Conservation Physiology

Volume 1 • 2013 10.1093/conphys/cot008

SEB Society for Experimental Biology

Research article

Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon

Erika J. Eliason[1,*], Timothy D. Clark[1,2,3,†], Scott G. Hinch[3] and Anthony P. Farrell[1,2]

[1]Department of Zoology, University of British Columbia, Vancouver, Canada V6T 1Z4
[2]Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada V6T 1Z4
[3]Department of Forest Sciences, University of British Columbia, Vancouver, Canada V6T 1Z4;

*__Corresponding author__: Tel: +1 604 822 1969; fax: +1 604 822 9102. Email: eliason@zoology.ubc.ca

Elevated summer river temperatures are associated with high in-river mortality in adult sockeye salmon (*Oncorhynchus nerka*) during their once-in-a-lifetime spawning migration up the Fraser River (British Columbia, Canada). However, the mechanisms underlying the decrease in whole-animal performance and cardiorespiratory collapse above optimal temperatures for aerobic scope (T_{opt}) remain elusive for aquatic ectotherms. This is in part because all the relevant cardiorespiratory variables have rarely been measured directly and simultaneously during exercise at supra-optimal temperatures. Using the oxygen- and capacity-limited thermal tolerance hypothesis as a framework, this study simultaneously and directly measured oxygen consumption rate (MO_2), cardiac output ($\dot{V}_b$), heart rate (f_H), and cardiac stroke volume (V_s), as well as arterial and venous blood oxygen status in adult sockeye salmon swimming at temperatures that bracketed T_{opt} to elucidate possible limitations in oxygen uptake into the blood or internal delivery through the oxygen cascade. Above T_{opt}, the decline in MO_{2max} and aerobic scope was best explained by a cardiac limitation, triggered by reduced scope for f_H. The highest test temperatures were characterized by a negative scope for f_H, dramatic decreases in maximal $\dot{V}_b$ and maximal V_s, and cardiac dysrhythmias. In contrast, arterial blood oxygen content and partial pressure were almost insensitive to supra-optimal temperature, suggesting that oxygen delivery to and uptake by the gill were not a limiting factor. We propose that the high-temperature-induced *en route* mortality in migrating sockeye salmon may be at least partly attributed to physiological limitations in aerobic performance due to cardiac collapse via insufficient scope for f_H. Furthermore, this improved mechanistic understanding of cardiorespiratory collapse at high temperature is likely to have broader application to other salmonids and perhaps other aquatic ectotherms.

Key words: Aerobic scope, cardiovascular, climate change, heart rate, migration, oxygen- and capacity-limited thermal tolerance

Editor: Steven Cooke

Received 2 March 2013; Revised 30 March 2013; Accepted 31 March 2013

Conserv. Physiol. (2013) **1**: doi: 10.1093/conphys/cot008

Introduction

Temperature has been coined the 'ecological master factor' for ectotherms because of its influence on the biochemistry, physiology, behaviour, and ecology of animals (Fry, 1971). The optimal temperature (T_{opt}) window for performance may be narrow in stenotherms, such as sockeye salmon (*Oncorhynchus nerka*), or broad in eurytherms, such as killifish (*Fundulus heteroclitus*; Eliason *et al.*, 2011; Healy and Schulte, 2012). Outside of the T_{opt} window, whole-animal performance declines until death eventually occurs. The T_{opt} for aerobic scope has been established as a useful metric for the successful return of adult sockeye salmon to their natal spawning areas in the Fraser River watershed

[†]Present address: Australian Institute of Marine Science, PMB 3, Townsville MC, QLD 4810, Australia.

(British Columbia, Canada; Farrell *et al.*, 2008). Indeed, T_{opt} appears to be locally adapted among sockeye salmon populations to the temperatures that they are likely to encounter once they enter the river (Eliason *et al.*, 2011). Peak summer river temperatures have increased by around 2°C since the 1950s (Patterson *et al.*, 2007), forcing salmon to migrate at temperatures warmer than their T_{opt} for aerobic scope. Biotelemetry studies have shown that high river temperature is correlated with excessive (>70%) mortality among migrating salmon (Crossin *et al.*, 2008; Farrell *et al.*, 2008; Mathes *et al.*, 2010; Martins *et al.*, 2011). Clearly, a conservation concern exists for future salmon migrations in this and other watersheds. As a result, there is great interest in the mechanism(s) underlying the decline of whole-animal performance and cardiorespiratory collapse above T_{opt}.

Physiological processes are critical in defining thermal limits and temperature-induced mortality (Wang and Overgaard, 2007). Aerobic scope, defined as the difference between maximal oxygen consumption (MO_{2max}) and resting oxygen consumption (MO_{2rest}; Fry, 1947), represents the amount of oxygen available for activity. By definition, aerobic scope remains largely unchanged from its maximum within a defined T_{opt} window. However, at the critical temperature (T_{crit}), when MO_{2rest} and MO_{2max} intersect, aerobic scope is zero and survival becomes passive, time limited, and supported by anaerobic metabolism (Pörtner, 2001; Pörtner and Farrell, 2008), a situation clearly incompatible with a highly aerobic upriver migration. Here we were specifically interested in which process triggers the decline in aerobic scope at a supra-optimal temperature. In the context of the present study, the term T_{opt} refers to the optimal temperature for aerobic scope.

While it is unclear what determines the limits of upper thermal tolerance in ectotherms, one leading possibility is the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis, which attributes the decline in aerobic scope above T_{opt} to capacity limitations of the organ systems delivering oxygen to the tissues (Pörtner, 2001; Pörtner and Knust, 2007). Several studies with aquatic ectotherms have found support for the OCLTT hypothesis (e.g. fish and invertebrates; Frederich and Pörtner, 2000; Mark *et al.*, 2002; Lannig *et al.*, 2004; Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Eliason *et al.*, 2011; Verberk and Calosi, 2012), while recent studies with pink salmon (*Oncorhynchus gorbuscha*), an intertidal snail (*Echinolittorina malaccana*), and an air-breathing toad (*Rhinella marina*) found little support for the OCLTT hypothesis (Clark *et al.*, 2011; Marshall *et al.*, 2011; Overgaard *et al.*, 2012). According to the OCLTT hypothesis, any one of the five major steps in the oxygen cascade from the environment to the mitochondria might trigger the collapse above T_{opt} (i.e. oxygen delivery by gill ventilation, oxygen diffusion across the gills, oxygen transport via the circulatory system, oxygen diffusion across tissue capillaries, and oxygen use by mitochondria; Weibel, 1984). However, simultaneous and preferably direct measurements of MO_2, cardiac output ($\dot{V}_b$), heart rate (f_H), and cardiac stroke volume (V_s), as well as arterial and venous blood oxygen status [partial pressure (P_{O2}) and content (C_{O2})] are required to discover the initial limitation in the oxygen cascade that triggers the decline in aerobic scope above T_{opt}. For example, a limitation in either oxygen delivery to or oxygen diffusion across the gill would be manifest by a decrease in arterial P_{O2} (P_{aO2}) and arterial C_{O2} (C_{aO2}). Alternatively, a cardiac limitation would be indicated by a failure of $\dot{V}_{bmax}$ to increase to match the increased tissue oxygen demand above T_{opt}. Finally, a limitation in oxygen diffusion to mitochondria in the working muscles would be indicated by venous P_{O2} (P_{vO2}) remaining constant with increased tissue oxygen demand above T_{opt}. However, support for a single limiting site in the oxygen cascade of fish (gills, heart, or locomotory muscle) remains incomplete despite considerable analysis (Brett, 1971; Heath and Hughes, 1973; Taylor *et al.*, 1996, 1997; Farrell, 2007a; Steinhausen *et al.*, 2008; Farrell *et al.*, 2009). This data gap exists because most studies examining temperature effects on aerobic scope in fish have not directly measured all the required variables (e.g. Fry, 1947; Brett, 1971; Taylor *et al.*, 1996), and the more comprehensive studies only used resting fish, thus failing to examine aerobic scope (e.g. Heath and Hughes, 1973; Cech *et al.*, 1976; Sartoris *et al.*, 2003; Gollock *et al.*, 2006; Clark *et al.*, 2008b; Keen and Gamperl, 2012). In fact, only Steinhausen *et al.* (2008) have directly measured all the required variables as a function of temperature in exercising fish.

The objective of this study was to examine the mechanism(s) that triggers the decline in MO_{2max} and aerobic scope above T_{opt} in sockeye salmon. Previous work suggested that sockeye salmon thermal limits are set by physiological limitations in aerobic performance, which may possibly be due to cardiac collapse at high temperatures (Steinhausen *et al.*, 2008; Eliason *et al.*, 2011). However, Eliason *et al.* (2011) did not systematically examine all the possible mechanisms leading to cardiorespiratory collapse (only aerobic scope, cardiac scope, scope for heart rate, MO_{2rest}, and MO_{2max} were reported as a function of temperature), and Steinhausen *et al.* (2008) only swam sockeye salmon up to ~75% of their critical swim speed (U_{crit}). Thus, the present study reports the first simultaneous and direct measurements of all the pertinent oxygen transport variables in adult sockeye salmon swimming maximally below, at, and above $T_{opt.}$ Novel data on MO_2, $\dot{V}_b$, f_H, and V_s, as well as arterial and venous P_{O2}, C_{O2}, and blood chemistry are presented. We hypothesize that a limitation on f_H triggers cardiorespiratory collapse when temperature exceeds T_{opt} for aerobic scope.

Materials and methods

Fish collection

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. Wild adult sockeye salmon ($n = 55$; body mass, 2380 ± 60 g; fork length, 59.6 ± 0.4 cm) were collected in 2007, 2008, and 2009 early in their upriver spawning migration (~100 km upstream of the Fraser River mouth) and transported to the Fisheries and Oceans Canada Cultus Lake Research Laboratory (Cultus Lake, BC, Canada), where experiments were conducted. All fish were given a unique PIT (Passive Integrated Transponder; Biomark Inc., Boise, ID, USA) tag for individual identification, a scale was removed, and <0.1 g of the adipose fin was clipped for population identification via DNA analysis (Beacham *et al.*, 2005). Only fish from Early Stuart ($n = 21$), Chilko ($n = 22$), and Quensel ($n = 12$) populations were used in the present study. Fish were held at 11–12°C for 1–4 weeks in outdoor 8000–12 000 l circular aquaria under seasonal photoperiod.

Surgical procedures

Individual fish were anaesthetized with buffered tricaine methanesulfonate in freshwater (0.2 g l^{-1} $NaHCO_3$ and 0.1 g l^{-1} MS-222; Sigma, St Louis, MO, USA), weighed, and transferred onto wet foam on a surgical table, where their gills were continuously 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; Eliason *et al.*, 2011). Fish were instrumented with a 3 mm SB flowprobe (lateral cable exit; Transonic Systems, Ithaca, NY, USA) around the ventral aorta (Steffensen and Farrell, 1998) to measure $\dot{V}_b$, f_H, and V_s. Cannulae made of PE-50 tubing were inserted into the dorsal aorta (Soivio *et al.*, 1973) and sinus venosus (Farrell and Clutterham, 2003) to sample arterial and venous blood, respectively, for measurement of oxygen status and blood chemistry.

Swim challenge to U_{crit}

Three days prior to the swimming test, most fish were placed in a 1400 l circular tank, and the temperature was progressively increased from the holding temperature (11–12°C) to the test temperature (12–22°C) by no more than 5°C day^{-1}. The fish were maintained at their test temperature for 24–48 h before surgery was conducted. Following surgery, the fish were placed individually in one of two Brett-type swim tunnels (described by Lee *et al.*, 2003b; Steinhausen *et al.*, 2008) and allowed to recover overnight (>8 h) at their test temperature (12–22°C) at a low water velocity of ~0.39 body lengths per second (bl s^{-1}). Resting measurements of all variables were made following overnight recovery from surgery in the swim tunnel. Fish then underwent two sequential ramp-U_{crit} swim protocols with a 45 min recovery in between (Jain *et al.*, 1997; Lee *et al.*, 2003b; Eliason *et al.*, 2011). Only data from the first swim are used here.

Fish could not be held at the temperature extremes due to logistical constraints on temperature regulation in the holding tanks. Therefore, some fish recovered overnight (>8 h) from surgery in the swim tunnel at the holding temperature (12°C); initial resting values were measured at this temperature in the morning, and then the water temperature was increased or decreased by 4°C h^{-1} to the test temperature of either 8–10 or 22–26°C. One hour after reaching the test temperature, resting variables were recorded, and the fish underwent a single ramp-U_{crit} swim challenge.

The MO_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 (version 6.0; National Instruments, Austin, TX, USA). The $\dot{V}_b$ was measured continuously at 200 Hz throughout the swim trials by connecting the flowprobe to a flowmeter (Transonic Systems) interfaced with Biopac hardware and Acknowledge software (Biopac Systems, Santa Barbara, CA, USA). The value of $\dot{V}_b$ was calculated as the mean of three to six segments of continuous 30 s traces. Blood was strategically sampled prior to the swim test (rest), during steady-state swimming (steady), once the fish had transitioned to burst-and-coast swimming (burst), and immediately following the swim test within 5 min of fatigue (fatigue).

