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# Effects of experimentally elevated egg cortisol on offspring traits in two species of wild Pacific salmon

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**Abstract** In fishes, elevated levels of cortisol in eggs can have carry-over effects on phenotypic and performance traits early in life. How responses to elevations in egg cortisol differ among species remains poorly understood. Using wild populations of chum salmon (*Oncorhynchus keta*) and sockeye salmon (*O. nerka*), we investigated whether experimentally-elevated concentrations of cortisol in newly fertilized eggs had effects on offspring morphology and/or burst swimming capacity. Immediately following fertilization, eggs were incubated for 2 h with water dosed with 0 ng/mL or 1000 ng/mL of cortisol. Embryos were reared to the fry life stage (complete yolk sac absorption). Morphology and burst swimming performance of fry were then assessed. Sockeye salmon fry reared from cortisol-treated eggs were smaller overall (i.e., smaller body, fins and eyes) compared to conspecifics reared from untreated eggs. In contrast, the morphology of chum salmon fry was not affected by the experimental elevation of egg cortisol. In both species, burst swimming duration was unaffected by egg cortisol treatment, while offspring reared from the cortisol-treated eggs initiated fewer bouts of burst swimming. Our results demonstrate that closely-related species can respond differently to elevations in egg cortisol, and not all offspring traits may be affected by these elevations in cortisol. Further efforts to establish links among offspring quality, maternal stress, and egg composition need to consider the potential for divergent responses among species and examine multiple measures of phenotype and performance throughout development.

**Keywords** Glucocorticoid · *Oncorhynchus* · Swimming · Morphology · Fish · Survival

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## Introduction

Repeated exposure to stressors that elevate levels of maternal cortisol, the primary glucocorticoid in fishes (e.g., handling stressors, Barton 2002; competitive interactions, Filby et al. 2010), can also elevate concentrations of cortisol in eggs (e.g., Stratholt et al. 1997; McCormick 2009). These naturally-occurring elevations in egg cortisol can result in smaller offspring (McCormick 2009). Experimental elevations in egg

cortisol, carried out to mimic the increases in egg cortisol observed in stressor-exposed females (e.g., via injection of females with cortisol [Eriksen et al. 2011] or bathing of unfertilized/fertilized eggs with cortisol-dosed solutions [Li et al. 2010; Sopinka et al. 2015]), have revealed that egg cortisol can influence a range of offspring attributes across a range of species. For example, exogenously-elevated cortisol concentrations in eggs are linked with reduced hatching success but enhanced growth in rainbow trout (*Oncorhynchus mykiss*, Li et al. 2010), and elevated oxygen consumption (Sloman 2010) and subordinate social status in brown trout (*Salmo trutta*, Burton et al. 2011). In zebrafish (*Danio rerio*), exogenously-elevated egg cortisol alters activity of the hypothalamus-pituitary-interrenal (HPI) axis (Nesan and Vijayan 2016) and impairs cardiac function (Nesan and Vijayan 2012). Fishes are increasingly being exposed to multiple stressors (e.g., extreme temperatures, Chadwick et al. 2015; aquatic contaminants, Pratap and Wendelaar Bonga 1990; catch-and-release fisheries, Marcalo et al. 2006) that can increase circulating cortisol and thus, potentially, concentrations of cortisol in eggs. As important populations of fishes have declined while under pressure from multiple stressors (e.g., Hutchings and Reynolds 2004; Crain et al. 2008), new knowledge on the role that egg cortisol plays in effecting intergenerational change is of fundamental and applied relevance.

Knowledge on whether phenotypic responses of offspring to elevated egg cortisol can vary between closely related species is limited. Identifying inter-specific patterns in offspring responses to elevated cortisol in eggs could reveal useful insight into which species are likely to be tolerant or vulnerable to stressor-induced increases in egg cortisol. Inter-specific differences in stressor-induced plasma cortisol production have been documented in fishes (e.g., Pottinger 2010), including salmonids. Barton (2000) found variation in post-stressor plasma cortisol levels among trout within a genus (e.g., between Lake trout [*Salvelinus namaycush*] and brook trout [*S. fontinalis*]) and among genera within a family (e.g., between rainbow trout and brown trout). Working with wild-caught Pacific salmon (*Oncorhynchus* spp.), Donaldson et al. (2014) found that post-stressor plasma cortisol levels were higher in pink salmon (*Oncorhynchus gorbuscha*) than in sockeye salmon (*Oncorhynchus nerka*). Whether these inter-specific differences in organismal stress variables correlate with egg cortisol content is not fully understood. Within a species, egg cortisol concentration is reported to be no different between low and high stress-reactivity strains of rainbow trout (Andersson et al. 2011). Still, this finding does not preclude the potential for chronic maternal stress to affect egg cortisol differently among species, nor does it preclude the possibility of inter-specific differences in offspring phenotype/performance resulting from elevations in egg cortisol. To date, species-specific intergenerational effects in salmonids have mostly been limited to embryonic offspring that are typically immobile (e.g., Campbell et al. 1994; Essington et al. 2000), and have not considered maternally-derived egg cortisol as a mechanistic factor.

