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RESEARCH ARTICLE

Elevated temperatures dampen the innate immune capacity of developing lake sturgeon (*Acipenser fulvescens*)

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ABSTRACT

Chronic exposure to high temperatures may leave freshwater fishes vulnerable to opportunistic pathogens, particularly during early life stages. Lake sturgeon, *Acipenser fulvescens*, populations within the northern expanse of their range in Manitoba, Canada, may be susceptible to high temperature stress and pathogenic infection. We acclimated developing lake sturgeon for 22 days to two ecologically relevant, summer temperatures (16 and 20°C). Individuals from both acclimation treatments were then exposed to 0, 30 and 60 µg ml^{-1} bacterial lipopolysaccharides (endotoxins), as an immune stimulus, for 48 h and sampled 4 and 48 h during trial exposures and following a 7 day recovery period. We then measured whole-body transcriptional (mRNA) responses involved in the innate immune, stress and fatty acid responses following acute exposure to the bacterial endotoxins. Data revealed that overall levels of mRNA transcript abundance were higher in 20°C-reared sturgeon under control conditions. However, following exposure to a bacterial stimulus, lake sturgeon acclimated to 16°C produced a more robust and persistent transcriptional response with higher mRNA transcript abundance across innate immune, stress and fatty acid responses than their 20°C-acclimated counterparts. Additional whole-animal performance metrics (critical thermal maximum, metabolic rate, cortisol concentration and whole-body and mucosal lysozyme activity) demonstrated acclimation-specific responses, indicating compromised metabolic, stress and enzymatic capacity following the initiation of immune-related responses. Our study showed that acclimation to 20°C during early development impaired the immune capacity of developing lake sturgeon as well as the activation of molecular pathways involved in the immune, stress and fatty acid responses. The present study highlights the effects of ecologically relevant, chronic thermal stress on seasonal pathogen susceptibility in this endangered species.

KEY WORDS: Innate immunity, Sturgeon, Conservation, Lipopolysaccharides, Environmental stress

INTRODUCTION

Globally, temperature change, flow alteration and extreme weather events have led to decreased productivity and imperiled fish populations in freshwater systems (Milly et al., 2008; Reid et al., 2019; Dudgeon, 2019). In response to environmental changes, fishes may show plasticity to alterations in their thermal environment (Earhart et al., 2022); however, this phenotypic plasticity may potentially decrease their ability to compensate during other physiological challenges. Additional stressors such as the effects of pathogenic infection may shape developmental and evolutionary trajectories for population and species dynamics as phenotypes change and mortality occurs (Dittmar et al., 2014; Schade et al., 2014; Strowbridge et al., 2021). These environmental changes are often accompanied by alterations in pathogen populations, abundance and virulence, which could leave freshwater species more vulnerable to the compounding stressors of temperature and disease (Marcos-López et al., 2010; Paull and Johnson, 2011; Dittmar et al., 2014; Miller et al., 2014). However, until recently, little research has focused on the effects of these combined stressors under laboratory conditions, especially in species of conservation concern (Bugg et al., 2021a). As host–pathogen–environment interactions may determine survival outcomes for fish in wild environments (Jeffries et al., 2014; Teffer et al., 2022), it is important to study the interactions of these stressors in fishes of conservation concern.

Sturgeons are members of the most critically endangered group of animals on the planet (IUCN, 2022) and are often reared in hatcheries for subsequent release to enhance wild populations. Recent research has indicated that in both wild and hatchery environments, sturgeons are susceptible to a variety of fungal, viral and bacterial pathogens (Coleman et al., 2018; Fujimoto et al., 2018; Mugetti et al., 2020; Brocca et al., 2022; Costábile et al., 2022; Stilwell et al., 2022; Soto et al., 2022) with the potential for thermal stress to compromise immune capacity, as revealed by full transcriptome studies (Penny et al., 2023; Bugg et al., 2023 preprint). This may be exacerbated among populations in the northern range for these species, such as lake sturgeon (*Acipenser fulvescens*) in Manitoba, Canada, as they may be more susceptible to the effects of increasing temperatures and inhabit areas where temperatures are projected to increase by 2.1–3.4°C by 2050 (Manitoba Hydro, 2015). Currently, temperatures rise above 20°C during the summer (May to October) for many rivers throughout Manitoba, a critical period for early development in lake sturgeon (Bugg et al., 2020, 2021b).

