REVIEW PAPER

Glucocorticoids in Fish Eggs: Variation, Interactions with the Environment, and the Potential to Shape Offspring Fitness*

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ABSTRACT

Wild and captive vertebrates face multiple stressors that all have the potential to induce chronic maternal stress (i.e., sustained, elevated plasma glucocorticoids), resulting in embryo exposure to elevated maternally derived glucocorticoids. In oviparous taxa such as fish, maternally derived glucocorticoids in eggs are known for their capacity to shape offspring phenotype. Using a variety of methodologies, scientists have quantified maternally derived levels of egg cortisol, the primary glucocorticoid in fishes, and examined the cascading effects of egg cortisol on progeny phenotype. Here we summarize and interpret the current state of knowledge on egg cortisol in fishes and the relationships linking maternal stress/state to egg cortisol and offspring phenotype/fitness. Considerable variation in levels of egg cortisol exists across species and among females within a species; this variation is hypothesized to be due to interspecific differences in reproductive life history and intraspecific differences in female condition. Outcomes of experimental studies manipulating egg cortisol vary both inter- and intraspecifically. Moreover, while exogenous elevation of egg cortisol (as a proxy for maternal stress) induces phenotypic changes commonly considered to be maladaptive (e.g., smaller offspring size), emerging work in other taxa suggests that there can be positive effects on fitness when the offspring's environment is taken into account. Investigations into (i) mechanisms by which egg cortisol elicits phenotypic change in offspring (e.g., epigenetics), (ii) maternal and offspring buffering capacity of cortisol, and (iii) factors driving natural variation in egg cortisol and how this variation affects offspring phenotype and fitness are all germane to discussions on egg glucocorticoids as signals of maternal stress.

Keywords: oocyte, cortisol, intergenerational effects, phenotype, maternal stress, match/mismatch.

1. Introduction

Animals in the wild regularly encounter multiple stressors and have evolved adaptations to cope with ecological challenges such as perturbations in the abiotic environment (e.g., fire, flooding), predators, resource limitation, and intra- and interspecific competition (Boonstra 2013). One of the coping mechanisms encompassed within the vertebrate stress response is the activation of the hypothalamic-pituitary-adrenal (HPA) axis in mammals, birds, and reptiles (see fig. 1 in Boonstra 2013) and the hypothalamic-pituitary-interrenal (HPI) axis in fishes (Wendelaar Bonga 1997; fig. 1*A*), resulting in the production of glucocorticoids (GCs). The elevation of circulating GCs in response to an environmental stressor is considered to be adaptive, initiating physiological and behavioral changes that function to promote survival (Wingfield et al. 1998; Sapolsky et al. 2000; McEwen and Wingfield 2003). Accordingly, elevated GCs are the most pervasive physiological indicator that an animal has been exposed to a stressor (Cooke and O'Connor 2010). However, compounded with ecological stressors, wildlife now encounter human-induced rapid environmental change (HIREC; e.g., habitat degradation, climatic change; Sih et al. 2011), and animals must now cope with novel stressors and unique combinations of stressors not previously encountered in their evolutionary history (Sih et al. 2011). Under these circumstances, energetically costly processes such as reproduction may be sacrificed for increased chances of survival, with these trade-offs being mediated by GCs (Ricklefs and Wikelski 2002). Such trade-offs not only affect the organism itself but also result in increased exposure of its offspring to maternal GCs and intergenerational phenotypic programming (Love et al. 2013).

Populations of fishes regularly encounter stressors and challenging conditions that can elevate circulating levels of the GC, cortisol (e.g., predation threat [Rehnberg and Schreck 1987] and intraspecific competition [Ejike and Schreck 1980]). Stressor ex-

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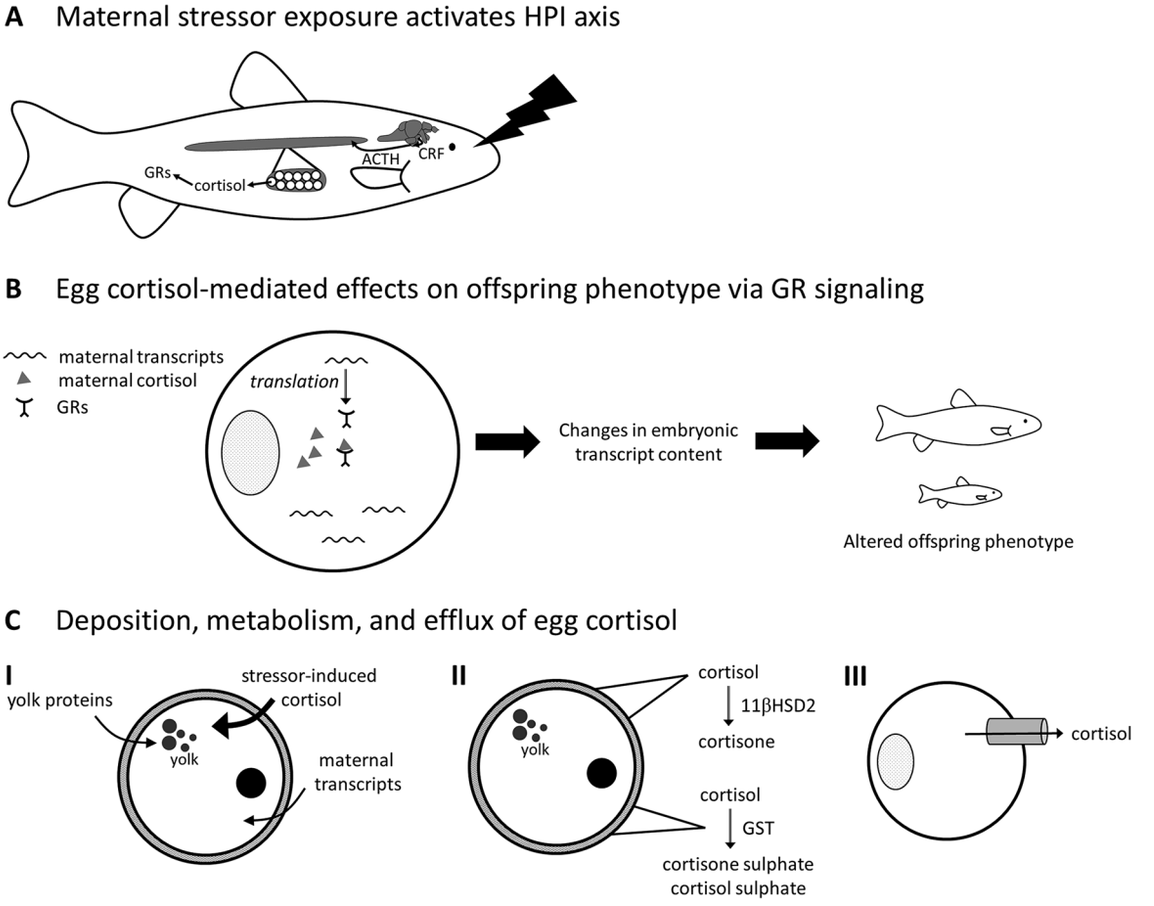

Figure 1. Schematic of effects of maternal stressor exposure and egg cortisol on offspring phenotype. *A*, Maternal stressor exposure activates the hypothalamic-pituitary-interrenal (HPI) axis. The hypothalamus releases corticotropin-releasing factor (CRF), which stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary. ACTH binds to receptors on the interrenal cells in the head kidney, initiating a biochemical cascade that results in the synthesis of cortisol. Circulating cortisol binds to glucocorticoid receptors (GRs) on target tissues and also reaches developing follicles in the female's ovaries. *B*, Within the fertilized egg, maternally derived cortisol is thought to bind to GRs translated from maternally derived GR transcripts. Once bound, the ligand-activated GR is hypothesized to induce changes in the abundance of embryonic transcripts associated with developmental pathways, resulting in altered offspring phenotype (sec. 3.1). *C*, Stressor-induced increases in circulating maternal cortisol can be associated with increased concentrations of ovarian or egg cortisol (*I*; sec. 5.3). Maternal transcripts, including transcripts for GRs, also enter the vitellogenic follicle. *C*, There is evidence for metabolism of cortisol to cortisone in the thecal/granulosa layer of follicles by 11β-hydroxysteroid dehydrogenase 2 (11βHSD2), as well as metabolism of cortisol to cortisol and cortisone sulphates by glucocorticoid sulphotransferase (GST; *II*; sec. 3.2). *C*, Efflux of cortisol via transmembrane ATP-binding cassette transporters is also observed in newly fertilized eggs (*III*; sec. 3.2).

