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

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Early life stress can lead to long-term behavioural 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 °C (low-stress) or 18 °C (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 behaviour 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 °C 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 °C had significantly higher activity levels during behaviour trials and significantly higher survival rates when exposed to crayfish predation, indicating that behavioural 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.

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

Physiological and behavioural responses to stress are mediated by glucocorticoids (e.g. cortisol) (Schreck, Olla, & Davis, 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 behavioural 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, Sapolsky, & McEwen, 1985), which impairs the negative feedback loop of the HPI axis and perpetuates chronic cortisol elevation (Jeanneteau et al., 2012). During early life stages,

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chronic stress can alter HPI axis function with long-term consequences for behaviour (Lukkes, Mokin, Scholl, & Forster, 2009; Turner et al., 2010).

HPI hyperactivity has been associated with altered behavioural phenotypes (Flandreau, Ressler, Owens, & Nemeroff, 2012). In rodents, chronic stress results in persistent expression of anxious behaviours (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).

Behavioural 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, Patterson, & Hahn, 2008). For example, HPI hyperactivity resulting from chronic stress can intensify antipredator behaviour (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 behavioural phenotypes, including antipredator behaviour (Ebbesson & Braithwaite, 2012; Galhardo & Oliveira, 2009; Wishingrad, Ferrari, & Chivers, 2014; Wishingrad, Sloychuk, Ferrari, & Chivers, 2014). 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 behaviour 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 behavioural alterations that affect survival (Crossman, Scribner, Forsythe, & Baker, 2018; Wishingrad, Musgrove, Chivers, & Ferrari, 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, Vecsei, & Jennings, 2007). At hatch, free embryos immediately burrow into substrate and emerge as larvae once yolk sac reserves have been depleted (Hastings, Bauman, Baker, & Scribner, 2013). At the larval drift stage, lake sturgeon begin exogenous feeding and disperse downstream from spawning areas to suitable larval rearing habitat (Duong, Scribner, Crossman, Forsythe, & Baker, 2011).

Behaviour 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, Baker, & Scribner, 2018). Antipredator behavioural phenotypes in sturgeon larvae have been shown to affect predation, survival and recruitment (McAdam, 2011). Sturgeon are known to alter antipredator behaviours 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 behavioural 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 3e 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, Poursaeid, Shakoorian, & Barton, 2009; Li, Liu, & Xie, 2012; Simontacchi et al., 2009; Wuertz et al., 2006).

Warm water temperature has been shown to be a stressor during early ontogenetic stages (Bates, Boucher, & Shrimpton, 2014; Dammerman, Steibel, & Scribner, 2016; Van Eenennaam, Linares-Casenave, Deng, & Doroshov, 2005; Zubair, Peake, Hare, & Anderson, 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 behavioural 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 °C by the end of the century (Hayhoe, VanDorn, Croley, II Schlegal, & Wuebbles, 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 behaviours on lake sturgeon, as well as consequences for survival of predation during the vulnerable larval stage. Behavioural 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 behaviours 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 °C. 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 × M48, F66 × M40, F69 × M48, F43 × 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 °C) and cold (10 ± 1 °C) 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 °C are within the typical range in the Upper Black River during the spawning season and thus are ecologically relevant, we considered 18 °C 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 °C) to predict developmental stages:

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

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, Ginsburg, & Schmalhausen, 1993; Simontacchi et al., 2009). Free embryo cortisol samples, measurements and behaviour trials were conducted at approximately 130 CTUs (approximately half-way through the free embryo stage). Larval cortisol samples, measurements and behaviour trials were conducted at the onset of exogenous feeding at approximately 206 CTUs (Table 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).

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 °C) and cold (10 °C) 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/litre 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

Table 1
Standardization of sampling schedule based on stage calculated by cumulative thermal units (CTUs)

Stage	CTU range	Days postfertilization	
		18 °C	10 °C
Fertilized egg (approximately half-way through egg incubation)	36–46	3 days	9 days
Hatch	72–74	6 days	14 days
Free embryo (approximately half-way 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

HPI: hypothalamic–pituitary–interrenal. Sampling and trials for each treatment were taken on days indicated using equation (1), using water temperature (x_i) and a constant (k, 5.8 °C) to predict developmental stages (Kempinger, 1998; Smith & King, 2005).

