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The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*)

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SUMMARY

The mechanism underlying the decrease in aerobic scope in fish at warm temperatures is not fully understood and is the focus of this research. Our study examined oxygen uptake and delivery in resting, swimming and recovering sockeye salmon while water temperature was acutely increased from 15°C to 24°C in 2°C h^{-1} increments. Fish swam at a constant speed during the temperature change. By simultaneously measuring oxygen consumption ($\dot{M}_{O_2}$), cardiac output ($\dot{Q}$) and the blood oxygen status of arterial and venous blood, we were able to determine where in the oxygen cascade a limitation appeared when fish stopped sustained swimming as temperature increased. High temperature fatigue of swimming sockeye salmon was not a result of a failure of either oxygen delivery to the gills or oxygen diffusion at the gills because oxygen partial pressure (P_{O_2}) and oxygen content (C_{O_2}) in arterial blood did not decrease with increasing temperature, as would be predicted for such limitations. Instead, arterial oxygen delivery ($T a_{O_2}$) was initially hampered due to a failure to adequately increase $\dot{Q}$ with increasing temperature. Subsequently, lactate appeared in the blood and venous P_{O_2} remained constant.

Key words: Pacific salmon, cardiac output, heart rate, oxygen consumption, respiration, temperature.

INTRODUCTION

The effect of oxygen limitation on the energy allocation of fishes at extreme temperature is well established (Brett, 1971; Neill and Bryan, 1991; Claireaux and Lagardere, 1999; Claireaux et al., 2000; Lee et al., 2003; Lefrancois and Claireaux, 2003). This limitation has been suggested to lead to the use of anaerobic metabolism, exhaustion, a restriction of whole-animal tolerance to temperature extremes and even mortality (Pörtner, 2002; Pörtner and Knust, 2007). Aerobic scope, which is defined as the difference between routine and maximal metabolic rates (Fry, 1971; Priede, 1977), is often used to estimate the maximum energy available for activity and assess the impact of temperature on energy allocation. Aerobic scope peaks at an optimum temperature (T_{opt}) but decreases with increasing temperature until it becomes minimal at the critical temperature (T_{crit}) when anaerobic metabolism takes over. T_{crit} arises in part because routine oxygen demand continues to increase above T_{opt} and in part because maximum oxygen delivery declines abruptly above T_{opt}.

The temperature dependence of aerobic scope has been successfully applied to the upriver spawning migration performed by sockeye salmon in the Fraser River, British Columbia, Canada, which can be exceptionally energy demanding because of the long migration distance (up to 1000 km) and major hydraulic challenges (e.g. Hell's Gate). T_{opt} for a given stock of sockeye salmon has been demonstrated to be similar to the river temperature encountered during migration (Lee et al., 2003), suggesting that the physiology of adult spawning salmon may be optimised to the environmental temperature conditions so that the next generation of salmon is secured. Correspondingly, when river temperature has been higher than normal and exceeds T_{opt}, high fish mortality has been observed in the river (Farrell et al., 2008). The temperature dependence of aerobic scope is also being used as a tool to assess potential impacts of global climate change on fishes (Pörtner and Knust, 2007; Wang and Overgaard, 2007; Farrell et al., 2008).

Despite these broad applications of aerobic scope, the exact mechanism leading to the decline in maximum aerobic scope above T_{opt} remains unresolved. Potential mechanisms (gill-related, cardiac-related and muscle-related limitations) have received theoretical consideration elsewhere (Brett, 1971; Taylor et al., 1997; Farrell, 1997; Farrell, 2002; Pörtner, 2002) but evidence supporting any one of these explanations is sparse and incomplete [see summary by Farrell (Farrell, 2007a)]. Considerable evidence points to a cardiac-related limitation, largely because of the similarity of the T_{opt} values for swimming performance, maximum $\dot{M}_{O_2}$ and maximum $\dot{Q}$ in salmonids (Brett, 1971; Farrell, 1997). However, Farrell noted the need to further examine whether or not an oxygen diffusion limitation exists at the gill at temperatures above T_{opt} (Farrell, 2007a; Farrell, 2007b). A key observation in this regard is the demonstration that acute increases in water temperature decreased both arterial and venous blood oxygen content (C_{O_2}) in resting rainbow trout (*Oncorhynchus mykiss* Linnaeus) (Heath and Hughes, 1973). In addition, Taylor and colleagues suggested that an oxygen diffusion limitation at skeletal muscle in swimming fish was important (Taylor et al., 1997).

In view of this uncertainty, the present study greatly extended on the earlier study by Heath and Hughes (Heath and Hughes, 1973) by undertaking simultaneous measurements of $\dot{M}_{O_2}$, $\dot{Q}$ and oxygen status in arterial and venous blood of resting and exercising adult sockeye salmon (*Oncorhynchus nerka* Walbaum) subjected to an acute increase in water temperature. Given the excellent swimming ability of sockeye salmon, we reasoned that any limitation in oxygen delivery during the temperature increase would be better revealed when fish were swimming near their maximum aerobic capacity rather than while resting. Consequently, for the exercise component of the study, salmon were swum continuously at a speed where $\dot{M}_{O_2}$ was approaching its maximum [around 75% of their critical swimming speed (U_{crit}) (Brett, 1971; Burgetz et al., 1998; Lee et

al., 2003)] while temperature was increased. Thus, as temperature increased at a constant swimming speed, we expected $\dot{M}_{O_2}$ to increase only due to temperature-related effects until fish could no longer support swimming at elevated temperature. We predicted that if there was either a limitation in oxygen delivery to the gills or an oxygen diffusion limitation at the gills, then we would observe a decrease in arterial P_{O_2} when salmon stopped swimming at elevated temperature. By contrast, if cardiac limitation appeared at elevated temperature, this would be manifest as a failure to increase $\dot{Q}$ with increasing temperature whereas an oxygen diffusion limitation to skeletal muscles would be manifest as a constant venous P_{O_2}.

MATERIALS AND METHODS

Experimental animals

Adult (2.2–2.9 kg) sockeye salmon were intercepted during their return migration to the Fraser River. They were caught by a commercial seine vessel between the 28th and 30th August, 2006 in the Strait of Georgia (salinity=30‰, temperature=12.5°C) near the mouth of the Fraser River, BC, Canada. The fish were held in the livehold of the vessel for a couple of hours while being transferred to and unloaded at the Centre for Aquaculture and Environmental Research (CAER), West Vancouver, BC, Canada. Prior to being placed in the holding tank each fish was identified with a cinch tag, and a scale and a DNA-clip (0.5 g) from the adipose fin were taken for stock identification. The stock origin of the fish was determined by DNA and scale analyses (Beacham et al., 1995; Beacham et al., 2004). Only fish identified as being from the Lower Adams River stock complex were used for the present experiments. Salmon were initially held in a 12,000 l holding tank supplied with full strength seawater at a temperature of 12.5°C. On the afternoon of the 30th August, the salinity of the water was decreased over a 4 h period to iso-osmotic (salinity~9‰, temperature=13.5°C) by mixing freshwater and seawater supplies, and the following day the holding tank was switched to pure freshwater. Freshwater temperature in the holding tank was initially 14.5°C and declined seasonally during the experimental period to 12.0°C by October when the experiments were completed. These changes in salinity and temperature simulated those experienced by sockeye salmon when they enter the Fraser River for their spawning migration. In general, one resting and one exercising fish were tested each day. Therefore, individual fish were held in freshwater for a minimum of two days and up to a maximum of 28 days before experimentation. All experimental procedures were approved by the Animal Care Committee of the University of British Columbia in accordance with the Canadian Council on Animal Care.

Surgical procedures for cardiorespiratory measurements

Each fish was prepared so that arterial and venous blood could be routinely sampled from in-dwelling cannulae without disturbing the fish and $\dot{Q}$ could be monitored continuously with a flow probe. For surgery, the fish was first anaesthetised with MS-222 in buffered freshwater (75 mg l^{-1} $NaHCO_3$+75 mg l^{-1} MS-222, Sigma-Aldrich, St Louis, MO, USA) until opercular movements ceased. Body mass and length were measured and the fish was placed ventral side up on water-soaked foam on a surgery table. During surgery, the gills were irrigated with aerated, chilled water containing a lower dose of anaesthetic (50 mg l^{-1} $NaHCO_3$+50 mg l^{-1} MS-222, Sigma-Aldrich). To sample arterial blood, a PE-50 cannula filled with a 0.9% saline solution containing heparin (150 i.u. ml^{-1}) was inserted into the dorsal aorta using an internal trochar. The cannula was anchored in place at the roof of the mouth using a 3-0 silk suture and exteriorized *via* a hole in the snout (Soivio et al., 1973). To measure $\dot{Q}$, the ventral aorta was exposed with a 0.5 cm incision on the right side of the isthmus and dissected free (Steffensen and Farrell, 1998). A Transonic transit-time blood flow probe (3 mm SB, Transonic systems, Ithaca, NY, USA) was positioned around the ventral aorta, proximal to the bulbus arteriosus, for measurements of absolute blood flow. Great care was taken during this procedure to leave the pericardium intact and avoid damage to the coronary artery. To sample venous blood, a PE-50 cannula filled with the heparinised saline was inserted into the ductus of Cuvier, which was carefully exposed and dissected free with an incision between the cleithrum and the fourth branchial arch (Farrell and Clutterham, 2003). A small portion of the vessel was lifted and secured with a vascular clamp and subsequently secured with a 4-0 suture, allowing the vessel to be gently lifted during the cannulation procedure. A small nick was made in the lifted vessel and a PE-50 cannula, with 2–3 side-holes and a bubble 1.5 cm from the tip was inserted towards the heart (Sandblom et al., 2006). A 4-0 suture was used to close the vessel wall around the catheter leaving the bubble on the luminal side of the vessel. The leads and cannulae were secured together, sutured onto the body wall with multiple sutures and led off the leading edge of the dorsal fin. The entire surgical procedure took approximately 1 h. After being placed in a Brett-type swim tunnel, the fish recovered from anaesthesia extremely quickly (~30–60 s), as indicated by their ability to maintain an upright position in the water current and ventilate at a regular frequency. After the first hour of recovery, the water temperature was ramped up from the holding tank temperature (12.0–14.5°C) to the experimental starting temperature of 15°C at a rate of 1°C h^{-1}. This experimental temperature was expected to be very close to T_{opt} for aerobic scope based on previous studies with sockeye salmon (Brett, 1971; Lee et al., 2003). Each fish recovered overnight (~10–12 h) in a swim tunnel at a temperature of 15°C with the water velocity set at a low flow rate of about 0.20 m s^{-1} [approximately 0.35 body lengths s^{-1} (BL s^{-1})]. At this modest water speed, salmon faced into the water flow and maintained station without any tail beats.

