Timothy D. Clark, Erik Sandblom, Georgina K. Cox, Scott G. Hinch and Anthony P. Farrell

You might find this additional information useful...

This article cites 54 articles, 21 of which you can access free at:
http://ajpregu.physiology.org/cgi/content/full/295/5/R1631#BIBL

Updated information and services including high-resolution figures, can be found at:
http://ajpregu.physiology.org/cgi/content/full/295/5/R1631

Additional material and information about *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* can be found at:
http://www.the-aps.org/publications/ajpregu

This information is current as of November 4, 2008 .

Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*)

Timothy D. Clark,[1] Erik Sandblom,[1,2] Georgina K. Cox,[3] Scott G. Hinch,[4] and Anthony P. Farrell[1,3]

[1]*Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada;* [2]*Department of Zoology, University of Gothenburg, Gothenburg, Sweden;* [3]*Department of Zoology, University of British Columbia, Vancouver, Canada; and* [4]*Department of Forest Sciences, University of British Columbia, Vancouver, Canada*

Clark TD, Sandblom E, Cox GK, Hinch SG, Farrell AP.
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, 2008. First
published September 3, 2008; doi:10.1152/ajpregu.90461.2008.—
This study was undertaken to provide a comprehensive set of data
relevant to disclosing the physiological effects and possible oxygen
transport limitations in the Chinook salmon (*Oncorhynchus tshaw-
ytscha*) during an acute temperature change. Fish were instrumented
with a blood flow probe around the ventral aorta and catheters in the
dorsal aorta and sinus venosus. Water temperature was progressively
increased from 13°C in steps of 4°C up to 25°C. Cardiac output
increased from 29 to 56 ml·min^{-1}·kg^{-1} between 13 and 25°C
through an increase in heart rate (58 to 105 beats/min). Systemic
vascular resistance was reduced, causing a stable dorsal aortic blood
pressure, yet central venous blood pressure increased significantly at
25°C. Oxygen consumption rate increased from 3.4 to 8.7
mg·min^{-1}·kg^{-1} during the temperature increase, although there were
signs of anaerobic respiration at 25°C in the form of increased blood
lactate and decreased pH. Arterial oxygen partial pressure was main-
tained during the heat stress, although venous oxygen partial pressure
(Pv_{O_2}) and venous oxygen content were significantly reduced. Cardiac
arrhythmias were prominent in three of the largest fish (>4 kg) at
25°C. Given the switch to anaerobic metabolism and the observation
of cardiac arrhythmias at 25°C, we propose that the cascade of venous
oxygen depletion results in a threshold value for Pv_{O_2} of around 1 kPa.
At this point, the oxygen supply to systemic and cardiac tissues is
compromised, such that the oxygen-deprived and acidotic myocar-
dium becomes arrhythmic, and blood perfusion through the gills and
to the tissues becomes compromised.

blood pressure; blood respiratory properties; cardiac output; hemato-
crit; hemoglobin; heart rate; blood plasma; rate of oxygen consump-
tion; oxygen equilibrium curves; Pacific salmon; stroke volume;
vascular resistance

WITH CURRENT TRENDS IN CLIMATE change, it is becoming increas-
ingly important to understand both the behavioral and physio-
logical responses of animals to perturbations in temperature
(41). The highly efficient counter-current arrangement between
blood and respiratory water at fish gills means that any acute
change in ambient water temperature rapidly changes body
temperature and modifies tissue oxygen requirements (10–13,
43, 58). A change in the rate of oxygen consumption ($\dot{M}_{O_2}$)
then requires a parallel change in oxygen delivery from the
gills to tissues by the circulatory system, which is determined
by the product of cardiac output (Q) and the difference between
arterial (Ca_{O_2}) and venous (Cv_{O_2}) oxygen content (Ca_{O_2}–Cv_{O_2})
according to the Fick equation:

$$\dot{M}_{O_2} = Q \cdot (Ca_{O_2} - Cv_{O_2}) \tag{1}$$

Q is the product of heart rate (f_H) and cardiac stroke volume
(V_S), while Ca_{O_2}–Cv_{O_2} is dependent on oxygen partial pressure
and capacitance of the blood for oxygen. Blood oxygen capac-
itance itself is reliant on mean corpuscular hemoglobin con-
centration (MCHC), oxygen binding characteristics, and the
degree of saturation of the hemoglobin molecule (62). Thus,
changes in circulatory oxygen delivery resulting from varia-
tions in body temperature can be modulated by changes in one
or any combination of these factors (18). In the present study,
we were particularly interested in circulatory oxygen delivery
because it has been suggested that circulatory performance of
fishes may be the first process to cause oxygen deficiency
during heat stress (40, 50). However, while many studies have
investigated components of the cardiorespiratory response to
heat stress in fish, the composite response has rarely been
measured in a single study. Furthermore, a range of responses
are reported, perhaps reflecting species variability and/or subtle
but important differences in the experimental protocols.

The primary cardiovascular responses to an acute increase in
water temperature involve an increase in Q, which is typically
mediated by a rise in f_H. Stroke volume has been reported to
either remain unchanged (8, 12, 29, 57) or decrease (3, 7, 48).
Ca_{O_2}–Cv_{O_2} is typically reported to remain unchanged or is
increased due to a reduction in Cv_{O_2} because of greater tissue
oxygen extraction (33, 50). An increase in Ca_{O_2} may also
contribute to an increase in Ca_{O_2}–Cv_{O_2}, resulting from greater
gill ventilation and an associated increase in arterial oxygen
partial pressure (Pa_{O_2}), and/or an increase in circulating eryth-
rocytes following splenic contraction (48). Substantial de-
creases in venous oxygen partial pressure (Pv_{O_2}) and Cv_{O_2} are
of concern for proper cardiac function, given that the heart
(specifically, the spongy myocardium) is the last organ to be
supplied with oxygen from systemic blood (14, 17).

While interspecific variability may be expected in the circu-
latory response to an acute temperature increase, the differ-
ences documented for the same species are surprising. Recent
studies with Atlantic cod (*Gadus morhua*) provide a good
example of divergent findings. Lannig et al. (36) found that
dorsal aortic blood flow in North Sea Atlantic cod, as measured
indirectly by flow-weighted magnetic resonance imaging, did
not match the continued increase in f_H with temperature but

rather yielded a hyperbolic regression, reflecting a strong increase in blood flow in the lower and a moderate increase in the upper temperature range. Conversely, Gollock et al. (29) found that Q, measured directly with Transonic flow probes on the ventral aorta, increased in parallel with f_H in Newfoundland Atlantic cod until water temperature reached the animals' critical thermal maximum. On the basis of oxygen equilibrium curves, they suggested, as did Heath and Hughes (33) for rainbow trout, that a decreased blood oxygen binding capacity hampered metabolic rate at high temperatures (29) and that cardiac function, therefore, was unlikely to limit aerobic metabolism in cod. Lacking from Gollock et al.'s study (29), however, were in vivo measurements of blood oxygen content to substantiate this claim. Indeed, the difficulty associated with obtaining simultaneous arterial and venous blood samples from cannulated vessels is one of the primary reasons why very few studies have concurrently investigated the full suite of cardio-respiratory variables and in vivo blood oxygen binding properties during an acute temperature change in fish. Studies of thermal limitations must necessarily measure a range of variables pertinent to circulatory function to discover the relationships between inadequate tissue oxygen delivery and circulatory collapse proposed for heat stress.

