The Journal of Experimental Biology 206, 487-496

doi:10.1242/jeb.00100

On-line venous oxygen tensions in rainbow trout during graded exercise at two acclimation temperatures

A. P. Farrell* and S. M. Clutterham

Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia, V5A 1S6, Canada

*Author for correspondence (e-mail: farrell@sfu.ca)

Accepted 23 October 2002

Summary

For most teleost fish, the majority of the myocardial oxygen supply is provided by the oxygen reserve remaining in venous blood after other tissues have extracted oxygen. We examined the effect of graded exercise and water temperature on this venous blood oxygen supply to the heart (termed the cardiac circulation) by performing novel on-line measurements of venous partial pressure of oxygen ($P_{\mathrm{v}O_2}$) using a fibreoptic micro-optode implanted in the ductus Cuvier of rainbow trout (*Oncorhynchus mykiss*). As expected, $P_{\mathrm{v}O_2}$ decreased progressively and significantly as swimming velocity approached the critical swimming speed (U_{crit}). Unsteady swimming behaviours during the graded exercise, however, caused abrupt and generally short-lived decreases in $P_{\mathrm{v}O_2}$. For the cold-acclimated (6–10°C) fish, $P_{\mathrm{v}O_2}$ reached a minimum plateau value of 15.3±3.7 torr (1 torr=133.3 Pa) before U_{crit} was reached, and so increased swimming effort near to U_{crit} did not reduce $P_{\mathrm{v}O_2}$ further. Warm-acclimated fish had a significantly higher $P_{\mathrm{v}O_2}$ (28.9±3.5 torr) at U_{crit}. Despite this difference in the $P_{\mathrm{v}O_2}$ at U_{crit}, we estimated that there was little difference between warm- and cold-acclimated fish in terms of oxygen supply in the cardiac circulation because of a right-shift in the haemoglobin–oxygen dissociation curve at warm temperatures. Furthermore, although $P_{\mathrm{v}O_2}$ decreased significantly at U_{crit}, our estimates suggest that the expected increase in cardiac output would easily maintain the oxygen supply in venous blood at a level similar to that found in resting fish. Although unsteady swimming behaviours decreased $P_{\mathrm{v}O_2}$, unsteady swimming rarely decreased the minimum $P_{\mathrm{v}O_2}$ value observed at U_{crit} by more than 10%. The findings are discussed in terms of a threshold $P_{\mathrm{v}O_2}$ required to maintain adequate rates of oxygen diffusion from the cardiac circulation to the myocardial tissues.

Key words: venous oxygen tension, exercise, temperature, cold acclimation, warm acclimation, $P_{\mathrm{v}O_2}$, heart, swimming speed, rainbow trout, *Oncorhynchus mykiss*, teleost.

Introduction

The vertebrate heart needs oxygen to survive. Avian and mammalian hearts receive oxygen almost exclusively *via* the coronary circulation, and the sixfold increase in myocardial oxygen consumption as cardiac work increases during exercise is supported by a proportionate increase in coronary blood flow (Feigl, 1983). In contrast to birds and mammals, fish such as trout and salmon rely on a dual oxygen supply to the myocardium. A coronary circulation carries oxygenated blood directly from the gills to the outer compact ventricular myocardium, while a cardiac circulation (i.e. luminal circulation) carries deoxygenated venous blood to the inner spongy myocardium (Davie and Farrell, 1991; Santer, 1985; Tota, 1983). Because the hearts of trout and salmon have a greater mass of spongy myocardium than compact myocardium (Farrell et al., 1988), the oxygen delivery *via* the cardiac circulation is presumed to be the larger of the two supplies. Furthermore, due to a complete lack of a coronary circulation in many teleost fish, the cardiac circulation is the heart's only source of oxygen. Thus, for most teleost fish, the majority, if not all, of the myocardial oxygen needs are precariously provided by a venous oxygen reserve, i.e. blood that has first served the needs of other tissues in the body (Farrell, 1993).

The venous blood oxygen supply to the fish myocardium apparently becomes precarious during exercise. For example, in rainbow trout (*Oncorhynchus mykiss*), exercise will cause cardiac output to increase by threefold and ventral aortic pressure to increase by 58% (Kiceniuk and Jones, 1977), which in combination will increase the oxygen needs of the heart by over fourfold (Farrell and Jones, 1992). While this increased myocardial oxygen demand is met, in part, by up to a 2.5-fold increase in coronary blood flow to the compact myocardium during swimming (Axelsson and Farrell, 1993; Gamperl et al., 1995), the oxygen content in the venous blood decreases

considerably because locomotory muscles extract a greater proportion of oxygen from the blood to support their increased workload.

This unfavourable change in myocardial oxygen demand compared with oxygen supply *via* the cardiac circulation has led to the idea of a threshold value for the venous partial pressure of oxygen ($P\mathrm{v}_{\mathrm{O}_2}$) that would then guarantee an adequate oxygen supply to the working myocardium (Davie and Farrell, 1991; Farrell, 1993; Steffensen and Farrell, 1998). Davie and Farrell (1991) reviewed available data for $P\mathrm{v}_{\mathrm{O}_2}$ in swimming fish and suggested that this threshold might be between 6 torr and 16 torr (1 torr=133.3 Pa), i.e. the $P\mathrm{v}_{\mathrm{O}_2}$ values when fish quit swimming. Steffensen and Farrell (1998) subsequently swam fish under progressively hypoxic conditions and found that fish quit swimming with a $P\mathrm{v}_{\mathrm{O}_2}$ of 7–8 torr. In addition, when the coronary circulation was ligated to eliminate coronary blood flow to the compact myocardium, the increase in ventral aortic blood pressure normally observed during swimming did not occur when $P\mathrm{v}_{\mathrm{O}_2}$ had decreased to 13–14 torr. They concluded that this $P\mathrm{v}_{\mathrm{O}_2}$ might be the venous oxygen threshold for adequately supplying the inner spongy myocardium. Furthermore, Jones (1986) suggested that a $P\mathrm{v}_{\mathrm{O}_2}$ value of 10 torr is likely to be the absolute limit at which cardiac cells can extract sufficient oxygen for their needs. However, beyond the theoretical considerations, all of the data to support the idea of a $P\mathrm{v}_{\mathrm{O}_2}$ threshold during swimming have involved taking a single venous blood sample while fish are in the final stages of a critical swimming speed test. We reasoned that on-line measurement of $P\mathrm{v}_{\mathrm{O}_2}$ might provide a much better resolution of whether or not a $P\mathrm{v}_{\mathrm{O}_2}$ threshold exists in exercising fish.

A further issue surrounding a $P\mathrm{v}_{\mathrm{O}_2}$ threshold concerns the effect of temperature on the venous oxygen reserve. Heath and Hughes (1973) reported that an acute increase in temperature produced a decline in the venous blood oxygen concentration in rainbow trout at a temperature of 24–25°C, and, at these temperatures, cardiac arrhythmias also developed. One interpretation of these data is that when temperature is elevated to near the upper lethal limit, there is a depletion of the venous oxygen reserve in the cardiac circulation, which results in a catastrophic cardiac hypoxic collapse (Farrell, 2002). The right-shift in the oxygen haemoglobin curve, which is known to occur in fish blood with temperature acclimation (Perry and Reid, 1994), of course would serve to increase the partial pressure gradient of venous blood reaching the heart, and this could be advantageous for the myocardial oxygen supply. Consequently, if a venous oxygen threshold does exist in fish, then the prediction is that the $P\mathrm{v}_{\mathrm{O}_2}$ threshold would increase with acclimation temperature. Thus, the objective of the present study was to measure $P\mathrm{v}_{\mathrm{O}_2}$ on line in rainbow trout acclimated to two acclimation temperatures to provide support for these ideas.