Analysis of whole blood and plasma

Partial pressure of oxygen (P_{O2}), oxygen content (C_{O2}), haemoglobin concentration ([Hb]), and haematocrit (Hct) were measured using whole blood samples. 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). The [Hb] was measured using either a handheld haemoglobin analyser (HemoCue 201$^+$; Ängelholm, Sweden) calibrated for fish blood (Clark *et al.*, 2008a) or the spectrophotometer method with Drabkin's solution (Drabkin and Austin, 1935). Haematocrit was measured in duplicate using microhaematocrit 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 analyser), sodium and potassium (Cole-Parmer, model 41, single channel flame photometer) and chloride (Haake Buchler digital chloridometer) were measured using techniques outlined previously (Farrell *et al.*, 2001).

Data analysis and statistics

The U_{crit} was calculated using established methods (Brett, 1964), after accounting for the solid blocking effect as outlined by Bell and Terhune (1970). Stroke volume (V_s) was calculated as follows: $V_s = \dot{V}_b \div f_H$. Cost of transport (COT) was calculated as follows: $COT = MO_2/(U \times 60)$, where U is the swimming speed in metres per second. Net cost of transport (COT_{net}) was calculated as follows: $COT_{net} = (MO_2 - MO_{2rest})/(U \times 60)$. Likewise, 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 calculated as follows: A–$V_{O2} = C_{aO2} - C_{vO2}$. Arterial oxygen transport (T_{aO2}) to the tissues was calculated as follows: $T_{aO2} = \dot{V}_b \times C_{aO2}$. Venous oxygen transport (T_{vO2}) to the spongy myocardium and gills was calculated as follows: $T_{vO2} = \dot{V}_b \times C_{vO2}$. Mean corpuscular haemoglobin concentration (MCHC) was calculated as follows: MCHC = [Hb]/(Hct/100).

In order to maximize statistical power, data were pooled for Early Stuart, Chilko, and Quesnel populations, because they undergo similar long, difficult river migrations, have a similar T_{opt} for aerobic scope (~17°C) and have a similar maximal aerobic scope, cardiac scope and scope for heart rate (Eliason *et al.*, 2011). Moreover, Eliason *et al.* (under review) clearly show that MO_2, $\dot{V}_b$, f_H, and V_s for these three populations do not differ at rest, when swimming, or during recovery at T_{opt} for aerobic scope.

In order to permit statistical comparisons, individual fish were sorted into four temperature groupings based on performance relative to the maximal aerobic scope for a population (Fig. 1). The T_{opt} grouping (15–20°C; $n = 28$) combined individuals that attained 90–100% of the population-specific maximal aerobic scope. The assumption here is that the T_{opt} window is >90% of maximal aerobic scope. Bracketing the T_{opt} window were groupings of individuals that attained only 50–90% of the population-specific maximal aerobic scope. Thus, the grouping below T_{opt} was designated $T_{min50–90}$ ($n = 8$; 12°C for Early Stuart, and 9–10°C for Chilko; no Quesnel fish were swum in this grouping), while the grouping above T_{opt} was designated $T_{max50–90}$ ($n = 11$; 22–23°C for Early Stuart and Quesnel, and 24–25°C for Chilko). The $T_{max0–50}$ grouping ($n = 8$) combined fish that attained 0–50% of the population-specific maximal aerobic scope at a supra-optimal temperature (23–26°C for Early Stuart and Quesnel, and 25–26°C for Chilko). In addition, a limited analysis was performed with the Chilko population alone to examine whether or not pooling data in the manner described above obscured or changed major cardiorespiratory trends.

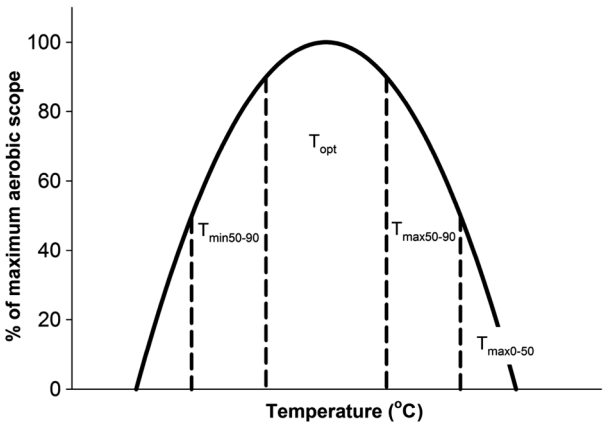

Figure 1: a schematic representation of the four temperature groupings used to analyse cardiorespiratory performance with temperature. The 50 and 90% cut-offs were assigned to individual fish based on their performance relative to T_{opt} for the population (see main text for details).

Grouped data are presented as means ± SEM, unless otherwise indicated. Values of $P < 0.05$ were considered statistically significant. Independent data were compared using one-way ANOVA. Dependent data were compared using Student's paired *t*-test, a 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, data were compared using appropriate non-parametric tests (e.g. Mann–Whitney *U*-test, Kolmogorov–Smirnov test, or Kruskal–Wallis test). The appropriate *post hoc* test (Holm–Sidak or Dunn's) was used to test for differences among groups. A polynomial quadratic equation was fitted to maximal and scope data for MO_2, $\dot{V}_b$, V_s, and f_H, and an exponential equation was fitted to the resting data for MO_2, $\dot{V}_b$, and f_H for individual Chilko sockeye salmon. A quadratic equation was fitted through the individual data for resting and fatigue venous P_{O2} and C_{O2}.

Results

Cardiorespiratory and swimming performance

As intended from the grouping procedure, aerobic scope was highest for the T_{opt} grouping, significantly lower and similar for the $T_{min50–90}$ and $T_{max50–90}$ groupings, and lowest for $T_{max0–50}$ grouping (Fig. 2E). Thus, the four performance groupings created the equivalent of a Fry curve for aerobic scope (Fry, 1947; Farrell, 2009). The Fry aerobic scope curve was a result of MO_{2rest} increasing progressively from the lowest to the highest temperature grouping (Fig. 2A), while MO_{2max} increased significantly between $T_{min50–90}$ and T_{opt}, was unchanged between T_{opt} and $T_{max50–90}$, and then decreased significantly at $T_{max0–50}$ (Fig. 2A). Indeed, swimming to fatigue significantly increasing MO_{2max} for the $T_{min50–90}$, T_{opt}, and $T_{max50–90}$ groupings, while maximal swim speed and its corresponding MO_{2max} were significantly lower for the $T_{max0–50}$ grouping compared with the other groupings (Fig. 3A).

Cardiac output varied among temperature groupings and with swimming, following similar patterns to MO_2 (Figs 2 and 3). The resting cardiac output increased progressively with increasing temperature groupings, plateauing between $T_{max50–90}$ and $T_{max0–50}$, while $\dot{V}_{bmax}$ increased significantly between $T_{min50–90}$ and T_{opt}, was unchanged between T_{opt} and $T_{max50–90}$, and then decreased significantly at $T_{max0–50}$. As a result, cardiac scope was lowest for the $T_{max0–50}$ grouping (Fig. 2F).

Stroke volume at rest was independent of temperature except for a quantitatively small, but statistically significant

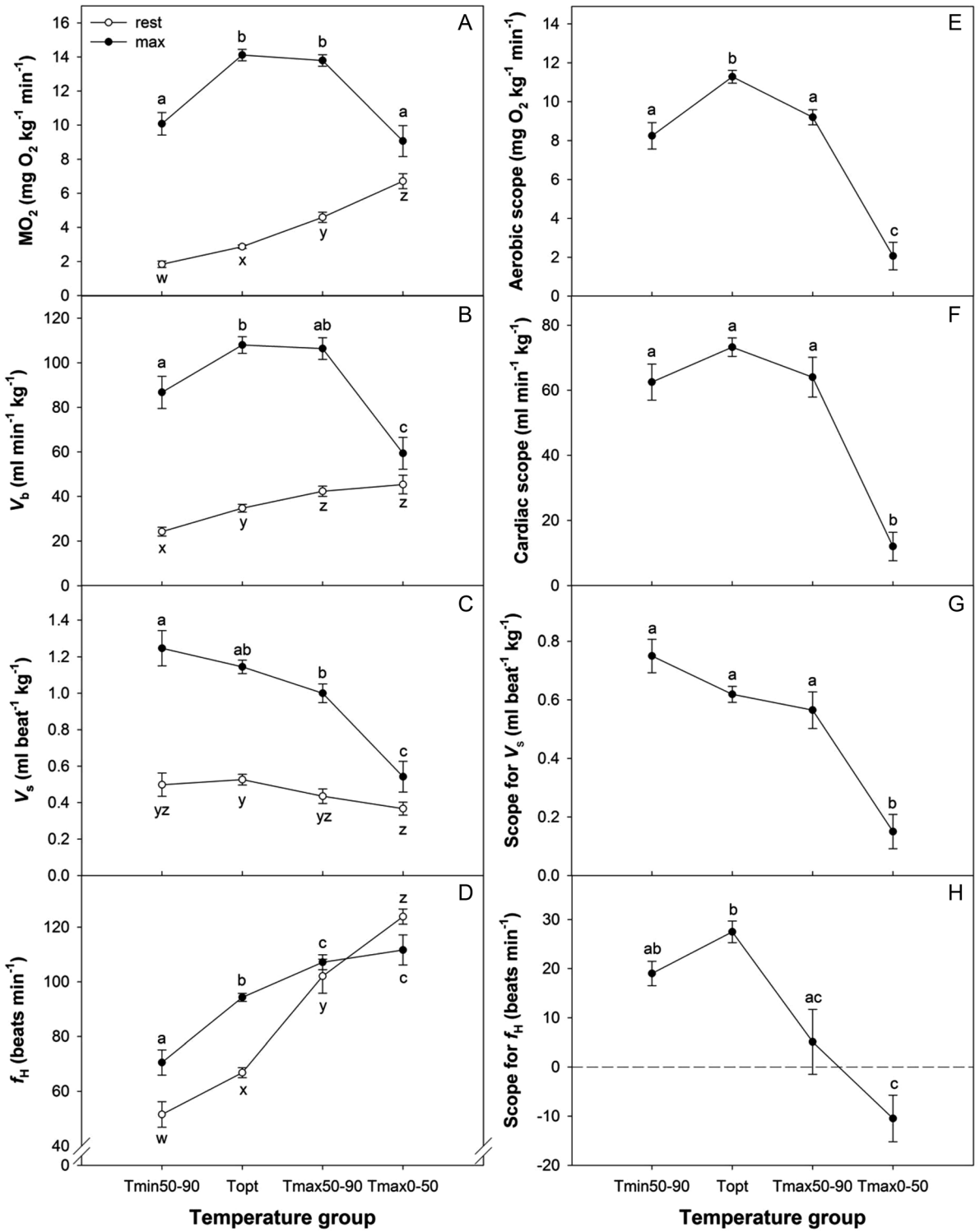

Figure 2: resting and maximal oxygen consumption rate (MO_2; **A**), cardiac output ($\dot{V}_b$; **B**), stroke volume (V_s; **C**), and heart rate (f_H; **D**) at the four temperature categories. Scope for MO_2 (**E**), $\dot{V}_b$ (**F**), V_s (**G**), and f_H (**H**) are shown. All values are presented as means ± SEM. Significant differences among temperature groupings are indicated by differing letters ($P < 0.05$).

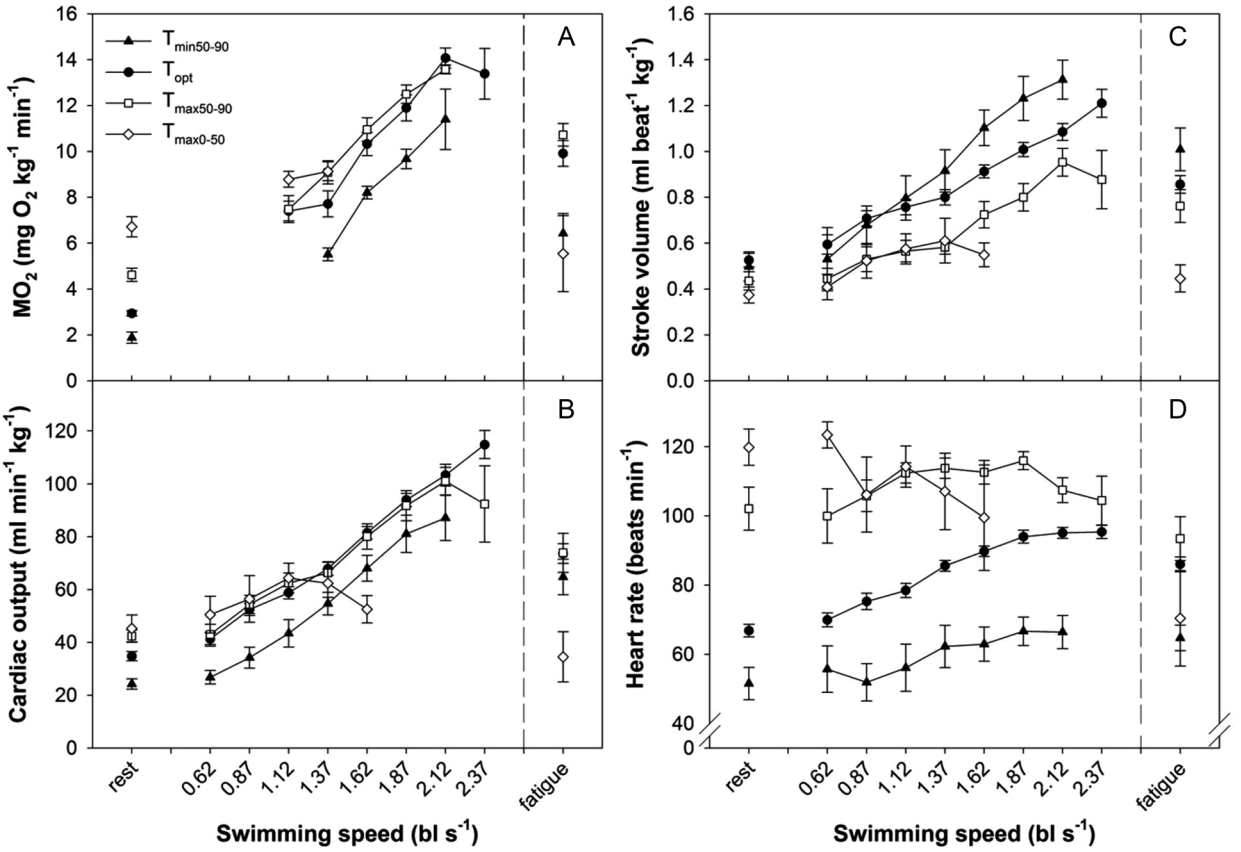

Figure 3: changes in oxygen consumption rate (**A**), cardiac output (**B**), cardiac stroke volume (**C**), and heart rate (**D**) as a function of swimming speed among the four temperature groupings. Fatigue values were obtained within 5 min after the fish stopped swimming. Means ± SEM are shown.

decrease at $T_{max0–50}$ when compared with T_{opt} (Fig. 2C). Scope for V_s was maintained between the $T_{min50–90}$ and $T_{max50–90}$ groupings, but decreased significantly for the $T_{max0–50}$ grouping when V_{smax} also decreased significantly (Fig. 2C and G).

