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Endogenous cortisol production and its relationship with feeding transitions in larval lake sturgeon (*Acipenser fulvescens*)

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A R T I C L E I N F O

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A B S T R A C T

Our understanding of the importance of cortisol in the development of fishes largely stems from teleosts and in particular the zebrafish, *Danio rerio*. However, studies examining the ontogeny of the cortisol endocrine axis in acipenseriformes (sturgeon and paddlefish) have demonstrated similar general patterns during early development. Beginning with maternal deposition of cortisol in the egg, followed by development of *de novo* synthesis, a hypo-responsive period, and finally the ability of the fish to appropriately increase whole-body levels of cortisol in response to a stressor. In the present study, we demonstrate a similar pattern of ontogeny in the cortisol response in lake sturgeon over two-year classes. Whole-body levels of cortisol were examined over two cohorts and found to be different in both concentration and timing of endogenous production. The 2016 cohort were found to have relatively high levels of cortisol and developed to first feeding approximately six days faster than the 2017 cohort with lower levels of cortisol. In the 2017 cohort, mRNA expression of *steroidogenic acute regulatory protein* (*StAR*) and *glucocorticoid receptor 1* (*GR1*) increased just prior to the increase in cortisol and associated onset of exogenous feeding. Treatment in metyrapone, an inhibitor of 11β-hydroxylase, significantly inhibited cortisol production and resulted in the inability of the fish to appropriately transition to exogenous feeding. Data suggest a potential key role for cortisol in lake sturgeon as they transition between diets during early life history.

1. Introduction

Ontogeny of the hypothalamic-pituitary-interrenal (HPI) axis in fishes has received significant attention in recent years (Faught et al., 2016a; Winberg et al., 2016; Nesan and Vijayan, 2016). Although it is recognized that the timing of development in the HPI axis is highly variable between species, a typical profile of whole-body cortisol in developing teleosts begins with high levels of maternally derived cortisol in the egg, followed by a decrease to near undetectable levels during embryogenesis, then the appearance of *de novo* synthesis post-hatch. *De novo* synthesis in teleosts is often followed by a period of hypo-responsiveness when the necessary molecular machinery is present for steroidogenesis. However, the fish lacks the ability to elevate circulating levels of cortisol following exposure to a stressor (Barry et al., 1995; Stouthart et al., 1998; Feist and Schreck, 2001; Jentoft et al., 2002; Pepels and Balm, 2004; Alderman and Bernier, 2009; Alsop and Vijayan, 2009; Tsalafouta et al., 2014).

Similar changes in egg, embryo, and whole-body concentrations of cortisol during embryogenesis and larval development have been documented in the acipenseriformes (sturgeons and paddlefish), and a hypo-responsive period during early development has been reported in white sturgeon, *Acipenser transmontanus* (Simontacchi et al., 2009), lake sturgeon, *Acipenser fulvescens* (Zubair et al., 2012), and Persian sturgeon, *Acipenser persicus* (Falahatkar et al., 2014). Interestingly, in both the white and lake sturgeon studies, while endogenous cortisol production begins at hatch, there is an additional increase in baseline values of whole-body cortisol coincidental with the transition from yolk to exogenous feeding (Simontacchi et al., 2009; Zubair et al., 2012).

Cortisol, the primary glucocorticoid in teleosts and acipenseriformes, is known to be involved in the regulation of organ maturation, developmental transitions, and metamorphosis in teleosts (Nesan and Vijayan, 2013). Manipulation of cortisol levels during embryogenesis in the zebrafish, *Danio rerio*, has been shown to result in mesodermal and organ defects, behavioural changes, and increases in mortality (Nesan et al., 2012; Nesan and Vijayan, 2012; Best et al., 2017). Conversely, glucocorticoids have been used to accelerate organ development in

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premature human infants (Chrousos and Gold, 1992) and in combination with thyroid hormones, corticosteroids are known to be important for appropriate regulation of amphibian metamorphosis (Norris and Dent, 1989) and larval fish development (de Jesus et al., 1991). A combination of cortisol and triiodothyronine (T_3) administration by immersion, in newly hatched Pacific threadfin, *Polydactylus sexfilus*, resulted in accelerated development of the gastrointestinal tract with the authors hypothesizing that cortisol may be involved in the promotion of nutrient uptake across the intestine during the critical time of first feeding (Brown and Kim, 1995), a period that is often coincidental with the onset of a fish's ability to mount an appropriate cortisol response following exposure to a stressor. Interestingly, the glucocorticoid receptor, *GR1*, was first detected in the intestine of the European seabass, *Dicentrarchus labrax*, at 12 days post-hatch (DPH) (Di Bella et al., 2008) a period coincidental with first feeding (approximately 9DPH) and appearance of an acute but transient response in whole-body cortisol following exposure to a chasing and disturbance stressor in this species (Tsalafouta et al., 2014). The expression of *GR1* is vital for proper development in zebrafish (Faught et al., 2016a, b) and has also been linked to feeding and weight maintenance in multiple species (Bernier and Peter 2001; Landys et al., 2004), suggesting the expression of *GR1* during early development could play a role in first feeding.

Initial growth and development in newly hatched oviparous fishes is sustained by the yolk, and after yolk absorption, individuals then undergo a brief larval phase prior to the juvenile phase. During this period of time there is significant morphological and functional development of the gastrointestinal tract that tends to occur in increments as opposed to a gradual developmental process (Wilson and Castro, 2010) and is often associated with changes in diet. In lake sturgeon these increments have been characterized as three distinct periods based on the appearance and activity levels of digestive enzymes. The first is as a yolk-sac larvae when the gastrointestinal tract is differentiating and digestive enzyme activities are low between 1-16DPH; the second when exogenous feeding commences and gastric secretions first occur between 14-18DPH; and the third when the gastrointestinal tract has taken on an adult phenotype and is fully functional between 24-30DPH (Buddington, 1985). In a hatchery, this third phase is often coincidental with a second dietary transition from a zooplanktivorous diet (e.g. artemia) to a carnivorous benthivore diet (bloodworm). Furthermore, in hatchery environments these periods of dietary change during early life history are known to be associated with high mortality in a number of fishes and are particularly prevalent in sturgeons (Buddington and Christofferson, 1985; Conte et al., 1988; Gisbert and Williot, 1997; Nilo et al., 1997; Boglione et al., 1999; Gessner et al., 2009).

In the present study, we examined the ontogenetic development of the cortisol response in developing lake sturgeon in two year classes, 2016 and 2017. In the first year of study, baseline levels of whole-body cortisol were monitored for up to 57DPH encompassing both the second and third dietary transition as described by Buddington (1985). As the 2016 study progressed it was evident baseline cortisol levels were increasing during feeding transitions. This formed the hypothesis for the 2017 study: that changes in whole-body cortisol may be related to dietary transitions during early development. To test this hypothesis, we assessed baseline levels of cortisol alongside transcriptional changes in *steroidogenic acute regulatory protein* (*StAR*) and *GR1* and whole-body levels of triglycerides, glucose and protein in control and metyrapone treated fish. Metyrapone was used to inhibit 11-βhydroxylase and therefore the production of endogenous cortisol (Wilson et al., 2013). Specifically, we expected a functioning HPI-axis stress response at the time of feeding and changes in protein, glucose and triglycerides to be reflective of dietary shifts. Furthermore, we predicted an increase in cortisol during dietary shifts and that increase would be blocked in the metyrapone treated fish resulting in inappropriate transition from yolk to exogenous feeding. Finally, we predicted that the increases in whole-body cortisol would be reflected in similar changes in mRNA expression of key genes involved in cortisol production and signaling, namely *StAR* and *GR1*.