Materials and methods

Fish husbandry

Rainbow trout *Oncorhynchus mykiss* Walbaum (840±112 g, 41.2±1.6 cm) were transported from Sun Valley Trout Farm (Mission, BC, Canada) to Simon Fraser University, where they were kept outside in 1000 l fibreglass aquaria at seasonal water temperatures. The experiments were performed at two acclimation temperatures: cold-acclimated fish in spring 2001 (6–10°C, N=6), and warm-acclimated fish in summer 2001 (13–15°C, N=5). All fish were acclimated to local conditions for at least two weeks prior to experimentation and were fed daily.

Experimental protocol

The purpose of the experiments was to monitor $P\mathrm{v}_{\mathrm{O}_2}$ in rainbow trout during a graded exercise challenge. $P\mathrm{v}_{\mathrm{O}_2}$ was determined using a fibreoptic micro-optode connected to a Microx 1 oxygen meter from Presens GmbH (www.presens.de) chronically implanted into the ductus Cuvier. To implant the optode, fish were first anaesthetized (0.1 mg l^{-1} MS222 buffered with 0.1 mg l^{-1} $\mathrm{NaHCO_3}$) and placed on an operating table, where the gills were continuously supplied with aerated water containing diluted anaesthetic (0.05 mg l^{-1} of MS222 and $\mathrm{NaHCO_3}$). The right operculum and gills were carefully retracted to expose the cleithrum, where a dorso-ventral incision was made to expose the ductus Cuvier. A small incision was made in the vein to advance the optode approximately 1 cm retrograde into the ductus Cuvier. The incision in the vessel was closed around the optode with a suture (3-0 silk) and minimal blood loss. A second suture was used to further secure the optode in place. The optode lead was then secured with sutures placed in the cleithrum, under the right pectoral fin and anterior to the dorsal fin. The incision in the cleithrum was then sutured and penicillin was spread on the wound. The gills were irrigated with fresh water until muscle tone was observed before moving the fish into the swim tunnel. Each micro-optode was calibrated in oxygen-free water and air-saturated water, as per manufacturer instructions, and the optode tip was soaked in a 100 IU ml^{-1} heparin solution for 5–10 min prior to surgery. The Microx 1 oxygen meter was set to continuously measure % air saturation of oxygen in the blood, recording a value every 1 s *via* a serial connection to a computer.

Fish were allowed to recover for 2 h in a Brett-type respirometer swim tunnel (as described in Jain et al., 1997) with a nominal water velocity of 9.6 cm s^{-1}, which helped orientate the fish while it rested on the bottom of the swim chamber. The water velocity (cm s^{-1}) in the swim chamber was calibrated to the frequency readings for the pump before both sets of experiments using a current meter (Valeport Marine Scientific Ltd., Dartmouth, UK). A shocking grid, affixed to the rear of the swimming chamber, provided an electric pulse (3–6 V) to discourage the fish from resting on the rear grid when the water velocity was increased. Each fish was subjected to two ramp-critical speed (U_{crit}) tests (Jain et al., 1997). Our initial concern was that the optode would not be robust enough to survive overnight recovery, and therefore the first swim test was performed 2 h after surgery. However, this concern proved to be unfounded and a second swim test was possible after a 24-h recovery period post-surgery. Both swim tests used the

same protocol. The ramp phase of the swim test consisted of seven increments of 5 cm s^{-1} every 5 min to bring to the fish to approximately 50% of the anticipated U_{crit} value. Subsequently, each velocity increment of 5 cm s^{-1} lasted 15 min or until the fish fatigued, as indicated by either the tail or the entire fish impinging on the rear shocking grid for 10 s. During each test, unsteady swimming was usually noted, categorized as either 'burst-and-coast swimming' or 'fighting', and related to the P_{O_2}. Burst-and-coast swimming consisted of an increase in swimming speed with directed forward motion, usually from the back to either the middle or the front of the working section of the tunnel. A fight was classified as more erratic and longer movement, often lacking direct forward motion. P_{O_2} was recorded continuously every 1 s during the 2-h recovery period post-surgery, immediately prior to and during both swim tests, and during a 2-h recovery period following each of the swim tests. After the recovery period of the second swim test, the fish was re-fatigued using a high water velocity, removed from the swim tunnel and re-anaesthetized (0.1 mg l^{-1} MS222 and $NaHCO_3$). The optode was removed and the wound sutured, after which the fish was revived with fresh water and returned to the outdoor tank.

Data analysis

Values of P_{O_2} were recorded as % air saturation. Using equations and a spreadsheet provided by Presens GmbH, these values were adjusted for minor temperature differences and for ambient atmospheric pressure. These values were then converted to P_{O_2} (torr; 1 torr=133.3 Pa) using the following equation:

$$P_{O_2} = \frac{[P_{atm} - (7.16\times10^{(T_m/17.09)})]\ (\%\ \text{air saturation}/100)\ (0.2095)}{1.33322}, \quad (1)$$

where P_{atm} (mbar) is the atmospheric pressure and T_m (°C) is water temperature.

Mean P_{O_2} for each step was determined by averaging the P_{O_2} values from steady-state swimming during the final 25% of each velocity increment. This task was made easier because it turned out that unsteady swimming behaviours were typically associated with unsteady P_{O_2} values (see below). P_{O_2} values during unsteady swimming were omitted from the calculation of mean values and were dealt with separately by reporting the minimum P_{O_2} due to a burst-and-coast swimming or fighting behaviour. U_{crit} (cm s^{-1}) was calculated using the equation:

$$U_{crit} = \text{speed of last completed step} + (\text{speed of exhaustion step}) \times (\text{portion of step completed/total time of step}). \quad (2)$$

Water velocities were adjusted for the solid blocking effect (Bell and Terhune, 1970) using the length (l), width (w) and area (A) of the fish. The fractional uncorrected swimming speed (F_s)=0.5(l/w), and the proportional error due to solid blocking (E_s)=(0.8F_s)(A/324.3)$^{1.5}$. All water velocities values were multiplied by (1+E_s), standardized to body length s^{-1} for comparisons and presentation, and presented as means ± S.E.M. for each swim. Statistical comparison of U_{crit} values was performed using a one-way analysis of variance (ANOVA). For comparisons between groups, mean incremental velocities were determined and presented as a percentage of the U_{crit}. P_{O_2} values for similar %U_{crit} values were pooled and averaged. All water velocities, %U_{crit} and P_{O_2} values are presented as means ± S.E.M. Statistical comparisons between the control and U_{crit} values for the first and second swim tests were performed using a paired t-test (Sigma Stat 2.0). The comparisons within a graded swim test were performed using a repeated-measures ANOVA. For this comparison, measurements between 11% and 39% U_{crit} and 10% and 29% U_{crit} for cold- and warm-acclimated fish, respectively, were excluded because many of the fish did not show steady-state swimming. Also, in each acclimation group, one fish was removed from the comparison because of missing steady-state data, making repeated measures impossible. A Student's t-test was used for comparisons between acclimation temperatures. Statistical significance was assigned when P<0.05. For the three recovery periods, a recovery curve for P_{O_2} was generated by pooling values for each fish at common times. For the warm-acclimated fish, the recovery curves represent values (means ± S.E.M.) taken at 1 s intervals for the entire 2-h recovery period. Similar data are presented for cold-acclimated fish, with the exception that the data were recorded only every minute for the final 30 min of the recovery from the initial swim.

Results

Recovery

On-line P_{O_2} recordings during the recovery from surgery and the two U_{crit} swim tests are presented in Fig. 1. It took cold-acclimated fish approximately 1 h post-surgery to reach a steady-state P_{O_2}, and this was maintained until the first swim test was performed (Fig. 1). The recovery of P_{O_2} after the first swim test (Fig. 1A) was more protracted than that after the second swim test (Fig. 1B,C), and for the latter, recovery was completed in approximately 30 min. Nevertheless, the overnight recovery from the first swim (data not shown) ensured that the control P_{O_2} values were the same at the outset of the two swim tests. Warm-acclimated fish showed a slightly different pattern of recovery for P_{O_2}. Recovery from surgery was more protracted than that for either of the swim tests (Fig. 1D–F). As a result, the control P_{O_2} was significantly lower for the first swim test than for the second swim test. In addition, recovery from the second swim test resulted in a P_{O_2} that was significantly higher than the control P_{O_2} (Fig. 1D,F).

