© 2016. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2016) 219, 2963 doi:10.1242/jeb.148452

CORRECTION

Correction: Programming of the hypothalamic-pituitary-interrenal axis by maternal social status in zebrafish (*Danio rerio*)

Jennifer D. Jeffrey and Kathleen Gilmour

There were two errors published in *J. Exp. Biol.* **219**, pp. 1734-1743.

In Table 1, the primers listed for the bottom two genes, ß-actin and ubiquitin, are identical. The ubiquitin primers are incorrect – they should be:

F: gag cct tct ctc cgt cag tta g

R: cgc agg ttg ttg gtg tgt c

The accession number listed in Table 1 for ubiquitin is correct but the reference provided, Alsop and Vijayan (2008), is incorrect and should be Sun et al. (2010).

Sun, Y., Liu, Z. and Zhang, S. (2010). Tissue distribution, developmental expression and up-regulation of p8 transcript on stress in zebrafish. *Fish Shellfish Immunol.* **28**, 549-554.

The authors apologise to the readers for any inconvenience this may have caused.

© 2016. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2016) 219, 1734-1743 doi:10.1242/jeb.138826

RESEARCH ARTICLE

Programming of the hypothalamic-pituitary–interrenal axis by maternal social status in zebrafish (*Danio rerio*)

Jennifer D. Jeffrey[*,‡] **and Kathleen M. Gilmour**

ABSTRACT

The present study examined the effects of maternal social status, with subordinate status being a chronic stressor, on development and activity of the stress axis in zebrafish embryos and larvae. Female zebrafish were confined in pairs for 48 h to establish dominant/subordinate hierarchies; their offspring were reared to 144 h post-fertilization (hpf) and sampled at five time points over development. No differences were detected in maternal cortisol contribution, which is thought to be an important programmer of offspring phenotype. However, once zebrafish offspring began to synthesize cortisol *de novo* (48 hpf), larvae of dominant females exhibited significantly lower baseline cortisol levels than offspring of subordinate females. These lower cortisol levels may reflect reduced hypothalamic-pituitary–interrenal (HPI) axis activity, because *corticotropin-releasing factor* (*crf*) and *cytochrome p450 side chain cleavage enzyme* (*p450scc*) mRNA levels also were lower in larvae from dominant females. Moreover, baseline mRNA levels of HPI axis genes continued to be affected by maternal social status beyond 48 hpf. At 144 hpf, stress-induced cortisol levels were significantly lower in offspring of subordinate females. These results suggest programming of stress axis function in zebrafish offspring by maternal social status, emphasizing the importance of maternal environment and experience on offspring stress axis activity.

KEY WORDS: Dominance hierarchy, HPI axis, Fish, Offspring, Cortisol, Stress

INTRODUCTION

Competition over resources that are limited in an environment, such as food, advantageous territory and access to mates, drives the formation of social hierarchies in a wide range of animal taxa, including teleost fish. The formation of social hierarchies has been well studied across a range of teleost species (Johnsson et al., 2006). Among juvenile teleost fish, such as juvenile salmonids, competition for food-rich territory, and thus monopolization of food resources, is high. Among adult teleosts, competition for reproductive opportunities and reproductive territories becomes more significant. The development of social hierarchies tends to lower aggression levels within groups and these relationships generally remain linear and stable, although reversals are possible (Abbott and Dill, 1985; Johnsson, 1997).

The social status acquired by an individual (i.e. dominant or high social status versus subordinate or low social status) can impact its physiological status. For example, within pairs of juvenile salmonids, dominant fish tend to exhibit a transient increase in circulating levels of catecholamine (Thomas and Gilmour, 2006) and cortisol [the main glucocorticoid (GC) in teleosts; Øverli et al., 1999] stress hormones during the initial period of hierarchy formation. Dominant fish also exhibit longer-term benefits from the monopolization of food (e.g. Metcalfe et al., 1989; McCarthy et al., 1992; Øverli et al., 1998), and changes such as increases in liver glycogen stores (Gilmour et al., 2012) and transcript levels of cortisol steroidogenic genes (Jeffrey et al., 2012) that suggest an enhanced ability to deal with subsequent stressors ('pre-adaptation'). Subordinate fish, in contrast, experience chronic social stress, as indicated by sustained elevation of circulating cortisol levels accompanied by reduced reactivity to additional stressors (Jeffrey et al., 2014), reduced growth rates, which also reflect low food intake and changes in digestive function (DiBattista et al., 2006), and increased standard metabolic rate (Sloman et al., 2000). Thus, there are negative impacts of low social status, but also potential benefits for those individuals that maintain dominant status. Whether these differences at the individual level translate into consequences for subsequent generations remains understudied, in part owing to challenges posed by the life history of salmonid fish.

Zebrafish (*Danio rerio*) provide a useful species in which to study the potential impacts of social status on offspring. Adult zebrafish form social hierarchies, with one fish becoming dominant through aggressive interactions (Larson et al., 2006; Filby et al., 2010; Paull et al., 2010; Dahlbom et al., 2011; Pavlidis et al., 2011). The physiological consequences of social stress have been evaluated to some extent in zebrafish. Dominant fish tended to be larger (Filby et al., 2010; Paull et al., 2010), and dominant males exhibited a higher growth rate compared with subordinate fish (Filby et al., 2010). As in salmonids, subordinate zebrafish exhibited elevated cortisol levels and subordinate males displayed higher telencephalic *corticotropin-releasing factor* (*crf*) and *glucocorticoid receptor* (*gr*) mRNA levels on the first day of interaction (Filby et al., 2010). Furthermore, preoptic area (POA) levels of arginine vasotocin (AVT), a mediator of social behaviour and stress axis activity, differed between dominant and subordinate fish (Larson et al., 2006). Subordinate fish also exhibited increased brain serotonergic activity as indicated by elevated hindbrain 5-hydroxyindoleacetic acid (5-HIAA) to serotonin (5-hydroxytryptamine, 5-HT) ratios following 5 days of interaction (Dahlbom et al., 2012), and social status influenced reproductive success and mate choice (Paull et al., 2010). Together, these studies clearly display the impacts of low social status or chronic social stress in adult zebrafish. The goal of the present study, then, was to determine whether social stress experienced by an adult female zebrafish has consequences for her offspring, specifically with respect to development of the stress response.

Department of Biology, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5.
*Present address: Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL 61802, USA.

‡Author for correspondence (jjeffrey@illinois.edu)

J.D.J., 0000-0001-6694-4563

Received 9 February 2016; Accepted 17 March 2016

List of abbreviations

11β-HSD2	11β-hydroxysteroid dehydrogenase type 2
11β-hyd	11β-hydroxylase
crf	corticotropin-releasing factor
crf-r	corticotropin-releasing factor receptor
GC	glucocorticoid
HPA	hypothalamic-pituitary–adrenal
hpf	hours post-fertilization
HPI	hypothalamic-pituitary–interrenal
P450scc	cytochrome P450 side chain cleavage enzyme
POA	preoptic area
S-QPCR	semi-quantitative real-time RT-PCR
star	steroidogenic acute regulatory protein

Maternal stress has been reported to affect egg quality (e.g. Campbell et al., 1992, 1994; Giesing et al., 2011; Mileva et al., 2011), as well as the size, physiology and behaviour of offspring (e.g. McCormick, 1998, 2006, 2009; Giesing et al., 2011; McGhee et al., 2012; Roche et al., 2012; Mommer and Bell, 2013; Eaton et al., 2015; Sopinka et al., 2015). Maternally derived cortisol, which is deposited to oocytes around the time of vitellogenesis, has been suggested as a potential mediator of the effects of maternal stress on offspring. Maternal cortisol plays an important organizational role during early development in fish, and cortisol treatment of eggs or embryos alters both physiology and behaviour of the developing young (e.g. Sloman, 2010; Burton et al., 2011; Li et al., 2012; Nesan and Vijayan, 2012; Colson et al., 2015; Sopinka et al., 2015). However, programming of offspring is probably more complex than the action of cortisol alone, because previous studies have found effects of maternal stress even in the absence of increased embryo cortisol levels – for example, on egg size in *Neolamprologus pulcher* (Mileva et al., 2011) and on swim performance in *Oncorhynchus nerka* (Sopinka et al., 2014).

