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Are there intergenerational and population-specific effects of oxidative stress in sockeye salmon (*Oncorhynchus nerka*)?

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ABSTRACT

Intergenerational effects of stress have been reported in a wide range of taxa; however, few researchers have examined the intergenerational consequences of oxidative stress. Oxidative stress occurs in living organisms when reactive oxygen species remain unquenched by antioxidant defense systems and become detrimental to cells. In fish, it is unknown how maternal oxidative stress and antioxidant capacity influence offspring quality. The semelparous, migratory life history of Pacific salmon (Oncorhynchus spp.) provides a unique opportunity to explore intergenerational effects of oxidative stress. This study examined the effects of population origin on maternal and developing offspring oxidative stress and antioxidant capacity, and elucidated intergenerational relationships among populations of sockeye salmon (*Oncorhynchus nerka*) with varying migration effort. For three geographically distinct populations of Fraser River sockeye salmon (British Columbia, Canada), antioxidant capacity and oxidative stress were measured in adult female plasma, heart, brain, and liver, as well as in developing offspring until time of emergence. Maternal and offspring oxidative stress and antioxidant capacity varied among populations but patterns were not consistent across tissue/developmental stage. Furthermore, maternal oxidative stress and antioxidant capacity did not affect offspring oxidative stress and antioxidant capacity across any of the developmental stages or populations sampled. Our results revealed that offspring develop their endogenous antioxidant systems at varying rates across populations; however, this variability is overcome by the time of emergence. While offspring may be relying on maternally derived antioxidants in the initial stages of development, they rapidly develop their own antioxidant systems (mainly glutathione) during later stages of development.

1. Introduction

The evolution of life history involves the concepts of trade-offs (Stearns, 1989). Such trade-offs have a physiological basis in that they involve resource allocation (Zera and Harshman, 2001; Ricklefs and Wikelski, 2002). The allocation of limiting resources to one trait has negative consequences to other traits requiring the same resource (Stearns, 1989; Zera and Harshman, 2001). Stress affects resource allocation in that it diminishes the total amount of resources available to allocate to any given trait (Ricklefs and Wikelski, 2002). The response to stress involves the shifting of resources towards metabolic reorganization, energy allocation, healing and repair (Monaghan et al., 2009). The greater the stress, the greater the allocation of resources to these processes. This detracts from resources required for growth, reproduction, foraging, immunity and other essential life traits (Ricklefs and Wikelski, 2002). Thus organisms attempt to minimize or avoid stress to maximize their energy allocation to these essential traits.

Oxidative stress has been receiving increased attention as a stress involved in trade off in organism life histories (reviewed in Costantini, 2008; Dowling and Simmons, 2009; Monaghan et al., 2009; Isaksson et al., 2011). Oxidative stress occurs when the balance between prooxidant reactive oxygen species (ROS) and antioxidants is tipped in favor of ROS (Halliwell and Gutteridge, 2002). Oxidative stress contributes to energy imbalance in organisms as induction of antioxidant enzyme production, synthesis of low molecular weight antioxidants such as glutathione, elimination of damaged cellular components

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(protein, lipids, DNA) and repair of damage due to ROS are all ATP-dependent processes. The reallocation of energy resources to combat and protect against oxidative stress reduces the overall fitness of the organism (Monaghan et al., 2009).

Fish encounter oxidative stress from a variety of environmental factors including pollutants, diet and temperature (reviewed by Stoliar and Lushchak, 2012). Female fish allocate antioxidant resources to their eggs in the form of carotenoids, flavonoids, and α-tocopherol (Garner et al., 2010). Deposits of sufficient antioxidants in eggs means greater defense against oxidative stress in the early stages of development in fish and represents a major contribution of parent to offspring fitness.

Semelparous Pacific salmon (*Oncorhynchus spp.*) that have a life history which is a delicate balance of resource allocation (Fleming and Gross, 1989; Quinn, 2005; Hinch et al., 2006) and that cease uptake of dietary-derived antioxidants, due to fasting during spawning migration (Welker and Congleton, 2005). Pacific salmon are anadromous, migrating up to 1000 km in distance and 1 km in elevation from the Pacific Ocean to their natal freshwater river to spawn before dying (Crossin et al., 2004; Hinch et al., 2006). Some species, such as sockeye salmon (*Oncorhynchus nerka*), exhibit extreme levels of philopatry which promotes extensive intraspecific variation and population differentiation related to local adaptation and minimal gene flow among populations (Lin et al., 2008). In addition to the natural obstacles and effort required for traversing such great distances, Pacific salmon also face a number of anthropogenic stressors including aquatic chemical pollution (Ross et al., 2013), capture by recreational, commercial and aboriginal fisheries (Donaldson et al., 2011), and unpredictable flows, potentially lethal river water temperatures, and temperature-mediated pathogen infections (Cooke et al., 2008; Crozier et al., 2008; Jeffries et al., 2012). The oxidative stress-related costs of migration can include increased muscular activity during migration and reproduction (Leeuwenburgh and Heinecke, 2001; Monaghan et al., 2009), increased riverine temperatures (Parihar and Dubey, 1995; Heise et al., 2006; Lushchak and Bagnyukova, 2006), and dwindling dietary-derived antioxidants due to cessation of feeding (Pascual et al., 2003; Morales et al., 2004). Research investigating oxidative stress processes in Pacific salmon is currently limited. When subjected to low-water stress after starvation, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) experienced higher oxidative stress than control fish (Welker and Congleton, 2004). Wilson et al. (2014) found that oxidative stress occurred in a tissue-specific manner during migration in pink salmon (*Oncorhynchus gorbuscha*), and that some tissues may be differentially protected to ensure successful spawning. However, the aforementioned studies do not address how oxidative stress varies intraspecifically or in an intergenerational manner.

The present study examines the relationship between maternal and offspring antioxidant content in sockeye salmon from three geographically distinct populations [Harrison River (short distance/low elevation), Chilko River and Stellako River (long distance/high elevation)] in the Fraser River watershed. Metrics of oxidative stress and antioxidant protection were also assessed in offspring at five stages of early development to provide insight into the extent to which female salmon contribute antioxidant capacity to their eggs. We hypothesized that females completing more arduous migrations (i.e., Chilko River and Stellako River) would have higher levels of oxidative stress and reduced antioxidant capacity, and that these levels would be reflected in the offspring.

2. Methods

2.1. Ethical note

Research conformed to the protocols approved by Animal Care Committees at the University of British Columbia (#A11 0215), Simon Fraser University (#926R-94), and Carleton University (#B10-06) and met the Canadian Council for Animal Care guidelines. Moreover, scientific collection permits were obtained from Fisheries and Oceans Canada.

