Sex-specific Differences in Cardiac Control and Haematology of Sockeye Salmon

(Oncorhynchus nerka) Approaching Their Spawning Grounds.

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Running head: Cardiovascular sex-differences in sockeye salmon

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ABSTRACT

Some male salmonids (e.g. rainbow trout) display profound cardiovascular adjustments during sexual maturation, including cardiac growth and hypertension, and tachycardia has been observed in free-ranging male salmonids near their spawning grounds. Here, we investigated cardiac control, dorsal aortic blood pressure, cardiac morphometrics and haematological variables in wild, sexually maturing sockeye salmon (Oncorhynchus nerka) with a particular aim to decipher any sex-specific differences. Routine heart rate ($f_H$) was significantly higher in females (52 vs. 43 beats min$^{-1}$), which was due to significantly lower cholinergic tone (28 vs. 46%) because there were no differences in adrenergic tone or intrinsic heart rate between sexes. No differences in blood pressure were observed despite males possessing an 11% greater relative ventricular mass. Concomitant with higher routine heart rates, female sockeye had significantly higher levels of cortisol, testosterone and $17\beta$-estradiol, while 11-ketotestosterone was higher in males. There were no differences in haematocrit or haemoglobin concentration between the sexes. The findings of this study highlight the importance of considering sex as a variable in research fields such as conservation biology, and when modeling the consequences of local and global climate change. Indeed, this study helps to provide a mechanistic basis for the significantly higher rates of female mortality observed in previous studies of wild-caught sockeye salmon.
INTRODUCTION

Cardiovascular oxygen transport capacity is considered to be one of the main determinants of environmental tolerance in fishes (45). Therefore, a thorough understanding of the function and control of the heart in fishes may benefit a wide range of researchers, including comparative physiologists, conservation biologists, and theoretical biologists modeling the consequences of local and global climate change. While it is evident that cardiac function, and thus resilience to environmental perturbations, can differ considerably among species and even between strains within a species (e.g. 30), few studies have examined for intraspecific and sex-related differences (9, 34).

The heart in most teleost fishes receives a dual input from the autonomic nervous system via excitatory adrenergic fibers, and inhibitory cholinergic fibers in the vagus nerve (43, 49). The adrenergic nerves release the catecholamines adrenaline and/or noradrenaline that bind to $\beta$-adrenoceptors, while the cholinergic nerves release acetylcholine that binds to muscarinic receptors. An additional adrenergic control is provided by catecholamines released into the blood stream from chromaffin tissue (27, 43). The autonomic nervous system responds faster than the humoral system and provides beat-to-beat regulation of heart rate ($f_{\text{H}}$), as well as rapid changes such as vagally mediated bradycardia that follows stimulation of oxygen- (35, 46, 47) and baro-receptors (26, 48). In addition, the humoral and neural systems are likely tonically active and provide the cholinergic and adrenergic tonus that sets the routine $f_{\text{H}}$.

The relative importance of the cholinergic and the adrenergic systems in setting routine $f_{\text{H}}$ can be determined pharmacologically by sequential injections of cholinergic
(i.e. atropine) and adrenergic (e.g. propranolol) blockers, and the subsequent change in $f_{ih}$ is used to determine routine cholinergic and adrenergic tones (see 2). The heart rate after blockade of both control systems is often called the intrinsic heart rate and reflects the pacemaker rate in the absence of any cholinergic or adrenergic input (27). This approach has been used on a wide variety of teleost species, revealing a large variability among species for the relative cholinergic and adrenergic tone (for references see 5). However, the functional significance of the species variability in the relative cholinergic and adrenergic tone remains uncertain. For example, unusually high cholinergic tones ($\geq 100\%$) have been reported for as ecologically and phylogenetically distinct species as skipjack tuna (*Katsuwonus pelamis*, 38), goldfish (*Carassius auratus*, 10) and two Antarctic species (*Pagothenia borchgrevinki* and *Trematomus bernachii*, 6).

