

Egg cortisol treatment affects the behavioural response of coho salmon to a conspecific intruder and threat of predation



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Knowledge continues to emerge on how maternally derived egg hormones influence progeny behaviour in oviparous taxa. Of particular interest is how glucocorticoids (GCs) in eggs, as a signal of maternal stress, affect offspring behavioural traits. To date, research has focused on effects of egg GCs on offspring behaviours within a single experimental context. Little is known regarding how egg GCs affect relationships among behaviours and across ecological contexts (i.e. behavioural syndromes). We explored how exogenously increased egg cortisol shapes behavioural syndromes of juvenile coho salmon, *Oncorhynchus kisutch*. Social dominance during a territory intrusion and boldness following a simulated predator attack were recorded for juvenile coho salmon reared from cortisol-treated and untreated eggs. When exposed to a conspecific intruder, coho salmon treated with cortisol in ovo increased dominance behaviours, whereas coho salmon reared from untreated eggs reduced these behaviours. Following exposure to a simulated predator attack, coho salmon reared from cortisol-treated eggs increased boldness (i.e. increased activity and feeding; reduced shelter use), whereas coho salmon reared from untreated eggs reduced boldness. A dominance–boldness relationship was observed for coho salmon reared from cortisol-treated, but not untreated, eggs; dominance during the territory intrusion positively correlated with boldness under threat of predation. Our results highlight the complex influence of egg GCs on offspring behaviour and behavioural syndromes. Continued investigation into the outcomes of egg GCs on offspring will broaden our understanding of intergenerational components of the hormone–behaviour nexus.

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How elevated egg hormone levels modify progeny, and whether these effects are adaptive, is an emerging topic of interest in behavioural and evolutionary ecology (reviewed in Dufty et al., 2002; Meylan, Miles, & Clobert, 2012). Among oviparous taxa, maternal hormones are thought to be passively absorbed into developing eggs (birds, Groothuis, Müller, von Engelhardt, Carere, & Eising, 2005; reptiles, Radder, 2007; fishes, Mommer, 2013; but see Groothuis & Schwabl, 2008; Moore & Johnston, 2008 for discussion on maternal and embryonic control of yolk hormone deposition). Maternal stressor exposure can elevate concentrations

of stress hormones, or glucocorticoids (GCs), in eggs (McCormick, 2006; Saino, Romano, Ferrari, Martinelli, & Møller, 2005). Exogenous elevation of egg GCs is used to mimic predicted outcomes of maternal stress. When egg GCs are experimentally elevated, behavioural effects on offspring can be profound, and also variable. Yellow-legged gulls, *Larus michahellis*, reared from GC-treated eggs did not beg as loudly or as frequently (Rubolini et al., 2005). In another species, European starlings, *Sturnus vulgaris*, chicks reared from GC-treated eggs begged at a higher intensity (Love & Williams, 2008). Running speeds of lizards (*Amphibolurus muricatus* and *Bassiana duperreyi*) were not affected by manipulation of egg GCs (Warner, Radder, & Shine, 2009). In brown trout, *Salmo trutta*, exogenously elevating egg GC concentration can increase juvenile aggression toward a mirror image (Sloman, 2010) and decrease aggression toward a conspecific (Burton, Hoogenboom,

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Armstrong, Groothuis, & Metcalfe, 2011). Although much is gleaned from studies to date that exogenously elevate egg GCs and quantify behavioural effects in a single environmental context, it is unclear how fluctuations in maternally derived egg GCs may be influencing correlations between behaviours displayed under different ecological contexts (i.e. behavioural syndromes sensu Sih & Bell, 2008; Sih, Bell, & Johnson, 2004).

There is now an intensifying interest in the proximate mechanisms underlying behavioural syndromes (Bell & Aubin-Horth, 2010; Careau & Garland, 2012; Coppens, de Boer, & Koolhaas, 2010). Of particular interest is how the production and regulation of GCs contribute to behavioural syndromes, given that hormones can modulate 'suites of correlated traits' (McGlothlin & Ketterson, 2008). In fishes, for example, endogenous GC synthesis in response to a stressor has an instrumental role in predicting an array of correlated behavioural traits (e.g. competitive ability, foraging, locomotion; reviewed in Øverli et al., 2007). Rainbow trout, *Oncorhynchus mykiss*, with an attenuated plasma GC stress response tended to be more dominant displaying increased aggression, activity levels and feeding (Pottinger & Carrick, 2001). Similar relationships between stressor-induced plasma GCs and correlated behaviours are also observed in birds (reviewed in Cockrem, 2007). Great tits, *Parus major*, with lower GC responses to stressors explored a novel environment more quickly and were more aggressive toward an intruder (Cockrem, 2007). Do maternally inherited GCs have a role in programming behavioural syndromes as well? Examination of how maternally derived egg GCs influence behavioural syndromes in progeny would complement the advances in our understanding of how circulating, stressor-induced GCs predict behavioural coupling.