Consequently, the present study was undertaken to provide a comprehensive set of data relevant to disclosing the physiological effects and possible oxygen transport limitations in a fish during an acute temperature change. Here, we simultaneously examined the cardiovascular, hematological and respiratory responses of adult Chinook salmon (*Oncorhynchus tshawytscha*) belonging to the Chilliwack River population in British Columbia, Canada. The large size of the individuals used here permitted repetitive blood sampling as temperature was increased incrementally. This species has a coronary circulation that supplies oxygen to a portion of the ventricle (the compact myocardium), while the venous blood being pumped by the heart supplies oxygen to the atrium and the remainder of the ventricle (the spongy myocardium). The results likely have direct ecological relevance for this population of Chinook salmon since they expose themselves to rapid and broad changes in water temperature during their migration from the open ocean to freshwater spawning grounds during summer months (5, 6, 18). While juvenile Chinook salmon have been described as the most tolerant of the Pacific salmon species to elevated temperatures (6), comparatively little is known of the high-temperature tolerance of adults, except that adult fish tend to have upper lethal temperatures a few degrees lower than their juvenile counterparts (38).

MATERIALS AND METHODS

Animals and holding conditions. Ten male Chinook salmon (*Oncorhynchus tshawytscha*) in the size range of 2.1–5.4 kg body mass and 58–79-cm fork length were caught by dip-net in August 2007 as they completed their migration from the ocean to their spawning site at the Chilliwack River Hatchery, British Columbia, Canada (~100 km upstream). They were transported 20 km by truck to the Cultus Lake Salmon Research Laboratory and held outdoors in 10,000-liter circular tanks containing circulating lake water at a temperature of 13–14°C. Temperature control was achieved by mixing lake water from two different depths [7 m (21°C) and 40 m (9°C)]. The holding temperature was chosen to match the temperature conditions in the Chilliwack River at the time of capture. The fish were

not fed while in captivity (2 wk; migrating Chinook salmon do not normally feed *en route* to spawning grounds). All protocols were approved by the Animal Care Committee of the University of British Columbia in accordance with the Canadian Council on Animal Care (A05-0007).

Surgical procedures. Individual fish were netted from the holding tanks and anesthetized in water containing tricaine methanesulfonate (MS-222; 100 mg/l; Sigma, St. Louis, MO) buffered with sodium bicarbonate (NaHCO₃; 200 mg/l). They were weighed and placed on wet foam on a surgery table. The gills were continuously irrigated with cooled (9–11°C) and aerated water containing NaHCO₃-buffered MS-222 at a lower dose (150 mg/l and 75 mg/l, respectively). The ventral aorta was dissected free in the isthmus anterior to the pericardium using sharp and blunt dissection and an appropriately sized blood flow probe (Transonic Systems, Ithaca, NY) was fitted around the vessel (12, 46). Great care was taken to avoid damaging the pericardium during this procedure. The ductus of Cuvier was cannulated with a polyethylene (PE)-50 catheter advanced toward the sinus venosus using the method described by Sandblom et al. (49), and the dorsal aorta was cannulated with PE-50 catheter using the method described by Soivio et al. (54). The catheters were filled with heparinized (150 IU/ml) saline and were sutured together with the flow probe lead to the skin of the fish with several silk sutures.

Following surgery, each fish was transferred to either *1*) an opaque experimental holding tube (length, 120 cm; diameter, 30 cm; wire mesh at each end, with a slit in the top to externalize catheters and the flow probe lead) submersed in a larger outer tank (*n* = 6), or *2*) one of two Brett-type respirometers [as described in (22, 37)] (*n* = 4). The holding tubes and respirometers were covered to minimize visual disturbance of the fish. Water temperature in the holding tubes was controlled by balancing the inflow of shallow and deep lake water, as described above, and a heating element was used to assist in obtaining the highest experimental temperature of 25°C. The respirometers were equipped with their own 9-kW titanium heating elements [(M. F. Steinhausen, E. Sandblom, E. J. Eliason, C. Verhille, A. P. Farrell, unpublished data)]. The temperature for post-surgical recovery was the same as the holding temperature (13°C). Water velocity both through the holding tubes and the swim respirometers was maintained at 20–30 cm/s for the entire experimental period, the former by utilizing submersible pumps and the latter by utilizing an inbuilt motor and propeller. The fish were allowed to recover from surgery overnight (>10 h).

Experimental protocol. Experiments began in the morning with routine cardiovascular variables being recorded for 1 h at 13°C. Oxygen consumption rate (M_{O_2}) was measured during the final 20-min period of the stable temperature exposure, and blood samples were taken from the arterial and venous catheters at the end of this period. Afterward, a new temperature was attained by gradually raising the temperature 4°C over 1 h. Water temperature was stabilized for 1 h at each of 17, 21, and 25°C, during which time the cardiorespiratory measurements were repeated. Following the final temperature challenge at 25°C, the temperature was decreased over 1 h to a stable temperature of 13°C. Recordings of the recovering fish were repeated after 1 h and 2 h at 13°C following the heat stress. Upon conclusion of experiments, fish were euthanized by a cranial blow. Hearts were excised, emptied of blood, dried, weighed, and placed in 70% ethanol for subsequent separation and quantification of the two (compact and spongy) ventricular myocardial layers (for details, see Ref. 23).

Measured and calculated variables. Blood flow and blood pressure measurements were made on all 10 fish. The blood flow probe was connected to a Transonic blood flow meter (Model T206; Transonic Systems) to continuously record cardiac output (Q), and flows were corrected for temperature following experiments by using a thermostatically controlled water bath and the methods outlined in the product user manual (temperature-correction increased flows by 8–20%). Central venous (P_{CV}) and dorsal aortic (P_{DA}) blood catheters were connected to pressure transducers (model DPT-6100, pvb;

Medizintechnik, Kirchseeon, Germany) that were calibrated against static water columns. The signals from the transducers were amplified using a 4-channel amplifier (Somedic AB, Hörby, Sweden). Blood flow and blood pressure signals were sampled at 50 Hz using a Biopac module (Biopac Systems, Santa Barbara, CA) connected to a laptop computer. Heart rate (f_H) was calculated from blood flow traces from the flow probe. Cardiac stroke volume (V_S) was calculated as Q/f_H. Systemic vascular resistance (R_{SYS}) was calculated from the drop in blood pressure between the dorsal aorta and the central veins as (P_{DA}-P_{CV})/Q.

The rate of oxygen removal from the water (=oxygen consumption rate; $\dot{M}_{O_2}$) over a 10–20 min period was obtained for the four fish that were placed in the water-tight respirometer swim tunnels. $\dot{M}_{O_2}$ was calculated from the decline in water oxygen saturation during sealed periods (accounting for temperature effects on oxygen capacitance), and the respirometers were flushed with fresh, filtered (sand and ultraviolet filtration), and aerated water at all times in between $\dot{M}_{O_2}$ measurements. The respirometers were bleached prior to the introduction of fish to preclude significant microbial oxygen consumption.