2.2. Sample collection

This study was conducted using three geographically distinct sockeye salmon populations from the Fraser River watershed in British Columbia, Canada. Harrison River (Table 1), with a migratory effort of 25 (migratory effort = 0.001 * Lower Fraser discharge ($m^3\ s^{-1}$) * migration distance (km), as per Eliason et al., 2011), was used in this study to represent a short distance. Chilko River and Stellako River (migratory effort 219 and 325, respectively, Table 1) were used to study longer distance migrations of varying efforts. Sockeye salmon were collected at the three sites in the watershed while staging for spawning (Chilko River on September 24, 2012, Stellako River on September 26, 2012, and Harrison River on November 6 and 9, 2012). At the spawning grounds, sockeye salmon breeding pairs were collected by either angling (landed within 30 s, Chilko River ($n = 15$)) or by beach seine (Harrison River ($n = 7$), Stellako River ($n = 15$)), immediately euthanized by cerebral percussion and sampled. Blood was sampled from each of the collected females using caudal venipuncture into heparinized vacutainers, inverted, and centrifuged at 1162 ×*g* for 5 min (Clay Adams Compact II Centrifuge, Becton-Dickson; Sparks, MD) to separate erythrocytes and plasma. Plasma, along with the entire brain, and samples of roughly 500 mg taken from the end of the liver (Chilko and Stellako River only), and the lower portion of the heart from the same females were flash frozen in liquid nitrogen and stored at −80 °C until analysis. All females and an equal number of males were stripped of their eggs and milt respectively, which were stored on ice in clean, dry, containers until fertilization. Care was taken to avoid contamination of the milt and eggs with water or excreta. A sample of three pre-fertilized (PF) eggs from each female was also flash frozen in liquid nitrogen and stored at −80 °C until analysis. When all sampling was complete, morphometrics measured including the weight (to nearest 0.01 kg), fork length (to nearest 0.1 cm) and gonadosomatic index (GSI; gonad weight (to nearest 0.001 g)) as a percentage of total body weight (Neat et al., 1998).

Collected eggs and milt were transported on ice in coolers to Simon Fraser University in Burnaby (British Columbia, Canada) for fertilization. In triplicate, 15 g of eggs from each female was transferred to clean, dry mason jars and 1 mL milt from each male was added to create full sibling crosses (i.e., within a population, each female was crossed with each male once). After milt application, 30 mL of distilled water was added and swirled gently to activate milt and jars were topped off with an additional 400 mL of distilled water. After incubation (1 h for Harrison River, and overnight for Chilko River and Stellako River), separated by population and family ID, fertilized eggs were transferred to flow through baskets and distributed in Heath stacks with circulating de-chlorinated municipal water at ambient temperature (4.8–10.7 °C). Wet and dry egg weight was determined for each female. Three replicates of ten eggs were weighed wet and after drying for 24 h in an oven (65 °C) to the nearest 0.0001 g. Baskets were checked daily and dead embryos removed.

At 24 h post-fertilization (24 HPF) a sample of eggs from each cross was flash frozen in liquid nitrogen and stored at −80 °C until analysis. This same sampling procedure was also performed at eyed stage (EYE), hatch (HA), and emergence (EMG, full yolk sac absorption) for all crosses from each of the three populations. For offspring from Chilko River, days post fertilization (dpf) were 37, 80, and 133, Stellako River offspring dpf were 35, 71, and 131, and Harrison River offspring dpf were 64, 93, and 160 for EYE, HA, and EMG respectively.

2.3. Antioxidant capacity

All samples were ground over liquid nitrogen and homogenized on ice in 1:5 lysis buffer (20 mM Tris–HCl, 137 mM NaCl, 1% NP-40, 10% glycerol, 2 mM EDTA) using a handheld Tissue Master 125 (Omni

Table 1
Population-specific migration characteristics for the three study populations of *Oncorhynchus nerka*, including adult migration distance, elevation, effort and temperature, a description of where the rearing areas are for fry, the direction and distance fry must migrate to reach these areas from spawning grounds following emergence (Eliason et al., 2011; Sopinka et al., 2013; Whitney et al., 2013).

Population	Adult				Offspring		
	Migration distance (km)	Elevation (m)	Mean spawning temperature (°C)	Migratory Effort (0.0001* discharge* distance)	Migration direction	Rearing area	Maximum distance to rearing area (km)
Harrison River	121	10	8.50	25	Downstream	Lower Fraser River & estuary	100
Chilko River	642	1174	9.95	219	Upstream	Chilko Lake	10
Stellako River	976	664	10.95	328	Downstream	Fraser Lake	10

International, Kennesaw, GA). Sample lysate was centrifuged at 18,500 ×*g* for 5 min at 4 °C in a Hermle Labnet Z216MK (Mandel, Guelph, ON) and supernatant was stored at −80 °C until resistance to free radical attack was measured using the Oxygen Radical Absorbance Capacity (ORAC) assay as described in Lucas-Abellán et al. (2008); Wilson et al. (2012). The ORAC analyses were completed using a Fluostar Optima microplate reader (BMG Labtech; Offenburg, Germany) and black 96-Well Costar microplates. Fluorescence was measured with an excitation wavelength of 485 nm and emission of 520 nm. Fluorescence data were analyzed using Optima software (9.15.31, Optima Technology Corporation, Rio Rico, Arizona).

Each reaction well contained 20 μL of either sample, blank (75 mM potassium phosphate (pH 7.4)), standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox); 0–400 μM), or antioxidant positive control (Rutin; 200 μM), and 3.82 μM fluorescein in 75 mM potassium phosphate (pH 7.4). The plate was incubated at 37 °C for 30 min before rapidly adding 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) to a final concentration of 79.83 mM. The plate was placed immediately in the microplate reader and the fluorescence was read every 35 s for 60 min. The area under the fluorescence decay curve (AUC) was determined for the samples and Trolox standards to determine the Trolox equivalents. Total protein of samples was determined using the Bradford assay (Bradford, 1976) and final values were reported in Trolox equivalents/mg total protein.

2.4. Glutathione

All samples were ground over liquid nitrogen and homogenized on ice in 1:5 5% sulfosalicylic acid solution (previously bubbled with N_2) using a handheld Tissue Master 125 (Omni International, Kennesaw, GA). Sample lysate was centrifuged at 18,500 ×*g* for 5 min at 4 °C in a Hermle Labnet Z216MK (Mandel, Guelph, ON) and supernatant was used to assess total glutathione (TGSH) and oxidized glutathione (GSSG) [TGSH = GSH + 2GSSG]. Glutathione assays were performed using a SpectraMax 340PC microplate reader with SoftMax Pro 4.8 data analysis software (Molecular Devices, Sunnyvale, CA) and clear 96-Well Costar microplates. Glutathione assay was performed as previously described in Hermes-Lima and Storey (1996) by following the rate of reduction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) by GSH at 412 nm compared to a standard curve of GSH.