In fishes, behavioural traits incorporated into behavioural syndromes commonly include dominance over conspecifics and boldness under threat of predation (reviewed in Conrad, Weinersmith, Brodin, Saltz, & Sih, 2011). Social dominance and boldness are commonly reported to be positively correlated within individuals (Aubin-Horth, Deschênes, & Cloutier, 2012; Bell, 2005; Bell & Sih, 2007; Dingemans et al., 2007; Huntingford, 1976). In three-spined sticklebacks, *Gasterosteus aculeatus*, the dominance–boldness syndrome is evident in high- but not low-predation populations (Dingemans et al., 2007). Furthermore, within a generation, this behavioural syndrome manifests following exposure to a predator (Bell & Sih, 2007). Ecological stressors can thus select for the coupling of behaviours if adaptive under particular environmental conditions (Dall, Houston, & McNamara, 2004). Between generations, elevated egg GCs, as a maternal signal of a challenging environment, could be another potential driver of the dominance–boldness syndrome. Social dominance in brown trout (Burton et al., 2011; Sloman, 2010) and predator avoidance in three-spined sticklebacks (McGhee, Pintor, Suhr, & Bell, 2012) appear to be influenced by egg GC levels, which can be elevated following maternal exposure to a stressor (McCormick, 2006; Stratholt, Donaldson, & Liley, 1997). However, how inherent hormonal properties of eggs shape coupling of these behaviours within individuals remains largely unknown. We experimentally increased the concentration of cortisol (the primary GC in fishes) in coho salmon, *Oncorhynchus kisutch*, eggs, mimicking the predicted outcomes of maternal stressor exposure. We examined how elevated egg cortisol influenced social dominance in response to a conspecific intruder, boldness under threat of predation, and the relationship between interindividual differences in dominance and boldness (i.e. presence or absence of a behavioural syndrome).

The early life history of coho salmon provides an ideal opportunity to investigate how egg hormones affect the occurrence of behavioural syndromes. Following emergence from spawning

ground gravel, juvenile coho salmon inhabit freshwater streams for 1–2 years before migrating out to the ocean as smolts (Sandercock, 1991). During this rearing period the threat of predation by avian (e.g. great blue heron, *Ardea herodias*) and piscivorous predators is significant (Clements, Stahl, & Schreck, 2012; Sandercock, 1991). While evading predators, juvenile coho salmon must acquire sufficient resources to fuel growth necessary for successful downstream migration to sea. In contrast to other juvenile Pacific salmon (e.g. sockeye salmon, *Oncorhynchus nerka*, chum salmon, *Oncorhynchus keta*, pink salmon, *Oncorhynchus gorbuscha*), juvenile coho salmon do not readily school, are more aggressive toward conspecifics and defend feeding territories in streams (Dill, Ydenberg, & Fraser, 1981; Hutchison & Iwata, 1997). The two ecological scenarios (i.e. predator evasion and territory defence) theoretically require an individual to be behaviourally plastic; increased activity and feeding can assert dominance in a competitive interaction (Abbott & Dill, 1985), but such behaviours would increase predation risk in the presence of a predator (reviewed in Lima & Dill, 1990). Increased shelter occupancy is suitable for both competitive (Faria, Almada, & do Carmo Dunes, 1998) and predatory (Lima & Dill, 1990) scenarios. Behavioural syndromes can thus constrain this behavioural plasticity if dominance and boldness are coupled within an individual (Sih et al., 2004). However, behavioural syndromes can emerge if the coupled behaviours confer a fitness advantage in a particular environment (Dall et al., 2004).

We predicted that coho salmon reared from cortisol-treated eggs would (1) be less socially dominant (reduced activity, feeding, shelter occupancy and aggression) when interacting with a conspecific intruder (Burton et al., 2011) and (2) act in a bold or risk-taking manner following a simulated predator attack (increased activity and feeding, reduced shelter occupancy; McGhee et al., 2012). Elevations in egg cortisol as a signal of a challenging maternal environment (e.g. high predation pressure) could be a selective factor for behavioural syndromes (Bell, 2005; Bell & Sih, 2007; Dingemans et al., 2007). We predicted that correlational strength between dominance and boldness would be greater in coho salmon reared from cortisol-treated eggs.

METHODS

Egg Exposure and Offspring Rearing

Sperm and eggs were taken from 15 ripe male and 15 ripe female coho salmon that had migrated to the Fisheries and Oceans Canada (DFO) Chilliwack River Hatchery in Chilliwack, British Columbia, Canada. Sperm and eggs were then transported to the University of British Columbia (UBC) within ~2 h. In duplicate, 15 g of eggs from each female were fertilized with sperm from a male to create full-sibling crosses (i.e. each female was paired once with a male). Facility water (30 ml) was added to the sperm–egg mixture to activate sperm motility. Four hundred millilitres of water dosed with 1000 ng/ml cortisol (H4001; Sigma) dissolved in 95% ethanol (0.002% final concentration) was then added to one replicate and 400 ml of control water (0 ng/ml cortisol) was added to the other replicate with the same concentration of ethanol as cortisol-treated eggs. The concentration of cortisol was chosen based on plasma concentrations detected in mature female Pacific salmon (Hruska et al., 2010), and has been used previously to exogenously elevate salmonid egg GCs (Auperin & Geslin, 2008). Using enzyme immunoassay (EIA; see Sopinka, Hinch, Middleton, Hills, & Patterson, 2014 for protocols), egg cortisol concentrations 2 and 24 h post-fertilization were determined for each full-sibling cross and each hormone treatment (untreated, 0 ng/ml; cortisol-treated, 1000 ng/ml). Intra- and interplate variability was 5.3% and 8.6%, respectively. Egg cortisol concentrations were significantly higher after 2 h