The present study aimed to quantify any differences in cardiac autonomic control and hematology between wild, sexually maturing male and female sockeye salmon (*Oncorhynchus nerka*). There were several reasons for selecting a salmonid species as the model for this between-sex comparison. Firstly, some salmonid species undergo phenomenal morphological and physiological alterations immediately prior to spawning, which are clearly illustrated in sexually mature male sockeye salmon that develop a kype (extended jaw) and a large dorsal hump to deter rival males and impress prospective female mates. There are concomitant sex-specific haematological changes in salmonids, including elevated levels of thyroid hormone, gonadal steroid hormone, plasma insulin and cortisol (37, 40, 52, 54), which may be associated with differences in cardiovascular control between sexes. Indeed, the androgen 11-ketotestosterone has been linked with cardiac growth in rainbow trout (*Oncorhynchus mykiss*), such that relative ventricular
mass (RVM) may be up to threefold higher in sexually mature males than females (13, 14, 32, 33). The increase in RVM of male fish has been linked with factors such as hypervolemia, hypertension, increased cardiac afterload and increased oxygen transport requirements (14, 16, 33). Furthermore, cortisol has been reported to have a sex-dependent positive inotropic effect on cardiac tissue of rainbow trout (25), and there is evidence that this glucocorticoid is differentially expressed in mature male and female sockeye salmon approaching their spawning grounds (21). Finally, there appear to be sex-specific differences in \( f_H \) of mature free-swimming salmonids near their spawning grounds, where males tend to maintain higher rates for greater proportions of time (3, 16, 41).

Thus, this study was undertaken to quantify any differences in cardiac control and haematology between sexually maturing male and female sockeye salmon. In particular, we sought to investigate if the previously observed differences in \( f_H \) in male and female salmon are associated with (or possibly explained by) differences in intrinsic heart rate and/or cardiac sympathovagal tone. Furthermore, we measured RVM and dorsal aortic blood pressure to determine whether cardiac hypertrophy is associated with hypertension in sockeye salmon, as has been reported for rainbow trout (14). Blood sampling allowed us to quantify sex-specific differences in haematology and hormone titers, and investigate possible interactions with cardiovascular control.
MATERIALS AND METHODS

Animals
Between September 15 and 18, 2008, sexually maturing sockeye salmon (*Oncorhynchus nerka*) were intercepted by beach seine in the Harrison River, British Columbia, Canada during their river ascent to their nearby spawning grounds. A sample of tissue (~0.1 g) from the adipose fin was taken for subsequent DNA stock (=population) analysis (7, 8), and each fish was equipped with identifying tags (Peterson discs; Floy Tag, www.floytag.com) inserted into the dorsal tissue, or Passive Integrated Transponder (PIT) tags inserted into the ventral tissue posterior to the pelvic fins. The fish were then transported to Chehalis River Hatchery (~10 min drive), where they were held in a large outdoor holding channel (length 50 m, width 5 m, water depth 0.6 m) supplied by through-flowing freshwater. The water temperature varied between 11.0°C and 13.2°C at the time of the experiments, and maximally by 0.4°C during a single experiment. The fish were not fed, as they had ceased feeding naturally before entering the river system, and they were allowed a minimum of 5 d following capture before being used in any experiments. Protocols were approved by the Animal Care Committee of the University of British Columbia in accordance with the Canadian Council on Animal Care.

Instrumentation
Individual fish were netted from the holding channel and anaesthetized in sodium bicarbonate-buffered (NaHCO₃; 200 mg l⁻¹) water containing tricaine methanesulfonate (MS-222; 100 mg l⁻¹; Sigma, St Louis, MO, USA). First, a caudal blood sample (~2-3 ml) was taken using a vacutainer from the lightly anaesthetised fish for subsequent analysis.
(termed ‘acute sample’). The fish was then weighed and placed on wet foam on a surgery
table where the gills were continuously irrigated with cooled (~8°C), aerated freshwater
containing NaHCO₃-buffered MS-222 (150 mg l⁻¹ and 75 mg l⁻¹, respectively). The dorsal
aorta was cannulated with PE-50 tubing using the method described by Soivio et al. (51).
The catheter enabled subsequent recordings of dorsal aortic mean blood pressure (P_{da}),
systolic pressure (P_{da syst}), diastolic pressure (P_{da dia}), pulse pressure (P_{da puls}) and heart rate
(f_{H}), and sampling of arterial blood from unanaesthetized fish. The catheter was secured
with one 4-0 silk suture in the mouth and one 2-0 silk suture to the back of the fish. The
entire procedure typically took about 10 min. Following instrumentation, the fish was
transferred to one of several opaque experimental holding tubes (diameter 20-23 cm) that
were submersed in an enclosed section of the holding channel. The holding tubes were
sealed at both ends with plastic mesh and they had a slit at the top to externalize the
catheter. The fish was placed in the tube with the head facing into the mild current, which
ensured a continuous supply of oxygen rich water to the fish, but without the need for
active swimming to maintain position. A post-instrumentation recovery period of
approximately 24 h was allowed before experimentation commenced.

