RESEARCH ARTICLE

Prenatal Stress Exposure Generates Higher Early Survival and Smaller Size without Impacting Developmental Rate in a Pacific Salmon

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ABSTRACT

Prenatal exposure to elevated glucocorticoids can act as a signal of environmental stress, resulting in modifications to offspring phenotype. While "negative" phenotypic effects (i.e., smaller size, slower growth) are often reported, recent research coupling phenotype with other fitness-related traits has suggested positive impacts of prenatal stress. Using captive Chinook salmon (*Oncorhynchus tshawytscha*), we treated eggs with biologically relevant cortisol levels—low (300 ng mL^{-1}), high (1,000 ng mL^{-1}), or control (0 ng mL^{-1})—to examine the early-life impacts of maternally transferred stress hormones on offspring. Specifically, we measured early survival, rate of development, and multiple measures of morphology. Low and high cortisol dosing of eggs resulted in significantly higher survival compared to controls (37% and 24% higher, respectively). Fish reared from high dose eggs were structurally smaller compared to control fish, but despite this variation in structural size, exposure to elevated cortisol did not impact developmental rate. These results demonstrate that elevations in egg cortisol can positively influence offspring fitness through an increase in early survival while also altering phenotype at a critical life-history stage. Overall, these results suggest that exposure to prenatal stress may not always produce apparently negative impacts on offspring fitness and further proposes that complex phenotypic responses should be examined in relevant environmental conditions. *J. Exp. Zool. 00:1–10, 2017.* © 2017 Wiley Periodicals, Inc.

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INTRODUCTION

Maternally derived hormones play a major role in the early development of vertebrates (Groothius and Schwabl, 2008; Nesan and Vijayan, 2013), and determining how they shape offspring phenotype as well as how this phenotype interacts with environmental variation to affect fitness is a rapidly emerging field of research (Meylan et al., 2012). In particular, glucocorticoid (GC) hormones manage energy balance at baseline levels (Landys et al., 2006) and facilitate homeostatic return in response to acute stressors (Sapolsky et al., 2000; Barton, 2002). Circulating levels of GCs in reproductive females depend on individual condition and environment (Love et al., 2005; McCormick, 2006; Sheriff et al., 2009, 2011), thereby providing a link among maternal state, reproduction, and quality of progeny (Schreck et al., 2001; Love et al., 2005). Indeed, stressful environments due to natural or anthropogenic perturbations have the potential to shape offspring phenotype through hormonal mechanisms (Leatherland et al., 2010; Meylan et al., 2012; Love et al., 2013), such as maternal transfer of elevated levels of GCs to offspring (Saino et al., 2005; McCormick, 2006; Dantzer et al., 2013).

Exposure to elevated maternal GCs is known to impact offspring phenotypic traits (e.g., smaller size, slower growth, morphological changes) and outcomes are typically deemed maladaptive for offspring (Saino et al., 2005; Eriksen et al., 2006). However, less work has examined how and why this phenotypic variation impacts fitness metrics such as early survival and performance (Sheriff and Love, 2013). It has been suggested that phenotypic traits such as small size and slower growth may actually be adaptive responses in preparation for expected lowered resources in the future environment (i.e., predictive adaptive responses (PARs); Gluckman et al., 2005). Evolutionary ecologists have extended the PAR framework from laboratory mammals and humans to free-living systems (i.e., maternal or environmental matching; Love et al., 2005; Love and Williams, 2008) to test the adaptive potential of maternal stress (Sheriff and Love, 2013). For example, European starlings (*Sturnus vulgaris*) exposed to elevated embryonic GCs (a prenatal stress signal) were smaller at independence (Love and Williams, 2008), but had improved flight performance, presumably in response to expected increased predation risk (Chin et al., 2009). In addition, Dantzer and colleagues (2013) found that wild red squirrels (*Tamiasciurus hudsonicus*) with experimentally elevated GCs produced faster growing offspring, which is positively associated with fitness in this population when high densities occur. Despite these well-rooted hypotheses, further research needs to be conducted at more specific life-history stages and in species where mothers and offspring share a common environment to appreciate how complex phenotypic responses interact with environmental variation to impact fitness (Sheriff and Love, 2013).

