INVESTIGATIONS INTO EFFECTS OF ENVIRONMENTAL STRESSORS ON LAKE STURGEON PHYSIOLOGY, BEHAVIOR, AND SURVIVAL DURING EARLY ONTOGENY

By

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ABSTRACT

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Environmental stressors experienced by wildlife can have profound impacts on behavior and physiology that may have consequences for survival. My dissertation investigates how early life stress influences physiology, behavior, and survival in lake sturgeon (*Acipenser fulvescens*), an ancient chondrostean fish species. Lake sturgeon are regionally threatened, and therefore exploring the mechanisms by which stressors influence fitness is important in informing conservation efforts. My dissertation examines behavioral and physiological outcomes of four potential stressors encountered by lake sturgeon larvae: high temperature, maternal-offspring environmental mismatch, captive rearing, and predator interaction.

In Chapter 1, I examined effects of temperature by comparing lake sturgeon reared at 10℃ (low stress) and 18℃ (high stress). During the free embryo stage, individuals reared at 18C exhibited a smaller cortisol elevation in response to an acute stressor, indicating lower physiological reactivity to stress. At the larval stage, individuals reared at 18C had higher levels of swimming activity and higher survival rates when exposed to a crayfish predator. Findings suggest that physiological and behavioral phenotypes induced by early life stress may be adaptive during subsequent life stages in high-stress contexts such as exposure to predators

In Chapter 2, I further explored the adaptive potential of stress-related phenotypes. Since stressed females can provision eggs with elevated cortisol that potentially prepares offspring for high-stress conditions, I investigated outcomes of a match or mismatch between egg cortisol and

offspring stress levels. Individuals that experienced both high egg cortisol and high stress had reduced cortisol reactivity to an acute stressor, but only in one of two families. Results suggest that family (genetic) effects may mediate the interaction of maternal and offspring stress experiences. The largest differences in swimming activity occurred between the "mismatch" treatments, indicating that the combination of egg cortisol and offspring stress is more important in determining offspring behavior than is egg cortisol or offspring stress alone.

In Chapter 3, I evaluated the role of stress in conservation programs by comparing stress levels, behavior, and predation rates for hatchery-produced and wild-caught lake sturgeon larvae. Cortisol levels did not indicate that hatchery-produced individuals were more stressed, but cortisol reactivity to an acute stressor disappeared for both hatchery-produced and wild-caught larvae after 9 days in the hatchery. Predation rates increased over time for larvae from both treatments, suggesting that the hatchery environment may inhibit survival even though individuals do not exhibit high stress. Results highlight that effects of captive rearing become evident after only a short duration spent in captivity during early ontogeny.

In Chapter 4, I investigated stress-related effects of encounters with aquatic insects commonly found in stream substrate during the free embryo stage. Lake sturgeon experienced stress proportional to the amount of predation risk experienced. Individuals that encountered Perlidae (obligate predators) had high mortality as well as elevated cortisol and cortisol reactivity to an acute stressor. Individuals that encountered Isonychiidae (facultative predators) had slightly elevated mortality compared to individuals that encountered no insects, and had slightly elevated cortisol levels and cortisol reactivity. Results indicate early life stress as a mechanism by which nonlethal predator interactions impact lake sturgeon, indicating the importance of community-level ecological context in predicting outcomes for species of conservation concern.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

cm	centimeter
ng	nanogram
mg	milligram
ml	milliliter
µl	microliter
min	minute
℃	degrees Celsius
CTU	Cumulative Thermal Units
HPLC-MS/MS	high-performance liquid chromatography-tandem mass spectrometry

INTRODUCTION

Stress is a pervasive and widely studied phenomenon among species, with a variety of potential outcomes that are often by default considered to be negative (Sheriff & Love 2013). However, as stated by Robert Green Ingersoll (1882): "There are in nature neither rewards nor punishments; there are consequences." (p. 71). Similarly, in an ecological and evolutionary framework, outcomes are not necessarily positive or negative. Rather, the adaptive or maladaptive value of stress to the individual, as well as significance at the population level, must be interpreted in the context of the environmental conditions in which they are expressed. The physiological and behavioral outcomes of stress may have adaptive value in certain environments, increasing individuals' fitness by promoting survival in high-stress conditions (Lynn et al. 2010). Understanding stress therefore necessitates evaluating its role within relevant ecological contexts (Sheriff & Love 2013).

Since stress outcomes must be understood within relevant contexts, wildlife species are valuable and important model systems for studying stress. Stress has been extensively studied in a lab setting using a rodent models (Hammels et al. 2015), and this has provided considerable insight into the physiological and epigenetic mechanisms that produce stress-related phenotypes. However, evolutionarily, stress as a biological system exists in a broader and more complex ecological setting than can be easily replicated in lab experiments. Therefore, studying stress in wildlife species provides opportunities to gain a more complete picture of the role of stress and the consequences of stress-induced phenotypes. Threatened wildlife species are especially useful model systems, since in light of global change, stress is an important mechanism by which environmental factors influence species of conservation concern. Wildlife stress responses can

be studied in terms of fitness within species' natural environment, thus offering greater insight into the adaptive or maladaptive value of stress outcomes.

My dissertation investigates stress using lake sturgeon (*Acipenser fulvescens*) as a model system. Lake sturgeon are an excellent model system for studying stress within relevant contexts. As poikilotherms, lake sturgeon physiology, phenotypes, and behaviors are closely tied to environmental conditions. Lake sturgeon also experience high early life mortality so fitness consequences can be quantified within a reasonable experimental period during early life stages, despite extreme longevity and delayed sexual maturity. Finally, lake sturgeon a regionally threatened wildlife species with populations that have been severely impacted by environmental factors, particularly those related to anthropogenic change. Therefore, understanding how stress influences fitness for lake sturgeon is relevant to informing to conservation and management efforts in the Great Lakes region, and also offers insight into the ecological role of stress for vulnerable wildlife species. I examine four contexts:

- In Chapter 1, I investigate the context of the environment under climate change, specifically high temperature as a stressor. Prior research has documented that lake sturgeon larvae are stressed by high temperatures (Zubair et al. 2012), but effects on fitness have not been quantified. Lake sturgeon early developmental rates are determined by temperature (Kempinger 1988), and adults rely on temperature cues for spawning (Smith & King 2005). Since temperatures in the Great Lakes region are predicted to rise 3-5 ℃ by the end of the century (Hayhoe et al. 2010), understanding effects of high temperature stress on lake sturgeon phenotypes, and how stress-related phenotypes will impact fitness, is important for predicting survival.

- In Chapter 2, I investigate the context of transgenerational mismatch, specifically a match or mismatch in stress levels between maternal and offspring environments. In lake sturgeon and other fish species, females experiencing stress provision eggs with elevated cortisol that can influence offspring developmental trajectories and prepare offspring for high-stress conditions (Giesing et al. 2010). However, if conditions change rapidly, maternal environment may not match offspring environment. Understanding how a match or mismatch between offspring stress levels and maternally-provisioned cortisol influences offspring fitness is important in understanding how wildlife may respond to rapidly changing environments.
- In Chapter 3, I investigate the context of conservation, specifically the hatchery conservation program as a potential source of stress. Reintroduction programs, including fish hatcheries, are important tools for wildlife conservation. However, captivity is a well-documented source of stress for individuals, and may result in phenotypes that are maladapted to wild environments after individuals are released (Berger-Tal et al. 2016). Understanding effects of captivity on lake sturgeon stress, behavior, and survival is important for informing conservation efforts.
- In Chapter 4, I investigate the context of ecological community, specifically inter-species interaction. Lake sturgeon obligately share habitat with a variety of benthic invertebrates during early life stages, some of which are potential predators (Bournaud et al. 1998). Encounters with these species may be important early life experiences that affect stress-related phenotypes and survival during subsequent life stages. Understanding the role of stress in interactions with other species is essential to gaining insight into the ecological context of stress.

For each of these four contexts, I focus on early life stages of lake sturgeon. Experiences during early ontogeny can induce long-term phenotypic changes, with consequences for fitness. I primarily focus on quantifying stress using cortisol levels, observing function of the stress axis, analyzing stress-related behaviors, and quantifying fitness using predation rates.

Together, these studies highlight the value and importance of exploring stress within an ecological framework. Quantifying context-dependent fitness consequences is necessary for advancing a scientific understanding of stress beyond the dichotomous paradigm of positive and negative and gaining insight into the evolutionary and ecological role of stress.

REFERENCES

REFERENCES

Berger-Tal, O., Blumstein, D. T., Carroll, S., Fisher, R. N., Mesnick, S. L., Owen, M. A., et al. (2016). A systematic survey of the integration of animal behavior into conservation. *Conservation Biology, 30*(4), 744-753.

Bournaud, M., Tachet, H., Berly, A., & Cellot, B. (1998). Importance of microhabitat characteristics in the macrobenthos microdistribution of a large river reach. *Annales de Limnologie-International Journal of Limnology, 3*4(1), 83-98

Giesing, E. R., Suski, C. D., Warner, R. E., & Bell, A. M. (2010). Female sticklebacks transfer information via eggs: effects of maternal experience with predators on offspring. *Proceedings of the Royal Society B: Biological Sciences, 278*(1712), 1753-1759.

Hammels, C., Pishva, E., De Vry, J., van den Hove, D. L., Prickaerts, J., van Winkel, R., ... & van Os, J. (2015). Defeat stress in rodents: from behavior to molecules. *Neuroscience & Biobehavioral Reviews, 59*, 111-140.

Hayhoe, K., VanDorn, J., Croley II, T., Schlegal, N., & Wuebbles, D. (2010). Regional climate change projections for Chicago and the US Great Lakes. *Journal of Great Lakes Research, 36*, 7-21.

Ingersoll, R. G. (1882). The Christian Religion: An Enquiry. United Kingdom: imprinted for B.E. and W.L.S.

Kempinger, J.J. (1988). Spawning and early life history of lake sturgeon in the Lake Winnebago System, Wisconsin. In R. D. Hoyt (Ed.), *Proceedings of the 11th Annual Larval Fish Conference: Symposium 5, Houghton, Michigan, 1–3 June 1987* (pp. 110–122). Bethesda, MD: American Fisheries Society.

Lynn, S. E., Stamplis, T. B., Barrington, W. T., Weida, N., & Hudak, C. A. (2010). Food, stress, and reproduction: short-term fasting alters endocrine physiology and reproductive behavior in the zebra finch. *Hormones and behavior, 58*(2), 214-222.

Sheriff, M. J., & Love, O. P. (2013). Determining the adaptive potential of maternal stress. *Ecology letters, 16*(2), 271-280.

Smith, K. M., & King, D. K. (2005). Dynamics and extent of larval lake sturgeon *Acipenser fulvescens* drift in the Upper Black River, Michigan. *Journal of Applied Ichthyology, 21*(3), 161-168.

Zubair, S. N., Peake, S. J., 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). *Environmental biology of fishes*, *93*(4), 577-587.

CHAPTER 1:

High-stress rearing temperature in *Acipenser fulvescens* affects physiology, behavior, and predation rates

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ABSTRACT

Early life stress can lead to long-term behavioral and physiological phenotypic alterations that impact fitness. Understanding effects of environmental stressors on wildlife is important to predict individual and population-level responses to stressors associated with climate change. Lake sturgeon, *Acipenser fulvescens*, are a regionally threatened fish species that experience high predation rates during larval stages. To investigate effects of a high temperature stressor, we exposed lake sturgeon eggs from four families to 10 ℃ (low-stress) or 18 ℃ (high-stress) rearing temperatures. At egg, free embryo and larval stages, we quantified stress levels for individuals from each treatment using whole-body cortisol analysis at baseline and after an acute stressor. At the larval stage, we videorecorded behavior trials to quantify swimming activity, and we conducted predation trials to quantify survival outcomes for individuals from high-stress and low-stress temperature treatments. Free embryos reared at 18 ℃ had a significantly smaller cortisol response after exposure to an acute stressor, indicating that chronic high temperature stress may reduce stress reactivity in lake sturgeon. In addition, larvae reared at 18 ℃ had significantly higher activity levels during behavior trials and significantly higher survival rates when exposed to crayfish predation, indicating that behavioral alterations induced by early life stress may be adaptive in high-stress contexts such as predation. These findings illustrate the need to experimentally evaluate fitness effects of stressors within ecologically relevant contexts in order to predict population- and community-level outcomes of climate change.

INTRODUCTION

The life history, physiology and behavior of poikilothermic vertebrates are heavily influenced by the environment. Early ontogenetic stages are especially susceptible, as environmental stressors may alter physiological and behavioral development, with profound consequences for survival (Biro et al., 2003; Sopinka et al., 2017). Quantifying effects of early life chronic stress on physiological and behavioral development is essential to understand the consequences of climate change and other anthropogenic disturbances at individual and population levels (Baker et al., 2013; Hofer & East, 1998).

Physiological and behavioral responses to stress are mediated by glucocorticoids (e.g. cortisol) (Schreck et al., 1997). In response to an acute stressor, the hypothalamic-pituitary-interrenal (HPI) stress axis stimulates production of cortisol (Lovallo & Thomas, 2000). Cortisol is important in regulating metabolism, immune system function (acting as an anti-inflammatory), the cardiovascular system and other physiological systems. An increase in cortisol levels after experiencing an acute stressor initiates behavioral responses as well as physiological responses to enable the individual to react to and survive the source of stress (Dickerson & Kemeny, 2004).

If stressors are experienced continuously, this chronic stress can trigger long-term HPI hyperactivity, in which the stress axis maintains a high level of activity that may outlast the initial source of stress. HPI hyperactivity occurs when the HPI stress axis is unable to regulate itself via the negative feedback loop, and instead continues to release elevated levels of corticotropin-releasing hormone from the hypothalamus, adrenocorticotropic hormone from the anterior pituitary and cortisol from the interrenal gland (Pariante & Lightman, 2008). Dysregulation can occur when sustained elevated cortisol levels result in downregulation of corticosteroid receptors in brain tissue (Meaney et al., 1985), which impairs the negative

feedback loop of the HPI axis and perpetuates chronic cortisol elevation (Jeanneteau et al., 2012). During early life stages, chronic stress can alter HPI axis function with long-term consequences for behavior (Lukkes et al., 2009; Turner et al., 2010).

HPI hyperactivity has been associated with altered behavioral phenotypes (Flandreau et al., 2012). In rodents, chronic stress results in persistent expression of anxious behaviors (Sterlemann et al., 2008). Similar outcomes are seen in poikilothermic vertebrates exposed to chronic stress. In zebrafish, *Danio rerio*, a 2-week unpredictable chronic stress regime resulted in reduced activity, lower swimming height in the water column and decreased social cohesion, along with elevated cortisol levels (Piato et al., 2011). Early life stress (1 min removal from water repeated during three early stages) in rainbow trout, *Oncorhynchus mykiss*, affected later HPI axis function (Auperin & Geslin, 2008). In sticklebacks (*Gasterosteus aculeatus*), chronic stress from predation risk defines long-term individual personality, based on associations between boldness and aggression (Bell & Sih, 2007).

Behavioral and physiological outcomes of stress can directly impact fitness (Cook et al., 2014). Stress axis hyperactivity decreases the likelihood of reproduction and survival (MacDougall-Shackleton et al., 2009; Romero & Wikelski, 2001), although the adaptive value of stress reactivity varies in response to environmental conditions. Thus, fitness consequences of stress may be context dependent (Breuner et al., 2008). For example, HPI hyperactivity resulting from chronic stress can intensify antipredator behavior (Schreck et al., 1997), which may increase survival in a high-risk environment (Boonstra, 2013). Fish are especially sensitive to environmental stressors, and early life environments have been shown to adaptively influence long-term behavioral phenotypes, including antipredator behavior (Ebbesson & Braithwaite, 2012; Galhardo & Oliveira, 2009; Wishingrad et al., 2014a; Wishingrad et al., 2014b).

Immediate survival benefits may thus represent a trade-off with the long-term costs of stress. Therefore, researchers have suggested that quantifying the adaptive or maladaptive potential of stress-mediated phenotypes requires incorporating tests of fitness in ecologically relevant contexts (Boonstra, 2013; Sheriff & Love, 2013; Sopinka et al., 2016).

Understanding fitness effects of early life chronic stress is especially important for wildlife species exposed to environmental stressors associated with anthropogenic disturbance such as climate change (Baker et al., 2013; Hofer & East, 1998). Stress-related alterations to important functions such as antipredator behavior and reproduction may have negative impacts on threatened populations. Lake sturgeon, *Acipenser fulvescens*, are a regionally threatened fish species that are susceptible to environmental stressors during early life stages and may express behavioral alterations that affect survival (Crossman et al., 2018; Wishingrad et al., 2015). After overexploitation and habitat disturbance caused declines in populations across North America (Ferguson & Duckworth, 1997), lake sturgeon are now a priority for conservation in the Great Lakes basin. Lake sturgeon reach sexual maturity after approximately 20 years and congregate in rivers to spawn during the spring (Peterson et al., 2007). At hatch, free embryos immediately burrow into substrate and emerge as larvae once yolk sac reserves have been depleted (Hastings et al., 2013). At the larval drift stage, lake sturgeon begin exogenous feeding and disperse downstream from spawning areas to suitable larval rearing habitat (Duong et al., 2011).

Behavior and survival during the larval period is of particular interest since predation during this period can negatively affect recruitment in fishes (Dudley & Matter, 2000; Silbernagel & Sorensen, 2013). Predation on lake sturgeon during the period of larval drift accounts for a large portion of the high level of mortality experienced during the first year of life (Waraniak et al., 2018). Antipredator behavioral phenotypes in sturgeon larvae have been shown

to affect predation, survival and recruitment (McAdam, 2011). Sturgeon are known to alter antipredator behaviors according to environmental factors during early ontogenetic stages (Crossman et al., 2018; Wishingrad et al., 2015). Therefore, the role of early life environmental stressors in programming behavioral development, especially related to antipredator responses, may be critical in determining survival (Biro et al., 2003), with implications for threatened populations.

In lake sturgeon, chronic stress experienced during early life stages may trigger developmental changes that influence probability of mortality during periods of high larval predation. Embryonic production of cortisol, the primary circulating glucocorticoid in sturgeon, begins around 3 days after egg fertilization (De Jesus, 1991). The HPI axis is functional by the third day posthatch, as seen by increases in cortisol in response to an acute stressor (Falahatkar et al., 2009; Li et al., 2012; Simontacchi et al., 2009; Wuertz et al., 2006).

Warm water temperature has been shown to be a stressor during early ontogenetic stages (Bates et al., 2014; Dammerman et al., 2016; Van Eenennaam et al., 2005; Zubair et al., 2012). Therefore, warm temperature during egg incubation and during the free embryo stage immediately after hatch may represent a chronic stressor and may have physiological and behavioral effects that influence larval susceptibility to predation. Effects of temperature stress on lake sturgeon development is particularly concerning in the current era of climate change, which is expected to have profound impacts on fish populations.

Lake sturgeon are an important model system for investigating mechanisms of plasticity in the context of changing environmental conditions. Since lake sturgeon take over 20 years to attain reproductive maturity, they lack the ability to evolve rapidly, limiting their ability to respond genetically to environmental change. Historic overharvest has already numerically

bottlenecked lake sturgeon populations, reducing genetic variation, and stressors associated with climate change will most likely continue to negatively affect recruitment for threatened populations. In the Great Lakes region, air temperatures are expected to increase by 3-5 ℃ by the end of the century (Hayhoe et al., 2010), and habitat suitability for *Acipenseridae* is predicted to decrease by 5.5% as a result of climate change (Comte et al., 2012). Warming stream temperatures cause shifts in the distribution of fish populations, but these shifts do not occur at a fast enough pace to avoid detrimental effects of climate change (Comte & Grenouillet, 2013). Understanding how temperature stress during early ontogeny affects larval survival is important to predict effects of climate change on lake sturgeon population recruitment and long-term viability.

This study seeks to quantify effects of temperature on HPI axis development and stress-related behaviors on lake sturgeon, as well as consequences for survival of predation during the vulnerable larval stage. Behavioral consequences of environmental stressors are particularly important to consider within the ecologically relevant context of predation (Sheriff & Love, 2013; Storm & Lima, 2010). Understanding the effect of environmental stressors on fitness will provide insight into the adaptive or maladaptive nature of stress-related behaviors and will help inform conservation efforts for lake sturgeon and other threatened wildlife species.

METHODS

We collected gametes from four female lake sturgeon and two male lake sturgeon spawning in the Upper Black River in Onaway, MI in May 2016. All males and females were captured on the same day (3 May 2016), during the first spawning period of the spawning season while the water temperature was around 10 ℃. During capture and gamete collection from spawning adults, stress was minimized by ensuring that each individual's head and gills remained

in the water at all times, and each individual was only handled for approximately 4 min while gametes were extruded noninvasively before being returned to the stream to resume spawning. Eggs were fertilized that same day according to standard hatchery procedures (Crossman et al., 2011), with sperm from each of the two males used to fertilize eggs from two of the females according to the following crosses: F60 x M48, F66 x M40, F69 x M48, F43 x M40. The nested paternal half-sib design (1:2 male:female cross ratio) was chosen with the limitations of egg and sperm availability from adult sturgeon captured that day, to avoid any confounding effects of different start dates for different families included in the experiment. Fertilized eggs from each of the four females were divided between warm (18 ± 1 ℃) and cold (10 ± 1 ℃) recirculating tank systems that were temperature-controlled using a heater (SmartOne Heater: 1000 W, Model S1T1111, Process Technology, Willoughby, OH, U.S.A.) and chillers (chiller 1: JBJ Arctica DBE-200 3000 BTU/h Chiller (¼ hp, 180 W), Transworld Aquatic Enterprises, Inglewood, CA, U.S.A.; chiller 2: Pacific Coast Imports ½ hp C-0500 Aquarium Chiller (1700 W, 6000 BTU/h), Transworld Aquatic Enterprises). Although water temperatures of 10-18 ℃ are within the typical range in the Upper Black River during the spawning season and thus are ecologically relevant, we considered 18 ℃ to be a high-stress treatment based on prior studies (Bates et al., 2014; Van Eenennaam et al., 2005; Zubair et al., 2012).

Six replicates, each containing 286 eggs, were used per family in each of the two temperature treatments (a total of 48 biological replicates). Earlier research pertaining to growth responses to temperature (Dammerman et al., 2016) was a guide for selection of sample sizes. Fertilized eggs were randomly allocated into each replicate and treatment by hatchery technicians who were blind to treatment. Each of the recirculating tank systems included a heath tray stack. Each replicate was contained in a 4-inch (10.16 cm) diameter coupling made of PVC

plastic and mesh, and couplings were randomly assigned to trays in the heath tray stack. Trays were rotated every 2 days throughout the experiment to avoid any effects of tray levels on offspring. Since each tank system was supplied with stream water from the same source, any biologically active compounds that may have been recirculating through tanks would have been universally in contact with eggs and offspring of all families in both warm and cold treatments. In addition, tank systems were thoroughly disinfected using dilute citric acid and betadyn solutions and rinsed thoroughly prior to the experiment, in order to prevent the presence of any biologically active agents that could have diffused among replicates. The recirculating tank system used for this experiment has been successfully used for previous studies on effects of different temperature regimes on sturgeon (Dammerman et al., 2016).

Since lake sturgeon developmental rates vary depending on water temperature, sampling schedules were based on cumulative thermal units (CTUs) calculated for each temperature treatment (Kempinger, 1988; Smith & King, 2005). CTUs use mean daily water temperature in degrees Celsius (x_i) and a constant (k, 5.8 ℃) to predict developmental stages:

$$\text{CTU} = \sum_{i=1}^{n} (x_i - k)$$

Fertilized egg cortisol samples were taken at approximately 36 CTUs (approximately half-way through egg incubation), at which point all maternally derived cortisol has diffused out of the egg and any cortisol present is expected to have been produced by the developing embryo (Detlaff et al., 1993; Simontacchi et al., 2009). Free embryo cortisol samples, measurements and behavior trials were conducted at approximately 130 CTUs (approximately half-way through the free embryo stage). Larval cortisol samples, measurements and behavior trials were conducted at the onset of exogenous feeding at approximately 206 CTUs (Table 1.1). For each sampling period, approximate developmental stage for each temperature treatment was confirmed based on

examination of morphological features. For fertilized eggs at 36 CTUs, the closed neural tube was visible; for free embryos at 130 CTUs, eyes were visible and yolk sac was still present; for larvae at 206 CTUs, the yolk sac was depleted and the anal plug was shed (Detlaff et al., 1993; Kempinger, 1988).

Table 1.1. Standardization of sampling schedule based on stage calculated by CTU.
Sampling and trials were taken on days indicated using CTU equation for each treatment, using water temperature (xi) and a constant (k, 5.8°C) to predict developmental stages ((Kempinger 1998, Smith and King 2005).

Stage	CTU Range	Days Post Fertilization	
		18°C	10°C
fertilized egg (approximately halfway through egg incubation)	36-46	3 days	9 days
hatch	72-74	6 days	14 days
free embryo (approximately halfway through free embryo stage, after HPI axis functional)	130-134	11 days	24 days
larva (emergence from substrate, onset of exogenous feeding, beginning of drift period)	206-207	17 days	37 days

Throughout the experiment, care was given to animal welfare by following hatchery protocol for sturgeon rearing in order to minimize incidental stress. Since the warm (18 ℃) and cold (10 ℃) temperatures chosen for experimental treatments reflect the typical range of water temperatures occurring during the spawning season in the Black River, sturgeon were not exposed to temperature stressors beyond what would have been encountered in their natural environment. All sturgeon in the experiment were housed in couplings made of PVC plastic and mesh that ensured adequate water flow-through. Dead eggs were removed daily during

incubation. After hatch, shells were removed to avoid impediment of water flow-through, and free embryos were supplied with 2.54 cm^3 Bio Balls (Pentair No. CBBI-5, Pentair Aquatic Eco-Systems, Cary, NC, U.S.A.) to simulate substrate for burrowing. Since lake sturgeon are negatively phototaxic until reaching the larval stage, couplings were kept in darkness and light exposure was limited to daily removal of mortalities. All rearing and experimental protocols were conducted according to approved Michigan State University Animal Use and Care guidelines under Animal Use and Care project 05-16-056-00.

Body size

Size measurements were obtained using ImageJ software (ImageJ, National Institutes of Health, Bethesda, MD, U.S.A., http:// rsbweb.nih.gov/ij/) to measure total body length for hatchlings, free embryos and larvae. Photos used for ImageJ analysis were taken with a digital camera and included a ruler for size calibration. Six individuals per replicate were measured across all three stages, including each family and temperature treatment, for a total of 144 biological replicates (864 individuals) represented in the size data set. Individuals used for measurements were sedated with 25 mg/liter MS 222 using approved Michigan State University Animal Use and Care protocols and then removed from the experiment. Measurements of body length at hatch were taken immediately after hatch at approximately 72 CTUs. Free embryo size measurements were taken at approximately 130 CTUs (approximately half-way through the free embryo stage). Larval size measurements were conducted at the onset of exogenous feeding at approximately 206 CTUs. We used a Shapiro-Wilk test to assess normality for the body size data set. Generalized linear models were fitted using the *glm* function in R v.3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Variables present in the AICc-selected model (Cavanaugh, 1997) were further explored using ANOVA.

Cortisol analysis

We preserved samples for cortisol analysis at the stages of unfertilized egg, fertilized egg, free embryo and larva. For unfertilized eggs, we took three replicate samples from each of the four females. We took fertilized egg samples from each replicate in the experiment, including both temperature treatments and all four families, for a total of 48 samples. Egg samples contained 1 ml of eggs per sample (approximately 52 unfertilized eggs or 25 fertilized eggs). Free embryo and larvae samples contained six individuals per sample that were euthanized using an overdose of MS-222. While sturgeon begin producing cortisol during egg development, the HPI axis becomes functional after hatch, enabling individuals to increase cortisol levels in response to a stressor (Simontacchi et al., 2009). Therefore, for free embryo and larval stages, samples were taken at baseline, with no stressor applied, or 30 min after individuals were exposed to an acute stressor to capture levels of cortisol elevation during HPI response to the stressor. The acute stressor used was exposure to odorant created from whole-body homogenization of sacrificed sturgeon larvae, as subdermal tissue homogenate from conspecifics causes a physiological and behavioral response in fishes (Wagner et al., 2011; Wishingrad et al., 2014a; Wishingrad et al., 2014b). Free embryo and larval samples were both taken from each replicate in the experiment and at a state of either baseline or post-stress, for a total of 96 free embryo samples and 96 larval samples. All cortisol samples were stored in cryotubes and immediately submerged in liquid nitrogen for preservation.

We conducted cortisol analysis of samples using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Samples were homogenized prior to liquid-liquid extraction using ethyl acetate as a solvent. After the organic layer was extracted and evaporated, it was reconstituted in methanol and stored at -18 ℃ until analysis. HPLC-

MS/MS analysis was conducted using a Waters Xevo TQ-S mass spectrometer (Waters, Millford, MA, U.S.A.) (Bussy et al., 2017).

We used Shapiro-Wilk tests to assess normality. The cortisol data set was not normally distributed, so we log-transformed the data before analysis. We fitted generalized linear models using the *glm* function in R v.3.2.2. Variables present in the AICc-selected model (Cavanaugh, 1997) were further evaluated using ANOVA and post hoc Tukey tests.

Behavior

We quantified effects of temperature treatment on behavior by observing escape behaviors in response to perceived threats (Lehtiniemi, 2005). Loligo v.4.0 tracking software (Loligo Systems, Viborg, Denmark; https://www.loligosystems.com/software) was used to record activity of six individuals in a 6-inch (15.24 cm) petri dish for 5 min. Observed variables included velocity (cm/s), acceleration (cm/s^2), percentage of time active, number of seconds active and total distance travelled (cm) (Sakamoto et al., 2016). Loligo tracking software records pixel-based measurements that are converted to desired units (cm) and here reported to two significant digits. During behavior trials, larvae were exposed to either an odorant created from whole-body homogenization of euthanized sturgeon larvae simulating a predator cue (Wagner et al., 2011; Wishingrad et al., 2014a; Wishingrad et al., 2014b) or water as a control. Control and odorant trials were run simultaneously for each replicate in the experiment and analysed using Loligo tracking software. We used Shapiro-Wilk tests to assess normality for the data set of each behavioral variable. We fitted generalized linear models using the *glm* function in R v.3.2.2. Variables present in the AICc-selected model (Cavanaugh, 1997) were further evaluated using ANOVA and post hoc Tukey tests.

Predation trials

At the larval stage, we quantified survival rates in predation trials using rusty crayfish, *Orconectes rusticus*, which are known to prey upon larval sturgeon (Crossman et al., 2018). In the presence of crayfish, sturgeon larvae show predator avoidance by occupying more time in water column than on substrate, unlike their responses to other predators (Crossman et al., 2018). We placed 20 sturgeon larvae from each replicate in tanks supplied with flowing stream water, for a total of 48 replicated predation trials. Flow-through tanks could not be temperature controlled, so predation trials took place at ambient stream water temperature (17 ℃). Since ambient stream temperature for predation trials was closer to the warm temperature treatment (18 ℃) than to the cold temperature treatment (10 ℃), care was taken to acclimate larvae from both treatments to ambient water temperature prior to predation trials. Tank dimensions were 42 30 cm and water depth was 12 cm. We measured carapace length (cm) of each crayfish and placed one crayfish in each tank. After 5 h, we removed the crayfish and counted the surviving larvae in each tank. Stress associated with the predation trial was minimized to the extent possible for larvae by acclimating them to ambient water temperature beforehand, supplying trial tanks with stream water at a flow rate of 15 gallons/h (56.78 liters/h) to ensure adequate oxygenation, and removing surviving larvae immediately upon completion of the trial to avoid further interaction with the crayfish predator. We fitted generalized linear models using a Poisson distribution for the data set of survival counts using the *glm* function in R v.3.2.2. Variables present in the AICc-selected model (Cavanaugh, 1997) were further evaluated using a chi-square test.

RESULTS

Body size

The AIC-selected model included temperature treatment, female (family), stage, the interaction of temperature and female, the interaction of temperature and stage, and the interaction of female and stage (AICc = 128.83) (Table 1.2). ANOVA indicated that the main effect was significant for temperature ($p = 0.0102$), female ($p < 0.0001$) and stage ($p < 0.0001$). There were significant interactions of temperature), stage ($p < 0.0001$), and female*stage ($p = 0.0147$), but the interaction of female*temperature was not significant ($p = 0.2982$). Tukey HSD showed that while families differed significantly in body size at hatch and free embryo stages, by the larval stage there were no significant differences in body size among families. At hatch, individuals from the warm treatment were significantly smaller (mean length ± SE = 11.54 ± 0.07 mm) than hatchlings from the cold treatment (12.05 ± 0.07 mm) ($F_{1,45} = 28.00$, $p < 0.0001$; Figure 1.1). At the free embryo stage, individuals from the warm treatment were significantly larger (16.53 ± 0.08 mm) than those from the cold treatment (16.13 ± 0.1 mm) ($F_{1,43} = 20.22$, $p < 0.0001$; Figure 1.1). At the larval stage, individuals from the warm treatment (22.31 ± 0.1 mm) were significantly larger than those from the cold treatment (21.56 ± 0.09 mm) ($F_{1,46} = 30.79$, $p < 0.0001$; Figure 1.1).

Cortisol analysis

Mean cortisol in unfertilized eggs was 543.40 ± 56.1 pg/g, with no significant differences between eggs from the four females ($p = 0.079$). For fertilized eggs, the AIC-selected model contained temperature treatment, female (family), and the interaction of female and temperature (AICc = 362.03) (Table 1.3). An ANOVA did not indicate a significant difference between mean cortisol for fertilized eggs from the warm treatment (mean ± SE = 48.19 ± 4.26 pg/g) and from

the cold treatment (mean = 39.20 ± 2.38 pg/g) ($F_{1,42} = 3.59$, $p = 0.065$; Figure 1.2). Family had a significant effect ($F_{3,43} = 15.47$, $p < 0.001$), as did the interaction of family and temperature ($F_{3,39} = 3.38$, $p = 0.028$; Figure 1.3).

Table 1.2. Models for size at hatch, free embryo, and larvae.

