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Research article

Sex-specific differences in swimming, aerobic metabolism and recovery from exercise in adult coho salmon (*Oncorhynchus kisutch*) across ecologically relevant temperatures

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Adult female Pacific salmon can have higher migration mortality rates than males, particularly at warm temperatures. However, the mechanisms underlying this phenomenon remain a mystery. Given the importance of swimming energetics on fitness, we measured critical swim speed, swimming metabolism, cost of transport, aerobic scope (absolute and factorial) and exercise recovery in adult female and male coho salmon (*Oncorhynchus kisutch*) held for 2 days at 3 environmentally relevant temperatures (9°C, 14°C, 18°C) in fresh water. Critical swimming performance (U_{crit}) was equivalent between sexes and maximal at 14°C. Absolute aerobic scope was sex- and temperature-independent, whereas factorial aerobic scope decreased with increasing temperature in both sexes. The full cost of recovery from exhaustive exercise (excess post-exercise oxygen consumption) was higher in males compared to females. Immediately following exhaustive exercise (i.e. 1 h), recovery was impaired at 18°C for both sexes. At an intermediate time scale (i.e. 5 h), recovery in males was compromised at 14°C and 18°C compared to females. Overall, swimming, aerobic metabolism, and recovery energetics do not appear to explain the phenomenon of increased mortality rates in female coho salmon. However, our results suggest that warming temperatures compromise recovery following exhaustive exercise in both male and female salmon, which may delay migration progression and could contribute to *en route* mortality.

Key words: fish, salmon, temperature, thermal stress, metabolisn, swim performance

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Introduction

Sex-specific mortality is a conservation concern for sexually dimorphic species (Fleming and Gross, 1989; Miles *et al.*, 2000; Grüebler *et al.*, 2008; Minke-Martin *et al.*, 2018), including the iconic Pacific salmon. Phenotypic differences between males and females can place one sex at a higher risk of mortality as a result of unequal predation risk (Hassell *et al.*, 2012), immune function (Klein and Flanagan, 2016), stressor tolerance and energy balance (Srean *et al.*, 2017; reviewed by Hanson *et al.*, 2008). Recent studies have shown that female Pacific salmon have higher rates of mortality than males during their once in a lifetime upriver migration to spawn (Hinch et al., 2021, and that this vulnerability is exacerbated by thermal stress (Ytrestøyl *et al.*, 2001; Crossin *et al.*, 2008; Jeffries *et al.*, 2012; Martins *et al.*, 2012; Raby *et al.*, 2016; Keefer *et al.*, 2017; Minke-Martin *et al.*, 2018). This has been reported in both field (e.g. tagging and dam passage studies; Roscoe *et al.*, 2011; Martins *et al.*, 2012; Burnett *et al.*, 2014a; Keefer *et al.*, 2017; Minke-Martin *et al.*, 2018) and laboratory holding studies (Jeffries *et al.*, 2012; Raby *et al.*, 2016). However, it is unclear what the underlying physiological mechanisms driving female-biassed mortality in Pacific salmon are and how they will interact with rising temperatures under climate change.

Pacific salmon's upriver spawning migration is an aerobically challenging feat during which swimming and reproductive maturation occur simultaneously and are possibly causing metabolic trade-offs (Calow, 1985; Eliason and Farrell, 2016; Williams and Brett, 1987). Absolute aerobic scope (AAS), or the difference between the resting metabolic rate (RMR) and maximum metabolic rate (MMR), represents an individual's capacity for any aerobic performance beyond baseline maintenance (e.g. predator avoidance, locomotion, reproduction, etc.; Fry, 1971). As temperatures rise, exponential increases in RMR combined with limitations on MMR begin to constrict AAS, presumably limiting performance (Claireaux and Lagardère, 1999; Eliason and Farrell, 2016; Farrell, 2016; Norin and Clark, 2016). In salmon, reproductive maturation must be considered a routine metabolic cost during migration. Indeed, female ovaries grow to ∼18% of their total body mass, 4–5 times larger than the male testes (Groot and Margolis, 1991). Salmon do not feed during their spawning migration, and yet female salmon use ∼50% of their finite gross somatic energy reserve on reproductive development, whereas males invest closer to 4% (Crossin *et al.*, 2004). Sexual maturation is also known to increase ventricle size in males (Gamperl and Farrell, 2004) and female Pacific salmon generally have smaller hearts than males (e.g. Sandblom *et al.*, 2009; Clark *et al.*, 2011). In pink salmon (*Oncorhynchus gorbuscha*), females displayed lower maximum cardiac output likely because of their smaller heart size, which may have limited their AAS relative to their male counterparts (Clark *et al.*, 2011). These differences suggest that MMR may be constrained by cardiovascular capacity in female salmon (i.e. limiting factor on AAS) at the same time as RMR is higher in females due to elevated reproductive costs (i.e. loading factor on AAS). With temperature amplifying these potential limiting and loading factors, we hypothesized that females would suffer reduced AAS at high temperatures.

An added concern with the elevated cost of female reproductive development during river migration is that all tissues cannot be simultaneously and maximally perfused. This leads to trade-offs between performance measures. Energetically demanding tissues (i.e. muscle, gonads, intestines if feeding) are selectively perfused with oxygenated blood according to their relative needs and the total capacity of the heart (Thorarensen and Farrell, 2006; Altimiras *et al.*, 2008; Ekström *et al.*, 2017). For example, juvenile rainbow trout (*Oncorhynchus mykiss*) and Chinook salmon (*Oncorhynchus tshawytscha*) have lower maximum swimming performance after feeding compared to starved individuals (Alsop and Wood, 1997; Thorarensen and Farrell, 2006). This is because the fish that are actively digesting a meal must perfuse both the intestines and swimming muscles while the starved fish are able to divert a greater proportion of blood flow to swimming muscles (Alsop and Wood, 1997; Thorarensen and Farrell, 2006). For migrating female salmon, which have ceased feeding, selective blood perfusion may result in an energetic trade-off between gonadal development and swimming performance. Prioritizing oxygen delivery to the gonads, including during extreme exercise may explain migration failure in female salmon. Therefore, we hypothesized that female salmon would have reduced swim performance, particularly at high temperatures, compared to males.

To overcome fast-flowing sections of the river or escape predators, salmon must use burst swimming (Black et al., 1966; Geist et al., 2003; Lauritzen et al., 2005), which is supported by anaerobic metabolism (i.e. white muscle fibers). Furthermore, rising temperatures increase the overall reliance on anaerobic metabolism during swimming (Eliason et al., 2013a). For successful migration, salmon must recover from these anaerobic bouts of activity quickly. If females have lower AAS, they may have an increased reliance on less efficient, more costly glycolytic pathways and have impaired recovery performance. In the short term, physiological recovery following exhaustive exercise includes a rapid restoration of cellular oxygen and ATP stores (Milligan and Wood, 1986; Scarabello *et al.*, 1991; Cech *et al.*, 2004; Zhang *et al.*, 2018). This is followed by a long recovery phase (i.e. re-gaining cardiorespiratory, hormone and metabolite balance; Wood, 1991; Scarabello *et al.*, 1992; Wang *et al.*, 1994; Cech *et al.*, 2004; Zhang *et al.*, 2018). Pacific salmon have an extraordinary ability to repeat their swim performance after only 40–45 min of rest and despite the fact that metabolic rate has not fully recovered to routine levels (Farrell *et al.*, 1998, 2003; Jain *et al.*, 1998; Jain and Farrell, 2003; MacNutt *et al.*, 2006; Wagner *et al.*, 2006; Eliason *et al.*, 2013b). However, impaired recovery at high temperatures may mean consecutive swimming challenges are more taxing for female salmon, thereby increasing their physiological burden or delaying migration

compared to males. For instance, female sockeye salmon had elevated plasma lactate, cortisol and potassium levels relative to males after capture and tagging stressor (Eliason et al., 2020; Hinch et al., 2021), suggesting they had impaired recovery. We hypothesized that female salmon would take longer to recover (potentially with disrupted hormone, ion and metabolite levels) and have higher recovery costs after a critical swimming test and chase to exhaustion. If true, this could undermine their ability to tolerate multiple or repeat stressors *en route* to their spawning grounds (Burnett *et al.*, 2014a; Eliason and Farrell, 2016; Fenkes *et al.*, 2016).

The objective of this study was to make a comprehensive assessment of metabolic capacity, locomotor performance and recovery from exercise in coho salmon (*Oncorhynchus kisutch*) to determine whether sex-specific differences in the thermal sensitivity can explain female-biassed mortality in Pacific salmon. Coho salmon are an ecologically and economically important species (Brownscombe *et al.*, 2014) that have higher mortality rates in adult females (Spidle *et al.*, 1998). We measured aerobic scope (absolute and factorial), critical swim performance (U_{crit}) and the metabolic costs and duration of recovery from exhaustive exercise across three ecologically relevant temperatures (typical, 9°C; current maximum, 14°C; and a climate change scenario, 18°C) in fresh water. We predicted that females would be more sensitive to the negative effects of higher temperature than males, but that high temperatures would negatively affect both sexes. Specifically, we hypothesized that females would have (i) lower AAS (higher RMR and lower MMR), (ii) lower swimming performance and higher cost of transport (COT) and (iii) prolonged duration and higher metabolic costs of recovery from exercise relative to males.

Methods

Study animals and temperature treatments

Chilliwack coho salmon are a fall migrating population (spawning period: late August–November) with a ∼125 km freshwater migration up the Fraser River and subsequently Chilliwack River to reach the Chilliwack hatchery or their adjacent spawning grounds in British Columbia, Canada (Fig. 1). Sexually maturing adult coho salmon (females (F), $N = 64$; males (M), $N = 51$) returning from the ocean were dip-netted at the hatchery in October–November 2017 and transported 24 km by truck in a 1250-L tank containing aerated freshwater (dissolved oxygen, DO > 70% air saturation; stocking density, ≤27) to the Fisheries and Oceans Canada Cultus Lake Salmon Research laboratory (Chilliwack, BC, Canada; Fig. 1). Fish were collected across eight trips to the hatchery (between October 3 and November 10). We selected freshly arrived, silver fish to standardize maturity level to the best of our ability. During this time, Fraser River temperatures ranged from 14°C (early October) to 7°C (early November) and Chilliwack River temperatures ranged from 9°C to 11°C (data not shown). On arrival, fish were transferred to circular outdoor holding tanks (∼8000 L) supplied with sand-filtered and UV-sterilized water from Cultus Lake at 9°C. All tanks were equipped with air stones (DO, >87% air saturation) and a water pump to generate a circular current. 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).

Fish were haphazardly assigned to 9°C (typical), 14°C (current maximum) or 18°C ('climate change scenario' for this population and current maximum at other parts of the species range; Welsh *et al.*, 2001; Hayes *et al.*, 2011) treatment temperatures. Individuals were transferred to treatment tanks (diameter: 1.8 m; 3–6 fish per tank), and the water temperature was raised at a rate of 2–4°C h^{-1} from 9°C to the test temperature. Fish were held at their test temperature for 2 days before experiments began. In the wild, Pacific salmon routinely experience rapid changes in water temperature such that a 2-day holding period prior to experimentation is ecologically relevant. All tests were conducted outdoors under natural photoperiod. Each individual was used only once in this study, either for intermittent respirometry or the critical swim test.