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 behavioural response in fishes (Wagner, Stroud, & Meckley, 2011; Wishingrad, Ferrari et al., 2014; Wishingrad, Sloychuk et al., 2014). 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 °C until analysis. HPLC-MS/MS analysis was conducted using a Waters Xevo TQ-S mass spectrometer (Waters, Millford, MA, U.S.A.) (Bussy, Wassink, Scribner, & Li, 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.

Behaviour

We quantified effects of temperature treatment on behaviour by observing escape behaviours 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, Dew, Hecnar, & Pyle, 2016). Loligo tracking software records pixel-based measurements that are converted to desired units (cm) and here reported to two significant digits. During behaviour 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, Ferrari et al., 2014; Wishingrad, Sloychuk et al., 2014) 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 behavioural 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 °C). Since ambient stream temperature for predation trials was closer to the warm temperature treatment (18 °C) than to the cold temperature treatment (10 °C), 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 litres/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 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)

Table 2
Models for size at hatch, free embryo and larvae

Model	AICc	ΔAICc	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

than hatchlings from the cold treatment (12.05 ± 0.07 mm) ($F_{1,45} = 28.00$, $P < 0.0001$; Fig. 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) ($F_{1,43} = 20.22$, $P < 0.0001$; Fig. 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) ($F_{1,46} = 30.79$, $P < 0.0001$; Fig. 1).

Cortisol

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 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) ($F_{1,42} = 3.59$, $P = 0.065$; Fig. 2). Family had a significant effect ($F_{3,43} = 15.47$, $P < 0.001$), as did the interaction of family and temperature ($F_{3,39} = 3.38$, $P = 0.028$; Fig. 3).

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 4). ANOVA did not indicate a significant difference in mean baseline cortisol for free embryos from the warm treatment

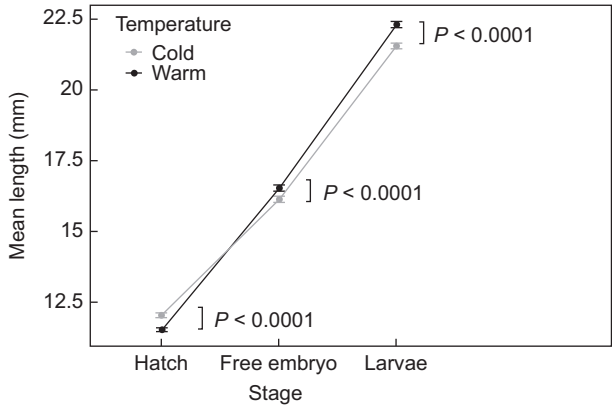

Figure 1. Mean ± SE length (mm) at hatch (130–134 cumulative thermal units, CTUs), free embryo stage (130–134 CTU) and larval stage (206–207 CTU). $N = 144$ biological replicates (864) individuals across all three stages, with six individuals measured per replicate at each stage.

Table 3
Models for fertilized egg cortisol

Model	AICc	ΔAICc	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

(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) ($F_{1,91} = 37.04$, $P < 0.0001$; Fig. 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 ($F_{3,134} = 0.69$, $P = 0.557$; Fig. 5) or of the interaction of temperature and stress state ($F_{2,126} = 1.10$, $P = 0.3360$; Fig. 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.

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 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) ($F_{1,43} = 3.36$, $P = 0.074$; Fig. 6). After exposure to an acute stressor, cortisol levels significantly increased for individuals from both temperature treatments ($F_{2,137} = 6.53$, $P = 0.006$; Fig. 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

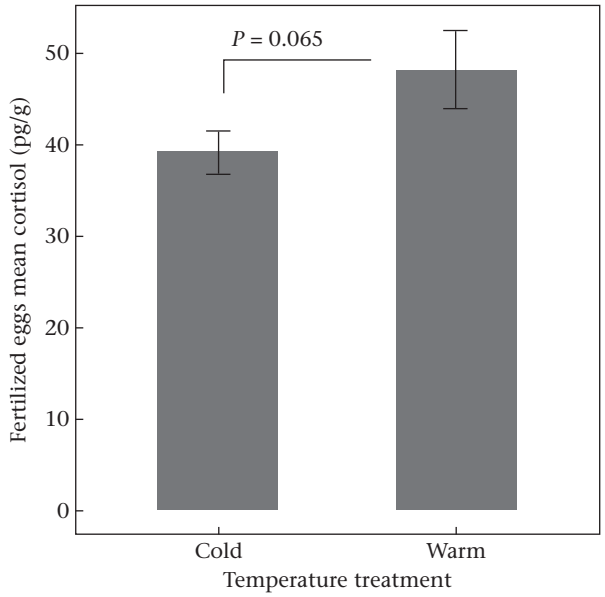

Figure 2. Mean ± SE cortisol levels in fertilized eggs in original (not log-transformed) scale. $N = 48$ samples, with 1 ml of eggs (~25 eggs) per sample. One sample was taken from each replicate in the range of 36–46 cumulative thermal units, CTUs (3 days postfertilization for 18 °C treatment, 9 days postfertilization for 10 °C treatment). *P* value from ANOVA.