2. Materials and methods

2.1. Fish Husbandry

In the spring of 2016 and 2017, eggs and sperm were collected from wild-caught, spawning female and male lake sturgeon from the Winnipeg River, Manitoba, and were transported to the University of Manitoba for fertilization. Approximately 50 ml of eggs from two and four females, in 2016 and 2017 respectively, were freely mixed with approximately 100 μl of sperm (Yoon et al., 2019). Multiple families were created each year using at least ten males to reduce potential confounding genetic effects on the resultant hatched larvae. De-adhesed embryos were incubated in tumbling jars at 12°C until hatch in early June (Monaco and Doroshov, 1983). Once larvae hatched, they were transferred to 16 9l aquaria with flow-through dechlorinated tap water maintained at 15 ± 1°C. Each tank had bio-balls for substrate until exogenous feeding began, then all substrate was removed over a 7-day period. 3–5 days prior to complete yolk-sac absorption, live artemia (Artemia International LLC, Texas, USA) were introduced to the tanks to begin familiarizing the larvae with an exogenous food source. After approximately two weeks (35–30 days post fertilization (DPF)), larvae were slowly transitioned to a bloodworm diet (Hikari USA, California, USA) over a 25-day period. Tanks were monitored three times a day, when mortalities were recorded and debris was removed. The highest mortality rates were observed during feeding transitions; approximately 25% mortality during the yolk-sac to artemia transition and approximately 30% from artemia to bloodworm transition. All animals used in this study were reared and sampled according to animal use and care guidelines established by the Canadian Council for Animal Care and approved by the Animal Care Committee at the University of Manitoba (Protocol #F15-007).

2.2. 2016 sampling

Samples were randomly selected beginning with the pre-fertilized egg until 57 days post-fertilization. (Fig. 1). To obtain baseline whole-body cortisol values, fish were transferred to an overdose of anesthetic (MS-222 0.5 g.l $^{-1}$) buffered with equal amounts of sodium bicarbonate within 10 s of netting from the tank. Samples were immediately transferred to −80°C for later analysis. The minimum detectable limit of the cortisol assay dictated the number of individuals required to obtain a measurable value for each sampling point. For example, unhatched embryos required combining a minimum of 5 individuals at each time point. Thus, for a sample size of eight at that developmental stage, a total of forty individuals were sampled. Post-hatch larvae required two to four individuals per sampling point. To avoid introducing bias due to diurnal changes in cortisol (Lankford et al., 2003), sampling was conducted between 11:00 am and 1:00 pm each day.

2.3. 2017 sampling

Samples were taken from randomly selected tanks from egg (pre-fertilization) to 25DPF as described above for baseline levels of whole-body cortisol and from 11DPF to 25DPF for analysis of mRNA expression of *StAR* and *GR1*. To determine the onset of endogenous cortisol production following exposure to a stressor, larvae were sampled after hatch from 11 to 25DPF. To elicit a cortisol response, larvae were transferred from their holding tank to a 500 ml glass bowl and chased for two minutes by gently prodding the tail with a blunt probe. They were then left undisturbed for 30 min, at which point the larvae were sampled by transfer to an overdose of anesthetic as described above. The post-stressor timepoint was selected as previous studies have shown elevated levels of whole-body cortisol at 20 min post-stressor in larval and juvenile lake sturgeon (Zubair et al., 2012; Allen et al.,

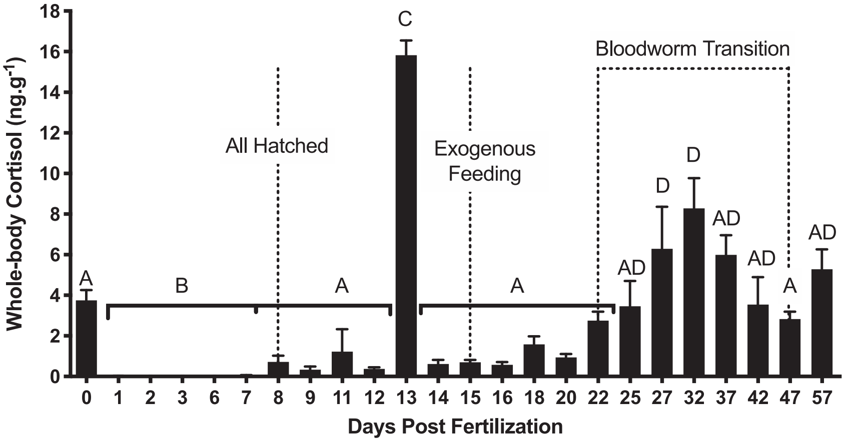

Fig. 1. Whole-body cortisol (ng.g^{-1} wet mass) pre-and post-fertilization in developing lake sturgeon raised in 2016. Data are expressed as a mean +/− SEM; n = 6–10, statistical significance designated by different letters where $p < .05$.

2009). Body mass and total length were then recorded for each individual from 11 to 25DPF, and samples were placed in a − 80°C freezer for later analysis.

2.4. *Metyrapone treatment*

At 13DPF, a subset of larvae was evenly divided between three experimental recirculating aquaria treated with 10 mg.l^{-1} metyrapone to inhibit activity of the enzyme 11β-hydroxylase and presumably endogenous cortisol production (Mommsen et al., 1999). 10 mg.l^{-1} metyrapone (approximately 44 μM) was used based on pilot immersion studies with 5, 10, 20 and 40 mg.l^{-1} over 24 h at 9 and 10DPH. Significant mortality occurred in the 20 and 40 mg.l^{-1} treatment, but none were recorded in the 5 and 10 mg.l^{-1} treatments. Interestingly, immersion of adult zebrafish for four days in 325.4 μM had no effect on mortality (Tea et al., 2019). Samples for whole-body cortisol analysis were randomly selected from the experimental treatment from 15DPF to 25DPF. Whole-body cortisol values for post-stressed metyrapone-treated fish are not reported because mortality in this treatment group exceeded 60% prior to completion of the 30-min, post-stress period, likely related to a failure to appropriately respond to the stressor. In addition to assessing the effects of metyrapone treatment on whole-body cortisol, we also assessed the effect of metyrapone treatment on whole-body glucose and protein content in comparison to control fish. Samples for analysis of whole-body protein were taken daily and samples for glucose were collected every second day from the metyrapone-treated and control fish between 13DPF and 19DPF. Due to high mortality in the metyrapone treated group sampling opportunity was limited, consequently frequency of sample collection was reduced for glucose analysis due to the number of fish required to obtain a measurable value. Samples for whole-body triglyceride analysis were only taken from the control fish between 12 and 27DPF as this assay required approximately 60 individuals to obtain a measurable level of triglyceride (see below).

2.5. *Whole-body cortisol*

Whole-body samples were homogenized with phosphate-buffered saline (PBS; 0.9% NaCl in PB and pH 7.4) and cortisol was extracted by solid phase extraction using C18-SEP-Pak cartridges (Waters Corporation; Milford, MA, USA) as previously described (Zubair et al., 2012; Deslauriers et al., 2018) or by liquid extraction using diethyl ether. Diethyl ether extractions were conducted as follows: 4 X volume of diethyl ether was added to each homogenized sample in a glass test tube and vortexed for 15 s, and the diethyl ether was transferred into a new tube; this was repeated three times for each sample and all samples were then dried down under a nitrogen stream at room temperature. Samples were then stored in a − 80°C freezer until further use. Each sample was individually reconstituted in 250 μl of radioimmunoassay (RIA) buffer (90 ml Milli-Q water, 10 ml PBS, 0.9 g NaCl, and 0.5 g bovine serum albumin) then split into duplicate 100 μl samples. 100 μl of tritiated cortisol (Perkin Elmer; 5000 disintegrations per minute (DPM)) and 100 μl of cortisol antibody (rabbit anti-cortisol polyclonal antibody; Fitzgerald Industries) were then added to each sample tube. Following vortexing, the samples were incubated at room temperature for one hour and then overnight at 4 °C. The following day, the assay was terminated by the addition of 100 μl charcoal separation buffer (1.25 g of charcoal and 0.125 g of dextran in 25 ml RIA buffer) to each sample. After vortexing, the samples were left on ice for 15 min and then centrifuged at 4°C for 30 min at 2500G (Hereaus Multifuge X3R, Thermo Fisher). The supernatant was then decanted into a 6 ml scintillation vial and 4 ml of scintillation fluid was added (Ultima Gold, Perkin Elmer). Each sample was counted on a liquid scintillation counter (Perkin Elmer; Tri-Carb 3110 TR) for 5 min. Unknown cortisol values were determined by interpolating against a standard curve, generated with each assay. Intra-assay and inter-assay variation were 13% and 7% respectively and extraction efficiency was 99.5 ± 1.32%. Serial dilution of samples showed good parallelism with the standard curve. Concentration of whole-body cortisol concentration using different extraction protocols was compared using a sample pool and confirmed to yield similar values.

