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RESEARCH ARTICLE

Reduced lactate dehydrogenase activity in the heart and suppressed sex hormone levels are associated with female-biased mortality during thermal stress in Pacific salmon

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ABSTRACT

Female-biased mortality has been repeatedly reported in Pacific salmon during their upriver migration in both field studies and laboratory holding experiments, especially in the presence of multiple environmental stressors, including thermal stress. Here, we used coho salmon (*Oncorhynchus kisutch*) to test whether females exposed to elevated water temperatures (18°C) (i) suppress circulating sex hormones (testosterone, 11-ketotestosterone and estradiol), owing to elevated cortisol levels, (ii) have higher activities of enzymes supporting anaerobic metabolism (e.g. lactate dehydrogenase, LDH), (iii) have lower activities of enzymes driving oxidative metabolism (e.g. citrate synthase, CS) in skeletal and cardiac muscle, and (iv) have more oxidative stress damage and reduced capacity for antioxidant defense [lower catalase (CAT) activity]. We found no evidence that a higher susceptibility to oxidative stress contributes to female-biased mortality at warm temperatures. We did, however, find that females had significantly lower cardiac LDH and that 18°C significantly reduced plasma levels of testosterone and estradiol, especially in females. We also found that relative gonad size was significantly lower in the 18°C treatment regardless of sex, whereas relative liver size was significantly lower in females held at 18°C. Further, relative spleen size was significantly elevated in the 18°C treatments across both sexes, with larger warm-induced increases in females. Our results suggest that males may better tolerate bouts of cardiac hypoxia at high temperature, and that thermal stress may also disrupt testosterone- and estradiol-mediated protein catabolism, and the immune response (larger spleens), in migratory female salmon.

KEY WORDS: Fish, Salmon, Temperature, Thermal stress, Metabolism, Oxidative stress, Hormones

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INTRODUCTION

Female-biased mortality represents an especially important conservation concern because female numbers typically determine the reproductive potential of a population, and subsequent male biases in operational sex ratios can be disproportionately costly for females (Hardy, 2002; Rankin and Kokko, 2006; Quinn, 1999; Garner et al., 2009). Over the past decade, female-biased mortality has been increasingly reported during upriver spawning migrations of Pacific salmon (e.g. Crossin et al., 2008; Jeffries et al., 2012; Martins et al., 2012; Keefer et al., 2008; Minke-Martin et al., 2018). This trend is especially apparent in the presence of multiple environmental stressors, including temperature (e.g. Jeffries et al., 2014). Although female-biased mortality has been demonstrated in both field (Burnett et al., 2014; Keefer et al., 2008; Martins et al., 2012; Minke-Martin et al., 2018; Roscoe et al., 2011) and laboratory holding studies (Jeffries et al., 2012; Raby et al., 2016), the underlying mechanisms that enhance female vulnerability during this once-in-a-lifetime migration are unknown. Understanding the physiological basis for female-biased mortality represents a crucial goal to help manage these ecologically and economically important species in a changing climate.

Thermal stress arguably represents the most pervasive factor influencing how populations will respond to the effects of climate change (e.g. Pörtner and Farrell, 2008). The current trend has seen a 1–2°C increase in peak summer water temperatures in the Fraser River basin (British Columbia, Canada), with future predictions estimating a 3-fold increase in the number of days above 19°C (a critical salmon threshold) over the next 50–100 years (Ferrari et al., 2007; Hague et al., 2011). Adult Pacific salmon are especially vulnerable to rising summer temperatures because this is when many populations migrate upstream, already encountering river temperatures near their upper thermal limits (Eliason and Farrell, 2016). Increasing water temperatures are known to compromise cardiorespiratory performance (Eliason et al., 2011; Eliason et al., 2013), upregulate the hypothalamic–pituitary–interrenal (HPI) axis (Strange et al., 1977; Raby et al., 2016) and promote the production of reactive oxygen species (ROS; Nakano et al., 2014). The present study aimed to determine the physiological differences that make female Pacific salmon more vulnerable to thermal stress than males.

There is some evidence that sex-specific differences in aerobic capacity may underlie female-biased mortality with rising temperatures. In pink salmon (*Oncorhynchus gorbuscha*), for instance, aerobic scope (the absolute difference between standard metabolic rate and maximum metabolic rate; AS) is lower in females than in males (Clark et al., 2011). In sockeye salmon (*O. nerka*), females rely more heavily on anaerobic metabolism during dam passage than males (Burnett et al., 2014), and have higher levels of cardiac lactate following handling stress (Eliason et al., 2020).

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In another salmonid, the rainbow trout (*O. mykiss*), females have significantly lower scope for coronary blood flow at high temperatures (Ekström et al., 2017). These combined data suggest that females may be more vulnerable to oxygen supply limitations during thermal stress, especially in the heart. Although upregulation of anaerobic metabolism may help offset oxygen limitations, this compensatory response is less efficient in terms of energy conversion and may carry other costs. Higher rates of glycolytically fuelled burst swimming, for instance, have been associated with upriver migration mortality (Burnett et al., 2014). We therefore hypothesized that skeletal and cardiac muscle of female Pacific salmon are more primed for anaerobic metabolism than their male counterparts.

A second hypothesis is that differences in the neuroendocrine stress response underlie female-biased mortality at high temperatures. Plasma cortisol levels typically peak during upriver migration (Hruska et al., 2007; Carruth et al., 2002), and female Pacific salmon are known to have far higher levels of circulating cortisol than their male counterparts (e.g. Schmidt and Idler, 1962; Hinch et al., 2006). Females are also known to experience elevated and more prolonged cortisol spikes in response to stress (Hruska et al., 2010; Cook et al., 2011; Donaldson et al., 2014; Raby et al., 2016). Increases in cortisol energetically prime fish to respond to acute stressors by increasing blood glucose, liver glycogen and branchial blood flow (Barton and Iwama, 1991; Mommsen et al., 1999). However, chronic elevation of plasma cortisol above baseline levels as a result of stress can disrupt intermediary metabolism (see Van Der Boon et al., 1991) and reproductive endocrine signalling (Carragher et al., 1989; Pankhurst and Van Der Kraak, 2000; McConnachie et al., 2012). This means that a cortisol-driven decrease in testosterone and estradiol can then potentially delay sexual maturation rates (Truscott et al., 1986; Jeffries et al., 2012), and has also been linked to river mortality in Pacific salmon (Cooke et al., 2006). We were therefore interested in how high temperature stress differentially affects glucose metabolism and sex hormone signalling in male and female Pacific salmon.

A third hypothesis is that females may be more susceptible to oxidative damage as temperatures rise. At a molecular level, ROS damage DNA, lipids and proteins (see Beckman and Ames, 1998). Importantly, it has been hypothesized that ROS production indirectly increases costs of reproduction and exercise (Beckman and Ames, 1998; Costantini, 2008). Cessation of feeding during the spawning migration means Pacific salmon only have finite antioxidant stores to protect against ROS (Wilson et al., 2014), and increased metabolic rates as a function of higher river temperatures are also likely to augment ROS production (Bagnyukova et al., 2007). There is some evidence, however, that females may be more vulnerable to ROS than males. High levels of testosterone have been linked to increased production of ROS (see Metcalfe and Alonso-Alvarez, 2010), and high levels of estradiol can compromise antioxidant activity (Thilagam et al., 2010). Plasma testosterone levels are relatively low and stable throughout the upriver migration in male Pacific salmon, where estradiol is virtually absent (Sower and Schreck, 1982; Truscott et al., 1986; Hruska et al., 2007). In females, however, both testosterone and estradiol naturally increase throughout the upriver migration, reaching levels between 2- and 3-fold higher than during their initial entry into the river (Sower and Schreck, 1982; Truscott et al., 1986; Hruska et al., 2007). We might thereby expect increased ROS damage in females, as climbing levels of testosterone and estradiol enhance ROS production and reduce rates of ROS clearance, respectively.

The aim of our study was to explore these three hypotheses that could potentially explain the elevated female mortality phenomenon in Pacific salmon. Specifically, we sought to determine whether differences in anaerobic versus oxidative metabolism, sex hormone signalling and oxidative stress might contribute to female-biased mortality in Pacific salmon exposed to thermal stress. We acclimated mature migratory coho salmon (*O. kisutch*) to two separate thermal regimes (9°C and 18°C). We then measured differences in metabolic enzyme activities (lactate dehydrogenase, LDH; citrate synthase, CS) in skeletal and cardiac muscles, blood chemistry (lactate, glucose, testosterone, estradiol and electrolytes), ROS damage [thiobarbituric acid reactive substances (TBARS)] and antioxidant enzyme activity (catalase, CAT) between male and females from each temperature treatment. We predicted that relative to males and females at 9°C and males at 18°C, females exposed to 18°C would have: (i) higher LDH but lower CS activities (i.e. females would be more primed for anaerobic exercise), especially in the heart, (ii) higher plasma glucose and lower levels of circulating sex hormones (testosterone and estradiol) and (iii) more ROS damage (higher TBARS concentrations) and reduced capacity for antioxidant defense (lower CAT activity).

