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Interaction of egg cortisol and offspring experience influences stress-related behaviour and physiology in lake sturgeon

Lydia Wassink [a, *], Belinda Huerta [b], Weiming Li [b], Kim Scribner [a, b]

[a] Department of Integrative Biology, Michigan State University, East Lansing, MI, U.S.A.
[b] Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, U.S.A.

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Quantifying transgenerational effects of stress is important to predict outcomes of anthropogenic disturbances for wildlife species. Maternal stress can programme physiological and behavioural phenotypes in offspring, which may be maladaptive if maternal and offspring environments are mismatched. We investigated effects of a match and mismatch between egg cortisol and offspring stress levels in lake sturgeon, *Acipenser fulvescens*, using artificially elevated egg cortisol levels (simulating maternal stress) and a chronic unpredictable stress regime for offspring after hatch. Offspring cortisol levels were quantified at baseline and after an acute stressor. Multiple measures of offspring swimming activity were assessed in behaviour trials. Individuals that experienced elevated egg cortisol and high offspring stress exhibited a diminished cortisol response to an acute stressor, but responses varied among offspring from different families. Results suggest that the interaction between maternal and offspring experience may cue an offspring phenotype that is adaptive in high-stress conditions. Principal components analysis characterizing interindividual variation in offspring behavioural variables showed that treatment significantly affected multivariate offspring response along the PC1 axis (associated with inactivity), and both treatment and family significantly affected response along the PC2 axis (associated with shorter distance moved). The largest differences for PC1 occurred between the 'mismatch' treatments (high egg cortisol and low offspring stress exhibiting lower activity; low egg cortisol and high offspring stress exhibiting higher activity), indicating that the combination of egg cortisol and offspring stress is more important in determining offspring behaviour than is egg cortisol or offspring stress alone. Findings suggest that family effects, such as genetic components or maternal experience, may mediate how the interaction of maternal and offspring stress influences offspring physiological and behavioural outcomes, and indicate the need for further research into environmental factors experienced by females that influence how offspring respond to egg cortisol and early life stress.

Environmental disturbances can induce chronic stress for wildlife (Clinchy, Zanette, Boonstra, Wingfield, & Smith, 2004), and have been quantified by increases in cortisol levels (Baker et al., 2013; Wingfield et al., 1997). In a variety of wildlife species, cortisol levels have been shown to increase in response to increased human activity (Creel et al., 2002; Thiel, Jenni-Eiermann, Braunisch, Palme, & Jenni, 2008; Wasser, Bevis, King, & Hanson, 1997), pollution (Hopkins, Mendonça, & Congdon, 1997) and interannual climate variation (Bechshøft et al., 2013). Quantifying fitness effects of physiological changes induced by environmental stressors is essential for predicting effects of climate change and other anthropogenic disturbances (Wikelski & Cooke, 2006).

Environmental stressors that affect individuals' cortisol levels can also impact subsequent generations via maternal effects. Exposure to maternal cortisol can epigenetically reprogramme developing offspring and alter phenotypic trajectories, especially traits related to physiological and behavioural stress reactivity (Brunton & Russell, 2010; Champagne & Meaney, 2006; Clarke & Schneider, 1993; Ho & Burggren, 2010; Weinstock, 2005). In Atlantic salmon, *Salmo salar*, artificially elevated maternal cortisol was associated with reduced offspring swimming activity in a novel environment 4 months after hatch (Espmark, 2008). Free-living European starlings, *Sturnus vulgaris*, experimentally subjected to chronic stress had offspring that exhibited increased physiological reactivity to an acute stressor, indicated by higher corticosteroid levels (Cyr & Romero, 2007).

Behavioural and physiological alterations induced by maternal stress may not necessarily be maladaptive, but in some cases may

* Correspondence: L. Wassink, Department of Fisheries and Wildlife, Michigan State University, 480 Wilson Road, East Lansing, MI, 48824-1222, U.S.A.
E-mail address: wassink3@msu.edu (L. Wassink).

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prepare offspring to function and survive in high-stress conditions (Gagliano & McCormick, 2009; Sheriff & Love, 2013). For example, in the tropical damselfish *Pomacentrus amboinensis*, high-density stress causes females to have offspring with reduced body sizes (but does not affect offspring yolk size); the smaller body:yolk ratio increases available energy reserves for offspring, enabling them to disperse farther from the high-density area (Gagliano, McCormick, & Meekan, 2007; McCormick, 2006). By preparing offspring to respond to a high-stress environment, maternal effects provide a mechanism for transgenerational phenotypic plasticity (Mousseau & Fox, 1998). However, for stress-induced offspring phenotypes to be adaptive, maternal stress must accurately predict the stress level of future environments. If the environment changes rapidly or unpredictably, resulting in a mismatch between stress-related maternal effects and stress levels actually experienced by offspring, offspring phenotypes may be maladaptive. Therefore, transgenerational stress effects occurring in the context of rapid environmental change creates the potential for ecological and evolutionary traps (Schlaepfer, Runge, & Sherman, 2002), which are especially important to consider for conservation and management of threatened wildlife (Robertson, Rehage, & Sih, 2013).

Since females of oviparous species lack an in utero stage during which offspring may be directly exposed to maternal cortisol, transgenerational effects of stress instead occur via egg provisioning. Egg provisioning, the supply of eggs during oogenesis with hormones, lipids, vitamins, mRNAs, proteins and other substances, is an important means by which females transmit information about the environment to offspring, especially for oviparous species that provide no postovulatory parental care (Berg et al., 2001; Nesan & Vijayan, 2013). For example, stressed female cod (*Gadus morhua*) have higher cortisol levels and deposit higher levels of cortisol into developing eggs, resulting in elevated egg cortisol postspawning (Kleppe et al., 2013). Female sticklebacks stressed by predation deposited more cortisol into eggs, and offspring showed an increase in antipredator shoaling behaviour (Giesing, Suski, Warner, & Bell, 2010). Compared to in utero exposure to maternal stress, egg provisioning involves a longer temporal gap between the female's experience of the stressor and the development of the embryo, resulting in more opportunity for mismatch between maternal and offspring environments.