Blood samples (0.3–0.8 ml from each catheter) were collected anaerobically into heparinized syringes and immediately refrigerated for subsequent blood analysis within 1 h. The cumulative blood volume collected throughout the experimental period never exceeded 10% of the assumed total blood volume of 4% of fish body mass (39). Occasionally, a blockage in the catheter prevented blood sampling, although at least seven fish were represented at all temperatures. Blood pH and oxygen partial pressure (P_{O_2}) were measured using a blood gas monitor (PHM 73, Radiometer, Copenhagen, Denmark) calibrated at each experimental temperature, and maintained at each temperature, using thermostatically controlled water jackets around the electrodes. Blood oxygen content was determined using a Tucker chamber (61) maintained at 37°C. Hematocrit (Hct) was determined using microhematocrit capillary tubes spun at 10,000 g for 7 min. Hemoglobin concentration ([Hb]) was always obtained using a handheld hemoglobin analyzer appropriately calibrated for fish blood [Hemocue 201$^+$, www.hemocue.com; (10)], and it was sometimes also measured spectrophotometrically at 540 nm using a solution of 10 μl of blood in 1 ml of Drabkin's solution (10, 15). The mean corpuscular hemoglobin concentration (MCHC) was calculated as [Hb]/(Hct/100). Remaining whole blood was subsequently spun in Eppendorf tubes at 7,000 g for 7 min, and then the plasma was collected, frozen in liquid nitrogen, and placed at −80°C for subsequent analyses. Plasma measurements were made of cortisol (Neogen ELISA with Molecular Devices Spectramax 240pc plate reader), lactate (YSI 2300 stat plus analyzer), osmolality (Advanced
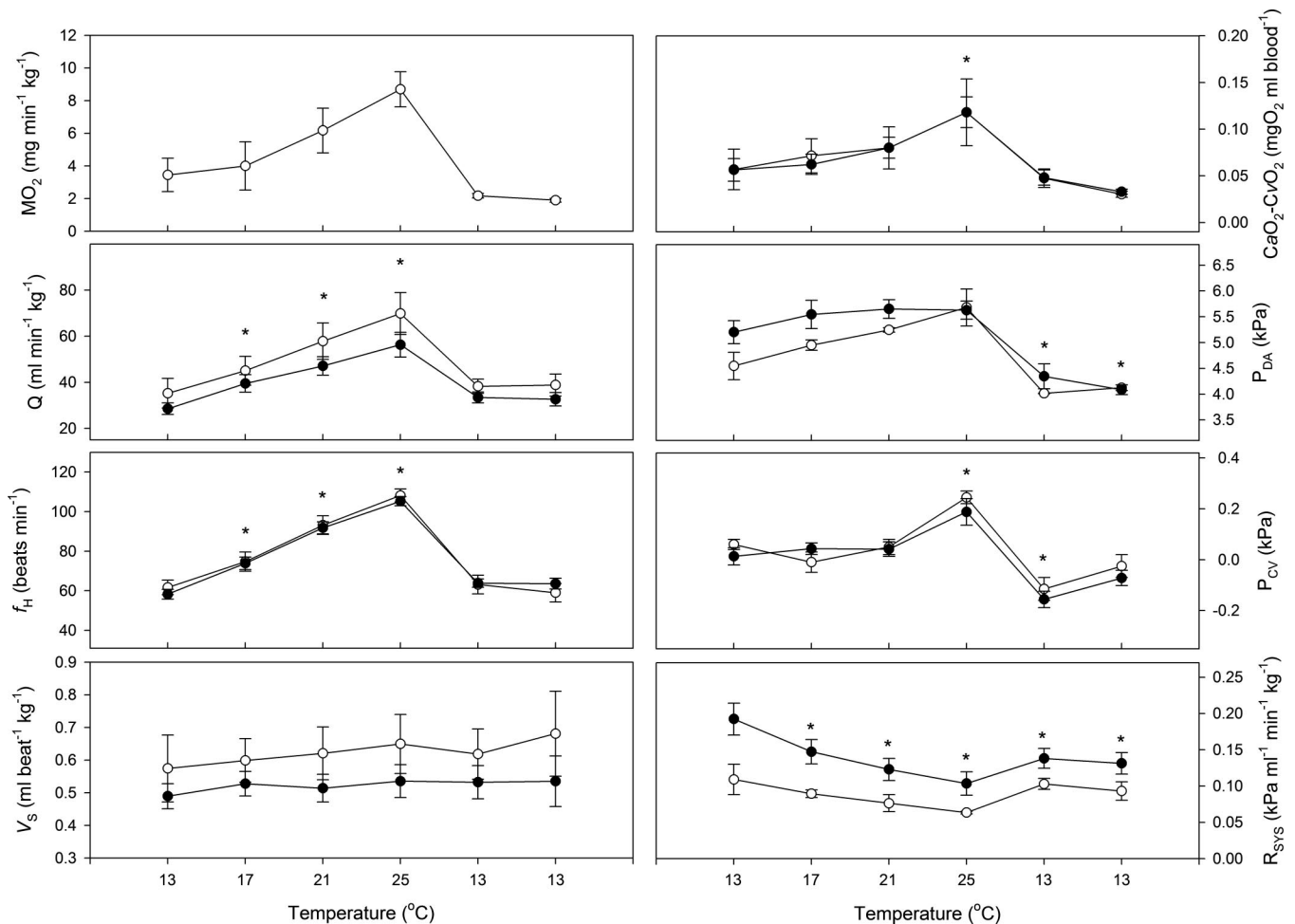

Fig. 1. Effects of acute temperature changes on cardiorespiratory variables of Chinook salmon (*Oncorhynchus tshawytscha*), including oxygen consumption rate ($\dot{M}_{O_2}$), cardiac output (Q), heart rate (f_H), stroke volume (V_S), arteriovenous oxygen content difference (Ca_{O_2}–Cv_{O_2}), dorsal aortic blood pressure (P_{DA}), central venous blood pressure (P_{CV}), and systemic vascular resistance (R_{SYS}). Dark symbols and lines include data from all fish (n = 7–10), while light symbols and lines include only those data from fish that were placed in respirometers and had $\dot{M}_{O_2}$ measured (n = 4). Values are expressed as means ± SE. Asterisks refer to dark symbols and denote statistically significant differences (P < 0.05) from the initial value at 13°C. Statistics were not performed on data in light symbols due to insufficient statistical power, although it is evident that data from these fish followed similar relationships to those determined for all fish combined.

Instruments 3320 freezing point osmometer), chloride (Haake Buchler digital chloridometer), and sodium and potassium (Cole-Parmer, model 410 single channel flame photometer) (see Ref. 21 for further details).

Statistical analyses. All fish generally remained calm during the majority of the experiments, and so it was possible to exclude active periods from the analysis such that only data from resting fish are presented. The effect of increasing temperature on mean ($\pm$SE) hematological and cardiovascular variables was assessed with one-way repeated-measures ANOVA followed by Dunnett's post hoc test to identify individual points that were significantly different from the initial value at 13°C. Significance was considered at $P < 0.05$.

RESULTS

Morphometrics. Body mass (M_b) was related to fork length (FL) as $M_b = 0.15 \times FL - 6.65$ ($r^2 = 0.94$), where M_b is in kilograms and FL is in centimeters. Total wet heart mass (atrium, ventricle, and bulbus) was 9.29 ± 0.86 g, and relative wet heart mass was $0.238 \pm 0.004\%$ of body mass. The dry relative ventricular mass was $0.028 \pm 0.001\%$ of wet body mass, and the ventricle was composed of $53.2 \pm 0.8\%$ compact myocardium based on the ratio of the dry masses of the spongy and compact myocardial layers.

Cardiovascular, hematological, and respiratory responses to temperature change. At 13°C, routine Q was 28.5 ± 2.5 ml·min^{-1}·kg^{-1}, f_H was 58.3 ± 2.5 beats/min, and V_S was 0.49 ± 0.04 ml·beat^{-1}·kg^{-1}. The dorsal aortic and central venous blood pressures were 5.20 ± 0.22 kPa and 0.01 ± 0.03 kPa, respectively (Fig. 1). Cardiovascular values measured for the fish in the respirometers were not different from these values (Fig. 1), and routine $\dot{M}_{O_2}$ was 3.4 ± 1.0 mg·min^{-1}·kg^{-1}. Hematological variables are summarized in Fig. 2 and Table 1.