Pacific salmon are an ideal group of species to explore impacts of stressors across generations, as adults undergo arduous migrations from the ocean to freshwater natal spawning grounds and encounter multiple anthropogenic stressors (e.g., fisheries capture, warming water temperatures) that can reduce migration success (Donaldson et al., 2012; Martins et al., 2012). While baseline levels of circulating cortisol (the primary GC in fish) are known to increase in mature female salmon during migration to spawning areas (Baker and Vynne, 2014), individuals remain responsive to acute stressors in the freshwater environment, and levels of circulating cortisol are linked to survival on spawning grounds (Cook et al., 2011; McConnachie et al., 2012). Furthermore, maternally derived cortisol is present in salmon eggs and can increase following maternal exposure to a repeated stressor (Stratholt et al., '97). Pacific salmon have a semelparous life history, undergoing a single breeding attempt, and provide little parental care (Groot and Margolis, '91); therefore, egg cortisol–mediated effects on offspring phenotype are expected to have significant fitness consequences for mothers, suggesting that maternal GC transfer should be under strong selection (Love et al., 2009, 2013). Moreover, studies in salmonid species that have experimentally elevated egg cortisol levels via egg immersion have shown that cortisol can be an important regulator of offspring phenotype (Auperin and Geslin, 2008; Li et al., 2010; Sloman, 2010; Burton et al., 2011; Colson et al., 2015; Sopinka et al., 2015, 2016).

In this study, we explore the effects of biologically relevant egg cortisol exposures (low dose, high dose, control) on three important early life-history traits in Chinook salmon (*Oncorhynchus tshawytscha*): offspring survival, developmental rate, and morphology. Survival was monitored to the embryonic stage (eyed egg stage) as well as to first feeding (fry stage), and rate of development (based on degree of yolk sac absorption) and morphology were measured at the fry stage. We focused on the egg-to-fry period since these three early life-history traits all contribute to determining the success of juvenile salmon in fresh water (Einum and Fleming, 2000; Jones et al., 2015). We hypothesized that a signal of prenatal stress (elevated egg cortisol) would lead to an accelerated developmental trajectory based on other fish studies showing higher heart rates (McCormick and Nechaev, 2002), as well as earlier hatching (Gagliano and McCormick, 2009) and faster yolk-sac absorption (Mathiyalagan et al., '96) in cortisol-dosed embryos. We also predicted that elevated egg cortisol would lead to smaller-sized fry (McCormick, '99; Burton et al., 2011), and that these developmental differences could also impact egg-to-fry survival. Finally, low and high cortisol exposure doses were chosen with the prediction that they could represent optimal and supra-optimal doses, respectively, since effects of stress are often nonlinear (Schreck, 2010). Therefore, we predicted that there would be phenotypic differences in offspring from the low and high cortisol dose groups, with the low dose showing positive effects and the high dose showing negative effects compared to controls (Li et al., 2010).

METHODS

Fish Origin

This study was completed at Yellow Island Aquaculture Ltd. (YIAL), a small Chinook salmon farm on Quadra Island in British Columbia, Canada. YIAL's domestic stock originated in 1985 from gametes taken from Robertson Creek and Big Qualicum hatcheries on Vancouver Island (see Lehnert et al., 2014, for further details). The females whose eggs were used in the current study were the progeny of a self-crossed fish that was bred in Autumn 2009 (Komsa, 2012). This breeding design allowed us to minimize the influence of genetic maternal effects on early survival and size (Burt et al., 2012) by using a more homogeneous pool of eggs. All research followed the University of Windsor Animal Care Committee guidelines for the care and use of animals and complied with those of the Canadian Council on Animal Care.

Egg Cortisol Treatment

In Autumn 2014, eggs were collected from seven females which were euthanized by cerebral percussion. Three unfertilized eggs were taken from each female and frozen at −80°C for cortisol analysis to compare naturally-occurring levels of prefertilization egg cortisol among females. For each female, we measured total body mass (mean ± SE: 3.41 ± 0.30 kg, range: 2.09–4.49 kg), fork length (62.2 ± 1.6 cm, 55.4–67.7 cm), and total ovary mass (849.65 ± 88.41 g, 476.49–1,159.16 g). Individual egg mass was measured by weighing three sets of 10 eggs, taking the average of the three sets, and dividing by 10. Mean ± SE individual egg mass was 0.29 ± 0.01 g and ranged from 0.27 to 0.32 g. Eggs from all seven females were pooled prior to fertilization to reduce female-specific maternal effects among egg cortisol treatment groups. Pooled eggs were split into 18 containers of 180 g of eggs (~600 eggs), with six replicate containers assigned to each of the three egg cortisol treatment groups. Milt was collected from five males by applying pressure to the abdomen and then pooled. Containers of eggs were fertilized with ~1 mL of pooled sperm, and egg-milt mixtures were left for 2 min before adding hatchery water (Shrimpton et al., 2012). After 2 min (at which time sperm would no longer be motile; Hoysak and Liley, 2001), egg-milt mixtures were immersed in water containing either a (1) low cortisol dose (300 ng mL^{-1}), (2) high cortisol dose (1,000 ng mL^{-1}), or (3) control dose (0 ng mL^{-1}) for a 2-hr treatment period (Sopinka et al., 2015, 2016). The low and high cortisol doses contained cortisol powder (H4001, Sigma-Aldrich Canada Co., Oakville, ON, Canada) dissolved in 90% ethanol (HPLC grade, Sigma-Aldrich Canada Co., Oakville, ON, Canada; 7.5×10^{-6} low dose final concentration, 2.5×10^{-5} high dose final concentration) and diluted in hatchery water, whereas the control solution contained water with the same concentration of ethanol as the low dose. Low and high cortisol dose concentrations were chosen based on previous work that used similar concentrations in salmonid species and showed that dosages result in biologically relevant elevations in egg cortisol content (Auperin and Geslin, 2008; Sopinka et al., 2015, 2016) and can lead to biphasic effects on offspring phenotype (Li et al., 2010).