Model	AICc	Delta AICc	Weight
hatch			
Size ~ Female + Temperature	2.77	0	0.92
Size ~ Female + Temperature + Female*Temperature	7.66	4.89	0.08
Size ~ Temperature	35.1	32.33	0
Size ~ Female	42.46	39.7	0
free embryo			
Size ~ Female + Temperature	32.07	0	0.84
Size ~ Female + Temperature + Female*Temperature	35.38	3.3	0.16
Size ~ Female	47.95	15.88	0
Size ~ Temperature	60.57	28.49	0
larvae			
Size ~ Temperature	68.45	0	0.55
Size ~ Female + Temperature	69.18	0.73	0.38
Size ~ Female + Temperature + Female*Temperature	72.88	4.43	0.06
Size ~ Female	93.99	25.54	0

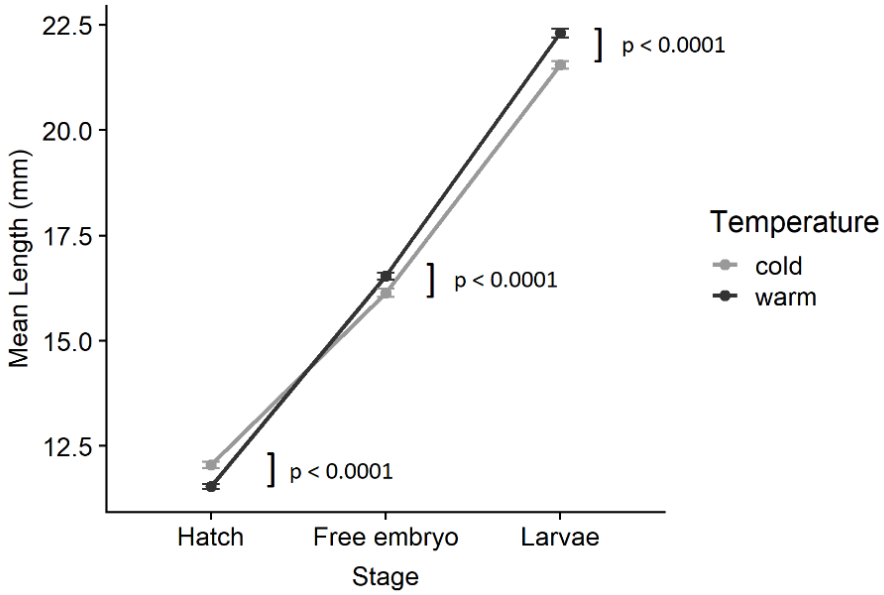

Figure 1.1. Mean length (mm) at hatch (130 to 134 CTU), free embryo stage (130 to 134 CTU), and larval stage (206 to 207 CTU).
N = 144 biological replicates (864 individuals) across all 3 stages, with 6 individuals measured per replicate at each stage. At hatch, individuals from the warm treatment were significantly smaller (mean length ± standard error = 11.54 ± 0.07 mm) than individuals from the cold treatment (12.05 ± 0.07 mm) ($F = 28.00$, $df = 1$, $p < 0.0001$). At the free embryo stage, individuals from the warm treatment were significantly larger (16.53 ± 0.08 mm) than cold treatment individuals (16.13 ± 0.1 mm) ($F = 20.22$, $df = 1$, $p < 0.0001$). At the larval stage, individuals from the warm treatment (22.31 ± 0.1 mm) were significantly larger than those from the cold treatment (21.56 ± 0.09 mm) ($F = 30.79$, $df = 1$, $p < 0.0001$). Error bars indicate standard error.

Table 1.3. Models for fertilized egg cortisol.

Model	AICc	Delta AICc	Weight
Cortisol ~ temperature + female + female*temperature	362.03	0	0.99
Cortisol ~ temperature + female	372.12	10.09	0.01
Cortisol ~ temperature	378.08	16.04	0
Cortisol ~ female	401.58	39.55	0

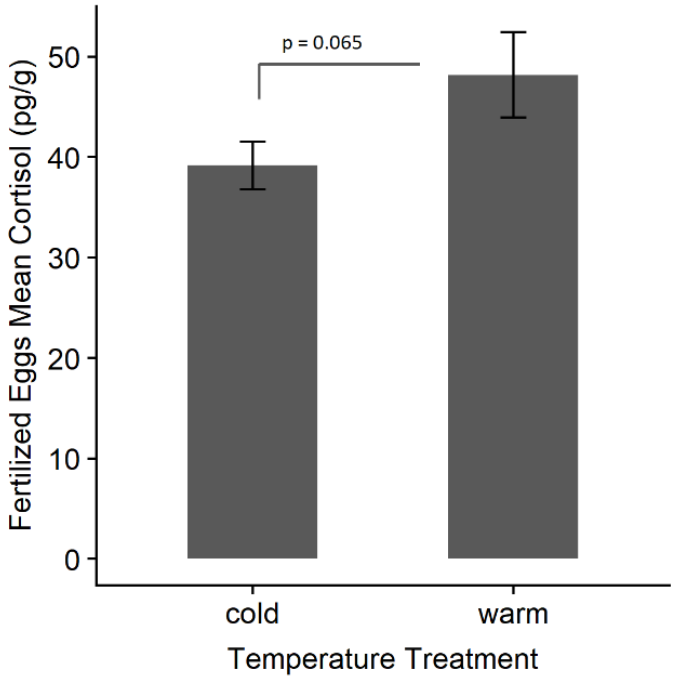

Figure 1.2. Mean cortisol in fertilized eggs in original (not log-transformed) scale.
N = 48 samples, with 1 ml of eggs (approximately 25 eggs) per sample. One sample was taken from each replicate in CTU range 36 to 46 (3 days post fertilization for 18°C treatment, 9 days post fertilization for 10°C treatment). Mean cortisol for fertilized eggs was higher for the warm treatment (mean ± standard error = 48.19 ± 4.26 pg/g) than for the cold treatment (mean = 39.20 ± 2.38 pg/g), but an ANOVA did not indicate a significant difference ($F = 3.59$, $df = 1$, $p = 0.065$). Errors bars indicate standard error.

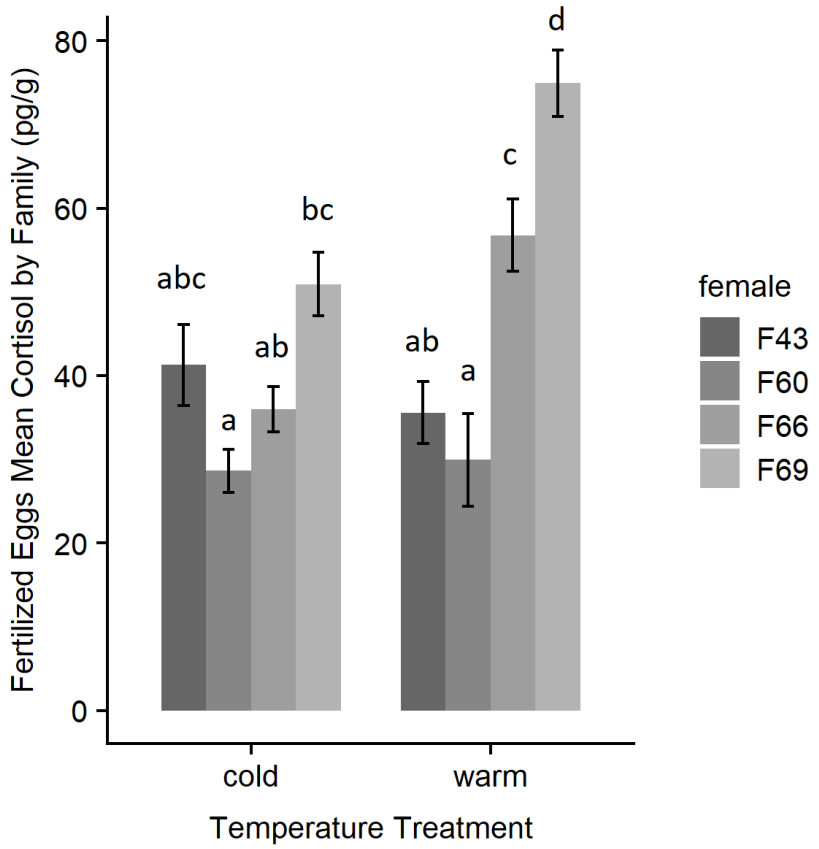

Figure 1.3. Fertilized egg cortisol levels by family in original (not log-transformed) scale. N = 48 samples, with 1 ml of eggs (approximately 25 eggs) per sample. In both temperatures, eggs from F69 had highest cortisol, eggs from F60 had lowest cortisol, and eggs from F43 and F66 were in between. Consistency of relative cortisol levels across treatments indicate that family effects (genetic or maternal effects) are important in determining embryonic cortisol production. F43 eggs in the warm treatment had lower cortisol, in contrast to the other families, which show increased egg cortisol in the warmer temperature. The main effect of family was significant ($F = 15.47$, $df = 3$, $p < 0.001$), as was the interaction of family and temperature ($F = 3.38$, $df = 3$, $p = 0.0278$) Letters indicate significant differences among families based on post-hoc Tukey results. Error bars indicate standard error.

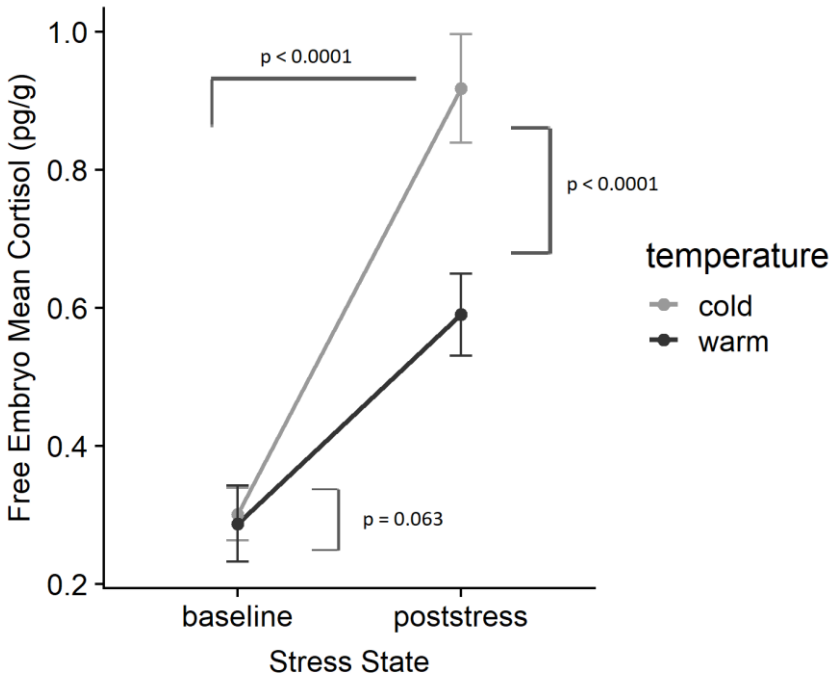

Figure 1.4. Mean cortisol levels in free embryos at baseline and after an acute stressor in original (not log-transformed) scale.
N = 96 samples, with 6 individuals per sample. Samples were taken from each replicate in CTU range 130 to 134 (11 days post fertilization for 18°C treatment, 24 days post fertilization for 10°C treatment). Baseline samples were taken after immediate euthanasia using an overdose of MS-222. Individuals in post stress samples were euthanized 30 minutes after exposure to an acute stressor. Mean baseline cortisol was lower for the warm treatment (0.29 ± 0.06 pg/g) than for the cold treatment (0.30 ± 0.04 pg/g), but ANOVA did not indicate a significant difference ($F = 3.67$, $df = 1$, $p = 0.063$). After exposure to an acute stressor, cortisol significantly increased for both treatments ($F = 93.60$, $df = 2$, $p < 0.0001$), but free embryos from the warm treatment had significantly lower post-stress cortisol levels (0.59 ± 0.06 pg/g) compared to free embryos from the cold treatment (0.92 ± 0.08 pg/g) ($F = 37.04$, $df = 1$, $p < 0.0001$). Error bars indicate standard error.

For free embryos, the AIC-selected model included temperature treatment, female (family), stress state (whether cortisol samples were taken at baseline or after an acute stressor), and the two-way and three-way interactions of these factors (AICc = -20.92) (Table 1.4). ANOVA did not indicate a significant difference in mean baseline cortisol for free embryos from the warm treatment (0.29 ± 0.06 pg/g) and free embryos from cold treatment (0.30 ± 0.04 pg/g) ($F_{1,43} = 3.67$, $p = 0.063$). After exposure to an acute stressor, cortisol significantly increased for

individuals from both treatments ($F_{2,137} = 93.60$, $p < 0.0001$). Free embryos from the warm treatment had significantly lower poststress cortisol levels (0.59 ± 0.06 pg/g) compared to free embryos from the cold treatment (0.92 ± 0.08 pg/g) ($F_{1,91} = 37.04$, $p < 0.0001$; Figure 1.4). Family had a significant effect on free embryo cortisol ($F_{3,140} = 22.78$, $p < 0.001$), as did the interaction of family and stress state ($F_{6,128} = 6.83$, $p < 0.001$), and the three-way interaction of family, stress state and temperature ($F_{6,120} = 6.29$, $p < 0.001$). There was no significant effect of the interaction of family and temperature ($F_{3,134} = 0.69$, $p = 0.557$; Figure 1.5) or of the interaction of temperature and stress state ($F_{2,126} = 1.10$, $p = 0.3360$; Figure 1.5) on free embryo cortisol levels. The increased cortisol levels following exposure to an acute stressor indicated HPI axis functionality as early as the free embryo stage.

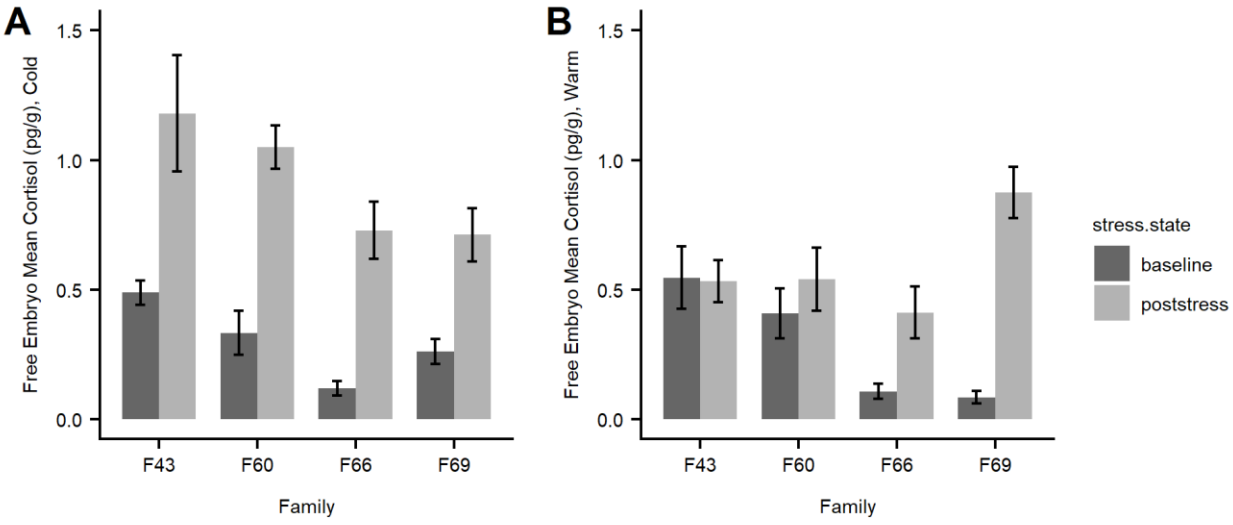

Figure 1.5. Free embryo cortisol levels by family at baseline and after an acute stressor (post stress) in original (not log-transformed) scale for cold treatment (a) and warm treatment (b).
N = 96 samples, with 6 individuals per sample. The main effect of family on free embryo cortisol was significant (F = 22.78, df = 3, $p < 0.0001$). Error bars indicate standard error.

Table 1.4. Models for free embryo cortisol.

Model	AICc	Delta AICc	Weight
Cortisol ~ female + temperature + stress.state + female*temperature + female*stress.state + temperature*stress.state + female*temperature*stress.state	-20.92	0	1
Cortisol ~ female + temperature + stress.state + female*temperature + female*stress.state	-1.71	19.2	0
Cortisol ~ female + temperature + stress.state + female*temperature + female*stress.state + temperature*stress.state	1.56	22.48	0
Cortisol ~ female + temperature + stress.state	10.85	31.76	0
Cortisol ~ female + temperature + stress.state + female*temperature	16.29	37.21	0
Cortisol ~ female + stress.state	30.69	51.61	0
Cortisol ~ temperature + stress.state	45.99	66.91	0
Cortisol ~ stress.state	60.68	81.6	0
Cortisol ~ female + temperature	100.48	121.4	0
Cortisol ~ female	110.22	131.13	0
Cortisol ~ temperature	117.28	138.2	0

For larvae, the AIC-selected model included temperature treatment, female (family), stress state (whether cortisol samples were taken at baseline or after an acute stressor), and the two-way interactions of these factors (AICc = 138.75; Table 1.5). An ANOVA indicated no significant difference between mean baseline cortisol levels for larvae from the warm treatment (1.42 ± 0.1 pg/g) and larvae from the cold treatment (1.22 ± 0.08 pg/g) ($F_{1,43} = 3.36$, $p = 0.074$; Figure 1.6). After exposure to an acute stressor, cortisol levels significantly increased for individuals from both temperature treatments ($F_{2,137} = 6.53$, $p = 0.006$; Figure 1.6). There was no significant difference between mean post-stress cortisol in larvae from the warm treatment (1.49 ± 0.11 pg/g) and larvae from the cold treatment (1.64 ± 0.1 pg/g) ($F_{1,91} = 0.28$, $p = 0.595$; Figure 1.6). Family had a significant effect on larval cortisol ($F_{3,140} = 8.16$, $p < 0.0001$), as did the interaction of family and stress state ($F_{6,128} = 6.32$, $p < 0.001$; Figure 1.7). There was no significant effect of the interaction of family and temperature ($F_{3,134} = 0.99$, $p = 0.3999$) or of the interaction of temperature and stress state ($F_{2,126} = 2.43$, $p = 0.0924$; Figure 1.7).

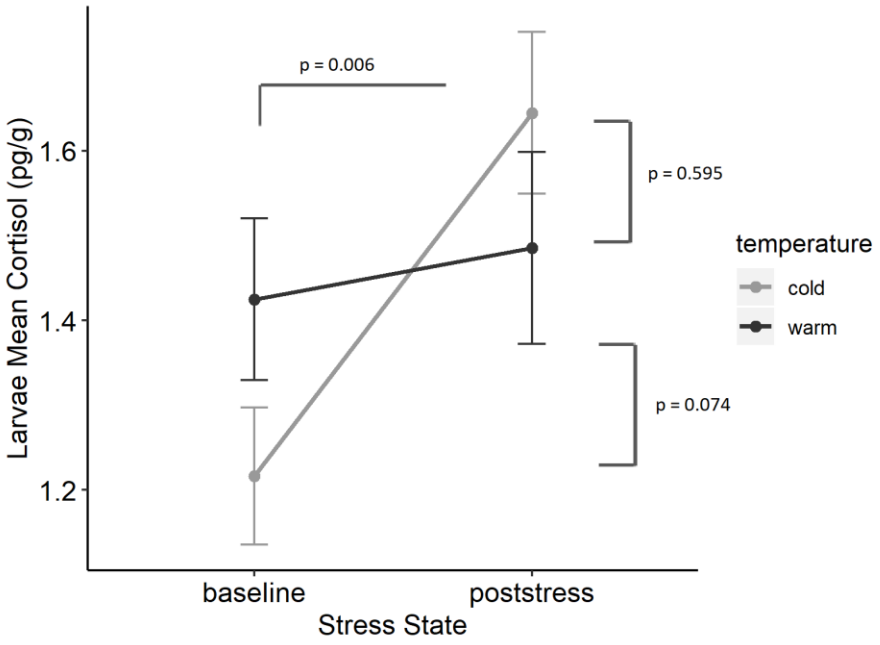

Figure 1.6. Mean cortisol levels in larvae at baseline and after an acute stressor in original (not log-transformed) scale.
N = 96 samples, with 6 individuals per sample. Samples were taken from each replicate in CTU range 206 to 207 (17 days post fertilization for 18℃ treatment, 37 days post fertilization for 10℃ treatment). Baseline samples were taken after immediate euthanasia using an overdose of MS-222. Individuals in post stress samples were euthanized 30 minutes after exposure to an acute stressor. Mean baseline cortisol was higher for the warm treatment (1.42 ± 0.1 pg/g) than for the cold treatment (1.22 ± 0.08 pg/g) but the difference was not significant ($F = 3.36$, $df = 1$, $p = 0.074$). After exposure to an acute stressor, cortisol significantly increased for both treatments ($F = 6.53$, $df = 1$, $p = 0.006$). Larvae from the warm treatment had lower post-stress cortisol (1.49 ± 0.11 pg/g) compared to larvae from the cold treatment (1.64 ± 0.1 pg/g), but ANOVA did not indicate a significant difference ($F = 0.28$, $df = 1$, $p = 0.595$). Error bars indicate standard error.

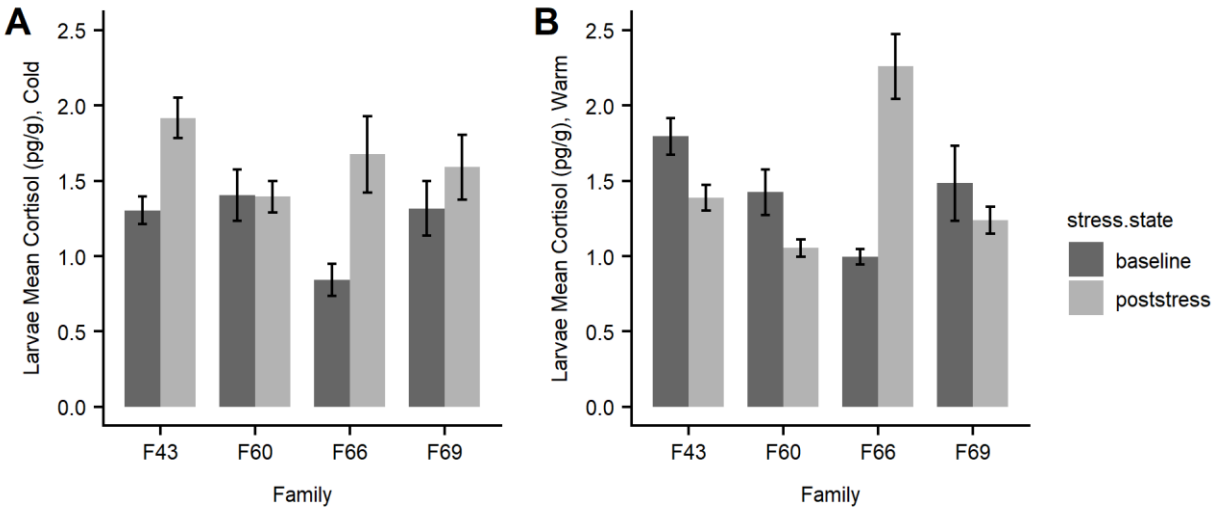

Figure 1.7. Larvae cortisol levels by family at baseline and after an acute stressor (post stress) in original (not log-transformed) scale for cold treatment (a) and warm treatment (b).
N = 96 samples, with 6 individuals per sample. The main effect of family on larval cortisol was significant ($F = 6.96$, $df = 3$, $p < 0.0001$). Error bars indicate standard error.

Male was not included as a factor in the models since male and female were not linearly independent (i.e. we used a 1:2 male:female fertilization ratio due to limitations of gamete availability). Examination of the raw data indicated that paternity was the driving factor in determining interfamily differences at the fertilized egg (Figure A1), free embryo (Figure A2) or larval stage (Figure A3).

Table 1.5. Models for larvae cortisol.

Model	AICc	Delta AICc	Weight
Cortisol ~ female + temperature + stress.state + female*temperature + female*stress.state + temperature*stress.state	-138.75	0	0.51
Cortisol ~ female + temperature + stress.state + female*temperature + female*stress.state	-138.58	0.17	0.47
Cortisol ~ female + temperature + stress.state + female*temperature + female*stress.state + temperature*stress.state + female*temperature*stress.state	-131.85	6.9	0.02
Cortisol ~ female + stress.state	-123.21	15.54	0
Cortisol ~ female + temperature + stress.state	-121.2	17.55	0
Cortisol ~ female	-117.07	21.68	0
Cortisol ~ female + temperature + stress.state + female*temperature	-116.78	21.97	0
Cortisol ~ female + temperature	-115.1	23.65	0
Cortisol ~ stress.state	-110.59	28.16	0
Cortisol ~ temperature + stress.state	-108.65	30.1	0
Cortisol ~ temperature	-103.64	35.11	0

Behavior

The AIC-selected model for larval velocity included temperature treatment, female (family) and odor treatment (exposed to either sturgeon larvae homogenate or water as a control) (AICc = 428.27; Table 1.6). An ANOVA did not indicate a significant difference in mean

velocity between larvae from the cold treatment (6.11 ± 0.42 cm/s) and larvae from the warm treatment (6.89 ± 0.25 cm/s) ($F_{1,91} = 2.46$, $p = 0.1216$). There was also no significant effect of family ($F_{3,88} = 0.94$, $p = 0.4309$) or odor treatment ($F_{1,87} = 3.62$, $p = 0.1460$; Figure 1.8a) on velocity.

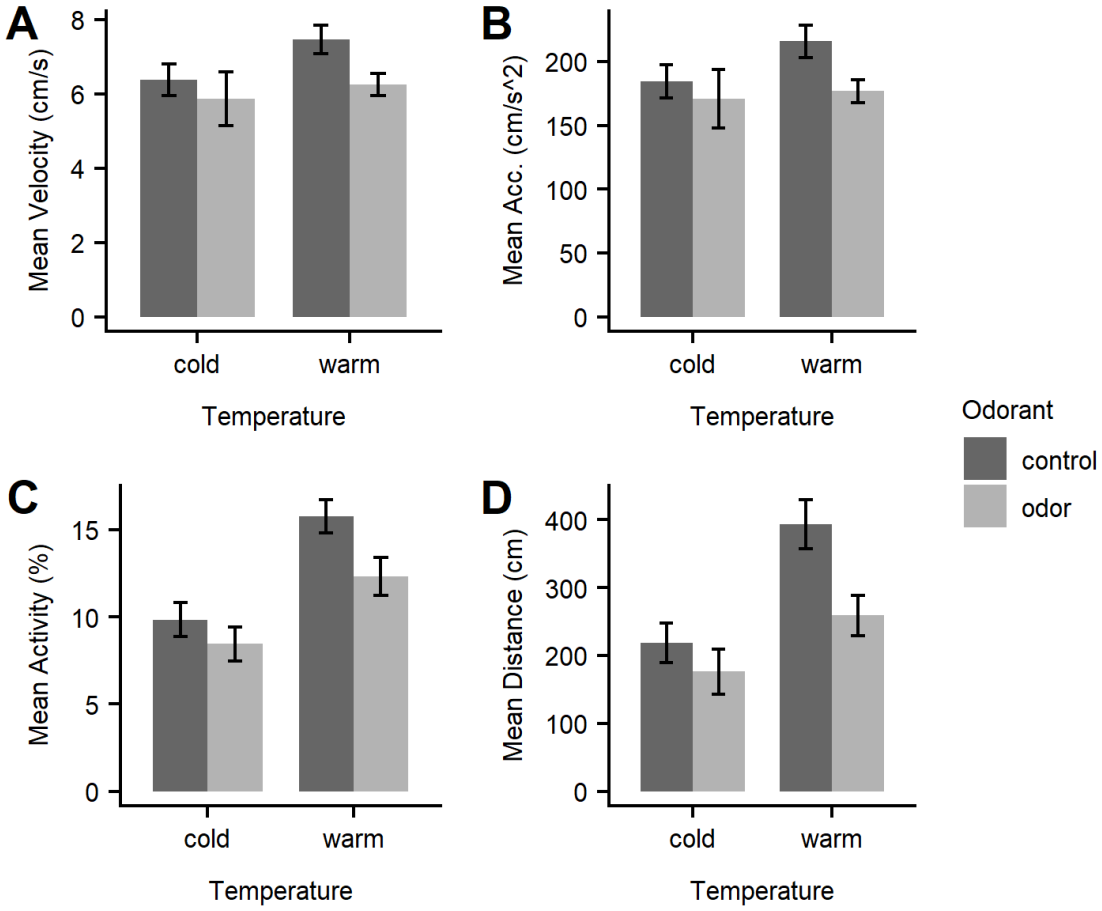

Figure 1.8. Activity parameters yielded by Lolitrack analysis of larval movement.
Six larvae were tracked in a 6-inch petri dish for 5 minutes during exposure to either alarm odor (made from sturgeon larvae homogenate) or control (water). There were no significant effects of temperature treatment or odorant on larval velocity (a) or acceleration (b). Larvae from the warm treatment had significantly higher percent activity (14 ± 0.77 %) compared to larvae from the cold treatment (9.12 ± 0.68 %) ($F = 29.55$, $df = 1$, $p < 0.0001$). Mean distance traveled was significantly higher for warm treatment larvae (326.03 ± 25.1 cm) compared to cold treatment larvae (196.77 ± 22.13cm) ($F = 16.95$, $df = 1$, $p < 0.0001$). Error bars indicate standard error.

Table 1.6. Models for larval velocity, acceleration, % activity, and distance.

Model	AICc	Delta AICc	Weight
velocity			
Velocity ~ temperature + female + odor.treatment	426.24	0	0.64
Velocity ~ temperature + female + odor.treatment + temperature*female	428.04	1.8	0.26
Velocity ~ temperature + female + odor.treatment + temperature*female + temperature*odor.treatment	430.16	3.92	0.09
Velocity ~ temperature + female + odor.treatment + temperature*female + temperature*odor.treatment + female*odor.treatment	435.11	8.87	0.01
Velocity ~ temperature + female + odor.treatment + temperature*female + temperature*odor.treatment + female*odor.treatment + temperature*female*odor.treatment	441.19	14.95	0
acceleration			
Acceleration ~ temperature + female + odor.treatment	1070.09	0	0.66
Acceleration ~ temperature + female + odor.treatment + temperature*female	1072.13	2.04	0.24
Acceleration ~ temperature + female + odor.treatment + temperature*female + temperature*odor.treatment	1074.09	4	0.09
Acceleration ~ temperature + female + odor.treatment + temperature*female + temperature*odor.treatment + female*odor.treatment	1078.4	8.31	0.01
Acceleration ~ temperature + female + odor.treatment + temperature*female + temperature*odor.treatment + female*odor.treatment + temperature*female*odor.treatment	1085.14	15.05	0
% activity			
%Activity ~ temperature + female + odor.treatment + temperature*female + temperature*odor.treatment + female*odor.treatment	550.82	0	0.35

Table 1.6 (Cont'd)

%Activity ~ temperature + female + odor.treatment + temperature*female	550.89	0.07	0.33
%Activity ~ temperature + female + odor.treatment	552.4	1.58	0.16
%Activity ~ temperature + female + odor.treatment + temperature*female + temperature*odor.treatment	552.44	1.62	0.15
%Activity ~ temperature + female + odor.treatment + temperature*female + temperature*odor.treatment + female*odor.treatment + temperature*female*odor.treatment	558.58	7.76	0.01
distance			
Distance ~ temperature + female + odor.treatment	1206.63	0	0.58
Distance ~ temperature + female + odor.treatment + temperature*female	1208.48	1.85	0.23
Distance ~ temperature + female + odor.treatment + temperature*female + temperature*odor.treatment	1209.03	2.4	0.17
Distance ~ temperature + female + odor.treatment + temperature*female + temperature*odor.treatment + female*odor.treatment	1213.73	7.1	0.02
Distance ~ temperature + female + odor.treatment + temperature*female + temperature*odor.treatment + female*odor.treatment + temperature*female*odor.treatment	1220.16	13.53	0

The AIC-selected model for larval acceleration included temperature treatment, female (family) and odor treatment (exposed to either death odor or water as a control) (AICc = 1072.06; Table 6). An ANOVA did not indicate a significant difference in mean acceleration between larvae from the cold treatment (177.59 ± 13.16 cm/s^2) and larvae from the warm treatment (196.35 ± 8.11 ± cm/s^2) ($F_{1,91}$ = 1.55, p = 0.2180). ANOVA also showed no significant

effect of family ($F_{3,88} = 0.96$, p = 0. 0.4187) or odor treatment ($F_{1,87} = 3.35$, p = 0.1613; Figure 1.8b) on acceleration.

The AICc-selected model for larval activity (percentage of time active) included temperature treatment, female (family), odor treatment, and the interaction of family and temperature (AICc = 553.07; Table 6). Larvae from the warm treatment had a significantly higher percentage of activity (14 ± 0.8%) compared to larvae from the cold treatment (9.12 ± 0.68%) ($F_{1,91} = 29.55$, $p < 0.0001$; Figure 1.8c). An ANOVA showed that all factors indicated by the model were significant: temperature ($F_{1,91} = 29.55$, $p < 0.0001$), female ($F_{3,88} = 5.89$, p = 0.0011), odor treatment ($F_{1,87} = 8.18$, p = 0.0206), temperature*female ($F_{3,84} = 2.80$, p = 0.0401; Figs. 8c and 9).

The AIC-selected model for distance included temperature treatment, female (family) and odor treatment (AICc = 1209.00; Table 6). Mean distance travelled was significantly higher for warm treatment larvae (326.03 ± 25.1 cm) compared to cold treatment larvae (196.77 ± 22.13 cm) ($F_{1,91} = 16.95$, $p < 0.0001$; Figure 1.8d). An ANOVA showed that all factors indicated by the model were significant: temperature ($F_{1,91} = 16.95$, $p < 0.0001$), female ($F_{3,88} = 2.90$, p = 0.0415), odor treatment ($F_{1,87} = 8.77$, p = 0.0159; Figure 1.8d).

Predation trials

The AIC-selected model for larval survival included temperature treatment, female (family), crayfish (carapace length), length (larval length), and the interaction of crayfish and length (AICc = 304.20; Table 1.7). Chi-square analysis of the AICc-selected generalized linear model showed that larvae from the warm temperature treatment had significantly higher survival in the presence of a crayfish predator ($\chi^2_1 = 80.68$, $p < 0.0001$; Figure 1.9). Mean ± SD numbers

of survivors (out of 20 individuals per replicate) was 3.75 ± 4.01 for larvae from the cold treatment and 10.54 ± 4.93 for larvae from the warm treatment.

There were no significant differences in survival among families ($\chi^2_3 = 4.52$, p = 0.2106; Figure 1.10). Neither crayfish carapace length ($\chi^2_1 = 2.51$, p = 0.1134) nor mean larval length ($\chi^2_1 = 0.32$, p = 0.5727) had a significant effect on survival. However, the interaction of crayfish length and larval length had a significant effect ($\chi^2_1 = 14.64$, p = 0.0001; Figure 1.11).

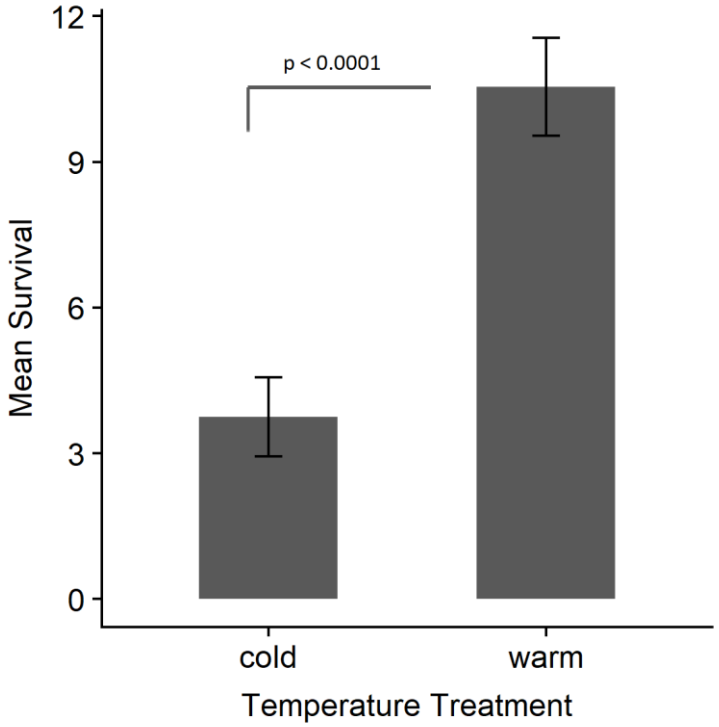

Figure 1.9. Mean number of surviving larvae after a 5 hour exposure to a crayfish predator.
Mean numbers of survivors (out of 20 individuals per replicate) was 3.75 for larvae from the cold treatment and 10.54 for larvae from the warm treatment, and Chi Square analysis indicated that the difference was significant ($\chi^2 = 80.68$, df = 1, p < 0.0001). Error bars indicate standard error.