2.6. *Whole-body gene expression*

Individuals were sacrificed in an overdose of anesthetic as described above, then fish were transferred to a vial containing 500 μl of RNA*later* (Invitrogen; Thermo Fisher Scientific; Lithuania). Samples were transferred to 4 °C for 24 h, then stored at −80 °C until RNA extraction and subsequent measurement of whole-body mRNA expression of *StAR* and *GR1* mRNA. Whole-body larvae were extracted for total RNA using a PureLink RNA Mini kit following the manufacturer's instructions (Invitrogen; Ambion Life Technologies; 12,183,025). RNA purity and concentration were assessed for all samples using a Nanodrop 2000c (Thermo Scientific) and RNA integrity was assessed by gel electrophoresis. Total RNA samples were then stored in −80°C until further use.

cDNA was synthesized from 1 μg of DNase treated RNA using a qScript cDNA Synthesis Kit following the manufacturers' instructions (Quantabio 95047-025). Briefly, 1 μg of RNA was added to a 96-well plate, on ice, and then DEPC-treated water was added to make a volume of 8 μl. The plate was sealed and briefly centrifuged before incubating at 25°C for 15 min in a thermocycler (SimpliAmp Thermal Cycler;

Table 1
List of reference genes, *StAR* and *GR1* forward and reverse primer sequences. Efficiencies listed as %.

Target gene	Forward primer	Reverse primer	Efficiency %
RPS6	CTGGCTGGATTCTGATTTGGATG	ATCTGATTATGCCAAGCTGCTG	96.9
RPL7	GAAGTCCAGGGCGACATAGC	TGAAGATCCTGACCGAGCGA	97.3
EF1α	GGTTGTCTTCCAGCTTCTTACCG	ACTGCTCACATTGCCTGCA	98.8
StAR	CCCGAGCAAAAAGGCTTCA	TTGGGCCGAAGAACAATACAG	99.7
GR1 (NR3C1)	GCCGGGATCAACAGCTACTT	CCAGGTGTGGACAACTCGAT	104.9%

Applied Biosystems). The plate was then put back on ice and 1 µl of 25 mM EDTA was added to all wells to inhibit the DNase I reaction. It was then sealed again and centrifuged before incubating at 65°C for 10 min. A master mix of nuclease-free water (5 µl per sample), reaction buffer (4 µl per sample), and reverse transcriptase (1 µl per sample) was made and 10 µl added to each well. A foil plate seal was then tightly adhered, the plate was centrifuged for 20 s, and run in the thermocycler (SimpliAmp Thermal Cycler; Applied Biosystems) under the following conditions: 1 cycle at 22°C for 5 min, 1 cycle at 42°C for 30 min, 1 cycle at 85°C for 5 min, and then held at 4°C. cDNA was then stored at −20°C until further use. qPCR reactions for each gene were completed with a master mix of 0.05 µl of both forward and reverse primers, 5 µl SsoAdvanced Universal SYBR Green Supermix, (BioRad, Hercules, CA, USA) and nuclease-free water to make a volume of 8 µl (Bio-Rad; 1725271), and 2 µl of cDNA was added to each well to make a final volume of 10 µl.

StAR primers were adapted from White Sturgeon primers, with the amplicon sharing over 60% homology with other vertebrates (Kusakabe et al., 2009; Table 1). Primers for *GR1* and the reference genes *Ribosomal Protein s6* (*RPs6*), *Ribosomal Protein L7* (*RPL7*) and, *elongation factor 1α* (*EF1α*) were created by using partial ovarian lake sturgeon sequences produced using pyrosequencing (Hale et al.,2009). Primer efficiency was assessed by using a 1:10 dilution standard curve for each primer set (Table 1). All reactions were completed using a QuantStudio 5 thermal cycler (Applied Biosystems; Thermo Fischer Scientific) on 384 well-plates and were run under the same cycling conditions: 2 min at 95°C, 40 cycles of 15 s at 95°C, 30 s at 60°C, and 30 s at 72°C. Melt curves for each qPCR reaction were run as follows: denaturation for 15 s at 95°C, a decrease for 1 min to 60 °C, and followed by a gradual increase of 0.075°C.second^{-1} back to 95°C. All expression data are normalized to the three reference genes, *RPS6, RPL7,* and *EF1α,* which were not significantly different across treatments or time points. Data are presented as ΔΔCt values and 11DPF unstressed control larvae were used as the control reference group.

2.7. Whole-body protein, glucose, and triglycerides

Oven-dried, whole-body larvae were homogenized in known volumes of 50 mM Tris buffer using a TissueLyser II (Qiagen, Hilden, Germany) for all three assays. Whole-body protein was measured in both control and metyrapone-treated fish using a commercially available Pierce Coomassie protein assay kit (Thermo Fisher, Waltham, MA, USA). To generate a standard curve, varying concentrations of BSA were combined with 150 µl 50 mM Tris buffer. The standards and samples were measured in triplicate in a 96-well plate and absorbance was quantified at 595 nm and 37°C. Absorbance values were measured using a PowerWave XS2 plate reader (BioTek, Winooski, VT, USA). Glucose levels were measured in both control and metyrapone-treated fish using a commercially available glucose kit (WAKO chemicals, Osaka, Japan). The standard curve was diluted 10× to ensure samples fell within the range of the standard curve. All standards and samples were measured in a 96-well plate, where absorbance was measured at 505 nm and 37°C, using the same instrument as above. Whole-body triglycerides required 60 larvae per measurement and as such were only measured in control fish given the high mortality rates observed in the metyrapone treated group. Triglyceride levels were measured using the LabAssay Triglyceride kit (WAKO chemicals, Osaka, Japan). All standards and samples were measured in triplicate in a 96-well plate. Absorbance values were obtained at 505 nm and 37°C using the same instrument as above.

2.8. Statistical analysis

One-way ANOVA followed by Tukey's post-hoc tests was used to test for significant differences over time within each measured variable and year. Two-way ANOVA followed by a Bonferroni's multiple comparison test was used to test for significant differences at each timepoint between control and stressed or metyrapone treated fish. Normality of data was assessed with Shapiro-Wilks test and Levenes test, and if the normality assumption was violated, data was log or rank transformed. Depending on the availability of fish, sample size ranged from $n = 5–10$ for each sampling point, except for body mass data which $n = 16–32$. Data are presented as mean ± SEM and were considered significant at $p < .05$. Statistical analyses were conducted using Graph Pad Prism 8 software.

3. Results

3.1. Changes in baseline whole-body cortisol levels 2016

Prior to fertilization, egg cortisol concentration was on average 3.74 ± 0.52 ng. g^{-1}. Following fertilization, cortisol values significantly dropped to negligible values (p-value < .001; 0.02 ± 0.01 ng.g^{-1}). *De novo* synthesis of cortisol began at hatch, significantly increasing at 8DPF from embryo values (p-value < .001; 0.631 ± 0.37 ng.g^{-1}). Baseline cortisol levels significantly increased two days before the onset of exogenous feeding (p-value < .001; 15.83 ± 0.72 ng.g^{-1}) at 15DPF (Fig. 1). At 22DPF, when bloodworms were introduced at a ratio of 1:10 bloodworm:artemia, cortisol values began to increase (2.75 ± 1.62 ng.g^{-1}) and peaked at 32DPF during this transition (9.69 ± 1.39 ng.g^{-1}) then returned to pre-transition levels by 37DPF when the food was approximately 50% bloodworm (2.84 ± 0.37 ng.g^{-1}) (Fig. 1).