Phenotypic development during early life history is a critical period involving a remarkable shift of biological function, organ development, narrowing energy reserves and often high levels of mortality (Sifa and Mathias, 1987; Wieser, 1991; Rombough, 1994). During this period, fish (and particularly sturgeons) must cope with environmental conditions due to limited swimming ability (Kopf et al., 2014; Brandt et al., 2021), making them particularly vulnerable to environmental perturbations, especially changes in temperature. Additionally, at hatch and during early development, many fishes have a limited adaptive immune response, leaving them reliant on their innate immune systems until later life stages (Chantanachookhin et al., 1991; Petrie-Hanson

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and Ainsworth, 2001; Magnadottir et al., 2006; Reyes-López et al., 2018). Thus, with limited mobility and immune capacity, exposure to thermal stress may compromise the defenses of developing larval freshwater species and leave them susceptible to the effects of opportunistic viral, bacterial and fungal pathogens, which are pervasive throughout freshwater ecosystems. Sturgeons are likely even more susceptible to opportunistic pathogenic infections in early development as their adaptive immune development is slower than that of many other fish species (Gradil et al., 2014a,b). While previous research has demonstrated that prolonged periods at temperatures of 20°C and above can be thermally stressful and have negative physiological consequences for developing lake sturgeon (Bugg et al., 2020; Bugg et al., 2023 preprint), there has been little evaluation of the immune capabilities of this species in early development or the immune capacity of sturgeons under thermal stress (Bugg et al., 2021a). Ultimately, in this critical period of early development, the ability to functionally sustain organismal responses against multiple stressors likely determines survival (Dittmar et al., 2014), and may influence population-level outcomes for northern sturgeon.

Innate immunity relies on two specific mechanisms to respond to pathogens: the detection of the immune stimulus (pathogen detection), resulting in an intracellular immune-stimulating cascade, and the subsequent transcriptional initiation, activating the production of immune-related compounds (immune response). The ability of the innate immune system to detect pathogens largely relies on pattern recognition receptors, such as toll-like receptor 4 (TLR4), which detects gram-negative bacteria by their outer lipopolysaccharide (LPS) structure and other associated peptidoglycans (Magnadottir, 2006; Amarante-Mendes et al., 2018). Once activated, TLR4 can respond through two different activation pathways: myeloid differentiation primary response 88 (MyD88)-dependent activation or toll-like receptor adaptor molecule 1 (TICAM-1) signaling, both of which induce the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) to translocate into the nucleus and initiate the transcription of mRNAs coding for pro-inflammatory cytokines and other immune compounds (Fig. 1A; Vaure and Liu, 2014; Srivastava et al., 2017; Amarante-Mendes et al., 2018). Immune-responsive proteins such as cytokines (TNFα, IL-1β and IL-8, involved in immune signaling, the inflammation response and macrophage recruitment; Turnbull and Rivier, 1999), antibodies (IgM; Lobo, 2016; Yu et al., 2020), complement activators (C3; Holland and Lambris, 2002) and enzymes (lysozyme; Saurabh and Sahoo, 2008) initiate a variety of immunomodulatory activities to suppress and destroy bacteria both inside and outside the body, but can also have detrimental impacts on survival if overexpressed (Anderberg et al., 2021). Additionally, the activation of innate immune responses can further induce changes in long-chain fatty acids (Fig. 1B) either through mitochondrial β-oxidation, limited by the mitochondrial transporter carnitine palmitoyltransferase I (CPT1; Coccia et al., 2014; Norambuena et al., 2015), or through the formation of immune precursors via cytokine-induced phospholipase A2 (PLA2)-mediated cleavage (Okamura et al., 2021; Nguyen et al., 2022). However, many of these innate immune mechanisms may be ultimately compromised by the effects of chronic thermal stress, associated allostatic load (i.e. physiological stress caused by abiotic or biotic environmental factors; Samaras et al., 2018), and the energetic costs related to elevation of the stress response (Schreck, 2010; Schreck and Tort, 2016).

Under environmental conditions with limited allostatic load, infection, triggering of the immune response and the detection of circulating cytokines by the hypothalamus can stimulate the hypothalamic-pituitary–interrenal (HPI) axis with an increased release of adrenocorticotropin (ACTH) from the pituitary, which ultimately increases the production of cortisol (Shintani et al., 1995;