MATERIALS AND METHODS

Animal collection and holding

Adult coho salmon [*Oncorhynchus kisutch* (Walbaum 1792)] were collected from the Chilliwack River Hatchery (British Columbia, Canada; hatchery water temperature 9°C during collection) and transported 23.7 km in a 1250 l tank (8.2–10.4°C; >90% air saturation) to the Fisheries and Oceans Canada Cultus Lake Research Laboratory (British Columbia, Canada). Fish were held in flow-through UV-sterilized and sand-filtered freshwater under natural photoperiod in 8000 l outdoor holding tanks (9°C; >90% air saturation; $n \leq 27$ fish per tank, mixed sex) for at least 36 h before experimentation to ensure recovery from handling stress. Fish were then moved to either 9°C or 18°C treatment tanks (1.8 m diameter; 3–6 fish per tank), where water either remained at 9°C, or was increased at a rate of 2–4°C h^{-1} until 18°C was reached. This represents an ecologically relevant rate of warming in Pacific salmon, which perform rapid vertical migrations both in the ocean and in lakes, during which they can encounter a 5–10°C temperature swing in less than an hour (Eliason and Farrell, 2016). Fish were held at 18°C for 4.77±0.34 days (mean±s.d.; range: 4.51–5.75 days) before they were sampled compared with their 9°C counterparts. We chose 9°C to approximate current river temperatures, whereas 18°C was used to represent a future climate change scenario for this population. It should be noted, however, that some populations across the southern range of the species already experience temperatures as high as 18°C (Welsh et al., 2001; Hayes et al., 2011).

Following these acclimation periods, fish were removed from their tanks and rapidly euthanized by cerebral concussion. A blood sample was immediately collected from the caudal vein and stored in an ice slurry before being centrifuged at 7000 ***g*** for 5 min. Plasma was stored at −80°C until analysis. A skeletal muscle sample (<0.5 cm thick), comprising both red and white muscle fibres, was removed from halfway between the dorsal and anal fin, and the ventricle was removed and bisected. The muscle and ventricle samples were blotted dry of blood, rapidly freeze-clamped in liquid nitrogen and stored at −80°C until analysis. Morphometric data (e.g. body mass, length and organ masses) were collected from the carcasses. In general, we acclimated N=12 males and N=12 females to 9°C, and N=12 males and N=12 females to 18°C. However, actual sample sizes vary between the different assays and are further noted in Tables 1 and 2. These sample sizes are known to provide sufficient

Table 1. Results and statistical (ANOVA) parameters for blood chemistry, sex hormones, relative organ sizes and thiobarbituric acid reactive substances (TBARS) of wild adult coho salmon acclimated to two test temperatures

	Test temperature				Statistical parameters								
					Sex			Acclimation temperature			Sex×Acclimation temperature		
	9°C		18°C		*F*	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
Plasma	Male (*N*=12)	Female (*N*=11)	Male (*N*=11)	Female (*N*=10)									
Lactate (μmol l^{-1})	2.15±1.59	1.66±1.00	3.92±2.02	3.10±1.87	0.79	1	0.380	13.16	1	<0.001	0.04	1	0.847
Glucose (μmol l^{-1})	4.44±0.64	4.62±0.65	5.06±1.57	5.18±1.05	0.24	1	0.628	3.50	1	0.069	0.01	1	0.928
Osmolality (mOsm kg^{-1})	313.42±9.88	308.18±9.35	307.55±8.57	308.10±7.34	0.76	1	0.389	1.23	1	0.275	1.16	1	0.288
Na^+ (μmol l^{-1})	148.22±5.53	143.82±5.39	141.52±8.54	143.60±5.12	0.37	1	0.547	3.28	1	0.078	2.89	1	0.097
K^+ (μmol l^{-1})	3.74±1.35	3.85±1.15	3.53±0.69	3.40±0.97	2.25e-4	1	0.988	1.01	1	0.321	0.13	1	0.721
Cl^- (μmol l^{-1})	134.71±3.85	133.36±7.59	131.36±8.53	129.25±7.05	0.68	1	0.413	3.19	1	0.082	0.03	1	0.855
Hormones (ng ml^{-1})	Male (*N*=12)	Female (*N*=11)	Male (*N*=11)	Female (*N*=11)									
Testosterone	104.22±142.95	383.57±132.31	29.12±39.32	80.85±81.01	46.27	1	<0.001	45.34	1	<0.001	12.08	1	**0.001**
Estradiol	0.26±0.13	30.54±15.81	0.23±0.11	7.21±6.70	174.90	1	<0.001	10.38	1	0.003	7.923	1	**0.007**
	Male (*N*=9)	Female (*N*=7)	Male (*N*=9)	Female (*N*=7)									
11-KT	106.28±59.76	5.50±1.47	52.18±35.68	2.58±1.12	119.55	1	<0.001	16.15	1	<0.001	1.17	1	0.288
Organ mass[a]	Male (*N*=11)	Female (*N*=10–11)	Male (*N*=11–12)	Female (*N*=10–11)									
GSI	5.39±1.34	19.81±2.52	4.93±1.12	17.51±2.21	346.91	1	<0.001	4.12	1	0.049	2.68	1	0.109
RVM	0.20±0.02	0.17±0.02	0.20±0.02	0.17±0.02	35.14	1	<0.001	0.14	1	0.706	0.06	1	0.810
SSI	0.23±0.08	0.12±0.05	0.27±0.13	0.32±0.36	12.27	1	0.001	7.57	1	0.009	4.31	1	**0.045**
HSI	1.57±0.36	2.45±0.31	1.51±0.23	1.91±0.31	43.97	1	<0.001	8.56	1	0.006	7.57	1	**0.009**
TBARS (μmol g^{-1})	Male (*N*=10–11)	Female (*N*=9–11)	Male (*N*=9–10)	Female (*N*=9–10)									
Red muscle	98.68±65.25	64.77±26.50	58.74±20.70	49.51±13.64	3.10	1	0.087	2.80	1	0.103	0.59	1	0.449
White muscle	12.33±12.87	17.15±29.56	8.62±10.32	8.86±7.45	0.21	1	0.651	2.20	1	0.286	0.17	1	0.682
Cardiac muscle	14.87±2.54	16.50±5.99	14.35±4.60	13.51±2.87	0.03	1	0.861	2.06	1	0.147	0.04	1	0.839

11-KT, 11-ketotestosterone; GSI, gonadosomatic index; RVM, relative ventricular mass; HSI, hepatosomatic index; SSI, splenosomatic index. Bold represents significant differences (P<0.05).
[a]Relative organ mass (organ mass/body mass×100).

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Table 2. Statistical (mixed-model ANOVA) parameters for activities of lactate dehydrogenase (LDH), citrate synthase (CS) and catalase (CAT)

		F	d.f.	Den d.f.	*P*
Red muscle					
LDH (*N*=10–12)	Sex	1.547	1	42.00	0.221
	Acclimation temperature	1.367	1	42.00	0.249
	Test temperature	212.022	4	168.00	**<0.001**
	Sex×Acclimation temperature	0.080	1	42.00	0.779
	Sex×Test temperature	1.547	4	168.00	0.191
	Acclimation temperature×Test temperature	0.389	4	168.00	0.816
	Sex×Acclimation temperature×Test temperature	0.662	4	168.00	0.619
CS (*N*=10–12)	Sex	0.054	1	42.00	0.818
	Acclimation temperature	0.027	1	42.00	0.871
	Test temperature	80.646	4	168.00	**<0.001**
	Sex×Acclimation temperature	2.730	1	42.00	0.106
	Sex×Test temperature	1.777	4	168.00	0.136
	Acclimation temperature×Test temperature	0.469	4	168.00	0.758
	Sex×Acclimation temperature×Test temperature	0.124	4	168.00	0.974
CAT (*N*=4–11)	Sex	0.028	1	43.45	0.869
	Acclimation temperature	9.382	1	43.45	**0.004**
	Test temperature	9.228	4	123.90	**<0.001**
	Sex×Acclimation temperature	0.068	1	43.45	0.795
	Sex×Test temperature	1.418	4	123.90	0.232
	Acclimation temperature×Test temperature	0.426	4	123.90	0.790
	Sex×Acclimation temperature×Test temperature	0.930	4	123.90	0.449
White muscle					
LDH (*N*=10–12)	Sex	0.125	1	42.00	0.726
	Acclimation temperature	0.571	1	42.00	0.454
	Test temperature	402.037	4	168.00	**<0.001**
	Sex×Acclimation temperature	2.121	1	42.00	0.153
	Sex×Test temperature	0.113	4	168.00	0.978
	Acclimation temperature×Test temperature	0.364	4	168.00	0.834
	Sex×Acclimation temperature×Test temperature	1.273	4	168.00	0.282
CS (*N*=10–12)	Sex	1.350	1	42.00	0.525
	Acclimation temperature	6.569	1	42.00	**0.024**
	Test temperature	65.208	4	168.00	**<0.001**
	Sex×Acclimation temperature	0.654	1	42.00	0.423
	Sex×Test temperature	0.643	4	168.00	0.633
	Acclimation temperature×Test temperature	1.615	4	168.00	0.173
	Sex×Acclimation temperature×Test temperature	0.340	4	168.00	0.851
CAT (*N*=8–12)	Sex	0.984	1	39.92	0.327
	Acclimation temperature	18.785	1	39.92	**<0.001**
	Test temperature	3.611	4	149.42	**0.008**
	Sex×Acclimation temperature	0.921	1	39.92	0.343
	Sex×Test temperature	0.285	4	149.42	0.887
	Acclimation temperature×Test temperature	2.561	4	149.42	**0.041**
	Sex×Acclimation temperature×Test temperature	0.631	4	149.42	0.641
Cardiac muscle					
LDH (*N*=11–12)	Sex	5.382	1	42.00	**0.025**
	Acclimation temperature	0.403	1	42.00	0.529
	Test temperature	225.469	4	168.00	**<0.001**
	Sex×Acclimation temperature	0.071	1	42.00	0.791
	Sex×Test temperature	2.143	4	168.00	0.078
	Acclimation temperature×Test temperature	0.745	4	168.00	0.562
	Sex×Acclimation temperature×Test temperature	1.232	4	168.00	0.299
CS (*N*=11–12)	Sex	3.151	1	42.00	0.083
	Acclimation temperature	0.144	1	42.00	0.706
	Test temperature	431.366	4	168.00	**<0.001**
	Sex×Acclimation temperature	0.410	1	42.00	0.525
	Sex×Test temperature	0.117	4	168.00	0.976
	Acclimation temperature×Test temperature	1.326	4	168.00	0.262
	Sex×Acclimation temperature×Test temperature	1.107	4	168.00	0.355
CAT (*N*=8–11)	Sex	2.885	1	39.06	0.097
	Acclimation temperature	1.222	1	39.06	0.276
	Test temperature	36.194	4	122.64	**<0.001**
	Sex×Acclimation temperature	2.457	1	39.06	0.125
	Sex×Test temperature	0.915	4	122.64	0.458
	Acclimation temperature×Test temperature	0.845	4	122.64	0.499
	Sex×Acclimation temperature×Test temperature	1.395	4	122.64	0.240