Experimental protocol

An arterial blood sample (0.4 ml) was first withdrawn into a heparinised syringe from the
quiescent fish to determine routine haematological status (termed ‘chronic sample’).
Cardiac adrenergic and cholinergic tones were thereafter determined using the protocol of
Altimiras et al. (2). Briefly, routine blood pressure and f_{H} were recorded for 20-60 min.
Atropine sulphate [1.2 mg kg body mass (M_{b})⁻¹; Sigma, St Louis, MO, USA] was then
injected through the catheter to block muscarinic receptors, and cardiovascular variables were allowed to stabilize before a recording was made after ~30 min. Finally, propranolol (3 mg kg $M_b^{-1}$; Sigma, St Louis, MO, USA) was injected to block β-adrenergic receptors, and cardiovascular variables were allowed to stabilize for at least 40 min before a final recording was made.

After the experiments, the fish was killed by a sharp blow to the head. The following morphometrics were taken from the dead fish. Fork length (tip of snout to fork) and post-orbital fork length (POFL, posterior edge of eye to fork to account for differences in morphology between males and females). The gonads and spleen were removed and weighed, and the heart was excised, emptied of blood, blotted dry and weighed. The bulbus and atrium were thereafter trimmed away from the ventricle, which was again blotted and weighed and then placed in 70% ethanol for subsequent separation and quantification of the two (compact and spongy) ventricular myocardial layers (for details see 29).

Data acquisition and blood analyses

The dorsal aortic catheter was connected to a pressure transducer (model DPT-6100, pvb Medizintechnik, Kirchseeon, Germany) that was calibrated against a static water column. The signal from the pressure transducer was amplified using a four-channel amplifier (Somedic AB, Hörby, Sweden). The blood pressure signal was sampled at 40 Hz using a PowerLab unit (ADInstruments Pty Ltd, Castle Hill, Australia) connected to a laptop computer running LabChart Pro software (v6.0; ADInstruments Pty Ltd, Castle Hill, Australia).
Haematocrit (Hct) was determined in duplicate using micro-haematocrit capillary tubes spun at 10,000 x g for 4 min. Haemoglobin concentration ([Hb]) was determined with a handheld haemoglobin analyser (Hemocue 201⁺; Ängelholm, Sweden) calibrated for fish blood according to Clark et al. (15). The mean cell haemoglobin concentration (MCHC) was calculated as [Hb]/(Hct/100). Remaining blood was spun and the plasma was collected and first stored in liquid nitrogen before being placed at -80°C for subsequent analyses.

Plasma cortisol, testosterone and 17β-estradiol were assayed in duplicate after appropriate dilution with a commercially available ELISA kit (enzyme-linked immunosorbent assay, Neogen Co. Lexington, KY). Plasma testosterone and 17β-estradiol were first ether extracted as outlined in Neogen ELISA kit instructions. 11-ketotestosterone was determined in duplicate using a commercially available EIA kit (enzyme immunoassay, Cayman Chemical, Ann Arbor, MI). Plasma measurements were also made of lactate, glucose (YSI 2300 stat plus analyser), chloride (Haake Buchler digital chloridometer), sodium and potassium (Cole-Parmer, model 410 single channel flame photometer). Sex hormones were determined only in the acute blood samples.

Calculations and statistical analyses
Dorsal aortic mean (P_{da}), systolic (P_{da syst}), diastolic (P_{da dia}) and pulse (P_{da pulse}) pressures were calculated using the blood pressure module in the LabChart Pro software. Heart rate (f_H) was obtained from the pulsatile blood pressure trace. Heart beat intervals [interval=(60/f_H)], rather than instantaneous heart rates, were used to calculate cholinergic and adrenergic tones as suggested by Altimiras et al. (2). Thus, relative cholinergic tone
was calculated as \((\text{Interval}_{\text{cont}} - \text{Interval}_{\text{atr}})/\text{Interval}_{\text{intr}}\) and relative adrenergic tone was calculated as \((\text{Interval}_{\text{intr}} - \text{Interval}_{\text{atr}})/\text{Interval}_{\text{intr}}\); where \(\text{Interval}_{\text{cont}}\) is the heart beat interval in untreated animals, \(\text{Interval}_{\text{atr}}\) is the heart beat interval after atropine treatment and \(\text{Interval}_{\text{intr}}\) is the heart beat interval after additional propranolol treatment. Fish typically remained calm during the majority of the experiments, and so it was possible to visually exclude active periods from the analysis so that only data from quiescent fish were used. Mean values for males and females were compared statistically using unpaired t-tests or a non-parametric Mann-Whitney test when the requirement for normal distribution could not be fulfilled after transformation of data. Wilcoxon signed-ranks tests and repeated measures ANOVA were used where paired comparisons were appropriate, such as comparisons between acute and chronic blood samples. Statistical significance was considered at \(p<0.05\).

