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Examining the relationships between egg cortisol and oxidative stress in developing wild sockeye salmon (*Oncorhynchus nerka*)☆

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ABSTRACT

Maternally-derived hormones in oocytes, such as glucocorticoids (GCs), play a crucial role in embryo development in oviparous taxa. In fishes, maternal stressor exposure increases circulating and egg cortisol levels, the primary GC in fishes, as well as induces oxidative stress. Elevated egg cortisol levels modify offspring traits but whether maternal oxidative stress correlates with circulating and egg cortisol levels, and whether maternal/egg cortisol levels correlate with offspring oxidative stress have yet to be determined. The objective of this study was to examine the relationships among maternal and egg cortisol, and maternal and offspring oxidative stress to provide insight into the potential intergenerational effects of stressor exposure in sockeye salmon (*Oncorhynchus nerka*). Antioxidant concentration and oxidative stress were measured in maternal tissues (plasma, brain, heart and liver) as well as offspring developmental stages (pre-fertilization, 24 h post-fertilization, eyed, and hatch), and were compared to both naturally-occurring and experimentally-elevated (via cortisol egg bath) levels of cortisol in eggs. Oxygen radical absorptive capacity of tissues from maternal sockeye salmon was measured spectrophotometrically and was not correlated with maternal or egg cortisol concentrations. Also, naturally-occurring and experimentally-elevated cortisol levels in eggs (to mimic maternal stress) did not affect oxidative stress or antioxidant capacity of the offspring. We conclude that the metrics of maternal stress examined in sockeye salmon (i.e., maternal/egg cortisol, maternal oxidative stress) are independent of each other, and that egg cortisol content does not influence offspring oxidative stress.

1. Introduction

For oviparous organisms a key characteristic is the critical presence of all nutrients and hormones needed to sustain a viable embryo at the time of oviposition (Blackburn, 1999). These necessary hormones and nutrients can include corticosteroids, sex and thyroid hormones, amino acids, lipids, carbohydrates, vitamins, minerals, and metals, all of which must be protected from turnover and degradation until needed by the developing embryos (e.g., birds, Sotherland and Rahn, 1987; Groothuis and Schwabl, 2008; reptiles, Thompson and Speake, 2002; Bowden et al., 2001; fishes, Brooks et al., 1997). In many teleost fishes, including salmonids, thyroid hormones (Greenblatt et al., 1989), sex steroids (Feist et al., 1990) and cortisol (the primary glucocorticoid (GC) in fishes, Hwang et al., 1992), are thought to be taken up by the oocyte during vitellogenesis, a process that involves the secretion of a yolk protein precursor, vitellogenin, by the maternal liver and its sequestration into the oocyte (Brooks et al., 1997; Tyler et al., 1991). This deposition of nutrients and hormones into eggs is critical for early larval development in fishes which are unable to endogenously produce certain enzymes, hormones, and growth factors until after hatching (Hwang et al., 1992; Mylonas et al., 1994; Tanaka et al., 1995).

Abbreviations: 24HPF, 24 h post fertilization; ANOVA, analysis of variance; AUC, area under the curve; CBP, corticosteroid-binding protein; DFO, Fisheries and Oceans Canada; DTNB, 5,5′-dithiobis-(2-nitrobenzoic) acid; EDTA, ethylenediaminetetraacetic acid; EIA, enzyme immunoassay; EMG, emergence; EYE, eyed; GC, glucocorticoid; GR, glutathione reductase; GSH, glutathione; GSSG, glutathione disulfide; HA, hatch; NADPH, nicotinamide adenine dinucleotide 2′-phosphate; NSERC, Natural Sciences and Engineering Research Council; ORAC, oxygen radical absorbance capacity; PF, pre-fertilization; ROS, reactive oxygen species; SE, standard error; SEM, standard error of mean; TGSH, total glutathione; UBC, University of British Columbia.