immersion in cortisol-dosed water (paired t test: $t_{14} = 7.20$, $P < 0.0001$; Fig. 1). Elevated egg cortisol concentrations (average = 33.03 ng/g, range 23.62–39.44 ng/g) were marginally outside the upper range of concentrations detected in unfertilized eggs of the 15 female coho salmon (range 1.10–30.45 ng/g). Elevated egg cortisol concentrations were comparable to those detected in unfertilized eggs from adult coho salmon stressed in captivity via chronic exposure (2 weeks) to a chase stressor (Stratholt et al., 1997). Cortisol concentrations reduced by 24 h postfertilization in both egg treatments (0 ng/ml: $t_{14} = -4.20$, $P = 0.001$; 1000 ng/ml: $t_{14} = -12.93$, $P < 0.0001$), although concentrations in cortisol-treated eggs still remained higher than concentrations in untreated eggs ($t_{14} = 2.50$, $P = 0.03$; Fig. 1).

Fertilized eggs were moved to individual flow-through baskets and incubated in heath stacks until emergence (full yolk sac absorption). Water temperature was 7–8 °C throughout incubation. Dead eggs and embryos were removed from heath stacks daily and stored in Stockard's solution (5% formaldehyde (40%), 4% glacial acetic acid, 6% glycerin, 85% water) to determine fertilization success, and survival to hatch and emergence. The 0 ng/ml full-sibling cross for one female was accidentally placed in Stockard's solution in its entirety and fertilization success and progeny survival could not be determined for this female. Fertilization success did not differ between eggs fertilized in control (0 ng/ml) and cortisol-treated (1000 ng/ml) water (Wilcoxon signed-ranks test: $Z = 0.94$, $N = 29$, $P = 0.35$). Emergent fish were transferred to 1000-litre flow-through troughs, separated by egg hormone treatment and families pooled. Light cycle was adjusted throughout rearing to match natal stream photoperiod at latitude 49°18'N. Water temperature in troughs ranged from 6 to 11 °C due to natural fluctuations in municipal water sources, but still matched the natural temperature range of the study population. Fish were fed fishmeal (EWOS Canada Ltd; www.ewos.com) daily ad libitum up until the day of behavioural trials.

Behavioural Trials

Fifty-eight behavioural trials were completed in a sectioned area (26 × 25 cm and 23 cm deep) of a 37.5-litre aquarium (50 × 25 cm and 31 cm high) filled with dechlorinated water. The area of the divided section of the aquarium was chosen based on territory size of juvenile coho salmon in the wild (Dill et al., 1981), and contained

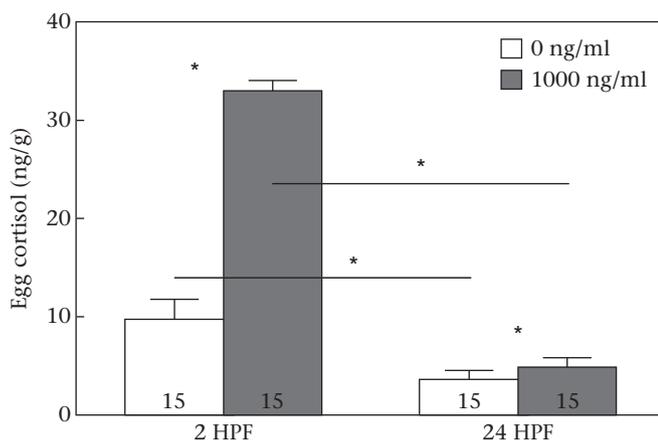


Figure 1. *Oncorhynchus kisutch* egg cortisol levels (mean ± SE) for eggs fertilized in control (0 ng/ml cortisol) and cortisol-dosed (1000 ng/ml) water at 2 h and 24 h postfertilization (2 HPF and 24 HPF, respectively). Asterisks and asterisks with bars denote statistical significance at $P < 0.05$ between egg hormone treatment groups (0 versus 1000 ng/ml) and fertilization time points (2 HPF versus 24 HPF), respectively. N is presented at the bottom of the bars.

a 2 cm layer of 1 cm gravel, a 21 cm aquarium plant and a 34 cm black PVC tube with 0.25 cm randomly drilled holes (Fig. 2). A perforated partition facilitated oxygen flow between the open area and the area containing an airstone (Fig. 2).