Lake sturgeon, *Acipenser fulvescens*, are an ancient chondrostean fish species that is regionally threatened and a priority for conservation in the Great Lakes basin. Egg provisioning occurs far in advance of spawning (Doroshov, Moberg, & Van Eenennaam, 1997), and thus stressors influencing maternal deposition of cortisol into egg yolk may not accurately predict the stress level of environments experienced by offspring after hatch. Lake sturgeon populations have been bottlenecked through historic overexploitation and habitat disturbance (Ferguson & Duckworth, 1997), and will likely continue to be threatened by environmental stressors associated with climate change (Comte, Buisson, Daufresne, & Grenouillet, 2013; Hayhoe, VanDorn, Croley, II Schlegal, & Wuebbles, 2010). Since lake sturgeon take approximately 20 years to reach sexual maturity, the ability of populations to respond genetically to environmental changes is limited, making them vulnerable to rapidly changing environmental conditions. Therefore, it is important to understand whether transgenerational plasticity mediated by maternal effects plays a role in determining survival and population viability. Maternal effects on the behaviour of lake sturgeon offspring may be especially important in the context of antipredator behaviours, which have been shown to affect survival and recruitment in this species (McAdam, 2011), as well as other fishes (Dudley & Matter, 2000; Silbernagel & Sorensen, 2013). During the larval stage, lake sturgeon are particularly vulnerable to predation, which contributes largely to high mortality rates during the first year of life (Waraniak, Baker, & Scribner, 2018). Therefore, alterations in offspring behaviour induced by maternally provisioned egg cortisol can potentially influence lake sturgeon larval survival and have downstream population-level effects.

Understanding effects of maternal stress requires assessing survival outcomes within ecologically relevant contexts (Sheriff & Love, 2013; Sopinka et al., 2014), an important component of which is match or mismatch between maternal and offspring environments (Sheriff et al., 2017). In fishes, maternal stress can be simulated by incubating eggs in a cortisol solution to elevate egg cortisol levels (Sopinka et al., 2015, 2017). Offspring stress levels can be manipulated using a chronic unpredictable stress regime (Lankford, Adams, Miller, & Cech, 2005; Piato et al., 2011). By combining these techniques, this study creates treatments that pair high egg cortisol with high offspring stress, low egg cortisol with low offspring stress, high egg cortisol with low offspring stress and low egg cortisol with high offspring stress (Fig. 1). In this study, we hypothesize that specific combinations of egg cortisol exposure and early life stress exposure, rather than egg cortisol alone or early life stress alone, will determine offspring stress axis function, which can be quantified by assessing physiological and behavioural reactivity (Weinstock, 2005). We specifically predicted that offspring that experience a mismatch between egg cortisol exposure and offspring stress would have increased physiological and behavioural reactions to stress (higher rises in cortisol levels post-stress and higher activity levels, respectively) compared to those that experience a match. Stress reactivity determines how individuals respond to threats such as predation risk (Vitousek, Jenkins, & Safran, 2014), and thus is important for predicting survival outcomes of transgenerational stress.

METHODS

Sturgeon eggs used in the experiment were collected from two female lake sturgeon spawning in the Upper Black River in Onaway, Michigan, U.S.A. Female 1 was captured on 29 April 2017, and Female 2 was captured on 20 May 2017. Stress was minimized for adult sturgeon during capture by ensuring that each individual's head and gills remained underwater during handling, extruding gametes noninvasively by applying gentle pressure to the abdomen. Each individual was handled for an average of around 5 min before release. Eggs were fertilized using a 1:1 female:male ratio, and sperm was obtained the same day as egg collection for each of the two families (Bauman, Woodward, Baker, Marsh, & Scribner, 2016; Crossman et al., 2011). PIT (passive integrated transponder) tags and RFID (radiofrequency identification) tags were used for identification in order to confirm that each of the females and males from which gametes were collected were unique individuals. There were six replicates per family in each of the four treatments (Fig. 1). Each replicate contained 11 ml of fertilized eggs (approximately 572 eggs), 4 ml (approximately 208 eggs) of which were used for samples within the first 24 h of development, leaving each replicate with 7 ml of eggs (approximately 364 eggs).

The four maternal/offspring treatments were high maternal stress and high offspring stress (S/S), high maternal stress and low offspring stress (S/C), low maternal stress and high offspring stress (C/S), and low maternal stress and low offspring stress (C/C). 'S' in the treatment name designates stress, 'C' designates control (or low stress) (Fig. 1). To simulate high maternal stress, eggs in S/S and S/C treatments were incubated in a cortisol solution made by dissolving cortisol (H4001, Sigma) in 95% ethanol and adding to 400 ml of water for a final cortisol concentration of 600 ng/ml (Sopinka et al., 2015). Eggs in the C/S and C/C treatments were incubated in a control solution, made using the same amount of ethanol and water

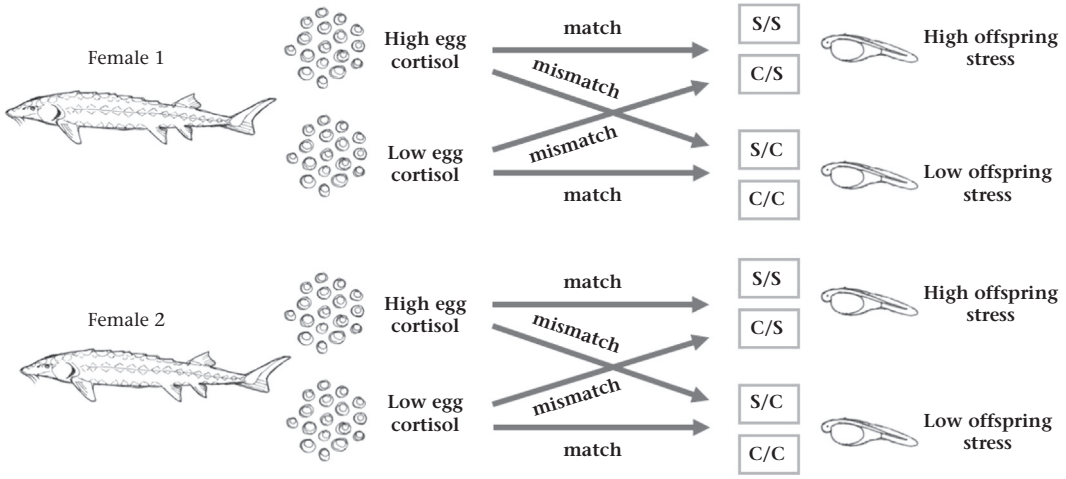

Figure 1. Experimental design. The four treatments were high egg cortisol (simulating high maternal stress) and high offspring stress (S/S), high egg cortisol and low offspring stress (S/C), low egg cortisol and high offspring stress (C/S), and low egg cortisol and low offspring stress (C/C). 'S' in the treatment name designates stress, 'C' designates control (or low stress). C/C and S/S treatments indicate a match between egg cortisol and offspring environment, and C/S and S/C treatments indicate a mismatch. Two families were included in the experiment, with eggs from each female divided into the four treatments as depicted.

but without cortisol. All eggs were incubated in cortisol or control solutions for 1 h immediately following fertilization. High offspring stress was later created after hatch by applying an unpredictable chronic stress regime (described below), and low offspring stress was created by withholding the unpredictable chronic stress regime.

