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Effect of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Atlantic cod (*Gadus morhua*)

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SUMMARY

Low water oxygen content (hypoxia) is a common feature of many freshwater and marine environments. However, we have a poor understanding of the degree to which diminished cardiac function contributes to the reduction in fish swimming performance concomitant with acute exposure to hypoxia, or how fish cardiorespiratory physiology is altered by, or adapts to, chronic hypoxia. Thus, we acclimated adult Atlantic cod (*Gadus morhua*) to either ~8–9 kPa O_2 (40–45% air saturation) or ~21 kPa O_2 (100% air saturation; normoxia) for 6–12 weeks at 10°C, and subsequently measured metabolic variables [routine oxygen consumption ($\dot{M}_{O_2}$), maximum $\dot{M}_{O_2}$, metabolic scope] and cardiac function (cardiac output, $\dot{Q}$; heart rate, f_H; and stroke volume, V_S) in these fish during critical swimming speed (U_{crit}) tests performed at both levels of water oxygenation. Although surgery (flow probe implantation) reduced the U_{crit} of normoxia-acclimated cod by 14% (from 1.74 to 1.50 $BL\,s^{-1}$) under normoxic conditions, exposure to acute hypoxia lowered the U_{crit} of both groups (surgery and non-surgery) by ~30% (to 1.23 and 1.02 $BL\,s^{-1}$, respectively). This reduction in swimming performance was associated with large decreases in maximum $\dot{M}_{O_2}$ and metabolic scope (≥50%), and maximum f_H and $\dot{Q}$ (by 16 and 22%), but not V_S. Long-term acclimation to hypoxia resulted in a significant elevation in normoxic metabolic rate as compared with normoxia-acclimated fish (by 27%), but did not influence normoxic or hypoxic values for U_{crit}, maximum $\dot{M}_{O_2}$ or metabolic scope. This was surprising given that resting and maximum values for $\dot{Q}$ were significantly lower in hypoxia-acclimated cod at both levels of oxygenation, because of lower values for V_S. However, hypoxia-acclimated cod were able to consume more oxygen for a given cardiac output. These results provide important insights into how fish cardiorespiratory physiology is impacted by short-term and prolonged exposure to hypoxia, and further highlight the tremendous capacity of the fish cardiorespiratory system to deal with environmental challenges.

Key words: hypoxia, heart, exercise, oxygen consumption, cardiac output, critical swimming speed.

INTRODUCTION

Hypoxia is a frequently occurring environmental phenomenon in many freshwater and coastal systems, and can be caused by either anthropogenic input, or naturally occurring biological and physical factors (Rosenberg et al., 1991; Pihl et al., 1992; Hoback and Barnhart, 1996; Wu, 1999). Recent studies show that hypoxia in marine waters is not restricted to localized areas, but is more extensive and longer-lasting (weeks to months) than previously thought (Diaz and Rosenberg, 1995; Wu, 1999; Weeks et al., 2002; Bell and Eggleston, 2005; Gilbert et al., 2005). This can create inhospitable habitats for fish and sedentary animals (Wu, 2002), and severe oxygen depletion of coastal waters has significant negative consequences for economically important fisheries, ecosystems and biodiversity (UN Global Environment Outlook Year Book, 2003). These problems become even more serious if large areas are affected by hypoxia for an extended time, as fish may not be able to leave these areas; avoidance being the predominant reaction to hypoxia (Pihl et al., 1991; Claireaux et al., 1995).

Locomotor performance is determined by the interaction of many organ systems, and is considered to be an integrated measure of an animal's physiological capacity in a particular environment (Nelson, 1989). Thus, measuring the locomotor performance of fishes could provide valuable information on their physiological response to hypoxia. Furthermore, cardiorespiratory adjustments are a prerequisite for vital functions (e.g. locomotion, digestion) and for appropriate responses to environmental changes (Farrell, 2002; Claireaux et al., 2005; Gollock et al., 2006; Clark and Seymour, 2006; Steinhausen et al., 2008), and an understanding of how chronic hypoxia affects both swimming performance and cardiovascular function could reveal important information about whether fish will survive, and how well they adapt to, hypoxic environments. At present, studies on the effects of chronic (weeks of) hypoxia have been conducted on a limited number of teleost species, and focused on a range of aspects such as food intake (Chabot and Dutil, 1999; Pichavant et al., 2000; Pichavant et al., 2001; Zhou et al., 2001), reproduction (Wu et al., 2003), oxygen carrying capacity (Greaney et al., 1980; Taylor and Miller, 2001; Pichavant et al., 2003), cardiomyocyte physiology (Lennard and Huddart, 1992; Paajanen and Vornanen, 2003) and circulating catecholamine levels (Butler et al., 1979; Montpetit and Perry, 1998). However, to our knowledge only two studies (Kutty, 1968; Bushnell et al., 1984) have investigated how chronic hypoxia affects fish swimming performance and metabolism, and only one study (Burleson et al., 2002) has examined the effect of chronic hypoxia on fish in *vivo* cardiovascular function. Furthermore, this lack of information is surprising given that: (1) acute exposure to reduced oxygen levels decreases metabolic scope (Claireaux et al., 2000; Evans, 2007) and swimming performance (Dahlberg et al., 1968; Kutty, 1968; Bushnell et al., 1984; Dutil et al., 2007); and (2) hypoxia induces bradycardia in many species, and stroke volume must increase if the fish is to maintain or elevate cardiac output (Saunders, 1963; Wood and Shelton, 1980; Fritsche and Nilsson, 1989; Gamperl et

al., 1994; Sandblom and Axelsson, 2005; Sandblom and Axelsson, 2006).

The Atlantic cod (*Gadus morhua* L.) is a demersal North Atlantic species of significant economic and cultural importance that has experienced dramatic population declines over the past several decades (Myers et al., 1996; Svedäng and Bardon, 2003; Hutchings and Reynolds, 2004). Further, this species has traditionally inhabited areas, such as the Baltic Sea (Gerlach, 1988) and the Gulf of St Lawrence (GSL) (D'Amours, 1993; Kiceniuk and Colbourne, 1997; Gilbert et al., 2005), where they are now likely to encounter environmental oxygen levels that may strongly affect their distribution, growth and reproduction, at least during part of their life history. For example, GSL cod are sensitive to hypoxia (D'Amours, 1993), completely avoid regions of water oxygen partial pressures (P_{wO_2}) below a threshold of ~6.6 kPa (Kiceniuk and Colbourne, 1997), and there are thus areas in the GSL that are below the threshold for survival (Plante et al., 1998; Gilbert et al., 2005).

Given the expanding threat of hypoxia for marine organisms including cod (Breitburg, 2002; Neuenfeldt, 2002; Wu et al., 2002; Gilbert et al., 2005), and our incomplete understanding of fish physiology when exposed to low oxygen conditions, the main goal of the present study was to determine whether Atlantic cod are able to adapt to chronic hypoxia. Thus, we acclimated adult Newfoundland cod to water oxygen levels (P_{wO_2}) of 8–9 kPa (hypoxia) and 21 kPa (normoxia) for 6–12 weeks, fitted them with Transonic® flow probes around their ventral aorta, and measured cardiac function and oxygen consumption during critical swimming speed (U_{crit}) tests conducted at both water oxygen levels.

MATERIALS AND METHODS

These studies were conducted in accordance with the guidelines of the Canadian Council on Animal Care, and approved by the Institutional Animal Care Committee of Memorial University of Newfoundland (Protocol # 05-03-KG).

Experimental animals

Experiments were performed on adult (0.62±0.03 kg; range=0.43–0.78 kg; N=31) Atlantic cod at the Ocean Sciences Centre (OSC; Memorial University, St John's, Newfoundland, Canada). Cod were obtained from stocks reared at the OSC's Aquaculture Research and Development Facility (ARDF), and subsequently held in sea cages at Hermitage Bay (Newfoundland, Canada) for approximately 18 months before being transported back to the OSC. At the OSC, the cod were originally held in a 12,000 l tank supplied with aerated seawater at 10°C for at least 2 months prior to being moved to acclimation tanks. During this period, the fish were fed a commercial cod diet (EWOS, Canada) three times a week, and photoperiod was maintained at 12 h:12 h light:dark.

Experimental conditions

Acclimation to normoxia

Prior to experiments, 20 fish from the holding tank were acclimated at a P_{wO_2} of 21 kPa for 6–12 weeks at 10±0.1°C in two ~1300 l tanks, each supplied with aerated seawater from a header tank at a flow-rate of ~6-8 l min^{-1}. The header tank was fitted with two submersible heaters (Process Technology, OH, USA; model NA15E-2) and connected to a separate heater/chiller (custom built by Technical Services, Memorial University of Newfoundland). Furthermore, a wooden lid was placed on the tank to reduce stress from external stimuli (presence of people, noise, etc.) and to reduce fluctuations in water temperature. The normoxia acclimation tank was fully aerated to ensure normoxic conditions (>20 kPa), and fish were fed three times a week with commercial cod pellets at a ration equal to that consumed by the hypoxic group.

Acclimation to hypoxia

The hypoxia acclimation tank was supplied with ambient seawater from its own header tank, and fitted with a wooden lid to reduce noise and fluctuations in water temperature, and to reduce the exchange of oxygen with the atmosphere. Temperature in the hypoxic tank was controlled by a small submersible rod-type heater (Process Technology; model DRAE15-1) fitted on the lid and by circulating water in the tank through a custom built heater/chiller (Technical Services, Memorial University of Newfoundland). These two systems were sufficient to maintain water temperature at 10±0.1°C.

Twenty fish were transferred from the holding tank to the hypoxia acclimation tank and held under normoxic conditions (>20 kPa) for 1 week before the oxygen level was reduced. A hypoxia level of ~8 kPa (~40% air saturation) was achieved over the time course of 1 week by: (1) slowly reducing the flow rate to the tank to 1–2 l min^{-1} (i.e. fish metabolism partially reduced the water O_2 content); and (2) using a custom designed solenoid valve system (Electronics Workshop, Memorial University of Newfoundland). This system continuously monitored the oxygen level in the tank by pumping water through an external circuit of tubing (Tygon Food, ser. 6-419, Cole Parmer, Montreal, QC, Canada) that contained a galvanic oxygen electrode (model CellOx 325, WTW, Weilheim, Germany) housed in a D201 flow cell (WTW). Further, the oxygen probe was connected to an oxygen meter (model Oxi 340, WTW), which was subsequently connected to two solenoid valves; one that bubbled pure N_2 into the tank when O_2 reached an upper limit of 9 kPa, and the other bubbled air into the tank when oxygen levels reached 7 kPa. This design allowed the oxygen level in the hypoxic tank to be kept within a narrow O_2 range (±1 kPa), and together with the reduced water flow, proved to be highly efficient in maintaining appropriate O_2 levels; average O_2 level 8.6±0.2 kPa over the 6–12 week acclimation period.

