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ORIGINAL PAPER

Sex differences in circulatory oxygen transport parameters of sockeye salmon (*Oncorhynchus nerka*) on the spawning ground

Timothy Darren Clark · S. G. Hinch · B. D. Taylor · P. B. Frappell · A. P. Farrell

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Abstract Upon reaching sexual maturity, several species of male salmonids possess a relative ventricular mass (rM_V) that may be up to 90% larger than females. This can increase maximum cardiac stroke volume and power output, which may be beneficial to increasing the oxygen transport capacity of male salmonids during the spawning period. It may be further hypothesized, therefore, that other variables within the circulatory oxygen transport cascade, such as blood oxygen-carrying capacity and heart rate, are similarly enhanced in reproductively mature male salmonids. To test this idea, the present study measured a range of circulatory oxygen transport variables in wild male and female sockeye salmon (*Oncorhynchus nerka*) during their spawning period, following a 150 km migration from the ocean. The rM_V of male fish was 13% greater than females. Conversely, the haemoglobin concentration ([Hb]) of female fish was 19% higher than males, indicative of a greater blood oxygen-carrying capacity (138 vs. 116 ml O_2 l^{-1}, respectively). Surgically implanted physiological data loggers revealed a similar range in heart rate for both sexes on the spawning ground (20–80 beats min^{-1} at 10°C), with a tendency for male fish to spend a greater percentage of time (64%) than females (49%) at heart rates above 50 beats min^{-1}. Male fish on average consumed significantly more oxygen than females during a 13-h respirometry period. However, routine oxygen consumption rates ($\dot{M}O_2$) ranged between 1.5 and 8.5 mg min^{-1} kg^{-1} for both sexes, which implies that males did not inherently possess markedly higher routine aerobic energy demands, and suggests that the higher [Hb] of female fish may compensate for the smaller rM_V. These findings reject the hypothesis that all aspects of the circulatory oxygen transport cascade are inherently superior in male sockeye salmon. Instead, it is suggested that any differences in $\dot{M}O_2$ between sexually mature male and female sockeye salmon can likely be attributed to activity levels.

Keywords Energetics · Heart rate · Haemoglobin concentration · Haematocrit · Oxygen consumption rate · Blood oxygen-carrying capacity · Relative ventricular mass

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T. D. Clark (✉) · A. P. Farrell
Faculty of Land and Food Systems, University of British Columbia, Vancouver V6T 1Z4, Canada
e-mail: timothy.clark.mail@gmail.com

S. G. Hinch
Department of Forest Sciences, University of British Columbia, Vancouver V6T 1Z4, Canada

B. D. Taylor · P. B. Frappell
Department of Zoology, La Trobe University, Melbourne 3086, Australia

Introduction

Pacific salmon (*Oncorhynchus* spp.) are anadromous fishes that return from the ocean to their natal freshwater spawning ground, typically at 2–5 years of age (species-dependent). They are semelparous and do not feed once the migration has commenced, and so their stored energy reserves must satisfy the energetic demands of the migration, primary and secondary sexual development, and the reproductive phase on the spawning ground prior to death (Brett 1995; Hinch and Rand 1998). Failure to appropriately allocate energy reserves and maintain physiological function during each of these periods of the life cycle will result in pre-spawn mortality and consequently zero lifetime fitness (Healey et al. 2003; Farrell et al. 2008).

While it is clear that female salmon invest more energy into gonadal development (up to 18% of body mass in females vs. about 4% in males) and males invest more energy into secondary sexual characteristics (e.g. growth of a kype and dorsal hump), there are other important sexual dimorphisms in salmon (Brett 1995). Upon reaching sexual maturity, several species of male salmonids possess a relative ventricular mass (rM_V) that may be up to 90% larger than females (Franklin and Davie 1992; Armstrong and West 1994). This anatomical change has been shown in the laboratory to increase maximum cardiac stroke volume and power output in rainbow trout (*Oncorhynchus mykiss*) (Franklin and Davie 1992), which are hypothesized to support increased functional demands placed on the hearts of male salmonids during the spawning period (Gamperl and Farrell 2004). Owing to a lack of cardiovascular data from salmonids on the spawning ground, the increased functional demands of male hearts can only be speculated, but have been linked with factors such as hypervolemia, hypertension, increased afterload and increased circulatory oxygen transport requirements to cope with increased aerobic metabolism (Altimiras et al. 1996; Thorarensen et al. 1996a; Clark and Rodnick 1999). The hypothesis that male salmonids require enhanced circulatory oxygen transport capacity during the spawning period is a matter of debate. In support of this hypothesis, higher heart rates in sexually-mature male Atlantic salmon (*Salmo salar*) have been proposed to support increased levels of circulatory oxygen transport (Lucas et al. 1993; Altimiras et al. 1996). On the other hand, this hypothesis is opposed by proximate analysis studies that suggest female sockeye salmon (*Oncorhynchus nerka*) have greater overall energy usage than males during the spawning period (548–649 kJ for females, 247–657 kJ for males, Healey et al. 2003).