Increasing water temperature gradually and significantly increased Q. This response was mediated entirely through tachycardia because V_S was statistically unchanged (Fig. 1). At 25°C, Q had increased by 97% to 56.3 ± 5.4 ml·min^{-1}·kg^{-1} and f_H had increased by 81% to 105.3 ± 2.3 beats/min. This resulted in a Q_{10} value of 1.8 for Q and 1.6 for f_H between 13 and 25°C. Associated with the increase in Q was a significant reduction in systemic vascular resistance (R_{SYS}), which resulted in P_{DA} being unchanged during heat stress. Similarly, P_{CV} was unchanged at 17 and 21°C but increased significantly to 0.19 ± 0.05 kPa at 25°C. Cardiovascular responses of the fish in the respirometers followed the same trends. $\dot{M}_{O_2}$ increased by 152% to 8.7 ± 1.1 mg·min^{-1}·kg^{-1} at 25°C, which represented a Q_{10} value of 2.2. The blood convection requirement ($Q/\dot{M}_{O_2}$) decreased from 10.2 ml/mg O_2 at 13°C to 8.0 ml/mg O_2 at 25°C.

Increasing temperature to 25°C significantly decreased MCHC in arterial and venous blood from about 316 g/l to about 279 g/l, resulting from a slight increase in Hct, likely due to erythrocyte swelling, while [Hb] was maintained (Fig. 2). Pa_{O_2} and Pv_{O_2} tended to decrease by similar amounts, although only Pv_{O_2} at 25°C was significantly lower than the initial 13°C value (Fig. 2). There was a decrease in Cv_{O_2} that was greater than the decrease in Ca_{O_2}, such that Ca_{O_2}–Cv_{O_2} increased with temperature and, along with increased Q, contributed to the more than doubling of $\dot{M}_{O_2}$. Interestingly, oxygen partial pressures and contents of arterial and venous blood typically scaled negatively with body mass across temperatures (Fig. 3). Plasma lactate concentration increased significantly at 25°C, while
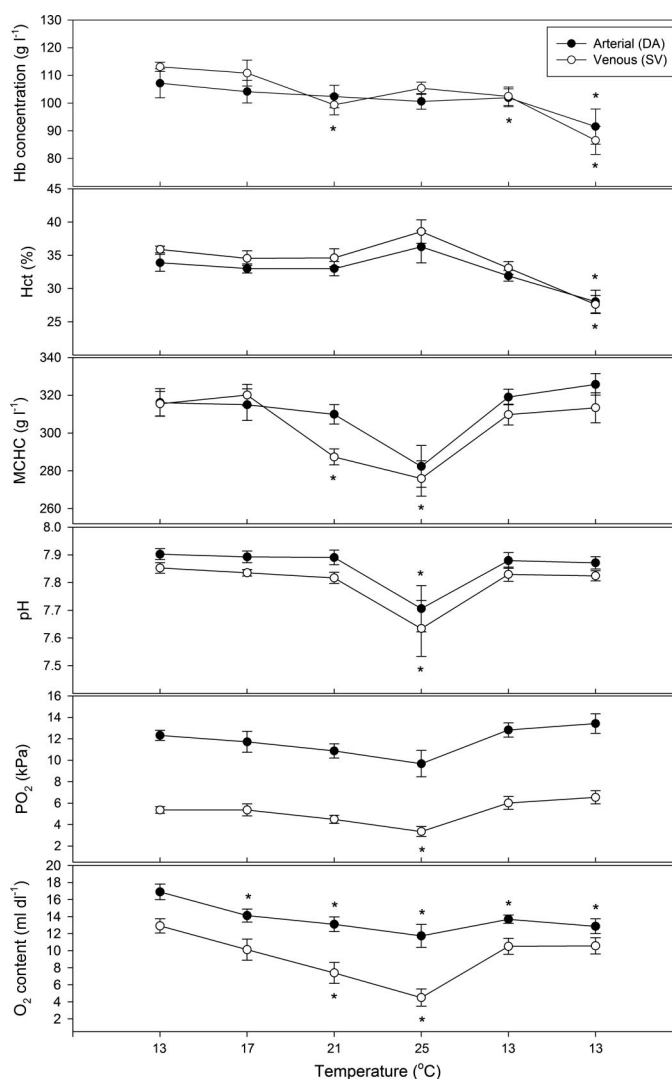

Fig. 2. Effects of acute temperature changes on hematological variables ($n = 7$–10) in Chinook salmon (*Oncorhynchus tshawytscha*). Both arterial (solid circles) and venous (open circles) blood was obtained from the dorsal aorta and the sinus venosus, respectively. The variables are hemoglobin concentration ([Hb]), hematocrit (Hct), mean corpuscular hemoglobin concentration (MCHC), pH, oxygen partial pressure (P_{O_2}), and oxygen content. Values are expressed as means $\pm$ SE. Asterisks denote statistically significant differences ($P < 0.05$) from the initial value at 13°C (arterial at top of figure, venous at bottom).

whole blood pH decreased significantly and there was a decrease in plasma glucose levels (Fig. 2; Table 1). Thus, despite the large increase in $\dot{M}_{O_2}$, there was evidence of insufficient oxygen delivery to the tissues at 25°C. Indeed, prominent cardiac arrhythmias at 25°C occurred in the first-, second-, and fourth-largest fish (>4 kg; Fig. 3), and one of these fish (second largest) lost equilibrium and failed to recover despite an immediate decrease in water temperature.

The progressive changes in oxygen content and partial pressure at each temperature are illustrated in the form of in vivo oxygen equilibrium curves in Fig. 4. Increasing temperature caused a modest right-shifting of the curve, and the calculated P_{50} values are given in Table 1. The estimated P_{50} values for 13°C may be underestimated because there were no data below 50% hemoglobin saturation at this temperature and

Table 1. *Hematological variables of Chinook salmon (Oncorhynchus tshawytscha) during acute changes in water temperature*

	Temperature (°C)											
	13		17		21		25		13		13	
Variable	DA	SV	DA	SV	DA	SV	DA	SV	DA	SV	DA	SV
Cortisol	229±51	218±56	178±55	175±60	205±45	200±52	220±29	236±42	250±55	231±61	297±54	240±82
Lactate	1.4±0.2	1.5±0.2	1.6±0.3	1.5±0.2	2.3±0.4	2.5±0.4	8.2±2.4*	6.8±2.3*	3.1±0.9	2.7±0.3	3.4±1.1	2.8±1.1
Glucose	4.2±0.3	4.2±0.4	3.7±0.4*	3.5±0.4*	3.1±0.3*	3.2±0.4*	3.2±0.4*	3.4±0.5*	4.0±0.3	4.0±0.2	4.0±0.3	3.9±0.3
Osmolality	289.9±2.6	290.6±2.4	283.1±5.0	282.9±3.3	286.8±4.1	284.6±3.0	295.8±3.3	298.5±2.2	292.1±2.8	288.9±2.8	290.1±3.4	290.8±1.4
Chloride	127.2±2.0	126.1±2.3	124.5±2.7	123.0±1.7	125.9±1.5	122.7±2.2	126.4±2.1	125.5±1.5	129.9±2.8	125.0±2.7	127.9±2.1	129.7±1.5
Potassium	4.6±0.3	2.9±0.2	6.0±0.4	3.9±0.5	7.0±0.7	3.6±0.4	6.0±1.3	2.9±0.4	8.1±1.5	3.0±0.4	6.0±1.3	3.1±0.4
Sodium	144.8±1.8	149.6±2.6	141.5±3.0	145.3±3.4	142.0±3.2	143.9±3.2	146.9±2.2	146.9±1.3	142.3±3.1	145.7±3.5	143.2±2.4	149.4±3.2
P_{50}	2.95		3.71		4.27		4.95		3.39		3.39	

Values are expressed as means $\pm$ SE; n = 7–10. DA, dorsal aorta blood (arterial); SV, sinus venosus blood (venous). Units are cortisol (ng/ml), lactate (mmol/l), glucose (mmol/l), osmolality (mOsm/kg) chloride (mmol/l), potassium (mmol/l), sodium (mmol/l). *Significantly different from the initial value at 13°C. P_{50} (kPa) predicted from regressions in Fig. 4, and no statistics were performed.

the P_{50} values were derived from extrapolation rather than interpolation.

