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Abstract

This study investigated the response to acute stress in Atlantic sturgeon (*Acipenser oxyrinchus*) and HPI axis regulation of glucocorticoid production during stress by examining the expression of key genes—corticotropin-releasing hormone (*crh*), corticotropin-releasing hormone receptor (*crhr*), proopiomelanocortin (*pomca/pomcb*), melanocortin 2 receptor (*mc2r*), and steroidogenic acute regulatory protein (*star*)—that are associated with hypothalamus/pituitary/interrenal (HPI) axis of bony fishes. Additionally, tyrosine hydrogenase (*th1* and *th2*) and phosphoenolpyruvate carboxykinase (*pepck*), found in catecholamine-producing cells in the head kidney of the sturgeon, were investigated as alternative pathways of stress regulation outside of the HPI axis. We sought to test the hypothesis that there is an upregulation of one or several components of the HPI axis in *A. oxyrinchus* to produce a corticosteroidogenic response to acute stress. Atlantic sturgeons were exposed to an acute stressor and were sampled at 1, 6, and 24 h during recovery, whilst maintaining an unstressed control. The fish were examined for plasma cortisol, plasma glucose, and the relative abundance of *crh*, *crhr*, *pomca*, *pomcb*, *mc2r*, *star*, *th1*, *th2*, and *pepck*. Our investigations demonstrated a significant elevation in plasma glucose and cortisol in response to stress, however, there was no significant transcriptional upregulation of the expected corticosteroidogenic HPI axis components *crh*, *crhr*, *pomca*, *pomcb*, *mc2r*, and *star*. However, the investigation revealed significant upregulation of *th2* and *pepck* in response to stress, which provides a possible explanation for elevated plasma glucose levels that were observed. These findings indicate that there may be alternative pathways for stress-regulation of plasma glucose in *A. oxyrinchus* and further investigation is necessary for a more comprehensive understanding. Future studies directed at the stress-responsive regulation of cortisol and catecholamines may offer a greater understanding of the neuroendocrine pathways regulating steroidogenesis and gluconeogenesis during stress in *A. oxyrinchus* as well as basal vertebrates in general.

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**INVESTIGATING THE REGULATION OF THE HYPOTHALAMIC-PITUITARY-INTERRENAL AXIS IN
ATLANTIC STURGEON (*Acipenser Oxyrinchus*) FOLLOWING AN ACUTE STRESSOR**

An Honors Thesis By

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ABSTRACT

This study investigated the response to acute stress in Atlantic sturgeon (*Acipenser oxyrinchus*) and HPI axis regulation of glucocorticoid production during stress by examining the expression of key genes— corticotropin-releasing hormone (*crh*), corticotropin-releasing hormone receptor (*crhr*), proopiomelanocortin (*pomca/pomcb*), melanocortin 2 receptor (*mc2r*), and steroidogenic acute regulatory protein (*star*)—that are associated with hypothalamus/pituitary/interrenal (HPI) axis of bony fishes. Additionally, tyrosine hydrogenase (*th1* and *th2*) and phosphoenolpyruvate carboxykinase (*pepck*), found in catecholamine-producing cells in the head kidney of the sturgeon, were investigated as alternative pathways of stress regulation outside of the HPI axis. We sought to test the hypothesis that there is an upregulation of one or several components of the HPI axis in *A. oxyrinchus* to produce a corticosteroidogenic response to acute stress. Atlantic sturgeons were exposed to an acute stressor, netting, and were sampled at 1, 6, and 24 h during recovery, whilst maintaining an unstressed control. The fish were examined for plasma cortisol, plasma glucose, and the relative abundance of *crh*, *crhr*, *pomca*, *pomcb*, *mc2r*, *star*, *th1*, *th2*, and *pepck*. Our investigations demonstrated a significant elevation in plasma glucose and cortisol in response to stress, however, there was no significant transcriptional upregulation of the expected corticosteroidogenic HPI axis components *crh*, *crhr*, *pomca*, *pomcb*, *mc2r*, and *star*. However, the investigation revealed significant upregulation of *th2* and *pepck* in response to stress, which provides a possible explanation for elevated plasma glucose levels that were observed. These findings indicate that there may be alternative pathways for stress regulation of plasma glucose in *A. oxyrinchus* and further investigation is necessary for a more comprehensive understanding. Our findings fail to explain the increase in plasma cortisol levels that was observed. Future studies directed at the stress-responsive regulation of cortisol and catecholamines may offer a greater understanding of the neuroendocrine pathways regulating steroidogenesis and gluconeogenesis during stress in *A. oxyrinchus* as well as basal vertebrates in general.

