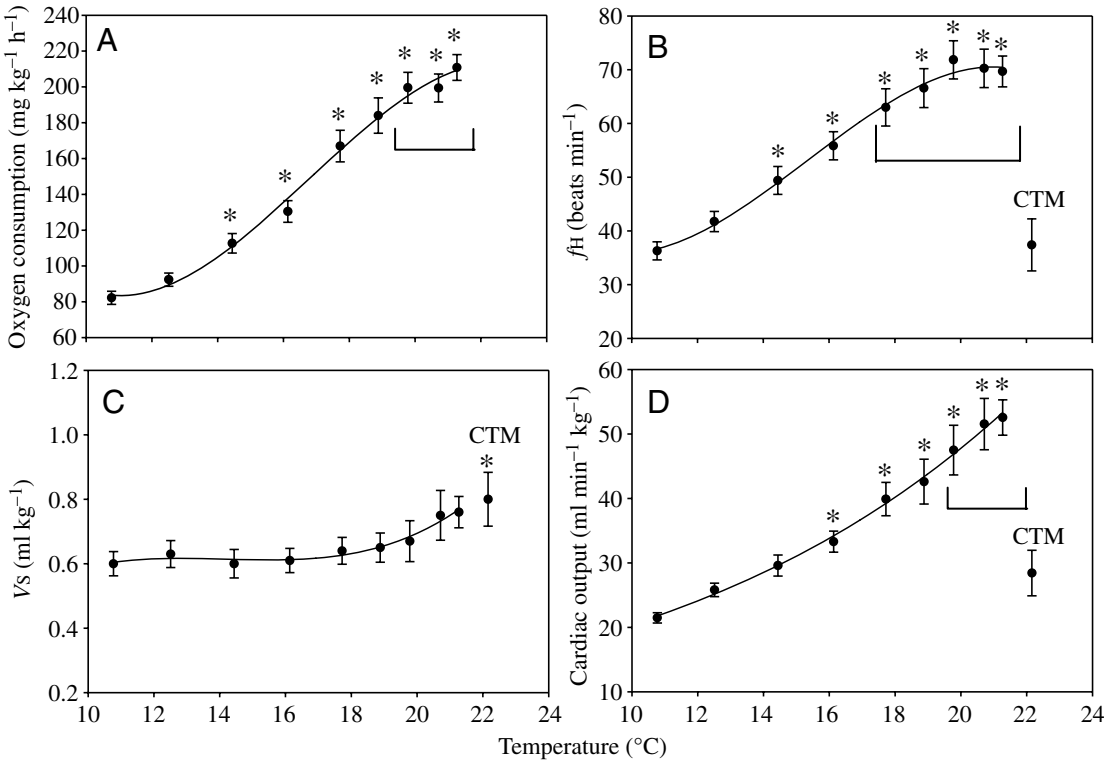

Fig. 2. (A) Rate of oxygen consumption, (B) heart rate f_H, (C) stroke volume V_S and (D) cardiac output of Atlantic cod acclimated to 10–11°C, and subsequently exposed to an acute temperature increase (at ~1.7°C h^{-1}) until they reached their critical thermal maximum (CTM). Values are means ± s.e.m. (N=6–9). *Value significantly different ($P<0.05$) from the baseline; horizontal bracket indicates the range of measurements that were not significantly different from the maximum value.

increased gradually as temperature rose, was significantly elevated above basal (10.0–11.0°C) values after 14.0°C, and reached a maximum value 2.56 times baseline just prior to the cod's CTM (22.2±0.2°C) (Table 1 and Fig. 2). A similar pattern was observed for $\dot{Q}$, where values became significantly elevated above basal values after 16.0°C ($\dot{Q}$=33.3±1.6 ml min^{-1} kg^{-1}) and the maximum value reached 2.45× baseline (Table 1, Fig. 2D). In contrast, both f_H and V_S showed different relationships with temperature (Fig. 2B,C). Heart rate rose significantly above baseline after 14.0°C (f_H=49.4±2.6 beats min^{-1}), peaked at a temperature of approx. 20.0°C (at 1.99× baseline), but had declined slightly (by approx. 3 beats min^{-1}) by the time cod reached their CTM. Although V_S appeared to be increasing at temperatures above 19.0°C, no significant elevation in V_S was observed prior to the fish reaching their CTM (Fig. 2C). Both f_H and $\dot{Q}$ declined rapidly when the fish reached their CTM, returning to values not significantly different from baseline. In contrast, V_S showed a significant increase (to 0.80±0.08 ml kg^{-1}) after CTM was reached (Fig. 2C).