While f_{Hrest} increased progressively with each temperature grouping (Fig. 2D), f_{Hmax} increased with temperature only up to the $T_{max50–90}$ grouping, plateauing between $T_{max50–90}$ and $T_{max0–50}$ (Fig. 2D). Therefore, scope for f_H was highest at T_{opt} (Fig. 2H). Notably, scope for f_H decreased precipitously at supra-optimal temperatures, approaching zero for the $T_{max50–90}$ grouping, and becoming negative for the $T_{max0–50}$ grouping (Fig. 2H).

In addition to the bradycardia during swimming and thus negative scope for f_H, every fish that swam at $T_{max0–50}$ exhibited an irregular heart beat immediately following the swim test, with 57% exhibiting cardiac dysrhythmias during the swim and shortly before fatigue (Fig. 4). Despite decreasing the temperature immediately following fatigue, 29% of the $T_{max0–50}$ fish died, even though minutes before many were swimming at 1.1–1.5 bl s^{-1}. Post-fatigue cardiac dysrhythmia was also present in the $T_{max50–90}$ grouping, but at a lower percentage (27% of the fish), and none died post-exhaustion. Thus, the difference between post-fatigue life and death at supra-optimal temperature was a matter of 2–3°C. No cardiac dysrhythmias or deaths accompanied or followed swimming to exhaustion at either $T_{min50–90}$ or T_{opt}.

Maximal scope occurred at T_{opt} for MO_2, $\dot{V}_b$, and f_H, but at $T_{min50–90}$ for V_s (Fig. 5). In comparison to these maximal values, scope for MO_2, $\dot{V}_b$, and V_s decreased by 13–25%, whereas scope for f_H plummeted by 80% for the $T_{max50–90}$ grouping. Clearly, the precipitous collapse of scope for f_H was a potential trigger for the general cardiorespiratory collapse, which became clear and profound for the $T_{max0–50}$ grouping, with scope for MO_2, $\dot{V}_b$, and V_s all declining to 16–20% of their maximal values, while scope for f_H collapsed to almost –40% of its maximal value (Fig. 5).

Cardiorespiratory and swimming performance of Chilko sockeye salmon

The pooling of populations accurately reflected the major cardiovascular trends seen for a single population, Chilko sockeye salmon swum at temperatures ranging from 8 to 26°C (Figs 2 and 6). Values of MO_2, $\dot{V}_b$, V_s, and f_H all

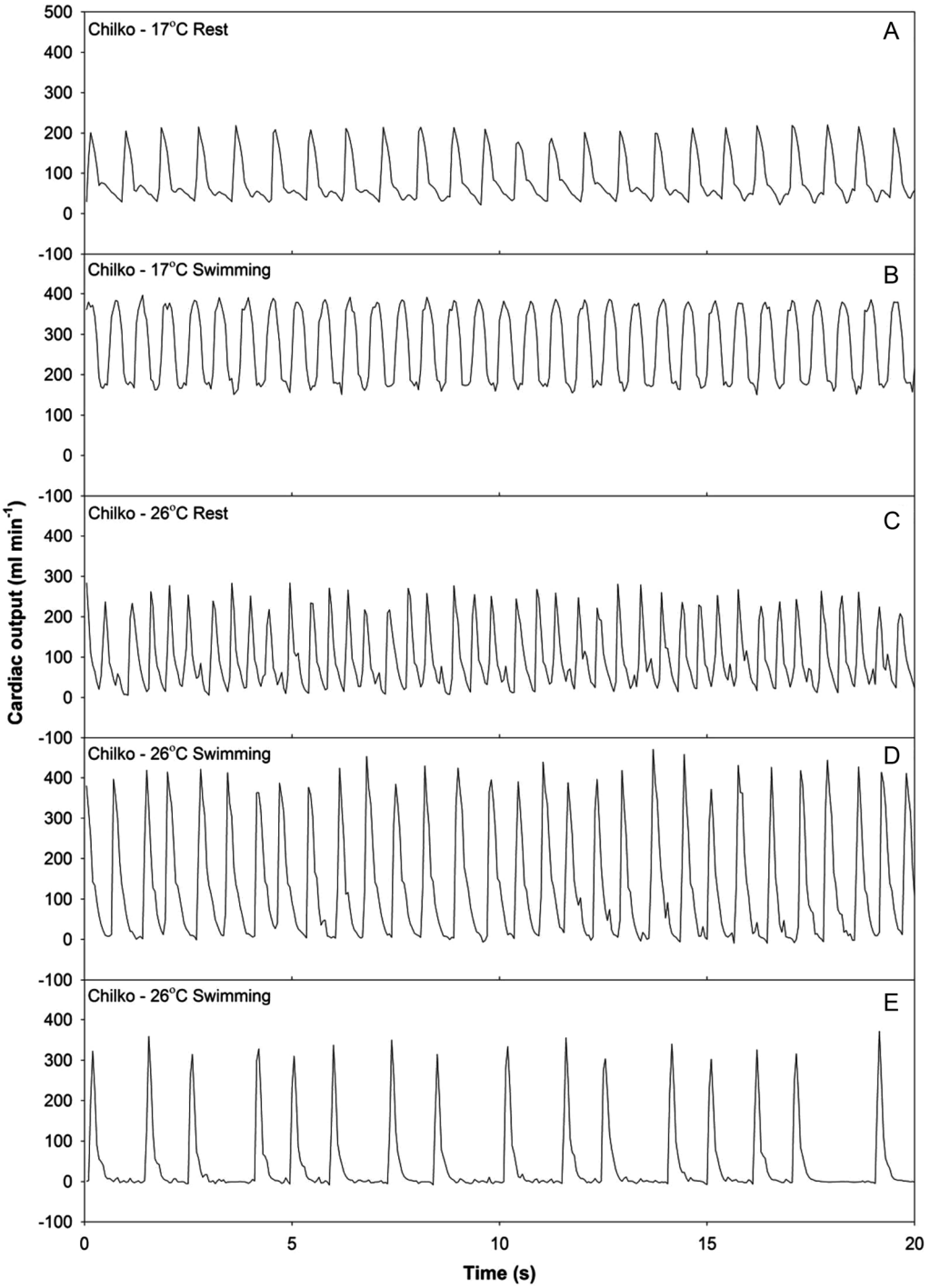

Figure 4: individual blood flow traces for two Chilko sockeye salmon at 17°C (T_{opt}) and 26°C ($T_{max0–50}$) at rest (**A** and **C**) and during swimming (**B**, **D**, and **E**). Swimming traces were recorded during the final swimming speed before each fish fatigued (measured at 2.3 and 1.5 bl s^{-1} for the 17 and 26°C fish, respectively). Trace D was recorded 5 min before trace E, at the same swimming speed.

changed as a function of temperature in a similar manner to those of the pooled populations that were analysed statistically (Figs 2 and 6). Resting MO_2, $\dot{V}_b$, and f_H all increased exponentially with increasing temperature (Fig. 6), whereas V_{srest} was insensitive to temperature. Both MO_{2max} and $\dot{V}_{bmax}$ increased with increasing temperature until T_{opt} was reached, and both declined at the warmest temperatures. Maximal heart rate increased with increasing

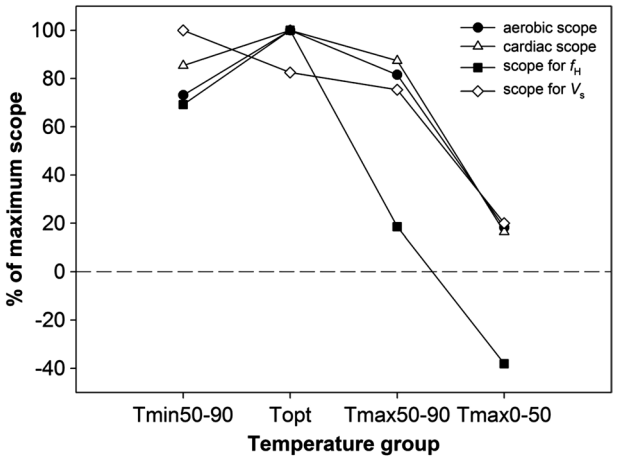

Figure 5: percentage of maximal aerobic scope, cardiac scope, scope for heart rate (f_H), and scope for stroke volume (V_s) for each temperature grouping.

temperatures and plateaued above T_{opt}, resulting in a negative scope for f_H at the warmest temperatures. Both V_{smax} and scope for V_s steadily declined with increasing temperatures (Fig. 6).

Cost of swimming

The oxygen cost of transport (COT) decreased with increasing swimming speed and reached a minimum of 0.12–0.26 mg O_2 kg^{-1} m^{-1} across temperature groupings (Fig. 7). Temperature effects on COT were primarily evident at low speeds (0.4 bl s^{-1} = 'rest'), with the warmer groupings ($T_{max50–90}$ and $T_{max0–50}$) tending to be twice as high compared with the colder groupings (T_{opt} and $T_{min50–90}$). The pattern was reversed for the net cost of transport (COT_{net}), which tended to be higher in the T_{opt} and $T_{min50–90}$ groupings.

The circulatory cost of transport (COT-$\dot{V}_b$) and net circulatory cost of transport for cardiac output (COT-$\dot{V}_{bnet}$) followed similar patterns with swimming and temperature to COT and COT_{net}, respectively (Fig. 7). The circulatory cost of transport decreased with increasing swimming speeds until ~0.87 bl s^{-1}, after which it was maintained at around 0.7–1.9 ml kg^{-1} m^{-1} for the $T_{min50–90}$, T_{opt}, and $T_{max50–90}$ groupings. In contrast, COT-$\dot{V}_b$ declined steadily in the $T_{max0–50}$ grouping until fish stopped swimming. Again, COT-$\dot{V}_b$ tended to be higher for warm temperature groupings; it was significantly lower at $T_{min50–90}$ compared with the $T_{max50–90}$ and $T_{max0–50}$ groupings at the slowest speeds (Fig. 7); however, COT-$\dot{V}_{bnet}$ tended to be higher in the T_{opt} and $T_{min50–90}$ groupings.

Oxygen uptake, transport, and removal

The oxygen status of arterial blood was assessed from P_{aO2} and C_{aO2}, which were found to be independent of temperature at all swimming speeds (Table 1, Fig. 8). In addition, resting P_{aO2} and C_{aO2} in the $T_{max50–90}$ or $T_{max0–50}$ groupings did not differ between the starting temperature (12°C) and the test temperature (22–26°C; Table 2). This result suggests that an acute temperature change while the fish were in the swim tunnel had no significant effect on arterial blood oxygen status. However, P_{aO2} and C_{aO2} tended to decrease relative to the resting values with swimming, as has been previously observed (Steinhausen *et al.*, 2008). Thus, oxygen transfer at the gills was unimpaired by supra-optimal temperature, even with the small effect of swimming on arterial oxygen status.

Haemoglobin concentration, haematocrit, and mean corpuscular haemoglobin concentration were also independent of temperature and swimming speed, except that MCHC decreased at fatigue in the $T_{max50–90}$ grouping relative to rest (Table 3). Given the stability of both C_{aO2} and [Hb] as a function of temperature, the changes in arterial oxygen transport (T_{aO2}) as a function of temperature (and swimming speed) were primarily a result of changes in $\dot{V}_b$. Notably, T_{aO2} for the $T_{max0–50}$ grouping was significantly lower for burst swimming and at fatigue when compared with T_{opt} (Table 1). Indeed, while T_{aO2} increased during burst swimming by 294, 153, and 80% over the resting values for the $T_{min50–90}$, T_{opt}, and $T_{max50–90}$ groupings, respectively, T_{aO2} was unchanged for the $T_{max0–50}$ grouping (Table 1). Thus, the ability of the circulatory system to transport blood to active locomotory muscles was greatly diminished at supra-optimal temperatures because of a decline in $\dot{V}_b$, not C_{aO2}.