Following the exposure period, eggs were washed thoroughly with hatchery water and placed in a flow-through, vertical-stack incubator. Eggs from each replicate container were split into three replicate cells (18 total replicates per treatment group) and randomly placed among four trays (each tray divided into 16 cells) within the stack. Three eggs were collected from each cell (54 total cells across all egg treatment groups) 2- and 24-hours postfertilization (hpf) and frozen at −80°C to determine the level and duration of cortisol elevation following the cortisol treatment. At the eyed stage, embryos were counted and dead embryos were removed from each replicate cell. Survival was then monitored every 3 days until the fry stage by removing and recording any dead embryos. Mean ± SE water temperature during incubation was 7.4 ± 0.2°C and ranged from 5.5 to 8.9°C. Mean ± SE dissolved oxygen concentration was 10.2 ± 0.2 mg L^{-1} and ranged from 9.1 to 11.3 mg L^{-1}.

Developmental Rate and Morphology

At 4 months postfertilization (fry life stage), we randomly sampled five fry from each replicate cell ($N = 90$ fry per egg cortisol treatment group), recorded their body mass (to the nearest 0.01 g), and took a photograph (Canon EOS Rebel XT, Canon Inc., Tokyo, Japan) for future morphometric analysis. Digital photographs were analyzed using ImageJ (http://imagej.nih.gov/ij/). Measurements taken included fork length (FL), gape (GAPE), three measures of body depth (depth from dorsal fin perpendicular to fish length (BD1), depth to widest point of yolk sac protrusion (BD2), depth from dorsal fin to anal fin (BD3)), caudal peduncle width (PED), and caudal fin width (CAUD) (Fig. 1 and Table 1). Additional body-depth measurements were taken to account for variation among individual fry in depth based on amount of residual yolk sac. From this variation in residual yolk sac, a measure of developmental rate was assessed from the digital photographs by assigning a 0 to 3 ranking for each fry based on protrusion of the yolk sac (Fig. 1). A rank of 0 indicated minimal yolk sac protrusion (higher degree of yolk sac absorption) and a rank of 3 indicated maximal yolk sac protrusion (lower degree of yolk sac absorption).

Egg Cortisol Assays

We determined egg cortisol levels with enzyme immunoassays (Cayman Chemicals, Ann Arbor, MI, USA) using methods modified from Sopinka et al. (2015). Briefly, three eggs per container replicate (or three eggs per female for unfertilized eggs) were homogenized with 1,200 μL of assay buffer, and 3 mL diethyl ether was added. Samples were vortexed for 30 sec and then centrifuged for 5 min at 4,000 rpm. Samples were allowed to settle for 30 min, flash frozen at −80°C for 30 min, and then the liquid

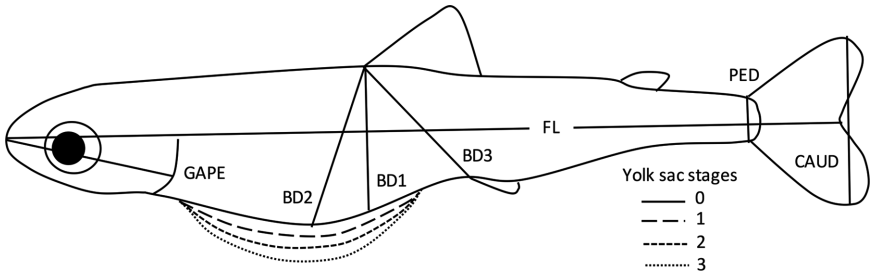

Figure 1. Morphological measurements taken from digital photographs of Chinook salmon (*O. tshawytscha*) at first feeding (fry). Measurements include fork length (FL), gape (GAPE), three measures of body depth (BD1, BD2, BD3), caudal peduncle width (PED), and caudal fin width (CAUD). The rate of development was assessed by assigning a 0–3 ranking for each fry based on protrusion of the yolk sac, where a rank of 0 indicated minimal yolk sac protrusion (higher degree of yolk sac absorption) and a rank of 3 indicated maximal yolk sac protrusion (lower degree of yolk sac absorption).