Fish (~3 months postemergence) were lightly anaesthetized with buffered tricaine methanesulfonate (MS-222) between 1500 h and 1900 h, weighed to the nearest 0.0001 g, and fork length was measured to the nearest 0.1 cm. To identify resident and intruder fish, nontoxic acrylic paint was injected subcutaneously on the left and right side of the fish anterior to the dorsal fin. Pairs of fish were size-matched (absolute size difference = 0.04 g, range 0.00–0.15 g; relative size difference = 4%, range 0–10%). Pairs consisted of one individual reared from cortisol-treated eggs and one individual reared from untreated eggs. For half of the trials conducted, the resident fish was reared from cortisol-treated eggs and the intruder was reared from untreated eggs. For the other half of the trials conducted, the resident fish was reared from untreated eggs and the intruder was reared from cortisol-treated eggs. Fish recovered in aerated water within 1 h of injection (as demonstrated by upright, vigorous swimming) and were placed in an aquarium; the resident fish was placed into the open sectioned area and the intruder fish placed into the PVC tube (Fig. 2). Both fish were then fed ~0.04 g (~4% body mass) of fishmeal.

Eighteen hours after placement in the aquarium, ~0.04 g of fishmeal was slowly strewn into a back corner of the open sectioned area. After 3 min the PVC tube was slowly lifted out of the aquarium, revealing the intruder (territory intrusion). Three minutes after introduction of the intruder, the intruder was removed from the aquarium, euthanized with an overdose of buffered MS-222, and body mass and fork length were measured. A 3 min interval was chosen based on previous studies observing aggressive behaviours in juvenile salmonids (Burton et al., 2011; Vøllestad & Quinn, 2003). Three hours later, ~0.04 g of fishmeal was again slowly strewn into a back corner of the open sectioned area. After 3 min, a model great blue heron was rapidly submerged into the open sectioned area three times (predator avoidance). Three minutes after the simulated predator attack the resident was removed from the aquarium and euthanized as above. All behavioural trials were videorecorded using a Canon EOS Rebel T3i digital camera (www.canon.com) at 60 frames/s.

We obtained behaviour of resident and intruder coho salmon from the digital videos for four 3 min intervals: before and after intruder introduction, and before and after the simulated predator attack. When applicable, we tabulated the following behaviours: activity (total seconds that the focal fish swam more than one body length), feeding (number of times focal fish bit at a particle of food), shelter occupancy (total seconds that the focal fish spent behind the plant), chase (number of rapid and directed movements by focal fish toward opposing fish that caused the opposing fish to flee rapidly) and displacement (number of times the focal fish slowly approached opposing fish causing the opposing fish to flee rapidly). Total aggression was calculated as the sum of chases and displacement. Trials with pairs of fish that did not display aggressive behaviours were categorized as 'no interaction'; trials with pairs of fish that displayed aggressive behaviours were categorized as 'pair interaction'. Average tabulation of resident behaviours are presented in Table 1. Video observers were blind to the egg treatment of the focal fishes.

Statistical Analyses

We generated composite behaviour scores (Bell, 2005) for resident and intruder coho salmon using three principal components analyses (PCA) with a correlation matrix. The use of composite scores versus individual behaviours avoids pitfalls of

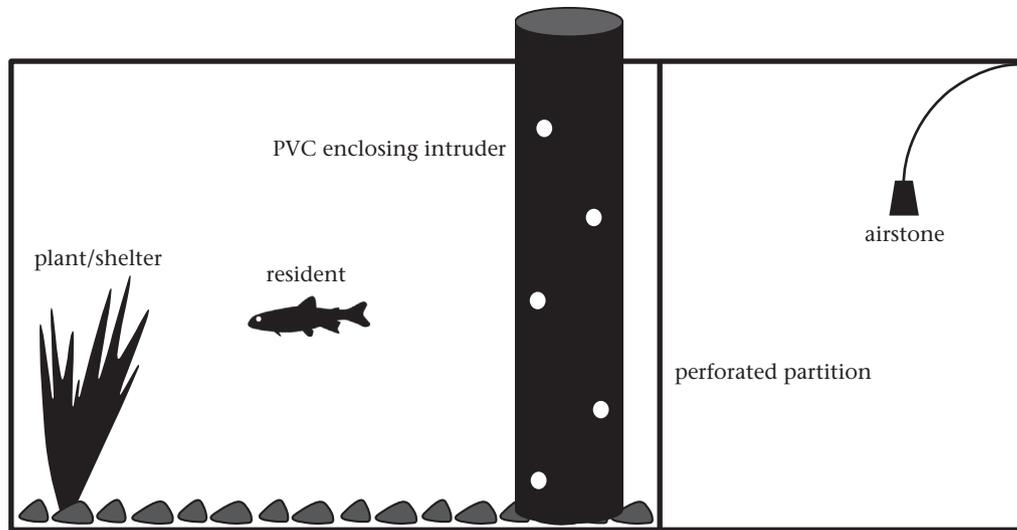


Figure 2. Initial experimental aquarium set-up. Area of the section containing the resident *Oncorhynchus kisutch* was chosen based on territory size of juvenile *O. kisutch* in the wild (Dill et al., 1981), and contained a 2 cm layer of 1 cm gravel, a 21 cm aquarium plant (for shelter) and a 34 cm black PVC tube with 0.25 cm randomly drilled holes. The conspecific intruder was contained within the PVC tube. The territory intrusion was initiated when the PVC tube was slowly lifted out of the aquarium and the intruder was revealed. The intruder was removed from the aquarium 3 h prior to the predator avoidance trial. Figure not to scale; see [Methods](#) for further details.