While the need for a greater cardiac mass in adult male salmon remains in some doubt, it may be expected that other variables within the circulatory oxygen transport cascade, such as blood oxygen-carrying capacity and heart rate, will be similarly enhanced in male fish. In order to better document any sex-specific differences in circulatory oxygen transport capacity of reproductively mature salmonids, the present study was undertaken to compare and quantify aspects of the circulatory oxygen transport pathway in male and female sockeye salmon sampled on the Weaver Creek spawning ground, British Columbia, Canada. In particular, this study examined for sex differences in relative ventricular mass, blood oxygen-carrying capacity and routine oxygen consumption rates ($\dot{M}O_2$) in reproductively mature fish. Additionally, surgically implanted data loggers were utilised to monitor, for the first time, heart rates of free-swimming sockeye salmon on the spawning ground. We hypothesized that male fish would have greater circulatory oxygen transport potential than females, reflected in a greater relative ventricular mass, greater blood oxygen-carrying capacity and higher heart rates, and that these would contribute to males having an inherently higher level of routine metabolism that shared little overlap with female values.

Materials and methods

Animals and spawning site

Healthy looking, newly arrived sockeye salmon (*Oncorhynchus nerka*) were individually dip-netted from the Weaver Creek spawning channel throughout October 2007, after they had completed their 150 km spawning migration from the ocean. All fish were 4–5 years old, and males and females were visibly distinguished by their morphology and colouration. The spawning channel had a gravel bottom, was about 50 cm deep and 5 m wide, and wound back and forth for about 3 km. Water flow through the spawning channel ranged from 0.1 to 0.4 m s^{-1}, and the fish could maintain position with little swimming effort. Sockeye salmon that arrive at the Weaver Creek spawning channel typically die (pre- or post-spawn) within 2–10 days (R. Stitt, Canadian Department of Fisheries and Oceans, personal communication).

Respirometry

Immediately following dip-netting from the spawning channel at about 19:00 hours, a fish was placed into one of two respirometers for 16 h. Oxygen consumption rates were monitored on ten male [mass range 2.5–3.4 kg, fork length (FL) range 63–69 cm] and eight female (2.2–3.3 kg, 58–63 cm) individual fish, typically using one fish of each sex per day. The respirometers were constructed from circular fibreglass tanks (diameter 150 cm, volume 880 l, depth ~50 cm; sufficiently large to allow fish to undergo exploratory behaviours) and were positioned beside the spawning channel. A pool liner, held in place by metal clamps, covered the water surface of each tank, and a Clark-type oxygen sensor (Oxyguard, Point Four Systems Inc., Richmond, Canada) recorded water oxygen concentration every 5 s to a personal computer. Water temperature in the respirometers during the measurement periods was the same as the spawning channel (mean ± SEM = 10 ± 0.1°C) and was archived every 2 h (iButton, Maxim Integrated Products, http://www.maxim-ic.com). Circular water velocity was regulated by a submersible pump at ~0.2 m s^{-1} to facilitate water mixing and provide a gentle current against which the fish could orient itself. A flush pump, connected to a timer, refreshed the respirometer water for 1 h in every two. Oxygen consumption

rates were calculated from the decline in water oxygen levels for alternating 1-h periods. Following $\dot{M}O_2$ measurements, each fish was removed by dip-net from the respirometer, anaesthetised, sampled for blood, weighed and measured as described below. Each fish was then returned to the spawning channel and assisted until it regained equilibrium and swam away. No further sampling was conducted on these fish, as they were to contribute to the spawning population.

Anaesthesia, blood sampling and morphological measurements

All anaesthesia was performed in a bath containing 100 mg l^{-1} of $NaHCO_3$-buffered tricaine methanesulfonate (Sigma, St Louis, MO, USA) in channel water. Prior to complete anaesthesia, but when the fish could be rolled into a supine position in the anaesthetic bath (i.e. <3 min after capture), a 1-ml blood sample was obtained by caudal venepuncture into a heparin-coated vacutainer. Each fish remained in the anaesthetic bath until it lost equilibrium and opercular movements became weak (<5 min after capture), and then the fish was weighed and the following morphological measurements were taken. FL, from the tip of the snout to the fork in the tail, was measured for each fish. Post-orbital to fork length (POFL) was characterised as a measure from the posterior wall of the eye to the fork in the tail, to account for differences in snout length (e.g. the male kype) between individuals. Body girth was characterised as the circumference of the fish immediately anterior to the dorsal fin. The metric POFL girth^{-1} was used as an index of ripeness in female fish, where a high value indicated relatively small girth for the length of the fish and was characteristic of less ripe females, and a low value indicated the reverse and was characteristic of ripe fish, in which the eggs had been released from the ovaries into the peritoneal cavity. [Hb] was measured using a HemoCue analyser calibrated for fish blood (see Clark et al. 2008a), haematocrit (Hct) was determined using micro-capillary tubes centrifuged at 10,000×*g* for 7 min, and mean corpuscular haemoglobin concentration (MCHC) was calculated as [Hb]/(Hct/100). Means of duplicate or triplicate samples were used.