Concentration of cortisol solution (600 ng/ml) was selected based on typical ranges chosen for similar studies in which fish eggs are incubated in cortisol solutions to elevate egg cortisol levels (Auperin & Geslin, 2008; Sopinka et al., 2017). Data on cortisol concentrations in unfertilized eggs of stressed sturgeon is lacking, and therefore we cannot compare cortisol levels in experimental eggs with naturally occurring ranges. However, mean cortisol levels in eggs immediately after incubation in cortisol solution were within the range of blood cortisol of stressed adult sturgeon (Baker, Peake, & Kieffer, 2008; Bayunova, Barannikova, & Semenkova, 2002), suggesting that egg cortisol in this experiment was not elevated beyond ecologically relevance. Furthermore, in nature, sturgeon eggs would be exposed to elevated maternal cortisol while in the ovaries prior to spawning and fertilization, whereas in this experiment cortisol treatment was applied to eggs immediately after fertilization, since sturgeon eggs are activated by water (hydrolysis and opening of micropyles allowing sperm to enter the egg). Water absorption during the water-hardening process, in which fish eggs absorb water after fertilization until hardening of the chorion membrane, allows uptake of cortisol from solution (Bouchard & Aloisi, 2002; Dettlaff, Ginsberg, & Schmalhausen, 1982; Zotin, 1958). Therefore, our experimental elevation of cortisol in fertilized sturgeon eggs is an artificial manipulation meant to approximate the natural condition, in which eggs are exposed to maternal cortisol well in advance of fertilization.

After fertilization and incubation in the cortisol or control solutions, eggs were rinsed thoroughly to remove any residual solution and placed in Heath trays (Heath Tecna-Plastics, Kent, WA, U.S.A.) in a recirculating tank supplied with filtered stream water from the Upper Black River. Prior to the experiment, the tank was thoroughly disinfected using dilute citric acid and betadyn solutions and rinsed. Any remaining biologically active agents recirculating through the tank system would have been in contact with eggs and offspring of all treatments and families and thus would not have affected experiment results. In addition, completion of the water-hardening process after fertilization would prevent further uptake of any residual cortisol or other chemicals (Bouchard & Aloisi, 2002; Dettlaff et al., 1982; Zotin, 1958). The tank was temperature controlled to remain at 13 ± 1 °C using a heater (SmartOne Heater, 1000 W, Model S1T1111, Process Technology, Willoughby, OH, U.S.A.) and chiller (½ HP C-0500 Aquarium Chiller, 1700 W, Pacific Coast Imports, Transworld Aquatic Enterprises, Inglewood, CA, U.S.A.). Mortalities were removed daily.

Offspring stress treatment (high stress for treatments C/S and S/S, low stress for treatments C/C and S/C) was implemented during the free embryo stage, starting 2 days after hatch and continuing until the larval stage at the onset of exogenous feeding. Free embryos in the S/S and C/S treatments were subjected to an unpredictable chronic stress regime (Lankford et al., 2005), while free embryos in the S/C and C/C treatments were left unstressed. The unpredictable chronic stress regime, which is designed to produce ongoing stress without habituation, consisted of a set of three stressors, two of which were randomly applied daily. Stressor 1 was exposure to light for 2 min using a flashlight, since sturgeon free embryos are negatively phototaxic (Richmond & Kynard, 1995, pp. 172–182). Stressor 2 was a thump on the table surface, produced by dropping a weight of 212 g onto the table from a height of 22 cm, repeated twice. The thump caused a visible startle response, indicating that it induced stress for free embryos (Davis, 2010). Stressor 3 was exposure to low water level for 2 min, during which water was drained to a depth of 2.54 cm. Low water level temporarily increases free embryo density, which has been shown to stress lake sturgeon free embryos (Bauman, Baker, Marsh, & Scribner, 2015). Daily stressors were selected using a random number generator and applied at 1100 h and 1400 h. Applying randomly selected stressors twice daily has been shown to cause chronic stress in sturgeon (Lankford et al., 2005).

Care was taken to promote animal welfare by minimizing incidental stress unrelated to the set of three experimental stressors. Starting at the egg stage, individuals were housed in 4-inch (10.16 cm) diameter couplings made of PVC plastic and mesh that ensured adequate water flow-through. Eggs were treated with 500 µl/ml peroxide every 2 days during incubation following standard hatchery protocols to prevent fungal infection, and any mortalities were removed. After hatch, shells were removed from couplings to avoid impediment of water flow-through, and 2.54 cm^3 BioBalls (Pentair, No. CBBI-5, Pentair Aquatic Eco-Systems, Cary, NC, U.S.A.) were added to each coupling to simulate substrate

for burrowing free embryos. All protocols were conducted according to approved Michigan State University Animal Use and Care guidelines under Animal Use and Care project 04/17-071-00.

Body Size and Yolk Sac Area

To observe effects of treatment on offspring growth, we obtained body size (mm) and yolk sac area (mm^2) measurements at hatch and body size measurements at the larval stage using ImageJ software (National Institutes of Health, Bethesda, MD, U.S.A., http://rsbweb.nih.gov/ij/). Photos used for ImageJ analysis were taken with a digital camera and included six individuals per replicate, as well as a ruler for size calibration. Individuals used for measurements were sedated with 25 mg/litre of MS 222 using approved Michigan State University Animal Use and Care protocols and then removed from the experiment.

Cortisol Levels

For each of the two females, we preserved three replicates of unfertilized egg samples, each sample containing 1 ml or approximately 50 eggs, in liquid nitrogen for cortisol analysis to characterize baseline levels of cortisol provisioned in eggs before experimental treatment to elevate egg cortisol. After fertilization and incubation in the cortisol solution (for cortisol-treated eggs) or control solution, samples were taken at 0 h, 2 h, 4 h and 24 h postfertilization. All samples taken after fertilization contained 1 ml of eggs (approximately 52 eggs) taken from each replicate in the experiment (6 replicates per treatment per female). Larval cortisol samples were taken at the onset of exogenous feeding from each replicate (approximately 12 days posthatch), and included six individuals per sample. Baseline cortisol samples of larvae were preserved immediately after larvae were removed from the tank and euthanized, in order to capture cortisol levels without application of acute stressor. Post-stress cortisol samples of larvae were preserved 30 min after individuals were exposed to an acute stressor and then euthanized, in order to capture physiological response to acute stress. The acute stressor used for post-stress cortisol samples consisted of a thump on the table surface (produced by dropping a 212 g weight from height of 22 cm), which induces a startle response. All euthanasia was conducted using an overdose of MS-222 (>250 mg/litre), which acts quickly enough (<1 min) to avoid causing a cortisol increase in response.

All egg and larval samples to be used for cortisol analysis were preserved in liquid nitrogen and stored at −80 °C until analysis. Prior to cortisol extraction, samples were thawed and excess liquid was removed. All samples, containing either whole eggs or larvae, were homogenized using 600 µl of ethyl acetate as a solvent. The organic layer was extracted and evaporated before being reconstituted in methanol and stored at −80 °C until analysis. Cortisol levels of samples were determined using liquid chromatography tandem mass spectrometry using a Waters Xevo TQ-S mass spectrometer (Waters, Milford, MA, U.S.A.) (Bussy, Wassink, Scribner, & Li, 2017).