Table 1. Morphological traits (mean ± SE) of Chinook salmon (*O. tshawytscha*) at first feeding (fry) originating from eggs treated with a control dose, low cortisol dose, or high cortisol dose

Trait	Control ($n = 90$)	Low dose ($n = 90$)	High dose ($n = 90$)
Body mass (g)	0.44 ± 0.003	0.43 ± 0.004	0.42 ± 0.004
Fork length (mm)	36.13 ± 0.09	35.91 ± 0.07	35.69 ± 0.06
Gape (mm)	7.46 ± 0.02	7.43 ± 0.03	7.38 ± 0.02
Body depth 1 (mm)	6.53 ± 0.05	6.27 ± 0.05	6.30 ± 0.04
Body depth 2 (mm)	8.10 ± 0.05	7.80 ± 0.05	7.86 ± 0.04
Body depth 3 (mm)	6.46 ± 0.04	6.31 ± 0.03	6.28 ± 0.04
Caudal peduncle width (mm)	1.91 ± 0.02	1.96 ± 0.01	1.94 ± 0.01
Caudal fin width (mm)	7.04 ± 0.04	6.96 ± 0.04	6.90 ± 0.04

layer was decanted and evaporated overnight in a fume hood. Dried samples were reconstituted with 1,200 μL of assay buffer. To check for parallelism across multiple dilutions and determine an optimal dilution factor, a pooled sample was extracted as described above and a serial dilution performed using assay buffer (1:10, 1:20, 1:40, 1:57, 1:81, 1:116). Samples from the serial dilution were run on a single Cayman cortisol plate. Following this optimization, all samples were diluted using a 1:57 dilution factor and run in triplicate wells following kit insert instructions. Three plates were run and read with a plate reader at 412 nM wavelength. Intra- and interassay coefficients of variation were 4.5% and 10.0%, respectively.

Statistical Analyses

Statistical analyses were completed using R version 3.2.4 (R Core Team, 2016). Model assumptions were assessed by graphical inspection: residuals versus fitted values were plotted to verify homogeneity, and quantile–quantile plots and histograms of the residuals were plotted to verify normality. A two-way ANOVA with time (2, 24 hpf), egg cortisol treatment (control, low dose, high dose), and a time × egg cortisol treatment interaction was used to analyze differences in egg cortisol levels. Following a significant interaction term, post hoc slice tests were used to test for differences among groups for both fixed effects at the level of the other fixed effect using the lsmeans package (Lenth, 2016). Survival to eyed and fry stages were totaled per replicate cell (number dead and number alive), and data were converted to binary form with 0 representing a dead embryo and 1 representing an alive embryo (build binary function in the fullfact package; Houde and Pitcher, 2016). To examine survival differences to the eyed and fry stages, generalized linear mixed models for binary data were fitted with the logit link function (lme4 package; Bates et al., 2015). Replicate, treatment container, and incubation tray position were included in the models as random effects. Likelihood ratio tests were used to compare model fit and test significance of the fixed effect (egg cortisol treatment). To examine differences in fry morphology, a principal component analysis (PCA) was used. Morphological traits (mass, FL, GAPE, BD1, BD2, BD3, PED, CAUD; Fig. 1 and Table 1) were loaded into a PCA to reduce redundancies and find trends in fry body size and shape based on multiple measurements. Two components were extracted based on the Kaiser criterion

Table 2. PCA loadings (PC1, structural size; PC2, developmental rate) for morphological traits of Chinook salmon at first feeding (fry) where values in bold (>0.3) indicate loadings that contribute significantly to the PC scores

Trait	PC1 loading	PC2 loading
Eigenvalue	3.2	1.6
Percent variance explained	40	21
Body mass	**0.42**	−0.02
Fork length	**0.37**	**0.39**
Gape	0.28	**0.52**
Body depth 1	**0.42**	**−0.43**
Body depth 2	**0.36**	**−0.48**
Body depth 3	**0.40**	−0.05
Caudal peduncle width	0.12	**0.38**
Caudal fin width	**0.36**	0.13