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These hormones could also play a major role in directing development post-fertilization (Schreck et al., 2001). However, when in excess, maternally-derived gametic factors, such as cortisol, may prove to be disadvantageous to offspring development.

Maternal environment can influence gametic hormone content. In particular, stressors experienced by the mother can have varying effects on the levels of cortisol in eggs depending on severity and longevity of stressor, and when in the reproductive process the stressor is experienced (Schreck et al., 2001). Stratholt et al. (1997) showed that female coho salmon *(Oncorhynchus kisutch)* exposed to a physical stressor in the final two weeks of oogenesis had increased plasma cortisol (an established indicator of chronic stress in fishes, Wendelaar Bonga, 1997) and increased deposition of cortisol in their oocytes. This maternal effect was also observed in tropical damselfish *(Pomacentrus amboinensis)* where females exposed to increased conspecific aggression (McCormick, 2009) and females injected with cortisol 3–5 days pre-spawn (McCormick, 1998) had elevated levels of cortisol in their ovaries. There were intergenerational consequences of the observed elevation in ovarian cortisol. Offspring reared from stressor-exposed/manipulated females were smaller in size compared to undisturbed/unmanipulated females (McCormick, 1998, 2009), demonstrating a link between maternal stress, gametic cortisol and offspring traits. Egg hormone baths are a useful tool to mimic this variation in hormone deposition in fishes (e.g., Sloman, 2010), in order to explore the physiological mechanisms connecting exposure of fishes to stressors (e.g., elevated levels of circulating cortisol following fisheries capture (Donaldson et al., 2014), increased oxidative stress following exposure to elevated water temperature (Madeira et al., 2013) or aquatic pollution (Oakes et al., 2003)), and offspring development.

Several studies utilizing egg hormone baths to assess the effect of elevated egg cortisol on offspring development have revealed changes in offspring behavior and physiology. Behaviorally, experimentally-elevated egg cortisol levels have been shown to alter aggression in brown trout (*Salmo trutta*; Sloman, 2010; Burton et al., 2011), predator avoidance in coho salmon (Sopinka et al., 2015a) and response to a novel object in rainbow trout (Colson et al., 2015). Such behavioral alterations may be underpinned by egg cortisol-mediated changes to physiological processes. Indeed, exogenous egg cortisol treatment affects cardiac function (Nesan and Vijayan, 2012), metabolism (Sloman, 2010), immunity (Li et al., 2011) and the endocrine stress response (Auperin and Geslin, 2008). Another physiological process known to be linked to behavior is oxidative stress (Monaghan et al., 2009). Oxidative stress occurs in living organisms when the products of aerobic metabolism, reactive oxygen species (ROS), remain unquenched by the antioxidant defense systems and become detrimental to the cell (Metcalfe and Alonso-Alvarez, 2010; Monaghan et al., 2009). Although causal effects of oxidative stress on behavior in teleost fish remain unclear, studies suggest that oxidative stress experienced after metal exposure may be a contributing factor to behavioral changes (post-feeding behavior, Berntssen et al., 2003; gill morphology and Begum et al., 2006, swim performance, Vieira et al., 2009).

It is not yet clear how oxidative stress is affected by stressor exposure/increases in GCs, and little is known about the intergenerational effects of oxidative stress across taxa (Pamplona and Costantini, 2011). Administration of GCs in mammals (Behl et al., 1996) and birds (Lin et al., 2004) led to observed oxidative stress, feasibly through pathways that disrupt the balance of pro-oxidants and antioxidants (see Constantini et al., 2008). As mentioned previously, in fishes, environmental factors that elicit increases in circulating GCs (e.g., hypoxia, low food availability, thermal stress, chemical pollution) also elicit increases in oxidative stress (Welker and Congleton, 2004; Oakes et al., 2003; Madeira et al., 2013). Like the stressor-induced elevations detected in levels of circulating and egg cortisol, increases in oxidative stress following stressor-exposure may correlate with these traditional, hormonal signatures of stress in fishes, that are also known to influence offspring traits (e.g. juvenile growth and survival, Li et al., 2010; behavioral and physiological traits, see above). Further, these maternal metrics (i.e., maternal/egg cortisol, maternal oxidative stress) may also influence offspring oxidative stress (Taylor et al., 2015; Giordano et al., 2015). For example, it has been shown that exogenously elevated yolk GCs in domestic chickens (*Gallus domesticus*) increased progeny oxidative stress and accelerated telomere loss; both of which are linked to risk factors for disease and increased risk of mortality (Haussmann et al., 2012). However, in fishes, it has yet to be examined if maternal/egg cortisol influences offspring oxidative stress.