RESULTS

**DNA stock analysis, mass and length measurements**

The DNA stock analysis revealed that the fish used in this study were a mixture of Weaver Creek sockeye (5 males, 8 females) and Harrison sockeye (8 males, 6 females). Based on historic spawning dates, it is known that Weaver Creek sockeye are 2-4 weeks more reproductively advanced than Harrison sockeye during the time of year at which these experiments were conducted (D.A. Patterson, Canadian Department of Fisheries and Oceans, personal communication). Statistical analyses (t-tests) revealed differences between stocks (within a sex) for only a few variables, and so data are presented for each stock where these differences existed (indicated in text), but otherwise pooled.
There were no significant differences in body mass or length between males and females (Table 1). The RVM in males was 11% greater than in females, but the relative proportion of compact myocardium did not differ between the sexes. The ovaries in female sockeye constituted 12.9±0.8% of their total body mass, while the testes in males were significantly smaller and only constituted 3.5±0.3% of body mass. When the RVM was expressed as a percentage of body mass minus gonad mass (i.e. somatic mass) there was no difference in relative ventricular mass between the sexes (see sRVM in Table 1).

No significant correlations were found between RVM and RGM within either sex. Male sockeye had a greater relative spleen mass than females. Within each sex, Harrison sockeye had significantly greater relative spleen mass than Weaver sockeye (Table 1).

Heart rate, cardiac sympathovagal tone and blood pressures
Heart rate variables and sympathovagal tones are summarized in Fig. 1. Routine $f_H$ in male sockeye salmon was 43.2±1.4 beats min$^{-1}$ and this was significantly lower than the 52.4±3.8 beats min$^{-1}$ recorded in females. Atropine treatment increased $f_H$ significantly to 68.6±2.1 beats min$^{-1}$ in males and 69.4±1.9 beats min$^{-1}$ in females, while propranolol significantly decreased $f_H$ to 52.2±1.2 beats min$^{-1}$ in males and 49.5±2.1 beats min$^{-1}$ in females. Thus, the sex-specific difference in routine $f_H$ was due to a significantly higher cholinergic tone in males (46.2±4.9% vs. 27.8±6.1%), while the adrenergic tone did not differ between the sexes (23.6±1.4% in males vs. 25.4±1.6% in females). There was no significant difference in $f_{H\text{ intr}}$ between sexes (Fig. 1).

Correlation analysis suggested that routine $f_H$ in females was primarily determined by differences in cholinergic tone because there was a strong linear relationship between
heart beat interval and cholinergic tone (Fig. 2), but not adrenergic tone (Fig. 3). In males, however, the situation was different and routine \( f_{\text{HR}} \) appeared to be determined by a combination of differences in adrenergic and cholinergic tones, as the activity of both control systems was significantly correlated with routine heart beat interval (Figs. 2 and 3).

There were no sex-specific differences for any of the dorsal aortic blood pressure variables (Table 2). Furthermore, the antagonists that were used to determine cholinergic and adrenergic tones had no significant effect on \( \text{P}_{\text{da}} \) (data not shown), demonstrating that the recorded changes in \( f_{\text{HR}} \) were not chronotropic baroreflex responses due to changes in arterial blood pressure \((48)\). Within each sex, there were no significant correlations between RVM and any of the measured cardiovascular variables \((r^2<0.28, p>0.1)\). There were no stock-specific differences in any of the abovementioned variables.

**Blood variables**

Plasma hormones and other blood variables are presented in Table 3. The most striking differences at the time of acute caudal sampling were that females had approximately 14-fold higher \( \beta \)-estradiol concentration, 3-fold higher testosterone concentration, and double the concentration of cortisol compared with males, while 11-ketotestosterone concentration was approximately 8-fold higher in males. However, there were stock-specific differences in these blood variables. Within males and females, testosterone was significantly higher in Weaver fish than in Harrison fish, which corresponds with the advanced state of sexual maturation in Weaver fish. Similarly, Weaver males had significantly higher 11-ketotestosterone than Harrison males (details
given in Table 3). Linear regression analyses (combined sexes and stocks) revealed
significant positive correlations between cortisol from the chronic blood sample and each
of $f_{ti} (r^2=0.30, p=0.028)$ and $P_{da} (r^2=0.29, p=0.032)$.

There were no significant differences in Hct, [Hb] or MCHC between the sexes or
stocks within any of the sampling periods (i.e. acute or chronic). However, the acute
blood sampling likely provided an estimate of maximum Hct and [Hb] because these
variables were respectively 43-64% and 12-18% higher during the acute sampling
compared with the chronic blood samples that were collected from the dorsal aortic
catheter 24 h after instrumentation (Table 3). The fact that Hct was elevated more than
[Hb] in the acute blood sampling was reflected in a significantly lower MCHC for both
sexes in the acute blood samples compared with the chronic samples, suggesting that part
of the increase in Hct during the acute blood sampling was due to erythrocyte swelling.