Experimental protocols

The two experimental protocols were essentially identical in terms of the protocol to acutely increase temperature above T_{opt} but were performed on either resting or exercising fish. Both protocols began with measurements of routine $\dot{M}_{O_2}$ and $\dot{Q}$ over a 30–45 min period. At the end of this recording period, 0.7 ml of blood was withdrawn from the arterial and venous cannulae using heparinised syringes and the collected blood volume was replaced with 0.9% NaCl. The protocol for resting fish (N=18 fish) involved increasing the water temperature at a rate of 2°C h^{-1} to each of the following test temperatures: 17, 19, 21, 23 and 24°C. The cardiorespiratory measurements were repeated in duplicate, starting immediately after a stable temperature was reached. All resting fish completed the 24°C temperature exposure protocol, after which water temperature was decreased to 15°C over a 45 min period and the recovery of cardiorespiratory status was evaluated by repeating measurements after approximately 1 h.

The second group of fish (swimming fish; N=15) were first required to swim at a steady-state before they were tested with the same acute temperature protocol. Thus, after taking routine cardiorespiratory measurements, the water velocity in the swim tunnel was increased by 0.1 m s^{-1} every 2 min until a final velocity of 0.85 m s^{-1} (~1.35 BL s^{-1}) was reached. Preliminary experiments (data not shown) had shown that at this temperature, the fish were swimming at approximately 75% of U_{crit} and they could sustain this speed in excess of 4 h. By having the fish swimming at a fixed swimming speed (1.35 BL s^{-1}), we could use temperature as

a continuous factor in a repeated-measures statistical design for analysis of variance (RM ANOVA). The fish were maintained at the sustained swimming speed for 30 min to allow the physiological variables to reach a steady state before measuring the cardiorespiratory status of swimming fish at 15°C. The expectation was that 75% U_{crit} placed these fish near to their maximum aerobic scope (Lee et al., 2003) and that, with the subsequent increase in water temperature, the associated increase in tissue oxygen demand and the decrease in aerobic scope at a temperature above T_{opt} would cause the fish to eventually quit swimming. All fish swam at 19°C but only three fish swam at 24°C, the remainder quitting at intermediate water temperatures (Fig. 1). Regardless, of the final temperature, water flow was reduced to 0.2 m s^{-1} when the fish quit swimming and the water temperature was reduced to 15°C. The recovery of cardiorespiratory status was evaluated by repeating measurements after approximately 1 h, as with the resting fish.

Swim tunnels and heating system

Two Brett-type swim tunnels of slightly different sizes were used for the experiments. The larger swim tunnel contained 425 l of water and had a circular swimming section with a diameter of 25.4 cm and a length of 124.3 cm. The smaller version contained 220 l of water and the circular swimming section had a diameter of 22.2 cm and a length of 124.3 cm (Lee et al., 2003; Farrell et al., 2003). A shocking grid at the rear end of the tunnels was used to provide a mild electrical stimulation (2–10 V) to prevent the fish from resting on the grid during swimming. Water velocity was measured with an anemometer (Valeport Marine Scientific, Dartmouth, UK) and calibrated against the motor frequency for each tunnel (Farrell et al., 2003). Temperature was controlled by a custom-designed heating system. A 9 kW titanium heating element was installed directly into the swim tunnel vessel to provide controlled increases in water temperature (up to 12°C above ambient). During a measuring period, the water temperature never fluctuated by more than 0.5°C.

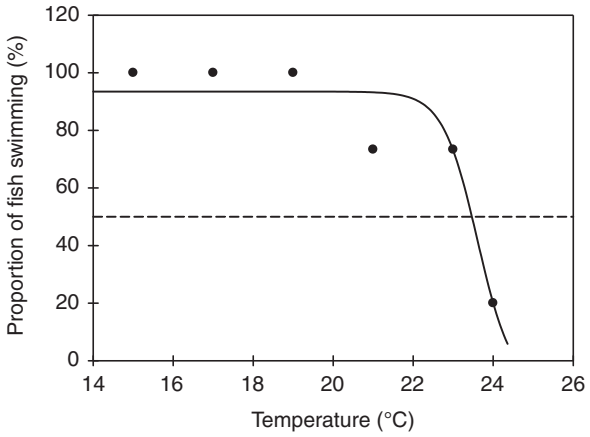

Fig. 1. The percentage of swimmers remaining at each acute temperature challenge (100%=15 salmon). The temperature for 50% failure is interpolated as 23.5°C. The line is fitted using a sigmoidal dose-response equation:

$$y = \min + \frac{\max - \min}{1 + 10^{(\log EC_{50} - x)}}, \quad (1)$$

where y is the proportion of fish swimming, x is temperature and the parameters min, max and EC_{50} are estimated from data.

$\dot{M}_{O_2}$ and $\dot{Q}$ measurements

$\dot{M}_{O_2}$ was measured by closing the water inflow and outflow from the swim tunnel. The decrease in water oxygen content was measured with an Oxyguard probe (Mark IV, Point Four Systems, Richmond, Canada) that measured oxygen levels with a precision of 3 mol l^{-1}. The oxygen probe was placed in a small chamber (~0.3 l) outside the swim tunnel and a pump (Eheim Universal 1046, Loligosystems, Tjele, Denmark) placed in the loop created a flow-through of water (5 l min^{-1}) over the probe. The probe reading was digitised *via* a Windaq box (Dataq instruments, Akron, ON, USA) and data were saved at a frequency of 0.2 Hz using software programmed in Labview 6.0 (National Instruments, Austin, TX, USA). The duration of the measuring period (ranging from 3–45 min) was determined by a suitable decrease in oxygen content (≥10 mol l^{-1}) and was dependent on the size of the fish and activity level, i.e. routine $\dot{M}_{O_2}$ measurements at the coldest temperature took the longest time, while those for the swimming fish were the shortest. The decline in water oxygen content over time resulted in a linear regression with r^2 values of ≥0.95. $\dot{M}_{O_2}$ (μmol min^{-1} kg^{-1}) was calculated as: $\dot{M}_{O_2} = \Delta[O_2]\nu M_b^{-1} t^{-1}$, where $\Delta[O_2]$ is measured in μmol l^{-1}, ν is the volume of the swim tunnel minus the fish volume in litres, M_b is body mass in kilograms and t is time measured in minutes.

$\dot{Q}$ was monitored simultaneously during $\dot{M}_{O_2}$ measurements. The flowprobe was connected to a Transonic flowmeter (Model T206, Transonic Systems, Ithaca, New York, USA). Blood flow data was sampled at 200 Hz using a Biopac module running Acknowledge 3.8.2, (Biopac Systems, Santa Barbara, CA, USA). $\dot{Q}$ (ml min^{-1} kg^{-1}) was calculated as a mean of three periods of 10–20 s when a stable cardiac rhythm was present. Heart rate (f_H; beats min^{-1}) was calculated from the intervals between systolic peaks in the flow trace and cardiac stroke volume (V_s; ml beat^{-1} kg^{-1}) was calculated as $V_s = \dot{Q} f_H^{-1}$.

As the swimming velocity was never increased until fish reached their maximum cardiorespiratory performance at a fatigue velocity, aerobic scope and cardiac scope were never measured directly in any fish. However, because fish were swimming near their maximum $\dot{M}_{O_2}$ at 75% U_{crit}, we therefore expected that ultimately the acute increase in temperature would create a situation where aerobic scope was reached, either because routine $\dot{M}_{O_2}$ had progressively increased, maximum $\dot{M}_{O_2}$ had declined above T_{opt}, or, more likely, some combination of the two. At this juncture, a further increase in water temperature would cause fish either to quit swimming aerobically or to compliment the aerobic effort with anaerobic swimming. To visualise such effects and to properly account for the temperature effect on routine metabolism, we calculated the difference (termed range) between the routine and swimming values for $\dot{M}_{O_2}$, $\dot{Q}$ and f_H. Because these values were derived from the mean values from two different groups of fish (resting and swimming) at each temperature, no statistical inferences can be made from these data. To simplify this presentation, range is presented as a percentage of the highest value obtained which was at 17°C for f_H and 19°C for $\dot{M}_{O_2}$ and $\dot{Q}$.