Ontogeny of the hypothalamic-pituitary–interrenal (HPI) axis has been well studied in zebrafish (Alsop and Vijayan, 2008; Alderman and Bernier, 2009; Wilson et al., 2013), providing the necessary groundwork for investigating effects of maternal social stress. Specific maternal mRNAs associated with the HPI axis are transferred to eggs and play a key role in embryogenesis before embryos begin to synthesize mRNAs (i.e. become transcriptionally active). Transcripts for *crf*, its binding protein and its receptors are present in eggs; however, their role in early development remains unclear (Alderman and Bernier, 2009). Maternal *gr* transcripts mediate cortisol-dependent actions during early development (Nesan and Vijayan, 2013). In addition, cytochrome P450 side-chain cleavage enzyme (P450scc)-synthesized pregnenolone is involved in early embryogenesis and epiboly (Hsu et al., 2009). As embryos become transcriptionally active, mRNA abundance of HPI axis genes increases (Alsop and Vijayan, 2009). At this point, zebrafish larvae possess all of the machinery necessary to synthesize cortisol *de novo*, and by 72 h post-fertilization (hpf), they begin to respond to stressors with an increase in cortisol levels (Alderman and Bernier, 2009; Wilson et al., 2013).

The present study tested the hypothesis that social status would influence the maternal contribution of cortisol and HPI axis-related mRNA to offspring (measured at 1 hpf), offspring cortisol levels and mRNA abundance of HPI axis genes over development, and the larval stress response. Previous studies reported that stressed female fish gave rise to offspring with a dampened cortisol response to a stressor (Sopinka et al., 2015), and that embryos exposed to cortisol exogenously exhibited a dampened stress response at later stages of development (Auperin and Geslin, 2008). Therefore, a similar attenuation of the stress response was predicted to occur in offspring of subordinate mothers experiencing chronic social stress. To investigate the mechanisms underlying such an effect, maternal contributions to offspring and mRNA abundance of key stress axis genes were examined during early development across offspring of sham-treated (females handled in the same way as paired fish, but not paired with a conspecific), dominant and subordinate fish.

MATERIALS AND METHODS

Experimental animals

Adult zebrafish (N=183; 0.588±0.015 g, mean±s.e.m.), purchased from AQUAlity Tropical Fish Wholesale in Mississauga, ON, Canada, were housed under a 14 h light:10 h dark photoperiod in 3 and 10 l flow-through polycarbonate tanks supplied with aerated, dechloraminated city of Ottawa tap water at 28°C. Fish were fed once or twice daily to satiation with No. 1 crumble-Zeigler (Aquatic Habitats, Apopka, FL, USA) as well as brine shrimp. Fish were acclimated to these holding conditions for at least 3 weeks prior to experimentation. Embryos were reared in 50 ml Petri dishes containing embryo medium (0.01% Methylene Blue, NaCl 0.275 g l^{-1}, KCl 0.012 g l^{-1}, $MgSO_4 \cdot 7H_2O$ 0.078 g l^{-1}, $CaCl_2 \cdot 2H_2O$ 0.046 g l^{-1}) and kept in a 28°C incubator. All experiments were conducted in compliance with the guidelines of the Canadian Council on Animal Care (CCAC) and after approval from the University of Ottawa Animal Care Committee (protocol BL-280).

Female adult zebrafish were tagged with a unique mark using Alcian Blue (Sigma-Aldrich, Oakville, ON, Canada). Fish were lightly anaesthetized in a buffered solution of MS-222 (0.24 mg ml^{-1} 3-aminobenzoic acid ethyl ester; 21 mmol l^{-1} Tris, pH 7; Sigma-Aldrich; Westerfield, 2000), blotted dry, weighed, measured for fork length, and marked. Preliminary experiments revealed that fish were less likely to provide good quality embryos following marking and social stress, a problem that was improved by allowing fish to recover for 2 days in their original tank prior to being paired. Fork length-matched females were then placed in pairs (N=74; difference in fork length 1.25±0.15%) or on their own for the sham treatment (N=35) in experimental tanks (4.5 l). A sham group was included in the experimental design to account for the potential effects of handling and other stressors (e.g. transfer to the experimental tank) experienced by fish over the course of the experiment. Fish were placed individually on either side of an opaque perforated divider (day 1) and on the morning of day 2, the divider was removed, allowing fish to interact. Each fish within a pair was observed twice daily for 3 min each time on days 2 and 3. Fish were assessed for position within the water column, feeding (first to feed), acts of aggression, and retreats/freezing behaviour. Dominant behaviours (acts of aggression and monopolizing of food and territory) received higher scores than subordinate behaviours (retreats and freezing). The fish within a pair with the higher overall score was assigned dominant status. Previous studies have used similar scoring systems (Filby et al., 2010; Paull et al., 2010). An interaction period of 48 h was chosen to maximize social interactions while minimizing decrements in physiological condition in subordinate fish that might impair reproduction. Preliminary experiments indicated that moving females to a breeding chamber with a male (separated from the male by a clear divider) prior to sampling improved the success of retrieving viable eggs from the females. Females were terminally anaesthetized in a buffered solution of MS-222 (as above but using 0.72 mg ml^{-1}) on the morning of day 4, weighed and stripped of their eggs before being flash frozen in liquid nitrogen. Each female's eggs were collected into a 50 ml Petri dish and fertilized with sperm, pooled from 11–20 males, collected in Hanks' solution (Sigma-Aldrich) (see Westerfield, 2000, for similar methods), and Petri dishes were placed in a 28°C incubator.

All embryos were counted to determine clutch size, where clutch size refers to the number of eggs stripped from the female. Clutches were reared to 1, 24, 48, 96 or 144 hpf. At each time point, groups of 25 embryos or larvae were collected for cortisol analysis, and groups of 100 (1 hpf) to 15 (144 hpf) embryos/larvae were collected for analysis of mRNA abundance; collected embryos were flash frozen in liquid nitrogen and stored at −80°C. A female's clutch constituted a single *N* for a time point.

After fertilization, embryo volume was determined using ImageJ (http://imagej.nih.gov/ij/) from pictures taken using a Micropublisher 3.3 RTV camera (Q Imaging, Surrey, BC, Canada) and an Olympus SZ61 dissecting microscope. The equation for an ellipsoid was used to estimate embryo volume. Survival to 24 hpf, hatching success (at 48 hpf) and survival over the developmental period were also assessed.

Maternal cortisol levels

Cortisol was extracted from a subset (N=12 per treatment group) of adult female zebrafish using a protocol adapted from Fuzzen et al. (2010, 2011). In brief, zebrafish were powdered in liquid nitrogen with a mortar and pestle prior to homogenization with a handheld homogenizer (PowerGen 125, Fisher Scientific, Ottawa, ON, Canada) on ice in 400 µl of homogenization buffer (80 mmol l^{-1} Na_2HPO_4, 20 mmol l^{-1} NaH_2PO_4, 100 mmol l^{-1} NaCl, 1 mmol l^{-1} EDTA). Homogenates were extracted three times with 1 ml of methanol each time. After each addition of methanol, samples were vortexed thoroughly and incubated at 4°C in the dark for 60 min (for the first extraction) or 30 min (for the second and third extractions). Samples were centrifuged at 3000 ***g*** for 5 min at 4°C and flash frozen at −80°C for 10 min. After each extraction, the supernatant was transferred to a 2 ml microtube and evaporated under forced air. Combined extractions were reconstituted in 2 ml of acetate buffer (2.35 ml glacial acetic acid and 1.23 g sodium acetate trihydrate per litre). Reconstituted samples were purified by passing them through a 500 mg C_{18} solid phase extraction column (Fisher Scientific) primed with methanol and milliQ water. Prior to elution, ultra-pure water (Caymen Chemical, Cedarlane, Burlington, ON, Canada) and hexane were passed through the column. Steroids were eluted slowly from the column four times with 1 ml of ethyl acetate (1% methanol) each time. The eluates were combined into a 2 ml microtube, dried under forced air and then reconstituted in 1 ml of extraction buffer [from Neogen cortisol enzyme-linked immunoassay (EIA) kit, Lexington, KY, USA]. Samples were heated at 60°C for 5 min, vortexed twice, and stored at −80°C until analysis by EIA (Neogen). Samples were diluted 2-fold and assayed in duplicate on a single plate where intra-assay variability was 5.6%. Extraction efficiency was determined by spiking homogenates with a known amount of ^{3}H-hydrocortisone and was 59%.