Unlike arterial blood, P_{vO2} and C_{vO2} both varied significantly with temperature and swimming (Table 1). The changes in C_{vO2} directly reflected changes in haemoglobin saturation, because [Hb] was unchanged (Table 3). The effect of temperature on resting P_{vO2} and C_{vO2} was profound. Indeed, resting P_{vO2} for the $T_{max0–50}$ grouping was 10 torr, 25% of the T_{opt} value, and resting C_{vO2} was only 16–20% of the C_{vO2} for the other three temperature groupings. When temperature was treated as a continuous independent variable, the significant polynomial relationships also revealed decreases in resting P_{vO2} and C_{vO2} at the highest temperature (Fig. 8).

Swimming increased muscle oxygen extraction from the blood, as reflected by decreases of P_{vO2} and C_{vO2} (Table 1). At fatigue, C_{vO2} and P_{vO2} were 114 and 77%, respectively, lower in the $T_{max50–90}$ grouping compared with T_{opt}. Likewise, the significant polynomial relationship with temperature for both P_{vO2} and C_{vO2} at fatigue further illustrates the point that tissue oxygen extraction must have increased above T_{opt} (Fig. 8). Tissue oxygen extraction ($A–V_{O2}$) was calculated only for individuals with paired, simultaneous arterial and venous samples. The tendency for $A–V_{O2}$ to increase with swimming and at warmer temperatures did not reach statistical significance, probably due to low statistical power (Table 1).

Even though the tissues extracted more oxygen from the blood with swimming, the amount of oxygen leaving the

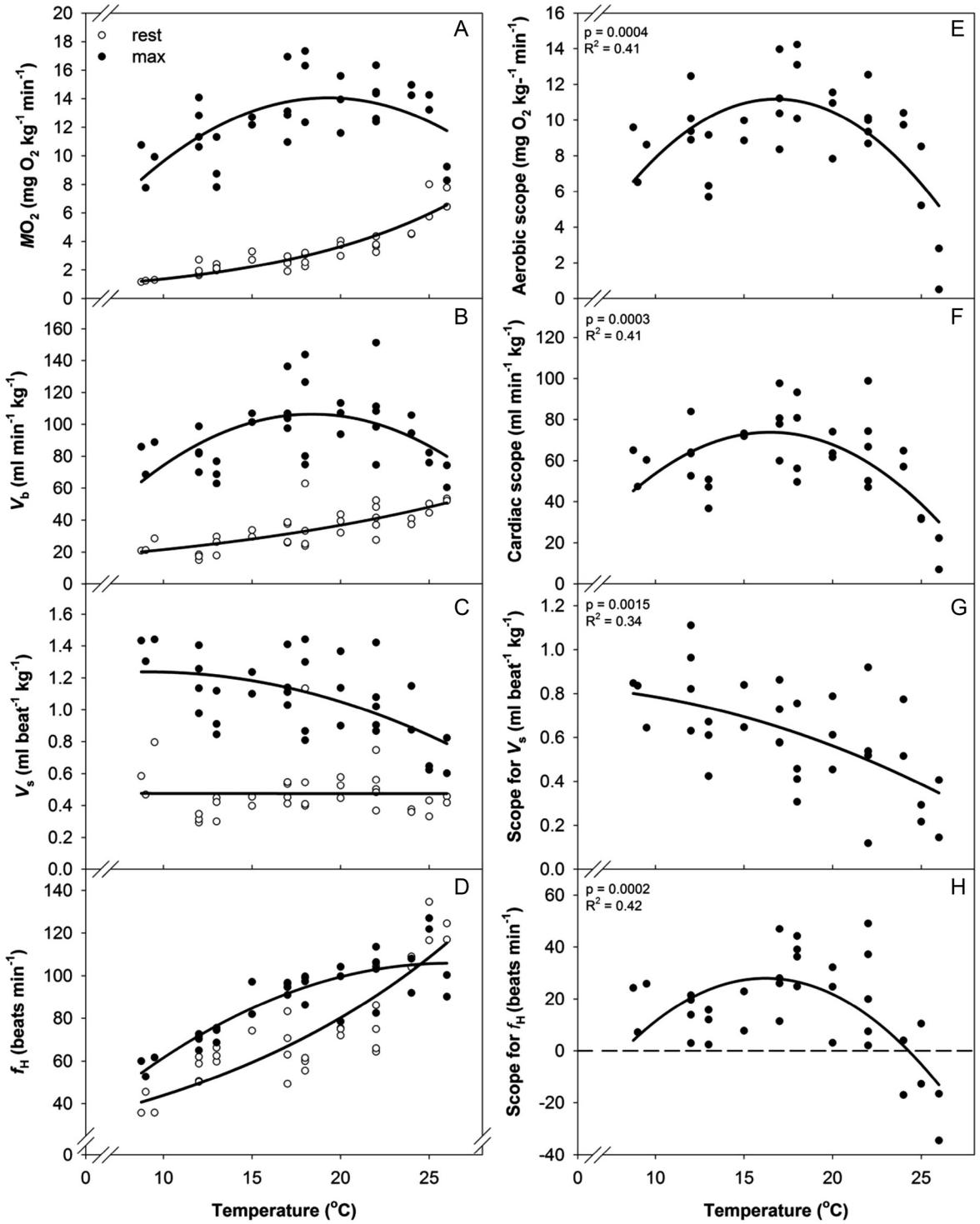

Figure 6: resting (open circles) and maximal values (filled circles) for oxygen consumption rate (MO_2; **A**), cardiac output ($\dot{V}_b$; **B**), stroke volume (V_s; **C**), and heart rate (f_H; **D**) in Chilko sockeye salmon. Each point corresponds to a single fish. Scope for MO_2 (**E**), $\dot{V}_b$ (**F**), V_s (**G**), and f_H (**H**) are shown. A polynomial quadratic equation was fitted to the maximum and scope data, an exponential equation was fitted to the resting data for MO_2, $\dot{V}_b$, and f_H, and no relationship was found with temperature for resting V_s. Data in panels A and E and the equations for the lines for F and H have been previously reported by Eliason *et al.* (2011).

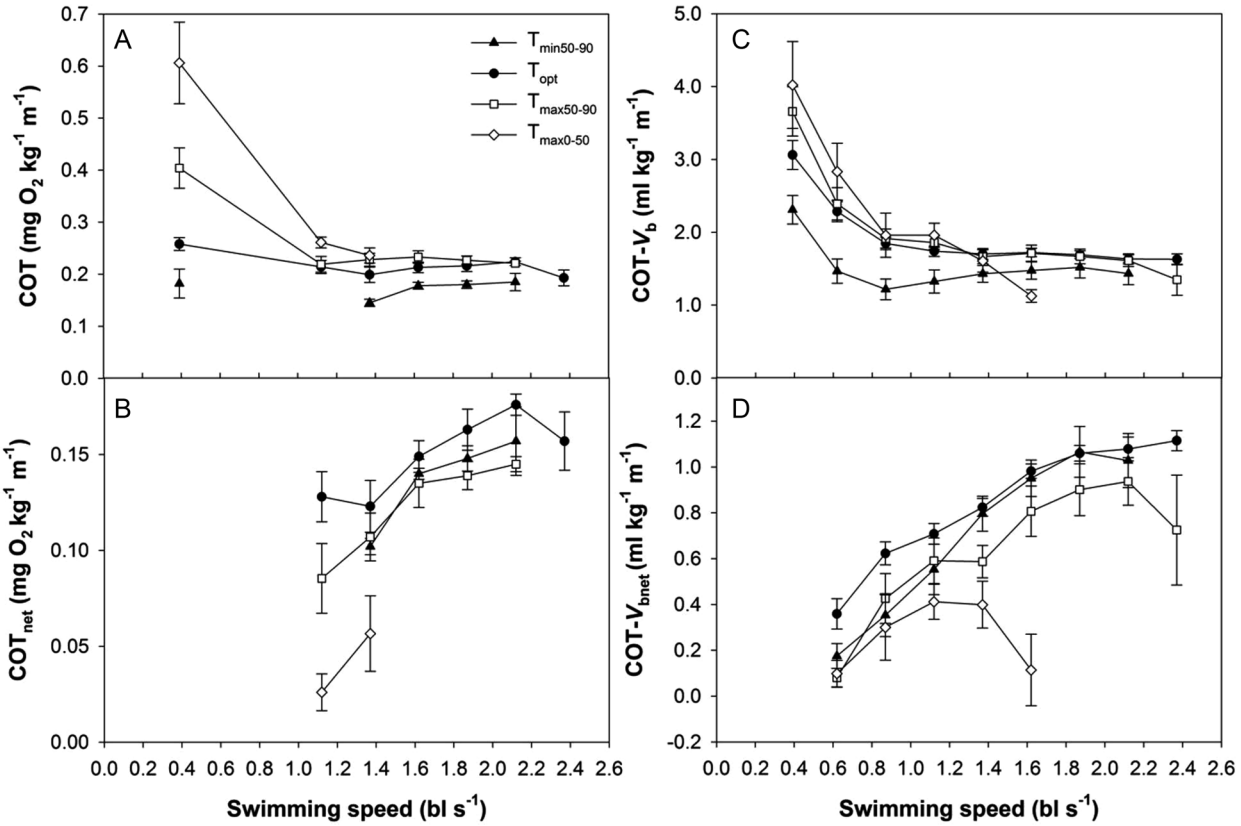

Figure 7: Cost of transport (COT; **A**), net cost of transport (COT_{net}; **B**), cardiovascular cost of transport (COT - $\dot{V}_b$; **C**), and net cardiovascular cost of transport (COT - $\dot{V}_{bnet}$; **D**) with swimming speed across the four temperature groupings. Means ± SEM are shown.

tissues and returning to the heart (venous oxygen transport; T_{vO2}) was independent of swimming effort at T_{opt} (Table 1). Thus, the increase in $\dot{V}_b$ matched the decrease in C_{vO2}. However, this situation changed at supra-optimal temperatures, because T_{vO2} significantly decreased at burst and fatigue for the $T_{max50–90}$ grouping (Table 1). Even at rest, T_{vO2} was reduced by over 4-fold in the $T_{max0–50}$ grouping compared with T_{opt} (Table 1).

Other blood variables

Plasma lactate varied significantly with both temperature and swimming (Table 3). The effect of supra-optimal temperatures on plasma lactate was profound, because resting plasma lactate was >3-fold higher in the $T_{max0–50}$ grouping compared with T_{opt} (Table 3). This result suggests that oxygen supply was insufficient to meet tissue oxygen demand even at rest for the highest test temperature. As expected, burst swimming to fatigue increased plasma lactate by 2- to 4-fold relative to resting levels for all temperature groupings. Moreover, plasma lactate was highest in fish at $T_{max50–90}$ at fatigue compared with the other groups (Table 3). Plasma glucose was independent of temperature and swimming, except for a significant decrease in plasma glucose with swimming for the $T_{max0–50}$ grouping (Table 3), perhaps reflecting a greater drain on this fuel for anaerobic metabolism.

Plasma sodium varied significantly with both temperature and swimming speed (Table 3), tending to be highest for the T_{opt} grouping and increasing with swimming speed for the T_{opt} and $T_{max50–90}$ groupings. Plasma chloride also varied significantly with temperature, but not with swimming speed (Table 3), and was significantly higher at T_{opt} relative to the $T_{max0–50}$ grouping. Plasma potassium did not vary significantly with temperature (Table 3), but tended to decrease with swimming speed, and was lowest at fatigue.

Discussion

In order to apply physiological knowledge to conservation problems, scientists require a mechanistic understanding and the support of guiding principles. The present study sought to make such a contribution by examining the mechanism of the decline in MO_{2max} and aerobic scope above T_{opt} in sockeye salmon using the OCLTT hypothesis as a framework. Earlier temperature studies with fishes either did not measure all the required variables (i.e. MO_2, $\dot{V}_b$, C_{aO2}, and C_{vO2}) or the fish

Table 1: oxygen status variables across the four temperature groupings and with swimming