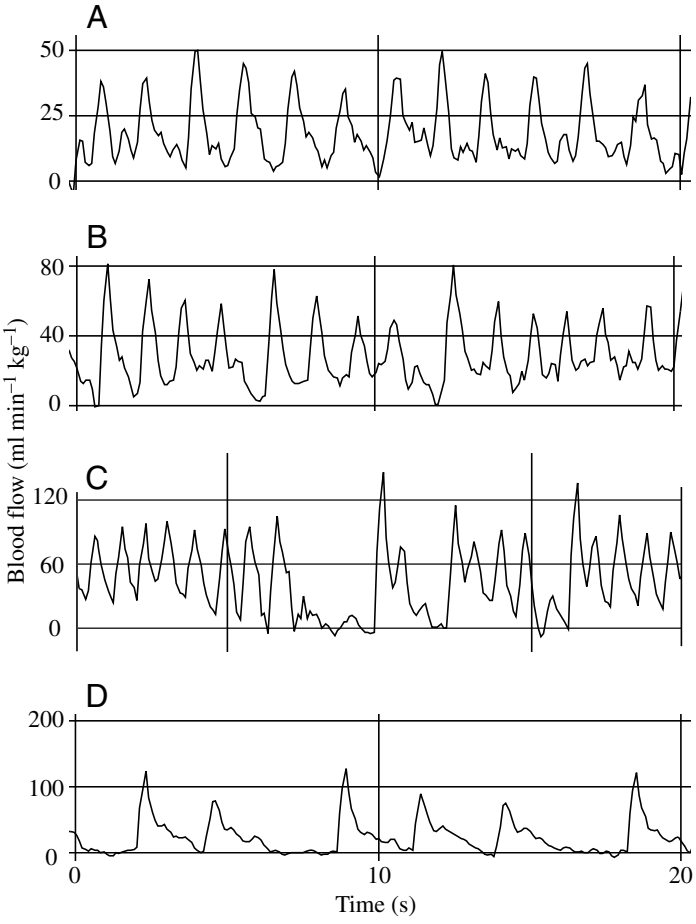

Fig. 3. Representative traces of blood flow obtained from an Atlantic cod (A) at baseline temperature (10.9°C), (B) exhibiting the onset of minor arrhythmias (17.1°C), (C) experiencing significant and prolonged arrhythmias (20.4°C) and (D) after losing equilibrium (22.3°C). Data was collected at 10 Hz. Note: different *y*-axis scales are used in each panel.

Although f_H increased steadily before 20.0°C, and was maintained close to maximum values prior to the fish's CTM, the heart became progressively arrhythmic as temperature increased (Fig. 3A–D). Changes in cardiac rhythmicity first became evident at a temperature of 18.1±0.1°C, where both inter-beat period and V_S became variable (Fig. 3B). This suggests that heart function was being negatively influenced approximately 4.0°C prior to the cod's CTM. This pattern became more pronounced just prior to CTM, where periods of rapid f_H were interspersed with ones where the heart stopped beating/missed beats (Fig. 3C). At CTM f_H declined rapidly and V_S increased (Fig. 3D); however, the response after this was variable. Some fish maintained this level of cardiac function for a prolonged period, while others showed an almost complete cessation of cardiac activity.

Fig. 4 shows the relationship between $\dot{M}_{O_2}$, and f_H (A) and $\dot{Q}$ (B), for all individuals used in this study. When the data are displayed as a scatter plot, it appears that the relationship between $\dot{M}_{O_2}$ and the two cardiac parameters is similar; a conclusion supported by the correlation coefficients for the two linear relationships (f_H, r=0.862; $\dot{Q}$, r=0.871). However, fitting third order regressions to the mean values recorded at each temperature revealed a subtle difference in the shape of the relationships. For example, the relationship between $\dot{M}_{O_2}$ and f_H appears exponential in nature, whereas that between $\dot{M}_{O_2}$ and $\dot{Q}$ is more sigmoidal, apparently because cardiac output was still increasing after $\dot{M}_{O_2}$ had plateaued.

In vitro haemoglobin-oxygen binding curves

Haemoglobin-oxygen binding curves (HBCs) generated at the three temperatures showed that haemoglobin-oxygen affinity and binding capacity were reduced when blood from 7–8°C acclimated cod was exposed to high temperatures (20.0 and 24.0°C) (Fig. 5). This finding was supported by the Hill plots (not shown), which revealed that although the blood's P_{50} value was significantly increased at both temperatures, oxygen binding was only reduced at 24.0°C (Table 2). This reduction in haemoglobin-oxygen binding capacity was associated with a significantly (approx. 24%) lower blood [Hb] (Fig. 6), that was evident at a P_{O_2} of 16 kPa and maintained as P_{O_2} was lowered to 1.2–2.5 kPa. This decrease in [Hb] was likely caused by erythrocyte swelling as MCHC was significantly lower (by approx. 15%) at 24.0°C as compared to both 20.0°C and 7.0°C, and we found no evidence of haemoglobin degradation or that the optimal wavelength or wavelength spectra of the haemoglobin assay were altered when blood was incubated at temperatures up to 26.0°C (data not shown).