For the measurement of TGSH, the reaction medium contained 10 μL of sample, 0.5 U/mL glutathione reductase (GR), 100 mM potassium phosphate buffer (pH 7.0), 0.25 mM nicotinamide adenine dinucleotide 2′-phosphate (NADPH), and 0.60 mM DTNB and the reduction of DTNB to TNB^{2-} was read for 20 min and compared to a GSH standard curve (0–20 μM). To quantify only GSSG, 50 μL of the initial supernatant and the GSSG standards (0–10 μM) were treated with 44.7 mM 2-vinylpyridine and 227.27 mM KPi in a total volume of 110 μL and allowed to incubate at room temperature for 90 min to derivatize the GSH to a chemically inert form. Once complete, the GSSG was measured in the same manner as TGSH using the methods described above. GSH values were calculated using the equation described above. Final values were reported in GSH/g wet weight and GSH/GSSG.

2.5. Statistical analysis

Statistical analyses were completed using RStudio (v. 0.98.501). Pearson's correlations were used to examine the association between maternal tissues and offspring at various stages in three populations with regard to oxidative stress and antioxidant capacity. To control for Type 1 errors, false discovery rate was calculated and significant p-values for antioxidant capacity and oxidative stress correlations were taken to be 0.011.

Mixed effects models were used to determine how maternal antioxidant capacity and oxidative stress were influenced by population, egg wet weight and GSI as predictor variables. An interaction term was included for egg weight and GSI. Additionally, fish ID and fish ID nested within population were tested as random variables. Akaike Information Criterion (AIC) was used to evaluate model fit. The best fitting model for each maternal tissue was identified as having the lowest AICs. Tukey post hoc tests with planned contrasts were used to examine the differences between populations, within maternal tissues.

Two-way ANOVAs were run to test the effects of both population origin and offspring developmental stage on antioxidant capacity, oxidative stress and reduced glutathione concentration. To further investigate the differences between stages and populations, Tukey post hoc tests were run. To control for Type 1 errors, false discovery rate was calculated and significant p-values for differences between populations and between offspring life stages are 0.011 and 0.010, respectively. Although data were taken from the same cohort through time, individuals were not sampled more than once so data were considered to be independent and thus we did not use repeated measures analyses.

3. Results

Antioxidant capacity, as measured by the ORAC assay, oxidative stress, as measured by the ratio of reduced glutathione to oxidized glutathione (GSH/GSSG), and reduced glutathione for all maternal and offspring tissues across the three populations are presented in Table 2.

3.1. Does maternal oxidative stress and antioxidant capacity vary with migration effort?

For all maternal tissues, both antioxidant capacity and oxidative stress did not correlate with GSI or egg weight. However, population was a significant predictor variable in the full factorial mixed effect model (ΔAIC = 0). Tukey post hoc tests revealed significant differences in antioxidant capacity and oxidative stress between populations for maternal brain and liver tissue only. Antioxidant capacity was significantly higher in brain tissue of Stellako River females compared to Chilko River ($z = 6.57$, $p < 0.01$) females, but no differences were detected between Chilko River and Harrison River ($z = 1.83$, $p = 0.793$) or Stellako River and Harrison River ($z = 3.41$, $p = 0.030$). There was also significantly higher liver antioxidant capacity in Stellako River females compared to Chilko River females ($z = 3.81$, $p < 0.01$). No differences in liver antioxidant capacity was detected between Chilko River and Harrison River females ($z = 0.39$, $p = 1.000$) or Stellako River

Table 2
ORAC (TE/mg protein), GSH/GSSH, GSH (nmol/g wet weight) levels in maternal tissues and offspring life stages in sockeye salmon. Data are means ± SEM, sample sizes n are in parentheses.

		Harrison River	Chilko River	Stellako River
Heart	ORAC	1.05 ± 0.05 (7)	0.92 ± 0.09 (15)	0.68 ± 0.04 (15)
	GSH/GSSG	3.20 ± 0.58 (4)	2.25 ± 0.32 (12)	2.71 ± 0.52 (15)
	GSH	517 ± 49 (7)	521 ± 32 (15)	921 ± 111 (15)
Liver	ORAC	0.32 ± 0.03 (7)	0.26 ± 0.04 (15)	0.69 ± 0.05 (15)
	GSH/GSSG	–	5.52 ± 1.16 (14)	1.41 ± 0.16 (15)
	GSH	–	341 ± 58 (15)	1005 ± 133 (15)
Brain	ORAC	0.84 ± 0.10 (7)	0.54 ± 0.09 (15)	1.47 ± 0.20 (15)
	GSH/GSSG	4.96 ± 1.78 (7)	2.95 ± 0.43 (14)	0.69 ± 0.09 (15)
	GSH	381 ± 27 (7)	515 ± 34 (15)	414 ± 33 (15)
Plasma	ORAC	0.73 ± 0.06 (6)	0.98 ± 0.12 (15)	0.76 ± 0.03 (15)
	GSH/GSSG	2.08 ± 0.22 (7)	1.19 ± 0.08 (15)	1.67 ± 0.10 (15)
	GSH	–	–	–
PF	ORAC	0.62 ± 0.07 (7)	0.61 ± 0.03 (15)	0.76 ± 0.03 (15)
	GSH/GSSG	1.50 ± 0.12 (7)	1.61 ± 0.05 (15)	1.67 ± 0.10 (15)
	GSH	25 ± 2 (7)	14 ± 1 (15)	–
24 HPF	ORAC	0.77 ± 0.06 (7)	0.51 ± 0.04 (14)	0.92 ± 0.05 (15)
	GSH/GSSG	4.37 ± 0.69 (7)	3.09 ± 0.23 (14)	0.54 ± 0.05 (13)
	GSH	18 ± 1 (7)	22 ± 1 (14)	4 ± 1 (13)
EYE	ORAC	0.52 ± 0.01 (7)	0.82 ± 0.08 (15)	0.66 ± 0.06 (15)
	GSH/GSSG	4.69 ± 0.75 (7)	1.49 ± 0.10 (15)	2.50 ± 0.23 (15)
	GSH	60 ± 4 (7)	31 ± 3 (15)	48 ± 4 (15)
HA	ORAC	2.05 ± 0.30 (7)	0.60 ± 0.04 (13)	0.68 ± 0.08 (15)
	GSH/GSSG	4.53 ± 0.58 (7)	5.49 ± 0.55 (13)	0.64 ± 0.07 (14)
	GSH	161 ± 11 (7)	222 ± 19 (13)	94 ± 12 (14)
EMG	ORAC	0.98 ± 0.07 (7)	0.25 ± 0.02 (14)	0.91 ± 0.10 (15)
	GSH/GSSG	7.27 ± 0.97 (7)	4.33 ± 0.52 (14)	1.81 ± 0.14 (15)
	GSH	297 ± 25 (7)	308 ± 13 (14)	288 ± 15 (15)