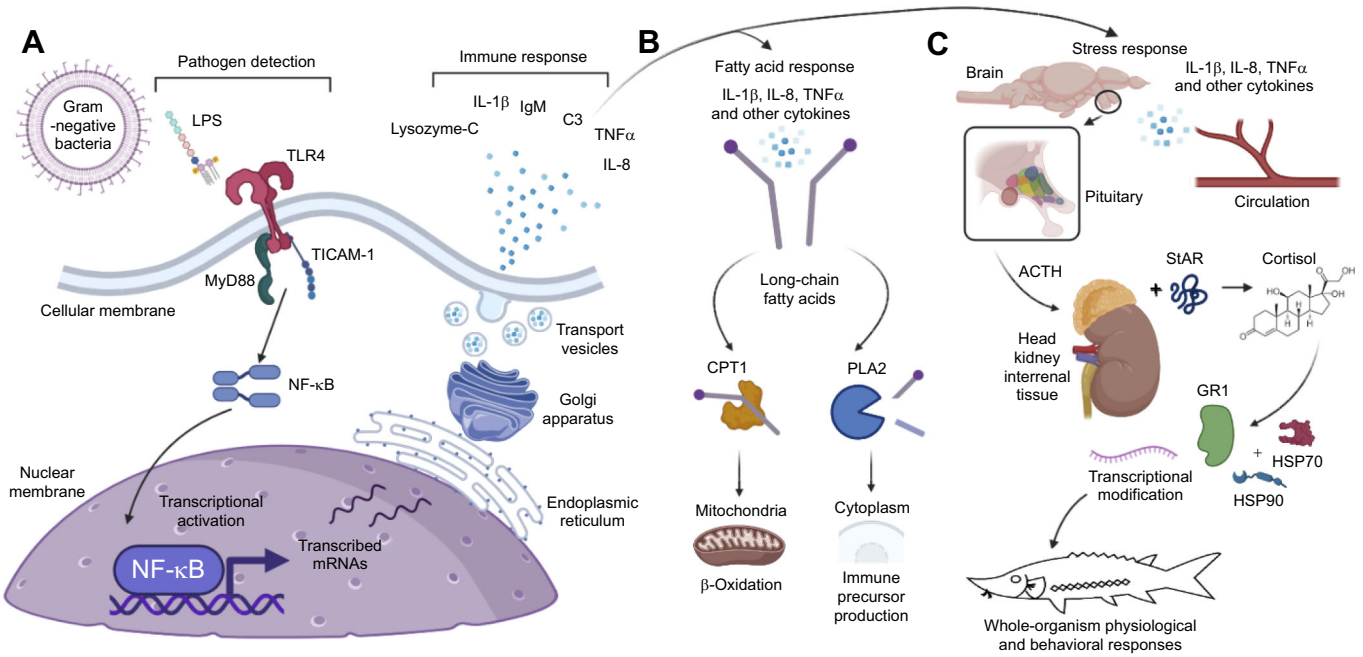

Fig. 1. Pathogen response in developing lake sturgeon, *Acipenser fulvescens*. Hypothetical pathogen detection, transcriptional activation and immune responses to bacterial lipopolysaccharides (LPS) (A) as well as stimulation of the fatty acid response (B) and stress response (C; picture credit: Miri E. Seo) via circulating cytokines. Note, StAR and cortisol production take place in the interrenal tissue of the head kidney of fish; pictured here is a mammalian kidney for graphical demonstration. The figure shows some hypothesized proteins and pathways that may influence the represented responses; however, it is not an exhaustive representation of all possible factors that could be potentially impacting these responses, and many, if not all, of these proteins have multiple biological roles. The components of this figure were created with BioRender.com and then compiled in Inkscape.

Turnbull and Rivier, 1999; Fig. 1C). This synthesis of cortisol is rate limited by steroidogenic acute regulatory protein (StAR; Stocco et al., 2005). Once cortisol enters the circulation and then cells, cytosolic glucocorticoid receptors (GRs) can then bind to it with the help of molecular chaperones and members of the general stress response (HSP70 and HSP90) (Hutchinson et al., 1994; Bamberger et al., 1996; Bekhbat et al., 2017). This complex acts as a transcription factor, transcriptionally regulating further cellular signaling mechanisms and physiological responses critical to the maintenance of homeostasis and organismal survival (Marchi and van Eeden, 2021). However, if stressful conditions are present before pathogen exposure, sub-lethal thermal thresholds may be exceeded, increasing GRs and cortisol levels. In these situations, increased GRs and cortisol levels may interfere with immune-stimulating transcription factors such as NF-κB (Bekhbat et al., 2017; Jeffries et al., 2018). Ultimately, this may result in the inactivation of the cytokine response, immunosuppression and subsequent infection by opportunistic pathogens (Tort, 2011; Alfonso et al., 2021; Aversa-Marnai et al., 2022), to which fish at early life stages are particularly vulnerable.

The goal of this study was to investigate the innate immune capacity of developing lake sturgeon when exposed to bacterial LPS under thermal stress. Using a transcriptional profiling approach (Jeffrey et al., 2020), we targeted genes involved in the innate immune, stress and fatty acid pathways responsive to bacterial infection. This strategy was then paired with metrics to assess the immune responses and physiology of developing lake sturgeon exposed to bacterial LPS following acclimation to ecologically relevant temperatures of 16 and 20°C. We tested the hypothesis that chronic thermal stress will impact energetic balance, transcriptional plasticity and whole-animal physiology, and that it will specifically alter the activation of the innate immune system and related processes. Thus, we predicted that a heightened stress response associated with acclimation to increased temperatures would reduce energetic stores and elevate glucocorticoid (cortisol) and receptor levels (GR1), impairing the transcriptional activation of the innate immune system and downstream processes following exposure to bacterial LPS. We also predicted that the immune responses of 16°C-acclimated sturgeon would involve a greater transcriptional response than their 20°C-acclimated counterparts, following exposure to the bacterial stimuli. Finally, we predicted that these compounding impacts from both elevated temperature and immune stimulus would extend to measurements of lysozyme activity and whole-organism physiology [hepatosomatic index (HSI), critical thermal maximum (CT_{max}) and metabolic rates].

MATERIALS AND METHODS

Lake sturgeon husbandry

In May 2021, gametes from wild-caught female and male lake sturgeon (*Acipenser fulvescens* Rafinesque 1817) were harvested from individuals at the Pointe du Bois Generating Station on Winnipeg River, MB, Canada (50°17′52″N, 95°32′51″W). Once collected, eggs and sperm were transported to the University of Manitoba animal holding facility. Immediately upon arrival, fertilization was carried out using the eggs from three females and the diluted sperm from six males to produce three maternal families. After 1 min of fertilization, embryos were washed 3 times with dechlorinated water and immediately placed on mesh egg mats. Once the eggs had adhered to the egg mats, flow of well-oxygenated water at 12°C was applied over them (Earhart et al., 2020b).