Table 1
Summary of resident coho salmon behaviours during the territory intrusion and the predator avoidance observation periods

	Activity (s)	Feeding attempts	Shelter occupancy (s)	Aggressive acts
Territory intrusion	83±11 (0–285)	3±1 (0–43)	74±16 (0–360)	3±1 (0–27)
Predator avoidance	112±15 (0–360)	5±1 (0–40)	52±15 (0–360)	–

Means ± SE are presented, with ranges in parentheses.

multiple comparisons including type I error. The composite scores generated were (1) resident dominance during the territory intrusion (total aggression, change in activity, feeding and shelter occupancy), (2) resident boldness following the simulated predator attack (change in activity, feeding and shelter occupancy) and (3) intruder dominance during the territory intrusion (total aggression, activity, feeding and shelter occupancy). Changes in resident activity, feeding and shelter occupancy were calculated as follows: (behaviour after intruder introduction/simulated predator attack) – (behaviour before intruder introduction/simulated predator attack). During the territory intrusion, change in resident activity, feeding and shelter occupancy and total aggression positively correlated with the principal component of the first PCA (Table 2). These composite scores were defined as resident dominance. Following the simulated predator attack, the principal component of the second PCA was positively correlated with change in activity and feeding, and negatively correlated with shelter occupancy (Table 2). These composite scores were defined as resident boldness. The third PCA summarized behaviour of intruder coho salmon during the territory intrusion; activity level, shelter occupancy and aggression were positively correlated with the principal component, and feeding was negatively correlated with the principal component (Table 2). These composite scores were defined as intruder dominance.

To determine differences in dominance and boldness of resident coho salmon reared from untreated and cortisol-treated eggs, generalized linear mixed models (GLMM) and generalized linear models (GLM) were fitted in R with the package ‘nlme’ (Pinheiro & Bates, 2000) following the methods outlined in Zuur, Ieno, Walker, Saveliev, and Smith (2009). For the GLMM, resident dominance scores were used as the response variable, with egg cortisol treatment (untreated, 0 ng/ml; cortisol-treated, 1000 ng/ml), pair

interaction (no interaction versus pair interaction, see above) and the pair interaction*egg cortisol treatment interaction as categorical fixed effects. To account for the possible role of intruder behaviour, a categorical variable was fitted as a random intercept (intruder behaviour was classified into the following four percentile groups based on PC scores: 0 to 25, >25 to 50, >50 to 75 and >75 to 100). The inclusion of the random effects intercept based on intruder PC percentile group was found to be a better fit to the data than the model with fixed effects only (AICΔ = –4.11), with the

Table 2
Results of principal components analyses (PCA) on resident dominance during the territory intrusion trial, resident boldness during the predator avoidance trial and intruder dominance during the territory intrusion trial

Behavioural context	Behaviour	PC loading
Resident dominance	Δ Activity level	0.65
	Δ Shelter occupancy	0.22
	Δ Feeding	0.57
	Aggression	0.46
	Variance explained	39.2%
Resident boldness	Δ Activity level	0.66
	Δ Shelter occupancy	–0.46
	Δ Feeding	0.60
	Variance explained	60.7%
	Intruder dominance	Activity level
Shelter occupancy		0.49
Feeding		–0.39
Aggression		0.50
Variance explained		52.3%

Δ represents change in a behaviour (activity level, shelter occupancy, feeding) and was calculated as behaviour after the intruder/simulated predator attack minus behaviour before the intruder/simulated predator attack. Only loadings with eigenvalues >1.0 are presented.

standard deviation of the variance for the intercept and residual variance of 0.61 and 1.08, respectively. After establishing the optimum random effects structure, the most parsimonious fixed effect structure was identified using backwards selection based on conditional F tests at a significance level of $P < 0.05$, and model estimate significance was determined using the Wald t test.

For the GLM, resident boldness scores were used as the response variable, and categorical fixed effects again included egg cortisol treatment and pair interaction status (defined during the territory intrusion). In this model, to determine whether intruder behaviour influenced resident antipredator behaviour, intruder behaviour percentile groups (see above) were included in the fixed effect structure and no random effects structure was incorporated. All possible two-way interactions between the three categorical fixed effects were included in the beyond optimal model. Backwards selection procedure was again applied to determine the optimal fixed effect structure, this time using AIC comparison (Zuur et al., 2009). Where significant interaction terms were identified, post hoc multiple comparison procedure using the false detection ratio correction was applied to compare between interaction levels (Bretz, Hothorn, & Westfall, 2010). All models were fitted with Gaussian responses, and model assumptions including normality and homogeneity of variance were established using residual plotting techniques (Zuur et al., 2009).

Correlations between dominance and boldness were assessed using Spearman rank correlations. PCA and Spearman rank correlations were performed using JMP 10 (SAS Institute Inc., Cary, NC, U.S.A.; www.jmp.com).