Over the past two decades, sockeye salmon (*Oncorhynchus nerka*) in the Fraser River (British Columbia, Canada) have experienced a substantial decline in productivity (adults produced per spawner) (Peterman et al., 2012). Declines have been associated with various environmental and anthropogenic stressors, but data are deficient as to whether intergenerational processes may be contributing to declines (Cohen, 2012). Fraser River sockeye salmon thus are a relevant species to study the relationships between maternal oxidative stress and maternal/egg cortisol, as well as the relationships between maternal/egg cortisol and offspring oxidative stress, in an effort to provide insight into the potential intergenerational effects of stress. The objectives of this study were to examine how 1) maternal oxidative stress (measured as a ratio of reduced to oxidized glutathione) and antioxidant concentration in plasma, heart, brain, and liver are related to the concentration of cortisol in eggs and 2) experimentally-elevated egg cortisol, as a proxy for stressor-induced increases in maternal deposition of egg cortisol, influences offspring oxidative stress parameters at 24 h post fertilization (24HPF), eyed (EYE), and hatch (HA) stages. We hypothesized that maternal oxidative stress would correlate with egg cortisol and that exogenously elevated egg cortisol would alter offspring oxidative stress. We predicted that 1) mothers with higher oxidative stress and reduced antioxidant capacity would deposit higher levels of cortisol into their eggs, and 2) eggs exposed to experimentally-elevated levels of cortisol would develop into offspring with higher oxidative stress and lower antioxidant capacity.

2. Materials and methods

2.1. Ethical note

Research conformed to protocols approved by Animal Care Committees at the University of British Columbia (#A11 0215) and Carleton University (#B10-06) and met the Canadian Council on Animal Care guidelines.

2.2. Collection

This study was conducted using a population of sockeye salmon that migrate ~100 km upstream in the Fraser River to spawning grounds in the Harrison River in British Columbia, Canada (49°17′5N, 121°54′27W). Sockeye salmon were collected on November 6 and 9, 2012 by beach seine net and immediately euthanized by cerebral percussion. Blood was sampled immediately from each of the collected females ($n = 7$) using caudal venipuncture into heparinized vacutainers, inverted, and centrifuged at 3200 rpm for 5 min (Clay Adams Compact II Centrifuge, Becton-Dickson; Sparks, Maryland, USA) to separate erythrocytes and plasma. Plasma, along with samples of liver, heart and brain 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. Samples of three pre-fertilized (PF) eggs from each female were also flash frozen in liquid nitrogen and stored at −80 °C until further analysis.