Embryo/larval baseline and stress-induced cortisol levels

Baseline, whole-body cortisol levels were assessed at all time points, whereas stress-induced cortisol levels in response to a 'swirling' stressor were determined at 96 and 144 hpf according to Alsop and Vijayan (2008). Cortisol was extracted from pooled embryos/larvae using a protocol modified from Sopinka et al. (2014). In brief, samples were partially thawed on ice, and homogenized in 200 µl of extraction buffer (Neogen) in a 1.5 ml microtube using a battery-operated pestle grinder (Kimble Chase Kontes, Rockwood, TN, USA). Samples were extracted three times with 1 ml of diethyl ether (Fisher Scientific) each time. After each addition of diethyl ether, samples were vortexed thoroughly, incubated at room temperature for 30 min (15 min for the second and third extractions), centrifuged at 3000 ***g*** for 5 min, and flash frozen at −80°C for 30 min. The liquid

Table 1. Oligonucleotide primer sets for semi-quantitative real-time RT-PCR in zebrafish (*Danio rerio*)

Gene	Primer (5′–3′)	Accession no.	Reference*
crf	F: gcc gcg caa agt tca aaa R: gcgaggagaatctgtgcgtaa	BC085458	Alsop and Vijayan, 2008
crf-r1	F: gaa atg cca cct ggt tcg tg R: agc ctg cac cag atc aca tt	XM691254	
crf-r2	F: aat ggt gag gtt cgg tct gc R: tgt ggg aat gga cat cgc tc	ENSDART 00000055713	
star	F: ttg aac aag ctc tcc gga cc R: cac tgt atg tct cct cgg ca	NM131663	
p450scc	F: agg gcc atc acc cca ata g R: cca ggc ctt ccc ttc ttt tag	NM152953	Alderman and Bernier, 2009
11β-hyd	F: gct cat gca cat tct gag ga R: tgt gct gaa ggt gat tct cg	NM001080204	Ings and Van Der Kraak, 2006
11β-hsd2	F: cac gac tta ccc tcc tgc aa R: aca agc ccc cac aaa tct ct	NM212720	
gr	F: aca gct tct tcc agc ctc ag R: ccg gtg ttc tcc tgt ttg at	NM001020711	Alsop and Vijayan, 2008
mr	F: ccc att gag gac caa atc ac R: agt aga gca ttt ggg cgt tg	EF567113	Alsop and Vijayan, 2008
18s	F: ggc ggc gtt att ccc atg acc R–ggt ggt gcc ctt ccg tca att c	FJ915075	
β-actin	F–tgt ccc tgt atg cct ctg gt R: aag tcc aga cgg agg atg g	AF025305	Alsop and Vijayan, 2008
ubiquitin	F: tgt ccc tgt atg cct ctg gt R: aag tcc aga cgg agg atg g	BC105746	Alsop and Vijayan, 2008

*Where no reference is provided, primers were designed for the present study using Primer3 or Primer3Plus, and primer specificity was verified by sequencing (GenScript, Piscataway, NJ, USA).

crf, *corticotropin-releasing factor*; *crf-r*, *crf receptor*; *star*, *steroidogenic acute regulatory protein*; *11β-hyd*, *11β-hydroxylase*; *11β-hsd2*, *11β-hydroxysteroid dehydrogenase 2*; *gr*, *glucocorticoid receptor*; *mr*, *mineralocorticoid receptor*.

phases of each extraction were combined, transferred to a clean 1.5 ml microtube, and evaporated under forced air. The extract was reconstituted in 250 µl of extraction buffer (Neogen), vortexed, heated at 65°C for 5 min twice, and stored at −80°C until analysis by EIA (Neogen). Samples were assayed in duplicate over four plates where inter-assay variability was 3.1% and intra-assay variability was 7.8%. Because solid phase extraction was not used, a subset of samples was used to generate dilution curves that were found to be parallel to the standard curve. Extraction efficiency was determined by spiking homogenates with a known amount of ^{3}H-hydrocortisone and was 85% for embryos at 1 hpf and 79% for larvae at 144 hpf.

Embryo mRNA abundance

Total RNA was extracted from pools of 15–100 embryos/larvae using TRIzol reagent (Invitrogen, Burlington, ON, Canada) according to the manufacturer's protocol. Embryos/larvae were homogenized in 0.5 ml of TRIzol reagent using a battery-operated pestle grinder (Kimble Chase Kontes). Extracted RNA was quantified using a NanoDrop® ND-2000c UV-Vis Spectrophotometer (Thermo Scientific, Ottawa, ON, Canada). Next, cDNA was synthesized from 0.5 µg of RNA using QuantiTect Reverse Transcription Kit (Qiagen, Toronto, ON, Canada) and following the manufacturer's protocol with the exception that half reaction volumes, with a final volume of 10 µl, were used.

Semi-quantitative real-time RT-PCR (S-QPCR) was used to assess relative mRNA levels of HPI axis genes (Table 1). All genes were assessed over 1–144 hpf except *11β-hydroxysteroid dehydrogenase 2* (*11β-hsd2*), which was measured over 48–144 hpf because mRNA levels are not detectable in zebrafish until after 25 hpf (Alsop and Vijayan, 2008). All S-QPCR reactions were performed in a similar manner using Rotor-Gene SYBR Green PCR Kit (Qiagen) and a Rotor-Gene Q real-time PCR system (Qiagen), following the manufacturer's protocol with the exception that reaction volumes were scaled to 10 µl instead of 25 µl. Standard curves using serial dilutions of pooled cDNA were performed to optimize reaction compositions; the efficiency for each primer set was between 0.92 and 1.10. Negative controls, including no-template controls (where cDNA was replaced with water) and no-reverse transcriptase controls (where RNA was treated as for other cDNA reactions but reverse transcriptase was replaced with water), were used to confirm that primers did not bind to genomic DNA.

Because more than five genes were assessed in the present study, the NORMA-gene approach of Heckmann et al. (2011) was used to normalize *Ct* values. Normalized *Ct* values were then expressed relative to the sham 1 hpf mean [for *crf*, *crf-receptor 1* (*crf-r1*), *crf-r2*, *p450scc* and *gr*], the sham 24 hpf mean [for *steroidogenic acute regulatory protein* (*star*), *11β-hydroxylase* (*11β-hyd*) and *mineralocorticoid receptor* (*mr*)] or the sham 48 hpf mean (for *11β-hsd2*) using the $-\Delta Ct$ method (Livak and Schmittgen, 2001). Genes were expressed relative to a group other than the sham 1 hpf when expression levels for the gene were undetectable at 1 hpf.

Table 2. Clutch size and offspring viability (i.e. embryo volume, survival at 24 hpf and hatching at 48 hpf) for sham-treated, dominant and subordinate female zebrafish (*Danio rerio*)

	Maternal social status			
	Sham-treated	Dominant	Subordinate	*P*-value
Clutch size	353.0±28.9 (28)	363.2±24.3 (40)	333.5±30.8 (38)	*0.181*
Embryo volume at 1 hpf (mm^3)	0.0729± 0.0030 (20)	0.0738± 0.0028 (18)	0.0767± 0.0031 (23)	0.627
Survival to 24 hpf (%)	81.7±3.6 (26)	70.1±4.7 (36)	63.8±5.0 (38)	*0.098*
Hatching at 48 hpf (%)	52.3±6.8 (20)	41.9±6.4 (28)	37.1±6.6 (27)	*0.349*

Data are presented as means±s.e.m. (*N*). Effects of maternal status were analysed by one-way ANOVA (on ranks indicated by italics). Clutch size refers to the number of eggs stripped from a female.

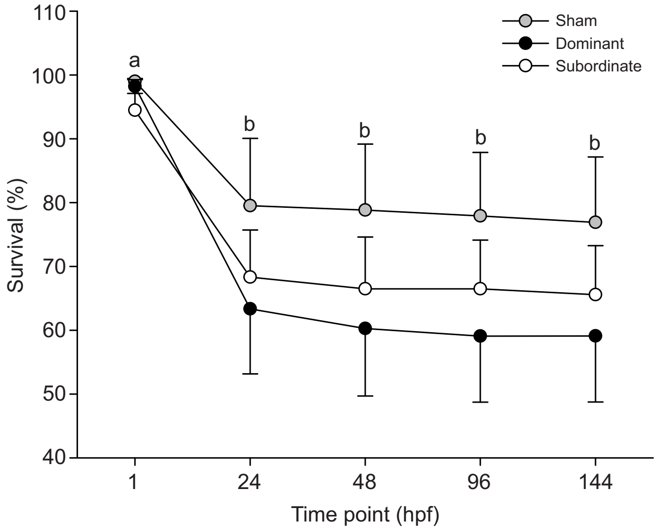

Fig. 1. Survival of offspring of sham-treated (*N*=8), dominant (*N*=12) and subordinate (*N*=13) female zebrafish (*Danio rerio*) over 1–144 h post-fertilization (hpf). Values are presented as means±s.e.m. No significant effect of maternal treatment was found. Time points that do not share a letter are significantly different from one another (two-way RM ANOVA; *P*=0.475 for maternal status, *P*<0.001 for time point, *P*=0.546 for treatment×time).