Blood analyses

Blood samples were placed on ice and immediately analysed for P_{O_2}, O_2 content (C_{O_2}), pH, haemoglobin concentration (Hb) and haematocrit (Hct). The remaining blood was spun down and the plasma was frozen in liquid nitrogen and stored at –80°C for subsequent analysis. Blood P_{O_2} was measured with a Radiometer P_{O_2} electrode (E101, Loligosystems, Tjele, Denmark), thermostatted in a D616 cell at the respirometer temperature, and

displayed on a Radiometer PHM 71 acid–base analyzer. The electrode was calibrated with air-saturated water several times daily and in accordance with the experimental temperature changes from 15–24°C. C_{O_2} of whole blood was measured according to Tucker (Tucker, 1967). Arteriovenous O_2 delivery ($A\dot{V}_{O_2}$) was calculated as the difference between arterial and venous C_{O_2} using data only from fish where both blood samples were successfully drawn and analysed. Arterial O_2 delivery (Ta_{O_2}) was calculated as the product of $\dot{Q}$ and arterial C_{O_2}. Blood pH was measured from 0.2 ml of blood, which was injected into a custom designed plastic chamber housing a snugly fit pH probe (Symphony 14002-764, VWR, PA, USA). The blood was thermostatted to the experimental temperature and whole blood pH values were read on a handheld pH meter (Symphony SP301, VWR, PA, USA). Haemoglobin concentration was determined spectrophotometrically on 10 µl of blood using a Randox total haemoglobin assay kit (HG980, Randox Laboratories, Antrim, UK). Plasma lactate, potassium and cortisol concentrations were measured on samples that were thawed immediately before use and vortexed for 30 s before use in accordance with the methods fully described by Farrell and colleagues (Farrell et al., 2001). Lactate was measured in duplicates using an YSI 2300 lactate/glucose analyzer (Yellow Springs Instruments, OH, USA) that was automatically calibrated every five measurements to a precision of 0.2 mmol l^{-1}. Potassium was measured in duplicate on a flame photometer (Cole Parmer model 2655-00, Vernon Hills, IL, USA) after dilution of the plasma (deionized water 1:200). The flame photometer was calibrated prior to use with a four-point calibration and standards were checked every 10 samples. Plasma cortisol concentrations were measured in duplicates using an ELISA kit (Neogen, Lexington, KY, USA). Only arterial values for Hb, lactate, potassium and cortisol are presented because we did not expect large differences with the venous values and there were lower numbers of successful venous samples.

Statistics

Given that no significant differences were found between male and female fish, the data were pooled. All data were analysed using a two-way (swimming *vs* resting or arterial *vs* venous) RM (temperature) ANOVA and Tukeys' test was used for all pairwise comparisons. In some cases the ANOVA design was disconnected, which did not permit a two-way test and so the data were analysed using a one-way RM ANOVA followed by a Student's *t*-test to separately compare either swimming *vs* resting fish or arterial *vs* venous blood samples. A Spearman correlation test was used to analyse the correlation between rank performance (based on different cardiorespiratory parameters) and the temperature at which fish quit swimming. All values are means ± s.e.m. and statistical difference was assigned for a P-value<0.05.

RESULTS

Fish behaviour

Resting fish remained on the bottom of the swim chamber, with the exception of occasional exploratory movements. These exploratory movements were more noticeable when water temperature reached 19°C but were not quantified and were never excessive at any temperature.

Fish swam steadily and continuously at water temperatures between 15°C and 19°C, as would be expected for sustained swimming near to maximum aerobic performance. However, at water temperatures of 19°C and above, the swimming mode included an increasing contribution of burst and coast activity, which is indicative of a switch to anaerobically powered swimming using white muscle fibres.

All fish completed the swimming protocol at 19°C. However, above this temperature, the number of fish able to maintain station at the imposed swimming speed declined with increasing temperature (Fig. 1) and only three out of 15 fish completed the swimming protocol at 24°C. The temperature for a 50% failure to swimming was interpolated from Fig. 1 as 23.5°C. Therefore, increasing temperature induced a change in swimming behaviour and caused some fish to quit swimming, as predicted. These behavioural changes occurred at and above 19°C, and were associated with important physiological changes (as shown below). Nevertheless, these changes did not prevent the fish from making a rapid recovery when returned to 15°C (as shown below).

Because of these behaviours, the physiological data presented in subsequent figures necessarily represent only the fish that swam at a given temperature, i.e. a decreasing N value above 19°C. Implicit for such presentations is that only the temperature-tolerant or best performers are represented for the highest temperature, creating a potential bias. Therefore, the cardiorespiratory variables at fatigue, regardless of the temperature at which fatigue occurred, are summarised for all fish in Table 1. These values are representative of the average physiological state at fatigue following an acute temperature challenge. Given the fact that U_{crit} varies among individuals, a further difficulty with a fixed speed swimming protocol and progressive increases in temperature is distinguishing between temperature tolerance and best performers. Therefore, to shed light on whether fish quit swimming because of the increase in temperature *per se* or because the imposed swimming speed was closer to their real U_{crit}, we examined for relationships between the temperature at which a fish quit and the ranking of various key cardiorespiratory variables (routine $\dot{M}_{O_2}$, routine $\dot{Q}$, $\dot{M}_{O_2}$ swimming at 15°C and $\dot{Q}$ swimming at 15°C). No significant relationships were found.

Oxygen uptake

In resting sockeye salmon, routine $\dot{M}_{O_2}$ increased significantly with each temperature increment (Fig. 2A), resulting in a 3.2-fold increase between 15°C and 24°C (an overall $\dot{Q}_{10}$ value of 3.6). The $\dot{Q}_{10}$ values between each of the test temperatures varied from the lowest $\dot{Q}_{10}$ value of 3.2 between 19°C and 21°C to the highest $\dot{Q}_{10}$ value of 4.2 between 17°C and 19°C. When fish were returned to a temperature of 15°C for 1 h, routine $\dot{M}_{O_2}$ was largely restored to the initial routine $\dot{M}_{O_2}$ value but remained significantly elevated by 29% (Fig. 2A).

Routine $\dot{M}_{O_2}$ at 15°C for the group of swimming fish prior to swimming was identical to that measured at 15°C for the resting fish group (Fig. 2A). Swimming at approximately 75% of U_{crit} at 15°C significantly increased $\dot{M}_{O_2}$, such that active $\dot{M}_{O_2}$ was 6.5-times higher than routine $\dot{M}_{O_2}$ (Fig. 2A; Table 1). Increasing water temperature initially increased active $\dot{M}_{O_2}$ significantly (Fig. 2A). However, active $\dot{M}_{O_2}$ was not significantly different between 19°C and 24°C, and active $\dot{M}_{O_2}$ at 24°C was no different to that measured at 17°C (Fig. 2A). Thus, active $\dot{M}_{O_2}$ reached a maximum and showed indications of a decline at 24°C, unlike the continuous increase in $\dot{M}_{O_2}$ in the resting fish group.

When fish stopped swimming and the temperature was returned to 15°C, $\dot{M}_{O_2}$ decreased significantly but remained significantly elevated by 65% above the initial routine $\dot{M}_{O_2}$ value at 15°C (Fig. 2A; Table 1).

Across all temperatures, the increase in routine $\dot{M}_{O_2}$ was not matched with the change in active $\dot{M}_{O_2}$, as shown by the range for

Table 1. Cardiorespiratory parameters for sockeye salmon at rest, swimming at 1.35 $BL\,s^{-1}$ and during recovery

	Resting at 15°C	Swimming at 15°C	Fatigue at 21–24°C	Recovery at 15°C
$\dot{M}_{O_2}$ (μmol min^{-1} kg^{-1})	56.3±3.1a (15)	365.6±18.8b (15)	453.1±12.5c (13)	93.8±9.4d (14)
$\dot{Q}$ (ml min^{-1} kg^{-1})	25.3±1.8a (13)	58.0±2.2b (12)	67.8±2.8c (11)	33.8±2.1d (13)
f_H (beats min^{-1})	65.2±2.5a (12)	81.4±1.7b (13)	104.5±2.6c (11)	82.0±1.3b (13)
V_s (ml beat^{-1} kg^{-1})	0.38±0.03a (12)	0.69±0.03b (11)	0.63±0.04b (11)	0.41±0.03a (13)
Pa_{O_2} (Torr)	97.3±6.1a (11)	74.4±6.3b (11)	102.0±4.4a (10)	101.3±5.1a (12)
Pv_{O_2} (Torr)	36.9±3.6q* (7)	21.9±2.8r* (5)	27.5±2.9r* (5)	41.9±3.0q* (7)
Ca_{O_2} (μmol ml^{-1})	6.59±0.38a (7)	5.22±0.34b (7)	5.54±0.22b (7)	6.08±0.25ab (7)
Cv_{O_2} (μmol ml^{-1})	4.80±0.40a* (5)	2.21±0.17b* (5)	1.11±0.09c* (5)	3.71±0.44a* (5)
Ta_{O_2} (μmol min^{-1} kg^{-1})	163.4±15.8a (7)	314.1±21.4b (7)	358.5±12.5b (7)	200.9±20.7a (7)
$A\dot{V}_{O_2}$ (μmol ml^{-1})	1.29±0.24a (3)	2.99±0.44b (5)	4.76±0.48c (5)	2.57±0.26b (5)
pHa	7.74±0.05ac (9)	7.67±0.03ab (9)	7.62±0.02b (9)	7.78±0.02c (9)
pHv	7.74±0.05q (5)	7.65±0.04qrs (5)	7.54±0.02r* (5)	7.73±0.04qs (5)
Hct a (%)	30.0±1.5a (13)	31.4±1.4a (14)	32.0±1.8a (12)	30.4±1.6a (14)
Hct v (%)	28.9±0.7q (9)	31.1±0.8q (8)	30.9±0.9q (6)	29.3±2.0q (8)
Hb a (mg ml^{-1})	107.1±8.1a (11)	104.8±12.9a (12)	118.5±7.9a (8)	102.3±4.6a (10)
Lactate (mmol l^{-1})	1.9±0.3a (9)	3.0±0.4ac (9)	5.5±0.5b (9)	4.0±0.6c (9)
Potassium (mmol l^{-1})	3.9±0.5a (7)	5.1±0.5a (7)	7.4±0.5b (7)	5.6±0.7ab (7)
Cortisol (ng ml^{-1})	45.2±5.6a (11)	206.3±25.3b (12)	559.6±25.5c (11)	271.3±41.6b (10)

