# Evaluating cardiac oxygen limitation as a mechanism for female-biased mortality in coho salmon (*Oncorhynchus kisutch*)

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## Abstract

Female-biased mortality has been consistently reported in Pacific salmon during their adult upriver migration. We collected coho salmon (*Oncorhynchus kisutch* (Walbaum, 1792)) upon arrival at their spawning grounds to test whether females are more prone to cardiac oxygen limitations following exercise stress. We used a surgical approach to periodically sample arterial and venous blood over 48 h following recovery from a chase protocol to induce maximum metabolic rate. We found no significant differences in arterial or venous partial pressures of $O_2$ between males and females. Female salmon had significantly elevated plasma cortisol levels but there were no effects of sex on either plasma lactate or $K^+$. Our data show that female coho salmon do not suffer oxygen limitations to the spongy myocardium after a single exercise event at moderate temperatures ($14\,^\circ$C)—at least not when arriving to their spawning grounds. This study found no clear support for a cardiac oxygen limitation underlying elevated female mortality in Pacific salmon. Neither, however, does our study design nor specific findings allow us to rule out cardiac limitations in these fish. Future work should address whether potential oxygen limitations to the spongy myocardium at high temperatures or oxygen limitations to the compact myocardium via coronary blood flow contribute to female-biased mortality earlier on the migratory route.

**Key words:** fish, cardiac, oxygen limitation, partial pressure, cortisol, $PO_2$, handling, stress, *Oncorhynchus kisutch*, coho salmon

## Introduction

Female-biased mortality represents a grave conservation concern for many animal systems (Rankin and Kokko 2006). Absolute declines in female abundance directly limit the reproductive potential of entire populations, and subsequent male biases in operational sex ratios can have dire consequences for population health (Watters 2005; Rankin and Kokko 2006; Garner et al. 2010). Over the past couple of decades, work on adult Pacific salmon (*Oncorhynchus* sp.) has consistently demonstrated female-biased mortality during upriver migrations to their spawning grounds, especially in the presence of secondary stressors (see Hinch et al. 2021 for review). Across these studies, female mortality rates were on average more than two-fold higher than those of males, with the highest reported female mortality rates reaching eight-fold greater than those of males (Hinch et al. 2021). Despite the remarkable scale of this female-biased mortality, however, the physiological mechanisms that underlie female vulnerability during this key life stage are not known. However,

mechanistic hypotheses include aerobic/cardiac collapse, energy exhaustion, compromised immunity, differential stress responses, or a combination thereof (Hinch et al. 2021).

Adult Pacific salmon are sexually dimorphic, where males can develop obvious secondary sex characteristics, including a hump and a kype, over the course of their spawning migration (Groot and Margolis 1991). Stark differences in reproductive investment are also apparent. In particular, the relative gonadal mass (gonadal somatic index (GSI) = gonad mass/total body mass) is approximately 11%–20% in reproductive females, as opposed to 1%–4% in males (e.g., Idler and Clemens 1959; Little et al. 2020a). We postulated that the larger gonads in female salmon would be more energetically expensive and have higher aerobic demands (i.e., greater oxygen uptake from the blood) than male gonads. In fish, oxygen is supplied to the heart's avascular spongy myocardium (i.e., the inner part of the ventricle) via residual oxygen in the returning venous blood (Farrell 2002). Female salmon may develop a cardiac oxygen limitation during maximal exercise

if the metabolic demands of the gonads and exercising muscles leave insufficient venous oxygen reserve to supply the spongy myocardium (Eliason et al. 2020). Indeed, female sockeye salmon (*O. nerka* (Walbaum, 1792)) had elevated ventricle lactate relative to males following equivalent capture, handling, and tagging stress (Eliason et al. 2020), suggesting female hearts had an increased reliance on anaerobic energy metabolism. Female coho salmon also had lower activities of cardiac lactate dehydrogenase, especially at high temperatures (Little et al. 2020a). This suggests females may also be less equipped to overcome bouts of cardiac hypoxia than their male counterparts (Little et al. 2020a).

Our aim was to determine whether cardiac oxygen limitations following exercise stress differed in mature coho salmon (*O. kisutch* (Walbaum, 1792)) sampled from their spawning grounds. We used a surgical approach to periodically sample blood from the dorsal aorta and sinus venosus over a period of 48 h after a 3 min exercise treatment at 14 °C, which represents an optimal temperature for swimming in this population (Kraskura et al. 2020). We predicted that females would show greater signs of cardiac collapse than males. Specifically, we hypothesized that females would have lower venous $O_2$ partial pressure ($P_vO_2$), reflecting a reduced ability to oxygenate the spongy myocardium during and following exercise. We also hypothesized prolonged elevations in plasma lactate and plasma $K^+$ in female salmon, reflecting a greater reliance on anaerobic metabolism and greater ion imbalance during exercise. Acidosis and hyperkalemia compound cardiac stress in salmonids (Hanson et al. 2006). Additionally, excessive plasma cortisol can prolong spikes in plasma lactate and glucose (Milligan et al. 2000; Milligan 2003; Eliason et al. 2020), potentially reflecting prolonged recovery from exercise. In salmonids, cortisol can also lead to cardiac pathologies and increases in systemic pressure in ways that may compromise overall cardiac function (Johansen et al. 2017). While we expected these measures would return to baseline levels after 48 h in all fish (Zhang et al. 2018), we predicted that females would require prolonged recovery compared with males.