Statistical analysis

Cortisol concentrations and mRNA abundance of HPI axis genes have been well characterized throughout development in zebrafish (Alsop and Vijayan, 2008; Alderman and Bernier, 2009; Wilson

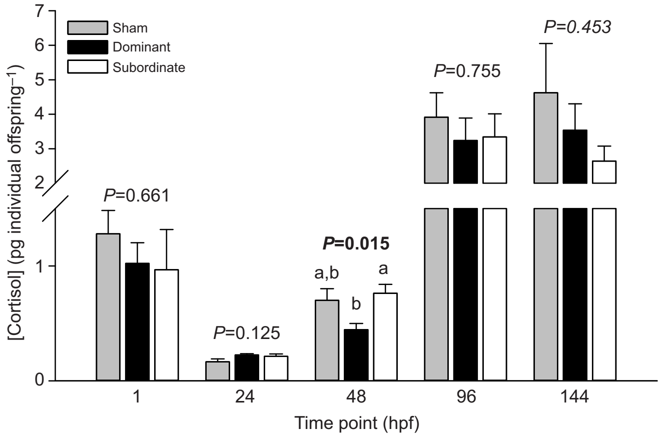

Fig. 2. Baseline whole-body cortisol levels for offspring of sham-treated (*N*=5–6), dominant (*N*=6–8) and subordinate (*N*=6–8) female zebrafish over 1–144 hpf. Independent samples were pools of 25 offspring from a single female. Values are expressed per individual offspring and presented as means+s.e.m. Maternal status effects were analysed by one-way ANOVA (on ranks indicated by italics, and significant values are in bold). Within a time point, groups that do not share a letter are significantly different from one another.

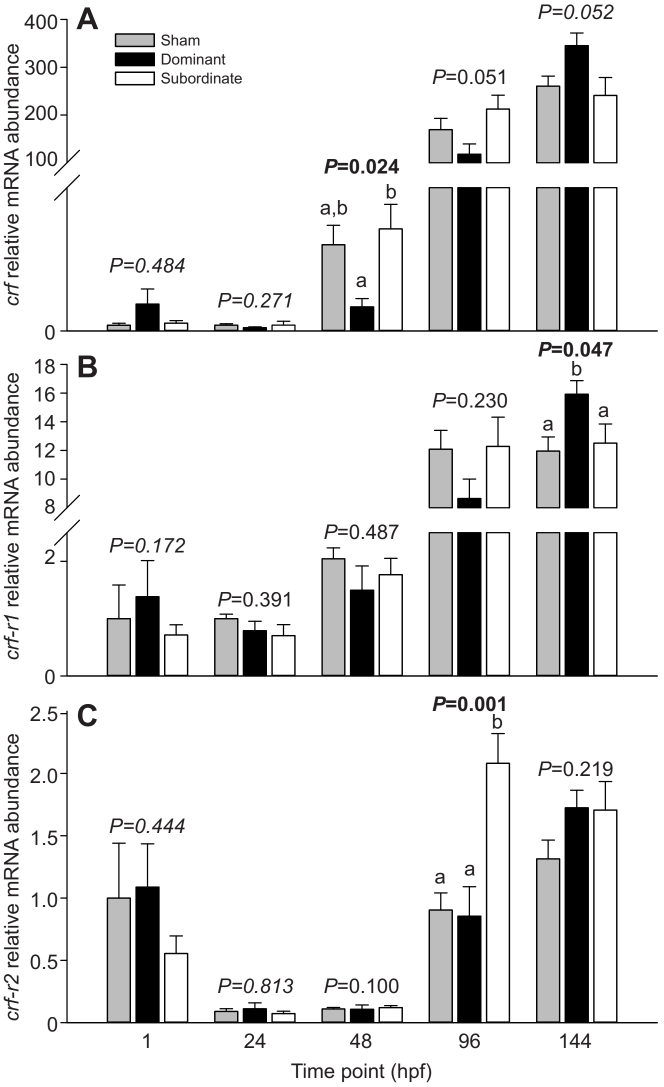

Fig. 3. Relative mRNA abundance of *corticotropin-releasing factor*, *crf-receptor 1* and *crf-r2* in offspring of sham-treated (*N*=5–6), dominant (*N*=6) and subordinate (*N*=4–6) female zebrafish over 1–144 hpf. (A) *corticotropin-releasing factor* (*crf*); (B) *crf-receptor 1* (*crf-r1*); and (C) *crf-r2*. Levels of mRNA were measured by semi-quantitative real-time RT-PCR using 15–100 pooled offspring from a single female as a single *N*. Values are presented as means+s.e.m. All data were expressed relative to the 1 hpf group from sham-treated females. Maternal status effects were analysed by one-way ANOVA (on ranks indicated by italics, and significant values are in bold). Within a time point, groups that do not share a letter are significantly different from one another.

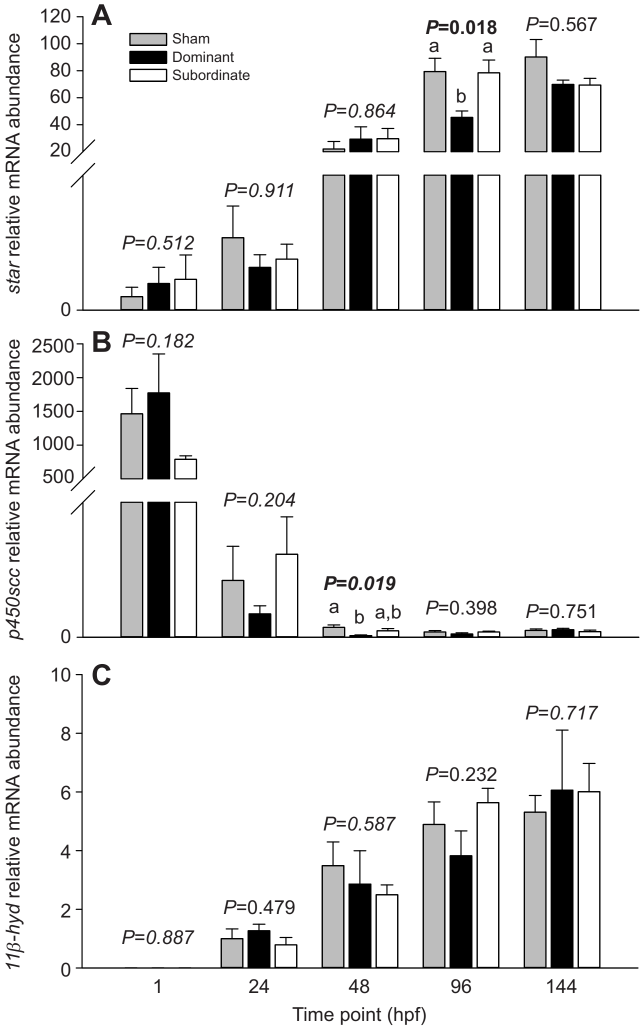

Fig. 4. Relative mRNA abundance of *steroidogenic acute regulatory protein*, *cytochrome p450 side chain cleavage enzyme* and *11β-hydroxylase* in offspring of sham-treated, dominant and subordinate female zebrafish over 1–144 hpf. (A) *steroidogenic acute regulatory protein* (*star*); (B) *cytochrome p450 side chain cleavage enzyme* (*p450scc*); and (C) *11β-hydroxylase* (*11β-hyd*). Levels of mRNA were measured by semi-quantitative real-time RT-PCR using 15–100 pooled offspring from a single female as a single *N*. Values are presented as means+s.e.m. (*N*=5–6 for all groups). Data were expressed relative to the 24 hpf group from sham-treated females for *star* and *11β-hyd*, and relative to the 144 hpf group from sham-treated females for *p450scc*. Maternal status effects were analysed by one-way ANOVA (on ranks indicated by italics, and significant values are in bold). Within a time point, groups that do not share a letter are significantly different from one another.

et al., 2013). Because patterns for these variables in the present study were similar to those reported previously (Figs 2–5), and the goal of the present study was to evaluate effects of maternal social status, only maternal effects within a developmental time point were evaluated statistically.

All data are expressed as mean values±1 s.e.m. A Pearson's χ^2 test was used to evaluate the effect of maternal social status on the production of a viable clutch. Maternal cortisol levels as well as maternal treatment effects on embryo size, survival at 24 hpf, hatching, baseline or stress-induced cortisol levels, and mRNA abundance were analysed by one-way ANOVA. Paired Student's *t*-tests were used to analyse differences between baseline and stress-induced cortisol levels. Survival over the developmental period was analysed by two-way repeated measures (RM) ANOVA. Where data were not normally distributed, equivalent non-parametric tests were used. The level of significance (α) was 0.05. All analyses were carried out using JMP V.11.