Physiological data logging

Heart rate and body temperature were monitored in a separate group of fish to those described above. Data were obtained from four males (mass, 2.3–4.2 kg; FL, 62–71 cm) and seven females (2.5–3.8 kg; 62–69 cm). A further four male and six female fish were used, but data were not obtained due to technical difficulties with the data loggers. The surgical procedure was as follows. A physiological data logger (iLogR, mass 23 g in air; La Trobe University, Melbourne, Australia) that has been described previously (Clark et al. 2008c), but modified to use electrocardiogram (ECG) electrodes rather than a piezoelectric sensor to measure heart beats, was surgically implanted into the body cavity of each fish. Fish were individually anaesthetised, sampled for blood, weighed and measured, as described above, prior to being placed ventral side down on a plastic-covered foam operating table. The gills were continuously irrigated with cold water (<10°C) that contained a maintenance dose of buffered anaesthetic (70 mg l^{-1}). Identifying tags (Peterson discs; Floy Tag, http://www.floytag.com) were inserted into the dorsal tissue, approximately 10 mm ventral and anterior of the dorsal fin, and then the fish was rolled into a supine position. A 30–40 mm incision was made along the ventral midline to access the peritoneal cavity, just anterior to the ventral fins and associated cartilage. A logger that had been soaked in iodine antiseptic (Proviodine, Rougier, http://www.rougier.com), and was programmed to record for 10 s in every 10 min for a 6-week duration, was inserted through the incision using uterine forceps and positioned with the ECG electrodes ventral to the liver and as close as possible to the pericardial cavity (the pericardium is not breached during this procedure; see Clark et al. 2008c for further details). Once in position, the body of the logger was loosely sutured to the peritoneal wall and associated ventral tissue to prevent it from moving away from the heart. The incision was closed with silk sutures, the number of which was dependent on the sex of the fish. Male fish had a thick layer of ventral tissue that sealed tightly with 5–7 sutures. Female fish possessed a thin layer of ventral tissue, and so 12–16 sutures were used with the intention to seal the wound tightly to prevent water leaking into the peritoneal cavity and consequent damage of the eggs. Tissue adhesive (Vetbond^{TM}, 3M) was applied to the wound to assist with the seal before placing the fish back into the spawning channel and assisting its recovery as described above. The entire procedure took about 20 min. Tagged fish were monitored 3–4 times a day in the spawning channel until they died using observation periods of 20–30 min per fish.

Post-mortem

Following death of each tagged fish (8 males, 13 females), it was re-weighed and re-measured as above, and a dorsal section of muscle tissue (100–300 g) was removed from each individual for calorimetric analysis of gross energy density (this method provides an accurate measure of whole body gross energy density; D.A. Patterson, Canadian Department of Fisheries and Oceans, unpublished data). The ventricle of each fish was excised, emptied of blood,

blotted and weighed in order to calculate relative ventricular mass. It was necessary, for the following reasons, to use the initial body mass measured for each individual at the time of logger implantation for the rM_V calculations, rather than the body mass at death. Firstly, female fish lost their egg mass (equivalent to 12–18% of their initial body mass) during spawning, and so using the post-spawn body mass greatly exaggerated rM_V of female fish. Secondly, moribund and dead fish lose osmoregulatory ability and gain water, which overestimates body mass and likely underestimates rM_V. Thus, we believe that this provided the best method of calculating rM_V of the tagged fish used in this study. Nevertheless, we assessed rM_V in a newly arrived group of fish (termed 'controls'; six males, four females) that were euthanized by a cranial blow immediately upon arrival at the spawning ground. We obtained body mass measurements from these fish, and we excised, prepared and weighed the ventricle as above in order to calculate rM_V.

Data analysis and statistics

A combination of *t* tests, one-way ANOVA, two-way ANOVA, and ANCOVA were used, where appropriate to compare between male and female fish. Significance was considered at $P < 0.05$.

Results

On arrival at the spawning ground, [Hb] was 18.8% higher in females (102.9 ± 4.1 g l^{-1}) than males (86.6 ± 1.9 g l^{-1}), which reflected a 13.5% higher Hct and a similar MCHC (Fig. 1b–d). On the basis that 1 g of haemoglobin combines with 1.34 ml of oxygen, the theoretical maximum arterial oxygen-carrying capacities of female and male blood were 138 and 116 ml O_2 l^{-1}, respectively. [Hb], Hct and MCHC were correlated with POFL girth^{-1} in female fish (Fig. 2), with [Hb] providing the strongest linear relationship ($P < 0.001$). Combined with the fact that POFL varied by less than 15% among females, while girth varied by up to 30%, this result suggests that [Hb] was highest in females that were less ripe (ovaries intact, somewhat restricting the stomach from bulging) as opposed to very ripe females, in which the girth was increased by eggs being released from the ovaries into the peritoneal cavity. There was no similar relationship for males between [Hb] and POFL girth^{-1} ($r^2 < 0.1$, $P > 0.5$).

Measurements of $\dot{M}O_2$ were discarded for the first 3 h of residency in the respirometer to allow recovery of the fish from handling. Fish were observed to explore the respirometer, probing the structure with their nose and occasionally undergoing vigorous periods of activity.