Bold represents significant differences (P<0.05).

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statistical power to detect effect sizes expected from previous work in Pacific salmon or closely related species. 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 no. A17-0160).

Blood chemistry

Plasma metabolites were analyzed according to established methods (Farrell et al., 2001; Richards and Hultin, 2002). Briefly, sodium and potassium were determined using an XP Five-channel Flame Photometer (BWB Technologies, UK), chloride was measured with a Chlorochek Digital Chloridometer (EliTech Group, France), and osmolality was determined using a 3320 Freezing Point Osmometer (Advanced Instruments, USA). Lactate and glucose were measured with a 2300 stat plus glucose and L-lactate analyzer (YSI, USA). All samples were run in duplicate.

Sex hormones

Testosterone, 11-ketotestosterone and 17B-estradiol were extracted using ethyl ether, and quantified using commercially available ELISA kits (Testosterone and 17B-estradiol, Neogen, USA; 11-ketotestosterone, Cayman Chemical, USA) according to manufacturer instructions. All plates were read on a FLUOstar Omega multi-mode microplate reader (BMG Labtech, USA). Measurements for testosterone and 17B-estradiol were made in duplicate, whereas those for 11-ketotestosterone were made in duplicate across at least two dilutions to test for interference. Assays for 11-ketotestosterone were performed approximately 2 years after tissue collection. Some samples therefore were rejected owing to high intra- and inter-assay variation in concentrations.

Enzyme assays

Enzyme activities for LDH (anaerobic), CS (oxidative) and CAT (oxidative repair) were measured in homogenates of ventricle, red muscle and white muscle tissues to determine thermal performance across 9, 14, 18, 22 and 25°C test temperatures according to previously described methods (LDH, Martínez et al., 2006; CS, Moyes et al., 1997; CAT, Ghanizadeh Kazerouni et al., 2016). In short, tissues were homogenized in homogenization buffer (LDH and CS: 0.1% Triton, 50 mmol l^{-1} Hepes, 1 mmol l^{-1} EDTA, pH 7.4; CAT: 100 mmol l^{-1} K phosphate buffer, 100 mmol l^{-1} KCl, 1 mmol l^{-1} EDTA, pH 7.4) on ice. All assays were run in triplicate on a SpectraMax iD3 Multi-Mode Microplate Reader (Molecular Devices, USA) using a wavelength of 340 nm to measure the disappearance of NADH for LDH activity, a wavelength of 412 nm to measure the production of 2-nitro-5-thiobenzoic acid as a proxy of CS activity, or a wavelength of 240 nm to measure the disappearance of H_2O_2 for CAT activity. Absorbance readings were automatically normalized to 1 cm via the integrated PathCheck sensor technology (Molecular Devices, USA). Extinction coefficients were 6.22, 14.1 and 40 l $mmol^{-1}$ cm^{-1} for LDH, CS and CAT, respectively.

Oxidative damage

TBARS were quantified separately for tissue from the ventricle, red skeletal muscle and white skeletal muscle to identify levels of lipid peroxidation using a commercially available colorimetric assay kit (Cayman Chemical). Briefly, tissues were homogenized in RIPA buffer and treated with thiobarbituric acid under high temperature (100°C) and acidity. TBARS were then quantified by comparing optical densities at 535 nm to simultaneously determined standard curves for malondialdehyde. All samples were run in duplicate.

Statistical analysis

All statistical analyses were done using the free and open software JAMOVI (Version 0.9; GAMLj module; https://www.jamovi.org). Categorical variables [blood chemistry (lactate, glucose, Cl^-, osmolality, Na^+, K^+), sex hormones (17B-estradiol, testosterone, 11-ketotestosterone), organ masses (gonads, liver, ventricle and spleen)] and TBARS were analyzed with general linear models and ANOVA. Acclimation temperature, sex and their interaction term were used as predictor variables in all models. In models examining organ masses, we included body mass as a covariate. Kinetic enzyme assays were analyzed with mixed models with effect terms: sex, acclimation temperature, test temperature and their interaction terms (sex×acclimation temperature, sex×test temperature, acclimation temperature×test temperature, and a three-way interaction sex×acclimation temperature×test temperature). Fish ID was used as a cluster variable to account for repeated measures across the different test temperatures. Metrics were investigated for normality using quantile-quantile plots. Non-normal data were transformed (log_{10}, square root, Box–Cox) prior to statistical analysis. Heteroskedasticity was subsequently assessed via Levene's test. All data are displayed with untransformed values. Significance levels were set at 0.05, and data are presented as means±s.e.m. in figures and as means±s.d. in tables. Thermal performance curves for enzyme activity are presented with either quadratic or linear curves, depending on best fit (Akaike's information criterion).

RESULTS

Relative organ size

Females had a significantly larger GSI than males, and both sexes in the 18°C treatment had significantly smaller gonads than at 9°C (9°C: males 5.4±1.3%, females 19.8±2.5%; 18°C: males 4.9±1.1%, females: 17.5±0.8%; Table 1). There was no significant effect of acclimation temperature on mean ventricle size relative to body mass (relative ventricular mass; RVM). However, there was a significant effect of sex, where RVM was approximately 12% higher in males (Table 1). There was a significant interaction between acclimation temperature and sex on relative spleen size (splenosomatic index, SSI; Table 1). Specifically, mean female SSI was approximately half that of males at 9°C but increased more drastically with temperature so that it was nearly 20% larger at 18°C. Specifically, SSI was elevated in the 18°C treatment for both sexes (Table 1). Also, a significant interaction existed between sex and acclimation temperature on relative liver size (hepatosomatic index, HSI), with mean HSI being lower in females held at 18°C but no different in males (Table 1).

Blood chemistry

Plasma lactate levels were significantly higher in the 18°C treatments. This effect was independent of sex, as plasma lactate did not significantly differ between males and females at either temperature (Table 1). There were no significant effects of sex or acclimation temperature on plasma osmolality or the plasma concentrations of glucose, Na^+, K^+ or Cl^- (Table 1).