Critical swimming test

Critical swim speed (U_{crit}) was assessed using a Brett-type swim tunnel respirometer (diameter, 25.4 cm; volume, 450 L) (Farrell *et al.*, 2003; Lee *et al.*, 2003b) and a ramp-U_{crit} protocol (Jain *et al.*, 1997). Swim performance tests were conducted on 26 males (9°C, $N = 9$; 14°C, $N = 9$; 18°C, $N = 8$) and 34 females (9°C, $N = 12$; 14°C, $N = 12$; 18°C, $N = 10$). Briefly, fish were transferred from their treatment tank to the tunnel with minimal air exposure and handling stress (transfer time, <30 s) and were held for 1 h at ∼0.25 BL s^{-1} water velocity prior to the swim test. During the test, water velocity was increased every 5 min by ∼0.26 BL s^{-1} until 1.5 BL s^{-1} was reached. Thereafter, the water velocity was increased by 0.26 BL s^{-1} every 20 min until the fish were unable to maintain their position in the water column and rested against the back grid for >30 s. On average, the U_{crit} test lasted 80 min. MO_2 was measured at each 20-min interval, typically during the second 10-min period. As soon as fish fatigued, the water flow was lowered to 0.26 BL s^{-1} and the fish's MO_2 was followed over a 1-h recovery period (short-term recovery). Oxygen levels in the tunnel were measured using a fiber optic oxygen sensor (Pyroscience, Germany) placed immediately after the fish swimming area. DO was maintained above 70% air saturation and temperature was kept within ± 1°C of their treatment temperature. Background respiration was measured once per day and determined to be negligible. No fish lost equilibrium during any test. Upon completion of the U_{crit} test and 1 h recovery, fish were removed from the tunnel and anesthetized in 100 mg L^{-1} MS-222 buffered with 200 mg L^{-1} $NaHCO_3^-$ (Sigma Aldrich Co., Oakville,

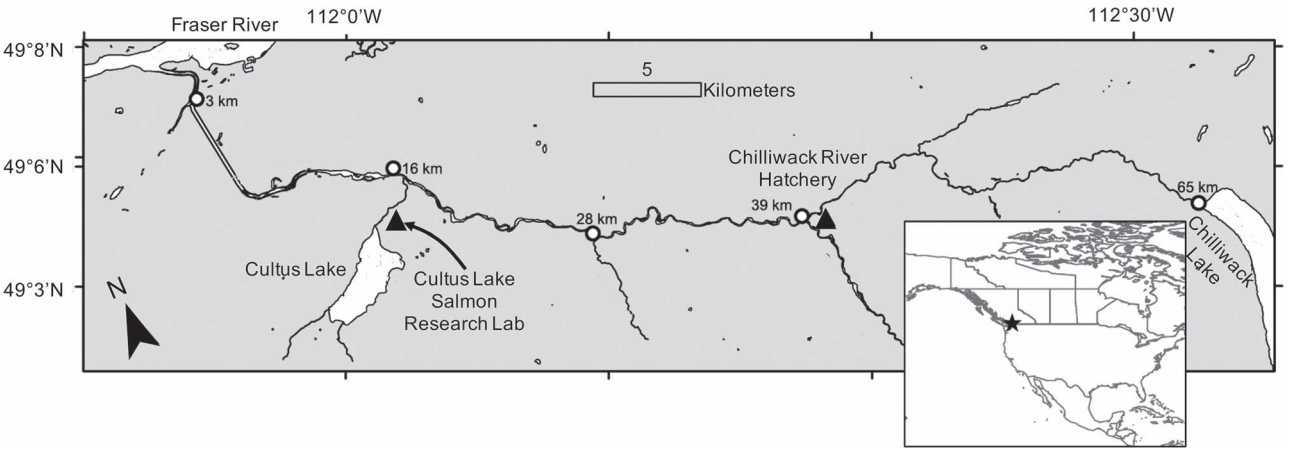

Figure 1: Map of the 2017 study area. Triangles indicate the Cultus Lake Salmon Research Laboratory, where all experiments were conducted, and the Chilliwack River Hatchery, where fish were collected. Map modified from Bass et al., 2019.

Ontario, Canada), then measured and weighed for fork length (FL), depth and maximum circumference.

Intermittent flow respirometry

Eight respirometers (volumes 33.3 L and 57.9 L) constructed with clear 20.3-cm (8″) and 25.4-cm (10″) polyvinyl chloride tubes, respectively, housed individual fish during intermittent flow respirometry. Each had a fixed and a removable end to load fish. Water was continuously recirculated through each respirometer with a water pump (600 L h^{-1} or 1200 L h^{-1} Eheim Universal Aquarium Pump, Eheim, Germany) and a 1200-L h^{-1} pump flushed water from the immersion tank through all chamber after a MO_2 measurement cycle to reoxygenate the water inside the respirometer. Four respirometers were placed in each of two temperature-controlled, flow-through immersion tanks (diameter, 181.6 cm; depth, 41.9 cm). Dissolved oxygen was measured continuously in each respirometer using a robust oxygen probe and Firesting oxygen optical oxygen meter (Pyroscience, Germany).

Respirometry tests were conducted on 25 males (9°C, $N = 8$; 14°C, $N = 9$; 18°C, $N = 8$) and 30 females (9°C, $N = 12$; 14°C, $N = 9$; 18°C, $N = 9$). Fish were introduced to a 'chase tank' (diameter, 1.8 m; 2000 L, filled to ∼660 L), where 4 people manually motivated the fish to burst swim continuously without allowing time for recovery, simulating predator evasion (Donaldson *et al.*, 2010) or 'catch and release' fisheries interactions (Gale *et al.*, 2011) by making quick movements with their hands under the water, often lightly touching the fish's caudal fin. Chases occurred between 11:30 and 13:00 and lasted for 3 min, followed by 1-min air exposure, before fish were placed into a respirometer (Robinson *et al.*, 2013; Little *et al.*, 2020a). Dissolved oxygen recordings were initiated as soon as the respirometer lid was sealed and the chamber was flushed of all air bubbles (within 50–120 s post-chase). Shade cloth was then placed over the holding tanks to minimize potential disturbance. The first 1–4 MO_2 measurements consisted of a 4-min closed DO measurement period followed by a 6-min flush period. As peak MO_2 subsided, the measurement cycle was automated at a 4–5 min measurement period every 15 min for a recovery period of ∼20 h during which fish were maintained within ± 1°C of their test temperature under a natural photoperiod. Background respiration (found to be negligible) was measured daily. Respirometers were disinfected weekly using Virkon, according to the manufacturer's instructions (du Pont de Nemours and Company, Wilmington, DE, USA).

On completion, fish were removed from the respirometer, anesthetized in 100 mg L^{-1} MS-222 buffered with 200 mg L^{-1} $NaHCO_3^-$, weighed and measured for FL, depth and maximum circumference. A blood sample (∼3 ml) was removed from the caudal vasculature using heparinized vacutainers (BD Vacutainer®, BD, Franklin Lake, NJ, USA) and a PIT tag (Passive Integrated Transponder, Biomark Inc., Boise, ID, USA) was implanted in the dorsal musculature for individual identification.

Blood sampling and analysis

Plasma and blood cells (from samples taken 24 h post-chase) were separated by centrifugation at 1200 *g* for 5 min. Haematocrit was determined using a Microhematocrit Centrifuge Reader Card. Plasma was stored at −80°C for metabolite (lactate, glucose), ion (K^+, Na^+, Cl^-) and hormone (testosterone and oestradiol) analyses. Chloride was analyzed using an ELITech Chlorochek Digital Chloridometer (ELITech Group, Puteaux, France). Sodium and potassium concentrations were determined using a BWB XP Five-channel Flame Photometer (BWB Technology, Newberry, England). Lactate and glucose concentrations were determined using a YSI 3200 Stat Plus (Xylem Inc., Rye Brook, NY, USA) and osmolality was determined using an Advanced Instruments 3320 Freezing Point Osmometer (Advanced Instruments, Norwood, MA, USA), as outlined in Farrell *et al.*, 2001. Testosterone and 17β-

oestradiol were extracted using ethyl ether and analyzed following protocols outlined by the Neogen hormone kits (Neogen Life Sciences, Lexington, KY, USA).

End sampling

The fish were not euthanized immediately following these experiments because they were used for subsequent experiments (not included here). Between 0 and 23 days after the conclusion of all experiments, fish were euthanized to confirm sex by cerebral concussion followed by severing the spinal cord. Accordingly, gonad mass at the time of the experiment for individual fish is unknown. Therefore, we calculated gonadal somatic index (GSI) on fish that were dissected within 5 days of experiment tests (F, $N = 32$; M, $N = 31$; respirometry and swim test combined) to get an estimate of approximate mean GSI for these fish.

Data analyses

A reliable MO_2 measurement required a linear decrease in DO with an $R^2 > 0.9$ during the 4–5-min cycle. For intermittent flow respirometry, only individual fish with >75% of the MO_2 measurements (i.e. >60 MO_2 measurements per fish) meeting this quality control were included in the analysis. Standard metabolic rate, or the minimum oxygen uptake rate measured in a post-absorptive, non-reproductively active, at rest individual (Chabot *et al.*, 2016), is typically used as the baseline for calculating AAS (Clark *et al.*, 2013). However, migratory salmon are reproductively active and so we applied the term RMR to the average of the lowest 10% of MO_2 values measured over ~20 h recovery period and used this to calculate AAS (Chabot *et al.*, 2016; Rosewarne *et al.*, 2016). MMR was calculated from the slope during the steepest 60 s from the first measurement period (Norin and Clark, 2016; Little *et al.*, 2020a). However, if the metabolic rate was higher at any point during the recovery phase, the MO_2 value of that entire measurement cycle was used to represent MMR instead (14 of 56 fish). AAS was calculated as the difference between MMR and RMR. Factorial aerobic scope (FAS) was calculated as MMR divided by RMR. The temperature coefficient (Q_{10}) was calculated as $Q_{10} = (R_2/R_1)^{10/(T2-T1)}$ where R_1 and R_2 represent the average metabolic rate measurements of each sex at temperature T1 and T2, respectively.

Critical swimming speed (U_{crit}) was calculated as $U_{crit} = U_F + (t_f/t_i U_i)$ (Brett, 1964) where U_f is speed of the velocity of the last fully completed increment, t_f is the time spent in the speed increment at which the fish reached fatigue, t_i is the time of each completed interval (20 min) and U_i is the velocity increment (~0.26 BL s^{-1}; adjusted for each individual based on their FL). Velocity variables were corrected for solid blocking effects using the equation $U_F = U_T(1 + \varepsilon_s)$ (Bell and Terhune, 1970; Lee *et al.*, 2003b), where U_F is the corrected flow, U_T is the flow in the tunnel without the fish and ε_s is the fractional error due to solid blocking effect specific to each fish obtained from $\varepsilon_s = \tau\lambda(A_o/A_T)^{1.5}$; τ is a swim tunnel specific parameter (0.8 from Lee *et al.*, 2003b, λ is ½ body length divided by body thickness and A_o and A_T are cross-sectional areas of fish and tunnel, respectively. Oxygen uptake rate (MO_2, mgO_2 kg^{-1} min^{-1}) and COT (MO_2 divided by U_F; mgO_2 kg^{-1} m^{-1}) were calculated for each speed increment (using both cm s^{-1} and BL s^{-1}). Only regressions with $R^2 > 0.9$ were included in swimming metabolism and COT analysis. We could not calculate net COT (i.e. $MO_2 - MO_{2rest}$ divided by U_F) because we did not measure resting MO_2 for fish in the swim tunnel experiments.

MMR_{swim} was measured as the steepest decline in dissolved oxygen recorded over any 3-min measurement period within either of the last two U_{crit} test increments (Little *et al.*, 2020a). $MMR_{recovery}$ was calculated from the first 10 min of recovery data (i.e. immediately after the U_{crit} test was completed). To obtain $MMR_{recovery}$ we used the steepest decline in dissolved oxygen over any 2-min period (if $R^2 < 0.9$, timing was increased to 3 min). All files were assessed visually and only regressions with $R^2 > 0.9$ were used for $MMR_{recovery}$ analysis.

We analyzed post-exercise recovery using several approaches. First, we calculated excess post-exercise oxygen consumption (EPOC) after the chase. To do so, we used all MO_2 values recorded during the first hour of the static respirometry experiment and the minimum MO_2 value for each subsequent hour. This allowed us to minimize MO_2 values representing spontaneous activity (Eliason *et al.*, 2007). These MO_2 measurements were smoothed using a cubic smoothing spline function (smooth.spline, R package 'stats') for each fish. The duration of EPOC was the time when the curve intercepted 20% of RMR. Total EPOC was the integrated area of smoothed MO_2 recovery curve capturing all MO_2 values for this period minus the integrated area of RMR.

Second, to more closely analyze the initial phase of recovery (5 hours after chase), we used two measurement metrics: (i) hourly EPOC defined as the total excess oxygen consumed during each hour (i.e. hour 0–1 post-chase, hour 1–2 post-chase, etc.; cut-off at 5 h) and (ii) cumulative hourly EPOC, calculated as the additive amount of excess oxygen consumed at each hour post-chase (i.e. hour 0–1 post-chase, hour 0–2 post-chase, etc.). These intermediate recovery metrics were not calculated post-U_{crit} because we did not have RMR data for fish in the swim tunnel experiments.

Finally, we calculated a novel estimate of short-term recovery from exercise: the time to recover to 50% of MMR, termed $time_{MMR50}$. Pacific salmon are well known to be able to repeat maximum swim performance after only a short recovery duration (e.g. 30–60 min)—well before they have fully recovered back to baseline metabolism (Farrell *et al.*, 1998, 2003; Jain *et al.*, 1998; Jain and Farrell, 2003; MacNutt *et al.*, 2006; Wagner *et al.*, 2006; Eliason *et al.*, 2013b). These studies suggest that salmon recover to 30–70% of MMR and are then able to repeat their swim performance. Therefore, we

selected time to reach 50% of MMR as a threshold to evaluate short-term recovery capacity. Additionally, we calculated the % MMR at time points 10, 20, 30, 40 and 50 min after exercise, both post-chase and post-U_{crit} (the raw value of MO_2 during the first measurement was used as 100% MMR). We included all measured MO_2 values from the first hour post-chase and all MO_2 values from every 5-min interval post-U_{crit} (using ≥3 min regression slope; $R^2 > 0.85$).