Fish were fed three times a week with commercial cod pellets, and most fish were feeding from the first day of reaching 8–9 kPa; average food consumption over the period of acclimation 1.4% body mass day^{-1}. To avoid the build up of carbon dioxide and nitrogen that may have affected the hypoxic fish, we tested water quality (total nitrogen, un-ionized ammonia, P_{CO_2} and pH) in the hypoxic tank once a week throughout acclimation.

Surgical procedures

With the exception of the 'non-surgery' group, the fish were netted and anaesthetized in seawater containing tricaine methane sulphonate (MS-222; 0.1 g l^{-1}) until ventilatory movements ceased. Then the fish were weighed and measured before being transferred to an operating table where chilled (4°C) oxygenated seawater, containing a lower dose of MS-222 (0.05 g l^{-1}), was continuously pumped over the fish's gills.

To allow for the direct measurement of cardiac function (cardiac output, $\dot{Q}$; heart rate f_H; and stroke volume, V_S), a 2S or 2.5S Transonic® flow probe was fitted around the ventral aorta of each cod as previously described by Gollock et al. (Gollock et al., 2006). After the flow probe had been carefully placed around the vessel, it was connected to a flow meter (Transonic Systems Inc., Ithaca, NY, USA; Model TS-420) to test for correct placement of the probe, and the flow probe lead was secured to the cod's skin at three locations using silk suture (3-0, American Cyanamid Company, Pearl River, NY, USA): one location close to the incision, a second ventral to the pectoral fin, and a third close to the dorsal fin.

Once surgery had been completed, the fish were transferred to the swim-tunnel, and all fish commenced ventilation within <2 min. The water velocity in the swim-tunnel was set at 0.25 body lengths per second ($BL\,s^{-1}$; a velocity at which the fish did not swim actively, but had no trouble orienting themselves), and all fish were allowed at least 18 h of recovery in normoxic water prior to the first swim trial.

Critical swimming speed tests

Critical swimming speed (U_{crit}) tests were performed in a 81 l Blazka-type swim-tunnel respirometer (University of Waterloo, Biotelemetry Institute, Waterloo, ON, USA) with an internal diameter of 25 cm and a 90 cm long working section. The front of the respirometer was fitted with a plastic grid, which created uniform water flow in the swimming section of the respirometer (Taylor and McPhail, 1985), and the rear of the tunnel was fitted with a stainless steel grid connected to an external electrical circuit. This stainless steel grid could be electrified with a small current (<5 V, ~0.2 A) to discourage the fish from resting on the grid during swimming trials. Furthermore, the tunnel was covered with black plastic to provide the fish with a dark refuge, and to minimize stress from external stimuli (i.e. investigator's presence).

Water (21 or 8–9 kPa) was supplied to the swim-tunnel from a temperature-controlled 270 l water reservoir that was maintained at 10±0.1°C using a heater/chiller (Memorial University of Newfoundland, Technical Services). The O_2 content of the water was controlled by bubbling pure N_2 into the reservoir at rates predetermined to achieve the desired O_2 level.

Experimental protocol

Resting and active oxygen consumption and cardiac function, and swimming performance, of individual fish were initially measured under normoxic conditions using a critical swimming speed test (Brett, 1964). After measuring cardiac function and oxygen consumption (see below) at the baseline speed of $0.25\,BL\,s^{-1}$, swimming speed was increased in $0.125\,BL\,s^{-1}$ increments every 20 min until the fish were exhausted; exhaustion was determined as the inability of the fish to move away from the electric grid after three successive mild (5 V) shocks. Thereafter, water velocity was returned to $0.25\,BL\,s^{-1}$ and the fish left overnight to recover. During the morning of the second day, the oxygen level in the tunnel was reduced over a period of 3 h by bubbling pure N_2 into the reservoir to reduce the oxygen level in the tunnel to 16 kPa in the first hour, 12 kPa in the second hour, and 8–9 kPa by the end of the third hour. The oxygen level in the swim-tunnel was then maintained at 8–9 kPa for 1 h before the hypoxic U_{crit} trial was performed. The hypoxic U_{crit} trial was identical to that performed during normoxia, and fish swum under hypoxic conditions were also allowed to recover under normoxic conditions; water $P_{w}O_2$ increased from 8–9 kPa to ~19 kPa during the first 20 min of recovery. Fish swum under hypoxic conditions ($P_{w}O_2$ 8–9 kPa) were not recovered at this oxygen level because preliminary experiments on normoxia-acclimated fish showed that some individuals were having trouble righting themselves or swimming constantly even more than 2 h post-exercise. This is also the main reason that the fish were swum in the same order; normoxia, then hypoxia.

For both normoxic and hypoxic swim trials, U_{crit} was calculated as:

$$U_{crit} = \mathbf{v} + \left(\left(\frac{t_f}{t_i} \right) \times \mathbf{v}_i \right) , \quad (1)$$

where $\mathbf{v}$ is the highest velocity at which the fish swam for the entire time increment ($BL\,s^{-1}$); $\mathbf{v}_i$ is velocity increment ($BL\,s^{-1}$); t_f is time elapsed from the last change in current velocity to fatigue (min); and t_i is time increment, the time between step increases in velocity (20 min).

Measurements of cardiac function and metabolism

Cardiac output ($\dot{Q}$) was continuously measured during the U_{crit} trial and for ~2 h after the fish became exhausted [i.e. measurements were taken immediately after the fish stopped swimming (0 min), and at 25 min, 50 min, 75 min, 100 min and 125 min of recovery]. $\dot{Q}$ was measured by connecting the flow meter to a MP100A-CE data acquisition system and a laptop running AcqKnowledge software (BIOPAC Systems Inc., Santa Barbara, CA, USA). Data were recorded at a frequency of 20 Hz, and values of cardiac output were obtained during the last 5 min at each swimming speed and during the first 5 min of each 25 min period during recovery. Cardiac output ($\dot{Q}$, in $ml\,min^{-1}\,kg^{-1}$) was calculated by dividing the raw data ($ml\,min^{-1}$) by the mass of the fish (kg). Heart rate (f_H; $beats\,min^{-1}$) was calculated by measuring the time required for 20 systolic peaks, and multiplying this value by 60 divided by the measurement period (s). Stroke volume (V_S; $ml\,kg^{-1}$) was calculated as $\dot{Q}/f_H$. Maximum values of $\dot{Q}$, V_S and f_H were measured as the highest value that each individual fish achieved. Finally, the absolute scope for cardiac variables ($\dot{Q}$, V_S and f_H) was calculated by subtracting routine (resting) values from maximum values.

Water temperature and oxygen concentration ($mg\,O_2\,l^{-1}$) in the swim tunnel were continuously measured via an external circuit containing an oxygen probe housed in a D201 flow through cell (see description for the hypoxia acclimation tank). Oxygen consumption ($\dot{M}_{O_2}$) of the cod was measured over 10 min intervals at rest, at each swimming speed, and at 0, 25, 50, 75, 100 and 125 min of recovery by stopping the flow of water into the swim-tunnel, recording the drop in water-oxygen concentration in the swim-tunnel, and using the following equation (Cech, 1990):

$$\dot{M}_{O_2} = \frac{((C_i - C_f) \times V_c) \times 60}{M \times t} , \quad (2)$$

where C_i is water oxygen concentration ($mg\,O_2\,l^{-1}$) at the start of $\dot{M}_{O_2}$ measurement; C_f is oxygen concentration ($mg\,O_2\,l^{-1}$) at the end of $\dot{M}_{O_2}$ measurement; V_c is volume of the respirometer and external circuit (81 l); M is fish mass (kg); and t is time required to make $\dot{M}_{O_2}$ measurement (10 min). Note that water oxygen concentration only dropped by ~0.1–0.3 $mg\,l^{-1}$ over the measurement period.

Standard oxygen consumption was obtained from a semi-log plot of swimming speed ($BL\,s^{-1}$) *vs* $\log \dot{M}_{O_2}$, and using the derived linear regression to extrapolate back to $0\,BL\,s^{-1}$. Maximum oxygen consumption ($\dot{M}_{O_2max}$) was measured as the highest oxygen consumption that each individual fish achieved, and absolute metabolic scope was then calculated by subtracting routine (that of fish resting quietly in the tunnel) $\dot{M}_{O_2}$ from $\dot{M}_{O_2max}$. Finally, each fish's total excess post-exercise oxygen consumption (EPOC; in $mg\,O_2\,kg^{-1}$; a measure of the non-aerobic cost of exercise) was obtained by integrating the area underneath the $\dot{M}_{O_2}$–time curve until $\dot{M}_{O_2}$ returned to routine values (see Lee et al., 2003).

The routine metabolic rate of fishes, including cod, scales allometrically with body mass with a slope of ~0.8–0.85 (Saunders, 1963; Post and Lee, 1996; Killen et al., 2007). However, isometrically scaled metabolic rate data (i.e. $mg\,O_2\,h^{-1}\,kg^{-1}$) are reported, because to our knowledge no metabolic scaling exponents have been reported for fish cardiovascular function, and we wanted to report all the variables using common units. Furthermore, the

Table 1. Physical characteristics of the Atlantic cod used in the various experiments

	Treatment		
	No surgery	Normoxia-acclimated	Hypoxia-acclimated
Mass (kg)	0.55±0.03*	0.68±0.03	0.57±0.04
Length (cm)	39.7±0.8	41.9±0.9	40.4±0.8
Condition factor (K)	0.90±0.04	0.94±0.03	0.86±0.03

*Significant difference between non-surgery and normoxia-acclimated groups. N=9, 10 and 12 for the three groups. Values are means±s.e.m.

range of mass of the fish used was small, and thus the error in using isometric units would be minimal. However, we do provide allometrically scaled metabolic rates in the discussion when comparing our data with the literature.

Effect of surgery on swimming performance and metabolism

We did not want to give the hypoxic fish an extended period of exposure to normoxic water prior to the initial U_{crit} test. However, we were also cognisant of the fact that surgery and/or the post-surgical recovery period can potentially affect swimming and cardiovascular performance (e.g. Butler et al., 1989; Campbell et al., 2004) [also see figure 7 in Webber et al. (Webber et al., 1998)]. Thus, normoxic and hypoxic swim trials were also performed on nine (0.55±0.03 kg) normoxia-acclimated cod that did not undergo surgery or anaesthesia (non-surgery group). These fish were placed directly in the swim tunnel after being netted from their acclimation tank and allowed ~18 h to recover.