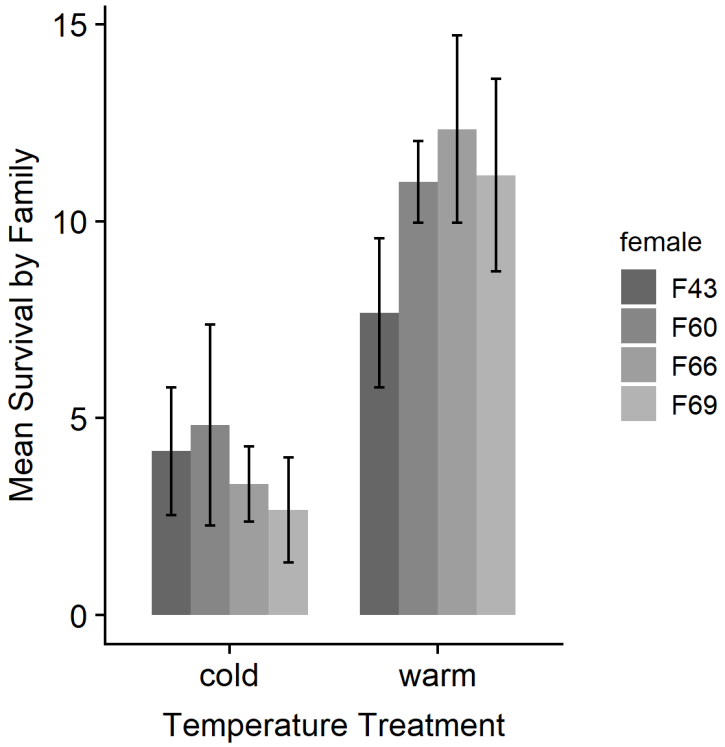

Figure 1.10. Mean number of survivors in each family during predation trials.

Surviving larvae are out of 20 larvae total after a 5 hour exposure to a crayfish predator. Chi Square analysis indicated no significant differences in survival among families (χ^2 = 4.52, df = 3, p = 0.2106).

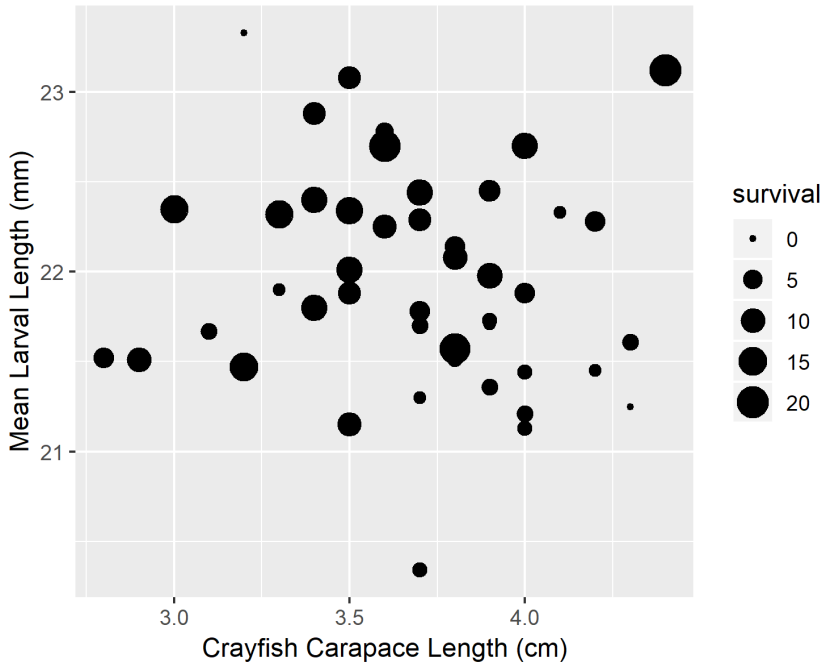

Figure 1.11. Larval survival after crayfish encounter as a function of mean larval length and crayfish carapace length.
Dot size indicates number of surviving larvae (out of 20) after a 5 exposure to a crayfish predator. Crayfish carapace length and mean larval length did not have a significant effect on survival (χ^2 = 2.51, df = 1, p = 0.1134; χ^2 = 0.32, df = 1, p = 0.5727, respectively); however, the interaction of crayfish length and larval length did have a significant effect (χ^2 = 14.64, df = 1, p = 0.0001). Larval survival is lower when larval size is small and crayfish size is large.

Table 1.7. Models for survival.

Model	AICc	Delta AICc	Weight
Survival ~ female + temperature + crayfish + length + crayfish*length	304.2	0	0.69
Survival ~ female + temperature + crayfish + length + temperature*crayfish + crayfish*length	306.92	2.72	0.18
Survival ~ female + temperature + crayfish + length + female*temperature + crayfish*length	308.38	4.19	0.08
Survival ~ female + temperature + crayfish + length + temperature*crayfish	311.84	7.64	0.02
Survival ~ female + temperature + crayfish + length + female*temperature + temperature*crayfish + crayfish*length	311.93	7.73	0.01
Survival ~ female + temperature	313.4	9.2	0.01
Survival ~ female + temperature + crayfish	313.51	9.32	0.01
Survival ~ temperature + crayfish + length	315.29	11.09	0
Survival ~ female + temperature + crayfish + length	315.95	11.75	0
Survival ~ female + temperature + crayfish + length + female*temperature + temperature*crayfish	316.07	11.87	0
Survival ~ female + temperature + crayfish + length + female*temperature	316.35	12.15	0
Survival ~ crayfish + length	360.28	56.08	0
Survival ~ female	391.58	87.38	0

DISCUSSION

In both temperature treatments, cortisol present in unfertilized eggs greatly decreased immediately following fertilization, and then gradually increased during egg incubation, as observed in other fish species (Paitz et al., 2015). In contrast to results in lake sturgeon from Zubair et al. (2014), which showed no consistent cortisol response to a chase stressor until the larval stage, in the present study cortisol was significantly higher after an acute stressor in free embryos ($p < 0.001$), confirming that the HPI stress axis began functioning during this early ontogenetic stage. In the present study, cortisol levels were considerably lower than those observed in prior studies with lake sturgeon (Zubair et al., 2012) and white sturgeon, *Acipenser transmontanus* (Simontacchi et al., 2009), which is most likely explained by the use of HPLCMS/MS rather than radioimmunoassay for cortisol samples. HPLCMS/MS has been shown to yield significantly lower measures of cortisol levels compared to radioimmunoassay, due to higher selectivity (Vieira et al., 2014).

Temperature treatment was an important factor contributing to cortisol levels in AICc-selected models. Differences in predation rates and multiple measures of swimming activity demonstrated that temperature influenced HPI axis function and associated fitness-related traits. One important effect of warm rearing temperature was the smaller poststress cortisol increase observed during the free embryo and larval stages. Studies on other fish species including tilapia, *Oreochromis niloticus*, Atlantic salmon, *Salmo salar*, and rainbow trout also documented a 'dampening' effect of chronic stress on the HPI axis, in which chronic stress reduces or eliminates the cortisol response to an acute stressor, rather than the hyperactivity observed in other model systems (Barcellos et al., 1999; Barton et al., 1987; Madaro et al., 2015). Auperin and Geslin (2008) observed reduced cortisol response to stressors in 5-month-old trout that had

been stressed during larval stages, showing that stress sensitivity can be modified by environmental variables experienced during early life stages. Similarly, Vallée et al. (1999) saw that postnatal handling stress in rats caused a decreased corticosterone response to stress that persisted throughout adulthood, indicating that early life stress may be able to program more efficient stress recovery for individuals. This effect could represent an adaptive response to chronic stress, limiting an individuals' physiological reaction to additional stressors to avoid perpetuating HPI hyperactivity.

Family also affected cortisol levels between temperature treatments. Family was indicated as an important factor in AICc-selected models for cortisol levels at the fertilized egg, free embryo and larval stages, as well as in models for behavior variables and predation. Due to the 1:2 male:female fertilization ratio, shared paternity may have increased offspring similarity among families. Despite the potential increase in offspring similarity due to paternal effects, there were still significant interfamily differences among mean cortisol for offspring at the fertilized egg stage, free embryo stage and larval stage. There were also significant differences in activity level for offspring from different families. Family*treatment interactions were frequently found to be significant. Therefore, family-specific factors are important in determining phenotypic responses of offspring to stress, and both maternal and paternal effects should be considered in future studies on stress-related development. Dammerman et al. (2016) observed phenotypic variation among families reared in different temperature regimes, indicating that genetic factors influence developmental responses to temperature. In studies on humans, genetic and environmental factors interact to determine the cortisol response to an acute stressor, as well as longterm stress-related behavioral phenotypes (Alexander et al., 2009).

Chronic stress associated with warm rearing temperature increased activity levels in larval lake sturgeon, as indicated by a significantly higher percentage of activity ($p < 0.001$) and total distance travelled ($p < 0.001$) compared to individuals reared in the cold temperature. In multiple fish species, acute temperature stressors of upper and lower extremes have caused increased swimming activity (Schreck et al., 1997). Similarly, chronic stress has been shown to increase activity in an open field test in Norway rats, *Rattus norvegicus* (Grønli et al., 2005). In contrast, Piato et al. (2011) saw reduced locomotion in chronically stressed zebrafish, suggesting that behavioral outcomes of stress may be species specific.

Behavioral outcomes of exposure to a high-stress environment during early ontogenetic stages have been proposed to be adaptive by reducing predation risk (Sih, 2011). The increased activity levels of larvae reared in warm temperatures reduced vulnerability to crayfish predation and thus represents an adaptive behavioral response to early life stress. Larger mean size may also have contributed to higher survival rates for larvae reared at 18 ℃ in the presence of a crayfish predator, since larger body size has been associated with lower predation rates in lake sturgeon (Wishingrad et al., 2014a; Wishingrad et al., 2014b). Note, however, that predation trials were conducted at ambient stream temperature (17 ℃), which was closer to the warm temperature treatment (18 ℃) than to the cold temperature treatment (10 ℃), and thus larvae from the cold treatment may have been affected by encountering a warm temperature and this may have influenced their higher predation rates. However, since care was taken to acclimate larvae from each treatment to ambient stream temperature prior to predation trials, interpretation of predation results as being primarily affected by rearing temperature seems warranted. For lake sturgeon, increased activity may be a behavioral outcome of early life stress that is adaptive in the short term, while larvae are vulnerable to predators during the larval drift period. However,

further research is needed to ascertain whether lake sturgeon and other threatened wildlife species experience a long-term cost to developmental alterations associated with early life stress. In addition, examining stressors that do not have as profound an effect on growth rates (for example, high rearing density, which has been shown to create chronic stress for lake sturgeon) may help disentangle the roles of size and stress in avoiding predation (Falahatkar et al., 2009; Li et al., 2012; Wuertz et al., 2006).

Further research into family differences in stress responses will be useful in exploring mechanisms of individual plasticity and population-level effects. The interaction between genetic and environmental factors in developmental responses to temperature indicate that population genetic structure and levels of diversity are important in predicting how populations will respond to environmental stressors such as high temperatures. Parental experiences, which were unknown in this experiment, may partially explain differences in offspring development among families through maternal and paternal effects. For example, temperature stressors experienced by parents influence stress-related development (Mills et al., 2015). Maternal effects play an important role in programming stress responses (Sopinka et al., 2014), and different stressors experienced by parents may have influenced offspring physiological and behavioral responses to temperature treatment. Exploring transgenerational effects of environmental stressors by incorporating parental experiences will give further insight into how vulnerable wildlife species respond to climate change (Sheriff & Love, 2013), especially in utilizing studies on offspring stress to predict population- and community-level effects (Love et al., 2013).

Assessing early life developmental alterations in response to environmental stressors, especially those related to warming temperatures, is important for predicting how threatened wildlife species will respond to climate change. The adaptive or maladaptive potential of

physiological and behavioral outcomes need to be investigated within ecologically relevant contexts (Sheriff & Love, 2013), such as predation, in order to make inferences about how environmental stressors will affect vulnerable wildlife species. Fitness effects of stress depend on environmental context, and thus rapidly changing environments can create ecological or evolutionary traps for individuals and populations (Schlaepfer et al., 2002). Quantifying ecological effects of stress and the potential for individual plasticity can help predict how populations and communities will respond evolutionarily to climate change (Reed et al., 2010; Woodward et al., 2010). Therefore, this study highlights the importance of understanding responses to environmental stressors within contexts that can predict fitness.

APPENDIX

APPENDIX

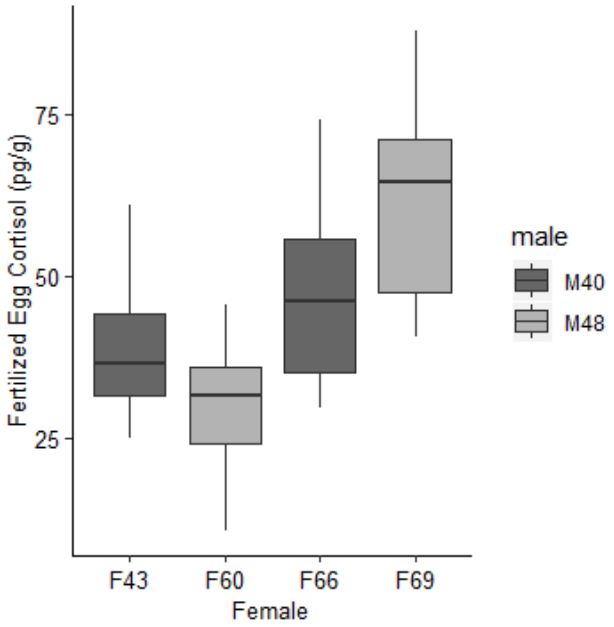

Figure A1. Cortisol dataset in original scale for fertilized eggs, showing influence of maternity and paternity.

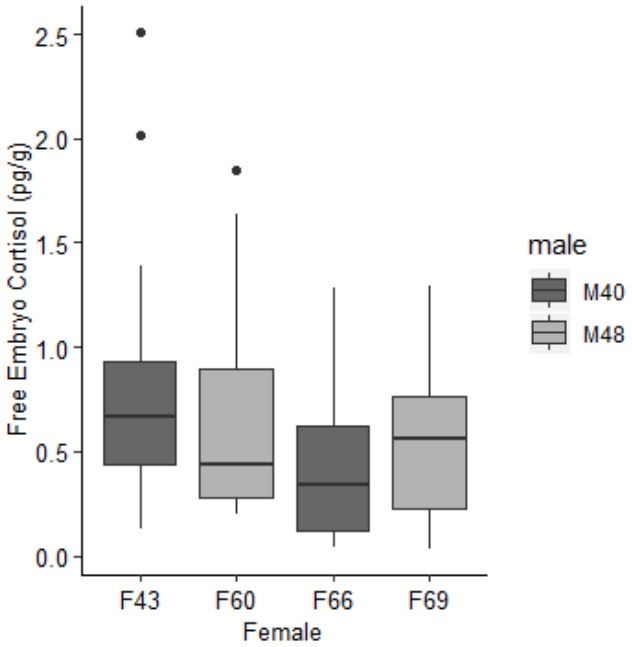

Figure A2. Cortisol dataset in original scale for free embryos, showing influence of maternity and paternity.

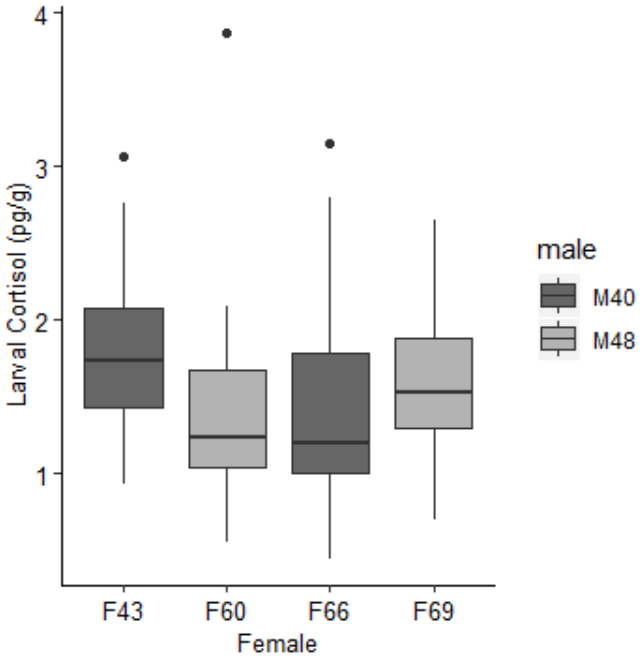

Figure A3. Cortisol dataset in original scale for larvae, showing influence of maternity and paternity.

REFERENCES

REFERENCES

Alexander, N., Kuepper, Y., Schmitz, A., Osinsky, R., Kozyra, E., & Hennig, J. (2009). Gene–environment interactions predict cortisol responses after acute stress: Implications for the etiology of depression. *Psychoneuroendocrinology*, *34*(9), 1294–1303.

Auperin, B., & 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. *General and Comparative Endocrinology*, *158*(3), 234–239.

Baker, M. R., Gobush, K. S., & Vynne, C. H. (2013). Review of factors influencing stress hormones in fish and wildlife. *Journal for Nature Conservation*, *21*(5), 309–318.

Barcellos, L. J. G., Nicolaiewsky, S., De Souza, S. M. G., & Lulhier, F. (1999). Plasmatic levels of cortisol in the response to acute stress in Nile tilapia, *Oreochromis niloticus (L.)*, previously exposed to chronic stress. *Aquaculture Research, 30*(6), 437-444.

Barton, B. A., Schreck, C. B., & Barton, L. D. (1987). Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. *Diseases of aquatic organisms, 2*(3), 173-185.

Bates, L. C., Boucher, M. A., & Shrimpton, J. M. (2014). Effect of temperature and substrate on whole body cortisol and size of larval white sturgeon (*Acipenser transmontanus* Richardson, 1836). *Journal of Applied Ichthyology, 30*(6), 1259-1263.

Bell, A. M., & Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). *Ecology Letters*, *10*(9), 828–834.

Biro, P. A., Post, J. R., & Parkinson, E. A. (2003). From individuals to populations: Prey fish risk-taking mediates mortality in whole-system experiments. *Ecology, 84*(9), 2419–2431.

Boonstra, R. (2013). Reality as the leading cause of stress: Rethinking the impact of chronic stress in nature. *Functional Ecology, 27*(1), 11–23.

Breuner, C. W., Patterson, S. H., & Hahn, T. P. (2008). In search of relationships between the acute adrenocortical response and fitness. *General and Comparative Endocrinology, 157*(3), 288–295.

Bussy, U., Wassink, L., Scribner, K. T., & Li, W. (2017). Determination of cortisol in lake sturgeon (*Acipenser fulvescens*) eggs by liquid chromatography tandem mass spectrometry. *Journal of Chromatography B, 1040*, 162–168.

Cavanaugh, J. E. (1997). Unifying the derivations for the Akaike and corrected Akaike information criteria. *Statistics & Probability Letters*, *33*(2), 201–208.

Comte, L., Buisson, L., Daufresne, M., & Grenouillet, G. (2013). Climate-induced changes in the distribution of freshwater fish: Observed and predicted trends. *Freshwater Biology*, *58*(4), 625–639.

Comte, L., & Grenouillet, G. (2013). Do stream fish track climate change? Assessing distribution shifts in recent decades. *Ecography*, *36*(11), 1236–1246.

Cook, K. V., Crossin, G. T., Patterson, D. A., Hinch, S. G., Gilmour, K. M., & Cooke, S. J. (2014). The stress response predicts migration failure but not migration rate in a semelparous fish. *General and Comparative Endocrinology*, *202*, 44–49.

Crossman, J. A., Scribner, K. T., Forsythe, P. S., & Baker, E. A. (2018). Lethal and non-lethal effects of predation by native fish and an invasive crayfish on hatchery-reared age-0 lake sturgeon (*Acipenser fulvescens* Rafinesque, 1817). *Journal of Applied Ichthyology*, *34*(2), 322–330.

Crossman, J. A., Scribner, K. T., Yen, D. T., Davis, C. A., Forsythe, P. S., & Baker, E. A. (2011). Gamete and larval collection methods and hatchery rearing environments affect levels of genetic diversity in early life stages of lake sturgeon (*Acipenser fulvescens*). *Aquaculture*, *310*(3–4), 312–324.

Dammerman, K. J., Steibel, J. P., & Scribner, K. T. (2016). Increases in the mean and variability of thermal regimes result in differential phenotypic responses among genotypes during early ontogenetic stages of lake sturgeon (*Acipenser fulvescens*). *Evolutionary Applications*, *9*(10), 1258–1270.

De Jesus, E.G., Hirano, T., & Inui, Y. (1991). Changes in cortisol and thyroid hormone concentrations during early development and metamorphosis in Japanese flounder, *Paralichthys olivaceus*. *General and Comparative Endocrinology*, *82*, 369–376.

Dettlaff, T. A., Ginsburg, A. S., & Schmalhausen, O. I. (1993). *Sturgeon fishes: Developmental biology and aquaculture*. Berlin, Germany: Springer Science & Business Media.

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin*, *130*(3), 355–391.

Dudley, R. K., & Matter, W. J. (2000). Effects of small green sunfish (*Lepomis cyanellus*) on recruitment of Gila chub (*Gila intermedia*) in Sabino Creek, Arizona. *Southwestern Naturalist*, *45*, 24–29.

Duong, T. Y., Scribner, K. T., Crossman, J. A., Forsythe, P. S., & Baker, E. A. (2011). Environmental and maternal effects on embryonic and larval developmental time until dispersal of lake sturgeon (*Acipenser fulvescens*). *Canadian Journal of Fisheries and Aquatic Sciences*, *68*(4), 643–654.

Ebbesson, L. O. E., & Braithwaite, V. A. (2012). Environmental effects on fish neural plasticity

and cognition. *Journal of Fish Biology*, *81*(7), 2151–2174.

Falahatkar, B., Poursaeid, S., Shakoorian, M., & Barton, B. (2009). Responses to handling and confinement stressors in juvenile great sturgeon *Huso huso*. *Journal of Fish Biology*, *75*(4), 784–796.

Ferguson, M. M., & Duckworth, G. A. (1997). The status and distribution of lake sturgeon, *Acipenser fulvescens*, in the Canadian provinces of Manitoba, Ontario and Quebec: A genetic perspective. In V. J. Birstein, J. R. Waldman, & W. E. Bemis (Eds.), *Sturgeon biodiversity and conservation* (pp. 299–309). New York, NY: Kluwer Academic/Plenum.

Flandreau, E. I., Ressler, K. J., Owens, M. J., & Nemeroff, C. B. (2012). Chronic overexpression of corticotropin-releasing factor from the central amygdala produces HPA axis hyperactivity and behavioral anxiety associated with gene-expression changes in the hippocampus and paraventricular nucleus of the hypothalamus. *Psychoneuroendocrinology*, *37*(1), 27–38.

Galhardo, L., & Oliveira, R. F. (2009). Psychological stress and welfare in fish. *Annual Review of Biomedical Sciences*, *11*, 1–20.

Grønli, J., Murison, R., Fiske, E., Bjorvatn, B., Sørensen, E., Portas, C. M., et al. (2005). Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions. *Physiology & Behavior*, *84*(4), 571–577.

Hastings, R. P., Bauman, J. M., Baker, E. A., & Scribner, K. T. (2013). Post-hatch dispersal of lake sturgeon (*Acipenser fulvescens*, Rafinesque, 1817) yolk-sac larvae in relation to substrate in an artificial stream. *Journal of Applied Ichthyology*, *29*(6), 1208–1213.

Hayhoe, K., VanDorn, J., Croley, T., II, Schlegal, N., & Wuebbles, D. (2010). Regional climate change projections for Chicago and the US Great Lakes. *Journal of Great Lakes Research*, *36*, 7–21.

Hofer, H., & East, M. L. (1998). Biological conservation and stress. *Advances in the Study of Behavior*, *27*, 405–525.

Jeanneteau, F. D., Lambert, W. M., Ismaili, N., Bath, K. G., Lee, F. S., Garabedian, M. J., et al. (2012). BDNF and glucocorticoids regulate corticotrophin-releasing hormone (CRH) homeostasis in the hypothalamus. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(4), 1305–1310.

Kempinger, J. J. (1988). Spawning and early life history of lake sturgeon in the Lake Winnebago System, Wisconsin. *American Fisheries Society Symposium Series*, *5*, 110–122.

Lehtiniemi, M. (2005). Swim or hide: Predator cues cause species specific reactions in young fish larvae. *Journal of Fish Biology*, *66*(5), 1285–1299.

Li, D., Liu, Z., & Xie, C. (2012). Effect of stocking density on growth and serum concentrations

of thyroid hormones and cortisol in Amur sturgeon, *Acipenser schrenckii*. *Fish Physiology and Biochemistry*, 38(2), 511–520.

Lovallo, W. R., & Thomas, T. L. (2000). Stress hormones in psychophysiological research: Emotional, behavioral, and cognitive implications. In J. T. Cacioppo (Ed.), *Handbook of psychophysiology* (pp. 465–494). Cambridge, U.K.: Cambridge University Press.

Love, O. P., McGowan, P. O., & 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. *Functional Ecology*, *27*(1), 81–92.

Lukkes, J. L., Mokin, M. V., Scholl, J. L., & Forster, G. L. (2009). Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses. *Hormones and Behavior*, *55*(1), 248–256.

MacDougall-Shackleton, S. A., Dindia, L., Newman, A. E. M., Potvin, D. A., Stewart, K. A., & MacDougall-Shackleton, E. A. (2009). Stress, song and survival in sparrows. *Biology letters*, *5*(6), 746–748.

Madaro, A., Olsen, R. E., Kristiansen, T. S., Ebbesson, L. O., Nilsen, T. O., Flik, G., et al. (2015). Stress in Atlantic salmon: Response to unpredictable chronic stress. *Journal of Experimental Biology*, *218*(16), 2538–2550.

McAdam, S. O. (2011). Effects of substrate condition on habitat use and survival by white sturgeon (*Acipenser transmontanus*) larvae and potential implications for recruitment. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*(5), 812–822.

Meaney, M. J., Sapolsky, R. M., & McEwen, B. S. (1985). The development of the glucocorticoid receptor system in the rat limbic brain. I. Ontogeny and autoregulation. *Developmental Brain Research*, *18*(1), 159–164.

Mills, S. C., Beldade, R., Chabanet, P., Bigot, L., O'Donnell, J. L., & Bernardi, G. (2015). Ghosts of thermal past: Reef fish exposed to historic high temperatures have heightened stress response to further stressors. *Coral Reefs*, *34*(4), 1255–1260.

Paitz, R. T., Mommer, B. C., Suhr, E., & Bell, A. M. (2015). Changes in the concentrations of four maternal steroids during embryonic development in the threespined stickleback (*Gasterosteus aculeatus*). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *323*(7), 422–429.

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: Classical theories and new developments. *Trends in Neurosciences*, *31*(9), 464–468.

Peterson, D. L., Vecsei, P., & Jennings, C. A. (2007). Ecology and biology of the lake sturgeon: A synthesis of current knowledge of a threatened North American Acipenseridae. *Reviews in Fish Biology and Fisheries*, *17*(1), 59–76.

Piato, Â. L., Capiotti, K. M., Tamborski, A. R., Oses, J. P., Barcellos, L. J., Bogo, M. R., et al. (2011). Unpredictable chronic stress model in zebrafish (*Danio rerio*): Behavioral and physiological responses. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(2), 561–567.

Reed, T. E., Waples, R. S., Schindler, D. E., Hard, J. J., & Kinnison, M. T. (2010). Phenotypic plasticity and population viability: The importance of environmental predictability. *Proceedings of the Royal Society of London B: Biological Sciences*, *277*(1699), 3391–3400.

Romero, L. M., & Wikelski, M. (2001). Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Nino events. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(13), 7366–7370.

Sakamoto, K., Dew, W. A., Hecnar, S. J., & Pyle, G. G. (2016). Effects of lampricide on olfaction and behavior in young-of-the-year lake sturgeon (*Acipenser fulvescens*). *Environmental Science & Technology*, *50*(7), 3462–3468.

Schlaepfer, M. A., Runge, M. C. & Sherman, P. W. (2002). Ecological and evolutionary traps. *Trends in Ecology & Evolution*, *17*, 474–480.

Schreck, C. B., Olla, B. L., & Davis, M. W. (1997). Behavioral responses to stress. *Fish Stress and Health in Aquaculture*, *62*, 145–170.

Sheriff, M. J., & Love, O. P. (2013). Determining the adaptive potential of maternal stress. *Ecology Letters*, *16*(2), 271–280.

Sih, A. (2011). Effects of early stress on behavioral syndromes: An integrated adaptive perspective. *Neuroscience & Biobehavioral Reviews*, *35*(7), 1452–1465.

Silbernagel, J. J., & Sorensen, P. W. (2013). Direct field and laboratory evidence that a combination of egg and larval predation controls recruitment of invasive common carp in many lakes of the Upper Mississippi River Basin. *Transactions of the American Fisheries Society*, *142*(4), 1134–1140.

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. *Aquaculture International*, *17*(1), 7–14.

Smith, K. M., & King, D. K. (2005). Dynamics and extent of larval lake sturgeon *Acipenser fulvescens* drift in the Upper Black River, Michigan. *Journal of Applied Ichthyology*, *21*(3), 161–168.

Sopinka, N. M., Capelle, P. M., Semeniuk, C. A. D., & Love, O. P. (2017). Glucocorticoids in fish eggs: Variation, interactions with the environment, and the potential to shape

offspring fitness. *Physiological and Biochemical Zoology*, *90*(1), 15–33.

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

Sopinka, N. M., Hinch, S. G., Middleton, C. T., Hills, J. A., & 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*(2), 493–500.

Sterlemann, V., Ganea, K., Liebl, C., Harbich, D., Alam, S., Holsboer, F., et al. (2008). Long-term behavioral and neuroendocrine alterations following chronic social stress in mice: Implications for stress-related disorders. *Hormones and Behavior*, *53*(2), 386–394.

Storm, J. J., & Lima, S. L. (2010). Mothers forewarn offspring about predators: A transgenerational maternal effect on behavior. *American Naturalist*, *175*(3), 382–390.

Turner, J. D., Alt, S. R., Cao, L., Vernocchi, S., Trifonova, S., Battello, N., et al. (2010). Transcriptional control of the glucocorticoid receptor: CpG islands, epigenetics and more. *Biochemical Pharmacology*, *80*(12), 1860–1868.

Vallée, M., Maccari, S., Dellu, F., Simon, H., Le Moal, M., & Mayo, W. (1999). Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: A longitudinal study in the rat. *European Journal of Neuroscience*, *11*(8), 2906–2916.

Van Eenennaam, J. P., Linares-Casenave, J., Deng, X., & Doroshov, S. I. (2005). Effect of incubation temperature on green sturgeon embryos, *Acipenser medirostris*. *Environmental Biology of Fishes*, *72*(2), 145–154.

Vieira, J. G. H., Nakamura, O. H., & Carvalho, V. M. (2014). Determination of cortisol and cortisone in human saliva by a liquid chromatography-tandem mass spectrometry method. *Arquivos Brasileiros de Endocrinologia & Metabologia*, *58*(8), 844–850.

Wagner, C. M., Stroud, E. M., & Meckley, T. D. (2011). A deathly odor suggests a new sustainable tool for controlling a costly invasive species. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*(7), 1157–1160.

Waraniak, J. M., Baker, E. A., & Scribner, K. T. (2018). Molecular diet analysis reveals predator–prey community dynamics and environmental factors affecting predation of larval lake sturgeon *Acipenser fulvescens* in a natural system. *Journal of Fish Biology*, *93*(4), 616–629.

Wishingrad, V., Ferrari, M. C., & Chivers, D. P. (2014). Behavioural and morphological defences in a fish with a complex antipredator phenotype. *Animal Behaviour*, *95*, 137–143.

Wishingrad, V., Musgrove, A. B., Chivers, D. P., & Ferrari, M. C. (2015). Risk in a changing world: Environmental cues drive anti-predator behaviour in lake sturgeon (*Acipenser fulvescens*) in the absence of predators. *Behaviour*, *152*(5), 635–652.

Wishingrad, V., Sloychuk, J. R., Ferrari, M. C. O., & Chivers, D. P. (2014). Alarm cues in lake sturgeon *Acipenser fulvescens* Rafinesque, 1817: Potential implications for life-skills training. *Journal of Applied Ichthyology*, *30*(6), 1441–1444.

Woodward, G., Perkins, D. M., & Brown, L. E. (2010). Climate change and freshwater ecosystems: Impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1549), 2093–2106.

Wuertz, S., Lutz, I., Gessner, J., Loeschau, P., Hogans, B., Kirschbaum, F., et al. (2006). The influence of rearing density as environmental stressor on cortisol response of shortnose sturgeon (*Acipenser brevirostrum*). *Journal of Applied Ichthyology*, *22*(Suppl. 1), 269–273.

Zubair, S. N., Peake, S. J., 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). *Environmental biology of fishes*, *93*(4), 577-587.

CHAPTER 2:

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

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ABSTRACT

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

INTRODUCTION

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

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

Behavioral and physiological alterations induced by maternal stress may not necessarily be maladaptive, but in some cases may prepare offspring to function and survive in high-stress conditions (Gagliano & McCormick, 2009; Sheriff & Love, 2013). For example, in the tropical damselfish *Pomacentrus amboinensis*, high-density stress causes females to have offspring with

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

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

female's experience of the stressor and the development of the embryo, resulting in more opportunity for mismatch between maternal and offspring environments.

Lake sturgeon, *Acipenser fulvescens*, are an ancient chondrostean fish species that is regionally threatened and a priority for conservation in the Great Lakes basin. Egg provisioning occurs far in advance of spawning (Doroshov et al. 1997), and thus stressors influencing maternal deposition of cortisol into egg yolk may not accurately predict the stress level of environments experienced by offspring after hatch. Lake sturgeon populations have been bottlenecked through historic overexploitation and habitat disturbance (Ferguson & Duckworth, 1997), and will likely continue to be threatened by environmental stressors associated with climate change (Comte et al., 2013; Hayhoe et al., 2010). Since lake sturgeon take approximately 20 years to reach sexual maturity, the ability of populations to respond genetically to environmental changes is limited, making them vulnerable to rapidly changing environmental conditions. Therefore, it is important to understand whether transgenerational plasticity mediated by maternal effects plays a role in determining survival and population viability. Maternal effects on the behavior of lake sturgeon offspring may be especially important in the context of antipredator behaviors, which have been shown to affect survival and recruitment in this species (McAdam, 2011), as well as other fishes (Dudley & Matter, 2000; Silbernagel & Sorensen, 2013). During the larval stage, lake sturgeon are particularly vulnerable to predation, which contributes largely to high mortality rates during the first year of life (Waraniak et al., 2018). Therefore, alterations in offspring behavior induced by maternally provisioned egg cortisol can potentially influence lake sturgeon larval survival and have downstream population-level effects. Understanding effects of maternal stress requires assessing survival outcomes within ecologically relevant contexts (Sheriff & Love, 2013; Sopinka et al., 2014), an important

component of which is match or mismatch between maternal and offspring environments (Sheriff et al., 2017). In fishes, maternal stress can be simulated by incubating eggs in a cortisol solution to elevate egg cortisol levels (Sopinka et al., 2015, 2017). Offspring stress levels can be manipulated using a chronic unpredictable stress regime (Lankford et al., 2005; Piato et al., 2011). By combining these techniques, this study creates treatments that pair high egg cortisol with high offspring stress, low egg cortisol with low offspring stress, high egg cortisol with low offspring stress and low egg cortisol with high offspring stress (Figure 2.1).