3.2. Changes in baseline and peak whole-body cortisol levels 2017

Prior to fertilization, egg cortisol values were 0.51 ± 0.23 ng.egg^{-1}, substantially lower than the equivalent values in the previous year (Fig. 2A). Following fertilization, cortisol values during embryogenesis dropped to negligible values (0.026 ± 0.04 ng.g^{-1}). *De novo* synthesis of whole-body cortisol began at hatch, significantly increasing at 8DPF (p-value < .0001; 1.04 ± 0.40 ng.g^{-1}). Baseline whole-body cortisol levels significantly increased one day prior to exogenous feeding (p-value < .0001; 4.00 ± 0.63 ng.g^{-1}) at 20DPF (Fig. 2A). Following exogenous feeding, values decreased to 0.757 ± 0.14 ng.g^{-1} and stress-induced whole-body cortisol levels were consistently higher than baseline whole-body cortisol levels, although this was not statistically significant until 25DPF (mean baseline levels = 0.76 ± 0.14 ng.g^{-1}; mean peak levels = 1.31 ± 0.52 ng.g^{-1}) (Fig. 2B), indicating a period of hyporesponsiveness to a stressor between 11 and 25DPF in this cohort.

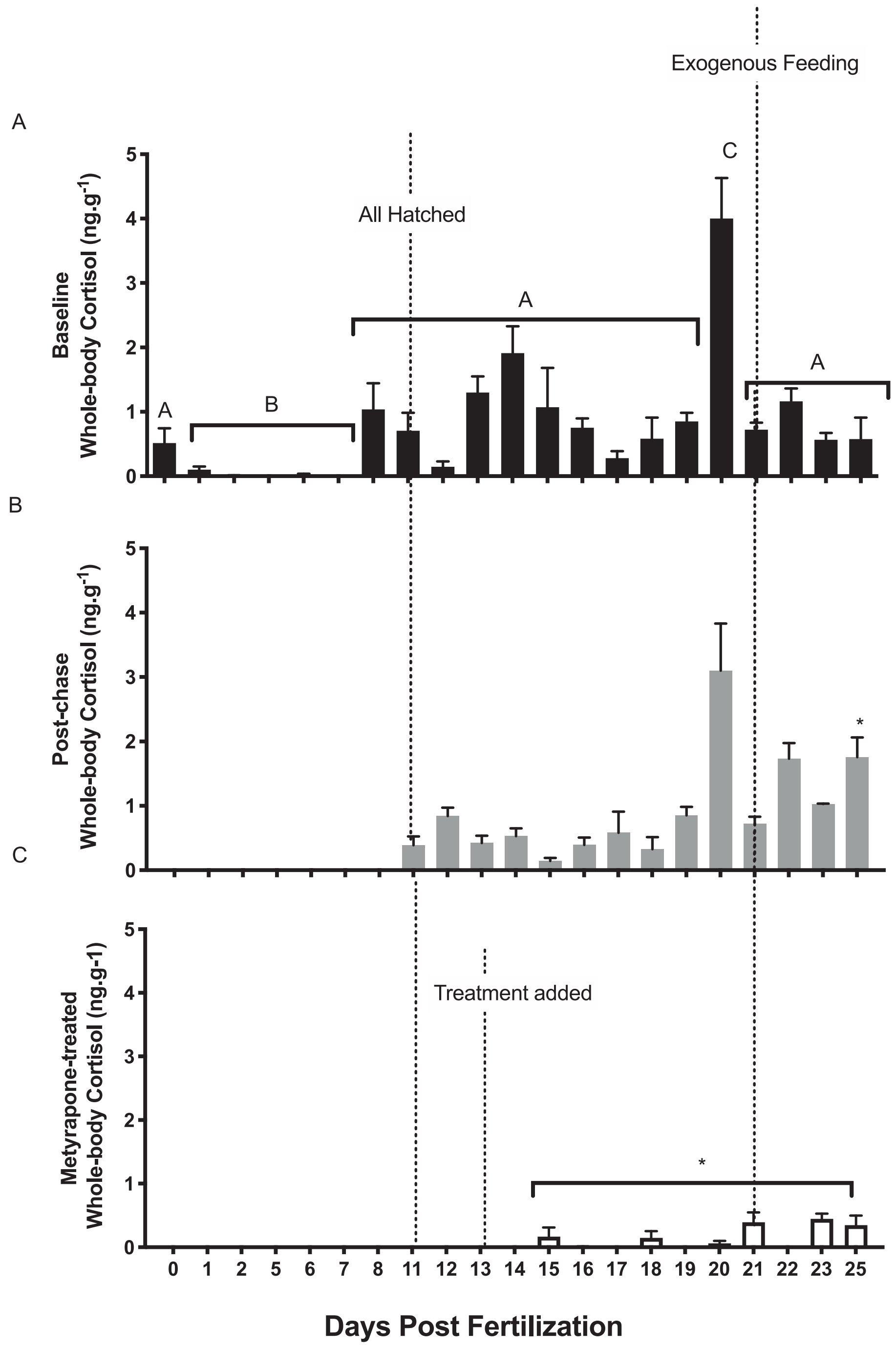

Fig. 2. (A) Whole-body cortisol (ng.g^{-1} wet mass) pre- and post-fertilization in developing lake sturgeon raised in 2017; (B) Whole-body cortisol (ng.g^{-1} wet mass) thirty minutes following an acute stressor in post-hatched lake sturgeon raised in 2017; and (C) Whole-body cortisol (ng.g^{-1} wet mass) in larval lake sturgeon raised in 2017 immersed in metyrapone-(10 mg.L^{-1}) from 13DPH to 25DPH. Data are expressed as a mean +/− SEM; n = 5–8; n = 5 for 18, 23, 25. Statistical significance within treatments is represented by different letters where $p < .05$. Statistical significance between treatments at comparable timepoints is represented by an asterisk where $p < .05$.

3.3. Effects of metyrapone treatment on development and whole-body cortisol levels

Metyrapone-treated fish had significantly lower whole-body cortisol (p-value < .05; 0.157 ± 0.05 ng.g^{-1}) from 15DPF to the end of the experiment when compared with baseline whole-body cortisol in control fish (1.06 ± 0.34 ng.g^{-1}) (Fig. 2C). In general, developmental rate was slower in metyrapone-treated fish. During the course of the experiment, metyrapone-treated fish never expunged their anal plugs suggesting a significant effect on gut development in this treatment. Further, developmental abnormalities including large edemas around the heart and abdominal organs and deformed rostrums were frequently observed in the metyrapone-treated fish (data not shown). Compared with control larvae, metyrapone-treated larvae had lower body mass overall (p-value < .0001), over time (p-value < .0001) and their interaction (p-value < .0001). These differences were apparent at days 13, 17, 19, 21, 23, and 25DPF (p-value < .05) (Fig. 3). Whole-body glucose did differ between control and metyrapone treated fish (p-value < .05) across comparable timepoints (p-value < .0001) (Fig. 4A) with 13DPF larvae in both treatments, control and metyrapone treated, exhibiting significantly higher values than all other timepoints. Whole-body protein was significantly different across timepoints (p-value < .00001) between control and metyrapone treated fish (p-value < .0001) and their interaction (p-value < .00001) (Fig. 4B). Whole-body triglycerides in the control fish increased significantly following exogenous feeding (p-value < .0001) (Fig. 4C).