## Methods

### Animal collection and holding

Adult coho salmon returning from their ~125 km freshwater migration up the Fraser River, and subsequently Chilliwack River, were collected from the Chilliwack River Hatchery (BC, Canada) and transported 24 km in a 1250 L tank (8.2–10.4 °C; >90% air saturation) to the Fisheries and Oceans Canada Cultus Lake Research Laboratory (BC, Canada). Coho salmon are found throughout the North Pacific, ranging from Japan to Russia to California and Alaska (Sandercock 1991). This population was selected for several reasons— it is found roughly in the middle of the North American range, migration distance is intermediate (~125 km) relative to other coho salmon populations in the Fraser River drainage (up to 1100 km), spawning occurs late August to November, the hatchery is located relatively close to the Fisheries and Oceans Canada research laboratory, and the pop-

ulation is healthy and not listed or vulnerable. We selected freshly arrived silver fish that were ~2 kg to standardize maturity level to the best of our ability. Fish were held in flow-through UV-sterilized and sand-filtered freshwater under natural photoperiod in circular 8000 L outdoor holding tanks (9 °C; >90% air saturation; $n \leq 27$ fish per tank, mixed sexes) for at least 36 h before experimentation to ensure recovery from handling stress. Coho salmon were periodically collected over a period of 2 months (October and November 2017) for several concurrent studies (see Kraskura et al. 2020; Little et al. 2020a, 2020b); for this work, 14 males and 13 females were selected for surgery and all experiments occurred within a period of 10 days. Individuals were transferred to treatment tanks (1.8 m diameter; 3–6 fish per tank), and the water temperature was increased at a rate of 2–4 °C h$^{-1}$ until it reached 14 °C. Fish were held at 14 °C for 36–50 h, depending on the time of day that surgeries were performed. All experimental protocols were approved by the Animal Care Committee at the University of British Columbia in accordance with the Canadian Council on Animal Care (protocol # A17-0160).

### Animal surgeries

From the 27 fish selected for surgery, 22 were retained in the full study and analysis (5 were excluded because of blocked cannulae or mortality following surgery). We cannulated both the dorsal aorta (to sample arterial blood shortly after oxygenation at the gills) and the sinus venosus (to sample venous blood re-entering the heart) in 11 males (body mass 1.89 ± 0.16 kg; fork length 55.65 ± 1.58 cm; GSI 5.06% ± 0.30%; means ± SEM) and 11 females (body mass 2.12 ± 0.12 kg; fork length 56.67 ± 0.86 cm; GSI 14.15% ± 1.26%; means ± SEM). Fish were netted from their treatment tanks, anesthetized in buffered tricaine methane sulfonate (0.1 g L$^{-1}$ MS-222 and 0.2 g L$^{-1}$ NaHCO$_3$, Sigma), and transferred to a surgery table where their gills were continuously irrigated with a recirculating chilled, aerated maintenance anesthetic bath (0.08 g L$^{-1}$ MS-222 and 0.16 g L$^{-1}$ NaHCO$_3$). A PE-50 cannula was inserted into the dorsal aorta using a stainless steel trocar (Soivio et al. 1973; Eliason et al. 2013) and exited via the roof of the mouth through a PE-190 sleeve. To cannulate the sinus venosus (Steinhausen et al. 2008; Eliason et al. 2013), a PE-50 cannula was prepared by cutting the tip on a 45° angle and cutting several side holes along the first 2 cm. The ductus of Cuvier was carefully dissected free and lifted with a pair of vascular clamps. A second clamp was placed adjacent to the first and a silk suture was loosely tied below both clamps. The PE-50 cannula was inserted into a small hole made between the clamps and directed into the sinus venosus. The silk suture was closed around a bubble in the cannula and the clamps were removed (Steinhausen et al. 2008; Eliason et al. 2013). Both cannulas were secured along the dorsal line of the fish using 2-0 silk. The paired cannulas were coiled together in a loop and secured near the dorsal fin. The cannulas were filled with heparinized saline solution (150 IU mL$^{-1}$). The fish were then supported by hand against the flow of water in their treatment tank (1.8 m diameter, 0.25 m height) at 14 °C until they

recovered from anesthesia and were able to maintain equilibrium on their own. They were allowed to freely swim throughout the tank overnight.