Statistical analyses

A one-way ANOVA was used to examine whether fish mass, length and condition factor were affected by chronic acclimation to hypoxic conditions (Table 1). Two-way ANOVAs with repeated measures were used to determine at which swimming speeds or times during recovery $\dot{M}_{O_2}$ and cardiovascular variables were different between: (1) surgery *vs* non-surgery groups (Fig. 1); and (2) normoxia- *vs* hypoxia-acclimated cod (Fig. 2). This analysis was also used to determine at which values of water O_2 saturation variables were different between normoxia- and hypoxia-acclimated cod during the step-down period (Fig. 3). Furthermore, Dunnett's *post-hoc* tests were performed to examine if/when variables became different from values at 100% air saturation (Fig. 3). Two-way ANOVAs with repeated measures, followed by paired (normoxic *vs* hypoxic swim) or unpaired (normoxia- *vs* hypoxia-acclimated or surgery *vs* non-surgery) *t*-tests were used to identify differences in metabolic and cardiovascular variables (see Tables 2 and 3). Finally Pearson's correlation analysis was carried out to define the strength of the relationship between oxygen consumption and cardiac output during the normoxic and hypoxic swims and graded hypoxia (Fig. 4). All data presented in figures, tables and the text are means ± standard error (s.e.m.). Statistical analyses were carried out using SPSS (v.13.0; SPSS, Chicago, IL, USA) and a difference was considered significant when $P<0.05$.

RESULTS

Water quality in the hypoxic tank did not deteriorate during the 6–12 weeks of acclimation to hypoxia. Mean values (N=10–12) for total nitrogen, un-ionized ammonia, carbon dioxide and pH were 0.03±0.01 p.p.m.: 0.0003±0.0 p.p.m., 2.3±0.6 p.p.m. and 7.8±0.1, respectively. Further, although the mass of hypoxia-acclimated cod was ~15% lower as compared with those held under normoxic conditions, neither this variable or condition factor were significantly different between the two groups (Table 1).

Effects of anaesthesia and surgery

Surgery resulted in a 14% decrease in the normoxic U_{crit} value (from 1.74 to 1.50 $BL\,s^{-1}$). However, the effect of surgery was similar when the fish were swum under hypoxia (17% decrease), and as a result, the reduction in U_{crit} between normoxic and hypoxic conditions was similar for both groups (non-surgery, 29%; surgery, 32%; Table 2). This pattern of change in swimming performance was not reflected by most metabolic variables: (1) cod fitted with flow probes had significantly lower routine and standard metabolic rates (by ~25%) when measured under normoxic conditions; (2) surgery had no significant effect on normoxic values of $\dot{M}_{O2max}$ or metabolic scope; and (3) $\dot{M}_{O2max}$ and metabolic scope decreased to a much greater extent in the surgery group compared with the non-surgery group when they were swum at 8–9 kPa (57% *vs* 43% and 80% *vs* 55%, respectively; Table 2, Fig. 1). Finally, EPOC was significantly ($P<0.05$) lower in the surgery group when swum under normoxia and hypoxia (by 42% and 46%, respectively; Table 2).

Normoxic U_{crit} test

In normoxic water, routine f_H, V_S, $\dot{Q}$ and $\dot{M}_{O_2}$ averaged 32.9±2.2 beats min^{-1}, 0.73±0.07 ml kg^{-1}, 23.1±1.8 ml min^{-1} kg^{-1} and 63.5±2.4 mg O_2 h^{-1} kg^{-1}, respectively for the 10 normoxia-acclimated

Table 2. The effect of surgery and anaesthesia on swimming performance and oxygen consumption of Atlantic cod measured under normoxia ($P_{W_{O_2}}$ ~20 kPa) and hypoxia ($P_{W_{O_2}}$ ~8–9 kPa)

	Non-surgery		Surgery (normoxia-acclimated group)	
	Normoxic swim	Hypoxic swim	Normoxic swim	Hypoxic swim
SMR	64.3±8.8[a,*]	53.6±7.4	47.4±2.0	55.3±4.1
Routine $\dot{M}_{O_2}$	82.5±7.7[a]	65.3±5.4	63.5±2.4	63.6±3.2
Max $\dot{M}_{O_2}$	234.6±21.2[a]	133.1±8.8*	214.1±10.6[a]	92.7±3.4
Scope	152.1±20.7[a]	67.8±8.0*	150.6±10.0[a]	29.2±4.1
EPOC	73.1±11.9[a,*]	47.7±3.4*	42.6±4.3	28.0±7.7
U_{crit}	1.74±0.06*	1.23±0.05*	1.50±0.04[a]	1.02±0.03

Values are means±s.e.m., N=9 for non-surgery fish and 10 for fish that underwent surgery prior to the U_{crit} tests. [a]A significant difference between the normoxic and hypoxic swim within each acclimation condition. *A significant difference between groups (non-surgery *vs* surgery) within a particular test condition.

Table 3. Metabolic and cardiac variables, and swimming performance, in normoxia- and hypoxia-acclimated Atlantic cod subjected to critical swimming speed (U_{crit}) tests under both normoxic (Pw_{O_2} ~20 kPa) and hypoxic (Pw_{O_2} ~8–9 kPa) conditions

	Normoxia-acclimated								Hypoxia-acclimated							
	Normoxic swim				Hypoxic swim				Normoxic swim				Hypoxic swim			
	$\dot{M}_{O_2}$	f_H	V_S	$\dot{Q}$	$\dot{M}_{O_2}$	f_H	V_S	$\dot{Q}$	$\dot{M}_{O_2}$	f_H	V_S	$\dot{Q}$	$\dot{M}_{O_2}$	f_H	V_S	$\dot{Q}$
Routine	63.5*±2.4	32.9±2.2	0.73*±0.07	23.1*±1.8	63.6±3.2	33.0±1.7	0.73*±0.04	23.2±0.9	80.9[a]±3.4	35.2±1.9	0.49[a]±0.04	17.0±1.1	72.0±4.3	35.9±2.4	0.56±0.04	19.8±1.5
Max	214.1[a]±10.6	46.3[a]±0.9	0.99*±0.07	44.5[a,*]±2.7	92.7±3.4	38.8*±1.5	0.99*±0.06	34.6±1.7	231.4[a]±12.3	50.1±0.8	0.74±0.04	34.2±2.2	100.6±5.1	48.5±3.0	0.71±0.04	31.3±1.8
Scope	150.6[a]±10.0	13.4[a]±1.9	0.26±0.04	21.5[a]±2.2	29.2±4.1	5.8*±1.0	0.27*±0.04	11.4±1.5	150.6[a]±12.5	14.9±2.0	0.25[a]±0.03	17.3[a]±1.9	28.6±2.8	12.6±1.3	0.15±0.03	11.5±1.8
SMR	47.4*±2.0	–	–	–	55.3±4.1	–	–	–	68.3±4.0	–	–	–	63.2±4.1	–	–	–
EPOC	42.6±4.3	–	–	–	28.0±7.7	–	–	–	33.0±6.8	–	–	–	22.3±3.9	–	–	–
U_{crit}	1.50[a]±0.04				1.02±0.03				1.51[a]±0.07				1.02±0.05			

$\dot{M}_{O_2}$, oxygen consumption (mg O_2 h^{-1} kg^{-1}); f_H, heart rate (beats min^{-1}); V_S, stroke volume (ml kg^{-1}); $\dot{Q}$, cardiac output (ml min^{-1} kg^{-1}) and U_{crit} (*BL* s^{-1}). Routine refers to values measured at rest (0.25 *BL* s^{-1}). SMR (standard metabolic rate) was obtained by plotting log $\dot{M}_{O_2}$ of individual fish against swimming speed and extrapolating to 0 *BL* s^{-1}. EPOC (excess post-exercise oxygen consumption). Values are means±s.e.m., *N*=10 for both groups. [a]A significant difference between normoxic and hypoxic swims within each acclimation condition. *A significant difference between groups (normoxia- *vs* hypoxia-acclimation) within a particular test condition.

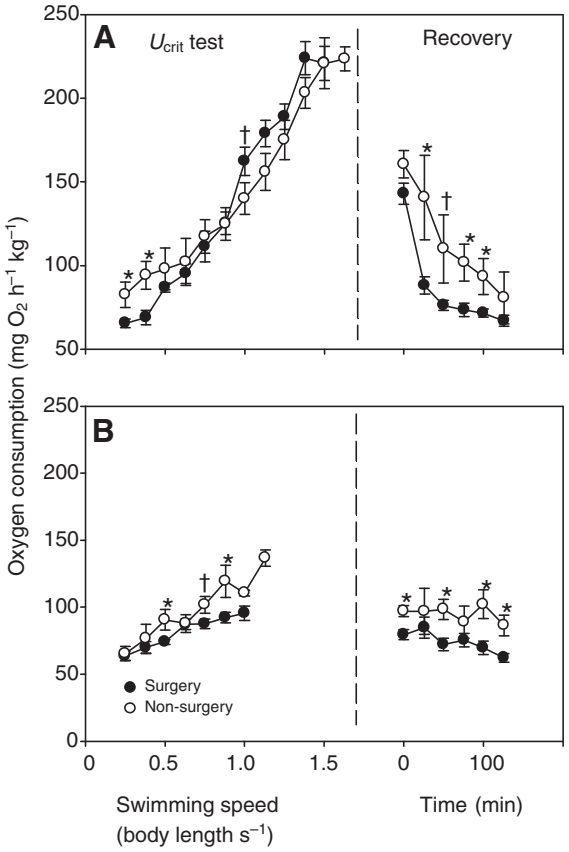

Fig. 1. The effect of anaesthesia and surgery (Transonic® flow probe placement around ventral aorta) on the oxygen consumption of cod during normoxic (A) and hypoxic (B; Pw_{O_2} 8–9 kPa) critical swimming speed tests, and during post-exercise recovery. Non-surgery fish were netted from their holding tank, and placed directly into the swim-tunnel respirometer. All fish were swum in normoxic water on day 1 and hypoxic water on day 2, but recovery was performed in normoxic water for all swims. *N*=9 for non-surgery fish, and *N*=10 for fish that underwent surgery. *Significant differences between non-surgery and surgery groups at *P*<0.05; †differences at (*P*<0.10). See Table 2 for a statistical analysis of differences in maximum swimming speed (U_{crit}) and metabolic variables between groups.

fish (Fig. 2, Table 3). Although resting f_H was similar in hypoxia-acclimated cod (also *N*=10), $\dot{M}_{O_2}$ was significantly higher (by 40%) in this group, despite the fact that both $\dot{Q}$ and V_S were significantly lower (by 26% and 30%, respectively).