Fish fatigued at different temperatures ranging from 21–24°C (see Fig. 1) and the measurements presented were taken at the temperature when fatigue occurred. Values are means ± s.e.m., and *N*-values are presented in brackets. Different letters indicate significant differences within groups and * indicates a significant difference between arterial and venous blood samples. $\dot{M}_{O_2}$, metabolic rate (rate of O_2 consumption); $\dot{Q}$, cardiac output; f_H, heart rate; V_s, cardiac stroke volume; Pa_{O_2}, partial pressure of O_2, arterial; Pv_{O_2}, partial pressure of O_2, venous; Ca_{O_2}, O_2 concentration of arterial blood; Cv_{O_2}, O_2 concentration of venous blood; Ta_{O_2}, arterial O_2 delivery; $A\dot{V}_{O_2}$, arteriovenous O_2 delivery (O_2 extraction); pHa, pH of arterial blood; pHv, pH of venous blood; Hct a, haematocrit, arterial; Hct v, haematocrit, venous; Hb a, haemoglobin, arterial.

$\dot{M}_{O_2}$ (Fig. 3). The range for $\dot{M}_{O_2}$ was maximal at 19°C (362.5 μmol min^{-1} kg^{-1}). This means that range for $\dot{M}_{O_2}$ increased modestly with an increase in temperature from 15°C to 19°C, was maintained at 21°C and then declined at temperatures of 23°C and 24°C.

Cardiac performance

In resting fish, routine $\dot{Q}$ increased significantly with temperature (Fig. 2B), increasing by 70% between 15°C and 24°C (an overall $\dot{Q}_{10}$ value of 1.7). The values between each of the test temperatures varied from the lowest $\dot{Q}_{10}$ value of 1.1 between 23°C and 24°C to the highest $\dot{Q}_{10}$ value of 2.2 between 19°C and 21°C. When fish were returned to a temperature of 15°C for 1 h, routine $\dot{Q}$ was almost restored to the initial $\dot{Q}$ value but remained significantly elevated by 17% (Fig. 2B).

While the temperature-induced increase in routine $\dot{Q}$ paralleled that in $\dot{M}_{O_2}$ (Fig. 2), there was an important difference. Routine $\dot{Q}$ reached a plateau at 23°C (Fig. 2B) whereas routine $\dot{M}_{O_2}$ did not (Fig. 2A). Notably, the temperature-induced increase in routine $\dot{Q}$ was brought about through f_H increasing from 64 beats min^{-1} to over 100 beats min^{-1} (Fig. 4A), and little change in V_S from the routine value of 0.38 ml beat^{-1} kg^{-1} (Fig. 4B). Routine f_H was almost restored during recovery at 15°C, but remained significantly 16% higher during recovery (Fig. 4A).

Routine $\dot{Q}$ at 15°C for fish resting before swimming was identical to that measured in the resting fish at 15°C. Swimming at 15°C increased $\dot{Q}$ by 2.3-times (Table 1; Fig. 2B). Active $\dot{Q}$ increased significantly with the initial temperature increase (Fig. 2B) but did not increase subsequently between 17°C and 24°C (Fig. 2B). When fish stopped swimming and recovered at 15°C for 1 h, $\dot{Q}$ decreased significantly but remained significantly elevated by 33% compared with the initial routine $\dot{Q}$ value (Fig. 2B; Table 1).

Across all temperatures, the increase in routine $\dot{Q}$ was not matched with the change in active $\dot{Q}$, as shown by the range for $\dot{Q}$ (Fig. 3). The range for $\dot{Q}$ was maximal at 19°C. This means that the range for $\dot{Q}$ increased modestly with an increase in temperature from 15°C to 19°C was maintained up to 21°C and declined at temperatures of 23°C and 24°C.

Prior to swimming at 15°C, routine f_H and V_s (Fig. 4) were identical to those measured at 15°C for the resting fish group. Fish swimming at 15°C elevated f_H by 20% (Fig. 4A) and V_s by 80% (Fig. 4B). The temperature-induced increase in active $\dot{Q}$ came about solely though increased f_H (Fig. 4A), as in resting fish. Although there was no increase in V_s with temperature, V_s remained significantly elevated above that measured in resting fish (Fig. 4B). Noticeably, f_H did not increase significantly as temperature was increased above 19°C and, as a result, f_H for swimming and resting fish converged at just over 100 beats min^{-1} when water temperature reached 24°C. Consequently, active f_H was significantly higher than resting f_H at all temperatures except 24°C (Fig. 4A). Thus, the range for f_H was greatest at 17°C but declined to near zero at 24°C (Fig. 3). In addition, cardiac rhythm became more variable at high temperatures (data not shown), which may have contributed to the increased variability around the mean values for f_H at 23 and 24°C (Fig. 4). While V_s was fully restored after a 1 h at 15°C, f_H remained significantly elevated by 25% compared with the resting fish (Fig. 4A).

Hb, Hct, pH, lactate, potassium and cortisol

Hb and Hct (Table 1) were unchanged during the acute temperature change, indicating that anaemia did not develop despite repeated blood sampling.

In resting fish, arterial and venous blood pH values were not significantly different at any temperature (Fig. 5A). However, at 24°C venous blood pH was significantly reduced compared with the routine values at 15°C and this corresponded with a significant increase in the plasma lactate concentration (Table 1; Fig. 6A). Plasma cortisol (Fig. 6B), but not plasma potassium (Fig. 6C), increased significantly with temperature in resting fish and all these variables were restored after a 1 h recovery at 15°C.

The anticipated effect of swimming on plasma pH was not statistically resolved as there was no difference between arterial

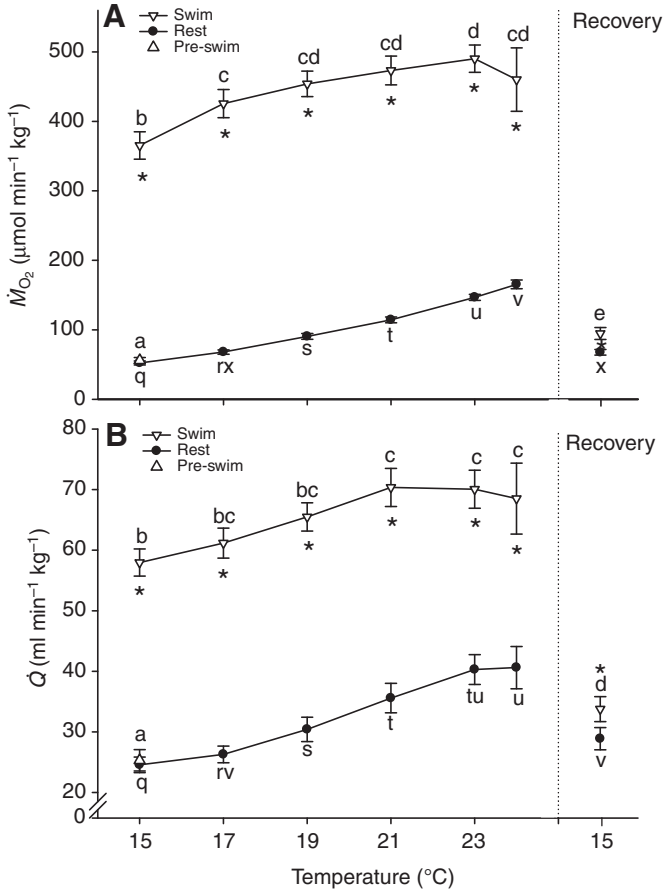

Fig. 2. (A) Oxygen consumption ($\dot{M}_{O_2}$) and (B) cardiac output ($\dot{Q}$) of resting (●) and swimming (▽) sockeye salmon during an acute temperature increase of 2°C h^{-1}. For swimming fish, an initial resting value at 15°C was obtained prior to swimming (△). At the end of the acute temperature increment, water velocity for the swimming fish was immediately decreased to the resting level and the temperature was decreased to 15°C over an hour before a recovery measurement was recorded for both groups. All values are means ± s.e.m. Different letters indicate significant differences between temperatures within the same group. An asterisk indicates a significant difference between resting and swimming fish at a given temperature.