Collected eggs and milt were transported (~2 h) to the University of British Columbia in Vancouver, Canada for fertilization. For each female (n = 7), two replicates of fifteen grams of eggs were each transferred to a clean, dry mason jar and mixed with 1 mL milt from males to create full sibling crosses (i.e., each female paired once with a male). After milt application, 30 mL of water was added to each jar and swirled gently to activate sperm. Following Sopinka et al. (2015a), one mason jar was topped off with an additional 400 mL of water with 1000 ng/mL cortisol that was initially dissolved in 95% ethanol (0.002% final ethanol concentration). The other mason jar was topped off with an additional 400 mL of water with 0 ng/mL cortisol and the same concentration of ethanol as the cortisol-treated eggs (0.002%). The concentration of cortisol was chosen based on baseline and stressor-induced plasma cortisol levels in mature Pacific salmon that can approach and exceed 1000 ng/mL (Hruska et al., 2010; Cook et al., 2011; McConnachie et al., 2012). Also, this concentration is known to elevate egg cortisol levels within physiologically-relevant ranges in salmonids (Auperin and Geslin, 2008; Li et al., 2010; Sopinka et al., 2015a). After a two-hour incubation, fertilized eggs were rinsed thoroughly with facility water, transferred to flow-through baskets (separated by cross and cortisol-treatment), and distributed randomly in Heath stacks with semi re-circulating de-chlorinated municipal water maintained between 8 and 10 °C. Baskets were checked daily and dead embryos were removed. Offspring were sampled at 24 HPF, EYE, and HA for each cross and flash frozen in liquid nitrogen and stored at −80 °C until analysis.

Egg cortisol concentrations were quantified with an enzyme immunoassay according to the manufacturer's instructions (EIA, Neogen Corporation, http://www.neogen.com; see Sopinka et al., 2014 for further details). Briefly, three PF eggs were weighed to the nearest 0.01 g, homogenized in assay buffer, vortexed with diethyl ether, centrifuged and then flash frozen at −80 °C. The liquid phase was poured off, evaporated under nitrogen and reconstituted in 1200 μL of assay buffer. Reconstituted samples were warmed for 10 min at 65 °C, and a 250 μL subsample was removed and stored at −80 °C for use on the EIA plate. In duplicate, samples were run on two plates with intra- and inter-assay coefficients of variation 4.3% and 5.9%, respectively. Average extraction efficiency (n = 4 samples of extracted and unextracted eggs) was determined to be 83% (range 75–98%), comparable to Li et al. (2010) which also utilized an ether extraction. Maternal plasma cortisol was measured with EIA (Neogen Corporation) using methods validated previously in sockeye salmon (e.g., Jeffries et al., 2011). Samples were run in duplicate on two plates. Intra- and inter-assay coefficients of variation were 2.4% and 3.7%, respectively. After the 2 h incubation in mason jars, cortisol concentrations of eggs treated with 1000 ng/mL cortisol were significantly higher (mean ± SD, range; 33.71 ± 3.80 ng/g, 27.97–38.67 ng/g) compared to eggs treated with 0 ng/mL cortisol (10.83 ± 11.76 ng/g, 2.40–29.69 ng/g), $t = -4.899$, $p < 0.001$, Welch's Two Sample *t*-test). There were no differences in fertilization success or embryonic survival (to yolk sac absorption) between the egg cortisol treatments (0 ng/mL cortisol *versus* 1000 ng/mL cortisol) (Sopinka et al. unpublished data).

2.3. Antioxidant capacity

All maternal (liver, heart, brain) and offspring (24 HPF, EYE, HA) tissue samples were ground and homogenized using a handheld Tissue Master 125 (Omni International, Kennesaw, Georgia, USA) as described in Taylor et al. (2015). Sample lysate was centrifuged at 13,000 rpm for 5 min at 4 °C in a Hermle Labnet Z216MK (Mandel, Guelph, Ontario, Canada) and supernatant was stored at −80 °C until the Oxygen Radical Absorbance Capacity (ORAC) assay as described in Wilson et al. (2012) and Lucas-Abellán et al. (2008). 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, USA).

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 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 the pro-oxidant 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. Sample lysate was centrifuged at 13,000 rpm for 5 min at 4 °C in a Hermle Labnet Z216MK 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, California, USA) and clear 96-Well Costar microplates. The glutathione assay was performed as previously described in Hermes-Lima and Storey (1996) by following the rate of reduction of DTNB by GSH at 412 nm compared to a standard curve of GSH.