Cardiovascular, hematological and respiratory responses during recovery from heat stress. After just 1 h recovery at 13°C following the heat stress, $\dot{M}_{O_2}$, Q, and f_H were all restored to their initial control values at 13°C (Fig. 1). Similarly, nearly all hematological variables, with the exception of Ca_{O_2}, [Hb], and Hct, were restored to their initial control values at 13°C after 1 h of recovery and remained at normal levels through to 2 h of recovery (Fig. 2; Table 1). There was a significant arterial hypotension because the reduction in R_{SYS} persisted throughout the second hour of recovery. P_{CV} also decreased significantly while fish were recovering at 13°C, and this resulted in a transient negative P_{CV} (-0.16 ± 0.03 kPa) at the first hour of recovery, which is indicative of the heart functioning with a *vis-a-fronte* filling mechanism.

DISCUSSION

The large wild salmon were observed to remain calm in the test apparatus, given that there were very few movement artifacts on the recordings from the blood flow probe. Indeed, the ranges for routine f_H and $\dot{M}_{O_2}$ at 13°C in the present study (54–64 beats/min and 1.9–3.4 mg·min^{-1}·kg^{-1}, respectively) compare favorably with those reported in previous studies with smaller Chinook salmon at similar or lower temperatures (27, 34, 44, 59). Although long postsurgical recovery times are desirable, this finding suggests that the relatively short recovery period (at least 10 h) for the routine surgical procedures used in the present study was an acceptable compromise between recovery time and the need to quickly perform the experiments before the Chinook salmon fully matured and deteriorated as their spawning date approached (about 3 wk after capture).

While routine f_H and $\dot{M}_{O_2}$ are comparable to other studies, the initial routine P_{DA} of 5.2 kPa is 13–62% higher than previous measurements of P_{DA} in juvenile Chinook (3.2–4.6 kPa) (27, 34, 44, 59). This systemic hypertension could reflect a stress (e.g., elevated sympathetic tone causing systemic vasoconstriction) that was not expressed as an elevated $\dot{M}_{O_2}$, or it may reflect the advanced sexual maturity of these adult male Chinook salmon. Indeed, male rainbow trout are known to become hypertensive during sexual maturation due to cardiac hypertrophy and hypervolemia (9). We measured dry relative ventricular mass as 0.028%, which converts to a wet relative

ventricular mass of 0.18% [if we assume 84% water content (53)]. This value is almost twice that measured in immature Chinook (27, 59), and it suggests a state of cardiac hypertrophy in our fish of a level previously observed in mature male rainbow trout (9, 28, 60).

Measurements of the response of systemic vascular resistance to temperature change in fish are scarce because very few studies have measured blood pressures and flows directly and simultaneously during acute temperature changes. Values of R_{SYS} at 13°C in the present study bracketed what has been reported previously for juvenile Chinook (27). The present study probably exposed Chinook salmon to temperatures very close to their upper thermal limit since we saw evidence of a mismatch between oxygen supply and demand in the form of cardiac arrhythmias, as well as acidosis and lactate appearance in the blood at 25°C. Increasing temperature to 25°C saw a gradual and significant decrease in R_{SYS} that was simultaneous with the increase in Q such that P_{DA} did not change. This response is consistent with systemic vasodilatation in muscle tissue and is consistent with total vascular resistance decreasing during an acute heat stress in the Antarctic fish *Pagothenia bernachii* (3). At temperatures closer to the optimum for rainbow trout (acute increase from 10°C through 13°C to 16°C), R_{SYS} did not change significantly but did tend to decrease with temperature (48). Combined with the decrease in Pv_{O_2}, we can speculate that this vasodilatation reflects increased tissue perfusion of parallel vascular beds to reduce oxygen diffusion distances. There was a pronounced arterial and venous hypotension that persisted for at least 2 h after fish had been returned to 13°C following the acute heat stress. The arterial hypotension may be indicative of a metabolite-induced response lingering while energy stores are restored following anaerobic metabolism. A similar arterial hypotension is seen during recovery from the anaerobic effort involved in critical swimming speed tests with juvenile Chinook salmon (27). To what degree a temperature-related decrease in blood viscosity (25, 30, 31) might contribute to the decrease in R_{SYS} during heat stress is unclear but would not be a factor when 13°C conditions were restored. Regardless of the triggers or their mechanisms, all but the second largest fish (Fig. 3) recovered quickly and well from the acute heat stress. In fact, while the increase in P_{CV} at 25°C perhaps can be interpreted to indicate cardiac insufficiency at this temperature, the subambient pres-
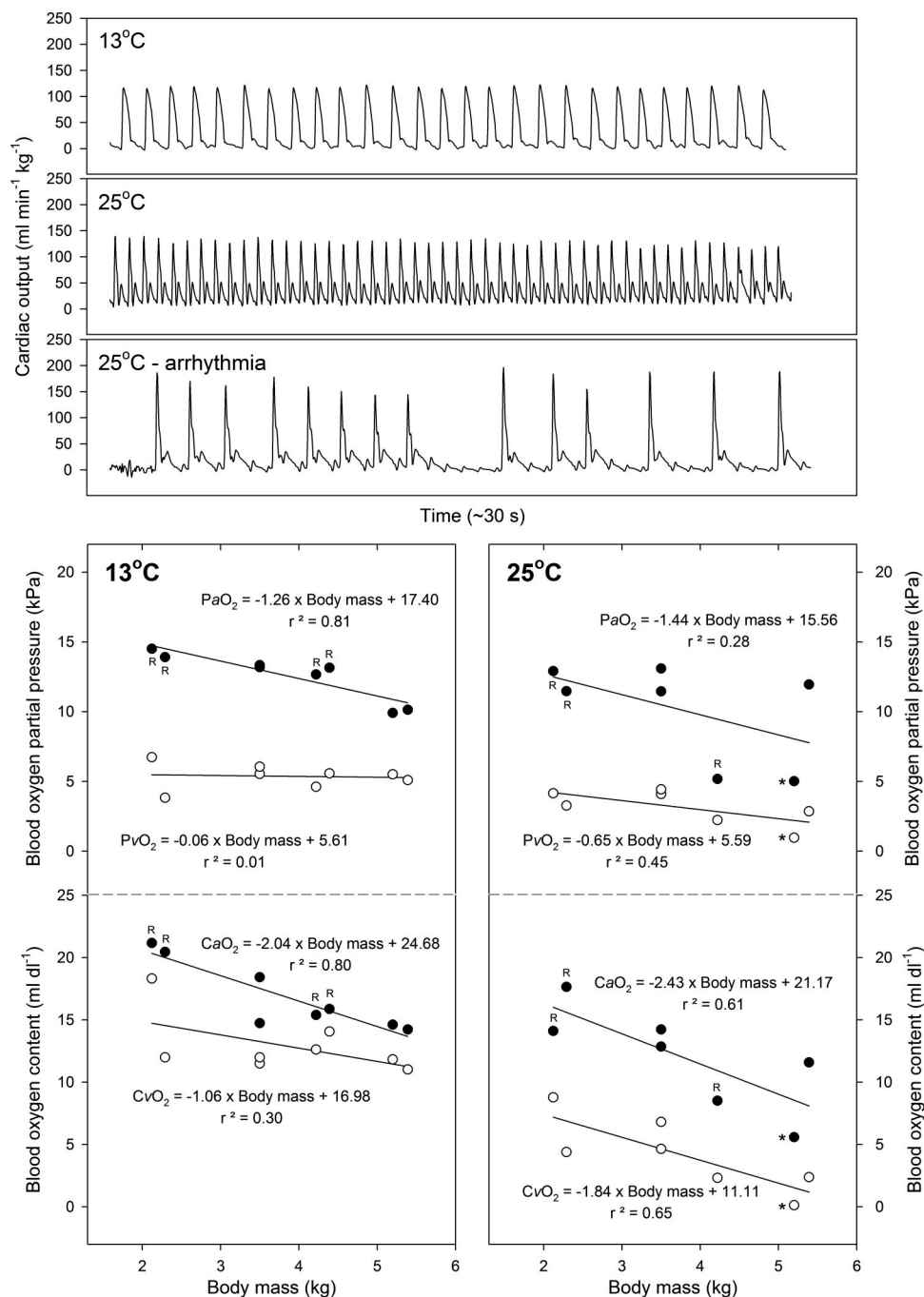
Fig. 3. *Top*: individual blood flow traces through the ventral aorta of Chinook salmon (*Oncorhynchus tshawytscha*) at 13°C, immediately upon reaching 25°C, and after being exposed to 25°C for 1 h. *Bottom*: arterial (closed circles) and venous (open circles) blood oxygen partial pressure (Pa_{O_2} and Pv_{O_2}, respectively) and oxygen content (Ca_{O_2} and Cv_{O_2}, respectively) as a function of body mass in Chinook salmon at 13°C (*left*) and when acutely exposed to 25°C (*right*) for 1 h ($n = 7$–8). R represents those fish that were housed in the respirometers (includes the labeled arterial value and the corresponding venous value at the same body mass), whereas all other fish were housed in holding tubes (see MATERIALS AND METHODS). Missing data points were due to blocked blood catheters. Asterisks indicate the individual displaying arrhythmias in the top panel of this figure.