U_{crit} for warm-acclimated fish (1.98±0.19 body lengths s^{-1} and 2.05±0.17 body lengths s^{-1} for first and second swim tests, respectively) was significantly higher than that for cold-acclimated fish (1.32±0.07 body lengths s^{-1} and 1.47±0.08 body lengths s^{-1} for first and second swim tests,

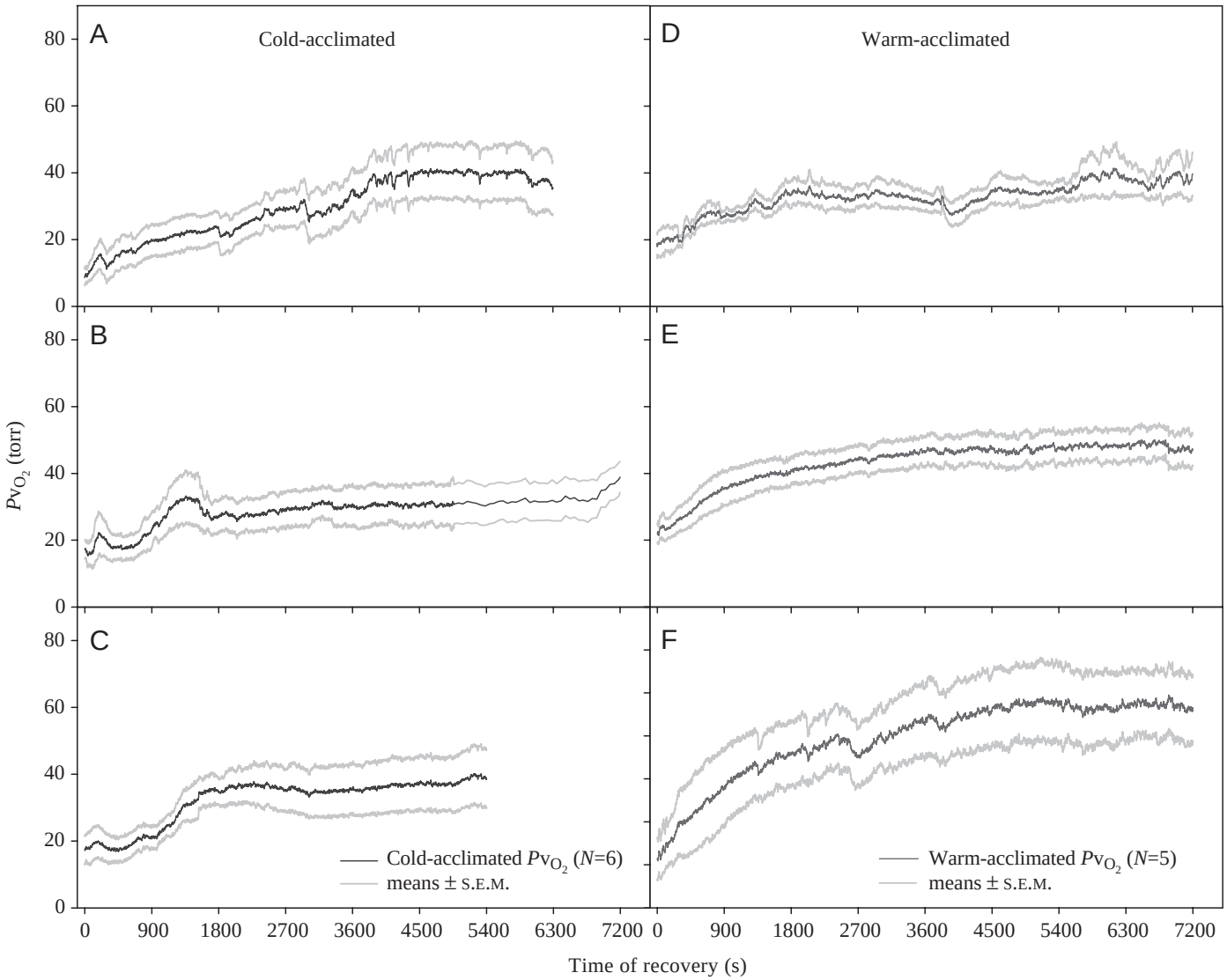

Fig. 1. On-line venous blood oxygen partial pressure ($P\mathrm{v}_{\mathrm{O}_2}$) measurements in the ductus Cuvier of cold-acclimated and warm-acclimated rainbow trout (A,D) during the post-operative recovery period, (B,E) following the first swim test and (C,F) following the second swim test. Mean values (black line; grey lines are the S.E.M.) were derived by pooling data for all fish as a function of time. 1 torr=133.3 Pa.

respectively). Therefore, to facilitate comparisons of steady-state $P\mathrm{v}_{\mathrm{O}_2}$ values, swimming velocities were expressed as a percentage of the respective U_{crit} value (Fig. 2). It was clear from the mean values, as well as individual recordings and visual observations, that swimming performance and $P\mathrm{v}_{\mathrm{O}_2}$ during the first swim test (inset Fig. 2) were more erratic compared with the second swim test because of a greater proportion of burst-and-coast-type swimming behaviours. Consequently, the first test was regarded as a habituation swim and the comparisons made below relate only to the results for the second swim test.

Steady-state swimming

For both cold- and warm-acclimated fish, $P\mathrm{v}_{\mathrm{O}_2}$ decreased significantly with increasing swimming speed (Fig. 2). The decrease in $P\mathrm{v}_{\mathrm{O}_2}$ was statistically significant at 69% U_{crit} for the cold-acclimated fish but not until 100% U_{crit} for the warm-acclimated fish (Fig. 2). In addition, $P\mathrm{v}_{\mathrm{O}_2}$ tended to be higher for warm-acclimated fish throughout the swim test, although statistical significance was reached only at velocities of ≥49% U_{crit}. This situation came about because $P\mathrm{v}_{\mathrm{O}_2}$ tended to decrease at a lower $\%U_{\mathrm{crit}}$ for cold-acclimated fish than for warm-acclimated fish. Importantly, warm-acclimated fish quit swimming at a significantly higher $P\mathrm{v}_{\mathrm{O}_2}$ than did cold-acclimated fish (Fig. 2) despite the fact that they were swimming at a higher velocity.

The examples of on-line $P\mathrm{v}_{\mathrm{O}_2}$ recordings for individual fish (Fig. 3) illustrate several important points. Foremost, as fish approached U_{crit}, there was a minimum value for $P\mathrm{v}_{\mathrm{O}_2}$. In fact, $P\mathrm{v}_{\mathrm{O}_2}$ reached a plateau for both swims with cold-acclimated fish (Fig. 3A,B; see also Fig. 2) and for the first swim with warm-acclimated fish (Fig. 3C; see also inset in Fig. 2).