Larval Behaviour

The effect of treatment on larval behaviour was investigated by observing larval swimming activity during a 5 min trial that began by administering a startle cue. Six larvae from each replicate were placed in a 6-inch (15.24 cm) diameter Petri dish filled with water from the tank system and allowed to acclimate for 2 min. After acclimation, a 5 min video was recorded. The weight was dropped at 2 min to characterize behaviour prior to and following the startle cue.

Loligo v.4.0 tracking software (Loligo Systems, Viborg, Denmark; https://www.loligosystems.com/software) was used to simultaneously track activity of the six individuals in each video. A centre zone was defined that excluded a 1-inch (2.54 cm) perimeter around the Petri dish edge to determine whether edge-seeking behaviour varied among larvae from different treatments. Variables quantified from video analysis included velocity (cm/s), acceleration (cm/s^2), percentage of time active, total distance travelled (cm), number of visits to centre zone and time spent in centre zone (s), following Sakamoto, Dew, Hecnar, and Pyle (2016).

Statistical Analysis

We assessed normality for each data set using a Shapiro–Wilk test in R v.3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). The cortisol data set was not normally distributed and was log-transformed prior to analysis. Generalized linear models were fitted to quantify factors associated with body size, yolk sac area and cortisol data sets using the 'glm' function in R v.3.2.2. Models with delta AIC < 2 were considered competitive for the top model (Burnham & Anderson, 1998). If multiple models were competitive, we took a maximization of parsimony approach and chose the model with the fewest variables. For yolk area and size data sets, candidate models included main effects of treatment and family, as well as the interaction of treatment and family to assess whether cortisol treatment had a family-specific effect. For the fertilized egg cortisol data set, candidate models included main effects of treatment, family and stage, as well as the interaction of treatment and family (to assess whether treatment had a family-specific effect on egg cortisol), the interaction of stage and treatment (to assess whether cortisol content of eggs depended on developmental stage), and the interaction of family and stage (to assess whether families had differing rates of cortisol absorption or efflux from eggs). Candidate models for the larval cortisol data set included the main effects of treatment, family and stress state (whether larvae were euthanized at baseline stress level or after an acute stressor), as well as the interaction of treatment and family (to assess whether treatment had a family-specific effect on larval cortisol), the interaction of family and stress state (to assess whether family influenced physiological response to an acute stressor), and the interaction of treatment and stress state (to assess whether treatment altered the physiological response to an acute stressor). For each AICc selected model (Cavanaugh, 1997), we used ANOVA to run *F* tests on the model output and determine which variables were significant. Post hoc Tukey HSD tests were conducted for significant variables.

We used principal components analysis (Hotelling, 1933) to examine behavioural variables in order to reduce dimensionality of the data set by compressing dependent variables (percentage of time active, acceleration, velocity, distance travelled, zone time and zone visits) into a composite behavioural measure (Ballew et al., 2017). The broken stick method was used to determine that PC1 and PC2 were significant. We selected generalized linear models for PC1 and PC2 using AICc model selection, and used ANOVA to run *F* tests on the model output and determine which variables were significant. Factor loadings above 0.5 were examined to characterize behavioural relevance of each principal component.

RESULTS

Body Size and Yolk Sac Area

Since only cortisol or control incubation had been applied prior to hatch, and the stress regime was not implemented until after hatch, we included only egg treatment (S for cortisol-treated, C for

control) during model selection for hatchling yolk sac area and body size data sets. The top two AICc-selected models for yolk sac area at hatch were competitive, so we chose the most parsimonious model, which included family as the only factor (delta AICc = 1.28) (Table 1). ANOVA indicated that free embryos from Family 1 had a significantly larger yolk sac area (mean ± SD: C treatment: 6.97 ± 0.90 mm^2; S treatment: 7.28 ± 0.90 mm^2) than did free embryos from Family 2 (C treatment: 6.95 ± 0.75 mm^2; S treatment: 6.75 ± 1.17 mm^2) ($P = 0.01283$; Fig. 2).

The AICc-selected model for body size at hatch included family only (Table 2). ANOVA indicated that free embryos from Family 2 were significantly larger (12.46 ± 0.31 mm) than free embryos from Family 1 (11.79 ± 0.26 mm) ($P < 0.0001$; Fig. 2).

At the larval stage, the AICc-selected model for body size included family only (Table 2). ANOVA indicated that larvae from Family 1 were significantly larger (21.78 ± 0.48 mm) than larvae from Family 2 (21.25 ± 0.50 mm) ($P = 0.0006$; Fig. 3).

Cortisol Levels

For unfertilized eggs (prior to incubation in cortisol solution), mean egg cortisol was much higher in eggs from Female 2 (mean ± SD = 8.27 ± 0.21 ng/g) than for eggs from Female 1 (mean ± SD = 4.77 ± 0.23 ng/g).

Since only cortisol or control incubation had been applied at the fertilized egg stage, and the stress regime was not implemented until after hatch, only egg treatment (S for cortisol-treated, C for control) was included during model selection for the fertilized egg cortisol data set. The AICc-selected model for fertilized egg cortisol included egg development stage, egg treatment, family, the interaction of egg treatment and family, the interaction of egg development stage and egg treatment, and the interaction of egg development stage and family (Table 3). ANOVA indicated that stage had a significant effect ($P < 0.0001$; Figs. 4, 5). ANOVA also indicated that eggs in the S treatment had significantly higher cortisol (89.58 ± 165.19 ng/g) than eggs in the C treatment (3.64 ± 5.17 ng/g) ($P < 0.0001$). The interaction of treatment and family had a significant effect ($P = 0.0003$), with eggs from Family 1 having higher mean cortisol than eggs from Family 2 in the C treatment but lower mean cortisol in the S treatment. The interaction of stage and egg treatment had a significant effect ($P < 0.0001$), with S treatment eggs showing a sudden increase in cortisol that then decreased by 24 h after fertilization to levels comparable to those of C treated eggs. The interaction of stage and family had a significant effect, with eggs from Family 1 having lower cortisol than eggs from Family 2 at 0 h and 2 h after fertilization but higher cortisol 4 h and 24 h after fertilization ($P < 0.0001$; Fig. 4).