Enzyme assays

In the ventricle, there was a main effect of sex and test temperature on LDH activity (Fig. 1A, Table 2). Males had higher ventricular rates of LDH activity. LDH activity increased with test temperature in both sexes, with a marginally significant interaction between sex and test temperature, where enzyme activity appears to increase more quickly with test temperature in males than in females (Table 2). In contrast, neither sex nor acclimation temperature had

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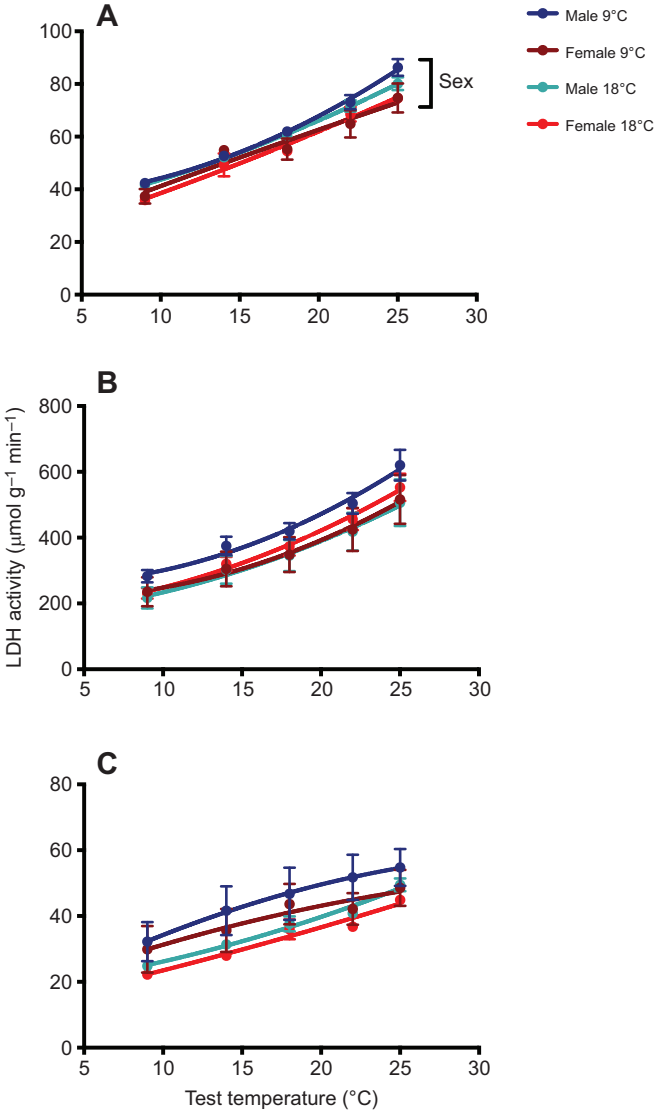

Fig. 1. Lactate dehydrogenase (LDH) activity in skeletal and cardiac muscle in coho salmon. (A) Cardiac muscle, (B) white muscle and (C) red muscle. A statistically significant (P<0.05) main effect of sex is noted in A. Test temperature represented a significant main effect in all panels. Each sample represents a biological replicate and all samples were run in triplicate. Data are means±s.e.m.

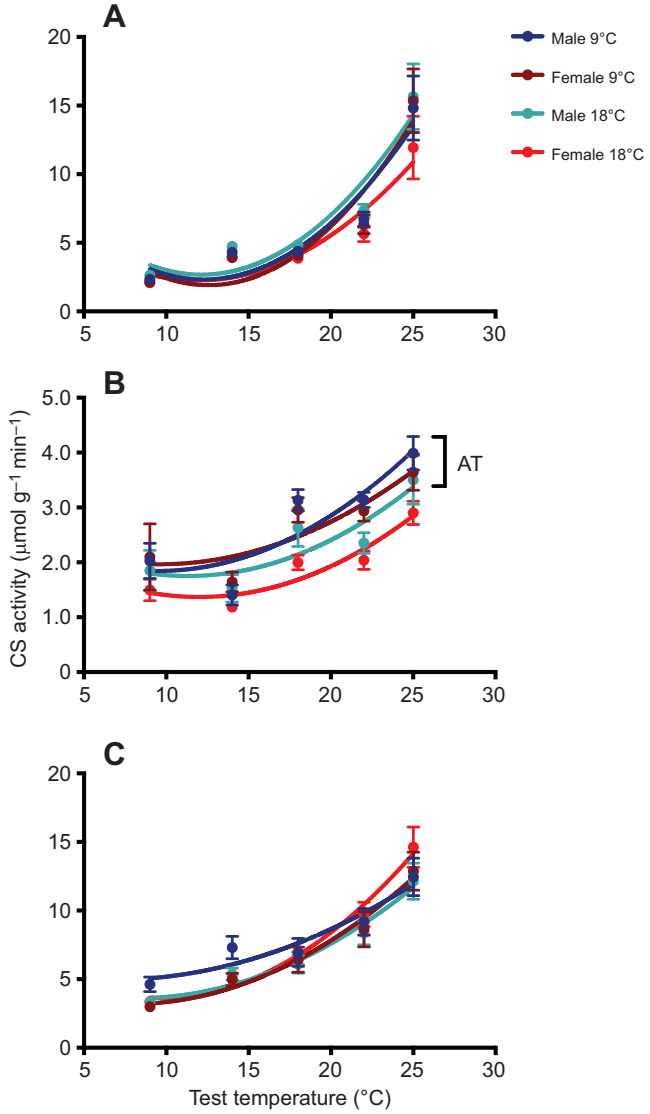

Fig. 2. Citrate synthase (CS) activity in skeletal and cardiac muscle in coho salmon. (A) Cardiac muscle, (B) white muscle and (C) red muscle. A statistically significant (P<0.05) main effect of acclimation temperature (AT) is noted in B. Test temperature represented a significant main effect in all panels. Each sample represents a biological replicate and all samples were run in triplicate. Data are means±s.e.m.

significant effects on maximal LDH activity in white or red skeletal muscle, although LDH activity similarly increased with test temperature in both tissues (Fig. 1B,C, Table 2). There were no significant effects of sex or acclimation temperature on CS activity in ventricular, white muscle or red muscle tissues (Fig. 2, Table 2). For the antioxidant enzyme activities, acclimation temperature and test temperature had significant effects on CAT activity in red muscle (Fig. 3C, Table 2). CAT activity was significantly higher in fish acclimated to 9°C, regardless of sex, and increased with test temperature. A significant interaction existed between acclimation temperature and test temperature in CAT activity in white muscle, where CAT activity was significantly higher in 9°C-acclimated fish at the higher test temperatures (Fig. 3B, Table 2). Also, test temperature significantly affected CAT activity in the ventricle, and there was a trend (P=0.097) for ventricular CAT activity to be higher in males (Fig. 3A, Table 2).

Sex hormones

A significant interaction existed between sex and acclimation temperature on plasma testosterone levels (Table 1). Although mean testosterone levels were significantly lower in the 18°C treatment in both sexes, the difference was much more pronounced in females (Table 1). Males had significantly higher levels of 11-ketotestosterone than females, and warmer temperatures significantly decreased 11-ketotestosterone concentrations in both sexes (Table 1). A significant interaction existed between sex and acclimation temperature on plasma estradiol levels (Table 1), with estradiol levels decreasing by nearly 75% in females in the 18°C treatment, but not in males, where the estradiol level was negligible (Table 1).

Oxidative damage

There were no significant effects of sex or acclimation temperature on the concentration of TBARS in red muscle, white muscle or in

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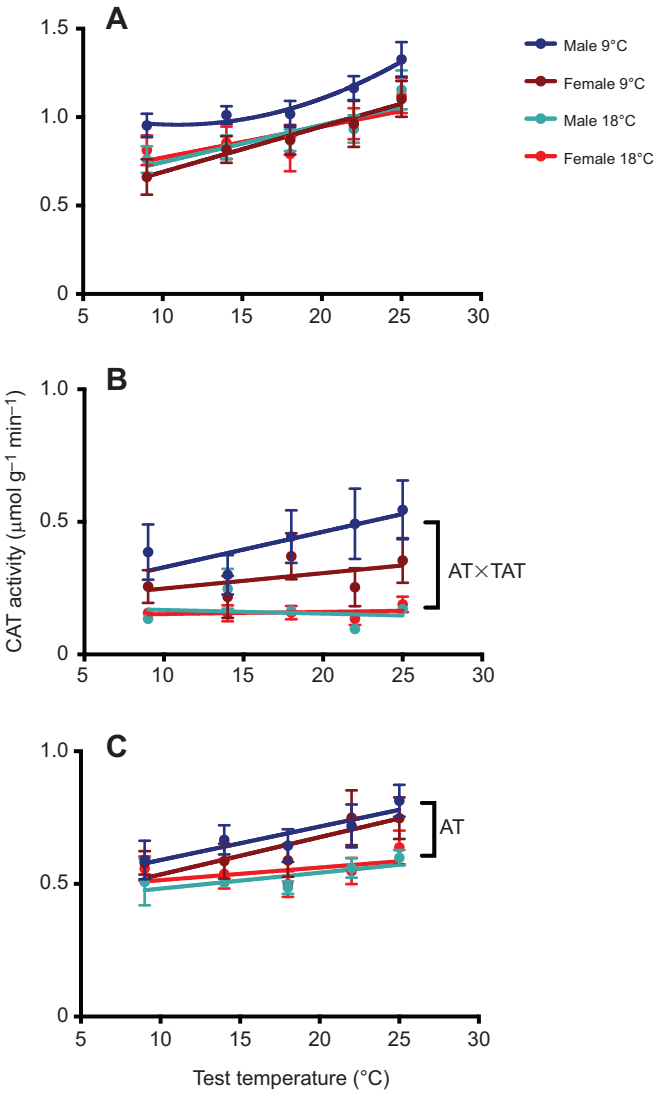

Fig. 3. Catalase (CAT) activity in skeletal and cardiac muscle in coho salmon. (A) Cardiac muscle, (B) white muscle and (C) red muscle. Statistically significant (P<0.05) effects are represented by AT (main effect of acclimation temperature) and AT×TT (interaction between acclimation temperature and test temperature). Test temperature represented a significant main effect in all cardiac (A) and red muscle (C). Each sample represents a biological replicate and all samples were run in triplicate. Data are means±s.e.m.

the ventricle (Table 1). However, the effect of sex on TBARS concentrations approached statistical significance in red muscle (P=0.087), where males tended to have higher levels in red muscle than females (Table 1).