INTRODUCTION

In mammals, the hypothalamic-pituitary-adrenal (HPA) axis is an endocrine relationship between the hypothalamus, pituitary gland, and adrenal glands with the primary function of regulating the body's stress response by controlling glucocorticoid production (Romero and Butler, 2007). When a stressful event occurs, stimulation of the sympathetic nervous system occurs where epinephrine and norepinephrine are released from the adrenal medulla and are released quickly into circulation (Shaughnessy & McCormick, 2021). Concurrently, the HPA axis is also stimulated. The hypothalamus releases corticotropin-releasing hormone (CRH) into a portal vein network connecting the hypothalamus and the pituitary gland, allowing the hypothalamus to communicate with the anterior pituitary (Clifton & Steiner, 2009). At the anterior pituitary, CRH binds to the corticotropin-releasing hormone receptor (Crhr), stimulating corticotropic cells in the anterior pituitary to release ACTH (adrenocorticotrophic hormone, a cleavage product of the proopiomelanocortin (POMC) precursor protein), which travels through the bloodstream to the adrenal cortex (Wendelaar Bonga, 1997), where ACTH binds to the melanocortin 2 receptor (Mc2r). When bound by ACTH, the Mc2r upregulates cAMP via its association with G α s protein and leads to the activation of protein kinase A. This ultimately results in the transcriptional upregulation and phosphorylation of steroidogenic acute regulatory protein (Star) (Abdel-Malek, 2001), the rate-limiting step in the biosynthesis and release of corticosteroids, involving a serial enzymatic conversion of cholesterol and transport from the outer to the inner mitochondrial membrane.

There is no HPA axis in the bony fishes as members of this group lack distinct adrenal glands like those present in mammals. Instead, bony fishes utilize an HPI axis (hypothalamic-pituitary-interrenal axis), wherein the interrenal tissue of the head kidney region is the putative site of corticosteroid production and release in bony fishes (Shaughnessy et al., 2023). Thus, in this way, the head kidney of the bony fish is analogous to the adrenal gland in mammals. The HPI axis regulates glucocorticoid production via an endocrine signaling pathway similar to the HPA axis found in mammals, including the expression of CRH in the hypothalamus, Crhr, and POMC/ACTH in the corticotropic cells of the anterior pituitary, and Mc2r and Star in the head kidney. In the HPI axis, the hypothalamus releases CRH at the median eminence, which binds to Crhr on corticotropic cells within the pituitary gland and stimulates the release of POMC-derived ACTH. Then, ACTH binds to Mc2r in the head kidney, stimulating the production of corticosteroids including upregulation of the rate-limiting step of Star transcription and phosphorylation.

Although much is known about the arrangement and stress-responsiveness of the HPI axis of derived bony fishes (i.e., teleosts), relatively little is known about the HPI axis of basal bony fishes, such as sturgeons (Acipenseridae). In sturgeons, components of an HPI axis have been identified (Shaughnessy et al., 2023), and cortisol has been identified as the predominate glucocorticoid and its circulating concentration in the blood has been shown to be stress-responsive (Shaughnessy & McCormick, 2021). However, whether and how an HPI axis regulates stress-responsive production of cortisol has not been adequately investigated. In this study, we examined the response to acute stress in Atlantic sturgeon (*Acipenser oxyrinchus*),

with a particular focus on HPI axis regulation of glucocorticoid production during stress by examining the expression of key genes that are believed to be involved in the signaling pathway of the HPI axis (Shaughnessy et al., 2023). These included the genes of *crh*, *crhr*, *pomca*, *pomcb*, *mc2r*, and *star*. Additionally, we investigated two genes encoding a tyrosine hydrogenase (*th1* and *th2*), which are involved with catecholamine synthesis in the head kidney, as well as a gene encoding phosphoenolpyruvate carboxykinase (*pepck*), which is a hepatic gluconeogenic enzyme. As there is very little known about the HPI axis in the lineages of fishes that predate the sturgeons, including the cartilaginous fishes (Chondrichthyes) and the jawless fishes (Agnatha), our work represents an investigation into the stress-responsiveness of an HPI axis of one of the most basal vertebrates studied to date.