Post-hatch, larvae were transferred to three 9 l flow-through aquaria with aeration and bio-balls as substrate, with each maternal family initially reared in a separate tank. Starting at 13 days post-fertilization (dpf), the rearing temperature was increased by 1°C day^{-1} until 16°C. Once temperatures reached 16°C at 17 dpf, developing sturgeon were transferred into five replicate 9 l flow-through aquaria. Each aquarium had 100 sturgeon larvae from each maternal family (n=300 sturgeon per replicate tank to reduce stress and mortality by decreasing rearing density; Aidos et al., 2020). Beginning at 19 dpf, freshly hatched *Artemia* (Artemia International LLC, Fairview, TX, USA) were provided as a starting diet before yolk sac absorption had been completed, and tank substrate was removed over a 7 day period (Earhart et al., 2020a). Lake sturgeon were fed freshly hatched *Artemia* to satiation 3 times daily until LPS challenges began (59–63 dpf).

At 33 dpf, acclimation began by reducing the stocking density of sturgeon further. For each acclimation temperature (16 and 20°C), four replicate 9 l tanks were filled with approximately 170 sturgeon each, evenly distributed from the five replicate initial rearing tanks for a total of 680 sturgeon per acclimation temperature. Sturgeon remained in these tanks until they were moved to the LPS trial experimental setup at 52 and 56 dpf for 20 and 16°C treatments, respectively. Throughout initial rearing and acclimation, mortality and rearing temperature was monitored at least twice daily. All animals in this study were reared and sampled under guidelines established by the Canadian Council for Animal Care and approved by the animal care committee at the University of Manitoba under protocol #F15-007.

Acclimation

Acclimation and experimental trials were staggered by 4 days to make accumulated thermal exposure similar across the two treatment groups (degree days at the beginning of the trials: 16°C, 950; 20°C, 980; a difference of approximate 2 developmental days at 16°C). In addition to temperature acclimation, developing lake sturgeon were also acclimated to the environment of the LPS trial experimental tanks for 1 week prior to experimentation (beginning at 56 and 52 dpf for 16 and 20°C treatments, respectively), to avoid possible stress-related effects of handling and transfer (Bugg et al., 2021a). Acclimation for the 20°C treatment began at 34 dpf, increasing the temperature 1°C day^{-1} until 20°C was reached at 37 dpf; this temperature was maintained until the end of the study. LPS trials began 22 days later (59 dpf; Fig. 2), with a 48 h LPS exposure (61 dpf) and ended following the 7 day recovery period (68 dpf). For sturgeon acclimated to 16°C, this temperature was maintained from 16 dpf until the beginning of the LPS trials (63 dpf), throughout the 48 h trial (65 dpf), and during the 7 day recovery period (72 dpf).

Each LPS trial experimental tank was 30×25×7 cm L×W×H with drainage holes cut into the side at the 3.3 l volume mark to allow for water to flow out of the unit during acclimation. A total of approximately 75 sturgeon (selected from the four acclimation tanks) were stocked into each of six experimental tanks for each acclimation temperature (16 and 20°C), with duplication for each of the LPS treatments (i.e. two replicate tanks for each concentration of 0, 30 and 60 μg ml^{-1} LPS for each temperature, n=12 tanks total). Throughout experimental tank acclimation, developing sturgeon were provided with flow-through water and aeration. During the first 6 days of acclimation, sturgeon were fed *Artemia* to satiation, and were fasted for 24 h before the initiation of LPS trials. Throughout acclimation and LPS trials, water temperature was recorded every 15 min by HOBO Water Temperature Pro v2 Data Loggers (Onset Computer Corporation, Bourne, MA, USA).

LPS trials and sampling

The LPS trials conducted on developing lake sturgeon were based on previously established protocols (Dalmo et al., 2000; Novoa

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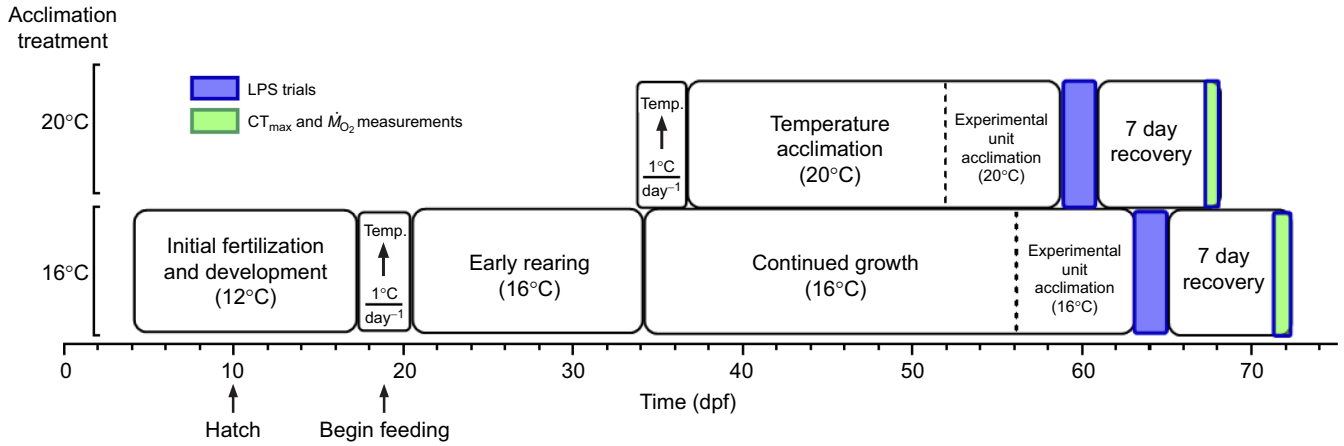

