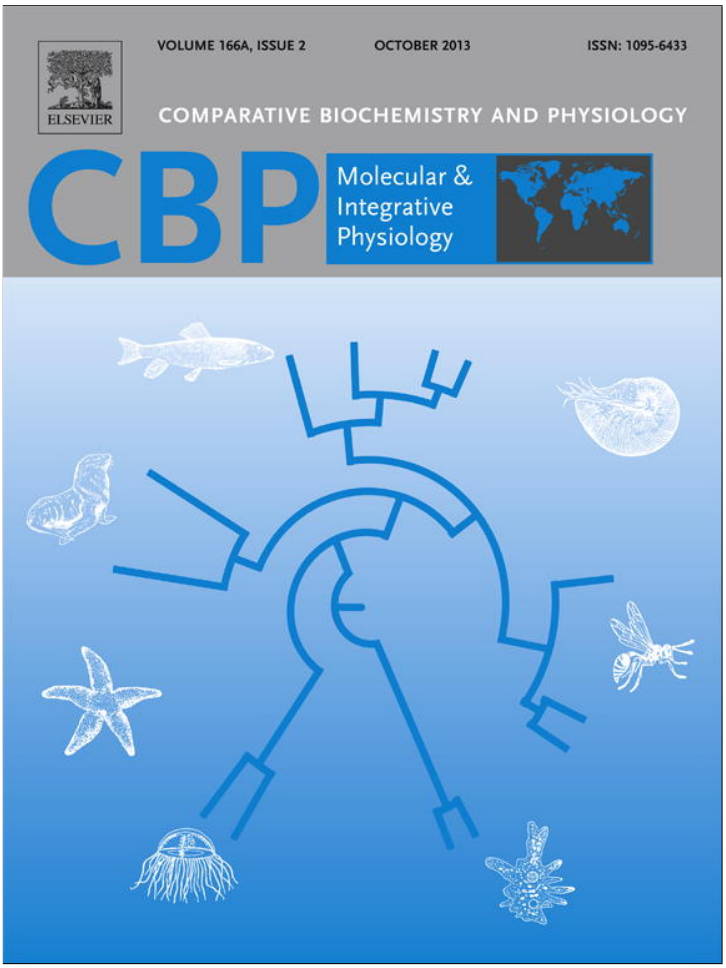

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Cardiorespiratory performance and blood chemistry during swimming and recovery in three populations of elite swimmers: Adult sockeye salmon

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ABSTRACT

Every year, millions of adult sockeye salmon (*Oncorhynchus nerka*) perform an arduous, once-in-a-lifetime migration up the Fraser River (BC, Canada) to return to their natal stream to spawn. The changes in heart rate, stroke volume, and arterio-venous oxygen extraction (i.e., factors determining rates of oxygen delivery to the tissues by the cardiovascular system) have never been directly and simultaneously measured along with whole animal oxygen uptake in a maximally swimming fish. Here, such measurements were made using three sockeye salmon populations (Early Stuart, Chilko and Quesnel), which each performed two consecutive critical swimming speed (U_{crit}) challenges to provide a comprehensive quantification of cardiovascular physiology, oxygen status and blood chemistry associated with swimming and recovery. Swim performance, oxygen uptake, cardiac output, heart rate and stroke volume did not significantly vary at rest, during swimming or during recovery between populations or sexes. Despite incomplete metabolic recovery between swim challenges, all fish repeated their swim performance and similar quantitative changes in the cardiorespiratory variables were observed for each swim challenge. The high maximum cardiorespiratory performance and excellent repeat swim performance are clearly beneficial in allowing the salmon to maintain steady ground speeds and reach the distant spawning grounds in a timely manner.

1. Introduction

Cardiorespiratory support of locomotion can be represented by the Fick equation for circulatory oxygen convection in which the rate of oxygen delivery to tissues is determined by the product of cardiac output ($\dot{V}_b$) and the difference between arterial and venous blood oxygen contents (C_{aO2} and C_{vO2}, respectively), the latter being termed the tissue oxygen extraction ($A\text{-}V_{O2} = C_{aO2} - C_{vO2}$). Despite an extensive literature on swimming and cardiovascular performance of fish (e.g. Stevens and Randall, 1967; Brett, 1971; Kiceniuk and Jones, 1977; Lai et al., 1990; Thorarensen et al., 1996a; Korsmeyer et al., 1997b; Gallaugher et al., 2001), to date no study has simultaneously and directly measured all the components of the Fick equation in any fish swimming maximally. Steinhausen et al. (2008) did make all the critical measurements, but with fish swimming only up to ~75% of their critical swimming speed (U_{crit}). Previous studies have either assumed or indirectly calculated one or more of the variables in the Fick equation. For example, two classic studies with salmonids (Brett, 1971; Kiceniuk and Jones, 1977) calculated $\dot{V}_b$ using the Fick equation. However, such calculations do not account for any oxygen directly consumed by the gills (thought to be 10–30% of routine oxygen uptake; see Thorarensen et al., 1996b), which likely results in an overestimate of cardiac output. Such uncertainty concerning a key cardiorespiratory variable is unacceptable if adaptations and mechanistic limitations of maximum exercise capacity are to be properly understood in fishes. Therefore, the objective of this study was to measure the integrated cardiorespiratory response of maximally swimming fish by directly and simultaneously measuring all the variables associated with

Abbreviations: $A\text{-}V_{O2}$, tissue oxygen extraction ($A\text{-}V_{O2} = C_{aO2} - C_{vO2}$); C_{aO2}, arterial blood oxygen content; C_{vO2}, venous blood oxygen content; COT, cost of transport ($COT = \dot{M}O_2 / (U \times 60)$); COT_{net}, net cost of transport ($COT_{net} = (\dot{M}O_2 - \dot{M}O_{2rest}) / (U \times 60)$); $COT\text{-}V_b$, cardiovascular cost of transport ($COT\text{-}V_b = V_b / (U \times 60)$); $COT\text{-}V_{bnet}$, net cardiovascular cost of transport ($COT\text{-}V_{bnet} = (V_b - V_{brest}) / (U \times 60)$); EPOC, excess post-exercise oxygen uptake; f_H, heart rate; [Hb], haemoglobin concentration; Hct, haematocrit; MCHC, mean corpuscular haemoglobin concentration ($MCHC = [Hb] / (Hct / 100)$); $\dot{M}O_2$, rate of oxygen uptake; P_{aO2}, arterial blood partial pressure of oxygen; P_{vO2}, venous blood partial pressure of oxygen; RR, recovery ratio for consecutive swim challenges ($RR = U_{crit}\,2 \div U_{crit}\,1$); T_{opt}, optimal temperature for aerobic scope; T_{aO2}, arterial blood oxygen transport ($T_{aO2} = V_b \times C_{aO2}$); T_{vO2}, venous blood oxygen transport ($T_{vO2} = V_b \times C_{vO2}$); U_{crit}, critical swimming speed; V_b, cardiac output; V_s, stroke volume ($V_s = V_b \div f_H$).

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oxygen delivery to the tissues by the cardiovascular system as described above.

Fraser River sockeye salmon (*Oncorhynchus nerka*) were chosen as the model organism for two reasons. Foremost, we wished to examine a fish with a remarkable swimming prowess, an elite athlete in the piscine world. Every year, a new generation of millions of sockeye salmon returns from the ocean to the Fraser River (British Columbia, Canada) to perform a physically demanding upriver migration to reach spawning grounds. During this highly aerobic and energetically expensive migration, sockeye salmon swim continuously against a fast flowing river for several weeks at ground speeds of 20 to 40 km day^{-1} (Hinch and Rand, 1998; English et al., 2005). The salmon have a finite amount of time (typically 3–6 weeks) and energy to complete the river migration because they cease feeding in the ocean and must spawn around a specific date. Upriver swimming, reproductive maturation (secondary sexual characteristics, gonadal growth) and spawning are fuelled entirely by limited endogenous energy stores. Given the time constraint, it is critical that sockeye salmon recover rapidly from exhaustive exercise (often used in rapids) to prevent a delay in their continuous upriver migration.

The second reason for studying Fraser River sockeye salmon is their remarkable fidelity to return to their natal stream to spawn (Burgner, 1991), which has resulted in over 100 genetically and geographically distinct populations (Beacham et al., 2005). These populations experience highly variable environmental conditions during the spawning migration depending on spawning location and river entry timing. Migration distance varies 10-fold (100 to >1100 km), elevation gain varies 100-fold (10 to 1200 m), river temperature varies from 9° to 22 °C and river flow varies 5-fold [2000 to 10,000 $m^3\ s^{-1}$ (Eliason et al., 2011)]. Furthermore, because sockeye salmon are semelparous (only spawn once), individual fish have a single opportunity to complete the journey to their spawning grounds in order to reproduce. As a corollary, there is likely strong selection pressure for successful upstream migration, which then leads to the possibility that adult sockeye salmon have adapted to the particular set of river migration challenges that they face.

The present experiments focused on the cardiorespiratory performance of three interior sockeye salmon populations (Early Stuart, Chilko and Quesnel). All three populations must traverse major hydrological barriers in the Fraser Canyon (including Hells Gate, located 180 km upstream from the mouth of the Fraser River). Hells Gate and its adjacent river reaches are the most energetically costly sections of the river and likely require maximum aerobic scope as well as anaerobic swimming to be negotiated (Hinch et al., 1996; Hinch and Rand, 1998; Hinch and Bratty, 2000). These three populations travel between 642 and 1071 km upstream to spawn at elevations of between 690 and 1174 m while encountering similar modal and median (16–17 °C) river temperatures (Eliason et al., 2011). Previous work demonstrated that these populations have a similar optimal temperature for aerobic scope (T_{opt}: ~17 °C), and similar maximum aerobic scope, cardiac scope and scope for heart rate (Eliason et al., 2011). However, Eliason et al. (2011) did not systematically report on how these populations mechanistically support their impressive swimming performance and aerobic scope.

We performed a detailed investigation of how the sockeye salmon cardiorespiratory system supports swimming performance during two sequential U_{crit} critical swimming speed challenges at T_{opt} for aerobic scope. In addition to investigating the general question of how cardiac function is affected by repeated bouts of exercise, we examined five specific hypotheses: 1) oxygen delivery to the tissues is supported by equivalent increases in V_b and $A\text{-}V_{O2}$; 2) increased cardiac stroke volume is the primary means of increasing arterial oxygen delivery to tissues; 3) arterial oxygen saturation is maintained during maximal swimming; 4) the concentration of oxygen returning in venous blood to the heart remains well in excess of the oxygen needs of the heart when it is working maximally to support locomotion; and 5) cardiorespiratory support of aerobic scope is similar among three populations facing a long, arduous migratory challenge.