DISCUSSION

Here, we considered potential physiological mechanisms that could contribute to female-biased mortality in response to thermal stress during the spawning migration in Pacific salmon. Although our results render it difficult to pinpoint a specific mechanism, we revealed that (i) sex-specific differences in anaerobic metabolism in the heart, and (ii) changes in sex hormone signalling might play roles in the elevated female mortality rates observed at high temperatures.

Anaerobic metabolism

We found no evidence that females rely more on anaerobic metabolism at rest during high temperature exposure than males. At a tissue level, we predicted that females would be more vulnerable to cardiac oxygen limitations at high temperatures and would therefore upregulate anaerobic pathways. Contrary to this hypothesis, however, ventricular LDH activity was significantly higher in males than in females. Because LDH is needed for a tissue to cope with oxygen limitations, the higher LDH activities in male hearts suggests a greater capacity to cope with hypoxia. This could be due to genetic differences that result in higher constitutive LDH in males or a plastic response to prior hypoxia stress. Increased LDH activities may also indicate that males may have a greater capacity to metabolize lactate (Chippari-Gomes et al., 2005; Crans et al., 2015; Borowiec et al., 2016), which appears to be a preferred extracellular substrate to glucose in salmonids (Lanctin et al., 1980; Farrell et al., 1988). The nearly significant interaction between sex and test temperatures (Table 2) indicates that LDH activities in male ventricles increase more quickly as a function of ambient temperature than in females. In fact, ventricular LDH activities in males were more than 10% higher than for females at the 18°C test temperatures. This represents a departure from previous work in sexually immature hatchery-reared rainbow trout, where female ventricles had higher LDH activities than males (Battiprolu et al., 2007). Moreover, male Pacific salmon are known to have a larger RVM than females (Clark et al., 2008; Sandblom et al., 2009). In the present study, mean ventricular mass in males was approximately 16% bigger than in females (male RVM, 0.199; female RVM, 0.171; Table 1). Earlier work suggested that a cardiac oxygen limitation may contribute to higher mortality in female Pacific salmon during stress (Eliason et al., 2020). Ventricles of female rainbow trout are also more hypoxia tolerant (Battiprolu et al., 2007; Battiprolu and Rodnick, 2014), suggesting that they may experience bouts of hypoxia more routinely – though this idea has not been tested. Our work indicates that a reduced reliance on anaerobic metabolism combined with smaller ventricles in female hearts may undermine cardiac performance as temperatures rise. Collectively, these studies suggest that a cardiac limitation might contribute to heightened female mortality.

Stress response

Migratory female salmon are known to have elevated plasma cortisol (e.g. Schmidt and Idler, 1962; Kubokawa et al., 2001) with higher and more prolonged cortisol spikes in response to stress (e.g. Raby et al., 2016; Hruska et al., 2010; Cook et al., 2011; Donaldson et al., 2014; Eliason et al., 2020). We therefore predicted that downstream pathways (i.e. sex hormone signalling and carbohydrate metabolism) would be disrupted in females exposed to thermal stress (i.e. the 18°C treatment). We did find evidence that female-biased mortality in Pacific salmon may stem from the disruption of sex hormone signalling. As predicted, testosterone levels were markedly higher in females, and the effect of temperature was much more pronounced. Although testosterone drives gonadal development and secondary sex characteristics in male Pacific salmon, it also plays crucial roles in migratory females, including the regulation of oocyte development and final maturation (Truscott et al., 1986). Reductions in testosterone levels may thereby delay rates of maturation in female salmon at high temperatures, which potentially explains the reduced rates of final maturation observed in female pink and sockeye salmon held at 19°C (Jeffries et al., 2012). Supporting this suggestion, we found reduced GSIs in both sexes of the 18°C treatment relative to their 9°C counterparts. We also found reduced mean liver size in females, but relatively no effect in males. Because estradiol signals liver synthesis of vitellogenin and eggshell proteins (Korsgaard et al.,

1986; Berg et al., 2004; Larsen et al., 1992), thereby increasing liver size, the relatively low HSI of females at 18°C may also represent a delay in maturation, or an overall reduction in egg mass, as a consequence of reduced estradiol levels. Alternatively, a lower HSI is also consistent with reduced energy reserves (i.e. glycogen; Arndt et al., 1996), potentially indicating substrate limitations in female salmon exposed to high temperatures.

Although the mechanisms remain unknown, lower levels of circulating testosterone have also been linked to upper river mortality in sockeye salmon (Cooke et al., 2006). However, the large decrease in 11-ketotestosterone suggests that males may face their own sex-specific challenges with warming temperatures. 11-Ketotestosterone represents a primary androgen in male salmon (Idler et al., 1961), where it promotes sexual maturation and upstream migratory behaviours (Munakata et al., 2001). This means that maturation may also be delayed in males exposed to high migratory temperatures. Although circulating levels of estradiol were virtually non-detectable in males at either temperature, they plummeted by approximately 75% in females held in the 18°C treatment when compared with the 9°C treatment. Again, this suggests that low estradiol may contribute to delays in female maturation as a function of temperature. These warm temperature effects were seen for other sex hormones, with females appearing to suffer more drastic reductions in sex hormones than do males at warm temperatures. For example, plasma testosterone concentrations, which were more than 3-fold higher in females at 9°C, fell by roughly 80% in females exposed to 18°C and by 70% in males exposed to the same temperature (Table 1). Also, the 75% decrease in estradiol concentration in females during warming is comparable to the 50% decrease 11-ketotestosterone in males during warming. Although it is difficult to reconcile how concentration differences in these three hormones impact each sex comparatively, it is clear that females suffer greater absolute and factorial reductions in sex hormones at high temperatures. Both testosterone and estradiol levels have also been correlated with faster and more successful migrations (Sato et al., 1997; Crossin et al., 2008; Cooke et al., 2008), although mechanisms underlying these effects are not known. However, both testosterone and estradiol increase rates of protein utilization, which likely underlie the repartitioning of white muscle proteins to the gonads (Cleveland and Weber, 2011), and facilitate white muscle as an energy source once lipid reserves become depleted (Ando, 1986). A caveat may be that female Pacific salmon suffer substrate limitations during migration, as high temperatures reduce plasma testosterone and estradiol, potentially compromising these important signalling pathways for protein catabolism.

Contrary to our expectations, there were no significant differences in plasma lactate, glucose or electrolytes (i.e. Na^+, K^+ or Cl^-) between males and females at either acclimation temperature. This reflects a divergence from previous work in pink salmon (*O. gorbuscha*) and sockeye salmon, where plasma osmolality and Cl^- were elevated following exposure to 19°C, and both plasma glucose and lactate were elevated in females (Jeffries et al., 2012). Although a substantial body of work in rainbow trout suggests that cortisol mediates glucose and lactate metabolism (e.g. Eros and Milligan, 1996; Milligan, 1996, 1997; Milligan et al., 2000), the link between circulating cortisol and plasma lactate varies greatly across teleost species (see Mommsen et al., 1999), and can even differ among salmonids (Barton, 2000). Previous work in female pink salmon supplemented with exogenous cortisol during their upriver migration also found no evidence elevated cortisol influences blood glucose levels (McConnachie et al., 2012).

Cortisol is also known to disrupt the immune response (Maule et al., 1987; 1989; Maule and Schreck, 1990), and is positively associated with disease-induced mortality (Pickering and Pottinger, 1989). This means that females may be at greater risk of infection than males. Although we did not set out to test this hypothesis, the higher relative spleen size in males relative to females may represent enhanced immunocompetence. For example, SSI is known to positively correlate with resistance to infections in rainbow trout (Hadidi et al., 2008). Further, the increase in SSI in both sexes in the warm treatment, where aquatic infections likely intensify (Snieszko, 1974; McCullough, 1999; Materna, 2001; Crossin et al., 2008), may signify a mounting immune response. Together, this may mean that the higher cortisol levels and relatively smaller spleens of female Pacific salmon reflect a heightened susceptibility to infection at high temperature. In fact, recent work shows that higher intensities of infection at elevated temperatures at least partly account for higher mortality rates in female coho salmon following handling stress in warm water (Teffer et al., 2019). Differences in spleen size could also reflect differences in the ability to mobilize sequestered red blood cells (Gallaugher et al., 1992), where males have the greater ability.