Fig. 2. Experimental design and time line. Fertilization, early rearing, acclimation, LPS exposure and measurement of critical thermal maximum (CT_{max}) and metabolic rates ($\dot{M}_{O_2}$) following a 7 day recovery. All time periods are given as days post-fertilization (dpf).

et al., 2009; Bugg et al., 2021a). Developing lake sturgeon were exposed to concentrations of 0, 30 and 60 µg ml^{-1} of LPS from *Pseudomonas aeruginosa* (extracted to >97% purity by phenol extraction; Sigma-Aldrich, St Louis, MO, USA) for 48 h. Each tank was dosed once with the designated concentration of LPS diluted with ultrapure water. Following introduction of LPS into the experimental tanks, each tank was monitored every 15 min for the first 8 h and then at least every 2 h for the following 40 h. As flow-through water was removed for the duration of the trials, ammonia was also measured every 6 h to monitor any potential accumulation and found to be below 1 mg l^{-1} in all tanks throughout the study. Following the 48 h LPS exposure, flow-through water was then returned, and fish remained in these flow-through units for 7 days to recover from their LPS exposure.

Throughout the experiment, sturgeon were sampled at 4 h and 48 h during LPS exposure, as well as following 7 days of recovery post-LPS exposure. At these time points, 5 developing lake sturgeon from each replicate experimental tank were euthanized by immersion in 250 mg l^{-1} solution of MS-222 buffered with an equal amount of sodium bicarbonate. Body mass (to 0.0001 g) and length (measured to the nearest 1 mm) were quickly taken from each fish and then whole sturgeon were immediately flash frozen in liquid nitrogen, after which they were stored at −80°C until processing for measurement of mRNA transcript abundance, cortisol concentration and lysozyme activity. Additionally, throughout the duration of the trials, moribund fish were removed and euthanized by immersion in an overdose of MS-222, and their body mass (in g), length (in cm) and liver wet mass (in g) were recorded. These measurements were then used to calculate Fulton's condition factor (K; Fulton, 1911) for each sampled fish:

$$K = \frac{\text{Body mass}}{\text{Total length}^3} \times 100, \quad (1)$$

as well as condition factor and HSI for each individual mortality as follows:

$$\text{HSI} = \frac{\text{Liver mass}}{\text{Body mass}} \times 100. \quad (2)$$

At the beginning of LPS trials, 20 sturgeon from each acclimation treatment were sampled directly from acclimation tanks, taking measurements of body mass, length and liver mass as described above, 10 for measurement of energy density and 10 for preservation of whole-body samples to be used in analysis of mRNA transcript abundance and additional physiological metrics as described above. Whole-body energy density was estimated from the dry-to-wet mass ratio of sample sturgeon using a previously established model (Yoon et al., 2019). These samples represent sturgeon that experienced thermal acclimation but did not go through the experimental unit acclimation and will be henceforth referred to as negative controls. In contrast, sturgeon that went through both thermal and experimental unit acclimation, were exposed to 0 µg ml^{-1} LPS at the beginning of LPS trials, and were sampled 4 h, 48 h and after a 7 day recovery period, will be referred to as handling controls.

An additional 40 fish from each treatment with surviving fish (20 replicates per experimental tank) were sampled pre-trial, at the end of the 48 h trial and 7 days post-trial during recovery for collection of skin mucus samples. Sturgeon were euthanized as described above, carefully patted dry to remove excess water, and then mucus was collected using Puritan PurFlock Ultra Flocked swabs (Puritan, Guilford, ME, USA). Prior to collecting the sample, each swab was individually weighed (to 0.0001 g). Swabs were then firmly rubbed across the dorsal and ventral surfaces of each sturgeon with the mucus from four sturgeon collected with one sample swab in order to amass an adequate mucus sample for detection of lysozyme activity based on results from preliminary trials (samples >0.25 mg performed well for enzyme detection). Following mucus collection, each swab was reweighed to determine the amount of mucus collected in the sample. Next, the stem of the swab was snipped off and the head of the swab was place in a CryoELITE cryogenic vial (DWK Life Sciences, Millville, NJ, USA) and immediately flash frozen in liquid nitrogen, after which it was stored at −80°C until analysis for lysozyme activity.