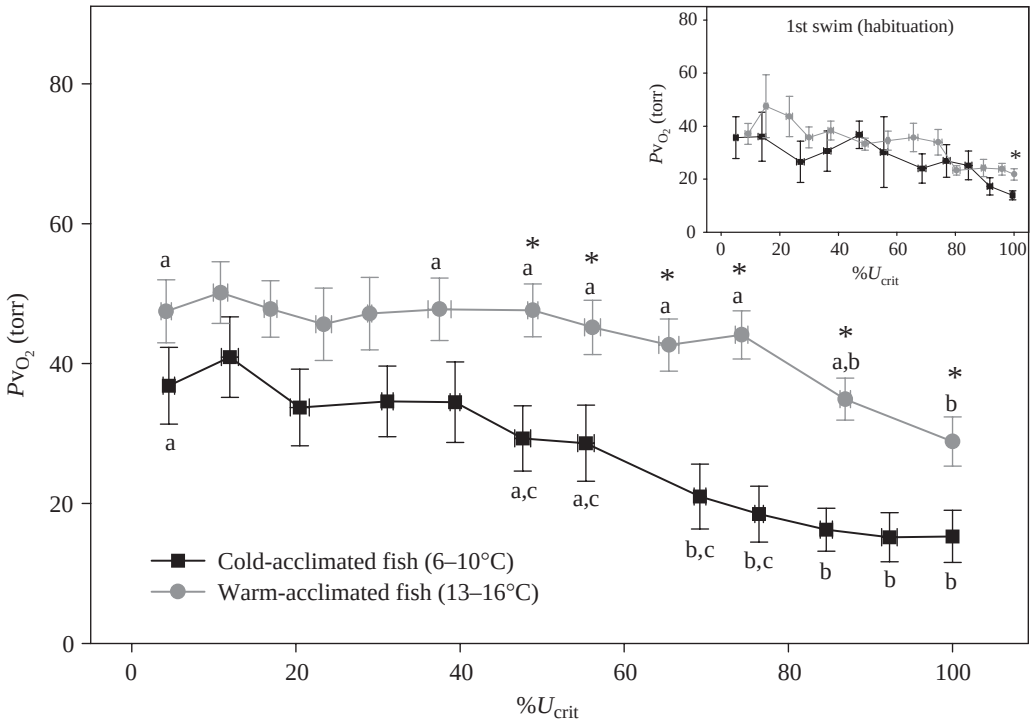

Fig. 2. Mean venous blood oxygen partial pressure ($P\bar{v}_{O_2}$) measurements in the ductus Cuvier for cold-acclimated and warm-acclimated rainbow trout during graded exercise. Values were derived from periods of steady-state swimming at each velocity increment. To permit comparisons between the cold- and warm-acclimated fish, swimming velocity is expressed as a percentage of the critical swimming speed (%U_{crit}). Horizontal and vertical bars indicate the S.E.M. Dissimilar letters represent statistical difference within a swim. Values without letters were not included in the repeated-measures analysis of variance (ANOVA). Asterisks represent significant difference between the acclimation temperatures at similar %U_{crit}. The inset presents the data for the first (habituation) swim. 1 torr=133.3 Pa.

Second, the more erratic nature of the first swim compared with the second swim can be seen clearly by comparing Fig. 3A with Fig. 3B. Finally, the individual $P\bar{v}_{O_2}$ recordings reflected the cessation of swimming behaviour as fish approached fatigue, i.e. the fish resting on the rear grid. This resting behaviour caused a modest but progressive increase in $P\bar{v}_{O_2}$ just prior to the termination of the test (Fig. 3A–C).

Unsteady swimming

Burst-and-coast swimming and, in particular, fighting behaviours produced characteristically abrupt decreases in $P\bar{v}_{O_2}$ of varying magnitude (Fig. 3). Such behaviours often occurred whenever there was an incremental increase in the water velocity. While Fig. 3A shows the most extreme effect on $P\bar{v}_{O_2}$ that was observed for either swimming behaviour, overall the associated decrease in $P\bar{v}_{O_2}$ rarely went below the minimum $P\bar{v}_{O_2}$ observed at U_{crit}. This point is illustrated in Fig. 4, where minimum $P\bar{v}_{O_2}$ values associated with unsteady swimming are presented as a percentage of the minimum $P\bar{v}_{O_2}$ at U_{crit}. Of the 37 instances that $P\bar{v}_{O_2}$ decreased to within 60% of the minimum $P\bar{v}_{O_2}$ at U_{crit}, there were only two instances where $P\bar{v}_{O_2}$ decreased to more than 10% below the minimum $P\bar{v}_{O_2}$ at U_{crit} (Fig. 4B). Both instances were for cold-acclimated fish and neither occurred when fish were swimming at a velocity greater than 80% U_{crit}. A similar pattern was seen for the first swim (Fig. 4A), despite the more erratic swimming behaviours. Collectively, these data lead us to conclude that the minimum $P\bar{v}_{O_2}$ values that were recorded near or at U_{crit} are threshold $P\bar{v}_{O_2}$ values.

Discussion

To the best of our knowledge, these are the first on-line measurements of $P\bar{v}_{O_2}$ during graded exercise in fish. Previous measurements of $P\bar{v}_{O_2}$ in fish have used either a blood withdrawal technique from a vein (i.e. pre-heart) or the ventral aorta (i.e. post-heart) or an extracorporeal circulation. The use of an extracorporeal circulation has provided valuable continuous recordings of blood oxygen status in fish despite the inherent recording delays associated with moving blood through a cannula to the oxygen electrode and the response time of the oxygen electrode. Such studies have measured either arterial blood oxygen status during graded exercise (e.g. Thomas et al., 1987) or arterial and venous blood oxygen status during hypercapnia and hypoxia (e.g. Thomas et al., 1994; Thomas and Hughes, 1982; Thomas and Le Ruz, 1982). Venous oxygen status has not been monitored during graded exercise with an extracorporeal circulation to our knowledge but, in resting normoxic rainbow trout at 8–10°C, Thomas et al. (1994) reported that $P\bar{v}_{O_2}$ measured in an afferent gill artery