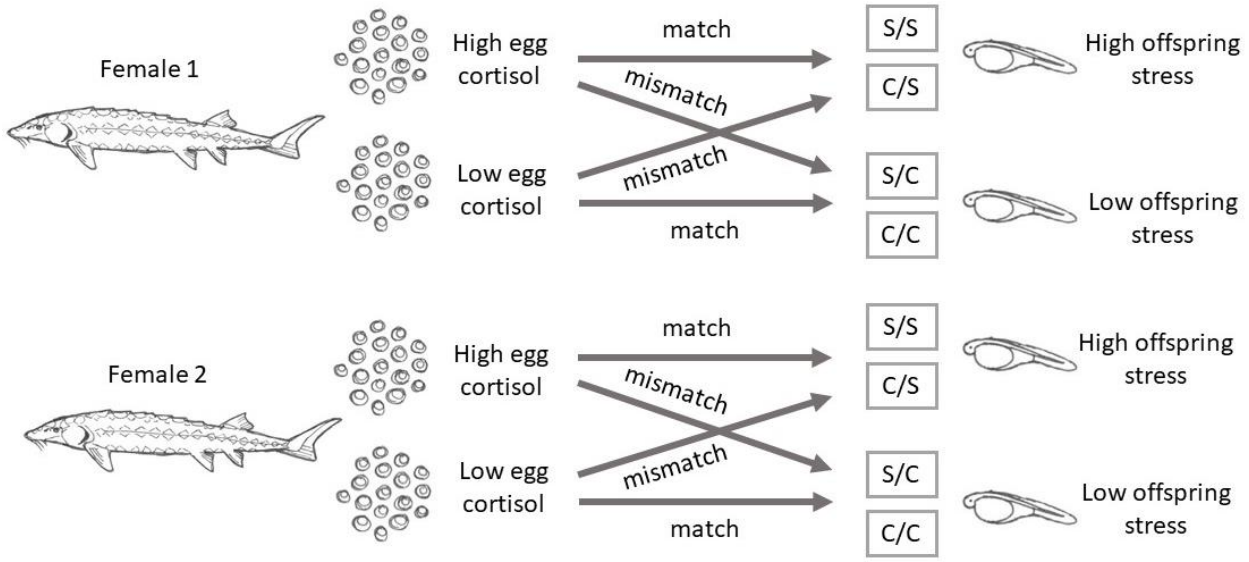

Figure 2.1. Experimental design.
The four treatments were high egg cortisol (simulating high maternal stress) and high offspring stress(S/S), high egg cortisol and low offspring stress (S/C), low egg cortisol and high offspring stress (C/S), and low egg cortisol and low offspring stress (C/C). "S" in the treatment name designates stress, "C" designates control (or low stress). C/C and S/S treatments indicate a match between egg cortisol and offspring environment, and C/S and S/C treatments indicate a mismatch. Two families were included in the experiment, with eggs from each female divided into the four treatments as depicted.

In this study, we hypothesize that specific combinations of egg cortisol exposure and early life stress exposure, rather than egg cortisol alone or early life stress alone, will determine offspring stress axis function, which can be quantified by assessing physiological and behavioral reactivity (Weinstock, 2005). We specifically predicted that offspring that experience a

mismatch between egg cortisol exposure and offspring stress would have increased physiological and behavioral reactions to stress (higher rises in cortisol levels poststress and higher activity levels, respectively) compared to those that experience a match. Stress reactivity determines how individuals respond to threats such as predation risk (Vitousek et al., 2014), and thus is important for predicting survival outcomes of transgenerational stress.

METHODS

Sturgeon eggs used in the experiment were collected from two female lake sturgeon spawning in the Upper Black River in Onaway, Michigan, U.S.A. Female 1 was captured on 29 April 2017, and Female 2 was captured on 20 May 2017. Stress was minimized for adult sturgeon during capture by ensuring that each individual's head and gills remained underwater during handling, extruding gametes noninvasively by applying gentle pressure to the abdomen. Each individual was handled for an average of around 5 min before release. Eggs were fertilized using a 1:1 female:male ratio, and sperm was obtained the same day as egg collection for each of the two families (Bauman et al., 2016; Crossman et al., 2011). PIT (passive integrated transponder) tags and RFID (radiofrequency identification) tags were used for identification in order to confirm that each of the females and males from which gametes were collected were unique individuals. There were six replicates per family in each of the four treatments (Figure 2.1). Each replicate contained 11 ml of fertilized eggs (approximately 572 eggs), 4 ml (approximately 208 eggs) of which were used for samples within the first 24 h of development, leaving each replicate with 7 ml of eggs (approximately 364 eggs).

The four maternal/offspring treatments were high maternal stress and high offspring stress (S/S), high maternal stress and low offspring stress (S/C), low maternal stress and high offspring stress (C/S), and low maternal stress and low offspring stress (C/C). ‘S’ in the

treatment name designates stress, ‘C’ designates control (or low stress) (Figure 2.1). To simulate high maternal stress, eggs in S/S and S/C treatments were incubated in a cortisol solution made by dissolving cortisol (H4001, Sigma) in 95% ethanol and adding to 400 ml of water for a final cortisol concentration of 600 ng/ml (Sopinka et al., 2015). Eggs in the C/S and C/C treatments were incubated in a control solution, made using the same amount of ethanol and water but without cortisol. All eggs were incubated in cortisol or control solutions for 1 h immediately following fertilization. High offspring stress was later created after hatch by applying an unpredictable chronic stress regime (described below), and low offspring stress was created by withholding the unpredictable chronic stress regime.

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

our experimental elevation of cortisol in fertilized sturgeon eggs is an artificial manipulation meant to approximate the natural condition, in which eggs are exposed to maternal cortisol well in advance of fertilization.

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

Offspring stress treatment (high stress for treatments C/S and S/ S, low stress for treatments C/C and S/C) was implemented during the free embryo stage, starting 2 days after hatch and continuing until the larval stage at the onset of exogenous feeding. Free embryos in the S/S and C/S treatments were subjected to an unpredictable chronic stress regime (Lankford et al., 2005), while free embryos in the S/C and C/C treatments were left unstressed. The unpredictable chronic stress regime, which is designed to produce ongoing stress without habituation, consisted of a set of three stressors, two of which were randomly applied daily. Stressor 1 was exposure to

light for 2 min using a flashlight, since sturgeon free embryos are negatively phototaxic (Richmond & Kynard, 1995, pp. 172-182). Stressor 2 was a thump on the table surface, produced by dropping a weight of 212 g onto the table from a height of 22 cm, repeated twice. The thump caused a visible startle response, indicating that it induced stress for free embryos (Davis, 2010). Stressor 3 was exposure to low water level for 2 min, during which water was drained to a depth of 2.54 cm. Low water level temporarily increases free embryo density, which has been shown to stress lake sturgeon free embryos (Bauman et al., 2015). Daily stressors were selected using a random number generator and applied at 1100 h and 1400 h. Applying randomly selected stressors twice daily has been shown to cause chronic stress in sturgeon (Lankford et al., 2005). Care was taken to promote animal welfare by minimizing incidental stress unrelated to the set of three experimental stressors. Starting at the egg stage, individuals were housed in 4-inch (10.16 cm) diameter couplings made of PVC plastic and mesh that ensured adequate water flow-through. Eggs were treated with 500 µl/ml peroxide every 2 days during incubation following standard hatchery protocols to prevent fungal infection, and any mortalities were removed. After hatch, shells were removed from couplings to avoid impediment of water flow-through, and 2.54 cm^3 BioBalls (Pentair, No. CBBI-5, Pentair Aquatic Eco-Systems, Cary, NC, U.S.A.) were added to each coupling to simulate substrate for burrowing free embryos. All protocols were conducted according to approved Michigan State University Animal Use and Care guidelines under Animal Use and Care project 04/17-071-00.

Body Size and Yolk Sac Area

To observe effects of treatment on offspring growth, we obtained body size (mm) and yolk sac area (mm^2) measurements at hatch and body size measurements at the larval stage using ImageJ software (National Institutes of Health, Bethesda, MD, U.S.A., http://rsbweb.nih.gov/ij/).

Photos used for ImageJ analysis were taken with a digital camera and included six individuals per replicate, as well as a ruler for size calibration. Individuals used for measurements were sedated with 25 mg/liter of MS 222 using approved Michigan State University Animal Use and Care protocols and then removed from the experiment.

Cortisol levels

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

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

Larval behavior

The effect of treatment on larval behavior was investigated by observing larval swimming activity during a 5 min trial that began by administering a startle cue. Six larvae from each replicate were placed in a 6-inch (15.24 cm) diameter Petri dish filled with water from the tank system and allowed to acclimate for 2 min. After acclimation, a 5 min video was recorded. The weight was dropped at 2 min to characterize behavior prior to and following the startle cue. Loligo v.4.0 tracking software (Loligo Systems, Viborg, Denmark; https://www.loligosystems.com/software) was used to simultaneously track activity of the six individuals in each video. A centre zone was defined that excluded a 1-inch (2.54 cm) perimeter around the Petri dish edge to determine whether edge-seeking behavior varied among larvae from different treatments. Variables quantified from video analysis included velocity (cm/s), acceleration (cm/s^2), percentage of time active, total distance travelled (cm), number of visits to centre zone and time spent in centre zone (s), following Sakamoto et al. (2016).

Statistical analysis

We assessed normality for each data set using a Shapiro-Wilk test in R v.3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). The cortisol data set was not normally

distributed and was log-transformed prior to analysis. Generalized linear models were fitted to quantify factors associated with body size, yolk sac area and cortisol data sets using the *glm* function in R v.3.2.2. Models with delta AIC < 2 were considered competitive for the top model (Burnham & Anderson, 1998). If multiple models were competitive, we took a maximization of parsimony approach and chose the model with the fewest variables. For yolk area and size data sets, candidate models included main effects of treatment and family, as well as the interaction of treatment and family to assess whether cortisol treatment had a family-specific effect. For the fertilized egg cortisol data set, candidate models included main effects of treatment, family and stage, as well as the interaction of treatment and family (to assess whether treatment had a family-specific effect on egg cortisol), the interaction of stage and treatment (to assess whether cortisol content of eggs depended on developmental stage), and the interaction of family and stage (to assess whether families had differing rates of cortisol absorption or efflux from eggs). Candidate models for the larval cortisol data set included the main effects of treatment, family and stress state (whether larvae were euthanized at baseline stress level or after an acute stressor), as well as the interaction of treatment and family (to assess whether treatment had a family-specific effect on larval cortisol), the interaction of family and stress state (to assess whether family influenced physiological response to an acute stressor), and the interaction of treatment and stress state (to assess whether treatment altered the physiological response to an acute stressor). For each AICc selected model (Cavanaugh, 1997), we used ANOVA to run F tests on the model output and determine which variables were significant. Post hoc Tukey HSD tests were conducted for significant variables.

We used principal components analysis (Hotelling, 1933) to examine behavioral variables in order to reduce dimensionality of the data set by compressing dependent variables

(percentage of time active, acceleration, velocity, distance travelled, zone time and zone visits) into a composite behavioral measure (Ballew et al., 2017). The broken stick method was used to determine that PC1 and PC2 were significant. We selected generalized linear models for PC1 and PC2 using AICc model selection, and used ANOVA to run F tests on the model output and determine which variables were significant. Factor loadings above 0.5 were examined to characterize behavioral relevance of each principal component.

RESULTS

Body size and yolk sac area

Since only cortisol or control incubation had been applied prior to hatch, and the stress regime was not implemented until after hatch, we included only egg treatment (S for cortisol-treated, C for control) during model selection for hatchling yolk sac area and body size data sets. The top two AICc-selected models for yolk sac area at hatch were competitive, so we chose the most parsimonious model, which included family as the only factor (delta AICc = 1.28) (Table 2.1). ANOVA indicated that free embryos from Family 1 had a significantly larger yolk sac area (mean ± SD: C treatment: 6.97 ± 0.90 mm2 ; S treatment: 7.28 ± 0.90 mm2) than did free embryos from Family 2 (C treatment: 6.95 ± 0.75 mm2 ; S treatment: 6.75 ± 1.17 mm2) ($p = 0.01283$; Figure 2.2).

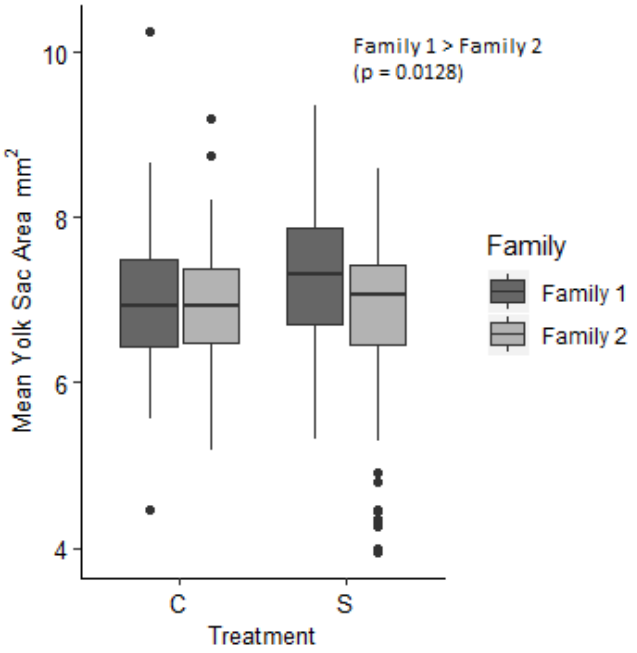

Figure 2.2. Offspring yolk sac area at hatch for each treatment and family.
Whiskers indicate minimum and maximum values, excluding data points that lie further than 1.5 times the interquartile range from the upper or lower quartile. "C" treatment refers to eggs incubated in control solution to simulate low maternal stress, "S" treatments refers to eggs incubated in cortisol solution to simulate high maternal stress. Individuals from Family 1 had a significantly larger yolk sac area than did individuals from Family 2 ($p = 0.0128$). Eggs were incubated in respective solutions for one hour immediately after fertilization.

Table 2.1. Models for yolk sac area at hatch.
Since the top two models were competitive with a delta AICc < 2, the model containing family only was chosen as the most parsimonious model. Akaike weight indicates conditional probability of each model (Wagenmakers & Farrell 2004).

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

The AICc-selected model for body size at hatch included family only (Table 2.2). ANOVA indicated that free embryos from Family 2 were significantly larger (12.46 ± 0.31 mm) than free embryos from Family 1 (11.79 ± 0.26 mm) ($p < 0.0001$; Figure 2.2).

At the larval stage, the AICc-selected model for body size included family only (Table 2.2). ANOVA indicated that larvae from Family 1 were significantly larger (21.78 ± 0.48 mm) than larvae from Family 2 (21.25 ± 0.50 mm) ($p = 0.0006$; Figure 2.3).

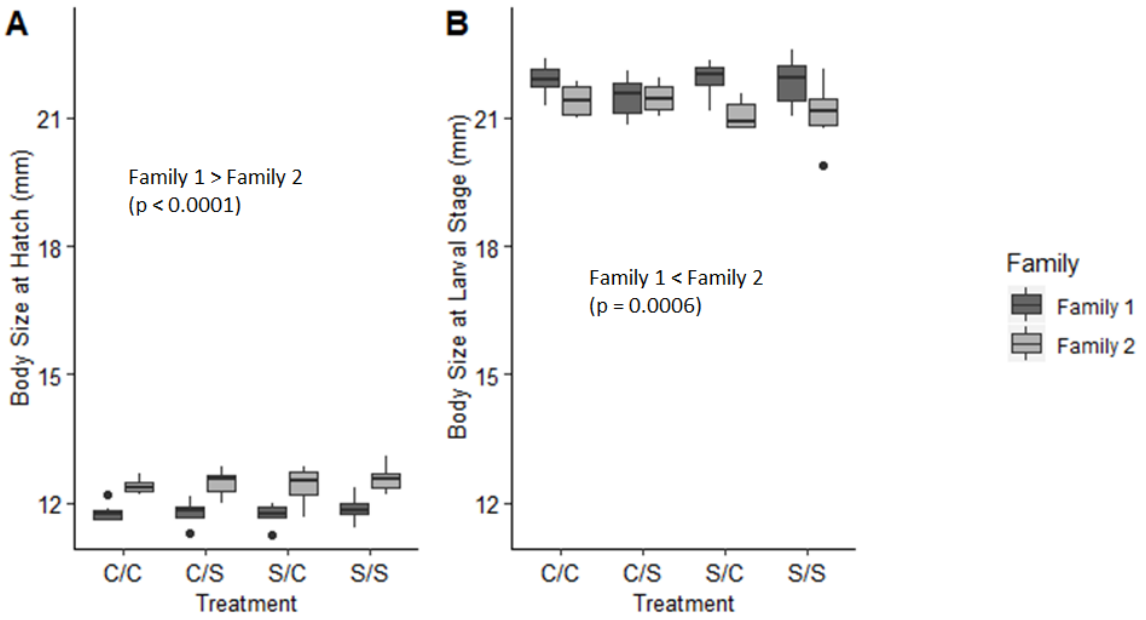

Figure 2.3. Body size at hatch (A) and larval stage (B) for each treatment and family. Whiskers indicate minimum and maximum values, excluding data points that lie further than 1.5 times the interquartile range from the upper or lower quartile. At hatch, individuals from Family 2 were significantly larger than free embryos from Family 1 ($p < 0.0001$). At the larval stage, individuals from Family 1 were significantly larger than individuals from Family 2 ($p = 0.0006$)

Table 2.2. Models for body size at hatch and larval stage.
Akaike weight indicates conditional probability of each model (Wagenmakers & Farrell 2004).

Model	AICc	Delta AICc	Akaike Weight
hatch			
Size ~ Family	21.92	0	0.95
Size ~ Family + Treatment	27.69	5.78	0.05
Size ~ Family + Treatment + Family*Treatment	36.14	14.22	0
(null model) Size ~ 1	61.51	39.59	0
Size ~ Treatment	67.95	46.04	0
larvae			
Size ~ Family	72.25	0	0.95
Size ~ Family + Treatment	78.55	6.3	0.04
Size ~ Family + Treatment + Family*Treatment	81.38	9.13	0.01
(null model) Size ~ 1	82.46		0.01
Size ~ Treatment	88.7	16.45	0

Cortisol levels

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

Since only cortisol or control incubation had been applied at the fertilized egg stage, and the stress regime was not implemented until after hatch, only egg treatment (S for cortisol-treated, C for control) was included during model selection for the fertilized egg cortisol data set. The AICc-selected model for fertilized egg cortisol included egg development stage, egg treatment, family, the interaction of egg treatment and family, the interaction of egg development stage and egg treatment, and the interaction of egg development stage and family (Table 2.3).

Table 2.3. Models for cortisol at fertilized egg stage and larval stage, including top three models and null model.
Akaike weight indicates conditional probability of each model (Wagenmakers & Farrell 2004).

Model	AICc	Delta AICc	Akaike Weight
fertilized egg stage			
Cortisol ~ stage + treatment1 + family + treatment1*family + stage*treatment1 + stage*family	-257.84	0	1
Cortisol ~ stage + treatment1 + family + treatment1*family+stage*treatment1	-36.02	221.82	0
Cortisol ~ stage + treatment1	183.39	441.23	0
(null) Cortisol ~ 1	449.19	707.03	0
larval stage			
Cortisol ~ treatment + family + stress state + treatment*family + treatment*stress state + family*stress state	-215.14	0	1
Cortisol ~ treatment + family + stress state + treatment*family + treatment*stress state	-182.31	32.84	0
Cortisol ~ treatment + family + stress state + treatment*family	-170.65	44.49	0
(null) Cortisol ~ 1	-62.25	152.89	0

ANOVA indicated that stage had a significant effect ($p < 0.0001$; Figs. 4, 5). ANOVA also indicated that eggs in the S treatment had significantly higher cortisol (89.58 ± 165.19 ng/g) than eggs in the C treatment (3.64 ± 5.17 ng/g) ($p < 0.0001$). The interaction of treatment and family had a significant effect ($p = 0.0003$), with eggs from Family 1 having higher mean cortisol than eggs from Family 2 in the C treatment but lower mean cortisol in the S treatment. The interaction of stage and egg treatment had a significant effect ($p < 0.0001$), with S treatment eggs showing a sudden increase in cortisol that then decreased by 24 h after fertilization to levels comparable to those of C treated eggs. The interaction of stage and family had a significant

effect, with eggs from Family 1 having lower cortisol than eggs from Family 2 at 0 h and 2 h after fertilization but higher cortisol 4 h and 24 h after fertilization ($p < 0.0001$; Figure 2.4).

For larval cortisol, the AICc-selected model included treatment, family, stress state (whether larvae were euthanized at baseline stress level or after an acute stressor), the interaction of treatment and family, and the interaction of treatment and stress state (Table 2.3). ANOVA indicated that treatment had a significant effect ($p = 0.0001$), family had a significant effect ($p < 0.0001$) and 'poststress' state had significantly higher cortisol than 'baseline' stress state ($p < 0.0001$). The interaction of treatment and family had a significant effect ($p < 0.0001$), with larvae from Family 2 having higher cortisol than larvae in Family 1 for all treatments except S/S. The interaction of treatment and stress state had a significant effect ($p < 0.0001$), with larvae in the S/S treatment having a smaller poststress increase in cortisol compared to other treatments. The interaction of family and stress state had a significant effect ($p < 0.0001$), with larvae from Family 2 having a higher post-stress cortisol increase across treatments than larvae from Family 1. Tukey HSD conducted for larvae from Family 1 indicated that in all treatments post-stress cortisol levels were significantly higher (5.33 ± 0.84 ng/g) than baseline (3.90 ± 0.69 ng/g). Tukey HSD conducted for larvae from Family 2 indicated that in all treatments post-stress cortisol levels were significantly higher (9.40 ± 2.99 ng/ g) than baseline (4.36 ± 0.74 ng/g). There were no significant differences among families at baseline, but at post-stress, larvae from Family 2 had significantly higher cortisol than did larvae from Family 1, except in the S/S treatment (Figure 2.6).

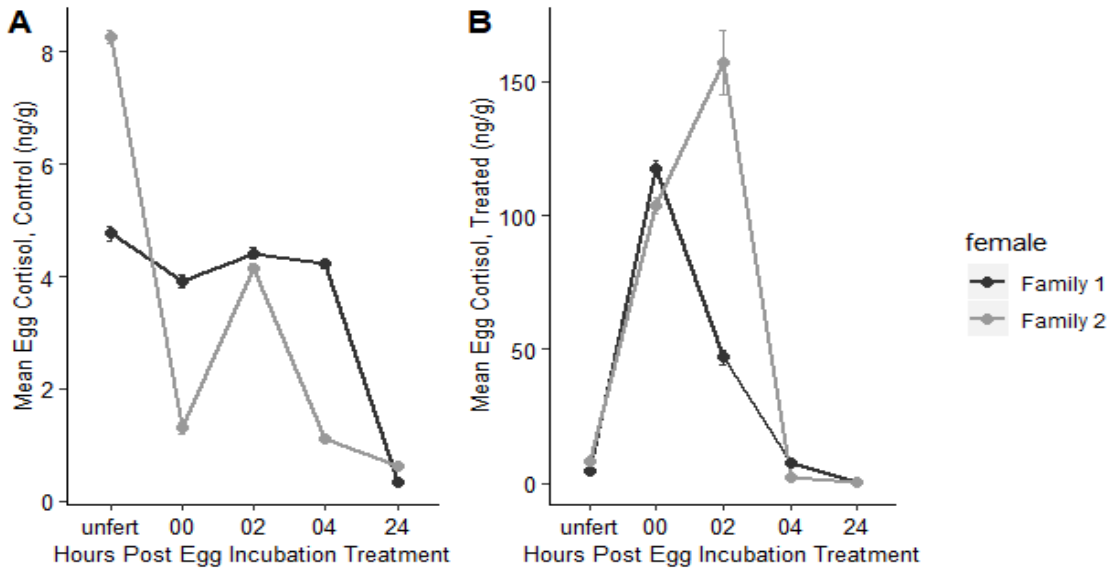

Figure 2.4. Cortisol levels in fertilized eggs, shown for "control" treatment eggs not incubated in cortisol solution (A) and for "stress" treatment eggs incubated in cortisol solution to simulate maternal stress (B).
Egg incubation treatment started immediately after fertilization and lasted one hour. Samples of fertilized eggs were taken starting immediately after incubation treatment (00). Eggs in the S treatment had significantly higher cortisol than eggs in the C treatment ($p < 0.0001$). Error bars show one standard error.

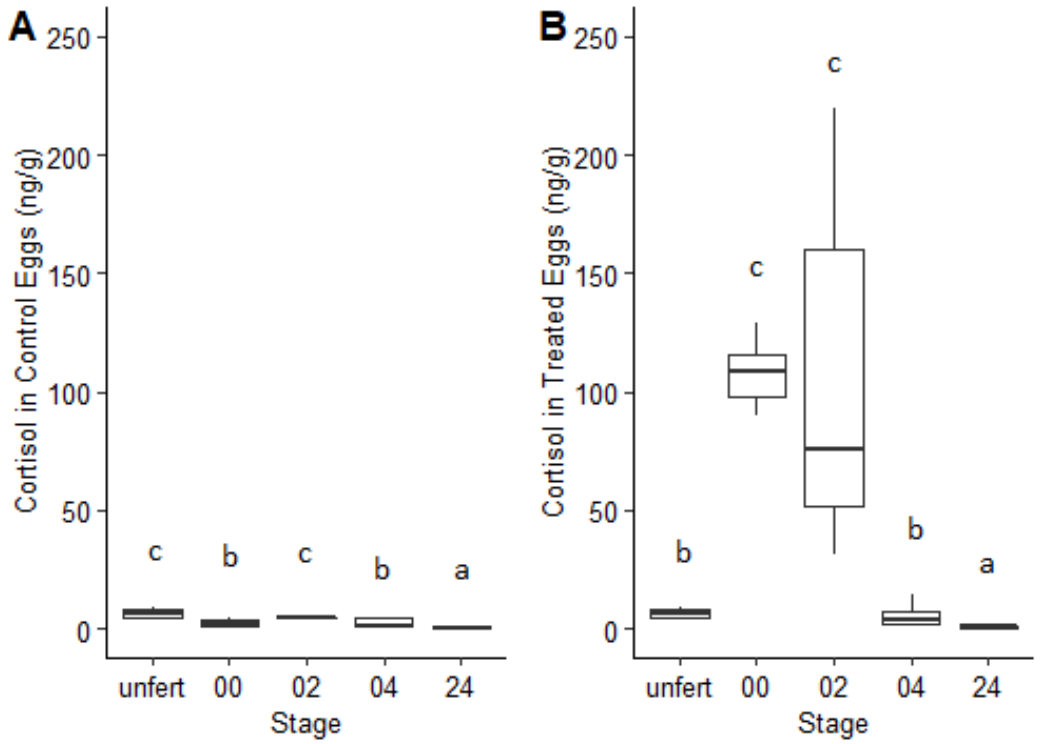

Figure 2.5. Cortisol levels in fertilized eggs, shown for "control" treatment eggs not incubated in cortisol solution (A) and for "stress" treatment eggs incubated in cortisol solution to simulate maternal stress (B).
Whiskers indicate minimum and maximum values, excluding data points that lie further than 1.5 times the interquartile range from the upper or lower quartile. Means for each stage are averaged across family. Stage had a significant effect ($p < 0.0001$), as did the interaction of treatment and female ($p = 0.0003$), the interaction of stage and egg treatment ($p < 0.0001$), and the interaction of stage and family ($p < 0.0001$). Letters indicate results of Tukey HSD test showing interaction of treatment and stage.

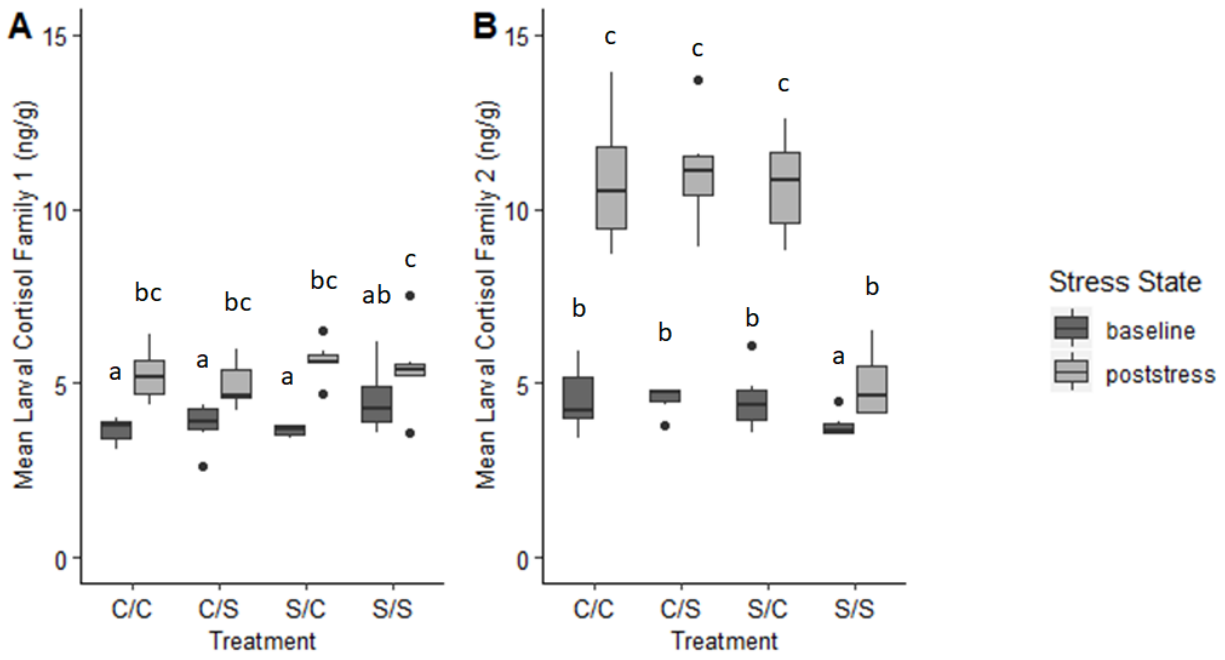

Figure 2.6. Cortisol levels at baseline levels and after exposure to an acute stressor, shown separately for Family 1 (A) and Family 2 (B).
Letters indicate results of Tukey HSD test showing interaction of treatment and stress state. Whiskers indicate minimum and maximum values, excluding data points that lie further than 1.5 times the interquartile range from the upper or lower quartile. Treatment C/C (control-control) is low maternal stress, low offspring stress; treatment C/S (control/stress) is low maternal stress, high offspring stress; treatment S/C (stress/control) is high maternal stress, low offspring stress; treatment S/S (stress/stress) is high maternal stress, high offspring stress. Stress state is either at baseline (no acute stressor applied) or post stress (30 minutes after acute stressor applied). There were significant effects of treatment, family, stress state, and the two-way interactions of those. Post-stress cortisol levels were significantly higher than baseline for both families ($p = 0.0001$). There were no significant differences among families at baseline, but at post-stress larvae from Family 2 had significantly higher post-stress cortisol than did larvae from Family 1, except in the S/S treatment ($p < 0.0001$). The interaction of treatment and stress state had a significant effect ($p < 0.0001$), with larvae in the S/S treatment having a smaller post-stress increase in cortisol compared to other treatments.

Larval behavior

Behavioral variables (percentage of time active, velocity, acceleration, distance travelled, zone time and zone visits) were reduced into two components using PCA. Factor loadings indicated that the most important variable contributing to variation along PC1 was the percentage of time active, and the most important variable contributing variation along PC2 was distance

travelled (Table 2.4). PC1 was negatively associated with the percentage of time active and therefore is interpreted as a measure of inactivity; PC2 was negatively associated with total distance travelled (cm) and therefore is interpreted as a measure of reduced movement during trials. PC1 explained 57.3% of the variation in the data and PC2 explained 21.1% of the variation in the data (Figure 2.6). Model selection was conducted using PC1 and PC2 in generalized linear models. For PC1, the AICc-selected model included treatment, family, and the interaction of treatment and family (Table 2.5). For PC2, the AICc-selected model included treatment, family, and the interaction of treatment and family (Table 2.5).

ANOVA for PC1 indicated that treatment was significant ($p = 0.016$) and that there was no significant difference between families ($p = 0.7318$). The interaction of treatment and family was significant ($p = 0.0002$), with larvae from Family 1 having lower mean PC1 scores (higher activity levels) than larvae from Family 2 in all treatments except C/C. Tukey HSD indicated that the mean PC1 score for treatment S/C (mean PC1 score = 0.40) was significantly higher than that for treatment C/S (mean PC1 score = - 0.53). Since PC1 was negatively associated with the percentage of time active, results indicate higher activity levels for individuals from treatment C/S than for individuals from treatment S/C. There were no other significant pairwise differences between treatments (mean for C/C = 0.16, mean for S/S = - 0.02).

Table 2.4. Factor loadings and eigenvalues for principal components analysis of behavioral variables.

	factor loadings						eigen-value	variance %	cumulative variance %
	velocity	*accel.*	*zone time*	*% activity*	*distance*	*zone visits*			
PC1	0.4499	0.4569	-0.2114	-0.5013	-0.3464	-0.4158	3.438	57.299	57.299
PC2	-0.47	-0.4418	0.0708	-0.0605	-0.6134	-0.446	1.266	21.107	78.405
PC3	0.1247	0.1377	0.8972	-0.1726	-0.2581	0.2531	1.017	16.952	95.358
PC4	-0.1586	-0.2137	-0.2957	-0.6329	-0.1643	0.6437	0.18	2.994	98.351
PC5	0.1338	0.2377	-0.2405	0.5578	-0.6378	0.387	0.094	1.57	99.922
PC6	-0.7198	0.6892	-0.0025	-0.0605	0.057	0.0053	0.005	0.0779	100

Table 2.5. Models for principal components. Akaike weight indicates conditional probability of each model (Wagenmakers & Farrell 2004).

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

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

DISCUSSION

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

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

Initial differences in unfertilized egg cortisol prior to experimental treatment may also have influenced offspring growth, evidenced by differences in yolk sac area between families at hatch. In fishes, yolk sac area can be reduced by maternal stress (Erikson et al., 2006; McCormick, 1998) due to increased metabolic rates (Mccormick & Nechaev, 2002). For Family 2, individuals from cortisol-treated eggs had increased growth during embryonic development, possibly due to increased metabolic rates associated with faster yolk sac absorption, resulting in hatchlings with smaller remaining yolk sacs and larger body sizes. While offspring from both families hatched within the expected time span based on calculations of developmental stage using cumulative temperature units (CTU) (Kempinger, 1998; Smith & King, 2005), offspring from Family 1 hatched at a slightly earlier developmental stage. Family 1 hatched around CTU 54-62 and Family 2 hatched around CTU 65-76. Earlier hatch time confirms that increased metabolic rate most likely speeded growth for Family 1. By the larval stage, individuals from Family 1 had grown larger than individuals from Family 2 despite starting with smaller hatch

sizes. Larger larval size has been shown to be advantageous in avoiding predation (Wassink et al., 2019; Wishingrad et al., 2014a). Family was the only factor indicated as an important predictor of larval size based on AICc model selection, highlighting the importance of interfamily variation in developmental trajectories, which may have down-stream influences on predation rates.