posure activates the HPI axis, resulting in a biochemical cascade that initiates at the hypothalamus and concludes in the interrenal cells of the head kidney with the synthesis and elevation of cortisol (fig. 1*A*). Elevations in circulating cortisol are hypothesized to also elevate egg cortisol in reproductive females (fig. 1*C*), with the potential for downstream effects on offspring (fig. 1*B*). Marine and freshwater fishes now additionally face novel types of anthropogenic stressors that are outside their evolutionary history, including climate change–mediated elevations in water temperature (e.g., Chadwick et al. 2015), interactions with fisheries (e.g., Marçalo et al. 2009), and deteriorated habitats due to human activities (e.g., sediment loading; Awata et al. 2011). Domesticated and farmed fishes are also subjected to stressors associated with husbandry and aquaculture (e.g., handling, confinement, and transport [Barton and Iwama 1991] and noise [Anderson et al. 2011]). These stressors can all elevate circulating levels of maternal cortisol, which may result in altered offspring phenotypes via elevations in egg cortisol, calling into question the ability of the next generation to cope with its environment. As such, determining the evolutionary role of variation in maternally derived GCs has become a topic of great interest in the field of integrative ecology (Sheriff and Love 2013). For example, information on variation in egg GCs has the potential to be translated into metrics of broodstock health and production quality for aquaculture and stock enhancement initiatives. Moreover, measurements of GCs in wild animals have the potential to

act as indicators of population stress for conservation practitioners (Madliger and Love 2014; Sopinka et al. 2015*b*). Despite research spanning almost two decades, patterns of cortisol investment in fish eggs have yet to be comprehensively synthesized and integrated with prevailing assumptions of intergenerational mechanisms of stress.

Here we synthesize the current knowledge base on the relationships among maternal stress, maternally derived egg GCs, and offspring phenotype in fishes. First, we briefly review research regarding maternal GCs and oviparity in avian systems, as it can help guide research conducted in fishes (sec. 2). Next, studies of maternal stress and egg GCs in fishes are reviewed, including detailed descriptions of the mechanisms of action of egg GCs (sec. 3.1) and the metabolism of egg GCs pre- and postfertilization (sec. 3.2). We then summarize and interpret literature on cortisol concentrations in fish eggs, focusing on the cascading effects of natural variation in egg cortisol (sec. 4) and experimentally induced variation in egg cortisol (sec. 5) on offspring phenotype and fitness. Based on this information, we suggest pertinent avenues of future research (sec. 6) and provide conclusions (sec. 7).

2. Maternal GCs and Oviparity: What Do We Know from Birds?

Considerable research within the biomedical realm, showing that stressor-induced and exogenously manipulated maternal GCs are robustly linked with maladaptive offspring phenotypes in humans and rodents (reviewed in Seckl 2004; Cottrell and Seckl 2009), has guided predictions regarding the effects of maternal GCs on offspring in nonmammalian taxa such as birds. After mammals, intergenerational effects of stress are most extensively studied in birds, whereby maternal GCs are deposited into eggs (reviewed in Henriksen et al. 2011). The concentration of egg GCs can vary by timing of breeding and laying order (Love et al. 2008), and these patterns can additionally differ by life-history strategy (Love et al. 2009) and breeding-selection regime (high- and low-plasma GC response [Hayward et al. 2005] and fast and slow growth [Ahmed et al. 2013]). Importantly, maternal stressor exposure (Okuliarová et al. 2010) and experimental manipulation of maternal plasma GCs (Hayward and Wingfield 2004; Love et al. 2005) both result in eggs with elevated levels of GCs compared to unexposed or nonmanipulated females. A number of studies have then directly manipulated egg GCs (as a proxy for maternal stress) to examine effects on offspring phenotype. For some avian studies, results align with mammalian models of maternal stress; prenatal (i.e., in the egg) exposure to elevated GCs produces offspring with phenotypes commonly interpreted as maladaptive (e.g., reduced body size/feather growth [Saino et al. 2005], reduced competitive ability [Janczak et al. 2006], and reduced begging intensity [Rubolini et al. 2005]). However, as Henriksen et al. (2011) conclude, there is notable variation in the directionality of effects egg GCs have on offspring phenotype (e.g., increased body size [Tilgar et al. 2016], enhanced flight performance [Chin et al. 2009], and increased begging intensity [Love and Williams 2008]). In turn, this growing body of literature in avian systems has supported and enhanced the interpretation of the effects of maternal GCs on offspring fitness in other oviparous taxa, including fish.

3. Egg GCs in Fishes

Similar to avian species, GCs (cortisol) in eggs of fishes are maternally derived (table 1) and are necessary for proper offspring development (Nesan and Vijayan 2013*a*, 2013*b*). Cortisol, a lipophilic steroid, is reported to be incorporated into eggs during vitellogenesis, a late stage of oogenesis whereby glycolipoproteins (e.g., vitellogenins) are taken up by the follicle and processed into yolk (fig. 2; see Brooks et al. 1997; Jalabert 2005; Lubzens et al. 2010 for further details on teleost oogenesis). Hormones enter the vitellogenic follicle by diffusion along a concentration gradient (Tagawa et al. 2000) or possibly via coentry with vitellogenins (Brooks et al. 1997) and accumulate in the yolk (fig. 2). In vitro incubation of follicles in media with and without radio-labeled cortisol also suggests bidirectional movement of cortisol between follicles and maternal circulation (Tagawa et al. 2000; fig. 2).

While the biochemical and physiological relationships among cortisol, the stress response (HPI axis; fig. 1*A*), and reproductive parameters (e.g., circulating levels of sex steroids, egg size, fecundity, and fertilization success) in fishes have been extensively addressed (e.g., Iwama et al. 1997; Wendelaar Bonga 1997; Mommsen et al. 1999; Milla et al. 2009), studies on the intergenerational effects of stressor-induced GCs in fishes are not as abundant. Pioneering studies by Campbell et al. (1992, 1994) found that female salmonids that were chronically stressor exposed had elevated plasma cortisol levels, eggs of smaller size, and reduced survival of embryonic offspring. Schreck et al. (2001) were among the first to synthesize known maternal effects of stress in fishes at a time when there was still limited knowledge of the intergenerational effects of stress and only a handful of new studies (e.g., Contreras-Sánchez et al. 1996, 1998; Stratholt et al. 1997; McCormick 1998). These new studies did, however, provide important insight into a potential mechanism underlying maternal stressor-induced offspring change—namely, elevated egg cortisol concentrations. Stressor-induced elevations in maternal plasma cortisol (and proxies thereof via intraperitoneal injection of cortisol; McCormick 1998) can result in hypercortisolism of fish eggs (fig. 1*C*; Stratholt et al. 1997). Since then, research across species has demonstrated how elevations in egg cortisol shape offspring phenotype (table 2).

3.1. *Mechanisms of Action*

When relationships are detected between egg cortisol and offspring phenotype, how do these hormonally mediated phenotypes manifest? In adult fishes, cortisol binds to tissue-specific GC receptors (GRs), and this intracellular ligand-receptor complex moves to a cell's nucleus. In the nucleus, the ligand-receptor complex binds to GC response elements on DNA and induces transcription (Bury and Sturm 2007). Maternal transcripts for