For the measurement of TGSH, the reaction media contained 10 μL of sample, 0.5 U/mL glutathione reductase (GR), 100 mM potassium phosphate buffer (potassium phosphate; pH 7.0), 0.25 mM nicotinamide adenine dinucleotide 2′-phosphate (NADPH), and 0.60 mM 5.5′-dithiobis(2-nitrobenzoic acid) (DTNB) and reduction 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 potassium phosphate in a total volume of 110 μL and allowed to incubate at room temperature for 90 min to derivatize the GSH. 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 the ratio of reduced to oxidized glutathione (GSH/GSSG; larger values represent lower oxidative stress).

2.5. Statistical analysis

Statistical analyses were completed using R 3.0.2. Pearson's correlation was used to examine the relationships between maternal plasma and PF egg cortisol levels, and maternal (plasma, liver, heart, brain) and offspring (24 HPF, EYE, HA) antioxidant concentration and oxidative stress (measured as GSH/GSSG). To control for Type 1 errors, false discovery rate was calculated and significant p-values for antioxidant concentration and oxidative stress correlations are 0.019.

Two-way ANOVAs were run to test the effects of egg cortisol treatment (0 *versus* 1000 ng/mL) and offspring developmental stage (24 HPF, EYE, HA) on offspring antioxidant concentration, reduced glutathione concentration, and oxidative stress. Significant main effects were investigated using Tukey *post hoc* tests. To control for Type 1 errors, false discovery rate was calculated and significant p-values for differences between egg cortisol treatments and among developmental stages were 0.027 and 0.020, respectively. Data were considered to be independent as the same individuals were not sampled more than once throughout the duration of the experiment. Accordingly repeated measures analyses were not used.

3. Results

Naturally-occurring egg cortisol concentrations (mean ± SD, 17.35 ± 12.47 ng/g, range 7.62–36.10 ng/mL) did not correlate with oxidative stress or antioxidant concentration in any maternal tissues or offspring whole body homogenate (Table 1). Naturally-occurring maternal plasma cortisol (mean ± SD, 197.90 ± 41.21 ng/mL, range 148.68–259.33 ng/mL) initially correlated positively with PF antioxidant concentration ($r = 0.85$, $p = 0.016$, Table 1) but correlated negatively with offspring antioxidant capacity 24 h later at 24HPF ($r = -0.90$, $p = 0.007$, Table 1) and with offspring GSH/GSSG ratio at HA ($r = -0.99$, $p = 0.008$, Table 1). Maternal plasma cortisol did not correlate with egg cortisol concentrations ($r = -0.60$, $p = 0.129$).

Two-way ANOVA revealed neither egg cortisol treatment ($F_{1,26} = 4.014$, $p = 0.056$) nor developmental stage ($F_{2,26} = 4.028$, $p = 0.030$) influenced antioxidant concentration (Fig. 1A). Offspring developmental stage ($F_{2,26} = 105.391$, $p < 0.001$), but not egg cortisol treatment ($F_{1,26} = 2.807$, $p = 0.106$), affected concentration of reduced glutathione (two-way ANOVA, Fig. 1B). Tukey *post hoc* tests revealed that HA had reduced glutathione that differed significantly from levels at 24HPF ($p < 0.001$) and EYE ($p < 0.001$), but there were no differences in reduced glutathione between 24HPF and EYE ($p = 0.801$, Fig. 1B). Similarly, offspring oxidative stress varied across developmental stage ($F_{2,26} = 10.717$, $p < 0.001$), but not between egg cortisol treatments ($F_{1,26} = 0.105$, $p = 0.748$) (two-way ANOVA, Fig. 1C). Tukey *post hoc* tests revealed that there were significant differences in offspring oxidative stress between 24HPF and EYE ($p < 0.001$), but not between 24HPF and HA ($p = 0.068$) or EYE and HA ($p = 0.116$, Fig. 1C).