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

and behavior may therefore be due to maternal stress-related effects already at play in eggs prior to cortisol incubation treatment.

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

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

In contrast, larvae that experienced high egg cortisol but low offspring stress (S/C treatment) exhibited lower activity levels compared to larvae from the C/S treatment. Increased maternal cortisol in salmon has been shown to decrease offspring swimming activity (Espmark et al., 2008). Maternal stress and associated elevation in egg cortisol may therefore be related to a reduction in offspring activity levels. However, in this study the effect of egg cortisol on activity levels may have been mediated by early life experience, since larvae that experienced both elevated egg cortisol and high early life stress (S/S treatment) did not have lower activity.
For PC2, which was associated with total distance travelled during trials, larvae from the C/C treatment moved greater distances than larvae in either of the treatments that experienced high

early life stress (C/S and S/S). Family also had a significant effect on PC2, as larvae from Family 1 moved greater distances during trials than larvae from Family 2. While behavioral trials in petri dishes bears limited applicability to behavior in the wild, a reduction in movement due to early life stress may influence larval drift, when larvae emerge from the substrate and disperse downstream (Smith & King, 2005). Past research in dispersal behavior of larvae from this population documented large variability in the timing of dispersal by larvae from different females that spawned within the same day (Duong et al., 2011). Further research could explore whether early life stress and family-specific parental effects may reduce the rate at which larvae drift downstream, and whether this represents an adaptive behavioral response to high-stress conditions.

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

Further research on transgenerational stress should consider interfamily variation in how offspring respond to different combinations of maternal and offspring environments. Phenotypic

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

REFERENCES

REFERENCES

Auperin, B., & 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. *General and comparative endocrinology*, *158*(3), 234-239.

Baker, D.W., Peake, S.J., & Kieffer, J.D. (2008). The effect of capture, handling, and tagging on hematological variables in wild adult lake sturgeon. *North American Journal of Fisheries Management, 28*(1), 296–300.

Ballew, N. G., Mittelbach, G. G., & Scribner, K. T. (2017). Fitness consequences of boldness in juvenile and adult largemouth bass. *The American Naturalist, 189*(4), 396-406.

Bauman, J. M., Baker, E. A., Marsh, T. L., & Scribner, K. T. (2015). Effects of rearing density on total length and survival of Lake Sturgeon free embryos. *North American journal of aquaculture*, *77*(4), 444-448.

Bauman, J. M., Woodward, B. M., Baker, E. A., Marsh, T. L., & Scribner, K. T. (2016). Effects of Family, Feeding Frequency, and Alternate Food Type on Body Size and Survival of Hatchery-Produced and Wild-Caught Lake Sturgeon Larvae. *North American Journal of Aquaculture*, *78*(2): 136–144.

Bayunova, L., Barannikova, I., & Semenkova, T. (2002). Sturgeon stress reactions in aquaculture. *Journal of Applied Ichthyology*, *18*(4-6), 397-404.

Bechshøft, T. Ø., Sonne, C., Rigét, F. F., Letcher, R. J., Novak, M. A., Henchey, E., et al. (2013). Polar bear stress hormone cortisol fluctuates with the North Atlantic Oscillation climate index. *Polar Biology*, *36*(10), 1525-1529.

Berg, O. K., Hendry, A. P., Svendsen, B., Bech, C., Arnekleiv, J. V., & Lohrmann, A. (2001). Maternal provisioning of offspring and the use of those resources during ontogeny: variation within and between Atlantic salmon families. *Functional Ecology*, *15*(1), 13-23.

Blas, J., Bortolotti, G. R., Tella, J. L., Baos, R., & Marchant, T. A. (2007). Stress response during development predicts fitness in a wild, long lived vertebrate. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(21), 8880-8884.

Bouchard, H. J., III, & Aloisi, D. B. (2002). Investigations in concurrent disinfection and de-adhesion of lake sturgeon eggs. *North American Journal of Aquaculture*, *64*(3), 212-216.

Brunton, P. J., & Russell, J. A. (2010). Prenatal social stress in the rat programmes neuroendocrine and behavioural responses to stress in the adult offspring: sex-specific effects. *Journal of Neuroendocrinology*, *22*(4), 258-271.

Burnham, K. P., & Anderson, D. R. (1998). Practical use of the information-theoretic approach. *Model Selection and Inference* (pp. 75-117). New York, NY: Springer

Bussy, U., Wassink, L., Scribner, K. T., & Li, W. (2017). Determination of cortisol in lake sturgeon (*Acipenser fulvescens*) eggs by liquid chromatography tandem mass spectrometry. *Journal of Chromatography B, 1040,* 162-168.

Cavanaugh, J. E. (1997). Unifying the derivations for the Akaike and corrected Akaike information criteria. *Statistics & Probability Letters*, *33*(2), 201-208.

Champagne, F. A., & Meaney, M. J. (2006). Stress during gestation alters postpartum maternal care and the development of the offspring in a rodent model. *Biological Psychiatry, 59*(12), 1227-1235.

Clarke, A., & Schneider, M. L. (1993). Prenatal stress has long-term effects on behavioral responses to stress in juvenile rhesus monkeys. *Developmental Psychobiology*, *26*(5), 293-304.

Clinchy, M., Zanette, L., Boonstra, R., Wingfield, JC., Smith, J.N.M. (2004). Balancing food and predator pressure induces chronic stress in songbirds. *Proceedings of the Royal Society B: Biological Sciences 271*:2473–2479

Comte, L., Buisson, L., Daufresne, M., & Grenouillet, G. (2013). Climate-induced changes in the distribution of freshwater fish: observed and predicted trends. *Freshwater Biology, 58*(4), 625-639.

Creel, S., Fox, J.E., Hardy, A., Sands, J., Garrott, B., & Peterson, R.O. (2002) Snowmobile activity and glucocorticoid stress responses in wolves and elk. *Conservation Biology, 16*:809–814

Crossman, J. A., Scribner, K. T., Yen, D. T., Davis, C. A., Forsythe, P. S., & Baker, E. A. (2011). Gamete and larval collection methods and hatchery rearing environments affect levels of genetic diversity in early life stages of lake sturgeon (*Acipenser fulvescens*). *Aquaculture*, *310*(3-4), 312-324.

Cyr, N., & Romero, L.M. (2007) Chronic stress in free-living European starlings reduces corticosterone concentrations and reproductive success. *General and Comparative Endocrinology, 151*:82–89

Davis, M. W. (2010). Fish stress and mortality can be predicted using reflex impairment. *Fish and Fisheries, 11*(1), 1-11.

Dettlaff, T.A., Ginsberg, A.C., & Schmalhausen, O.I. (1982). Development of sturgeon: Maturation of eggs, fertilization, development of embryos and prelarvae. *Canadian Translation of Fisheries and Aquatic Sciences, 4843,* 1–19.

Doroshov, S. I., Moberg, G. P., & Van Eenennaam, J. P. (1997). Observations on the reproductive cycle of cultured white sturgeon, *Acipenser transmontanus*. *Environmental Biology of Fishe*s, *48*(1-4), 265-278.

Dudley, R.K., & Matter, W.J. (2000). Effects of small green sunfish (*Lepomis cyanellus*) on recruitment of Gila chub (*Gila intermedia*) in Sabino Creek, Arizona. *Southwestern Naturalist*, *45*, 24–29.

Duong, T.Y., Scribner, K.T., Crossman, J.A., Forsythe, P.S., Baker, E.A., Kanefsky, J., et al. (2011). Relative larval loss among females during dispersal of lake sturgeon (*Acipenser fulvescens*). *Environmental Biology of Fishes*, *91*(4), 459.

Eriksen, M. S., Bakken, M., Espmark, Å., Braastad, B. O., & Salte, R. (2006). Prespawning stress in farmed Atlantic salmon *Salmo salar*: maternal cortisol exposure and hyperthermia during embryonic development affect offspring survival, growth and incidence of malformations. *Journal of Fish Biology*, *69*(1), 114-129.

Espmark, Å. M., Eriksen, M. S., Salte, R., Braastad, B. O., & Bakken, M. (2008). A note on pre-spawning maternal cortisol exposure in farmed Atlantic salmon and its impact on the behaviour of offspring in response to a novel environment. *Applied Animal Behaviour Science*, *110*(3), 404-409.

Ferguson, M.M., & Duckworth, G.A. (1997). The status and distribution of lake sturgeon, *Acipenser fulvescens*, in the Canadian provinces of Manitoba, Ontario and Quebec: A genetic perspective. *Environmental Biology of Fishes*, *48*(1), 299–309.

Forsythe, P. S., Crossman, J. A., Bello, N. M., Baker, E. A., & Scribner, K. T. (2011). Individual-based analyses reveal high repeatability in timing and location of reproduction in lake sturgeon (*Acipenser fulvescens*). *Canadian Journal of Fisheries and Aquatic Sciences*, *69*(1), 60-72.

Gagliano, M., & McCormick, M.I. (2009). Hormonally mediated maternal effects shape offspring survival potential in stressful environments. *Oecologia*, *160*, 657–665.

Gagliano, M., McCormick, M. I., & Meekan, M. G. (2007). Survival against the odds: ontogenetic changes in selective pressure mediate growth-mortality trade-offs in a marine fish. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1618), 1575-1582.

Giesing, E. R., Suski, C. D., Warner, R. E., & Bell, A. M. (2010). Female sticklebacks transfer information via eggs: effects of maternal experience with predators on offspring. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1712), 1753-1759.

Hayhoe, K., VanDorn, J., Croley, T., II, Schlegal, N., & Wuebbles, D. (2010). Regional climate change projections for Chicago and the US Great Lakes. *Journal of Great Lakes Research*, *36*, 7–21.

Ho, D. H., & Burggren, W. W. (2010). Epigenetics and transgenerational transfer: a physiological perspective. *Journal of Experimental Biology*, *213*(1), 3-16.

Hopkins, W.A., Mendonça, M.T., & Congdon, J.D. (1997). Increased circulating levels of testosterone and corticosterone in southern toads, *Bufo terrestris*, exposed to coal combustion waste. *General and Comparative Endocrinology*, *108*(2), 237–246.

Kempinger, J.J. (1988). Spawning and early life history of lake sturgeon in the Lake Winnebago System, Wisconsin. In R. D. Hoyt (Ed.), *Proceedings of the 11th Annual Larval Fish Conference: Symposium* 5, *Houghton, Michigan, 1–3 June 1987* (pp. 110–122). Bethesda, MD: American Fisheries Society.

Kleppe, L., Karlsen, Ø., Edvardsen, R.B., Norberg, B., Andersson, E., Taranger, G.L., et al. (2013). Cortisol treatment of prespawning female cod affects cytogenesis related factors in eggs and embryos. *General and Comparative Endocrinology*, *189*, 84–95.

Koolhaas, J. M., De Boer, S. F., Coppens, C. M., & Buwalda, B. (2010). Neuroendocrinology of coping styles: towards understanding the biology of individual variation. *Frontiers in neuroendocrinology*, *31*(3), 307-321.

Lankford, S. E., Adams, T. E., Miller, R. A., & Cech Jr, J. J. (2005). The cost of chronic stress: impacts of a nonhabituating stress response on metabolic variables and swimming performance in sturgeon. *Physiological and Biochemical Zoology*, *78*(4), 599-609.

McCormick, M. I. (1998). Behaviorally induced maternal stress in a fish influences progeny quality by a hormonal mechanism. *Ecology*, *79*(6), 1873-1883.

McCormick, M. I. (2006). Mothers matter: crowding leads to stressed mothers and smaller offspring in marine fish. *Ecology*, *87*(5), 1104-1109.

Mccormick, M. I., & Nechaev, I. V. (2002). Influence of cortisol on developmental rhythms during embryogenesis in a tropical damselfish. *Journal of Experimental Zoology*, 293(5), 456-466.

Mommer, B. (2013). *The neuroendocrine and molecular mechanisms underlying stress-induced maternal effects in the threespine stickleback* (Doctoral dissertation). Champaign, IL: University of Illinois at Urbana-Champaign.

Mousseau, T. A., & Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends in Ecology & Evolution*, *13*(10), 403-407.

Nesan, D., & Vijayan, M. M. (2013). Role of glucocorticoid in developmental programming: evidence from zebrafish. *General and comparative endocrinology*, *181*, 35-44.

Paitz, R.T., Bukhari, S.A., & Bell, A.M. (2016). Stickleback embryos use ATP-binding cassette transporters as a buffer against exposure to maternally derived cortisol. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1826), 20152838.

Piato, Â.L., Capiotti, K.M., Tamborski, A.R., Oses, J.P., Barcellos, L.J., Bogo, M.R., et al. (2011). Unpredictable chronic stress model in zebrafish (*Danio rerio*): Behavioral and physiological responses. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(2), 561–567.

Richmond, A.M., & Kynard, B. (1995). Ontogenetic behavior of shortnose sturgeon, *Acipenser brevirostrum*. *Copeia*, *1995*, 172–182.

Robertson, B.A., Rehage, J.S., & Sih, A. (2013). Ecological novelty and the emergence of evolutionary traps. *Trends in Ecology & Evolution*, *28*(9), 552–560.

Sakamoto, K., Dew, W.A., Hecnar, S.J., & Pyle, G.G. (2016). Effects of lampricide on olfaction and behavior in young-of-the-year lake sturgeon (*Acipenser fulvescens*). *Environmental Science & Technology*, *50*(7), 3462–3468.

Sampath-Kumar, R., Byers, R.E., Munro, A.D., & Lam, T.J. (1995). Profile of cortisol during the ontogeny of the Asian seabass, *Lates calcarifer*. *Aquaculture*, *132*(3–4), 349–359.

Schlaepfer, M.A., Runge, M.C., & Sherman, P.W. (2002). Ecological and evolutionary traps. *Trends in Ecology & Evolution*, *17*(10), 474–480.

Schreck, C.B., Olla, B.L., & Davis, M.W. (1997). Behavioral responses to stress. *Fish Stress and Health in Aquaculture*, *62*, 145–170.

Sheriff, M.J., Bell, A., Boonstra, R., Dantzer, B., Lavergne, S.G., McGhee, K.E., et al. (2017). Integrating ecological and evolutionary context in the study of maternal stress. *Integrative and Comparative Biology*, *57*(3), 437–449.

Sheriff, M.J., & Love, O.P. (2013). Determining the adaptive potential of maternal stress. *Ecology Letters*, *16*(2), 271–280.

Silbernagel, J.J., & Sorensen, P.W. (2013). Direct field and laboratory evidence that a combination of egg and larval predation controls recruitment of invasive common carp in many lakes of the Upper Mississippi River Basin. *Transactions of the American Fisheries Society*, *142*(4), 1134–1140.

Smith, K.M., & King, D.K. (2005). Dynamics and extent of larval lake sturgeon *Acipenser fulvescens* drift in the Upper Black River, Michigan. *Journal of Applied Ichthyology*, *21*(3), 161–168.

Sopinka, N.M., Capelle, P.M., Semeniuk, C.A., & Love, O.P. (2017). Glucocorticoids in fish eggs: Variation, interactions with the environment, and the potential to shape offspring fitness. *Physiological and Biochemical Zoology*, *90*(1), 15–33.

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

Sopinka, N.M., Hinch, S.G., Middleton, C.T., Hills, J.A., & 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*(2), 493–500.

Thiel, D., Jenni-Eiermann, S., Braunisch, V., Palme, R., Jenni, L. (2008). Ski tourism affects habitat use and evokes a physiological stress response in capercaillie *Tetrao urogallus*: A new methodological approach. *Journal of Applied Ecology, 45*, 845–853.

Vitousek, M.N., Jenkins, B.R., & Safran, R.J. (2014). Stress and success: Individual differences in the glucocorticoid stress response predict behavior and reproductive success under high predation risk. *Hormones and Behavior*, *66*(5), 812–819.

Wagenmakers, E.J., & Farrell, S. (2004). AIC model selection using Akaike weights. *Psychonomic Bulletin & Review*, *11*(1), 192–196.

Waraniak, J.M., Baker, E.A., & Scribner, K.T. (2018). Molecular diet analysis reveals predator–prey community dynamics and environmental factors affecting predation of larval lake sturgeon *Acipenser fulvescens* in a natural system. *Journal of Fish Biology*, *93*(4), 616–629.

Wasser, S.K., Bevis, K., King, G., & Hanson, E. (1997). Noninvasive physiological measures of disturbance in the northern spotted owl. *Conservation Biology, 11*, 1019–1022.

Weinstock, M. (2005). The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain, Behavior, and Immunity, 19*, 296–308.

Wikelski, M., & Cooke, S.J. (2006). Conservation physiology. *Trends in Ecology & Evolution*, *21*(1), 38–46.

Wingfield, J.C., Hunt, K., Breuner, C.R., Dunlap, K.E., Fowler, G.S., Freed, L.E., et al. (1997). Environmental stress, field endocrinology, and conservation biology. In J. R. Clemmons & R. Buchholz (Eds.), *Behavioral approaches to conservation in the wild* (pp. 95–131). Cambridge, U.K.: Cambridge University Press.

Wishingrad, V., Ferrari, M.C., & Chivers, D.P. (2014). Behavioural and morphological defences in a fish with a complex antipredator phenotype. *Animal Behaviour, 95*, 137–143.

Zotin, A.I. (1958). The mechanism of hardening of the salmonid egg membrane after fertilization or spontaneous activation. *Development, 6*(4), 546–568.

CHAPTER 3:

Hatchery and wild larval lake sturgeon experience effects of captivity on stress reactivity, behavior, and predation risk

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ABSTRACT

Reintroduction programs are important tools for wildlife conservation. However, captive rearing environments may lead to maladaptive behavior and physiological alterations that inhibit survival after release. For captive rearing programs that raise individuals captured from the wild during early ontogeny for later release, there is a lack of information about when during ontogeny the detrimental effects of captive rearing may become evident. In this study we compared cortisol levels, predation rates, and swimming behavior between hatchery-produced and wild-caught larval lake sturgeon (*Acipenser fulvescens*), a threatened fish species, at three times over 9 days. Cortisol levels did not indicate that hatchery-produced individuals were more stressed, but cortisol reactivity to an acute stressor disappeared for both hatchery-produced and wild-caught larvae after 9 days in the hatchery. Swimming activity levels decreased over time for hatchery-produced larvae but increased over time for wild-caught larvae, suggesting that behavioral trajectories may be programmed prior to the larval stage. Neither increasing nor decreasing activity levels was advantageous for survival, as predation rates increased over time in captivity for larvae from both treatments. Results suggest that physiological and behavioral phenotypes may not accurately predict survival for individuals released from reintroduction programs, and that the captive environment may inhibit transition to the wild even if cortisol levels do not indicate high stress. Findings emphasize that even a short amount of time in captivity during early ontogeny can affect phenotypes of individuals captured from wild populations, which may impact success of reintroduction programs.

INTRODUCTION

Reintroduction programs, including captive breeding and rearing programs that release individuals to increase numerical abundance and persistence of wild populations, are important tools for wildlife conservation (Clark & Westrum, 1989). However, release of captive individuals can be counterproductive to conservation goals by reducing fitness of wild populations, as traits adaptive in captive environments may be maladaptive in the wild (McPhee, 2004). Studies that focus on conditions in fish hatcheries have demonstrated due to domestication selection, mean population fitness declines proportionally with the amount of time spent in a captive environment (Lynch & O'Hely, 2001; Ford, 2002). Hatchery-produced fish often experience higher risk of predator-based mortality compared to wild fish as a result of altered antipredator behavior (Berejikian, 1995; Stunz & Minello, 2001; Alvarez & Nicieza, 2003; Huntingford, 2004) as well as lower reproductive success (Berejikian et al., 1997; Araki et al., 2009). While the multi-generational effects of captivity have been well documented, there is still a need to understand within-generational effects of captive rearing on individuals in captive rearing or headstarting programs. Captive rearing methods used for wildlife reintroduction programs raise wild-caught individuals for release, in order to maximize survival during vulnerable early life stages while avoiding the multi-generational effects of captivity. However, stress induced by captivity may result in maladaptive behaviors that reduce survival post-release, thus hindering the goals of conservation programs seeking to numerically expand wild populations (McDougall et al., 2006; Berger-Tal et al., 2016). To inform conservation efforts, it is essential to understand how and when during early ontogeny individual phenotypes are affected by captive environments, so that maladaptive effects of captivity can be reduced.

While captive rearing programs may protect individuals from mortality during early life stages, captive rearing may also expose individuals to stress during important developmental periods. Stress experienced during early life stages can cause long-term alteration to hypothalamic-pituitary-adrenal (HPA) stress axis function, which can affect stress-related behaviors (Auperin & Geslin, 2008; Lukkes et al., 2009; Turner et al., 2010). Chronic stress (stressors experienced continuously) causes chronic elevation of cortisol levels, which can lead to inhibition of the negative feedback loop of the stress axis and result in stress axis hyperactivity (Schreck et al.. 1997, Pariante & Lightman, 2008, Jeanneteau et al., 2012). Stress axis hyperactivity is characterized by elevated cortisol levels as well as altered behavior (Piato et al., 2011). Features of the captive environment, such as high density in fish hatcheries, has been shown to increase cortisol levels in individuals, indicating increased stress (Falahatkar et al., 2009; Li et al., 2012). In captive rearing programs, behavior has been shown to be important for predator avoidance, dispersal, foraging, and reproduction (Harvey et al., 2002; Kreger et al., 2006; Okuyama et al., 2010), but chronic stress inhibits development of these important behaviors and negatively affects transition to the wild and survival post-release (Olla et al., 1998; Evans et al., 2014). Therefore, even if captive-reared individuals were produced from wild parents and thus are genetically adapted to the wild environment, stress experienced during early life stages in captivity can have profound behavioral effects that result in maladaptation when subsequently introduced to the wild.

Lake sturgeon (*Acipenser fulvescens*) are a regionally threatened fish species. Lake sturgeon conservation also is of cultural value for First Nations people in the Great Lakes region (LRBOI 2008, Mann et al., 2011). Populations have been dramatically reduced by historic over-exploitation and habitat disturbance (Ferguson & Duckworth, 1997), and populations remain

vulnerable to environmental stressors associated with climate change and other anthropogenic threats (Hayhoe et al., 2010; Comte et al., 2013), leading to a need for hatchery conservation programs. Currently, lake sturgeon are a management priority in the Great Lakes (Hayes & Caroffino, 2012).

Streamside rearing facilities raise larval lake sturgeon for release into local populations (Brown & Day, 2002; Holtgren et al., 2007). In lake sturgeon, the stress axis is functional by the third day post hatch, showing a physiological response to acute stress evidenced by an increase in cortisol (Simontacchi et al., 2009). Early life stress due to factors associated with captive rearing environments, such as high density, has been shown to increase cortisol levels in fish, indicating higher stress (Falahatkar et al., 2009; Li et al., 2012). Features of hatchery rearing have been shown to cause stress for lake sturgeon, such as incubating eggs in McDonald jars (Earhart et al., 2020) and rearing larvae at high densities after hatch (Bauman et al., 2015). Early life stress induced by hatchery rearing may impact survival, since early life stress influences antipredator behaviors in larval lake sturgeon (Wassink et al., 2019, Biro et al., 2003). Much of the high mortality lake sturgeon experience during the first year of life is caused by predation during the early larval stage (Waraniak et al., 2018). Therefore, the impact of hatchery stress on lake sturgeon larval behavior and predation has important conservation implications.

The Black Lake Sturgeon Facility in Cheboygan Co., MI was used to investigate how early rearing environments shaped behavior, stress, and survival in lake sturgeon. Eggs and sperm were collected from spawning adults in the Upper Black River and used to produce larvae for release. Additionally, drifting larvae (10-30 days post hatch) are captured from the river using drift nets and then reared captively in the hatchery. Since lake sturgeon are not bred in captivity due to long generation times, there is no possibility of transgenerational domestication effects.

The Black Lake system enables a direct comparison between early rearing environments, specifically egg incubation and free embryo rearing in natural stream vs. hatchery conditions, for individuals produced from the same wild population.

To examine how early rearing environment affects stress, behavior, and predation rates, we conducted a study comparing hatchery-reared and wild-caught sturgeon larvae. We focused on the early larval stage to investigate whether even short durations in captivity may detectably stress individuals, since stress during early ontogeny is especially likely to alter behavioral and physiological developmental trajectories, and since husbandry practices during early stages are of interest to captive rearing programs. Research objectives were to: 1) determine whether the hatchery environment influences stress and stress reactivity in larval lake sturgeon, and whether wild-caught lake sturgeon experience these effects after spending time in the hatchery, and 2) investigate whether rearing environment influences predation rates and ability to learn predator avoidance.

METHODS

Lake sturgeon larvae used in the experiment were produced in the hatchery from gametes collected from spawning adults were collected as wild larvae from the Upper Black River during the period of larval dispersal in May 2018. Data collection was initiated approximately 8 days post-hatch for both hatchery-produced and wild-caught groups. For hatchery-produced larvae, eggs were collected from three female lake sturgeon and sperm was collected from three male lake sturgeon spawning in the Upper Black River in Onaway, MI. Full-sibling offspring were produced using one-to-one crosses using standard lake sturgeon culture procedures (Bauman et al., 2016, Crossman et al., 2011). Fertilized eggs were incubated in McDonald jars, with 5 mls of eggs (approximately 260 eggs) per jar, supplied with flowing stream water at a rate of 56.78

liters/hour until hatch. After hatch, free embryos were moved to 3-liter aquaria supplied with flowing stream water at a rate of 56.78 liters/hour and provided with 2.54 cm^3 sinking Bioballs (N=32, CBB1-S, Pentair AES) as artificial substrate until reaching the larval stage. For wild-caught larvae, individuals dispersing downstream from spawning areas at night were collected using 1,000 micron D-frame drift nets and transported to the hatchery in a cooler supplied with aerated stream water. At the hatchery, wild-caught larvae were placed in 3-liter aquaria supplied with flowing stream water at a rate of 56.78 liters/hour.

We used equal numbers of hatchery-produced and wild-caught larvae, with six replicates of hatchery-produced larvae from each of three families and six replicates of wild larvae from each of three drift nights. Each replicate contained 132 larvae for a total of 6336 larvae in the entire experiment. To avoid overcrowding, larvae from each replicate were divided among 3-liter aquaria, with no more than 100 larvae per aquarium. Each 3-liter aquarium was supplied with 50 micron filtered stream water at ambient stream temperature flowing at a rate of 56.78 liters/hour. Both hatchery-produced and wild-caught larvae were episodically fed premium grade brine shrimp (*Artemia sp.*, BSEP16Z, Brine Shrimp Direct) four times a day as per Bauman et al. (2016), beginning at the onset of exogenous feeding (approximately 10 days after hatch) and continuing throughout the duration of the experiment.

Trials were repeated three times over a total duration of 9 days after wild-caught individuals were brought to the hatchery. The goals of this experiment were to determine whether behavioral and physiological phenotypes of wild-caught lake sturgeon larvae would change over the 9 day period of captivity to phenotypes of hatchery-reared larvae. The first 9 days of the larval stage encompassed the period of larval dispersal in the wild (Duong et al., 2011), during which larvae initiate exogenous feeding and drift downstream from spawning

locations. Stress-related behavior is particularly important during this stage due to the high rate of predation larvae encounter during dispersal (Waraniak et al., 2018). Cortisol sampling, measurements, behavior trials, and predation trials were conducted at three sequential time periods during early larval development (hereafter referred to as stages): stage A (day one of the larval stage for hatchery-produced larvae, or the day after capture for wild-caught larvae), stage B (day five of the larval stage), and stage C (day nine of the larval stage). Stage A, the beginning of the larval stage, was defined for hatchery-produced larvae based on emergence from the substrate and the onset of exogenous feeding. Individuals captured from the stream were assumed to be at the beginning of the larval stage, since the drift period begins upon emergence from the substrate. No individuals were used more than once for samples or trials.

Analyses of larval body size

Total body length was quantified for each of six larvae per replicate at each of the three stages using ImageJ software (National Institutes of Health, Bethesda, MD, U.S.A., http://rsbweb.nih.gov/ij /). Photos of larvae were taken using a digital camera, including a ruler for body size calibration.

Analyses of larval cortisol levels

Cortisol levels were quantified to determine whether the hatchery environment induces stress in larval lake sturgeon. Whole-body cortisol levels were quantified for larvae from each replicate at stages A, B, and C. At each stage, samples were taken at baseline (meaning no acute stressor was applied), or 30 minutes after individuals were exposed to an acute stressor to document levels of cortisol elevation as a physiological response to the stressor. The acute stressor was the novel environment behavioral trial, which involved placing larvae into a 15.24 cm diameter petri dish to record swimming activity for 4 minutes, and has been shown to be a

stressor for larval sturgeon (Wassink et al., 2019). Each cortisol sample contained 6 individuals. Individuals were euthanized using an overdose of MS-222 according to approved Michigan State University Animal Use and Care protocols. Whole body levels of cortisol were estimated from samples using liquid chromatography tandem mass spectrometry. LC-MSMS analysis was conducted using a Waters Xevo TQ-S mass spectrometer (Waters, Millford, MA, USA) as described previously for Black River lake sturgeon (Bussy et al., 2017).

Analyses of larval behavior

Three types of behavior trials were conducted to measure larval behavioral responses to different stimuli.

1) Novel environment trials: Larval swimming activity in the petri dish was recorded with no additional stimulus. Encountering the novel environment of the petri dish has been shown to be a stressor for sturgeon larvae (Wassink et al., 2019).
2) Thump trials: Larval swimming activity in the petri dish was recorded after a 212 g weight was dropped onto the table surface from a height of 22 cm to induce a startle response.
3) Odor trials: Larval swimming activity in the petri dish was recorded after larvae were exposed to odor created from whole-body homogenization of sacrificed sturgeon larvae, as alarm cues in tissue homogenate from conspecifics has been shown to cause a physiological and behavioral response in sturgeon (Wishingrad et al., 2014). 1 ml of the odor homogenate was added to the center of the petri dish using a pipette at the start of the trial.

One of each type of behavioral trial was conducted using 6 individuals from each of 6 replicates in each treatment (hatchery and wild). Trials took place in a 6-inch petri dish and

behaviors of all individuals were recorded using a Go-Pro Hero 4 camera (GoPro, Inc) for a duration of 4 minutes. Behavior trials employing the same acute stimuli were conducted for individuals at each of the three stages (A, B, and C).

Loligo® software was used to simultaneously track activity of the six individuals in each replicated trial, following Sakamoto et al. (2016). A center zone was defined that excluded a one-inch perimeter around the petri dish edge to quantify edge-seeking behavior. Variables quantified from the entire 4 minute video period included each individual's velocity (cm/s), acceleration (cm/s^2), percent time active, total distance traveled (cm), number of visits to the center zone, and time (s) spent in the center zone (Wassink et al., 2019, Wassink et al., 2020).

Predation

Predation rates were quantified using rusty crayfish (*Orconectes rusticus*), an important predator of larval lake sturgeon (Crossman et al. 2018). One set of predation trials was conducted using lake sturgeon larvae naïve to crayfish, and a second set of trials was conducted using larvae conditioned to crayfish odor combined with dead conspecific alarm cues, in order to observe whether rearing environment affected ability to learn predator odor. Larval sturgeon have an innate antipredator response to alarm cues released from the skin of injured conspecifics that facilitates learning of predator odors (Wishingrad et al., 2014, Sloychuk et al., 2016), an ecologically important cognitive function.

Predation trials were conducted at each of the three stages (A, B, and C) and included a set of trials with larvae naïve to predator odor and a set of trials with larvae conditioned to predator odor combined with alarm cues. No larvae were used for more than one trial. One naïve predation trial and one conditioned predation trial was conducted per replicate. Conditioned predation trials were conducted 24 hours after naïve predation trials at each stage.

Larvae were removed to a separate 3-liter tank for conditioning to predator odor. Conditioning odor was created by combining crayfish odor (30 mls of water from a 15.24 cm by 22.86 cm container housing 3 crayfish for one hour) and lake sturgeon death odor (20 mls of water containing sturgeon larvae homogenate). Sturgeon larvae homogenate was created from whole-body homogenization of sacrificed sturgeon larvae, using approximately 15 individuals of the same age and size as experimental larvae. 4 mls of conditioning odor was added to each 3-liter tank housing larvae the night before the predation trial, and an additional 4 mls of conditioning odor was added to each tank the following morning approximately eight hours prior to the predation trial.

Predation trials took place in tanks that measured 42 cm by 30 cm, with a water depth of 12 cm (volume = 15.12 liters). Tanks were supplied with flowing stream water at a rate of 56.78 liters/hour, at ambient stream temperature (mean daily temperature ± standard deviation = 17.1 °C ± 1.07). Rusty crayfish (*Orconectes rusticus*) were collected from the Upper Black River using minnow traps, and carapace length of each individual was measured. For each trial, ten sturgeon larvae were placed in a tank and allowed to acclimate for 50 minutes. After acclimation, one crayfish was added to each tank and then removed after 2.5 hours. Surviving larvae were counted and removed from the tank.

Statistical analysis

Normality for body size and cortisol datasets was assessed using a Shapiro-Wilk test in R v 3.2.2. The body size and cortisol datasets were not normally distributed and therefore were log-transformed prior to analysis. Generalized Linear Models were fit using the *glm* function in R v 3.2.2. Models with delta AIC < 2 were considered competitive for top model (Burnham & Anderson, 1998). The variables included in the body size models were treatment (hatchery-

produced or wild-caught), stage (A, B, and C), and the interaction of treatment and stage. The variables included in the cortisol models were treatment (hatchery-produced or wild-caught), stress state (baseline or post stress), and the interaction of treatment and stress state. Predictor variables in the AICc selected models were further evaluated using ANOVA.

For behavior datasets, dependent variables (percent activity, acceleration, velocity, distance, zone time, and zone visits) were compressed into a composite behavioral measure using Principal Components Analysis (Ballew et al., 2017). The broken stick method was used to determine that PC1, PC2, and PC3 were significant (Jackson, 1993). Factor loadings above 0.5 were used to determine behavioral relevance of each principal component. Generalized Linear Models were selected for the three principal components using AICc model selection. ANOVA was used to conduct F-tests on the model output and determine which variables were significant ($p < 0.05$).

For predation datasets, Generalized Linear Models using a Poisson distribution were fit for the dataset of surviving larvae per tank using the *glm* function in R v 3.2.2. Variables in the models included treatment (hatchery-produced or wild-caught), stage (A, B, or C) conditioning treatment (naïve or conditioned to odor), and the two-way factor interactions. Variables present in the AICc selected model were further evaluated using a Chi-Square test.

Animal welfare considerations

All experiments were conducted under approved Michigan State University Animal Use and Care protocols (04/17-071-00). Incidental stress was minimized for all lake sturgeon in the experiment to the extent possible. Captured adults were handled for only about 4 minutes each, and care was taken to ensure heads and gills remained underwater. In the hatchery, free embryos were provided with 2.54 cm^3 sinking Bioballs (N=32, CBB1-S, Pentair AES) as artificial

substrate until emergence. At the onset of exogenous feeding at the beginning of the larval stage, larvae were supplied with food *ad libitum*. Tanks housing free embryos and larvae were cleaned daily, and mortalities were removed daily. Flow rate of filtered stream water was maintained at 56.78 liters/hour to ensure adequate oxygenation. During the dispersal period or wild larvae, individuals were kept in a large cooler filled with stream water that was oxygenated using an aerator. Larvae used for behavior trials were supplied with oxygenated water and only left in the petri dish for the duration of the 4 minute trial. Any larvae sacrificed for samples or to create odor were euthanized using an overdose of MS-222 according to approved Michigan State University Animal Use and Care protocols.