During the acute sampling, females had a significantly higher glucose
concentration than males, which was solely the result of high levels in Harrison rather
than Weaver females (Table 3). However, there was no difference in glucose between the
sexes or stocks in the chronic sampling (i.e. 24 h after instrumentation). While there were
no sex-specific differences in lactate at any sampling period, the lactate level within
females was significantly lower at the chronic sampling compared with the acute
sampling. Chloride and sodium were significantly lower in males at the chronic sampling
compared with the acute sampling, whereas potassium was significantly elevated at the
chronic sampling in both sexes. The only significant difference in ion levels between
the sexes was a significantly higher sodium concentration in females at the time of
chronic sampling.
DISCUSSION

Sex-differences in heart rate and autonomic control

Sex is an often neglected variable when analyzing cardiovascular data from non-mammalian vertebrates (9, 34). To the best of our knowledge, this is the first study to investigate sex-specific differences in autonomic nervous control of cardiac function in any species of fish. While the intrinsic heart rate was similar for both sexes, routine $f_H$ was 21% higher in female sockeye (52 beats min$^{-1}$) compared with males (43 beats min$^{-1}$). When the data are pooled for both sexes, the mean $f_H$ was 48 beats min$^{-1}$ at ~12°C, which is lower than previous reports for sockeye at similar temperatures and under similar conditions (53), and thus suggests that the fish used in the present study were likely calm and resting at the time of the measurements. Furthermore, the male and female sockeye used in the present study were clearly approaching an advanced state of sexual maturation. This was emphasized by the early signs of secondary sex characteristics in males of both stocks (e.g. red coloration, a kype and dorsal hump), and the fact that the relative gonadal mass of ~13% in females in this study (combined stocks) was within the range (12-18%) previously reported for fully mature Weaver sockeye on their spawning ground in the month of October (16).

Previous studies, using various heart rate telemetry and logging devices in free-swimming fish, have reported that sexually mature male salmonids spend a greater proportion of time at high heart rates (3, 16, 41). However, the opposite was true for the cannulated and confined fish investigated in the present study. The significantly lower routine $f_H$ in male sockeye was due to a significantly higher cholinergic tone (Fig. 1). This finding of a higher vagal tone in males is consistent with studies on rats (12) and
humans (1, 19), although female rats may also have a higher adrenergic tone and intrinsic heart rate (12), which was clearly not the case in the salmon studied here. Regardless of the proximate causes and the functional significance of the higher routine $f_{H}$ in females, this study suggests that the higher $f_{H}$ previously recorded in free-swimming male salmonids is not due to physiological differences, but more likely due to a more active behaviour in males on the spawning ground as previously suggested (3, 16, 41).

We do not know if routine $f_{H}$ and cardiac control differs between the sexes prior to maturation, or if cardiac autonomic control is modulated by such factors as endocrine signals during sexual maturation, as has been demonstrated to occur in mammals during certain periods including the estrous cycle (22). Pharmacological experiments on chronically hormone-treated fish (e.g. 23, 55) could be instrumental in resolving these possibilities.

Functional significance of cardiac morphology in sexually maturing sockeye

Sexually mature male rainbow trout undergo ventricular enlargement during sexual maturation (13, 14, 32, 33), which is associated with an increased maximum stroke volume and cardiac output, and a heightened cardiac pressure-generating ability (32, 33). The difference in RVM between male and female sockeye (~11%) in the present study was relatively small compared with reports of up to threefold larger ventricles in sexually mature male rainbow trout relative to mature females and immature males (14, 32). In fact, the RVM of 0.13% for mature male sockeye salmon reported here is about 25% lower than reports for mature male rainbow trout (20, 32). For this reason, it is perhaps not surprising that the mean dorsal aortic blood pressure for sockeye salmon in the
The present study did not differ between sexes (4.9-5.0 kPa). There are few reports on blood pressures in wild sexually mature Pacific salmonids, but the values reported here for sockeye are comparable to previous studies on sexually mature male Chinook salmon, *Oncorhynchus tshawytscha* (17).

Furthermore, RVM positively correlates with the proportion of compact myocardium in rainbow trout (13), but this relationship did not exist for sockeye in the present study. When the RVM of sockeye was expressed as a percentage of body mass minus gonad mass (i.e. sRVM, Table 1) there was no difference between the sexes, suggesting that the slightly larger RVM in males was an effect of their smaller gonads (16). Nevertheless, no study has measured gonadal blood flow or oxygen consumption in any salmonid species. As such, the gonads must be considered as a metabolically active aerobic tissue and therefore we cannot justify the removal of gonad mass in calculations of RVM. Within each sex, no significant correlations were found between any of the measured cardiovascular variables and RVM or RGM, or between RVM and RGM themselves. These findings contrast with the significant correlations found in sexually mature rainbow trout (14, 32).