During the normoxic U_{crit} test, $\dot{M}_{O_2}$ and all cardiovascular variables increased with swimming speed, and differences between normoxia- and hypoxia-acclimated cod were generally retained (Fig. 2, Table 3). For example, standard metabolic rate (SMR) was 30% higher in the hypoxia-acclimated group (68 *vs* 47 mg O_2 h^{-1} kg^{-1}), and there were no differences in the scope for $\dot{M}_{O_2}$ (~150 mg O_2 h^{-1} kg^{-1}) or any of the cardiac variables (f_H, ~14 beats min^{-1}; V_S, ~0.25 ml kg^{-1}; $\dot{Q}$, 17 *vs* 21 ml min^{-1} kg^{-1}). However, there were some notable differences. First, f_H became significant elevated in the hypoxia-acclimated group, as compared with normoxia-acclimated fish, at swimming speeds between 1.0 and 1.38 *BL* s^{-1}. Second, although $\dot{M}_{O_2}$, f_H, V_S and $\dot{Q}$ generally increased in normoxia-acclimated fish until exhaustion, these variables either plateaued or decreased slightly in hypoxia-acclimated fish after 1.25 *BL* s^{-1}. Given that there were no differences

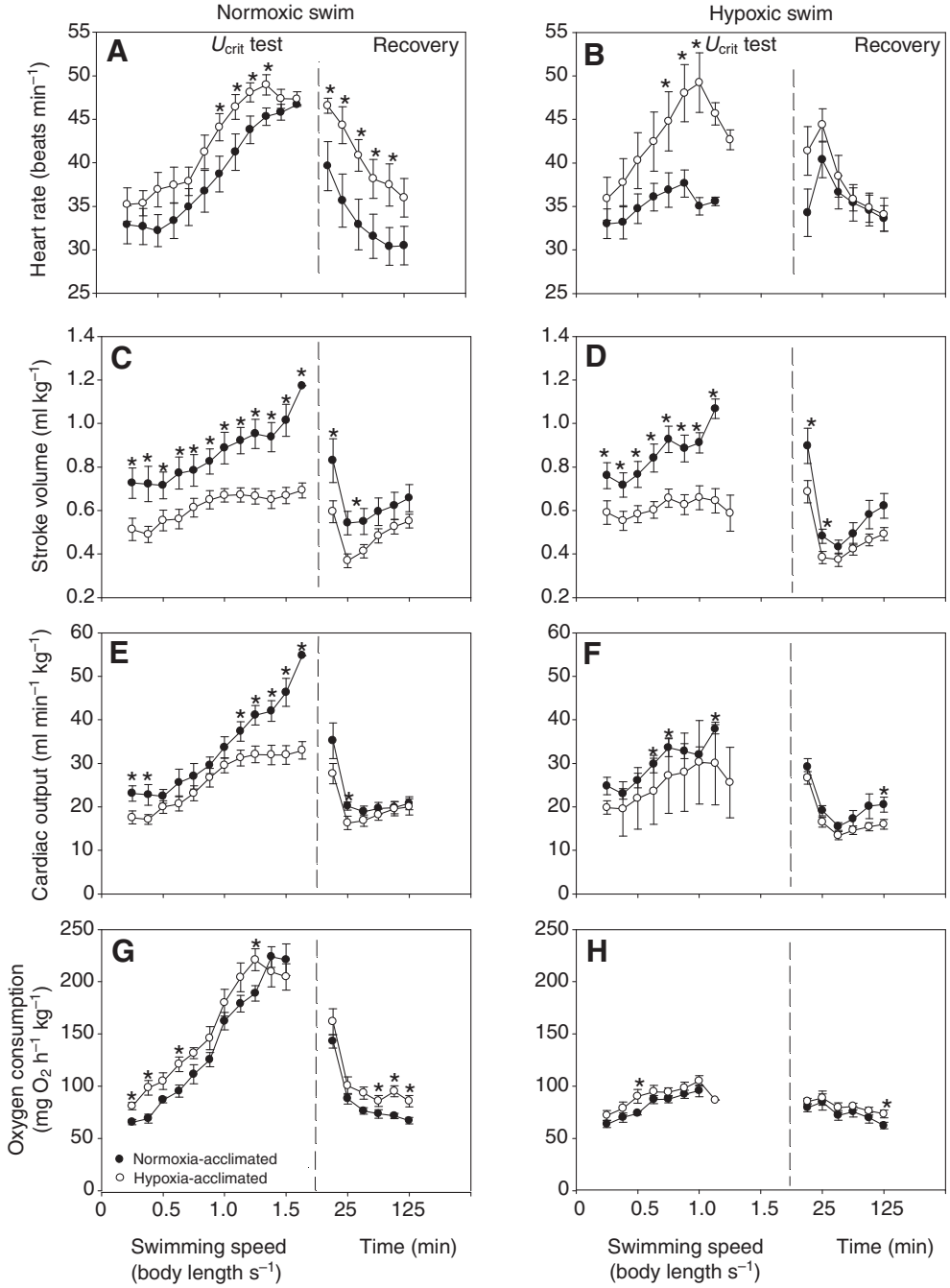

Fig. 2. (A,B) Heart rate, (C,D) stroke volume, (E,F) cardiac output and (G,H) oxygen consumption in normoxia- (N=10) and hypoxia-acclimated (N=12) cod during critical swimming speed (U_{crit}) tests, and during post-exercise recovery. All fish were swum in normoxic water on day 1 and hypoxic water on day 2, but recovery was performed in normoxic water for all swims. *A significant difference (P<0.05) between the normoxia- and hypoxia-acclimated groups at a particular swimming speed.

in metabolic scope or the scope for cardiac variables, it was not surprising that U_{crit} (~1.5 BL s^{-1}) was identical in the two groups.

During recovery from the U_{crit} test, $\dot{M}_{O_2}$ fell rapidly in both groups, and there was no significant difference in EPOC (P=0.31; Table 3), although it was 30% higher in the normoxia-acclimated group. Interestingly, the post-exercise pattern of change for f_H was different than for $\dot{Q}$ and V_S. The f_H fell slowly after the cod were exhausted, whereas both $\dot{Q}$ and V_S decreased rapidly (i.e. within 25 min) to values comparable to, or below, routine levels and then rebounded (Fig. 2).

Graded hypoxia and the hypoxic U_{crit} test

At the start of the second day (i.e. at P_{wO_2} 21 kPa; Fig. 3) $\dot{M}_{O_2}$ and values for cardiac function were very similar to those measured at the beginning of day 1 (i.e. prior to the normoxic U_{crit} test, Fig. 2), and the differences between groups were maintained. For example, routine $\dot{M}_{O_2}$ was slightly (by ~10%) higher, and $\dot{Q}$ and V_S were again significantly lower (by ~22 and 30%, respectively), in the hypoxia-acclimated group (Fig. 3). There were very few changes in $\dot{M}_{O_2}$ or cardiovascular variables as water O_2 partial pressure was lowered from 21 kPa to 8–9 kPa. However, f_H and $\dot{Q}$ did increase slightly, and significantly, after 1 h of exposure to water of 8–9 kPa O_2 in the normoxia-acclimated cod.

The pattern of change in $\dot{M}_{O_2}$ and cardiac variables during the hypoxic U_{crit} test was qualitatively similar to that seen during the normoxic swim (Fig. 2). However, U_{crit} for both groups (1.0 BL s^{-1}) was only approximately two-thirds of that measured during normoxia (1.5 BL s^{-1}), and this diminished swimming performance

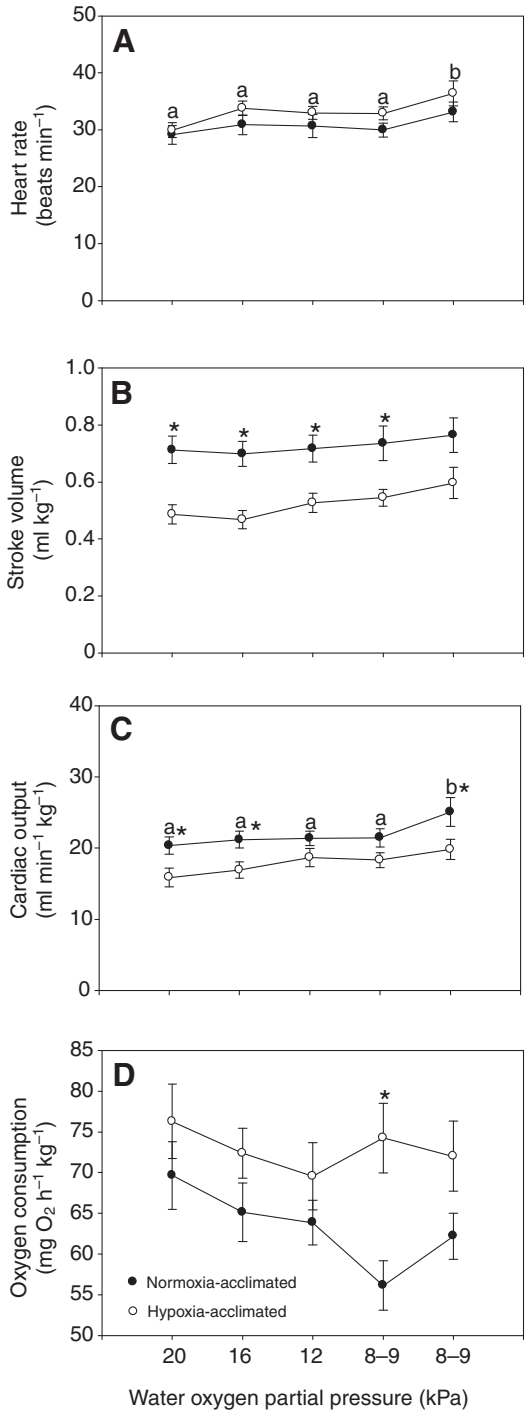

Fig. 3. Cardiac function and metabolism when normoxia- and hypoxia-acclimated cod were exposed to graded hypoxia. (A) Heart rate, (B) stroke volume, (C) cardiac output and (D) oxygen consumption. Oxygen levels were dropped from a $P_{W_{O_2}}$ of 20 kPa to 16 kPa in the first hour, from 16 to 12 kPa in the second hour and finally from 12 to 8–9 kPa in the third hour. Dissimilar letters indicate significant (P<0.05) differences from 20 kPa and other oxygen levels within the normoxic group. *Indicates values significantly (P<0.05) different between normoxia- and hypoxia-acclimated fish at a particular $P_{W_{O_2}}$.

was associated with important differences in how cardiorespiratory variables in normoxia- and hypoxia-acclimated cod responded to the exercise regimen. First, as compared with the normoxic swim, maximum $\dot{M}_{O_2}$ was greatly reduced in both groups (214 and 231 mg O_2 h^{-1} kg^{-1} *vs* 93 and 101 mg O_2 h^{-1} kg^{-1}), and this resulted in a dramatically reduced metabolic scope (to ~30 mg O_2 h^{-1} kg^{-1}). Second, although the scope for $\dot{Q}$ was reduced to a similar degree in both groups (to ~11.5 ml min^{-1} kg^{-1}) when the cod were swum under hypoxic conditions as compared with normoxia, the reason for the reduced scope for $\dot{Q}$ was different. In the normoxia-acclimated group, the scope for $\dot{Q}$ was diminished because the scope for f_H was reduced by 50% (normoxia 13.4 beats min^{-1}; hypoxia 5.8 beats min^{-1}). In contrast, the scope for V_S fell from 0.25 to 0.15 ml kg^{-1} in the hypoxia-acclimated group when the cod were swum at an oxygen level of 8–9 kPa (Table 3).