2. Materials and methods

2.1. Fish collection

Wild adult sockeye salmon were collected in 2007, 2008 and 2009 from the lower Fraser River while en-route to their spawning grounds using a beach seine or gill net. The salmon were collected early in their river migration (after only ~100 km), which translates to 1–3 days after entry into freshwater from temperatures of ~10–12 °C in the ocean. Following capture, the fish were transported 25–75 km by land to the Department of Fisheries and Oceans Cultus Lake Salmon Research Laboratory (Cultus Lake, BC, Canada). All fish were given a unique PIT (Passive Integrated Transponder, Biomark Inc., Boise, Idaho, USA) tag for individual identification, and <0.1 g of the adipose fin was clipped for population identification via DNA analysis (Beacham et al., 2005). Since the DNA analysis takes several days to complete, experiments commenced without knowledge of the specific populations to which the fish belonged. Many different populations of sockeye salmon enter the Fraser River at the same time, co-migrate upriver and cannot be visually distinguished, thus it is impossible to guarantee in advance which population will be captured. While it would have been desirable to compare populations across a broader range of migration difficulties, sufficient fish were captured from only three interior populations with highly challenging upriver migrations: Early Stuart population (N = 9, distance: 1071 km, elevation: 690 m), Chilko population (N = 13, distance: 642 km, elevation: 1174 m) and Quesnel population (N = 6, distance: 796 km, elevation: 728 m). Other characteristics associated with each of the adult migrations (e.g. peak Fraser River entry, peak spawning ground arrival, migration rate, migration duration, work, river slope, migration effort) are detailed in Eliason et al. (2011). All procedures were approved by the University of British Columbia's Animal Care Committee (Animal use protocols A06-0328 and A08-0388) in accordance with guidelines recommended through the Canadian Council on Animal Care.

Fish were held at 11–12 °C for 1–4 weeks in outdoor 8000–12,000 L circular aquaria supplied with filtered and UV sterilised freshwater (LS-PermaBead Filtration System, Integrated Aqua Systems Inc., Escondido, CA, USA) under seasonal photoperiod. The fish were not fed because they had ceased feeding naturally before entering the Fraser River. Three days before the swimming challenge, fish were placed in 1400 L circular aquaria and the temperature was progressively increased to the test temperature (15–20 °C) by no more than 5 °C day^{-1}. The fish were maintained at this temperature for 24–48 h before experiments.

2.2. Surgical procedures

Individual fish were anaesthetised with buffered tricaine methanesulfonate in freshwater (0.2 g L^{-1} $NaHCO_3$ and 0.1 g L^{-1} MS-222, Sigma-Aldrich, St. Louis, MO, USA), weighed and transferred onto wet foam on a surgical table where their gills were continually irrigated with aerated, chilled freshwater with a lower dose of buffered anaesthetic (0.15 g L^{-1} $NaHCO_3$ and 0.075 g L^{-1} MS-222). Surgical procedures have been detailed elsewhere (Steinhausen et al., 2008). To sample arterial blood, a PE-50 cannula was inserted into the dorsal aorta (Soivio et al., 1973). To measure $\dot{V}_b$, a 3 mm SB flow probe (lateral cable exit, Transonic Systems, Ithaca, NY, USA) was positioned around the ventral aorta without opening the pericardium (Steffensen and Farrell, 1998). To sample venous blood, a PE-50 cannula was inserted into the ductus of Cuvier and advanced towards the heart into the sinus venosus (Farrell and Clutterham, 2003). Both cannulae were filled and regularly flushed with heparinised saline solution (150 IU mL^{-1}). The flow probe and cannulae leads were secured together and sutured to the dorsal line of the fish's body using 2-0 silk. The fish were placed individually in one of two Brett-type swim tunnels (described in Lee et al., 2003b; Steinhausen et al., 2008) and allowed to recover overnight at their test temperature

at a low water velocity of ~0.39 body lengths per second (bl s^{-1}), a velocity that provided the fish an orientation without inducing it to actively swim.

2.3. U_{crit} critical swimming challenge

Following overnight recovery (>8 h) from surgery in the swim tunnel at their test temperature (15–20 °C), resting values for oxygen uptake rate from the water ($\dot{M}O_2$) and cardiac output ($\dot{V}_b$) were measured at a water velocity of ~0.39 bl s^{-1} and arterial and venous blood samples were taken (see below). Then the fish underwent a U_{crit} critical swimming speed challenge (Jain et al., 1997; Lee et al., 2003b). The velocity of the water was increased every 5 min until approximately 50% of U_{crit} was reached (~1.0 bl s^{-1}). Thereafter, the water velocity was increased by approximately 0.25 bl s^{-1} every 20 min until the fish no longer swam continuously and rested on the back grid for >30 s. The water velocity was immediately reduced to the resting velocity (fatigue), and then the fish were allowed a 45-min recovery before the same U_{crit} critical swimming speed challenge was repeated. Hereafter, the U_{crit} critical swimming speed challenges are referred to as the first swim challenge and second swim challenge. Following the second swim challenge, fish remained in the swim tunnel for 2 h before being removed and sacrificed by a cranial blow. To test the impact of surgery on swimming performance, four additional Early Stuart fish were not instrumented and were tested in exactly the same way as the other fish. Data from these non-instrumented fish are only reported in Table 1.

$\dot{V}_b$ was measured continuously at 200 Hz throughout the swim trials by connecting the flow probe to a flowmeter (Transonic Systems, Ithaca, NY, USA) interfaced with BIOPAC hardware and Acknowledge software (BIOPAC Systems, Santa Barbara, CA, USA). $\dot{V}_b$ was calculated as the mean of 3 to 6 segments of continuous 30-s traces. Heart rate (f_H) was measured from the same $\dot{V}_b$ flow trace using automated software, which was confirmed with manual counting. Stroke volume (V_s) was calculated as $V_s = \dot{V}_b \div f_H$.

$\dot{M}O_2$ was measured during the second half of every 20-min velocity interval using an OxyGuard probe (Point Four Systems, Richmond, BC, Canada) attached to a WinDaq box (DATAQ Instruments, Akron, ON, USA) interfaced with LabVIEW software (6.0, National Instruments, Austin, TX, USA). The duration of the measurement was sufficient so that the dissolved oxygen decreased by at least 0.3 mg O_2 L^{-1}, which resulted in a linear regression with r^2 values typically >0.95. If the dissolved oxygen levels approached 70%, an $\dot{M}O_2$ measurement was deliberately suspended to ensure a near normoxic environment in the swim tunnel. Microbial (background) $\dot{M}O_2$ was measured after each swim trial and determined to be negligible.

Table 1
Measurements of oxygen uptake ($\dot{M}O_2$), critical swimming speed (U_{crit}) and recovery ratio (RR) in Early Stuart sockeye salmon swum at T_{opt} for aerobic scope that had (with leads) and had not (no leads) been instrumented with a flowprobe and catheters to measure cardiovascular variables. Mean ± SEM are presented, an asterisk indicates a statistically significant difference between fish with leads and those without ($p < 0.05$).

$\dot{M}O_2$ (mg O_2 kg^{-1} min^{-1})	n	No leads	n	With leads
Rest	4	2.6 ± 0.2	8	3.2 ± 0.2
Maximum	4	14.4 ± 1.4	8	14.7 ± 0.4
Scope	4	11.9 ± 1.3	7	11.7 ± 0.3
Fatigue 1	3	8.6 ± 0.2	7	10.0 ± 0.8
Fatigue 2	4	9.0 ± 2.3	7	8.9 ± 0.8
45-min recovery 1	4	4.2 ± 0.9	7	6.6 ± 0.3*
45-min recovery 2	4	6.3 ± 1.4	7	5.4 ± 0.8
2-h recovery 2	4	4.0 ± 0.7	7	3.9 ± 0.4
U_{crit} 1 (bl s^{-1})	4	2.41 ± 0.13	9	2.02 ± 0.06*
U_{crit} 2 (bl s^{-1})	4	2.35 ± 0.19	8	1.91 ± 0.06*
U_{crit} 1 (cm s^{-1})	4	144.1 ± 7.6	9	122.1 ± 4.1*
U_{crit} 2 (cm s^{-1})	4	140.6 ± 12.1	8	114.4 ± 3.5*
RR	4	0.97 ± 0.05	8	0.95 ± 0.02

Blood samples (~0.7 mL per sample) were strategically collected: (1) at rest; (2) during the second half of the first 20-min swim interval during steady swimming ("steady": mean speed = 1.18 ± 0.02 bl s^{-1}, or 55.8 ± 0.9% of maximum swim speed); (3) when the fish exhibited burst-and-coast swimming near exhaustion ("burst": mean speed = 2.05 ± 0.06 bl s^{-1} or 92.6 ± 1.7% of maximum swim speed); (4) immediately after the fish quit swimming (fatigue); (5) after 45 min of recovery; and (6) a final sample after a 2-h recovery period following the second swim challenge before the fish were sacrificed. Each blood sample was replaced by an equivalent volume of saline.

2.4. Whole blood and plasma analyses

Whole blood samples were collected anaerobically through the arterial and venous cannulae and used to measure partial pressure of oxygen (P_{O2}; 1 Torr = 0.133 kPa), oxygen content (C_{O2}), haemoglobin concentration ([Hb]) and haematocrit (Hct). The blood samples were held in gas-tight syringes at 4 °C and analysed within 4 h after collection. Blood P_{O2} was measured using a blood gas monitor (PHM 73, Radiometer, Copenhagen, Denmark), which was calibrated and maintained at the experimental temperature using a water jacket. Blood C_{O2} was measured according to the method of Tucker (1967). [Hb] was measured using either a handheld haemoglobin analyzer (HemoCue 201$^+$, Ängelholm, Sweden) calibrated for fish blood (Clark et al., 2008a) or the spectrophotometer method with Drabkin's solution (Drabkin and Austin, 1935). Hct was measured in duplicate using microhematocrit capillary tubes spun at 10,000 *g*. The remaining blood was centrifuged at 7000 *g* and the plasma was flash frozen in liquid nitrogen and stored at −80 °C for subsequent analyses. Plasma glucose and lactate (YSI 2300 STAT Plus analyzer), sodium and potassium (Cole-Parmer, model 41-single channel flame photometer) and chloride (Haake Buchler digital chloridometer) were measured as described previously (Farrell et al., 2001).

2.5. Data analysis and statistics

While additional fish were swum over a larger range of temperatures for accompanying experiments [9–26 °C (Eliason et al., 2011)], the present study examines fish swum only at temperatures within their T_{opt} window [90–100% of population-specific maximum aerobic scope = 15–20 °C for Early Stuart, Chilko and Quesnel, see Eliason et al. (2011)]. Maximum aerobic scope, cardiac scope and scope for heart rate at T_{opt} for these three populations are presented in Eliason et al. (2011).

Since no statistically significant differences between sexes were detected for any of the cardiorespiratory variables, data from males and females were pooled to improve statistical power for the population comparisons.