RESULTS

Larval body size

The AICc selected model for body size included treatment, stage, and the interaction of treatment and stage (Table 3.1). ANOVA indicated that hatchery-produced larvae had a larger mean body size than wild-caught larvae ($p < 0.0001$), body size significantly increased at each stage for both groups ($p < 0.0001$), and the interaction of stage and treatment was significant ($p = 0.0280$). Tukey HSD indicated that at stages A and C, hatchery-produced larvae were significantly larger than wild-caught larvae (Cohen's D = 1.11 and 0.48, respectively), but that there was no significant difference in size between treatments at stage B (Figure 3.1).

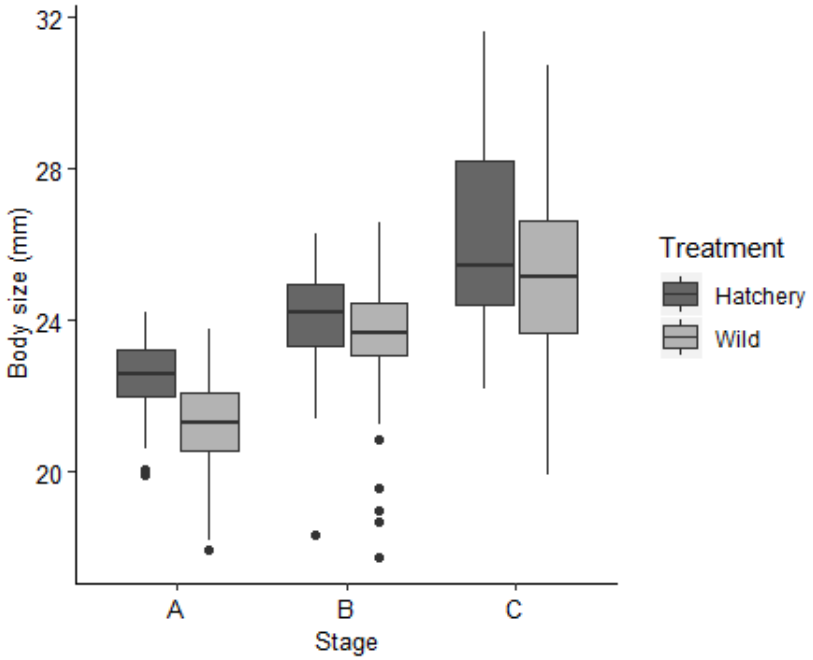

Figure 3.1. Body size (data on original scale, prior to log-transformation) across all three stages for both treatments.
Stage A (day one of the larval stage for hatchery-produced larvae, or the day after capture for wild-caught larvae), stage B (day five of the larval stage), and stage C (day nine of the larval stage). Size increased significantly across stages ($p < 0.0001$). Tukey HSD indicated that hatchery larvae were significantly larger than wild larvae at stages A and C, but not at stage B. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5 x the interquartile range for the upper and lower quartiles.

Table 3.1. AICc selected models for body size, including predictor variables of treatment (hatchery or wild) and stage (A, B, or C)

Model	AICc	Delta AICc	Weight
Length ~ Treatment + Stage + Treatment*Stage	-2727.8	0	0.83
Length ~ Treatment + Stage	-2724.67	3.13	0.17
Length ~ Treatment	-2672.54	55.26	0
Length ~ Stage	-2309.88	417.92	0
null model	-2282.63	445.17	0

Larval cortisol levels

For stage A (day 1 of the larval stage), the AICc selected model included treatment and stress state (Table 3.2). ANOVA indicated wild-caught larvae had significantly higher whole-body cortisol than hatchery-produced larvae at stage A ($p < 0.0001$, Cohen's D = 1.26) (Figure 3.2). Post stress cortisol levels were also significantly higher than baseline cortisol levels at stage A for both treatments ($p < 0.0001$, Cohen's D = 1.11) (Figure 3.2). For stage B (day 5 of the larval stage), the AICc selected model included stress state only (Table 3.2). ANOVA indicated that post stress cortisol levels were significantly higher than baseline ($p = 0.0008$, Cohen's D = 0.83) (Figure 3.2). For stage C (day 9 of the larval stage), the AICc selected model was the null model (Table 3.2), indicating that no variables were important in explaining variation in cortisol levels at this stage (Table 3.3).

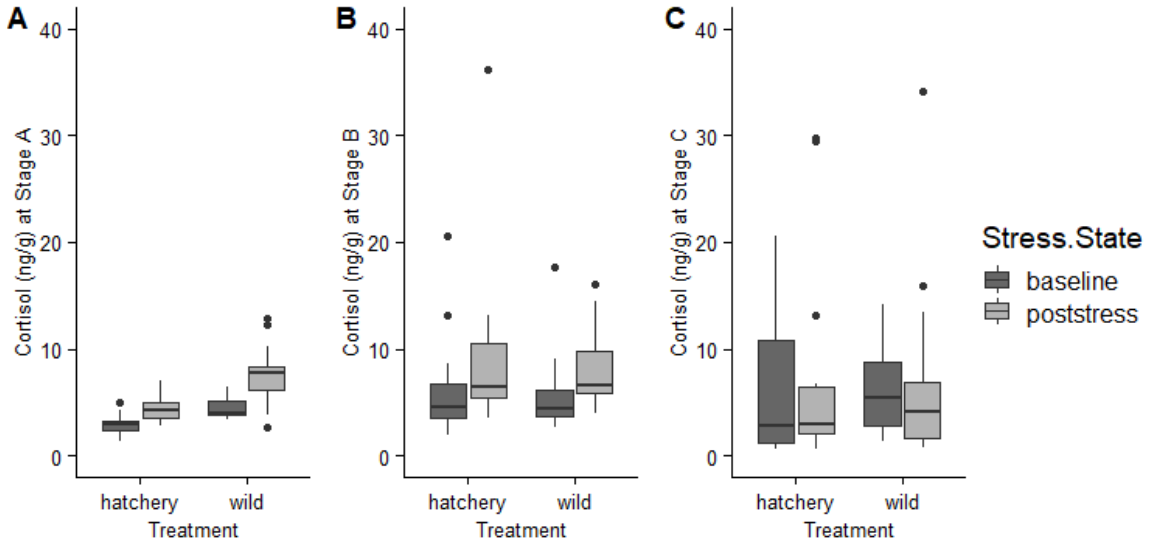

Figure 3.2. Cortisol (data on original scale, prior to log-transformation) during each of three stages for both treatments (hatchery and wild) and stress states.
At stage A, mean cortisol of wild larvae was significantly higher than for hatchery larvae ($p < 0.0001$), and mean post stress cortisol was significantly higher than baseline ($p < 0.0001$). At stage B, treatment was not significant, and mean post stress cortisol was significantly higher than baseline ($p = 0.0008$). At Stage C, neither treatment nor stress state was significant. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5 x the interquartile range for the upper and lower quartiles. Outliers over 40 ng/g, of which there were 9 in Stage C, were excluded from the boxplot but included in the analysis.

Larval behavior

Behavioral traits associated with swimming activity (percent activity, velocity, acceleration, distance, zone time, and zone visits) were reduced into three components using PCA. Factor loadings indicated that the most important variable contributing to variation along PC1 was percent activity (Figure 3.3) (Table 3.4).

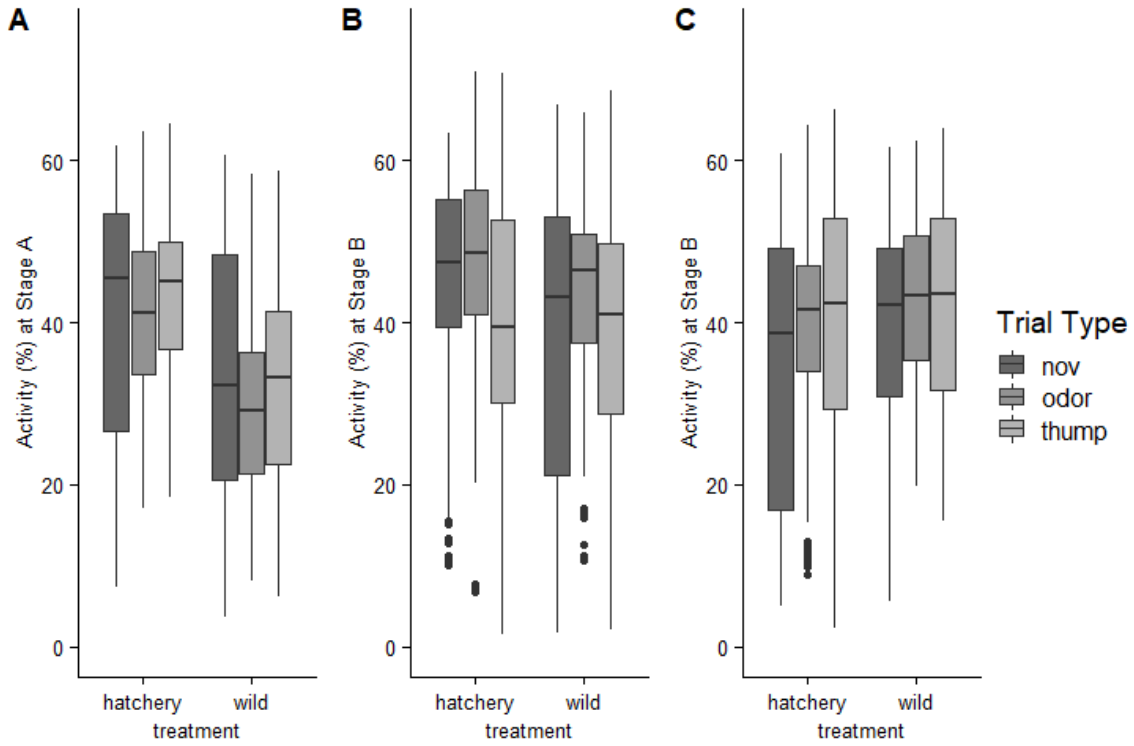

Figure 3.3. Percent activity for hatchery and wild larvae at all three stages (A, B, and C), for all three behavior trial types.
Percent activity was the most important factor informing PC1. Stage A was day one of the larval stage for hatchery-produced larvae, or the day after capture for wild-caught larvae. Stage B was day five of the larval stage. Stage C was day nine of the larval stage. Novel environment trials (nov) involved larvae being placed in the petri dish with no additional stimulus. Odor trials (odor) involved 1 ml of odor, created from whole-body homogenization of conspecifics containing alarm cues, being added to the center of the petri dish at the start of the trial. Thump trials (thump) involved a 212 g weight being dropped onto the table surface from a height of 22 cm to induce a startle response. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5 x the interquartile range for the upper and lower quartiles.

Table 3.2. AICc selected models for whole body cortisol levels at each stage (A, B, and C), using log-transformed cortisol dataset, including predictor variables of treatment (hatchery or wild) and stress state (baseline or post acute stressor)

Model	AICc	Delta AICc	Weight
Stage A			
Cortisol ~ Treatment + Stress State	-81.36	0	0.73
Cortisol ~ Treatment + Stress State + Treatment*Stress State	-79.38	1.98	0.27
Cortisol ~ Treatment	-53.03	28.33	0
Cortisol ~ Stress State	-48.3	33.06	0
null model	-30.92	50.44	0
Stage B			
Cortisol ~ Stress State	-11.02	0	0.69
Cortisol ~ Treatment + Stress State	-8.86	2.17	0.23
Cortisol ~ Treatment + Stress State + Treatment*Stress State	-6.57	4.46	0.07
null model	-1.55	9.48	0.01
Cortisol ~ Treatment	0.57	11.59	0
Stage C			
null model	132.02	0	0.42
Cortisol ~ Treatment	132.7	0.69	0.3
Cortisol ~ Stress State	134.04	2.02	0.15

Table 3.2 (Cont'd)

Cortisol ~ Treatment + Stress State	134.8	2.78	0.1
Cortisol ~ Treatment + Stress State + Treatment*Stress State	137.02	5.01	0.03

Table 3.3. Mean (± SD) cortisol levels (in original scale, prior to log-transformation) for hatchery-produced and wild-caught sturgeon larvae at each stage (A, B, and C) and for each stress state (baseline or post stress).

Stage	Treatment	Cortisol	
		baseline	post stress
A	hatchery	2.97 ± 0.94	4.32 ± 1.10
	wild	4.46 ± 0.93	7.53 ± 2.69
B	hatchery	5.98 ± 4.53	9.07 ± 7.42
	wild	5.48 ± 3.46	7.81 ± 3.37
C	hatchery	13.81 ± 25.34	11.72 ± 22.40
	wild	19.22 ± 32.14	17.44 ± 25.09

PC1, which explained 45.67% of the variation in the data, was negatively associated with percent activity and can be characterized as "inactivity." The AICc selected model for PC1 included treatment, stage, trial, and the interactions of treatment and trial (novel environment, thump, or odor), trial and stage, and treatment and stage (Table 3.5). ANOVA indicated that all terms included in the model significantly affected PC1: treatment ($p < 0.0001$), stage ($p = 0.0008$), trial ($p = 0.0040$), treatment*trial ($p = 0.0066$), stage*trial ($p = 0.0028$), and treatment*stage ($p < 0.0001$). Hatchery-produced larvae had lower mean estimates of PC1 than did wild-caught larvae at all stages (averaged across trial type) (Cohen's $D = 0.17$), indicating that hatchery-produced larvae were more active than wild-caught larvae regardless of

developmental stage. At each stage, hatchery-produced larvae showed decreasing activity levels (indicated by an increase in mean PC1 scores), while wild-caught larvae increased their activity levels (indicated by a decreased in mean PC1 scores).

Factor loadings indicated that the most important variable contributing to variation along PC2 was distance traveled (Figure 3.4) (Table 3.4). PC2, which explained 28.82% of the variation in the data, was positively associated with distance and can be characterized as "total distance moved." The AICc selected model for PC2 included treatment, stage, trial, the interaction of treatment and trial, and the interaction of trial and stage (Table 3.5). ANOVA indicated that all terms included in the model significantly affected PC2: treatment ($p < 0.0001$), stage ($p < 0.0001$), trial ($p = 0.6411$), treatment*trial ($p = 0.0398$), and stage*trial ($p < 0.0001$). Hatchery-produced larvae moved greater distances (higher PC2 means) than wild-caught larvae at all stages (averaged across trial type) (Cohen's $D = 0.29$).

Factor loadings indicated that the most important variable contributing to variation along PC3 was "zone time," or time spent in the center zone of the petri dish (Figure 3.5) (Table 3.4). PC3, which explained 18.34% of the variation in the data, is negatively associated with zone time and can be characterized as "zone avoidance" (Table 3.4) (Figure 3.6). The AICc selected model for PC3 included treatment, stage, trial, the interaction between treatment and trial, and the interaction between trial and stage (Table 3.5). ANOVA indicated that all terms included in the model significantly affected PC3: Treatment ($p < 0.0001$), stage ($p < 0.0001$), trial ($p = 0.0142$), treatment*trial ($p = 0.0140$), and stage*trial ($p = 0.0104$). Wild-caught larvae exhibited significantly higher center zone avoidance, or more time spent along the one-inch perimeter of

the petri dish outside of the center zone, compared to hatchery-reared larvae at all three stages (averaged across trial type) (Cohen's D = 0.28) ($p < 0.0001$).

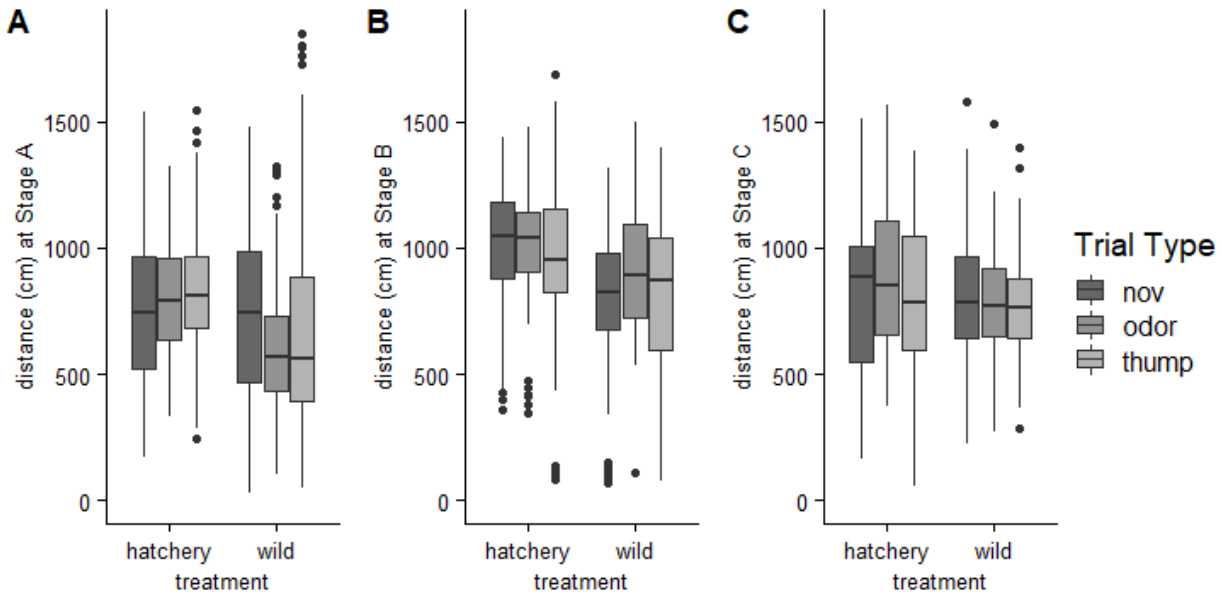

Figure 3.4. Total distance traveled (cm) during a four minute behavior trial for hatchery and wild larvae at all three stages (A, B, and C), for all three behavior trial types. Distance was the most important factor informing PC2. Stage A was day one of the larval stage for hatchery-produced larvae, or the day after capture for wild-caught larvae. Stage B was day five of the larval stage. Stage C was day nine of the larval stage. Novel environment trials (nov) involved larvae being placed in the petri dish with no additional stimulus. Odor trials (odor) involved 1 ml of odor, created from whole-body homogenization of conspecifics containing alarm cues, being added to the center of the petri dish at the start of the trial. Thump trials (thump) involved a 212 g weight being dropped onto the table surface from a height of 22 cm to induce a startle response. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5 x the interquartile range for the upper and lower quartiles.

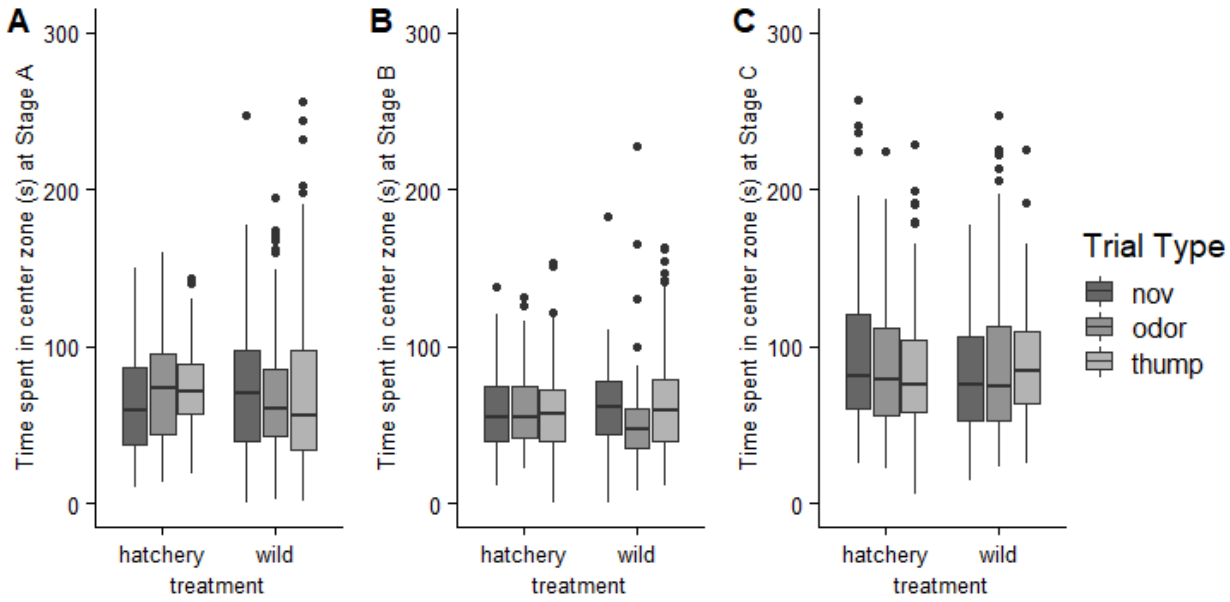

Figure 3.5. Time spent in center zone (s) for hatchery and wild larvae at all three stages (A, B, and C), for all three behavior trial types.
The center zone was defined by excluding a one-inch perimeter around the edge of the petri dish. Zone time was the most important factor informing PC3. Stage A was day one of the larval stage for hatchery-produced larvae, or the day after capture for wild-caught larvae. Stage B was day five of the larval stage. Stage C was day nine of the larval stage. Novel environment trials (nov) involved larvae being placed in the petri dish with no additional stimulus. Odor trials (odor) involved 1 ml of odor, created from whole-body homogenization of conspecifics containing alarm cues, being added to the center of the petri dish at the start of the trial. Thump trials (thump) involved a 212 g weight being dropped onto the table surface from a height of 22 cm to induce a startle response. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5 x the interquartile range for the upper and lower quartiles.

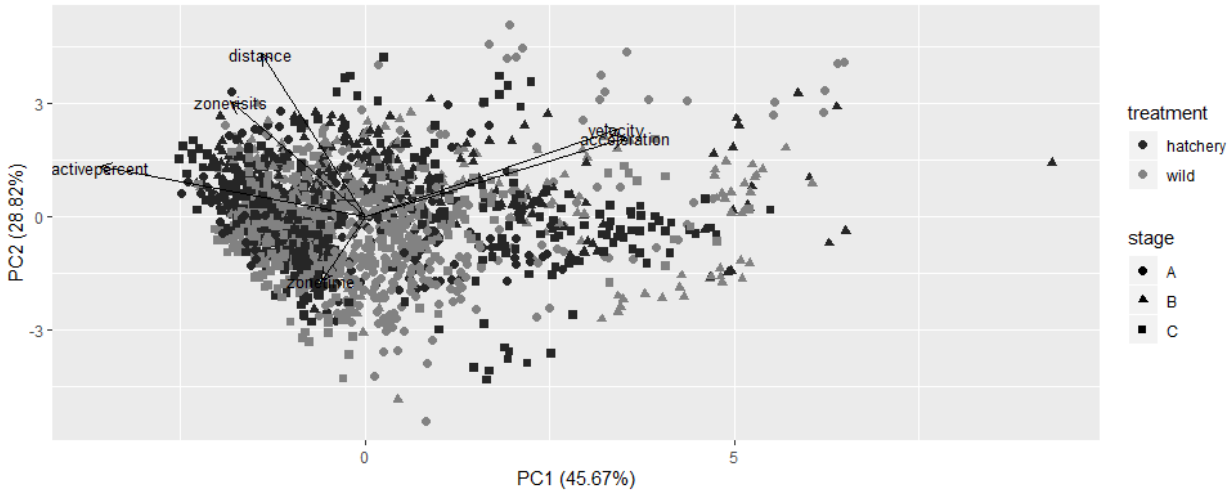

Figure 3.6. Principal Components Analysis showing ordination of all larval sturgeon (hatchery and wild-caught) over the three stages (A, B, and C) based on behavioral variables.

Behavioral variables quantified during each four minute video included percent activity (percent of the trial spent active), velocity (mean velocity during the trial, cm/s), acceleration (mean acceleration during the trial, cm/s2), distance (total distance traveled during the trial, cm), zone time (amount of time spent in center zone, s), and zone visits (numbers of visits to center zone). The center zone of the petri dish excluded a one-inch perimeter around the petri dish edge.

Table 3.4. Factor loadings and eigenvalues for principal components analysis of behavioral variables.

	factor loadings						eigen-value	variance %	cumulative variance %
	velocity	*accel.*	*zone time*	*% activity*	*distance*	*zone visits*			
PC1	0.5247	0.5396	-0.0907	-0.545	-0.2158	-0.2763	2.7399	45.6651	45.6652
PC2	0.3582	0.3198	-0.2607	0.2025	0.6642	0.4683	1.7293	28.8209	74.4861
PC3	-0.1005	-0.1043	-0.8485	0.1587	0.0612	-0.4797	1.1002	18.3367	92.8228
PC4	0.1121	0.0657	0.4502	0.2493	0.494	-0.6886	0.3342	5.5696	98.3924
PC5	0.2547	0.3232	0.0338	0.7543	-0.5105	0.0011	0.0912	1.5196	99.912
PC6	0.7133	-0.6978	-0.0048	0.0167	-0.0616	0.0083	0.0053	0.0879	100

Table 3.5. AICc selected models for principal components associated with behavioral measurements, including only top four competitive models and null model, and predictor variables of treatment (hatchery or wild), stage (A, B, or C) and trial type (novel environment, odor, or thump)

Model	AICc	Delta AICc	Weight
PC1			
PC1 ~ treatment + stage + trial + treatment*trial + trial*stage + treatment*stage	7136.86	0	0.98
PC1 ~ treatment + stage + trial + treatment*trial + treatment*stage	7144.71	7.85	0.02
PC1 ~ treatment + stage + trial + treatment*stage	7150.93	14.07	0
PC1 ~ treatment + stage + trial + treatment*trial + trial*stage	7207.65	70.79	0
null model	7248.5	111.63	0
PC2			
PC2 ~ treatment + stage + trial + treatment*trial + trial*stage	6180.05	0	0.47
PC2 ~ treatment + stage + trial + treatment*trial + trial*stage + treatment*stage	6180.48	0.43	0.38
PC2 ~ treatment + stage + trial + trial*stage	6182.39	2.34	0.15
PC2 ~ treatment + stage	6206.65	26.59	0
null model	6381.42	201.36	0
PC3			
PC3 ~ treatment + stage + trial + treatment*trial + trial*stage	5147.46	0	0.54
PC3 ~ treatment + stage + trial + treatment*trial + trial*stage + treatment*stage	5148.42	0.97	0.33
PC3 ~ treatment + stage + trial + trial*stage	5151.91	4.46	0.06

Table 3.5 (Cont'd)

PC3 ~ treatment + stage + trial + treatment*trial	5152.62	5.17	0.04
null model	5529.48	382.02	0

Levels of larval predation

For predation trials, the AICc selected model included stage and treatment (Table 3.6). Both treatment and stage were significant ($p = 0.0033$, $p < 0.0001$, respectively). Wild-caught larvae had a significantly lower survival rate than hatchery-produced larvae at stage A and B ($p = 0.0033$, Cohen's $D = 0.22$ for Stage A and 0.40 for Stage B). By stage C, after wild-caught larvae fish have been in the hatchery for 10 days, there is no significant difference in mean survival rates between treatments (Figure 3.7). Survival rates significantly decreased at each stage ($p < 0.0001$).

Table 3.6. AICc selected models for larval lake sturgeon predation data, including the top four competitive models and null model, and predictor variables of treatment (hatchery or wild), stage (A, B, or C) and carapace (crayfish carapace length as a proxy of crayfish size)

Model	AICc	Delta AICc	Weight
Mortalities ~ Stage + Treatment	790.58	0	0.4
Mortalities ~ Treatment + Carapace + Stage	791.48	0.91	0.26
Mortalities ~ Treatment + Carapace + Stage + Exposure	792.37	1.8	0.16
Mortalities ~ Treatment + Carapace + Stage + Exposure + Treatment*Stage	794.57	3.99	0.05
null model	851.72	61.15	0

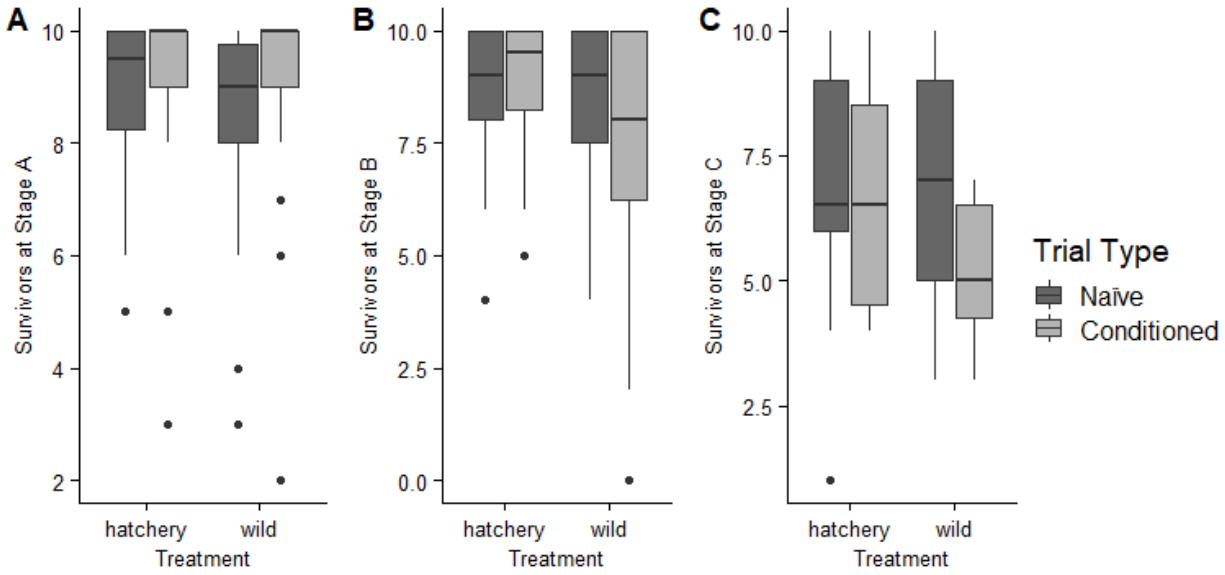

Figure 3.7. Survival of hatchery-produced and wild-caught larvae across three stages, including naïve to predator (first exposure) treatment and conditioned to predator odor (second exposure) treatment.
Stage A was day one of the larval stage for hatchery-produced larvae, or the day after capture for wild-caught larvae, stage B was day five of the larval stage, and stage C was day nine of the larval stage. Predation trials were conducted for each replicate, one trial using larvae that were naïve to predator odor and one using larvae that were conditioned to predator odor combined with alarm cues. For conditioning, larvae were exposed to crayfish odor combined with conspecific homogenate containing alarm cues. 4 mls of conditioning odor was added to each 3-liter tank housing larvae the night before the predation trial, and an additional 4 mls of conditioning odor was added to each tank the following morning approximately eight hours prior to the predation trial. For each predation trial, 10 larvae were placed in a 15.12 liter tank supplied with flowing stream water with one crayfish per tank for 2.5 hours. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5 x the interquartile range for the upper and lower quartiles.

DISCUSSION

Findings demonstrate that stress-related phenotypes may develop over very short periods of time following the onset of captive rearing. Both hatchery-produced and wild-caught sturgeon experienced effects of the hatchery environment as indicated by physiological and behavioral differences among stages. Wild-caught larvae initially exhibited elevated physiological responses to an acute stressor as evidenced by higher whole-body cortisol (Figure 3.2). Behaviorally, wild-caught larvae exhibited greater tendencies to maximize concealment (lower

activity, lower total distance traveled, and less time spent in the center of the petri dish). Wild-caught larvae experienced lower survival compared to hatchery-reared larvae until stage C, likely due to their relative proclivity for inactivity when predators were experimentally introduced into a small tank. At stage C (after 9 days in the hatchery environment), larvae from both rearing environments had similarly low survival. Inferentially, a modest extension of 3 additional days in a hatchery environment (6 days s. 9 days) was sufficient exposure to the captive regime to alter behavior (i.e., increase activity) to the point where mortality of wild-caught larvae was at comparative levels with hatchery-produced larvae (Figure 3.7). Low cortisol levels were likewise expressed by larvae from both treatments by stage C, with no post-stress elevation of cortisol levels (Figure 3.2). Wild-caught sturgeon, despite having spent early ontogenetic stages in the wild, were still affected by the captive rearing environment over a short period to the extent that their stress axis function was indistinguishable from that of hatchery-produced larvae after only 9 days.

It is possible that the higher baseline cortisol levels observed in wild-caught larvae compared to hatchery-produced larvae at Stage A may be a result of stress associated with capture and transitioning to a new food type. However, prior research supports the likelihood of hatchery environments affecting larval cortisol. A study by Earhart et al. (2020) on cortisol in hatchery-produced lake sturgeon indicated that larvae incubated as eggs using a tumbling regime in McDonald jars, a standard hatchery practice, had lower cortisol levels and delayed onset of cortisol production than non-tumbled individuals. The decrease in physiological reactivity to stress over time for both treatment groups in this study suggests that hatchery rearing impacts stress axis function. Cortisol elevation in response to an acute stressor decreased over time for larval lake sturgeon from both treatments, and was no longer evident by stage C (day 9 of the

larval stage) (Figure 3.2). A prior experiment with lake sturgeon larvae observed that a decrease in physiological reactivity was associated with chronic stress (Wassink et al., 2019). While it has been proposed that lower physiological reactivity to threats may be adaptive in some contexts (Blas et al., 2007), this experiment showed that predation rates increased over time coincident with a decrease in cortisol reactivity. Therefore, decreasing physiological reactivity to stress does not appear to improve fitness by helping lake sturgeon larvae avoid predation. Further research could investigate the role cortisol responses play in fitness by defining in what situations (i.e., captivity or wild environment) a lack of physiological reactivity is adaptive.

Overall, hatchery-produced sturgeon were more active and moved greater distances (Figure 3.3, Figure 3.4), while wild-caught sturgeon spent more time avoiding the center zone (Figure 3.5). In addition, hatchery larvae showed decreasing activity levels over time, while wild larvae showed increasing activity levels (Figure 3.3). The opposite change in activity level over time in hatchery-reared and wild-caught sturgeon suggests that behavioral trajectories may be program prior to the larval stage (Dammerman et al., 2015). Behavioral trajectories could be determined in response to factors such as genetic differences, maternal effects, egg incubation environment, or free embryo experience prior to emergence from the substrate. For example, prior work with lake sturgeon free embryos suggests that maternal effects are reflected in familial differences in yolk sac egg provisioning, metabolism, and subsequent activity levels after hatch (Wassink et al., 2019). Egg incubation environment has also been shown to influence development (Walquist et al., in review, Dammerman et al., in review), which could have downstream effects on stress-related behavior. Furthermore, behavioral phenotype may not be directly linked to physiological phenotype, since differences in physiological reactivity between treatments abated over time while behavioral differences remained. Collectively, findings

indicate that further investigations into behavior programming mechanisms other than stress in the early life environment are warranted.