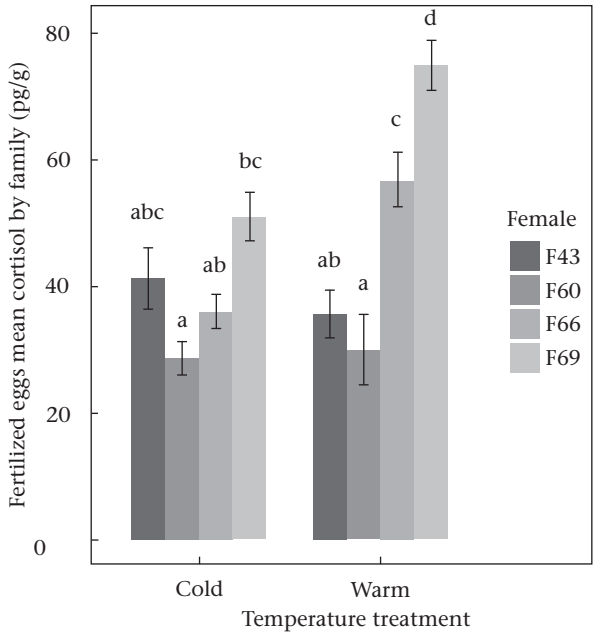

Figure 3. Mean ± SE cortisol levels in fertilized eggs by family in original (not log-transformed) scale. $N = 48$ samples, with 1 ml of eggs (~25 eggs) per sample. Letters indicate significant differences between families based on post hoc Tukey results.

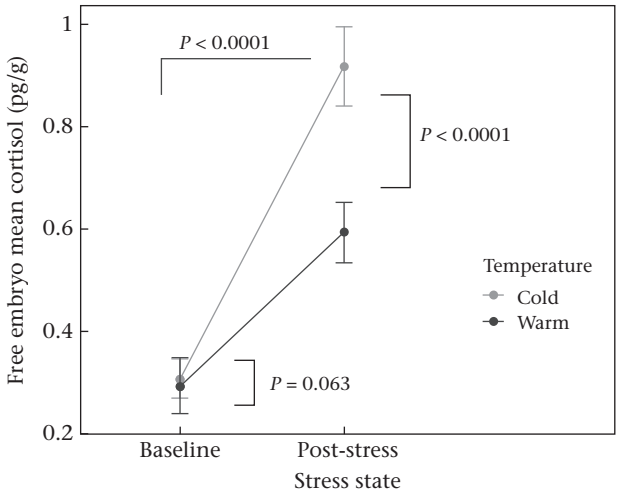

Figure 4. Mean ± SE cortisol levels in free embryos at baseline and after an acute stressor in original (not log-transformed) scale. $N = 96$ samples, with six individuals per sample. Samples were taken from each replicate in the range of 130–134 cumulative thermal units, CTUs (11 days postfertilization for the cold (18 °C) treatment, 24 days postfertilization for the warm (10 °C) treatment). Baseline samples were taken after immediate euthanasia using an overdose of MS-222. Individuals in post-stress samples were euthanized 30 min after exposure to an acute stressor. *P* values from ANOVA.

treatment (1.64 ± 0.1 pg/g) ($F_{1,91} = 0.28$, $P = 0.595$; Fig. 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 ($F_{6,128} = 6.32$, $P < 0.001$; Fig. 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 ($F_{2,126} = 2.43$, $P = 0.0924$; Fig. 7).

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 (Fig. A1), free embryo (Fig. A2) or larval stage (Fig. A3).

Table 4
Models for free embryo cortisol

Model	AICc	ΔAICc	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

Behaviour

The AIC-selected model for larval velocity included temperature treatment, female (family) and odour treatment (exposed to either sturgeon larvae homogenate or water as a control) (AICc = 428.27; Table 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 odour treatment ($F_{1,87} = 3.62$, $P = 0.1460$; Fig. 8a) on velocity.