Post-sampling processing

Whole-body samples of sturgeon collected both prior to and during LPS trials were homogenized to measure the mRNA transcript abundance, cortisol concentration and lysozyme activity from each individual sturgeon sampled. Each whole fish was individually homogenized using a pestle and mortar in liquid nitrogen. All homogenized samples were then returned to storage at −80°C until further analysis.

Primer design

Primers were designed to target genes involved in the innate immune, stress and fatty acid responses of lake sturgeon to the

combined effects of elevated temperature and bacterial infection (Table 1). Many primers were sourced from previous studies conducted on the lake sturgeon immune response (*MyD88*, *IL-1β*; Bugg et al., 2021a), stress response (*StAR*, *GR1*, *HSP70*, *HSP90a*; Bugg et al., 2020; Earhart et al., 2020a) and fatty acid metabolism response (*PLA2*, *CPT1*; Yoon et al., 2022). Additionally, primers for other targets in immune-responsive pathways (*TLR4*, *TICAM-1*, *NF-κB*, *TNFα*, *IL-8*, *C3*, *Lysozyme-C*, *IgM*) and potential reference genes (*RPL13a*, *eEF1A1* and *RPL4*) were designed from lake sturgeon (Thorstensen et al., 2022 preprint) and white sturgeon, *Acipenser transmontanus* (Doering et al., 2016), tissue-specific transcriptomes. All results from transcriptome searches were aligned against publicly available transcripts using NCBI BLAST (Johnson et al., 2008), with primers designed over conserved regions between the query and the search result(s). Original transcript sources, results from related species with highly conserved regions, percentage identities and accession numbers for each publicly available transcript from NCBI BLAST results are listed in the Supplementary Materials and Methods.

RNA extraction, cDNA synthesis and qPCR

Total RNA was extracted from the whole-body homogenates of developing lake sturgeon from all treatment groups using RNeasy Plus Mini Prep Kits (Qiagen, Germantown, MD, USA) following the manufacturer's instructions. Whole-body homogenates were additionally homogenized in 500 µl of lysis buffer for 5 min at 50 Hz using a TissueLyser II (Qiagen). Total concentration, integrity and purity of RNA for each sample were assessed using a Nanodrop One (Thermo Fisher Scientific, Waltham, MA, USA) and gel electrophoresis. Synthesis of cDNA was conducted using a SuperScript IV First-Strand Synthesis System with ezDNase Enzyme (Quantbio, Beverly, MA, USA) from 500 ng of total RNA following the manufacturer's instructions. Genomic DNA was first removed using 1 µl of ezDNAse Enzyme prior to cDNA synthesis. Synthesis of cDNA was then performed using 1 µl of 50 ng µl^{-1} random hexamers to anneal to the template RNA, followed by reverse transcription using 1 µl of SuperScript IV Reverse Transcriptase. Synthesis was conducted using a SimpliAmp Thermal Cycler (Thermo Fisher Scientific) with cycling conditions of one cycle of 23°C for 10 min, one cycle of 55°C for 10 min and one cycle of 88°C for 10 min, with a final hold at 4°C.

Real-time quantitative polymerase chain reactions (qPCR) for each gene of interest (*TLR4*, *MyD88*, *TICAM-1*, *NF-κB*, *TNFα*, *IL-8*, *IL-1β*, *C3*, *Lysozyme-C*, *IgM*, *StAR*, *GR1*, *HSP70*, *HSP90a*, *PLA2* and *CPT1*) and potential reference genes (*RPL13a*, *eEF1A1*, *RPL7*, *RPS6*, *β-actin* and *RPL4*) were conducted using 5 µl of PowerUp SYBR Green Master Mix (Applied Biosystems, Bedford, MA, USA), 0.1–0.025 µl of each 100 µmol l^{-1} forward and reverse primer, 5–10 µl 1:10 nuclease-free water-diluted cDNA per well, with additional nuclease-free water adjusted for each assay to bring the total volume of each well to 12–16 µl based on the amount of cDNA included. Assays for potential reference genes *RPS6* and *RPL7* included 0.1 µl each of forward and reverse primer per well, while all other assays included 0.025 µl each of forward and reverse primers per well. All assays used 5 µl 1:10 diluted cDNA per well and had a total reaction volume of 12 µl except for the *StAR* assay, which used 10 µl of 1:10 diluted cDNA per well and a total volume of 16 µl.

As potential reference genes were not stable across acclimation treatments and LPS exposure concentrations, NORMA-Gene, a robust method for qPCR normalization based on the mRNA transcript abundance of target genes, was used for normalization of mRNA transcript abundance, inputting the abundance of all 22 assayed genes to produce the lowest theoretical variance in normalization (Heckmann et al., 2011). Post-normalization, abundance was then analyzed after applying the $2^{-\Delta\Delta Ct}$ method as described by Livak and Schmittgen (2001). The mRNA transcript abundance of all target genes was then normalized to the abundance of the pre-trial 16°C (negative) control group.