4. Discussion

We explored how maternal oxidative stress relates to maternal and egg cortisol, and in turn, how egg cortisol alters offspring oxidative metrics. Maternal plasma cortisol did not correlate with egg cortisol levels in this study. The disconnect between maternal and egg cortisol may be due to low sample sizes that do not encompass a broad enough range of cortisol values; plasma cortisol levels detected in the 7 females sampled in this study did not exceed 300 ng/mL. It is also possible that a relationship between these two traits emerges (or are revealed *sensu;* Killen et al., 2013) when females are exposed to stressors (e.g., Stratholt et al., 1997). Although experimental manipulation of cortisol levels is a common approach in experimental biology (Sopinka et al., 2015b; Crossin et al., 2016) it fails to mimic the entirety of the stress response (e.g., the sensory component and entire neuroendocrine cascade). There was no support for the prediction that maternal oxidative stress levels were correlated with circulating cortisol levels or cortisol concentration in PF eggs. Accordingly, it appears that although these metrics independently can be indicative of maternal stress, they are not correlated with each other in females not experimentally exposed to a stressor. The tissues used in this study were collected from females caught on spawning grounds following migration from the ocean to freshwater. Again, a relationship between maternal oxidative stress and maternal/egg cortisol may only manifest when females are exposed to stressors (Killen et al., 2013). Also, other egg components, known to alter offspring phenotype (e.g., genetic material, sex/thyroid hormones, lipids) may be coupled with maternal oxidative stress. Future studies incorporating experimental manipulation of females (e.g., exposure to a stressor known to induce oxidative stress, Welker et al., 2004), multiple concentrations of cortisol when exogenously treating eggs (Auperin and Geslin, 2008; Li et al., 2010), or investigation of other egg constituents can further our understanding of the interactions between maternal stress/state and gamete quality.

Interestingly, maternal plasma cortisol did correlate with offspring oxidative stress metrics at different developmental stages. Mothers with higher levels of plasma cortisol produced eggs with higher antioxidant concentration. The adaptive potential of maternal responses to stress was recently reviewed by Sheriff and Love (2013). If maternal cortisol is indicative of maternal stress/stressful environment (e.g., Stratholt et al., 1997; McCormick, 2006), increased antioxidant concentration in eggs of females with higher cortisol may be adaptive and function to increase offspring survival. At 24HPF maternal cortisol negatively correlated with embryo antioxidant capacity suggesting that maternally-derived antioxidant stores of offspring reared from mothers with elevated cortisol may have been utilized shortly after fertilization. At hatch, when offspring are able to endogenously respond to stressors via activation of the hypothalamic-pituitary-interrenal (HPI) axis (Feist and Schreck, 2001), maternal plasma cortisol was negatively correlated with oxidative stress suggesting increased maternal plasma cortisol may benefit offspring at developmental stages out of the scope of this study. Ultimately, the functional significance of these correlations remains speculative and further work encompassing a broader range of maternal cortisol levels is required.

Contrary to predictions, experimentally elevated egg cortisol levels did not affect either oxidative stress or antioxidant concentration in offspring, compared to levels observed in offspring reared from unmanipulated eggs. In fishes, negative effects of elevated egg GCs on offspring traits are the findings predominantly emphasized in a given study (e.g., reduced competitive ability (Burton et al., 2011; Eriksen et al., 2011), size (McCormick, 1999), and survival (Gagliano and McCormick, 2009)). However, these same traits, and others, are also found to not be affected by elevated egg GCs (e.g., metabolism (Burton et al., 2011), survival (Stratholt et al., 1997; Sloman, 2010; Sopinka et al., 2015a), and size (Colson et al., 2015)). Differing findings may be attributable to a number of factors including experimental design (e.g., rearing environment, wild-caught *versus* domesticated females) and variation in life history of study species (e.g., iteroparous *versus* semelparous). For example, exogenously-elevated cortisol in eggs from semelparous Pacific salmon, that reproduce once before dying, does not affect offspring survival (Stratholt et al., 1997; Sopinka et al.,

Table 1
Pearson correlations for maternal tissue and offspring whole-body homogenate ORAC values, reduced glutathione (GSH) and GSH/GSSG with egg cortisol pre-fertilization and maternal plasma cortisol. Listed are r values, p values are shown in parentheses. *p* values <0.019 (false discovery rate) are considered significant.