Discussion

The results from this study provide a number of important insights into how high environmental temperature affects the cardiac function, arterial oxygen

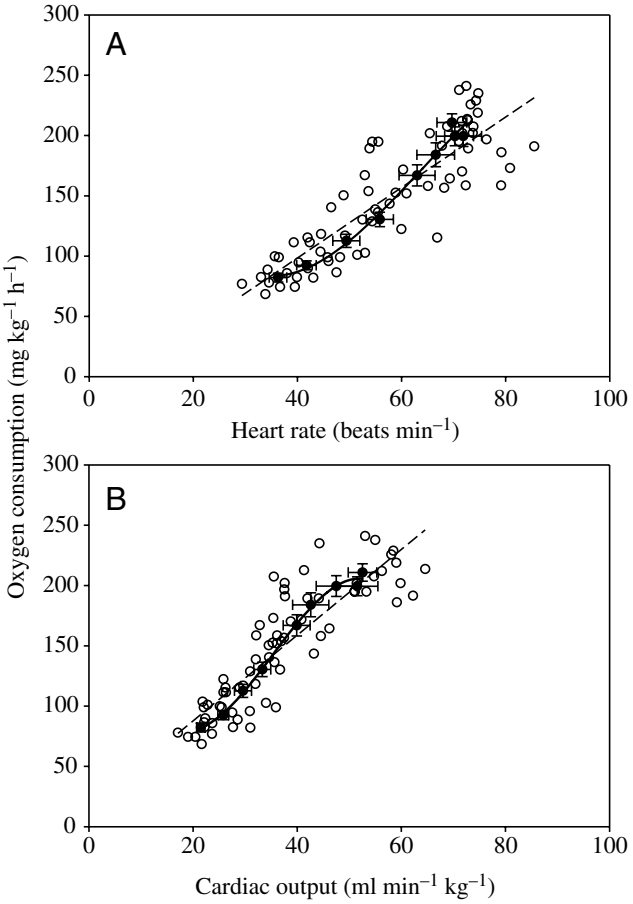

Fig. 4. Relationship between oxygen consumption, and (A) heart rate and (B) cardiac output, when Atlantic cod acclimated to 10–11°C were exposed to an acute temperature increase of ~1.7°C h^{-1}. Open circles represent data for individual fish; filled circles represent mean values (± s.e.m.) recorded at particular temperatures. The broken lines define the linear regressions that were fitted to the individual data (heart rate: y=2.92x–18.5, r^2=0.744; cardiac output: y=3.55x+16.7, r^2=0.759). The solid lines are third order regressions that were fitted to the mean data to show the general trends in cardiac parameters with temperature (heart rate: y=–18.7x+0.4x^2+0.0019x^3+372.5, r^2=0.986; cardiac output: y=–20.0x+0.7x^2+0.0069x^3+242.8, r^2=0.995).

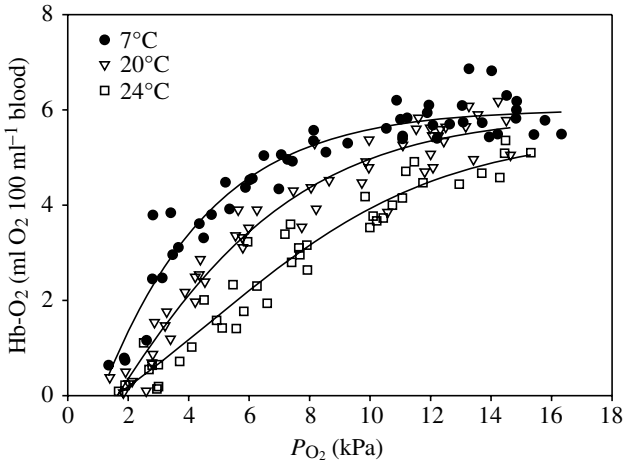

Fig. 5. *In vitro* haemoglobin-oxygen binding curves for cod blood incubated at the fish's acclimation temperature (7°C), or after incubation temperature was increased to 20 or 24°C. Haematocrit was initially set at 20%, and changes in P_{O_2} were made every 30 min. The lines were fitted to the data for each temperature using a 4-parameter sigmoidal function. Six individuals were used to generate each curve. See Table 2 for statistical analyses of parameters that define Hb-O_2 affinity and binding capacity.

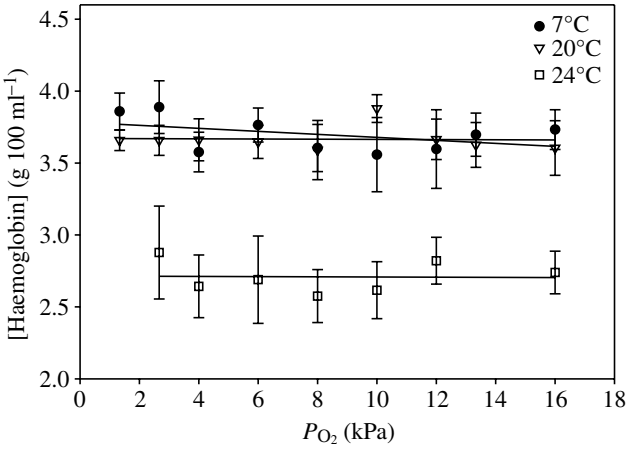

Fig. 6. *In vitro* haemoglobin concentrations in the cod blood used to generate the haemoglobin-oxygen binding curves presented in Fig. 5. For the 20 or 24°C experiments, temperature was increased from 7°C (acclimation temperature) to these temperatures over a 1 h period. Haematocrit was initially set at 20%, and changes in P_{O_2} were made every 30 min. Values are means ± s.e.m. (N=5–6). A two-way ANOVA revealed that haemoglobin levels at 24°C were significantly (P<0.05) lower than measured at the other two temperatures.