## Exercise protocol and blood sampling

The following day, individual fish were netted from the recovery tank and introduced to a 14 °C doughnut-shaped "chase tank" (1.8 m diameter, 0.25 m height), where they were subjected to a 3 min chase plus 1 min air exposure (Little et al. 2020b). Importantly, recovery from this chase protocol elicits similarly high levels of oxygen consumption ($MO_2$) for maximum metabolic rate similarly to longer critical swim speed ($U_{crit}$) and maximum swim speed ($U_{max}$) tests (Little et al. 2020b). The fish were immediately transferred to separate opaque experimental holding chambers (PVC tubing; length, 92.2 cm; diameter, 21.59 cm; wire mesh at each end, with a slit in the top to externalize catheters). A blood sample (~0.8 mL) was immediately collected from both the dorsal aorta and sinus venosus (time 0). The fish remained in the holding chambers for a 48 h recovery period and subsequent blood samples were collected at 0.25, 1, 4, 24, and 48 h time points. The actual time that the blood was collected was also recorded (occasionally a cannula was blocked or was slow to collect a sample).

## Blood oxygen measures

For measures of partial pressure of $O_2$ from the arterial ($P_aO_2$) and venous blood ($P_vO_2$), we used a microcell respirometer (MC100; Strathkelvin Instruments, Scotland) fitted with an optical oxygen probe (OXROB10; Pyroscience, Germany) connected to a FireStingO2 optical oxygen meter (Pyroscience, Germany). We circulated chilled water through the glass water jacket to maintain the 70 μL microflow cell at 14 °C for all readings. We injected blood directly through the microflow cell at a constant pressure while recording $PO_2$. The microflow cell was rinsed with deionized water between samples. For measures of oxygen content from the arterial ($C_aO_2$) and venous blood ($C_vO_2$), we used a Tucker chamber (TC500; Strathkelvin Insturments, Scotland; Tucker 1967) according to the manufacturer's instructions. Briefly, we filled the sample chamber with degassed ferricyanide, added 10 μL of blood with a Hamilton syringe, and recorded the subsequent increase in $PO_2$. Throughout the analyses, we circulated water through the glass water jacket to maintain the sample chamber at 37 °C.

## Blood chemistry measures

Blood samples were centrifuged, and plasma was aliquoted to fresh collection tubes, flash frozen in liquid nitrogen, and stored at −80 °C. All plasma variables were measured using the procedures outlined in Farrell et al. (2001). Briefly, $Na^+$ and $K^+$ were measured in duplicate on a BWB XP flame photometer (BWB Technologies, USA). Lactate and glucose were measured with a YSI 2300 STAT PLUS glucose and ʟ-Lactate analyzer. Cortisol measurements were determined via ELISA kits (Neogen, USA), where samples were diluted 501× in extraction buffer and run in duplicate. All plates were read first at 650 nm, and then at 450 nm after 50 μL of 1N HCl solu-

tion was added to each well on a FLUOstar Omega multimode microplate reader (BWB technologies, USA). All calculations were made using the 450 nm wavelength.

## Data presentation and statistical analysis

All data were analyzed using linear mixed effects models (LMMs) to account for repeated sampling over time (JAMOVI v.0.9; GAMLj module; https://www.jamovi.org). We analyzed $P_aO_2$, $P_vO_2$, $C_aO_2$, and $C_vO_2$ in separate models using (i) the respective experimental time bins in which the fish were sampled (i.e., 0, 15, and 60 min) and (ii) the exact sampling times. Sex, time bin (or exact time point), and their interaction term were used as predictor variables and fish ID was used as a random effect. There were no qualitative statistical differences between these models and data have therefore been expressed in experimental time bins for results and graphical representation. For analyses of cortisol, glucose, lactate, $Na^+$, and $K^+$, we used sex, time bin, vessel type (dorsal aorta versus sinus venosus), and the sex × time bin interaction term as fixed effects and fish ID as a random effect. With the exception of plasma $K^+$ levels, there were no significant differences between vessel type—we therefore pooled these data (i.e., used the mean between vessels) for graphical representations. In the case of plasma $K^+$ levels, which were significantly different between vessel types (Table 1), we further stratified the model by vessel type. All metrics were investigated for normality using quantile–quantile plots. Non-normal data were transformed (Box–Cox) prior to statistical analysis. However, measures for plasma lactate violated assumptions of normality regardless of transformation attempts (e.g., Box–Cox, $log_{10}$, SQRT, etc.). All data are displayed with untransformed values. We used a significance threshold of $\alpha = 0.05$ for all statistical tests. All values are presented as mean ± SEM.

# Results

## Blood oxygen measures

Males and females did not significantly differ in $P_vO_2$, $P_aO_2$, $C_aO_2$, or $C_vO_2$ throughout the experiment (Table 1; Figs. 1a–1d). However, $P_vO_2$ and $C_aO_2$ changed over the course of the experiment, where $P_vO_2$ increased at 0.25 h and $C_aO_2$ fell at 1 h (Table 1; Figs. 1b and 1c).