METHODS

Live Animal Care

The Atlantic Sturgeon (*A. Oxyrinchus*) that were used in this investigation were handled and cared for following procedures that were approved by the Institutional Animal Care and Use Committees at the University of Massachusetts (Protocol No. 20160009) and U.S. Geological Survey (Protocol No. C0907). Atlantic Sturgeon juveniles were obtained 14 d after hatching from the Bears Bluff National Fish Hatchery (USFWS, Wadmalaw Island, SC, USA) and were reared at the Conte Anadromous Fish Research Laboratory (USGS, Tumers falls, MA, USA). The fish were held in 1.5 m diameter tanks supplied with 4 L min⁻¹ of dechlorinated municipal water with natural photoperiod and ambient temperature. The Atlantic Sturgeon were fed a progression of diets as they grew, including only live brine shrimp, then a mixture of bloodworms and a fine commercial pellet (Otohime, Reed Mariculture, Inc., USA), and then a diet of only a standard commercial pellet (Bio-Oregon, USA).

Experimentation and Tissue Sampling

For experimentation, the individual Atlantic sturgeon (~1-year post-hatch; 22.7 ± 3.3 cm total length; 39.9 ± 16.4 g mass) were randomly assigned to 'stressed' or 'unstressed' (control) groups. The procedure for the acute stress treatment consisted of 5 consecutive repetitions of 1 min netting (approximately 5 fish per 1.6 m² net) followed by 5 min confinement (5 fish confined to 15 L tank), lasting 30 min in total. After the acute stressor, fish were placed back in rearing tanks to recover and sampled at 1, 6, and 24 h during recovery. Unstressed fish were sampled alongside stressed fish as time-matched controls.

During sampling, the Atlantic Sturgeon were euthanized using a lethal dose of MS-222 (200 mg L⁻¹ buffered using NaHCO₃, pH 7.4). The fish were measured for body length and mass and then sampled for tissues. The tissues sampled included the brain (hypothalamus region), pituitary, head kidney (anterior-most region of the kidney), and liver. The tissues were immediately frozen and stored at -80 °C to be used in later RNA extraction. Blood was collected from the caudal vasculature into heparinized capillary tubes. Plasma was separated from the blood by centrifugation at 2000 g for 5 min.

Plasma Glucose and Cortisol

The levels of plasma glucose were determined against a standard curve in an assay using the enzymatic coupling of hexokinase and glucose 6-phosphate dehydrogenase (Stein, 1963). Plasma cortisol was determined by enzyme immunoassay (Carey and McCormick, 1998).

Gene Expression Analysis

The total RNA was extracted from frozen tissue using the TRIzol method by following the manufacturer's instructions (Molecular Research Center, Inc.). It was quantified and analyzed for purity using a Take3 micro volume plate reader (BioTek Instruments, Inc.). Only the high purity samples ($A_{260}/A_{280} > 1.9$) were used for the cDNA synthesis and real-time PCR analysis. A set of samples was examined electrophoretically to confirm the batch-level RNA integrity. The first strand cDNA was synthesized by using a high-capacity reverse transcription kit by following the manufacturer's instructions (Applied Biosystems Inc.). Real-time quantitative PCR was performed in 10 μ l reactions that contained 2 ng cDNA, 150 nmol L⁻¹ forward and reverse primers, and 1 \times SYBRselect master mix and by following the manufacturer's instructions (ThermoFisher Inc.). The thermal profile of the reactions was first 2 min at 50 °C, then 2 min at 95 °C (holding and activation), then 40 cycles of 15 s at 95 °C, then 1 min at 60 °C, 30 s at 72 °C (cycling), and finally, a ramp from 60 to 95 °C (melt curve analysis) was used to ensure there was a single product in each reaction. The relative abundance of *crh*, *crhr*, *pomca*, *pomcb*, *mc2r*, *star*, *th1*, *th2*, and *pepck* was calculated using the comparative method ($\Delta\Delta$ CT; Pfaffl, 2001) using *actb* as a reference gene.