In salmonids, the swimming performance of fry (the life stage that follows complete yolk absorption) is influenced by body size (Taylor and McPhail 1985), differs among species (Hawkins and Quinn 1996), can be shaped by maternal effects (Burt et al. 2012a) and has been correlated with maternal, but not egg, cortisol (Tierney et al. 2009). Upon yolk sac absorption, Pacific salmon fry must quickly transition from reliance on their yolk sac to exogenous food sources by migrating to rearing areas to feed and grow before migrating to the ocean as smolts (Quinn 1999). During this period of growth, young fish must successfully evade predators. It remains unclear how egg cortisol influences metrics related to predator escape (e.g., burst swimming capacity and hydrodynamic morphology). Sopinka et al. (2015) found differential behavioural responses to a simulated predator attack between juvenile (~3 months post-yolk sac absorption) coho salmon (*Oncorhynchus kisutch*) reared from untreated eggs and those reared from eggs with exogenously elevated cortisol levels. However, there have been few direct between-species comparisons of the effects of elevated egg cortisol on offspring performance.

The diverse life histories of Pacific salmon provide a useful model for understanding the inter-specific effects of elevated egg cortisol on offspring phenotype and performance. Species-level differences could be considered by conservation practitioners trying to understand animal sensitivity to stressors (Donaldson et al. 2012). Research that illustrates functional differentiation among closely related species can help inform conservation actions aimed at protecting diversity, consistent with the stated principles of Pacific salmon management (in Canada; DFO 2005). Accordingly, we examined the hypothesis that elevation of egg cortisol would alter fry morphometrics and burst swimming performance in a

species-specific manner. Eggs were collected from wild chum salmon (*Oncorhynchus keta*) and sockeye salmon (*O. nerka*) migrating in the Harrison River in British Columbia, Canada. Concentrations of egg cortisol were then experimentally elevated immediately following fertilization in the laboratory. Following incubation of embryos, morphometrics and burst swimming performance of chum salmon and sockeye salmon fry were assessed.

## Methods

### Offspring rearing

Ripe, wild chum salmon and sockeye salmon were collected in November 2012 from spawning areas along the Harrison River (49°17′5″ N, 121°54′27″ W) in the Fraser River watershed in British Columbia, Canada. Following capture via beach seine and after fish were euthanized via cerebral percussion, eggs and milt were collected. Gametes were fertilized following Sopinka et al. (2015) at the University of British Columbia (UBC), generating nine full sibling crosses (each female's eggs were fertilized once with a male's milt) of chum salmon and seven full sibling crosses of sockeye salmon. For each full sibling cross, four replicates of 15 g of eggs were mixed with a few drops of milt each and then 30 mL of dechlorinated water was added to each replicate to activate sperm. After 2 min, 400 mL of water was added to each milt-egg mixture and left to stand for 2 h. Two replicates received water with 1000 ng/mL cortisol (H4001, Sigma, www.sigmaaldrich.com) that was initially dissolved in 95 % ethanol (0.002 % final ethanol concentration), and the other two replicates (0 ng/mL cortisol) received water with the same concentration of ethanol as the cortisol-treated eggs (0.002 %). Given that duration of sperm motility of Pacific salmon is <1 min (Hoysak and Liley 2001), it is unlikely that any sperm that fertilized the eggs were exposed to cortisol. Cortisol dose was chosen based on 1) previous studies that increased egg cortisol concentrations within naturally-occurring levels in salmonids (Auperin and Geslin 2008), and 2) evidence that stressor-induced (Cook et al. 2011) and baseline (Hruska et al. 2010; McConnachie et al. 2012) plasma levels of maternal cortisol can approach and exceed 1000 ng/mL in mature Pacific salmon. After 2 h of incubation (following Burton et al. 2011), fertilized eggs were rinsed thoroughly with dechlorinated freshwater and transferred to semi-recirculating vertical-stack incubators (Heath stacks). Families and replicates were incubated separately within the Heath stacks. Mean (± SD) temperature of the dechlorinated freshwater circulating through the Heath stacks was 8.2 ± 0.6 °C. Water temperature was maintained using aquarium heaters and chillers, and digital controls (ReefKeeper Lite, Digital Aquatics, www.digitalaquatics.com). Dissolved oxygen was maintained above 85 % and the flow of water through each Heath stack/tray was maintained at ~10 L/min. Stacks were checked every other day to remove any dead eggs/embryos. Dead eggs/embryos were stored in Stockard's solution (5 % formaldehyde, 4 % glacial acetic acid, 6 % glycerin, 85 % water) to enable assessment of fertilization success. At the fry life stage (complete yolk sac absorption), were pooled and fry were transferred to 1000 L flow-through troughs separated by species and cortisol treatment. Family-level effects are important contributors to offspring performance (e.g., Burt et al. 2012a, b). However, the focus of this study was on treatment-level differences and due to logistical constraints of the study design and rearing infrastructure, family-level effects were not fully investigated. Throughout the experiment, photoperiod in the laboratory was adjusted to mimic the photoperiod at latitude 49°18′N. Water temperature in the flow-through troughs ranged from 5 to 9 °C due to natural fluctuations in the municipal water source that was used. Fry were fed powdered fishmeal (EWOS Canada Ltd., www.ewos.com) ad libitum twice daily until 24 h prior to burst swimming trials.