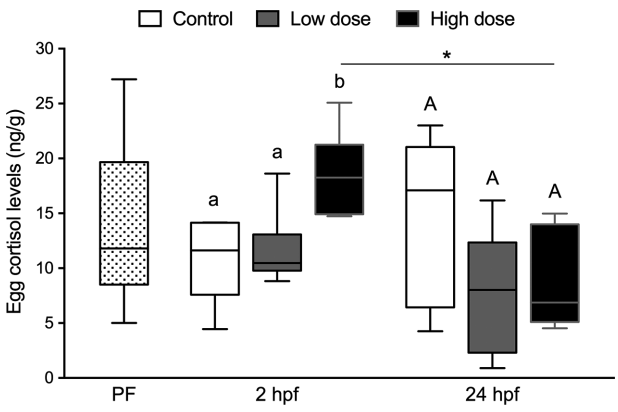

Figure 2. Egg cortisol concentrations in prefertilization eggs ($N = 7$ females), and eggs 2 and 24 hours postfertilization (hpf) that were treated with a control dose (0 ng mL^{-1}; $N = 6$ pooled egg samples), low cortisol dose (300 ng mL^{-1}; $N = 6$ pooled egg samples), or high cortisol dose (1,000 ng mL^{-1}; $N = 6$ pooled egg samples) immediately postfertilization. The middle line represents the median egg cortisol concentration, the boxes represent the first and third quantiles, and the whiskers represent the maximum and minimum values. Different lower case letters represent a significant difference (control dose–high dose; $P < 0.05$) and marginally significant difference (low dose–high dose; $P = 0.052$) between egg cortisol groups at 2 hpf based on slice tests following a significant time by egg cortisol treatment interaction. The asterisk represents a significant difference ($P < 0.05$) between egg cortisol levels 2 and 24 hpf in the high dose group, based on a slice test following the significant time by egg cortisol treatment interaction.

(eigenvalue >1) and visual inspection of variance plots (Table 2). PC1 (eigenvalue 3.2) explained 40% of the variation and represented structural size (PC1 positively correlated with mass, FL, CAUD, BD1, BD2, and BD3). PC2 (eigenvalue 1.6) explained an additional 21% of the variation and represented developmental rate (PC2 positively correlated with FL, GAPE, and PED, and negatively correlated with BD1 and BD2). Effects of egg cortisol treatment on PC1 and PC2 were assessed using linear mixed models (lme4 package; Bates et al., 2015). Categorical yolk sac rankings were compared among egg cortisol treatments using a cumulative link mixed model for ordinal data (ordinal package; Christensen, 2015). All models included replicate, treatment container, and incubation tray position as random effects. Significance of the fixed effect was tested using likelihood ratio tests fit with maximum likelihood, and then final models were fitted with restricted maximum likelihood estimation. For significant models ($P < 0.05$), pairwise differences between treatment groups were assessed using Tukey post hoc tests (lsmeans package; Lenth, 2016).

RESULTS

Egg Cortisol Levels

Egg cortisol treatment interacted with time to affect levels of egg cortisol ($F_{2,30} = 5.07$, $P = 0.01$; Fig. 2). Eggs that were treated with the high cortisol dose had higher cortisol levels immediately following the exposure period (2 hpf) compared to control dose eggs ($P = 0.03$) and marginally higher levels than the low dose eggs ($P = 0.052$). Cortisol levels in the high dose eggs were elevated within one standard deviation of the cortisol levels measured in nonmanipulated, unfertilized eggs (mean 14.07 ng g^{-1}; SD 7.45 ng g^{-1}; range 5.02–27.21 ng g^{-1}), confirming that the high dose treatment represented a biologically relevant elevation in egg cortisol. We found no difference in egg cortisol levels between the low dose eggs and control eggs ($P = 0.95$) immediately following the exposure period. Egg cortisol levels no longer differed among the three treatment groups 24 hr after the exposure period (high versus low: $P = 0.95$, high versus control: $P = 0.20$, low versus control: $P = 0.11$; Fig. 2); however, the high dose treatment showed a significant decrease in egg cortisol levels from 2 to 24 hpf ($P = 0.002$), whereas levels in the control dose and low dose groups did not change from 2 to 24 hpf (control dose: $P = 0.31$; low dose: $P = 0.19$).