	Egg cortisol			Maternal cortisol		
	ORAC	GSH	GSH/GSSH	ORAC	GSH	GSH/GSSH
Maternal						
Plasma	−0.09 (0.856)		0.30 (0.569)	−0.26 (0.566)		0.02 (0.974)
Brain	0.17 (0.752)	−0.53 (0.223)	−0.53 (0.222)	0.23 (0.655)	−0.17 (0.713)	0.27 (0.555)
Liver	0.36 (0.430)			0.08 (0.866)		
Heart	−0.21 (0.645)	−0.40 (0.380)	−0.12 (0.792)	0.08 (0.867)	0.16 (0.729)	0.00 (0.995)
PF	−0.47 (0.282)	−0.78 (0.039)	−0.67 (0.097)	0.27 (0.564)	0.85 (0.016)	0.27 (0.554)
Offspring						
24HPF	0.38 (0.406)	−0.37 (0.411)	0.58 (0.172)	−0.89 (0.007)	0 (0.992)	−0.57 (0.182)
Eyed	0.18 (0.777)	0.01 (0.988)	−0.49 (0.402)	−0.34 (0.577)	−0.03 (0.958)	0.44 (0.453)
Hatch	0.37 (0.631)	0.77 (0.231)	0.98 (0.022)	−0.12 (0.877)	−0.68 (0.321)	−0.99 (0.008)