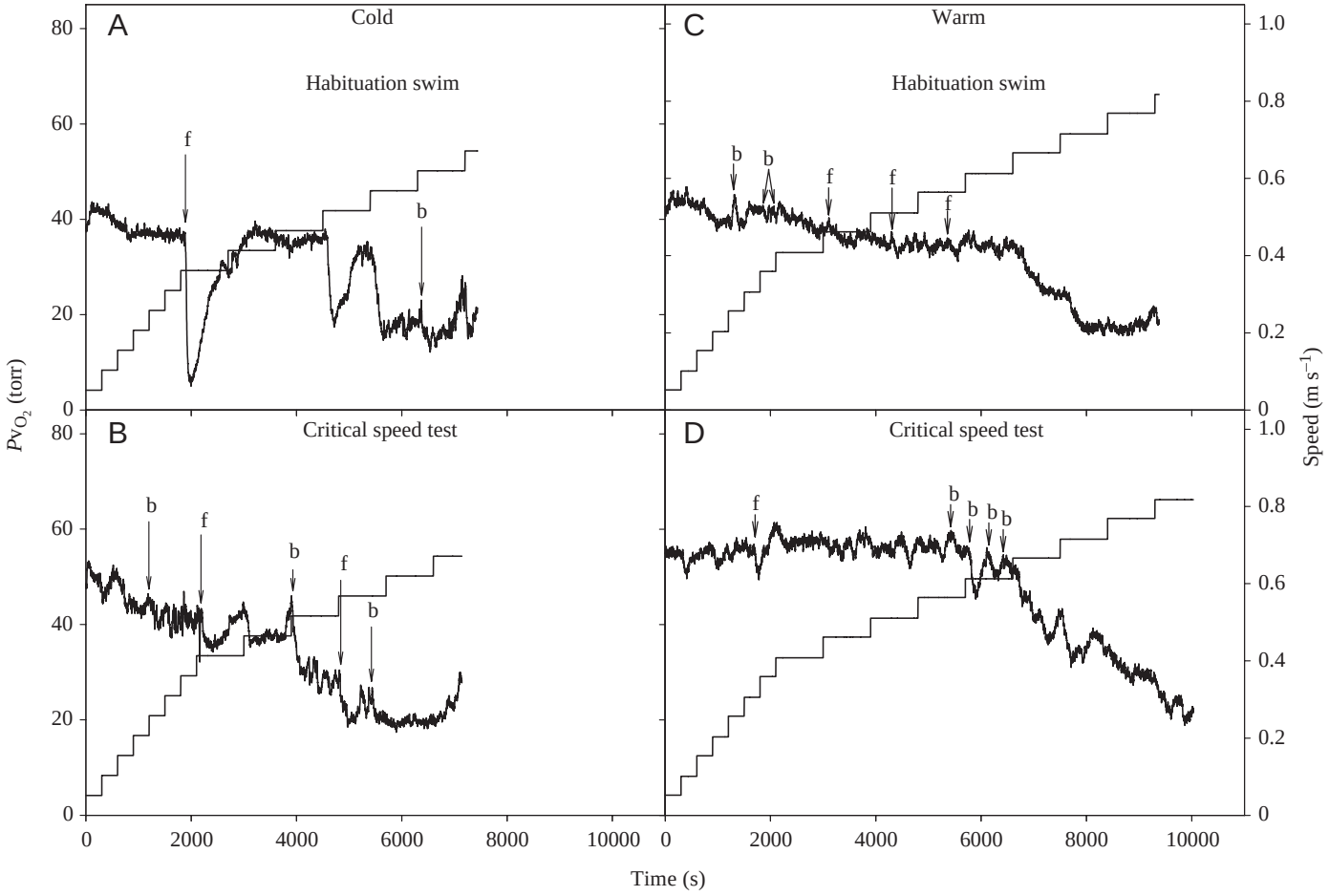

Fig. 3. Examples of on-line venous blood oxygen partial pressure ($P\mathrm{v}_{\mathrm{O}_2}$) measurements in the ductus Cuvier of (A,B) cold-acclimated and (C,D) warm-acclimated rainbow trout during the first (habituation) and second (critical speed) swim tests. The incremental increases in water velocity are also shown for reference. The symbols 'f' and 'b' refer to fighting and burst-and-coast swimming behaviours, respectively, which were visually observed. 1 torr=133.3 Pa.

(= ventral aorta) was 20–22 torr (1 torr=133.3 Pa), a value that is lower than our values.

Measurement of $P\mathrm{v}_{\mathrm{O}_2}$ in the ventral aorta is easier than in veins because fish veins typically collapse when blood is withdrawn (Capra and Satchell, 1977). Nevertheless, there is very good agreement between the control $P\mathrm{v}_{\mathrm{O}_2}$ measured in the ductus Cuvier of resting fish in the present study (36.9 torr and 47.6 torr; Fig. 2) and several ventral aortic $P\mathrm{v}_{\mathrm{O}_2}$ measurements made previously with rainbow trout (44 torr, Kiceniuk and Jones, 1977; 31 torr, Eddy et al., 1977; 36 torr, Steffensen and Farrell, 1998). The exception is a pre-exercise ventral aortic $P\mathrm{v}_{\mathrm{O}_2}$ of 19 torr reported by Stevens and Randall (1967). Based on the above comparisons with previous studies, we are confident that the optode system provided reliable on-line measurements of $P\mathrm{v}_{\mathrm{O}_2}$ in rainbow trout during graded exercise and that reliable conclusions can be drawn concerning the idea of a $P\mathrm{v}_{\mathrm{O}_2}$ threshold.

The minimum $P\mathrm{v}_{\mathrm{O}_2}$ values at U_{crit} were 15.3 torr for cold-acclimated fish and 28.9 torr for warm-acclimated fish. Similar to the present study, Kiceniuk and Jones (1977) reported that $P\mathrm{v}_{\mathrm{O}_2}$ decreased to 21 torr at U_{crit} for normoxic rainbow trout performing a U_{crit} test at 11°C. (However, it is not explicitly stated whether the measurement was made with blood sampled from the cardinal vein or the ventral aorta.) By contrast, Stevens and Randall (1967) observed no appreciable change in the pre-exercise $P\mathrm{v}_{\mathrm{O}_2}$ (19 torr) when rainbow trout were exercised for 15 min at 4–8°C. Environmental hypoxia can also lower $P\mathrm{v}_{\mathrm{O}_2}$. For example, $P\mathrm{v}_{\mathrm{O}_2}$ decreased to 5 torr with hypoxia (water oxygen tension, 30 torr; Thomas et al., 1994). Similarly, rainbow trout quit swimming (at approximately 70% U_{crit}) when progressive hypoxia had reduced $P\mathrm{v}_{\mathrm{O}_2}$ to 7 torr (Steffensen and Farrell, 1998).

While the above comparisons are important to make, a cautionary note is that, at a given level of tissue oxygen demand and arterial blood saturation, $P\mathrm{v}_{\mathrm{O}_2}$ is determined in part by cardiac output and blood haemoglobin concentration, as well as the oxygen saturation level. Therefore, until this additional information is available, comparison of $P\mathrm{v}_{\mathrm{O}_2}$ values must remain rather superficial. This is particularly the case when different protocols are used. For example, when rainbow trout were swum at a constant speed and made progressively hypoxic until they quit swimming (Steffensen and Farrell,

1998), cardiac performance was probably not as high as in the present experiments where the fish were swum to U_{crit} under normoxic conditions. With a lower cardiac work and myocardial oxygen demand, the P_{vO_2} at which the heart can no longer maintain maximum performance is expected to be lower. Such considerations help explain why Davie and Farrell (1991) suggested a rather large range for threshold P_{vO_2} of 6–16 torr when all available data were considered, while Steffensen and Farrell (1998) suggested a narrower threshold P_{vO_2} of 8.6–11.1 torr for hypoxic rainbow trout acclimated to 15°C and swimming at 70% U_{crit}. The present study suggests that for normoxic rainbow trout swum to U_{crit} at 6–10°C, the threshold P_{vO_2} was 15 torr (Fig. 2).

The P_{vO_2} plateau of 15 torr for cold-acclimated fish suggests to us that swimming at velocities in excess of approximately 85% U_{crit} involved a recruitment of only white glycolytic fibres. [The present data cannot eliminate the possibility of cardiac output being increased further, but Gallaugher et al. (2001) showed that adjustments to cardiac output were very small in Chinook salmon (*Oncorhynchus tschawytscha*) at swimming velocities greater than 80% U_{crit}.] Consistent with this suggestion are the findings of Burgetz et al. (1998), who showed that anaerobic metabolism is required to support swimming speeds greater than 70% U_{crit} in rainbow trout. At 70% U_{crit} and 80% U_{crit}, anaerobic metabolism was estimated to contribute approximately 25% of the oxygen consumption, and this value increased to 77% at 100% U_{crit}. Rome et al. (1985) used electromyogram recordings to show that the initial recruitment of white muscle in carp occurred at lower velocities in cold-acclimated fish compared with warm-acclimated fish. The present results appear to be consistent with this finding because the plateau for P_{vO_2} occurred much closer to 100% U_{crit} in warm-acclimated compared with cold-acclimated rainbow trout. However, we do not know if the initial recruitment of white muscle is aerobic and whether or not white muscle is capable of a greater range of aerobic performance at warmer temperatures. In fact, Taylor et al. (1997), who measured regional blood flow in exercising rainbow trout, reported that blood flow to white muscle was significantly higher in 18°C-acclimated fish compared with 11°C-acclimated fish.