### Cortisol concentrations

To assess the efficacy of the egg cortisol treatment, cortisol concentrations of unfertilized and newly fertilized eggs were quantified with enzyme immunoassay (EIA, Neogen Corporation, www.neogen.com, product #402710) following Sopinka et al. (2015). Three unfertilized eggs were collected from each female, and three fertilized eggs were collected from each replicate 2 and 24 h post fertilization (hpf). Collected tissue was frozen in liquid nitrogen and transferred to a − 80 °C freezer for storage. Using predictive models of Pacific salmon development (IncubWin, Fisheries and Oceans Canada), with the temperature of water ranging from 8 to 10 °C, fertilized eggs 2 hpf are not developed beyond the 1st cleavage, and fertilized eggs 24 hpf are 32 cells. Endogenous cortisol production (in response to a stressor) does not occur until after hatch in Pacific salmon (e.g., Feist and Schreck 2001). Thus, the concentrations of cortisol detected in the collected tissue samples are thought to be maternally-

derived (i.e., in the yolk) and not reflect endogenously produced hormone. In the laboratory, unfertilized/fertilized eggs were thawed, weighed (to the nearest 0.01 g) and homogenized in 1200 μL of assay buffer. The homogenate was vortexed with 3 mL of diethyl ether and flash-frozen by placing it in a − 80 °C freezer for 30 min. The liquid phase was poured off, evaporated under nitrogen and reconstituted in 1200 μL of assay buffer. Reconstituted samples were warmed for 10 min in an incubator set at 65 °C, after which a 50 μL subsample was removed for use on the EIA plate. Samples were run in duplicate on four assay plates with intra- and inter- assay coefficients of variation of 4 % and 5 %, respectively. Concentrations of cortisol in unfertilized/fertilized eggs were calculated as ng/g and incorporate dilution factors.

## Burst swimming performance

For a detailed description of the swim trial equipment and protocol we used, see Sopinka et al. (2013, 2014). Briefly, fry that were two months post-yolk sac absorption were randomly selected from a trough and placed in a swim flume with fixed water speed of 39 and 34 cm/s for chum and sockeye salmon, respectively. Water speed was chosen to achieve 8–9 fork lengths per s (mean ± SE fry fork length, 4.88 ± 0.04 cm and 3.81 ± 0.04 cm for chum and sockeye salmon, respectively). These speeds have previously resulted in salmon fry swimming for ~30 s before exhaustion (Sopinka et al. 2013), a duration that falls within general definitions of burst swimming (speeds maintained for ~20 s, Beamish 1978), but that is longer in duration than a startle response (i.e., <1 s). Exposure to these water speeds recruits anaerobic metabolic pathways (Brett 1964) and elicits the maximal swimming effort that could be necessary for predator evasion (Taylor and McPhail 1985). Each trough held up to 1000 fry pooled from all families within a species/egg cortisol treatment and random selection of fry was employed to reduce likelihood of pseudoreplication within a single family. The front of the swim flume was shaded and a light shone on the back section of the flume to encourage fish to swim forward. All trials were recorded with a digital camera (Canon EOS Rebel T3i, www.canon.com). Exhaustion, and the end of the swim trial, was defined as failure of a fish to move from the back of the working section of the flume despite being prodded three times with a blunt instrument. Exhausted fish were removed from the flume and sacrificed with an overdose of buffered MS-222. Body mass was recorded to the nearest 0.001 g. Finally, a photograph was taken of each fish against graph paper (for scale) with a digital camera (Nikon D40, www.nikonusa.com) for morphological analyses (see below).

Burst swimming duration and burst swimming rate were quantified from recorded videos. In total, 171 fish were swum (79 sockeye salmon [0 ng/mL, $n = 29$; 1000 ng/mL, $n = 50$]; 92 chum salmon [0 ng/mL, $n = 42$; 1000 ng/mL, n = 50]). Fish either 1) swam continuously in one bout of burst swimming (i.e., remained at the front, shaded part of the flume until exhaustion), or 2) swam multiple bouts of burst swimming (i.e., re-initiated swimming to the front, shaded part of the flume after falling to the back, un-shaded portion of the working section prior to exhaustion). Total number of bouts before exhaustion were tallied and burst swimming duration was calculated as the summed duration (in s) a fish was swimming in the front, shaded part of the flume (summation of all burst swimming bouts). Burst swimming rate was calculated as the total number of bouts completed per 10 s of swimming. A fish was classified as having failed to swim if, after transfer to the flume and three probes with a blunt instrument, it did not initiate swimming.

## Morphology

Following Pon et al. (2007), morphological traits (Fig. 1) were measured from digital images using ImageJ (imagej.nih.gov/ij/). Eleven measurements were taken from each fish: fork length (FL), fin size, which included pectoral fin length (PEC, left fin only), caudal fin length (upper segment [CAUD1], lower segment [CAUD 2]), caudal fin height (CAUD 3) and caudal fin area (CAUD 4), body depth, which comprised three measurements (distance between dorsal and pelvic fins [DORPEL], distance between adipose and anal fins [ADAN], and caudal peduncle height [PED]), and eye area, which was calculated using the formula: $\pi$ x (0.5 x eye width [EW]) x (0.5 x eye length [EL]) (Neff 2004).

## Data analyses and statistics

All statistical tests were conducted using JMP 10.0.2 (SAS Institute Inc.). Data were $\log_{10}$ (cortisol concentration), cubed-root (burst swimming duration) or logit (fertilization success) transformed to achieve normality and thus enable use of parametric statistics. If data could not be transformed to achieve normality, non-parametric tests were used. Two-way ANOVAs with egg cortisol treatment (0 ng/mL cortisol versus 1000 ng/mL cortisol) and time post fertilization (2 hpf versus 24 hpf) as fixed