RESULTS

Effects of maternal social status on egg production and early embryo development

Maternal whole-body cortisol levels measured at the end of the experiment did not differ with social status (sham-treated, 7.74±1.31 ng g^{-1} body mass; dominant, 8.03±1.40 ng g^{-1} body mass; subordinate, 6.17±1.29 ng g^{-1} body mass; one-way ANOVA, *P*=0.574). Both dominant (56.8%; *N*=74) and subordinate

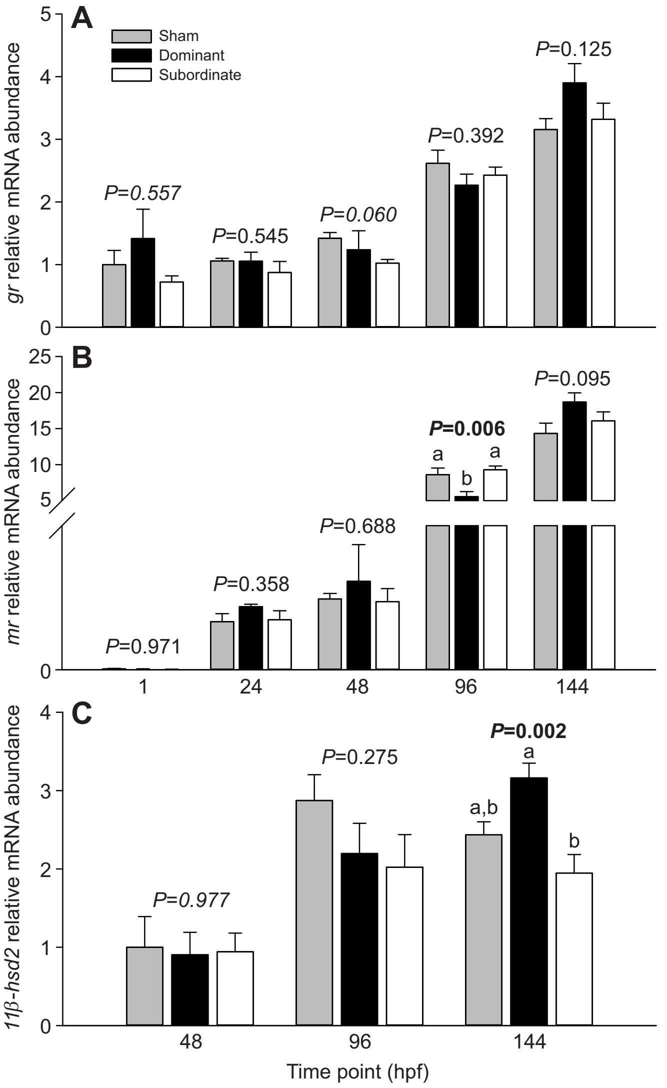

Fig. 5. Relative mRNA abundance of *glucocorticoid receptor*, *mineralocorticoid receptor* and *11β-hydroxysteroid dehydrogenase type 2* in offspring of sham-treated (*N*=5–6), dominant (*N*=5–6) and subordinate (*N*=6) female zebrafish. Data are shown over 1–144 hpf for (A) *glucocorticoid receptor* (*gr*) and (B) *mineralocorticoid receptor* (*mr*); and over 48–144 hpf for (C) *11β-hydroxysteroid dehydrogenase type 2* (*11β-hsd2*; see Materials and methods). Levels of mRNA were measured by semi-quantitative real-time RT-PCR using 15–100 pooled offspring from a single female as a single *N*. Values are presented as means+s.e.m. Data were expressed relative to 1 hpf offspring from sham-treated females for *gr*, relative to 24 hpf offspring from sham-treated females for *mr*, and relative to 48 hpf offspring from sham-treated females for *11β-hsd2*. Maternal status effects were analysed by one-way ANOVA (on ranks indicated by italics, and significant values are in bold). Within a time point, groups that do not share a letter are significantly different from one another.

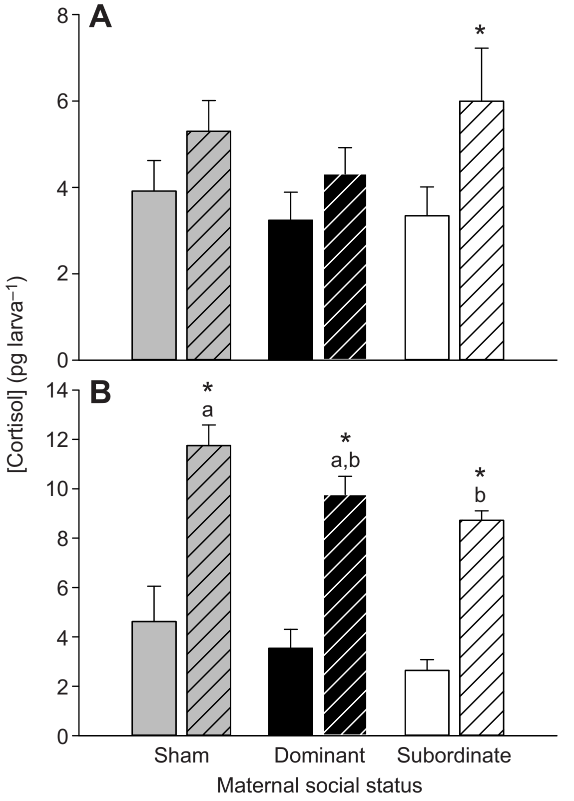

Fig. 6. Whole-body cortisol levels following exposure of offspring of sham-treated (*N*=6), dominant (*N*=7–8) and subordinate (*N*=6–8) female zebrafish to a stressor. Exposure was for (A) 96 hpf and (B) 144 hpf. Groups of 25 larvae were sampled before (baseline; solid bars) or 5 min after exposure to a 30 s swirling stressor (stressed; hatched bars). Independent samples were pools of offspring from a single female. Values are expressed per individual offspring and presented as means+s.e.m. Baseline values are repeated from Fig. 2 for ease of comparison (see Fig. 2 for statistical analysis). An asterisk represents a significant difference between baseline and stressed values within a maternal status (paired Student's *t*-tests; *P*=0.061, 0.183 and 0.009 for 96 hpf offspring of sham-treated, dominant and subordinate females, respectively; *P*=0.003, 0.006 and 0.031 for 144 hpf offspring of sham-treated, dominant and subordinate females, respectively). Stress-induced cortisol levels did not differ with maternal social status at 96 hpf (one-way ANOVA; *P*=0.426). Stressed groups of 144 hpf offspring that do not share a letter are significantly different from one another (one-way ANOVA; *P*=0.030).

(59.5%; *N*=74) females yielded viable clutches less often than sham-treated females (91.4%; *N*=35; Pearson's χ^2 test, *P*=0.010). Maternal social status had no significant effect on clutch size or early embryo viability (i.e. survival, embryo volume at 1 hpf, hatching at 48 hpf; Table 2, Fig. 1).

Offspring baseline cortisol levels and mRNA abundance of HPI axis genes

Baseline whole-body cortisol levels (Fig. 2) and mRNA abundance of HPI axis genes (Figs 3–5) were unaffected by maternal treatment until 48 hpf. At 48 hpf, cortisol levels were significantly lower in offspring of dominant females compared with those of subordinate females (Fig. 2). Similarly, mRNA abundance of *crf* and *p450scc* was significantly lower at 48 hpf in offspring of dominant females compared with offspring of subordinate and sham-treated females, respectively (Figs 3A and 4B).

Although baseline cortisol levels were unaffected by maternal social status after 48 hpf (Fig. 2), mRNA abundance of HPI axis genes continued to be modulated by maternal status. At 96 hpf, *crf* mRNA abundance tended to be lower in offspring of dominant females (Fig. 3A). Similarly, transcript levels of *star* (Fig. 4A) and *mr* (Fig. 5B) were significantly lower in offspring of dominant females than in offspring of sham-treated or subordinate females at 96 hpf. Conversely, *crf-r2* mRNA levels were elevated in offspring of subordinate females compared with offspring of sham-treated or dominant females at 96 hpf (Fig. 3C). At 144 hpf, *crf* mRNA abundance tended to be elevated (Fig. 3A) and *crf-r1* mRNA abundance was significantly elevated in offspring of dominant females (Fig. 3B). Likewise, maternal dominance resulted in significantly higher *11β-hsd2* mRNA levels compared with those of offspring of subordinate females at this time point (Fig. 5C). Overall, no significant effect of maternal treatment on *11β-hyd* (Fig. 4C) or *gr* (Fig. 5A) mRNA abundance was detected.

Offspring stress-induced cortisol levels

Cortisol levels were measured 5 min after exposure to a 30 s swirling stressor (stressed) for groups of 25 larvae at 96 and 144 hpf (Fig. 6). At 96 hpf, only larvae of subordinate females exhibited significantly elevated cortisol levels (Fig. 6A; paired Student's *t*-test; P=0.009); overall, however, stressed cortisol levels did not differ significantly with maternal treatment at 96 hpf (Fig. 6A; one-way ANOVA; P=0.426). At 144 hpf, all larvae significantly elevated cortisol levels in response to the swirling stressor (Fig. 6B; paired Student's *t*-tests; P=0.003, 0.006 and 0.031 for offspring of sham-treated, dominant and subordinate females, respectively), but stress-induced cortisol levels were significantly lower in larvae of subordinate females compared with offspring of sham females (Fig. 6B; one-way ANOVA; P=0.030).