In contrast to prior studies in which larger size was advantageous for avoiding predation for lake sturgeon larvae aged 8 to 16 weeks (Crossman et al., 2018), the current experiment found that predation rates increased over time (Figure 3.7). Larger size may explain the initially higher survival of hatchery-produced larvae compared to wild-caught larvae at stages A and B (Figure 3.1). However, the increasing size experienced by both treatment groups over time was associated with decreased, rather than increased, survival during predation trials. Higher activity levels have been associated in prior studies with increased survival of larval lake sturgeon during predation trials with crayfish (Wassink et al., 2019). However, in this study wild-caught larvae increased activity levels over time with no associated increase in survival. Thus, the negative effect of the captive environment on anti-predator abilities may override advantages that would typically promote survival for larval lake sturgeon.

Exposure to predator odor did not help lake sturgeon avoid predation by crayfish (Figure 3.7). Our results contrast with those of Wishingrad et al. (2014), who observed that lake sturgeon larvae dramatically increase activity levels in response to predator odor combined with conspecific homogenate. Lake sturgeon larvae associate predator odor with alarm cues released from the skin of conspecifics, to which they have an innate reaction (Wishingrad et al., 2014). One possible explanation is that in this experiment, lake sturgeon larvae increased swimming activity as a stress response to conditioning odor, thereby depleting energy prior to predation trials. A second possibility is that larval lake sturgeon do not as readily learn the odor of a predator they did not co-evolve with, as rusty crayfish are an invasive species. A third possibility is that the hatchery environment inhibited learning for both hatchery-produced and

wild-caught individuals. It has been suggested that the capacity for learning is cued by variability in the environment, since plasticity is usually adaptive in situations where individuals encounter new stimuli (Kotrschal & Taborsky, 2010; Mettke-Hofmann, 2014). If the hatchery setting creates a homogenous rearing environment through predictability of surroundings, food availability, and lack of predator encounters, learning may not be promoted. This finding highlights the importance of studies linking cognitive ecology to conservation, in order to develop a more complete understanding of what environmental factors promote or inhibit learning. Conservation programs would benefit from an understanding of how animal cognition is impacted by captive environments, as cognitive abilities are likely important for post-release survival (Teixeira et al., 2007).

Inter-individual variation in cortisol concentrations and predation rates appears to increase by stage C, illustrating the importance of individuality in studying effects of captive rearing environments (Figure 3.2, Figure 3.7). This finding is consistent with research showing that captive environments increased inter-individual variation in oldfield mice (*Peromyscus polionotus subgriseus*) (McPhee, 2004). Personality (temperament) is important in studies on conservation in predicting success of captive breeding, captive rearing, and reintroduction programs (McDougall et al., 2006). For example, amphibians exhibit consistent individual differences in boldness, exploration, and activity, all of which are important in both captive breeding success and survival after reintroduction (Kelleher et al., 2018) in contexts such as predation, foraging, and dispersal (Cote et al., 2010). Research investigating the mechanisms driving inter-individual differences in behavior could be applied to conservation efforts to predict or promote success in released individuals (Healy & Jones 2002; Powell & Gartner 2011; Greggor et al., 2014; Ballew et al., 2017).

Parentage of wild-caught larvae is unknown, but it is likely that genetic effects played a role in determining how individuals responded developmentally to rearing environment (Dammerman et al., 2015, Dammerman et al., 2016). There was likely higher genetic variation in wild-caught fish than in hatchery-reared fish, which was only represented by three full-sib families. Non-genetic maternal effects are also important for lake sturgeon, as prior research has suggested that both maternal and offspring experience are important in programming offspring stress axis function (Wassink et al., 2020). Thus, additive genetic and maternal effects likely explain some of the inter-individual variation in behavior and physiology expressed during experimental trials. Genetic differences between hatchery-reared and wild-caught larvae could also explain behavioral differences.

Overall, this study illustrates that even captive rearing programs that use individuals captured from wild populations may impose a rearing environment that results in physiological and behavioral changes that decrease survival after release. The cortisol stress response of wild-caught lake sturgeon larvae became similar to that of hatchery-produced larvae after only 9 days in the hatchery. Even though cortisol levels did not indicate that the hatchery environment induced chronic stress, predation rates were still high, suggesting that physiological measures of stress may not accurately predict success of individuals after release. Similarly, physiological phenotype may not predict behavioral responses to threats, as behavioral differences between hatchery-reared and wild-caught lake sturgeon did not appear to be linked to stress physiology.

Negative effects of captivity on predator avoidance may override advantages, such as larger size and higher activity levels, that would typically promote survival. Both hatchery-produced and wild-caught lake sturgeon larvae experienced increasing predation mortality over time, with larger size and higher activity levels failing to convey an advantage for predator

avoidance as expected from prior studies (Wassink et al., 2019, Crossman, 2018). Therefore, even behaviors not directly induced by stress (or, conversely, not directly related to welfare) are of concern for reintroduction programs. This finding suggests that reintroduction programs could utilize research in cognitive ecology to create environments that behaviorally and cognitively prepare individuals for facing challenges in the wild (Greggor et al., 2014). While re-creating features of the wild environment within captive environments may not be logistically feasible, programs could instead focus on promoting learning, behavioral plasticity, and adaptation to novelty, to prepare individuals to navigate the transition to the wild and the high variability in environments that will be encountered (Teixeira et al., 2007; Kotrschal & Taborsky, 2010; Mettke-Hofmann, 2014). Implementing this strategy would require a more detailed understanding of which specific features of early rearing environments promote learning and resilience to environmental variability, as well as the developmental mechanisms involved.

REFERENCES

REFERENCES

Alvarez, D., & Nicieza, A. G. (2003). Predator avoidance behaviour in wild and hatchery-reared brown trout: the role of experience and domestication. *Journal of Fish Biology*, *63*(6), 1565-1577.

Araki, H., Cooper, B., & Blouin, M. S. (2009). Carry-over effect of captive breeding reduces reproductive fitness of wild-born descendants in the wild. *Biology Letters*, *5*(5), 621-624.

Auperin, B., & 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. *General and Comparative Endocrinology*, *158*(3), 234-239.

Ballew, N. G., Mittelbach, G. G., & Scribner, K. T. (2017). Fitness consequences of boldness in juvenile and adult largemouth bass. *The American Naturalist, 189*(4), 396-406.

Bauman, J. M., Baker, E. A., Marsh, T. L., & Scribner, K. T. (2015). Effects of rearing density on total length and survival of Lake Sturgeon free embryos. *North American journal of aquaculture, 77*(4), 444-448.

Bauman, J. M., Woodward, B. M., Baker, E. A., Marsh, T. L., & Scribner, K. T. (2016). Effects of Family, Feeding Frequency, and Alternate Food Type on Body Size and Survival of Hatchery-Produced and Wild-Caught Lake Sturgeon Larvae. *North American Journal of Aquaculture*, *78*(2): 136–144.

Berejikian, B. A. (1995). The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. *Canadian Journal of Fisheries and Aquatic Sciences*, *52*(11), 2476-2482.

Berejikian, B. A., Tezak, E. P., Schroder, S. L., Knudsen, C. M., & Hard, J. J. (1997). Reproductive behavioral interactions between wild and captively reared coho salmon (*Oncorhynchus kisutch*). *ICES Journal of Marine Science*, *54*(6), 1040-1050.

Berger-Tal, O., Blumstein, D. T., Carroll, S., Fisher, R. N., Mesnick, S. L., Owen, M. A., et al. (2016). A systematic survey of the integration of animal behavior into conservation. *Conservation Biology*, *30*(4), 744-753.

Biro, P. A., Post, J. R., & Parkinson, E. A. (2003). From individuals to populations: prey fish risk-taking mediates mortality in whole-system experiments. *Ecology, 84*(9), 2419-2431.

Blas, J., Bortolotti, G. R., Tella, J. L., Baos, R., & Marchant, T. A. (2007). Stress response during development predicts fitness in a wild, long lived vertebrate. *Proceedings of the National Academy of Sciences*, *104*(21), 8880-8884.

Brown, C., & Day, R. L. (2002). The future of stock enhancements: lessons for hatchery practice from conservation biology. *Fish and Fisheries, 3*(2), 79-94.

Burnham, K. P., & Anderson, D. R. (1998). Practical use of the information-theoretic approach. *Model Selection and Inference* (pp. 75-117). Springer, New York, NY.

Bussy, U., Wassink, L., Scribner, K. T., & Li, W. (2017). Determination of cortisol in lake sturgeon (*Acipenser fulvescens*) eggs by liquid chromatography tandem mass spectrometry. *Journal of Chromatography B, 1040,* 162-168.

Clark, T. W., & Westrum, R. (1989). High-performance teams in wildlife conservation: a species reintroduction and recovery example. *Environmental Management, 13*(6), 663-670.

Comte, L., Buisson, L., Daufresne, M., & Grenouillet, G. (2013). Climate-induced changes in the distribution of freshwater fish: observed and predicted trends. *Freshwater Biology, 58*(4), 625-639.

Cote, J., Clobert, J., Brodin, T., Fogarty, S., & Sih, A. (2010). Personality-dependent dispersal: characterization, ontogeny and consequences for spatially structured populations. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1560), 4065-4076.

Crossman, J. A., Scribner, K. T., Yen, D. T., Davis, C. A., Forsythe, P. S., & Baker, E. A. (2011). Gamete and larval collection methods and hatchery rearing environments affect levels of genetic diversity in early life stages of lake sturgeon (*Acipenser fulvescens*). *Aquaculture*, *310*(3-4), 312-324.

Crossman, J. A., Scribner, K. T., Forsythe, P. S., & Baker, E. A. (2018). Lethal and non-lethal effects of predation by native fish and an invasive crayfish on hatchery-reared age-0 lake sturgeon (Acipenser fulvescens Rafinesque, 1817). Journal of Applied Ichthyology, *34*(2), 322-330.

Dammerman, K.J., J.P. Steibel, and K.T. Scribner. Shifts in the influence of microhabitat incubation conditions and genetic parentage effects on offspring traits during early ontogeny in lake sturgeon *(Acipenser fulvescens*). *Environmental Biology of Fishes*. In review.

Dammerman, K. J., Steibel, J. P., & Scribner, K. T. (2015). Genetic and environmental components of phenotypic and behavioral trait variation during lake sturgeon (*Acipenser fulvescens*) early ontogeny. *Environmental Biology of Fishes, 98*(6), 1659-1670.

Dammerman, K. J., Steibel, J. P., & Scribner, K. T. (2016). Increases in the mean and variability of thermal regimes result in differential phenotypic responses among genotypes during early ontogenetic stages of lake sturgeon (*Acipenser fulvescens*). *Evolutionary Applications, 9*(10), 1258-1270.

Duong, T. Y., Scribner, K. T., Crossman, J. A., Forsythe, P. S., & Baker, E. A. (2011). Environmental and maternal effects on embryonic and larval developmental time until dispersal of lake sturgeon (*Acipenser fulvescens*). *Canadian Journal of Fisheries and Aquatic Sciences*, *68*(4), 643-654.

Earhart, M. L., Bugg, W. S., Wiwchar, C. E., Kroeker, J. R., Jeffries, K. M., & Anderson, W. G. (2020). Shaken, rattled and rolled: The effects of hatchery-rearing techniques on endogenous cortisol production, stress-related gene expression, growth and survival in larval Lake Sturgeon, *Acipenser fulvescens*. *Aquaculture*, *522*, 735116.

Evans, M. L., Wilke, N. F., O'Reilly, P. T., & Fleming, I. A. (2014). Transgenerational Effects of Parental Rearing Environment Influence the Survivorship of Captive-Born Offspring in the Wild. *Conservation Letters*, *7*(4), 371-379.

Falahatkar, B., Poursaeid, S., Shakoorian, M., & Barton, B. (2009). Responses to handling and confinement stressors in juvenile great sturgeon *Huso huso*. *Journal of Fish Biology*, *75*(4), 784-796.

Ferguson, M. M., & Duckworth, G. A. (1997). The status and distribution of lake sturgeon, *Acipenser fulvescens*, in the Canadian provinces of Manitoba, Ontario and Quebec: a genetic perspective. In *Sturgeon Biodiversity and Conservation* (pp. 299-309). Springer Netherlands.

Ford, M. J. (2002). Selection in captivity during supportive breeding may reduce fitness in the wild. *Conservation Biology*, *16*(3), 815-825.

Greggor, A. L., Clayton, N. S., Phalan, B., & Thornton, A. (2014). Comparative cognition for conservationists. *Trends in Ecology & Evolution*, *29*(9), 489-495.

Harvey, N. C., Farabaugh, S. M., & Druker, B. B. (2002). Effects of early rearing experience on adult behavior and nesting in captive Hawaiian crows (Corvus hawaiiensis). *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association*, *21*(1), 59-75.

Hayes, D. B., & Caroffino, D. C. (2012). Michigan's Lake Sturgeon rehabilitation strategy. Michigan Department of Natural Resources, Fisheries Division Special Report, 62.

Hayhoe, K., VanDorn, J., Croley II, T., Schlegal, N., & Wuebbles, D. (2010). Regional climate change projections for Chicago and the US Great Lakes. *Journal of Great Lakes Research*, *36*, 7-21.

Healy, S. D., & Jones, C. M. (2002). Animal learning and memory: an integration of cognition and ecology. *Zoology*, *105*(4), 321-327.

Holtgren, J. M., Ogren, S. A., Paquet, A. J., & Fajfer, S. (2007). Design of a portable streamside rearing facility for lake sturgeon. *North American Journal of Aquaculture*, *69*(4), 317-323.

Huntingford, F. A. (2004). Implications of domestication and rearing conditions for the behaviour of cultivated fishes. *Journal of Fish Biology*, *65*(1), 122-142.

Jackson, D. A. (1993). Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology*, *74*(8), 2204-2214.

Jeanneteau, F. D., Lambert, W. M., Ismaili, N., Bath, K. G., Lee, F. S., Garabedian, M. J., et al. (2012). BDNF and glucocorticoids regulate corticotrophin-releasing hormone (CRH) homeostasis in the hypothalamus. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(4), 1305-1310.

Kelleher, S. R., Silla, A. J., & Byrne, P. G. (2018). Animal personality and behavioral syndromes in amphibians: a review of the evidence, experimental approaches, and implications for conservation. *Behavioral Ecology and Sociobiology*, *72*(5), 79.

Kotrschal, A., & Taborsky, B. (2010). Environmental change enhances cognitive abilities in fish. *PLoS biology*, *8*(4), e1000351.

Kreger, M. D., Hatfield, J. S., Estevez, I., Gee, G. F., & Clugston, D. A. (2006). Behavioral profiles of the captive juvenile whooping crane as an indicator of post-release survival. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association*, *25*(1), 11-24.

Li, D., Liu, Z., & Xie, C. (2012). Effect of stocking density on growth and serum concentrations of thyroid hormones and cortisol in Amur sturgeon, *Acipenser schrenckii*. *Fish Physiology and Biochemistry*, *38*(2), 511-520.

LRBOI (Little River Band of Ottawa Indians). 2008. Nmé (Lake Sturgeon) stewardship plan for the Big Manistee River and 1836 reservation, Manistee, Michigan: LRBOI Natural Resources Department, Special Report 1.

Lukkes, J. L., Mokin, M. V., Scholl, J. L., & Forster, G. L. (2009). Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses. *Hormones and Behavior*, *55*(1), 248-256.

Lynch, M., & O'Hely, M. (2001). Captive breeding and the genetic fitness of natural populations. *Conservation Genetics*, *2*(4), 363-378.

Mann, K. A., Holtgren, J. M., Auer, N. A., & Ogren, S. A. (2011). Comparing size, movement, and habitat selection of wild and streamside-reared lake sturgeon. *North American Journal of Fisheries Management*, *31*(2), 305-314.

McDougall, P. T., Réale, D., Sol, D., & Reader, S. M. (2006). Wildlife conservation and animal temperament: causes and consequences of evolutionary change for captive, reintroduced, and wild populations. *Animal Conservation*, *9*(1), 39-48.

McPhee, M. E. (2004). Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. *Biological conservation*, *115*(1), 71-77.

Mettke-Hofmann, C. (2014). Cognitive ecology: ecological factors, life-styles, and cognition. *Wiley Interdisciplinary Reviews: Cognitive Science*, *5*(3), 345-360.

Okuyama, J., Shimizu, T., Abe, O., Yoseda, K., & Arai, N. (2010). Wild versus head-started hawksbill turtles Eretmochelys imbricata: post-release behavior and feeding adaptions. *Endangered Species Research*, *10*, 181-190.

Olla, B. L., Davis, M. W., & Ryer, C. H. (1998). Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. *Bulletin of Marine Science*, *62*(2), 531-550.

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: Classical theories and new developments. *Trends in Neurosciences*, *31*(9), 464-468.

Piato, A. L., Capiotti, K. M., Tamborski, A. R., Oses, J. P., Barcellos, L. J., Bogo, M. R., et al. (2011). Unpredictable chronic stress model in zebrafish (*Danio rerio*): Behavioral and physiological responses. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(2), 561-567.

Powell, D. M., & Gartner, M. C. (2011). Applications of personality to the management and conservation of nonhuman animals. *From genes to animal behavior.* Springer, Tokyo, 185-199.

Sakamoto, K., Dew, W. A., Hecnar, S. J., & Pyle, G. G. (2016). Effects of lampricide on olfaction and behavior in young-of-the-year lake sturgeon (*Acipenser fulvescens*). *Environmental science & technology*, *50*(7), 3462-3468.

Schreck, C. B., Olla, B. L., & Davis, M. W. (1997). Behavioral responses to stress. *Fish Stress and Health in Aquaculture*, *62*, 145-170.

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. *Aquaculture international*, *17*(1), 7-14.

Sloychuk, J. R., Chivers, D. P., & Ferrari, M. C. (2016). Juvenile lake sturgeon go to school: life-skills training for hatchery fish. *Transactions of the American Fisheries Society*, *145*(2), 287-294.

Stunz, G. W., & Minello, T. J. (2001). Habitat-related predation on juvenile wild-caught and hatchery-reared red drum Sciaenops ocellatus (Linnaeus). *Journal of Experimental Marine Biology and Ecology*, *260*(1), 13-25.

Teixeira, C. P., De Azevedo, C. S., Mendl, M., Cipreste, C. F., & Young, R. J. (2007). Revisiting translocation and reintroduction programmes: the importance of considering stress. *Animal Behaviour, 73*(1), 1-13.

Turner, J. D., Alt, S. R., Cao, L., Vernocchi, S., Trifonova, S., Battello, N., et al. (2010). Transcriptional control of the glucocorticoid receptor: CpG islands, epigenetics and more. *Biochemical Pharmacology, 80*(12), 1860e1868.

Walquist, R.W., K.T. Scribner, J. Waraniak, J.M. Bauman, T.L. Marsh, and J. Kanefsky. Aquatic insect functional feeding groups differentially affect lake sturgeon larval phenotypes and egg surface microbial communities. *Ecology*. In Review.

Waraniak, J. M., Baker, E. A., & Scribner, K. T. (2018). Molecular diet analysis reveals predator–prey community dynamics and environmental factors affecting predation of larval lake sturgeon *Acipenser fulvescens* in a natural system. *Journal of Fish Biology, 93*(4), 616-629.

Wassink, L., Bussy, U., Li, W., & Scribner, K. (2019). High-stress rearing temperature in *Acipenser fulvescens* affects physiology, behaviour and predation rates. *Animal Behaviour, 157*, 153-165.

Wassink, L., Huerta, B., Li, W., & Scribner, K. (2020). Interaction of egg cortisol and offspring experience influences stress-related behaviour and physiology in lake sturgeon. *Animal Behaviour, 161*, 49-59.

Wishingrad, V., Sloychuk, J. R., Ferrari, M. C. O., & Chivers, D. P. (2014). Alarm cues in Lake Sturgeon *Acipenser fulvescens* Rafinesque, 1817: potential implications for life-skills training. *Journal of Applied Ichthyology, 30*(6), 1441-1444.

CHAPTER 4:

Early life interactions with aquatic insects elicits physiological and behavioral stress responses in lake sturgeon (*Acipenser fulvescens*)

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ABSTRACT

Inter-species interactions, including predator encounters, during early life stages can elicit behavioral and physiological responses that in turn can have important consequences for populations. In threatened lake sturgeon (*Acipenser fulvescens*), newly hatched free embryos co-occupy stream substrate with potential predators including aquatic insect larvae. This study investigated stress effects on lake sturgeon larvae after encounters with aquatic insects by quantifying mortality, body size, cortisol levels, and swimming behavior. Free embryos were exposed to either Perlidae (stonefly obligate predators) or Isonychiidae (mayfly filterers and facultative predators). Free embryos that encountered Perlids exhibited high mortality as well as elevated cortisol and cortisol reactivity to an acute stressor compared to individuals in the control treatment (no insects). Free embryos that encountered Isonychiids exhibited slightly elevated mortality compared to individuals in the control treatment (no insects) and had slightly elevated cortisol and cortisol reactivity. Findings indicate that lake sturgeon free embryos are stressed by exposure to benthic environments during early life stages that include predation of nearby conspecifics in proportion to the amount of predation experienced. Lake sturgeon larvae also exhibited alterations to swimming behavior, with individuals that encountered isonychiids exhibiting lower activity levels, and individuals that encountered perlids exhibiting slower swimming speed. Behavioral outcomes suggest that even encounters with aquatic insects associated with low predation (isonychiids) have the potential to alter behavioral trajectories, potentially as an adaptive response that will reduce predation rates in subsequent life stages. Our results contribute to a broader understanding of how inter-species interaction with obligately co-occurring benthic invertebrate communities may impact lake sturgeon populations, with the potential to inform management and conservation efforts.

INTRODUCTION

Interactions among members of different trophic levels play an important role in ecosystems. In particular, nonlethal predator effects can have notable influences on individuals, populations, and communities (Lima, 1998; Werner & Anholt, 1996; Skelly & Werner, 1990; McKauley et al., 2011). Even in the absence of predator-induced mortality, the presence of predators can alter prey behavioral and physiological phenotypes and impact survival during subsequent life stages (Werner & Anholt, 1996, Skelly & Werner, 1990). For example, predator cues can alter the rate of early development (Mirza et al., 2001), or determine responses to subsequent predation cues (Ferrari & Chivers, 2009). One mechanism by which predator presence can influence individuals is by creating early life stress (McKauley et al., 2011). Early life stress can have profound impacts on downstream behavior and physiology, with implications for future survival (Chen et al., 2014; Liesenjohann & Krause, 2012; Middlemis et al., 2013). Therefore, understanding the role of early life predator-related stress in individual development is important to predict population-level effects.

Lake sturgeon (*Acipenser fulvescens*) are a priority for management and conservation in the Great Lakes because populations have experienced prolonged periods of over-harvest and loss and degradation of spawning habitat (Hayes & Caroffino, 2012; Ferguson & Duckworth, 1997). During the free embryo and larval stages, lake sturgeon experience high levels of predation by invertebrates and fishes (Waraniak et al., 2018). In response to conspecific predation, lake sturgeon are known to respond to alarm cues from the skin of conspecifics during early ontogeny (Wishingrad et al., 2014; Sloychuk et al. 2016). Alarm cues are released from injured or predated conspecifics, allowing nearby individuals to perceive and adaptively respond to predator presence (Cao & Li, 2020; Mourabit et al., 2010; Laurila et al., 1997). In fishes,

exposure to conspecific alarm cues can be important by enabling individuals to recognize predators and respond appropriately to potential threats (Smith, 1999; Vilhunen & Hirvonen, 2003; Holmes & McCormick, 2010). During the lake sturgeon free embryo stage, first the olfactory and subsequently visual perceptive abilities become functional (Dettlaff et al., 1993), enabling individuals to sense and responding to predator presence However, effects of predator presence on important stress-related physiology and behavior during early life stages on lake sturgeon development has not been extensively investigated.

Considering the ecological importance of phenotypic alteration related to nonlethal predator effects (Lima, 1998), understanding implications of early life exposure to predators for lake sturgeon survival and recruitment is essential for informing conservation efforts. The hypothalamic-pituitary-interrenal (HPI) stress axis becomes functional during the free embryo stage in sturgeon (Falahatkar et al., 2012; Simontacchi et al., 2009), so individuals likely experience predator presence and associated alarm cues as a stressor. The stress axis is responsible for mediating physiological response to stressors via the release of cortisol, the stress hormone (Auperin & Geslin, 2008; Lukkes et al., 2009). For lake sturgeon free embryos experiencing predator presence and associated alarm cues, stress can be quantified using cortisol levels. Early life stress can alter stress axis function, resulting in longterm changes in physiological and behavioral development (Piato et al., 2011; Schreck et al., 1997; Pariante & Lightman, 2008; Jeanneteau et al., 2012). As a consequence, early life predator-induced stress may have important downstream effects on future threat responses, especially during the larval drift period when lake sturgeon are particularly vulnerable to predation (Waraniak et al., 2018). Studies with other species have shown that nonlethal predation effects can change growth trajectories and size at age (Middlemis et al., 2013; Skelly & Werner, 1990), which in sturgeon

have been shown to be important for survival during subsequent periods of high predation (Wishingrad et al., 2014; Crossman et al., 2011; Wassink et al., 2019). Early life exposure to predators that induces stress may be important for triggering adaptive responses to predators during the period of larval dispersal from spawning areas to rearing habitat downstream. Lake sturgeon larvae that experience chronic temperature stress have lower cortisol reactivity and higher activity levels, which have been shown to be associated with higher survival in the presence of a crayfish predator (Wassink et al., 2019), suggesting that early life stress has the potential to induce compensatory adaptive physiological and behavioral phenotypes in lake sturgeon.

Lake sturgeon free embryos and aquatic insect larvae are likely to interact due to overlap of obligate habitats (Hamilton, 2004). Lake sturgeon adults spawn on hard gravel substrate, and free embryos burrow into interstitial spaces immediately after hatch until yolk sac reserves are depleted (Kempinger, 1988). Larval stages of diverse aquatic benthic insect communities also occupy gravel substrate in streams (Bournaud et al., 1998; Jähnig & Lorenz, 2008) and thus co-occur with lake sturgeon free embryos. However, effects of invertebrate community composition on lake sturgeon populations are not well understood. Prior research with lake sturgeon eggs suggest that the presence of aquatic insects influences timing and body size at hatch time depending on functional feeding group (Walquist et al., in review), but an understanding of how aquatic insects affect lake sturgeon after hatch and effects during subsequent life stages is limited.

Abundance and biodiversity of aquatic insects are heavily influenced by water quality and other environmental factors (Dijkstra et al., 2014; Hershey et al., 2010). Therefore, differing environments likely generate variation in taxonomic groups of aquatic insects encountered by

lake sturgeon free embryos, as well as insect abundance and the frequency of such encounters. Predatory groups like members of the stonefly family Perlidae are predator of larval fish (Claire & Phillips, 1968). Members of the mayfly family Isonychiidae are collector-filterers and facultative predators (Merritt & Cummins, 2008). Therefore, depending on function, isonychiids could either potentially reduce stress of fish larvae by improving water quality (Menzie, 1980; Morin et al., 1988), or increase stress and reduce survival via predation. Considering the extensive co-occurrence between members of aquatic invertebrate communities and lake sturgeon during early life stages, lake sturgeon are likely to have developed mechanisms for reacting adaptively to exposure to predaceous aquatic insects. Understanding the adaptive physiological and behavioral outcomes of inter-species interactions across trophic levels is important in informing lake sturgeon management and conservation.

This study investigated both direct and indirect effects on lake sturgeon of encounters with aquatic insect larvae during early ontogenetic stages. Specifically, we quantified mortality rates, stress physiology, and swimming behavior of lake sturgeon larvae that encountered either perlids, isonychiids, or no insects during the free embryo stage. We hypothesized that exposure to perlids (predators) would cause alterations in behavioral and physiological reactivity since individuals were expected to encounter an environment of high predation-related stress. We predicted that lake sturgeon larvae exposed to perlids would have higher mortality rates, higher cortisol levels, and increased behavioral reactivity to alarm cue odor compared to larvae exposed to isonychiids or control larvae. Understanding how inter-species interactions during early life stages impact lake sturgeon development will contribute to a broader understanding of proximal and long term effects of biotic community interactions on wildlife species like lake sturgeon whose populations are highly vulnerable to environmental changes.

METHODS

Eggs and sperm were collected from adult sturgeon spawning in the Upper Black River in Onaway, MI on May 11, 2018. Eggs were fertilized using standard lake sturgeon hatchery procedure (Bauman et al., 2016, Crossman et al., 2011). All individuals used for the experiment were taken from one full sibling family, based on gamete fertilization using a single male and female to reduce variation due to family (genetic or maternal) effects (Wassink et al., 2020). Eggs were reared using a tumbling regime in McDonald egg-hatching jars (Pentair J32, Apopka, FL) until hatch. Free embryos were then moved to 3-liter flow-through tanks. Tanks were supplied with stream water at ambient temperature flowing at a rate of 56.78 liters/hour.

Experimental treatments using free embryo lake sturgeon began at 5 days after hatch, approximately 3 days away from reaching the larval stage at the beginning of exogenous feeding. Each of the three treatments (control, isonychiid, and perlid) included six replicates, each containing 25 free embryos, with one replicate per tank. All aquatic insect larvae were captured from the Upper Black River using either a kick-net or a D-frame drift net. The drift net was moved downstream while sediment was disturbed in front of it for approximately 1 minute. Free embryos held in 3-liter aquaria were provided with Bioballs (2.54 cm^3 BioBalls Pentair #CBBI-5) as artificial stream substrate. Food was not provided to free embryos, since lake sturgeon utilize yolk sac reserves during the free embryo stage and only begin exogenous feeding at the onset of the larval stage (approximately 8 days after hatch). In the control treatment, no insects were added to the 3-liter tanks. In the isonychiid and perlid treatments, four aquatic insect larvae were added to each of the six replicate tanks in each treatment. For the isonychiid and perlid treatments, insects were left to interact with free embryos for three days and then removed from tanks.

Free embryo mortality

After insects were removed from replicate tanks, all surviving free embryos were counted to determine the mortality rate for each tank. Proportion of mortalities was calculated for each replicate. A beta regression and pairwise contrasts were used to determine if proportions of mortality differed among perlid, isonychiid, and control treatments using the *betareg* function in program R v 3.2.2.

Larval body size

At the beginning of the lake sturgeon larval stage, total body length (mm) of six individuals from each replicate in each of the three treatments were measured. Photos of lake sturgeon larvae were taken using a digital camera, and ImageJ software (National Institutes of Health, Bethesda, MD, U.S.A., http://rsbweb.nih.gov/ij /). Images were used to quantify total body length for each individual using a ruler in each photo for calibration. A Shapiro-Wilk test indicated that the body size dataset was normally distributed ($p = 0.1423$). A One-way ANOVA was conducted using the *aov* function in program R v 3.2.2. to determine differences in body size among treatments.

Larval cortisol levels

At the onset of the larval stage, larval samples for cortisol analysis were taken at baseline (with no acute stressor applied) or after an acute stressor in order to quantify stress levels as well as physiological reactivity of the stress axis to an acute stressor. The acute stressor was a 20 second period of removal from the water and exposure to air which is a known stressor in sturgeon (Eslamloo & Falahatkar, 2014). Afterwards individuals were placed back in water for a rest period of 30 minutes before being euthanized. Each cortisol sample contained 6 individuals. One baseline and one post-stress sample were taken from each replicate tank in the experiment.

Individuals were euthanized using an overdose of MS-222 according to approved Michigan State University Animal Use and Care protocols. Whole body cortisol levels were estimated using liquid chromatography tandem mass spectrometry with a Waters Xevo TQ-S mass spectrometer (Waters, Millford, MA, USA) as developed by our group for lake sturgeon (Bussy et al. 2017).

The cortisol data were not normally distributed, so log-transformation was applied prior to analysis. A Shapiro-Wilk test confirmed that the log-transformed dataset was normally distributed ($p = 0.8180$). Generalized Linear Models were fit using the *glm* function in program R v 3.2.2. Predictor variables in the models included invertebrate treatment, stress state (baseline or after the acute stressor was applied), and the interaction of treatment and stress state. Variables present in the AICc selected model were further evaluated using an ANOVA and post hoc Tukey HSD tests.

Larval behavior

Behavior trials were conducted at the beginning of the larval stage for 6 individuals from each of the 6 replicate tanks in each treatment. Larvae were placed into a 15.24 cm diameter petri dish filled with filtered stream water, and swimming activity was video recorded for 4 minutes using a Go-Pro Hero 4 camera (GoPro, Inc), without any additional stimuli added. After 4 minutes, video recording was paused and 1 ml of odor made from whole-body homogenization of conspecifics was added to the center of the petri dish using a pipette. Video recording was resumed for an additional 4 minutes to capture individuals' swimming activity in reaction to odor. Conspecific tissue homogenate has been shown to cause a physiological and behavioral response in sturgeon, which have an innate reaction to conspecific alarm cues released from skin (Wishingrad et al., 2014).

Loligo v.4.0 tracking software (Loligo Systems, Viborg, Denmark; https://www.loligosystems.com/software) was used to simultaneously track activity of the six individuals in each replicated trial, following Sakamoto et al. (2016). A center zone was defined that excluded a one-inch perimeter around the petri dish edge to quantify edge-seeking behavior. Variables quantified from the entire 4 minute video period included each individual's velocity (cm/s), acceleration (cm/s^2), percent time active, total distance traveled (cm), number of visits to center zone, and time (s) spent in center zone (Wassink et al., 2019; Wassink et al., 2020).

Behavioral variables (percent activity, acceleration, velocity, distance, zone time, and zone visits) were compressed into a composite behavioral measure using Principal Components Analysis (Ballew et al., 2017). Datasets from videos prior to addition of alarm cue odor, with no stimulus, and datasets from videos after the addition of alarm cue odor were analyzed separately, since the same individuals were present in both sets of videos and thus datapoints were not independent. The broken stick method was used to determine that PC1, PC2, and PC3 were significant for the no stimulus dataset, and PC1 and PC2 were significant for the alarm cue dataset (Jackson, 1993). Factor loadings above 0.5 were used to determine behavioral relevance of each principal component. ANOVAs were conducted using the *aov* function in program R v 3.2.2 for the principal components to determine whether there were significant differences among principal component scores of the perlid, isonychiid, and control treatments.

Animal welfare

All experiments were conducted under approved Michigan State University Animal Use and Care protocols (04/17-071-00). To the extent possible, stress was minimized for all individuals in the experiment. Adults captured for gamete collection were handled for about 4 minutes each, while head and gills remained underwater. In the hatchery, Bioballs were

provided as artificial substrate for free embryos until emergency. Once reaching the larval stage and beginning exogenous feeding, individuals were supplied with food (premium grade brine shrimp Artemia sp., BSEP16Z, Brine Shrimp Direct) *ad libitum*. Flow rate of filtered stream water in tanks was maintained at 56.78 liters/hour to ensure adequate oxygenation. During the 4 minute behavior trials in petri dishes, larvae were supplied with oxygenated stream water for the duration of the trial. Any larvae sacrificed for cortisol samples or for alarm cue odors were euthanized according to approved Michigan State University Animal Use and Care protocols using an overdose of MS-222.

RESULTS

Free embryo mortality

Mean percent mortalities among the six replicate tanks for the control treatment was 5.33%, for the isonychiid treatment was 7.33%, and for the perlid treatment was 18.67% (Figure 4.1). A beta regression and pairwise contrasts indicated that the perlid treatment experienced a significantly higher mortality rate than did the control treatment ($p = 0.0025$). No significant differences in mortality rate existed between the isonychiid treatment and either of the other treatments.