## Blood chemistry measures

Females had significantly elevated mean plasma cortisol relative to males at the 0, 0.25, and 1 h time points (time × sex interaction; Table 1; Fig. 2a). Mean cortisol levels were highest at the 1 h time point in both sexes, where female cortisol peaked at approximately $605.78 \pm 58.18$ ng mL$^{-1}$ and mean male cortisol peaked at approximately $291.775 \pm 29.45$ ng mL$^{-1}$ (SEM). Mean plasma lactate levels did not significantly differ between the sexes but shifted throughout the course of the experiment (Table 1; Fig. 2b). In both sexes, mean lactate levels peaked at 1 h (males: $19.35 \pm 0.98$ mmol L$^{-1}$; females: $16.96 \pm 0.85$ mmol L$^{-1}$) before recovering to resting levels by 24 h (Supplementary File S1). Males had significantly higher

**Table 1.** Transformations and test statistics for the linear mixed effects models (LMMs) used in this study.

| Variable | Model | Transformation | $F$ | Num df | Den df | $p$ |
|---|---|---|---|---|---|---|
| $P_aO_2$ | LMM | None | | | | |
|   Sex | | | 0.43 | 1 | 19.68 | 0.520 |
|   Time | | | 1.19 | 2 | 37.46 | 0.317 |
|   Sex × time | | | 1.10 | 2 | 37.46 | 0.902 |
| $P_vO_2$ | LMM | None | | | | |
|   Sex | | | 0.22 | 1 | 20.02 | 0.642 |
|   Time | | | 11.79 | 2 | 38.58 | **<0.001** |
|   Sex × time | | | 0.63 | 2 | 38.58 | 0.538 |
| $C_aO_2$ | LMM | None | | | | |
|   Sex | | | 0.57 | 1 | 19.12 | 0.461 |
|   Time | | | 7.27 | 2 | 36.00 | **0.002** |
|   Sex × time | | | 1.97 | 2 | 36.00 | 0.154 |
| $C_vO_2$ | LMM | None | | | | |
|   Sex | | | 0.66 | 1 | 20.44 | 0.426 |
|   Time | | | 1.87 | 2 | 36.12 | 0.169 |
|   Sex × time | | | 0.68 | 2 | 36.12 | 0.511 |
| Cortisol | LMM | None | | | | |
|   Sex | | | 19.006 | 1 | 25.258 | **<0.001** |
|   Time | | | 58.534 | 5 | 197.523 | **<0.001** |
|   Vessel | | | 0.077 | 1 | 198.167 | 0.781 |
|   Sex × time | | | 11.106 | 5 | 197.829 | **<0.001** |
| Lactate | LMM | None | | | | |
|   Sex | | | 1.837 | 1 | 26.578 | 0.187 |
|   Time | | | 210.828 | 5 | 197.465 | **<0.001** |
|   Vessel | | | 0.478 | 1 | 197.892 | 0.49 |
|   Sex × time | | | 0.554 | 5 | 197.662 | 0.735 |
| Glucose | LMM | Box–Cox $\lambda = -0.6$ | | | | |
|   Sex | | | 5.416 | 1 | 34.546 | **0.026** |
|   Time | | | 37.579 | 5 | 197.125 | **<0.001** |
|   Vessel | | | 8.34E-04 | 1 | 196.813 | 0.977 |
|   Sex × time | | | 1.197 | 5 | 196.972 | 0.312 |
| $Na^+$ | LMM | Box–Cox $\lambda = 4$ | | | | |
|   Sex | | | 0.013 | 1 | 22.401 | 0.91 |
|   Time | | | 1.581 | 5 | 205 945.1 | 0.162 |
|   Vessel | | | 0.921 | 1 | 150 628.7 | 0.337 |
|   Sex × time | | | 1.595 | 5 | 176 201.7 | 0.157 |
| $K^+$ | LMM | Box–Cox $\lambda = 0.45$ | | | | |
|   Sex | | | 4.359 | 1 | 21.334 | **0.049** |
|   Time | | | 30.627 | 5 | 200.102 | **<0.001** |
|   Vessel | | | 30.51 | 1 | 202.018 | **<0.001** |
|   Sex × time | | | 0.679 | 5 | 201.446 | 0.639 |
| DA $K^+$ | LMM | None | | | | |
|   Sex | | | 0.626 | 1 | 18.996 | 0.439 |
|   Time | | | 27.55 | 5 | 79.079 | **<0.001** |
|   Sex × time | | | 0.361 | 5 | 79.79 | 0.874 |
| SV $K^+$ | LMM | None | | | | |
|   Sex | | | 8.879 | 1 | 108 | **0.004** |
|   Time | | | 13.927 | 5 | 108 | **<0.001** |
|   Sex × time | | | 1.261 | 5 | 108 | 0.286 |

**Note:** Data for lactate did not meet assumptions of normality. DA, dorsal aorta and SV, sinus venosus. Bolded values represent $p < 0.05$.