DISCUSSION

Effects of maternal social status on egg production and early embryo development

Social stress did not affect whole-body cortisol levels in the adult female zebrafish of the present study. Filby et al. (2010) reported that cortisol levels were elevated as a result of low social status for zebrafish held in groups of four fish (two females and two males per group). However, Pavlidis et al. (2011) found no difference in whole-trunk cortisol levels between dominant and subordinate zebrafish after 5 days of interaction, although the levels in both dominant and subordinate fish were elevated above those of control fish. In the present study, female fish were moved to breeding tanks more than 12 h prior to sampling (see Materials and methods) and this time may have been sufficient for social status-induced differences in cortisol levels to be masked by cortisol metabolism. The metabolic clearance of cortisol depends on the rate of tissue uptake and steroid catabolism (Mommsen and Walsh, 1988), which do not appear to have been measured in zebrafish. However, Faught et al. (2016) reported that a bolus of cortisol administered via food intake was not detectable after 12 h, suggesting that cortisol clearance is rapid in this species. Elevation of cortisol levels is not, however, the only consequence of social interactions or social stress.

Competition for dominance requires fish to invest energy in agonistic contests (Johnsson et al., 2006), and thus energy may have been reallocated from reproduction to social contests, resulting in dominant and subordinate females yielding viable clutches less often than sham-treated females (where clutch refers to the eggs stripped from the female). For the viable clutches obtained, maternal social status had no apparent effect on clutch size or offspring viability during early development (i.e. embryo size, survivability or hatching at 48 hpf). However, the possibility that females of different social status could produce clutches of different size or at different frequencies if allowed to spawn naturally cannot be excluded. The results of previous studies have been mixed, with evidence for maternal stress effects on offspring size and survival in rainbow trout (*Oncorhynchus mykiss*) and/or brown trout (*Salmo trutta*) (Campbell et al., 1994; Contreras-Sánchez et al., 1998), damselfish (*Pomacentrus amboinensis*) (McCormick, 2006), *Neolamprologus pulcher* (Mileva et al., 2011) and three-spined stickleback (*Gasterosteus aculeatus*) (Giesing et al., 2011), but no effect on gamete quality in coho salmon (*Oncorhynchus kisutch*) (Stratholt et al., 1997), or on egg size or early offspring survival in sockeye salmon (*O. nerka*) (Sopinka et al., 2014). This variability may reflect, at least in part, differences in the severity of the stressor experienced by the female fish; in the present study, subordinate social stress may not have been sufficiently severe to induce effects beyond those resulting from the demands of social interaction. Moreover, the timing of the stressor relative to oocyte development may play a role in determining the quality of the offspring. Zebrafish are asynchronous breeders and therefore social stress would have occurred during the final stages of oocyte development for the eggs examined in the present study.

Maternal social status affected cortisol and HPI axis function during early development

Egg (1 hpf embryo) cortisol levels were not affected by social status in the present study. In fish, maternal cortisol contribution to the egg occurs around the time of vitellogenesis (Selman et al., 1993; Mommsen et al., 1999). In asynchronously breeding zebrafish, cortisol deposition to the developing oocyte is actively controlled through ovarian 11β-HSD-2 activity and probably occurs only during a short window (Faught et al., 2016). Consequently, increases in embryo cortisol levels as a result of maternal stress will not be visible in zebrafish embryos until about 9 days after the period of maternal stress (Faught et al., 2016), i.e. after the period of egg collection used in the present study. Even in the absence of increased maternal cortisol transfer to embryos, Sopinka et al. (2014) reported that maternal stress caused differences in offspring swim performance, suggesting that cortisol is not the only mediator of offspring programming by maternal stress. Similarly, the present study found evidence of effects of maternal social status on development of the HPI axis in zebrafish offspring without differences in maternal contribution of cortisol or mRNA levels to eggs, suggesting that maternal social status may affect oocytes even during their late stages of maturation. These effects were first observed at 48 hpf, the time of hatching and the point in development at which zebrafish begin to synthesize cortisol *de novo*.

De novo synthesis of cortisol and activation of the stress response are delayed in teleost fish until after hatch (Alsop and Vijayan, 2008; Alderman and Bernier, 2009). At 48 hpf, offspring of dominant females exhibited lower baseline cortisol levels than offspring of subordinate females. Correspondingly, mRNA levels of both *crf* and *p450scc* were lower in offspring of dominant females. Lower cortisol levels, perhaps owing to lower mRNA levels of these key HPI axis genes, might reflect a delay in the *de novo* synthesis of cortisol in offspring of dominant females. Cortisol affects hatching rate in zebrafish and blocking cortisol synthesis either by morpholino knockdown of *11β-hyd* or with metyrapone (an 11β-hyd enzyme inhibitor) decreased the proportion of embryos hatched by 72 hpf (Wilson et al., 2013). The lower baseline cortisol levels in embryos of dominant females did not appear to delay hatching rate in the present study. However, hatching was measured only at 48 hpf, which may have hindered the detection of differences; Wilson et al. (2013) detected significant effects of metyrapone and *cyp11b1* knockdown at 72 but not 48 hpf. It is interesting that this impact of maternal status on offspring cortisol levels during an important point in development was associated with dominant rather than subordinate social status. It adds another instance where high social status has distinct physiological consequences (for other examples, see Gilmour et al., 2012; Jeffrey et al., 2012) and emphasizes the importance of considering maternal environment and experience broadly in examining effects on offspring.

Effects of maternal social status on HPI axis genes were also observed at 96 and 144 hpf, even though offspring baseline cortisol levels did not differ significantly with maternal treatment at these time points. Similar to the results at 48 hpf, *crf* mRNA abundance tended to be lower in offspring of dominant females at 96 hpf, as were mRNA levels of *star*, effects with the potential to lower cortisol levels; the absence of any impact on cortisol levels suggests either compensation by other elements of the stress axis or a mismatch between mRNA and protein levels. Interestingly, by 144 hpf, *crf* mRNA abundance tended to be elevated and *crf-r1* transcript levels were significantly so in offspring of dominant females, which might have been expected to increase baseline cortisol levels. However, transcript levels of the cortisol breakdown enzyme 11β-HSD2 also were significantly elevated in these offspring, which may account for the absence of an effect on baseline cortisol. Together, these results suggest that maternal dominance affected baseline expression of genes associated with stress axis function during early points in development, even in the absence of changes in baseline cortisol levels beyond 48 hpf. The mechanisms through which maternal social status late in oocyte development affect stress axis development remain to be determined. In addition, the question of whether these effects of maternal dominance have functional consequences for older offspring warrants investigation.

Cortisol production in response to a stressor is affected by maternal social status

Larvae exhibited a robust cortisol response to a swirling stressor only at 144 hpf. Although stress-induced elevation of cortisol has been observed as early as 72 hpf in zebrafish larvae (Alderman and Bernier, 2009; Wilson et al., 2013), these studies used different, perhaps more severe, stressors than the swirling stressor used in the present study. Notably, Alsop and Vijayan (2008) using the same swirling stressor detected an acute cortisol response only at 97 hpf (in their study, fish were not tested beyond 97 hpf). No effect of maternal social status was detected at 96 hpf in the present study, although it is possible that assessing the cortisol response over a longer time scale (i.e. beyond 5 min post-stressor) might have revealed differences in the dynamics of the stress response that were not captured in the present study (e.g. Nesan and Vijayan, 2016). Stressor-induced cortisol levels were significantly lower in the offspring of subordinate females at 144 hpf. This apparent plasticity of the HPI axis during early development also has emerged in other studies. For example, developing rainbow trout exposed to either a stressor (air exposure/cold water) or cortisol treatment during early development (Auperin and Geslin, 2008) and sockeye salmon reared from mothers subjected to a chasing stressor (Sopinka et al., 2015) exhibited attenuated stress responses. In addition, offspring of female three-spined stickleback stressed by exposure to a predator exhibited a cortisol response different to that of offspring of unstressed females (Mommer and Bell, 2013). Similarly, manipulation of cortisol content in freshly fertilized embryos influenced the stress response in 72 hpf larvae (Nesan and Vijayan, 2016). An antibody against cortisol or exogenous cortisol was injected into single-cell embryos to sequester maternally deposited cortisol or increase cortisol levels, respectively (Nesan and Vijayan, 2016). Decreased cortisol bioavailability during early development increased the stress response of larvae, whereas increased embryo cortisol attenuated larval stress responsiveness (Nesan and Vijayan, 2016). Collectively, these studies demonstrate that maternal stress affects offspring HPI axis programming in fish. Effects have been observed in both synchronous and asynchronous breeders and during different points of oocyte development, suggesting that the stress axis may be quite sensitive to maternal experience.