Statistical analyses

All data were statistically analyzed using R version 3.5.1 (2018). We used a significance level $\alpha = 0.05$ for all statistical tests. Metrics were investigated for normality using Shapiro–Wilk test and quantile–quantile plots and for heteroscedasticity using Levene's test. $Time_{MMR50}$ (for post-chase only), oestradiol and testosterone data were non-normal and therefore, log10-transformed before statistical analysis. MMR, RMR, AAS, FAS, time to recovery, EPOC, U_{crit} (cm s^{-1}), U_{crit} (BL s^{-1}), MMR_{swim}, $MMR_{recovery}$, $time_{MMR50}$ (post-chase) and blood parameters were analyzed using ANOVAs and Tukey's HSD post hoc tests when appropriate (R package 'emmeans'; Lenth, 2019); residuals of all tests were visually assessed for normality. To directly test our hypothesis, we included the interaction between sex and temperature in all ANOVAs (type III testing for significant interaction). When not significant, the interaction was excluded and main effects, sex and temperature were tested for significance without the interaction (R package 'car', Fox and Weisberg 2019; type II for relationships without interaction term). Body size (kg) was added as a covariate when analysing U_{crit} (cm s^{-1}; absolute swim speed). All individual fish were approximately the same size and body size was not a significant factor influencing any other performances. Data are reported as means and standard error (SEM) unless otherwise specified. All data are displayed and reported with untransformed values.

To investigate the aerobic metabolic costs of swimming, we constructed nonlinear mixed effect models for each sex at each temperature using nlmer function (R package 'lme4'; Bates *et al.*, 2015). In each model, we accounted for non-independence by including individual as a random effect. In other words, this allowed us to include individual-specific performance curves when evaluating one descriptive recovery relationship that was specific to each sex and temperature treatment. Here, the best-fit relationship was defined by (i) the model with the lowest AIC scores, (ii) normally distributed residual plots and (iii) and regressions fitted on raw data. No statistical tests were applied here; instead, we report the non-linear relationships. COT data varied substantially in both males and females across all temperatures and all swim speeds; this limited us from robustly determining any representative relationships.

Medium and short-term recovery data (i.e. hourly EPOC, cumulative hourly EPOC and $\%MMR_{time}$) were non-independent across time and were analyzed using a repeated measured ANOVA. We used mixed models to account for individual-specific recovery trends (R package 'lme4', function lmer; Bates *et al.*, 2015). The variances of cumulative hourly EPOC varied across timepoints, and therefore, this measurement was log10-transformed before analysis to comply with parametric test assumptions. In all recovery models, individual fish was added as a random effect to account for repeated measures across timepoints. The timepoints (hours or 10-min intervals, analyzed as discrete timepoints), temperature, sex and a three-way interaction between these factors were all included as fixed effects. The significance of this interaction was tested using type III ANOVAs.

Results

There were no observed size differences between male and female coho salmon at the time of testing: male fork length (FL, cm) = 56.4 ± 3.66, body mass (BM, kg) = 1.98 ± 0.38; female FL = 56.5 ± 5.04, BM = 2.16 ± 0.45 (mean ± SEM). Gonadosomatic index (GSI) for fish euthanized within 5 days of the experiment was 18.64 ± 0.57% for females and 4.33 ± 0.18% for males (mean ± SEM).

Swim performance

Both absolute U_{crit}, critical swimming speed (cm s^{-1}; Fig. 2A) and relative U_{crit} (BL $s^{-1;}$ Fig. 2B) were significantly different across temperatures, but not between the sexes (Fig. 2; Supplementary Table 1). Generally, both females and males achieved higher U_{crit} swim speeds at 14°C than at either 9°C or 18°C (Fig. 2). Absolute U_{crit} (in cm s^{-1}) was higher in larger fish (body mass (kg): $F = 8.96$, df = 1, $P = 0.004$).

Oxygen uptake rates increased with increasing swimming speed, with small differences between sexes (Fig. 3A–C). Females performed worse at 14°C than the males (but not at 9°C or 18°C) (Fig. 3A–C). Females at 18°C increased MO_2 in a more linear manner until they reached exhaustion (Fig. 3C). However, males at 18°C increased their MO_2 more exponentially (Fig. 3C). MMR from the U_{crit} tests (MMR_{swim}) were higher (but not significantly so) in males compared to females ($P = 0.082$) and significantly increased with temperature ($P < 0.001$, Fig. 3A–C; Table 1; Supplementary Table 1). The post hoc analysis revealed that MMR_{swim} was higher at 14°C and 18°C than at 9°C (Supplementary Table 1).

The COT varied substantially among individuals at all temperatures and with all swim speeds (Fig. 3D–F). The variance among males was consistently higher than in females at all temperatures, with the coefficient of variation (CV) reaching 30.2% in males and 23.9% CV in females (all temperatures combined).

Metabolism

There were significant effects of temperature and sex on RMR (Fig. 4A; Supplementary Table 1). RMR increased

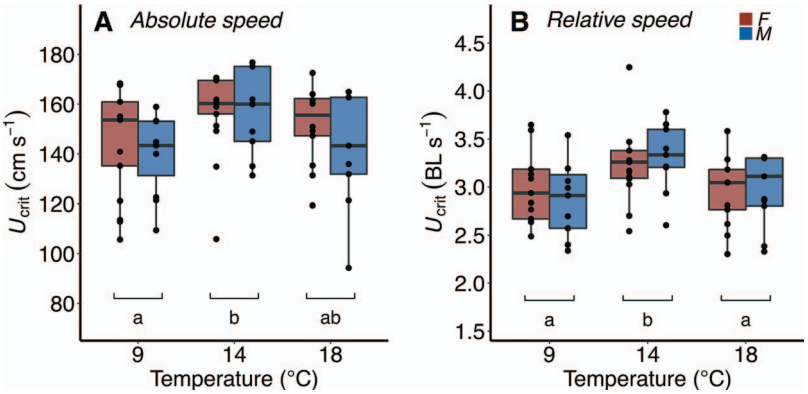

Figure 2: (**A**) Absolute critical swim speed (U_{crit}; cm s^{-1}) and (**B**) relative U_{crit} (body lengths per second, BL s^{-1}) during an incremental swimming protocol for male (M, blue) and female (F, red) coho salmon at 9°C (F, $N = 12$; M, $N = 9$), 14°C (F, $N = 12$; M, $N = 9$) and 18°C (F, $N = 10$; M, $N = 8$). Lower case letters indicate a significant difference ($P < 0.05$) between temperature treatment groups as revealed by post hoc HD test (see Table S2 for 2-way ANOVA outputs). Box plots represent interquartile ranges (boxes and whiskers), median values (solid lines) and outliers (>1.5 beyond interquartile range) are plotted as data points outside the whiskers.

Table 1: Statistics for critical swim speed test (U_{crit}) and chase test performances

Swim performance: U_{crit}				
		df	*F*-value	*P*-value
MMR_{swim} (mg O_2 kg^{-1} min^{-1})	Sex	1	3.132	0.082
	Temp	2	19.341	<**0.001**
U_{crit} (BL s^{-1})	Sex	1	0.029	0.865
	Temp	2	5.266	**0.008**
U_{crit} (cm s^{-1})	Sex	1	0.964	0.330
	Temp	2	5.478	**0.007**
Metabolic performance: chase				
		df	*F*-value	*P*-value
RMR (mg O_2 kg^{-1} min^{-1})	Sex	1	6.5062	**0.014**
	Temp	2	39.857	<**0.001**
MMR (mg O_2 kg^{-1} min^{-1})	Sex	1	6.506	0.137
	Temp	2	39.857	0.582
AAS (mg O_2 kg^{-1} min^{-1})	Sex	1	1.187	0.281
	Temp	2	0.090	0.914
FAS (MMR/RMR)	Sex	1	1.040	0.313
	Temp	2	22.085	<**0.001**

Represented are ANOVA results and significant ($P < 0.05$) factors are highlighted in grey. Temp = temperature; df = degrees of freedom.

significantly with increasing temperature and females had lower RMR than males independent of test temperature. The Q_{10} for RMR from 9°C to 18°C was 2.03 in females and 2.13 in males. MMR_{chase} and AAS did not significantly differ across temperatures or between sexes (Fig. 4; Supplementary Table 1). FAS decreased with increasing temperature (Fig. 4D; Supplementary Table 1).

We also compared MMR elicited using three methods: the chase, during the U_{crit} test and fatigued fish immediately after

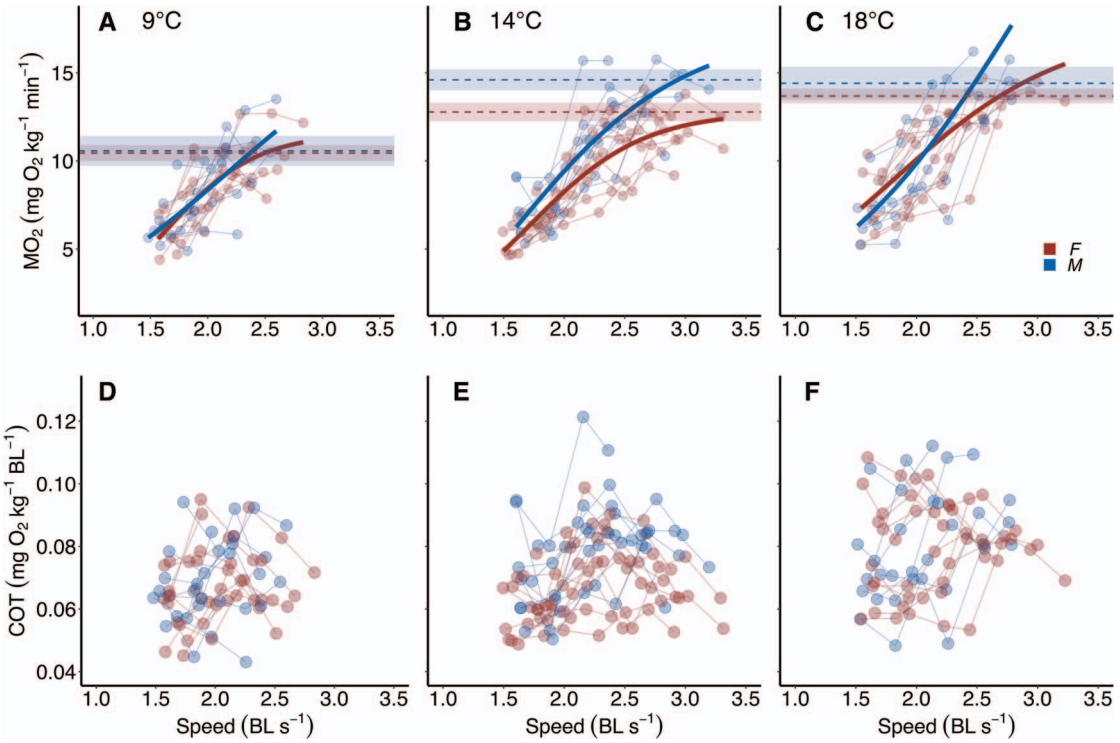

Figure 3: Oxygen uptake rate (MO_2; mg O_2 kg^{-1} min^{-1}) during swimming and COT (mg O_2 kg^{-1} m^{-1}). The top panels show MO_2 as a function of relative swimming speed (BL s^{-1}) in male (M, blue) and female (F, red) coho salmon at 9°C (F, $N = 12$; M, $N = 9$), 14°C (F, $N = 12$; M, $N = 9$) and 18°C (F, $N = 10$; M, $N = 8$) (**A, B, C**, respectively). The mean maximum MO_2 achieved during swim (MMR_{swim}; mg O_2 kg^{-1} min^{-1}) is indicated by dashed lines for both sexes at each temperature (faded bands show standard errors) (no difference between sexes: $P = 0.08$). The coloured solid lines represent the mean best non-linear fit for each sex and at each temperature. For all mixed-models, individual included as a random effect. This allowed us to account for individual-specific performance curves when evaluating the overall relationship that was specific to each sex and temperature treatment. The model equations were as follows: females at 9°C, $MO_2 = 11.59/(1 + e^{(1.60-BLs^{-1})/0.41})$; females at 14°C,$MO_2 = 12.79/(1 + e^{(1.72-BLs^{-1})/0.47})$; females at 18°C, $MO_2 = 17.61/(1 + e^{(1.79-BLs^{-1})/0.72})$; males at 9°C, $MO_2 = 18.78/(1 + e^{(2.18-BLs^{-1})/0.83})$; males at 14°C,$MO_2 = 17.70e^{(-7.59\times0.28^{BLs^{-1}})}$; and males at 18°C, $MO_2 = 37.53/(1 + e^{(2.88-BLs^{-1})/0.86})$. The bottom panels represent the COT at each swim speed for individual fish of both sexes (**D, E, F**). Faded lines indicate individual specific performance.

U_{crit} test (Supplementary Fig. 4; ANOVA: $\chi^2 = 80.46$, df = 2, $P < 0.001$; sexes pooled). MMR_{chase} and MMR_{swim} were not significantly different (post hoc: $P = 0.875$) while $MMR_{fatigue}$ was lower than both MMR_{chase} and MMR_{swim} (post hoc for both: $P < 0.001$).

Recovery

The metabolic costs of full recovery after the chase (EPOC) were significantly higher in males ($P = 0.034$; Fig. 5, Table 2) but did not differ across temperatures (Table 2). The average time it took to recover from exercise (i.e. the duration of EPOC) did not differ between sexes or across temperatures (Fig. 5, Table 2) and lasted 6.06 ± 0.35 h (mean ± SEM for all fish).