Early Survival

There was a significant effect of egg cortisol treatment on survival to the eyed stage ($\chi^2 = 22.82$, $df = 2$, $P < 0.0001$; Fig. 3a), with all three treatment groups being significantly different from each other (high versus low: $P = 0.007$; high versus control: $P = 0.0005$; low versus control $P < 0.0001$). Survival to the eyed stage was highest for low cortisol dose eggs

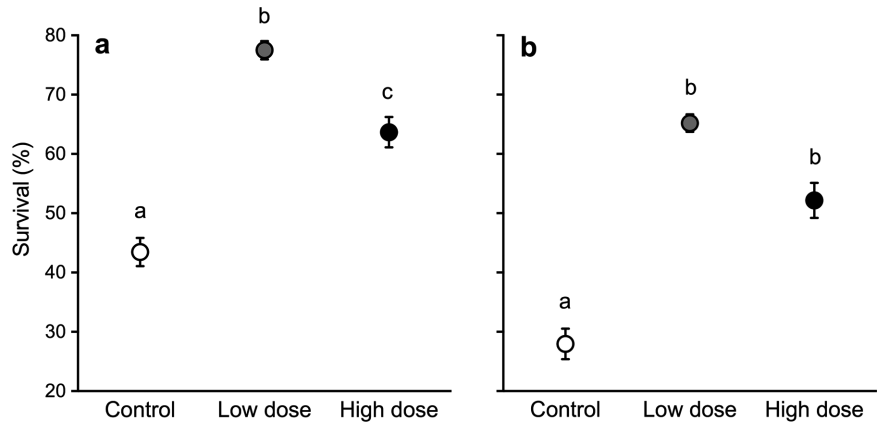

Figure 3. Percent survival (mean ± SE) to (a) embryonic (eyed) stage and (b) first feeding (fry) stage for Chinook salmon embryos/fry originating from eggs treated with a control dose, low cortisol dose, and high cortisol dose ($N = 18$ replicates per treatment). Data presented based on percent average survival per replicate cell, whereas statistical analyses were performed on binary response data (0, 1) per individual. Different letters represent significant differences based on Tukey post hoc tests ($P < 0.05$).

(mean ± SE: 77.5 ± 1.5%), intermediate for high cortisol dose eggs (63.7 ± 2.6%), and lowest for control dose eggs (43.4 ± 2.4%).

There was also a significant effect of egg cortisol treatment on survival to the fry stage ($\chi^2 = 22.55$, $df = 2$, $P < 0.0001$; Fig. 3b). Survival to the fry stage of low and high cortisol-treated eggs was significantly higher compared to survival of control dose eggs (both $P < 0.0001$). However, survival of low and high cortisol dose eggs did not differ from one another ($P = 0.07$). Survival to the fry stage was 65.2 ± 1.5 % for low cortisol dose eggs, 52.2 ± 2.9% for high cortisol dose eggs, and 28.0 ± 2.6% for control dose eggs.

Developmental Rate and Morphology

Egg cortisol treatment had a significant effect on structural size (PC1 scores, $\chi^2 = 6.99$, $df = 2$, $P = 0.03$; Fig. 4a), with fry from high cortisol dose eggs having a tendency to be structurally smaller compared to fry from control dose eggs ($P = 0.056$). However, fry from low cortisol dose eggs were not different in structural size compared to fry from high cortisol dose eggs ($P = 0.85$) or fry from control dose eggs ($P = 0.15$). Egg cortisol treatment did not have a significant effect on rate of development (PC2 scores, $\chi^2 = 5.02$, $df = 2$, $P = 0.08$; Fig. 4b). In addition, when assessed categorically based on yolk sac protrusion (see Fig. 1), developmental rate did not differ among egg cortisol treatments ($\chi^2 = 2.90$, $df = 2$, $P = 0.23$).

DISCUSSION

Importance of Early Survival for Future Success

Compared to offspring reared from control eggs, we found 37% and 24% higher survival of fry reared from eggs exposed to a low and high cortisol dose, respectively. While elevated egg cortisol levels have been reported to negatively affect embryonic survival (Eriksen et al., 2006; Li et al., 2010), offspring survival is often not affected when egg cortisol levels are elevated within a physiologically relevant range (Auperin and Geslin, 2008; Sloman, 2010; Burton et al., 2011; Colson et al., 2015; Sopinka et al., 2015, 2016). Our results are the first to our knowledge to demonstrate survival benefits following exposure to elevated cortisol immediately postfertilization, particularly relatively small increases (i.e., low dose here); however, the mechanisms by which exogenous cortisol applied postfertilization functions to mimic those observed in eggs with endogenously elevated maternally derived cortisol warrants further study.