Table 1: Egg cortisol concentrations of fishes

Species	No. females	Origin	Time point	Egg cortisol (ng g^{-1})	Reference
Brown trout (*Salmo trutta*)	3	Farmed	Unfertilized	46.2 ± 13.1	Sloman 2010
Brown trout (*S. trutta*)	15	Hatchery	Unfertilized	30.3 ± 9.3 (3.2–122.5)	Burton et al. 2011
Brook trout (*Salvelinus fontinalis*)	3	Farmed	Unfertilized	~3.5[a]	Ghio et al. 2016
Rainbow trout (*Oncorhynchus mykiss*)	5	Research broodstock	Unfertilized	60 ± 8[b]	Li et al. 2010
Rainbow trout (*O. mykiss*)	10, 10	Research broodstock	Unfertilized	23.1 ± 4.7[c]; 16.7 ± 3.1[d]	Andersson et al. 2011
Rainbow trout (*O. mykiss*)	3	Research broodstock	Unfertilized	5.1 ± .1	Ghaedi et al. 2013
Atlantic salmon (*Salmo salar*)	10, 15	Farmed	Unfertilized	13 ± 87[e]; 26 ± 46[e,f] (21–558)	Eriksen et al. 2013
Chinook salmon (*Oncorhynchus tshawytscha*)	23	Farmed	Unfertilized	20.6 ± 3.3 (2.4–57.1)	P. Capelle, unpublished data
Chinook salmon (*O. tshawytscha*)	12, 7	Wild caught	Unfertilized	12.1 ± 1.0[g] (6.3–17.2); 22.1 ± 8.9[h] (3.7–62.8)	P. Capelle, unpublished data
Coho salmon (*Oncorhynchus kisutch*)	7	Hatchery	Unfertilized	9.9 ± .9	Stratholt et al. 1997
Coho salmon (*O. kisutch*)	15	Hatchery	Unfertilized	9.4 ± 2.2 (1.1–30.5)	Sopinka et al. 2015*a*
Sockeye salmon (*Oncorhynchus nerka*)	17, 20	Wild caught	Unfertilized	8.7 ± 1.4 (2.7–32.8); 11.6 ± 1.7[i] (3.2–28.3)	Sopinka et al. 2014, 2016*b*
Persian sturgeon (*Acipenser persicus*)	1	Wild caught	Unfertilized	3.6 ± .7	Falahatkar et al. 2014
White sturgeon (*Acipenser transmontanus*)	2	Farmed	Unfertilized	21.5 ± 3.5	Simontacchi et al. 2009
Atlantic cod (*Gadus morhua*)	9	Research broodstock	Unfertilized[j]	~1[a]	Kleppe et al. 2013
Common carp (*Cyprinus carpio*)	1	Unknown	Unfertilized	~208[k]	Stouthart et al. 1998
Silver carp (*Hypophthalmichthys molitrix*)	1	Hatchery	Unfertilized	155.7 ± .5	Kausar et al. 2013
Three-spined stickleback (*Gasterosteus aculeatus*)	7	Wild caught	Unfertilized	12.6 ± 1.6	Paitz et al. 2015
Damselfish (*Pomacentrus amboinensis*)	150	Wild caught	Ovarian	(.3–76.0)	McCormick 1998
Fathead minnow (*Pimephales promelas*)	23	Research broodstock	Ovarian	190	DeQuattro et al. 2012
Great sturgeon (*Huso huso*)	5	Farmed	Ovarian[l]	13.5 ± 3.9	Poursaeid et al. 2012

Largemouth bass (*Micropterus salmoides*)	19	Wild caught	Ovarian	11.1 ± .8	O'Connor et al. 2013
Asian sea bass (*Lates calcarifer*)	~25	Farmed	At fertilization	2.20[m]; 1.20[n]; .62[o]	Sampath-Kumar et al. 1995
Chum salmon (*Oncorhynchus keta*)	1	Research broodstock	At fertilization	20	de Jesus and Hirano 1992
Japanese flounder (*Paralichthys olivaceus*)	...	Research broodstock	At fertilization	2.5	de Jesus et al. 1991
Rainbow trout (*Oncorhynchus mykiss*)	6, 6	Hatchery	At fertilization	1.4; 6.0	Barry et al. 1995
Yellow perch (*Perca flavescens*)	6	Research broodstock	At fertilization	~1[p]	Jentoft et al. 2002
Zebrafish (*Danio rerio*)	...	Commercial supplier	At fertilization	~600[q]	Nesan and Vijayan 2012
Red drum (*Sciaenops ocellatus*)	...	Research broodstock	1 h postfertilization	.2 ± .2	Applebaum et al. 2010

Note. The common methodology utilized to quantify cortisol content of eggs is homogenization of pooled eggs, followed by extraction of the hormone using diethyl ether or ethyl acetate. Extracted samples are then analyzed with radioimmunoassay or enzyme-linked immunosorbent assay. A new methodology for quantifying egg cortisol content is liquid chromatography tandem mass spectrometry (see Bussy et al., forthcoming). Mean ± SE and range (in parentheses) of egg cortisol concentration (ng g^{-1}; see the other footnotes for exceptions and conversions) are presented where possible. If values were not presented in the text of published articles, average values were estimated from figures. Ellipses indicate that data were not reported. Commercial supplier = females obtained from a commercial breeder; farmed = females obtained from a fish farm; hatchery = females held and bred in a hatchery facility; no. females = the number of individuals that eggs were obtained from; research brood stock = females held and bred in a laboratory facility; time point = the time at which cortisol concentration was quantified or that cortisol was measured in ovarian tissue; unknown = origin not reported; wild caught = females caught from a natural water source.

[a]Nanograms per milliliter.

[b]Converted from nanograms per egg to nanograms per gram based on an egg size of 0.05 g, estimated from Blom and Dabrowski (1995).

[c]Concentration in eggs collected from females selected for high stressor-induced plasma cortisol.

[d]Concentration in eggs collected from females selected for low stressor-induced plasma cortisol.

[e]Converted from nanograms per egg to nanograms per gram based on an egg size of 0.10 g from Berg et al. (2001).

[f]Females were injected with 1 mg kg^{-1} coconut oil (sham).

[g]Females migrated ~650 km from the ocean to reach freshwater spawning grounds.

[h]Females migrated ~800 km from the ocean to reach freshwater spawning grounds.

[i]Females were wild caught and held in captivity for ~6 wk.

[j]Females had a sham osmotic pump surgically inserted 3 wk before egg sampling.

[k]Converted from picograms per 20 eggs to nanograms per gram based on an egg size of 1.2 mg from Stouthart et al. (1998).

[l]Females were injected every 6 wk for 6 mo with two gelatinous intraperitoneal capsules containing 0.2 mL cocoa butter (sham); follicles within sampled ovaries were previtellogenic.

[m]Average concentration in eggs collected in July and October 1991 and March 1992.

[n]Concentration in eggs collected in January 1992.

[o]Concentration in eggs collected in February 1992.

[p]Converted from picograms per embryo to nanograms per gram based on an egg size of 0.04 g from Brown et al. (2009).

[q]Single-cell-stage embryos, converted from picograms per embryo to nanograms per gram based on an egg size of 0.006 mg from Markovich et al. (2007).

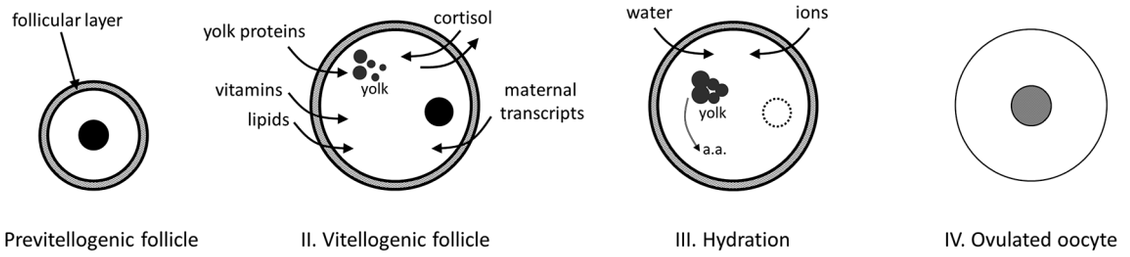

Figure 2. Schematic of oogenesis. *I*, The previtellogenic follicle is surrounded by a follicular layer comprising thecal and granulosa cells and contains the germinal vesicle (filled circle). *II*, During vitellogenesis, yolk proteins (e.g., vitellogenins) are incorporated into the developing follicle and processed into yolk. Cortisol, a lipophilic steroid, enters the vitellogenic follicle and accumulates in the yolk via diffusion or coentry with yolk proteins. The movement of cortisol between the follicle and maternal circulation is thought to be bidirectional. Lipids, vitamins, and maternal transcripts are also incorporated into the follicle during vitellogenesis. Due to the incorporation of lipids and proteins during vitellogenesis, the follicle undergoes significant growth. *III*, Hydration of the follicle occurs postvitellogenesis, whereby water and ions are taken up into the mature follicle and the germinal vesicle breaks down (dotted circle). Yolk proteins are also hydrolyzed into free amino acids (a.a.). *IV*, Following hydration, ovulation occurs, whereby the mature oocyte containing an oil droplet (gray circle) is released from the follicle into the abdominal cavity. The ovulated oocyte contains nutritional, molecular, and hormonal (e.g., cortisol) components necessary for proper embryo development. This figure is adapted from Cerdà et al. (2008).