**Fig. 1.** Oxygen levels in arterial and venous blood following exercise in male (blue) and female (red) coho salmon (*Oncorhynchus kisutch*). Arterial partial pressure of oxygen ($P_aO_2$) (A), venous partial pressure of oxygen ($P_vO_2$) (B), arterial oxygen content ($C_aO_2$) (C), and venous oxygen content ($C_vO_2$) (D) sampled at 0, 0.25, and 1 h following a 3 min exhaustive exercise chase. Error bars represent SEM; $n = 8$–11 per sex per time point; rectangular boxes indicate time as a significant main effect; and $p < 0.05$.
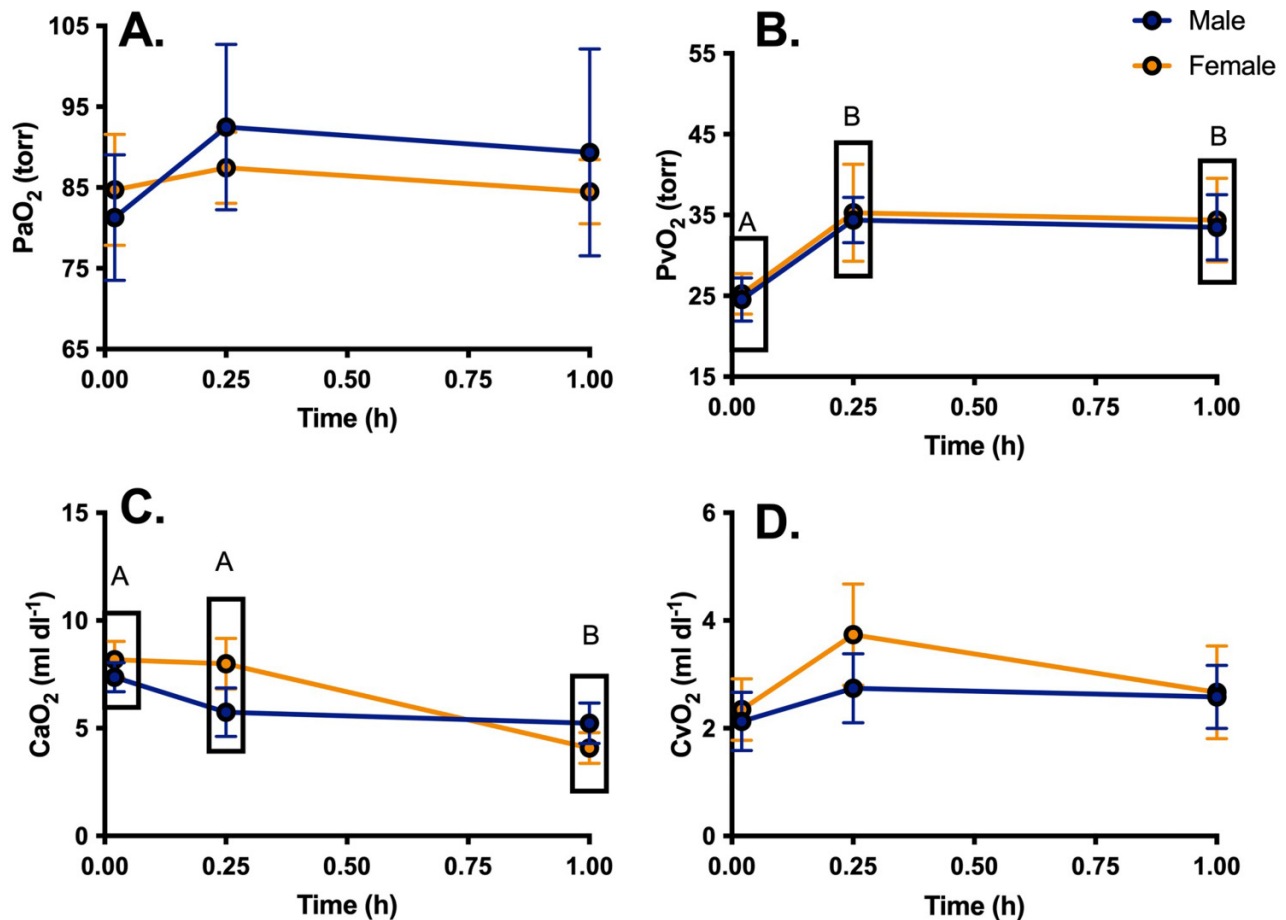


mean plasma glucose than females, which peaked in both sexes at 4 h (Table 1; Fig. 2c; males: $7.14 \pm 0.46$ mmol L$^{-1}$; females: $5.61 \pm 0.42$ mmol L$^{-1}$). Mean plasma Na$^+$ levels did not significantly differ between the sexes and remained stable (134–148 mmol L$^{-1}$) throughout the experiment (Table 1; Fig. 2d). Vessel type (i.e., dorsal aorta versus sinus venosus) had a significant effect on mean plasma K$^+$ levels (Table 1). After stratifying the model by vessel type, we found that mean plasma K$^+$ levels were not significantly different between sexes in the dorsal aorta (Table 1; Fig. 2e), whereas females had significantly lower mean plasma K$^+$ levels relative to males in the sinus venosus (Table 1; Fig. 2f). Mean plasma K$^+$ levels peaked in both vessels (and sexes) at 4 h. Peaks were higher in the dorsal aorta (male: $7.12 \pm 2.67$ mmol L$^{-1}$; female: $7.18 \pm 1.27$ mmol L$^{-1}$) than the sinus venosus (males: $5.42 \pm 1.14$ mmol L$^{-1}$; females: $3.32 \pm 1.10$ mmol L$^{-1}$).