effects were used, separately for each species, to determine differences in egg cortisol concentrations.Linear mixed models with egg cortisol treatment as a fixed effect and female ID nested within egg cortisol treatment as a random effect were used to determine differences in fertilization success and embryo survival. Fertilization success was calculated as: total number of fertilized eggs/total number of eggs. Embryo survival was calculated as: total number of surviving fry/total number of fertilized eggs. Values for fertilization success and embryo survival inputted into the models were averaged for the two replicate crosses per egg cortisol treatment (see above). Fry morphological data (eye area, body mass, and traits in Fig. 1, excluding EW and EL) were loaded into a principal component analysis (PCA). PC1 explained 80 % of the variation (eigenvalue = 8.9) and positively correlated with all contributed metrics (all positive eigenvectors). PC1 thus represented a general trend of body size and depth, eye size, and fin size, and was used in subsequent analyses. Three chum salmon (0 ng/mL, $n = 1$; 1000 ng/mL, $n = 2$) and four sockeye salmon (1000 ng/mL, $n = 4$) were excluded from the PCA analyses as one of the metrics included in the PCA could not be obtained from the digital images. The interactive effect of species (chum salmon versus sockeye salmon) and egg cortisol treatment (0 ng/mL cortisol versus 1000 ng/mL cortisol) on fry PC1 was analyzed using a two-way ANOVA. Separately for each species, Student's *t*-tests, with a Bonferroni correction ($P < 0.005$), were used to further assess which of the 11 morphometric variables (see above and Fig. 1) were driving any observed differences between egg cortisol treatments. Separately for each species, Chi-squared tests were used to determine whether the proportion of fish that failed to swim differed between egg cortisol treatments. A two-way ANOVA with species and egg cortisol treatment as fixed effects was used to examine effects of egg cortisol treatment on absolute burst swimming duration. A two-way ANCOVA with PC1 as a covariate, and species and egg cortisol treatment as fixed effects, was then used to assess effects of egg cortisol treatment on burst swimming duration, correcting for body size. Egg cortisol treatment effects on number of bouts of burst swimming and burst swimming rate were quantified with a Wilcoxon signed-rank test separately for each species. Non-significant interactions ($P > 0.05$) were removed from statistical models.

## Results

### Cortisol concentrations

Two-hour incubation in the cortisol-dosed solution (1000 ng/mL) significantly elevated cortisol levels in the newly fertilized eggs of chum salmon (Two-way ANOVA, egg treatment: $F_{1,32} = 112.11$, $P < 0.0001$; hpf: $F_{1,32} = 125.33$, $P < 0.0001$; hpf x egg treatment: $F_{1,32} = 18.23$, $P = 0.0002$; Fig. 2a) and of sockeye salmon (egg treatment: $F_{1,24} = 24.25$, $P < 0.0001$; hpf: $F_{1,24} = 17.51$, $P = 0.0003$; hpf x egg treatment: $F_{1,24} = 5.78$, $P = 0.02$; Fig. 2b). Experimentally-elevated cortisol levels of chum salmon (mean ± SE; 22.3 ± 1.4 ng/g) and sockeye salmon (33.7 ± 1.4 ng/g) fertilized eggs were similar to those detected in previous studies using salmonids (55.0 ± 5.4 ng/g, brown trout, Burton et al. 2011; 33.0 ± 0.9 ng/g , coho salmon, Sopinka et al. 2015) and were similar to the mean concentration detected in eggs of coho salmon chased daily for two weeks prior to spawning (25.3 ± 0.8 ng/g; Stratholt et al. 1997). Thus, the elevated concentrations of cortisol our manipulation achieved approximate

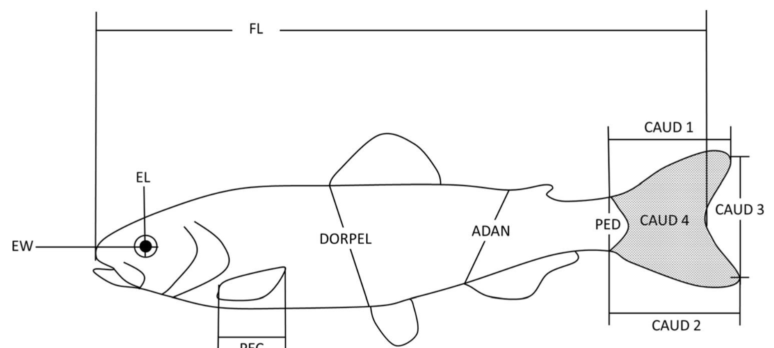



**Fig. 1** Body size (FL), depth (DORPEL, ADAN, PED) and fin (PEC, CAUD 1–4) metrics measured for each fish. Eye area was calculated using the formula: $\pi$ x [0.5 x eye width (EW)] x [0.5 x eye length (EL)]. See Methods for details

ecologically-relevant levels, rather than being elevated to pharmacological or pathological levels (e.g., 699.0 ± 46.4 ng/g, Sloman 2010).