Although few studies have assessed HPI responsiveness in fish as a result of maternal stress, studies on avian and mammalian species are more abundant (reviewed by Love et al., 2013). Maternal programming of hypothalamic-pituitary–adrenal (HPA) axis responsiveness in mammals has been well studied for both the prenatal (by maternal stress, e.g. Welberg and Seckl, 2001; Emack et al., 2008) and postnatal (e.g. by the level of maternal care delivered to rats during early development; Weaver, 2009) periods and epigenetic modification of brain GR has emerged as a key modulator of HPA axis function (Mueller and Bale, 2008; Weaver, 2009). Similarly, both prenatal maternal stress and quality of the postnatal environment (i.e. provisioning rates) influenced responsiveness of the HPA axis in fledglings of European starling (*Sturnus vulgaris*) (Love and Williams, 2008). Elevated maternal GC resulted in increased transfer of maternal GC to the egg in birds (e.g. Hayward and Wingfield, 2004; Saino et al., 2005), but differences in the phenotypes of offspring that resulted from maternal stress or exogenous elevation of egg GC suggest that factors beyond GC probably also affected programming of the stress axis, in agreement with the findings of the present study.

Collectively, these data suggest that maternal experience programmes HPA/HPI function across a number of vertebrate species, in part but not entirely by maternal GC, with other, as yet unidentified factors probably also playing a role. At least in certain cases, maternal experience may take advantage of developmental plasticity to produce a phenotype that may be beneficial in a future life-history stage (i.e. 'predictive adaptive response'; Love and Williams, 2008). Put another way, predictive programming of the HPI axis may be beneficial for offspring that are likely to experience a stressful environment. Further investigation is required to determine whether the lower level of stress-induced cortisol in offspring of subordinate mothers is maintained in older fish and, if so, under what conditions this response might be advantageous in zebrafish.

Acknowledgements

Thanks are extended to J. Dupuis and J. D'Silva for their help with preliminary experiments. In addition, special thanks to A. Castle, M.-È. Bélair-Bambrick, M. Brannen and E. Ashour for their help with sampling and sample extractions, and to B. Fletcher and V. Saxena for their excellent care of the fish in the aquatic facility. Thanks also to P. Walsh for access to the qPCR machine and to N. Sopinka for her thoughts and discussion. Finally, thanks to the referees for their thoughtful and helpful comments.

Competing interests

The authors declare no competing or financial interests.

Author contributions

J.D.J. and K.M.G. contributed equally to the experimental design, analysis and writing of the manuscript. J.D.J. performed all experiments.

Funding

This work was supported by Natural Sciences and Engineering Research Council of Canada Discovery and Research Tools & Instruments Grants to K.M.G. sectors.

References

Abbott, J. C. and Dill, L. M. (1985). Patterns of aggressive attack in juvenile steelhead trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci.* **42**, 1702-1706.

Alderman, S. L. and Bernier, N. J. (2009). Ontogeny of the corticotropin-releasing factor system in zebrafish. *Gen. Comp. Endocrinol.* **164**, 61-69.

Alsop, D. and Vijayan, M. M. (2008). Development of the corticosteroid stress axis and receptor expression in zebrafish. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294**, R711-R719.

Alsop, D. and Vijayan, M. M. (2009). Molecular programming of the corticosteroid stress axis during zebrafish development. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **153**, 49-54.

Auperin, B. and Geslin, M. (2008). Plasma cortisol response to stress in juvenile rainbow trout is influenced by their life history during early development and by egg cortisol content. *Gen. Comp. Endocrinol.* **158**, 234-239.

Burton, T., Hoogenboom, M. O., Armstrong, J. D., Groothuis, T. G. G. and Metcalfe, N. B. (2011). Egg hormones in a highly fecund vertebrate: do they influence offspring social structure in competitive conditions? *Funct. Ecol.* **25**, 1379-1388.

Campbell, P. M., Pottinger, T. G. and Sumpter, J. P. (1992). Stress reduces the quality of gametes produced by rainbow trout. *Biol. Reprod.* **47**, 1140-1150.

Campbell, P. M., Pottinger, T. G. and Sumpter, J. P. (1994). Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. *Aquaculture* **120**, 151-169.

Colson, V., Valotaire, C., Geffroy, B. and Kiilerich, P. (2015). Egg cortisol exposure enhances fearfulness in larvae and juvenile rainbow trout. *Ethology* **121**, 1191-1201.

Contreras-Sánchez, W. M., Schreck, C. B., Fitzpatrick, M. S. and Pereira, C. B. (1998). Effects of stress on the reproductive performance of rainbow trout (Oncorhynchus mykiss). *Biol. Reprod.* **58**, 439-447.

Dahlbom, S. J., Lagman, D., Lundstedt-Enkel, K., Sundström, L. F. and Winberg, S. (2011). Boldness predicts social status in zebrafish (*Danio rerio*). *PLoS ONE* **6**, e23565.

Dahlbom, S. J., Backström, T., Lundstedt-Enkel, K. and Winberg, S. (2012). Aggression and monoamines: effects of sex and social rank in zebrafish (*Danio rerio*). *Behav. Brain Res.* **228**, 333-338.

DiBattista, J. D., Levesque, H. M., Moon, T. W. and Gilmour, K. M. (2006). Growth depression in socially subordinate rainbow trout *Oncorhynchus mykiss*: more than a fasting effect. *Physiol. Biochem. Zool.* **79**, 675-687.

Eaton, L., Edmonds, E. J., Henry, T. B., Snellgrove, D. L. and Sloman, K. A. (2015). Mild maternal stress disrupts associative learning and increases aggression in offspring. *Horm. Behav.* **71**, 10-15.

Emack, J., Kostaki, A., Walker, C. D. and Matthews, S. G. (2008). Chronic maternal stress affects growth, behaviour and hypothalamo-pituitary-adrenal function in juvenile offspring. *Horm. Behav.* **54**, 514-520.

Faught, E., Best, C. and Vijayan, M. M. (2016). Maternal stress-associated cortisol stimulation may protect embryos from cortisol excess in zebrafish. *R. Soc. Open Sci.* **3**, 160032.

Filby, A. L., Paull, G. C., Bartlett, E. J., Van Look, K. J. W. and Tyler, C. R. (2010). Physiological and health consequences of social status in zebrafish (*Danio rerio*). *Physiol. Behav.* **101**, 576-587.

Fuzzen, M. L. M., Van Der Kraak, G. and Bernier, N. J. (2010). Stirring up new ideas about the regulation of the hypothalamic-pituitary-interrenal axis in zebrafish (*Danio rerio*). *Zebrafish* **7**, 349-358.

Fuzzen, M. L. M., Bernier, N. J. and Van Der Kraak, G. (2011). Differential effects of 17β-estradiol and 11-ketotestosterone on the endocrine stress response in zebrafish (*Danio rerio*). *Gen. Comp. Endocrinol.* **170**, 365-373.

Giesing, E. R., Suski, C. D., Warner, R. E. and Bell, A. M. (2011). Female sticklebacks transfer information via eggs: effects of maternal experience with predators on offspring. *Proc. R. Soc. B Biol. Sci.* **278**, 1753-1759.

Gilmour, K. M., Kirkpatrick, S., Massarsky, A., Pearce, B., Saliba, S., Stephany, C.-É. and Moon, T. W. (2012). The influence of social status on hepatic glucose metabolism in rainbow trout *Oncorhynchus mykiss*. *Physiol. Biochem. Zool.* **85**, 309-320.

Hayward, L. S. and Wingfield, J. C. (2004). Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. *Gen. Comp. Endocrinol.* **135**, 365-371.

Heckmann, L. H., Sorensen, P. B., Krough, P. H. and Sorensen, J. G. (2011). NORMA-Gene: a simple and robust method for qPCR normalization based on target gene data. *BMC Bioinformatics* **12**, 250.

Hsu, H.-J., Lin, J.-C. and Chung, B.-C. (2009). Zebrafish cyp11a1 and hsd3b genes: structure, expression and steroidogenic development during embryogenesis. *Mol. Cell. Endocrinol.* **312**, 31-34.

Ings, J. S. and Van Der Kraak, G. J. (2006). Characterization of the mRNA expression of StAR and steroidogenic enzymes in zebrafish ovarian follicles. *Mol. Reprod. Dev.* **73**, 943-954.

Jeffrey, J. D., Esbaugh, A. J., Vijayan, M. M. and Gilmour, K. M. (2012). Modulation of hypothalamic–pituitary–interrenal axis function by social status in rainbow trout. *Gen. Comp. Endocrinol.* **176**, 201-210.