## Discussion

In contrast to our predictions, we found no evidence that cardiac oxygen limitations underlie female-biased mortality following exercise stress in coho salmon. In fish, a car-diac oxygen limitation can develop if the $P_vO_2$ is too low to meet the oxygen demands of the spongy myocardium (Farrell 2002). Here, however, there was no evidence that males and females differed in $PO_2$ or $CO_2$ in either the arterial supply or the venous return (Figs. 1a–1d). Immediately following exercise, mean $P_vO_2$ of the fish used in this study ($24.41 \pm 1.80$ Torr or $3.25 \pm 0.24$ kPa.) and $C_vO_2$ ($2.24 \pm 0.39$ mL dL$^{-1}$) is consistent with those previously measured in fatigued sockeye salmon (e.g., ~20–25 Torr and 1–3 mL dL$^{-1}$, respectively; Steinhausen et al. 2008; Eliason et al. 2013). Notably, these levels did not approach the $P_vO_2$ threshold (~10 Torr) known to induce cardiac collapse in salmonids (see Davie and Farrell 1991; Hanson and Farrell 2007; Clark et al. 2008). Together, these data suggest that despite the much higher gonadal somatic indices of females (GSI 14.15% $\pm$ 1.26%, as opposed to GSI 5.06% $\pm$ 0.30% in males), systemic aerobic demands were similar between both sexes. Indeed, recent work in the same population of coho salmon found no significant sex differences in aerobic scope (Kraskura et al. 2020).

As expected, female salmon demonstrated a more pronounced cortisol response to exercise stress compared with

**Fig. 2.** Blood chemistry measurements following 3 min exhaustive exercise in male (blue) and female (red) coho salmon (*Oncorhynchus kisutch*). Measurements were pooled from samples from the dorsal aorta and sinus venosus of each fish to determine plasma cortisol (A), lactate (B), glucose (C), and Na$^+$ (D). Separate measurements for dorsal aorta and sinus venosus K$^+$ are shown in panels (E) and (F), respectively. Error bars represent SEM; $n$ = 9–11 per sex, per time point; rectangular boxes with letters indicate time as a significant main effect; asterisks represent sex as a significant main effect; and asterisks and letters represent $p < 0.05$.
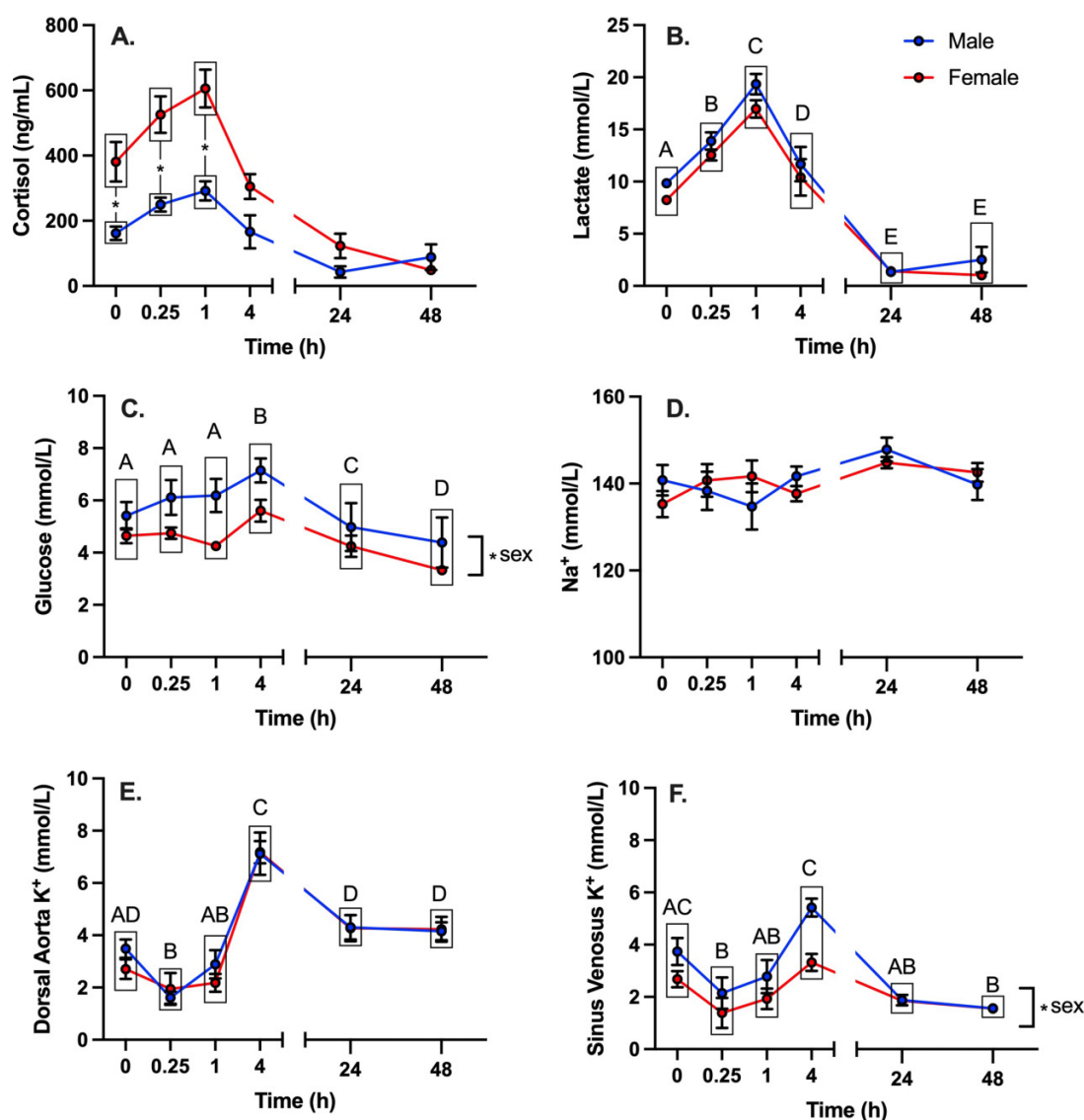