The AIC-selected model for larval acceleration included temperature treatment, female (family) and odour treatment (exposed to either death odour 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 odour treatment ($F_{1,87} = 3.35$, $P = 0.1613$; Fig. 8b) on acceleration.

The AIC-selected model for larval activity (percentage of time active) included temperature treatment, female (family), odour 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%) ($F_{1,91} = 29.55$, $P < 0.0001$; Fig. 8c). An ANOVA showed that all factors indicated by the model were significant: temperature ($F_{1,91} = 29.55$, $P < 0.0001$), female ($F_{3,88} = 5.89$, $P = 0.0011$), odour 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 odour 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) ($F_{1,91} = 16.95$, $P < 0.0001$; Fig. 8d). An ANOVA showed that all factors indicated by the model were significant: temperature ($F_{1,91} = 16.95$, $P < 0.0001$), female ($F_{3,88} = 2.90$, $P = 0.0415$), odour treatment ($F_{1,87} = 8.77$, $P = 0.0159$; Fig. 8d).

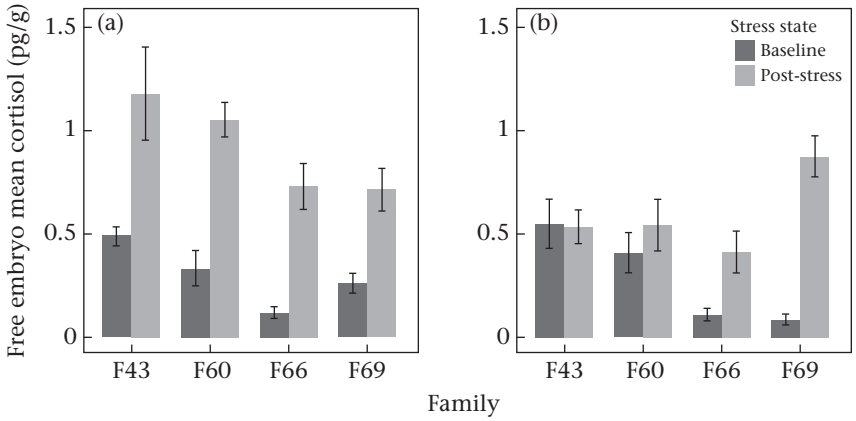

Figure 5. Mean ± SE cortisol levels in free embryos by family at baseline and after an acute stressor (post-stress) in original (not log-transformed) scale for (a) cold and (b) warm treatments. $N = 96$ samples, with six individuals per sample.

Table 5
Models for larvae cortisol

Model	AICc	ΔAICc	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

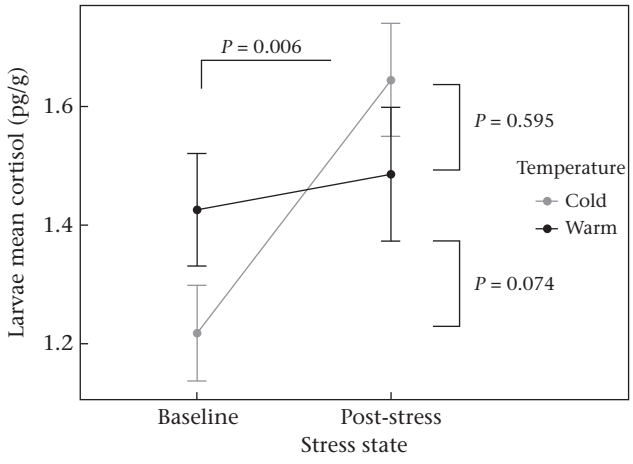

Figure 6. Mean ± SE cortisol levels in larvae at baseline and after an acute stressor in original (not log-transformed) scale. $N = 96$ samples, with six individuals per sample. Samples were taken from each replicate in the range of 206–207 cumulative thermal units, CTUs (17 days postfertilization for the warm (18 °C) treatment, 37 days postfertilization for the cold (10 °C) treatment). Baseline samples were taken after immediate euthanasia using an overdose of MS-222. Individuals in post-stress samples were euthanized 30 min after exposure to an acute stressor.

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 7). Chi-square analysis of the AIC-selected generalized linear model showed that larvae from the warm temperature treatment had significantly higher survival in the presence of a crayfish predator ($\chi^2_1 = 80.68$, $P < 0.0001$; Fig. 10). 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 ($\chi^2_3 = 4.52$, $P = 0.2106$; Fig. 11). 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 ($\chi^2_1 = 14.64$, $P = 0.0001$; Fig. 12).