Jeffrey, J. D., Gollock, M. J. and Gilmour, K. M. (2014). Social stress modulates the cortisol response to an acute stressor in rainbow trout (*Oncorhynchus mykiss*). *Gen. Comp. Endocrinol.* **196**, 8-16.

Johnsson, J. I. (1997). Individual recognition affects aggression and dominance relations in rainbow trout, *Oncorhynchus mykiss*. *Ethology* **103**, 267-282.

Johnsson, J., Winberg, S. and Sloman, K. (2006). Social interactions. In *Fish Physiol*, Vol. 24 (ed. K. A. Sloman, R. W. Wilson and S. Balshine), pp. 151-196. San Diego, CA: Academic Press.

Larson, E. T., O'Malley, D. M. and Melloni, R. H., Jr (2006). Aggression and vasotocin are associated with dominant–subordinate relationships in zebrafish. *Behav. Brain Res.* **167**, 94-102.

Li, M., Leatherland, J. F., Vijayan, M. M., King, W. A. and Madan, P. (2012). Glucocorticoid receptor activation following elevated oocyte cortisol content is associated with zygote activation, early embryo cell division, and IGF system gene responses in rainbow trout. *J. Endocrinol.* **215**, 137-149.

Livak, K. J. and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}$T method. *Methods* **25**, 402-408.

Love, O. and Williams, T. (2008). Plasticity in the adrenocortical response of a free-living vertebrate: the role of pre- and post-natal developmental stress. *Horm. Behav.* **54**, 496-505.

Love, O. P., McGowan, P. O. and Sheriff, M. J. (2013). Maternal adversity and ecological stressors in natural populations: the role of stress axis programming in individuals, with implications for populations and communities. *Funct. Ecol.* **27**, 81-92.

McCarthy, I. D., Carter, C. G. and Houlihan, D. F. (1992). The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Biol.* **41**, 257-263.

McCormick, M. I. (1998). Behaviorally induced maternal stress in a fish influences progeny quality by a hormonal mechanism. *Ecology* **79**, 1873-1883.

McCormick, M. I. (2006). Mothers matter: crowding leads to stressed mothers and smaller offspring in marine fish. *Ecology* **87**, 1104-1109.

McCormick, M. I. (2009). Indirect effects of heterospecific interactions on progeny size through maternal stress. *Oikos* **118**, 744-752.

McGhee, K. E., Pintor, L. M., Suhr, E. L. and Bell, A. M. (2012). Maternal exposure to predation risk decreases offspring antipredator behaviour and survival in threespined stickleback. *Funct. Ecol.* **26**, 932-940.

Metcalfe, N. B., Huntingford, F. A., Graham, W. D. and Thorpe, J. E. (1989). Early social status and the development of life-history strategies in Atlantic salmon. *Proc. R. Soc. B Biol. Sci.* **236**, 7-19.

Mileva, V. R., Gilmour, K. M. and Balshine, S. (2011). Effects of maternal stress on egg characteristics in a cooperatively breeding fish. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **158**, 22-29.

Mommer, B. C. and Bell, A. M. (2013). A test of maternal programming of offspring stress response to predation risk in threespine sticklebacks. *Physiol. Behav.* **122**, 222-227.

Mommsen, T. P. and Walsh, P. J. (1988). Vitellogenesis and oocyte assembly. In *Fish Physiology: The Physiology of Developing Fish Eggs and Larvae*, Vol. 11 (ed. W. S. Hoar and D. J. Randall), pp. 347-406. California: Academic Press.

Mommsen, T. P., Vijayan, M. M. and Moon, T. W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Rev. Fish Biol. Fish.* **9**, 211-268.

Mueller, B. R. and Bale, T. L. (2008). Sex-specific programming of offspring emotionality after stress early in pregnancy. *J. Neurosci.* **28**, 9055-9065.

Nesan, D. and Vijayan, M. M. (2012). Embryo exposure to elevated cortisol level leads to cardiac performance dysfunction in zebrafish. *Mol. Cell. Endocrinol.* **363**, 85-91.

Nesan, D. and Vijayan, M. M. (2013). Role of glucocorticoid in developmental programming: evidence from zebrafish. *Gen. Comp. Endocrinol.* **181**, 35-44.

Nesan, D. and Vijayan, M. M. (2016). Maternal cortisol mediates hypothalamus-pituitary-interrenal axis development in zebrafish. *Sci. Rep.* **6**, 22582.

Øverli, Ø., Winberg, S., Damsgård, B. and Jobling, M. (1998). Food intake and spontaneous swimming activity in Arctic char (*Salveliunus alpinus*): role of brain serotonergic activity and social interaction. *Can. J. Zool.* **76**, 1366-1370.

Øverli, Ø., Harris, C. A. and Winberg, S. (1999). Short-term effects of fights dominance and the estabilshment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. *Brain Behav. Evol.* **54**, 263-275.

Paull, G. C., Filby, A. L., Giddins, H. G., Coe, T. S., Hamilton, P. B. and Tyler, C. R. (2010). Dominance hierarchies in zebrafish (*Danio rerio*) and their relationship with reproductive success. *Zebrafish* **7**, 109-117.

Pavlidis, M., Sundvik, M., Chen, Y.-C. and Panula, P. (2011). Adaptive changes in zebrafish brain in dominant–subordinate behavioral context. *Behav. Brain Res.* **225**, 529-537.

Roche, D. P., McGhee, K. E. and Bell, A. M. (2012). Maternal predator-exposure has lifelong consequences for offspring learning in threespined sticklebacks. *Biol. Lett.* **8**, 932-935.

Saino, N., Romano, M., Ferrari, R. P., Martinelli, R. and Møller, A. P. (2005). Stressed mothers lay eggs with high corticosterone levels which produce low-quality offspring. *J. Exp. Zool. A Comp. Exp. Biol.* **303A**, 998-1006.

Selman, K., Wallace, R. A., Sarka, A. and Qi, X. (1993). Stages of oocyte development in the zebrafish, *Brachydanio rerio*. *J. Morphol.* **218**, 203-224.

Sloman, K. A. (2010). Exposure of ova to cortisol pre-fertilisation affects subsequent behaviour and physiology of brown trout. *Horm. Behav.* **58**, 433-439.

Sloman, K. A., Motherwell, G., O'Connor, K. I. and Taylor, A. C. (2000). The effect of social stress on standard metabolic rate (SMR) of brown trout, Salmo trutta. *Fish Physiol. Biochem.* **23**, 49-53.

Sopinka, N. M., Hinch, S. G., Middleton, C. T., Hills, J. A. and Patterson, D. A. (2014). Mother knows best, even when stressed? Effects of maternal exposure to a stressor on offspring performance at different life stages in a wild semelparous fish. *Oecologia* **175**, 493-500.

Sopinka, N. M., Hinch, S. G., Healy, S. J., Harrison, P. M. and Patterson, D. A. (2015). Egg cortisol treatment affects the behavioural response of coho salmon to a conspecific intruder and threat of predation. *Anim. Behav.* **104**, 115-122.

Sopinka, N. M., Jeffrey, J. D., Burnett, N. J., Patterson, D. A., Gilmour, K. M. and Hinch, S. G. (2015). Maternal programming of offspring hypothalamic-pituitary-interrenal axis in wild sockeye salmon (*Oncorhynchus nerka*). *Gen. Comp. Endocrinol.*

Stratholt, M. L., Donaldson, E. M. and Liley, N. R. (1997). Stress induced elevation of plasma cortisol in adult female coho salmon (*Oncorhynchus kisutch*), is reflected in egg cortisol content, but does not appear to affect early development. *Aquaculture* **158**, 141-153.

Thomas, J. B. and Gilmour, K. M. (2006). The impact of social status on the erythrocyte β-adrenergic response in rainbow trout, *Oncorhynchus mykiss*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **143**, 162-172.

Weaver, I. C. G. (2009). Epigenetic effects of glucocorticoids. *Semin. Fetal Neonatal Med.* **14**, 143-150.

Welberg, L. A. M. and Seckl, J. R. (2001). Prenatal stress, glucocorticoids and the programming of the brain. *J. Neuroendocrinol.* **13**, 113-128.

Westerfield, M. (2000). *The Zebrafish Book. A Guide for the Laboratory use of Zebrafish (Danio rerio)*. Eugene: University of Oregon Press.

Wilson, K. S., Matrone, G., Livingstone, D. E. W., Al-Dujaili, E. A. S., Mullins, J. J., Tucker, C. S., Hadoke, P. W. F., Kenyon, C. J. and Denvir, M. A. (2013). Physiological roles of glucocorticoids during early embryonic development of the zebrafish (*Danio rerio*). *J. Physiol.* **591**, 6209-6220.