During recovery from the hypoxic U_{crit} test, the pattern of change in $\dot{M}_{O_2}$, $\dot{Q}$ and V_S was similar to that observed after the normoxic swim. However, the pattern of change in f_H was quite different. Heart rate increased in both groups between 0 and 25 min post-exercise, before declining to pre-swim levels (Fig. 2). This was probably due to the fact that these fish were recovered in normoxic, not hypoxic water. As with the normoxic swim, there was no difference in EPOC values between the two groups. However, for both groups, EPOC was ~35% lower than the values for cod swum under normoxic conditions (Table 3).

Relationship between oxygen consumption and cardiac output

During the normoxic swim (Fig. 4A) there was a strong linear relationship between cardiac output and oxygen consumption in both the normoxic (r^2=0.97) and hypoxic groups (r^2=0.95). However, the relationship for the hypoxia-acclimated fish was shifted decidedly upwards, and this resulted in a substantially greater $\dot{M}_{O_2}$ for a given $\dot{Q}$ in hypoxia- as compared with normoxia-acclimated fish. There was no clear relationship between $\dot{M}_{O_2}$ and $\dot{Q}$ when the fish were exposed to graded hypoxia. However, the $\dot{M}_{O_2}$ of hypoxia-acclimated fish was generally above that of the normoxia-acclimated fish, and this elevated level of $\dot{M}_{O_2}$ was achieved at reduced levels of $\dot{Q}$ (Fig. 4B). Finally, although the relationship between $\dot{M}_{O_2}$ and $\dot{Q}$ was not as strong during the hypoxic swim (r^2=0.83 and 0.84), and the data for the hypoxia-acclimated group was much more variable, it was again apparent that the hypoxia-acclimated fish consumed more O_2 for a given cardiac output (Fig. 4C).

DISCUSSION

The cod in this study were swum to exhaustion, first under normoxia, and then at a $P_{W_{O_2}}$ of ~8–9 kPa after being allowed to recover from the initial U_{crit} test for ~24 h. This experimental design has some limitations. Most importantly, the possibility that different degrees of recovery from surgery (18 *vs* ~42 h) or effects related to the initial U_{crit} test, might have obscured some effects of acute hypoxia on swimming performance, cardiac function and metabolic capacity. We believe that these were minimal given how closely our data fit with those in the literature, that fish recover quickly (within 2 h) from exhaustive exercise under normoxic conditions (Jain and Farrell, 2003; Jain et al., 1998), and that the reduction in U_{crit} with acute hypoxia was similar in fish that were simply placed into the swim-tunnel (non-surgery) *vs* those that were implanted with flow probes (see below). Furthermore, it is unlikely that the experimental design significantly affected our major findings with respect to the effects of acclimation to hypoxia on cod cardiorespiratory function. This is because our results are very similar to those obtained in a later study where cod were given a graded hypoxic challenge ~24 h after recovering from surgery

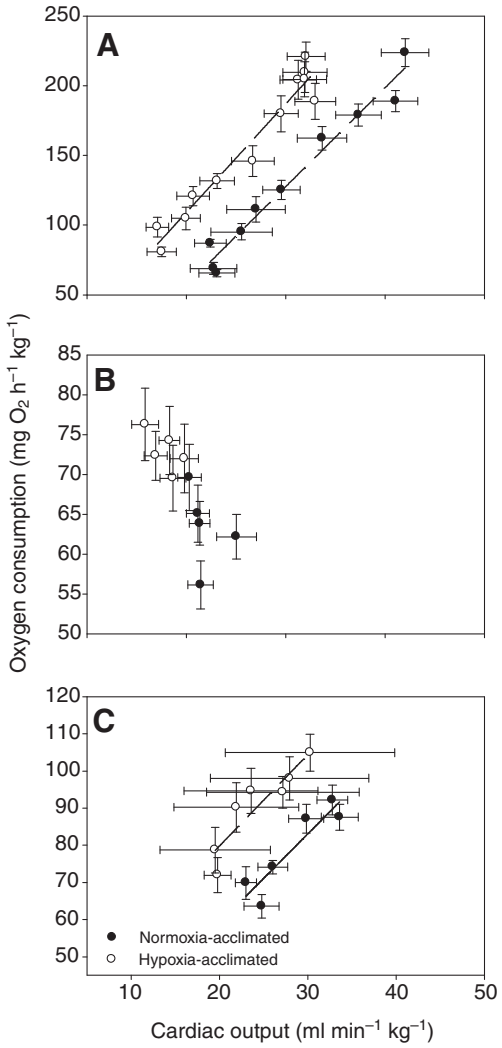

Fig. 4. Relationship between oxygen consumption and cardiac output in the normoxia- and hypoxia-acclimated cod during the normoxic U_{crit} test (A), when exposed to graded hypoxia (B), and finally during the hypoxic U_{crit} test (C). Dashed lines define the linear regressions that were fitted to the data. Normoxic swim: normoxia-acclimated group (y=7.1x–85.48, r^2=0.97), hypoxia-acclimated group (y=7.79x–0.95, r^2=0.95). Hypoxic swim: normoxia-acclimated group (y=2.40x+11.14, r^2=0.84), hypoxia-acclimated group (y=2.46x+30.62, r^2=0.83).

(L.H.P. and A.K.G., unpublished) and to those recently reported by Lamarche et al. (Lamarche et al., 2009).

Effects of surgery

The reduction in normoxic swimming performance (from 1.74 to 1.5 $BL\,s^{-1}$) associated with surgery and/or anaesthesia was not unexpected (see Butler et al., 1989; Campbell et al., 2004; Bell and Eggleston, 2005). Nonetheless, it is difficult to ascribe a definitive cause for the diminished U_{crit} under normoxic conditions. This is because Butler et al. (Butler et al., 1989) report that U_{crit} in cod is diminished by 13.5% following sham surgery (a similar decrease to that reported here, 14%), and thus the presence of the flow probe and/or drag associated with the probe lead are unlikely to explain the reduced U_{crit} in fish that underwent surgery. Furthermore, maximum $\dot{M}_{O_2}$ and metabolic scope were similar between groups (~220 and 150 $mg\,O_2\,h^{-1}\,kg^{-1}$; ~198 and 135 $mg\,O_2\,h^{-1}\,kg^{-0.8}$; Table 1), and the scope for $\dot{Q}$ (21.5 $ml\,min^{-1}\,kg^{-1}$) was almost identical to that obtained for cod fitted with Doppler flow probes that were subjected to a U_{crit} test following at least 48 h of recovery [23 $ml\,min^{-1}\,kg^{-1}$; Webber et al. (Webber et al. 1998)]. There are, however, two potential explanations. Cod in poor condition (condition factor 0.68) start burst-coast swimming (i.e. switch to a mix of aerobic and anaerobic powered swimming) at lower speeds, and become exhausted at a reduced U_{crit} (Lapointe et al., 2006). However, we feel that an earlier initiation of burst-coast swimming is unlikely in this study as there were no significant (P<0.05) differences in $\dot{M}_{O_2}$ between the two groups of fish at swimming speeds above 0.38 $BL\,s^{-1}$; this finding suggesting that the cost of transport was similar between the two groups. In addition, EPOC [an index of the non-aerobic costs of exercise (Lee et al., 2003)] was significantly lower in the surgery group. This latter result suggests that U_{crit} was reduced not because burst-coast swimming was initiated at a lower velocity, but because the number or intensity of burst-coast events prior to exhaustion was diminished. Such a reduction in swimming effort associated with recovery from anaesthesia/surgery would be consistent with the conclusions of McFarlane and McDonald (MacFarlane and MacDonald, 2002) and Peake and Farrell (Peake and Farrell, 2006). These authors indicate that the cessation of activity and decreased swimming performance in U_{crit} tests is often a behavioural response (i.e. fish are disinclined to perform).

Effects of acute hypoxia and acclimation to hypoxia: measurements at rest

Acclimation to chronic hypoxia (~8 kPa) resulted in elevated routine (i.e. measured at 0.25 $BL\,s^{-1}$) and standard metabolic rates (Table 3, Fig. 2) in normoxia as compared with normoxia-acclimated cod, despite lower values of V_S and $\dot{Q}$ (0.49 *vs* 0.73 $ml\,kg^{-1}$ and 17.0 *vs* 21.3 $ml\,min^{-1}\,kg^{-1}$, respectively). The higher routine $\dot{M}_{O_2}$ in hypoxia-acclimated cod contrasts with the findings of Bushnell et al. (Bushnell et al., 1984) who reported that acclimation to hypoxia did not influence, or significantly reduced, normoxic $\dot{M}_{O_2}$ values in resting rainbow trout. The disparity in results between our study and that of Bushnell et al. (Bushnell et al., 1984) could be related to methodological differences. Bushnell et al. (Bushnell et al., 1984) only acclimated their trout to hypoxia for 3 weeks, the level of hypoxia during acclimation (~5.6 kPa) was more severe than utilized in the present study, and the hypoxia-acclimated trout were only allowed a brief period at normoxic levels of oxygen prior to measurements of $\dot{M}_{O_2}$. However, it may also be due to inter-specific differences in how teleost fish are affected by chronic hypoxia. For example, Lomholt and Johansen (Lomholt and Johansen, 1979) report that carp (*Cyprinus carpio*) acclimated to a P_{O_2} of 4 kPa for 4 weeks had a reduced $\dot{M}_{O_2}$ when they were returned to high oxygen conditions, whereas Pichavant et al. (Pichavant et al., 2000) showed that acclimation to hypoxia (45 days at a P_{O_2} ~10 kPa) did not alter the routine $\dot{M}_{O_2}$ of turbot (*Scophthalmus maximus*) at any water P_{O_2} level. This study did not attempt to elucidate the factors responsible for the elevated routine $\dot{M}_{O_2}$ in hypoxia-acclimated fish. However, it is unlikely that the higher $\dot{M}_{O_2}$ was stress related. This is because circulating catecholamines in resting hypoxia-acclimated cod at 18–24 h post surgery are ≤5 $nmol\,l^{-1}$ when measured under normoxia, and similar to those measured in normoxia-acclimated fish (L.H.P. and A.K.G., unpublished).