transport and $\dot{M}_{O_2}$ of Atlantic cod from Newfoundland. We show that these cod are able to increase $\dot{Q}$ in response to an acute temperature increase until ~2.0°C prior to the cod losing equilibrium, but that heart function (based on the appearance of cardiac arrhythmias) is negatively influenced ~4.0°C before the fish's CTM. Our results show that V_S was not compromised even at the highest measured f_H, a result that contrasts with what would be expected based on the negative force–frequency relationship exhibited by *in vitro* heart muscle (Shiels et al., 2002a) and *in situ* studies, which suggest that limitations on cardiac filling may contribute to decreases in stroke volume at high heart rates/temperatures (Graham and Farrell, 1990; Farrell et al., 1996). This latter finding suggests that *in vivo* homeostatic mechanisms compensate for the negative effects of temperature and increased contraction frequency on myocardial performance, and enable cod to increase routine cardiac output to a level (53 ml min^{-1} kg^{-1}) greater than that measured at maximal exercise at 10°C [35 ml min^{-1} kg^{-1} (Webber et al., 1998); 34 ml min^{-1} kg^{-1} (L.H.P. and A.K.G., unpublished)]. Finally, we provide *in vitro* data, which suggests that decreased blood oxygen binding capacity may contribute to the plateau in oxygen consumption prior to the fish's CTM.

Table 2. *In vitro haematological parameters in samples taken from Atlantic cod acclimated to 7°C and equilibrated at 7, 20 and 24°C*

	Acclimation temperature (°C)		
	7	20	24
Final haematocrit (%)	18.7±0.4[a]	17.3±0.2[b]	16.9±0.6[b]
Final MCHC (g 100 ml^{-1})	20.8±0.7[a]	21.2±0.6[a]	17.8±0.6[b]
P_{50} (kPa)	3.3±0.4[a]	5.1±0.3[b]	6.0±0.4[b]
n	2.3±0.3[a]	3.7±0.4[b]	3.9±0.1[b]
Hb-O_2 (ml O_2 100 ml^{-1} blood) at 13 kPa	5.58±0.20[a]	5.03±0.16[a]	4.12±0.14[b]

Values shown are mean ± s.e.m. (N=5–6 at each temperature). Dissimilar letters within each row indicate values that are significantly different (P<0.05).

As expected, $\dot{M}_{O_2}$ also rose in response to the acute temperature increase, and there was a tight relationship between $\dot{M}_{O_2}$ and $\dot{Q}$. However, it is unclear from our results whether there was a mismatch between blood oxygen delivery ($\dot{Q}\times$blood oxygen content) and the cod's oxygen demand as temperatures approached CTM. First, we did not measure demand, only oxygen consumption, and it is possible that tissue oxygen utilization fell as the high temperatures began to impair cellular functions. Second, from Fig. 4B, it appears that $\dot{Q}$ was still rising when $\dot{M}_{O_2}$ was beginning to plateau. Finally, the acute temperature increase negatively affected *in vitro* [Hb], and both haemoglobin oxygen affinity and binding capacity. A decrease in haemoglobin oxygen affinity and binding capacity at temperatures approaching the cod's CTM could have limited oxygen uptake at the gills, and consequently resulted in reduced arterial oxygen content (Jensen et al., 1998) and a plateau in $\dot{M}_{O_2}$. However, these *in vitro* results do not take into account the potential benefits of catecholamine release on red blood cell oxygen-carrying capacity and gill perfusion (Perry et al., 1995; Reid et al., 1998), and thus it is unknown whether blood oxygen-carrying capacity was diminished *in vivo*.

In our study, the cod's CTM was 22.2±0.2°C, Q_{10} values for $\dot{M}_{O_2}$, $\dot{Q}$ and f_H were 2.78, 2.48 and 2.12, respectively, and cardiac function and metabolic rate showed similar patterns of increase until approx. 2.0°C below the fish's CTM. The CTM of the Atlantic cod used in this study is in the range of that determined for North Sea cod (19–22°C) (Sartoris et al., 2003), and similar increases in metabolic rate and f_H have been recorded in salmonids exposed to an acute temperature increase (Heath and Hughes, 1973; Brodeur et al., 2001; Rodnick et al., 2004). However, few studies have examined the effects of an acute temperature increase to CTM (or near CTM) on $\dot{Q}$ and V_S *in vivo*. In North Sea cod, it was suggested that above 7.0°C, although f_H increases, $\dot{Q}$ does not, because V_S is reduced (Lannig et al., 2004). In contrast, we found that V_S was maintained in Atlantic cod at high temperatures, and that this allowed $\dot{Q}$ to increase until just prior to the fish's CTM (22°C; Figs 2 and 4). These differing results may not be surprising considering that cod from different habitats have equivalent exercise performances, but achieve these by different physiological mechanisms (Nelson et al., 1994). Furthermore, Webber et al. highlighted differences between the cardiac responses to exercise in cod from the Scotian Shelf, and those from the North Sea (Webber et al., 1998). For example, the percentage increase in $\dot{Q}$ from 0 to 0.67 BL s^{-1} was 100% in the Scotian Shelf cod (Webber et al., 1998), compared to 47% and 57% in North Sea cod (Axelsson and Nilsson, 1986; Axelsson, 1988).