While cardiac hypertrophy in males has also been demonstrated during sexual maturation in other fish species (4, 42), this study on sockeye suggests that the profound cardiac hypertrophy observed in male rainbow trout during sexual maturation is not a general trait among all salmonid species. It is possible that differences in life history between sockeye and rainbow trout may explain these discrepancies. Perhaps, potential differences in cardiac morphology due to sex-specific differences in behaviour in sockeye are overridden by the need for an optimized oxygen convection system in both sexes due
to the unusually long and arduous migration of this species. Nonetheless, more work is clearly needed to resolve the interplay between sexual maturation, migration difficulty and cardiac remodeling in fish.

Haematology and hormones

The most outstanding sex differences in the variables measured in the blood were the higher levels of testosterone, 17β-estradiol and cortisol in female fish, and the higher level of 11-ketotestosterone in male fish (Table 3). Testosterone is highly correlated with aggressiveness and restlessness across taxa (24), and so the higher levels in female fish may have played a role in initiating higher heart rates (Fig. 1). Testosterone and 17β-estradiol have been reported to reach peak levels around 1-2 weeks prior to spawning in rainbow trout (44), and so it is perhaps not surprising that sex hormone titers were relatively low in the sockeye used in the present study, which were about 3-6 weeks from spawning (cf. 36, 44). Indeed, the low level of 17β-estradiol in females in comparison with previous studies is likely to be a reflection of low aromatase activity and thus incomplete maturation (31, 36, 44).

There was a notable elevation in cortisol of female fish, which failed to subside even 24 hours after handling (Table 3). Although cortisol titers may have been elevated in both sexes due to holding and handling conditions, this finding agrees with previous studies of sockeye that have reported a chronic elevation of cortisol in female fish throughout the entire migration (11, 21, 39, 50). To our knowledge, this is the first study of a Pacific salmonid to confirm that sex-specific differences in cortisol persist in cannulated and quiescent fish (cf. acute caudal sample which requires fish capture and
restraint). High cortisol loads in mature male and female salmonids have been hypothesized to result from the stress associated with upriver migration, but a study on kokanee salmon (land-locked sockeye salmon) refutes this hypothesis (11). Given that kokanee salmon are landlocked as juveniles, upon reaching sexual maturation they face relatively easy migrations to their spawning grounds rather than having to endure seawater to freshwater transition and a lengthy upstream journey. The discovery of elevated cortisol loads in male and female kokanee salmon during the spawning season suggests that this is a consequence of endogenous mechanisms associated with reproductive maturation (e.g. gonadal development) rather than the stress of the migration itself (11, 39). Nevertheless, cortisol has been shown to have a positive inotropic effect on rainbow trout cardiac tissue in vitro (25), and in the present study there was a positive linear correlation between cortisol and each of $f_H$ and $P_{da}$. However, a previous study failed to see any major effect of cortisol on the performance of perfused trout hearts (28).

The finding of similar Hct and [Hb] between the sexes is consistent with a previous study that measured Hct in the same population of sockeye salmon at a similar level of reproductive maturation (21), but is in contrast to measurements made 2-3 weeks later when Weaver sockeye arrived at their spawning ground. Using the same acute sampling method as used in the present study, Clark et al. (16) found significantly higher Hct and [Hb] in female sockeye upon arrival at the Weaver Creek spawning ground. These results suggest that females may undergo increased erythropoiesis in the final 2-3 weeks prior to spawning.
Perspectives and significance

The present study has revealed several differences in cardiovascular status between male and female sockeye salmon. We do not know whether these sex-specific differences are present throughout the lifecycle of sockeye salmon, although we suspect that they become increasingly pronounced as sexual maturation progresses (e.g. the case for [Hb]), and as testosterone and cortisol increase. While knowledge about sex-specific differences in the physiology of fishes is still very limited, this study highlights the importance of considering sex as a variable in research fields such as conservation biology, and when modeling the consequences of local and global climate change. For example, the higher heart rates found here in female fish may indicate a reduction in the available scope in \( f_{\text{RVM}} \), which may translate to a reduced aerobic metabolic scope and a reduced ability to endure particular perturbations (45). Indeed, it has continually been noted with long-term holding experiments of sockeye salmon that females have significantly higher mortality rates (21), which was emphasized in one study where 60% mortality of female fish occurred after 5 days at 13°C, whereas male mortality was only 18% (K.M. Jeffries, unpublished data). In this regard, the somewhat lower RVM, and presumably lower cardiac stroke volume, of female fish may further reduce the available scope in cardiovascular oxygen transport. To confirm these possibilities, studies must simultaneously measure the cardiovascular physiology and aerobic energy expenditure of sockeye salmon under the conditions of interest. The use of biologging and biotelemetry techniques to measure \( f_{\text{RVM}} \) in free-swimming fish while faced with various environmental perturbations is likely to prove a fruitful direction for future research (16, 18).
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FIGURE LEGENDS