Standard and routine metabolic rates decreased slightly (non-surgery and hypoxia-acclimated fish) or were unchanged (normoxia-acclimated group) when the cod were acutely exposed to a P_{wO_2} of 8–9 kPa (Tables 2 and 3). These results are consistent with recent

data on juvenile cod at 10°C where both trends have been reported prior to fish reaching their critical oxygen tension (Steffensen et al., 1994; Schurmann and Steffensen, 1997; Gamperl et al., 2009). When normoxia-acclimated cod were exposed to acute hypoxia, V_S did not change significantly, although $\dot{Q}$ and f_H did increase slightly after 1 h at 8–9 kPa, as compared with the value recorded at the start of day 2 (Table 3, Fig. 3). The lack of hypoxic bradycardia, and a concomitant increase in V_S, in this study as compared with that of Fritsche and Nilsson (Fritsche and Nilsson, 1989) is most likely due to the depth of hypoxia, as: (1) Fritsche and Nilsson exposed their cod to ~5 kPa O_2 as compared to 8–9 kPa in the present study; (2) the S_{crit} for Atlantic cod (i.e. the water O_2 saturation at which they transitioned from being an oxyregulator to an oxyconformer) at 10°C (Schurmann and Steffensen, 1997; Gamperl et al., 2009) is at or below the lowest level of hypoxia used in this study; and (3) Petersen and Gamperl (L.H.P. and A.K.G., unpublished) have shown that f_H falls (and V_S increases) rapidly in normoxia- and hypoxia-acclimated cod when Pw_{O_2} falls below ~5 kPa (f_H and V_S in normoxia-acclimated cod ~20 beats min^{-1} and 1.3 ml kg^{-1}, respectively, at 2.7 kPa). However, we cannot exclude the possibility that the rapid induction of hypoxia in the Fritsche and Nilsson study (Fritsche and Nilsson, 1989) (i.e. over ~60 s), *vs* exposure to graded hypoxia over a period of 3 h (present study), contributed to the contrasting effects of hypoxia on f_H between the two studies.

Effects of acute hypoxia: exercise

When the two normoxia-acclimated groups (i.e. surgery and non-surgery) were exposed to a Pw_{O_2} of 8-9 kPa, U_{crit} was reduced by 33 and 29%, respectively. This decrease in U_{crit} is very similar to that reported by Dutil et al. (Dutil et al., 2007) for 7°C cod swum at 8.5 kPa (1.72 *vs* 1.26 *BL* s^{-1}), and comparable to those for rainbow trout [~25% at 5.6 kPa; Bushnell et al. (Bushnell et al., 1984)] and coho salmon [*Oncorhynchus kisutch*; 27% at ~8.5 kPa; Dahlberg et al. (Dahlberg et al., 1968)] swum at 15 and 20°C, respectively. However, it is much greater than that recorded for largemouth bass (*Micropterus salmoides*) at 25°C (10% at ~8.5 kPa) (Dahlberg et al., 1968) and mullaway (*Argyrosomus japonicus*) at 21–23°C (17% at both ~10 and 5 kPa) (Fitzgibbon et al., 2007). When combined, these data suggest that there are considerable inter-specific differences in the sensitivity of swimming performance to reduced oxygen levels, and that this variation is related to the likelihood that a species will encounter hypoxic conditions during its life history. For example, most salmonids inhabit well-aerated fluvial environments and cod normally avoid water oxygen levels less than 9 kPa (40–45% air saturation) (Claireaux et al., 2005). By contrast, largemouth bass prefer shallow, warm, weedy areas (Heidinger, 1975) and the mullaway spends its early life in estuaries (Fitzgibbon et al., 2007), both environments where water quality and oxygenation can vary considerably.

It is clear that reduced metabolic scope (by 55 and 80% in non-surgery and surgery groups, respectively; Table 2) restricted the swimming performance of our normoxia-acclimated cod during acute hypoxic exposure; and that this was, in large part, related to a diminished maximum cardiac performance. The dependence of fish swimming performance on metabolic scope is well established (e.g. Fry, 1971; Arnott et al., 2006; Chatelier et al., 2006), and the magnitude of the decreases in metabolic scope when the cod were swum under hypoxic conditions are consistent with other studies that have examined the relationship between metabolic scope and reduced water oxygen levels in this species. For example, at a Pw_{O_2} of 8–10 kPa cod forced to swim in respirometers to U_{crit} show reductions in metabolic scope ranging from 65–75% (Claireaux et al., 1995; Dutil et al., 2007), whereas scope calculated on free-living fishes using limiting oxygen concentration curves (Claireaux and Lagardére, 1999) falls by ~53% (Claireaux et al., 2000). Furthermore, myocardial performance is a primary factor limiting metabolic rate and U_{crit} in many active teleosts (Farrell, 2002; Claireaux et al., 2005; Clark et al., 2005), and maximum $\dot{Q}$ was reduced by 47% when normoxia-acclimated cod were swum under hypoxic conditions. Although a diminished maximum $\dot{Q}$ under hypoxic as compared with normoxic conditions (from 45.5 to 34.6 ml min^{-1} kg^{-1}) was not unexpected (e.g. Hanson et al., 2006), we were surprised to find that the reduction in $\dot{Q}$ was solely related to a lower maximum f_H (and scope for f_H); maximum V_S reaching the same value as measured under normoxia (0.99 ml kg^{-1}). This is because, in contrast to the rainbow trout (e.g. Faust et al., 2004; Gamperl et al., 2004), acute exposure of the *in situ* cod heart to severe hypoxia does not affect f_H during resting or maximum levels of cardiac performance (Petersen and Gamperl, 2010), and maximum f_H and scope for f_H were not different in hypoxia-acclimated cod when swum under hypoxic and normoxic conditions (Fig. 2, Table 3). Furthermore, it is unlikely that the lowered f_H was the result of the stimulation of branchial O_2 receptors as this level of hypoxia (8–9 kPa) did not elicit bradycardia under resting conditions. This raises the distinct possibility that venous O_2 receptors located at or before the heart, as proposed by Barrett and Taylor (Barrett and Taylor, 1984), were stimulated by severe hypoxemia associated with exercise under hypoxic conditions and mediated a reduction in heart rate through the efferent limb of the cardiac vagus.

Effects of acclimation to hypoxia: exercise

Cod chronically acclimated to moderate hypoxia (8–9 kPa) had values of maximum $\dot{M}_{O_2}$, metabolic scope and U_{crit} under normoxia and hypoxia that were not significantly different from those measured in normoxia-acclimated fish (Table 3). These results are in agreement with the results of Bushnell et al. (Bushnell et al., 1984) and Kutty (Kutty, 1968), who showed that neither the swimming speed–oxygen consumption relationship or U_{crit} values were altered when rainbow trout and goldfish, respectively, were acclimated to hypoxic conditions. Collectively, these data indicate that the swimming performance of fish living in areas impacted by prolonged hypoxia will be diminished significantly, and that they may be more vulnerable to predation. This would be especially true if they encountered mammalian (e.g. seals, cetaceans) or avian (e.g. diving birds) predators whose aerobic requirements are not dependent upon water oxygen levels.

The similar values for metabolic variables and U_{crit} in the two groups was surprising given that maximum $\dot{Q}$ was lower in hypoxia-acclimated fish at all swimming speeds in both U_{crit} tests, and that maximum $\dot{Q}$ during the normoxic swim test was 23% lower in hypoxia-acclimated cod as compared with normoxia-acclimated individuals due to diminished values for V_S (Fig. 2, Table 3). This apparent discrepancy is resolved, however, when the relationship between $\dot{Q}$ and oxygen consumption is examined for the two groups (Fig. 4). During both the normoxic and hypoxic swims, hypoxia-acclimated cod consumed more oxygen for a given cardiac output. For example, at a $\dot{Q}$ of 30 ml min^{-1} kg^{-1}, hypoxia-acclimated cod consumed ~185 ml O_2 h^{-1} kg^{-1} when swimming under normoxic conditions as compared with ~130 ml O_2 h^{-1} kg^{-1} in normoxia-acclimated fish. This upward shift in the relationship between $\dot{Q}$ and oxygen consumption following long-term acclimation to moderate hypoxia was probably due to increases in both blood oxygen transport capacity and tissue O_2 extraction efficiency

because: (1) several authors have reported increases in haematocrit, blood haemoglobin levels and/or haemoglobin oxygen affinity in response to hypoxia acclimation (e.g. Bushnell et al., 1984; Driedzic et al., 1985; Timmerman and Chapman, 2004); and (2) subsequent experiments in our lab have shown that tissue O_2 extraction efficiency is significantly enhanced (by 15%) in hypoxia-acclimated cod under normoxic conditions, while blood haemoglobin levels are slightly or significantly (depending on water P_{O2}) higher in hypoxia-acclimated cod at rest (L.H.P. and A.K.G., unpublished). Furthermore, although cod heart function does not appear to be modulated by circulating catecholamines (Axelsson, 1988), these hormones activate Na^+/H^+ exchange in cod red blood cells [and presumably improve blood oxygen carrying capacity (Berenbrink and Bridges, 1994)], and hypoxia-acclimated cod have significantly higher stress-induced catecholamine levels (Petersen and Gamperl, 2010).

One of the major findings of this study was that hypoxia-acclimated cod had significantly lower values for resting and maximum V_S and $\dot{Q}$ in both swim tests, and a significantly lower scope for V_S when swum under hypoxic conditions, as compared with the normoxia-acclimated group (Fig. 2, Table 3). Given that neither cod heart size or relative ventricular mass (RVM) are affected by exposure to chronic hypoxia (Petersen and Gamperl, 2010), the most obvious explanation for this diminished cardiac function is that long-term acclimation to hypoxia had a direct negative effect on the heart's pumping capacity. Indeed, this hypothesis is directly supported by the results of a recent study that investigated the effects of chronic hypoxia on *in situ* cardiac function (present study). These authors showed that acclimation to hypoxia reduced maximum *in situ* $\dot{Q}$ and V_S by 19 and 28%, respectively, under oxygenated conditions. However, whether this reduced pumping capacity is due myocardial stunning (Bolli and Marban, 1999), myocardial necrosis (e.g. Lennard and Huddart, 1992) or remodelling of the myocardium that resulted in a smaller ventricular lumen and outflow tract (Marques et al., 2008) has not been resolved.