It has been shown that the haemoglobin isotype expressed can have a significant effect on the physiology of cod (Brix et al., 2004; Petersen and Steffensen, 2003). Atlantic cod caught off the coast of Newfoundland were found to be ⩾90% HbI 2-2 – the 'low temperature' isoform (Sick, 1965) – whereas fish caught from the German Bight are likely to be composed of >55% HbI 1-1 – the 'high temperature' isoform (Brix et al., 2004). Data suggest that the occurrence of the 'high temperature' isoform (HbI 1-1) may have a beneficial effect on *in vivo* oxygen transport when the fish are exposed to elevated temperatures (Brix et al., 2004). Thus, it is possible that differences in haemoglobin isotype and associated physiological characteristics, as well as the prolonged exposure to differing temperature profiles in the wild prior to being held in lab conditions, allowed the German Bight cod used by Lannig et al. (Lannig et al., 2004) to meet the metabolic demands concomitant with elevated temperatures without having to increase $\dot{Q}$. It should also be noted, however, that different techniques were used to measure blood flow (cardiac output) in the two studies, and this may also account for some of the observed differences. Magnetic resonance imaging, used by Lannig et al. (Lannig et al., 2004), provided a relative measure of blood flow in the caudal vein and dorsal aorta. In contrast, the Transonic® flow probes used in the present study provided a direct and accurate measure of $\dot{Q}$. Clearly, future research should focus on the degree of intra-specific variation in cardiac function between cod populations, its relation to haemoglobin isotype, and the influence of both these factors on the thermal tolerance and biology of cod.

As indicated by the large decreases in $\dot{Q}$ and f_H, cardiac function collapsed at the cod's CTM. There are several possible reasons why this occurred. Bradycardia at high temperatures might occur as an adaptive response to either internal or external hypoxia when fish are exposed to high temperatures (Heath and Hughes, 1973). The concept that slowing of the heart is an adaptive response to temperature extremes has also been promoted by Rantin et al. (Rantin et al., 1998), who suggest that a controlled decrease in f_H may provide protection by maintaining low intracellular Ca^{2+} levels. However, we did not observe a decrease in f_H until very close to, or at, the cod's CTM, and the decrease in $\dot{Q}$ would have resulted in a considerable mismatch between the fish's metabolic demands and blood oxygen transport. This strongly suggests that the decrease in f_H was not adaptive, but an indication that the fish was reaching its thermal limit, and that homeostasis could no longer be maintained.

It is also possible that heart function was compromised just prior to CTM due to a temperature-dependent increase in peripheral tissue oxygen demand, and thus insufficient oxygen to supply the heart's needs. For example, the oxygen gradient between the red muscle and the blood is maintained in rainbow trout even during hypoxia (McKenzie et al., 2004), and if a similar situation occurs during an acute temperature increase, venous blood oxygen levels reaching the heart could be limiting. Moreover, a right shift in the HBC, as we observed at the higher temperatures, would allow oxygen to be unloaded more efficiently to the tissues (Jensen et al., 1998). For fish with a coronary blood supply, this right shift in the HBC could be beneficial (Farrell and Clutterham, 2003). However, for fish such as gadids, that do not have a coronary circulation and rely on returning venous blood for the heart's oxygen supply, this would be detrimental. Venous P_{O_2} values of between 0.7 and ~4 kPa, depending on water oxygen saturation, activity and acclimation temperature, have been suggested as the minimum that will allow *O. mykiss* to maintain oxygen supply to the myocardium (Kiceniuk and Jones, 1977; Steffensen and Farrell, 1998; Farrell and Clutterham, 2003). Although no values are given, we estimate from the work of Lannig et al. (Lannig et al., 2004), that Pv_{O_2} declines to ~2.3 kPa at 19.0°C in North Sea cod, and expect that levels would decline further as temperature approached the fish's CTM (mean 22.2°C). More importantly, applying the 2.3 kPa estimate of venous P_{O_2} to our 20.0°C and 24.0°C *in vitro* HBCs, suggests that Hb-O_2 would be <0.25 ml O_2 100 ml^{-1} blood just prior to CTM, severely limiting oxygen supply to the heart. The fact that $\dot{Q}$ stabilized or declined during the ~10 min prior to loss of equilibrium in most of the fish used in the study would support this argument.