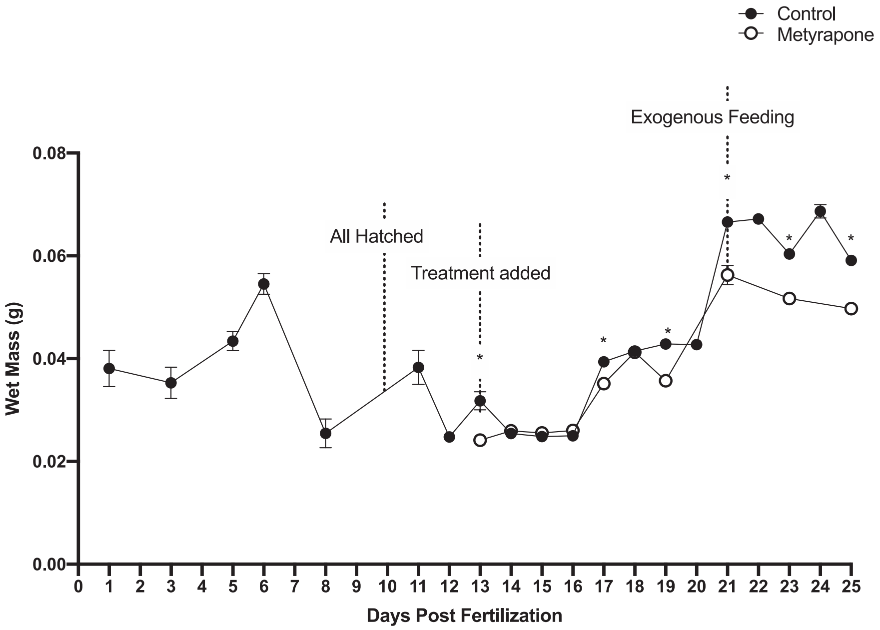

Fig. 3. Wet mass (g) of control (black circles) and metyrapone- treated larvae (open circles) raised in 2017. * indicates significant difference between control and metyrapone treated fish where $p < .05$. Data are expressed as a mean +/− SEM; n = 16.

3.4. mRNA expression

In the 2017 baseline control group, the relative expression of *StAR* following hatch remained constant until 16DPF when there was a transient increase in expression. At 18DPF *StAR* expression began to increase and was significantly higher by 19DPF (p-value < .005), 2 days prior to the onset of exogenous feeding. After 21DPF, the expression of *StAR* remained significantly higher at all time points compared to pre-feeding levels with the exception of 16DPF (Fig. 5). *GR1* expression followed a similar pattern throughout development as *StAR*, however, unlike *StAR*, there was no significant effect of time for *GR1* expression (Fig. 5).

4. Discussion

In this study, the overall pattern in both years of endogenous baseline whole-body cortisol production in lake sturgeon larvae followed the general pattern previously reported in teleosts and sturgeon (de Jesus et al., 1991; Gessner et al., 2009; Simontacchi et al., 2009; Nesan and Vijayan, 2012; Zubair et al., 2012; Falahatkar et al., 2014; Tsalafouta et al., 2014). Interestingly, absolute values between years were significantly different with the 2016 cohort having 3 to 4-fold higher cortisol values compared to the 2017 cohort. Why this is the case is unknown but likely the result of differential provisioning of yolk in eggs between females in the different years of study. The threshold of maternally derived cortisol that is absolutely necessary for appropriate embryogenesis and development (Nesan and Vijayan, 2013) *versus* excessive amounts that can lead to abnormal development (Hillegass et al., 2008; Nesan and Vijayan, 2012) is not known in sturgeons, but the between year values reported in the present study may have influenced the timing of diet transition from yolk to exogenous feeding.

Comparing 2016 and 2017 to previous years in our own research, 2017 first-feeding was more typical than what was observed in 2016. Thus, despite adults and developing fish being handled in a similar manner between years, the 2016 cohort had an accelerated development trajectory. A relationship between increased cortisol and earlier feeding has been reported for the Atlantic sturgeon, *Acipenser oxyrinchus*, where larvae raised over no substrate exhibited the highest whole-body cortisol values and began feeding earlier compared to larvae raised over a gravel substrate (Gessner et al., 2009). While it is evident that elevated cortisol may lead to accelerated development in sturgeon, why this is the case is not known. The timing of spawn in the females used in this study between years is not known, but it is tempting to speculate that differential provisioning of yolk between early and late female spawners may dictate developmental time and thus competition for resources at hatch; a phenomenon that has been used to explain differences in yolk sac conversion efficiency in brown trout displaying anadromous or resident life histories (Van Leeuwen et al., 2017).

The differences in brood year could also be explained by environmental conditions that year or the physiological status of the individual females. As the females are wild-caught our level of control over these parameters is very limited and it is important to recognize these may lead to differences year to year. Despite individual variability, wild-caught individuals are typically preferred by hatcheries over brood-stock individuals, as re-introductions completed using wild individuals are more successful than those using captive individuals (Fischer and Lindenmayer, 2000). Thus, these differences in developmental rate across individuals, whether driven by environmental or maternal physiological changes could be problematic in future hatchery settings. For example, if a hatchery spawns females with different egg cortisol levels in the same year, the subsequent offspring may feed at different times. In this case a standardized feeding approach could be detrimental to the faster developing individuals if food is not introduced at the appropriate time. In addition to timing of first feeding, the differences in cortisol between the two cohorts could have lasting effects on other behavioural phenotypes such as boldness (Moisiadis and Matthews, 2014). Boldness as a behavioural trait is of high importance to hatcheries as it is commonly linked to social dominance (Sundström et al., 2004) and predation risk (McLeod and Huntingford, 1994). Behavioural responses to high levels of cortisol are species-specific, as cortisol has been linked to both increased (Best et al., 2017) and decreased (Raoult et al., 2012) boldness. While cortisol production has been measured in many sturgeon species, whether cortisol has an effect on the development of behavioural phenotypes in sturgeon is unknown.

Lake sturgeon in both years showed an increase in baseline cortisol prior to exogenous feeding, similar to previous reports for lake, white, and Atlantic sturgeon (Gessner et al., 2009; Simontacchi et al., 2009;