Statistics

One-way ANOVA analyses was used to determine the significant difference over time between the control and stress groups. Individual T-tests were performed at each time point to determine significance between the control and stress groups at any given time point. An α -value of 0.05 was selected to denote statistical significance in all analyses and all P-values are presented in figures or figure captions.

RESULTS

Stress-Responsive Regulation of Plasma Glucose and Cortisol

Unstressed levels of plasma cortisol were at ~ 2.7 ng mL⁻¹ (Fig. 1A) and did not significantly change over time ($P = 0.64$, one-way ANOVA). In response to stress, plasma cortisol levels at the 1 h and 6 h time points increased significantly ($P_{1h} < 0.001$; $P_{6h} < 0.05$; t-test). At the 1 h time point, the plasma cortisol levels were at 24.2 ng mL⁻¹. At the 6 h time point, the plasma cortisol levels were at 9.9 ng mL⁻¹. By the 24 h time point, the plasma cortisol levels were no longer significantly increased ($P_{24h} = 0.41$; t-test) and were at 3.8 ng mL⁻¹. Unstressed plasma glucose levels were at ~ 4.8 mM (Fig. 1B) and did not significantly increase over time ($P = 0.24$; one-way ANOVA). In response to stress, plasma glucose levels at the 1 h and 6 h time points increased significantly, ($P_{1h} < 0.001$; $P_{6h} < 0.001$; t-test). At the 1 h time point, the plasma

glucose levels were at 9.9 mM. At the 6 h time point, the plasma cortisol levels were at 7.2 mM. By the 24 h time point, the plasma glucose levels were no longer significantly increased ($P_{24h} = 0.75$; t-test) and were at 4.9 mM.

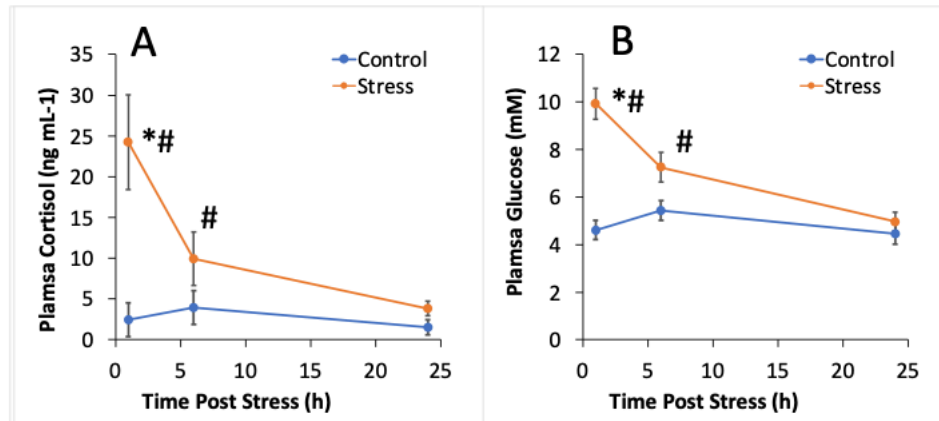


Fig. 1. Stress response in Atlantic sturgeon. Plasma concentrations of cortisol (A) and glucose (B) in Atlantic sturgeon at time 1, 6 and 24 h post stress. Data represent means \pm s.e.m. Asterisks indicates difference from control and stress at each respective time point. Pound symbol indicates difference from the mean control value.