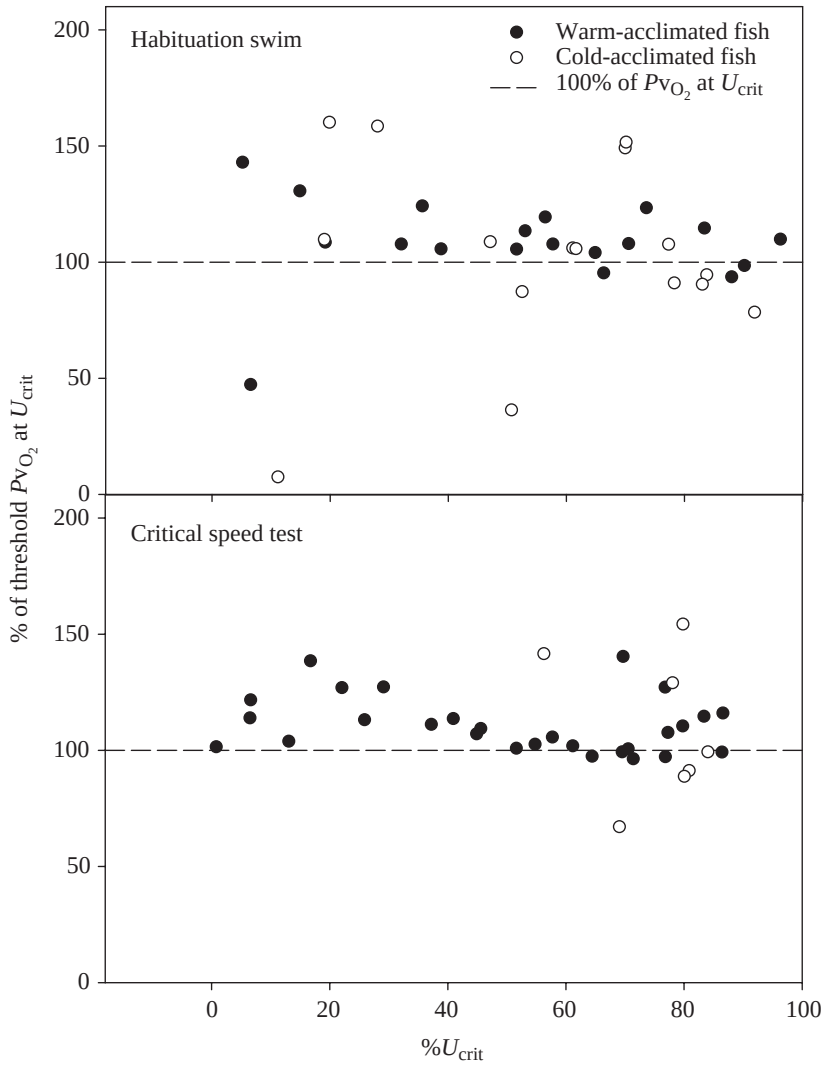

Fig. 4. A summary of the effects of unsteady (fighting and burst-and-coast) swimming behaviours on the venous blood oxygen partial pressure (P_{vO_2}). The minimum P_{vO_2} observed at 100% critical swimming speed (U_{crit}) was assigned a value, and the minimum P_{vO_2} as a result of the unsteady swimming behaviour was expressed as a percentage. For clarity, only P_{vO_2} values within 60% of the threshold are presented.

A new idea that is now explored is the possibility that the switch from red oxidative muscle fibres to glycolytic muscle fibres during high-speed swimming in rainbow trout is not only orderly but also serves to preserve a reserve of oxygen in the venous blood. The underlying mechanisms that could bring this about are a matter for speculation. However, two possibilities are worth exploring. First, a diffusion limitation for oxygen exchange at the skeletal muscle may develop as fish swim faster and this would then set an upper limit for oxygen extraction in locomotory tissues. This could occur because either the transit time through capillaries in red muscle becomes too short as red muscle perfusion increases with increasing cardiac output or (and probably in addition to) white glycolytic muscle fibres have a lower capillary density (Taylor et al., 1997), which creates longer diffusion distances. Thus, the anatomical arrangement of capillaries in fish skeletal muscle probably represents a perfusion-limited system for oxygen under routine conditions, but one that approaches or becomes diffusion-limited as fish exercise at levels near to their U_{crit}. This diffusion limitation for oxygen may be such that

sufficient oxygen remains in venous blood to supply the heart adequately. The other possibility would involve oxygen receptors on the venous side of the circulation that could, through central integration, produce an efferent neural output to constrict muscle arterioles and thereby limit muscle blood flow when $P\mathrm{v}_{\mathrm{O}_2}$ is near the threshold.

Rather than invoking some anatomical design feature or physiological regulatory mechanism to explain the venous oxygen reserve, a simple alternative is that the portion of cardiac output that perfuses non-locomotory tissues provides the venous oxygen reserve. Limited data on the regional distribution of blood flow in rainbow trout allow us to explore this alternative possibility, although the outcome seems to be that oxygen extraction by skeletal muscle is far from complete. Randall and Daxboeck (1982) reported that in resting rainbow trout approximately 52% of blood flow was directed to locomotory muscles (8.9% to red lateral muscle; 37.4% to white muscle; 5.2% to pink muscle) and 48% to the rest of the body. At 80% U_{crit}, when cardiac output had tripled, approximately 69% of the blood flow was now directed to locomotory muscles (42% to red lateral muscle; 1.1% to white muscle; 25.5% to pink muscle) and 31% to the rest of the body. If we assume that oxygen supply in the venous circulation returning from the rest of the body at U_{crit} was the same as that measured in the ductus Cuvier of resting fish, i.e. there was no net change in oxygen extraction in non-locomotory tissues, then this 31% of cardiac output would return $3.3\,\mathrm{ml\,O_2\,s^{-1}}$ to the heart (i.e. 30% of $11\,\mathrm{ml\,O_2\,s^{-1}}$; see below and Fig. 5). Because this amount is less than the $5\,\mathrm{ml\,O_2\,s^{-1}}$ that we estimate was returning to the heart during exercise (Fig. 5), blood leaving the locomotory muscles must make up the difference of $1.7\,\mathrm{ml\,O_2\,s^{-1}}$. This would mean that the venous oxygen content of blood leaving locomotory muscles during exercise would be approximately 73% lower than that in the resting fish. Nevertheless, these theoretical calculations, which suggest that only one-third of the venous oxygen reserve comes from blood leaving locomotory muscle, probably underestimate this contribution based on the following concern.

The blood flow distributions used in the above analysis were based on a microsphere injection methodology, but it has been suggested that this is an unreliable methodology for estimating splanchnic blood flow in fish (Farrell et al., 2001). When gut blood flow was simultaneously measured with an ultrasonic flow probe and with microspheres, there was very poor agreement between the two methodologies under a variety of conditions (Crocker et al., 2000). Two additional findings suggest that perhaps the entire blood flow distribution pattern as revealed by the microsphere methodology should be treated with caution, and, if anything, the estimate that non-locomotory tissues receive 31% of cardiac output in exercising rainbow trout (which is actually an increase in total blood flow from $6.2\,\mathrm{ml\,min^{-1}\,kg^{-1}}$ to $11.8\,\mathrm{ml\,min^{-1}\,kg^{-1}}$; Randall and Daxboeck, 1982) is too high. First, Neumann et al. (1983) used the microsphere method to estimate that 30% of cardiac output in rainbow trout went to red muscle and 68.2% went to white muscle 5 min after exhaustive activity. Thus, with <2% of cardiac output going to non-locomotory tissues, a 10-fold discrepancy exists between the two studies in the estimates of non-locomotory tissue blood flow. Second, Thorarensen et al. (1993) measured a 60% decrease in gut blood flow in exercising Chinook salmon at U_{crit} using Doppler flow probes. Consequently, given a decrease in gut blood flow with exercise and the fact that gut blood flow normally represents 30% of cardiac output (Thorarensen et al., 1993), it seems unlikely that non-locomotory muscle would only decrease from 48% to 31% of cardiac output. Why more blood flow was not diverted to locomotory muscles will remain a mystery until further studies on blood flow distribution and its control are performed on fish. For the present purpose, it is suffice to say that a protected venous oxygen reserve does exist when rainbow trout approach U_{crit} and, minimally, one-third of this oxygen reserve has escaped being used by the locomotory muscles, although these muscles were using glycolysis to power contractions.