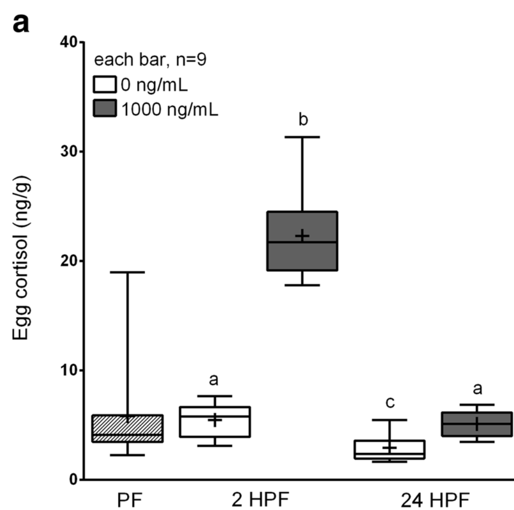
The mean magnitude of cortisol elevation following egg hormone treatment (mean [treated eggs] – mean [untreated eggs]) did not differ between the two species (Wilcoxon signed-rank test, Z = 1.16, $n$ = 16, $P$ = 0.24). However, variation in cortisol concentration among untreated (and pre-fertilized) eggs of individual sockeye salmon was evident (Fig. 2b). Of the seven sockeye salmon females sampled, five females had pre-fertilized cortisol concentrations between 7.6 ng/g and 11.9 ng/g. The other two females had concentrations of 34.8 ng/g and 36.1 ng/g. Despite these higher pre-fertilized concentrations, concentrations in the fertilized eggs of these two females remained elevated 2 hpf within the range of concentrations observed for the other five females (Fig. 2b). Cortisol concentrations of hormone-treated eggs were significantly lower 24 hpf compared to 2 hpf for both chum salmon and sockeye salmon (Fig. 2a, b). In chum salmon, egg cortisol levels of hormone-treated eggs were still elevated after 24 hpf relative to cortisol levels of untreated eggs (Fig. 2a). At 24 hpf, cortisol concentrations of eggs treated with 0 ng/mL cortisol solution (controls) were similar to (sockeye salmon) or lower than (chum salmon) concentrations at 2 hpf for the same treatment (Fig. 2a, b).

Fertilization success and embryonic survival

For both chum salmon (Linear mixed model, $F_{1,16}$ = 2.18, $P$ = 0.16) and sockeye salmon ($F_{1,10}$ = 1.48, $P$ = 0.25), fertilization success did not differ between the egg cortisol treatments (0 ng/mL cortisol versus 1000 ng/mL cortisol). Likewise, embryonic survival did not differ between the egg cortisol treatments in either chum salmon ($F_{1,16}$ = 0.18, $P$ = 0.68) or sockeye salmon ($F_{1,12}$ = 0.18, $P$ = 0.83). Embryos survived to the fry life stage from all nine chum salmon females. Two of the seven sockeye salmon females did not have any embryos survive to the fry life stage.

Morphology

Egg cortisol treatment had effects on morphometric measurements in sockeye salmon fry but not in chum salmon. Sockeye salmon fry reared from cortisol-treated eggs (1000 ng/mL cortisol) had lower PC1 scores compared to fry reared from untreated eggs (0 ng/mL cortisol), indicating smaller body size, shallow body shape and smaller fins and eyes (two-way ANOVA, egg treatment x species: $F_{1,160}$ = 5.75, $P$ = 0.02, Fig. 3). Indeed, further analyses on individual morphometric traits revealed that sockeye salmon fry body size (mass, FL) and body depth (ADAN, DORPEL) measurements differed between egg cortisol treatments. Chum salmon fry morphology was not



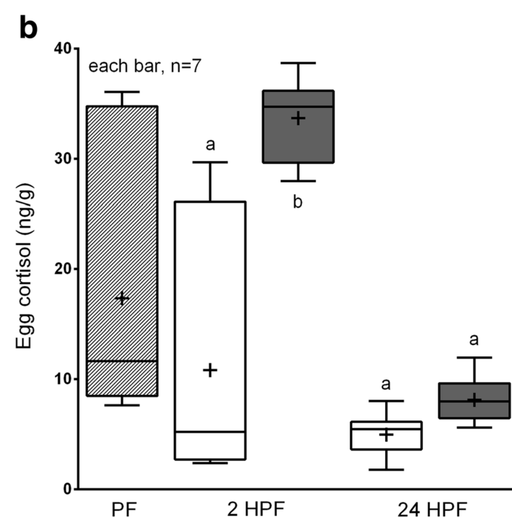




**Fig. 2** Egg cortisol concentrations of chum salmon (**a**) and sockeye salmon (**b**) pre-fertilized eggs (hatched boxes), and untreated (0 ng/mL, open boxes) and cortisol-treated (1000 ng/mL, filled boxes) eggs at 2 and 24 h post-fertilization (hpf). Lines within boxes are the median, and crosses (+) are the mean. Boxes represent the 25th and 75th percentile and whiskers represent the minimum and maximum. Different letters denote significant differences at $P < 0.05$

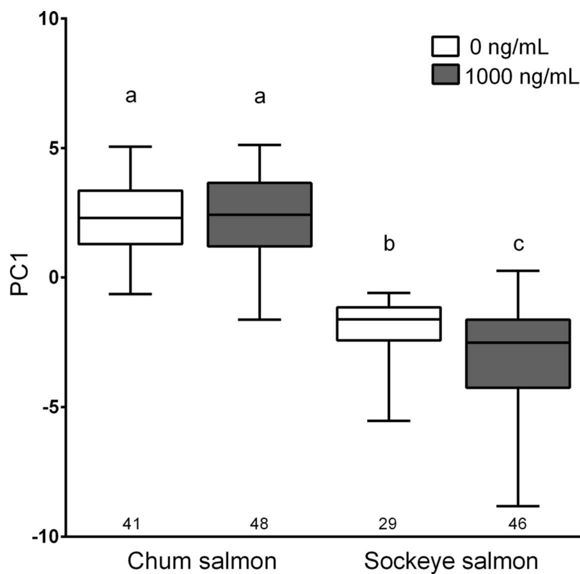



**Fig. 3** Comparison of principal component output (PC1) of chum salmon and sockeye salmon reared from untreated (0 ng/mL, open boxes) and cortisol-treated (1000 ng/mL, filled boxes). PC1 was generated using body size, eye size, 3 metrics of body depth and 5 measures of fin size. See Fig. 1 and Methods for details. Lines within boxes are the median. Boxes represent the 25th and 75th percentile and whiskers represent the minimum and maximum. Different letters denote significant differences at $P < 0.05$

affected by egg cortisol treatment (Fig. 3). Across treatments, chum salmon fry were significantly larger than sockeye salmon fry (species: $F_{1,160} = 305.82$, $P < 0.0001$, Fig. 3). See Table 1 for mean (± SE) values for all fry morphometric features measured. Results of the principal component analysis (PCA) on fry morphometric features which generated PC1 scores are shown in Table 2.