Finally, there are a number of other factors that could have independently, or in concert, led to the observed loss of cardiac function. It is possible that the decline in f_H and subsequent $\dot{Q}$ observed just prior to loss of equilibrium, rendered the brain hypoxic, and subsequently resulted in neural dysfunction. For example, it has been suggested that impaired neural circuit function is a more likely factor in death caused by exposure to environmental extremes than accumulating cell death in organs (Robertson, 2004). High temperatures may have disrupted signal production and/or transduction of the heart's pacemaker. In the present study, arrhythmias initially occurred at ~18.0°C, and increased in frequency and duration as temperature increased. Further, Lennard and Huddart suggest that as a result of changes in membrane fluidity, cessation of trans-membrane ion transport causes the fish heart to cease beating (Lennard and Huddart, 1991). It has been shown that acclimation to different temperatures, and acute temperature increases, affect the duration of the action potential in both isolated plaice (*Pleuronectes platessa*) pacemaker cells (Harper et al., 1995) and *O. mykiss* ventricular myocardium strips (Coyne et al., 2000). Changes in action potential duration have been shown to negatively affect intracellular Ca^{2+} flux in atrial myocytes during depolarisation (Shiels et al., 2002b), and this would potentially affect cod myocardial contractility, considering the pronounced negative effect that temperature has on the myocardium's force–frequency relationship [(Shiels et al., 2002a), adapted from Shiels and Farrell (Shiels and Farrell, 1997)]. However, in the present study, a decrease in *V*s was not observed with increased temperature. Although the catecholamine sensitivity of trout atrial myocytes is reduced when exposed to an acute increase in temperature from 14.0 to 21.0°C, high levels of adrenaline (e.g. 1 μmol l^{-1}) still cause a 1.6-fold increase in *L*-type Ca^{2+} channel current (Shiels et al., 2003). Thus, it is possible that the cod released large amounts of catecholamines during our CTM experiments, and that these hormones had a positive inotropic effect on the heart that facilitated the maintenance of *V*s.

In conclusion, our results show that the cardiorespiratory system of Atlantic cod (*Gadus morhua*) from the waters surrounding Newfoundland is able to cope with an acute temperature increase to near the fish's CTM by increasing f_H, and concomitantly $\dot{Q}$. Indeed both $\dot{Q}$ and f_H proved to be tightly correlated with metabolic rate (Fig. 4) during the acute temperature exposure. Although the relationship between f_H and $\dot{M}_{O_2}$ has been shown to vary with a number of factors (Lucas et al., 1993; Lefrançois et al., 1998), our data and that of Webber et al. (Webber et al., 1998) show tight relationships between both f_H and $\dot{Q}$, and $\dot{M}_{O_2}$, when cod are exposed to acute temperature increases and exercise tests, respectively. These data indicate that cardiac parameters may be valuable for telemetered studies of metabolism in this species. Finally, based on our *in vitro* studies, it appears that as temperature approaches/reaches the cod's CTM, the blood's capacity to take up oxygen decreases due to reductions in both haemoglobin-oxygen affinity and binding capacity. This was particularly evident at 24.0°C, where cell swelling occurred, and the haemoglobin-oxygen binding curve was shifted considerably downward and to the right. However, we are unsure whether blood oxygen-carrying capacity is compromised at high temperatures *in vivo*, or whether $\dot{M}_{O_2}$ declined prior to CTM because of a decrease in blood oxygen transport or a decline in tissue oxygen demand. Clearly, more *in vivo* experiments must be performed before the inter-relationships between blood oxygen transport, tissue oxygen demand and thermal tolerance in this species can be understood.

List of symbols and abbreviations

BL s^{-1}	body lengths per second
CTM	critical thermal maximum
f_H	heart rate
Hb	haemoglobin
Hb-O_2	blood oxygen content
Hct	haematocrit
MCHC	mean corpuscular haemoglobin content
$\dot{M}_{O_2}$	rate of oxygen consumption
MRI	magnetic resonance imaging
HBC	haemoglobin-oxygen binding curve
P_{O_2}	partial pressure of oxygen
$\dot{Q}$	cardiac output
*V*s	stroke volume

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References

Axelsson, M. (1988). The importance of nervous and humoral mechanisms in the control of cardiac performance in the Atlantic cod *Gadus morhua* at rest and during non-exhaustive exercise. *J. Exp. Biol.* **137**, 287-303.

Axelsson, M. and Nilsson, S. (1986). Blood-pressure control during exercise in the Atlantic cod, *Gadus morhua*. *J. Exp. Biol.* **126**, 225-236.

Beitinger, T. L., Bennett, W. A. and McCauley, R. W. (2000). Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environ. Biol. Fishes* **58**, 237-275.

Björnsson, B., Steinarsson, A. and Oddgeirsson, M. (2001). Optimal temperature for growth and feed conversion of immature cod (*Gadus morhua* L.). *ICES J. Mar. Sci.* **58**, 29-38.