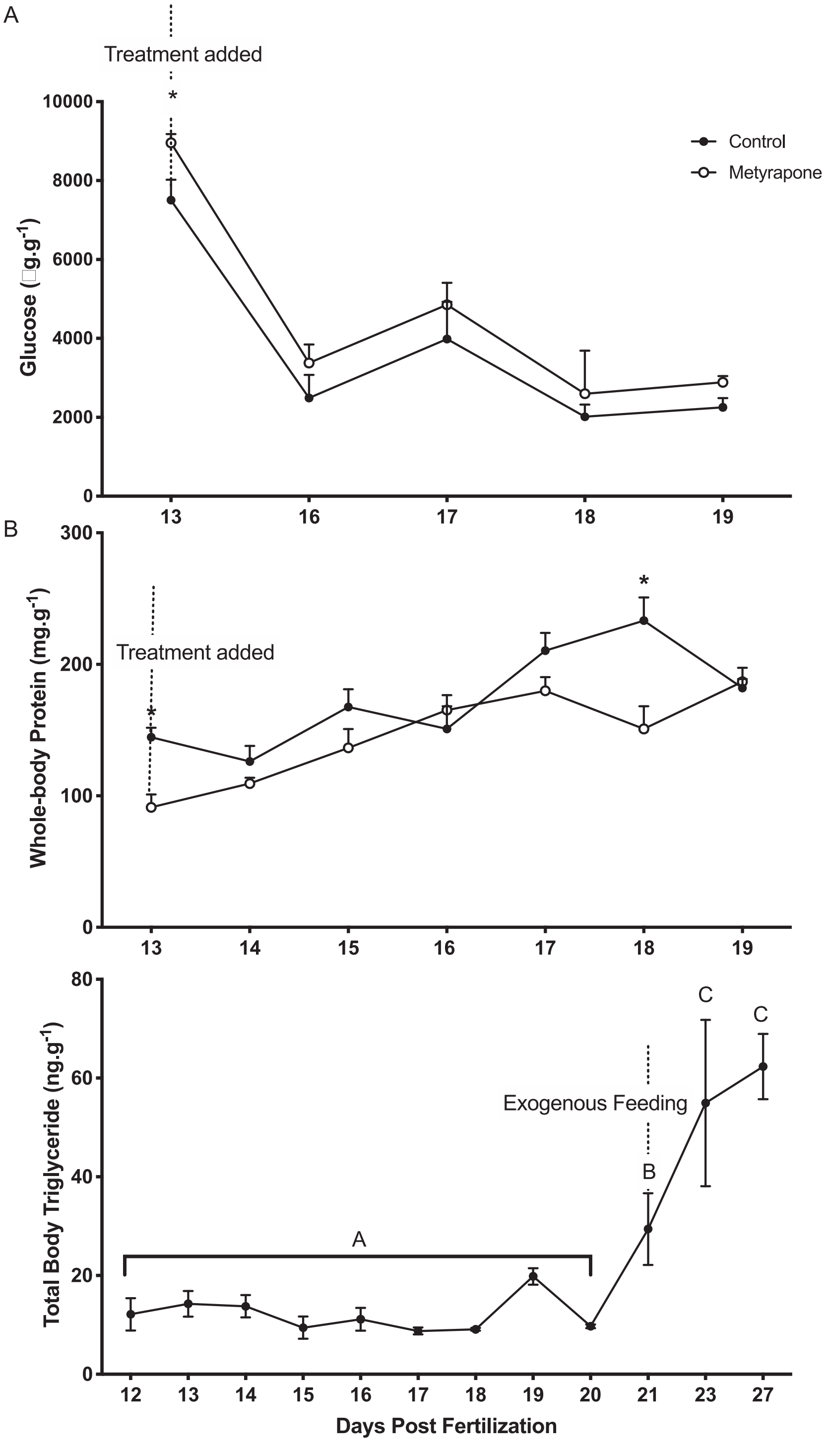

Fig. 4. (A) Whole-body glucose (μg.g^{-1}) (B) Protein (ng.g^{-1}) and (C) triglyceride (ng.g^{-1}) in control (black circles) and metyrapone-treated larvae (open circles) in lake sturgeon raised in 2017. Data are expressed as a mean +/− SEM; n = 5–10. * indicates significant difference between control and metyrapone treated fish where $p < .05$; different letters indicate significant difference between days post fertilization within treatment where $p < .05$.

Zubair et al., 2012). The increase in cortisol in all studies is coincidental with the switch from endogenous (yolk) to exogenous (artemia) feeding and presumably a significant remodeling of gut function. A role for cortisol during development of gut function in fishes has been implicated in the carp, *Catla catla*, where immersion treatment of 4DPH larvae in cortisol or a combination of cortisol and triiodothyronine (T_3) resulted in significant increases in growth and digestive enzyme activity for up to 30 days post-treatment (Khangembam et al., 2017). Similarly, this hormone combination resulted in increased gut differentiation rates and survival up to 29 days in Pacific Threadfin, *Polydactjlus sexfilis*, (Brown and Kim, 1995). While T_3 was not measured in this study, the increase in baseline levels of cortisol just prior to exogenous feeding, and during the switch from artemia to bloodworm, suggest a link between cortisol and dietary shifts in lake sturgeon that may be associated with known changes in gut development at these times (Buddington, 1985).

This conclusion is supported by our results from the metyrapone treated fish. Metyrapone is a known inhibitor of 11-β hydroxylase, the

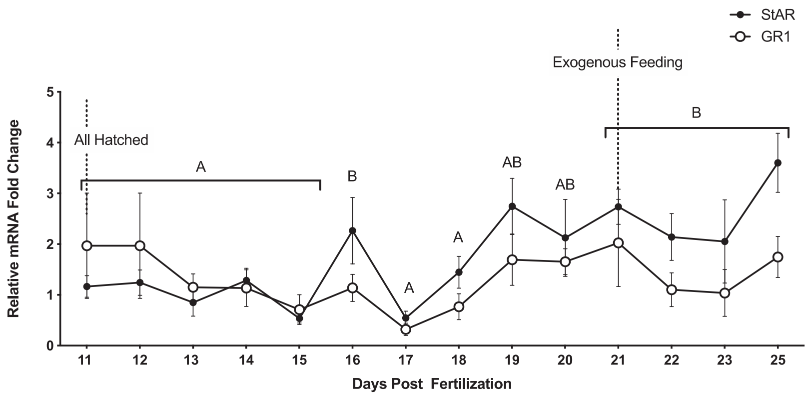

Fig. 5. Relative mRNA fold change of *StAR* (black circles) and *GR1* (open circles) in post-hatch larval lake sturgeon. *StAR* expression significance overtime is indicated by letters where $p < .05$. Control *GR1* expression not significantly different overtime. Data are expressed as a mean $+/-$ SEM; $n = 8$.

enzyme responsible for conversion of 11-deoxycortisol to cortisol (Mommsen et al., 1999), and has been used in a number of studies to inhibit cortisol production in fish, delivered by implants, (Bennet and Rhodes, 1986) injection, (Rodela et al., 2009; Rodela and Walsh, 2011; McConnachie et al., 2012) or immersion (Wilson et al., 2013; Tea et al., 2019). Baseline whole-body cortisol was measurable but significantly lower in the metyrapone-treated group compared to the control fish suggesting the treatment was effective at reducing endogenous cortisol production. A similar response was observed in zebrafish immersed in metyrapone during embryogenesis, with endogenous cortisol levels significantly lower than control even after hatch (Wilson et al., 2013). Interestingly, metyrapone also affected individual zebrafish swimming ability with decreases in both swim speed and activity (Wilson et al., 2013). Similar actions of metyrapone on swimming in lake sturgeon, in the present study, may therefore have impacted their foraging ability at first feed, alongside key sensory components such as electrosensing (Zhang et al., 2012) or olfaction (Brittebo et al., 1986; Kasumyan, 1999). Indeed, in rainbow trout, *Salmo gairdneri*, olfactory rosettes treated with metyrapone decreased binding affinity of the rosette epithelia to radio-labelled carcinogens (Brittebo et al., 1986). The authors concluded that the olfactory rosettes have cytochrome P450-dependent enzyme activity (Brittebo et al., 1986), which could be inhibited by metyrapone.

Unfortunately, we were unable to assess the cortisol responsiveness in the metyrapone treated fish due to high mortality following our 2-min chase protocol in this treatment. Conversely, larval zebrafish treated with metyrapone did not experience high levels of mortality following a stressor, but were unable to increase cortisol past baseline levels (Wilson et al., 2013). Despite the lack of appropriate development to feeding in the metyrapone treated fish, whole-body glucose and protein were largely similar between control and treated groups, likely as a result of provisioning from the yolk as opposed to exogenous food sources over the timeframe of the experiment as none of the metyrapone treated fish expelled their anal plugs compared to all the control fish expelling their plugs; a process that has been shown to indicate first feeding in sturgeon (Gisbert and Williot, 1997). Further, the control fish successfully transitioned to exogenous feeding, as evidenced by the rapid increase in whole-body triglycerides around the time of first feeding.

Understanding the association between the appearance of key molecular components in cortisol synthesis and endocrine signaling during early development is critical to understanding the role cortisol plays in directing development (Faught et al., 2016b). Here we measured two HPI-axis genes, *StAR*, the protein involved in the rate limiting step of steroidogenesis, translocating cholesterol from the outer to the inner mitochondrial membrane (Stocco et al., 2005); and *GR1*, the primary cortisol receptor for mediating glucocorticoid actions in teleosts (Faught et al., 2016b). *StAR* mRNA expression first occurs in the interrenal tissue at 24 h post-fertilization in the developing zebrafish embryo and has been shown to increase in response to cortisol antibody injections in larval zebrafish (Nesan and Vijayan, 2016). *GR1* transcripts are maternally deposited in the egg of zebrafish and are responsible for changes in developmental programming (Nesan and Vijayan, 2013). For example, morpholino knock-down of *GR1* in zebrafish induced neural, vascular, and visceral organ malformations (Nesan et al., 2012). The mineralocorticoid receptor (MR), has received less attention than glucocorticoid receptors in the context of development in fishes, however, it has also been implicated in developmental processes in rainbow trout, *Oncorhynchus mykiss* (Kiilerich et al., 2018), and European seabass (Tsalafouta et al., 2014). It is unknown what role, if any, MR may play in sturgeon HPI-axis development.