When EPOC was assessed over the first 5 hours of recovery, there was a three-way interactive effect of temperature, sex and timepoint (hour) for both hourly EPOC (time * sex * temperature: $P = 0.001$, $\chi^2 = 25.86$, df = 8; Fig. 5, Table 3) and cumulative EPOC (time * sex * temperature: $P = 0.036$, $\chi^2 = 16.46$, df = 8; Supplementary Table 2). Generally, hourly EPOC increased with temperature in both sexes (Fig. 6). In the 14°C and 18°C treatments, however, EPOC was initially higher and remained elevated for longer in males relative to females (Fig. 6B,C; Supplemental Table 2).

Short-term recovery profiles (first hour) were notably impaired after the U_{crit} test compared to the chase method (expressed as % MMR across time in min; Fig. 6A,B; Supplemental Fig. 5). Following the chase, there were no differences in short-term recovery between sexes; however, we found a significant interaction between time (10-min timepoints) and temperature (time * temperature: $P < 0.001$, $\chi^2 = 62.00$, df = 10; Fig. 7A,B; Table 3). Short-term recovery was much faster at cooler temperatures for both chase and U_{crit} tests (Fig. 7A,B; Table 2). Fish reached $time_{MMR50}$ in 30.4 min at 9°C, 54.4 min at 14°C and did not reach

Table 2: Recovery performance of female and males coho salmon at 9, 14, 18°C

				Mean ± SEM		%CV		Sex			Temp		
Performance	Temperature (°C)	Female	Male	Female	Male	Female	Male	df	*F*-value	*P*-value	df	*F*-value	*P*-value
Time to EPOC following chase (min)	9	12	8	391.25 ± 42.10	431.12 ± 85.81	37.27	56.30	1	0.058	0.810	2	1.276	0.288
	14	9	9	354.33 ± 74.07	349.44 ± 37.64	62.71	32.32						
	18	9	8	360.00 ± 31.29	287.00 ± 19.60	26.08	19.31						
EPOC following chase (mg O_2 kg^{-1})	9	12	8	888.62 ± 216.72	1464.73 ± 453.19	84.49	87.51	1	4.748	**0.034**	2	0.587	0.560
	14	9	9	755.69 ± 163.37	1103.33 ± 174.28	64.86	47.39						
	18	9	8	870.37 ± 24.15	1115.68 ± 116.55	8.32	29.55						
Time to reach 50% of MMR following chase (min)	9 (A)	12	8	39.25 ± 21.97	17.00 ± 3.52	193.86	58.49	1	0.082	0.775	2	11.042	<**0.001**
	14 (A)	9	9	49.22 ± 17.59	59.67 ± 21.24	107.20	106.81						
	18 (B)	9	8	106.22 ± 34.41	136.50 ± 35.42	97.19	73.39						

Represented are means and standard error values for each test group and ANOVA results. Performance metrics that were significantly affected by temperature and sex are highlighted in grey, and uppercase letters indicate significant post hoc HD results ($P < 0.05$). Temp = temperature; chase = chase and air exposure protocol; df = degrees of freedom.

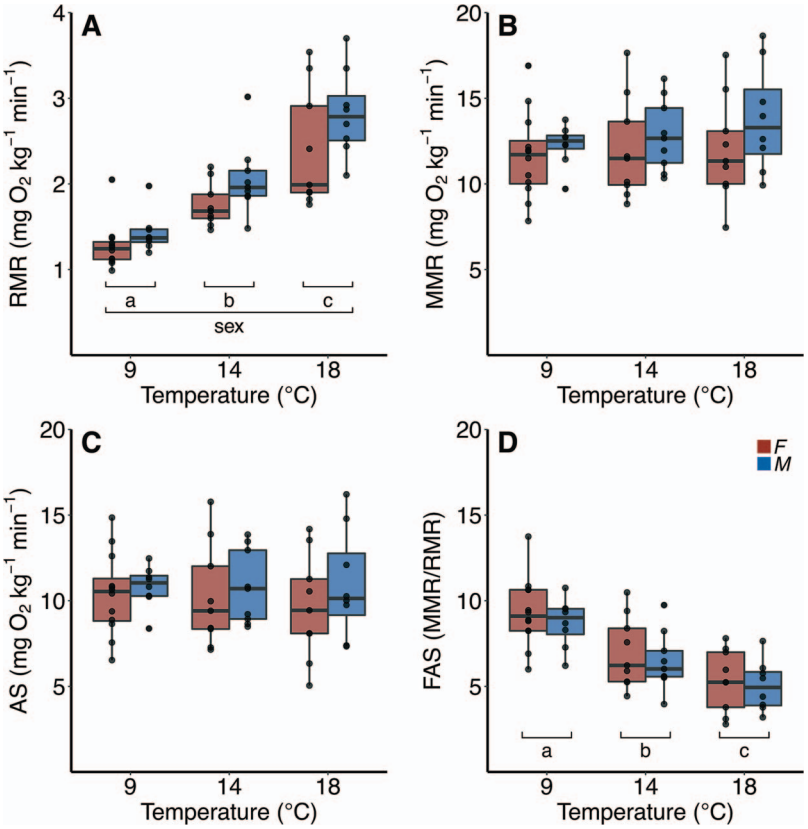

Figure 4: Oxygen uptake rate (MO_2; mg O_2 kg^{-1} min^{-1}) of female (F, red) and male (M, blue) coho salmon after an exhaustive chase with air exposure protocol at 9°C (F, $N = 12$; M, $N = 8$), 14°C (F, $N = 9$; M, $N = 9$) and 18°C (F, $N = 9$; M, $N = 8$). Presented are RMR (**A**), MMR (**B**), AAS (MMR-RMR) (**C**) and FAS (MMR/RMR) (**D**). Lower case letters indicate significant difference ($P < 0.05$) between treatment groups where applicable (see Table S2 for 2-way ANOVA outputs); the significant difference of RMR between sexes (**A**) is indicated by the line labelled 'sex'. Box plots represent interquartile ranges (boxes and whiskers), median values (solid lines) and outliers (>1.5 beyond interquartile range) are plotted as data points outside the whiskers.

$time_{MMR50}$ at all within the first 60 min at 18°C. In fact, fish at 18°C only reached $time_{MMR50}$ at 120.0 min (Table 2, males and females combined; Fig. 5).

Following the U_{crit} swim test, fish at warmer temperatures recovered more slowly as indicated by the significant interaction between time and temperature in short-term recovery (time * temperature: $P < 0.001$, $\chi^2 = 33.76$, df = 10; Fig. 7C,D; Table 3). Sex also influenced short-term recovery following U_{crit} swimming (sex * time: $P = 0.016$, $\chi^2 = 13.92$, df = 2; Fig. 6C,D; Table 3). Female recovery post-swimming was clearly impaired at 18°C compared to 9 and 14°C, while male recovery was more variable (Fig. 7C,D).

Individual variability in EPOC decreased with increasing temperature in both males and females, as indicated by coefficients of variation (Table 2, Fig. 5). This was true regardless of how the EPOC was expressed (i.e. time of recovery, full EPOC, hourly EPOC, cumulative hourly EPOC; Table 2; Supplementary Table 2).

Blood physiology

Lactate, osmolality, chloride, sodium and haematocrit levels at 20 h post-chase were not significantly different between sexes or across temperatures (Table 4). Potassium was significantly higher at 18°C compared to the other temperatures, but there was no effect of sex (Table 4). There were significant effects of both temperature and sex on the sex hormone titers (Table 4). Oestradiol and testosterone significantly differed among temperatures, with a general trend of decreasing hormone levels with increasing temperatures (Table 4). Males displayed lower levels of both oestradiol (as expected) and testosterone compared to females.

Discussion

This study aimed to investigate the underlying mechanisms driving high female mortality of Pacific salmon during their spawning migration. We predicted that females would have

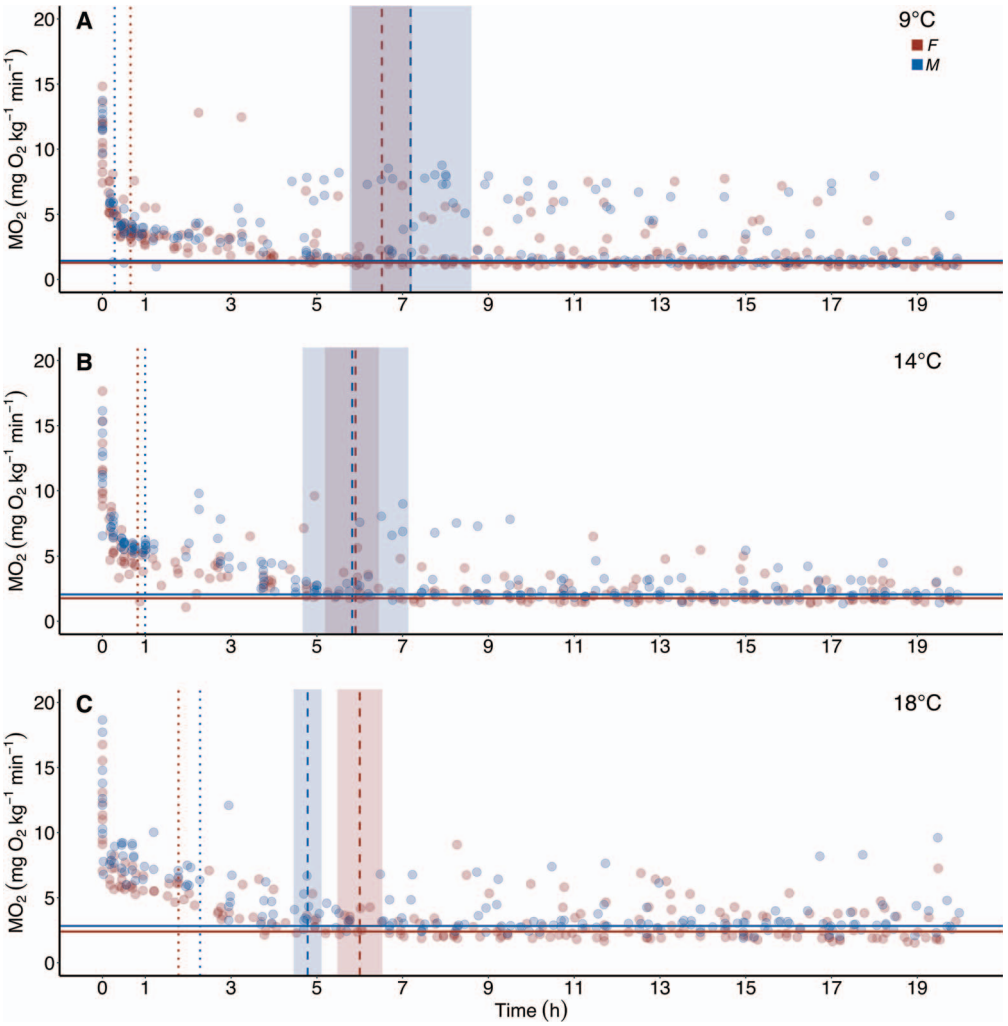

Figure 5: Full recovery of oxygen consumption rate trends (MO_2; mg O_2 kg^{-1} min^{-1}) over 20 h after exhaustive chase and air exposure in female (F, red) and male (M, blue) coho salmon at 9°C, 14°C and 18°C (**A, B, C**, respectively). $Time_{MMR50}$ (dotted line; see Fig. 6 for short-term recovery relationships), RMR, mean duration (coloured dashed lines) and standard error (faded area) for EPOC for each sex at each temperature are shown.

lower AAS, higher energetic costs of swimming and higher costs of recovery from exhaustion, particularly at high temperatures. In contrast to our prediction, females had lower RMR than males across temperatures, suggesting that they are more energetically conservative. However, females had no mean difference in AAS. We found no evidence that females have impaired swim performance or recovery capacity compared to males. In fact, males had higher EPOC compared to females. Thus, this study found no new evidence to explain female-biassed mortality rates and much work remains to be done to explain this mysterious phenomenon. However, this work did identify an important physiological constraint with conservation implications. Both sexes have impaired short-term and intermediate recovery from exhaustive exercise (higher energetic costs and longer duration) at warm temperatures. Impaired recovery may have been a consequence of reduced FAS at high temperatures. This finding is relevant for salmon conservation, as prolonged recovery may cause migration delays, increase predation risk and ultimately prevent successful migration to the spawning grounds.