and Harrison River females ($z = 2.65$, $p = 0.244$). Oxidative stress was highest in brain tissue of Harrison River females, which was higher than levels in Stellako ($z = -4.49$, $p < 0.001$), but not Chilko River females ($z = 2.09$, $p = 0.3654$). There were no differences in brain oxidative stress between Chilko River and Stellako River females ($z = -2.93$, $p = 0.110$). Liver oxidative stress was higher in Chilko River females compared to Stellako River females ($z = -5.33$, $p < 0.001$).

3.2. Does offspring oxidative stress and antioxidant capacity vary with developmental stage and maternal migration effort?

There was a significant interaction between population and offspring life stage for offspring antioxidant capacity ($F_{8,166} = 18.853$, $p < 0.001$), indicating variable effects of population across developmental stages. For all life stages except EYE, Tukey post hoc tests revealed population differences in antioxidant capacity ($p < 0.011$; Fig. 1A, Table 3). Antioxidant capacity of Harrison River offspring was higher than Chilko River offspring at HA and EMG, and higher than Stellako River offspring at HA (Table 3). Antioxidant capacity was lower in Chilko River offspring compared to Stellako River offspring at PF, 24 HPF, and EMG (Fig. 1A, Table 3). Within each population, differences in antioxidant capacity were observed among developmental stages ($p < 0.010$, Fig. 1A, Table 4). Within a population, Harrison River HA had higher antioxidant capacity than PF, 24 HPF, EYE, and EMG, Chilko River EMG had lower antioxidant capacity than EYE, and Stellako River HA had lower antioxidant capacity than PF, 24 HPF, and EMG (Fig. 1A, Table 4).

Similarly, a significant interaction effect was detected between population and development stage on concentration of reduced glutathione ($F_{8,165} = 9.349$, $p < 0.001$). Tukey post hoc tests revealed that at PF, 24 HPF, EYE and EMG there were no significant differences in concentration of reduced glutathione among populations ($p < 0.011$; Fig. 1B, Table 3). Only at HA were there population-level differences in offspring reduced glutathione; Chilko River and Harrison River offspring levels were higher than those measured in Stellako River offspring, but did not differ from each other (Fig. 1B, Table 3). Tukey post hoc tests also revealed differences in concentrations of reduced glutathione among developmental stages, within a population ($p < 0.010$, Fig. 1B, Table 4). For the Harrison and Chilko River populations, reduced glutathione was higher at HA than at PF, 24 HPF and EYE, with concentrations highest at EMG compared to all other stages. Offspring from Stellako River had higher concentrations of reduced glutathione at HA than at PF and 24 HPF, and the highest reduced glutathione concentrations at EMG compared to all other earlier life stages (Fig. 1B, Table 4).

A significant interaction effect was also detected between population and offspring stage on oxidative stress ($F_{8,163} = 15.871$, $p < 0.001$). Tukey post hoc tests revealed that at PF there were no population-level differences in oxidative stress ($p < 0.011$; Fig. 1C, Table 3). Harrison River offspring had higher oxidative stress than offspring from Chilko River at EYE and EMG, and offspring from Stellako River at 24 HPF, EYE, HA, and EMG. Offspring from Chilko River had higher oxidative stress than offspring from Stellako River at 24 HPF, HA, EMG (Fig. 1C, Table 3). Differences in oxidative stress among developmental stages were also observed ($p < 0.010$, Fig. 1C, Table 4). Within the Harrison River population, PF oxidative stress was lower than all other stages and EMG oxidative stress was higher than all other stages (Fig. 1C, Table 4). In the Chilko River population, oxidative stress in offspring at HA was higher than oxidative stress at PF, 24 HPF, and EMG, and at EMG oxidative stress was higher than PF and EYE. Stellako River offspring at EYE had higher oxidative stress than levels measured at 24 HPF and HA (Fig. 1C, Table 4).

3.3. Does maternal oxidative stress and antioxidant capacity influence offspring oxidative stress and antioxidant capacity?

Despite evidence for population differences in maternal and offspring oxidative stress and antioxidant capacity (ORAC), there were few significant correlations between maternal and offspring tissues. Antioxidant capacity in heart tissues sampled from Chilko River females positively correlated with offspring antioxidant capacity (ORAC values in μmol TE/mg protein) at emergence (Pair-wise Pearson Correlation matrix with p-values < 0.011 (false discovery rate) as significant; $r = 0.78$, $p = 0.001$). No other differences were found in ORAC values between maternal tissue and offspring were found (data not shown). Likewise, comparing GSH/GSSG ratios between maternal and offspring tissues, only maternal liver tissue sampled from Stellako River females positively correlated offspring oxidative stress at 24 HPF (Pair-wise Pearson Correlation matrix with p-values < 0.011 (false discovery rate) as significant; $r = 0.70$, $p = 0.008$). No other differences were found in GSH/GSSG ratios between maternal and offspring tissues (data not shown).

4. Discussion

The semelparous life history of sockeye salmon involves a delicate balance of resource allocation, the importance of which is highlighted during their final migration to natal freshwater rivers to spawn (Fleming and Gross, 1989; Quinn, 2005; Hinch et al., 2006). Salmon must distribute their finite resources (such as time and energy) in order to maximize the fitness benefits of growth, maintenance and reproduction (Fleming and Gross, 1989). For example, females must face trade-offs having to allocate a fixed amount of energy to egg production, osmoregulatory transition from seawater to freshwater, migration to and from breeding competition on spawning grounds (Fleming and Gross, 1989). Contrary to our predictions, population origin had no clear effect on either maternal oxidative stress or antioxidant capacity, though some evidence emerged for tissue protection in Stellako River mothers that migrate the furthest of the populations studied. Offspring oxidative stress and antioxidant capacity was affected in a population-dependent manner; however, these differences were not related to maternal oxidative stress nor antioxidant capacity.