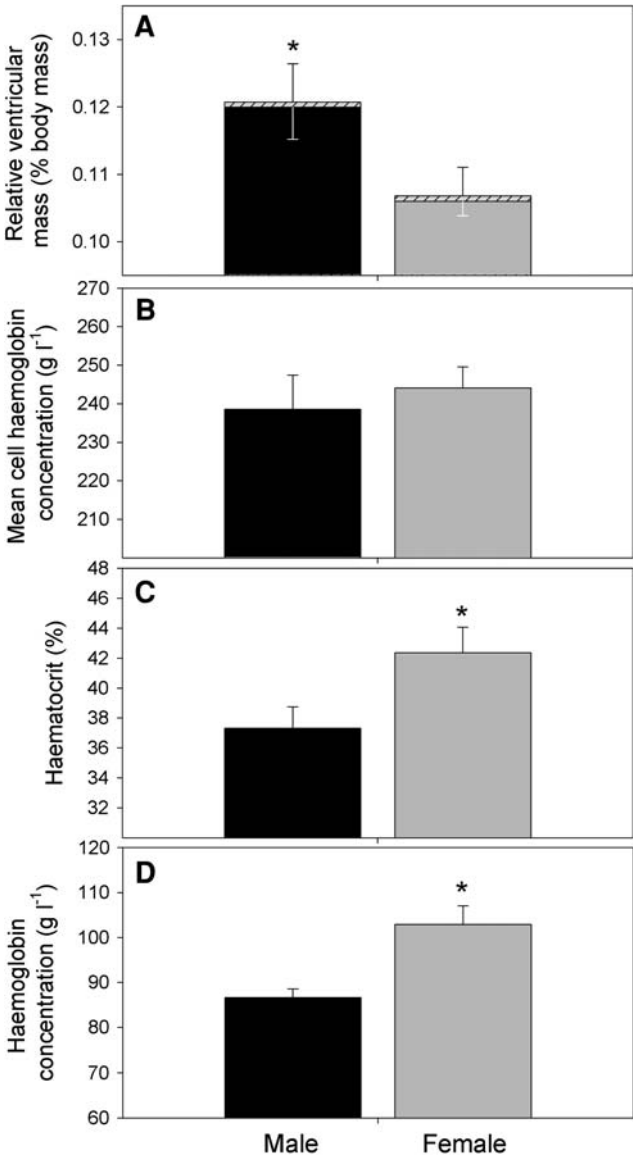

Fig. 1 **a** Relative ventricular mass (rM_V; $N = 8$ males, 13 females for tagged fish (*black* and *grey bars*; *downward error bars*); $N = 6$ males, 4 females for control fish (*hatched bars* at back)), **b** mean cell haemoglobin concentration (MCHC), **c** haematocrit (Hct), and **d** haemoglobin concentration ([Hb]) of Weaver Creek sockeye salmon (*Oncorhynchus nerka*) upon arrival at their spawning ground ($N = 18$ males, 21 females for the latter three variables). *Asterisk*: significantly higher than the corresponding value for the opposite sex, where rM_V is 13.2% higher in males (referring to tagged fish), and Hct and [Hb] are, respectively 13.5% and 18.8% higher in females (*t* test, $P < 0.05$)

A frequency histogram revealed a similar range in $\dot{M}O_2$ ($\sim$1.5–8.5 mg min^{-1} kg^{-1}) between male (mean 4.0 mg min^{-1} kg^{-1}) and female (mean 3.3 mg min^{-1} kg^{-1}) fish at a standardised water temperature of 10°C (Fig. 3). A cumulative frequency plot indicated that females spent 50% of their time at $\dot{M}O_2$ below 2.7 mg min^{-1} kg^{-1}, while the 50% threshold for males was at a higher $\dot{M}O_2$ of 3.6 mg min^{-1} kg^{-1} (inset Fig. 3). The average total