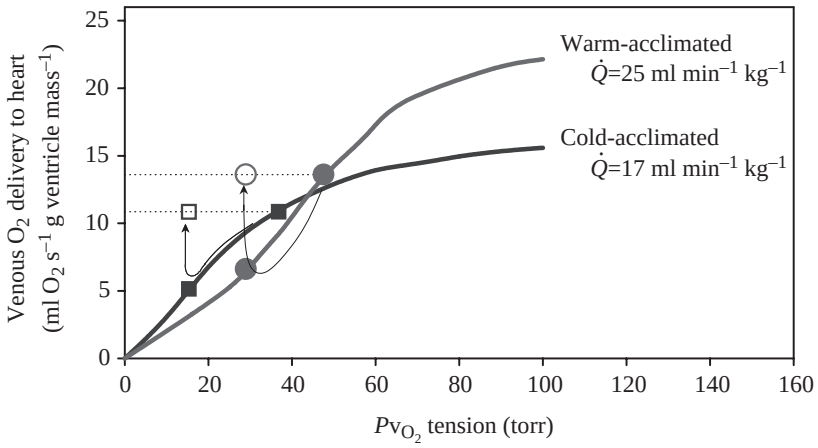

Fig. 5. A theoretical estimate of the venous oxygen delivery to the heart *via* the cardiac circulation for cold- and warm-acclimated rainbow trout (see Discussion for an explanation of the derivation of the oxygen-delivery curves). The filled squares (cold-acclimated rainbow trout) and circles (warm-acclimated trout) represent the venous oxygen delivery at the measured venous blood oxygen partial pressures for rainbow trout under resting conditions and at 100% critical swimming speed (U_{crit}) with cardiac output ($\dot{Q}$) at the routine levels indicated. Because cardiac output would have increased at U_{crit}, the curve underestimates the true venous oxygen delivery. The open symbols, which account for the effect of a 2.1-fold increase in $\dot{Q}$, indicate that the increase in $\dot{Q}$ would have restored venous oxygen delivery for both cold- and warm-acclimated fish. 1 torr=133.3 Pa.

Although we can only speculate on a mechanism to explain the venous oxygen reserve, it is important to note that the effect of unsteady swimming behaviours on $P\mathrm{v}_{\mathrm{O}_2}$ provided further support for a protected venous oxygen reserve at high swimming speeds. $P\mathrm{v}_{\mathrm{O}_2}$ would decrease dramatically with unsteady swimming behaviours that are known to recruit white muscle

fibres, but rarely did these swimming behaviours decrease $P\mathrm{v}_{\mathrm{O}_2}$ below the threshold level (Fig. 4). Furthermore, once the fish had reached the threshold $P\mathrm{v}_{\mathrm{O}_2}$, burst-and-coast swimming seemed to have little impact on $P\mathrm{v}_{\mathrm{O}_2}$. In fact, whenever the fish rested temporarily on the rear screen of the swim chamber, $P\mathrm{v}_{\mathrm{O}_2}$ tended to increase, presumably because oxygen extraction by the muscle decreased.

As predicted, the minimum $P\mathrm{v}_{\mathrm{O}_2}$ was higher for the warm-acclimated fish and this reflected a general increase in $P\mathrm{v}_{\mathrm{O}_2}$ for the warm-acclimated fish, although statistical significance was reached only at swimming velocities greater than 50% U_{crit}. In contrast to the cold-acclimated fish, however, a plateau in $P\mathrm{v}_{\mathrm{O}_2}$ was not maintained over a substantial range of the higher swimming velocities, except during the habituation swim when the final $P\mathrm{v}_{\mathrm{O}_2}$ was slightly lower than the second swim (i.e. 23.8 torr *versus* 28.9 torr) but still higher than the minimum $P\mathrm{v}_{\mathrm{O}_2}$ for cold-acclimated fish. Thus, it is possible that warm-acclimated fish only approached, and did not quite reach, a threshold $P\mathrm{v}_{\mathrm{O}_2}$.

The higher minimum $P\mathrm{v}_{\mathrm{O}_2}$ for warm-acclimated compared with cold-acclimated fish may translate to an improved cardiac oxygen supply for the warm-acclimated fish, because rates of oxygen diffusion could be faster (due to a larger partial pressure gradient). This could help support the faster rate of cardiac contraction and the higher level of cardiac work also associated with elevated temperature (Aho and Vornanen, 1999). However, this benefit might be negated if venous oxygen content was not preserved. As neither venous oxygen content nor oxygen dissociation curves were measured in the present study, we have used literature values to generate theoretical curves for venous oxygen delivery to the heart at the two acclimation temperatures (Fig. 5). An oxygen–haemoglobin dissociation curve for venous blood was taken from Thomas et al. (1994) for 10°C rainbow trout. A Bohr coefficient was used to adjust an oxygen–hemoglobin dissociation curve for arterial blood at 15°C (taken from Perry and Reid, 1994) and generate a venous curve for the warm-acclimated fish. The haemoglobin concentration was assumed to be such that fully saturated blood contained 10 vols % oxygen (Gallaugher et al., 1995), routine cardiac output was assumed to be 17 ml $\mathrm{min}^{-1}\,\mathrm{kg}^{-1}$ for cold-acclimated fish (Kiceniuk and Jones, 1977) and 25 ml $\mathrm{min}^{-1}\,\mathrm{kg}^{-1}$ for warm-acclimated fish, and a 1 kg fish was assumed to have a 1 g ventricle (Farrell et al., 1988). These assumptions allowed venous oxygen delivery to the heart (ml $\mathrm{O}_2\,\mathrm{s}^{-1}$ g ventricle mass^{-1}) to be calculated from the product of cardiac output (ml $\mathrm{O}_2\,\mathrm{s}^{-1}$ g ventricle mass^{-1}) and venous oxygen concentration (mg $\mathrm{O}_2\,\mathrm{ml}^{-1}$; calculated from % haemoglobin saturation at a given partial pressure). The results show that at the $P\mathrm{v}_{\mathrm{O}_2}$ values measured in resting fish (36.9 torr and 47.6 torr for cold- and warm-acclimated fish, respectively), the venous oxygen delivery to the heart was quite similar despite the differences in $P\mathrm{v}_{\mathrm{O}_2}$ (11.0 ml $\mathrm{O}_2\,\mathrm{s}^{-1}$ g ventricle mass^{-1} and 13.5 ml $\mathrm{O}_2\,\mathrm{s}^{-1}$ g ventricle mass^{-1}, respectively). The $P\mathrm{v}_{\mathrm{O}_2}$ at 100% U_{crit} (15.3 torr and 28.9 torr for cold- and warm-acclimated fish, respectively) obviously reduced venous oxygen content, but a 2.1-fold increase in cardiac output would have been adequate to maintain venous oxygen delivery to the heart at the level estimated for resting fish (see open symbols in Fig. 5). As cardiac output can increase by 2.5-fold to 3.0-fold at U_{crit} (Kiceniuk and Jones, 1977; Thorarensen et al., 1996), these theoretical estimates lead to the conclusion that venous blood oxygen delivery *via* the cardiac circulation is similar in warm-acclimated and cold-acclimated rainbow trout whether they are resting or swimming at 100% U_{crit}. However, for exercising fish, the oxygen partial pressure gradient driving oxygen diffusion to the myocardium decreases approximately 2-fold, while myocardial oxygen demand increases approximately 4-fold. In addition, any increase in heart rate associated with exercise will mean that the residence time of blood in the lumen of heart decreases proportionately. These changes suggest that, in exercising fish, the rate of oxygen diffusion to the myocardium is likely to be far more precarious than the rate of oxygen supply by the cardiac circulation. This then argues for the need of a $P\mathrm{v}_{\mathrm{O}_2}$ threshold to ensure an adequate rate of oxygen diffusion from the cardiac circulation to support the myocardial oxygen demand during exercise and prevent hypoxic cardiac collapse near U_{crit}.