Gene Expression in the Brain and Pituitary: *crh*, *crhr*, *pomca*, and *pomcb*

The relative abundance of *crh* mRNA in the hypothalamus (Fig. 2A) of the stressed fish were not significantly different from the unstressed fish at any of the time points 1 h, 6 h, and 24 h ($P_{1h} = 0.82$, $P_{6h} = 0.21$, $P_{24h} = 0.53$; t-test). The relative abundance of *crhr* mRNA in the pituitary (Fig. 2B) did have a significant decrease in response to stress at the 6 h time point when compared with the unstressed fish ($P_{6h} < .001$; t-test). The relative abundance of *crhr* mRNA in the pituitary (Fig. 2B) did not significantly increase in response to stress at the time points 1 h and 24 h when compared with the unstressed fish ($P_{1h} = 0.054$, $P_{24h} = 0.89$; t-test). The relative abundance of *pomca* mRNA in the pituitary (Fig. 2C) did not significantly increase in response to stress at the time points 1 h, 6 h, and 24 h when compared with the unstressed fish ($P_{1h} = 0.074$, $P_{6h} = 0.74$, $P_{24h} = 0.58$; t-test). The relative abundance of *pomcb* mRNA in the pituitary (Fig. 2D) did have a significant decrease in response to stress at the 1 h time point when compared with the unstressed fish ($P_{1h} < .05$; t-test). The relative abundance of *pomcb* mRNA in the pituitary (Fig. 2D) did not significantly increase in response to stress at the time points 6 h and 24 h when compared with the unstressed fish ($P_{6h} = 0.66$, $P_{24h} = 0.16$; t-test).

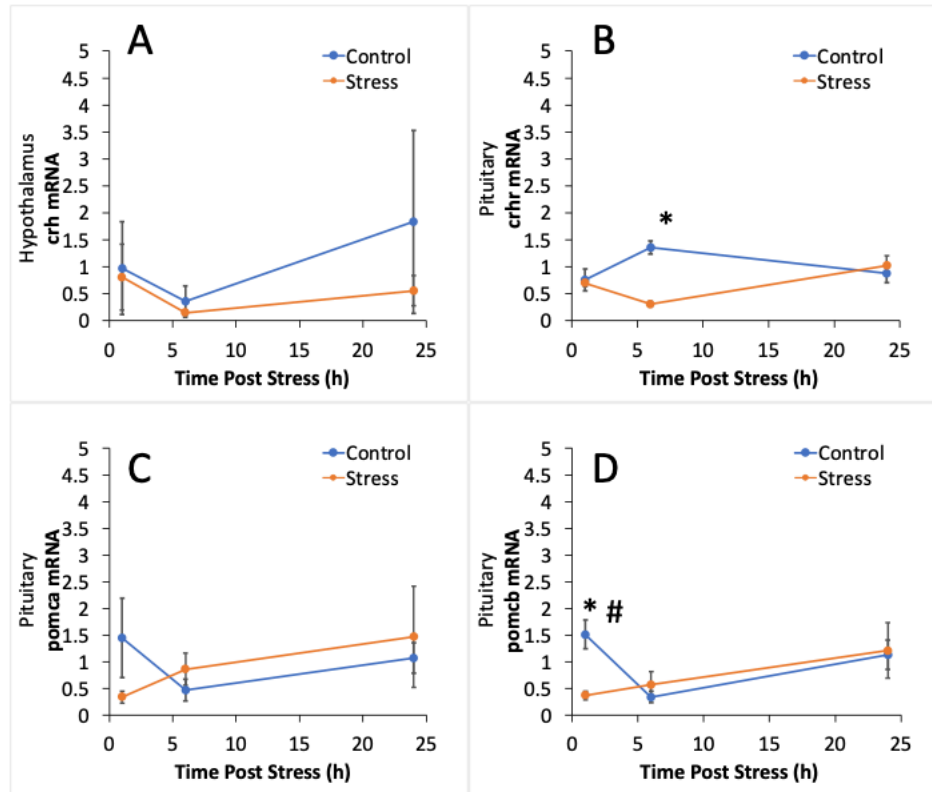


Fig. 2. Stress response in Atlantic sturgeon. mRNA concentrations in Atlantic sturgeon of crh in the hypothalamus (A), crhr in the pituitary (B), pomca in the pituitary (C), and pomcb in the pituitary (D) after 1, 6, and 24 h post stress. Data represent means \pm s.e.m. Asterisks indicates difference from control and stress at each respective time point. Pound symbol indicates difference from the mean control value.