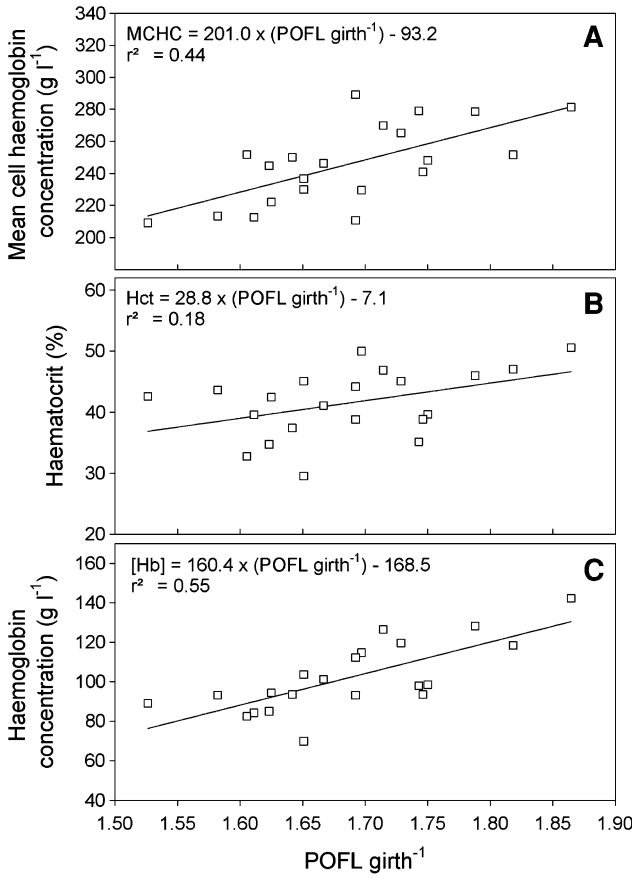

Fig. 2 Mean cell haemoglobin concentration (MCHC), haematocrit (Hct) and haemoglobin concentration ([Hb]) of reproductively mature, pre-spawn female sockeye salmon (*Oncorhynchus nerka*) as a function of post-orbital to fork length (POFL) × girth^{-1} (see "Materials and methods" for measurement details). Fish ranged in body mass from 2.15 to 3.78 kg. Linear regression equations were significant for MCHC ($P = 0.001$) and [Hb] ($P < 0.001$), but not Hct ($P = 0.055$). $N = 21$

oxygen consumed during the 13-h post-recovery period was significantly higher for males (3,015 ± 343 mg kg^{-1}) than females (2,528 ± 374 mg kg^{-1}) ($P = 0.044$; *t* test, data log-transformed for normality).

In the natural, thermal oscillations of the spawning ground water (range 8.5–12.5°C), there was a general temperature-dependency of heart rate in individual free-swimming male and female fish (e.g. Fig. 4a, d). Heart rate data were standardised to a water temperature of 10°C (using Q_{10} of 2; Clark et al. 2008b; Steinhausen et al. 2008), and a period of 12 h was excluded from the start of each data set to minimise the influence of post-surgical recovery on the results. A frequency histogram of temperature-standardised heart rate data revealed a range of 20–80 beats min^{-1} for both sexes (Fig. 5), although male fish tended to spend a greater, yet non-significant, percentage of time than females at heart rates above 50 beats min^{-1} (64 ± 20 vs. 49 ± 15%, respectively; *t* test, $P = 0.559$; Fig. 5).

Post-mortem analysis of fish that were implanted with data loggers indicated a 13.2% greater rM_V in male fish, which is the same trend that existed for control fish that were destructively sampled immediately upon arrival at the spawning ground (male rM_V 13.0% greater than females; Fig. 1a). The method used to calculate rM_V of tagged fish based on initial body mass measurements at the time of logger implantation did not significantly underestimate rM_V in comparison with control fish (Fig. 1a). The average gross energy density at death, irrespective of time on the spawning ground, was statistically similar for males (3.24 ± 0.14 MJ kg^{-1}) and females (3.59 ± 0.10 MJ kg^{-1}) ($N = 8$ males, 13 females; ANCOVA with body mass as covariate, $P = 0.093$). Gross energy density at death had a negative, linear relationship with time spent on the spawning ground in male fish, but the relationship was not as clear for females (Fig. 6).

Discussion

The results of this study confirm the presence of a greater rM_V in male Weaver Creek sockeye salmon upon arrival at their spawning ground. This sexual dichotomy is found in other reproductively mature salmonids (Franklin and Davie 1992; Graham and Farrell 1992; Armstrong and West 1994) and some non-salmonid species (Luk'yanenko and Raspopov 1972). There are conflicting reports on whether this cardiac growth is due to hypertrophy (the enlargement of existing cells) or hyperplasia (the production of new cells; Graham and Farrell 1992; Bailey et al. 1997; Clark and Rodnick 1998), but it is generally accepted that the growth is largely stimulated by the elevated levels of androgens (testosterone, 11-ketotestosterone) in male compared with female fish (Thorarensen et al. 1996a, 1996b; Davie and Thorarensen 1997). Testosterone concentrations of sockeye upon arrival at the Weaver Creek spawning ground have been reported as 37 ng ml^{-1} in males, but only 21 ng ml^{-1} in females (Hruska et al. 2007). Other factors, such as hypervolemia, hypertension, and activity associated with migration and reproduction (i.e. a training effect), also may be important in increasing rM_V in male salmonids (Thorarensen et al. 1996a; Clark and Rodnick 1999). Given the large difference in gonad mass between male and female fish, and assuming that the gonads contribute little to total body oxygen demand, it may be argued that rM_V should be calculated on the basis of somatic mass (i.e. body mass minus gonad mass), in which case, there would be no intersexual difference in rM_V in the sockeye used in this study (0.12% for both sexes). The alternate, and generally accepted, argument is that the gonads contribute to the oxygen demands of the fish and therefore should be included in the calculations.