GRs are detected in newly fertilized zebrafish (*Danio rerio*) eggs, and extensive work on this model species has revealed the mechanistic actions of maternally derived cortisol and GR transcripts in mediating offspring development (e.g., regulating development of the stress axis; Nesan and Vijayan 2013*a*, 2013*b*). Pikulkaew et al. (2011) postulated that in zebrafish, binding of maternally derived cortisol to GRs, translated from maternal GR transcripts, was possible shortly after fertilization (fig. 1*B*). Recently, Nesan and Vijayan (2016) used microinjection of a cortisol antibody to sequester maternally derived cortisol from single-cell zebrafish embryos and found that the cortisol stress response of embryos 72 h postfertilization was heightened (i.e., higher poststressor whole-body cortisol levels compared to control embryos) and that transcript abundance of HPI axis genes in embryos 48 h postfertilization was altered (see table 2). The authors concluded that maternal cortisol is integral to the formation of the stress/HPI axis (Nesan and Vijayan 2016). Following knockdown of GR protein content in zebrafish embryos using morpholino oligonucleotides, Pikulkaew et al. (2011) and Nesan et al. (2012) found that growth, swim bladder and craniofacial development, and survival of larval offspring were altered. In addition, transcript abundance of genes for extracellular matrix remodeling, bone morphogenesis, and myogenesis and cell proliferation were also altered in larval offspring (Pikulkaew et al. 2011; Nesan et al. 2012). Thus, maternal cortisol is thought to affect offspring development via GR signaling effects on transcript abundance (Pikulkaew et al. 2011; Nesan et al. 2012; Nesan and Vijayan 2013*a*, 2013*b*; fig. 1*B*). Furthermore, knockdown of GR protein content also alters transcript abundance and behavioral phenotypes in adult zebrafish (Wilson et al. 2015, 2016). Using mutant zebrafish (gr^{s357}) with nonfunctional GRs, Griffiths et al. (2012) and Ziv et al. (2013) found that the startle response, locomotor activity, and exploratory and social behaviors were altered compared to control fish. Again, these whole-organism changes suggest that effects of maternally derived cortisol on offspring phenotype are mediated by GR signaling (fig. 1*B*). It is noted that zebrafish have only a single GR gene. Indeed, Alsop and Vijayan (2009:65) posit whether other fishes with multiple GR genes (e.g., rainbow trout [*Oncorhynchus mykiss*]) "have different mechanisms or abilities to cope with stressors" compared to zebrafish. For example, in rainbow trout, offspring transcription levels of genes associated with GRs, nuclear receptor superfamily proteins, and insulin-like growth factor can be altered when ovarian follicles or eggs are treated with the GR antagonist Mifepristone/RU486 (Li et al. 2012*b*; Ferris et al. 2015). Continued extension of the genomic and physiological tools available for use in zebrafish to other teleost species is warranted.

Cortisol-mediated epigenetic changes are also thought to account for changes in offspring phenotype (Li et al. 2010; Pikulkaew et al. 2011; Nesan and Vijayan 2013*a*). Drawing largely on what is known from mammalian literature, Li and Leatherland (2013) and Love et al. (2013) highlight epigenetic programming as a viable mechanism whereby maternal stress or GCs can cause phenotypic change in the offspring of oviparous taxa. Again, GR signaling is implicated in this mechanistic pathway, as GRs are subject to maternally mediated epigenetic programming in mammals (Weaver 2009). In embryonic three-spined stickleback (*Gasterosteus aculeatus*), Mommer and Bell (2014) found that variation in the expression of DNA methyltransferase and histone genes depended on whether their mothers were stressor exposed (i.e., under threat of predation) or left undisturbed. In birds, in ovo injection of GCs increased DNA methylation of the hypothalamic GR gene promoter (Ahmed et al. 2014). The interactions among maternal stress, egg hormones, and epigenetic regulation are complex, and efforts to further understand the proximate mechanisms of action of egg cortisol will be relevant in predicting how early-life effects contribute to phenotypic change of offspring.

3.2. Pre- and Postfertilization Metabolism of Egg GCs

In fishes, in vitro and in vivo studies indicate that developing ovarian follicles, fertilized eggs, and prehatch embryos are capable of metabolizing steroid hormones, including cortisol. These findings match what is known in other oviparous taxa such as birds (Vassallo et al. 2014) and reptiles (Paitz and Bowden 2013). Based on observations that maternal plasma cortisol levels are significantly higher than those measured in eggs and therefore indicative of blood-egg buffering, Schreck et al. (2001) proposed a progeny-protecting system. Along with attenuation of maternal HPI activity as sexual maturation progresses (i.e., attenuated plasma cortisol response to a stressor) and corticosteroid-binding proteins restricting transfer of free cortisol from maternal circulation to eggs, Schreck et al. (2001) also hypothesized that enzymes capable of metabolizing cortisol, such as 11β-hydroxysteroid dehydrogenase 2 (11βHSD2), which metabolizes GCs in the mammalian placenta (Benediktsson et al. 1997), are present in ovarian follicles. Indeed, Tagawa et al. (2000) detected metabolism of cortisol (to cortisone, the biologically inactive GC in fishes; Bury and Strum 2007) in the thecal/granulosa layer of tilapia (*Oreochromis mossambicus*) follicles following in vitro incubation with radio-labelled cortisol. More recently, Li et al. (2012*a*, 2014) showed that rainbow trout ovarian follicles metabolize cortisol to cortisone as well as cortisol and cortisone sulphates (fig. 1*C*). Li et al. (2012*a*, 2014) inferred this metabolism to indicate the presence and activity of 11βHSD2 and GC sulphotransferase (sulfonation as a buffering pathway of maternally derived steroid hormones in oviparous taxa is reviewed in Paitz and Bowden 2013; fig 1*C*). Indeed, Kusakabe et al. (2003) detected 11βHSD2 transcripts in the thecal and granulosa cells of rainbow trout ovaries and found that transcript abundance increased throughout sexual maturation/vitellogenesis. Recently, Faught et al. (2016) found that 11βHSD2 transcript abundance in zebrafish follicles also increased following incubation with cortisol in vitro, suggesting that, in response to maternal stress, there is upregulation of enzymes in ovaries that reduce cortisol levels in eggs. There are therefore multiple biochemical pathways to examine as potential prefertilization mechanisms that control excess cortisol in fish eggs.

Regarding the potential for postfertilization buffering, Leatherland et al. (2010:102) focused on the interconnectedness of cortisol and the HPI and HP-ovary axis, concluding that egg cortisol has "a relatively minor influence on early ontogeny" and that this may be due in part to the "ability of embryos to metabolize cortisol to form steroids that have a low biological activity." In fishes, the onset of endogenous cortisol production in response to a stressor is observed prehatch (Stouthart et al. 1998), at hatch (Barry et al. 1995; Jentoft et al. 2002), and at first feeding (Alsop and Vijayan 2008). Other components of the HPI axis (e.g., upregulation of genes associated with cortisol production) can be responsive to a stressor before differences in cortisol are detected (Fuzzen et al. 2011). Yet, in much-earlier stages of progeny development, steroid hormone levels are dynamic. Across species, newly fertilized eggs are able to clear maternally derived cortisol, as indicated by significant reductions in cortisol concentrations within 24 h postfertilization (e.g., coho salmon [*Oncorhynchus kisutch*; Sopinka et al. 2015*a*], Japanese flounder [*Paralichthys olivaceus*; de Jesus et al. 1991], silver carp [*Hypophthalmichthys molitrix*; Kausar et al. 2013], white sturgeon [*Acipenser transmontanus*; Simontacchi et al. 2009], and zebrafish [Nesan and Vijayan 2012]). In rainbow trout, Li et al. (2012*a*) found conversion of cortisol to other metabolites in ovulated oocytes and embryos 25–58 d postfertilization (dpf; but see Paitz et al. 2016 for absence of metabolism in embryonic three-spined sticklebacks). However, the extent of metabolism was less than that observed in ovarian follicles (Li et al. 2012*a*). Recently, Paitz et al. (2016) found evidence for excretion of cortisol from newly fertilized three-spined stickleback eggs via ATP-binding cassette transporters (fig. 1*C*), which are transmembrane transport proteins associated with the uptake of xenobiotics in fishes (Luckenbach et al. 2014). There remains much to be gleaned regarding postfertilization buffering mechanisms in fishes.

The consensus is that ovarian follicles, eggs, and embryos are neither "passive recipients of maternal steroids" (Vassallo et al. 2014:4) nor "passive responders to the levels of steroids present in eggs" (Paitz and Bowden 2013:6). Moore and Johnston (2008) address numerous questions regarding the deposition, regulation, and metabolism of yolk steroids in oviparous taxa. These notions have implications for experimental design (i.e., sampling time points) and data interpretation. Although cortisol is thought to accumulate in egg yolk, the capacity for follicles to metabolize cortisol and for newly fertilized eggs to transport cortisol out of the embryo demonstrates that if concentrations are measured at only one life stage (e.g., unfertilized eggs), the concentrations represent a snapshot in time of a fluctuating hormone. Further, GR density and affinity in species with multiple GRs, as well as the percentage of bound versus unbound cortisol in maternal circulation, are apt to influence egg cortisol–mediated effects. These notions can have implications with regard to the predicted roles of egg cortisol in fishes.

4. Natural Variation in Egg Cortisol

The presence of interindividual variation in egg GCs sets the stage for natural selection to act on mothers and offspring in response to environmental variation (Love and Williams 2008). We focus here on studies that report variation in concentrations of maternally derived cortisol in ovarian tissue, unfertilized eggs, and eggs sampled at fertilization.