Table 1. Primer sequences for lake sturgeon, *Acipenser fulvescens*

Gene	Forward	Reverse	Efficiency (%)
TLR4	AAGCTGACGGTTGTGGATAC	GCTGTGCCAAGTGACTGATA	103.4
MyD88	CACATGCGTCACTGTCAAGG	AGCATCACCAGCGAACTCAT	96.0
TICAM-1	GAAGCTCGCTAGAAGGACATAC	GAGAAGGATGCTCTGAGAAATGA	101.0
NF-κB	CAGAGCTTGCACTACAGCCT	TGGGTTCACTCAATGGCAGG	92.1
TNFα	AGGAGCGGTCTCTACTTCGT	TGTGCGACAGATATACGGGC	94.4
IL-8	CAGGCAGATCCAGAATGTAGAG	CCAGATTTCAAAGTGGCAATGA	100.9
IL-1β	CACCAGCGAGATCTTTGACTT	GCTCATCTTGCGTTCTCTTCT	99.9
C3	AGGGCTCTCTCATCCTTTACT	CAGACCCACTTCAAACTCCTT	91.0
Lysozyme-C	CTGCCAAACTGGGTGTGTCT	TGTTGTGGTTCACTGCCTGA	98.3
IgM	GGTGTTTCTCCTCTCGCCAT	GTCAGGCTAACTTCCCCGTT	97.7
StAR	CCGAGCAAAAAGGCTTCA	TTGGGCCGAAGAACAATACAG	92.1
GR1	TTTGCAGCTCCCACATGTAA	TCTTGTGTGCTCGGATGAAG	96.2
HSP70	CGTTCACTCGGACTTTAACTTTAATTT	AACTGTCCTAAAGAACTGCCTTATCC	98.7
HSP90a	GATCACACGAGCGGATTTGC	ATGTTGTGCTCTGTCCTGCG	92.9
PLA2	GCGGGCACAGTTAATACCCA	CCCTAACCCACAGTAGCAGC	96.9
CPT1	CAGAAGAAAGCTGGACAGAGAG	CATACGCTCATACTGGGAAGTG	92.3
RPL13a	TGAAGTACCTTGCGTTCCTG	TCTCACTGTCCTCCAGAAGAT	96.2
eEF1a1	TCAAGTATGCCTGGGTGTTG	GAGGGAGATGTCAATGGTGATG	97.3
RPL7	TGCTTAGGATTGCTGAGCCG	GATCTTTCCGTGACCCCGTT	100.2
RPS6	CTGGCTGGATTCTGATTTGGATG	ATCTGATTATGCCAAGCTGCT	98.7
β-actin	GAAGTCCAGGGCGACATAGC	TGAAGATCCTGACCGAGCGA	99.8
RPL4	CCGAGGGAGTCCTAAGCGAA	GGAGCCTTGAAGACAGCAGG	92.1

Target genes (*TLR4* to *CPT1*) were selected based on their roles in innate immune, stress and fatty acid responses to pathogenic infection. Reference genes–ribosomal protein L13a (*RPL13a*), eukaryotic translation elongation factor 1 alpha 1 (*eEF1a1*), ribosomal protein L7 (*RPL7*), ribosomal protein s6 (*RPS6*), *β-actin* and ribosomal protein L4 (*RPL4*) – were chosen as candidates based on their stability in other pathogen-based experiments; however, in the current experiment, they were largely unstable under the compounding effects of both temperature and bacterial LPS.

Cortisol and lysozyme analysis

Cortisol was assayed in whole-body homogenates as previously described in lake sturgeon (Earhart et al., 2020a), while lysozyme assays were conducted using EnzChek™ Lysozyme Assay Kits as per the manufacturer's instructions (Invitrogen, Thermo Fisher Scientific; see Supplementary Materials and Methods for further details).

Post-trial metabolism and thermal tolerance

During the 7 day recovery from LPS trials, measurements of both the metabolic rates (routine and forced maximum) and CT_{max} of sturgeon surviving the trials were taken. Metabolic rates were measured 6 days post-trial, while the CT_{max} of the same fish were measured the following day, 7 days post-trial.

Whole-body metabolic rate ($\dot{M}_{O_2}$) was measured by intermittent flow respirometry (Loligo Systems, Viborg, Denmark) following previously established protocols with some modifications (Yoon et al., 2021). The respirometry system consisted of 16 borosilicate metabolic chambers with oxygen sensor spots (PreSens, Regensburg, Germany) in two water baths (400×225×140 mm L×W×H) on which fiber optic cables were situated in order to read oxygen saturation as percentage air saturation at 1 Hz using a Witrox 4 Oxygen Meter (Loligo Systems). Water temperature was regulated by room temperature while the respirometry system was connected to AutoResp 4.1 on a PC (Loligo Systems). The oxygen probe was calibrated with 0% (2% sodium sulfite) and 100% dissolved oxygen (fully air saturated). The tubing was non-oxygen permeable (Tygon). The volume of the chambers and tubing was 44.60 ±3.58 ml and 3.51±0.90 ml (mean±s.d.), respectively. The ratio between the metabolic chamber and fish mass was 80.8±22.7 (mean ±s.d.). Before the experiment, fish were fasted overnight (approximately 12 h), and at each sampling point, fish (n=8 per treatment) were haphazardly taken from rearing tanks and chased for 15 min to induce forced maximum metabolic rate (FMR). Then, fish were immediately placed into chambers, and $\dot{M}_{O_2}$ was measured for the next 6 h. A black curtain was hung around the respirometry chamber to minimize visual disturbance, while lights were on for the duration of the experiment.