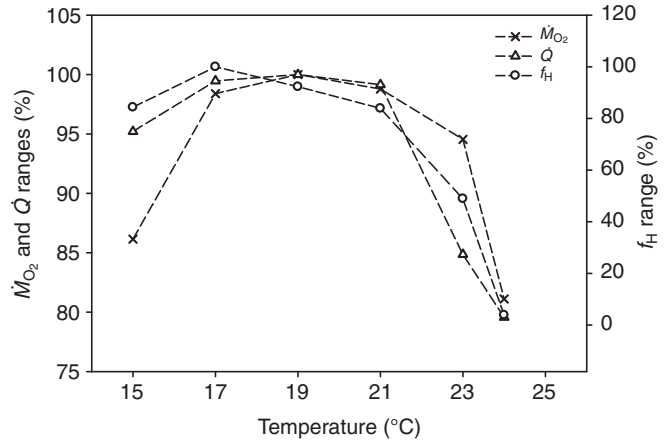

Fig. 3. The ranges for $\dot{M}_{O_2}$ (x), $\dot{Q}$ (△) and f_H (○) from the mean values presented in Figs 2 and 4A. Range was calculated as the difference between the routine and active value at a given temperature. Then to allow comparisons between the three variables, range was expressed as a percentage of the maximum value (either 17°C or 19°C).

and venous plasma pH. However, there was a significant decrease in venous blood pH at 23°C (Fig. 5B) and at fatigue for all fish (Table 1). Plasma lactate and potassium concentrations increased significantly at fatigue (Table 1; Fig. 6A,C). Swimming at 15°C elevated plasma cortisol, which increased further when the temperature was increased above 19°C (Fig. 6B), as in resting fish. A 1 h recovery period at 15°C was associated with a restoration of normal blood pH and plasma potassium and while plasma lactate and cortisol values decreased significantly, they remained significantly elevated above routine levels (Table 1; Fig. 6).

Arterial and venous blood $\dot{P}_{O_2}$ and C_{O_2} values

In resting fish, both venous and arterial P_{O_2} increased significantly with temperature (Fig. 7A). However, there were no corresponding increases in the C_{O_2} of arterial or venous blood (Fig. 8A), probably because of a right-shift in the HbO_2 dissociation curve with increasing temperature. Routine P_{O_2} values were restored after a 1 h recovery period at 15°C (Fig. 7A).

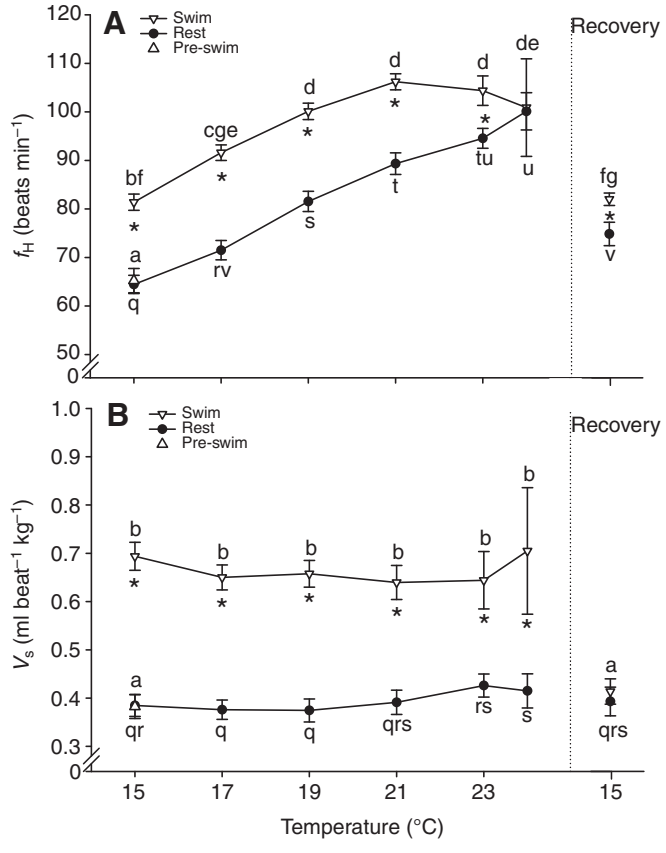

Fig. 4. (A) Heart rate (f_H) and (B) stroke volume (V_s) of resting (●) and swimming (▽) sockeye salmon during an acute temperature increase of 2°C h^{-1}. Before swimming, a resting value was obtained at 15°C (△). At the end of the acute temperature increments, the water velocity for the swimming fish was immediately decreased to the resting value and the temperature was decreased to 15°C over an hour for both groups before a recovery measurement was conducted. All values are means ± s.e.m. Different letters indicate significant differences between temperatures within a group, whereas an asterisk indicates a significant difference between resting and swimming fish at a given temperature.

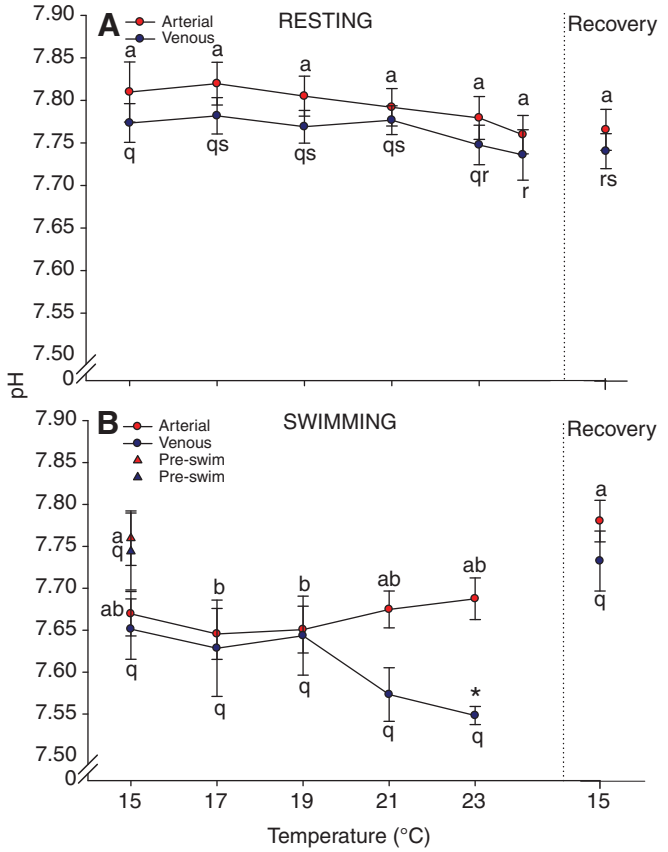

Fig. 5. Blood pH for (A) resting and (B) swimming fish during an acute temperature increase of 2°C h^{-1}. All values are means ± s.e.m. Different letters indicate significant differences between temperatures within a group, whereas an asterisk indicates a significant difference between the arterial and venous values at a given temperature.

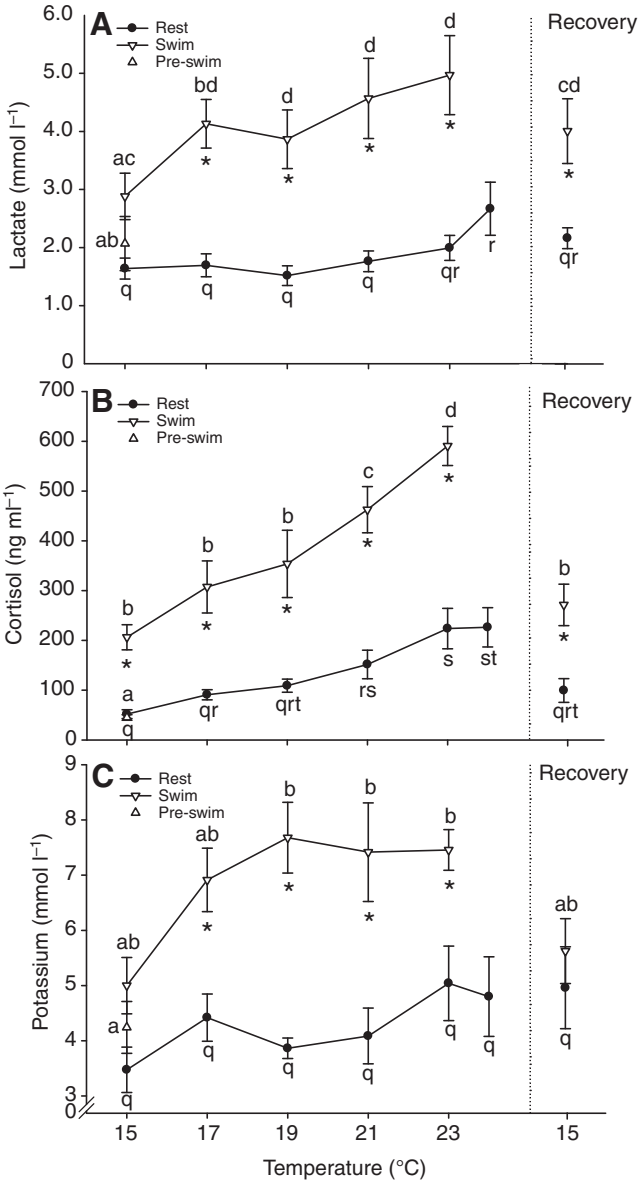

Fig. 6. (A) Plasma lactate levels, (B) plasma cortisol concentrations and (C) plasma potassium concentrations in resting (×) and swimming (▽) sockeye salmon during an acute temperature increase. All values are means ± s.e.m. Different letters indicate significant differences between temperatures within a group, whereas an asterisk indicates a significant difference between resting and swimming fish at a given temperature.

Venous and arterial routine P_{O_2} values for swimming group resting at 15°C were identical to those measured at 15°C for the resting fish group (Fig. 7). Swimming at 15°C induced significant decreases in venous P_{O_2} (Table 1; Fig. 7B) and venous C_{O_2} (Table 1; Fig. 8B), the latter indicating an increased extraction of oxygen from the blood by the skeletal muscles. Swimming at 15°C also induced significant decreases in arterial P_{O_2} (Table 1; Fig. 7B) and arterial C_{O_2} (Table 1; Fig. 8B).