Linear mixed effects models of maternal tissues described only population as a predictor for both antioxidant capacity and oxidative stress in the brain and the liver. Our results found that brain tissue from

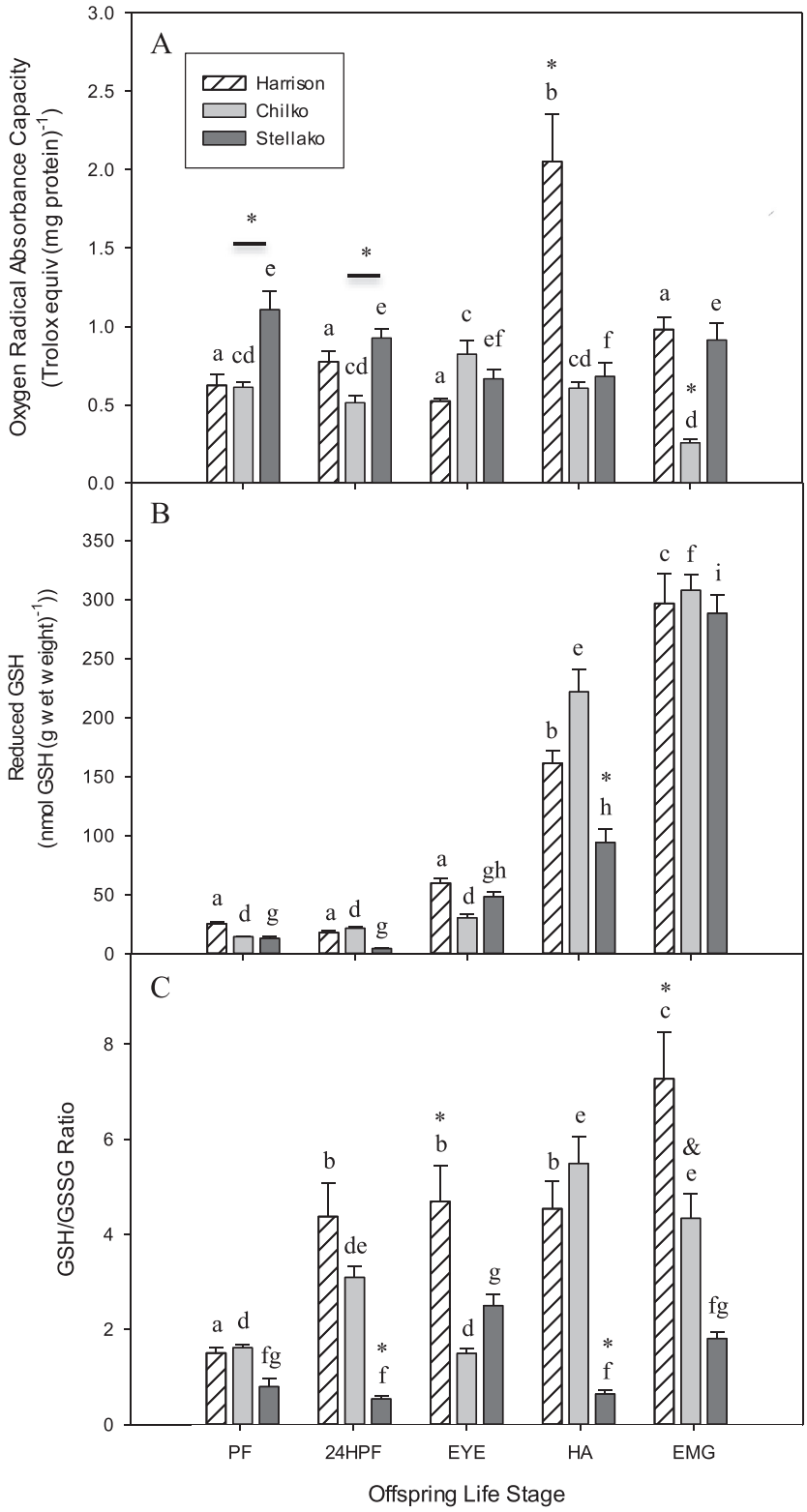

Fig. 1. Levels of antioxidant capacity shown as Trolox equivalents (TE)/mg protein (A), reduced glutathione (nmol/g wet weight) (B), and oxidative stress represented as a ratio of reduced glutathione to oxidized glutathione (GSH/GSSG) (C) for pre-fertilized eggs (PF), eggs at 24 h post-fertilization (24 HPF), eyed embryos (EYE), hatchlings (HA) and emergent fry (EMG, full yolk sac absorption) reared from female sockeye salmon captured at Harrison River (hashed bars), Chilko River (light gray bars), or Stellako River (dark gray bars) spawning sites. Letters represent significant differences between offspring developmental stages ($p < 0.010$, false discovery rate) and symbols represent significant differences between populations ($p < 0.011$, false discovery rate).

Stellako River females had higher antioxidant capacity and experienced lower oxidative stress than females from Chilko River and Harrison River, which are the populations with shorter migration distances. Similar results have been shown in pink salmon sampled at both the Strait of Georgia (prior to fasting and freshwater entrance) and at Weaver Creek spawning grounds to compare oxidative stress pre- and post-spawning migration (Wilson et al., 2014). Wilson et al. (2014) found that brain tissue had both higher antioxidant capacity and lower DNA

Table 3
Summary of Tukey post hoc tests for within stage differences for ORAC, GSH/GSSH and reduced GSH. p-Values are shown and bolded to show significance ($p < 0.010$, false discovery rate).

	ORAC				GSH/GSSG				GSH			
	24 HPF	EYE	HA	EMG	24 HPF	EYE	HA	EMG	24 HPF	EYE	HA	EMG
Harrison												
PF	1.000	1.000	<0.001	0.778	0.001	<0.001	<0.001	<0.001	1.000	0.872	<0.001	<0.001
24 HPF	–	0.984	<0.001	0.998	–	1.000	1.000	0.001	–	0.627	<0.001	<0.001
EYE	–	–	<0.001	0.376	–	–	1.000	0.008	–	–	<0.001	<0.001
HA	–	–	–	<0.001	–	–	–	0.003	–	–	–	<0.001
EMG	–	–	–	–	–	–	–	–	–	–	–	–
Chilko												
PF	1.000	0.909	1.000	0.210	0.074	1.000	<0.001	<0.001	1.000	0.995	<0.001	<0.001
24 HPF	–	0.416	1.000	0.764	–	0.034	<0.001	0.298	–	1.000	<0.001	<0.001
EYE	–	–	0.917	0.001	–	–	<0.001	<0.001	–	–	<0.001	<0.001
HA	–	–	–	0.282	–	–	–	0.453	–	–	–	<0.001
EMG	–	–	–	–	–	–	–	–	–	–	–	–
Stellako												
PF	1.000	0.148	0.003	1.000	1.000	0.013	1.000	0.590	1.000	0.268	<0.001	<0.001
24 HPF	–	0.182	0.004	1.000	–	0.003	1.000	0.266	–	0.048	<0.001	<0.001
EYE	–	–	0.996	0.108	–	–	0.005	0.963	–	–	0.038	<0.001
HA	–	–	–	0.002	–	–	–	0.373	–	–	–	<0.001
EMG	–	–	–	–	–	–	–	–	–	–	–	–