Gene Expression in the Head Kidney: *mc2r*, *star*, *th1*, and *th2*

The relative abundance of *mc2r* mRNA in the head kidney (Fig. 3A) did not significantly increase in response to stress at the time points 1 h, 6 h, and 24 h when compared with the unstressed fish ($P_{1h}=0.97$, $P_{6h}=0.62$, $P_{24h}=0.19$; t-test). The relative abundance of *star* mRNA in the head kidney (Fig. 3B) did not significantly increase in response to stress at the time points 1 h, 6 h, and 24 h when compared with the unstressed fish ($P_{1h}=0.66$, $P_{6h}=0.21$, $P_{24h}=0.099$; t-test). The relative abundance of *th1* mRNA in the head kidney (Fig. 3C) did have a significant increase in response to stress at the 6 h time point when compared with the unstressed fish ($P_{6h}<.05$; t-test). The relative abundance of *th2* mRNA in the head kidney (Fig. 3D) did not significantly increase in response to stress at the time points 1 h, 6 h, and 24 h when compared with the unstressed fish ($P_{1h}=0.38$, $P_{6h}=0.73$, $P_{24h}=0.32$; t-test).

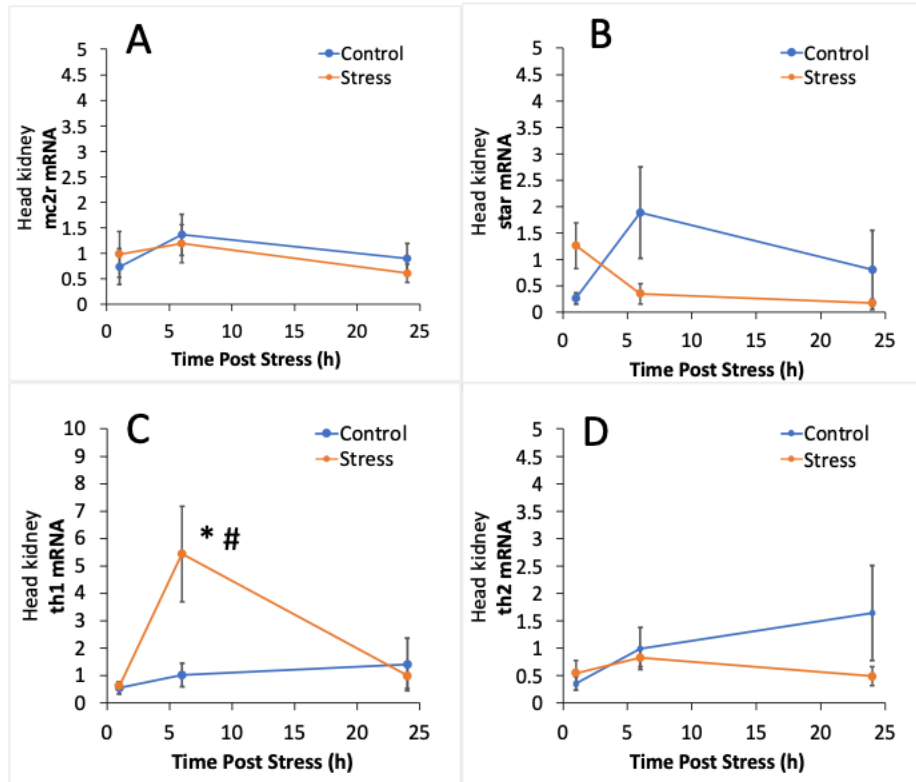


Fig. 3. Stress response in Atlantic sturgeon. mRNA concentrations in Atlantic sturgeon of mc2r in the head kidney (A), star in the head kidney (B), th1 in the head kidney (C), and th2 in the head kidney (D) after 1, 6 and 24 h post stress. Data represent means \pm s.e.m. Asterisks indicates difference from control and stress at each respective time point. Pound symbol indicates difference from the mean control value.

Gene Expression in the Liver: *pepck*

The relative abundance of *pepck* mRNA in the liver (Fig. 4) did have a significant increase in response to stress at the 1 h and 24 h time points when compared with the unstressed fish ($P_{1h} < .001$, $P_{24h} < .05$; t-test) The relative abundance of *pepck* mRNA in the liver (Fig. 4) did not significantly increase in response to stress at the time point 6 h when compared with the unstressed fish ($P_{6h} = 0.06$; t-test).