U_{crit} was calculated using established methods (Brett, 1964) after accounting for the solid blocking effect as outlined in Bell and Terhune (1970). The recovery ratio (RR) for sequential swim challenges was calculated as RR = U_{crit} 2 ÷ U_{crit} 1 (Jain et al., 1998) to determine how the first U_{crit} compared to the second U_{crit}.

Cost of transport (COT) was calculated as: COT = $\dot{M}O_2$ / (U × 60) where $\dot{M}O_2$ was measured in mg O_2 kg^{-1} min^{-1} and U was the swimming speed in m s^{-1}. Net cost of transport (COT_{net}) was calculated as: COT_{net} = ($\dot{M}O_2$ − $\dot{M}O_{2rest}$) / (U × 60). Similarly, cost of transport for cardiac output (COT-$\dot{V}_b$) and net cost of transport for cardiac output (COT-$\dot{V}_{bnet}$) were calculated.

Oxygen extraction (A-V_{O2}) was assessed only for fish that had simultaneous arterial and venous samples, and was calculated as arterial blood oxygen content (C_{aO2}) − venous blood oxygen content (C_{vO2}). Arterial oxygen transport (T_{aO2}) to the tissues was calculated as the product of $\dot{V}_b$ and C_{aO2}. Venous oxygen transport (T_{vO2}) to the spongy myocardium was calculated as the product of $\dot{V}_b$ and C_{vO2}. Mean

corpuscular haemoglobin concentration (MCHC) was calculated as [Hb] / (Hct / 100).

Aerobic scope and cardiac scope were determined as the difference between individual resting and maximum values. Scope for f_H and scope for V_s were similarly determined as the difference between the resting values and those measured at maximum $\dot{V}_b$.

All data are presented as mean ± SEM, unless otherwise indicated. p-Values less than 0.05 were considered statistically significant. Independent data were compared using a t-test, one-way ANOVA or two-way ANOVA, as appropriate. Dependent data were compared using a paired t-test, one-way repeated measures ANOVA or a two-way repeated measures ANOVA, as appropriate. When the requirement for normal distribution and equal variance could not be met after transformation, the data were compared using the appropriate non-parametric test (e.g. Mann–Whitney U-test, Kolmogorov–Smirnov test, Kruskal–Wallis test). A post-hoc Holm–Sidak or Dunn's test was used to test for differences among groups.

3. Results

Body mass and fork length (mean ± SEM) were similar for the three populations: Early Stuart 2.36 ± 0.05 kg, 60.3 ± 0.8 cm; Chilko 2.33 ± 0.18 kg, 59.5 ± 1.2 cm; and Quesnel 2.53 ± 0.17 kg, 60.0 ± 1.5 cm.

3.1. Effect of surgical intervention on performance

Early Stuart sockeye salmon swimming without the encumbrance of leads had an 18–23% significantly higher U_{crit} compared to salmon that had undergone surgery and swam with leads (Table 1). However, there were no significant differences in $\dot{M}O_{2rest}$, $\dot{M}O_{2max}$ or aerobic scope between these groups, suggesting that fish had recovered their routine $\dot{M}O_2$ following surgery and performed to their maximum aerobic scope. Fish with leads appeared to recover slower after the first swim challenge since $\dot{M}O_2$ was significantly higher after the first 45-min recovery period, but not after the 45-min or 2-h recovery periods following the second swim challenge (Table 1).

3.2. First swim challenge

U_{crit} did not significantly differ among populations or between sexes (Table 2). Active $\dot{M}O_2$, $\dot{V}_b$, V_s and f_H were not significantly different among populations or between sexes for any swimming speed (Figs. 1, 3, Tables 3, 4). Swimming maximally increased $\dot{M}O_{2rest}$ by ~5-fold for all populations (Fig. 1). Notably, COT did not display the characteristic U-shape, but instead it plateaued between swimming speeds of 1.12 and 2.37 bl s^{-1} (Fig. 2). During the first swim challenge, the ~3-fold increase in $\dot{V}_b$ was supported by a ~2-fold increase in V_s and 50% increase in f_H (Fig. 3).

Blood gas and chemistry were characterised using pooled data from the three populations. P_{aO2}, P_{vO2}, C_{aO2} and C_{vO2} all decreased significantly during swimming when compared with the rest (Fig. 4). P_{vO2} and C_{vO2} decreased to 17.6 ± 2.8 Torr and 25 ± 3 mL L^{-1}, respectively, during burst swimming and were unchanged at fatigue (Fig. 4).

The tendency for A-V_{O2} (calculated from paired blood samples from individual fish, i.e., fish that had both cannulae working simultaneously) to increase during swimming did not reach statistical significance (Fig. 5). Arterial transfer of oxygen to the tissues (T_{aO2}), which integrates changes in $\dot{V}_b$ and C_{aO2} ($T_{aO2} = \dot{V}_b \times C_{aO2}$), increased 2.5-fold during burst swimming (Fig. 5). In contrast, venous transfer of oxygen to the spongy myocardium ($T_{vO2} = \dot{V}_b \times C_{vO2}$) remained unchanged throughout the entire swimming protocol (Fig. 5).

When only paired blood samples for an individual fish were analysed, arterial and venous blood [Hb] were similar, except at steady 1 (Table 5). During burst swimming and at fatigue, Hct remained higher and MCHC lower in venous compared to arterial blood (Table 5). Plasma lactate, glucose, chloride and sodium varied minimally between arterial and venous blood (Fig. 4, Table 5). In contrast, plasma potassium was highly variable between arterial and venous blood samples. In general, plasma potassium was higher in arterial relative to venous blood (Table 5). None of the blood or plasma variables varied from resting levels during the first swim challenge, except for plasma lactate, which became significantly elevated at fatigue, and plasma potassium significantly decreased during burst swimming (Fig. 4, Table 5).

3.3. First swim challenge vs. second swim challenge

All fish repeated their swim performance following the 45-min recovery period. U_{crit} 1 did not significantly differ from U_{crit} 2 (overall mean maximum U_{crit} = 2.04 ± 0.04 bl s^{-1}) and RR was ≥0.95 (Table 2).

Active $\dot{M}O_2$, COT and COT_{net} did not differ significantly between the first and second swim challenges (Figs. 1, 2, Table 3). Active $\dot{V}_b$ was not significantly different between the two swim challenges (Fig. 3, Tables 3, 4), except at the first swim speed (0.62 bl s^{-1}) which was during the ramping phase of the U_{crit} protocol. Consequently, COT-$\dot{V}_b$ and COT-$\dot{V}_{bnet}$ only significantly differed between the two swim challenges at 0.62 bl s^{-1} (Fig. 2). Active V_s was not significantly different for the two swim challenges (Fig. 3, Tables 3, 4), although scope for V_s was significantly higher in the second swim challenge for Quesnel fish (Table 4). Notably, f_H was significantly higher at the lower swim speeds of the second swim challenge (Fig. 3). Maximum f_H and scope for f_H did not differ for the two swim challenges (Table 4).

P_{aO2}, P_{vO2}, C_{aO2}, C_{vO2}, T_{aO2}, and T_{vO2} did not differ between the two swim challenges (Figs. 4, 5). In order to investigate the possibility of progressive anaemia via haemodilution, fish with two functioning cannulae (n = 10), which had twice as much blood removed (~20 blood samples collected total, ≅14 mL of blood), were compared with those with one functioning venous cannula (~10 blood samples, ≅7 mL of blood) in Fig. 6. Fish with two cannulae had a significantly lower [Hb] and Hct during the second swim challenge but not the first swim challenge compared to fish with one functioning cannula (Fig. 6). Haemodilution during the second swim challenge had no significant effect on P_{vO2}, but C_{vO2} was lower during the final recovery period for fish with two cannulae relative to those with one cannula (Fig. 6).

Plasma lactate remained significantly elevated from the first swim challenge during steady swimming in the second swim challenge, resulting in a significant difference between the first and second swim challenges (Fig. 4). Notably, plasma lactate tended to recover by the time fish started burst swimming during the second swim challenge (Fig. 4). Plasma chloride and sodium were lower during the second swim challenge compared to the first (Table 5). However, these differences in plasma chloride and sodium may have been due to haemodilution since they were not present when all blood

Table 2
Measurements of critical swimming speed (U_{crit}) and the recovery ratio (RR) in three populations of sockeye salmon at their T_{opt}. Mean ± SEM are presented, there were no significant differences in U_{crit} between sexes, among populations or between swim 1 and swim 2 ($p > 0.05$).

Population	n	U_{crit} (bl s^{-1})		U_{crit} (cm s^{-1})		RR
		U_{crit} 1	U_{crit} 2	U_{crit} 1	U_{crit} 2	
Early Stuart	8–9	2.02 ± 0.06	1.91 ± 0.06	122.1 ± 4.1	114.4 ± 3.5	0.95 ± 0.02
Chilko	9–13	1.99 ± 0.08	1.95 ± 0.09	117.8 ± 3.8	114.2 ± 4.9	0.97 ± 0.03
Quesnel	6	2.02 ± 0.11	1.98 ± 0.04	121.1 ± 6.3	118.7 ± 3.2	0.99 ± 0.05

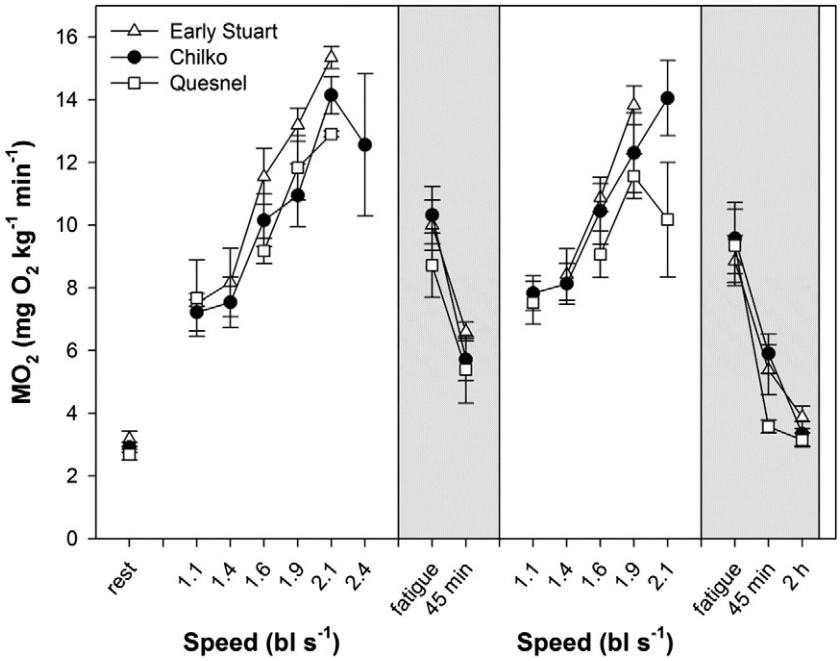

Fig. 1. Oxygen uptake rate ($\dot{M}O_2$) with swimming speed over two consecutive critical swimming speed challenges in three populations of sockeye salmon (Early Stuart n = 9; Chilko n = 13; Quesnel n = 6). Swimming speed at rest and during recovery (fatigue, 45 min, 2 h) was ~0.39 bl s^{-1} and mean ± SEM are presented. Shaded areas indicate the recovery periods, starting with the fatigue value collected immediately following the U_{crit} swim challenge. There were no significant differences among populations or between sexes.

samples were analysed (data not shown). Plasma potassium was highly variable between swim challenges (in both paired and unpaired blood samples). Plasma potassium tended to be higher during the second swim challenge compared to the first (Table 5).