In the wild, egg-to-fry survival of Pacific salmon ranges from ~7% to 20% (Bradford, '95), meaning that the fry stage is a critical period that determines future success of juveniles migrating to the ocean (Groot and Margolis, '91). As such, our results suggest that variation in egg cortisol among female salmon contributes to early survival differences, yet little is known about the extent of egg cortisol variation within and between individuals in a population, or the ecological/evolutionary underpinnings for such variation (Sopinka et al., 2017). Moreover, these findings highlight the complex nature of hormonally mediated effects and that impacts of increasing doses are not consistently linear (Gagliano and McCormick, 2009; Li et al., 2010), reaffirming the importance of using multiple exposure doses to properly represent the spectrum of phenotypic effects. Although our breeding design (i.e., self-crossed strain of females) was specifically used to reduce maternal effects, the design may be responsible for the low (28%) survival of control dose

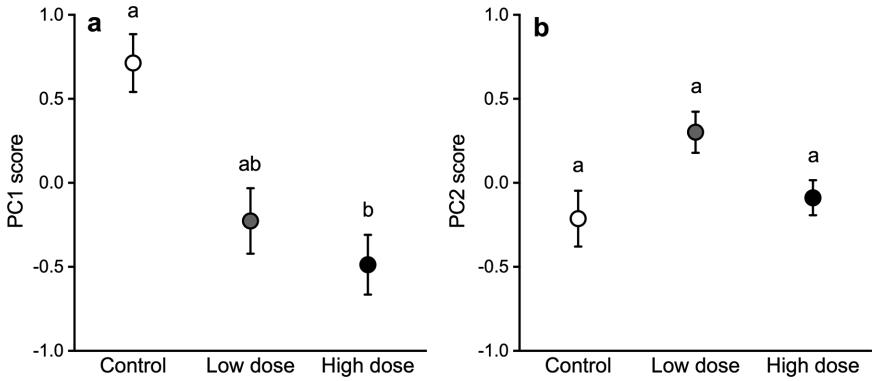

Figure 4. Mean ± SE values for (a) PC1 scores as a measure of structural size and (b) PC2 scores as a measure of developmental rate in Chinook salmon at first feeding (fry) from eggs treated with a control dose, low cortisol dose, and high cortisol dose ($N = 90$ per treatment group). Different letters represent a marginally significant difference in PC1 scores based on a Tukey post hoc test (control dose–high dose; $P = 0.056$).

offspring. Pure crosses using this strain led to reduced survival and growth at 7, 11, and 17 months postfertilization, possibly indicative of inbreeding depression, although gamete quality was not assessed (Komsa, 2012). Hybrid crosses performed between the self-crossed female strain and YIAL males in Autumn 2015 (i.e., same breeding design as the current study) showed that egg-to-fry survival for untreated eggs (i.e., not exposed to cortisol) was again low (38%), indicating a repeatable degree of survival (P. Capelle, unpublished data).

Mechanisms Underlying Variation in Survival

While only the high cortisol dose (1,000 ng mL^{-1}) successfully elevated egg cortisol within a biologically relevant range following the 2-hr exposure period, we argue that the low cortisol dose (300 ng mL^{-1}) may have cleared from the eggs rapidly but still provided a prenatal stress signal to the developing embryo, given the early survival differences. While initial research assumed that steroid hormones in eggs of oviparous species moved passively due to their lipophilic nature, recent work has shown that the process can instead be highly dynamic (Moore and Johnston, 2008). In fishes, ovarian follicles and fertilized eggs/embryos metabolize cortisol to cortisone and cortisol- and cortisone sulfates (Tagawa et al., 2000; Li et al., 2012), and cortisol metabolites were detectable in vitro within 4 hr in ovarian follicles coincubated with radio-labeled cortisol (Li et al., 2014). In zebrafish (*Danio rerio*), in vitro cortisol treatment of ovarian follicles for 4 hr increased expression of 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2), an enzyme that rapidly metabolizes cortisol to its inactive form, cortisone (Faught et al., 2016). Cortisol may also be actively transported out of the egg via ATP-binding cassette transporters that are known to regulate cortisol uptake within 6 hpf in three spined stickleback (*Gasterosteus aculeatus*) eggs immersed in a cortisol solution (Paitz et al., 2016). We found decreases in egg cortisol from 2 to 24 hpf in the high dose group, which is generally observed in fish eggs postfertilization (e.g., Sopinka et al., 2016). We did not find decreases in control or low dose groups, which is also reported in some egg cortisol exposure studies (Auperin and Geslin, 2008), but could have been due to low sample sizes and pooling of eggs across females.