Maternally derived cortisol is detected in unfertilized and newly fertilized eggs of many fish species (table 1). Within a species, variation in egg cortisol content is observed across studies; egg cortisol levels in rainbow trout range from 5 to 60 ng g^{-1} (table 1). This type of variation is potentially driven by differences in genetic or environmental factors associated with different suppliers or strains of females, or laboratory rearing practices, respectively. Although not frequently reported in the literature, the absolute ranges of egg cortisol levels detected among females within a given study can be quite substantial in

Table 2: Effects of direct (egg: microinjection or bath) and indirect (female: intraperitoneal injection, osmotic pump, or soaked food pellet) manipulations of egg cortisol levels

Species	Manipulation (dose; duration)	Untreated egg concentration (ng g^{-1})	Treated egg concentration (ng g^{-1})	Embryonic survival effects	Body size effects	Other phenotypic effects	Reference
Brown trout (*Salmo trutta*)	Bath (0 or 470 ± 185 ng mL^{-1}; ovarian fluid, 3 h)	46.2 ± 13.1	699.0 ± 46.4	No effect	No effect (28, 43, 58, 87, and 292 dpf)	Elevated oxygen consumption and ammonia excretion rate; more aggressive displays; different response to a maze	Sloman 2010
Brown trout (*S. trutta*)	Bath (0 or 200 ng mL^{-1}; at fertilization, 2 h)	11.1 ± .7	55.0 ± 5.4	…	No effect (alevin); smaller (fry)	Reduced aggression and lower social status score; no effect on standard metabolic rate, territory quality, or competitive ability	Burton et al. 2011
Brook trout (*Salvelinus fontinalis*)	Bath (500 ng mL^{-1}; salt solution prefertilization, 3 h)	~3.5[a]	~200[a]	…	…	No effect on boldness, spatial learning or memory, or neophobia	Ghio et al. 2016
Rainbow trout (*Oncorhynchus mykiss*)	Bath (0, 200, or 1,000 ng mL^{-1}; at fertilization, 1 h)	~4	~7; ~7	…	…	Attenuated plasma cortisol response	Auperin and Geslin 2008
Rainbow trout (*O. mykiss*)	Bath (0, 100, or 1,000 ng mL^{-1}; ovarian fluid, 3 h)	64 ± 6[b]	94 ± 6[b]; 144 ± 9[b]	No effect (low dose); reduced (high dose)	No effect (fry); larger (low dose, 102 and 117 dpf); no effect (high dose, 102 and 117 dpf)	Ontogenetic variation in lysozyme activity, intelectin levels, and transcript abundance of genes for growth and immunity	Li et al. 2010, 2011, 2012*b*
Rainbow trout (*O. mykiss*)	Bath (0 or 20,000 ng mL^{-1}; at fertilization, 55 min)	74 ± 13[b]	725 ± 148[b]	No effect	No effect (146 dpf)	Ontogenetic variation in startle response; lower basal plasma cortisol	Colson et al. 2015
Atlantic salmon (*Salmo salar*)	Injection (0, 50, or 100 mg kg^{-1}; 6 d)	37.6 ± 28.7[a]	123.1 ± 26.9[a]; 123.7 ± 25.4[a]	No effect	Smaller (alevin and fry); larger (high dose, 4 mph); no effect (8.5 and 10 mph)	Increased fluctuating asymmetry; less active in novel environment	Eriksen et al. 2006, 2007, 2008; Espmark 2008
Atlantic salmon (*S. salar*)	Injection (0 or 100 mg kg^{-1}; 7 d)	26 ± 46[c]	681 ± 55[c]	…	Smaller (7 mph); no effect (11 mph)	Variation in response to confinement (more active: 4 mph; less active: 18 mph); increased occurrence of morphological malformations; no effect on feeding or social status	Eriksen et al. 2011, 2013

Chum salmon (*Oncorhynchus keta*)	Bath (0 or 1,000 ng mL^{-1}; at fertilization, 2 h)	5.5 ± .5	22.3 ± 1.4	No effect	No effect (178 dpf)	No effect on swimming duration	Sopinka et al. 2016*a*
Coho salmon (*Oncorhynchus kisutch*)	Bath (0 or 600 ng mL^{-1}; at fertilization, 2 h)	37.0 ± 5.4	232.7 ± 13.9	No effect	No effect (alevin)	…	Stratholt et al. 1997
Coho salmon (*O. kisutch*)	Bath (0 or 1,000 ng mL^{-1}; at fertilization, 2 h)	9.3 ± 2.2	33.0 ± 1.0	No effect	…	Higher dominance and boldness scores	Sopinka et al. 2015*a*
Sockeye salmon (*Oncorhynchus nerka*)	Bath (0 or 1,000 ng mL^{-1}; at fertilization, 2 h)	10.8 ± 4.4	33.7 ± 1.4	No effect	Smaller (182 dpf)	No effect on swimming duration	Sopinka et al. 2016*a*
Atlantic cod (*Gadus morhua*)	Osmotic pump (0 or 30 mg; 3 wk)	~1[a]	~20[a]	No effect	…	Variation in expression of genes for cytogenesis	Kleppe et al. 2013
Damselfish (*Pomacentrus amboinensis*)	Injection (0, 25, or 50 $\mu g\ g^{-1}$; 5 d)	~2[d]	~6 [d]; ~8[d]	…	Smaller (larval)	…	McCormick 1998
Zebrafish (*Danio rerio*)	Microinjection (0 or 32,000 ng mL^{-1}; at single-cell stage)	…	…	No effect	…	Reduced transcript abundance of genes for cardiac development; resting and poststressor exposure heart rate	Nesan and Vijayan 2012
Zebrafish (*D. rerio*)	Microinjection (0 or 32,000 ng mL^{-1}; at single-cell stage)	…	…	No effect	Larger (48 and 72 h postfertilization); no effect on head-trunk angle	Higher whole-body cortisol content; absence of stress-induced changes in whole-body cortisol; altered transcript abundance of stress axis genes; no effect on transcript abundance of corticosteroid receptor genes	Nesan and Vijayan 2016
Zebrafish (*D. rerio*)	Soaked food pellet (25 $\mu g\ g^{-1}$ body mass; 5 d)	23.1 ± 6.3[d]	48.0 ± 6.6[d]	…	…	…	Faught et al. 2016

Note. dpf = days postfertilization; mph = months posthatch.

[a]Nanograms per milliliter.

[b]Converted from nanograms per egg to nanograms per gram based on an egg size of 0.05 g, estimated from Blom and Dabrowski (1995).

[c]Converted from nanograms per egg to nanograms per gram based on an egg size of 0.1 g, estimated from Berg et al. (2001).

[d]Ovarian tissue.

both freshwater (brown trout [*Salmo trutta*]: 3.22–122.47 ng g^{-1}; Burton et al. 2011) and marine (damselfish [*Pomacentrus amboinensis*]: 0.3–76.0 ng g^{-1}; McCormick 1998) species.

4.1. Drivers of Variation

Examining the environmental and ecological drivers of variation in maternal GCs during reproduction, and therefore the potential for maternally derived GCs to act as maternal signals (Nesan and Vijayan 2013*a*) linking mother and offspring environments, has become an important focus in studies of maternal stress (Love et al. 2013; Crossin et al. 2016). For a given study, there are several questions to consider when hypothesizing what factors may be influencing interfemale variation in egg cortisol.

1. Does population-specific life history dictate egg cortisol content, as has been suggested in other taxa (e.g., Love et al. 2009)? Egg cortisol levels vary among geographically distinct populations of Chinook salmon (*Oncorhynchus tshawytscha*) that differ in the distance adults migrate from the ocean to reach freshwater spawning grounds (table 1); egg cortisol levels are higher in females that swim a longer distance to reach spawning areas. In Pacific salmon, egg number and size appear to be selected for on the basis of population-specific migration distances (Beacham and Murrary 1993). Egg cortisol levels may be another trait selected for based on population-specific environmental conditions; however, without a greater understanding of how natural variation in egg cortisol influences offspring phenotype, this explanation lacks an evolutionary basis. Egg cortisol content also varies between farmed and wild stocks of Chinook salmon (table 1). It is well known that numerous traits vary between domesticated and wild salmonids (Weber and Fausch 2003). Differences in egg cortisol between farmed and wild stocks are likely the outcome of selection regimes, but the traits targeted for selection that are linked with egg cortisol are not presently known. On a finer spatial scale, cortisol levels detected in laid clutch masses did not significantly vary between benthic and limnetic populations of three-spined sticklebacks (Foster et al. 2015). The ecotypes are distinguished by their foraging mode, and one might predict that a reproductive trait such as egg cortisol would not vary between populations. To address these hypotheses, studies quantifying population-specific variation in egg cortisol and relating this variation to variation in maternal or offspring fitness are needed.