3.4. Recovery

$\dot{M}O_2$ was not fully recovered by the end of the 45-min recovery period after either swim challenge, but $\dot{M}O_{2rest}$ was restored after the 2-h recovery period following the second swim challenge for all three populations (Table 3). V_b did not fully recover during any of the recovery periods (Table 3). V_s fully recovered after a 45-min recovery with both swim challenges (Table 3). Notably, f_H failed to recover at all after the first swim challenge (Fig. 2, Table 3). Instead, f_{Hmax} was maintained at an elevated level throughout the recovery period and second swim challenge, except for a brief decrease at fatigue (Fig. 3, Table 3). f_H remained elevated above resting levels at the 2-h recovery period (Table 3).

Both P_{aO2} and P_{vO2} returned to resting levels after each 45-min recovery period (Fig. 4), suggesting that oxygen status of the blood recovered more rapidly than f_H and V_b. However, following the second swim challenge, C_{aO2} and C_{vO2} remained depressed below resting levels (Fig. 4), likely due to haemodilution as a result of blood sampling. T_{aO2} returned back to resting levels after both 45-min recovery periods, while T_{vO2} remained steady throughout the swimming protocol and recovery periods (Fig. 5).

Plasma lactate was elevated above resting levels at fatigue and during the 45-min recovery periods following both swim challenges,

Table 3
Recovery measurements for oxygen uptake rate ($\dot{M}O_2$), cardiac output (V_b), heart rate (f_H) and stroke volume (V_s) in three sockeye salmon populations after two consecutive U_{crit} critical swimming speed challenges. Measurements were made at rest, immediately after the fish quit swimming (fatigue), 45 min after fatigue (45-min recovery) and 2 h after the conclusion of the second swim challenge (2-h recovery). Mean ± SEM are presented. There were no significant differences between sexes or among populations. Significant differences from rest are indicated by an asterisk (*), significant differences between swim 1 and swim 2 are indicated by a double dagger (‡).

	n	Rest	Fatigue 1	Fatigue 2	45-min recovery 1	45-min recovery 2	2-h recovery 2
$\dot{M}O_2$ (mg O_2 kg^{-1} min^{-1})							
Early Stuart	5–8	3.2 ± 0.2	10.0 ± 0.8*	8.9 ± 0.8*	6.6 ± 0.3*	5.4 ± 0.8*	3.9 ± 0.4
Chilko	8–13	2.9 ± 0.2	10.3 ± 0.9*	9.6 ± 1.1*	5.7 ± 0.7*	5.9 ± 0.6*	3.3 ± 0.4
Quesnel	5–6	2.7 ± 0.2	8.7 ± 1.0*	9.3 ± 1.2*	5.4 ± 1.1*	3.6 ± 0.2*	3.1 ± 0.2
V_b (mL min^{-1} kg^{-1})							
Early Stuart	7–9	34.8 ± 2.7	72.0 ± 4.3*	79.9 ± 5.8*	45.3 ± 2.5*	49.7 ± 3.7*	47.9 ± 2.7*
Chilko	9–13	34.8 ± 2.9	77.0 ± 6.4*	80.0 ± 8.2*	49.8 ± 4.9*	56.2 ± 5.9*	53.6 ± 6.2*
Quesnel	5–6	34.7 ± 3.9	67.6 ± 8.1*	89.2 ± 9.0*‡	49.6 ± 6.6*	55.4 ± 4.4*	51.4 ± 6.5*
V_s (mL $beat^{-1}$ kg^{-1})							
Early Stuart	7–9	0.49 ± 0.03	0.83 ± 0.05*	0.92 ± 0.07*	0.46 ± 0.02	0.55 ± 0.04	0.59 ± 0.05
Chilko	9–13	0.53 ± 0.05	0.91 ± 0.06*	0.93 ± 0.08*	0.56 ± 0.05	0.60 ± 0.06	0.62 ± 0.07
Quesnel	5–6	0.57 ± 0.06	0.76 ± 0.08	0.96 ± 0.04*	0.53 ± 0.05	0.57 ± 0.03	0.54 ± 0.04
f_H (beats min^{-1})							
Early Stuart	7–9	70.1 ± 2.3	87.6 ± 3.5*	88.0 ± 5.0*	99.6 ± 3.6*	90.3 ± 3.7*	82.8 ± 2.8*
Chilko	9–13	67.3 ± 2.7	84.0 ± 2.7*	85.7 ± 2.0*	89.2 ± 2.3*	94.0 ± 3.2*	86.3 ± 3.1*
Quesnel	5–6	60.9 ± 4.7	88.5 ± 7.4*	92.6 ± 6.2*	93.1 ± 8.5*	97.7 ± 7.2*	95.1 ± 7.7*

Table 4
Maximum measurements for oxygen uptake rate ($\dot{M}O_2$), cardiac output ($\dot{V}_b$), heart rate (f_H) and stroke volume (V_s) in three sockeye salmon populations taken over two consecutive U_{crit} critical swimming speed challenges. Scope is the difference between maximum and resting values for each individual fish. Mean ± SEM are presented. There were no significant differences between sexes or among populations. Significant differences between swim 1 and swim 2 are indicated by a double dagger (‡).

	n	Max 1	Max 2	Scope 1	Scope 2
$\dot{M}O_2$ (mg O_2 kg^{-1} min^{-1})					
Early Stuart	6–7	14.5 ± 0.4	13.8 ± 0.6	11.4 ± 0.3	10.9 ± 0.5
Chilko	7–12	13.3 ± 0.6	14.0 ± 0.9	10.4 ± 0.5	10.9 ± 0.8
Quesnel	5	13.3 ± 0.5	12.0 ± 0.5	10.8 ± 0.5	9.5 ± 0.5
$\dot{V}_b$ (mL min^{-1} kg^{-1})					
Early Stuart	8–9	100.3 ± 5.2	104.1 ± 6.4	65.5 ± 4.7	68.2 ± 5.3
Chilko	9–13	105.0 ± 4.9	103.2 ± 9.3	70.2 ± 3.2	68.0 ± 6.5
Quesnel	5–6	101.9 ± 9.2	117.7 ± 12.1‡	67.2 ± 6.6	83.6 ± 7.6‡
V_s (mL $beat^{-1}$ kg^{-1})					
Early Stuart	8–9	1.08 ± 0.05	1.10 ± 0.06	0.58 ± 0.04	0.60 ± 0.04
Chilko	9–13	1.11 ± 0.05	1.12 ± 0.09	0.59 ± 0.04	0.57 ± 0.07
Quesnel	5–6	1.09 ± 0.10	1.28 ± 0.11	0.52 ± 0.07	0.69 ± 0.04‡
f_H (beats min^{-1})					
Early Stuart	8–9	93.1 ± 2.2	94.6 ± 2.8	23.0 ± 3.7	23.5 ± 3.3
Chilko	9–13	94.4 ± 1.9	92.0 ± 3.2	27.1 ± 3.5	27.4 ± 5.4
Quesnel	5–6	94.0 ± 3.6	91.3 ± 3.6	33.1 ± 3.6	34.6 ± 2.2

verifying the transition to anaerobic swimming (Fig. 4). Plasma glucose did not differ significantly from rest until a significant elevation during the second recovery period (Table 5).

3.5. General trends with swimming

By pooling the cardiorespiratory responses for the three populations (Fig. 7), it is possible to describe the generalised response of swimming to fatigue at T_{opt}. For the first swim challenge, $\dot{V}O_2$ increased 5-fold, primarily due to a 3-fold increase in $\dot{V}_b$ and secondarily through increased A-V_{O2} (Fig. 7A). A ~2-fold increase in V_s and a ~50% increase in f_H (Fig. 7B) supported the increase in $\dot{V}_b$, while a decrease in C_{vO2} (Fig. 7C) generated the increase in A-V_{O2} and compensated for the slight decrease in C_{aO2} (Fig. 7C). T_{aO2} increased by 2.5-fold because of the large increase in $\dot{V}_b$ (Fig. 7D). In contrast, T_{vO2} changed little because the increase in $\dot{V}_b$ offset the decrease in C_{vO2} (Fig. 7E). A 45-min recovery following fatigue was insufficient for full recovery of $\dot{V}O_2$, $\dot{V}_b$, f_H and C_{vO2}, but V_s, Ca_{O2}, T_{aO2}, P_{aO2}, and P_{vO2} all recovered.

A second swim challenge to the same U_{crit} with almost identical cardiorespiratory changes was possible despite incomplete cardiorespiratory recovery following the first swim challenge and a progressive haemodilution during the second swim challenge. The major difference between the first and second swim challenges was that f_H never recovered from its elevated rate during the entire second swim challenge and recovery. While $\dot{V}O_2$ recovered back to resting levels by 2 h after the second swim challenge, $\dot{V}_b$ and f_H did not, possibly as compensation for reduced A-V_{O2} during recovery due to haemodilution (Fig. 7A, B & C).