Oxidative stress

At odds with our hypothesis, we found little evidence that differences in oxidative damage explain female-biased mortality in migratory Pacific salmon despite the fact that oxidative stress markers are regarded as sensitive measures (Birnie-Gauvin et al., 2017). Females did not have elevated evidence of ROS damage (i.e. TBARS), nor did they appear to be investing in antioxidant defences (i.e. CAT activity) more than males in any of the tissues we examined. Red muscle had the highest level of TBARS, presumably owing to its high aerobic capacity (Wilhelm Filho, 2007; Mortelette et al., 2010). Contrary to our prediction, oxidative damage was not more pronounced with acclimation temperature in any of the tissues studied here. However, increased rates of CAT activity in red muscle of fish acclimated to 9°C may indicate a compensatory response to increased ROS challenge at cold temperatures. And it is also possible that reductions in plasma testosterone and estradiol at 18°C (see above) contribute to decreased oxidative defences (Thilagam et al., 2010). Previous work in pink salmon highlights the importance of tissue type and stage of migration when looking for effects of oxidative stress, where oxidative stress in the heart was found to increase over the course of migration, while red and white muscle had no significant changes (Wilson et al., 2014). It should be noted that fish used for our study were collected at their spawning ground (i.e. Chilliwack River hatchery), as opposed to along their migratory route. This means that our sampling may have been biased for the most resilient females by not targeting females at a potentially more vulnerable state earlier in migration.

Conclusions

Our results have helped exclude one hypothesis previously used to explain female-biased mortality at high temperatures. We found no evidence that a higher susceptibility to ROS damage contributes to female-biased mortality at warm temperatures. Our results, however, do bolster other hypotheses. Potential avenues for future work include testing whether high temperatures (i) disproportionately undermine the metabolic capacity of the heart, (ii) disrupt testosterone- and estradiol-mediated protein catabolism and (iii) affect immune responses (larger increases in spleen size) in migratory female salmon. Investigation into these processes, especially earlier along the migratory route, will undoubtedly help

to identify whether they contribute to female-biased mortality at high temperatures.

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Competing interests
The authors declare no competing or financial interests.

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Author contributions
Conceptualization: A.P.F., S.J.C., D.A.P., S.G.H., E.J.E.; Methodology: A.G.L., E.H., K.K., T.D., T.S.P., B.H., E.J.E.; Validation: E.J.E.; Formal analysis: A.G.L., K.K., E.J.E.; Investigation: A.G.L., E.H., K.K., T.D., T.S.P., B.H., E.J.E.; Resources: J.N.P., A.P.F., S.J.C., D.A.P., S.G.H., E.J.E.; Data curation: E.H.; Writing - original draft: A.G.L., E.J.E.; Writing - review & editing: A.G.L., E.H., K.K., T.D., T.S.P., B.H., J.N.P., A.P.F., S.J.C., D.A.P., S.G.H.; Supervision: A.G.L., J.N.P., A.P.F., S.J.C., D.A.P., S.G.H., E.J.E.; Project administration: E.J.E.; Funding acquisition: J.N.P., A.P.F., S.J.C., D.A.P., S.G.H., E.J.E.

References

Ando, S. (1986). Studies on the food biochemical aspects of changes in chum salmon, *Oncorhynchus keta,* during spawning migration: mechanisms of muscle deterioration and nuptial coloration. Mem. Fac. Fish. Hokkaido Univ., 33, 1-95.

Arndt, S. K. A., Benfey, T. J. and Cunjak, R. A. (1996). Effect of temporary reductions in feeding on protein synthesis and energy storage of juvenile Atlantic salmon. *J. Fish Biol.* **49**, 257-276. doi:10.1111/j.1095-8649.1996.tb00022.x

Bagnyukova, T. V., Lushchak, O. V., Storey, K. B. and Lushchak, V. I. (2007). Oxidative stress and antioxidant defense responses by goldfish tissues to acute change of temperature from 3 to 23°C. *J. Therm. Biol.* **32**, 227-234. doi:10.1016/j.jtherbio.2007.01.004

Barton, B. A. (2000). Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. *N. Am. J. Aquacult.* **62**, 12-18. doi:10.1577/1548-8454(2000)062<0012:SFDITC>2.0.CO;2

Barton, B. A. and Iwama, G. K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annu. Rev. Fish Dis.* **1**, 3-26. doi:10.1016/0959-8030(91)90019-G

Battiprolu, P. K. and Rodnick, K. J. (2014). Dichloroacetate selectively improves cardiac function and metabolism in female and male rainbow trout. *Am. J. Physiol. Heart Circ. Physiol.* **307**, H1401-H1411. doi:10.1152/ajpheart.00755.2013

Battiprolu, P. K., Harmon, K. J. and Rodnick, K. J. (2007). Sex differences in energy metabolism and performance of teleost cardiac tissue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R827-R836. doi:10.1152/ajpregu.00379.2006

Beckman, K. B. and Ames, B. N. (1998). The free radical theory of aging matures. *Physiol. Rev.* **78**, 547-581. doi:10.1152/physrev.1998.78.2.547

Berg, A. H., Westerlund, L. and Olsson, P. E. (2004). Regulation of Arctic char (*Salvelinus alpinus*) egg shell proteins and vitellogenin during reproduction and in response to 17β-estradiol and cortisol. *Gener. Comp. Endocrinol*, **135**, 276-285. doi:10.1016/j.ygcen.2003.10.004

Birnie-Gauvin, K., Costantini, D., Cooke, S. J. and Willmore, W. G. (2017). A comparative and evolutionary approach to oxidative stress in fish: a review. *Fish Fisheries* **18**, 928-942. doi:10.1111/faf.12215

Borowiec, B. G., Crans, K. D., Khajali, F., Pranckevicius, N. A., Young, A. and Scott, G. R. (2016). Interspecific and environment-induced variation in hypoxia tolerance in sunfish. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **198**, 59-71. doi:10.1016/j.cbpa.2016.04.010

Burnett, N. J., Hinch, S. G., Braun, D. C., Casselman, M. T., Middleton, C. T., Wilson, S. M. and Cooke, S. J. (2014). Burst swimming in areas of high flow: delayed consequences of anaerobiosis in wild adult sockeye salmon. *Physiol. Biochem. Zool.* **87**,587-598. doi:10.1086/677219

Carragher, J. F., Sumpter, J. P., Pottinger, T. G. and Pickering, A. D. (1989). The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta* L. and *Salmo gairdneri* Richardson. *Gener. Comp. Endocrinol.* **76**, 310-321. doi:10.1016/0016-6480(89)90163-9

Carruth, L. L., Jones, R. E. and Norris, D. O. (2002). Cortisol and Pacific salmon: a new look at the role of stress hormones in olfaction and home-stream migration. *Integr. Comp. Biol.* **42**, 574-581. doi:10.1093/icb/42.3.574

Chippari-Gomes, A. R., Gomes, L. C., Lopes, N. P., Val, A. L. and Almeida-Val, V. M. F. (2005). Metabolic adjustments in two Amazonian cichlids exposed to hypoxia and anoxia. *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* **141**, 347-355. doi:10.1016/j.cbpc.2005.04.006

Clark, T. D., Sandblom, E., Cox, G. K., Hinch, S. G. and Farrell, A. P. (2008). Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **295**, R1631-R1639. doi:10.1152/ajpregu.90461.2008

Clark, T. D., Jeffries, K. M., Hinch, S. G. and Farrell, A. P. (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. doi:10.1242/jeb.060517

Cleveland, B. M. and Weber, G. M. (2011). Effects of sex steroids on indices of protein turnover in rainbow trout (*Oncorhynchus mykiss*) white muscle. *Gener. Comp. Endocrinol.* **174**, 132-142. doi:10.1016/j.ygcen.2011.08.011

Cook, K. V., McConnachie, S. H., Gilmour, K. M., Hinch, S. G. and Cooke, S. J. (2011). Fitness and behavioral correlates of pre-stress and stress-induced plasma cortisol titers in pink salmon (*Oncorhynchus gorbuscha*) upon arrival at spawning grounds. *Hormones Behav.* **60**, 489-497. doi:10.1016/j.yhbeh.2011.07.017

Cooke, S. J., Hinch, S. G., Crossin, G. T., Patterson, D. A., English, K. K., Healey, M. C., Shrimpton, J. M., Van Der Kraak, G. and Farrell, A. P. (2006). Mechanistic basis of individual mortality in Pacific salmon during spawning migrations. *Ecology* **87**, 1575-1586. doi:10.1890/0012-9658(2006)87[1575:MBOIMI]2.0.CO;2

Cooke, S. J., Hinch, S. G., Farrell, A. P., Patterson, D. A., Miller-Saunders, K., Welch, D. W., Donaldson, M. R., Hanson, K. C., Crossin, G. T., Mathes, M. T. et al. (2008). Developing a mechanistic understanding of fish migrations by linking telemetry with physiology, behavior, genomics and experimental biology: an interdisciplinary case study on adult Fraser River sockeye salmon. *Fisheries* **33**, 321-339. doi:10.1577/1548-8446-33.7.321

Costantini, D. (2008). Oxidative stress in ecology and evolution: lessons from avian studies. *Ecol. Lett.* **11**, 1238-1251. doi:10.1111/j.1461-0248.2008.01246.x

Crans, K. D., Pranckevicius, N. A. Scott, G. R. (2015). Physiological tradeoffs may underlie the evolution of hypoxia tolerance and exercise performance in sunfish (Centrarchidae). *J. Exp. Biol.* **218**, 3264-3275. doi:10.1242/jeb.124602