For larval cortisol, the AICc-selected model included treatment, family, stress state (whether larvae were euthanized at baseline stress level or after an acute stressor), the interaction of treatment and family, and the interaction of treatment and stress state (Table 3). ANOVA indicated that treatment had a significant effect ($P = 0.0001$), family had a significant effect ($P < 0.0001$) and 'post-stress' state had significantly higher cortisol than 'baseline' stress state ($P < 0.0001$). The interaction of treatment and family had a significant effect ($P < 0.0001$), with larvae from Family 2 having higher cortisol than larvae in Family 1 for all treatments except S/S. The interaction of treatment and stress state had a significant effect ($P < 0.0001$), with larvae in the S/S treatment having a smaller post-stress increase in cortisol compared to other treatments. The interaction of family and stress state had a significant effect ($P < 0.0001$), with larvae from Family 2 having a higher post-stress cortisol increase across treatments than larvae from Family 1. Tukey HSD conducted for larvae from Family 1 indicated that in all treatments post-stress cortisol levels were significantly higher (5.33 ± 0.84 ng/g) than baseline (3.90 ± 0.69 ng/g). Tukey HSD conducted for larvae from Family 2 indicated that in all treatments post-stress cortisol levels were significantly higher (9.40 ± 2.99 ng/g) than baseline (4.36 ± 0.74 ng/g). There were no significant differences among families at baseline, but at post-stress, larvae from Family 2 had significantly higher cortisol than did larvae from Family 1, except in the S/S treatment (Fig. 6).

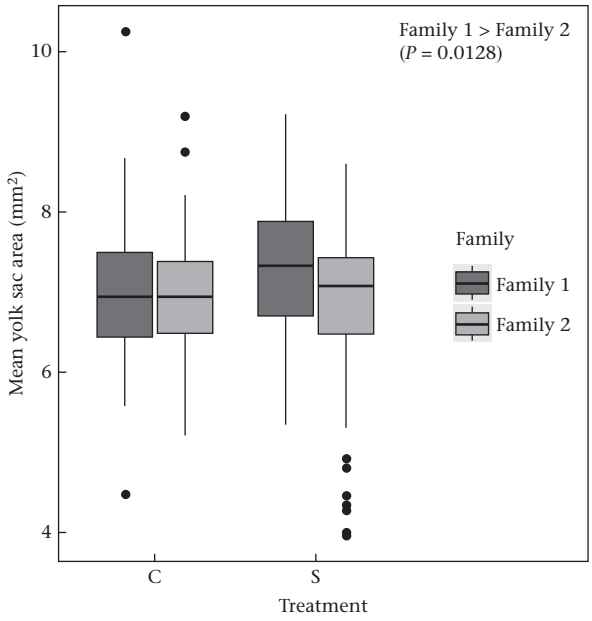

Figure 2. Offspring yolk sac area at hatch for each treatment and family. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5× the interquartile range for the upper and lower quartiles. C: eggs incubated in control solution to simulate low maternal stress; S: eggs incubated in cortisol solution to simulate high maternal stress. Eggs were incubated in their respective solutions for 1 h immediately after fertilization.

Larval Behaviour

Behavioural variables (percentage of time active, velocity, acceleration, distance travelled, zone time and zone visits) were reduced into two components using PCA. Factor loadings indicated

Table 1
Models for yolk sac area at hatch

Model	AICc	Delta AICc	Akaike weight
Area ~ family + treatment 1 + family*treatment1	787.86	0	0.55
Area ~ family	789.14	1.28	0.29
Area ~ family + treatment 1	790.98	3.13	0.11
(Null model) Area ~ 1	793.27	5.42	0.04
Area ~ treatment 1	795.11	7.25	0.01

Since the top two models were competitive with a delta AICc < 2, the model containing family only was chosen as the most parsimonious model. Akaike weight indicates conditional probability of each model (Wagenmakers & Farrell, 2004).

Table 2
Models for body size at hatch and larval stage

Model	AICc	Delta AICc	Akaike weight
Hatch			
Size ~ family	21.92	0	0.95
Size ~ family + treatment	27.69	5.78	0.05
Size ~ family + treatment + family*treatment	36.14	14.22	0
(Null model) Size ~ 1	61.51	39.59	0
Size ~ Treatment	67.95	46.04	0
Larvae			
Size ~ family	72.25	0	0.95
Size ~ family + treatment	78.55	6.3	0.04
Size ~ family + treatment + family*treatment	81.38	9.13	0.01
(Null model) Size ~ 1	82.46		0.01
Size ~ treatment	88.7	16.45	0

Akaike weight indicates conditional probability of each model (Wagenmakers & Farrell, 2004).

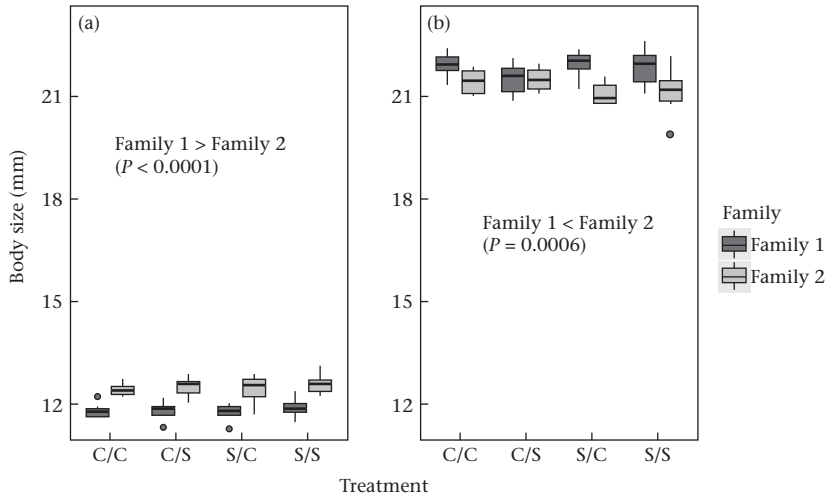

Figure 3. Body size (a) at hatch and (b) during the larval stage for each treatment and family. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5× the interquartile range for the upper and lower quartiles. Abbreviations as in Fig. 1.

Table 3
Models for cortisol at fertilized egg stage and larval stage, including the top three models and the null model

Model	AICc	Delta AICc	Akaike weight
Fertilized egg stage			
Cortisol ~ stage + treatment 1 + family + treatment1*family + stage*treatment1 + stage*family	−257.84	0	1
Cortisol ~ stage + treatment 1 + family + treatment1*family + stage*treatment1	−36.02	221.82	0
Cortisol ~ stage + treatment 1	183.39	441.23	0
(Null model) Cortisol ~ 1	449.19	707.03	0
Larval stage			
Cortisol ~ treatment + family + stress state + treatment*family + treatment*stress state + family*stress state	−215.14	0	1
Cortisol ~ treatment + family + stress state + treatment*family + treatment*stress state	−182.31	32.84	0
Cortisol ~ treatment + family + stress state + treatment*family	−170.65	44.49	0
(Null model) Cortisol ~ 1	−62.25	152.89	0

Akaike weight indicates conditional probability of each model (Wagenmakers & Farrell, 2004).

that the most important variable contributing to variation along PC1 was the percentage of time active, and the most important variable contributing variation along PC2 was distance travelled (Table 4). PC1 was negatively associated with the percentage of time active and therefore is interpreted as a measure of inactivity; PC2 was negatively associated with total distance travelled (cm) and therefore is interpreted as a measure of reduced movement during trials. PC1 explained 57.3% of the variation in the data and PC2 explained 21.1% of the variation in the data (Fig. 6). Model selection was conducted using PC1 and PC2 in generalized linear models. For PC1, the AICc-selected model included treatment, family, and the interaction of treatment and family (Table 5). For PC2, the AICc-selected model included treatment, family, and the interaction of treatment and family (Table 5).