Metabolism and swim performance

Contrary to our hypothesis, there was no evidence that females suffer reduced aerobic capacity compared to males, even at high temperatures. However, relative differences in RMR and MMR indicate that underlying tradeoffs may be more complex. The lower RMR in females suggests that female gonadal maintenance and development do not

Table 3: Statistics for intermediate (5-h) and short-term (1-h) recovery of female and male coho salmon at 9°C, 14°C and 18°C

		df	X^2	*P*-value
Hourly EPOC following chase (mg O_2 kg^{-1}; timepoint in h)	Timepoint	4	25.938	<0.001
	Sex	1	0.162	0.687
	Temp	2	6.079	0.048
	Timepoint*Sex	4	7.6816	0.104
	Timepoint*Temp	8	12.295	0.139
	Sex∗Temp	2	1.749	0.417
	Timepoint*Sex*Temp	8	**25.863**	**0.001**
Short-term recovery as a function % MMR following chase (timepoint in min)	Timepoint	5	1037.200	<0.001
	Sex	1	0.719	0. 397
	Temp	2	5.920	0.0518
	Timepoint*Sex	5	5.441	0.364
	Timepoint*Temp	10	**61.997**	**<0.001**
	Sex*Temp	2	1.876	0.391
	Timepoint*Sex*Temp	10	13.902	0.178
Short-term recovery as a function % MMR following U_{crit} (timepoint in min)	Timepoint	5	54.740	<0.001
	Sex	1	1.990	0.158
	Temp	2	9.598	0.008
	Timepoint*Sex	5	13.923	0.016
	Timepoint*Temp	10	**33.763**	**<0.001**
	Sex*Temp	2	2.972	0.226
	Timepoint*Sex*Temp	10	13.587	0.193

Represented are ANOVA results and significant ($P < 0.05$) factors are highlighted in grey. Temp = temperature; chase = chase and air exposure protocol; df = degrees of freedom.

represent a loading factor relative to males at this point in their migration. All of the fish in this study had just reached their spawning ground; therefore, gonadal development was largely complete. We do not currently know what drives the sex-specific differences in RMR but we suspect it may be driven by (i) a shift to gonad maintenance (as opposed to presumably more energetically expensive gonad development) in mature females or (ii) spontaneous activity in males during overnight recovery (Fig. 4). Males are known to be more aggressive and spontaneously active on the spawning ground (McVeigh *et al.*, 2007; Clark *et al.*, 2013; Raby *et al.*, 2016) and such activity may have resulted in overestimates of RMR if this led to increased arousal in the respirometers; although RMR values in our study (female averages ranged from 1.27 to 2.40 mgO_2 kg^{-1} min^{-1} between 9°C and 18°C; male averages ranged from 1.43 to 2.83 mgO_2 kg^{-1} min^{-1} between 9°C and 18°C) were similar to the reported values from Pacific salmon populations with comparable migration distances (1.3–2.7 mgO_2 kg^{-1} min^{-1} between 10°C and 15°C; Lee *et al.*, 2003b; Raby *et al.*, 2016). It is also interesting to consider that, in contrast to our hypotheses, the gonads may have a low oxygen demand and that at this advanced reproductive stage (oogenesis was largely complete), females may be allocating minimal blood to their gonads. To evaluate this scenario, we calculated RMR, MMR and AAS for fish with mean GSI removed (Supplementary Fig. 5, 6) and found that there was no longer a significant effect of sex on RMR. However, the outcome for MMR and AAS was virtually the same.

Our results revealed no convincing evidence that MMR differed between the sexes. This was unexpected because some female salmonids have lower MMR, AAS and cardiac output (pink salmon; Clark *et al.*, 2011), lower scope for heart rate (sockeye salmon, Sandblom *et al.*, 2009) and coronary blood flow (rainbow trout, Ekström *et al.*, 2017) and lower cardiac lactate dehydrogenase (LDH) activity (coho salmon, Little

Table 4: Sex-specific blood biochemistry parameters in coho salmon 20-h post-chase and air exposure at 9°C, 14°C and 18°C

		Mean ± SEM		%CV		Sex			Temp		
Performance	Tempe-rature (°C)	Female	Male	Female	Male	df	*F*-value	*P*-value	df	*F*-value	*P*-value
Glucose (mmol L^{-1})	9	7.96 ± 1.27	5.92 ± 0.45	53.04	20.07	1	2.745	0.104	2	3.346	**0.044**
	14	5.67 ± 0.51	5.05 ± 0.40	26.80	22.37						
	18	5.58 ± 0.44	5.09 ± 0.37	23.74	17.61						
Lactate (mmol L^{-1})	9	2.58 ± 0.27	2.13 ± 0.29	34.78	36.52	1	0.046	0.832	2	1.651	0.203
	14	2.85 ± 0.35	2.49 ± 0.43	37.24	48.89						
	18	2.78 ± 0.31	3.53 ± 0.58	33.84	40.44						
Osmolality (mOsm kg^{-1})	9	309.64 ± 2.65	308.43 ± 4.70	2.84	4.03	1	0.134	0.709	2	2.101	0.134
	14	312.56 ± 3.19	312.38 ± 3.69	3.06	3.34						
	18	314.11 ± 3.30	319.80 ± 1.77	3.15	1.24						
Testosterone (ng mL^{-1})	9 (A)	176.58 ± 23.92	22.10 ± 6.77	44.93	81.05	1	26.408	< **0.001**	2	11.123	< **0.001**
	14 (A)	99.07 ± 27.85	14.97 ± 3.97	79.52	74.98						
	18 (B)	57.63 ± 28.96	8.24 ± 3.32	150.75	98.54						
Oestradiol (ng mL^{-1})	9 (A)	12.74 ± 2.98	0.25 ± 0.02	77.56	24.72	1	76.581	< **0.001**	2	4.920	**0.012**
	14 (AB)	6.05 ± 2.16	0.16 ± 0.03	100.96	47.96						
	18 (B)	2.32 ± 0.75	0.14 ± 0.03	96.45	56.73						
Haematocrit (%)	9	53.88 ± 2.15	54.31 ± 1.65	13.83	8.61	1	0.003	0.959	2	0.433	0.651
	14	52.39 ± 3.01	51.94 ± 3.55	17.21	20.48						
	18	51.72 ± 2.89	52.07 ± 1.54	16.75	7.81						
Sodium, Na^+ (mmol L^{-1})	9	151.00 ± 2.34	155.71 ± 1.94	5.14	3.30	1	2.151	0.149	2	1.098	0.300
	14	150.41 ± 2.22	152.52 ± 1.73	4.43	3.21						
	18	154.90 ± 1.50	156.93 ± 3.97	2.90	6.20						
Potassium, K^+ (mmol L^{-1})	9 (A)	1.31 ± 0.19	1.66 ± 0.32	48.89	51.57	1	0.376	0.543	2	5.627	**0.007**
	14 (A)	1.26 ± 0.39	1.23 ± 0.30	92.16	67.92						
	18 (B)	2.20 ± 0.39	2.36 ± 0.29	53.25	29.97						
Chloride, Cl^- (mmol L^{-1})	9	130.50 ± 2.32	135.71 ± 1.92	5.89	3.74	1	0.102	0.751	2	2.614	0.084
	14	137.56 ± 2.89	133.94 ± 1.73	6.31	3.65						
	18	137.78 ± 1.97	137.92 ± 2.11	4.28	3.74						

Represented are means and standard error values for each test group, and ANOVA results. Performance metrics that were significantly affected by temperature and sex are highlighted in grey, and uppercase letters indicate significant post hoc HD results ($P < 0.05$). Temp = temperature, CV = coefficient of variation; df = degrees of freedom.

et al., 2020b) relative to males. This provided a rationale for our hypothesis that female salmon suffer a limitation in oxygen delivery to aerobic tissues compared to males, which is exacerbated at higher temperatures (e.g. Clark *et al.*, 2011; Anttila *et al.*, 2014). On the other hand, there appears to be no differences in haematocrit (Table 2) (Sandblom *et al.*, 2009) or cardiac sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) activity (Anttila *et al.*, 2019) or citrate synthase activity in cardiac, red and white muscle tissues (Little *et al.*, 2020b) between male and female salmon. Therefore, the evidence for sex-specific differences in oxygen transport remains equivocal and more mechanistic research on sex-specific differences in cardiorespiratory physiology in adult Pacific salmon is clearly warranted.

Swimming, recovery and reproduction are the primary activities that are partitioned within AAS for adult migrating salmon (Martin *et al.*, 2015). In contrast to our hypothesis, U_{crit} swimming performance and COT did not differ between sexes. This further suggests that larger gonads are not imposing an energetically demanding burden on the female fish. Others have similarly reported no sex differences in U_{crit} in sockeye salmon (Farrell *et al.*, 2003; Makiguchi *et al.*, 2008; Eliason *et al.*, 2013b; Wilson *et al.*, 2013), though swim performance did differ between sexes in pink salmon (U_{max}: Clark *et al.*, 2011, U_{crit}: Williams and Brett, 1987; Makiguchi *et al.*, 2017). Several studies have found that females rely more heavily on anaerobic metabolism than males in the field (Hinch and Rand, 1998; Standen *et al.*, 2002; Burnett *et al.*, 2014a, 2014b), though the relative roles of behaviour and physiology underlying this phenomenon are unknown. A subtle sex-specific trend did emerge in our swim performance data that supports this idea (Fig. 3). It appeared that as the swimming speeds increased, oxygen consumption tended to plateau at lower speeds in females across all temperatures, whereas in males, oxygen consumption rates tended to increase until they reached fatigue (Fig. 3). This suggests that females recruited glycolytic muscle for continuous swimming at lower swim speeds. However, these subtle trends were not supported by the recovery data. Greater anaerobic effort typically causes greater recovery costs (total EPOC), especially during later phases of recovery (Zhang *et al.*, 2018, Wood *et al.*, 1983, Wood, 1991), which was not detected in female fish in this study post-swim. Notably, we only measured recovery from U_{crit} for 1 h, so recovery differences may have been revealed during longer measurements. Several field studies have also shown that female salmon are more energetically efficient swimmers compared to males (i.e. lower COT; Hinch and Rand, 1998; Standen *et al.*, 2002). Similarly, females had a lower COT during U_{crit} tests in a more distant pink salmon population from the Shibetsu River in Japan (Makiguchi *et al.*, 2017). To date, only one study has examined how swimming performance changes with the state of maturity in Pacific salmon, where less mature fish were stronger swimmers (pink salmon males and females; Williams and Brett, 1987). The relative energetic cost to develop and maintain the gonads throughout migration is not well understood in salmon (Williams and Brett, 1987; Crossin *et al.*, 2003, 2004; Fenkes *et al.*, 2016). Future research should, therefore, focus on sex differences in swim performance, metabolism, immune function and regional blood distribution at different stages in migration and in response to sub-optimal temperatures.

Recovery

We found no direct evidence to support our hypothesis that female salmon have compromised recovery compared to males, particularly at high temperatures. The consequences of impaired recovery can have both immediate and delayed effects. To account for both possibilities, we measured short-term (1 h) and intermediate (5 h), as well as the full EPOC recovery costs. If the full cost of physiological recovery (EPOC) is increased, then fish could deplete their finite tissue energy reserves and be unable to reach their spawning grounds (Macdonald, 2000). Here, females had lower EPOC relative to males and there were no differences between sexes in the duration of EPOC after the exhaustive chase (mean overall duration was 6.06 h). Previous work on adult salmon reported at least a 4–6-h recovery time (time to EPOC; Lee *et al.*, 2003a; Clark *et al.*, 2012). Poor recovery performance may be indicated by an inability to recover blood chemistry to homeostatic levels following exercise. Here, there were no differences between sexes in the blood biochemistry parameters measured at ~20 h after the chase (except for sex hormones, discussed below), and most parameters returned to expected baseline levels within that timeframe (i.e. lactate, sodium, chloride, osmolality, glucose; Milligan and Wood, 1986; Cooke *et al.*, 2006; Crossin *et al.*, 2008; Mathes *et al.*, 2010; Donaldson *et al.*, 2014; Minke-Martin *et al.*, 2018). EPOC measurements are influenced by both physiology and behaviour but their relative contributions are difficult to parse. Because of the 'restlessness' of the coho salmon in respirometers, we observed substantial variability in EPOC. However, this variability was always greater in males than females and decreased with temperature. Thus, the elevated EPOC observed in males here may have been a behavioural artefact, rather than indicative of increased costs for physiological recovery. Another possibility is that males have a higher muscle mass compared to females (body size did not differ, but females had much larger gonads and thus males have greater somatic mass, most of which is composed of muscle), and thus the males may have incurred a great homeostatic imbalance in response to exhaustive exercise.

Full physiological recovery from exhaustive exercise is a dynamic and complex process that typically takes many hours in fish, involving: recovery of oxygen stores; re-synthesis of high energy phosphates; restoration of ionic, osmotic, metabolite and hormonal levels; and recovery of cardiovascular parameters (Wood, 1991; Scarabello *et al.*, 1992; Lee *et al.*, 2003a; Zhang *et al.*, 2018). When recovery was assessed over an intermediate timescale (5 h), interactive effects between temperature and sex were observed. Recovery costs, as determined by the hourly EPOC or cumulative

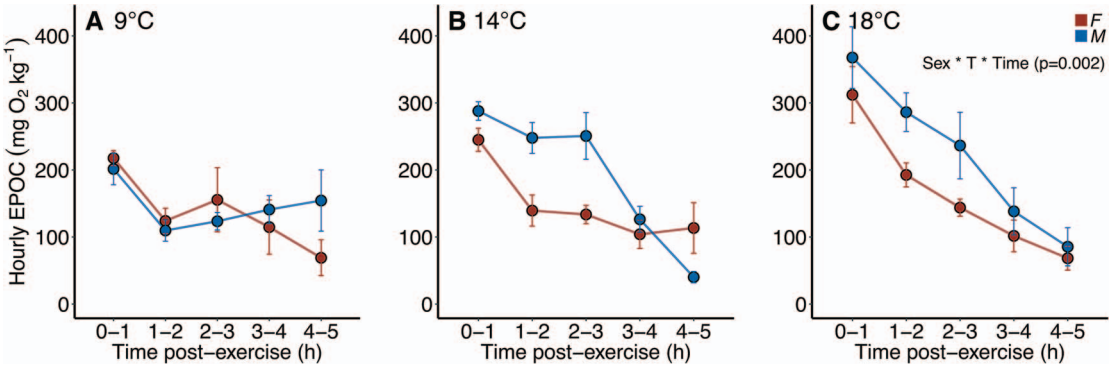

Figure 6: EPOC (mg O_2 kg^{-1}) as a function of 1-h time blocks for female (F, red) and male (M, blue) coho salmon at 9°C, 14°C and 18°C (**A**, **B**, **C**, respectively). Significant three-way interaction sex (Sex) * temperature (T) * timepoint (Time) (ANOVA: $P = 0.002$; Table 2) denotes statistical results for all panels.