Crossin, G. T., Hinch, S. G., Cooke, S. J., Welch, D. W., Patterson, D. A., Jones, S. R. M., Lotto, A. G., Leggatt, R. A., Mathes, M. T., Shrimpton, J. M. 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. doi:10.1139/Z07-122

Donaldson, M. R., Hinch, S. G., Jeffries, K. M., Patterson, D. A., Cooke, S. J., Farrell, A. P. and Miller, K. M. (2014). Species- and sex-specific responses and recovery of wild, mature pacific salmon to an exhaustive exercise and air exposure stressor. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* **173**, 7-16. doi:10.1016/j.cbpa.2014.02.019

Ekström, A., Axelsson, M., Gräns, A., Brijs, J. and Sandblom, E. (2017). Influence of the coronary circulation on thermal tolerance and cardiac performance during warming in rainbow trout. *Am. J. Physiol. Reg., Integr. Comp. Physiol.* **312**, R549-R558. doi:10.1152/ajpregu.00536.2016

Eliason, E. J. and Farrell, A. P. (2016). Oxygen uptake in Pacific salmon *Oncorhynchus* spp.: when ecology and physiology meet. *J. Fish Biol.* **88**, 359-388. doi:10.1111/jfb.12790

Eliason, E. J., Clark, T. D., Hague, M. J., Hanson, L. M., Gallagher, Z. S., Jeffries, K. M., Gale, M. K., Patterson, D. A., Hinch, S. G. and Farrell, A. P. (2011). Differences in thermal tolerance among sockeye salmon populations. *Science*, **332**, 109-112. doi:10.1126/science.1199158

Eliason, E. J., Clark, T. D., Hinch, S. G. and Farrell, A. P. (2013). Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon. *Conserv. Physiol.* **1**, cot008. doi:10.1093/conphys/cot008

Eliason, E. J., Dick, M., Patterson, D. A., Robinson, K. A., Lotto, J., Hinch, S. G. and Cooke, S. J. (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*. In press.

Eros, S. K. and Milligan, C. L. (1996). The effect of cortisol on recovery from exhaustive exercise in rainbow trout (*Oncorhynchus mykiss*): potential mechanisms of action. *Physiol. Zool.* **69**, 1196-1214. doi:10.1086/physzool.69.5.30164253

Farrell, A. P., MacLeod, K. R. and Scott, C. (1988). Cardiac performance of the trout (*Salmo gairdneri*) heart during acidosis: effects of low bicarbonate, lactate and cortisol. *Comp. Biochem. Physiol. A: Physiol.* **91**, 271-277. doi:10.1016/0300-9629(88)90417-3

Farrell, A. P., Gallaugher, P. E. and Routledge, R. (2001). Rapid recovery of exhausted adult coho salmon after commercial capture by troll fishing. *Can. J. Fish. Aquat. Sci.* **58**, 2319-2324. doi:10.1139/f01-188

Ferrari, M. R., Miller, J. R. and Russell, G. L. (2007). Modeling changes in summer temperature of the Fraser River during the next century. *J. Hydrol.* **342**, 336-346. doi:10.1016/j.jhydrol.2007.06.002

Gallaugher, P., Axelsson, M. and Farrell, A. P. (1992). Swimming performance and haematological variables in splenectomized rainbow trout, *Oncorhynchus mykiss*. *J. Exp. Biol.* **171**, 301-314.

Garner, S. R., Bortoluzzi, R. N., Heath, D. D. and Neff, B. D. (2009). Sexual conflict inhibits female mate choice for major histocompatibility complex dissimilarity in Chinook salmon. *Proc. Biol. Sci.* **277**, 885-894. doi:10.1098/rspb.2009.1639

Ghanizadeh Kazerouni, E., Franklin, C. E. and Seebacher, F. (2016). UV-B radiation interacts with temperature to determine animal performance. *Funct. Ecol.* **30**, 584-595. doi:10.1111/1365-2435.12520

Hadidi, S., Glenney, G. W., Welch, T. J., Silverstein, J. T. and Wiens, G. D. (2008). Spleen size predicts resistance of rainbow trout to *Flavobacterium psychrophilum* challenge. *J. Immunol.* **180**, 4156-4165. doi:10.4049/jimmunol.180.6.4156

Hague, M. J., Ferrari, M. R., Miller, J. R., Patterson, D. A., Russell, G. L., Farrell, A. P. and Hinch, S. G. (2011). Modelling the future hydroclimatology of the lower Fraser River and its impacts on the spawning migration survival of sockeye salmon. *Glob. Change Biol.* **17**, 87-98. doi:10.1111/j.1365-2486.2010.02225.x

Hardy, I. C. (2002). Sex ratios. *Concepts Res. Methods*. doi:10.1017/CBO9780511542053

Hayes, S. A., Bond, M. H., Wells, B. K., Hanson, C. V., Jones, A. W. and MacFarlane, R. B. (2011). Using archival tags to infer habitat use of central California steelhead and coho salmon. In Proceedings of the 2nd International Symposium on Advances in Fish Tagging and Marking Technology. American Fisheries Society.

Hinch, S. G., Cooke, S. J., Healey, M. C. and Farrell, A. P. (2006). Behavioural physiology of fish migrations: salmon as a model approach. *Fish Physiol.* **24**, 239-295. doi:10.1016/S1546-5098(05)24007-4

Hruska, K. A., Hinch, S. G., Healey, M. C. and Farrell, A. P. (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. In American Fisheries Society Symposium, vol. 54, p. 17.

Hruska, K. A., Hinch, S. G., Healey, M. C., Patterson, D. A., Larsson, S. and Farrell, A. P. (2010). Influences of sex and activity level on physiological changes in individual adult sockeye salmon during rapid senescence. *Physiol. Biochem. Zool.* **83**, 663-676. doi:10.1086/652411

Idler, D. R., Bitners, I. I. and Schmidt, P. J. (1961). 11-Ketotestosterone: an androgen for sockeye salmon. *Can. J. Biochem. Physiol.* **39**, 1737-1742. doi:10.1139/o61-191

Jeffries, K. M., Hinch, S. G., Sierocinski, T., Clark, T. D., Eliason, E. J., Donaldson, M. R., Li, S., Pavlidis, P. and Miller, K. M. (2012). Consequences of high temperatures and premature mortality on the transcriptome and blood physiology of wild adult sockeye salmon (*Oncorhynchus nerka*). *Ecol. Evol.* **2**, 1747-1764. doi:10.1002/ece3.274

Jeffries, K. M., Hinch, S. G., Sierocinski, T., Pavlidis, P. and Miller, K. M. (2014). Transcriptomic responses to high water temperature in two species of Pacific salmon. *Evol. App.* **7**, 286-300. doi:10.1111/eva.12119

Keefer, M. L., Peery, C. A. and Heinrich, M. J. (2008). Temperature-mediated en route migration mortality and travel rates of endangered Snake River sockeye salmon. *Ecol. Freshw. Fish* **17**, 136-145. doi:10.1111/j.1600-0633.2007.00267.x

Korsgaard, B., Mommsen, T. P. and Saunders, R. L. (1986). The effect of temperature on the vitellogenic response in Atlantic salmon post-smolts (*Salmo salar*). *Gener. Comp. Endocrinol.* **62**, 193-201. doi:10.1016/0016-6480(86)90109-7

Kubokawa, K., Yoshioka, M. and Iwata, M. (2001). Sex-specific cortisol and sex steroids responses in stressed sockeye salmon during spawning period. *Zool. Sci.* **18**, 947-955. doi:10.2108/zsj.18.947

Lanctin, H. P., McMorran, L. E. and Driedzic, W. R. (1980). Rates of glucose and lactate oxidation by the perfused isolated trout (*Salvelinus fontinalis*) heart. *Can. J. Zool.* **58**, 1708-1711. doi:10.1139/z80-234

Larsen, H. E., Celander, M. and Goksøyr, A. (1992). The cytochrome P450 system of Atlantic salmon (*Salmo salar*): II. Variations in hepatic catalytic activities and isozyme patterns during an annual reproductive cycle. *Fish Physiol. Biochem.* **10**, 291-301. doi:10.1007/BF00004478

Martínez, M. L., Landry, C., Boehm, R., Manning, S., Cheek, A. O. and Rees, B. B. (2006). Effects of long-term hypoxia on enzymes of carbohydrate metabolism in the Gulf killifish, *Fundulus grandis*. *J. Exp. Biol.* **209**, 3851-3861. doi:10.1242/jeb.02437

Martins, E. G., Hinch, S. G., Patterson, D. A., Hague, M. J., Cooke, S. J., Miller, K. M., Robichaud, D., English, K. K. and Farrell, A. P. (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. doi:10.1139/f2011-154

Materna, E. (2001). *Issue Paper 4: temperature interaction*. EPA-910-D-01-004. Seattle, WA: Environmental Protection Agency.

Maule, A. G., Schreck, C. B. and Kaattari, S. L. (1987). Changes in the immune system of coho salmon (Oncorhynchus kisutch) during the parr-to-smolt transformation and after implantation of cortisol. *Can. J. Fish. Aquat. Sci.* **44**, 161-166.