sure on recovery is clearly indicative that the ventricular contractions are sufficiently strong to generate the *vis-a-fronte* cardiac filling that may be characteristic of the salmonid heart under routine conditions (24).

It has often been suggested that the decreasing oxygen content of the water and limited gill ventilation could contribute to the failure of gill oxygen uptake to meet the increased tissue oxygen demand with increasing temperature (5, 33, 58), although others have recently argued against this (16). The present study showed that five out of seven Chinook salmon maintained high Pa_{O_2} during heat stress, even at the point where oxygen delivery to tissues was compromised (Fig. 2; Fig. 3). Consistent with our results is the finding that Pa_{O_2} did

not decrease in exercising sockeye salmon at high temperatures (M. F. Steinhausen, E. Sandblom, E. J. Eliason, C. Verhille, A. P. Farrell, unpublished data). The maintenance of Pa_{O_2} found for Chinook and sockeye is possible only if the conditions for oxygen diffusion across the gills and the ventilatory delivery of oxygen to the gills did not become limited under the present experimental conditions of heat stress. However, a diffusion limitation at the gills could be suggested for those larger individuals that did not maintain Pa_{O_2} at 25°C (Fig. 3; Fig. 4). This may be related to increased gill diffusion distances in larger Chinook, and so the allometry of gill thickness and oxygen uptake in different species of fish would be an interesting avenue for future research.
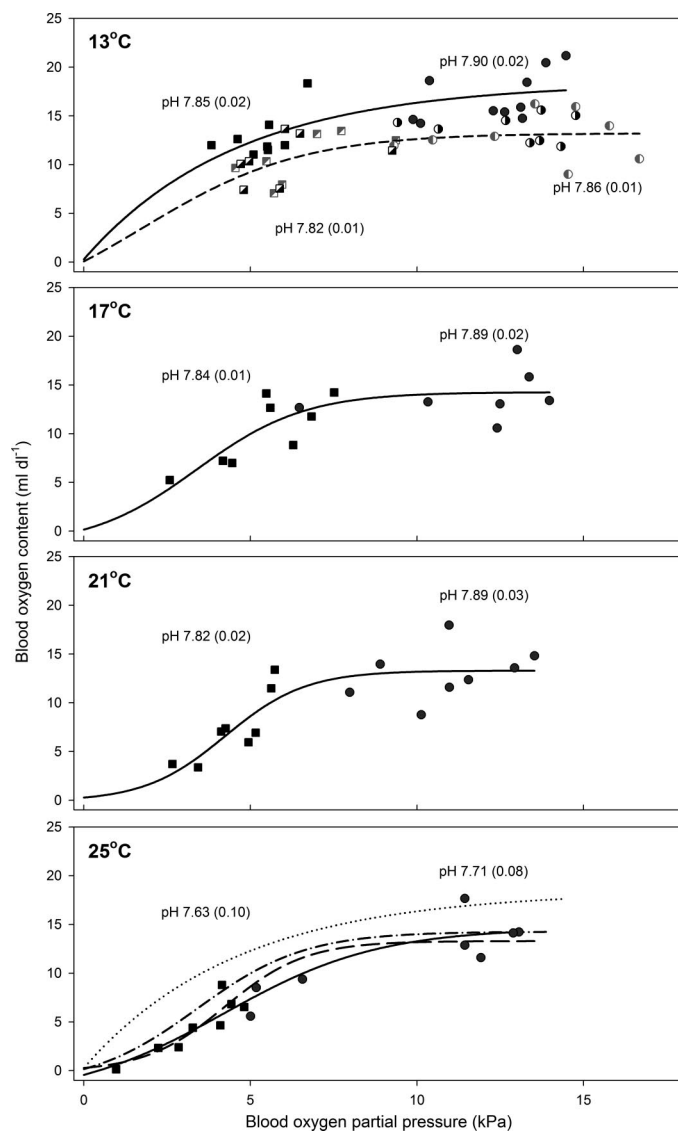
Fig. 4. Oxygen equilibrium curves at 13, 17, 21, and 25°C, as determined from arterial (circles) and venous (squares) blood samples taken from catheters at each experimental treatment. Also presented are the recovery data at 13°C; 1 h (half-filled black symbols on right) and 2 h (half-filled gray symbols on left) recovery data have been combined to construct the dashed curve. All curves were constructed using a 4-parameter sigmoid function in SigmaPlot. pH values (SE in parentheses) for arterial (*right*) and venous (*left*) data are given. All curves are presented on the 25°C panel for easy comparison: dotted line = 13°C; dashed-dotted line = 17°C; dashed line = 21°C; solid line = 25°C.

Notably, Hct, [Hb], and Ca_{O_2} were depressed during recovery, perhaps because resequestering of red blood cells in the spleen revealed the impact of withdrawing numerous blood samples that totaled <10% of blood volume if we assume a blood volume of 4%. Evidence against this possibility lies in the fact that the apparently chronic decrease in Ca_{O_2} occurred immediately when the fish were exposed to the first temperature increment of 17°C (Fig. 2; Fig. 4), at which point the cumulative blood volume withdrawn was small. It may be that the decrease in Ca_{O_2} at 17°C was associated with allosteric modulation of hemoglobin-oxygen binding, but this cannot be confirmed without further experimentation. Nevertheless, in vivo oxygen equilibrium curves of Chinook salmon did not

substantially right-shift with increasing temperature (Fig. 4), which contrasts with the suggestion of Gollock et al. (29) and Heath and Hughes (33) that oxygen binding capacity is limited at high temperatures in Atlantic cod and rainbow trout, respectively.