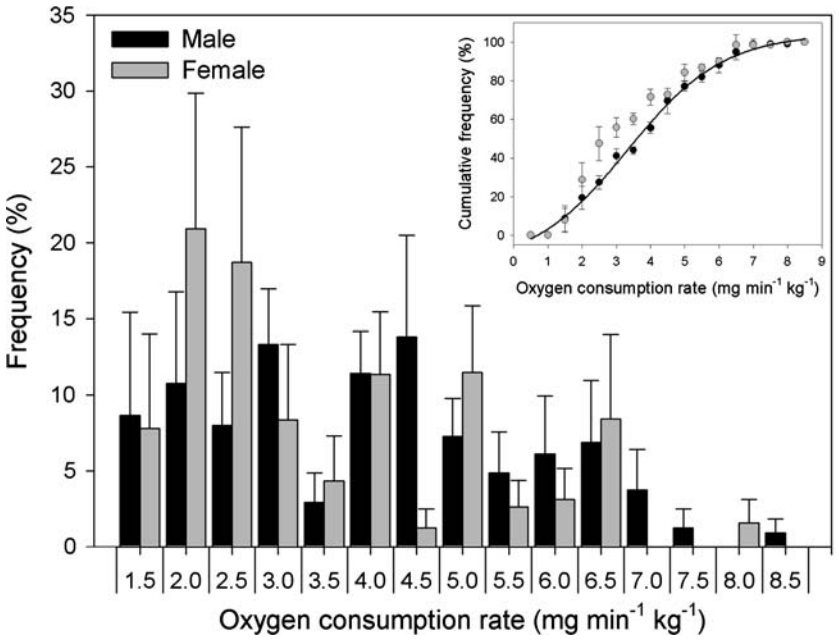

Fig. 3 Frequency histogram (means ± SEM) of oxygen consumption rates for reproductively mature male and female Weaver Creek sockeye salmon (*Oncorhynchus nerka*) measured in a large respirometer (diameter 150 cm, volume 880 l). Data have been standardised to a common temperature of 10°C using a Q_{10} of 3 (Clark et al. 2008b; Steinhausen et al. 2008). $N = 10$ males, 8 females. *Inset* displays the cumulative frequency of $\dot{M}O_2$ measurements from 0.5 to 8.5 mg min^{-1} kg^{-1} for male and female fish. Sigmoidal regression is displayed for male fish to allow more easy comparison with female values

To our knowledge, no study has quantified gonadal blood flow and oxygen demand in mature salmonids, and so in the absence of these data, we cannot justify the removal of gonad mass from calculations of rM_V.

The enhanced rM_V of reproductively mature male rainbow trout allows increased maximum cardiac stroke volume and power output (Franklin and Davie 1992), and it may be reasonable to assume that this is the case in all male salmonids that experience cardiac growth at maturation (Graham and Farrell 1989, 1990). Given this, we can speculate that circulatory oxygen transport of male sockeye in the present study benefited from a greater capacity to increase cardiac output through larger stroke volumes, as well as an increased ability of the heart to work against higher output pressures. Nevertheless, this dichotomy did not translate into a sex difference in modal values for heart rate (50–59 beats min^{-1}) or the range (20–80 beats min^{-1}) exhibited by sockeye salmon on the spawning ground (Fig. 5). Instead, males tended to spend a slightly larger proportion of time at high heart rates above 50 beats min^{-1}, which may complement greater cardiac stroke volume and translate to higher cardiac outputs than females during the spawning period. In any event, this study confirms what may have been predicted from behavioural observations, in that both sexes of sockeye salmon typically work at high rates during the extremely competitive and aggressive period on the spawning ground prior to programmed death.

There are few published heart rate data for large, wild salmonids like those used in the present study, and even fewer that examine for sex-related differences (Lucas et al. 1993; Altimiras et al. 1996; Clark et al. 2008b; Steinhausen et al. 2008). Altimiras et al. (1996) report heart rate ranges of 22–36 and 16–28 beats min^{-1} for mature male and female Atlantic salmon, respectively ($N = 2$ from each sex), while free-swimming in the natural environment. These are lower and narrower ranges than presented here for sockeye salmon, and are likely a result of cooler water temperatures during the Atlantic salmon study (water temperature not reported, but was likely to be <5°C; J. Altimiras, personal communication). Steinhausen et al. (2008) pooled data for mature male and female sockeye salmon and report mean resting and swimming heart rates of 65 and 81 beats min^{-1}, respectively, in fish that were instrumented with a ventral aortic blood flow probe and housed in a swim tunnel respirometer at 15°C. The mean heart rate of swimming fish compares favourably to the maximum values recorded in the present study for male and female sockeye that were free-swimming on their spawning ground at 8.5–12.5°C. The resting heart rate reported in Steinhausen et al. (2008), however, is in the upper range of values reported in the present study (Fig. 5). Apart from the obvious Q_{10} effect of temperature, this disparity may result from methodological differences in the measurement of heart rate, specifically that the fish in the present study were not tethered to recording equipment and were able to free-swim in their natural environment. The difference may also be related to differences in the natural physiological state of the fish used in each study. Fish in Steinhausen et al. (2008) were from a population of sockeye (Lower Adams River stock complex, B.C., Canada) that migrates further than Weaver sockeye (c.f. 500 vs. 150 km, respectively), and they were captured in the marine environment prior to river entry during the