4. Discussion

The present study set out to directly and simultaneously measure all of the variables affecting rates of oxygen delivery to the tissues by the cardiovascular system (i.e., $\dot{V}_b$, f_H, V_s, C_{aO2} and C_{vO2}) for the first time in any maximally swimming fish. Cardiorespiratory performance was characterised before, during and after two exhausting swim challenges performed in rapid succession for three populations of adult Fraser River sockeye salmon that naturally face similar, difficult upriver migrations. Previous cardiorespiratory assessments during swimming with salmonids either have not swum the fish maximally to U_{crit} (Steinhausen et al., 2008), or have always relied on at least one

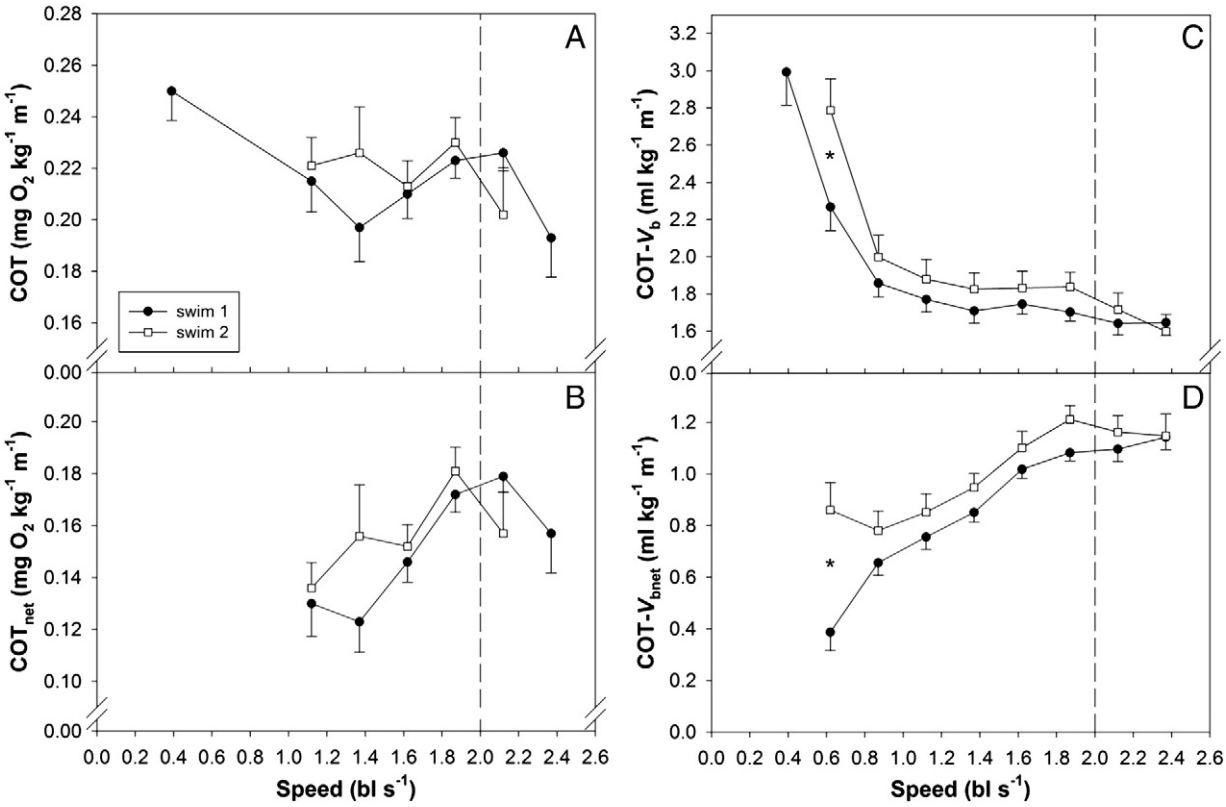

Fig. 2. (A) Cost of transport (COT), (B) net cost of transport (COT_{net}), (C) cardiovascular cost of transport (COT-$\dot{V}_b$) and (D) net cardiovascular cost of transport (COT-$\dot{V}_{bnet}$) over two consecutive critical swimming speed challenges. Since there were no significant differences in $\dot{M}O_2$ or $\dot{V}_b$ among populations, all populations were combined. Dashed line indicates typical swim speed at which the fish transitioned from steady swimming to burst swimming. Mean ± SEM are presented. There were no significant differences between swim 1 and swim 2 in COT or COT_{net}. Significant differences between swims in COT-$\dot{V}_b$ and COT-$\dot{V}_{bnet}$ are indicated by an asterisk.

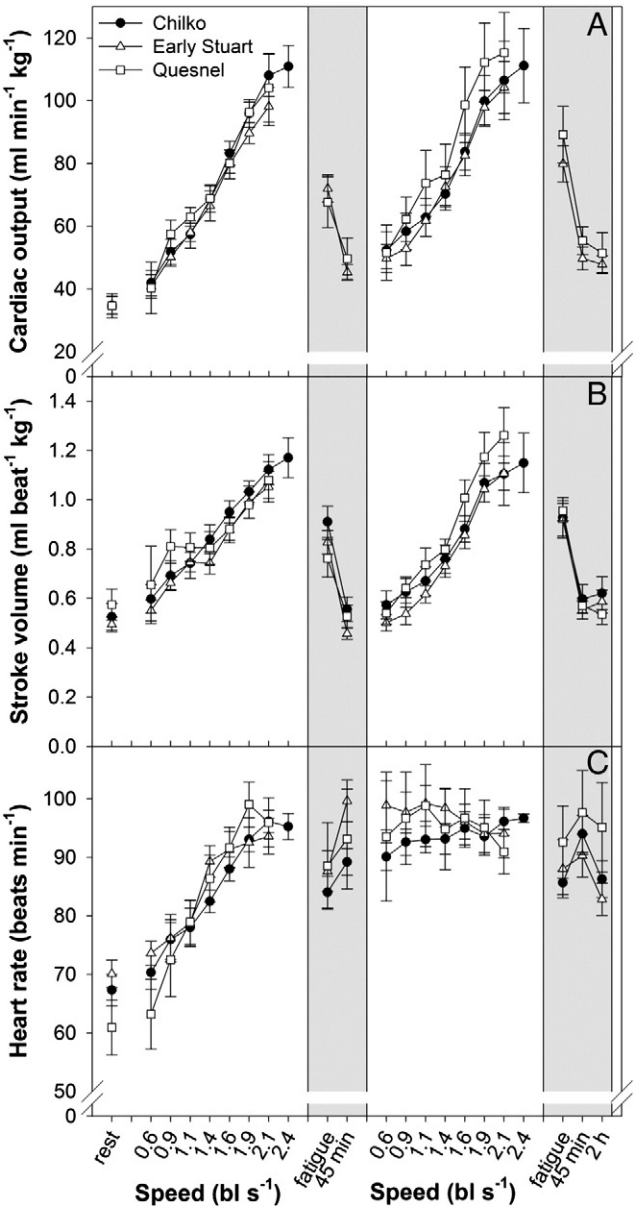

Fig. 3. (A) Cardiac output, (B) stroke volume and (C) heart rate with swimming speed over two consecutive critical swimming speed challenges in three populations of sockeye salmon (Early Stuart n = 9; Chilko n = 13; Quesnel n = 6). Swimming speed at rest and during recovery (fatigue, 45 min, 2 h) was ~0.39 bl s^{-1} and mean ± SEM are presented. There were no significant differences in cardiac output, stroke volume or heart rate among populations or between sexes.

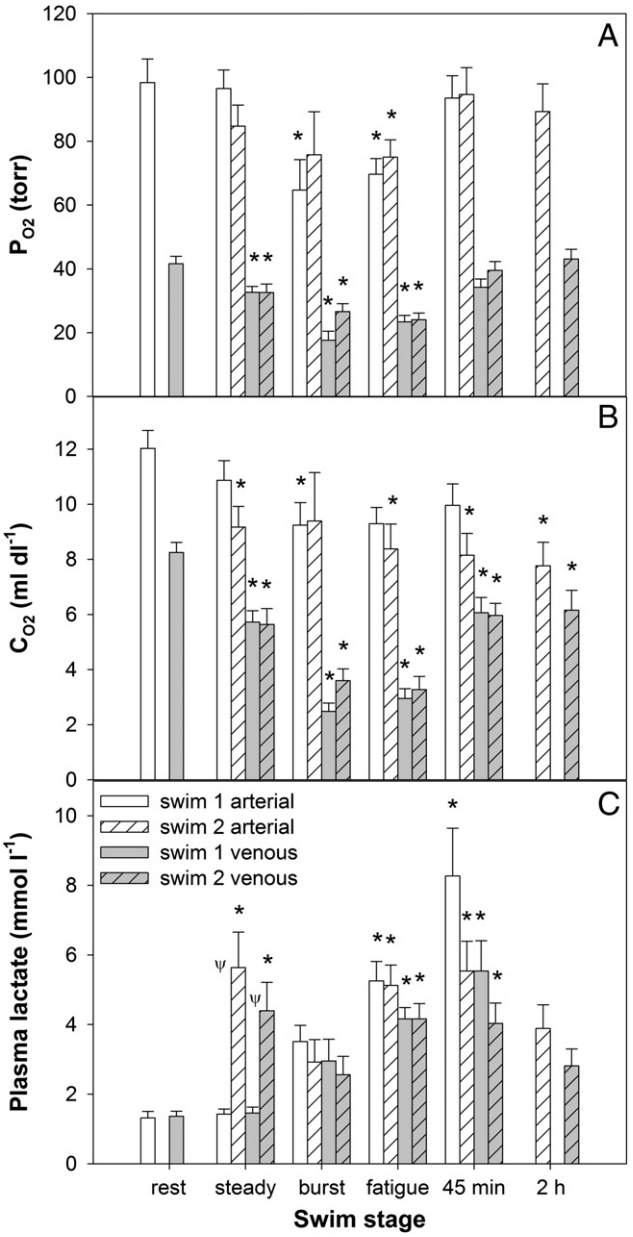

Fig. 4. Arterial and venous (A) partial pressure of oxygen (P_{O2}), (B) oxygen content (C_{O2}) and (C) plasma lactate levels in Early Stuart, Chilko and Quesnel populations combined (n = 25), over two consecutive critical swimming speed challenges. Mean ± SEM are presented, there were no significant differences between sexes. Significant differences from rest are indicated by an asterisk (*), significant differences between swims are indicated by the symbol (ψ). Lactate did not significantly differ between arterial and venous blood samples. P_{O2} and C_{O2} significantly differed between arterial and venous blood samples at every time point, except C_{O2} did not differ at 2 h recovery.

assumption to calculate cardiorespiratory variables (e.g. Stevens and Randall, 1967; Brett, 1971; Kiceniuk and Jones, 1977). By having direct measurements of cardiac performance, we can now explore adaptations and mechanistic limitations of maximum exercise capacity with greater confidence. In addition, these data may prove useful for bioenergetic models for this culturally, economically and ecologically important fish species.

4.1. Swimming and cardiorespiratory performance

Fish likely recovered well since $\dot{M}O_{2rest}$, $\dot{M}O_{2max}$ and aerobic scope did not significantly differ between Early Stuart sockeye salmon that had undergone surgery and those that had not. In addition, resting plasma lactate levels were low. The added drag of leads decreased U_{crit} by 18–23%, which means that our estimates of COT will be elevated by the same amount as a consequence.

Resting $\dot{M}O_2$, V_b, V_s and f_H values in the present study were comparable to previous studies with salmonids (Farrell et al., 1998, 2003; Jain et al., 1998; Gallaugher et al., 2001; Lee et al., 2003b; Claireaux et al., 2005; Wagner et al., 2005, 2006; Clark et al., 2008b; Steinhausen et al., 2008). The fish were given an overnight recovery in the swim tunnel, which might mean that aerobic scope was underestimated because $\dot{M}O_{2rest}$ was potentially elevated. Indeed, Farrell et al. (2003) demonstrated that a 48-h habituation period to the swim tunnel lowered $\dot{M}O_{2rest}$ compared with overnight recovery for wild salmon. While a longer recovery time would have been desirable in the present study, the trade-off would have been fewer fish tested since these fish naturally begin to senesce after just a few weeks in captivity.