During development in teleosts, both *StAR* and *GR1* mRNA expression have been shown to increase post-hatch until the time of exogenous feeding, followed by a decrease or maintenance at elevated levels (Alsop and Vijayan, 2008; Kiilerich et al., 2018). In lake sturgeon, *StAR* mRNA expression increased two to three days prior to the onset of exogenous feeding and remained high until at least 25DPH. Although not significant, the similar pattern in *GR1* mRNA expression up to first feed is suggestive of a fully functional and responsive stress axis. However, the lack of a significant increase in whole-body cortisol following exposure to a stressor until 25DPH would indicate that the larvae were hypo-responsive to stress until that time. A similar delay in the ability to mount a cortisol response to a stressor has been previously shown in the lake sturgeon (Zubair et al., 2012) and white sturgeon (Simontacchi et al., 2009) and may be the result of a lack of hypothalamic factors or inability to appropriately perceive a stressor (Alsop and Vijayan, 2009). However, this may be sturgeon specific as teleosts, such as zebrafish, are capable of eliciting an endocrine stress response prior to feeding when exposed to an acute stressor, although this pattern is stressor dependent (Alsop and Vijayan, 2008; Alderman and Bernier, 2009).

Importantly, *StAR* and *GR1* mRNA were both measured in whole-body homogenates; thus, we cannot exclude the role these genes may have played in the synthesis of other steroids such as estradiol and testosterone as reported during early development in white sturgeon (Simontacchi et al., 2009). In future studies, tissue specific expression of these genes, in particular the gastrointestinal tract during early development, would elucidate a specific role cortisol may play in directing tissue specific development.

5. Conclusion

In this study we have demonstrated cohort differences in the magnitude and timing of cortisol appearance in early development of lake sturgeon. These differences are most likely influencing the development rate and trajectory of the individual, in particular the gastrointestinal tract with immersion in metyrapone having a significant effect on whole-body cortisol and development to facilitate appropriate dietary transition in this species. The specific mechanisms by which cortisol may influence dietary transitions in lake sturgeon are not known and may include influences on foraging, prey capture, digestion and/or assimilation of nutrients. In the context of wild fish, if timing of first feeding is dependent on cortisol from maternal input, there could be increased or decreased competition between offspring for resources depending on developmental rate. In the context of hatchery fish, understanding the role of cortisol in the development of the gastrointestinal tract will significantly improve rearing practices in hatcheries and thus the success of conservation programs for this imperiled species.

Declaration of Competing Interest

The authors declare no conflict of interest.

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References

Alderman, S., Bernier, N., 2009. Ontogeny of the corticotropin-releasing factor system in zebrafish. Gen. Comp. Endocrinol. 164, 61–69.

Allen, P.J., Barth, C.C., Peake, S.J., Abrahams, M.V., Anderson, W.G., 2009. Cohesive social behaviour shortens the stress response: the effects of conspecifics on the stress response in lake sturgeon Acipenser fulvescens. Journal of Fish Biology 74 (1), 90–104.

Alsop, D., Vijayan, M.M., 2008. Development of the corticosteroid stress axis and receptor expression in zebrafish. Am. J. Phys. 294, R711–R719.

Alsop, D., Vijayan, M.M., 2009. Molecular programming of the corticosteroid stress axis during zebrafish development. Comp. Biochem. Physiol. A. 153, 49–54.

Barry, T.P., Malison, J.A., Held, J.A., Parrish, J.J., 1995. Ontogeny of the cortisol stress response in larval rainbow trout. Gen. Comp. Endocrinol. 97, 57–65.

Bennet, R.O., Rhodes, R.C., 1986. Evaluation of oral administration of cortisol and metyrapone, the serum cortisol in rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 83, 727–730.

Bernier, N.J., Peter, R.E., 2001. The hypothalamic–pituitary–interrenal axis and the control of food intake in teleost fish. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 129 (2-3), 639–644.

Best, C., Kurrasch, D.M., Vijayan, M.M., 2017. Maternal cortisol stimulates neurogenesis and affects larval behaviour in zebrafish. Sci. Rep. 7, 40905.

Boglione, C., Bronzi, P., Cataldi, E., Serra, S., Gagliardi, F., Cataudella, S., 1999. Aspects of early development in the Adriatic sturgeon, *Acipenser naccarii*. J. Appl. Icthyol. 15, 207–213.

Brittebo, E.B., Darnerud, P.O., Larsson, J., Svanberg, O., Brandt, I., 1986. O-dealkylation of phenacetin in the olfactory rosette in rainbow trout (*Salmo Gairdneri*). Acta Pharmacol. Toxicol. 58, 259–264.

Brown, C.L., Kim, B.G., 1995. Combined application of cortisol and triiodothyronine in the culture of larval marine finfish. Aquacult. 135, 79–86.

Buddington, R.K., 1985. Digestive secretions of lake sturgeon, *Acipenser fulvescens*, during early development. J. Fish Biol. 26 (2), 715–723.

Buddington, R.K., Christofferson, J.P., 1985. Digestive and feeding characteristics of the chondostreans. Environ. Bio. Fish. 14 (1), 31–41.

Chrousos, G.P., Gold, P.W., 1992. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. JAMA 267, 1244–1252.

Conte, F.S., Dorohov, S.I., Lutes, P.B., Strange, E.M., 1988. Hatchery manual for white sturgeon *Acipenser transmontanus*. Publication 3322. University of California, Oakland, California.

Deslauriers, D., Yoon, G.R., Earhart, M.L., Long, C., Klassen, C.N., Anderson, W.G., 2018. Over-wintering physiology of age-0 lake sturgeon (*Acipenser fulvescens*) and its implications for stocking programs. Enviro. Biol. Fish. (4), 623–637.

Di Bella, M.L., Vazzana, M., Vizzini, A., Parrinello, N., 2008. Glucocorticoid receptor (D1GR1) is expressed in pre-larval and larval stages of the teleost fish *Dicentrarchus labrax*. Cell Tissue Res. 333, 39–47.

Falahatkar, B., Akhavan, S.R., Ghaedi, G., 2014. Egg cortisol response to stress at early stages of development in Persian sturgeon *Acipenser persicus*. Aquacult. Int. 22, 215–223.

Faught, E., Best, C., Vijayan, M.M., 2016a. Maternal stress-associated cortisol stimulation may protect embryos from cortisol excess in zebrafish. R. Soc. Opensci. 3, 160032. https://doi.org/10.1098/rsos.160032.

Faught, E., Aluru, N., Vijayan, M.M., 2016b. The molecular stress response. In: Schreck, C.B., Tort, L., Farrel, A.P., Brauner, C.J. (Eds.), Fish Physiology - Biology of Stress in Fish. 35. Academic Press, New York, USA, pp. 113–150.

Feist, G., Schreck, C.B., 2001. Ontogeny of the stress response in Chinook salmon, *Oncorhynchus tshawytscha*. Fish Physiol. Biochem. 25, 31–40.

Fischer, J., Lindenmayer, D.B., 2000. An assessment of the published results of animal relocations. Biol. Conserv. 96, 1–11.

Gessner, J., Kamerichs, C.M., Kloas, W., Wuertz, S., 2009. Behavioural and physiological responses in early life phases of Atlantic sturgeon (*Acipenser oxyrinchus*, Mitchill 1815) towards different substrates. J. Appl. Icthyol. 25, 83–90.

Gisbert, E., Williot, P., 1997. Larval behaviour and effect of the timing of initial feeding on growth and survival of Siberian sturgeon (*Acipenser baeri*) larvae under small scale hatchery production. Aquacult. 156, 63–76.

Hale, M.C., McCormick, C.R., Jackson, J.R., DeWoody, J.A., 2009. Next-generation pyrosequencing of gonad transcriptomes in the polyploid lake sturgeon (Acipenser fulvescens): the relative merits of normalization and rarefaction in gene discovery. BMC Genomics 10 (1), 203.

Hillegass, J.M., Villano, C.M., Cooper, K.R., White, L.A., 2008. Glucocorticoids alter craniofacial development andincrease expression and activity of matrixmetalloproteinases in developing zebrafish (*Danio rerio*). Toxicol. Sci. 102, 413–424.

Jentoft, S., Held, J.A., Malison, J.A., Barry, T.P., 2002. Ontogeny of the cortisol stress response in yellow perch (*Perca flavescens*). Fish Physiol. Biochem. 26, 371–378.

de Jesus, E.G., Hirano, T., Inui, Y., 1991. Changes in cortisol and thyroid hormone concentrations during early development and metamorphosis in the Japanese flounder, *Paralichthys olivaceus*. Gen. Comp. Endocrinol. 82, 369–376.