2. For females that spawn multiple times a year, is egg cortisol content contingent on clutch order? Sampath-Kumar et al. (1995) found that egg cortisol concentrations in newly fertilized Asian sea bass (*Lates calcarifer*) eggs varied depending on when females spawned. Females that spawned in January and February had mean egg cortisol concentrations of 1.20 and 0.62 ng g^{-1}, respectively. Females that spawned in March of the same year had mean egg cortisol concentrations of 2.20 ng g^{-1}. These differences in egg cortisol of ~1–1.5 ng g^{-1} could elicit variation in offspring phenotype, given that differences in egg cortisol of ~3 ng g^{-1} elicit changes in offspring HPI function (Auperin and Geslin 2008). The farmed Asian sea bass used by Sampath-Kumar et al. (1995) spawn year-round. Egg cortisol content may vary temporally because of fluctuations in female condition, but what (and how) seasonal influences are modulating female condition are not known.

3. Is egg cortisol content driven by habitat choice? There is relatively little information currently available to answer this question. Due to high variability in ovarian cortisol among females within a nesting site, McCormick (1998) did not find that damselfish ovarian cortisol varied among different sites on a coral reef. However, for a given site, >20% of the variation in ovarian cortisol could be accounted for by the density of egg predators, suggesting that the threat of egg predation may influence egg cortisol levels.

4. Does maternal social status or stress-coping style regulate egg cortisol content? Egg cortisol content did not differ between dominant and subordinate zebrafish (Jeffrey and Gilmour 2016). This was contrary to the authors' predictions, given that subordinate fish have chronically elevated plasma cortisol (Sloman et al. 2001) and thus one would expect elevation of egg cortisol to align with elevated maternal cortisol (Stratholt et al. 1997). Rainbow trout bred for high and low responsiveness to an acute stressor yield offspring that differ with regard to yolk sac size and timing of emergence from spawning gravel (Andersson et al. 2011, 2013). However, egg cortisol content does not differ between the strains of rainbow trout (Andersson et al. 2011), suggesting that egg cortisol does not appear to be the mechanism coupling stress-coping style and other traits across generations. In contrast, Atlantic halibut (*Hippoglossus hippoglossus*) that were more resistant to handling (i.e., "unrested" and "requiring force to keep the fish on the table until it settled") had lower concentrations of cortisol in embryos collected 1 dpf (Skaalsvik et al. 2015:40). This finding suggests that maternal stress-coping style could be linked with egg cortisol, although comparison of cortisol levels in unfertilized versus recently fertilized eggs is required. Overall, the scope of variation among females described above suggests that allocation of egg cortisol has the potential to confer some influence on maternal fitness.

4.2. Relationships between Egg Cortisol Variation and Offspring Phenotype and Fitness

Ascertaining the impacts of variation in maternally derived GCs on offspring phenotype and fitness is often difficult to determine, given that underlying costs or benefits of exposure may be hidden without experimental manipulation (Crossin et al. 2016). As such, a dearth of knowledge still remains to be uncovered regarding the connections between interfemale variation in egg cortisol levels and offspring phenotype and fitness in fishes, especially since following egg cortisol analyses, embryos are not always reared long-term. In a coral reef damselfish, across females, higher concentrations of ovarian cortisol were associated with shorter larvae (McCormick 1998). A similar trend appeared to emerge in sockeye salmon (*Oncorhynchus nerka*), whereby offspring body condition decreased with increasing egg cortisol concentration (Sopinka et al. 2014). This same study did not find any correlation between egg cortisol and fertilization success or embryonic survival (Sopinka et al. 2014). An indication of how variation in cortisol may govern variation in the early development

of offspring can be gleaned from studies using embryos ~1 dpf. The occurrence of yolk sac edema increased with increased embryo cortisol content (1 dpf) in Atlantic halibut, although embryo cortisol content did not correlate with other parameters, including fertilization success and larval size (Skaalsvik et al. 2015). In smallmouth bass (*Micropterus dolomieu*), eggs were collected from nests, and eggs with higher cortisol had lower hatching success in the laboratory (Gingerich and Suski 2011). Cortisol levels measured in eyed embryos of masu salmon (*Oncorhynchus masou*) were negatively correlated with survival of a female's fertilized eggs to the eyed life stage (Mingist et al. 2007). Natural variation in egg cortisol thus has the potential to shape offspring phenotype and fitness, and yet research exploring the extent to which natural variation in egg cortisol dictates these offspring parameters later in development is limited. Long-term rearing of free-swimming offspring can be logistically challenging and require utilization of a marking system (e.g., passive integrated transponder or elastomer tagging) if offspring are to be segregated by maternal identity or egg cortisol content. Furthermore, given that aquatic ecosystems are subject to HIREC, this naturally occurring variation may be subject to novel selective pressures and accompanied by new modifications to offspring phenotype.

5. Experimental Manipulation of Egg Cortisol

5.1. Methodologies

Manipulation of egg GCs directly via exposure of eggs or indirectly via manipulating the female enables researchers to separate correlation from causation and illuminate the evolutionary significance of variation in maternally derived GCs (Meylan et al. 2012). There are several methods used to manipulate egg cortisol levels in vivo. Maternal environments can be altered and egg cortisol can be quantified (e.g., exposure of females to a physical stressor [Stratholt et al. 1997; Sopinka et al. 2014; Ghio et al. 2016], exposure of females to conspecific competition [McCormick 2006, 2009; Jeffrey and Gilmour 2016], and exposure of females to anthropogenic noise [Sierra-Flores et al. 2015]). Given that steroids are lipophilic, egg cortisol concentrations can be indirectly manipulated with an intraperitoneal injection of cortisol emulsified in cocoa butter or oil (Eriksen et al. 2006). However, caution should be heeded, as the injection medium itself (rather than the elevated egg cortisol per se) can also affect offspring size. Hoogenboom et al. (2011) found that, compared to unmanipulated female brown trout, egg and offspring size were smaller in females that were injected intraperitoneally with sham and cortisol-dosed cocoa butter. For larger-bodied fishes, osmotic pumps implanted into females offer an alternative to intraperitoneal injections (Kleppe et al. 2013). Food pellets soaked in cortisol-laced solutions are a viable option for species that are too small for surgery (e.g., zebrafish; Faught et al. 2016). Egg cortisol concentrations can be directly manipulated with microinjection of cortisol into eggs (zebrafish; Nesan and Vijayan 2012), bathing of unfertilized eggs in cortisol-dosed ovarian fluid (brown trout; Sloman 2010), or bathing of eggs in a cortisol solution at fertilization (coho salmon; Sopinka et al. 2015*a*). Each methodology has advantages and disadvantages (see Gamperl et al. 1994; Sopinka et al. 2015*b*) depending on the species and question of interest, and care should be taken when choosing an appropriate tool to manipulate egg cortisol.

5.2. Effects of Experimentally Elevated Egg Cortisol on Offspring Phenotype and Fitness

The array of phenotypic traits investigated following egg cortisol treatment is substantive, spanning from genomic to whole-animal responses (table 2). Collectively, there does not appear to be a consistent manner of change in offspring phenotype and fitness following experimentally elevated egg cortisol (table 2). For example, the effects of egg cortisol treatment on the size of offspring at first feeding (i.e., fry), a recognized predictor of performance in fishes, either are not reported or not detected or depend on the dose of egg cortisol treatment (table 2). Aggression and dominance are both increased (Sloman 2010; Sopinka et al. 2015*a*) and reduced (Burton et al. 2011) in salmonids reared from cortisol-treated eggs. Effects of exogenously elevated egg cortisol on activity levels in rainbow trout offspring vary across ontogeny; at 5 mo postfertilization, offspring from cortisol-treated eggs were more active than offspring from untreated eggs, but there were no differences in activity levels at 2 mo postfertilization (Colson et al. 2015). In Atlantic salmon (*Salmo salar*), effects of elevated egg cortisol on offspring response to a confinement stressor were also dependent on age (Eriksen et al. 2011, 2013). Four months posthatch, offspring reared from cortisol-manipulated females were more active during acute confinement compared to offspring reared from sham females (Eriksen et al. 2013), whereas 1.5-yr-posthatch offspring reared from cortisol-manipulated eggs were more inactive during acute confinement compared to controls (Eriksen et al. 2011). These differences may be due to differences in the time of confinement (20 vs. 30 min) and the size of the confinement tank (0.5 vs. 1.5 L). On a study-by-study basis, findings can be argued to be beneficial (e.g., dominance and increased offspring size) or detrimental (e.g., subordinance and decreased offspring size). However, without measuring fitness outcomes of a specific phenotype, phenotype alone cannot be affirmed as adaptive or maladaptive. Interpretation of the modified trait as adaptive or maladaptive, in conjunction with the hypothesis that elevated egg cortisol acts as a maternal signal to offspring (Nesan and Vijayan 2013*a*), must also consider whether the environment that offspring encounter or are tested in is matched or mismatched to the maternal environment (see sec. 6.2). Effects of elevated egg cortisol on correlates of offspring fitness (e.g., survival to first feeding) are restricted to early life stages (table 2). When reported, and with the exception of Li et al. (2010), elevated egg cortisol does not affect embryonic survival. It is possible that egg cortisol is affecting genomic or physiological pathways but not in a manner that results in embryo death.