Randall and Daxboeck (42) have suggested that oxygen exchange in rainbow trout gills was limited by blood perfusion during exercise. Here, we found that the increase in Q did not match the increase in $\dot{M}_{O_2}$ during heat stress in Chinook, as indicated by a reduction in the blood convection requirement, which is also consistent with a perfusion limitation at the gills during heat stress. This may be simply because Q has either reached a functional limit or a physiological limit relative to the experimental conditions. It is interesting, yet unclear why the increase in Q with temperature in this study was mediated by an increase in f_H, while a similar increase in Q during exercise trials at 8–10°C in juvenile Chinook was mediated primarily by an increase in V_S (27). Maximum Q in salmonids is reportedly able to reach 60–110 ml·min^{-1}·kg^{-1} (2, 19), and f_H to have a maximum of around 120 beats/min (20). Therefore, f_H may have approached a maximum during heat stress in the present study, and so it is curious why V_S of Chinook remained unchanged, as has been found in a number of previous studies (8, 12, 29, 57). V_S was not abnormally high, as maximum V_S is reported in the range of 1 ml·beat^{-1}·kg^{-1} for salmonids, and so this removes the possibility that V_S was already near its maximum. Three potential reasons come to mind why V_S does not increase concomitantly with f_H and further enhance arterial oxygen transport at elevated temperatures. First, cardiac contractility decreases with increasing contraction frequency [a negative force frequency relationship (51)] and high temperature per se (35). Therefore, end-systolic volume may increase with elevated f_H because of weaker ventricular contractions. Second, cardiac filling time and potentially filling pressure (i.e., P_{CV}) are negatively correlated with f_H and temperature (1, 47, 48). Temperature may passively increase venous compliance (32, 45, 52), possibly reducing circulating blood volume and decreasing adrenergically mediated venous vascular tone (48). However, Sandblom and Axelsson (48) demonstrated for rainbow trout that cardiac filling pressure is maintained constant due to a decrease in vascular capacitance, which mobilizes blood to the central venous compartment when f_H increases during a moderate (10–16°C) acute temperature increase. Also, P_{CV} was initially unchanged in Chinook salmon as f_H increased with temperature, suggesting that there was a compensatory decrease in venous vascular capacitance which mobilized blood to the central venous compartment and compensated for the tachycardia (47, 48). In fact, at 25°C in Chinook, P_{CV} was significantly increased, and yet V_S did not increase despite an increased cardiac filling pressure, an observation that is consistent with cardiac congestion and contractile insufficiency. The third possibility relates to the deleterious changes in the chemical composition of the blood that could weaken cardiac contractility in vivo (e.g., acidosis and hypoxemia), which has been used as an explanation why maximum Q for in situ working trout hearts receiving fresh perfusate is typically greater than that measured during exercise when the chemical composition of venous blood changes substantially (16, 18, 26). In a similar context, cardiac arrhythmias were present in three of the largest Chinook at 25°C, and one of these fish (second-largest) lost equilibrium and failed to

recover. This latter fish was the only individual to reach a Pv_{O_2} as low as 1 kPa (Fig. 3), and thus it may be suggested that this is the lower Pv_{O_2} threshold for sufficient cardiac function. This is in agreement with data from rainbow trout when exposed to hypoxia during a swimming challenge (55), and the associated limitation in cardiac function will lead to gill perfusion limitations as discussed above.

Perspectives and Significance

The present study has shown that this population of Chinook salmon relies on a combination of Q and Ca_{O_2}–Cv_{O_2} to meet the increased $\dot{M}_{O_2}$ required during heat stress. We have uncovered some potential effects of body mass on arterial oxygen saturation, which may be linked with greater gill diffusion distances in larger fish. This is a topic that warrants further investigation over a greater body mass range, preferably with a species that grows well in excess of 5 kg. Nevertheless, it seems that tolerance to high temperature decreases with body mass, as indicated by lower arterial and venous oxygen levels. Given the switch to anaerobic metabolism and the cardiac arrhythmias observed in large fish at 25°C, we propose that the cascade of venous oxygen depletion results in a threshold value for venous oxygen partial pressure of around 1 kPa (Fig. 3). At this point, the oxygen supply to systemic and cardiac tissues is compromised such that the oxygen-deprived and acidotic myocardium becomes arrhythmic and blood perfusion through the gills and to the tissues becomes compromised. We suggest that the critical thermal maximum for resting adult Chinook salmon is mass-dependent and lies around 25°C for large fish (>4 kg) and perhaps around 27°C for smaller adult individuals. There is likely to be negligible aerobic scope at these temperatures, and so swimming fish are likely to have a reduced critical thermal maximum to maintain satisfactory aerobic scope for locomotion.

REFERENCES

1. **Altimiras J, Axelsson M.** Intrinsic autoregulation of cardiac output in rainbow trout (*Oncorhynchus mykiss*) at different heart rates. *J Exp Biol* 207: 195–201, 2004.
2. **Altimiras J, Axelsson M, Claireaux G, Lefrancois C, Mercier C, Farrell AP.** Cardiorespiratory status of triploid brown trout during swimming at two acclimation temperatures. *J Fish Biol* 60: 102–116, 2002.
3. **Axelsson M, Davison W, Forster ME, Farrell AP.** Cardiovascular responses of the red-blooded Antarctic fishes *Pagothenia bernachii* and *P. borchgrevinki. J Exp Biol* 167: 179–201, 1992.
5. **Brett JR.** Energetic response of salmon to temperature. A study of some thermal relations in the physiology and fresh-water ecology of sockeye salmon (*Oncorhynchus nerka*). *Am Zool* 11: 99–114, 1971.
6. **Brett JR.** Temperature tolerance in young Pacific salmon, genus *Oncorhynchus. J Fish Res Board Can* 9: 265–323, 1952.
7. **Brodeur JC, Dixon DG, McKinley RS.** Assessment of cardiac output as a predictor of metabolic rate in rainbow trout. *J Fish Biol* 58: 439–452, 2001.
8. **Cech JJ, Bridges DW, Rowell DM, Balzer PJ.** Cardiovascular responses of winter flounder, *Pseudopleuronectes americanus* (Walbaum), to acute temperature increase. *Can J Zool* 54: 1383–1388, 1976.
9. **Clark JJ, Rodnick KJ.** Effects of ventricular hypertrophy on the coronary vasculature of male rainbow trout. *Comp Biochem Physiol A* 124: S120, 1999.
10. **Clark TD, Eliason EJ, Sandblom E, Hinch SG, Farrell AP.** Calibration of a hand-held haemoglobin analyser for use on fish blood. *J Fish Biol* in press, 2008.
11. **Clark TD, Ryan T, Ingram BA, Woakes AJ, Butler PJ, Frappell PB.** Factorial aerobic scope is independent of temperature and primarily modulated by heart rate in exercising Murray cod (*Maccullochella peelii peelii*). *Physiol Biochem Zool* 78: 347–355, 2005.
12. **Clark TD, Seymour RS.** Cardiorespiratory physiology and swimming energetics of a high-energy-demand teleost, the yellowtail kingfish (*Seriola lalandi*). *J Exp Biol* 209: 3940–3951, 2006.
13. **Crawshaw LI.** Effect of rapid temperature change on mean body temperature and gill ventilation in carp. *Am J Physiol* 231: 837–841, 1976.
14. **Davie PS, Farrell AP.** The coronary and luminal circulations of fishes. *Can J Zool* 69: 1993–2001, 1991.
15. **Drabkin DL, Austin JH.** Spectrophotometric studies II. Preparations from washed blood cells; nitric oxide hemoglobin and sulfhemoglobin. *J Biol Chem* 112: 51–65, 1935.
16. **Farrell AP.** Cardiorespiratory performance during prolonged swimming tests with salmonids: a perspective on temperature effects and potential analytical pitfalls. *Philos Trans R Soc Lond B Biol Sci* 362: 2017, 2007.
17. **Farrell AP.** Coronary flow in a perfused rainbow trout heart. *J Exp Biol* 129: 107–123, 1987.
18. **Farrell AP.** Effects of temperature on cardiovascular performance. In: *Global Warming: Implications for Freshwater and Marine Fish*, edited by Wood CM and McDonald DG. New York: Cambridge University, 1997, p. 135–158.
19. **Farrell AP.** Features heightening cardiovascular performance in fishes, with special reference to tunas. *Comp Biochem Physiol A* 113: 61–67, 1996.
20. **Farrell AP.** From hagfish to tuna: a perspective on cardiac function in fish. *Physiol Zool* 64: 1137–1164, 1991.
21. **Farrell AP, Gallaugher PE, Fraser J, Pike D, Bowering P, Hadwin AKM, Parkhouse W, Routledge R.** 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, 2001.
22. **Farrell AP, Lee CG, Tierney K, Hodaly A, Clutterham S, Healey M, Hinch S, Lotto A.** Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. *J Fish Biol* 62: 64–84, 2003.
23. **Farrell AP, Simonot DL, Seymour RS, Clark TD.** A novel technique for estimating the compact myocardium in fish reveals surprising results for an athletic air-breathing fish, the Pacific tarpon. *J Fish Biol* 71: 389–398, 2007.
24. **Farrell AP, Small S, Graham MS.** Effect of heart rate and hypoxia on the performance of a perfused trout heart. *Can J Zool* 67: 274–280, 1989.
25. **Fletcher GL, Haedrich RT.** Rheological properties of rainbow trout blood. *Can J Zool* 65: 879–883, 1987.
26. **Franklin CE, Davison W, Seebacher F.** Antarctic fish can compensate for rising temperatures: thermal acclimation of cardiac performance in *Pagothenia borchgrevinki. J Exp Biol* 210: 3068–3074, 2007.
27. **Gallaugher PE, Thorarensen H, Kiessling A, Farrell AP.** Effects of high intensity exercise training on cardiovascular function, oxygen uptake, internal oxygen transport and osmotic balance in chinook salmon (*Oncorhynchus tshawytscha*) during critical speed swimming. *J Exp Biol* 204: 2861–2872, 2001.
28. **Gamperl AK, Farrell AP.** Cardiac plasticity in fishes: environmental influences and intraspecific differences. *J Exp Biol* 207: 2539–2550, 2004.
29. **Gollock MJ, Currie S, Petersen LH, Gamperl AK.** Cardiovascular and haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature increase. *J Exp Biol* 209: 2961–2970, 2006.
30. **Graham MS, Fletcher GL.** Blood plasma viscosity of winter flounder: influence of temperature, red cell concentration, and shear rate. *Can J Zool* 61: 2344–2350, 1983.
31. **Graham MS, Fletcher GL.** On the low viscosity blood of two cold water, marine sculpins. *J Comp Physiol B* 155: 455–459, 1985.