ANOVA for PC1 indicated that treatment was significant ($P = 0.016$) and that there was no significant difference between families ($P = 0.7318$). The interaction of treatment and family was significant ($P = 0.0002$), with larvae from Family 1 having lower mean PC1 scores (higher activity levels) than larvae from Family 2 in all treatments except C/C. Tukey HSD indicated that the mean PC1 score for treatment S/C (mean PC1 score = 0.40) was significantly higher than that for treatment C/S (mean PC1 score = −0.53). Since PC1 was negatively associated with the percentage of time active, results indicate higher activity levels for

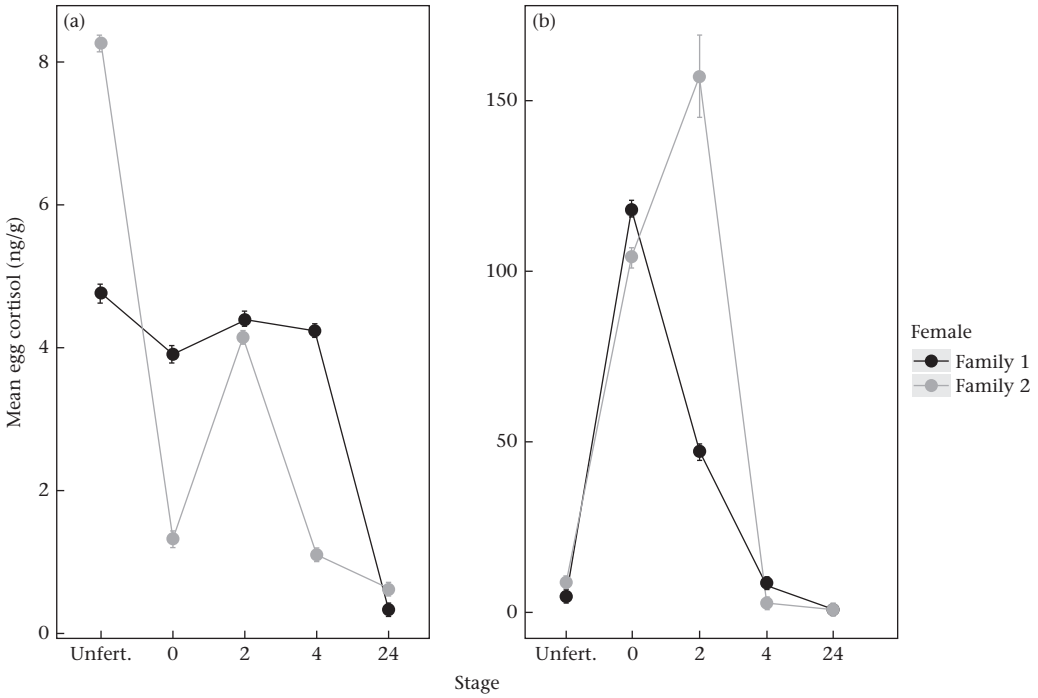

Figure 4. Cortisol levels in fertilized (a) control treatment eggs not incubated in cortisol solution and (b) stress treatment eggs incubated in cortisol solution to simulate maternal stress for each family. Egg incubation treatment started immediately after fertilization and lasted 1 h. Samples of fertilized eggs were taken immediately after the incubation treatment (0 h) and 2 h, 4 h and 24 h postfertilization. Error bars show 1 SE.

individuals from treatment C/S than for individuals from treatment S/C. There were no other significant pairwise differences between treatments (mean for C/C = 0.16, mean for S/S = −0.02).

An ANOVA conducted for PC2 indicated that treatment was significant ($P < 0.0001$) and that family was significant ($P < 0.0001$). The interaction of treatment and family was significant ($P < 0.0001$), with larvae from Family 1 having lower PC2 scores (greater distance moved) than larvae from Family 1 in all treatments except S/C. Tukey HSD indicated that for PC2, treatment C/C (mean PC2 score = −0.42) was significantly lower than treatments C/S (0.21) and S/S (mean = 0.27), and that Family 1 (mean = −0.46) was significantly lower than Family 2 (mean PC2 score = 0.47). Since PC2 was negatively associated with total distance travelled, results indicate that individuals from treatment C/C

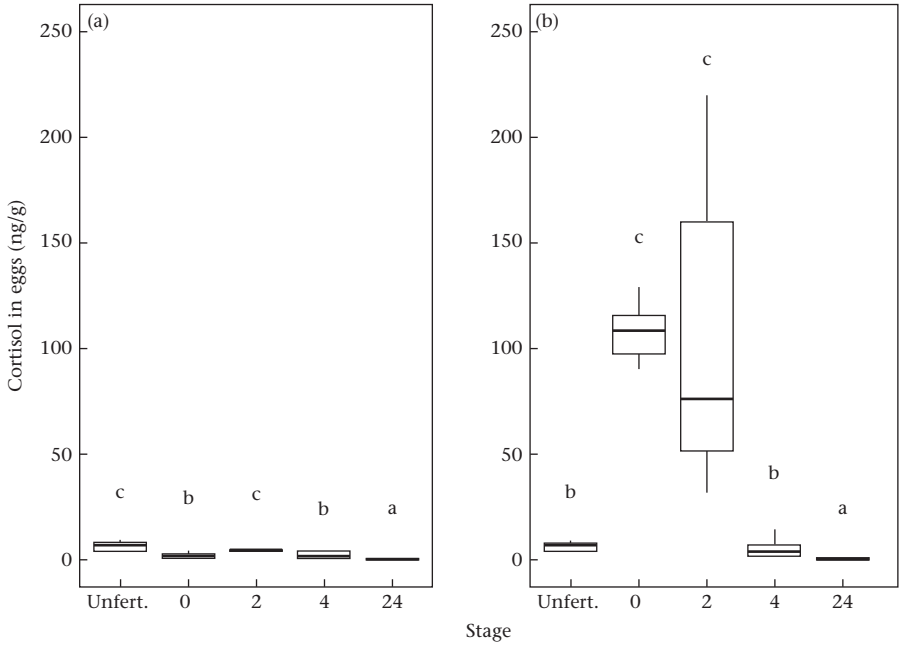

Figure 5. Cortisol levels in fertilized (a) control treatment eggs not incubated in cortisol solution and (b) stress treatment eggs incubated in cortisol solution to simulate maternal stress for both families combined. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5× the interquartile range for the upper and lower quartiles. Samples of fertilized eggs were taken immediately after the incubation treatment (0 h) and 2 h, 4 h and 24 h postfertilization. Means for each stage are averaged across family. Letters indicate results of Tukey HSD test showing the interaction of treatment and stage ($P < 0.0001$).