EPOC, were higher in males at warmer temperatures. Again, this may have been because male exhibit behavioural differences in the respirometers compared to females. Previous work with sockeye salmon found that males and females responded similarly to a beach seine and tagging stressor (equivalent plasma lactate levels at time 0 and 1 h), but females had impaired recovery as evidenced by elevated plasma lactate, potassium, cortisol levels and ventricular lactate levels 4 h after the stressor (Eliason *et al.*, 2020). More research is needed to determine why metabolic recovery is not impaired in females (present study) but metabolite recovery is compromised (Eliason *et al.*, 2020).

Salmon must be able to rapidly recover from anaerobic burst swimming to be able to negotiate challenging river reaches (e.g. turbulent rapids and high encounter velocities) and resume migration. Indeed, salmon have an outstanding capacity to repeat maximum swimming performance given only ~45 min to recover between tests and before they have physiologically recovered back to baseline levels (Eliason *et al.*, 2013b). Between tests, reported MO_2 values in salmon decreased an average of 30%–70% of their reported MMR, with most values being close to 50% of MMR (Farrell *et al.*, 1998, 2003; Jain *et al.*, 1998; Jain and Farrell, 2003; MacNutt *et al.*, 2006; Wagner *et al.*, 2006; Eliason *et al.*, 2013b). The recovery of metabolism from MMR to 50% of MMR value is suggested to coincide with the 'rapid' phase of physiological recovery (Zhang *et al.*, 2018) when muscle high energy phosphate levels are restored (Scarabello *et al.*, 1991; Eliason *et al.*, 2020), oxygen stores are replenished (McKenzie, 2004), catecholamines levels decrease (Nikinmaa and Tufts, 1989; Tufts and Randall, 1989) and cardiac output declines (Eliason *et al.*, 2013a). Therefore, recovery to ~50% of an individual's MMR may indicate the threshold of minimal recovery required to continue strenuous swimming. Because $time_{MMR50}$ is easy to measure experimentally, has links to repeat swimming performance and is clearly affected by temperature (e.g. here 17–120 min depending on temperature), we recommend it as an ecologically relevant estimate of recovery performance in migrating adult salmon. Future studies should investigate how blood and cellular recovery parameters relate to the % of MMR reached during recovery. If the rapid phase of recovery is impaired, then fish migration progress may be delayed and they may be less tolerant to repeat stressors. Here, there was no evidence of a difference in the rapid phase of recovery between sexes after either the U_{crit} or chase test, though temperature had an important impact on recovery (discussed in the next section below).

Temperature

Some studies have found that AAS is maximized across the range of temperatures that salmon populations historically encounter (e.g. Fraser River, Canada, sockeye salmon: Lee *et al.*, 2003b; Eliason *et al.*, 2011, 2013a; and Kitakami and Kasshi Rivers, Japan, chum salmon: Abe *et al.*, 2019), while other studies find that some salmon populations have maximum AAS at temperatures exceeding their historical range (e.g. Fraser River pink salmon; Clark *et al.*, 2011). When the thermal optimal window for aerobic scope ($T_{opt\text{-}AAS}$ window) is narrow, incremental increases in temperature (e.g. those associated with climate change) push individuals towards aerobic collapse (Lee *et al.*, 2003b; Eliason *et al.*, 2011, 2013a). RMR increases exponentially until the temperature tolerance threshold (T_{crit}) is reached, while MMR initially increases but often plateaus (or even decreases) before meeting RMR at T_{crit} (Claireaux and Lefrançois, 2007; Eliason *et al.*, 2011; Abe *et al.*, 2019). Thus, AAS tends to be reduced at thermal extremes. In the present study, AAS remained high across experimental temperatures, irrespective of sex (9°C is representative of a current average temperature, 14°C is a current warm temperature and 18°C represents a future climate change scenario). The ability of this population of Chilliwack River coho salmon to maintain 97% of maximum AAS and 89% of maximum swimming performance across this temperature range and regardless of sex was unexpected given that they rarely encounter temperatures above ~16°C

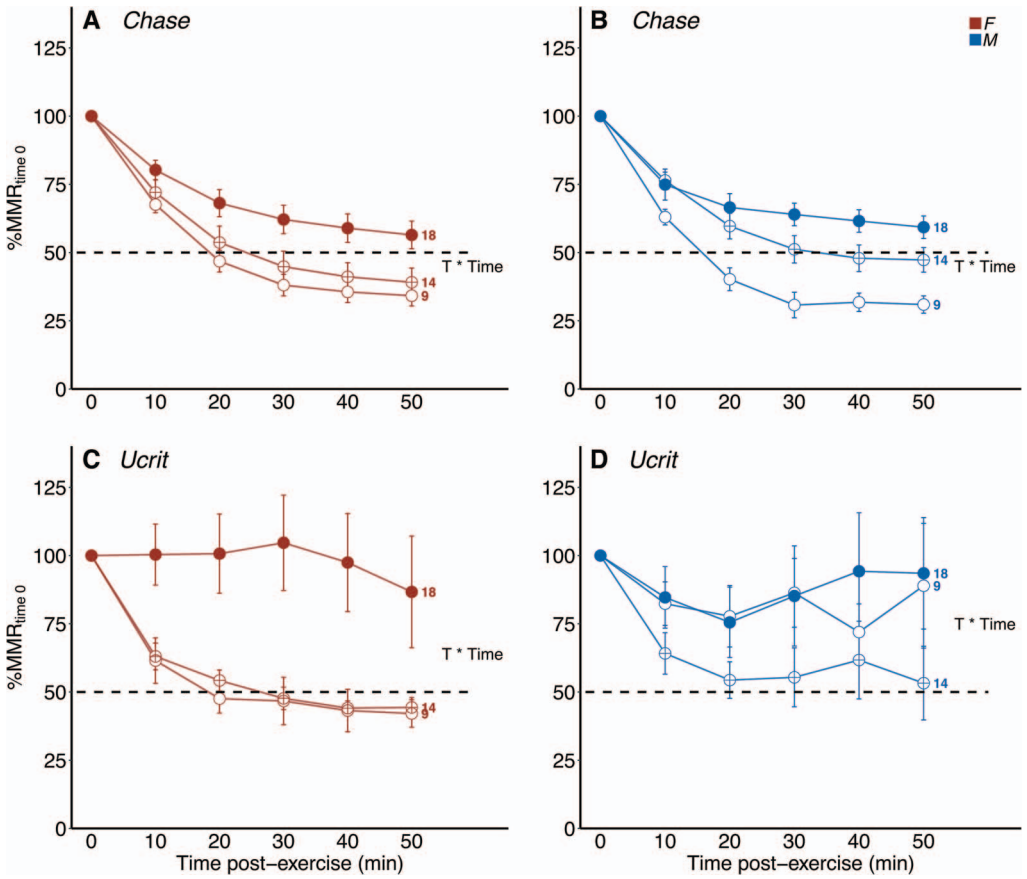

Figure 7: Short-term recovery profiles shown as the percentage of MMR achieved during the first hour following a chase and air exposure (**A**, **B**) and a U_{crit} swim test (**C**, **D**) in female and male salmon. Significant two-way interaction temperature (T) * timepoint (Time) (chase and U_{crit}: ANOVA: $P < 0.001$; Table 2) is indicated in each panel. The dashed lines show the mean resting metabolic rate (RMR; mg O_2 kg^{-1} min^{-1}) for each sex at each temperature (also expressed in % MMR); note that RMR indicated in (C) and (D) are from fish undergoing chase protocol. Shapes indicate the temperature treatment (9°C, open circle; 14°C, crossed circle; 18°C, filled circle). Data points represent mean ± SEM.

(personal communication from D.A.P., DFO; Bradford *et al.*, 2010). The geographic range for coho salmon historically extends as far south as San Francisco Bay California, where US populations are known to behaviourally avoid temperatures above 18°C (Final CCC Coho Salmon ESU Recovery Plan, 2012). This suggests that Chilliwack River coho salmon have a wide $T_{opt\text{-}AAS}$ window that extends beyond the temperatures they historically encounter during migration, similar to the findings of Clark et al. (2011) on pink salmon.

While AAS can be a useful indicator of energy available for feats of athleticism to overcome challenging situations, FAS better contextualizes how environmental stressors limit the more routine activities that are generally expressed relative to MR (e.g. specific dynamic action; Farrell, 2016). Here, FAS decreased with temperature, a trend that was predominantly driven by the swift increase in RMR at higher temperatures. Thus, while critical swimming capacity remained relatively high across the three temperatures, lower FAS may indicate a lower capacity or efficiency for physiological maintenance at higher temperatures (i.e. less proportional energy available for constitutive energy conversion, reproductive development and/or recovery (Clark *et al.*, 2013; Eliason and Farrell, 2016). In support, we identified that both short-term and intermediate recovery were impaired as temperatures increased. Our proposed threshold for continued migration ($time_{MMR50}$) was 4-fold longer for fish at 18°C (120 min) compared to 9°C (30 min). Fish encounter various stressors that require exhaustive effort during upriver migration (Hinch and Bratty, 2000). Prolonged recovery times would compromise their ability to overcome consecutive challenges, which could be catastrophic for migration success and timing. Thus, when AAS, FAS, swim performance and recovery are evaluated together as a function of temperature (Fig. 7), clear patterns emerge (Hvas *et al.*, 2017). Specifically, we see that drivers of acute athletic performance (i.e. AAS and U_{crit}) are relatively stable across temperatures, whereas recovery is constrained at high temperature.

In addition to prolonged recoveries, thermal stress also appears to compromise sex hormone signaling. Blood chem-

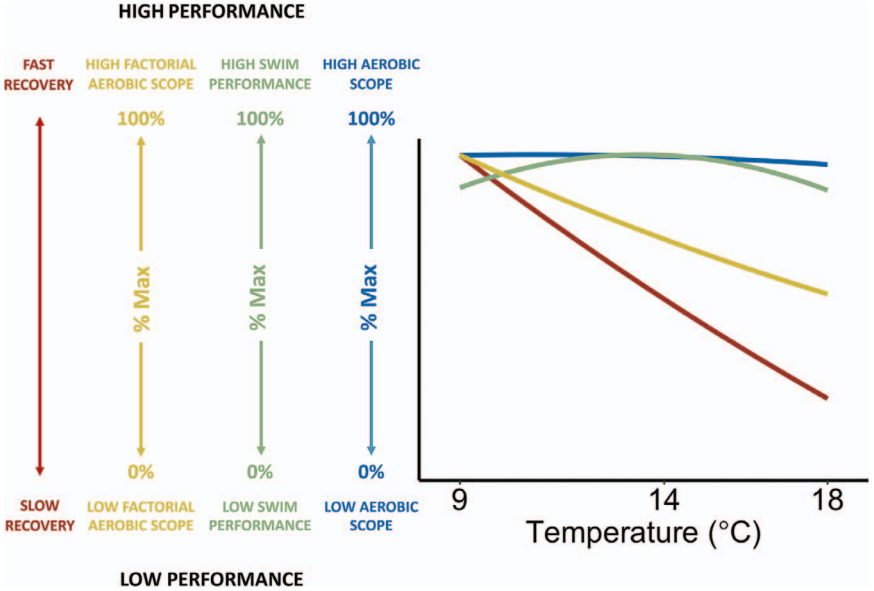

Figure 8: A conceptual representation of diverging metabolic performance capacities in coho salmon across temperatures. AAS, FAS, swim performance (U_{crit}) and short-term recovery performance ($time_{MMR50}$) are presented as the % max of the value across temperatures (average value at temperature/max value across temperatures) × 100 (used in AAS, FAS and swim performance) and as ($time_{MMR50}$ at the lowest value/$time_{MMR50}$ at each temperature) × 100 and data were subsequently fitted with a third-order polynomial. These metrics of metabolic performance were clearly not optimized at a single temperature (i.e. T_{opt}) in coho salmon. U_{crit} peaked at 14°C and decreased in warmer or cooler waters. AAS (maximum − routine metabolic rate) was maintained at all test temperatures while their FAS (maximum/routine metabolic rate) decreased with test temperature. Lastly, $time_{MMR50}$ clearly decreased with increasing test temperature Thus, all performance metrics except for AAS were compromised at some degree at 18°C.

istry analyses (taken 20 h post-chase) showed that rising temperatures decreased sex hormone levels (i.e. testosterone, oestradiol) mirroring previous work in pink and sockeye salmon (Jeffries *et al.*, 2012) and in the same population of coho salmon (Little *et al.*, 2020b). As expected, oestradiol and testosterone levels were higher in females than males (Truscott *et al.*, 1986; Sower *et al.*, 1982; Hruska *et al.*, 2007). Sex hormones serve multiple functions, including regulatory roles in sexual maturation and immune function (Milla *et al.*, 2011; Klein and Flanagan, 2016). Hormone levels can be disrupted under several conditions, including elevated temperature and exercise, which can have various effects on maturation. For example, decreased hormone levels at high temperatures can delay or inhibit maturation (Jeffries *et al.*, 2012), while exercise has been shown to accelerate maturation in female sockeye salmon (Patterson *et al.*, 2004). Thus, environmental temperature may interact with migration challenges, such as strenuous swimming, to alter maturation timing in salmon. The severity of these effects likely differs between sexes, although this requires further investigation.