	Rest	Steady	Burst	Fatigue
P_{aO2} (torr)				
$T_{min50-90}$	–	60.7 ± 9.3	62.9 ± 3.5	49.8 ± 2.4
T_{opt}	98.4 ± 7.4	96.5 ± 5.8	64.7 ± 9.6*	69.7 ± 4.9*
$T_{max50-90}$	84.6 ± 7.7	71.9 ± 3.8	58.1 ± 4.3	60.7 ± 5.7
$T_{max0-50}$	72.8 ± 4.8	70.8 ± 3.7	71.7 ± 7.4	75.4 ± 7.9
P_{vO2} (torr)				
$T_{min50-90}$	28.0 ± 1.3^{ab}	24.0 ± 1.1	–	17.5 ± 2.5
T_{opt}	41.6 ± 2.4^{a}	32.6 ± 1.9*	17.6 ± 2.8*	23.4 ± 2.0*
$T_{max50-90}$	28.5 ± 3.9^{b}	21.1 ± 3.3	11.6 ± 3.7*	13.2 ± 2.18*
$T_{max0-50}$	10.3 ± 4.6^{c}	–	–	–
C_{aO2} (ml dl^{-1})				
$T_{min50-90}$	10.3 ± 1.7	11.6 ± 1.1	11.9 ± 1.2	11.6 ± 1.8
T_{opt}	12.0 ± 0.6	10.9 ± 0.7	9.2 ± 0.8	9.3 ± 0.6
$T_{max50-90}$	12.8 ± 0.6	11.2 ± 0.6	10.2 ± 0.5	9.0 ± 0.7*
$T_{max0-50}$	10.8 ± 0.9	9.9 ± 1.6	9.2 ± 1.5	8.5 ± 0.5
C_{vO2} (ml dl^{-1})				
$T_{min50-90}$	7.7 ± 1.1^{a}	4.9 ± 1.2	–	3.5 ± 0.4^{a}*
T_{opt}	8.2 ± 0.4^{a}	5.7 ± 0.4*	2.5 ± 0.3 *	3.0 ± 0.4^{a}*
$T_{max50-90}$	6.9 ± 0.5^{a}	4.3 ± 0.8	1.0 ± 0.4*	1.4 ± 0.4^{b}*
$T_{max0-50}$	1.4 ± 0.6^{b}	–	–	–
A–V_{O2} (ml dl^{-1})				
$T_{min50-90}$	1.6 ± 1.6	–	–	6.8 ± 1.7*
T_{opt}	4.2 ± 0.8	4.8 ± 0.5	6.3 ± 1.0	6.0 ± 0.7
$T_{max50-90}$	6.1 ± 1.2	7.0 ± 0.9	9.2 ± 0.7	8.0 ± 1.4
$T_{max0-50}$	–	–	–	–
T_{aO2} (ml O_2 min^{-1} kg^{-1})				
$T_{min50-90}$	3.4 ± 0.5	8.6 ± 1.0	13.4 ± 0.1^{ab}*	11.1 ± 1.1^{a}*
T_{opt}	5.8 ± 0.4	9.4 ± 0.4*	14.7 ± 1.2^{a}*	10.7 ± 0.8^{a}*
$T_{max50-90}$	7.6 ± 0.3	9.8 ± 0.4	13.7 ± 2.0^{a}*	8.9 ± 1.7^{ab}
$T_{max0-50}$	7.2 ± 0.5	8.7 ± 1.2	7.4 ± 0.7^{b}	4.1 ± 1.4^{b}
T_{vO2} (ml O_2 min^{-1} kg^{-1})				
$T_{min50-90}$	2.8 ± 0.4^{b}	3.8 ± 0.7		3.6 ± 0.4
T_{opt}	4.1 ± 0.3^{a}	5.1 ± 0.4	3.7 ± 0.4	3.0 ± 0.4
$T_{max50-90}$	4.2 ± 0.4^{ab}	3.8 ± 0.7	1.4 ± 0.5*	1.6 ± 0.5*
$T_{max0-50}$	0.8 ± 0.6^{b}	–	–	–

Arterial and venous partial pressures of oxygen (P_{aO2} and P_{vO2}), oxygen content (C_{aO2} and C_{vO2}), oxygen extraction (A–V_{O2}), arterial oxygen transport (T_{aO2}), and venous oxygen transport (T_{vO2}) are indicated. Values are means ± SEM. Temperature groupings with different letters within a swimming speed are statistically different, and an asterisk indicates a statistically significant difference from rest within a temperature grouping ($P < 0.05$).

were not swum maximally, if at all. For example, many studies have examined the collapse of cardiorespiratory performance during warming of resting fish (Heath and Hughes, 1973; Cech *et al.*, 1976; Sartoris *et al.*, 2003; Gollock *et al.*, 2006; Clark *et al.*, 2008b; Keen and Gamperl, 2012), but their results relate to what is triggering events close to T_{crit} as opposed to the collapse of aerobic scope above T_{opt}.

Brett (1971) first described a parallel association of swimming performance, aerobic scope, and cardiac scope as a function of temperature between 5 and 22°C. He noted a decrease in aerobic scope, cardiac scope, and scope for heart rate above a T_{opt} of 15°C. However, $\dot{V}_b$ was calculated using the Fick principle, and Brett concluded that the cardiorespiratory collapse above T_{opt} was likely to be due to limited oxygen availability, even though blood gases were not reported (Brett, 1964, 1971). Taylor *et al.* (1996) examined aerobic swimming of rainbow trout at three acclimation temperatures (4, 11, and 18°C), and indirectly estimated $\dot{V}_b$ using microspheres. By coupling these cardiac measurements with an earlier study of blood gases (Taylor *et al.*, 1993), they concluded that aerobic swimming performance may have been limited at high temperature because of numerous factors, including reduced oxygen solubility in water and plasma, decreased arterial blood oxygen content and haematocrit, elevated energy expenditure for reproduction, reduced aerobic muscle mass or decreased cardiac scope, and reduced blood flow to swimming muscles. Steinhausen *et al.* (2008) was the first to measure MO_2, $\dot{V}_b$, C_{aO2}, and C_{vO2} simultaneously and directly in sockeye salmon as they were progressively warmed from 15 to 24°C while swimming steadily at ~1.35 bl s^{-1} or ~75% of maximum. They concluded that a cardiac limitation developed at warm temperatures in swimming fish, because $\dot{V}_b$, f_H, and T_{aO2} reached maxima and failed to continue to increase with increasing temperatures in order to meet the increased tissue oxygen demand.

The present study is the most comprehensive assessment to date of aerobic scope and the associated cardiovascular and blood gas variables across a range of temperatures in an aquatic ectotherm. We conclude that a limitation in maximal cardiac performance inhibits MO_{2max} and aerobic scope at supra-optimal temperatures in salmonids, which is apparently triggered by a precipitous decrease in scope for f_H. Thus, the present study with sockeye salmon found support for the OCLTT hypothesis, though we caution that the OCLTT hypothesis may not be broadly applicable to all ectotherms [e.g. pink salmon (Clark *et al.*, 2011), intertidal snails (Marshall *et al.*, 2011), and air-breathing toads (Overgaard *et al.*, 2012)] and warrants further investigation. Of particular conservation concern for sockeye salmon is the additional finding that post-fatigue mortality occurred at temperatures only 3–5°C above T_{opt}.

Swimming performance

Salmonids are known to increase their reliance on anaerobic swimming in U_{crit} tests at supra-optimal temperatures (Brett,

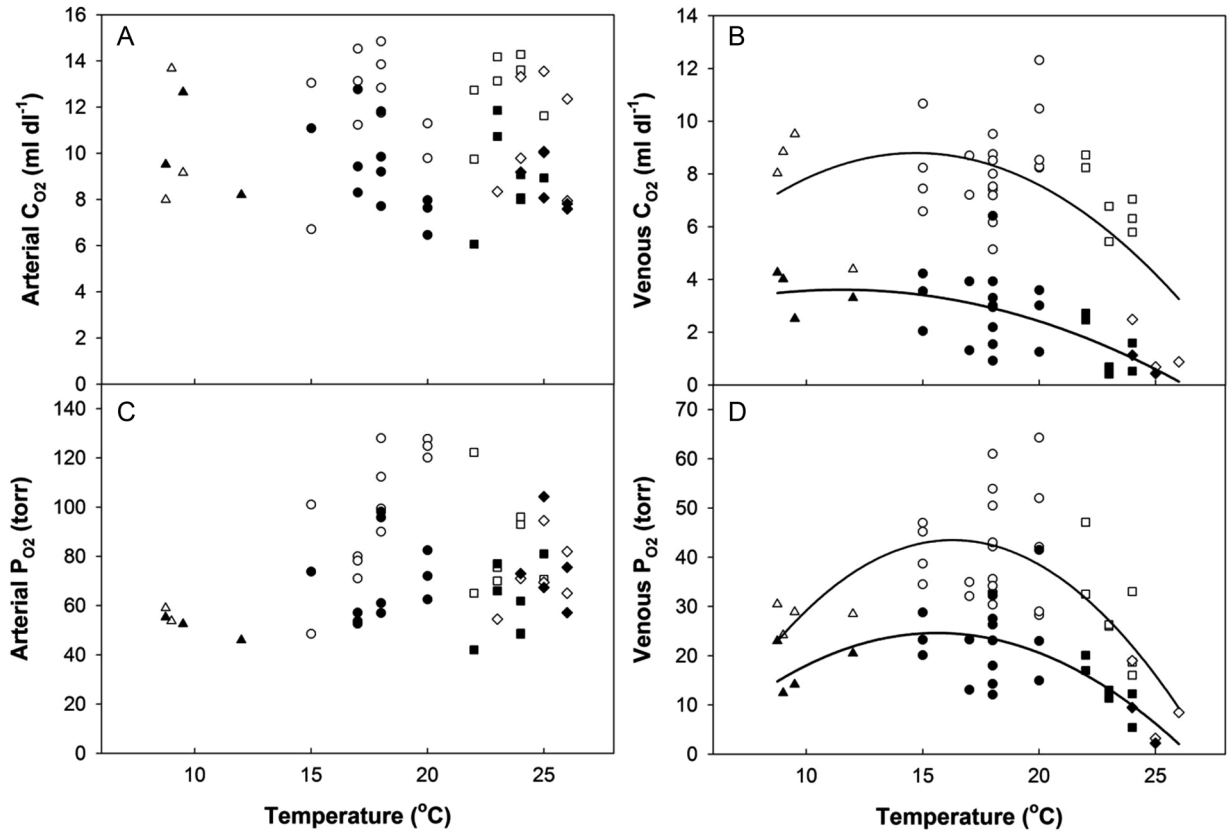

Figure 8: arterial and venous oxygen content (C_{O2}; **A** and **B**, respectively) and partial pressure of oxygen (P_{O2}; (**C** and **D**, respectively) at rest (open symbols) and fatigue (filled symbols) in four temperature groupings (triangles = $T_{min50–90}$; circles = T_{opt}; squares = $T_{max50–90}$; and diamonds = $T_{max0–50}$). Each data point corresponds to an individual fish. A quadratic equation was fitted through the venous data. For resting C_{vO2}, $R^2 = 0.37$, $P = 0.0007$; for fatigue C_{vO2}, $R^2 = 0.39$, $P = 0.002$; for resting P_{vO2}, $R^2 = 0.51$, $P < 0.0001$; and for fatigue P_{vO2}, $R^2 = 0.41$ $P = 0.001$.

Table 2: arterial partial pressure of oxygen (P_{aO2}) and oxygen content (C_{aO2}) in resting fish, measured at 12°C and at the test temperature

	n	P_{aO2} (torr)		C_{aO2} (ml dl^{-1})	
		12°C	Test temp.	12°C	Test temp.
$T_{max50–90}$	5	67.5 ± 3.3	81.0 ± 5.6	14.2 ± 0.6	13.4 ± 0.5
$T_{max0–50}$	7	63.5 ± 6.4	72.8 ± 4.8	12.0 ± 0.4	10.8 ± 0.9

Means ± SEM are presented. There were no significant differences within a temperature group ($P > 0.05$).

1964; Jain and Farrell, 2003), a finding that lends support to the OCLTT hypothesis. This was clearly evidenced here by the elevated lactate levels at fatigue in the warmest temperature grouping, supporting the idea that oxygen delivery to tissues in swimming fish becomes limited at temperatures near the critical maximum. A novel finding in the present study was the elevated plasma lactate even at rest and the depletion of plasma glucose with swimming in the highest temperature grouping, providing further indications that tissue oxygen delivery was compromised. The difficulty of swimming at supra-optimal temperature was evident from the increased COT and COT-$\dot{V}_b$ with temperature, indicating higher oxygen and cardiovascular costs to swimming at a given speed compared with cooler temperatures. In fact, the true COT and COT-$\dot{V}_b$ may be even higher, because the metabolic costs of anaerobic swimming require the characterization of excess post-exhaustion oxygen consumption (see Lee *et al.*, 2003a), which was not done here.

Gill oxygen delivery and uptake

Water oxygen content decreases by around 2% °C^{-1} with increasing water temperature (Dejours, 1975), which limits environmental oxygen availability. Furthermore, the affinity of haemoglobin for oxygen decreases with increasing temperature (Perry and Reid, 1994; Jensen *et al.*, 1998; Clark *et al.*, 2008b), which may hamper oxygen uptake at the gill and potentially prevent full haemoglobin saturation. Therefore, strong theoretical arguments exist for a limitation in oxygen uptake at the gills to explain the collapse of aerobic scope above T_{opt} (through either insufficient water delivery to

Table 3: arterial haematological variables across the four temperature groupings and with swimming