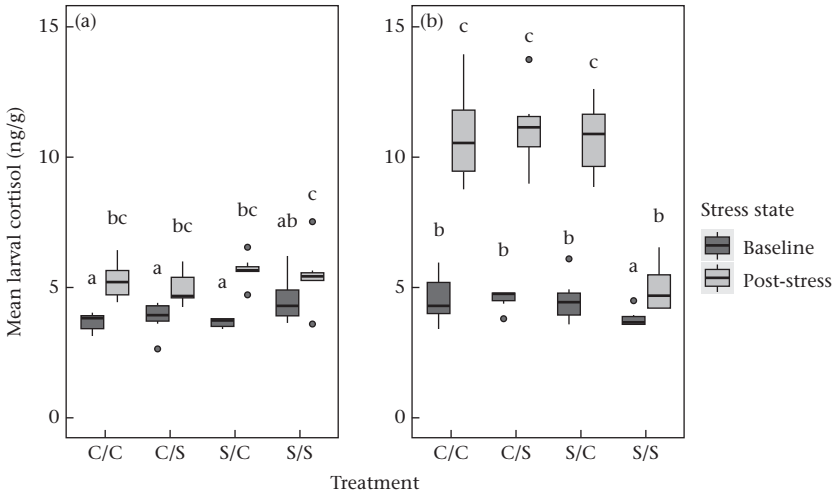

Figure 6. Cortisol levels in larvae when no acute stressor was applied (baseline) and 30 min after exposure to an acute stressor (post-stress) for (a) Family 1 and (b) Family 2. Treatment C/C (control-control) is low maternal stress, low offspring stress; treatment C/S (control/stress) is low maternal stress, high offspring stress; treatment S/C (stress/control) is high maternal stress, low offspring stress; treatment S/S (stress/stress) is high maternal stress, high offspring stress. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5× the interquartile range for the upper and lower quartiles. Letters indicate results of Tukey HSD test showing the interaction of treatment and stress state ($P = 0.0001$).

Table 4
Factor loadings and eigenvalues for principal components analysis of behavioural variables

	Factor loadings						Eigenvalue	Variance (%)	Cumulative variance (%)
	Velocity	Acceleration	Zone time	% Time active	Distance travelled	Zone visits			
PC1	0.4499	0.4569	−0.2114	−0.5013	−0.3464	−0.4158	3.438	57.299	57.299
PC2	−0.47	−0.4418	0.0708	−0.0605	−0.6134	−0.446	1.266	21.107	78.405
PC3	0.1247	0.1377	0.8972	−0.1726	−0.2581	0.2531	1.017	16.952	95.358
PC4	−0.1586	−0.2137	−0.2957	−0.6329	−0.1643	0.6437	0.18	2.994	98.351
PC5	0.1338	0.2377	−0.2405	0.5578	−0.6378	0.387	0.094	1.57	99.922
PC6	−0.7198	0.6892	−0.0025	−0.0605	0.057	0.0053	0.005	0.0779	100

Table 5
Models for principal components

Model	AICc	Delta AICc	Akaike weight
PC1			
PC1 ~ treatment + family + treatment*family	1119.57	0	1
PC1 ~ treatment	1131.58	12.01	0
PC1 ~ treatment + family	1133.56	13.99	0
(Null model) PC1 ~ 1	1135.26	15.69	0
PC1 ~ family	1137.2	17.63	0
PC2			
PC2 ~ treatment + family + treatment*family	775.05	0	1
PC2 ~ treatment + family	793.29	18.23	0
PC2 ~ treatment	807.18	32.13	0
PC2 ~ family	846.89	71.83	0
(Null model) PC2 ~ 1	857.63	82.58	0

Akaike weight indicates conditional probability of each model (Wagenmakers & Farrell, 2004).

exhibited more movement during trials than individuals from treatments C/S and S/S, and that individuals from Family 1 moved greater distances during trials than did individuals from Family 2.

DISCUSSION

Stress treatment influenced offspring growth, physiology and behaviour, although results varied among offspring of different families. Individuals from Family 1 had lower growth during egg incubation but larger larval size, lower physiological reactivity to an acute stressor and small cortisol differences among treatments. Individuals from Family 2 had higher growth during egg incubation but smaller larval size and high physiological reactivity to an acute stressor for larvae from all treatments except S/S. Treatment also influenced behaviour, with larvae that experienced low egg cortisol and high stress showing higher activity levels during trials than larvae that experienced high egg cortisol and low offspring stress. Larvae that experienced a match between low egg cortisol and low offspring stress also moved greater distances during trials than larvae that experienced high offspring stress (regardless of egg cortisol exposure). Interpretation of results is limited because we included only two families in the experiment and parental experiences were unknown. Differences in initial unfertilized egg cortisol and downstream physiology and behaviour may be

explained by differing environments experienced by parents. For example, spawn timing has been shown to impact egg provisioning of cortisol (Sampath-Kumar, Byers, Munro, & Lam, 1995), and Female 1 and Female 2 were early and late spawners, respectively (Forsythe, Crossman, Bello, Baker, & Scribner, 2011). Similarly, parents of each family could have experienced a variety of differing environmental stressors that influenced egg provisioning and triggered other parental effects to drive the differences seen in offspring throughout development. A larger sample size is needed to adequately assess the interfamily variation and family-specific effects of transgenerational stress. Nevertheless, this study highlights the complexity of transgenerational stress and the importance of both egg cortisol and early life stress in determining offspring physiology and behaviour.

Initial differences in unfertilized egg cortisol prior to experimental treatment may also have influenced offspring growth, evidenced by differences in yolk sac area between families at hatch. In fishes, yolk sac area can be reduced by maternal stress (Erikson et al., 2006; McCormick, 1998) due to increased metabolic rates (Mccormick & Nechaev, 2002). For Family 2, individuals from cortisol-treated eggs had increased growth during embryonic development, possibly due to increased metabolic rates associated with faster yolk sac absorption, resulting in hatchlings with smaller remaining yolk sacs and larger body sizes. While offspring from both families hatched within the expected time span based on calculations of developmental stage using cumulative temperature units (CTU) (Kempinger, 1998; Smith & King, 2005), offspring from Family 1 hatched at a slightly earlier developmental stage. Family 1 hatched around CTU 54–62 and Family 2 hatched around CTU 65–76. Earlier hatch time confirms that increased metabolic rate most likely speeded growth for Family 1. By the larval stage, individuals from Family 1 had grown larger than individuals from Family 2 despite starting with smaller hatch sizes. Larger larval size has been shown to be advantageous in avoiding predation (Wassink, Bussy, Li, & Scribner, 2019; Wishingrad, Ferrari, & Chivers, 2014). Family was the only factor indicated as an important predictor of larval size based on AICc model selection, highlighting the importance of interfamily variation in developmental trajectories, which may have down-stream influences on predation rates.