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, Mommer, Suhr, & Bell, 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 HPLC-MS/MS rather than radioimmunoassay for cortisol samples. HPLC-MS/MS has been shown to yield significantly lower measures of cortisol levels compared to radioimmunoassay, due to higher selectivity (Vieira, Nakamura, & Carvalho, 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

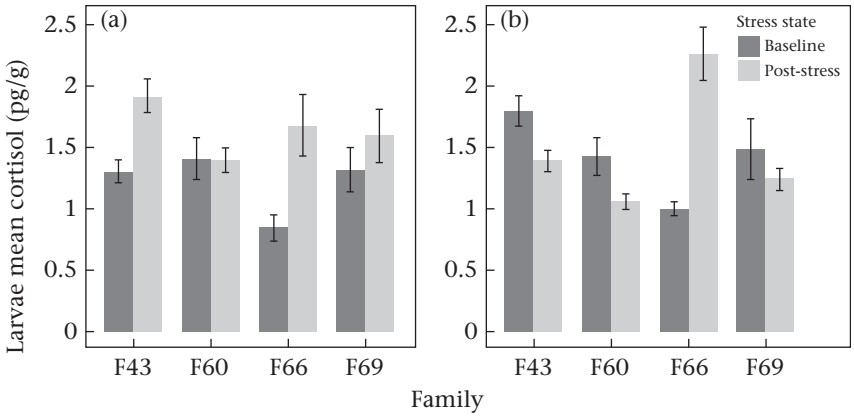

Figure 7. Mean ± SE cortisol levels in larvae by family at baseline and after an acute stressor (post-stress) in original (not log-transformed) scale for the (a) cold and (b) warm treatments. $N = 96$ samples, with six individuals per sample.

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, Nicolaiewsky, De Souza, & Lulhier, 1999; Barton, Schreck, & Barton, 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 programme 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 AIC-selected models for cortisol levels at the fertilized egg, free embryo and larval stages, as well as in models for behaviour 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

Table 6
Models for larvae velocity, acceleration, percentage of activity and distance

Model	AICc	ΔAICc	Weight
Velocity			
Velocity ~ temperature + female + odour.treatment	426.24	0	0.64
Velocity ~ temperature + female + odour.treatment + temperature*female	428.04	1.8	0.26
Velocity ~ temperature + female + odour.treatment + temperature*female + temperature*odour.treatment	430.16	3.92	0.09
Velocity ~ temperature + female + odour.treatment + temperature*female + temperature*odour.treatment + female*odour.treatment	435.11	8.87	0.01
Velocity ~ temperature + female + odour.treatment + temperature*female + temperature*odour.treatment + female*odour.treatment + temperature*female*odour.treatment	441.19	14.95	0
Acceleration			
Acceleration ~ temperature + female + odour.treatment	1070.09	0	0.66
Acceleration ~ temperature + female + odour.treatment + temperature*female	1072.13	2.04	0.24
Acceleration ~ temperature + female + odour.treatment + temperature*female + temperature*odour.treatment	1074.09	4	0.09
Acceleration ~ temperature + female + odour.treatment + temperature*female + temperature*odour.treatment + female*odour.treatment	1078.4	8.31	0.01
Acceleration ~ temperature + female + odour.treatment + temperature*female + temperature*odour.treatment + female*odour.treatment + temperature*female*odour.treatment	1085.14	15.05	0
% Activity			
% Activity ~ temperature + female + odour.treatment + temperature*female + temperature*odour.treatment + female*odour.treatment	550.82	0	0.35
% Activity ~ temperature + female + odour.treatment + temperature*female	550.89	0.07	0.33
% Activity ~ temperature + female + odour.treatment	552.4	1.58	0.16
% Activity ~ temperature + female + odour.treatment + temperature*female + temperature*odour.treatment	552.44	1.62	0.15
% Activity ~ temperature + female + odour.treatment + temperature*female + temperature*odour.treatment + female*odour.treatment + temperature*female*odour.treatment	558.58	7.76	0.01
Distance			
Distance ~ temperature + female + odour.treatment	1206.63	0	0.58
Distance ~ temperature + female + odour.treatment + temperature*female	1208.48	1.85	0.23
Distance ~ temperature + female + odour.treatment + temperature*female + temperature*odour.treatment	1209.03	2.4	0.17
Distance ~ temperature + female + odour.treatment + temperature*female + temperature*odour.treatment + female*odour.treatment	1213.73	7.1	0.02
Distance ~ temperature + female + odour.treatment + temperature*female + temperature*odour.treatment + female*odour.treatment + temperature*female*odour.treatment	1220.16	13.53	0