Increasing temperature during swimming had no significant effect on either venous P_{O_2} (Fig. 7B) or venous C_{O_2} (Fig. 8B). Venous C_{O_2} but not venous P_{O_2} was reduced significantly at fatigue (Table 1). Swimming at 19°C restored routine arterial P_{O_2} and C_{O_2} to the routine levels measured at 15°C and arterial P_{O_2} was then maintained at all temperatures above 19°C (Fig. 7B; Fig. 8B). Recovery for 1 h at 15°C fully restored routine blood O_2 status for the swimming group of fish (Fig. 7B, Fig. 8B).

Oxygen delivery across the gills and to tissues

Despite the inability of active $\dot{M}_{O_2}$ and active $\dot{Q}$ to increase further as temperature was acutely increased above 19°C, arterial P_{O_2} never decreased with increasing temperature in either resting or swimming sockeye salmon. These results support the conclusion that neither the delivery of water to the gills nor the diffusion of oxygen from water to the blood was a limiting factor.

Ta_{O_2} increased significantly when fish began to swim and this was a result of increased $\dot{Q}$ (Fig. 9A). However, increasing temperature during swimming did not increase Ta_{O_2} any further and at fatigue Ta_{O_2} was no higher than for fish swimming at 15°C (Table 1).

$A\dot{V}_{O_2}$ was significantly higher for fish at fatigue compared with fish swimming at 15°C (Table 1), indicating that the acute increase in water temperature at a constant swimming speed caused an increased extraction of oxygen from the blood by the tissues. This increase in $A\dot{V}_{O_2}$ at fatigue did not reach statistical significance when $A\dot{V}_{O_2}$ was plotted against temperature (Fig. 9B) but $A\dot{V}_{O_2}$ tended to increase with temperature when active $\dot{Q}$ had already reached a maximum.

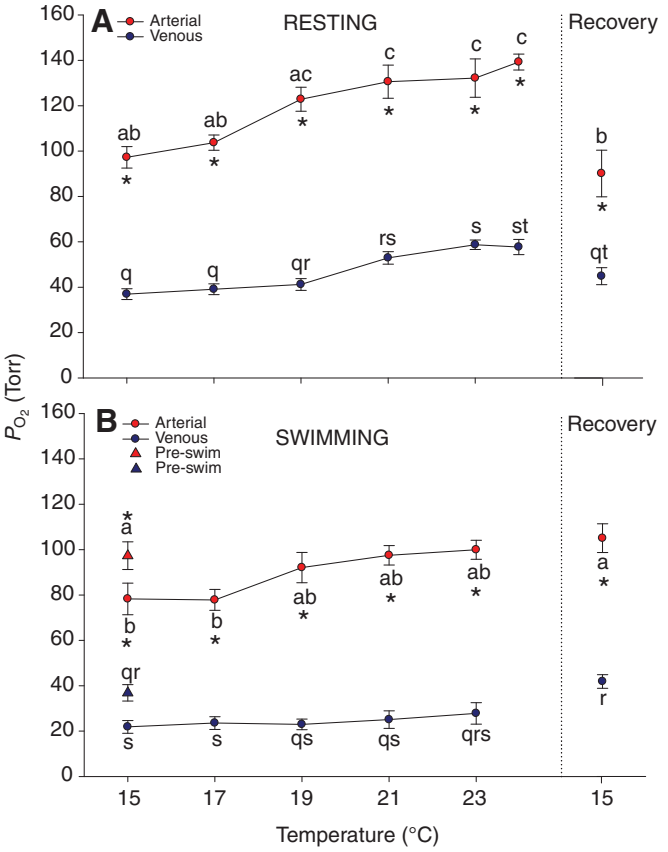

Fig. 7. Oxygen partial pressure (P_{O_2}) of arterial (red symbols) and venous blood (blue symbols) from (A) resting and (B) swimming sockeye salmon during acute temperature increase. All values are means ± s.e.m. Different letters indicate significant differences between temperatures within a group, whereas an asterisk indicates a significant difference between arterial and venous blood samples.

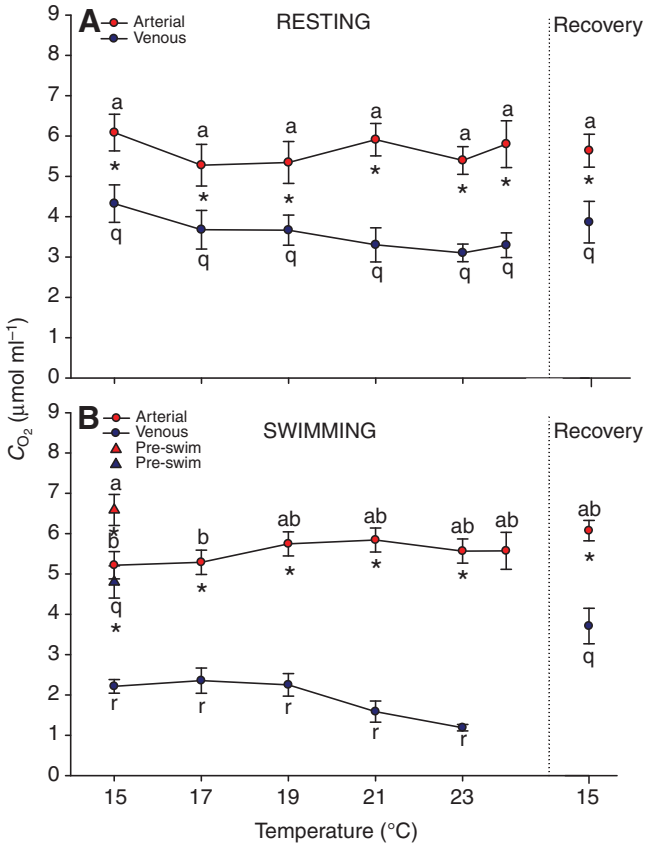

Fig. 8. Oxygen content (C_{O_2}) of arterial (red symbols) and venous blood (blue symbols) from (A) resting and (B) swimming sockeye salmon during an acute temperature increase. All values are means ± s.e.m. Different letters indicate significant differences between temperatures within a group, whereas an asterisk indicates a significant difference between arterial and venous blood samples at a given temperature.

DISCUSSION

The present study is the first to characterise a complete steady-state cardiorespiratory response of swimming fish to high temperature fatigue by measuring $\dot{M}_{O_2}$, $\dot{Q}$ and blood oxygen status simultaneously. The experiments were explicitly designed to swim fish above their T_{opt} and increase temperature to elicit the collapse in aerobic scope that has been demonstrated in previous studies (Lee et al., 2003; Farrell et al., 2008). Although we could not directly measure aerobic scope with the present experimental design, it was clear from the plateau in $\dot{M}_{O_2}$ for swimming fish above 19°C, the increased reliance on glycolytic swimming and the fact that most fish quit their sustained swimming effort at temperatures below 24°C (without any relationship to their rank order of cardiorespiratory performance) that aerobic scope was clearly compromised at temperatures above 19°C. Regardless, the primary purpose of the present study was not to define either this decline or T_{opt} but to provide insights into the underlying mechanisms for cardiorespiratory failure at high temperature.

At the outset we predicted that a decrease in arterial P_{O_2} with increasing temperature would indicate that there was either an oxygen diffusion limitation at the gills or a limit in oxygen delivery to the gills. This result was not obtained, as neither arterial P_{O_2} nor arterial C_{O_2} decreased in response to acute temperature increases for either resting or swimming sockeye salmon. By contrast, a cardiac limitation was manifest as revealed by the failure of swimming fish to progressively increase $\dot{Q}$ with increasing temperature, with f_H apparently reaching a maximum and Ta_{O_2} not being increased at fatigue. Given this temperature limitation on Ta_{O_2}, swimming fish were able to increase $A\dot{V}_{O_2}$ at fatigue (when temperature was above 19°C). However, this increase in $A\dot{V}_{O_2}$ came about because of a decrease in venous C_{O_2} and with a constant venous P_{O_2}. The most parsimonious explanation for this result is that the temperature-induced right-shift in the HbO_2 dissociation curve permitted greater oxygen unloading from the haemoglobin. This then suggests that either following or associated with temperature limitation on Ta_{O_2}, there is a limitation on oxygen diffusion into locomotory skeletal muscle, otherwise venous P_{O_2} would have decreased as tissue oxygen demand increased with increasing temperature.