males (Fig. 2*a*). Female plasma cortisol levels remained elevated above males for at least 1 h into recovery, whereas mean cortisol concentrations were more than double that of males at the 0, 0.25, and 1 h time points. Previous studies have found similarly high cortisol levels in migratory kokanee salmon (Carruth et al. 2000), female pink salmon (*O. gorbuscha* (Walkbaum, 1792)) following handling stress (Cook et al. 2006; Donaldson et al. 2014), and sockeye salmon (Cooke et al. 2006). While numerous studies have demonstrated elevated plasma cortisol in female salmonids (e.g., Schmidt and Idler 1962), the mechanisms underlying this sex-specific response are not known, nor are its precise functional consequences. Several studies, however, suggest that chronic elevation of plasma cortisol may also impair upriver migration and reproductive success in Pacific salmonids (e.g., Cooke et al. 2006; McConnachie et al. 2012; Little et al. 2020*a*).

We originally predicted that over the course of migration, the higher cortisol levels in females may promote oxygen limitations in the heart by constraining cardiac output (Johansen et al. 2017). While we found no evidence of oxygen limitations to the spongy myocardium, we also predicted that elevated cortisol would lead to a prolonged recovery duration for plasma lactate in females (Milligan et al. 2000; Milligan 2003; Eliason et al. 2020). However, we found no significant differences in lactate recovery dynamics between the sexes. This result contrasts previous work on sockeye salmon that discovered a prolonged recovery period for plasma lactate in females (Eliason et al. 2020). Plasma lactate in excess of 10–13 mmol L$^{-1}$ induces acidemia, thereby impairing swimming performance (Stevens and Black 1966; Farrell et al. 1998) and cardiac function (Hanson et al. 2006). At their 1 h peaks, mean plasma lactate concentrations

exceeded this threshold in both male and female fish (19.35 and 16.96 mmol L$^{-1}$, respectively), though they recovered to below threshold levels by 4 h. It is, therefore, unlikely that lactate recovery dynamics and any downstream effects on skeletal and cardiac muscle performance explain elevated female mortality in this system.

We also predicted that the higher plasma cortisol levels in females following exercise stress would elevate their blood glucose relative to males. Contrary to our hypothesis, however, plasma glucose levels were elevated in males throughout the course of the experiment. It is difficult to reconcile why higher plasma cortisol levels in females do not translate to higher plasma glucose levels, though it may possibly be related to differences in energy reserves between the sexes. Differential investment in gonads could lead to the exhaustion of energy reserves (Hruska et al. 2010). While increases in plasma cortisol can prime fish for acute stressors by mobilizing glucose stores, these effects vary greatly across teleost (and even salmonid) species (Barton and Iwama 1991; Mommsen et al. 1999; Barton 2000). Further, chronic elevations in plasma cortisol can disrupt intermediary metabolism (Van Der Boon et al. 1991) and lead to cardiac pathologies or increases in systemic pressure that constrain cardiac output (Johansen et al. 2017). In our study, the mean plasma cortisol levels of females returned to levels similar to males within 4 h of exercise, suggesting that baseline levels do not differ between the sexes. An important caveat, however, is that these baseline measurements were derived from experimental tanks built to minimize stress, as opposed to the arguably constant environmental stressors faced during migration.