## Burst swimming performance

Egg cortisol treatment did not significantly affect fry swim failure in either species of salmon (Chi-squared test, chum salmon: $\chi 2 = 3.86$, $n = 92$, $P = 0.05$; sockeye salmon: $\chi^2 = 3.10$, $n = 79$, $P = 0.08$, Table 3). The interaction between species and egg cortisol treatment was not significant ($P = 0.13$) in the two-way ANOVA used to assess absolute burst swimming duration. Sockeye salmon fry (egg cortisol treatments pooled) swam 20 s longer, on average, than did chum salmon fry (two-way ANOVA, species: $F_{1,135} = 34.55$, $P < 0.0001$, Table 3). Overall, absolute burst swimming duration did not differ between egg cortisol treatments (species pooled, egg treatment: $F_{1,135} = 0.27$, $P = 0.61$, Table 3). The interaction between species and egg cortisol treatment was also not significant ($P = 0.22$) in the two-way ANCOVA analyzing size-corrected burst swimming

**Table 1** Measures of fry morphological traits (mean ± SE). In addition to body mass, ten measurements were assessed for each fish; fork length (FL), fin size, which included pectoral fin length (PEC, left fin only), caudal fin length (upper segment [CAUD1], lower segment [CAUD 2]), caudal fin height (CAUD 3), and caudal fin area (CAUD 4), body depth, which comprised three measurements (distance between dorsal and pelvic fins [DORPEL], distance between adipose and anal fins [ADAN], and caudal peduncle height [PED]), and eye area, which was calculated using the formula: π x (0.5 x eye width [EW]) x (0.5 x eye length [EL]) (Neff 2004)

| Morphometric trait | Chum salmon | | Sockeye salmon | |
|---|---|---|---|---|
| | 0 ng/mL ($n = 41$) | 1000 ng/mL ($n = 48$) | 0 ng/mL ($n = 29$) | 1000 ng/mL ($n = 46$) |
| Body mass (g) | 0.858 ± 0.029 | 0.828 ± 0.022 | 0.523 ± 0.018 | 0.397 ± 0.019* |
| FL (cm) | 4.871 ± 0.052 | 4.879 ± 0.049 | 3.967 ± 0.042 | 3.711 ± 0.059* |
| PEC (cm) | 0.609 ± 0.018 | 0.530 ± 0.008* | 0.465 ± 0.007 | 0.444 ± 0.011 |
| CAUD 1 (cm) | 0.916 ± 0.009 | 0.908 ± 0.009 | 0.764 ± 0.010 | 0.725 ± 0.015 |
| CAUD 2 (cm) | 0.957 ± 0.012 | 1.019 ± 0.059 | 0.765 ± 0.009 | 0.736 ± 0.015 |
| CAUD 3 (cm) | 1.115 ± 0.019 | 1.249 ± 0.017* | 1.022 ± 0.017 | 0.938 ± 0.023 |
| CAUD 4 (cm) | 0.448 ± 0.011 | 0.469 ± 0.010 | 0.295 ± 0.008 | 0.265 ± 0.010 |
| DORPEL (cm) | 0.789 ± 0.011 | 0.778 ± 0.009 | 0.676 ± 0.010 | 0.604 ± 0.015* |
| ADAN (cm) | 0.711 ± 0.009 | 0.708 ± 0.007 | 0.592 ± 0.008 | 0.543 ± 0.011* |
| PED (cm) | 0.337 ± 0.004 | 0.331 ± 0.003 | 0.278 ± 0.004 | 0.262 ± 0.005 |
| Eye area ($mm^2$) | 0.910 ± 0.012 | 0.941 ± 0.010 | 0.638 ± 0.010 | 0.600 ± 0.014 |

*indicate significant differences ($P < 0.005$) between egg cortisol treatments, within a species. See Methods for further details

**Table 2** Results of the principal component analysis (PCA) on fry morphological traits. Body mass (g), fork length (FL, cm), eye area ($\pi$ x [0.5 x EW] x [0.5 x EL], $mm^2$), and metrics for fin size (PEC, CAUD 1, CAUD 2, CAUD 3, CAUD 4, cm) and body depth (DORPEL, ADAN, PED, cm; see Fig. 1) were loaded into the PCA (see Methods). Only loadings with an eigenvalue >1 are presented

| PCA | | |
|---|---|---|
| Morphometric variable | PC1 loading | Communality ($h^2$) |
| Eigenvalue | 8.9 | |
| % variance explained | 80 | |
| Body mass | 0.33 | 0.96 |
| FL | 0.33 | 0.98 |
| PEC | 0.23 | 0.44 |
| CAUD 1 | 0.32 | 0.87 |
| CAUD 2 | 0.20 | 0.29 |
| CAUD 3 | 0.28 | 0.63 |
| CAUD 4 | 0.32 | 0.90 |
| DORPEL | 0.32 | 0.97 |
| ADAN | 0.33 | 0.96 |
| PED | 0.32 | 0.94 |
| Eye area | 0.31 | 0.89 |