Fig. 1 Heart rate ($f_{H}$) and intrinsic heart rate ($f_{H \text{intr}}$) after blockade of muscarinic receptors with atropine (1.2 mg kg $M_b^{-1}$) and β-adrenoceptors with propranolol (3 mg kg $M_b^{-1}$) in male (♂) and female (♀) sockeye salmon (*Oncorhynchus nerka*). Cholinergic and adrenergic tones on routine heart rate (Chol tone and Adr tone, respectively) were calculated according to Altimiras et al. (2). Values are means (+S.E.) and an asterisk indicates significant difference between sexes.

Fig. 2 Heart beat interval as a function of cholinergic tone in sockeye salmon (*Oncorhynchus nerka*). Mean values (±S.E.) for heart beat interval and cholinergic tone in males (♂) and females (♀) are indicated by vertical and horizontal bars. Weaver stock is indicated with white crosses.

Fig. 3 Heart beat interval as a function of adrenergic tone in sockeye salmon (*Oncorhynchus nerka*). Mean values (±S.E.) for heart beat interval and cholinergic tone in males (♂) and females (♀) are indicated by vertical and horizontal bars. Weaver stock is indicated with white crosses. There was no significant relationship in female sockeye.
Table 1. Morphological measurements and weights from male and female sockeye salmon (*Oncorhynchus nerka*).

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mass (g)</th>
<th>Fork (cm)</th>
<th>POFL (cm)</th>
<th>RGM (%)</th>
<th>RSM (%)</th>
<th>RVM (%)</th>
<th>sRVM (%)</th>
<th>Compact (%)</th>
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<td>males</td>
<td>13</td>
<td>3038±154</td>
<td>67±1</td>
<td>59±1</td>
<td>3.5±0.3</td>
<td>0.32±0.04†</td>
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<td>42.1±1.4</td>
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<td>females</td>
<td>14</td>
<td>2979±112</td>
<td>64±1</td>
<td>59±1</td>
<td>12.9±0.8*</td>
<td>0.16±0.02†</td>
<td>0.11±0.00*</td>
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</tbody>
</table>

Values are means ± SEM. The abbreviations are post-orbital fork length (POFL), relative gonad mass (RGM), relative spleen mass (RSM), relative ventricular mass as a percentage of body mass (RVM), relative ventricular mass as a percentage of body mass minus gonad mass (sRVM), and percentage of compact myocardium. Measurements were taken post-mortem. * denotes statistically significant difference (p<0.05) between sexes. † denotes statistically significant difference between male Weaver (0.19±0.02%) and Harrison (0.40±0.03%) stocks of sockeye (p<0.001). † denotes statistically significant difference between female Weaver (0.10±0.01%) and Harrison (0.22±0.02%) stocks of sockeye (p<0.001).
Table 2. Dorsal aortic blood pressure variables in quiescent male and female sockeye salmon (*Oncorhynchus nerka*).

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>$P_{da}$ (kPa)</th>
<th>$P_{da\text{ syst}}$ (kPa)</th>
<th>$P_{da\text{ dia}}$ (kPa)</th>
<th>$P_{da\text{ puls}}$ (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>males</td>
<td>12</td>
<td>4.9±0.1</td>
<td>5.1±0.2</td>
<td>4.5±0.1</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>females</td>
<td>10-11</td>
<td>5.0±0.1</td>
<td>5.2±0.1</td>
<td>4.8±0.1</td>
<td>0.4±0.0</td>
</tr>
</tbody>
</table>

Values are means ± SEM. The variables are dorsal aortic blood pressure ($P_{da}$), dorsal aortic systolic blood pressure ($P_{da\text{ syst}}$), dorsal aortic diastolic blood pressure ($P_{da\text{ dia}}$) and dorsal aortic pulse pressure ($P_{da\text{ puls}}$). See Materials and Methods for a description of the calculation of dorsal aortic pressures. There were no significant differences between the sexes or between Weaver and Harrison stocks.
### Table 3. Haematological variables in male and female sockeye salmon (*Oncorhynchus nerka*).