Brix, O., Thorkildsen, S. and Colosimo, A. (2004). Temperature acclimation modulates the oxygen binding properties of the Atlantic cod (*Gadus morhua* L.) genotypes – *HbI* 1/1, HbI*1/2,* and *HbI* 2/2* – by changing the concentrations of their major haemoglobin components (results from growth studies at different temperatures). *Comp. Biochem. Physiol.* **138A**, 241-251.

Brodeur, J. C., Dixon, D. G. and McKinley, R. S. (2001). Assessment of cardiac output as a predictor of metabolic rate in rainbow trout. *J. Fish Biol.* **58**, 439-452.

Cherry, D. S., Dickson, K. L., Cairns, J., Jr and Stauffer, J. R. (1976). Preferred, avoided and lethal temperatures of fish during rising temperature conditions. *J. Fish. Res. Board Can.* **34**, 239-246.

Claireaux, G., Webber, D. M., Kerr, S. R. and Boutilier, R. G. (1995). Physiology and behaviour of free-swimming Atlantic cod (*Gadus morhua*) facing fluctuating temperature conditions. *J. Exp. Biol.* **198**, 49-60.

Claireaux, G., Webber, D. M., Lagardere, J. P. and Kerr, S. R. (2000). Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). *J. Sea Res.* **44**, 257-265.

Clark, D. S. and Green, J. M. (1991). Seasonal-variation in temperature preference of juvenile Atlantic cod (*Gadus morhua*), with evidence supporting an energetic basis for their diel vertical migration. *Can. J. Zool.* **69**, 1302-1307.

Coyne, M. D., Kim, C. S., Cameron, J. S. and Gwathmay, J. K. (2000). Effects of temperature and calcium availability on ventricular myocardium from rainbow trout. *Am. J. Physiol.* **278**, R1535-R1544.

Despatie, S.-P., Castonguay, M., Chabot, D. and Audet, C. (2001). Final thermal preferendum of Atlantic cod: effect of food ration. *Trans. Am. Fish. Soc.* **130**, 263-275.

Driedzic, W. R., Gesser, H. and Johansen, K. (1985). Effects of hypoxic adapation on myocardial performance and metabolism of *Zoarces viviparous*. *Can. J. Zool.* **63**, 821-823.

Farrell, A. P. and Clutterham, S. M. (2003). On-line venous oxygen tensions in rainbow trout during graded exercise at two acclimation temperatures. *J. Exp. Biol.* **206**, 487-496.

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

Graham, M. S. and Farrell, A. P. (1989). The effect of temperature acclimation and adrenaline on the performance of a perfused trout heart. *Physiol. Zool.* **62**, 38-61.

Harper, A. A., Newton, I. P. and Watt, P. W. (1995). The effects of temperature on spontaneous action potential discharge of the isolated sinus venosus from winter and summer plaice (*Pleuronectes platessa*). *J. Exp. Biol.* **198**, 137-140.

Heath, A. G. and Hughes, G. M. (1973). Cardiovascular and respiratory changes during heat stress in rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.* **59**, 323-338.

Jensen, F. B., Fago, A. and Weber, R. E. (1998). Hemoglobin structure and function. In *Fish Respiration* (ed. S. F. Perry and B. Tufts), pp. 1-40. New York: Academic Press.

Jobling, M. (1981). Temperature tolerance and the final preferendum – rapid methods for the assessment of optimum growth temperatures. *J. Fish Biol.* **19**, 439-455.

Jobling, M. (1988). A review of the physiological and nutritional energetics of cod, *Gadus morhua* L., with particular reference to growth under farmed conditions. *Aquaculture* **70**, 1-19.

Kiceniuk, J. W. and Jones, D. R. (1977). The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. Exp. Biol.* **69**, 247-260.

Lannig, G., Bock, C., Sartoris, F. J. and Pörtner, H. O. (2004). Oxygen limitation of thermal tolerance in cod, *Gadus morhua* L., studied by magnetic resonance imaging and on-line venous oxygen monitoring. *Am. J. Physiol.* **287**, R902-R910.

Lefrançois, C., Claireaux, G. and Lagardère, J.-P. (1998). Heart rate telemetry to study environmental influences on fish metabolic expenditure. *Hydrobiologia* **371/372**, 215-224.

Lennard, R. and Huddart, H. (1991). The effect of thermal stress on electrical and mechanical responses and associated calcium movements of flounder heart and gut. *Comp. Biochem. Physiol.* **98A**, 221-228.

Lucas, M. C., Johnstone, A. D. and Priede, I. M. (1993). Use of physiological telemetry as a method of estimating metabolism of fish in the natural environment. *Trans. Am. Fish. Soc.* **122**, 822-833.

McKenzie, D. J., Wong, S., Randall, D. J., Egginton, S., Taylor, E. W. and Farrell, A. P. (2004). The effects of sustained exercise and hypoxia upon oxygen tensions in the red muscle of rainbow trout. *J. Exp. Biol.* **207**, 3629-3637.