Ethical Note

All research protocols were approved by UBC's Committee on Animal Care (number A11 0215) and met the Canadian Council on Animal Care guidelines. Egg cortisol treatment was not found to have adverse effects on offspring hatching. Survival to hatch (Wilcoxon signed-ranks test: $Z = 1.59$, $N = 29$, $P = 0.11$) and emergence ($Z = 1.24$, $N = 29$, $P = 0.21$) did not vary between cortisol-treated and untreated offspring. Interactions between resident and intruder coho salmon were observed carefully and fish did not exhibit indices of distress. Intruder and resident fish were euthanized following the experiment as they would not be used in subsequent studies and could not be released into the wild, and due to space limitations.

RESULTS

Behavioural Response to a Conspecific Intruder

Opposing fish did not interact in 15 out of 29 (52%) trials when the resident coho salmon was reared from untreated eggs, and in 10 out of 29 (34%) trials when the resident coho salmon was reared from cortisol-treated eggs (chi-square test: $\chi^2_1 = 1.77$, $N = 58$, $P = 0.18$). Whether pairs of fish aggressively interacted or not did not affect resident social dominance (conditional F test: $F_{1,52} = 0.10$, $P = 0.75$; Fig. 3a). Resident coho salmon reared from cortisol-treated eggs showed increased dominance behaviours compared with resident coho salmon reared from untreated eggs (Wald t test: $t_{53} = -1.41$, $P = 0.01$; untreated eggs: PC1 estimate \pm SE = -0.39 ± 0.27 ; cortisol-treated eggs: PC1 estimate \pm SE = 0.38 ± 0.29 ; Fig. 3a).

Behavioural Response to a Simulated Predator Attack

Social interaction appeared to modify egg cortisol-mediated effects on antipredator behaviour (exclusion of the pair interaction *

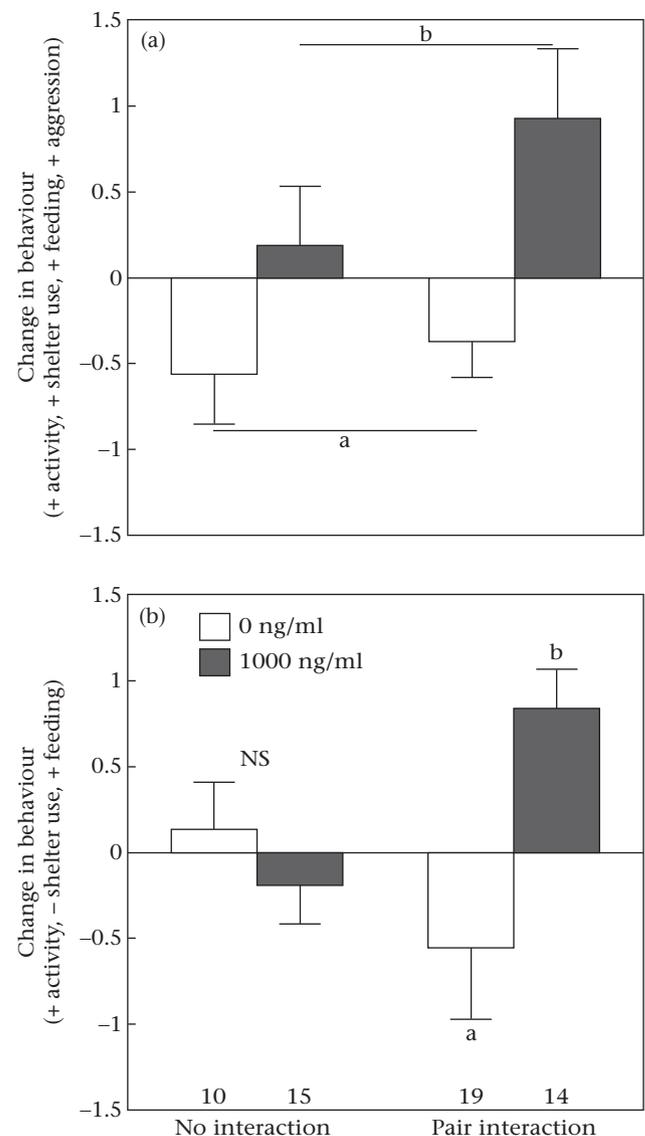


Figure 3. Comparison of mean \pm SE change in behaviour of resident *O. kisutch* reared from untreated (0 ng/ml cortisol) and cortisol-treated (1000 ng/ml cortisol) eggs, and exposed to a (a) conspecific intruder and (b) simulated predator attack. Change in behaviour was calculated as behaviour after the intruder/simulated predator attack minus behaviour before the intruder/simulated predator attack, and was assessed for resident *O. kisutch* that did (pair interaction) and did not (no interaction) interact with the intruder during the territory intrusion, as defined by the display of an aggressive behaviour (i.e. chase or displacement) by the resident and/or intruder. Behaviour scores were generated using principal components analysis (see Methods and Table 2). Different letters denote statistical significance ($P < 0.05$) between egg hormone treatments. N is presented below the bars in (b).