	Rest	Steady	Burst	Fatigue
[Hb] (g l^{-1})				
$T_{min50-90}$	92.3 ± 9.0	89.9 ± 10.6	93.5 ± 11.8	81.5 ± 12.6
T_{opt}	92.4 ± 4.6	90.3 ± 4.2	91.8 ± 5.4	87.3 ± 3.0
$T_{max50-90}$	100.3 ± 10.9	95.8 ± 2.7	89.3 ± 6.3	89.1 ± 6.3
$T_{max0-50}$	89.0 ± 5.0	98.3 ± 6.0	91.6 ± 7.5	91.8 ± 2.3
Hct (%)				
$T_{min50-90}$	30.7 ± 3.4	29.2 ± 1.6	30.6 ± 3.5	29.6 ± 5.2
T_{opt}	31.0 ± 1.7	30.4 ± 1.6	33.9 ± 2.5	31.1 ± 1.4
$T_{max50-90}$	32.9 ± 1.8	31.4 ± 0.8	34.2 ± 2.2	36.1 ± 1.4
$T_{max0-50}$	32.6 ± 1.5	32.1 ± 0.9	35.8 ± 3.4	37.5 ± 3.3
MCHC (g l^{-1})				
$T_{min50-90}$	302.2 ± 9.1	306.4 ± 19.7	305.1 ± 6.3	278.3 ± 10.9
T_{opt}	300.5 ± 6.6	299.4 ± 6.8	273.1 ± 8.1	282.3 ± 4.9
$T_{max50-90}$	308.4 ± 32.7	306.7 ± 13.5	267.7 ± 25.1	249.0 ± 22.0*
$T_{max0-50}$	274.5 ± 15.8	305.7 ± 11.7	259.1 ± 19.4	250.4 ± 16.4
Glucose (mmol l^{-1})				
$T_{min50-90}$	–	9.6 ± 0.7	9.0 ± 0.3	9.8 ± 0.7
T_{opt}	5.6 ± 0.5	5.1 ± 0.5	6.6 ± 0.5	6.2 ± 0.6
$T_{max50-90}$	7.6 ± 1.9	8.3 ± 1.2	6.9 ± 1.0	7.8 ± 1.1
$T_{max0-50}$	10.2 ± 2.0	–	5.4 ± 1.9*	7.3 ± 1.6*
Lactate (mmol l^{-1})				
$T_{min50-90}$	0.8 ± 0.4^{a}	1.3 ± 0.3	2.3 ± 0.5	4.0 ± 0.9^{a}
T_{opt}	1.3 ± 0.2^{a}	1.4 ± 0.2	3.5 ± 0.5	5.3 ± 0.6^{ab}*
$T_{max50-90}$	2.3 ± 0.5^{ab}	2.5 ± 0.4	5.0 ± 0.6	9.5 ± 0.9^{c}*
$T_{max0-50}$	4.5 ± 0.7^{b}	–	5.0 ± 1.4	8.0 ± 0.7^{b}*
Na^{+} (mmol l^{-1})				
$T_{min50-90}$	142.3 ± 2.9^{a}	138.1 ± 1.1	133.1 ± 6.0	–
T_{opt}	140.1 ± 1.7^{a}	143.8 ± 1.6	145.9 ± 2.1	152.9 ± 2.0^{a}*
$T_{max50-90}$	120.7 ± 6.8^{b}	140.4 ± 5.2*	138.9 ± 3.2*	139.6 ± 4.7^{ab}*
$T_{max0-50}$	137.7 ± 4.1^{ab}	–	135.9 ± 2.6	137.6 ± 2.6^{b}
K^{+} (mmol l^{-1})				
$T_{min50-90}$	3.3 ± 0.1	3.9 ± 0.5	4.3 ± 0.8	4.0 ± 1.8
T_{opt}	4.9 ± 0.4	4.6 ± 0.4	2.7 ± 0.7	2.6 ± 0.3*
$T_{max50-90}$	6.2 ± 1.0	4.5 ± 0.7	2.8 ± 0.6*	1.7 ± 0.3*
$T_{max0-50}$	3.8 ± 0.3	–	3.9 ± 0.9	1.7 ± 0.6
Cl^{-} (mmol l^{-1})				
$T_{min50-90}$	121.0 ± 2.0^{ab}	122.0 ± 5.2	117.4 ± 2.3^{ab}	123.6 ± 3.8^{ab}
T_{opt}	127.5 ± 0.9^{a}	129.9 ± 1.4	130.2 ± 1.3^{a}	133.3 ± 1.2^{a}
$T_{max50-90}$	119.3 ± 6.0^{ab}	119.8 ± 2.1	118.3 ± 4.5^{ab}	119.9 ± 5.2^{b}
$T_{max0-50}$	112.0 ± 3.1^{b}	–	113.4 ± 3.9^{b}	111.9 ± 2.7^{b}

Haemoglobin concentration ([Hb]), haematocrit (Hct), mean cell haemoglobin concentration (MCHC), plasma sodium (Na^{+}), plasma potassium (K^{+}), and plasma chloride (Cl^{-}) are indicated. Values are means ± SEM. Temperature groupings with different letters within a swimming speed are statistically different, and an asterisk indicates a statically significant difference from rest within a temperature grouping ($P < 0.05$).

the gills or reduced diffusion of oxygen across the gills). If this were the case, P_{aO2} and C_{aO2} would decrease and potentially reduce T_{aO2} at temperatures above T_{opt}.

There is considerable evidence for such changes in arterial oxygen status in resting, but not in exercising fishes. Keen and Gamperl (2012) showed a reduction in C_{aO2} and P_{aO2} by ~25 and 35%, respectively, in resting steelhead trout warmed from 12°C to their critical thermal maximum. However, they noted that a limited capacity for ram ventilation in the test apparatus could have been a contributing factor. Clark *et al.* (2008b) found that resting adult Chinook salmon slightly decreased C_{aO2} and P_{aO2} when acutely warmed, but this was more pronounced in larger individuals. Heath and Hughes (1973) showed a large and parallel decrease in C_{aO2} and C_{vO2} with acute heat stress up to 25°C in resting rainbow trout, but did not measure Hct or [Hb] to check for haemodilution with repetitive blood sampling. The only report of C_{aO2} becoming lower during swimming was for seasonally acclimatized rainbow trout at 18°C when compared with 4 and 11°C, but haematocrit was also halved at 18°C (Taylor *et al.*, 1993).

In contrast to these works, both the present study and that of Steinhausen *et al.* (2008) found that C_{aO2}, P_{aO2}, and Hct were maintained independent of temperature in swimming adult sockeye salmon. Likewise, Sartoris *et al.* (2003) found that P_{aO2} remained constant during acute warming in resting Atlantic cod. A telling discovery here was that C_{aO2} was maintained even when cardiac dyssynchrony and post-fatigue delayed mortality occurred at extremely warm temperatures. As such, the present study clearly provides no support for an oxygen limitation developing at the gills at warm temperatures.

Given the different conclusions that have been reached for the effects of temperature on oxygen delivery and uptake at the gill, future studies must pay close attention to the response of Hct and [Hb] to temperature, which directly affects C_{aO2}. Haematocrit has been shown to increase by up to 27% due to splenic contraction in acutely warmed resting rainbow trout (Sandblom and Axelsson, 2007), to decrease by 50% in warm-acclimated rainbow trout (Taylor *et al.*, 1993), and to be minimally affected by temperature (present study and see Farrell, 1997; Clark *et al.*, 2008b; Steinhausen *et al.*, 2008). Future studies must consider haemodilution, because repeated blood sampling can reduce Hct, [Hb], and C_{aO2}, though this was not a problem in the present study. In addition, future studies should carefully consider the role of water velocity in assisting gill ventilation (ram ventilation), as was possible here in the swim tunnels, and the possibility that fish holding devices may restrict buccal and opercular movements during gill ventilation.

Circulatory transport of oxygen

All studies that have measured $\dot{V}_b$ (directly or indirectly) and MO_2 as a function of temperature in swimming salmonids

have reported parallel declines in $\dot{V}_b$ and MO_2 above T_{opt} (Brett, 1971; Taylor *et al.*, 1996; Steinhausen *et al.*, 2008; Eliason *et al.*, 2011). Here we present additional evidence in support of a cardiac limitation developing at supra-optimal temperatures, because maximal T_{aO2}, MO_2, and $\dot{V}_b$ all failed to increase above T_{opt}. In addition, all three variables decreased at $T_{max0–50}$, at which point scope for f_H completely collapsed without any compensation by V_s. Therefore, we conclude that a perfusion limitation develops above T_{opt}, resulting in insufficient oxygen delivery to meet the increased locomotory tissue oxygen demand. Notably, scope for f_H collapsed at a lower temperature compared with scope for $\dot{V}_b$, aerobic scope, and scope for V_s (Fig. 5). Therefore, we propose that reduced scope for f_H is the triggering mechanism that limits $\dot{V}_{bmax}$ above T_{opt}, supporting an earlier proposal (Farrell, 2009). It is becoming clear that f_H is a central mechanism setting upper temperature tolerance and thus may be a key limiting factor regulating fish distribution globally.

Changes in $\dot{V}_b$ and T_{aO2} as a function of temperature are almost entirely mediated by changes in f_H in both resting and swimming fish (present study and Cech *et al.*, 1976; Gollock *et al.*, 2006; Sandblom and Axelsson, 2007; Clark *et al.*, 2008b, 2011; Steinhausen *et al.*, 2008), underscoring the central importance of f_H in determining cardiac performance and oxygen delivery with temperature. More broadly, the central importance of resting f_H in setting temperature tolerances for intertidal invertebrates is also gaining support (Somero, 2011). Direct effects of temperature on the cardiac pacemaker rate (Randall, 1970) are likely to account for temperature-induced cardiac acceleration. Here, resting f_H continued to increase until lethal temperatures were approached in the highest temperature group (range, 117–135 beats min^{-1}; mean, 123.9 beats min^{-1}). Moreover, f_{Hmax} plateaued above T_{opt}, decreasing from rest at the highest test temperatures. Therefore, we suggest that factors that (i) determine f_{Hrest} and (ii) limit f_{Hmax} may be of particular importance for future studies.

The bradycardia followed by dysrhythmia in fish swimming at the highest temperature is a novel observation that adds to the observation of irregular heart beats in resting rainbow trout, Atlantic cod, and Chinook salmon acutely warmed to their T_{crit} (Heath and Hughes, 1973; Gollock *et al.*, 2006; Clark *et al.*, 2008b). Here, severe dysrhythmias were associated with dramatic decreases in P_{vO2}, C_{vO2}, and T_{vO2}, although these sockeye salmon displayed an impressive tenacity to continue swimming, albeit at lower speeds and with a lower MO_2, $\dot{V}_b$, and T_{aO2}. Such tenacity, however, may have severe consequences, because there was significant post-fatigue mortality shortly after exhaustion despite cooling the salmon. At temperatures a few degrees cooler, there was no such immediate consequence. However, we cannot rule out the possibility of delayed mortality, because it can occur hours to days after exhaustive exercise, both in the laboratory (e.g. Black, 1957; Wood *et al.*, 1983) and in nature (e.g. Donaldson *et al.*, 2012), especially at elevated temperature.

The mechanisms limiting maximal f_H and triggering bradycardia and cardiac dysrhythmia remain unclear. Maximal f_H is clearly unable to continue increasing with a Q_{10} of ~2 at temperatures above T_{opt}. The initial bradycardia during swimming may have been vagally mediated and could act as a protective mechanism by (i) reducing $\dot{V}_b$ and cardiac oxygen demand (Farrell and Steffensen, 1987), (ii) increasing blood residence time in the heart to favour oxygen extraction by spongy myocardium (Farrell, 2007b), and (iii) reducing coronary vascular compression to favour a more continuous coronary blood flow to compact myocardium (Axelsson and Farrell, 1993; Gamperl *et al.*, 1995). The possibility of a centrally mediated bradycardia should be examined using atropine injection or vagotomy. Alternatively, synergistically or perhaps secondarily, the deleterious venous blood environment associated with anaerobic swimming at high temperature could have triggered cardiac problems, including the observed decrease in f_{Hmax} and V_{smax}. Specifically, anaerobic swimming makes venous blood acidotic (low pH), hypoxaemic (low P_{vO2}) and hyperkalaemic (high K^+; Kiceniuk and Jones, 1977; Holk and Lykkeboe, 1998), all of which become exacerbated at high temperature (Brett, 1964; Jain and Farrell, 2003; Steinhausen *et al.*, 2008). Notably, hyperkalaemia was absent in the present study. These extracellular changes can impair cardiac contractility (Driedzic and Gesser, 1994), and even more so at high temperature (Hanson and Farrell, 2007). At extremely high temperatures, the decreases in P_{vO2} and T_{vO2} at warm temperatures may not have guaranteed sufficient oxygen supply to the avascular spongy myocardium (Farrell and Clutterham, 2003; Farrell, 2007a), resulting in reduced contractility and perhaps dysrhythmias. In fact, the lowest P_{vO2} observed at the highest temperature (10 torr) is around the suggested lower limit for adequate oxygen delivery to the fish spongy myocardium (Davie and Farrell, 1991). In contrast, the compact myocardium (supplied with oxygen by the coronary circulation) probably would not have suffered in a similar manner, because C_{aO2} and P_{aO2} were maintained. All told, the exact mechanisms and sequence of events leading to the f_H limitation are unclear and warrant further study.

Why resting fish do not increase V_s with acute warming remains unknown (present study and Cech *et al.*, 1976; Brodeur *et al.*, 2001; Gollock *et al.*, 2006; Sandblom and Axelsson, 2007; Clark *et al.*, 2008b; Steinhausen *et al.*, 2008; Mendonça and Gamperl, 2010; Gamperl *et al.*, 2011). Elevated temperature, *per se*, does not prevent an increase in resting V_s, because zatebradine treatment, which reduces f_H at the level of the pacemaker, triggered a compensatory increase in V_s in resting rainbow and steelhead trout at high temperature (Gamperl *et al.*, 2011; Keen and Gamperl, 2012). All the same, supra-optimal temperatures can reduce V_{smax} in swimming salmon (present study and Clark *et al.*, 2011), something that was not revealed when sockeye salmon were swimming at ~75% of U_{crit} (Steinhausen *et al.*, 2008). The mechanism of the decline in V_{smax} at extremely warm temperatures is unknown, but may be related to the deleterious venous blood environment associated with anaerobic swimming at high temperature, as outlined above.