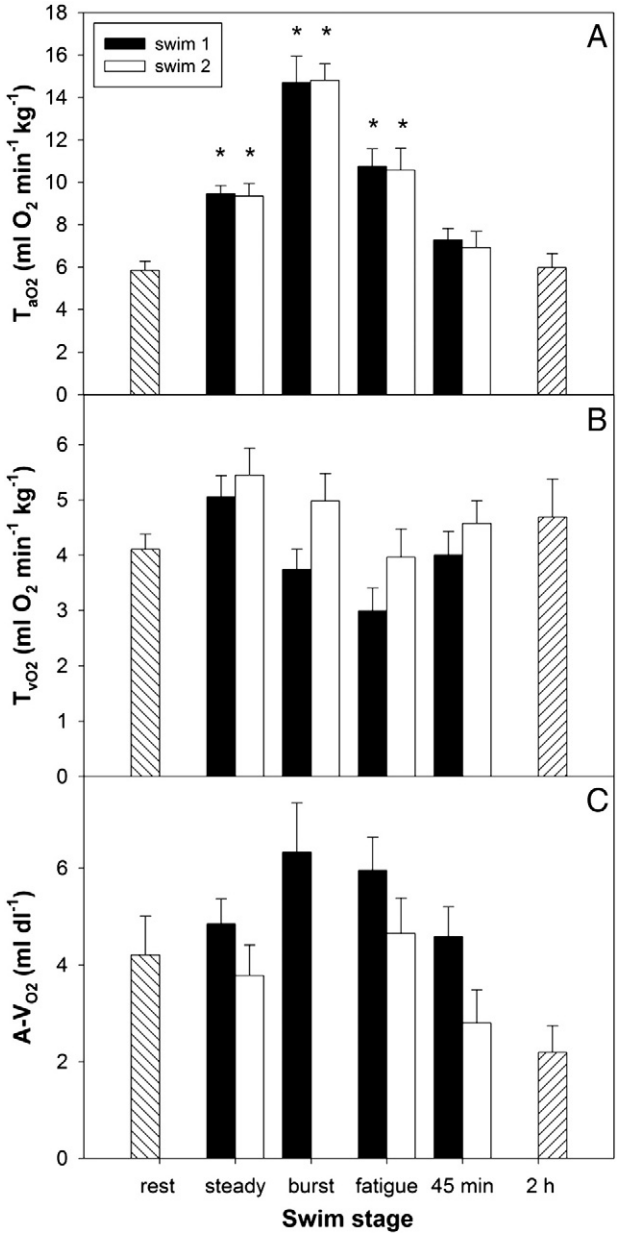

Fig. 5. (A) Arterial oxygen transport (T_{aO2}), (B) venous oxygen transport (T_{vO2}) and (C) tissue oxygen extraction (A-V_{O2}) in Early Stuart, Chilko and Quesnel populations combined (n = 25), over two consecutive critical swimming speed challenges. Measurements were made at rest, during steady swimming (steady; ~1.2 bl s^{-1}), and during burst swimming (burst; ~2.1 bl s^{-1}) immediately after the fish quit swimming (fatigue), 45 min after the fatigue (45 min) and 2 h after the conclusion of the second swim test (2 h). Mean ± SEM are presented, significant differences from rest are indicated by an asterisk (*), there were no significant differences between swim 1 and swim 2 or between sexes.

Maximum $\dot{M}O_2$, V_b, and V_s values for these wild salmon were considerably higher compared to previous values for hatchery and wild salmonids with a shorter river migration. Specifically, $\dot{M}O_{2max}$ (ranging from 10.6 to 17.3 mg O_2 kg^{-1} min^{-1}) is higher compared with coastal Fraser River and Vancouver Island sockeye salmon [9.7–12.3 mg O_2 kg^{-1} min^{-1} (Hinch et al., 1996; Jain et al., 1998; Farrell et al., 2003; Lee et al., 2003b)]. Previous reports for Early Stuart sockeye salmon [11–19 mg O_2 kg^{-1} min^{-1} (Lee et al., 2003b; MacNutt et al., 2006)] are in line with the present results. Among almonids, only Fraser River pink salmon have a superior $\dot{M}O_{2max}$ [10–22 mg O_2 kg^{-1} min^{-1} (MacNutt et al., 2006; Clark et al., 2011)]. Among adult fish, only tunas (Family Scombridae) have an appreciably higher $\dot{M}O_{2max}$ [estimated to be ~37–45 mg O_2 kg^{-1} min^{-1} at temperatures of ~24 °C (Dewar and Graham, 1994; Brill, 1996; Graham and Dickson, 2004)].

Maximum V_b (100–118 mL min^{-1} kg^{-1}) had never been directly measured for sockeye salmon swimming maximally. At ~75% of U_{crit}, V_b was directly measured as 68 mL min^{-1} kg^{-1} for Lower Adams sockeye salmon (a Fraser River population with intermediate migration difficulty) (Steinhausen et al., 2008). Here, V_b was 80 mL min^{-1} kg^{-1} at ~75% of U_{crit}. An earlier study indirectly calculated V_b in sockeye salmon (from an undisclosed origin) at 20 °C using the Fick equation and an assumed value for C_{vO2} as ~165 mL min^{-1} (Davis, 1968). If we assume a 2.3 kg body mass (body mass was not reported), V_{bmax} for the Davis study would be ~72 mL min^{-1} kg^{-1}, a value substantially lower than the present study. For hatchery-reared salmonids, V_{bmax} values are lower still: 42–69 mL min^{-1} kg^{-1} in rainbow trout at 10–18 °C (Kiceniuk and Jones, 1977; Taylor et al., 1996; Thorarensen et al., 1996a; Brodeur et al., 2001; Claireaux et al., 2005) and 66 mL min^{-1} kg^{-1} in immature Chinook salmon at 8–10 °C (Gallaugher et al., 2001). Notably, true V_{bmax} for some of these studies (e.g. Davis, 1968; Kiceniuk and Jones, 1977) is likely even lower since estimates of V_b using the Fick equation likely overestimate V_b by not accounting for gill oxygen consumption. Similarly, V_{smax} in the present study (1.08–1.28 mL $beat^{-1}$ kg^{-1}) is appreciably higher compared with 0.66–1.04 mL $beat^{-1}$ kg^{-1} in hatchery-reared rainbow trout and immature Chinook salmon (Kiceniuk and Jones, 1977; Gallaugher et al., 2001; Claireaux et al., 2005). Conversely, f_{Hmax} was not elevated relative to previous studies, ranging between 91 and 95 beats min^{-1} in the present study versus 81–105 beats min^{-1} for fish swimming at 13–16 °C [Stamp River sockeye salmon, Lower Adams sockeye salmon, rainbow trout and pink salmon (Smith et al., 1967; Claireaux et al., 2005; Steinhausen et al., 2008; Clark et al., 2011)]. Thus, the elevated $\dot{M}O_{2max}$ achieved by interior sockeye salmon relative to coastal and hatchery salmon is largely due to high V_{bmax} via enhanced V_{smax}. Notably, interior sockeye salmon have a significantly higher relative ventricular mass compared to coastal populations (Eliason et al., 2011), which may facilitate a higher V_{smax} and V_{bmax}.

4.2. Oxygen transport and removal by tissues

Few measurements of A-V_{O2} exist for swimming fish with which to make comparisons. This stems partly from a limited number of studies that have sampled venous blood. The maximum A-V_{O2} found here (6.3 ml dl^{-1}) is within the reported range for rainbow trout, yellowfin tuna and leopard shark [3.4–8.3 ml dl^{-1} (Kiceniuk and Jones, 1977; Lai et al., 1990; Korsmeyer et al., 1997a)]. Resting Hct, [Hb], MCHC, P_{aO2} and C_{aO2} were within the expected range for salmonids (Gallaugher et al., 1992; Thorarensen et al., 1993; Gallaugher et al., 2001; McKenzie et al., 2004; Clark et al., 2008b; Steinhausen et al., 2008; Clark et al., 2009; Sandblom et al., 2009). Thus, the exceptional aerobic swimming performance of interior sockeye salmon does not come as a result of a major enhancement of arterial oxygen carrying capacity. Instead, oxygen delivery to the tissues was primarily supported by a 3-fold increase in V_b and secondarily by a 50% increase in A-V_{O2}, and thus we reject our hypothesis that oxygen delivery to the tissues during maximal swimming is supported by equivalent increases in V_b and A-V_{O2}.

Arterial oxygen delivery to the tissues increased during swimming entirely via increased cardiac output, supporting our hypothesis. Specifically, the increase in T_{aO2} was accomplished primarily by increased V_s and secondarily by increased f_H, with no additional contribution from C_{aO2}. In fact, swimming decreased P_{aO2} and C_{aO2} (by 34 and 23%, respectively, during the first swim), thus we reject our hypothesis that arterial oxygen saturation is maintained during maximal swimming. While a decrease in P_{aO2} was observed in some previous swimming studies with salmonids (Gallaugher et al., 1992; Thorarensen et al., 1993; Farrell et al., 1998; Gallaugher et al., 2001; McKenzie et al.,

Table 5
Haematological variables from Early Stuart, Chilko and Quesnel populations combined over two consecutive U_{crit} critical swimming speed challenges. Only fish with paired arterial and venous cannulae were included. Arterial and venous blood samples were taken at rest, during steady state swimming (steady) and burst-and-coast swimming (burst), immediately after the fish quit swimming (fatigue), 45 min after the fatigue (45-min) and 2 h after the conclusion of the second swim test (2-h rec). Haemoglobin concentration (Hb), haematocrit (Hct) and mean cell haemoglobin concentration (MCHC) are indicated. Mean ± SEM are presented. Significant differences from rest are indicated by an asterisk (*), significant differences between swim 1 and swim 2 are indicated by a double dagger (‡) and significant differences between arterial and venous blood are indicated by different letters.