32. **Green JF, Jackman AP.** Mechanism of the increased vascular capacity produced by mild perfusion hypothermia in the dog. *Circ Res* 44: 411–419, 1979.

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

34. **Hill JV, Forster ME.** Cardiovascular responses of Chinook salmon (*Oncorhynchus tshawytscha*) during rapid anaesthetic induction and recovery. *Comp Biochem Physiol C* 137: 167–177, 2004.

35. **Hove-Madsen L.** The influence of temperature on ryanodine sensitivity and the force-frequency relationship in the myocardium of rainbow trout. *J Exp Biol* 167: 47–60, 1992.

36. **Lannig G, Bock C, Sartoris FJ, Pörtner HO.** 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, 2004.

37. **Lee CG, Farrell AP, Lotto A, MacNutt MJ, Hinch SG, Healey MC.** 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, 2003.

38. **McCullough DA.** A review and synthesis of effects of alterations to the water temperature regime on freshwater life stages of salmonids, with special reference to chinook salmon. Seattle, WA: U.S. Environmental Protection Agency, 1999, p. 279.

39. **Olson KR.** Blood and extracellular fluid volume regulation: role of the renin angiotensin system, kallikrein-kinin system, and atrial natriuretic peptides. In: *Fish Physiology*, edited by Randall DJ, Hoar WS, and Farrell AP. San Diego, CA: Academic, 1992, p. 135–254.

40. **Pörtner HO.** Climate variations and the physiological basis of temperature-dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comp Biochem Physiol A* 132: 739–761, 2002.

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

42. **Randall DJ, Daxboeck C.** Cardiovascular changes in the rainbow trout (*Salmo gairdneri* Richardson) during exercise. *Can J Zool* 60: 1135–1140, 1982.

43. **Reynolds WW.** Thermal equilibration rates in relation to heartbeat and ventilatory frequencies in largemouth blackbass, *Micropterus salmoides*. *Comp Biochem Physiol A* 56: 195–201, 1977.

44. **Rothwell SE, Black SE, Jerrett AR, Forster ME.** Cardiovascular changes and catecholamine release following anaesthesia in Chinook salmon (*Oncorhynchus tshawytscha*) and snapper (*Pagrus auratus*). *Comp Biochem Physiol A* 140: 289, 2005.

45. **Rubini A.** Effect of perfusate temperature on pulmonary vascular resistance and compliance by atrial and venous occlusion in the rat. *Eur J Appl Physiol* 93: 435–439, 2005.

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

47. **Sandblom E, Axelsson M.** The venous circulation: A piscine perspective. *Comp Biochem Physiol A* 148: 785, 2007.

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

49. **Sandblom E, Axelsson M, McKenzie DJ.** Venous responses during exercise in rainbow trout, *Oncorhynchus mykiss*: α-adrenergic control and the antihypotensive function of the renin-angiotensin system. *Comp Biochem Physiol A* 144: 401–409, 2006.

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

51. **Shiels HA, Vornanen M, Farrell AP.** Temperature dependence of cardiac sarcoplasmic reticulum function in rainbow trout myocytes. *J Exp Biol* 205: 3631–3639, 2002.

52. **Shoukas AA, Brunner MC.** Epinephrine and the carotid sinus baroreceptor reflex. Influence on capacitive and resistive properties of the total systemic vascular bed of the dog. *Circ Res* 47: 249–257, 1980.

53. **Simonot DL, Farrell AP.** Cardiac remodelling in rainbow trout *Oncorhynchus mykiss* Walbaum in response to phenylhydrazine-induced anaemia. *J Exp Biol* 210: 2574–2584, 2007.

54. **Soivio A, Nynolm K, Westman K.** A technique for repeated sampling of the blood of individual resting fish. *J Exp Biol* 63: 207–217, 1975.

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

57. **Stevens ED, Bennion GR, Randall DJ, Shelton G.** Factors affecting arterial pressure and blood flow from the heart in intact, unrestrained lingcod, *Ophiodon elongatus*. *Comp Biochem Physiol A* 43: 681–695, 1972.

58. **Taylor EW, Egginton S, Taylor SE, Butler PJ.** Factors which may limit swimming performance at different temperatures. In: *Global Warming: Implications for Freshwater and Marine Fish*, edited by Wood CM and McDonald DG. New York: Cambridge University, 1997, p. 105–134.

59. **Thorarensen H, Gallaugher PE, Kiessling AK, Farrell AP.** Intestinal blood flow in swimming chinook salmon *Oncorhynchus tshawytscha* and the effects of haematocrit on blood flow distribution. *J Exp Biol* 179: 115–129, 1993.

60. **Thorarensen H, Knight C, Davie PS.** Seasonal changes in 11-ketotestosterone and relative ventricle mass in wild rainbow trout, *Oncorhynchus mykiss*. *New Zeal J Mar Fresh Res* 30: 397–402, 1996.

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

62. **West JB.** *Respiratory Physiology: The Essentials.* Baltimore, MD: Lippincott Williams and Wilkins, 2000.