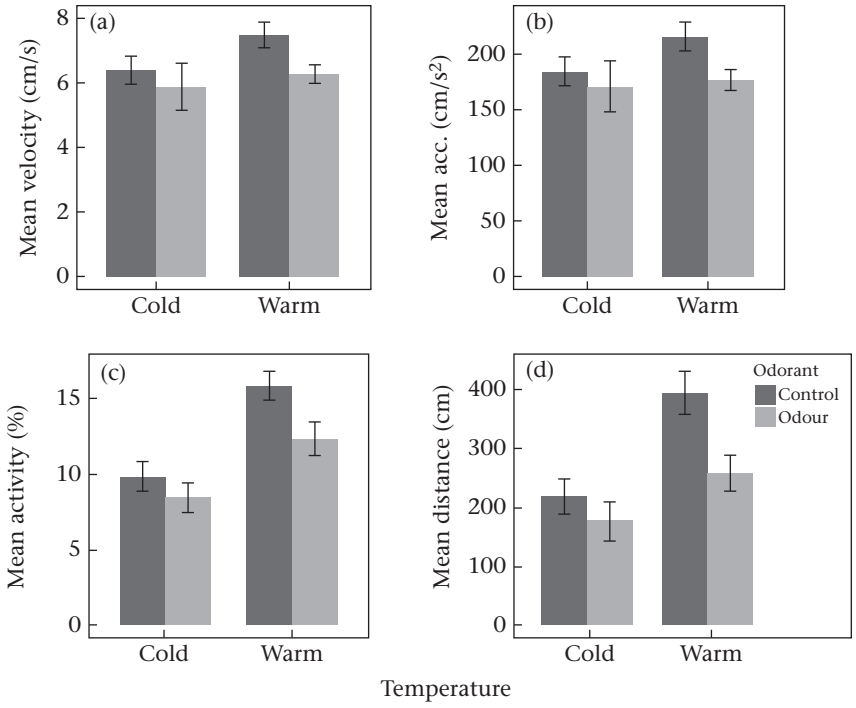

Figure 8. Mean ± SE activity levels yielded by Loligo analysis of larval movement. Six larvae were tracked in a 6-inch (15.24 cm) petri dish for 5 min during exposure to either alarm odour (made from sturgeon larvae homogenate) or control (water).

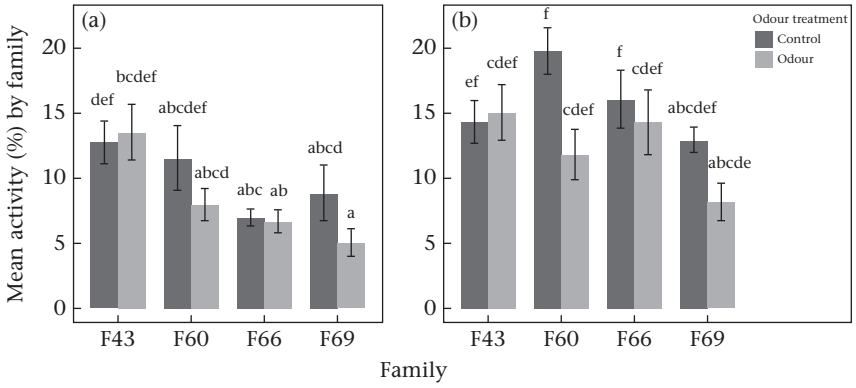

Figure 9. Mean ± SE activity levels (%) by family for trials with no odour added (control) and with odour for the (a) cold and (b) warm treatments. Letters indicate significant differences between families based on post hoc Tukey results.