Tissue oxygen extraction

The elevation in plasma lactate in both resting and swimming fish is a clear indication that the increased tissue oxygen demand was not being met by oxygen delivery at supra-optimal temperature. This mismatch between oxygen supply and demand could have resulted from a perfusion and/or a diffusion limitation to the locomotory muscles. The present study clearly showed that arterial oxygen transport was compromised above T_{opt} due to reduced $\dot{V}_b$, leading to a muscle capillary perfusion limitation. However, insufficient tissue oxygen extraction could also lead to increased anaerobic metabolism if a diffusion limitation for oxygen existed at the locomotory muscles. Factors leading to a diffusion limitation are inadequate capillary density, ineffective muscle cellular morphology (e.g. poor mitochondria density or location), and an insufficient driving force for oxygen diffusion (low P_{aO2}; Egginton and Cordiner, 1997; Taylor *et al.*, 1997; Egginton, 2002). Thus, a diffusion limitation for oxygen is more likely for white rather than red muscle given the attendant ~10 times lower capillary density of white compared with red muscle in salmonids (Mosse, 1978; Egginton and Sidell, 1989). However, none of these morphometric features was studied here, and so assessing the role of a diffusion limitation relative to a perfusion limitation is difficult with *in vivo* studies.

Nevertheless, Steinhausen *et al.* (2008) suggested that a constant P_{vO2} during acute warming of resting and swimming sockeye salmon was evidence for a diffusion limitation. However, P_{vO2} and C_{vO2} both decreased above T_{opt} in resting and maximally swimming sockeye salmon in the present study. Likewise, P_{vO2} and/or C_{vO2} decreased with warming to high temperature in resting rainbow trout, Atlantic cod, and Chinook salmon (Heath and Hughes, 1973; Sartoris *et al.*, 2003; Clark *et al.*, 2008b). Thus, a tissue diffusion limitation may not have occurred immediately, because the tissues were able to extract more oxygen from the blood at temperatures above T_{opt}. We caution, however, that the decrease in C_{vO2} may not have been proportional to the increased oxygen demand with warming (Wagner, 1996). Assessments of a diffusion limitation are complicated by the decrease in $\dot{V}_b$, which potentially slows capillary transit time for blood, favouring oxygen diffusion. Furthermore, the decrease in blood pH accompanying anaerobic metabolism, which may facilitate oxygen unloading into the tissues via Bohr and Root effects, would provide further temporary relief from a diffusion limitation (Rummer and Brauner, 2011). All told, further research examining the possibility of a tissue diffusion limitation at warm temperature is warranted. Specifically, studies on the role of muscle morphology in limiting oxygen diffusion at high temperature in exercising fish remain a ripe area for future research.

Limitations of the study

This study overcame the difficult challenge of swimming a sufficient number of large fish equipped with cannulae and probes to measure critical cardiorespiratory variables directly and simultaneously in order to resolve the subtle changes in the oxygen cascade when fish swim above supra-optimal temperature. Adult sockeye salmon have proved to be an effective model, because they are large enough to carry a flow probe and support repeated but strategic arterial and venous blood sampling without haemodilution. However, migrating adult sockeye salmon naturally senesce and die 4–6 weeks after they enter freshwater, which places a logistic constraint on the duration of recovery periods. Previous work has shown that MO_{2rest}, MO_{2max}, and aerobic scope did not differ significantly between sockeye salmon that had undergone surgery and those that had not (Eliason *et al.*, 2013), suggesting that sockeye salmon can recover quickly from surgery.

Another challenge with wild Fraser River sockeye salmon is that different populations co-migrate upstream, and individual fish are impossible to differentiate until scale and DNA analysis have been conducted several days later. Therefore, in order to ensure good statistical power, we pooled three sockeye salmon populations that all face long and difficult river migrations, have a similar T_{opt} for aerobic scope (~17°C), and have a similar maximal aerobic scope, cardiac scope, and scope for heart rate (Eliason *et al.*, 2011). These three populations do not differ in MO_2, $\dot{V}_b$, f_H, or V_s at rest, when swimming, or during recovery at T_{opt}. Also, great care was taken to create temperature groupings relative to the population-specific T_{opt} such that the temperature differences were only 1–3°C across the populations within a temperature grouping above T_{opt}. As a result, the variances for MO_{2rest}, MO_{2max}, and aerobic scope were small for each temperature grouping (Fig. 2), which would not be the case if large population differences existed. Moreover, the Chilko population had temperature responses that paralleled those for the four temperature groupings (Figs 2 and 6).

Given the above constraints, sex-specific differences were not considered, but each temperature grouping contained approximately equal numbers of male and female sockeye salmon. Previous work (Eliason *et al.*, 2013) found no sex-specific differences in cardiorespiratory physiology and blood oxygen status at T_{opt} for sockeye salmon, while a study on pink salmon showed that males maintain a higher MO_{2rest} and achieve greater MO_{2max}, $\dot{V}_{bmax}$, and aerobic scope (Clark *et al.*, 2011). Notably, female sockeye salmon suffer higher mortality compared with males in stressful migratory conditions, such as high temperature, elevated flow, and challenging migratory obstacles (Nadeau *et al.*, 2010; Roscoe *et al.*, 2011; Jeffries *et al.*, 2012; Martins *et al.*, 2012). Given that spawning success for a population is governed by females, this raises potential conservation concerns for any sex differences in the physiological response to temperature, which should be considered in future studies.

Summary and conclusions

The results from the present study greatly expand upon the idea of a 'death spiral' for salmon swimming at supra-optimal

temperatures (Farrell *et al.*, 2009), which proposes a mechanistic explanation of the failure of aerobic scope above T_{opt}. Here we provide definitive evidence that the collapse of aerobic scope above T_{opt} is associated with a cardiac limitation, triggered by a collapse in scope for f_H. Accordingly, a perfusion limitation for oxygen delivery to swimming muscles developed at temperatures above T_{opt}, resulting in a mismatch between oxygen supply and demand, as evidenced by elevated lactate levels. The severity of the cardiac constraint increased with temperature to the point that elevated resting lactate, cardiac dysrhythmias, a negative scope for f_H, and even post-exhaustion mortality were observed at the highest temperatures. Both P_{aO2} and C_{aO2} were unchanged with warming above T_{opt}, suggesting no limitation in oxygen uptake at the gill with the present experimental design. With P_{vO2} and C_{vO2} (and probably blood pH, given the elevation in plasma lactate) decreasing at the highest test temperature, the possibility of a diffusion limitation also developing at the muscle remains unclear and warrants further study. We propose that a noxious venous blood environment (low pH and low P_{vO2}) further impairs cardiac function (reducing cardiac oxygen delivery, contractility, and perhaps f_H), which could initiate a positive feedback loop to exacerbate the perfusion limitation to locomotory muscle and perhaps lead to delayed mortality at extremely warm temperatures.

In terms of salmon conservation, climate change-induced increases in Fraser River temperatures have been associated with massive mortality in sockeye salmon during their spawning migration. Nevertheless, a detailed mechanistic understanding of the causes of migration failure has remained elusive. Previous work suggests that the high *en route* mortality may be partly attributed to a decrease in aerobic scope (Farrell *et al.*, 2008; Eliason *et al.*, 2011). The present study expands on this idea and suggests that salmon are unable to swim at warm temperature due to a decrease in aerobic scope caused by a cardiac limitation via insufficient scope for heart rate. While some populations of sockeye salmon have an exceptionally wide T_{opt} window (e.g. from 13 to 21°C for Chilko sockeye salmon; Eliason *et al.*, 2011), all populations studied to date are currently experiencing temperatures that exceed their population-specific T_{opt} window, and this is expected only to worsen in the future (Eliason *et al.*, 2011). The central importance of f_H in determining cardiac performance and oxygen delivery at warm temperature and the possibility of f_{Hmax} setting upper temperature limits may provide a useful, but simple measurement to move physiology into the field (Casselman *et al.*, 2012) and improve our knowledge of temperature tolerance of fishes as we try to conserve the natural resources that are left.

Acknowledgements

A. Lotto, D. Patterson, G. Cox, C. Wilson, Z. Gallagher, C. Whitney, A. Collins, M. Drenner, K. Jeffries, M. Gale, E. Sandblom, S. Pieperhoff, J. Burt, M. Donaldson, E. Halttunen, M. Hamer, E. Martins, D. Roscoe, S. Tang, P. Parsons, M. Hague, J. Hills, A. Grant, M. Shimomura, J. Carter, V. Ives, D. Mckay, T. Nettles, L. Thompson, G. Toulec, and V. Baudry helped with field and laboratory work. K. Miller, N. Ginther, C. Wallace, and J. Candy conducted the DNA analysis, and S. Latham provided the scale analysis. B. Smith and the staff at the Department of Fisheries and Oceans Cultus Lake Salmon Research Laboratory provided valuable logistical support. Chehalis and Yale First Nations bands provided invaluable fishing assistance. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC; STPGP 372700, STPGP 350382, NETGP 375118, and RGPIN 170064 to A.P.F. and S.G.H.), a Canada Research Chair to A.P.F., BC Pacific Salmon Forum grants to E.J.E., A.P.F., and S.G.H., an NSERC postgraduate scholarship to E.J.E., and a Killam Postdoctoral Fellowship to T.D.C.

Appendix 1

Abbreviations

$A\text{–}V_{O2}$, tissue oxygen extraction ($A\text{–}V_{O2} = C_{aO2} - C_{vO2}$); C_{aO2}, arterial oxygen content; C_{vO2}, venous oxygen content; COT, cost of transport [$COT = MO_2/(U \times 60)$]; COT_{net}, net cost of transport [$COT_{net} = (MO_2 - MO_{2rest})/(U \times 60)$]; COT-$\dot{V}_b$, cardiovascular cost of transport [COT-$\dot{V}_b$ = $\dot{V}_b$ / $(U \times 60)$]; COT-$\dot{V}_{bnet}$, net cardiovascular cost of transport [COT-$\dot{V}_{bnet} = (\dot{V}_b - \dot{V}_{brest} / (U \times 60)$]; f_H, heart rate; [Hb], haemaglobin concentration; Hct, haematocrit; MCHC, mean corpuscular haemaglobin concentration [MCHC = [Hb]/(Hct/100)]; MO_2, rate of oxygen consumption; OCLTT, oxygen- and capacity-limited thermal tolerance; P_{aO2}, arterial partial pressure of oxygen; P_{vO2}, venous partial pressure of oxygen; T_{aO2}, arterial oxygen transport ($T_{aO2} = \dot{V}_b \times C_{aO2}$); T_{crit}, critical temperature; $T_{min50–90}$, group of fish swum at temperatures lower than T_{opt} at which 50–90% of maximal aerobic scope was attained; $T_{max50–90}$, group of fish swum at temperatures higher than T_{opt} at which 50–90% of maximal aerobic scope was attained; $T_{max0–50}$, group of fish swum at temperatures higher than T_{opt} at which 0–50% of maximal aerobic scope was attained; $_{opt}$, optimal temperature; T_{vO2}, venous oxygen transport ($T_{vO2} = \dot{V}_b \times C_{vO2}$); U_{crit}, critical swimming velocity; $\dot{V}_b$, cardiac output; V_s, stroke volume ($V_s = \dot{V}_b \div f_H$).

References

Axelsson M, Farrell AP (1993) Coronary blood flow in vivo in the coho salmon (*Oncorhynchus kisutch*). Am J Physiol Regul Integr Comp Physiol 264: R963–R971.

Beacham TD, Candy JR, McIntosh B, MacConnachie C, Tabata A, Kaukinen K, Deng L, Miller KM, Withler RE, Varnavskaya N (2005) Estimation of stock composition and individual identification of sockeye salmon on a Pacific Rim basis using microsatellite and major histocompatibility complex variation. Trans Am Fish Soc 134: 1124–1146.

Bell WH, Terhune LDB (1970) Water tunnel design for fisheries research. Fisheries Research Board of Canada. Technical Report No. 195, pp 1–69.

Black EC (1957) Alterations in the blood levels of lactic acid in certain salmonid fishes following muscular activity. I. Kamloops trout, *Salmo gairdneri*. J Fish Res Board Can 14: 117–134.

Brett JR (1964) The respiratory metabolism and swimming performance of young sockeye salmon. J Fish Res Board Can 21: 1183–1226.

Brett JR (1971) Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). Am Zool 11: 99–113.

Brodeur JC, Dixon DG, McKinley RS (2001) Assessment of cardiac output as a predictor of metabolic rate in rainbow trout. J Fish Biol 58: 439–452.

Casselman MT, Anttila K, Farrell AP (2012) Using maximum heart rate as a rapid screening tool to determine optimum temperature for aerobic scope in Pacific salmon *Oncorhynchus* spp. J Fish Biol 80: 358–377.

Cech JJ Jr, Bridges DW, Rowell DM, Balzer PJ (1976) Cardiovascular responses of winter flounder *Pseudopleuronectes americanus* (Walbaum), to acute temperature increase. Can J Zool 54: 1383–1388.

Clark TD, Eliason EJ, Sandblom E, Hinch SG, Farrell AP (2008a) Calibration of a hand-held haemoglobin analyser for use on fish blood. J Fish Biol 73: 2587–2595.

Clark TD, Sandblom E, Cox GK, Hinch SG, Farrell AP (2008b) Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). Am J Physiol Regul Integr Comp Physiol 295: R1631–R1639.

Clark TD, Jeffries KM, Hinch SG, Farrell AP (2011) Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. J Exp Biol 214: 3074–3081.

Crossin GT, Hinch SG, Cooke SJ, Welch DW, Patterson DA, Jones SRM, Lotto AG, Leggatt RA, Mathes MT, Shrimpton JM *et al.* (2008) Exposure to high temperature influences the behaviour, physiology, and survival of sockeye salmon during spawning migration. Can J Zool 86: 127–140.

Davie PS, Farrell AP (1991) The coronary and luminal circulations of the myocardium of fishes. Can J Zool 69: 1993–2001.

Dejours P (1975) Principles of Comparative Respiratory Physiology. Elsevier, New York, pp 1–253.