The right-shift in oxygen–haemoglobin dissociation curve with increasing temperature in fish is generally regarded as favouring oxygen unloading at the locomotory muscles but being unfavourable for oxygen extraction from water when it contains less oxygen than does colder water. However, what has not been considered previously is that the right-shift also favours oxygen delivery to the myocardial tissues *via* the cardiac circulation. In the present experiments, there was almost a doubling of this gradient at 100% U_{crit} for warm-acclimated fish. As an increase in temperature also increases the rate of diffusion of gases, and this cannot be avoided, perhaps the right-shift in the oxygen–haemoglobin dissociation curve co-evolved as a mechanism to also protect the oxygen supply to the heart *via* the cardiac circulation. While allowing unloading of oxygen from blood to muscles to increase in association with the higher workloads that are possible at warmer temperatures, the right-shift also resulted in an elevated partial pressure gradient for oxygen to cardiac tissues without compromising the overall venous oxygen delivery rate to the heart.

This research was supported by the Natural Sciences and Engineering Research Council of Canada. Dr David Randall is thanked for the loan of the Microx 1 system, without which this study would not have been possible.

References

Aho, E. and Vornanen, M. (1999). Contractile properties of atrial and ventricular myocardium of the heart of rainbow trout *Oncorhynchus mykiss*: effects of thermal acclimation. *J. Exp. Biol.* **202**, 2663-2677.

Axelsson, M. and Farrell, A. P. (1993). Coronary blood flow *in vivo* in the coho salmon (*Oncorhynchus kisutch*). *Am. J. Physiol.* **264**, R963-R971.

Bell, W. H. and Terhune, L. D. B. (1970). Water tunnel design for fisheries research. *J. Fish. Res. Bd. Can.* **195**, 1-169.

Burgetz, I. J., Rojas-Vargas, A., Hinch, S. and Randall, D. J. (1998). Initial

recruitment of anaerobic metabolism during sub-maximal swimming in rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **201**, 2711-2721.

Capra, M. F. and Satchell, G. H. (1977). The differential hemodynamic responses of the elasmobranch, *Squalus acanthias*, to the naturally occuring catecholamines, adrenaline and noradrenaline. *Comp. Biochem. Physiol. C* **58**, 41-47.

Crocker, C. E., Farrell, A. P., Gamperl, A. K. and Cech, J. J., Jr (2000). Cardiorespiratory responses of white sturgeon to environmental hypercapnia. *Am. J. Physiol. Reg. Int. Comp. Physiol.* **279**, R617-R628.

Davie, P. S. and Farrell, A. P. (1991). The coronary and luminal circulation of the fishes. *Can. J. Zool.* **69**, 1993-2000.

Eddy, F. B., Lomholt, J. P., Weber, R. E. and Johansen, K. (1977). Blood respiratory properties of rainbow trout (*Salmo gairdneri*) kept in water of high CO_2 tension. *J. Exp. Biol.* **67**, 37-47.

Farrell, A. P. (1993). Cardiac output in fish: regulation and limitations. In *The Vertebrate Gas Transport Cascade: Adaptions to Environment and Mode of Life* (ed. E. Bicudo), pp. 208-214. Boca Raton: CRC Press, Inc.

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

Farrell, A. P., Hammons, A. M., Graham, M. S. and Tibbits, G. F. (1988). Cardiac growth in rainbow trout, *Salmo gairdneri*. *Can. J. Zool.* **66**, 2368-2373.

Farrell, A. P. and Jones, D. R. (1992). The Heart. In *Fish Physiology*, vol. XIIA (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 1-88. San Diego: Academic Press Ltd.

Farrell, A. P., Thorarensen, H., Axelsson, M., Crocker, C. E., Gamperl, A. K. and Cech, J. J., Jr (2001). Gut blood flow in fish during exercise and severe hypercapnia. *Comp. Biochem. Physiol. A* **128**, 551-563.

Feigl, E. O. (1983). Coronary physiology. *Physiol. Rev.* **63**, 1-205.

Gallaugher, P., Thorarensen, H. and Farrell, A. P. (1995). Hematocrit in oxygen transport and swimming in rainbow trout (*Oncorhynchus mykiss*). *Respir. Physiol.* **102**, 279-292.

Gallaugher, P., Thorarensen, H., Kiessling, A. and Farrell, A. P. (2001). Effects of high intensity exercise training on cardiovascular function, oxygen uptake, internal oxygen transport and osmotic balance in chinook salmon (*Oncorhynchus tshawythcha*) during critical speed swimming. *J. Exp. Biol.* **204**, 2861-2872.

Gamperl, A. K., Axelsson, M. and Farrell, A. P. (1995). Effects of swimming and environmental hypoxia on coronary blood flow in rainbow trout. *Am. J. Physiol.* **269**, R1258-R1266.

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.

Jain, K. E., Hamilton, J. C. and Farrell, A. P. (1997). Use of a ramp velocity test to measure critical swimming speed in rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol. A* **117**, 441-444.

Jones, D. P. (1986). Intracellular diffusion gradients of O_2 and ATP. *Am. J. Physiol.* **250**, C663-C675.

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.

Neumann, P., Holeton, G. F. and Heisler, N. (1983). Cardiac output and regional blood flow in gills and muscles after exhaustive exercise in rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.* **105**, 1-14.

Perry, S. F. and Reid, S. G. (1994). The effects of acclimation temperature on the dynamics of catecholamine release during acute hypoxia in the rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* **186**, 289-307.

Randall, D. J. and Daxboeck, C. (1982). Cardiovascular changes in rainbow trout (*Salmo gairdneri* Richardson) during exercise. *Can. J. Zool.* **60**, 1135-1140.

Rome, L. C., Loughna, P. T. and Goldspink, G. (1985). Temperature acclimation: improved sustained swimming performance in carp at low temperatures. *Science* **228**, 194-196.

Santer, R. M. (1985). Morphology and innervation of the fish heart. *Adv. Anat. Embryol. Cell Biol.* **89**, 1-102.

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 progressive hypoxia. *Comp. Biochem. Physiol. A* **119**, 585-592.

Stevens, E. D. and Randall, D. J. (1967). Changes of gas concentrations in blood and water during moderate swimming activity in rainbow trout. *J. Exp. Biol.* **46**, 329-337.

Taylor, E. W., Egginton, S., Taylor, S. E. and Butler, P. J. (1997). Factors which may limit swimming performance at different temperatures. In *Global Warming: Implications for Freshwater and Marine Fish* (ed. C. M. Wood and D. G. McDonald), pp. 105-133. Cambridge: Cambridge University Press.

Thomas, S., Fritsche, R. and Perry, S. F. (1994). Pre- and post-brachial blood respiratory status during acute hypercapnia or hypoxia in rainbow trout, *Oncorhynchus mykiss*. *J. Comp. Physiol. B* **164**, 451-458.

Thomas, S. and Hughes, G. M. (1982). A study of the effects of hypoxia on acid-base status of rainbow trout using an extracorporeal blood circulation. *Respir. Physiol.* **49**, 371-382.

Thomas, S. and Le Ruz, H. (1982). A continuous study of rapid changes in blood acid-base status of trout during variations in water Pco_2. *J. Comp. Physiol. B* **148**, 123-130.

Thomas, S., Poupin, J., Lykkeboe, G. and Johansen, K. (1987). Effects of graded exercise on blood gas tensions and acid-base characteristics of rainbow trout. *Respir. Physiol.* **68**, 85-97.

Thorarensen, H., Gallaugher, P. E., Kiessling, A. K. and Farrell, A. P. (1993). Intestinal blood flow in swimming chinook salmon *Oncorhynchus tshawytscha* and the effects of haematocrit on blood flow distribution. *J. Exp. Biol.* **179**, 115-129.

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

Tota, B. (1983). Vascular and metabolic zonation in the ventricular myocardium of mammals and fishes. *Comp. Biochem. Physiol. A* **76**, 423-438.