While there were virtually no differences in plasma K$^+$ concentrations when sampled from the dorsal aorta, males had significantly higher plasma K$^+$ concentrations when sampled from the sinus venosus. Elevated plasma K$^+$ concentrations are typical following strenuous exercise and recovery, where K$^+$ is lost from the working muscles into the blood (Holk and Lykkeboe 1998). K$^+$ concentrations of 5 mmol L$^{-1}$ have been shown to disrupt normal cardiac function in rainbow trout (*O. mykiss* (Walbaum, 1792); Hanson et al. 2006). Mean plasma K$^+$ concentrations peaked at 4 h in males and females. While mean plasma K$^+$ in both sexes exceeded this 5 mmol L$^{-1}$ threshold when blood was sampled from the dorsal aorta (male: 7.12 mmol L$^{-1}$; female: 7.18 mmol L$^{-1}$), only males exceeded this threshold when blood was sampled from the sinus venosus (males: 5.42 mmol L$^{-1}$; females: 3.32 mmol L$^{-1}$). This is particularly surprising because it would suggest potentially reduced cardiac capacity in males. Thus, plasma K$^+$ effects on cardiac performance are unlikely to underlie high rates of female mortality.

It should be noted that several experimental limitations likely contributed to our findings. First, fish used for this study were collected at their spawning ground (i.e., the Chilliwack River Hatchery), as opposed to along their migratory route. This means that our sampling may be devoid of vulnerable females that perished en route to the spawning ground. Another caveat is that we may have missed the time frame when females are most vulnerable—particularly when they are investing heavily in gonad development and egg protein synthesis earlier in development. In addition, we tested

fish at a moderate temperature (14 °C) after a chase protocol, rather than during an aerobic ($U_{crit}$) swim test. A concurrent study discovered that 14 °C was the optimal temperature for swimming in this population (Kraskura et al. 2020). Thus, four caveats are that (*i*) our sampling at the spawning ground may have been biased for the most resilient females (see Raby et al. 2013); (*ii*) our sampling targeted more sexually mature females that may not have been in their most vulnerable state (i.e., during rapid gonadal growth and development); (*iii*) fish were tested in response to a single stressor (exercise), rather than the additional stressors (e.g., high temperature) they may encounter in the wild; and (*iv*) fish were tested during recovery from exercise, whereas acute effects may only be apparent during prolonged swimming. Thus, further work focusing on female Pacific salmon earlier in their migration and with secondary stressors will help resolve mechanisms underlying female-biased mortality.

In summary, we found no evidence that venous oxygen limitations, elevated plasma lactate, or elevated K$^+$ contributes to female-biased mortality in coho salmon in the wild. However, the relatively elevated cortisol levels in females are compelling. Together, these findings suggest that sex differences in other aspects of stress physiology are likely at play (see Little et al. 2020*a*; Hinch et al. 2021). However, our work here cannot totally rule out a cardiac limitation as a causal mechanism of female mortality. Future work should also investigate the possibility of an oxygen limitation to the outer compact myocardium. Coronary blood flow, in particular, supports cardiovascular function in rainbow trout during environmental extremes, such as hypoxia and warming (Morgenroth et al. 2021). Female rainbow trout, however, have reduced scope to enhance coronary blood flow relative to males as temperatures increase (Ekstrom et al.2017). Furthermore, female salmon may have reduced cardiac capacity and cardiac resilience to stressors (e.g., temperature, hyperkalemia) compared with males, particularly given their reduced cardiac size (relative ventricular mass is ~11%–13% smaller in females compared with males; Clark et al. 2009; Sandblom et al. 2009). For example, sexually mature male rainbow trout have larger ventricles and greater maximum cardiac power output compared with females (Franklin and Davie 1992). Plasma-accessible carbonic anhydrase has also been shown to promote cardiac oxygen delivery to support salmonid performance (Alderman et al. 2016; Harter et al. 2019); however, whether distributions and activities differ between the sexes is not known. Future work should also examine some of the other potential hypotheses not directly tested here. Specifically, females have shown evidence of increased susceptibility to disease and energy exhaustion after long and strenuous migrations (Hinch et al. 2021). Another possibility is that females have exhausted their energy reserves (Arndt et al. 1996; Hruska et al. 2010; Little et al. 2020*a*), perhaps because of a greater investment in gonadal development relative to males.

Negative feedback effects may mean that subsequent male biases in operational sex ratios further amplify costs for females (Watters 2005; Rankin and Kokko 2006; Garner et al. 2010), who govern the reproductive potential of the species. Over 60+ year time scale, for instance, several populations where females accounted for 60% or more of overall

spawners have now shifted to predominately males on the spawning ground (Hinch et al. 2021). The populations that show the largest relative declines in females represent those that have also been designated by COSEWIC (2017) as either threatened or endangered, and are now under assessment by the Species at Risk Act (see Hinch et al. 2021). Understanding the physiological basis of this female-biased mortality and the combination(s) of contributing factors thereby represents an urgent conservation goal to manage these environmentally, economically, and culturally important populations.

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## Article information

### History dates

### Copyright

### Data availability

All data are available in the supplementary material.

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