damage at the spawning ground than prior to their migration suggesting that brain may be preferentially protected over other tissues to ensure migratory success. Our results support this hypothesis of preferential tissue protection and suggest that populations with longer distance migrations have adapted to protect the brain from oxidative stress to ensure successful arrival at the spawning grounds despite the increased challenge. Our study showed that the liver might also be preferentially protected in populations making longer distance migrations, as antioxidant capacity was higher and oxidative stress lower in Stellako River compared to Chilko River. Wilson et al. (2014) also assayed liver tissue but found no difference between pre- and post-migration for oxidative stress, however the spawning migration studied in this case was only ~100 km and may not be long enough to observe the changes detected in the longer distance migrations.

Once sockeye salmon fry complete yolk-sac absorption and emerge from the gravel redds, they must migrate to rearing areas (Quinn, 2005). The direction and difficulty of this migration varies between populations of sockeye salmon and therefore fry require population-specific migration strategies (Sopinka et al., 2013). Specifically, Harrison River fry must migrate downstream to the Lower Fraser River to develop in estuaries, while Chilko River fry migrate upstream to Chilko Lake, and Stellako River fry migrate downstream to develop in Fraser Lake (see Table 1, Sopinka et al., 2013). While an upstream migration is taxing on the fry due to increased activity and risk of predation (Brannon, 1973), rearing in a riverine environment may also be challenging due to predation and flow rates, making the migration to the rearing area for the Harrison River fry arguably the most difficult of the three populations studied (Birtwell et al., 1987). Significant differences between populations at most offspring developmental stages were observed. In general, Harrison had the highest GSH/GSSG ratio indicative of the least amount of oxidative stress experienced, followed by Chilko River and finally Stellako River with the highest amount of oxidative stress. Given that rearing migration difficulty and offspring oxidative stress were inversely related, it is possible that in preparation for the upcoming migration, offspring have adapted to ensure their oxidative stress is at a level that will allow for the most successful migration and future life stages. This type of preparatory adaption has been seen in other migrant species including long distance migratory birds, the semipalmated sandpipers (*Calidris pusilla*), which prepare for their migration by priming their flight muscles by eating a diet rich in specific lipids as well as modifying dietary acids before storing them as fuel (Maillet and Weber, 2006). Although these types of adaptations prepare animals for their future migrations, it is a complex integration of multiple adjustments in morphology, biomechanics, behavior, nutrition and metabolism that ensure that individuals are set up for successful migrations (Weber, 2009). Although many factors will likely contribute to the health of the offspring as they prepare for their future migrations, the provision of antioxidants to offspring by adults represents one of these factors. It was interesting to note that offspring with the most difficult future migrations ahead had the lowest levels of oxidative stress as shown by GSH/GSSG levels. This suggested a higher provision of maternal antioxidants initially, possibly in the form of carotenoids.

Previous studies suggest that during early stages of embryogenic and larval development in fish, oxidant stress is high due to metamorphosis and rapid growth rates (Rudneva, 1999; Ciarcia et al., 2000; Kalaimani et al., 2008). During this period, antioxidant enzymes have been typically observed to increase, while a decrease is seen in low molecular weight antioxidants such as vitamins C and E, and GSH (Cowey et al., 1985; Kalaimani et al., 2008). GSH levels measured across development in this study remained low in all three populations until EYE stage, with significant increases seen at HA and continuing to EMG. These results coincide with those seen by Cowey et al. (1985) in Atlantic salmon

Table 4
Summary of Tukey post hoc tests for within population differences for ORAC, GSH/GSSH and reduced GSH. p-Values are shown and bolded to show significance ($p < 0.011$, false discovery rate).

	ORAC		GSH/GSSG		GSH	
	Stellako	Harrison	Stellako	Harrison	Stellako	Harrison
PF						
Chilko	0.009	1.000	0.870	1.000	1.000	1.000
Stellako	–	0.138	–	0.994	–	1.000
24 HPF						
Chilko	0.001	0.923	<0.001	0.595	0.992	1.000
Stellako	–	0.793	–	<0.001	–	1.000
Eye						
Chilko	1.000	0.801	0.606	<0.001	0.986	0.879
Stellako	–	0.993	–	0.009	–	1.000
Hatch						
Chilko	1.000	<0.001	<0.001	0.933	<0.001	0.022
Stellako	–	<0.001	–	<0.001	–	0.005
Emergence						
Chilko	<0.001	<0.001	<0.001	<0.001	0.975	1.000
Stellako	–	1.000	–	<0.001	–	1.000

(*Salmo salar*), who suggested that these temporal increases indicate that an efficient system for removal of peroxidase is established soon after hatch. In Asian Seabass (*Lates calcarifer*) a decrease in GSH was observed between 3 days post hatch (dph) and 20 dph (corresponding to yolk sac absorption and metamorphosis, respectively), however a drastic increase in GSH was observed at 25 dph (Kalaimani et al., 2008). This increase was synchronous with increases in catalase and glutathione peroxidase activity, which are involved in the oxidation of GSH, suggesting that secondary enzymes are active at this stage of development (Kalaimani et al., 2008). Our study indicates that glutathione concentration increases drastically at hatch, which may be attributed to the development of the larvae's endogenous antioxidant defense system. This is supported by the fact that antioxidant capacity remains relatively consistent across all stages, suggesting that in preparation for yolk sac absorption and the loss of maternal stores, larvae must compensate by increasing their endogenous antioxidants. The amount of glutathione in offspring varied among populations at HA. This among population plasticity in antioxidant development may allow for environmental variation and provide specific advantages at a population level (Quinn, 2005; Whitney, 2012). These population differences are all overcome by emergence (i.e., EMG) suggesting that regardless of degree of development at HA, antioxidant defense system efficiency is no longer population-dependent by the time the fry emerge.