Fish performance during an acute temperature increase

Routine $\dot{M}_{O_2}$ increased continuously with temperature, apparently following a simple $\dot{Q}_{10}$ effect up to 24°C. While all resting fish tolerated 24°C, the increased exploratory behaviour when the temperature reached 19°C and the increases in plasma lactate and cortisol concentrations above 19°C, which probably reflect an increased reliance on anaerobic metabolism, confound this interpretation somewhat. At temperatures above 19°C, fish also changed their swimming gait, perhaps because active $\dot{Q}$ and Ta_{O_2}

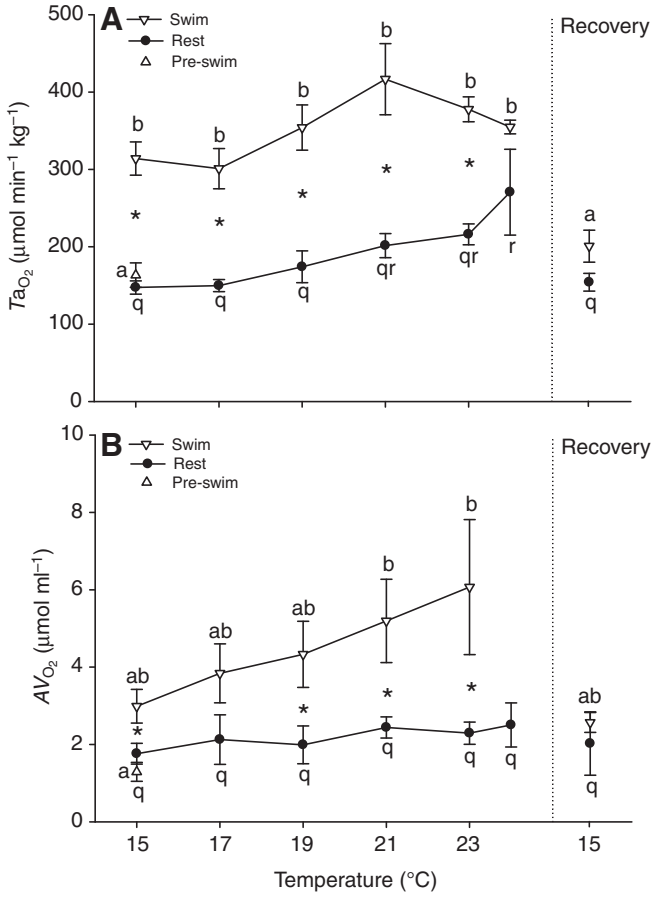

Fig. 9. (A) Arterial oxygen delivery (Ta_{O_2}) and (B) oxygen extraction ($A\dot{V}_{O_2}$) of resting (×) and swimming (▽) sockeye salmon during an acute temperature increase of 2°C h^{-1}. All values are means ± s.e.m. Different letters indicate significant differences between temperatures within a group, whereas an asterisk indicates a significant difference between resting and swimming fish at a given temperature.

had reached maximum values. Thus, for this particular stock of sockeye salmon, a temperature of 19°C appears to be a transition temperature for behaviour and physiology during an acute increase in water temperature. Only 20% of fish were able to swim at ~75% U_{crit} when water temperature was raised to 24°C.

In the present study, sockeye salmon swam at an estimated 75% of U_{crit} at 15°C and their $\dot{M}_{O_2}$ was approximately 70% of maximum $\dot{M}_{O_2}$. In addition, 70–75% of U_{crit} is generally considered to be near the swimming speed at which the onset of anaerobic metabolism begins (Burggetz et al., 1998; Holk and Lykkeboe, 1998; Lee et al., 2003). While we cannot be certain that all fish were close to their maximum $\dot{M}_{O_2}$, we can be certain that the active $\dot{M}_{O_2}$ and $\dot{Q}$ values measured here are among the highest literature values for salmonids (Farrell, 2002; Lee et al., 2003; Wagner et al., 2005).

We cannot be absolutely certain that the individual variability in U_{crit} coupled with the fixed swimming speed of 1.35 BL s^{-1} resulted in fish swimming at different percentages of U_{crit} and this factor, rather than temperature *per se*, contributed to fatigue (i.e. experimental duration and not increased temperature was the principal cause of fatigue). We suspect that this was not the case for a number of reasons. Foremost, these fish were captured before they entered the Fraser River and started an arduous upstream migration. Consequently, our experimental fish were likely well-provisioned and well-prepared for a sustained swim against a high velocity river system at a rate of 20–50 km per day for several weeks. Therefore, beyond the imposed temperature challenge, several hours in a swim tunnel was probably not an excessive challenge, as evidenced by the rapid rate of recovery at 15°C post-exercise. Indeed, preliminary experiments showed that these fish sustained the imposed level of swimming performance for >4 h at 15°C without any increase in plasma lactate levels. Furthermore, had fatigue been solely a result of the individual variation in swimming performance, we might have expected the poorer swimming fish (e.g. those with a lower maximum $\dot{M}_{O_2}$ and $\dot{Q}$ values) to fatigue at the lowest temperatures. However, this was not the case when we examined for relationships in rank performance and the fatigue temperature. A critical physiological observation was that, either at 24°C for resting fish or at the fatigue temperature for swimming fish, these sockeye salmon had exhausted their scope for increasing f_H.

Elevating temperature above T_{opt} was expected to increase the reliance on anaerobic metabolism (see Introduction). Similarly when sockeye salmon approach U_{crit}, they change swimming gait, activate glycolytic white muscles, produce lactate and decrease blood pH (Brauner et al., 2000). Increases in plasma cortisol are also recognised as a sensitive indicator of thermal stress in several fish species (Gamperl et al., 1994; Mommsen et al., 1999). Here, we observed most of these changes, with plasma lactate, cortisol and K^+ concentrations all increasing significantly at fatigue compared with swimming values at 15°C. The high plasma K^+ concentration reported in a number of fish studies (Nielsen and Lykkeboe, 1992; Nielsen et al., 1994; Holk and Lykkeboe, 1998) is due to the loss of K^+ from working muscles. High plasma K^+ levels may result in inexcitability of the muscle cells in general (i.e. skeletal and cardiac) and may have contributed to the fatigue of sockeye salmon at high temperatures.

Aerobic scope has been previously measured in temperature acclimation studies and shown to be 280 mol min^{-1} kg^{-1} at the T_{opt} of 15.0°C for the Weaver Creek and 336 mol min^{-1} kg^{-1} at 17.5°C for the Gates Creek stocks of sockeye salmon (Lee et al., 2003). Brett reported an aerobic scope of 400 mol min^{-1} kg^{-1} at a T_{opt} of 15°C for an undetermined stock of Fraser River sockeye salmon (Brett, 1971). Here, we used ranges to illustrate the temperature effects on the $\dot{M}_{O_2}$, $\dot{Q}$ and f_H. The various aerobic scope values measured among different studies and stocks of sockeye salmon compare favourably with the maximum $\dot{M}_{O_2}$ range measured here as 362 mol min^{-1} kg^{-1} for the Lower Adams River sockeye at 19°C. Thus, either the Lower Adams River sockeye have a considerably higher aerobic scope than other stocks or our experiments were extremely close to the revealing of a true maximum aerobic scope.

T_{crit} values have been estimated as 20.4°C for Weaver Creek and 24.4°C for Gates Creek sockeye salmon, respectively (Farrell et al., 2008), by extrapolation of the data on aerobic scope at acclimated temperatures (Lee et al., 2003). Again, we did not directly measure either T_{opt} or T_{crit} but only 20% fish tolerated swimming at 24°C, and if we assume that fish had zero aerobic scope at the temperature they quit swimming, a polynomial for the $\dot{M}_{O_2}$ range (Fig. 3) yields an intercept of 25°C, a temperature not unlike the T_{crit} for Gates Creek sockeye salmon.

The absence of a limitation in gill oxygen uptake

As oxygen saturation of water decreases by ~2% °C^{-1}, the availability of oxygen is lower in warm compared with cold water (Dejours, 1975). Increasing temperature also facilitates unloading rather than loading of oxygen as the HbO_2 dissociation curve is shifted down and to the right with elevated temperature (Jensen et

al., 1998). Consequently, a limitation on gill oxygen uptake (either delivery of water to or diffusion of oxygen across the gills) has been suggested to be a significant factor explaining high temperature fatigue in fish. In support of this idea, Heath and Hughes measured decreased C_{O_2} in arterial blood (and venous blood) of resting rainbow trout (*Oncorhynchus mykiss* Walbaum) when temperatures exceeded approximately 22°C (Heath and Hughes, 1973). By contrast, the present study showed that arterial C_{O_2} remained constant for resting and swimming, as well as fatigued sockeye salmon over the entire experimental temperature range. These results clearly demonstrate that oxygen uptake across the gills was not limited in the present experiments. In addition, arterial P_{O_2} for resting fish significantly increased with increasing temperature and was constant for swimming fish, which clearly demonstrates that neither water delivery to the gills nor oxygen diffusion across the gills were limited with acute temperature increases in sockeye salmon.

Why a difference exists between the present data and those for rainbow trout is unclear. The difference cannot reside with the fact that the previous work was only performed on resting rainbow trout, since we obtained similar results for both resting and swimming sockeye salmon. A possible explanation may reside with a difference between hatchery and wild fish. For example, the gills of wild sockeye salmon being better designed for oxygen transfer than the gills of hatchery-raised rainbow trout. Yet another explanation may rest with the experimental apparatus used to house fish. Although we do not know the nature of the aquarium used previously with rainbow trout, restrictive blackened square boxes were in vogue. In the present study, sockeye salmon took advantage of the water current in the swim tunnel, perhaps reducing ventilatory effort. Further studies investigating the role of ventilatory effort on a fish's ability to fully saturate arterial blood at high temperature are worth undertaking. Along these lines, a decline of arterial P_{O_2} in resting adult Chinook salmon (*O. tshawytscha* Walbaum) with increasing temperature was related directly to body size (Clark et al., 2008), perhaps because larger fish were more constrained than smaller fish and this limited or increased ventilation effort.

To our knowledge, the increase in arterial P_{O_2} that we observed with increasing temperature has not been previously demonstrated in fish but it is not unexpected given the decreased HbO_2 affinity (Bohr-effect) associated with warm temperatures in fish (Randall et al., 1997) or even an increase in the effectiveness of gill oxygen exchange. Arterial C_{O_2} was unchanged despite the increasing routine arterial P_{O_2} with temperature, suggesting that arterial blood was near full oxygen saturation. Physically dissolved oxygen contributes minimally to the arterial C_{O_2} as the presence of haemoglobin increases blood oxygen content many fold (Randall et al., 1997). Other alterations in the HbO_2 dissociation curve occur during long-term acclimation to high temperatures, such as a decreased level of organic phosphate in the erythrocyte, increased Hb concentration and changes in the synthesis of Hb components (Jensen et al., 1998) but were unlikely to be a factor in these short temperature exposures.