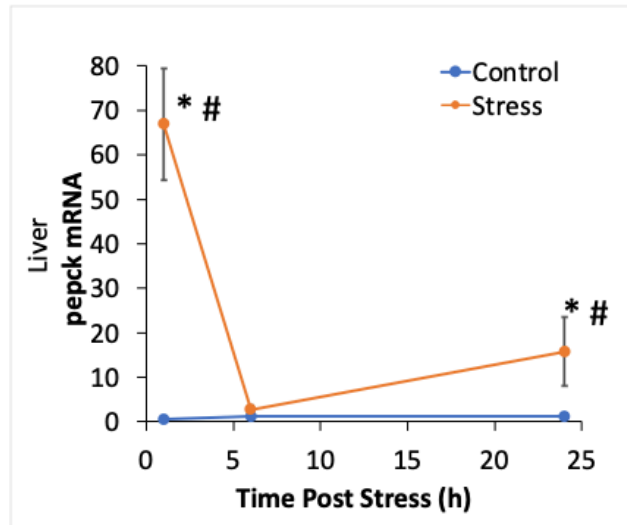


Fig. 4. Stress response in Atlantic sturgeon. mRNA concentrations in Atlantic sturgeon of *pepck* in the liver after 1, 6 and 24 h post stress. Data represent means±s.e.m. Asterisks indicates difference from control and stress at each respective time point. Pound symbol indicates difference from the mean control value.

DISCUSSION

This study aimed to investigate the stress-responsiveness of an HPI axis and to provide evidence of regulation of corticosteroidogenic response to acute stress in a basal bony fish, Atlantic sturgeon (*Acipenser oxyrinchus*). The plasma glucose and plasma cortisol levels were increased under stress, similar to previous studies of the HPI axis in fish (Jeffrey et al., (2012), Jeffrey et al., (2014), and liver *pepck* mRNA production was also significantly increased in response to acute stress, together demonstrating there was corticosteroidogenic and gluconeogenic acute stress responses in the Atlantic Sturgeon. If the HPI axis were regulating this gluconeogenic response, we would expect an upregulation of gene expression of one or several components of the HPI axis in *A. oxyrinchus* to produce a corticosteroidogenic response to acute stress. Similar studies show that in rainbow trout (*Oncorhynchus mykiss*) and Zebrafish (*Danio rerio*) there is upregulation of either or a combination of *pomca* (Pavlidis et al., (2015), Cortés et al., (2017), *pomcb* (Pavlidis et al., (2015), Cortés et al., (2017), *mc2r* (Pavlidis et al., (2015), Aluru & Vijayan, (2008) , Jeffrey et al., (2014), *crh* (Pavlidis et al., (2015), Jeffrey et al., (2012) , Jeffrey et al., (2014), or *crhr* (Pavlidis et al., (2015), and *star* (Jeffrey et al., (2012) , Jeffrey et al., (2014). However, in this study, there was no evidence of any upregulation of the investigated HPI axis gene components. The sturgeons subjected to the acute stress demonstrated similar levels of mRNA production of *pomca*, *pomcb*, *mc2r*, *crh*, *crhr*, and *star* to those of the control group, indicating there was no stress-responsive upregulation. Thus, our current results fail to support our hypothesis that the HPI axis of the Atlantic sturgeon is activated to promote corticosteroidogenesis in response to acute stress.

In derived bony fishes, an upregulation of certain HPI components to increase the corticosteroidogenic response after acute stress has been demonstrated. In rainbow trout