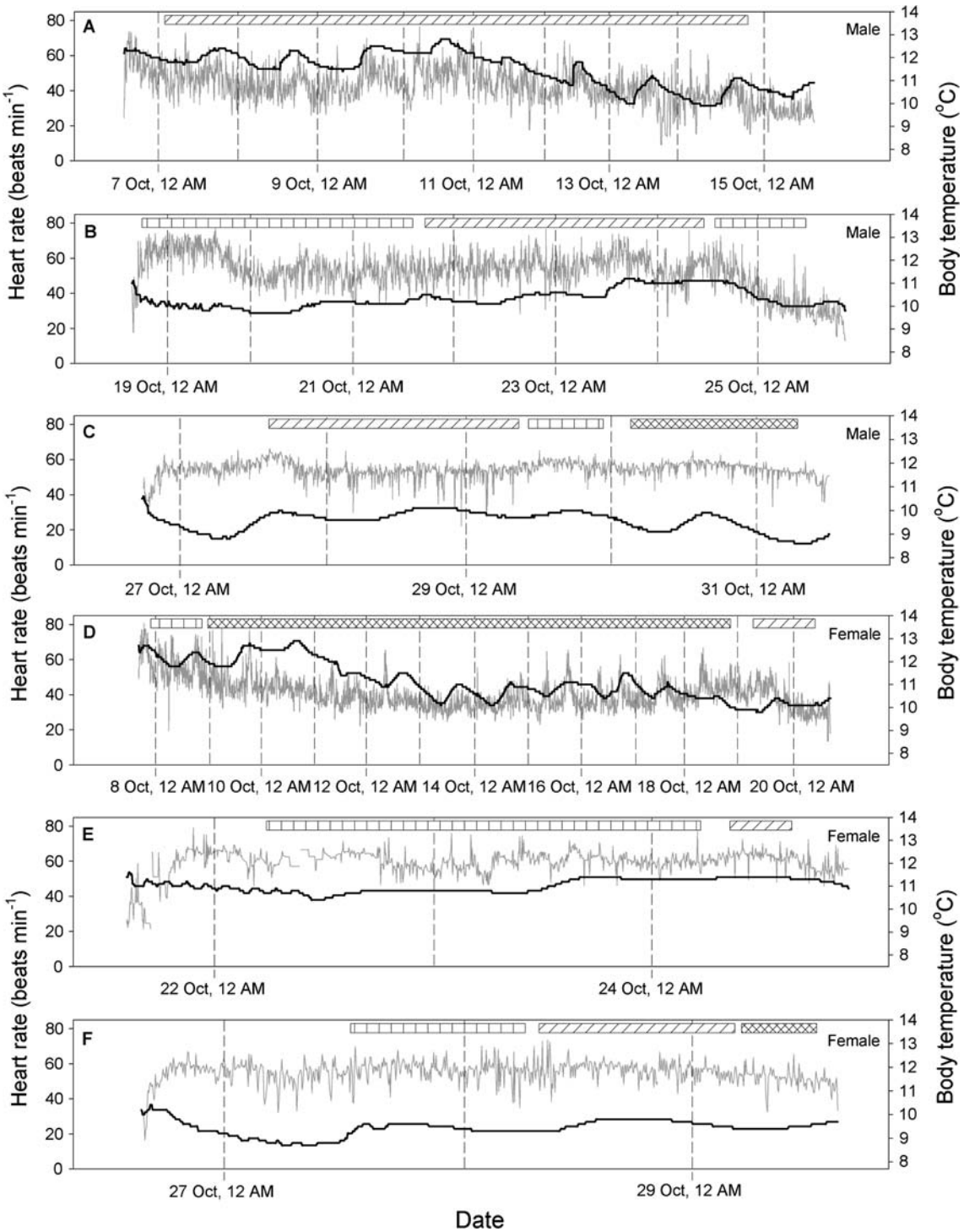

Fig. 4 Logged traces (3-s means) of heart rate (*grey solid lines*) and body temperature (*black solid lines*) obtained from six reproductively mature sockeye salmon (*Oncorhynchus nerka*), while free-swimming on the Weaver Creek spawning ground (each *panel* represents a different fish; **a**–**c** male fish, **d**–**f** female fish). Oscillations in body temperature are driven by daily fluctuations in water temperature. *Vertical dashed lines* indicate midnight of each day. *Rectangle panels* at the *top* of each plot indicate periods of various activities: large-range swimming and exploratory behaviours (*vertical lines*); small-range holding behaviours (*chequered lines*); and small-range spawning behaviours, including such things as redd-digging and conflicts with other individuals, but without necessarily spawning (*diagonal lines*). Note that fish survived less time on the spawning ground as the season progressed. Fish in **a**, **c**, **e** and **f** successfully spawned, whereas the others died without spawning