duration. Correcting for body size, sockeye salmon fry (egg cortisol treatments pooled) swam for longer durations than did chum salmon fry (two-way ANCOVA, species: $F_{1,135} = 55.23$, $P < 0.0001$). Correcting for body size, burst swimming duration was unaffected by egg cortisol treatment (species pooled, egg treatment: $F_{1,135} = 0.23$, $P = 0.64$; PC1: $F_{1,135} = 22.38$, $P < 0.0001$). Both chum salmon (Wilcoxon signed-rank test, $Z = 2.60$, $n = 69$, $P = 0.009$) and sockeye salmon ($Z = 3.89$, $n = 70$, $P = 0.0001$) reared from cortisol-treated eggs (1000 ng/mL cortisol) swam fewer bouts of burst swimming than did fry reared from untreated eggs (0 ng/mL cortisol). However, differences in the total number of bouts between fish from the two egg cortisol treatments were not large (see Table 3). Burst swimming rates were also lower in chum ($Z = 2.56$, $n = 69$, $P = 0.01$) and sockeye salmon fry ($Z = 2.19$, $n = 70$, $P = 0.03$) reared from cortisol-treated eggs relative to fish reared from untreated eggs; however, again, differences between egg treatments were not large (Table 3).

## Discussion

Exogenous elevation of cortisol in unfertilized or newly fertilized eggs is used to mimic natural increases in egg cortisol caused by maternal stress (e.g., Nesan and Vijayan 2012), whereby stressor-exposed mothers deposit increased amounts of cortisol into eggs (e.g., Stratholt et al. 1997; McCormick 2009). We detected species-specific effects of elevated egg cortisol on the morphology of wild Pacific salmon: sockeye salmon reared from cortisol-treated eggs were smaller but egg cortisol treatment had no morphological effects on chum salmon. Subtle differences in swimming performance between egg hormone treatments were detected (e.g., fewer swimming bouts). Similar to previous egg hormone studies using salmonids (Stratholt et al. 1997; Sloman 2010; Sopinka et al. 2015), fertilization success and embryonic survival were unaffected by cortisol treatment in our study. Although embryonic survival was not affected by egg hormone treatment, our results reveal that manipulating cortisol in newly fertilized eggs can have latent (i.e., 2 months post-yolk sac absorption), sub-lethal effects, highlighting the value of assessing intergenerational effects beyond embryonic life stages.

**Table 3** Mean (± SE) burst swimming characteristics of chum salmon and sockeye salmon fry reared from untreated (0 ng/mL) and cortisol-treated (1000 ng/mL) eggs. Ranges (minimum and maximum) are presented in brackets

| | Chum salmon | | Sockeye salmon | |
|---|---|---|---|---|
| | 0 ng/mL | 1000 ng/mL | 0 ng/mL | 1000 ng/mL |
| Failed to swim | 13/41 | 7/48 | 0/29 | 5/46 |
| Total burst swimming duration (s) | 35.1 ± 6.2 (4.0–134.6) | 39.6 ± 5.1$^{A}$ (1.0–117.1) | 64.1 ± 2.9 (37.8–92.7) | 53.2 ± 2.6$^{B}$ (10.0–90.3) |
| Total number of bouts | 4.7 ± 0.6 (1–15) | 2.9 ± 0.3$^{*}$ (1–8) | 2.4 ± 0.3 (1–6) | 1.3 ± 0.1$^{*}$ (1–3) |
| Burst swimming rate (bouts per 10 s) | 2.1 ± 0.3 (0.5–7.6) | 1.4 ± 0.3$^{*}$ (0.1–10.3) | 0.4 ± 0.0 (0.1–1.3) | 0.3 ± 0.0$^{*}$ (0.1–1.0) |

Different letters denote statistical differences ($P < 0.05$) between species, egg cortisol treatments pooled

*denote statistical differences ($P < 0.05$) between egg cortisol treatments, within a species. See Methods for further details

Following fertilization, cortisol concentrations of fertilized eggs declined over 24 h. This post-fertilization decline in concentration has been previously detected in many species (e.g., common carp, *Cyprinus carpio*, Stouthart et al. 1998; tilapia, *Oreochromis mossambicus*, Hwang et al. 1992; coho salmon, Sopinka et al. 2015). The cause of this decline could be because of endogenous metabolism of cortisol. Li et al. (2012) found that rainbow trout embryonic (eyed, hatchling and fry life stages) tissue incubated in radio-labeled cortisol contained cortisol sulphate, cortisone, and cortisone sulphate, suggesting that metabolism/sulphation of cortisol occurred. However, the degree of metabolism/sulphation was relatively low compared to levels of conjugated steroids detected in ovarian follicles (Li et al. 2012). The decline in cortisol concentration may also be occurring because of active transport of cortisol out of the fertilized egg. Paitz et al. (2016) found evidence for a transporter (i.e., ATP-binding cassette [ABC] transporter) in threespine stickleback (*Gasterosteus aculeatus*) that may be shuttling cortisol out of the fertilized eggs. Despite this rapid decline in cortisol concentration, the duration of elevation following the bathing of fertilized eggs (<24 h) was apparently sufficient to influence traits measured more than five months later in the resultant offspring.