(*Oncorhynchus mykiss*), when exposed to an acute stressor, there was a significant increase in plasma cortisol and plasma ACTH levels. Plasma cortisol and plasma ACTH levels peaked at 1 hour post stress, after which they declined to similar levels of the control at hour 4 and 24 (Aluru & Vijayan, 2008). This is a similar response to acute stress as the Atlantic sturgeon in this study. It was demonstrated that ACTH upregulates *mc2r* mRNA levels, which corresponded with higher transcript levels of *star* and elevated cortisol production, suggesting *mc2r* activation by ACTH is the primary signaling pathway to corticosteroid biosynthesis in teleosts (Aluru & Vijayan, 2008). In sturgeon, ACTH does activate *mc2r*, which is expressed alongside *star* in the head kidney (Shaughnessy et al., 2023). However, no significant up-regulation of *mc2r* mRNA after acute stress was observed in the Atlantic Sturgeon in this study. In Zebrafish (*Danio rerio*), when exposed to an acute stress, there was a significant increase in plasma cortisol levels post-acute stress, which significantly decrease with time (Pavlidis et al., 2015; Cortés et al., 2017). This is a similar response to acute stress as the Atlantic sturgeon in this study. There was also a significant post stress up-regulation of *pomcb* (Cortés et al., 2017), *pomca*, *mc2r*, *crh*, and *crhr* levels (Pavlidis et al., 2015). The upregulation of these transcripts suggests that *pomca*, *pomcb*, *mc2r*, *crh*, and *crhr*, and thus the HPI axis, may have a role in the increased plasma cortisol levels that were seen post-acute stress in Zebrafish (Pavlidis et al., 2015; Cortés et al., 2017). However, we observed no such significant up-regulation of *pomca*, *pomcb*, *mc2r*, *crh*, and *crhr* mRNA post-acute stress in the Atlantic Sturgeon in this study.

The increase in plasma glucose and plasma cortisol indicate there is function of a stress responsive neuroendocrine axis. However, the lack of upregulation of the expected corticosteroidogenic components raises the possibility that there are alternative pathways, other than the HPI axis, that Atlantic sturgeon use to regulate their response to acute stress. Catecholamines, such as epinephrine and norepinephrine, are another class of gluconeogenic hormones regulated by the sympathetic nervous system that can cause an increase in plasma glucose and plasma cortisol. Tyrosine hydroxylases, TH1 and TH2, are important enzymes in the biosynthesis of catecholamines in adrenal gland or the head kidney. In a previous study in zebrafish, an upregulation of the expression of *th2* was observed after exposure to acute social stress, while there was no significant change in *th1* (Pavlidis et al., 2011). In our current study we observed similar results to the previous study in Zebrafish, as the Atlantic Sturgeon exhibited an upregulation of *th1* in the head kidney post-acute stress, while there was no significant change in *th2*. While the particular TH ortholog that was upregulated in response to stress differed between zebrafish and Atlantic sturgeon, the upregulation of *th1* in response to acute stress suggests that regulation of catecholamines, rather than the HPI axis, may be driving gluconeogenesis post-acute stress in Atlantic Sturgeon, and thus may be responsible for the increases in liver *pepck* and plasma glucose that were observed. However, this interpretation still fails to explain what is regulating the increase in plasma cortisol after acute stress, if not the HPI axis. The lack of upregulation of the *crh*, *crhr*, *pomca*, *pomcb*, and *mc2r* genes does not necessarily indicate a complete absence of involvement of the HPI axis, but it may suggest that the Atlantic Sturgeon HPI axis is already at a high level of expression. The expression of the target genes may be at a high level, making it unnecessary for the upregulation of the genes in response to acute stress. This interpretation provides a possible explanation for the increase in plasma glucose and plasma cortisol and the lack of upregulation

of the target genes that were investigated. Further investigation into the stress responsive regulation of cortisol and catecholamines in Atlantic Sturgeon will provide greater insight into the neuroendocrine pathways regulating steroidogenesis and gluconeogenesis during stress in basal vertebrates.

Conclusions

In this study, we investigated the expression of key genes that are involved in the signaling pathway of the *A. oxyrinchus* HPI axis. Our investigations demonstrate a lack of upregulation of the expected corticosteroidogenic components (*crh*, *crhr*, *pomca*, *pomcb*, *mc2r*, and *star*). However, our investigation revealed that plasma glucose levels in response to acute stress may be regulated by alternate pathways due to the upregulation of *th2* and *pepck*. Nevertheless, there is a lack of explanation of the increase in plasma cortisol and future studies aimed at the stress responsive regulation of cortisol and catecholamines may offer greater understanding of the neuroendocrine pathways regulating steroidogenesis and gluconeogenesis during stress in *A. oxyrinchus* as well as basal vertebrates as a whole.

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