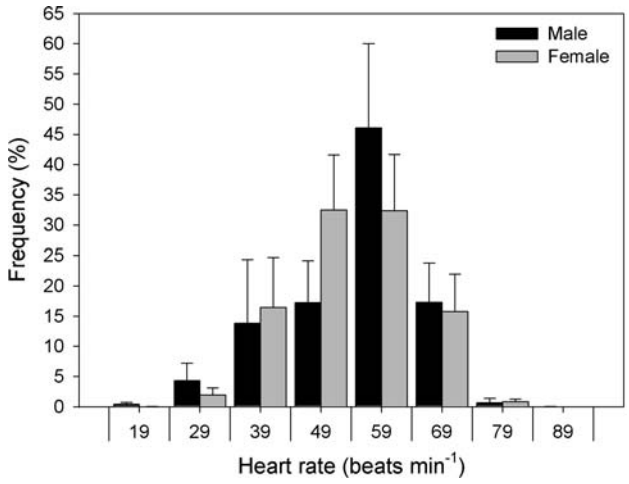

Fig. 5 Frequency histogram (means ± SEM) of heart rate for reproductively mature male and female sockeye salmon (*Oncorhynchus nerka*), while free-swimming on the Weaver Creek spawning ground. The first 12 h of data from each fish were excluded from this analysis to remove the influence of the post-surgical bradycardia or tachycardia displayed by some individuals (see Fig. 4). Data have been standardised to a common temperature of 10°C using a Q_{10} of 2 (Clark et al. 2008b; Steinhausen et al. 2008). $N = 4$ males, 7 females

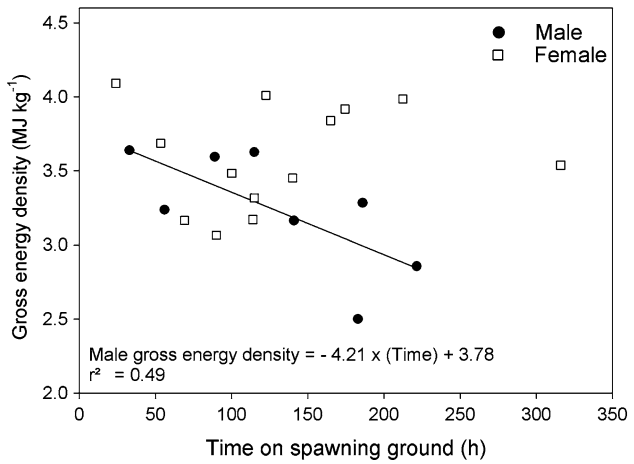

Fig. 6 Gross energy density of male and female sockeye salmon (*Oncorhynchus nerka*) at death as a function of time on the spawning ground. The relationship was significant for males (equation given; $P = 0.048$), but not females. $N = 8$ males, 13 females

spawning migration. In any event, we are confident that the values of heart rate reported in the present study are representative of wild, unstressed sockeye salmon during the spawning period, and we encourage the use of physiological data logging and biotelemetry where possible to answer questions relating to the normal physiological function of wild fish.

On the basis of the cardiac data alone, it is tempting to conclude that the reproductively mature male sockeye used in this study possess inherently greater circulatory oxygen transport rates than females. However, this conclusion is not supported by the blood oxygen-carrying capacity data. Fish were always active when captured, which is likely to have resulted in splenic contraction and release of erythrocytes into the circulating blood (Gallaugher and Farrell 1998). Thus, the values of [Hb] and Hct measured in this study are likely to represent maximum levels. Female sockeye had a significantly higher [Hb] than males, which was the result of an increased number of erythrocytes (i.e. increased Hct) rather than an increase in the density of haemoglobin within each erythrocyte (i.e. MCHC was constant across sexes). This sex-related dichotomy is the opposite of previous observations in mature fish and other vertebrates, where males typically have higher [Hb] (Pickering 1986; Pan and Habicht 1991). The reason for the sex difference in [Hb] in the present study is unclear. However, one possibility is that female sockeye compensate for the smaller rM_V through enhanced erythropoiesis and blood oxygen-carrying capacity. This would function to provide a high supply of oxygen to the developing eggs within the ovaries, perhaps at lower systemic pressures than in males. Rather than re-entering the circulating blood volume, erythrocytes may be trapped within the redundant ovarian sacs, as the eggs are progressively released into the peritoneal cavity, which would explain the lower levels of [Hb] in females that have a large girth in relation to their length (Fig. 2). Support for this stems from observations of blood clots in the body cavity and redundant ovaries of various fish species following spawning (Horvath 1986; Lefter et al. 2008).

In any event, these data do not support the proposition that all aspects of the circulatory oxygen transport cascade in male sockeye salmon are enhanced in similarity to rM_V. This is further emphasised by the finding of a similar range in $\dot{M}O_2$ between sexes (Fig. 3B). The results of this study clearly show a complete overlap of $\dot{M}O_2$ values between males and females when the fish were held in large tanks that allowed the fish to regulate their own level of swimming activity, and thus we reject the hypothesis that male sockeye possess an inherently higher routine level of aerobic metabolism than females. We suggest that the higher total oxygen consumption of male fish during the 13-h respirometry measurement is a reflection of slightly higher levels of activity in males during the spawning period. Quantification of activity levels of male and female fish on the spawning ground, using accelerometers for example (Wilson et al. 2006), would provide useful data in this regard.

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