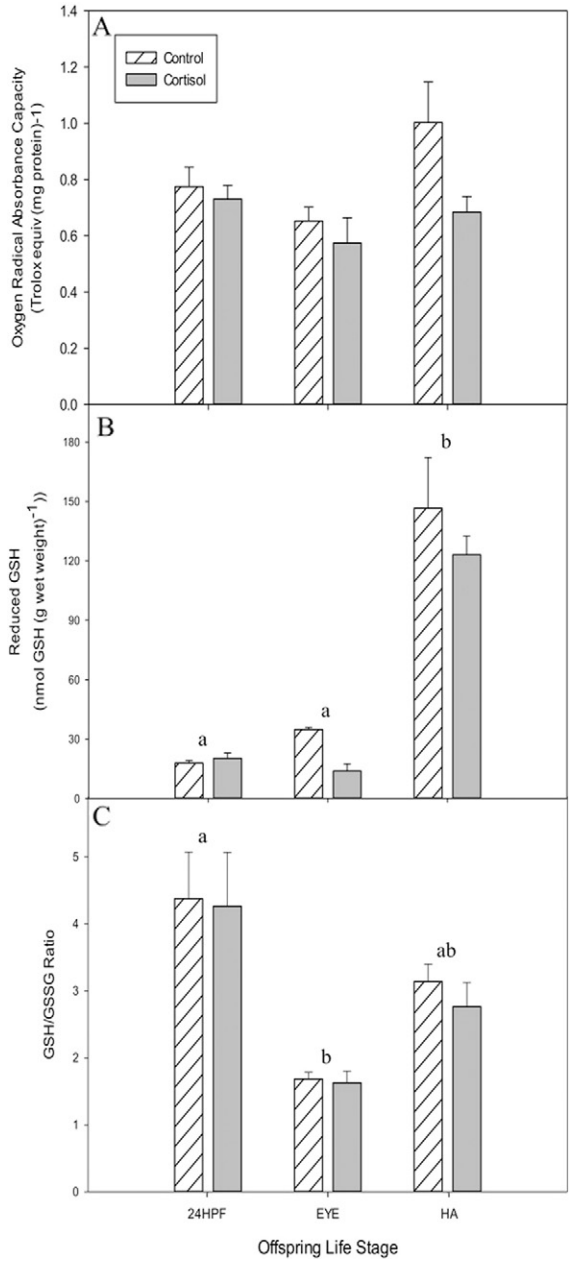

Fig. 1. Effect of offspring developmental stage and egg cortisol treatment on oxidative stress and antioxidant capacity. Levels of antioxidant capacity shown as Trolox equivalents (TE)/mg protein A), reduced glutathione (nmol/g wet weight) B) and oxidative stress represented as a ratio of reduced glutathione to oxidized glutathione (GSH/GSSG) C) for eggs at 24 h post-fertilization (24HPF), and eyed (EYE) and hatch (HA) embryos reared from female sockeye salmon captured at Harrison River. Offspring were reared from eggs fertilized with control water (0 ng/mL cortisol, hashed bars) or cortisol-dose water (1000 ng/mL, grey bars). Letters represent significant differences between offspring stages ($p < 0.020$, false discovery rate).

2015a; this study). In a tropical damselfish that repeatedly spawns within a 6-week breeding season, offspring survival is affected by egg cortisol treatment (Gagliano and McCormick, 2009). It is possible that when no effect of elevated egg cortisol is detected on offspring traits, that buffering of egg cortisol may be occurring. Indeed, in ovarian follicles and ovulated eggs of rainbow trout cortisol is converted to cortisone and GC sulphates, suggesting presence of enzymes that can metabolize cortisol to potentially, biologically inactive forms (Li et al., 2012). One such enzyme is 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2); mRNA transcripts of 11β-HSD2 are detected in pre-hatch embryos in zebrafish (*Danio rerio*) (Jeffrey and Gilmour, 2016) and European sea bass (*Dicentrarchus labrax*) (Tsalafouta et al., 2014). There is also evidence of active transport of cortisol out of newly fertilized eggs of threespined stickleback (*Gasterosteus aculeatus*) via ATP-binding cassette (ABC) transporters (Paitz et al., 2016), which could mediate effects of effects of elevated cortisol on offspring traits, including physiological pathways inducing phenotypic change (e.g., changes in gene expression *via* binding of cortisol to GC receptors, Bury and Sturm, 2007).

Here, we have shown that in sockeye salmon, physiological indicators of maternal stress are not always related, and these indicators do not necessarily correspond with biomarkers of stress in offspring (Taylor et al., 2015). Previous studies have demonstrated the importance of timing and severity of cortisol responses during reproduction in female fish (Schreck et al., 2001). Therefore, as individual and interacting stressors (see Johnson et al., 2012) such as climate change (Jeffries et al., 2012), fisheries interactions (Donaldson et al., 2011), aquatic pollution (Ross et al., 2013) and pathogen infections (Bradford et al., 2010; Miller et al., 2014) create additional challenges to migrating Pacific salmon, the effect of timing and severity of these stressors on intergenerational oxidative stress may be an interesting avenue for future research. Beaulieu et al. (2013) also highlighted the importance of life-history traits, life stages, and environmental conditions in future research when considering the use of oxidative markers as indicators of population health in conservation ecology. Although there appears to be no direct relationship between cortisol levels and oxidative metrics measured here in sockeye salmon, research on these metrics individually (e.g., Cook et al., 2014; Wilson et al., 2014) still provide insight into the long-term stability of this ecologically and economically relevant species. The potential for buffering of egg hypercortisolism by progeny (Paitz et al., 2016), and mother (e.g., Faught et al., 2016), is also a valuable avenue of future research. Collectively, although this study did not reveal correlational or causal effects of egg cortisol on progeny oxidative stress, separately, these traits still represent important forms of maternal investment in offspring.

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