Kasumyan, A.O., 1999. Olfaction and taste senses in sturgeon behavior. J. Appl. Icthyol. 15, 228–232.

Khangembam, B.K., Ninawe, A.S., Chakrabarti, R., 2017. Effect of cortisol and triiodothyronine bath treatments on the digestive enzyme profile and growth of *Catla catla* during ontogenic development. Aquac. Res. 48, 2173–2185.

Kiilerich, P., Servili, A., Peron, S., Valotaire, C., Goardon, L., Leguen, I., Prunet, P., 2018. Regulation of the corticosteroid signalling system in rainbow trout HPI axis during confinement stress. Gen. Comp. Endocrinol. 258, 184–193.

Kusakabe, M., Zuccarelli, M.D., Nakamura, I., Young, G., 2009. Steroidogenic acute regulatory protein in white sturgeon (*Acipenser transmontanus*): cDNA cloning, sites of expression and transcript abundance in corticosteroidogenic tissue after an acute stressor. Gen. Comp. Endocrinol. 162, 233–240.

Lankford, S.E., Adams, T.E., Cech Jr., J.J., 2003. Time of day and water temperature modify the physiological stress response in green sturgeon, *Acipenser medirostris*. Comp. Biochem. Physiol. A. 135, 291–302.

Landys, M.M., Ramenofsky, M., Guglielmo, C.G., Wingfield, J.C., 2004. The low-affinity glucocorticoid receptor regulates feeding and lipid breakdown in the migratory Gambel's white-crowned sparrow Zonotrichia leucophrys gambelii. Journal of Experimental Biology 207 (1), 143–154.

McConnachie, S.H., Cook, K.V., Patterson, D.A., Gilmour, K.M., Hinch, S.G., Farrell, A.P., Cooke, S.J., 2012. Consequences of acute stress and cortisol manipulation on physiology behavior and reproductive outcome of female Pacific salmon on spawning grounds. Horm. Behav. 62, 67–76.

McLeod, P.G., Huntingford, F.A., 1994. Social rank and predator inspection in sticklebacks. Anim. Behav. 47, 1238–1240.

Moisiadis, V.G., Matthews, S.G., 2014. Glucocorticoids and fetal programming part 2: mechanisms. Nat. Rev. Endocrinol. 10, 403–411.

Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts, dynamics, mechanisms of action and metabolic regulation. Rev. Fish Biol. Fisher. 9, 211–268.

Monaco, G., Doroshov, S.I., 1983. Mechanical de-adhesion and incubation of white sturgeon eggs (*Acipenser transmontanus Richardon*) in jar incubators. Aquacult. 35, 117–123.

Nesan, D., 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., Vijayan, M.M., 2013. Role of glucocorticoid in developmental programming, evidence from zebrafish. Gen. Comp. Endocrinol. 181, 35–44.

Nesan, D., Vijayan, M., 2016. Maternal cortisol mediates hypothalamus-pituitary-interrernal axis development in zebrafish. Sci. Rep. 6, 22582.

Nesan, D., Kamkar, M., Burrows, J., Scott, I.C., Marsden, M., Vijayan, M.M., 2012. Glucocorticoid receptor signaling is essential for mesoderm formation and muscle development in zebrafish. Endocrinol. 153, 288–1300.

Nilo, P., Dumont, P., Fortin, R., 1997. Climatic and hydrological determinants of first year-class strength of St. Lawrence River lake sturgeon (*Acipenser fulvescens*). Can. J. Fish. Aquat. Sci. 54, 774–780.

Norris, D.O., Dent, J., 1989. Neuroendocrine aspects of amphibian metamorphosis. In: Schreibman, M.P., Scanes, C.G. (Eds.), Hormones in Development, Maturation, and Senescence of Neuroendocrine Systems. A Comparative Approach. Academic Press, New York, pp. 63–90.

Pepels, P.P.L.M., Balm, P.H.M., 2004. Ontogeny of corticotropin-releasing factor and of hypothalamic–pituitary–interrenal axis responsiveness to stress in tilapia (*Oreochromis mossambicus*; Teleostei). Gen. Comp. Endocrinol. 139, 251–265.

Raoult, V., Brown, C., Zuberi, A., Williamson, J.E., 2012. Blood cortisol concentrations predict boldness in juvenile mulloway (Argyosomus japonicus). Journal of Ethology 30 (2), 225–232.

Rodela, T.M., Walsh, P.J., 2011. Evidence for transcriptional regulation of the urea transporter in the gill of Gulf toadfish, *Opsanus beta*. Comp. Biochem Physiol. B. 160, 72–80.

Rodela, T.M., McDonald, M.D., Walsh, P.J., Gilmour, K.M., 2009. The regulatory role of glucocorticoid and mineralcorticoid receptors in pulsatile urea excretion of the gulf toadfish, *Opsanus beta*. J. Exp. Bio. 212, 1849–1858.

Simontacchi, C., Negrato, E., Pazzaglia, M., Bertotto, D., Poltronieri, C., Radaelli, G., 2009. Whole-body concentrations of cortisol and sex steroids in white sturgeon (*Acipenser transmontanus*, Richardson 1836) during early development and stress response. Aquacult. Int. 17, 7–14.

Stocco, D.M., Wang, X., Jo, Y., Manna, P.R., 2005. Multiple signaling pathways regulating steroidogenesis and steroidogenic acute regulatory protein expression: more complicated than we thought. Mol. Endoncrinol. 19, 2647–2659.

Stouthart, A., Lucassen, E., van Strien, F., Balm, P., Lock, R., Wendelaar Bonga, S., 1998. Stress responsiveness of the pituitary–interrenal axis during early life stages of common carp (*Cyprinus carpio*). J. Endocrinol. 157, 127.

Sundström, L.F., Petersson, E., Höjesjö, J., Johnsson, J.I., Järvi, T., 2004. Hatchery selection promotes boldness in newly hatched brown trout (Salmo trutta): implications for dominance. Behavioral Ecology 15 (2), 192–198.

Tea, J., Alderman, S.L., Gilmour, K.M., 2019. Social stress increases plasma cortisol and reduces forebrain cell proliferation in subordinate male zebrafish (*Danio rerio*). J. Exp. Bio. 222, jeb194894. https://doi.org/10.1242/jeb.194894.

Tsalafouta, A., Papandroulakis, N., Gorissen, M., Katharios, P., Flik, G., Pavlidis, M., 2014. Ontogenesis of the HPI axis and molecular regulation of the cortisol stress response during early development in *Dicentrarchus labrax*. Sci. Rep. 4, 5525.

Van Leeuwen, T.E., Killen, S.S., Metcalfe, N.B., Adams, C.E., 2017. Differences in early developmental rate and yolk conversion efficiency in offspring of trout with alternative life histories. Ecol. Freshw. Fish 26, 371–382.

Wilson, J.M., Castro, L.F.C., 2010. Morphological diversity of the gastrointestinal tract in fishes. Fish Physiol. 30, 1–55.

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., Denvir, M.A., 2013. Physiological roles of glucocorticoids during early embryonic development of the zebrafish (*Danie rerio)*. J. Physiol. 591 (24).

Winberg, S., Höglund, E., Øyvind, Ø., 2016. Variation in the neuroendocrine stress response. Fish Physiol. 35, 35–74.

Yoon, G.R., Deslauriers, D., Enders, C.E., Treberg, J.R., Anderson, W.G., 2019. Effects of temperature, dissolved oxygen, and substrate on the development of metabolic phenotypes in age-0 lake sturgeon (*Acipenser fulvescens*): impacts for overwintering survival. Can. J. Fish. Aquat. Sci. 76, 1596–1607.

Zhang, X., Song, J., Fan, C., Guo, H., Wang, X., Bleckmann, H., 2012. Use of electrosense in the feeding behavior of sturgeons. Integr. Zool. 7, 74–82.

Zubair, S., Peake, S., Hare, J.F., Anderson, W.G., 2012. The effect of temperature and substrate on the development of the cortisol stress response in the lake sturgeon, *Acipenser fulvescens*, Rafinesque (1817). Env. Biol. Fish. 93, 577–587.