egg cortisol treatment interaction term resulted in $AIC\Delta = +4.32$). For resident coho salmon that did not interact with the intruder, the behavioural response of juveniles to the simulated predator attack did not differ between juveniles reared from cortisol-treated and untreated eggs (MCP estimate \pm SE = -0.31 ± 0.52 ; $Z = -0.601$, $N = 25$, $P = 0.55$; Fig. 3b). When opposing fish had interacted, resident coho salmon reared from cortisol-treated eggs showed increased boldness compared with resident coho salmon reared from untreated eggs (MCP estimate \pm SE = 1.40 ± 0.45 , $Z = 3.12$, $N = 33$, $P = 0.005$; Fig. 3b). Intruder behaviour during the territory intrusion did not account for a significant proportion of the variance in resident boldness (exclusion of intruder behaviour resulted in $AIC\Delta = -3.85$).

Behavioural Correlations

Social dominance during the territory intrusion and boldness following the simulated predator attack were positively correlated for resident coho salmon reared from cortisol-treated eggs that had aggressively interacted with the intruder; coho salmon reared from cortisol-treated eggs that were more dominant during the territory intrusion were also bolder following the simulated predator attack (Table 3).

DISCUSSION

We have shown that exogenous elevation in egg GCs modifies the behavioural responses of coho salmon progeny and generates a behavioural syndrome. Building on previous work examining how egg GCs influence offspring behaviour, we found that coho salmon reared from cortisol-treated eggs increased dominance behaviours following territory intrusion and increased boldness following a simulated predator attack, compared with coho salmon reared from untreated eggs. Between-individual differences in aggressiveness and boldness were positively correlated for juvenile coho salmon reared from cortisol-treated, but not untreated, eggs. Early developmental, or perinatal, stress has the potential to spawn adaptive behavioural syndromes (Sih, 2011). Our study provides novel insight into how intergenerational markers of prenatal stress shape behavioural syndromes.

In contrast to our first prediction, coho salmon reared from cortisol-treated eggs increased dominance behaviours following a territory intrusion. Social dominance imposes energetic costs such as increased oxygen consumption (Ros, Becker, & Oliveira, 2006) and reduced growth (Vøllestad & Quinn, 2003), but ultimately is associated with greater lifetime fitness (Ellis, 1995). Juvenile brown trout reared from cortisol-treated eggs were socially subordinate to juveniles reared from untreated eggs (Burton et al., 2011). Aggression levels are similar between brown trout and coho salmon (Hutchison & Iwata, 1997), and age at behavioural testing was similar between studies. Variation in methodology (i.e. use of dyadic versus triad interactions) and criteria for social dominance (i.e. only aggression versus activity, feeding, shelter occupancy and aggression) may account for discrepancies between the studies. Despite statistically controlling for effects of intruder behaviour on resident behaviour, the experiment design utilized in our study limits comprehensive interpretation of egg cortisol effects on social dominance. Although egg cortisol treatment appears to generate offspring that respond to conspecific intruders in a way that accords a fitness advantage (i.e. increasing social dominance), further research is needed to elucidate how egg GCs affect agonistic behaviours (e.g. using full reciprocal pairings of hormone treatment; DeNardo & Sinervo, 1994). Observing aggression under natural conditions (e.g. via telemetry; Harrison et al., 2015) and/or

seminatural conditions that mimic environmental and social aspects of progeny (e.g. experimental streams; Giannico & Hinch, 2003) would also be useful for understanding how egg cortisol affects progeny behaviour in the wild.

In line with our second prediction, coho salmon reared from cortisol-treated eggs increased risky behaviours following the simulated predator attack, suggestive of impaired predator avoidance. Here, increased boldness under threat of predation may be driven by a dominance–boldness syndrome selected for in offspring reared from cortisol-treated eggs, and thus, be an adaptive response (discussed below). Interestingly, behavioural responses to the simulated predator attack, irrespective of egg cortisol treatment, depended on whether resident coho salmon had displayed and/or received an act of aggression (i.e. chase and/or displacement) during the territory intrusion. A lack of behavioural change to the simulated predator attack in coho salmon that did not aggressively interact during the territory intrusion is also suggestive of suboptimal predator avoidance. Sensitivity or responsiveness to external stimuli is an inherent component of an animal's coping style and a key consideration when assessing behavioural syndromes (Bell, 2013; Sih & Bell, 2008). Theoretical models predict that, compared with unresponsive individuals, individuals responsive in one context are more likely to be responsive in future contexts when initial responsivity lowers costs of subsequent responsivity (Mathot, Wright, Kempnaers, & Dingemans, 2012; Wolf, Van Doorn, & Weissing, 2008). For example, if information accrued in one context (e.g. conspecific intruder) reduces uncertainty and/or is applicable in a subsequent context (e.g. threat of predation), then costs of responsivity are lowered and consistency of responsivity is maintained via positive feedback (Sih et al., 2015; Wolf et al., 2008). Resident coho salmon that physically interacted with the intruder potentially perceived being in an uncertain environment and accordingly used this information to respond to the second disturbance, the simulated predator attack (which may have been perceived as a secondary intrusion; Diaz-Uriarte, 1999). This hypothesis requires the assumption that an aggressive interaction provides the most meaningful information to an individual (and is the most meaningful measure of responsiveness) versus change in activity, feeding or shelter occupancy. Additional measures of social dominance (e.g. position in experimental arena) and measurement of offspring physiology following the territory intrusion (e.g. plasma GCs, metabolic rate) could provide further insight into what aspects of individual state are modifying an individual's decision to respond to subsequent stimuli.