Hypoxia-acclimated cod were able to increase f_H during the hypoxic swim to levels measured during normoxia (Fig. 2, Table 3). This resulted in them having a significantly greater scope for f_H (12.6 *vs* 5.8 beats min^{-1}), and allowed them to achieve the same maximum $\dot{Q}$, as compared with normoxia-acclimated fish when swum at 8–9 kPa O_2. The mechanism(s) resulting in the differential regulation of f_H in the two groups when swum under hypoxic conditions cannot be ascertained from the present study or the literature. However, this result, in combination with recent data showing that rainbow trout at 24°C can maintain $\dot{Q}$ even when f_H is halved using the pharmacological agent zatebradine (Gamperl et al., 2007), highlights the tremendous plasticity in how fish cardiorespiratory physiology responds to environmental challenges and that our understanding of control mechanisms that mediate myocardial function and adaptation in this taxa is far from complete.

Conclusion

In this study we made the first measurements of fish cardiorespiratory function during exercise under hypoxia (8–9 kPa O_2), and of how acclimation to this same level of hypoxia for 6–12 weeks influenced resting and exercise-induced *in vivo* cardiac function under both hypoxic and normoxic conditions. These studies revealed that: (1) when cod were swum under hypoxic conditions cardiac function was diminished, and this was associated with reduced aerobic scope, and a ~30% lower U_{crit}; (2) acclimation to hypoxia does not improve the cod's swimming capacity, maximum metabolic rate or scope for activity when swum to U_{crit} under either normoxic or hypoxic conditions; and (3) although the resting and maximal cardiac output of hypoxia-acclimated cod were diminished due to a reduced V_S, this does not significantly affect the swimming speed–O_2 consumption relationship, maximum $\dot{M}_{O2}$ or aerobic scope. These results offer novel insights into how cod cardiorespiratory physiology is impacted by short-term and prolonged exposure to environmental hypoxia. Furthermore, they provide indirect evidence that venous O_2 receptors may be important in regulating cardiac function in fishes, and are another important example of how incredibly flexible cardiac function, and its control, is in fishes. However, the fact that these experiments were performed with hatchery-reared (as opposed to wild) fish, and the paucity of data on the effects of exposure to hypoxia on fish cardiorespiratory physiology, leaves numerous questions unanswered. These include: to what extent are reductions in maximal exercise and cardiac pumping capacity in fish following exposure to hypoxia related to a species life history and/or hypoxia tolerance? Is the diminished pumping capacity of the cod heart following acclimation to hypoxia a consequence of myocardial damage or remodelling? What mechanisms allowed the cod's maximum oxygen consumption to be maintained following acclimation to hypoxia despite a considerable reduction in cardiac pumping capacity?

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REFERENCES

Arnott, S. A., Chiba, S. and Conover, D. O. (2006). Evolution of intrinsic growth rate: metabolic costs drive trade-offs between growth and swimming performance in *Menidia menidia*. *Evolution* **60**, 1269-1278.

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.

Barrett, D. J. and Taylor, E. W. (1984). Changes in heart rate during progressive hyperoxia in the dogfish *Scyliorhinus canicula* L.: evidence for a venous oxygen receptor. *Comp. Biochem. Physiol.* **78**, 697-703.

Bell, G. W. and Eggleston, D. B. (2005). Species-specific avoidance responses by blue crabs and fish to chronic and episodic hypoxia. *Mar. Biol.* **146**, 761-770.

Berenbrink, M. and Bridges, C. R. (1994). Catecholamine-activated sodium/proton exchange in the red blood cells of the marine teleost *Gadus morhua*. *J. Exp. Biol.* **192**, 253-267.

Bolli, R. and Marban, E. (1999). Molecular and cellular mechanisms of myocardial stunning. *Physiol. Rev.* **79**, 609-634.

Breitburg, D. (2002). Effects of hypoxia, and the balance between hypoxia and enrichment, on costal fishes and fisheries. *Estuaries* **25**, 767-781.

Brett, J. R. (1964). The respiratory metabolism of swimming performance of young sockeye salmon. *J. Fish. Res. Bd. Can.* **21**, 1183-1226.

Burleson, M. L., Carlton, A. L. and Silva, P. E. (2002). Cardioventilatory effects of acclimatization to aquatic hypoxia in channel catfish. *Resp. Physiol. Neurobiol.* **131**, 223-232.

Bushnell, P. G., Steffensen, J. F. and Johansen, K. (1984). Oxygen consumption and swimming performance in hypoxia-acclimated rainbow trout, *Salmo gairdneri*. *J. Exp. Biol.* **113**, 225-235.

Butler, P. J., Taylor, E. W., Capra, M. F. and Davison, W. (1979). The effect of hypoxia on the levels of circulating catecholamines in the dogfish *Scyliorhinus canicula*. *J. Comp. Physiol.* **127**, 325-330.

Butler, P. J., Axelsson, M., Ehrenström, F., Metcalfe, J. D. and Nilsson, S. (1989). Circulating catecholamines and swimming performance in the Atlantic cod, *Gadus morhua*. *J. Exp. Biol.* **141**, 377-387.

Campbell, H. A., Taylor, E. W. and Egginton, S. (2004). The use of power spectral analysis to determine cardiorespiratory control in the short-horned sculpin *Myxocephalus scorpius*. *J. Exp. Biol.* **207**, 1969-1976.

Cech, J. J., Jr (1990). Respirometry. In *Methods of fish Biology* (ed. C. B. Schreck and P. B. Moyle). Bethesda, MD: American Fisheries Society.

Chabot, D. and Dutil, J. D. (1999). Reduced growth of Atlantic cod in non-lethal hypoxic conditions. *J. Fish. Biol.* **55**, 472-491.

Chatelier, A., McKenzie, D. J., Prinet, A., Galois, R., Robin, J., Zambonino, J. and Claireaux, G. (2006). Associations between tissue fatty acid composition and physiological traits of performance and metabolism in the seabass (*Dicentrarchus labrax*). *J. Exp. Biol.* **209**, 3429-3439.

Claireaux, G. and Lagardère, J. P. (1999). Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. *J. Sea. Res.* **42**, 157-168.

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 salinity and oxygenation conditions. *J. Exp. Biol.* **198**, 61-69.

Claireaux, G., Webber, D. M., Lagardère, 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.

Claireaux, G., McKenzie, D. J., Genge, A. G., Chatelier, A., Aubin, J. and Farrell, A. P. (2005). Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow trout. *J. Exp. Biol.* **208**, 1775-1784.

Clark, T. D. and Seymour, R. S. (2006). Cardiorespiratory physiology and swimming energetics of a high-energy-demand teleost, the yellowtail kingfish (*Seriola lalandi*). *J. Exp. Biol.* **209**, 3940-3951.

Clark, T., Ryan, T., Ingram, B., Woakes, A., Butler, P. and Frappell, P. (2005). Factorial aerobic scope is independent of temperature and primarily modulated by heart rate in exercising Murray cod (*Maccullochella peelii peelii*). *Physiol. Biochem. Zool.* **78**, 347-355.

Dahlberg, M. L., Shumway, D. L. and Doudoroff, O. (1968). Influence of dissolved oxygen and carbon dioxide on swimming performance of largemouth bass and coho salmon. *J. Fish. Res. Bd. Can.* **25**, 49-70.

D'Amours, D. (1993). The distribution of cod (*Gadus morhua*) in relation to temperature and oxygen levels in the Gulf of St. Lawrence. *Oceanography* **2**, 24-29.

Diaz, R. J. and Rosenberg, R. (1995). Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceangr. Mar. Biol.* **33**, 245-303.

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

Dutil, J. D., Sylvestre, E. L., Gamache, L., Larocque, R. and Guderley, H. (2007). Burst and coast use, swimming performance and metabolism of Atlantic cod *Gadus morhua* in sub-lethal hypoxic conditions. *J. Fish. Biol.* **71**, 363-375.

Evans, D. O. (2007). Effects of hypoxia on scope-for-activity and power capacity of lake trout (*Salvelinus namaycush*). *Can. J. Fish. Aquat. Sci.* **64**, 345-361.

Farrell, A. P. (2002). Cardiorespiratory performance in salmonids during exercise at high temperature: Insights into cardiovascular design limitations in fishes. *Comp. Biochem. Physiol.* **132A**, 797-810.

Faust, H. A., Gamperl, A. K. and Rodnick, K. J. (2004). All trout are not created equal: intra-specific varaition in cardiac hypoxia tolerance. *J. Exp. Biol.* **207**, 1005-1015.

Fitzgibbon, Q. P., Strawbridge, A. and Seymour, R. S. (2007). Metabolic scope, swimming performance and the effects of hypoxia in the mulloway, *Argyrosomus japonicus* (Pisces: Sciaenidae). *Aquaculture* **270**, 358-368.

Fritsche, R. and Nilsson, S. (1989). Cardiovascular responses to hypoxia in the Atlantic cod, *Gadus morhua*. *Exp. Biol.* **48**, 153-160.

Fry, F. E. J. (1971). The effects of environmental factors on the physiology of fish. In *Fish Physiology*, Vol. 6 (ed. W. S. Hoar and D. J. Randall). New York: Academic Press.

Gamperl, A. K., Pinder, A. W., Grant, R. R. and Boutilier, R. G. (1994). Influence of hypoxia and adrenaline administration on coronary blood flow and cardiac performance in seawater rainbow trout *(Oncorhynchus mykiss). J. Exp. Biol.* **193**, 209-232.

Gamperl, A. K., Faust, H. A., Dougher, B. and Rodnick, K. J. (2004). Hypoxia tolerance and preconditioning are not additive in the trout (*Oncorhynchus mykiss*) heart. *J. Exp. Biol.* **207**, 2497-2505.

Gamperl, A. K., Swafford, B. L. and Rodnick, K. J. (2007). The impact of elevated water temperature and zatebradine-induced bradycardia on cardiovascular function in male and female rainbow trout (*Oncorhynchus mykiss*). *Integr. Comp. Biol.* **47** (S1), e182.

Gamperl, A. K., Busby, C. D., Hori, T. S. F., Afonso, L. O. B. and Hall, J. R. (2009). Haemoglobin genotype has minimal influence on the physiological response of Atlantic cod (*Gadus morhua*) to environmental challenges. *Physiol. Biochem. Zool.* **82**, 483-494.

Gerlach, S. A. (1988). The Baltic environment. Proceedings of the 10th symposium of the Baltic marine biologists. *Sonderheft Kieler Meeresforschungen.* **6**, 1-462.

Gilbert, D., Sundby, B., Gobeil, C., Mucci, A. and Tremblay, G. H. (2005). A seventy-two year record of diminishing deep water oxygen in the St. Lawrence estuary: The northwest Atlantic connection. *Limnol. Oceanogr.* **50**, 1654-1666.