5.3. Effects of Maternal Stressor-Induced Egg Cortisol on Offspring Phenotype and Fitness

Despite an enthusiastic interest in determining how elevations in egg cortisol shape offspring phenotype (table 2), the evidence

to date indicating that maternal stressor exposure modifies egg cortisol levels is ambiguous. There are as many studies that have found differences (e.g., Stratholt et al. 1997; McCormick 2006, 2009; Sierra-Flores et al. 2015) as there are that have not (e.g., Contreras-Sánchez 1996; Mileva et al. 2010; Sopinka et al. 2014; Jeffrey and Gilmour 2016; Ghio et al. 2016). There are physiological or biochemical (e.g., maternal and embryonic metabolism of cortisol; see sec. 3.2) and logistical (e.g., variation in stressor type and duration) reasons why a study may or may not detect differences in egg cortisol.

Variation in ovarian development across species could also affect incorporation and quantification of cortisol in the eggs of stressor-exposed females. For synchronous spawning females who have all eggs developing and ovulating at the same time (e.g., salmonids), eggs can be collected from stressor-exposed females at a single time point postovulation, and variation in cortisol levels can be interpreted as being stressor induced. However, the effects of timing of stressor application in relation to vitellogenesis and cortisol deposition are not yet clear. Contreras-Sánchez (1996) did not find variation in egg cortisol or embryo viability between undisturbed female rainbow trout and females exposed to a stressor treatment during early vitellogenesis, late vitellogenesis, or both early and late vitellogenesis. In contrast, in asynchronous fishes such as the zebrafish, all eggs of all stages of oogenesis are present in the female, and Faught et al. (2016) found temporal patterns in egg cortisol deposition in this species following a 5-d feeding period of cortisol-soaked food pellets. Accordingly, breeding synchronicity and strategy (reviewed in McBride et al. 2015), timing of stressor exposure, and timing of egg collection are important factors to consider when designing experiments, especially to facilitate comparison across studies.

Another common explanatory denominator threaded throughout these studies is the possibility that life-history variation could affect whether and how a female responds to a specific stressor and whether these responses would be selected for (Love et al. 2009). Accordingly, it is pertinent that (1) a species' evolutionary history be thought of a priori and (2) experimental egg manipulations are altering cortisol to levels that can be detected in a given species while it is under benign or disturbed conditions (i.e., within a biologically relevant range; Crossin et al. 2016). It is important to note that if maternal stressor exposure does not affect egg cortisol, this does not mean that (1) variation in egg cortisol does not have a role in phenotypic trajectories of offspring or (2) maternal stressor exposure does not affect phenotypic traits of offspring through other, nonhormonal mechanisms (e.g., epigenetics; sec. 3.1).

6. Future Research Directions

Cortisol's presence and functionality in the egg requires further exploration from several perspectives. From a methodological angle, transparent methods that target assessment of cortisol in ovarian follicles or tissue, unfertilized eggs, or newly fertilized eggs will ensure that maternally derived versus endogenous egg cortisol is being assessed and related to offspring phenotype, allowing for meaningful comparison of findings across studies. Combining multiple methodologies within a study is most powerful. For example, Ghio et al. (2016) reared offspring from female brook trout (*Salvelinus fontinalis*) fed cortisol-dosed food, repeatedly handled females, and eggs bathed in cortisol. There is also scope to investigate how synthetic GCs, such as the pharmaceutical prednisolone which is detected in bodies of water, influence offspring phenotype (McNeil et al. 2016). Other areas of future research include (1) conducting studies that adequately capture intra- and interspecific variation, (2) rearing and testing offspring in environments that do and do not match the maternal environment, and (3) assessing carryover effects on adult offspring phenotype and fitness.

6.1. Quantifying Multiple Levels of Variation

Our current understanding of how variation in egg cortisol within a female and among females correlates with offspring phenotypes remains limited, primarily due to low sample sizes and other logistical constraints (e.g., the difficulty of rearing maternal lines separately). In brown trout, Suter (2002) found evidence for intrafemale variation in egg cortisol depending on position within the ovary (anterior, middle, and posterior). Offspring phenotype of brown trout was also later found to vary according to position within the ovary (Burton et al. 2013*a*), but these data were not directly linked with egg cortisol. Interfemale variation in egg cortisol levels can be sizable for some species (table 1). Does this interfemale variation account for variation in offspring phenotype? Does this variation relate to variation in maternal condition? In birds, mothers in lower body condition are known to lay eggs with higher concentrations of GCs (Love et al. 2008). Similarly, analyses of female body condition and concentration of cortisol in unfertilized eggs of Pacific salmon show increasing egg cortisol with decreasing maternal condition in wild sockeye salmon (fig. 3). However, no statistically significant relationships were detected in coho salmon or farmed Chinook salmon (fig. 3).

Unresolved questions concerning how different levels of egg cortisol correlate with offspring phenotype may also be due to concentration thresholds of egg cortisol. As previously mentioned (sec. 5.3), the evidence amassed to date does not uphold the prevailing hypothesis that maternal stressor exposure consistently increases egg cortisol content in fishes. Moreover, the relationship between egg cortisol and offspring phenotype may not always be linear (fig. 4) and is expected to be under evolutionary constraints depending on the predictability and variability of the maternal and offspring environments (Burgess and Marshall 2014). Offspring performance may be optimal within an intermediate range of egg cortisol concentrations and be suboptimal at lower and higher concentrations of egg cortisol outside the range (fig. 4*A*). For example, Li et al. (2010) found nonlinear relationships between egg cortisol and offspring performance in rainbow trout. An egg cortisol treatment of 100 ng mL^{-1} (low dose) resulted in offspring larger than controls (0 ng mL^{-1}) and those reared from eggs treated with 1,000 ng mL^{-1} cortisol (high dose), which did not differ in size from controls (Li et al. 2010). This enhanced growth pattern in offspring treated with a low cortisol

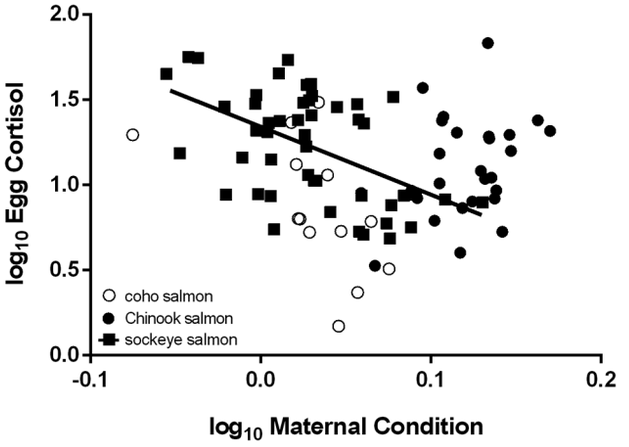

Figure 3. Log_{10} cortisol concentration in unfertilized eggs and log_{10} maternal body condition of Pacific salmon: hatchery coho salmon (*Oncorhynchus kisutch*; open circles; Pearson correlation: $r^2 = 0.30$, $n = 13$, $P = 0.05$), farmed Chinook salmon (*Oncorhynchus tshawytscha*; filled circles; $r^2 = 0.10$, $n = 27$, $P = 0.10$), and wild-caught sockeye salmon (*Oncorhynchus nerka*; squares; $r^2 = 0.26$, $n = 48$, $P = 0.0002$; line). Egg cortisol was quantified following Sopinka et al. (2014, 2015*a*). Maternal body condition was calculated following Fulton's condition factor *K*: (body mass (g)/fork length $(cm)^3)^{-1} \times 100\%$. See section 6.1 for further details.

dose was coupled with amplification of insulin-like growth factor transcripts (Li et al. 2010). Alternatively, there could be a negative linear relationship between offspring performance and concentrations of egg cortisol (fig. 4*B*; e.g., McCormick 1998). Offspring performance could also be maintained throughout a range of egg cortisol concentrations but decrease at concentrations beyond a specific threshold concentration (fig. 4*C*; e.g., Eriksen et al. 2006). When possible, implementation of multiple doses of cortisol when experimentally manipulating egg hormone levels can help determine concentration-dependent phenotypic effects.