<table>
<thead>
<tr>
<th></th>
<th>Acute sample</th>
<th></th>
<th>Chronic sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n males</td>
<td>females</td>
<td>n males</td>
</tr>
<tr>
<td>11-Ketotestosterone (ng ml⁻¹)</td>
<td>12-14</td>
<td>43.3±11.1</td>
<td>5.1±0.8*</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Testosterone (ng ml⁻¹)</td>
<td>12-14</td>
<td>11.9±3.0</td>
<td>33.8±9.8*†</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17β-estradiol (ng ml⁻¹)</td>
<td>12-14</td>
<td>0.13±0.01</td>
<td>1.77±0.16*</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cortisol (ng ml⁻¹)</td>
<td>12-14</td>
<td>107.1±20.2</td>
<td>195.0±23.0*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>78.6±21.4</td>
<td>181.9±30.9*</td>
</tr>
<tr>
<td>Glucose (mmol l⁻¹)</td>
<td>12-14</td>
<td>6.9±0.6</td>
<td>9.2±0.9*†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.3±1.8</td>
<td>10.3±1.1</td>
</tr>
<tr>
<td>Lactate (mmol l⁻¹)</td>
<td>12-14</td>
<td>2.6±0.2</td>
<td>3.5±0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.7±0.6</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td>Chloride (mmol l⁻¹)</td>
<td>12-14</td>
<td>126.6±1.9</td>
<td>124.2±1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110.1±5.1</td>
<td>118.2±2.1</td>
</tr>
<tr>
<td>Sodium (mmol l⁻¹)</td>
<td>12-14</td>
<td>151.2±1.6</td>
<td>151.6±2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>128.8±5.0</td>
<td>140.6±2.3*</td>
</tr>
<tr>
<td>Potassium (mmol l⁻¹)</td>
<td>12-14</td>
<td>1.3±0.3</td>
<td>1.6±0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.6±0.7</td>
<td>6.0±0.4*</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>13-14</td>
<td>37.3±1.3</td>
<td>41.4±1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26.0±1.7</td>
<td>25.3±1.4*</td>
</tr>
<tr>
<td>[Hb] (g l⁻¹)</td>
<td>13-14</td>
<td>88.9±4.6</td>
<td>91.5±3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>79.2±5.3</td>
<td>77.7±4.6*</td>
</tr>
<tr>
<td>MCHC (g l⁻¹)</td>
<td>13-14</td>
<td>237.3±6.1</td>
<td>221.9±5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>302.5±15.7</td>
<td>316.3±25.7*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Blood samples were obtained using acute caudal puncture in anaesthetized fish (Acute sample) or from cannulated fish 24 h after surgery (Chronic sample). Abbreviations are haematocrit (Hct), haemoglobin concentration ([Hb]) and mean cell haemoglobin concentration (MCHC). * denotes statistically significant difference (p<0.05) between sexes. † denotes statistically significant difference from the corresponding acute sampling value for a given sex. ‼ denotes statistically significant difference between Weaver and Harrison stocks of male sockeye: 11-Ketotestosterone (Weaver = 75.4±18.2 ng ml⁻¹, Harrison = 20.3±4.0 ng ml⁻¹ (p=0.006)); Testosterone (Weaver = 18.9±5.7 ng ml⁻¹, Harrison = 6.9±1.8 ng ml⁻¹ (p=0.045)); Sodium (Weaver = 146.4±1.2 mmol l⁻¹, Harrison = 154.7±1.7 mmol l⁻¹ (p=0.005)). ‡ denotes statistically significant difference between Weaver and Harrison stocks of female sockeye: Testosterone (Weaver = 65.1±15.0 ng ml⁻¹, Harrison = 6.2±1.9 ng ml⁻¹ (p=0.013)); Glucose (Weaver = 6.4±0.3 mmol l⁻¹, Harrison = 13.2±1.1 mmol l⁻¹ (p=0.003)).
REFERENCES


42. Luk’yanenko VI, and Raspopov VM. Sexual dimorphism and seasonal dynamics of the morphological parameters of the Russian sturgeon in the river period of life. (Summary of a review session of the Central Sturgeon Fishery Institute, Astrakhan 1972.


Fig. 1

- $f_H$ (beats min\(^{-1}\))
- $f_{H_{intr}}$ (beats min\(^{-1}\))
- Chol tone (%)
- Adr tone (%)

* Indicates statistical significance.
Fig. 2

- **Males**
  - Equation: $y = 0.01x + 1.09$
  - $R^2 = 0.59$
  - $p = <0.005$

- **Females**
  - Equation: $y = 0.02x + 0.77$
  - $R^2 = 0.97$
  - $p = <0.0001$
Fig. 3

\[ y = -0.02x + 1.90 \]

\[ R^2 = 0.44 \]

\[ p = <0.02 \]