Partially supporting our predictions, elevated cortisol in newly fertilized eggs did affect the morphology of fry, but effects were only evident for sockeye salmon. Lower PC1 values for sockeye salmon fry reared from cortisol-treated eggs indicated smaller body size, smaller fins and eyes and a more robust body shape. Interestingly, this finding did not result in impaired swimming performance for sockeye salmon fry reared from cortisol-treated eggs as absolute burst swimming duration (not controlling for body morphology/PC1) did not differ between egg cortisol treatments. Other swimming metrics such as fast-starts could be impaired but were not tested in this study. The mechanism by which elevated egg cortisol alters morphology remains speculative. In fishes, cortisol binds to intracellular glucocorticoid receptors (GRs) which, when bound, move to the nucleus, inducing transcription (Bury and Sturm 2007). Maternally-derived GR transcripts are present in newly fertilized eggs (Jeffrey and Gilmour 2016) and when translation of maternal GR transcripts is blocked effects on offspring phenotype are observed (Pikulkaew et al. 2011). One might hypothesize that differential cortisol concentrations in eggs induce changes in transcript abundance via GR signaling, resulting in effects on phenotype. Indeed, researchers that have exogenously manipulated egg cortisol have found differences in mRNA transcript abundance between embryos reared from untreated and cortisol-treated eggs. For example, Li et al. (2010) found higher abundance of mRNA transcripts of insulin-like growth factor and growth hormone in rainbow trout embryos reared from cortisol-treated eggs when compared against embryos reared from untreated eggs. This effect on mRNA transcript abundance was associated with variation in growth of offspring from the different egg treatment groups (Li et al. 2010). Coupling egg cortisol-mediated changes in morphology/performance with changes in abundance of transcripts related to these traits can reveal further insight into the potential mechanism by which egg cortisol manifests latent effects on offspring.

There is no obvious explanation for the divergent morphological effects on fry between the two species, particularly given that the fish were from the same natal watershed (the Harrison River). Species-specific effects of egg corticosterone treatment on juvenile growth have been reported in lizards, although the species tested were not geographically sympatric (Warner et al. 2009). Early life history is similar between Harrison River sockeye salmon and chum salmon. Chum salmon in the Harrison River demonstrate a typical life history for the species, migrating downstream to the ocean as fry (Salo 1991). Sockeye salmon in the Harrison River also migrate downstream to estuaries as fry (Birtwell et al. 1987), in contrast to the majority of other sockeye salmon populations in which fry rear in freshwater for 1–2 years before migrating to the ocean (Burgner 1991). Egg-fry/juvenile-adult survival are similar for chum salmon and sockeye salmon (Bradford 1995). The adult migration is also similar. Both species enter the Fraser River late September, migrate ~100 km upstream to the Harrison River system, and hold for approximately 6–12 weeks prior to spawning mid-November. Cortisol was higher and more variable in unfertilized sockeye salmon eggs compared to chum salmon. The apparent variation captured in this study is likely relatively pronounced due to low sample sizes. Following the egg cortisol dosing (i.e., 2 hpf), the concentration of cortisol in sockeye salmon eggs, which initially had higher cortisol concentrations, was elevated within the range of post-dosing concentrations in eggs with lower initial concentrations of cortisol. This result suggests a maximum threshold of cortisol content in eggs, and/or active transport of cortisol out of the egg (Paitz et al. 2016).

Species-specific sensitivity thresholds to exogenous cortisol may account for the differences in morphology we observed. However, very little is understood about the deposition, metabolism, or regulation of egg glucocorticoids in oviparous species (Moore and Johnston 2008), or about the physiological processes hormones target that could drive phenotypic changes (Lema 2014).

For sockeye salmon fry, morphological differences mediated by egg cortisol treatment apparently did not translate into large differences in burst swimming performance: there was no effect of egg hormone treatment on burst swimming duration and a small effect on bout number and burst swimming rate. These results suggest that despite changes to morphology, the physiological pathways that support burst swimming (e.g., oxygen consumption, lactate regulation, metabolic enzyme activity) were either unaffected or, to compensate for smaller body size, were enhanced by egg cortisol treatment. Chum salmon and sockeye salmon offspring reared from cortisol-treated eggs tended to initiate fewer bouts, which translated into lower burst swimming rates, a response which could compromise predator evasion (discussed in Sopinka et al. 2014) or migration success (Sopinka et al. 2013). Aspects of the way swimming performance was assessed in this study (e.g., single versus school of fish, no threat of predation) do limit the extent to which we can assess the ecological relevance of the altered traits. Future work should conduct observations in more complex behavioural arenas (e.g., Burton et al. 2011; Sopinka et al. 2015) to generate connections between egg cortisol and offspring behaviour and performance.

The hormonal composition of eggs has ecologically important effects in highly fecund animals. If egg hormones, such as cortisol, are reliable signals of ecosystem perturbation and maternal health, concentrations could be used in risk assessment and predictive modelling. However, the carry-over effects of egg cortisol can be inconsistent and multifaceted, particularly when conducting side-by-side comparisons using multiple traits or species. Caveats should be considered when drawing evolutionary conclusions and forming theoretical predictions regarding the adaptive (or maladaptive) effects of egg hormones (Sheriff and Love 2013). Given that egg hormones are maternally derived, and the environment a mother experiences can alter the hormonal composition of an egg, similar caveats apply to interpretation of maternal effects observed in experimental settings. The use of wild populations, in conjunction with domesticated species, will guide our understanding of how maternally-mediated processes (i.e., gametic cortisol deposition and its effects on phenotype/performance) occur in the context of rapid and human-mediated environmental change.

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