Nelson, J. A., Tang, Y. and Boutilier, R. G. (1994). Differences in exercise physiology between 2 Atlantic cod (*Gadus morhua*) populations from different environments. *Physiol. Zool.* **67**, 330-354.

Otterlei, E., Nyhammer, G., Folkvord, A. and Stefansson, S. O. (1999). Temperature- and size-dependent growth of larval and early juvenile Atlantic cod (*Gadus morhua*): a comparative study of Norwegian coastal cod and northeast Arctic cod. *Can. J. Fish. Aquat. Sci.* **56**, 2099-2111.

Overgaard, J., Stecyk, J. A. W., Gesser, H., Wang, T. and Farrell, A. P. (2004). Effects of temperature and anoxia upon the performance of *in situ* perfused trout hearts. *J. Exp. Biol.* **207**, 655-665.

Peck, M. A., Buckley, L. J., Caldarone, E. M. and Bengtson, D. A. (2003). Effects of food consumption and temperature on growth rate and biochemical-based indicators of growth in early juvenile Atlantic cod *Gadus morhua* and haddock *Melanogrammus aeglefinus*. *Mar. Ecol. Prog. Ser.* **251**, 233-243.

Perry, S. F., Daxboeck, C. and Dobson, G. P. (1985). The effect of perfusion flow rate and adrenergic stimulation on oxygen transfer in the isolated, saline-perfused head of the rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.* **116**, 251-269.

Petersen, M. F. and Steffensen, J. F. (2003). Preferred temperature of juvenile Atlantic cod *Gadus morhua* with different haemoglobin genotypes at normoxia and moderate hypoxia. *J. Exp. Biol.* **206**, 359-364.

Pörtner, H. O. (2002). Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comp. Biochem. Physiol.* **132A**, 739-761.

Rantin, F. T., Gesser, H., Kalinin, A. L., Guerra, C. D. R., De Freitas, J. C. and Driedzic, W. R. (1998). Heart performance, Ca^{2+} regulation and energy metabolism at high temperatures in *Bathygobius soporator*, a tropical marine teleost. *J. Therm. Biol.* **23**, 31-39.

Reid, S. G., Bernier, N. J. and Perry, S. F. (1998). The adrenergic stress response in fish: control of catecholamine storage and release. *Comp. Biochem. Physiol.* **120C**, 1-27.

Rodnick, K. J., Gamperl, A. K., Lizars, K. R., Bennett, M. T., Rausch, R. N. and Keeley, E. R. (2004). Thermal tolerance and metabolic physiology among redband trout populations in South-eastern Oregon. *J. Fish Biol.* **64**, 310-335.

Robertson, R. M. (2004). Modulation of neural circuit operation by prior environmental stress. *Integr. Comp. Biol.* **44**, 21-27.

Sartoris, F. J., Bock, C., Serendero, I., Lannig, G. and Pörtner, H. O. (2003). Temperature dependent changes in energy metabolism, intracellular

pH and blood oxygen tension in the Atlantic cod. *J. Fish Biol.* **62**, 1239-1253.

Saunders, R. L. (1963). Respiration of the Atlantic cod. *J. Fish. Res. Board Can.* **20**, 373-386.

Schurmann, H. and Steffensen, J. F. (1997). Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. *J. Fish Biol.* **50**, 1166-1180.

Shiels, H. A. and Farrell, A. P. (1997). The effect of temperature and adrenaline on the relative importance of the sarcoplasmic reticulum in contributing Ca^{2+} to force development in isolated ventricular trabeculae from rainbow trout. *J. Exp. Biol.* **200**, 1607-1621.

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

Shiels, H. A., Vornanen, M. and Farrell, A. P. (2002b). Effects of temperature on intracellular [Ca^{2+}] in trout atrial myocytes. *J. Exp. Biol.* **205**, 3641-3650.

Shiels, H. A., Vornanen, M. and Farrell, A. P. (2003). Acute temperature change modulates the response of I_{Ca} to adrenergic stimulation in fish cardiomyocytes. *Physiol. Biochem. Zool.* **76**, 816-824.

Sick, K. (1965). Haemoglobin polymorphism of cod in the North Sea and the North Atlantic Ocean. *Here* **54**, 49-69.

Steffensen, J. F. and Farrell, A. P. (1998). Swimming performance, venous oxygen tension and cardiac performance of coronary-ligated rainbow trout, *Oncorhynchus mykiss*, exposed to graded hypoxia. *Comp. Biochem. Physiol.* **119A**, 585-592.

Thorarensen, H., Gallaugher, P. E. and Farrell, A. P. (1996). Cardiac output in swimming rainbow trout, *Oncorhynchus mykiss*. *Physiol. Zool*. **69**, 139-153.

Tucker, V. (1967). Method for oxygen content and dissociation curves on microliter blood samples. *J. Appl. Physiol.* **23**, 410-414.

Webber, D. M., Boutilier, R. G. and Kerr, S. R. (1998). Cardiac output as a predictor of metabolic rate in cod *Gadus morhua*. *J. Exp. Biol.* **201**, 2779-2789.