Gollock, M. J., Currie, S., Petersen, L. H. and Gamperl, A. K. (2006). Cardiovascular and haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature increase. *J. Exp. Biol.* **209**, 2961-2970.

Greaney, G. S., Allen, R. P., Cashon, R. E., Smith, G. and Powers, D. A. (1980). Time course of changes in enzyme activities and blood respiratory properties of killifish during long-term acclimation to hypoxia. *Physiol. Zool.* **53**, 136-144.

Hanson, L. M., Obradovich, S., Mouniargi, J. and Farrell, A. P. (2006). The role of adrenergic stimulation in maintaining maximum cardiac performance in rainbow trout (*Oncorhynchus mykiss*) during hypoxia, hyperkalemia and acidosis at 10°C. *J. Exp. Biol.* **209**, 2442-2445.

Heidinger, R. C. (1975). Life history and biology of largemouth bass. *In Black Bass Biology and Management* (ed. R. H. Stroud and H. Clepper), pp. 11-20. Washington DC: Sport Fishing Institute.

Hoback, W. W. and Barnhart, M. C. (1996). Lethal limits and sublethal effects of hypoxia on the amphipod *Gammarus pseudolimnaeus*. *J. Nth. Amer. Benth. Soc.* **15**, 117-126.

Hutchings, J. A. and Reynolds, J. D. (2004). Marine fish population collapses: consequences for recovery and extinction risk. *Bioscience* **54**, 297-309.

Jain, K. E. and Farrell, A. P. (2003). Influence of seasonal temperature on the repeat swimming performance of rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* **206**, 3569-3579.

Jain, K. E., Birtwell, I. K. and Farrell, A. P. (1998). Repeat swimming performance of mature sockeye salmon following a brief recovery period: a sensitive measure of fish health and water quality. *Can. J. Zool.* **76**, 1488-1496.

Kiceniuk, J. W. and Colbourne, E. (1997). Relating oxygen levels in the Newfoundland offshore waters to the physiology of Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aqua. Sci.* **54**, 81-87.

Killen, S. S., Costa, I., Brown, J. A. and Gamperl, A. K. (2007). Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. *Proc. Royal. Soc. B Biol. Sci.* **274**, 431-438.

Kutty, M. N. (1968). Influence of ambient oxygen on the swimming performance of goldfish and rainbow trout. *Can. J. Zool.* **46**, 647-653.

Lamarche, M. C., Chabot, D. and Guderley, H. (2009). Effects of acute and chronic hypoxia on metabolic capacity of Atlantic cod (*Gadus morhua*) from the Gulf of St Lawrence. *Comp. Biochem. Physiol.* **153A**, S142-S142.

Lapointe, D., Guderley, H. and Dutil, J. D. (2006). Changes in the condition factor have an impact on metabolic rate and swimming performance relationships in Atlantic cod (*Gadus morhua* L.). *Physiol. Biochem. Zool.* **79**, 109-119.

Lee, C. G., Farrell, A. P., Lotto, A., Hinch, S. G. and Healy, M. C. (2003). Excess post-exercise oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon following critical speed swimming. *J. Exp. Biol.* **206**, 3253-3260.

Lennard, R. and Huddart, H. (1992). The effects of hypoxic stress on the fine structure of the flounder heart (*Platichthys flesus*). *Comp. Biochem. Physiol.* **101A**, 723-732.

Lomholt, J. P. and Johansen, K. (1979). Hypoxia acclimation in carp-how it affects O_2 uptake, ventilation and O_2 extraction from water. *Physiol. Zool.* **5**, 38-49.

MacFarlane, W. J. and MacDonald, D. G. (2002). Relating intramuscular fuel use to endurance in juvenile rainbow trout. *Physiol. Biochem. Zool.* **75**, 250-259.

Marques, I. J., Leito, J. T. D., Spaink, H. P., Testerink, J., Jaspers, R. T., Witte, F., van den Berg, S. and Bagowski, C. P. (2008). Transcriptome analysis of the response to chronic constant hypoxia in zebrafish hearts. *J. Comp. Physiol.* B **178**, 77-92.

Monpetit, C. J. and Perry, S. F. (1998). The effects of chronic hypoxia on the acute adrenergic stress response in the rainbow trout (*Oncorhynchus mykiss*). *Physiol. Zool.* **71**, 377-386.

Myers, R. A., Hutchings, J. A. and Barrowman, N. J. (1996). Hypotheses for the decline of cod in the North Atlantic. *Mar. Ecol. Prog. Ser.* **138**, 293-308.

Nelson, J. A. (1989). Critical swimming speeds of yellow perch *Perca flavescens*: comparison of populations from a naturally acidic lake and a circumneutral lake in acid and neutral water. *J. Exp. Biol.* **145**, 239-254.

Neuenfeldt, S. (2002). The influence of oxygen saturation on the distributional overlap of predator (cod, *Gadus morhua*) and prey (herring, *Clupea harengus*) in the Bornholm Basin of the Baltic Sea. *Fish. Ocean.* **11**, 11-17.

Paajanen, V. and Vornanen, M. (2003). Effect of hypoxia in inward rectifier K^+ current (I_{Ki}) in ventricular myocytes of crucian carp (*Carassius carassius*) heart. *J. Membr. Biol.* **194**, 119-127.

Peake, S. J. and Farrell, A. P. (2006). Fatigue is a behavioural response in respirometer confined smallmouth bass. *J. Fish. Biol.* **68**, 1742-1755.

Petersen, L. H. and Gamperl, A. K. (2010). *In situ* cardiac function in Atlantic cod (*Gadus morhua*): effects of acute and chronic hypoxia. *J. Exp. Biol.* **213**, 820-830.

Pichavant, K., Person-Le Ruyet, J., Le Bayon, N., Sèvère, A., Le Roux, A., Quèmèner, L., Maxime, V., Nonotte, G. and Bœuf, G. (2000). Effect of hypoxia on growth and metabolism of juvenile turbot. *Aquaculture* **188**, 103-114.

Pichavant, K., Person-Le-Ruyet, J., Le Bayon, N., Sèvère, A., Le Roux, A. and Bœuf, G. (2001). Comparative effects of long-term hypoxia on growth, feeding, and oxygen consumption in juvenile turbot and European sea bass. *J. Fish. Biol.* **59**, 875-883.

Pichavant, K., Maxime, V., Soulier, P., Boeuf, G. and Nonnotte, G. (2003). A comparative study of blood oxygen transport in turbot and sea bass: effect of chronic hypoxia. *J. Fish. Biol.* **62**, 928-937.

Pihl, L., Baden, S. P. and Diaz, R. J. (1991). Effects of periodic hypoxia on distribution of demersal fish and crustaceans. *Mar. Biol.* **108**, 349-360.

Pihl, L., Baden, S. P., Diaz, R. J. and Schaffer, L. C. (1992). Hypoxia-induced structural changes in the diet of bottom-feeding fish and crustacea. *Mar. Biol.* **112**, 349-361.

Plante, S., Chabot, D. and Dutil, J. D. (1998). Hypoxia tolerance in Atlantic cod. *J. Fish. Biol.* **53**, 1342-1356.

Post, J. R. and Lee, J. A. (1996). Metabolic ontogeny of teleost fishes. *Can. J. Fish. Aquat. Sci.* **53**, 910-923.

Rosenberg, R., Hellman, B. and Johansson, B. (1991). Hypoxic tolerance of marine benthic fauna. *Mar. Eco. Prog. Ser.* **79**, 127-131.

Sandblom, E. and Axelsson, M. (2005). Effects of hypoxia on the venous circulation in rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol.* **140A**, 233-239.

Sandblom, E. and Axelsson, M. (2006). Adrenergic control of venous capacitance during moderate hypoxia in the rainbow trout (*Oncorhynchus mykiss*): role of neural and circulating catecholamines. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **291**, R711-R718.

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

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

Steffensen, J. F., Schurmann, H. and Bushnell, P. G. (1994). Oxygen-consumption in four species of teleosts from Greenland – no evidence of metabolic cold adaptation. *Polar Biol.* **14**, 49-54.

Steinhausen, M. F., Sandblom, E., Eliason, E. J., Verhille, C. and Farrell, A. P. (2008). The effect of acute temperature increases on the cardiorespiratory

performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). *J. Exp. Biol.* **211**, 3915-3926.
Svedäng, H. and Bardon, G. (2003). Spatial and temporal aspects of the decline in cod (*Gadus morhua* L.) abundance in the Kattegat and eastern Skagerrak. *ICES J. Mar. Sci.* **60**, 32-37.
Taylor, E. B. and McPhail, J. D. (1985). Variation in burst and prolonged swimming performance among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. *Can. J. Fish. Aquat. Sci.* **42**, 2029-2033.
Taylor, J. C. and Miller, J. M. (2001). Physiological performance of juvenile southern flounder, *Paralichthys lethostigma* (Joran and Gilbert, 1884), in chronic and episodic hypoxia. *J. Exp. Mar. Biol. Ecol.* **258**, 195-214.
Timmerman, C. M. and Chapman, L. J. (2004). Hypoxia and interdemic variation in *Poecilia latipinna*. *J. Fish. Biol.* **65**, 635-650.
UN Global Environment Outlook Year Book (2003). United Nations Environment Programme. ISBN: 9280724150.
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.
Weeks, S. J., Bronwen, C. and Bakun, A. (2002). Satellite imaging: Massive emissions of toxic gas in the Atlantic. *Nature* **415**, 493-494.
Wood, C. M. and Shelton, G. (1980). The reflex control of the heart rate and cardiac output in the rainbow trout: interactive influences of hypoxia, haemorrhage and systemic vasomotor tone. *J. Exp. Biol.* **87**, 271-284.
Wu, R. S. S. (1999). Eutrophication, trace organics and water-borne pathogens and compounds: Environmental risks and challenges. *Mar. Poll. Bull.* **39**, 11-22.
Wu, R. S. S. (2002). Hypoxia: From molecular responses to ecosystem responses. *Mar. Poll. Bull.* **45**, 35-45.
Wu, R. S. S., Lam, P. K. S. and Wan, K. L. (2002). Tolerance to, and avoidance of, hypoxia by the penaeid shrimp (*Metapenaeus ensis*). *Environ. Poll.* **118**, 351-355.
Wu, R. S. S., Zhou, B. S., Randall, D. J., Woo, N. Y. S. and Lam, P. K. S. (2003). Aquatic hypoxia is an endocrine disruptor and impairs fish reproduction. *Environ. Sci. Technol.* **37**, 1137-1141.
Zhou, B. S., Wu, R. S. S., Randall, D. J. and Lam, P. K. S. (2001). Bioenergetics and RNA/DNA ratios in the common carp (*Cyprinus carpio*) under hypoxia. *J. Comp. Physiol. B* **171**, 49-57.