Donaldson MR, Hinch SG, Raby GD, Patterson DA, Farrell AP, Cooke SJ (2012) Population-specific consequences of fisheries-related stressors on adult sockeye salmon. Physiol Biochem Zool 85: 729–739.

Drabkin DL, Austin JH (1935) Spectrophotometric studies II. Preparations from washed blood cells; nitric oxide, hemoglobin and sulfhemoglobin. J Biol Chem 112: 51–65.

Driedzic WR, Gesser H (1994) Energy metabolism and contractility in ectothermic vertebrate hearts: hypoxia, acidosis, and low temperature. Physiol Rev 74: 221–258.

Egginton S, Sidell BD (1989) Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. Am J Physiol Regul Integr Comp Physiol 256: R1–R9.

Egginton S, Cordiner S (1997) Cold-induced angiogenesis in seasonally acclimatized rainbow trout (*Oncorhynchus mykiss*). J Exp Biol 200: 2263–2268.

Egginton S (2002) Temperature and angiogenesis: the possible role of mechanical factors in capillary growth. Comp Biochem Physiol A Mol Integr Physiol 132: 773.

Eliason EJ, Clark TD, Hague MJ, Hanson LM, Gallagher ZS, Jeffries KM, Gale MK, Patterson DA, Hinch SG, Farrell AP (2011) Differences in thermal tolerance among sockeye salmon populations. Science 332: 109–112.

Eliason EJ, Wilson SM, Farrell AP, Cooke SJ, Hinch SG (2013) Low cardiac and aerobic scope in a coastal population of sockeye salmon *Oncorhynchus nerka* with a short upriver migration. J Fish Biol [Epub ahead of print].

Farrell AP, Steffensen JF (1987) An analysis of the energetic cost of the branchial and cardiac pumps during sustained swimming in trout. Fish Physiol Biochem 4: 73–79.

Farrell AP (1997) Effects of temperature on cardiovascular performance. In CM Wood, DG McDonald, eds. Global Warming: Implications for Freshwater and Marine Fish. Cambridge University Press, Cambridge, pp 135–158.

Farrell AP, Gallaugher PE, Fraser JA, Pike D, Bowering P, Hadwin AK, Parkhouse WS, Routledge R (2001) Successful recovery of the physiological status of coho salmon on-board a commercial gillnet vessel by means of a newly designed revival box. Can J Fish Aquat Sci 58: 1932–1946.

Farrell AP, Clutterham SM (2003) On-line venous oxygen tensions in rainbow trout during graded exercise at two acclimation temperatures. J Exp Biol 206: 487–496.

Farrell AP (2007a) Cardiorespiratory performance during prolonged swimming tests with salmonids: a perspective on temperature effects and potential analytical pitfalls. Philos Trans R Soc B Biol Sci 362: 2017–2030.

Farrell AP (2007b) Tribute to P. L. Lutz: a message from the heart – why hypoxic bradycardia in fishes? J Exp Biol 210: 1715–1725.

Farrell AP, Hinch SG, Cooke SJ, Patterson DA, Crossin GT, Lapointe M, Mathes MT (2008) Pacific salmon in hot water: applying aerobic scope models and biotelemetry to predict the success of spawning migrations. Physiol Biochem Zool 81: 697–709.

Farrell AP (2009) Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. J Exp Biol 212: 3771–3780.

Farrell AP, Eliason EJ, Sandblom E, Clark TD (2009) Fish cardiorespiratory physiology in an era of climate change. Can J Zool 87: 835–851.

Frederich M, Pörtner HO (2000) Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja*

squinado. Am J Physiol Regul Integr Comp Physiol 279: R1531–R1538.

Fry FEJ (1947) Effects of the environment on animal activity. Publ Ontario Fish Res Lab 68: 1–62.

Fry FEJ (1971) The effect of environmental factors on the physiology of fish. In WS Hoar, DJ Randall, eds. Fish Physiology, Vol 6. Academic Press, New York, pp 1–98.

Gamperl AK, Axelsson M, Farrell AP (1995) Effects of swimming and environmental hypoxia on coronary blood flow in rainbow trout. Am J Physiol Regul Integr Comp Physiol 38: R1258–R1266.

Gamperl AK, Swafford BL, Rodnick KJ (2011) Elevated temperature, per se, does not limit the ability of rainbow trout to increase stroke volume. J Therm Biol 36: 7–14.

Gollock MJ, Currie S, Petersen LH, Gamperl AK (2006) Cardiovascular and haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature increase. J Exp Biol 209: 2961–2970.

Hanson LM, Farrell AP (2007) The hypoxic threshold for maximum cardiac performance in rainbow trout *Oncorhynchus mykiss* (Walbaum) during simulated exercise conditions at 18°C. J Fish Biol 71: 926–932.

Healy TM, Schulte PM (2012) Thermal acclimation is not necessary to maintain a wide thermal breadth of aerobic scope in the common killifish (*Fundulus heteroclitus*). Physiol Biochem Zool 85: 107–119.

Heath AG, Hughes GM (1973) Cardiovascular and respiratory changes during heat stress in rainbow trout (*Salmo gairdneri*). J Exp Biol 59: 323–338.

Holk K, Lykkeboe G (1998) The impact of endurance training on arterial plasma K^+ levels and swimming performance of rainbow trout. J Exp Biol 201: 1373–1380.

Jain KE, Hamilton JC, Farrell AP (1997) Use of a ramp velocity test to measure critical swimming speed in rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol A Mol Integr Physiol 117: 441–444.

Jain KE, Farrell AP (2003) Influence of seasonal temperature on the repeat swimming performance of rainbow trout *Oncorhynchus mykiss*. J Exp Biol 206: 3569–3579.

Jeffries KM, Hinch SG, Martins EG, Clark TD, Lotto AG, Patterson DA, Cooke SJ, Farrell AP, Miller KM (2012) Sex and proximity to reproductive maturity influence the survival, final maturation, and blood physiology of Pacific salmon when exposed to high temperature during a simulated migration. Physiol Biochem Zool 85: 62–73.

Jensen FB, Fago A, Weber RE (1998) Haemoglobin structure and function. In SF Perry, B Tufts, eds. Fish Physiology, Vol 17. Academic Press, San Diego, pp 1–40.

Keen AN, Gamperl AK (2012) Blood oxygenation and cardiorespiratory function in steelhead trout (*Oncorhynchus mykiss*) challenged with an acute temperature increase and zatebradine-induced bradycardia. J Therm Biol 37: 201–210.

Kiceniuk JW, Jones DR (1977) The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. J Exp Biol 69: 247–260.

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

Lee CG, Farrell AP, Lotto A, Hinch SG, Healey MC (2003a) Excess post-exercise oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon following critical speed swimming. J Exp Biol 206: 3253–3260.

Lee CG, Farrell AP, Lotto A, MacNutt MJ, Hinch SG, Healey MC (2003b) The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon stocks. J Exp Biol 206: 3239–3251.

Mark FC, Bock C, Pörtner HO (2002) Oxygen-limited thermal tolerance in Antarctic fish investigated by MRI and ^{31}P-MRS. Am J Physiol Regul Integr Comp Physiol 283: R1254–R1262.

Marshall DJ, Dong Y-w, McQuaid CD, Williams GA (2011) Thermal adaptation in the intertidal snail *Echinolittorina malaccana* contradicts current theory by revealing the crucial roles of resting metabolism. J Exp Biol 214: 3649–3657.

Martins EG, Hinch SG, Patterson DA, Hague MJ, Cooke SJ, Miller KM, Lapointe MF, English KK, Farrell AP (2011) Effects of river temperature and climate warming on stock-specific survival of adult migrating Fraser River sockeye salmon (*Oncorhynchus nerka*). Glob Change Biol 17: 99–114.

Martins EG, Hinch SG, Patterson DA, Hague MJ, Cooke SJ, Miller KM, Robichaud D, English KK, Farrell AP (2012) High river temperature reduces survival of sockeye salmon (*Oncorhynchus nerka*) approaching spawning grounds and exacerbates female mortality. Can J Fish Aquat Sci 69: 330–342.

Mathes MT, Hinch SG, Cooke SJ, Crossin GT, Patterson DA, Lotto AG, Farrell AP (2010) Effect of water temperature, timing, physiological condition, and lake thermal refugia on migrating adult Weaver Creek sockeye salmon (*Oncorhynchus nerka*). Can J Fish Aquat Sci 67: 70–84.

Mendonça PC, Gamperl AK (2010) The effects of acute changes in temperature and oxygen availability on cardiac performance in winter flounder (*Pseudopleuronectes americanus*). Comp Biochem Physiol A Mol Integr Physiol 155: 245–252.

Mosse PRL (1978) The distribution of capillaries in the somatic musculature of two vertebrate types with particular reference to teleost fish. Cell Tissue Res 187: 281–303.

Nadeau P, Hinch S, Hruska K, Pon L, Patterson D (2010) The effects of experimental energy depletion on the physiological condition and survival of adult sockeye salmon (*Oncorhynchus nerka*) during spawning migration. Environ Biol Fish 88: 241–251.

Overgaard J, Andersen JL, Findsen A, Pedersen PBM, Hansen K, Ozolina K, Wang T (2012) Aerobic scope and cardiovascular oxygen transport is not compromised at high temperatures in the toad *Rhinella marina*. J Exp Biol 215: 3519–3526.

Patterson DA, Macdonald JS, Skibo KM, Barnes DP, Guthrie I, Hills J (2007) Reconstructing the summer thermal history for the lower

Fraser River, 1941 to 2006, and implications for adult sockeye salmon (*Oncorhynchus nerka*) spawning migration. Canadian Technical Report of Fisheries and Aquatic Sciences 2724, pp 1–51.

Perry SF, Reid SG (1994) The effects of acclimation temperature on the dynamics of catecholamine release during acute hypoxia in the rainbow trout *Oncorhynchus mykiss*. J Exp Biol 186: 289–307.

Pörtner HO (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88: 137–146.

Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315: 95–97.

Pörtner HO, Farrell AP (2008) Ecology: physiology and climate change. Science 322: 690–692.

Randall DJ (1970) The circulatory system. In WS Hoar, DJ Randall, eds. Fish Physiology, Vol 4. Academic Press, New York, pp 133–172.

Roscoe DW, Hinch SG, Cooke SJ, Patterson DA (2011) Fishway passage and post-passage mortality of up-river migrating sockeye salmon in the Seton River, British Columbia. River Res Appl 27: 693–705.

Rummer JL, Brauner CJ (2011) Plasma-accessible carbonic anhydrase at the tissue of a teleost fish may greatly enhance oxygen delivery: *in vitro* evidence in rainbow trout, *Oncorhynchus mykiss*. J Exp Biol 214: 2319–2328.

Sandblom E, Axelsson M (2007) Venous hemodynamic responses to acute temperature increase in the rainbow trout (*Oncorhynchus mykiss*). Am J Physiol Regul Integr Comp Physiol 292: R2292–R2298.

Sartoris FJ, Bock C, Serendero I, Lannig G, Pörtner HO (2003) Temperature-dependent changes in energy metabolism, intracellular pH and blood oxygen tension in the Atlantic cod. J Fish Biol 62: 1239–1253.

Soivio A, Nynolm K, Westman K (1973) Technique for repeated sampling of blood of individual resting fish. J Exp Biol 63: 207–217.

Somero GN (2011) Comparative physiology: a "crystal ball" for predicting consequences of global change. Am J Physiol Regul Integr Comp Physiol 301: R1–R14.

Steffensen JF, Farrell AP (1998) Swimming performance, venous oxygen tension and cardiac performance of coronary-ligated rainbow trout, *Oncorhynchus mykiss*, exposed to progressive hypoxia. Comp Biochem Physiol A Mol Integr Physiol 119A: 585–592.

Steinhausen MF, Sandblom E, Eliason EJ, Verhille C, Farrell AP (2008) The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). J Exp Biol 211: 3915–3926.

Taylor SE, Egginton S, Taylor EW (1993) Respiratory and cardiovascular responses in rainbow trout (*Oncorhynchus mykiss*) to aerobic exercise over a range of acclimation temperatures. J Physiol 459: 19.

Taylor S, Egginton S, Taylor E (1996) Seasonal temperature acclimatisation of rainbow trout: cardiovascular and morphometric influences on maximal sustainable exercise level. J Exp Biol 199: 835–845.

Taylor EW, Egginton S, Taylor SE, Butler PJ (1997) Factors which may limit swimming performance at different temperatures. In CM Wood, DG McDonald, eds. Global Warming: Implications for Freshwater and Marine Fish. Cambridge University Press, Cambridge, pp 105–133.

Tucker VA (1967) Method for oxygen content and dissociation curves on microliter blood samples. J Appl Physiol 23: 410–414.

Verberk WCEP, Calosi P (2012) Oxygen limits heat tolerance and drives heat hardening in the aquatic nymphs of the gill breathing damselfly *Calopteryx virgo* (Linnaeus, 1758). J Therm Biol 37: 224–229.

Wagner PD (1996) Determinants of maximal oxygen transport and utilization. Annu Rev Physiol 58: 21–50.

Wang T, Overgaard J (2007) Ecology: the heartbreak of adapting to global warming. Science 315: 49–50.

Weibel ER (1984) The Pathway for Oxygen: Structure and Function in the Mammalian Respiratory System. Harvard University Press, Cambridge, MA, pp 1–425.

Wood CM, Turner JD, Graham MS (1983) Why do fish die after severe exercise? J Fish Biol 22: 189–201.