Table 7
Models for survival

Model	AICc	ΔAICc	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

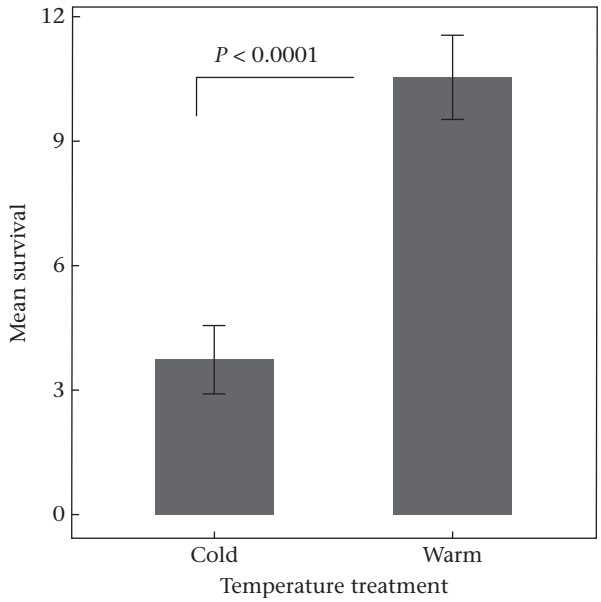

Figure 10. Mean ± SE number of surviving larvae after 5 h of exposure to a crayfish predator. *P* value from chi-square test.

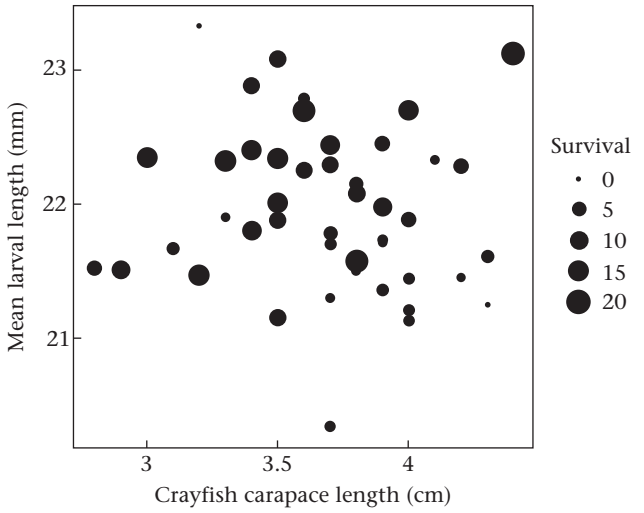

Figure 12. Larval survival after crayfish encounter as a function of mean larval length and crayfish carapace length. Dot size indicates number of surviving larvae (out of 20) after 5 h of exposure to a crayfish predator.

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 long-term stress-related behavioural 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 behavioural outcomes of stress may be species specific.

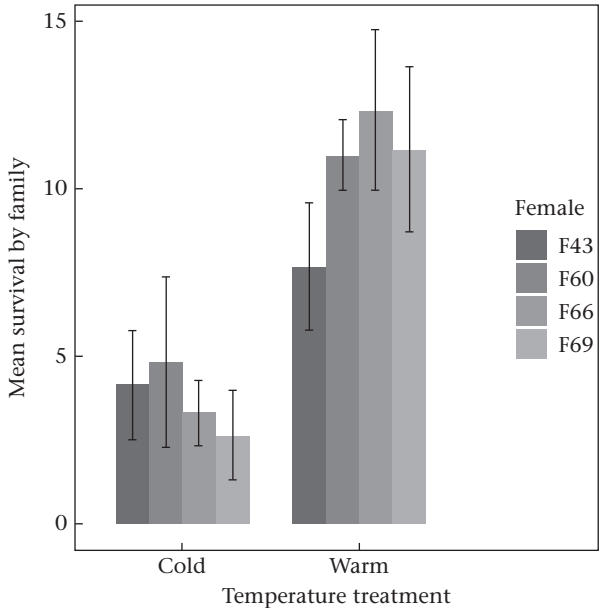

Figure 11. Mean ± SE number of survivors in each family during predation trials. Surviving larvae are out of 20 larvae total after 5 h of exposure to a crayfish predator.

Behavioural 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 behavioural response to early life stress. Larger mean size may also have contributed to higher survival rates for larvae reared at 18 °C in the presence of a crayfish predator, since larger body size has been associated with lower predation rates in lake sturgeon (Wishingrad, Ferrari et al., 2014; Wishingrad, Sloychuk et al., 2014). Note, however, that predation trials were conducted at ambient stream temperature (17 °C), which was closer to the warm temperature treatment (18 °C) than to the cold temperature treatment (10 °C), 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 behavioural 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, Hinch, Middleton, Hills, & Patterson, 2014), and different stressors experienced by parents may have influenced offspring physiological and behavioural 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, McGowan, & Sheriff, 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 behavioural 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, Runge, & Sherman, 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, Waples, Schindler, Hard, & Kinnison, 2010; Woodward, Perkins, & Brown, 2010). Therefore, this study highlights the importance of understanding responses to environmental stressors within contexts that can predict fitness.