Last, although not probed in detail in this article, interspecific variation in egg cortisol levels of fishes is also significant (table 1). While the reproductive life-history strategies of fishes are remarkably diverse and researchers have both predicted and demonstrated that variation in life histories affects the variability and adaptive potential of egg GCs in other taxa (Love et al. 2009, 2013; Sheriff and Love 2013), little is known about whether egg cortisol is associated with this variation in fishes. For example, among salmonids, there are semelparous Pacific salmon and iteroparous trout. One may predict that the buffering capacity of cortisol (sec. 3.2) in Pacific salmon would be superior to that in trout given that Pacific salmon have only one opportunity to reproduce before dying. Species undergoing long-distance migrations for spawning (e.g., Pacific salmon, American shad [*Alosa sapidissima*], and Pacific herring [*Clupea pallasii*]), and arguably a relatively more stressful reproduction, may deposit more or less cortisol into eggs compared to nonmigratory species. Species-specific variation in egg cortisol content may reflect interspecific variation in early offspring life history and rates of development (e.g., pelagic migration of larval marine fishes vs. overwinter rearing of juvenile freshwater fishes). Continuing to uncover answers to these questions regarding naturally occurring variation in egg cortisol can bolster support for the hypothesis that GCs act as maternally derived stress signals (Sheriff and Love 2013). In addition, establishing associations among maternal condition, egg cortisol, and offspring performance increases the validity of using egg cortisol as a metric of broodstock or population health.

6.2. Environmental Matching

From understanding the trajectory of diseases in humans as predictive adaptive responses (Gluckman and Hanson 2004) or analyzing the anticipatory parental effects in nonhuman animals and plants (Uller et al. 2013), there has been a recent surge in dialogue on the adaptive potential of maternal effects (i.e., responses of females induce offspring phenotypes that are deemed to be beneficial in anticipation of future circumstances). Dufty et al. (2002) postulated that the maternal endocrine system could mediate adaptive effects on offspring phenotype. Indeed, Meylan et al. (2012) focus on GCs as the candidate hormone for hormonally mediated maternal effects, and Sheriff and Love (2013) identify GCs as maternally derived stress (MDS) signals. Nesan and Vijayan (2013*a*:40) stated that the maternal deposition of cortisol and GR transcripts into fish eggs could serve as a "mechanism for transmitting [information] from stressed mothers to progeny." Increasingly, researchers have proposed that offspring exposure to MDS (via elevated prenatal GCs) can induce adaptive phenotypic outcomes in offspring if the stressful environment inducing MDS in the mother is shared temporally or spatially by offspring (i.e., environmental matching; Love and Williams 2008). To test the adaptive potential of MDS requires an environmentally relevant manipulation of MDS—that is, raising offspring in a matched stressful environment and then following fitness in the offspring (Sheriff and Love 2013). Selection for egg cortisol as an MDS signal will be favored in species where the maternal environment accurately predicts the environment of progeny in space or time (Love et al. 2013). Unfortunately, to date, the effects of elevated egg cortisol on offspring phenotype in fishes (table 2) have been predominantly tested under neutral or benign conditions (i.e., offspring are typically reared in unmanipulated common-garden environments). These conditions may be mismatched to the information transmitted via the MDS signal in the egg (i.e., elevated cortisol concentrations). There is a great opportunity to enrich our understanding of maternal effects by designing experiments that evaluate and interpret egg hormone–mediated offspring phenotypes in light of the quality of the offspring's future environment (see fig. 1 in Uller et al. 2013).

6.3. Adult Phenotype and Fitness

The majority of studies to date have focused on relationships between egg cortisol concentrations and offspring phenotype during early development versus the adult phenotype (table 2).

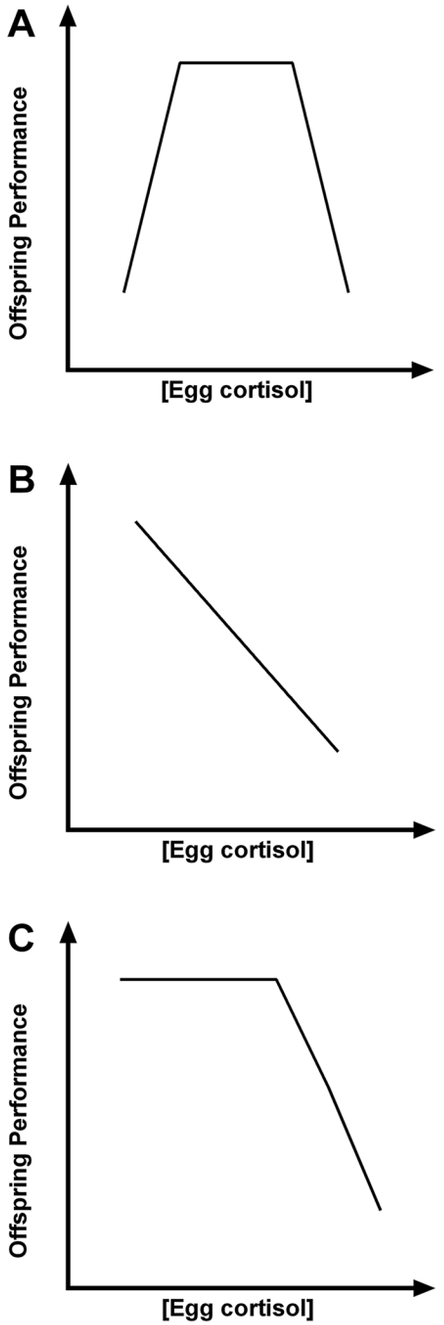

Figure 4. Proposed relationships between concentration of egg cortisol and offspring phenotypic performance. *A*, Offspring performance is optimal within an intermediate range of egg cortisol concentrations and suboptimal at both lower and higher concentrations of egg cortisol. *B*, Offspring performance is optimal at lower concentrations of egg cortisol and decreases linearly as egg cortisol concentrations increase. *C*, Offspring performance is maintained throughout a range of egg cortisol concentrations but decreases when concentrations increase above a specific threshold concentration. See section 6.1 for further details.

There are both logistical (e.g., complexity or expense of rearing offspring to sexual maturity) and phenological reasons for concentrating on effects of egg cortisol at early life stages. Still, it is worthwhile to investigate how egg cortisol–mediated effects on early offspring phenotype correlate with adult phenotype (and fitness). Phenotypic effects of experimentally elevated egg cortisol via maternal intraperitoneal injection were detected in farmed Atlantic salmon 1.5–2 yr after hatching (e.g., craniofacial and tissue abnormalities and reactivity to a confinement stressor; Eriksen et al. 2011, 2013). Egg cortisol–mediated effects may restrict phenotypic flexibility, and the offspring phenotype programmed early in life by egg cortisol titers may persist to adulthood. For example, exogenously elevating egg cortisol can reduce offspring size in salmonids (table 2), which may be linked with changes to growth early in embryogenesis (Li et al. 2010), and juvenile growth rates correlate with adult reproductive success (e.g., offspring survival; Burton et al. 2013*b*). However, the adult phenotype is likely an interaction between early-life hormonal influences (i.e., egg cortisol concentration or maternal signal) and the environment (see fig. 1 in Dufty et al. 2002). Evaluating how egg cortisol affects the phenotype of adult offspring will fill a current knowledge gap, but discerning the legacy effects of egg cortisol may be difficult due to inherent genetic effects and accumulating environmental input. Given that little information is available regarding the long-term effects of offspring exposure to elevated egg cortisol, it is perhaps not surprising that limited research has been conducted linking these effects to variation in adult fitness (e.g., survival to sexual maturation, age at reproduction, and gamete quality).

7. Conclusion

Glucocorticoids in the eggs of oviparous animals such as fish are not physiologically static entities; levels vary within and across mothers as well as across species. While the environmental and ecological pressures driving variation in GC concentrations remain elusive, this variation exerts diverse effects on biological functions of developing offspring, which have the potential to interact with environmental variation to contextually impact offspring fitness. An individual egg is but a fraction of a fish's mass, yet the contents of these globular morsels are the building blocks progeny are given to develop, grow, and survive to independence. To fully appreciate the phenotypic effects of egg GCs on the offspring of fishes, there are a number of theoretical and experimental factors to encompass into future scientific inquiry. An integrative approach to examining how egg GCs affect offspring phenotype can yield valuable insights into hormonally mediated intergenerational processes and population fitness.

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