		n	Hb (g L^{-1})	Hct (%)	MCHC (g L^{-1})	Glucose (mmol L^{-1})	Chloride (mmol L^{-1})	Sodium (mmol L^{-1})	Potassium (mmol L^{-1})
Rest	Arterial	9	89.5 ± 5.1	30.3 ± 2.1	298.0 ± 8.1	5.3 ± 0.6	127.7 ± 1.2	141.0 ± 2.2	4.4 ± 0.4[a]
	Venous	9	87.9 ± 4.7	30.6 ± 2.1	290.6 ± 9.6	5.1 ± 0.6	129.9 ± 2.1	145.8 ± 2.4	3.3 ± 0.4[b]
Steady 1	Arterial	9	88.5 ± 4.1[a]‡	29.9 ± 1.6‡	297.7 ± 8.1‡	4.6 ± 0.6	131.7 ± 1.9	146.2 ± 1.6	4.0 ± 0.3[a]‡
	Venous	9	83.3 ± 3.6[b]	28.8 ± 1.7	292.2 ± 8.7‡	5.0 ± 0.6	133.1 ± 1.6‡	150.8 ± 1.7‡	2.7 ± 0.4[b]‡
Steady 2	Arterial	8	79.0 ± 5.2‡	25.4 ± 2.3‡	316.9 ± 13.8‡	5.5 ± 0.7	128.0 ± 2.5	141.7 ± 3.0	7.6 ± 0.7[a*]‡
	Venous	8	78.7 ± 4.9	26.1 ± 2.4	308.3 ± 11.8‡	5.8 ± 0.7	128.6 ± 2.3‡	146.1 ± 2.3‡	4.1 ± 0.2[b]‡
Burst 1	Arterial	4	95.2 ± 2.2	36.6 ± 1.0[a]	260.4 ± 1.4[a]	6.8 ± 0.8	129.8 ± 1.9	148.3 ± 2.0	1.6 ± 0.2*
	Venous	4	94.8 ± 2.6	40.4 ± 1.6[b]	235.2 ± 4.8[b]	6.7 ± 0.7	127.0 ± 0.7	146.0 ± 2.8	1.0 ± 0.2
Fatigue 1	Arterial	8	88.9 ± 3.5‡	32.0 ± 1.6[a]‡	278.5 ± 4.8[a]‡	6.4 ± 0.8	134.2 ± 1.5[a]	154.7 ± 2.5*‡	2.4 ± 0.3‡
	Venous	8	89.0 ± 3.9‡	36.4 ± 2.0[b]‡	246.3 ± 6.0[b]‡	6.8 ± 0.6	133.1 ± 2.1[b]‡	155.8 ± 2.5‡	1.8 ± 0.5
Fatigue 2	Arterial	8	75.2 ± 5.0*‡	25.1 ± 2.3[a]‡	304.9 ± 9.7[a]‡	6.3 ± 0.9	130.5 ± 2.5[a]	147.1 ± 1.8‡	3.9 ± 0.4[a]‡
	Venous	8	75.4 ± 3.4*‡	27.5 ± 2.3[b]‡	281.3 ± 12.7[b]‡	6.5 ± 0.8	128.0 ± 3.0[b]‡	147.8 ± 3.2‡	2.0 ± 0.3[b]
45-min 1	Arterial	8	86.2 ± 5.5‡	28.9 ± 2.2‡	300.8 ± 9.3‡	5.5 ± 0.7	130.5 ± 2.2	147.0 ± 2.7	6.5 ± 0.7[a]
	Venous	8	84.4 ± 4.4‡	29.7 ± 2.0‡	286.3 ± 7.6‡	5.3 ± 0.6‡	129.7 ± 2.8	147.6 ± 3.8	3.2 ± 0.4[b]
45-min 2	Arterial	8	66.3 ± 4.4*‡	21.6 ± 1.6*‡	308.5 ± 4.9‡	6.3 ± 1.1*	129.7 ± 2.2	144.7 ± 2.1	5.7 ± 0.4[a]
	Venous	8	66.7 ± 4.8*‡	21.9 ± 2.0*‡	308.4 ± 11.0‡	7.0 ± 1.2*‡	126.6 ± 2.6	144.3 ± 3.2	3.9 ± 0.4[b]
2-h rec	Arterial	6	65.2 ± 7.2*	20.1 ± 2.8*	333.8 ± 19.0	7.4 ± 1.2*	128.0 ± 2.7	146.7 ± 2.1	4.1 ± 0.3
	Venous	6	63.8 ± 5.7*	20.6 ± 2.3*	315.1 ± 9.5	7.5 ± 1.3*	127.8 ± 2.2	146.4 ± 2.2	2.9 ± 0.3

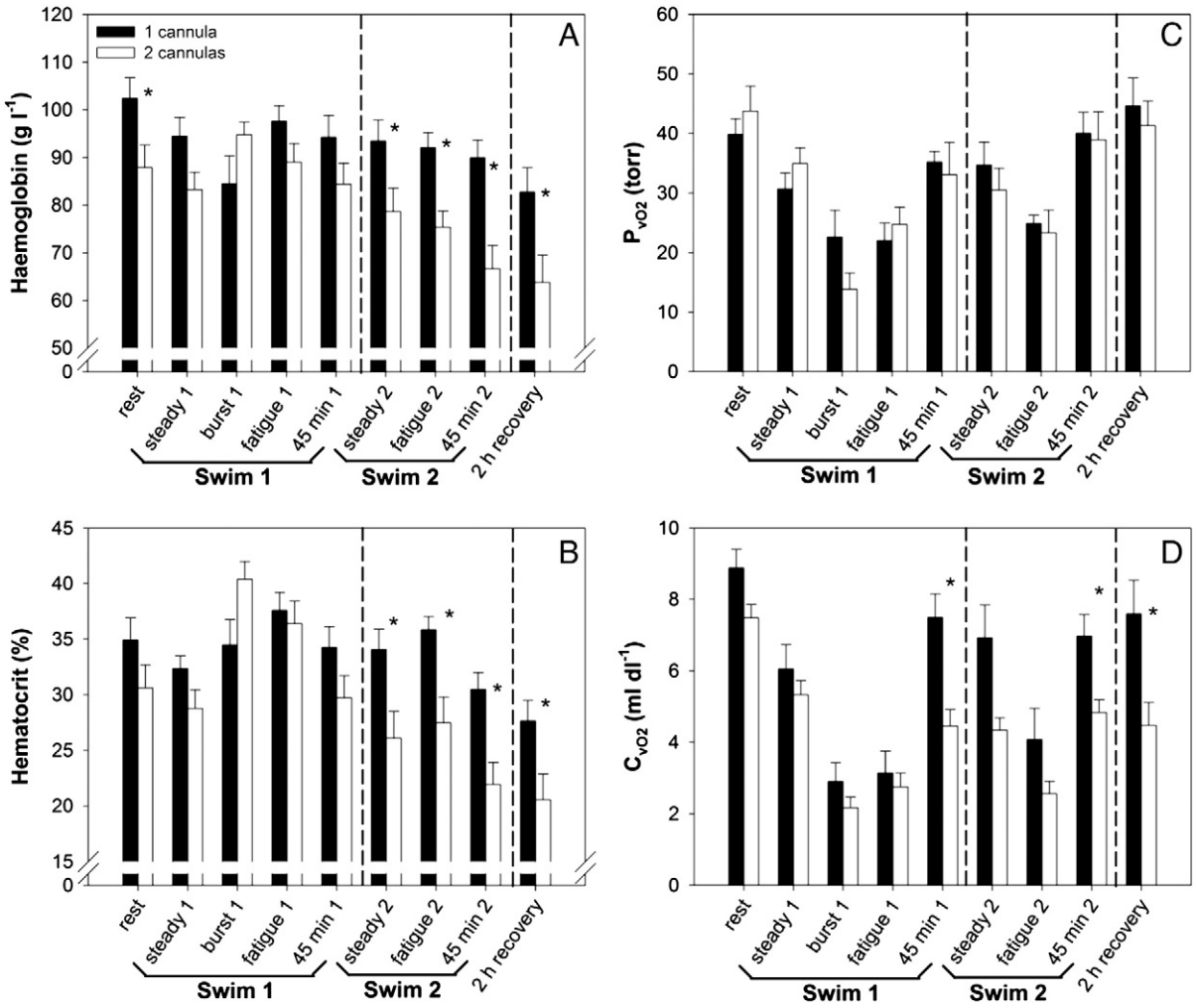

Fig. 6. Venous (A) haemoglobin, (B) haematocrit, (C) partial pressure of oxygen and (D) oxygen content in fish with both the arterial and venous cannulae operational (2 cannulae, n = 10) and fish with only the venous cannula functioning (1 cannula, n = 11), over two consecutive critical swimming speed challenges. Mean ± SEM are presented, significant differences between fish with 2 cannulae and those with 1 cannula working are indicated by an asterisk (*). Note that there were insufficient blood samples during burst swimming in the second swim to compare between groups.

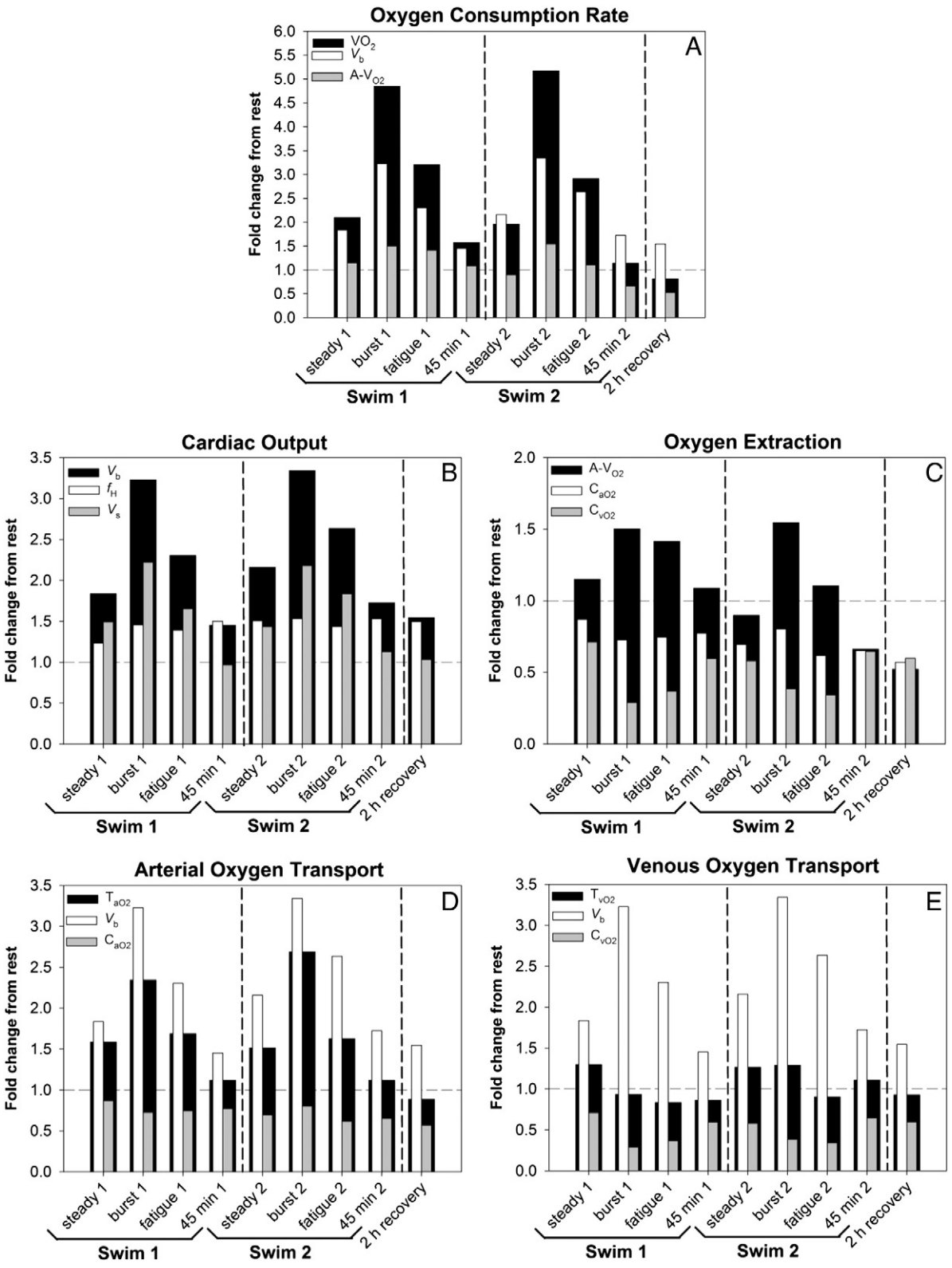

Fig. 7. Fold changes from rest for (A) oxygen uptake rate ($\dot{V}O_2 = V_b \times A\text{-}V_{O2}$), (B) cardiac output ($V_b = f_H \times V_s$), (C) tissue oxygen extraction ($A\text{-}V_{O2} = C_{aO2} - C_{vO2}$), (D) arterial oxygen delivery ($T_{aO2} = V_b \times C_{aO2}$) and (E) venous oxygen transport ($T_{vO2} = V_b \times C_{vO2}$). f_H = heart rate, V_s = stroke volume, C_{aO2} = arterial oxygen content, C_{vO2} = venous oxygen content. Only fish from Early Stuart, Chilko and Quesnel with both cannulae working were included in this analysis (n = 10).