In this study, neither antioxidant capacity nor oxidative stress observed in maternal tissues greatly influenced oxidative stress in offspring at the developmental stages that were examined. This result was consistent across the three populations of varying migration difficulties, with the exception of a correlation observed between maternal heart tissue and emergent offspring antioxidant capacity in the Chilko River population, and between maternal liver tissue and 24 HPF offspring oxidative stress in the Stellako River population. However, two results do not strongly infer the presence of intergenerational effects of oxidative stress. It may be that maternal antioxidants supplied to eggs consist of carotenoids which are soon depleted shortly after hatching. Carotenoids, as an antioxidant, are then replaced by the rapidly developing antioxidant system of the hatchling (in the form of glutathione). Such a strategy may be driven by the semelparous nature of Pacific salmon and the importance of offspring survival on their own fitness. Although unrelated to oxidative stress, a similar strategy is seen in other semelparous species such as *Parastrachia japonensis*, a species of shield bug (Filippi et al., 2001). Females of this species provide progressive provisioning to their brood until the third larval stadium at which time the female dies (Filippi et al., 2001). This reproductive strategy comes with large costs and risks and the female ceases feeding while provisioning her brood to ensure success because the female's entire reproductive effort is expressed in the success of that one brood (Filippi et al., 2001). Although female sockeye salmon must allocate their resources strategically to ensure their own survival to the spawning grounds (Fleming and Gross, 1989), it appears that they supply their young with sufficient antioxidants to combat potential oxidative stress which they may encounter in their initial stages of development until their own antioxidant defenses have been established.

Here we have shown that neither maternal antioxidant capacity nor oxidative stress is conferred to offspring and that oxidative stress appears to be related to population at specific life stages. We have provided further support to previous studies that have shown the brain to be protected from oxidative stress during sockeye salmon migration and also the importance of tissue-specific responses to oxidative stress (Wilson et al., 2014). The present study suggests that mothers transfer antioxidants, other than the ones measured in this study (i.e. possibly carotenoids), to their young without diminishing their own antioxidant capacity. To assess offspring oxidative stress specific in relation to maternal stress, experiments involving gametic cortisol exposure could be performed to simulate maternal deposition of cortisol into eggs (Auperin and Geslin, 2008). Our results showed that offspring develop their endogenous antioxidant system as they prepare to absorb their yolk sac at varying rates across populations, however this population-dependent variation is overcome by emergence just as all fry must transition to exogenous feeding. Overall, although maternal oxidative stress does not translate directly across generations; offspring are able to prepare themselves, in the context of oxidative stress, for future migrations based on the contributions of their mother despite stressors experienced during her migration. It is also important to consider year-to-year variance of migration conditions, as more stressful conditions may result in an intergenerational effect of oxidative stress. Based on the population-specific nature of the results in this paper, further investigation into whether these observations in offspring are based on adaptive transgenerational plasticity, genetics or a combination of both could provide insight into the long-term stability of this species in wake of climate change (Jeffries et al., 2012), fisheries (Donaldson et al., 2011), increase in aquatic pollution (Ross et al., 2013) and pathogen infections (Miller et al., 2014).

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References

Auperin, B., Geslin, M., 2008. Plasma cortisol response to stress in juvenile rainbow trout is influenced by their life history during early development and by egg cortisol content. Gen. Comp. Endocrinol. 158, 234–239.

Birtwell, I.K., Nassichuk, M.D., Beune, H., 1987. Underyearling sockeye salmon (*Oncorhynchus nerka*) in the estuary of the Fraser River. Can. Spec. Publ. Fish. Aquat. Sci. 9, a25–a35.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Brannon, E.L., 1973. Mechanisms controlling migration of sockeye salmon fry. International Pacific Salmon Fisheries Commission, New Westminster, BC.

Ciarcia, G., Paolucci, M., Guerriero, G., Cozzolino, G., Abrescia, P., 2000. Determination of vitamin E in eggs and during the larval development of the sea bass, *Dicentrarchus labrax* by high performance liquid chromatography. Biofactors 11, 19–21.

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., 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.

Costantini, D., 2008. Oxidative stress in ecology and evolution: lessons from avian studies. Ecol. Lett. 11, 1238–1251.

Cowey, C.B., Bell, J.G., Knox, D., Fraser, A., Youngson, A., 1985. Lipids and lipid antioxidant systems in developing eggs of salmon (*Salmo salar*). Lipids 20, 567–572.

Crossin, G.T., Hinch, S.G., Farrell, A.P., Higgs, D.A., Lotto, A.G., Oakes, J.D., Healey, M.C., 2004. Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. J. Fish Biol. 65, 788–810.

Crozier, L.G., Hendry, A.P., Lawson, P.W., Quinn, T.P., Mantua, N.J., Battin, J., Shaw, R.G., Huey, R.B., 2008. Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon. Evol. Appl. 1, 252–270.

Donaldson, M.R., Hinch, S.G., Patterson, D.A., Hills, J., Thomas, J.O., Cooke, S.J., Raby, G.D., Thompson, L.A., Robichaud, D., English, K.K., Farrell, A.P., 2011. The consequences of angling, beach seining, and confinement on the physiology, post-release behaviour and survival of adult sockeye salmon during upriver migration. Fish. Res. 108, 133–141.

Dowling, D.K., Simmons, L.W., 2009. Reactive oxygen species as universal constraints in life history evolution. Proc. R. Soc. B 276, 1737–1745.

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., Farrell, A.P., 2011. Differences in thermal tolerance among sockeye salmon populations. Science 332, 109–112.

Filippi, L., Hironaka, M., Nomakuchi, S., 2001. A review of the ecological parameters and implications of subsociality in *Parastrachia japonensis* (Hemiptera: Cydnidae), a semelparous species that specializes on a poor resource. Popul. Ecol. 43, 41–50.

Fleming, I.A., Gross, M.R., 1989. Evolution of adult female life history and morphology in a Pacific salmon (Coho: *Oncorhynchus kisutch*). Evolution 43, 141–157.

Garner, S.R., Neff, B.D., Bernards, M.A., 2010. Dietary carotenoid levels affect carotenoid and retinoid allocation in female Chinook salmon *Oncorhynchus tshawytscha*. J. Fish Biol. 76, 1474–1490.