Water temperature is a major conservation concern across Pacific salmon populations, with the southern range of coho salmon around San Francisco and interior Fraser river coho populations (Committee on the Status of Endangered Wildlife in Canada, COSEWIC 2017) listed as endangered and coho salmon populations in the lower Columbia River, Oregon coast, Southern Oregon and Northern California listed as threatened (Endangered Species Act, 1973). Rising temperatures (in some cases near species lethal levels), are one of the major contributors to habitat loss for coho salmon, and other ecologically and economically valued species (Kaczynski and Alvarado, 2006; Deutsch *et al.*, 2015). Overall, temperature is an incredibly strong selective agent shaping salmon physiology (Eliason *et al.*, 2011) and incorporating knowledge on the physiological ability of animals into species management plans is a vital process that leads to better conservation outcomes (Eliason and Farrell, 2016; Patterson *et al.*, 2016).

Conclusions

The present study assessed differences in the metabolism, swimming performance and recovery from exhaustive exercise between sexually maturing, migrating male and female coho salmon at three ecologically relevant temperatures (9°C, 14°C, 18°C). In contrast to our hypotheses, female salmon had lower RMR compared to males, with no strong evidence that MMR differs between the sexes. Additionally, AAS and U_{crit} were indistinguishable between sexes. Collectively, these results suggest that discrepancies in migration survivorship between the sexes in this species are not driven by a collapse in AAS or impaired swim performance at high temperatures. Our results indicate that male coho salmon are less efficient

than females at recovering from exercise in warm water, though some of this may be attributed to behavioural differences during the recovery period. One important finding was that recovery from exhaustive exercise was clearly impaired at high temperature for both sexes (Fig. 8). Under a climate change scenario (18°C), it could take a salmon an extra 90 min to sufficiently recover after passing challenging river reaches that require exhaustive exercise. Given that Pacific salmon have ceased feeding and must perform the upriver migration on finite energy stores, this could prolong and even doom their critically timed migration. These results highlight the complexity of the interactions between ecology, physiology and reproductive status in migrating salmon and the importance of investigating disparities in energy usage and performance in sexually dimorphic animals.

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References

Abe TK, Kitagawa T, Makiguchi Y, Sato K (2019) Chum salmon migrating upriver adjust to environmental temperatures through metabolic compensation. *J Exp Biol* 222:jeb186189. doi: 10.1242/jeb.186189.

Alsop DH, Wood CM (1997) The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout (*Oncorhynchus mykiss*). *J Exp Biol* 200: 2337–2346.

Altimiras J, Claireaux G, Sandblom E, Farrell AP, McKenzie DJ, Axelsson M (2008) Gastrointestinal blood flow and postprandial metabolism in swimming sea bass *Dicentrarchus labrax*. *Physiol Biochem Zool* 81: 663–672.

Anttila K, Farrell AP, Patterson DA, Hinch SG, Eliason EJ (2019) Cardiac SERCA activity in sockeye salmon populations: an adaptive response to migration conditions. *Can J Fish Aquat Sci* 76: 1–5.

Anttila K, Jorgensen SM, Casselman MT, Timmerhaus G, Farrell AP, Takle H (2014) Association between swimming performance, cardiorespiratory morphometry, and thermal tolerance in Atlantic salmon (*Salmo salar* L.). *Front Mar Sci* 1: 1–10.

Bass AL, Hinch SG, Teffer AK, Patterson DA, Miller KM (2019) Fisheries capture and infectious agents are associated with travel rate and survival of Chinook salmon during spawning migration. *Fish Res* 209: 156–166.

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67: 1–48.

Bell WH, Terhune LDB (1970) Water tunnel design for fisheries research. Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

Black EC, Bosomworth NJ, Docherty GK (1966) Combined effect of starvation and severe exercise on glycogen metabolism of rainbow trout, *Salmo gairdneri*. *J Fish Res Board Can* 23: 1461–1463.

Bradford MJ, Lovy J, Patterson DA, Speare DJ, Bennett WR, Stobbart AR, Tovey CP (2010) *Parvicapsula minibicornis* infections in gill and kidney and the premature mortality of adult sockeye salmon (*Oncorhynchus nerka*) from Cultus Lake, British Columbia. *Can J Fish Aquat* 67: 673–683.

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

Brownscombe JW, Bower SD, Bowden W, Nowell L, Midwood JD, Johnson N, Cooke SJ (2014) Canadian recreational fisheries: 35 years of social, biological, and economic dynamics from a national survey. *Fisheries* 39: 251–260.

Burnett NJ, Hinch SG, Braun DC, Casselman MT, Middleton CT, Wilson SM, Cooke SJ (2014a) Burst swimming in areas of high flow: delayed consequences of anaerobiosis in wild adult sockeye salmon. *Physiol Biochem Zool* 87: 587–598.

Burnett NJ, Hinch SG, Donaldson MR, Furey NB, Patterson DA, Roscoe DW, Cooke SJ (2014b) Alterations to dam-spill discharge influence sex-specific activity, behaviour and passage success of migrating adult sockeye salmon: influence of modified flow releases on sockeye salmon migration. *Ecohydrology* 7: 1094–1104.

Calow P (1985) Adaptive Aspects of Energy Allocation.. In P Tytler, P Calow, eds, *Fish Energetics: New Perspectives*. Springer, Netherlands, Dordrecht, pp. 13–31.

Cech JJ, McEnroe M, Randall DJ (2004) Coho salmon haematological, metabolic and acid-base changes during exercise and recovery in sea water. *J Fish Biol* 65: 1223–1232.

Chabot D, Steffensen JF, Farrell AP (2016) The determination of standard metabolic rate in fishes. *J Fish Biol* 88: 81–121.

Claireaux G, Lagardère J-P (1999) Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. *J Sea Res* 42: 157–168.

Claireaux G, Lefrançois C (2007) Linking environmental variability and fish performance: integration through the concept of scope for activity. *Philos Trans R Soc B Biol Sci* 362: 2031–2041.

Clark TD, Donaldson MR, Pieperhoff S, Drenner SM, Lotto A, Cooke SJ, Hinch SG, Patterson DA, Farrell AP (2012) Physiological benefits of being small in a changing world: responses of coho salmon (*Oncorhynchus kisutch*) to an acute thermal challenge and a simulated capture event. *PLoS One* 7: e39079. https://doi.org/10.1371/journal.pone.0039079.

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

Clark TD, Sandblom E, Jutfelt F (2013) Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *J Exp Biol* 216: 2771–2782.

Cooke SJ, Hinch SG, Crossin GT, Patterson DA, English KK, Healey MC, Shrimpton JM, Van Der Kraak G, Farrell AP (2006) Mechanistic basis of individual mortality in pacific salmon during spawning migrations. *Ecology* 87: 1575–1586.

COSEWIC (2016) COSEWIC Assessment and Status Report on the Coho Salmon, *Oncorhynchus Kisutch*, Interior Fraser Population in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. pp. xi + 50. http://www.registrelep-sararegistry.gc.ca/default.asp?lang=en&n=24F7211B-1.

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

Crossin GT, Hinch SG, Farrell AP, Higgs DA, Lotto AG, Oakes JD, Healey MC (2004) Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. *J Fish Biol* 65: 788–810.

Crossin GT, Hinch SG, Farrell AP, Whelly MP, Healey MC (2003) Pink salmon (*Oncorhynchus gorbuscha*) migratory energetics: response to migratory difficulty and comparisons with sockeye salmon (*Oncorhynchus nerka*). *Can J Zool* 81: 1986–1995.

Deutsch C, Ferrel A, Seibel B, Pörtner H-O, Huey RB (2015) Climate change tightens a metabolic constraint on marine habitats. *Science* 348: 1132–1135.

Donaldson MR, Clark TD, Hinch SG, Cooke SJ, Patterson DA, Gale MK, Frappell PB, Farrell AP (2010) Physiological responses of free-swimming adult coho salmon to simulated predator and fisheries encounters. *Physiol Biochem Zool* 83: 973–983.

Donaldson MR, Hinch SG, Jeffries KM, Patterson DA, Cooke SJ, Farrell AP, Miller KM (2014) Species- and sex-specific responses and recovery of wild, mature pacific salmon to an exhaustive exercise and air exposure stressor. *Comp Biochem Physiol* 173: 7–16.

Ekström A, Axelsson M, Gräns A, Brijs J, Sandblom E (2017) Influence of the coronary circulation on thermal tolerance and cardiac performance during warming in rainbow trout. *Am J Physiol Regul Integr Comp Physiol* 312: R549–R558.

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

Eliason EJ, Clark TD, Hinch SG, Farrell AP (2013a) Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon. *Conserv Physiol* 1: cot008.

Eliason EJ, Clark TD, Hinch SG, Farrell AP (2013b) Cardiorespiratory performance and blood chemistry during swimming and recovery in three populations of elite swimmers: adult sockeye salmon. *Comp Biochem Physiol* 166: 385–397.

Eliason EJ, Dick M, Patterson DA, Robinson KA, Lotto J, Hinch SG, Cooke SJ (2020) Sex-specific differences in physiological recovery and short-term behaviour following fisheries capture in adult sockeye salmon (*Oncorhynchus nerka*). *Can J Fish Aquat Sci*. 77: 1749–1757. doi: 10.1139/cjfas-2019-0258.

Eliason EJ, Farrell AP (2016) Oxygen uptake in Pacific salmon *Oncorhynchus* spp.: when ecology and physiology meet. *J Fish Biol* 88: 359–388.

Eliason EJ, Higgs DA, Farrell AP (2007) Effect of isoenergetic diets with different protein and lipid content on the growth performance and heat increment of rainbow trout. *Aquaculture* 272: 723–736.

Endangered Species Act of 1973, As Amended through the 108th Congress (1973) Department of the Interior U.S. Fish and Wildlife Service, Washington. *DC* 20240: 44.

Farrell AP (2016) Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. *J Fish Biol* 88: 322–343.

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

Farrell AP, Gamperl AK, Birtwell IK (1998) Prolonged swimming, recovery, and repeat swimming performance of mature sockeye salmon *Oncorhynchus nerka* exposed to moderate hypoxia and pentachlorophenol. *J Exp Biol* 201: 2183–2193.

Farrell AP, Lee CG, Tierney K, Hodaly A, Clutterham S, Healey M, Hinch SG, Lotto A (2003) Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. *J Fish Biol* 62: 64–84.

Fenkes M, Shiels HA, Fitzpatrick JL, Nudds RL (2016) The potential impacts of migratory difficulty, including warmer waters and altered flow conditions, on the reproductive success of salmonid fishes. *Comp Biochem Physiol A Mol Integr Physiol* 193: 11–21.

Fleming IA, Gross MR (1989) Evolution of adult female life history and morphology in a Pacific salmon (coho: *Oncorhynchus Kisutch*). *Evolution* 43: 141–157.

Final CCC Coho Salmon ESU Recovery Plan (No. Volume I of III) (2012). NOAA Fisheries, National Marine Fisheries Service, pp. 48–85.

Fox J, Weisberg S (2019) *An R Companion to Applied Regression*, EdEd 3. Sage, Thousand Oaks, CA, https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Fry FEJ (1971) The effect of environmental factors on the physiology of fish. In WS Hoar, DJ Randall, eds, *Fish Physiology*. Academic Press, New York, NY, p. 98.

Gale MK, Hinch SG, Eliason EJ, Cooke SJ, Patterson DA (2011) Physiological impairment of adult sockeye salmon in fresh water after simulated capture-and-release across a range of temperatures. *Fish Res* 112: 85–95.

Gamperl AK, Farrell AP (2004) Cardiac plasticity in fishes: environmental influences and intraspecific differences. *J Exp Biol* 207: 2539–2550.

Garner SR, Bortoluzzi RN, Heath DD, Neff BD (2010) Sexual conflict inhibits female mate choice for major histocompatibility complex dissimilarity in Chinook salmon. *Proc R Soc B Biol Sci* 277: 885–894.

Geist DR, Brown RS, Cullinan VI, Mesa MG, VanderKooi SP, McKinstry CA (2003) Relationships between metabolic rate, muscle electromyograms and swim performance of adult chinook salmon. *J Fish Biol* 63: 970–989.

Groot C, Margolis L (eds) (1991) *Pacific Salmon Life Histories*. UBC Press, Department of Fisheries and Oceans Biological Sciences Branch, Pacific Biological Station, Nanaimo British Columbia, Canada.