Our survival results are novel in showing a positive effect on early survival (both to the eyed and fry stages) following a biologically relevant and temporally short (i.e., <24 hr) exposure to exogenous cortisol. We propose that increased cortisol can adaptively program early development (and therefore survival to eyed/fry stages) through glucocorticoid receptor (GR) signaling in eggs. In zebrafish, maternally derived GR mRNA transcripts are abundant in unfertilized eggs and early-stage embryos (Pikulkaew et al., 2010). Maternal GR deficiency early in embryogenesis is associated with the up- and downregulation of hundreds of other mRNA transcripts and leads to deformities and compromised embryo viability (Pikulkaew et al., 2011). Moreover, experiments elevating embryonic cortisol and/or performing GR knockdowns have shown the essential role of cortisol-mediated GR signaling in cytogenesis, mesoderm formation, and muscle development (Hillegass et al., 2008; Nesan et al., 2012; Kleppe et al., 2013). While the negative developmental implications of overexposure to cortisol (outside of the normal physiological range) are clear, less is known about how biologically relevant elevations in egg cortisol can impact early survival. We propose that increased levels of cortisol bound to maternal GR led to changes in the regulation of other maternal mRNA transcripts during early embryogenesis (Pikulkaew et al., 2011), ultimately promoting embryo viability.

Effects of Elevated Cortisol on Offspring Developmental Rate and Size

We found that fry reared from eggs exposed to a high cortisol dose were structurally smaller than fry reared from control dose eggs, which is in agreement with previous work in oviparous taxa (McCormick, '99; Meylan and Clobert, 2005; Saino et al., 2005; Burton et al., 2011). Other studies have demonstrated that effects of elevated egg GCs on body size are not consistently linear (Li et al., 2010), occur at different life stages (Burton et al., 2011), and can be species specific (Sopinka et al., 2016). For example, nestlings exposed to elevated prenatal GCs were lighter at hatching but heavier at fledging due to compensatory growth (European starling, Love et al., 2005; Love and Williams, 2008; House wren (*Troglodytes aedon*), Strange et al., 2016). Our results can be interpreted as a result of cortisol-mediated GR signaling early in embryogenesis, as GR knockdown in zebrafish leads to delayed hatching and embryos with shorter body length (Nesan et al., 2012; Wilson et al., 2016). As predicted, we found that egg cortisol treatment affected size but had no impact on developmental rate. This result suggests that timing of yolk sac absorption should have been similar across treatment groups in the wild and fry would have been in competition for resources, with size therefore being a determinant of survival. Body size is an important fitness metric and fry length can be positively correlated with survival (Einum and Fleming, 2000), suggesting that fry reared from high dose eggs would have been at a disadvantage by being smaller. However, we cannot make conclusions about the outcome of a small body size without appreciating the future interactive role of environmental effects (Love and Williams, 2008; Sheriff and Love, 2013), since investing in offspring with a small phenotype may increase offspring fitness in stressful environments (e.g., high predation risk environment; Chin et al., 2009; Sheriff et al., 2009, 2011). For example, juvenile salmon that rear in freshwater streams with high predation may benefit from being small if they are less likely to be detected by predators, but a small phenotype may make juvenile salmon more susceptible to predation if they display lower swimming performance and are slower to escape predators (Lundvall et al., '99). As such, offspring phenotypic responses to elevated GCs during egg/embryo development and their expected fitness outcomes are complex and depend on environmental context (Sheriff and Love, 2013).

Conclusions

We show that a brief exposure of Chinook salmon eggs to biologically relevant concentrations of cortisol immediately postfertilization can lead to increased early survival and smaller body size, without changing developmental rate. Overall, we provide evidence for a biphasic effect of prenatal stress exposure, with positive effects observed following a low cortisol dose (increased early survival), and a positive impact coupled with an apparently negative effect following a high cortisol dose (increased early survival and smaller size). If egg cortisol levels function as a general representation of a female's stress load prior to reproduction, a short-term increase in egg cortisol may represent a female encountering acute stressors (Stratholt et al., '97). The low dose may have been an optimal prenatal stress signal that programmed an early survival benefit, whereas the high dose was supraoptimal by resulting in a small body-size phenotype (Schreck, 2010). Alternatively, the high dose may have signaled to developing embryos that future stream conditions were stressful, and the early survival benefit and small body-size phenotype could have been adaptive under challenging environmental conditions (Sheriff and Love, 2013). Future work should tease apart these possibilities by following individuals across life-history stages to assess performance in different environments that match and do not match the maternal environment. Advancing this field of reproductive ecology will ultimately help quantify the transgenerational fitness implications of hormonally mediated changes in offspring phenotype.

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