The release of red blood cells from the spleen can occur quickly during swimming (Gallaugher and Farrell, 1998) and with a moderate acute temperature increase (Sandblom and Axelsson, 2007). While we saw no increase in haemoglobin concentration, it is possible that the extensive blood sampling masked this effect.

A limitation on maximum cardiac performance at high temperature

The increased $\dot{M}_{O_2}$ with temperature was supported initially by increased $\dot{Q}$ and by increased $A\dot{V}_{O_2}$ near fatigue. However, in swimming fish Ta_{O_2} clearly reached a maximum, as did active $\dot{Q}$ and f_H with increasing temperature. Furthermore, these maxima corresponded to a temperature when sockeye salmon changed their swimming gait, increased plasma lactate and then started to fatigue as temperature was increased further.

The maximum f_H observed in the present study (106 min^{-1}) is close to the suggested maximum of 120 beats min^{-1} for active fish (Davie and Farrell, 1991). Resting and active f_H converged above 19°C and were identical at 24°C. These changes in f_H probably reflect direct temperature effects on the pacemaker even though the onset of swimming at 15°C increased routine f_H by about 25% and V_s by more than 80%. As a result, active f_H reached a maximum at a lower temperature than routine f_H. While, we know nothing of cardiac control mechanisms under such temperature situations, these results point to the upper temperature limits for maximum cardiac pumping ability being, in part, a result of a maximum f_H being reached.

The absence of any increase in V_s during an acute increase in temperature has been observed previously in resting fish (Gollock et al., 2006). The present observations add further intrigue to this observation, because apparently V_s was fixed at different levels, independent of f_H, for resting and swimming sockeye salmon. The basis for this response is a matter of speculation because of the multiple factors that can influence V_s. For example, acidosis inhibits cardiac contractility (Driedzic and Gesser, 1994), and so the observed decrease in venous pH with increasing temperature could have constrained cardiac inotropy during swimming. In addition to low pH, low venous oxygen and elevated plasma potassium have negative inotropic effects that could also limit maximum cardiac pumping capacity (Farrell et al., 1996; Hanson et al., 2006). These debilitating effects would be exacerbated if the protective effect of adrenaline on cardiac tissues was diminished at warm temperatures, as is the case in rainbow trout (Farrell et al., 1996; Hanson and Farrell, 2007). Furthermore, if increases in f_H are inevitable because of direct temperature effects on pacemaker cells, then cardiac filling time is progressively reduced and the negative force–frequency relationship common to many fish hearts (Shiels et al., 2002) may further constrain on V_s at high temperature. It can also be speculated that mobilization of blood from the venous capacitance vasculature is impaired at high temperatures that may result in a reduced cardiac filling pressure, which limits V_s (Sandblom and Axelsson, 2007). While all of these factors could contribute to greater or lesser extents in high temperature fatigue, central cardiac control cannot be excluded. We observed considerable cardiorespiratory synchrony at high temperature (data not shown). Randall reported that efferent bursting activity recorded from the cardiac vagus of tench (*Tinca tinca* Linnaeus) was synchronous with the mouth-opening phase of the respiratory cycle (Randall, 1966). Furthermore, recent data show that vagal burst activity can entrain f_H (E. W. Taylor, personal communication), opening the possibility for reflex control of f_H.

Limitations on tissue oxygen extraction

To compensate for the temperature independence of Ta_{O_2} for sockeye salmon swimming above 17°C, $A\dot{V}_{O_2}$ was increased near fatigue. Increased $A\dot{V}_{O_2}$ is a common response of salmonids during incremental velocity swimming so that the increased oxygen demand of locomotory muscles is satisfied (Stevens and Randall, 1967; Kiceniuk and Jones, 1977; Gallaugher et al., 2001; Brauner et al., 2000), and a similar plateau in venous P_{O_2} was observed for swimming rainbow trout just before U_{crit} was reached (Farrell and Clutterham, 2003). Heath and Hughes (Heath and Hughes, 1973) acutely warmed resting rainbow trout and observed a constant $A\dot{V}_{O_2}$

with increased temperature but this was because arterial and venous C_{O_2} both decreased in proportion, a response that was not observed in the present experiments. In itself, the modest 50% increase in $A\dot{V}_{O_2}$ observed here suggests that oxygen extraction by locomotory muscle can increase when sockeye salmon exceeded their aerobic threshold at high temperature and that tissue oxygen delivery does not become diffusion limited. However, the observed plateau in venous P_{O_2} with increasing temperature in swimming fish seems to argues for such a diffusion limitation, which would be in accord with the previous proposal for rainbow trout swimming at high temperature (Taylor et al., 1997). In fact, the plateau in venous P_{O_2} in swimming rainbow trout just before U_{crit} has been similarly interpreted as evidence for a diffusion limitation on tissue oxygen extraction (Farrell, 2007a). In the present study, the increase in $A\dot{V}_{O_2}$ without a decrease in P_{O_2} probably came about because increasing temperature causes a right-shift in the HbO_2 dissociation curve, i.e. there was greater unloading of oxygen from Hb. We then speculate that venous P_{O_2} could not decrease any further due to a diffusion limitation. Even so, other possibilities besides an oxygen diffusion limitation could explain the present results, the most likely being that tissue oxygen utilization decreases at high temperature. A decrease in tissue oxygen utilisation could arise because either the high temperatures begin to impair cellular function in swimming fish or the rate of ATP generation by oxidative phosphorylation is simply not fast enough for the tissue ATP demand and so they switch to a faster rate of ATP generation through glycolysis, much like the gait transition that salmonids undertake as they switch from red to white muscle powered locomotion when they swim faster.

If a diffusion limitation for oxygen extraction does exist in white muscle during locomotion, it may represent important cardioprotective mechanism because the venous blood still has to supply oxygen to the ventricle in fish such as salmon (Davie and Farrell, 1991) and this oxygen supply is thought to be ultimately limited by the venous P_{O_2} (Farrell, 1987). For swimming fish, venous P_{O_2} remained between 22 and 28 Torr with increasing temperature. Lower venous P_{O_2} values of around 10 Torr have been reported for resting hypoxic fish (Davie and Farrell, 1991), as well as for rainbow trout swimming moderately in hypoxic water (Steffensen and Farrell, 1998). A higher myocardial oxygen demand may account for a higher venous P_{O_2} for sockeye salmon exercising at high temperature (Farrell, 2007b). Indeed, Farrell and Clutterham (Farrell and Clutterham, 2003) found a higher venous P_{O_2} threshold value of 29 Torr for warm-acclimated (13–16°C) than the 15 Torr for cold-acclimated (6–10°C) swimming rainbow trout. In addition, the threshold oxygen concentration for the working perfused rainbow trout heart is greatly increased at high temperature if acidotic and high K^+ conditions exist (Hanson et al., 2006; Hanson and Farrell, 2007).

Recovery of sockeye salmon

Despite experiments lasting many hours, and a swim challenge in one group of fish, sockeye salmon had largely recovered their cardiorespiratory status after only 1 h at 15°C. $\dot{M}_{O_2}$ of resting fish was only 30% higher and $\dot{Q}$ and f_H were only 17% higher than routine values whereas swimming fish had 66% higher $\dot{M}_{O_2}$ and 33% higher $\dot{Q}$ and f_H values. All other parameters were restored to the initial routine levels, with only plasma lactate and cortisol as exceptions. The release of lactate from the exhausted muscles to the blood does occur over several hours (Milligan et al., 2000), thus explaining the elevated lactate concentration after just 1 h recovery. In addition, salmon recovered while resting and moderate activity keeps plasma lactate and cortisol levels down (Farrell et al., 2001; Milligan et al., 2000).

The type of physiological resilience exhibited here by sockeye salmon is probably needed while they experience large daily temperature fluctuations of up to 6°C (Lee et al., 2003) and up to 10°C over the course of an approximately three weeks upriver migration (Idler and Clemens, 1959). The rapid recovery observed here may be related to a faster metabolic recovery at higher temperatures as shown for other salmonids after being chased to exhaustion (Kieffer and Tufts, 1996; Galloway and Kieffer, 2003).

In summary, we conclude that high temperature fatigue in swimming sockeye salmon is due to a cardiac limitation resulting in insufficient oxygen delivery to working muscles. To compensate, oxygen extraction by skeletal muscle did increase modestly at fatigue but diffusion of oxygen to skeletal muscles was probably restricted. By contrast, oxygen uptake over the gills was not limited and is therefore insignificant in explaining high temperature fatigue of sockeye salmon.

LIST OF ABBREVIATIONS

$A\dot{V}_{O_2}$	arteriovenous O_2 delivery (O_2 extraction)
Ca_{O_2}	O_2 concentration of arterial blood
C_{O_2}	O_2 content
Cv_{O_2}	O_2 concentration of venous blood
f_H	heart rate
Hb a	haemoglobin, arterial
Hct a	haematocrit, arterial
Hct v	haematocrit, venous
$\dot{M}_{O_2}$	metabolic rate (rate of O_2 consumption)
Pa_{O_2}	partial pressure of O_2, arterial
pHa	pH of arterial blood
pHv	pH of venous blood
P_{O_2}	partial pressure of O_2
Pv_{O_2}	partial pressure of O_2, venous
$\dot{Q}$	cardiac output
RM ANOVA	repeated-measures analysis of variance
Ta_{O_2}	arterial O_2 delivery
T_{crit}	critical temperature
T_{opt}	optimum temperature
U_{crit}	critical swimming speed
V_s	cardiac stroke volume

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