Grüebler MU, Schuler H, Müller M, Spaar R, Horch P, Naef-Daenzer B (2008) Female biased mortality caused by anthropogenic nest loss contributes to population decline and adult sex ratio of a meadow bird. *Biol Conserv* 141: 3040–3049.

Hanson KC, Gravel MA, Graham A, Shoji A, Cooke SJ (2008) Sexual variation in fisheries research and management: when does sex matter? *Rev Fish Sci* 16: 421–436.

Hardy ICW (ed) (2002) *Sex Ratios: Concepts and Research Methods*. Cambridge University Press, Cambridge.

Hassell EMA, Meyers PJ, Billman EJ, Rasmussen JE, Belk MC (2012) Ontogeny and sex alter the effect of predation on body shape in a livebearing fish: sexual dimorphism, parallelism, and costs of reproduction. *Ecol Evol* 2: 1738–1746.

Hayes S, Bond MH, Wells BK, Hanson CV, Jones AW, Macfarlane RB (2011) Using archival tags to infer habitat use of central california steelhead and coho salmon. *American Fisheries Society Symposium* 76.

Hinch SG, Bett NN, Eliason EJ, Farrell AP, Cooke SJ, Patterson DA (2021) Exceptionally high mortality of adult female salmon: a large-scale pattern and a conservation concern. https://doi.org/101139/cjfas-2020-0385.

Hinch SG, Bratty J (2000) Effects of swim speed and activity pattern on success of adult sockeye salmon migration through an area of difficult passage. *Trans Am Fish Soc* 129: 598–606.

Hinch SG, Rand PS (1998) Swim speeds and energy use of upriver-migrating sockeye salmon (*Oncorhynchus nerka*): role of local environment and fish characteristics. *Can J Fish Aquat Sci* 55: 1821–1831.

Hruska KA, Hinch S, Healey M, Farrell AP (2007) Electromyogram telemetry, nondestructive physiological biopsy, and genetic markers: linking recent techniques with behavioral observations for the study of reproductive success in sockeye salmon mating systems. *American Fisheries Society Symposium* 54: 17–29.

Hvas M, Folkedal O, Imsland A, Oppedal F (2017) The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon, *Salmo salar*. *J Exp Biol* 220: 2757–2764.

Jain KE, Birtwell IK, Farrell AP (1998) Repeat swimming performance of mature sockeye salmon following a brief recovery period: a proposed measure of fish health and water quality. *Can J Zool* 76: 1488–1496.

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

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

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

Kaczynski VW, Alvarado F (2006) assessment of the southern range limit of North American coho salmon: difficulties in establishing natural range boundaries. *Fisheries* 31: 374–391.

Keefer ML, Jepson MA, Naughton GP, Blubaugh TJ, Clabough TS, Caudill CC (2017) Condition-dependent en route migration mortality of adult Chinook salmon in the Willamette River Main Stem. *N Am J Fish Manag* 37: 370–379.

Klein SL, Flanagan KL (2016) Sex differences in immune responses. *Nat Rev Immunol* 16: 626–638.

Lauritzen DV, Hertel F, Gordon MS (2005) A kinematic examination of wild sockeye salmon jumping up natural waterfalls. *J Fish Biol* 67: 1010–1020.

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

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

Lenth R (2019) Emmeans: Estimated Marginal Means, Aka Least-Squares Means. CRAN R package: https://cran.r-project.org/web/packages/emmeans/index.html.

Little AG, Dressler T, Kraskura K, Hardison E, Hendriks B, Prystay T, Farrell AP, Cooke SJ, Patterson DA, Hinch SG *et al.* (2020a) Maxed out: optimizing accuracy, precision, and power for field measures of maximum metabolic rate in fishes. *Physiol Biochem Zool* 93: 243–254.

Little AG, Hardison E, Kraskura K, Dressler T, Prystay TS, Hendriks B, Pruitt JN, Farrell AP, Cooke SJ, Patterson DA *et al.* (2020b) Reduced lactate dehydrogenase activity in the heart and suppressed sex hormone levels are associated with female-biased mortality during thermal stress in Pacific salmon. *J Exp Biol* 223: jeb214841. doi: 10.1242/jeb.214841.

Macdonald JS (2000) Mortality during the migration of Fraser River sockeye salmon (*Oncorhynchus nerka*): a study of the effect of ocean and river environmental conditions in 1997. *Can Tech Rep Fish Aquat Sci* 2315: 120.

MacNutt MJ, Hinch SG, Lee CG, Phibbs JR, Lotto AG, Healey MC, Farrell AP (2006) Temperature effects on swimming performance, energetics, and aerobic capacities of mature adult pink salmon (*Oncorhynchus gorbuscha*) compared with those of sockeye salmon (*Oncorhynchus nerka*). *Can J Zool* 84: 88–97.

Makiguchi Y, Nii H, Nakao K, Ueda H (2008) Migratory behaviour of adult chum salmon, *Oncorhynchus keta*, in a reconstructed reach of the Shibetsu River, Japan: migration of chum salmon. *Fish Manag Ecol* 15: 425–433.

Makiguchi Y, Nii H, Nakao K, Ueda H (2017) Sex differences in metabolic rate and swimming performance in pink salmon (*Oncorhynchus gorbuscha*): the effect of male secondary sexual traits. *Ecol Freshw Fish* 26: 322–332.

Martin BT, Nisbet RM, Pike A, Michel CJ, Danner EM (2015) Sport science for salmon and other species: ecological consequences of metabolic power constraints. *Ecol Lett* 18: 535–544.

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

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

McKenzie DJ (2004) The effects of sustained exercise and hypoxia upon oxygen tensions in the red muscle of rainbow trout. *J Exp Biol* 207: 3629–3637.

McVeigh BR, Healey MC, Wolfe F (2007) Energy expenditures during spawning by chum salmon *Oncorhynchus keta* (Walbaum) in British Columbia. *J Fish Biol* 71: 1696–1713.

Miles DB, Sinervo B, Frankino WA (2000) Reproductive burden, locomotor performance, and the cost of reproduction in free ranging lizards. *Evolution* 54: 1386–1395.

Milla S, Depiereux S, Kestemont P (2011) The effects of estrogenic and androgenic endocrine disruptors on the immune system of fish: a review. *Ecotoxicology* 20: 305–319.

Milligan CL, Wood CM (1986) Tissue intracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. *J Exp Biol* 123: 123–144.

Minke-Martin V, Hinch SG, Braun DC, Burnett NJ, Casselman MT, Eliason EJ, Middleton CT (2018) Physiological condition and migratory experience affect fitness-related outcomes in adult female sockeye salmon. *Ecol Freshw Fish* 27: 296–309.

Nikinmaa M, Tufts BL (1989) Regulation of acid and ion transfer across the membrane of nucleated erythrocytes. *Can J Zool* 67: 3039–3045.

Norin T, Clark TD (2016) Measurement and relevance of maximum metabolic rate in fishes: maximum metabolic rate in fishes. *J Fish Biol* 88: 122–151.

Patterson DA, Cooke SJ, Hinch SG, Robinson KA, Young N, Farrell AP, Miller KM (2016) A perspective on physiological studies supporting the provision of scientific advice for the management of Fraser River sockeye salmon (*Oncorhynchus nerka*). *Conserv Physiol* 4: cow026.

Patterson DA, Macdonald JS, Hinch SG, Healey MC, Farrell AP (2004) The effect of exercise and captivity on energy partitioning, reproductive maturation and fertilization success in adult sockeye salmon. *J Fish Biol* 64: 1039–1059.

Pinsky ML, Eikeset AM, McCauley DJ, Payne JL, Sunday JM (2019) Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature* 569: 108–111.

Quinn TP, Kinnison MT (1999) Size-selective and sex-selective predation by brown bears on sockeye salmon. *Oecologia* 121: 273–282.

Raby GD, Casselman MT, Cooke SJ, Hinch SG, Farrell AP, Clark TD (2016) Aerobic scope increases throughout an ecologically relevant temperature range in coho salmon. *J Exp Biol* 219: 1922–1931.

Rankin DJ, Kokko H (2006) Sex, death and tragedy. *Trends Ecol Evol* 21: 225–226.

Robinson KA, Hinch SG, Gale MK, Clark TD, Wilson SM, Donaldson MR, Farrell AP, Cooke SJ, Patterson DA (2013) Effects of post-capture ventilation assistance and elevated water temperature on sockeye salmon in a simulated capture-and-release experiment. *Conserv Physiol* 1. doi: 10.1093/conphys/cot015.

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

Rosewarne PJ, Wilson JM, Svendsen JC (2016) Measuring maximum and standard metabolic rates using intermittent-flow respirometry: a student laboratory investigation of aerobic metabolic scope and environmental hypoxia in aquatic breathers. *J Fish Biol* 88: 265–283.

Sandblom E, Clark TD, Hinch SG, Farrell AP (2009) Sex-specific differences in cardiac control and hematology of sockeye salmon (*Oncorhynchus*

nerka) approaching their spawning grounds. *Am J Physiol Reg Integr Comp Physiol* 297: R1136–R1143.

Scarabello M, Heigenhauser GJ, Wood CM (1991) The oxygen debt hypothesis in juvenile rainbow trout after exhaustive exercise. *Respir Physiol* 84: 245–259.

Scarabello M, Heigenhauser GJ, Wood CM (1992) Gas exchange, metabolite status and excess post-exercise oxygen consumption after repetitive bouts of exhaustive exercise in juvenile rainbow trout. *J Exp Biol* 167: 155–169.

Schulte PM (2015) The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J Exp Biol* 218: 1856–1866.

Sower SA, Schreck CB (1982) Steroid and thyroid hormones during sexual maturation of coho salmon (*Oncorhynchus kisutch*) in seawater of fresh water. *Gen Comp Endocrinol* 47: 42–53.

Spidle AP, Puinn TP, Bentzen P (1998) Sex-biased marine survival and growth in a population of coho salmon. *J Fish Biol* 52: 907–915.

Srean P, Almeida D, Rubio-Gracia F, Luo Y, García-Berthou E (2017) Effects of size and sex on swimming performance and metabolism of invasive mosquitofish *Gambusia holbrooki*. *Ecol Freshw Fish* 26: 424–433.

Standen EM, Hinch SG, Healey MC, Farrell AP (2002) Energetic costs of migration through the Fraser River Canyon, British Columbia, in adult pink (*Oncorhynchus gorbuscha*) and sockeye (*Oncorhynchus nerka*) salmon as assessed by EMG telemetry. *Can J Fish Aquat Sci* 59: 1809–1818.

Thorarensen H, Farrell AP (2006) postprandial intestinal blood flow, metabolic rates, and exercise in Chinook Salmon (*Oncorhynchus tshawytscha*). *Physiol Biochem Zool* 79: 688–694.

Truscott B, Idler DR, So YP, Walsh JM (1986) Maturational steroids and gonadotropin in upstream migratory sockeye salmon. *Gen Comp Endocrinol* 62: 99–110.

Tufts BL, Randall DJ (1989) The functional significance of adrenergic pH regulation in fish erythrocytes. *Can J Zool* 67: 235–238.

Urban MC (2015) Accelerating extinction risk from climate change. *Science* 348: 571–573.

Wagner GN, Kuchel LJ, Lotto A, Patterson DA, Shrimpton JM, Hinch SG, Farrell AP (2006) Routine and active metabolic rates of migrating adult wild sockeye salmon (*Oncorhynchus nerka* Walbaum) in seawater and freshwater. *Physiol Biochem Zool* 79: 100–108.

Wang Y, Heigenhauser GJ, Wood CM (1994) Integrated responses to exhaustive exercise and recovery in rainbow trout white muscle: acid-base, phosphogen, carbohydrate, lipid, ammonia, fluid volume and electrolyte metabolism. *J Exp Biol* 195: 227–258.

Welsh HH, Hodgson GR, Harvey BC, Roche MF (2001) Distribution of juvenile coho salmon in relation to water temperatures in tributaries of the Mattole River, California. *N Am J Fish Manag* 21: 464–470.

Williams IV, Brett JR (1987) Critical swimming speed of Fraser and Thompson River Pink Salmon (*Oncorhynchus gorbuscha*). *Can J Fish Aquat Sci* 44: 348–356.

Wilson SM, Hinch SG, Eliason EJ, Farrell AP, Cooke SJ (2013) Calibrating acoustic acceleration transmitters for estimating energy use by wild adult Pacific salmon. *Comp Biochem Physiol* 164: 491–498.

Wood CM (1991) Acid-base and ion balance, metabolism, and their interactions after exhaustive exercise in fish. *J Exp Biol* 160: 285–308.

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

Ytrestøyl T, Finstad B, McKinley RS (2001) Swimming performance and blood chemistry in Atlantic salmon spawners exposed to acid river water with elevated aluminium concentrations. *J Fish Biol* 58: 1025–1038.

Zhang Y, Claireaux G, Takle H, Jørgensen SM, Farrell AP (2018) A three-phase excess post-exercise oxygen consumption in Atlantic salmon *Salmo salar* and its response to exercise training. *J Fish Biol* 92: 1385–1403.