2004; Steinhausen et al., 2008), C_{aO2} remained unchanged in several of the studies (Kiceniuk and Jones, 1977; Thorarensen et al., 1993; Gallaugher et al., 2001; McKenzie et al., 2004). Using similar protocols as used here, C_{aO2} similarly decreased by 21% when sockeye salmon were swimming at ~75% of U_{crit} (Steinhausen et al., 2008). It is possible that C_{aO2} decreased during swimming in the two sockeye salmon studies because of a reduced gill surface area for diffusion due to either fourth gill arch filament damage that may have occurred via abrasion

from the flowprobe and cannula inside the opercular cavity, or possibly due to the inevitable fungal infection (*Saprolegnia* sp.) associated with adult Pacific salmon in freshwater.

Oxygen extraction by the tissues increased during swimming, decreasing C_{vO2} by 70% during the first swim challenge. As a result, P_{vO2} decreased to 18 Torr, which is comparable with previous studies with swimming rainbow trout [16 Torr at 10 °C (Kiceniuk and Jones, 1977); 15–16 and 29 Torr at 6–10 °C and 13–15 °C, respectively (Farrell and Clutterham, 2003)]. It has been proposed that the minimum value for P_{vO2} to adequately supply oxygen to the spongy myocardium is ~10 Torr during swimming (Davie and Farrell, 1991). Present values are above this estimated threshold, supporting our hypothesis that the concentration of oxygen returning in venous blood to the heart remains well in excess of the oxygen demand of the heart when it is working maximally at T_{opt}.

4.3. Repeat swim performance

The excellent repeat swimming ability of salmonids is well established (Farrell et al., 1998; Jain et al., 1998; Farrell et al., 2003; Lee et al., 2003b; MacNutt et al., 2004, 2006; Wagner et al., 2006). Such recovery potential has clear survival benefits and would also permit a timely migration to reach the spawning areas. The current study showed that physiological recovery was incomplete at the outset of the second swim challenge ($\dot{M}O_2$, $\dot{V}_b$, f_H, and plasma lactate were all elevated and C_{vO2} was depressed). Indeed, because f_H remained elevated at maximum levels throughout the recovery period, there was no scope to increase f_H during the second swim challenge. Yet, all three populations achieved the same U_{crit}, $\dot{V}_{bmax}$, V_{smax}, f_{Hmax}, and T_{aO2} during the second swim challenge. This feat was all the more impressive given the progressive haemodilution during the second swim challenge for fish with two functional cannulae. Gallaugher et al. (1995) have previously shown that U_{crit} did not become impaired in rainbow trout until Hct was experimentally reduced below 22%. Cardiorespiratory recovery was largely completed 2 h after the second swim challenge, with the exception of a depressed C_{aO2} and C_{vO2} and elevated f_H and $\dot{V}_b$, which may be attributed to haemodilution.

As fish transition to anaerobic metabolism near U_{crit} and accumulate lactate in the blood, venous blood also becomes acidic (CO_2 and H^+ accumulation), hypoxemic (low P_{vO2} and C_{vO2} due to increased tissue oxygen extraction) and hyperkalaemic (K^+ accumulation from working muscles) (Kiceniuk and Jones, 1977; Holk and Lykkeboe, 1998). Here, plasma K^+ was highly variable (1.0–7.6 mmol L^{-1}), decreasing during the first swim challenge, and increasing during the second swim challenge. Moreover, $[K^+]$ in arterial plasma was significantly higher relative to venous plasma, a difference possibly due to pH effects on haemoglobin-oxygen saturation. Specifically, red blood cells take up K^+ when blood pH and haemoglobin-oxygen saturation are low and lose K^+ when pH and haemoglobin-oxygen saturation are high (Nielsen and Lykkeboe, 1992). Increased plasma K^+ is associated with reduced excitability of muscle, which is suggested to contribute to muscle fatigue (both cardiac and skeletal) (Nielsen and Lykkeboe, 1992; Holk and Lykkeboe, 1998). The unusual decrease in plasma K^+ during the first swim challenge may be a protective mechanism to postpone muscle fatigue that may be unique to more athletic salmonids and warrants further study.

Repeat swimming performance did not involve a larger anaerobic contribution since plasma lactate was highest during the 45-min recovery period following the first swim, an observation consistent with sockeye salmon swum two or three times (Farrell et al., 1998; Jain et al., 1998). Plasma lactate level also remained <10–13 mmol L^{-1}, which has been proposed as the threshold beyond which repeat swimming performance is impaired (Stevens and Black, 1966; Farrell et al., 1998). Low swimming speed helps clear lactate and accelerates recovery in rainbow trout (Milligan et al., 2000), which may explain the decrease in plasma lactate between steady and burst swimming during the second swim challenge. In addition, the excellent repeat swim performance was not due to increased swimming efficiency since COT did not differ between swim challenges.

The upriver migration is energetically expensive, with interior populations consuming more than 50% of total somatic energy as they complete their upriver migration (Brett, 1995; Crossin et al., 2004). As such, energy-saving behaviours such as swimming at optimal swimming speeds and avoiding high velocity water flows may be important strategies for successful upriver migration (Hinch and Rand, 2000). Neither COT nor COT-$\dot{V}_b$ displayed the classic U-shaped curve with speed (Wakeman and Wohlschlag, 1982; Lee et al., 2003b). Instead, both plateaued at speeds higher than ~1 bl s^{-1}. Similarly, Gates sockeye salmon, an interior Fraser River population with an intermediate migration distance and elevation (364 km and 280 m, respectively), demonstrated a plateau in COT at speeds above ~1 bl s^{-1} (Lee et al., 2003b). Conversely, Weaver sockeye salmon, a coastal Fraser River population with a short, low elevation migration (117 km and 32 m), demonstrated a classic U-shaped curve in COT with swim speed (Lee et al., 2003b). These findings suggest that the effect of swimming speed on swimming efficiency varies among populations of sockeye salmon, which in turn may be related to known differences in body shape [coastal populations are larger and bulkier compared to interior populations (Crossin et al., 2004)].

It is important to note that the estimate of COT performed here did not incorporate excess post-exercise oxygen consumption (EPOC), which represents the $\dot{M}O_2$ cost to restore oxygen stores, high energy phosphates and glycogen and reverse biochemical, ionic and osmotic imbalances (Gaesser and Brooks, 1984; Scarabello et al., 1992). Therefore, the true COT, particularly at the highest swim speeds, was underestimated. An extended EPOC and prolonged rate of recovery could be detrimental to migrating sockeye salmon since they must migrate upstream in a timely manner. However, $\dot{M}O_2$ had returned to resting revels by 2 h after the second swim challenge in sockeye salmon from all three upriver populations, which is a comparable timeframe to an earlier study for sockeye salmon (Lee et al., 2003a), suggesting that EPOC may have been similar across these populations.

4.4. Differences among populations and between sexes

Accumulating evidence suggests that Fraser River adult sockeye salmon populations are locally adapted to their specific upriver migration environment (Crossin et al., 2004; Eliason et al., 2011). Populations with exceptionally challenging spawning migrations, including the three populations considered here, appear to have adaptations for high swim performance and energy conservation. Specifically, they have been shown to have a similar small, streamlined body shape, few eggs, high somatic energy, larger relative ventricular mass and a high aerobic scope compared to coastal and intermediate populations (Crossin et al., 2004; Eliason et al., 2011). Here we demonstrated that U_{crit} as well as resting, swimming and recovery cardiorespiratory variables ($\dot{M}O_2$, $\dot{V}_b$, V_s and f_H) were similar across the three populations. Thus, we found support for our hypothesis that cardiorespiratory support of aerobic scope is similar among populations facing a long, arduous migration.

Given the elevated $\dot{M}O_{2max}$ and $\dot{V}_{bmax}$ compared with coastal and hatchery-reared salmonids, it would appear that these interior populations return to the Fraser River with the enhanced cardiovascular capacity needed for the difficult upriver swim ahead of them. Furthermore, we predict that populations facing a less demanding upriver migration [e.g. Lower Adams (distance 480 km, elevation 346 m) or Weaver and Harrison (distance <121 km, elevation <32 m)] likely possess a reduced cardiac performance given that U_{crit} and aerobic scope are already known to be lower for these populations (Lee et al., 2003b; Eliason et al., 2011).

Sexual dimorphisms of cardiorespiratory performance and blood variables were not detected here or previously (Steinhausen et al., 2008) for adult sockeye salmon swimming at T_{opt} for aerobic scope.

However, neither study was designed specifically to test for such differences and a low statistical power in the present study may have precluded subtle differences being detected between sexes. Cardiorespiratory differences are reported for mature male and female pink salmon (Clark et al., 2011), but pink salmon are morphologically much more sexually dimorphic compared to sockeye salmon. Nevertheless, sex-specific differences are an important consideration in sockeye salmon because female sockeye salmon experience higher mortality under stressful river migration conditions and at warm temperatures in the laboratory (Nadeau et al., 2010; Roscoe et al., 2011; Jeffries et al., 2012; Martins et al., 2012). Thus, studies examining sex-specific differences in swimming and cardiorespiratory performance of adult salmon are warranted.

In summary, cardiorespiratory performance of interior populations of sockeye salmon has been fully characterised during swimming to fatigue and during recovery. The remarkable maximum performance and speed of recovery discovered for these populations suggest that they are well prepared for a difficult upstream migration to distant spawning areas.

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