For resident coho salmon that aggressively interacted with the intruder (i.e. responsive individuals), egg GC treatment generated a dominance–boldness syndrome. Dominance and boldness of responsive residents reared from untreated eggs were not correlated. When fitness prospects are low, it is thought that individuals are consistently risk-prone compared with individuals with high fitness expectations, which tend to be risk-averse, having more to lose (Wolf, Van Doorn, Leimar, & Weissing, 2007). Elevated egg GCs as a signal of a stressful maternal environment (e.g. predator, Saino et al., 2005; competition, McCormick, 2006; fisheries capture, Stratholt et al., 1997), with elevated risk of mortality/fitness loss, may select for a dominance–boldness syndrome. Ecologically challenging conditions (e.g. high predation) appear to select for individuals that are both aggressive and bold (Bell, 2005; Bell & Sih, 2007; Dingemans et al., 2007). Exhibiting behavioural flexibility may be costly in a stressful/unpredictable environment if individuals cannot afford to display an inappropriate phenotype for a given situation (Dall et al., 2004; Sih et al., 2004). Egg GC treatment generating a behavioural syndrome could thus be adaptive. For

Table 3

Spearman rank correlations (r_s) comparing dominance of resident coho salmon during the territory intrusion and boldness of resident coho salmon during the predator avoidance trials

Egg hormone treatment	Conspecific interaction ^a	r_s	P	N
0 ng/ml	No interaction	0.20	0.58	10
	Pair interaction	0.08	0.76	19
1000 ng/ml	No interaction	−0.42	0.12	15
	Pair interaction	0.61	0.02	14

Behaviours (activity level, shelter use, feeding, aggression) were transformed into composite scores using PCA (Table 2). Significant correlations ($P < 0.05$) are indicated in bold.

^a Conspecific interaction refers to whether pairs of coho salmon aggressively interacted during the territory intrusion (see Methods).

progeny reared from untreated eggs with no signal of a perturbed environment, emergence of behavioural flexibility would not be as consequential. To validate that stressor-induced changes to egg GCs drive behavioural syndromes, testing behaviour of offspring reared from stressor-exposed mothers is required. Furthermore, demonstrating that the dominance–boldness syndrome is adaptive in a stressful environment would require direct measurement of progeny fitness (e.g. exposure to a live predator; Bell & Sih, 2007).

Variation in endocrinology between offspring reared from cortisol-treated and untreated eggs may dictate behavioural syndromes. Hypothalamic–pituitary–adrenal (HPA; hypothalamic–pituitary–interrenal/HPI in fishes) axis function is linked to the dominance–boldness syndrome in birds (Kralj-Fiser, Weiß, & Kotrschal, 2010) and fishes (Aubin-Horth et al., 2012). HPA/I axis activity can differ between offspring reared from GC-treated and untreated eggs (Auperin & Geslin, 2008; Hayward, Richardson, Grogan, & Wingfield, 2006). Growth and food intake are positively correlated with boldness and aggression (Biro & Stamps, 2008). Effects of GC treatment on offspring growth are variable across species (reduced in fishes, Burton et al., 2011, and birds, Rubolini et al., 2005; increased in reptiles, Warner et al., 2009). Since coho salmon in this study were fed ad libitum, it is unlikely that growth rates varied between progeny reared from cortisol-treated and untreated eggs. Explicitly linking behavioural syndromes with juvenile growth and stress axis function will enhance our knowledge of how behavioural consistency reflects maternally derived individual state.

The implications of egg GCs as modulators of consistent inter-individual differences among offspring are applicable to both basic and applied research. Behavioural syndromes may evolve by way of maternal hormone signals (e.g. elevated egg GCs as indicators of maternal stress) that prime offspring with traits necessary for survival in a particular environment (Meylan et al., 2012). Behavioural syndromes are also an important component of animal husbandry (McDougall, Réale, Sol, & Reader, 2006). Understanding how egg hormones affect the behavioural profiles of progeny is especially relevant for breeding programmes. Adult holding conditions may influence deposition of egg GCs (e.g. high density, McCormick, 2006; captive versus free-living, Sopinka, Hinch, & Patterson, 2011). Egg GC concentrations also vary between strains of animals selected for growth (Ahmed et al., 2013) or stress responsiveness (Hayward, Satterlee, & Wingfield, 2005). All of these factors may contribute to behavioural variation in offspring, and are important to consider if offspring are to be released into the wild (McDougall et al., 2006). As research continues to divulge the underlying mechanisms of behavioural syndromes within a generation, tandem examination of variation in egg GCs will continue to reveal intergenerational drivers of these behavioural correlations.

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