In cortisol-treated eggs, cortisol initially absorbed from the solution decreased during the first 24 h after fertilization. In fishes, maternal cortisol decreases immediately after fertilization as maternal cortisol is ejected from the egg, and does not increase until developing embryos begin endogenous cortisol production (Sopinka et al., 2017). In this study, cortisol levels were unexpectedly higher at 2 h postfertilization, which may indicate individual variation in rates of cortisol efflux as samples represent separate eggs rather than repeated measures on the same individuals. Since water temperature was consistent during sampling, differences in cortisol efflux are more likely due to individual variation than to differences in developmental stages among sampled eggs. ATP-binding cassette (ABC) transporters facilitate the efflux of cortisol from the egg after fertilization, and may function to buffer offspring from effects of high maternal cortisol (Paitz, 2016). If higher unfertilized egg cortisol for Family 2 was due to higher maternal stress, other maternally mediated effects may have influenced uptake or efflux of cortisol during egg treatment. For example, maternal stress may influence egg provisioning of mRNAs and cause differential expression of ABC transporters in zygotes. Mommer (2013) suggested that maternal regulation of ABC transporter activity in order to mediate embryonic cortisol exposure is most likely dynamic and based on environmental influences encountered by females. The large differences observed between families in how treatment affected physiology and behaviour may therefore be due to maternal stress-related effects already at play in eggs prior to cortisol incubation treatment.

At the larval stage, treatment had a large impact on physiological response to an acute stressor, but only for one family. For Family 2, larvae in the S/S treatment had a greatly reduced cortisol response to an acute stressor, while larvae in the other three treatments had a significantly higher cortisol response to an acute stressor compared to larvae in Family 1. Individual variation in stress reactivity has been observed in many studies and most likely has a strong genetic basis (Koolhaas, De Boer, Coppens, & Buwalda, 2010). Interestingly, the S/S treatment represents a 'match' in which elevated egg cortisol accurately predicts a high-stress environment for offspring, but the other 'match' treatment (C/C) did not result in larvae showing the lower-reactivity phenotype. Since lower stress reactivity occurred for Family 2 only when elevated egg cortisol was combined with high offspring stress, it may indicate an interaction between maternal egg provisioning and offspring experience cuing an offspring phenotype that is adaptive in high-stress conditions. In prior research with lake sturgeon, larvae reared at a warm (high-stress) temperature did not show significant increases in baseline cortisol, but did show reduced cortisol responses to an acute stressor (Wassink et al., 2019). In storks (*Ciconia ciconia*), lower physiological stress reactivity predicted higher survival while baseline cortisol was not associated with survival, suggesting that lower stress reactivity may be an adaptive phenotype in some environmental contexts (Blas, Bortolotti, Tella, Baos, & Marchant, 2007). Future research on transgenerational stress should consider short-term advantages and long-term costs of phenotypes (Gagliano & McCormick, 2009) within ecologically relevant contexts (Sheriff & Love, 2013) in order to obtain a clearer picture of the adaptive value of different stress reactivity phenotypes.

Behavioural outcomes of different combinations of egg cortisol exposure and offspring stress are important for predicting survival and population-level consequences, especially if behaviours change predation rates. In this study, treatment affected behaviour primarily in the percentage of time active (negatively associated with PC1) and total distance moved during trials (negatively associated with PC2). For PC1, the largest difference was between the two 'mismatching' treatments (S/C and C/S), in which egg cortisol failed to match offspring environment. Individuals from treatment S/C had significantly higher scores for PC1 (indicating lower activity levels) than did individuals from C/S. Prior research has shown that lake sturgeon larvae reared at warmer (high-stress) temperatures are more active (Wassink et al., 2019), and stressed individuals of other fish species show increased swimming activity (Schreck, 1997). Higher activity levels exhibited by C/S larvae is therefore consistent with the higher level of early life stress they experienced. Interestingly, larvae in the S/S treatment did not have higher activity levels despite having experienced early life stress, suggesting that egg cortisol may play a role in mediating how stressors impact behavioural development.

In contrast, larvae that experienced high egg cortisol but low offspring stress (S/C treatment) exhibited lower activity levels compared to larvae from the C/S treatment. Increased maternal cortisol in salmon has been shown to decrease offspring swimming activity (Espmark, Eriksen, Salte, Braastad, & Bakken, 2008). Maternal stress and associated elevation in egg cortisol may therefore be related to a reduction in offspring activity levels. However, in this study the effect of egg cortisol on activity levels may have been mediated by early life experience, since larvae that experienced both elevated egg cortisol and high early life stress (S/S treatment) did not have lower activity.

For PC2, which was associated with total distance travelled during trials, larvae from the C/C treatment moved greater distances than larvae in either of the treatments that experienced high early life stress (C/S and S/S). Family also had a significant effect on PC2, as larvae from Family 1 moved greater distances during trials than

larvae from Family 2. While behavioural trials in petri dishes bears limited applicability to behaviour in the wild, a reduction in movement due to early life stress may influence larval drift, when larvae emerge from the substrate and disperse downstream (Smith & King, 2005). Past research in dispersal behaviour of larvae from this population documented large variability in the timing of dispersal by larvae from different females that spawned within the same day (Duong et al., 2011). Further research could explore whether early life stress and family-specific parental effects may reduce the rate at which larvae drift downstream, and whether this represents an adaptive behavioural response to high-stress conditions.

Overall, behaviour results indicate that the combination of maternal and offspring stress is more important in determining behaviour than is maternal stress alone or offspring stress alone. Higher activity levels in larval sturgeon have been associated with higher survival rates in the presence of a crayfish predator (Wassink et al., 2019), and therefore the combination of egg cortisol and early life environments experienced by sturgeon are probably important for larval survival. Additionally, behaviours that may influence dynamics of larval drift, such as the reduction in total distance moved, could also affect larval survival since larvae are particularly vulnerable to predation during the drift period (Waraniak et al., 2018). Therefore, rapid environmental changes resulting in a mismatch between maternal and offspring experiences has the potential to significantly alter offspring behaviour, which may ultimately have population-level consequences for recruitment by altering predation rates.

Further research on transgenerational stress should consider interfamily variation in how offspring respond to different combinations of maternal and offspring environments. Phenotypic variation, including stress reactivity, may play an important evolutionary and ecological role (Koolhaas et al., 2010). Interfamily differences in transgenerational stress effects may generate phenotypic variation and help populations escape evolutionary traps created by rapidly fluctuating environmental conditions. This would be especially important for threatened wildlife species such lake sturgeon, which have limited ability to respond genetically to environmental changes due to long generation times. Phenotypic variation induced by family-specific responses to maternal effects could therefore determine whether vulnerable wildlife species persist in the face of threats like climate change.

Declarations of Interest

None.

Acknowledgments

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