Declarations of Interest

None.

Acknowledgments

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Appendix

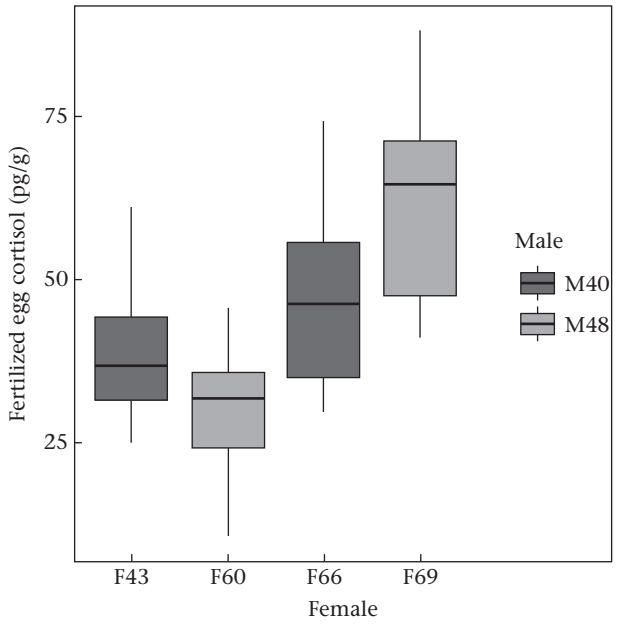

Figure A1. Cortisol data set in original scale for fertilized eggs, showing influence of maternity and paternity.

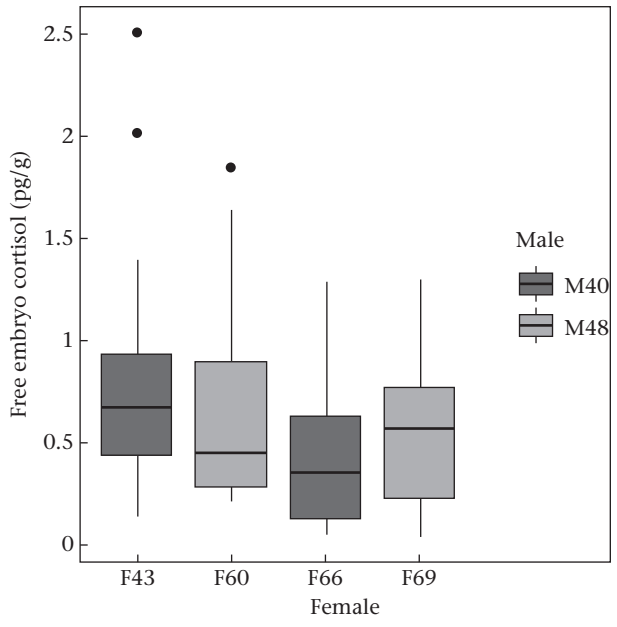

Figure A2. Cortisol data set in original scale for free embryos, showing influence of maternity and paternity.

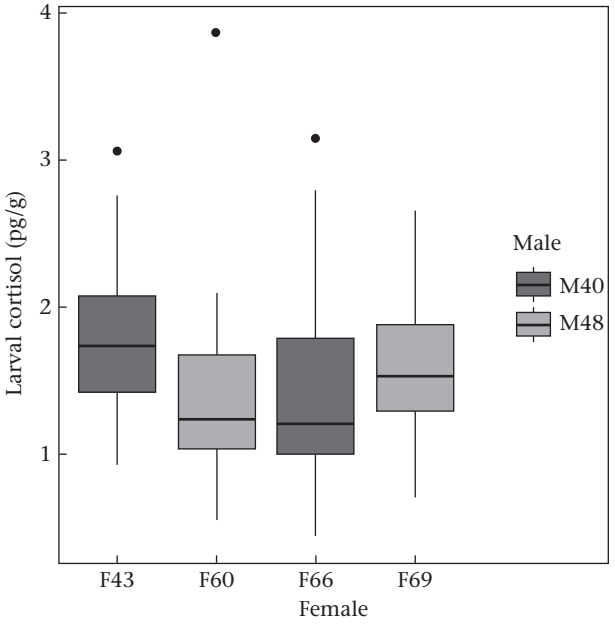

Figure A3. Cortisol data set in original scale for larvae, showing influence of maternity and paternity.