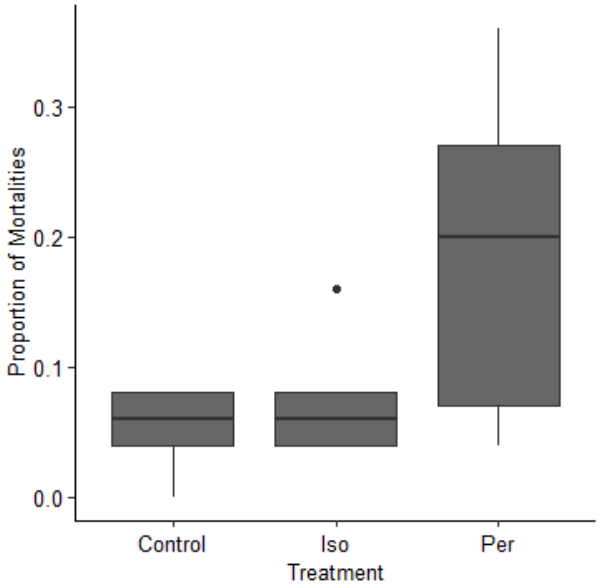

Figure 4.1. Mean proportion of mortality associated with each aquatic insect treatment. Surviving larvae were counted after exposure to either no insects (Control), Isonychiid mayflies (Iso), or Perlid stoneflies (Per) for three days. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5 x the range for upper and lower quartiles.

Larval body size

ANOVA indicated that treatment had a significant effect on body size at the beginning of the larval stage ($F_{2, 105} = 6.37$, $p = 0.0024$). Tukey HSD indicated that individuals in the perlid treatment were significantly larger (mean ± SD = 20.90 ± 1.63) than individuals from the other treatments (Figure 4.2). No significant difference existed between lengths of individuals from the control treatment (mean ± SD = 19.76 ± 1.39) and the isonychiid treatment (mean ± SD = 19.87 ± 1.46) (Figure 4.2).

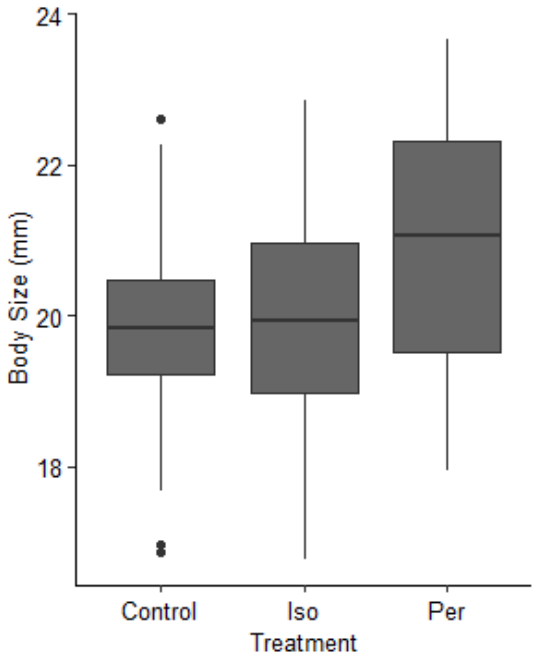

Figure 4.2. Mean length (mm) of lake sturgeon at the beginning of the larval stage after exposure to aquatic insect treatment (no insects (Control), Isonychiid mayflies (Iso), or Perlid stoneflies (Per)).
Sample sizes were 6 individuals per treatment, or 18 total larvae. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5 x the range for upper and lower quartiles.

Larval cortisol levels

For both baseline and post stress whole-body samples, larvae from the control treatment were characterized by the lowest cortisol levels, larvae from the isonychiid treatment showed intermediate levels, and larvae from the perlid treatment showed highest levels. The AICc-selected model included treatment, stress state, and the interaction of treatment and stress state (Table 4.1, Figure 4.3). ANOVA indicated a significant effect of treatment ($p < 0.0001$), stress state ($p < 0.0001$), and the interaction of treatment and stress state ($p = 0.0489$). Tukey HSD indicated that individuals in the perlid and isonychiid treatments had significantly higher cortisol after an acute stressor. Tukey HSD also indicated that baseline cortisol levels for individuals exposed to perlids were significantly higher than those for individuals in the control treatment.

Additionally, Tukey HSD indicated that post-stress cortisol levels for individuals exposed to perlids were significantly higher than cortisol levels from any other treatment or stress state (Figure 4.3).

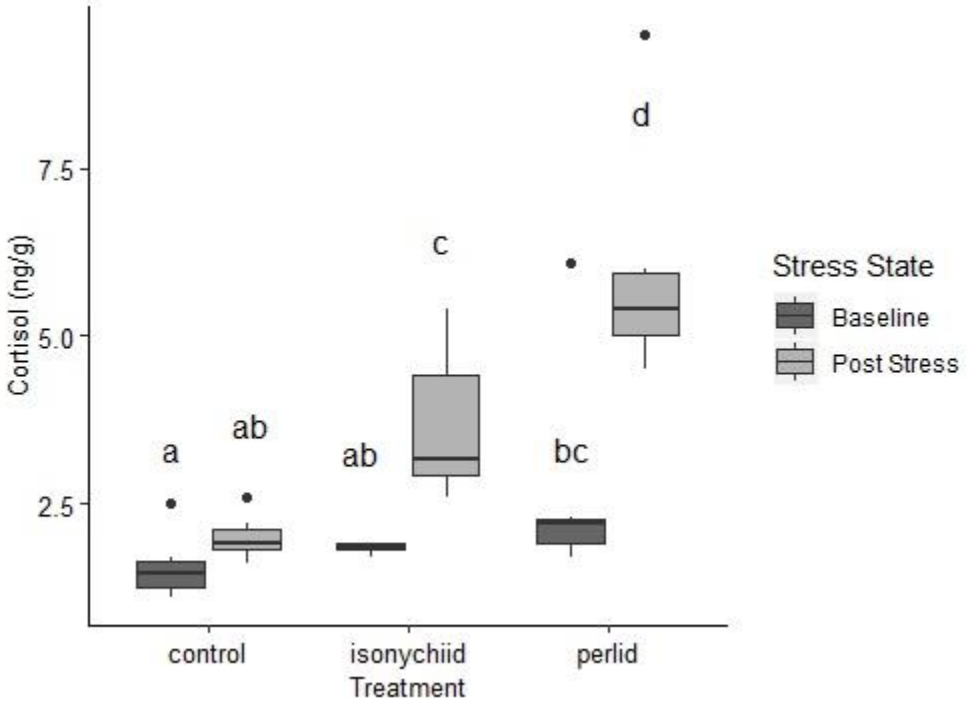

Figure 4.3. Cortisol levels presented in original scale, for lake sturgeon at the beginning of the larval stage after exposure to aquatic insect treatments (no insects (Control), Isonychiid mayflies (Iso), or Perlid stoneflies (Per)).
Cortisol levels are shown at baseline (pre exposure to air exposure as an acute stressor) and following exposure to the acute stressor. Letters indicate significant difference among treatment means based on Tukey HSD post hoc tests. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5 x the range for upper and lower quartiles.

Table 4.1. Models for whole-body cortisol levels for lake sturgeon larvae exposed to isonychiids, perlids, or no insect at baseline and after an acute stressor

Model	AICc	Delta AICc	Weight
Cortisol ~ Treatment + Stress State + Treatment * Stress State	-36.57	0	0.65
Cortisol ~ Treatment + Stress State	-35.33	1.24	0.35
Cortisol ~ Stress State	-12.14	24.44	0
Cortisol ~ Treatment	-11.87	24.7	0
null model	-0.29	36.28	0

Larval behavior

Behavioral traits associated with swimming activity (percent activity, velocity, acceleration, distance, zone time, and zone visits) were reduced into three components using PCA for the no stimulus and alarm cue datasets. The broken stick method was used to determine that PC1, PC2, and PC3 were significant for the no stimulus dataset, and PC1 and PC2 were significant for the alarm cue dataset (Jackson 1993).

For the dataset of behavioral responses with no stimulus applied, factor loadings indicated that the most important variable contributing to variation along PC1 was percent activity. PC1, which explained 55.93% of the variation in the dataset, was positively associated with percent activity. ANOVA and a post-hoc Tukey HSD test indicated that individuals from the perlid and control treatments were more active (higher mean PC1 scores) than individuals from the isonychiid treatment ($p = 0.0003$). PC2 explained 25.48% of the variation in the dataset and was negatively associated with velocity, acceleration, distance, and zone visits. ANOVA and a post-hoc Tukey HSD test indicated that individuals from the perlid and control treatments moved slower, traveled smaller distances, and avoided the center zone more (higher mean PC2 scores) compared to individuals from the isonychiid treatment ($p = 0.0017$) (Figure 4, Table 4.2).

Table 4.2. Factor loadings and eigenvalues for principal components analysis of swimming behavior with no stimulus applied and after the addition of alarm cue odor

	factor loadings						eigen-value	variance %	cumulative variance %
	velocity	*accel.*	*zone time*	*% activity*	*distance*	*zone visits*			
				No stimulus applied					
PC1	-0.4168	-0.4338	0.2308	0.5095	0.4003	0.4062	3.3560	55.9332	55.9332
PC2	-0.5139	-0.4841	-0.0993	-0.0028	-0.5001	-0.4915	1.5289	25.4812	81.4145
PC3	0.0267	0.0294	0.9599	-0.1936	-0.1914	-0.0550	0.8726	14.5436	95.9581
PC4	-0.2556	-0.2030	-0.1174	-0.7258	-0.0904	0.5871	0.1540	2.5666	98.5247
PC5	-0.1172	-0.1405	0.0417	-0.4188	0.7375	-0.4955	0.0858	1.4308	99.9555
PC6	-0.6945	0.7181	0.0081	0.0267	0.0313	-0.0147	0.0027	0.0445	100.0000
				Alarm cues added					
PC1	0.3901	0.4380	-0.3477	-0.3197	-0.4599	-0.4705	2.9495	49.1578	49.1578
PC2	-0.5943	-0.5240	-0.0570	-0.2115	-0.4215	-0.3828	1.5319	25.5317	74.6895
PC3	0.0594	0.0816	0.6960	-0.6952	-0.0556	0.1378	1.0654	17.7564	92.4458
PC4	-0.1060	-0.1004	-0.6182	-0.5987	0.3438	0.3462	0.3173	5.2880	97.7338
PC5	0.0300	-0.0496	0.0956	-0.1024	0.6956	-0.7023	0.1305	2.1745	99.9083
PC6	0.6921	-0.7172	-0.0116	-0.0290	-0.0754	0.0081	0.0055	0.0917	100.0000

For the dataset of behavioral responses after individuals were exposed to alarm cue odor, PC1 explained 49.16% of the variation in the dataset, and factor loadings indicated PC1 was negatively associated with distance and zonevisits. ANOVA and a post-hoc Tukey HSD test conducted for PC1 indicated that, in the presence of alarm cues, individuals from the isonychiid treatment traveled smaller distances and avoided the center zone more (higher mean PC1 score) compared to individuals from the perlid treatment ($p = 0.0142$). PC2 explained 25.53% of the variation in the dataset and was negatively associated with velocity and acceleration. ANOVA and a post-hoc Tukey HSD test conducted for PC2 indicated that individuals from the perlid treatment moved slower (higher mean PC2 score) than individuals from the isonychiid treatment, in the presence of alarm cues ($p = 0.0035$). PC3 explained 17.76% of the variation in the dataset and was positively associated with zone time and negatively associated with percent activity.

ANOVA conducted for PC3 indicated no significant differences among treatments (p = 0.134) (Figure 4.4, Table 4.3).

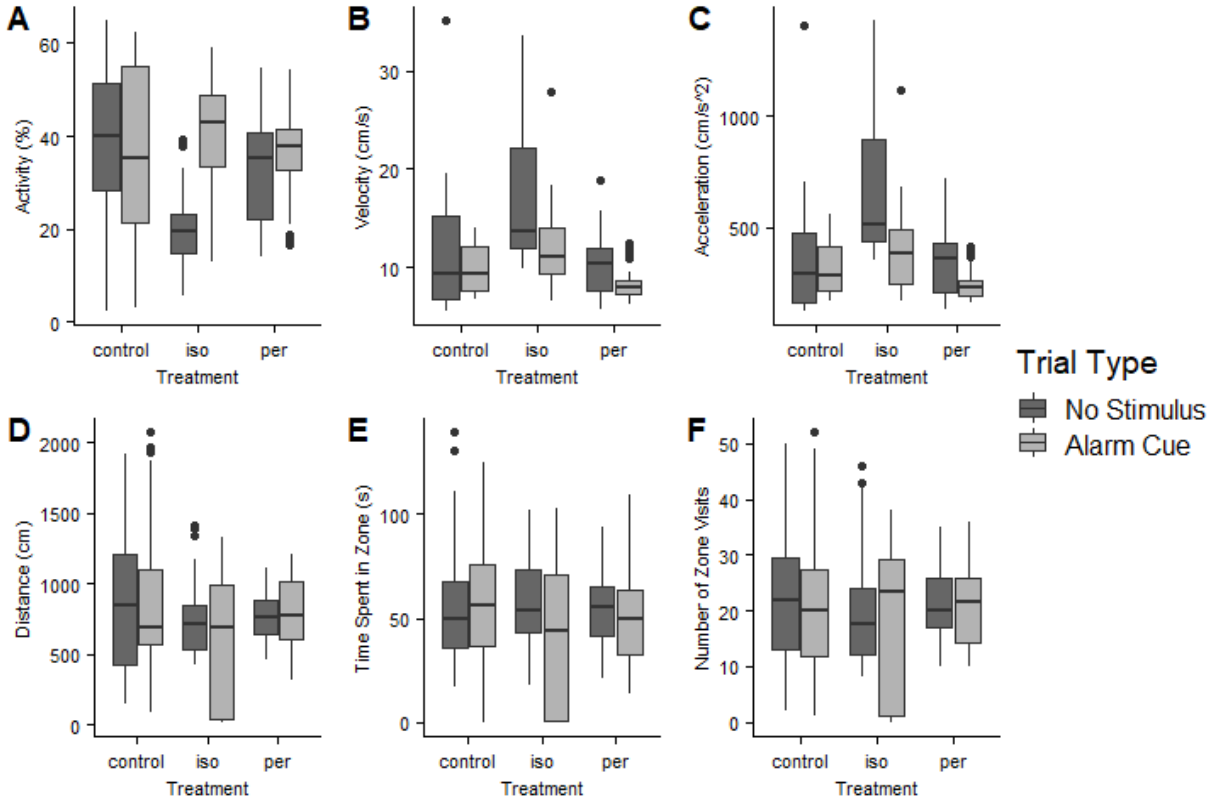

Figure 4.4. Behavioral responses of lake sturgeon at the beginning of the larval stage after exposure to aquatic insect treatments (no insects (Control), Isonychiid mayflies (Iso), or Perlid stoneflies (Per)), in the absence of stimuli and after the addition of an alarm cue odor.
Loligo software was used to extract % time active (A), velocity (cm/s) (B), acceleration (cm/s^2) (C), total distance traveled (cm) (D), time spent in center zone (s) (E), and number of visits to center zone (F) for each individual in each video. Whiskers indicate minimum and maximum values, excluding data points beyond 1.5 x the range for upper and lower quartiles.

DISCUSSION

This study demonstrates that lake sturgeon physiological and behavioral phenotypes are influenced by inter-species interactions, specifically encounters with aquatic insect larvae known to co-inhabit stream substrate with early sturgeon life stages. The presence of predaceous and herbaceous aquatic insect larvae has previously been shown to influence sturgeon hatch time

(Walquist et al., in review), and this study shows that aquatic insect presence has important effects on lake sturgeon physiology and behavior after hatch as well. Specifically, results showed that lake sturgeon free embryos experienced high mortality in the presence of perlids, confirming the importance of perlids as sturgeon predators. Additionally, the mortality rate of lake sturgeon free embryos in the presence of isonychiids implicates isonychiids as facultative sturgeon predators, though direct observation of a predation event in nature will be necessary for confirmation. Based on whole-body cortisol levels, lake sturgeon demonstrated alterations to physiological phenotypes that differed depending on the aquatic insect group encountered. Perlid encounters induced relatively high stress, with elevated baseline and post-stress cortisol levels, while isonychiid encounters elevate stress levels slightly compared to controls (Figure 4.3). Interaction with aquatic insects also influences larval swimming behavior in lake sturgeon, both with and without the presence of alarm cues (Figure 4). Findings collectively highlight the importance of community-level factors, including predator encounters, on phenotypes and survival. For threatened wildlife like lake sturgeon, conservation efforts should take be informed by environmental variables determining inter-species interactions, since these may have consequences for species of management concern.

Results indicate that cortisol levels and intensity of cortisol responses to an acute stressor varied in accordance with the level of predator-induced mortality being experienced by nearby conspecifics during an earlier life stage (Figure 4.3). Lake sturgeon larvae that encountered isonychiids as free embryos had lower mortality levels and a slight increase in baseline and post stress cortisol relative to larvae from the control (no insect) treatment. Larvae that encountered perlids as free embryos had a higher increase in baseline and post stress cortisol relative to larvae from the control treatment. Predator-induced stress reactivity has been documented elsewhere.

For example, in *Brachyrhaphis episcopi*, a freshwater fish, individuals from areas of higher predation risk had reduced physiological reactivity to stress, likely as an adaptation allowing individuals to function despite high stress environments (Archard et al., 2012). Similarly, tadpoles (*Rana sylvatica* and *Rana clamitans*) exposed to alarm cues showed lower physiological reactivity (suppression of the stress axis), likely as a means of promoting behavioral quiescence in order to avoid detection by predators (Fraker et al., 2009). In contrast, this study showed that lake sturgeon exposed to predation risk showed increased physiological reactivity to an acute stressor. This study used air exposure as a standardized acute stressor to observe stress axis function, and did not directly observe cortisol elevation in response to alarm cues. Therefore, it is possible that the intensified stress axis reactivity exhibited by lake sturgeon experiencing high predation risk applies to some acute stressors (such as air exposure) but not others (such as alarm cues). Research into differential responses of the stress axis to different threats likely to be encountered in nature could provide insight into physiological stress reactivity as a means of adaptation in response to environments experienced by individuals.

Interpretation of body length differences among lake sturgeon larvae from different treatments is limited, since length measurements prior to exposure to aquatic insects are not available. Without a before and after comparison, the significantly larger size of individuals from the perlid treatment cannot be conclusively linked to interaction with perlids despite the fact that all individuals were full-siblings. However, prior studies have shown that in larval lake sturgeon, larger size aids predator avoidance (Crossman et al., 2018; Wassink et al., 2019), supporting the interpretation that smaller individuals experienced higher rates of predation by perlids. Lake sturgeon body size during early ontogeny is closely tied to water temperature, with individuals reared in warm temperatures having much faster development but with smaller sizes

(Kempinger, 1988; Smith & King, 2005). Temperature could therefore influence lake sturgeon susceptibility to predation by aquatic insects. Specifically, warmer temperatures could increase rates of predation by decreasing mean size of lake sturgeon free embryos. Further investigation could investigate the inter-relationship of temperature, size, and fitness in the context of lake sturgeon and invertebrate communities (Kingsolver & Huey, 2008).

Interaction with aquatic insects during the free embryo stage also influenced swimming behavior of larval lake sturgeon (Figure 4.4). Comparison of individuals' behavior before and after addition of alarm cue odor to the petri dish is limited, since factor loadings indicated that principal components of the two analyses were primarily informed by different behavioral variables. However, results indicate that swimming behavior differs among lake sturgeon larvae from different treatments, both with and without the presence of alarm cue odor. Based on PC1 from analysis of behavior with no stimulus applied, individuals from the isonychiid treatment had lower activity levels, while individuals from perlid and control treatments had similar swimming activity. Prior studies have indicated that high activity levels are associated with higher survival in the presence of crayfish predators (Wassink et al., 2019), however, in this study the higher activity levels are unlikely to be an adaptive response to predator experience since they were observed in individuals from the control treatment. Notably, behavioral results did not match cortisol results, since intensity of behavioral differences was not proportional to the amount of predation experienced. One possibility is that the rate at which lake sturgeon encountered potential predators, resulting in direct interaction, was more important the amount of nearby conspecifics predation (indirect interaction) in determining behaviors. Individuals that encountered perlids frequently were more likely to be predated and thus removed from the sample population prior to behavior trials. Further investigation could examine whether

interaction with less-predatory species has a larger impact on a population's mean behavioral phenotypes due to higher survival rates for individuals that experience such interactions.

Based on PC1 from analysis of behavior in the presence of alarm cue odor, individuals from the isonychiid treatment traveled smaller distances and avoided the center zone more, suggesting they were more likely to avoid alarm cues which were added to the center of the petri dish. This result indicates that even when predator-induced mortality was low, as was the case with the isonychiid treatment, interaction with aquatic insects appears to affect larval lake sturgeons' reaction to alarm cues. Avoidance of alarm cues is likely an adaptive behavior in the presence of predators (Cao & Li, 2020; Mourabit et al., 2010; Laurila et al., 1997). Inter-species interaction may therefore induce adaptive behavioral development that allows individuals to avoid predation despite low prior experience with predation risk.

For individuals that did experience high levels of nearby conspecific predation moved slower (based on PC2 from analyses of behavior with no stimulus and with alarm cues) regardless of whether alarm cue door was present (Figure 4.4). Potentially, slower speeds could be an adaptive response to threat exhibited both in the novel environment of the petri dish, which has been shown to be a stressor for lake sturgeon larvae (Wassink et al., 2019; Wassink et al., 2020), as well as in response to alarm cues. Another possibility is that lake sturgeon larvae that had been actively avoiding perlids had reduced energy, resulting in slower movements during behavior trials. Conclusions about adaptive or maladaptive outcomes of interactions with aquatic insects requires further investigation that quantifies survival rates during subsequent life stages.

To gain further understanding of how aquatic insect larvae influence lake sturgeon survival, studies on how environmental factors influence aquatic insect predatory behavior would

be valuable. Perlids hunt as searchers under dark conditions, but as ambushers under light conditions (Sjöström, 1985). Since free embryos are burrowed into substrate, they are more likely to fall prey to perlids hunting as searchers in dark conditions, so abiotic factors such as moon phase and cloud cover could also influence rates of perlid predation on lake sturgeon. Further research could investigate under what conditions isonychiids switch from filter-feeding to predation, in order to predict effects on lake sturgeon free embryo survival. For example, temperature influences adult size of isonychiids (Wallace & Merritt, 1980), which could determine the feasibility of preying on lake sturgeon free embryos. Environmental variables are likely important for determining not only the abundance and biodiversity of aquatic insects encountered by lake sturgeon free embryos, but also whether such encounters result in predation.

Broadly, findings of this study indicate that interspecies interactions have important effects on sturgeon development, and thus a community-level perspective is important for understanding sources and outcomes of early life stress. Further research should explore how stress-altered physiological and behavioral phenotypes associated with exposure to aquatic insect larvae during the free embryo stage influence later predation rates of sturgeon. Understanding how community composition affects survival can help predict more precisely how threatened wildlife species like lake sturgeon respond to ecological factors. Specifically, inter-species interactions could be explored in the context of climate change and habitat disturbance. Freshwater stream biodiversity is highly vulnerable to climate change, and species are in danger of range shifts and extinction (Heino et al., 2009). For aquatic insects, land use, habitat disturbance, and climate change all have important impacts on abundance and biodiversity (Hershey et al., 2010; Sheldon, 2012), and by extension influence the species with which aquatic insects interact. Therefore, lake sturgeon populations could be affected by climate change and

habitat disturbance on multiple levels, experiencing not only direct effects on sturgeon (Hayhoe et al., 2010), but also indirect effects via the aquatic insects that influence survival rates and phenotypes of free embryos. Understanding implications of stress-related phenotypes for wildlife populations requires a thorough understanding of the interactions among biotic and physical components of ecological systems.

REFERENCES

REFERENCES

Archard, G. A., Earley, R. L., Hanninen, A. F., & Braithwaite, V. A. (2012). Correlated behaviour and stress physiology in fish exposed to different levels of predation pressure. *Functional Ecology*, *26*(3), 637-645.

Auperin, B., & 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. *General and Comparative Endocrinology*, *158*(3), 234-239.

Ballew, N. G., Mittelbach, G. G., & Scribner, K. T. (2017). Fitness consequences of boldness in juvenile and adult largemouth bass. *The American Naturalist, 189*(4), 396-406.

Bournaud, M., Tachet, H., Berly, A., & Cellot, B. (1998). Importance of microhabitat characteristics in the macrobenthos microdistribution of a large river reach. *Annales de Limnologie-International Journal of Limnology*, *34*(1), 83-98

Bussy, U., Wassink, L., Scribner, K. T., & Li, W. (2017). Determination of cortisol in lake sturgeon (*Acipenser fulvescens*) eggs by liquid chromatography tandem mass spectrometry. *Journal of Chromatography B, 1040,* 162-168.

Cao, X., & Li, W. (2020). Embryonic substances induce alarm response in adult zebrafish (*Danio rerio*). *Journal of Fish Biology,* 1-6

Chen, L. J., Shen, B. Q., Liu, D. D., & Li, S. T. (2014). The effects of early-life predator stress on anxiety-and depression-like behaviors of adult rats. *Neural plasticity, 2014.*

Claire, E. W., & Phillips, R. W. (1968). The stonefly *Acroneuria pacifica* as a potential predator on salmonid embryos. *Transactions of the American Fisheries Society*, *97*(1), 50-52.

Crossman, J. A., Scribner, K. T., Forsythe, P. S., & Baker, E. A. (2018). Lethal and non-lethal effects of predation by native fish and an invasive crayfish on hatchery-reared age-0 lake sturgeon *(Acipenser fulvescens* Rafinesque, 1817). *Journal of Applied Ichthyology*, *34*(2), 322-330.

Dettlaff, T.A., Ginsberg, A.C., & Schmalhausen, O.I. (1982). Development of sturgeon: Maturation of eggs, fertilization, development of embryos and prelarvae. *Canadian Translation of Fisheries and Aquatic Sciences, 4843*, 1–19.

Dijkstra, K. D. B., Monaghan, M. T., & Pauls, S. U. (2014). Freshwater biodiversity and aquatic insect diversification. *Annual review of entomology*, *59*, 143-163.

Eslamloo, K., & Falahatkar, B. (2014). Variations of some physiological and immunological parameters in Siberian sturgeon (*Acipenser baerii*, Brandt, 1869) subjected to an acute stressor. *Journal of Applied Animal Welfare Science, 17*(1), 29-42.

Falahatkar, B., Poursaeid, S., Shakoorian, M., & Barton, B. (2009). Responses to handling and confinement stressors in juvenile great sturgeon *Huso huso*. *Journal of Fish Biology, 75*(4), 784-796.

Ferguson, M.M., & Duckworth, G.A. (1997). The status and distribution of lake sturgeon, Acipenser fulvescens, in the Canadian provinces of Manitoba, Ontario and Quebec: A genetic perspective. *Environmental Biology of Fishes, 48*(1), 299–309.

Ferrari, M. C., & Chivers, D. P. (2009). Sophisticated early life lessons: threat-sensitive generalization of predator recognition by embryonic amphibians. *Behavioral Ecology, 20*(6), 1295-1298.

Fraker, M. E., Hu, F., Cuddapah, V., McCollum, S. A., Relyea, R. A., Hempel, J., & Denver, R. J. (2009). Characterization of an alarm pheromone secreted by amphibian tadpoles that induces behavioral inhibition and suppression of the neuroendocrine stress axis. *Hormones and behavior*, *55*(4), 520-529

Hamilton, I. M. (2004). A commitment model of reproductive inhibition in cooperatively breeding groups. *Behavioral Ecology, 15*(4), 585-591.

Hayes, D. B., & Caroffino, D. C. (2012). Michigan's Lake Sturgeon rehabilitation strategy. Michigan Department of Natural Resources, Fisheries Division Special Report, 62.

Hayhoe, K., VanDorn, J., Croley II, T., Schlegal, N., & Wuebbles, D. (2010). Regional climate change projections for Chicago and the US Great Lakes. *Journal of Great Lakes Research, 36*, 7-21.

Heino, J., Virkkala, R., & Toivonen, H. (2009). Climate change and freshwater biodiversity: detected patterns, future trends and adaptations in northern regions. *Biological Reviews, 84*(1), 39-54.

Hershey, A. E., Lamberti, G. A., Chaloner, D. T., & Northington, R. M. (2010). *Aquatic insect ecology. In Ecology and classification of North American freshwater invertebrates* (pp. 659-694). Academic Press.

Holmes, T. H., & McCormick, M. I. (2010). Smell, learn and live: the role of chemical alarm cues in predator learning during early life history in a marine fish. *Behavioural Processes, 83*(3), 299-305.

Jackson, D. A. (1993). Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology, 74*(8), 2204-2214.

Jähnig, S. C., & Lorenz, A. W. (2008). Substrate-specific macroinvertebrate diversity patterns following stream restoration. *Aquatic Sciences, 70*(3), 292-303.

Jeanneteau, F. D., Lambert, W. M., Ismaili, N., Bath, K. G., Lee, F. S., Garabedian, M. J., et al. (2012). BDNF and glucocorticoids regulate corticotrophin-releasing hormone (CRH) homeostasis in the hypothalamus. *Proceedings of the National Academy of Sciences of the United States of America, 109*(4), 1305-1310.

Kempinger, J. J. (1988). Spawning and early life history of lake sturgeon in the Lake Winnebago System, Wisconsin. American Fisheries Society Symposium Series, 5, 110-122.

Kingsolver, J. G., & Huey, R. B. (2008). Size, temperature, and fitness: three rules. *Evolutionary Ecology Research, 10*(2), 251-268.

Laurila, A., Kujasalo, J., & Ranta, E. (1997). Different antipredator behaviour in two anuran tadpoles: effects of predator diet. *Behavioral Ecology and Sociobiology, 40*(5), 329-336.

Liesenjohann, T., & Krause, E. T. (2012). Predation pressure and food abundance during early life alter risk-taking behaviour and growth of guppies (*Poecilia reticulata*). *Behaviour, 149*(1), 1-14.

Lima, S. L. (1998). Nonlethal effects in the ecology of predator-prey interactions. *Bioscience, 48*(1), 25-34.

Lukkes, J. L., Mokin, M. V., Scholl, J. L., & Forster, G. L. (2009). Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses. *Hormones and Behavior, 55*(1), 248-256.

McCauley, S. J., Rowe, L., & Fortin, M. J. (2011). The deadly effects of "nonlethal" predators. *Ecology*, 92(11), 2043-2048.

Menzie, C. A. (1980). Potential significance of insects in the removal of contaminants from aquatic systems. *Water, Air, and Soil Pollution, 13*(4), 473-479.

Merritt, R. W., Cummins, K. W., & Berg, M. B. (2008). An Introduction to the Aquatic Insects of North America, Kendall/Hunt Pub. *Co., Dubuque, IA*.

Middlemis Maher, J., Werner, E. E., & Denver, R. J. (2013). Stress hormones mediate predator-induced phenotypic plasticity in amphibian tadpoles. *Proceedings of the Royal Society B: Biological Sciences, 280*(1758), 20123075.

Mirza, R. S., Chivers, D. P., & Godin, J. G. J. (2001). Brook charr alevins alter timing of nest emergence in response to chemical cues from fish predators. *Journal of Chemical Ecology, 27*(9), 1775-1785.

Morin, A., Back, C., Chalifour, A., Boisvert, J., & Peters, R. H. (1988). Effect of black fly ingestion and assimilation on seston transport in a Quebec lake outlet. *Canadian Journal of Fisheries and Aquatic Sciences*, *45*(4), 705-714.

Mourabit, S., Rundle, S. D., Spicer, J. I., & Sloman, K. A. (2010). Alarm substance from adult zebrafish alters early embryonic development in offspring. *Biology letters*, *6*(4), 525-528.

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: Classical theories and new developments. *Trends in Neurosciences*, *31*(9), 464-468.

Piato, A. L., Capiotti, K. M., Tamborski, A. R., Oses, J. P., Barcellos, L. J., Bogo, M. R., et al. (2011). Unpredictable chronic stress model in zebrafish (*Danio rerio*): Behavioral and physiological responses. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(2), 561-567.

Schreck, C. B., Olla, B. L., & Davis, M. W. (1997). Behavioral responses to stress. *Fish Stress and Health in Aquaculture*, *62*, 145-170.

Sheldon, A. L. (2012). Possible climate-induced shift of stoneflies in a southern Appalachian catchment. *Freshwater Science*, *31*(3), 765-774.

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. *Aquaculture International*, *17*(1), 7-14.

Sjöström, P. (1985). Hunting behaviour of the perlid stonefly nymph *Dinocras cephalotes* (*Plecoptera*) under different light conditions. *Animal Behaviour*, *33*(2), 534-540.

Skelly, D. K., & Werner, E. E. (1990). Behavioral and life-historical responses of larval American toads to an odonate predator. *Ecology*, *71*(6), 2313-2322.

Sloychuk, J. R., Chivers, D. P., & Ferrari, M. C. (2016). Juvenile lake sturgeon go to school: life-skills training for hatchery fish. *Transactions of the American Fisheries Society*, *145*(2), 287-294.

Smith, R. J. F. (1999). What good is smelly stuff in the skin? Cross function and cross taxa effects in fish "alarm substances". In *Advances in chemical signals in vertebrates* (pp. 475-487). Springer, Boston, MA.

Smith, K. M., & King, D. K. (2005). Dynamics and extent of larval lake sturgeon *Acipenser fulvescens* drift in the Upper Black River, Michigan. *Journal of Applied Ichthyology*, *21*(3), 161-168.

Vilhunen, S., & Hirvonen, H. (2003). Innate antipredator responses of Arctic charr (*Salvelinus alpinus*) depend on predator species and their diet. *Behavioral Ecology and Sociobiology*, *55*(1), 1-10.

Walquist, R.W., Kim T. Scribner, J. Waraniak, J.M. Bauman, T.L. Marsh, and J. Kanefsky. Aquatic insect functional feeding groups differentially affect Lake Sturgeon larval phenotypes and egg surface microbial communities. *Ecosphere*. In Review.

Wallace, J. B., & Merritt, R. W. (1980). Filter-feeding ecology of aquatic insects. *Annual review of Entomology*, *25*(1), 103-132.

Waraniak, J. M., Baker, E. A., & Scribner, K. T. (2018). Molecular diet analysis reveals predator–prey community dynamics and environmental factors affecting predation of larval lake sturgeon *Acipenser fulvescens* in a natural system. *Journal of Fish Biology*, *93*(4), 616-629.

Wassink, L., Bussy, U., Li, W., & Scribner, K. (2019). High-stress rearing temperature in *Acipenser fulvescens* affects physiology, behaviour and predation rates. *Animal Behaviour*, *157*, 153-165

Wassink, L., Huerta, B., Li, W., & Scribner, K. (2020). Interaction of egg cortisol and offspring experience influences stress-related behaviour and physiology in lake sturgeon. *Animal Behaviour*, *161*, 49-59.

Wishingrad, V., Ferrari, M. C., & Chivers, D. P. (2014). Behavioural and morphological defences in a fish with a complex antipredator phenotype. *Animal Behaviour*, *95*, 137-143.

Wishingrad, V., Sloychuk, J. R., Ferrari, M. C. O., & Chivers, D. P. (2014). Alarm cues in Lake Sturgeon *Acipenser fulvescens* Rafinesque, 1817: potential implications for life-skills training. *Journal of Applied Ichthyology*, *30*(6), 1441-1444.