Halliwell, B., Gutteridge, J.M.C., 2002. Free Radicals in Biology and Medicine. Oxford University Press.
Heise, K., Puntarulo, S., Nikinmaa, M., Abele, D., Pörtner, H.-O., 2006. Oxidative stress during stressful heat exposure and recovery in the North Sea eelpout *Zoarces viviparus L.* J. Exp. Biol. 209, 353–363.
Hermes-Lima, M., Storey, K.B., 1996. Relationship between anoxia exposure and antioxidant status in the frog *Rana pipiens*. Am. J. Physiol. Regul. Integr. Comp. Physiol. 271, R918–R925.
Hinch, S.G., Cooke, S.J., Healey, M.C., Farrell, A.P., 2006. Behavioural physiology of fish migrations: salmon as a model approach. In: Sloman, K.A., Wilson, R.W., Balshine, S. (Eds.), Fish Physiology Volume 24: Behaviour and Physiology of Fish. Elsevier, San Diego, CA, pp. 239–295.
Isaksson, C., Sheldon, B.C., Uller, T., 2011. The challenges of integrating oxidative stress into life history biology. Bioscience 61, 194–202.
Jeffries, K.M., Hinch, S.G., Sierocinski, T., Clark, T.D., Eliason, E.J., Donaldson, M.R., Li, S., Pavlidis, P., 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.
Kalaimani, N., Chakravarthy, N., Shanmugham, R., Thirunavukkarasu, A.R., Alavandi, S.V., Santiago, T.C., 2008. Anti-oxidant status in embryonic, post-hatch and larval stages of Asian seabass (*Lates calcarifer*). Fish Physiol. Biochem. 34, 151–158.
Leeuwenburgh, C., Heinecke, J., 2001. Oxidative stress and antioxidants in exercise. Curr. Med. Chem. 8, 829–838.
Lin, J., Quinn, T.P., Hilborn, R., Hauser, L., 2008. Fine-scale differentiation between sockeye salmon ecotypes and the effect of phenotype on straying. Heredity 101 (4), 341–350.
Lucas-Abellán, C., Mercader-Ros, M.T., Zafrilla, M.P., Fortea, M.I., Gabaldón, J.A., Núñez-Delicado, E., 2008. ORAC-fluorescein assay to determine the oxygen radical absorbance capacity of resveratrol complexed in cyclodextrins. J. Agric. Food Chem. 56, 2254–2259.
Lushchak, V.I., Bagnyukova, T.V., 2006. Temperature increase results in oxidative stress in goldfish tissues. 1. Indices of oxidative stress. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 143, 30–35.
Maillet, D., Weber, J.-M., 2006. Performance-enhancing role of dietary fatty acids in a long-distance migrant shorebird: the semipalmated sandpiper. J. Exp. Biol. 209, 2686–2695.
Miller, K.M., Teffer, A., Tucker, S., Li, S., Schulze, A.D., Trudel, M., Juanes, F., Tabata, A., Kaukinen, K.H., Ginther, N.G., Ming, T.J., Cooke, S.J., Hipfner, M., Patterson, D.A., Hinch, S.G., 2014. Infectious disease, shifting climates and opportunistic predators: cumulative factors potentially impacting wild salmon declines. Evol. Appl. 7, 812–855.
Monaghan, P., Metcalfe, N.B., Torres, R., 2009. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecol. Lett. 12, 75–92.
Morales, A.E., Pérez-Jiménez, A., Hidalgo, M.C., Abellán, E., Cardenete, G., 2004. Oxidative stress and antioxidant defenses after prolonged starvation in *Dentex dentex* liver. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 139, 153–161.
Neat, F.C., Huntingford, F.A., Beveridge, M., 1998. Fighting and assessment in male cichlid fish: the effects of asymmetries in gonadal state and body size. Anim. Behav. 55, 883–891.
Parihar, P., Dubey, D., 1995. Lipid peroxidation and ascorbic acid status in respiratory organs of male and female freshwater catfish *Heteropneustes fossilis* exposed to temperature increase. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 112, 309–313.
Pascual, P., Pedrajas, P., Toribio, T., López-Barea, L.-B., Peinado, P., 2003. Effect of food deprivation on oxidative stress biomarkers in fish (*Sparus aurata*). Chem. Biol. Interact. 145, 191–199.
Quinn, T.P., 2005. The behavior and ecology of Pacific salmon and trout. American Fisheries Society. UBC Press, Vancouver.
Ricklefs, R.E., Wikelski, M., 2002. The physiology/life-history nexus. Trends Ecol. Evol. 17 (10), 462–468.
Ross, P.S., Kennedy, C.J., Shelley, L.K., Tierney, K.B., Patterson, D.A., Fairchild, W.L., MacDonald, R.W., 2013. The trouble with salmon: relating pollutant exposure to toxic effect in species with transformational life histories and lengthy migrations. Can. J. Fish. Aquat. Sci. 70 (8), 1252–1264.
Rudneva, I.I., 1999. Antioxidant system of Black Sea animals in early development. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 122, 265–271.
Sopinka, N.M., Hinch, S.G., Lotto, A.G., Whitney, C.K., Patterson, D.A., 2013. Does among-population variation in burst swimming performance of sockeye salmon *Oncorhynchus nerka* fry reflect early life migrations? J. Fish Biol. 83, 1416–1424.
Stearns, S.C., 1989. Trade-offs in life-history evolution. Funct. Ecol. 3 (3), 259–268.
Stoliar, O.B., Lushchak, V.I., 2012. Environmental pollution and oxidative stress in fish. In: Lushchak, Volodymyr (Ed.), Oxidative Stress — Environmental Induction and Dietary Antioxidants. InTech.
Weber, J.-M., 2009. The physiology of long-distance migration: extending the limits of endurance metabolism. J. Exp. Biol. 212, 593–597.
Welker, T.L., Congleton, J.L., 2004. Oxidative stress in juvenile chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). Aquac. Res. 35, 881–887.
Welker, T.L., Congleton, J.L., 2005. Oxidative stress in migrating spring Chinook salmon smolts of hatchery origin: changes in vitamin E and lipid peroxidation. Trans. Am. Fish. Soc. 134, 1499–1508.
Whitney, C.K., 2012. Variation in Embryonic Thermal Tolerance Among Populations of Sockeye Salmon: Offspring Survival, Growth, and Hatch Timing in Response to Elevated Incubation Temperature. University of British Columbia, Canada (M.Sc. Thesis).
Whitney, C.K., Hinch, S.G., Patterson, D.A., 2013. Provenance matters: thermal reaction norms for embryo survival among sockeye salmon *Oncorhynchus nerka* populations. J. Fish Biol. 82, 1159–1176.
Wilson, S.M., Gravel, M.-A., Mackie, T.A., Willmore, W.G., Cooke, S.J., 2012. Oxidative stress associated with paternal care in smallmouth bass (*Micropterus dolomieu*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 162, 212–218.
Wilson, S.M., Taylor, J.J., Mackie, T.A., Patterson, D.A., Cooke, S.J., Willmore, W.G., 2014. Oxidative stress in Pacific salmon (*Oncorhynchus* spp.) during spawning migration. Physiol. Biochem. Zool. 87, 346–352.
Zera, A.J., Harshman, L.G., 2001. The physiology of life history trade-offs in animals. Annu. Rev. Ecol. Syst. 95–126.