Maule, A. G., Tripp, R. A., Kaattari, S. L. and Schreck, C. B. (1989). Stress alters immune function and disease resistance in chinook salmon (Oncorhynchus tshawytscha). *J. Endocrinol.* **120**, 135-142.

Maule, A. G. and Schreck, C. B. (1990). Changes in numbers of leukocytes in immune organs of juvenile coho salmon after acute stress or cortisol treatment. *J. Aquat. Anim. Health* **2**, 298-304.

McConnachie, S. H., Cook, K. V., Patterson, D. A., Gilmour, K. M., Hinch, S. G., Farrell, A. P. and Cooke, S. J. (2012). Consequences of acute stress and cortisol manipulation on the physiology, behavior, and reproductive outcome of female Pacific salmon on spawning grounds. *Hormones Behav.* **62**, 67-76. doi:10.1016/j.yhbeh.2012.05.001

McCullough, D. A. (1999). A review and synthesis of effects of alterations to the water temperature regime on freshwater life stages of salmonids, with special reference to Chinook salmon. US Environmental Protection Agency, Region 10, pp. 1-291.

Metcalfe, N. B. and Alonso-Alvarez, C. (2010). Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death: Oxidative stress as a life-history constraint. *Funct. Ecol.* **24**, 984-996. doi:10.1111/j.1365-2435.2010.01750.x

Milligan, C. L. (1996). Metabolic recovery from exhaustive exercise in rainbow trout. *Comp. Biochem. Physiol. A: Physiol.* **113**, 51-60. doi:10.1016/0300-9629(95)02060-8

Milligan, C. L. (1997). The role of cortisol in amino acid mobilization and metabolism following exhaustive exercise in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Fish Physiol. Biochem.* **16**, 119-128. doi:10.1007/BF00004669

Milligan, C. L., Hooke, G. B. and Johnson, C. (2000). Sustained swimming at low velocity following a bout of exhaustive exercise enhances metabolic recovery in rainbow trout. *J. Exp. Biol.* **203**, 921-926.

Minke-Martin, V., Hinch, S. G., Braun, D. C., Burnett, N. J., Casselman, M. T., Eliason, E. J. and Middleton, C. T. (2018). Physiological condition and migratory experience affect fitness-related outcomes in adult female sockeye salmon. *Ecol. Freshw. Fish* **27**, 296-309. doi:10.1111/eff.12347

Mommsen, T. P., Vijayan, M. M. and Moon, T. W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Rev. Fish Biol Fish.* **9**, 211-268. doi:10.1023/A:1008924418720

Mortelette, H., Amérand, A., Sébert, P., Belhomme, M., Calvès, P. and Moisan, C. (2010). Effect of exercise training on respiration and reactive oxygen species metabolism in eel red muscle. *Resp. Physiol. Neurobiol.* **172**, 201-205. doi:10.1016/j.resp.2010.05.023

Moyes, C. D., Mathieu-Costello, O. A., Tsuchiya, N., Filburn, C. and Hansford, R. G. (1997). Mitochondrial biogenesis during cellular differentiation. *Am. J. Physiol., Cell Physiol.* **272**, C1345-C1351. doi:10.1152/ajpcell.1997.272.4.C1345

Munakata, A., Amano, M., Ikuta, K., Kitamura, S. and Aida, K. (2001). The involvement of sex steroid hormones in downstream and upstream migratory behavior of masu salmon. *Comp. Biochem. Physiol., B: Biochem. Mol. Biol.* **129**, 661-669. doi:10.1016/S1096-4959(01)00365-7

Nakano, T., Kameda, M., Shoji, Y., Hayashi, S., Yamaguchi, T. and Sato, M. (2014). Effect of severe environmental thermal stress on redox state in salmon. *Redox Biol.* **2**, 772-776. doi:10.1016/j.redox.2014.05.007

Pankhurst, N. W. and Van Der Kraak, G. (2000). Evidence that acute stress inhibits ovarian steroidogenesis in rainbow trout in vivo, through the action of cortisol. *Gener. Comp. Endocrinol.* **117**, 225-237. doi:10.1006/gcen.1999.7401

Pickering, A. D. and Pottinger, T. G. (1989). Stress responses and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol. *Fish Physiol. Biochem.* **7**, 253-258.

Pörtner, H. O. and Farrell, A. P. (2008). Physiology and climate change. *Science* **322**, 690-692. doi:10.1126/science.1163156

Quinn, T. P. and Kinnison, M. T. (1999). Size-selective and sex-selective predation by brown bears on sockeye salmon. *Oecologia* **121**, 273-282.

Raby, G. D., Casselman, M. T., Cooke, S. J., Hinch, S. G., Farrell, A. P. and Clark, T. D. (2016). Aerobic scope increases throughout an ecologically relevant temperature range in coho salmon. *J. Exp. Biol.* **219**, 1922-1931. doi:10.1242/jeb.137166

Rankin, D. J. and Kokko, H. (2006). Sex, death and tragedy. *Trends Ecol. Evol.* **21**, 225-226. doi:10.1016/j.tree.2006.02.013

Richards, M. P. and Hultin, H. O. (2002). Contributions of blood and blood components to lipid oxidation in fish muscle. *J. Agric. Food Chem.* **50**, 555-564. doi:10.1021/jf010562h

Roscoe, D. W., Hinch, S. G., Cooke, S. J. and Patterson, D. A. (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. doi:10.1002/rra.1384

Sandblom, E., Clark, T. D., Hinch, S. G. and Farrell, A. P. (2009). Sex-specific differences in cardiac control and hematology of sockeye salmon (*Oncorhynchus nerka*) approaching their spawning grounds. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R1136-R1143. doi:10.1152/ajpregu.00363.2009

Sato, A., Ueda, H., Fukaya, M., Kaeriyama, M., Zohar, Y., Urano, A. and Yamauchi, K. (1997). Sexual differences in homing profiles and shortening of homing duration by gonadotropin-releasing hormone analog implantation in lacustrine sockeye salmon (*Oncorhynchus nerka*) in Lake Shikotsu. *Zool. Sci.* **14**, 1009-1014. doi:10.2108/zsj.14.1009

Schmidt, P. J. and Idler, D. R. (1962). Steroid hormones in the plasma of salmon at various states of maturation. *Gener. Comp. Endocrinol.* **2**, 204-214. doi:10.1016/0016-6480(62)90005-9

Snieszko, S. F. (1974). The effects of environmental stress on outbreaks of infectious diseases of fishes. *J. Exp. Biol.* **6**, 197-208.

Sower, S. A. and Schreck, C. B. (1982). Steroid and thyroid hormones during sexual maturation of coho salmon (*Oncorhynchus kisutch*) in seawater or fresh water. *Gener. Comp. Endocrinol.* **47**, 42-53. doi:10.1016/0016-6480(82)90082-X

Strange, R. J., Schreck, C. B. and Golden, J. T. (1977). Corticoid stress responses to handling and temperature in salmonids. *Trans. Am. Fish. Soc.* **106**, 213-218. doi:10.1577/1548-8659(1977)106<213:CSRTHA>2.0.CO;2

Teffer, A. K., Hinch, S. G., Miller, K. M., Jeffries, K. M., Patterson, D. A., Cooke, S. J., Farrell, A. P., Kaukinen, K., Li, S. and Juanes, F. (2019). Sex-specific effects of thermal and fisheries stressors on pathogen development and early mortality of adult coho salmon (*Oncorhynchus kisutch*) in freshwater. *Physiol. Biochem. Zool.* DOI:10.1086/705125

Thilagam, H., Gopalakrishnan, S., Qu, H. D., Bo, J. and Wang, K. J. (2010). 17β estradiol induced ROS generation, DNA damage and enzymatic responses in the hepatic tissue of Japanese sea bass. *Ecotoxicology* **19**, 1258-1267. doi:10.1007/s10646-010-0510-3

Truscott, B., Idler, D. R., So, Y. P. and Walsh, J. M. (1986). Maturational steroids and gonadotropin in upstream migratory sockeye salmon. *Gener. Comp. Endocrinol.* **62**, 99-110. doi:10.1016/0016-6480(86)90098-5

Van Der Boon, J., Van Den Thillart, G. E. and Addink, A. D. (1991). The effects of cortisol administration on intermediary metabolism in teleost fish. *Comp. Biochem. Physiol., A: Mol. Integr. Physiol.* **100**, 47-53.

Welsh, Jr., H. H., Hodgson, G. R., Harvey, B. C. and Roche, M. F. (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. doi:10.1577/1548-8675(2001)021<0464:DOJCSI>2.0.CO;2

Wilhelm Filho, D. (2007). Reactive oxygen species, antioxidants and fish mitochondria. *Front. Biosci.* **12**, 1229-1237. doi:10.2741/2141

Wilson, S. M., Taylor, J. J., Mackie, T. A., Patterson, D. A., Cooke, S. J. and Willmore, W. G. (2014). Oxidative stress in Pacific salmon (*Oncorhynchus* spp.) during spawning migration. *Physiol. Biochem. Zool.* **87**, 346-352. doi:10.1086/674798