The measurement cycle for FMR consisted of 60 s waiting and 300 s measurement; that for routine metabolic rate (RMR) consisted of 300 s flushing, 60 s waiting and 300 s measurement. $\dot{M}_{O_2}$ slopes with $r^2 \geq 0.9$ were used to ensure linearity in oxygen consumption (96.2% of all slopes; Chabot et al., 2021). Before each experiment, $\dot{M}_{O_2}$ was measured for 15 min without fish to assess background respiration, and background respiration data were used to linearly interpolate over each $\dot{M}_{O_2}$ recording session (Rogers et al., 2016). Then, all $\dot{M}_{O_2}$ data were corrected by subtracting all corresponding background respiration data. The average ratio between background respiration and RMR was 5.3±5.8% (mean±s.d.). Maximum metabolic rate (MMR) was chosen as the highest $\dot{M}_{O_2}$ during the first three measurements, whereas RMR was estimated by averaging $\dot{M}_{O_2}$ of the last 2 h, excluding the first 4 h of acclimation. Because we measured RMR, we chose to report the difference ($\Delta\dot{M}_{O_2}$) and ratio (MMR/RMR) between routine and maximum metabolic rate as metabolic scope, both of which are analogous to absolute and factorial aerobic scope in the literature (Halsey et al., 2018).

After measurement of metabolic rates, CT_{max} was measured following previously established protocols with some modification (Bugg et al., 2020). At the end of metabolic rate measurements, the 8 sturgeon were transferred from metabolic chambers directly into the CT_{max} arena by placing them into individually labeled experimental units (9.5×5×4 cm L×W×H) with mesh-screened sides to allow water to flow through each unit; Yusishen et al., 2020) and well-oxygenated circulating water held at the respective acclimation temperature. An additional 16 fish, for which metabolic rate had not been measured, were added to each CT_{max} trial for a total of 24 fish per trial. The position of all fish in the experimental units in the CT_{max} arena was assigned via a random number generator. Temperature in the recirculating water bath was heated by approximately 0.3°C min^{-1} using a Isotemp recirculating heater (Fisher Scientific, Hampton, NH, USA) until fish were unable to right themselves after a physical disturbance. When sturgeon were unable to right themselves, the final CT_{max} temperature was recorded, the fish was euthanized, and mass and length measurements were recorded as described above.

Statistical analysis

Physiological data collected throughout acclimation, including body mass, length, condition factor, HSI and energy density, were analyzed using a two-factor ANOVA including acclimation treatment and developmental time (dpf) in the model as main effects. Importantly, our initial data analysis indicated that rearing tank did not influence the results and thus it was removed from the analysis.

Differences in mortality between acclimation treatments and LPS concentrations throughout trials were assessed using Cox proportional hazards models using the 'survival' and 'survminer' R packages (https://CRAN.R-project.org/package=survival; https://cran.r-project.org/web/packages/survminer/index.html), with covariates of both acclimation treatment and LPS concentration included in the model. A pairwise comparison was then conducted to compare mortality across both concentrations and acclimation temperatures, using the 'pairwise_survdiff' function in the 'survminer' package, as well as a Bonferroni correction to correct for the effects of multiple comparisons. Assumptions of the hazard model was evaluated using the 'cox.zph' function in the 'survival' package. Data collected from each mortality (body mass, total length, condition factor, HSI) were analyzed to determine whether there was a relationship with time to mortality using a Spearman's correlation to identify which physiological metrics were most indicative of time to survival in sturgeon exposed to LPS. Mortalities were only apparent in the 20°C acclimation treatment exposed to 60 µg ml^{-1} LPS; thus, these data are representative of mortality under these conditions (n=142).

The mRNA transcript abundance of all target genes, as well as whole-body cortisol, lysozyme and mucosal lysozyme activity, were analyzed using three-factor ANOVA to investigate changes in mRNA transcript abundance across treatments; this included acclimation treatment, LPS concentration and time in the model as main effects. A subset of the total mRNA transcript abundance data, including negative control samples following acclimation, 48 h exposure to 30 µg ml^{-1} LPS and a 7 day recovery, was then analyzed using two-factor ANOVA with acclimation treatment and gene as fixed effects in the model to focus on the sub-lethal tolerance thresholds of LPS exposure.

Principal component analysis (PCA) was conducted using the 'factomineR' (Le et al., 2008) and 'factoextra' (https://cran.r-project.org/package=factoextra) packages in R, including the subset of data with negative control samples following acclimation, 48 h exposure to 30 µg ml^{-1} LPS and a 7 day recovery (n=48). Contributions and vector directions of the variance in mRNA transcript abundance observed in PCA were illustrated using a contributions plot and variable plot, with only target genes that exceeded the expected average contributing towards variance from the overall PCA included.

Semi-partial Spearman's correlation was used to investigate the relationship between the mRNA transcript abundance of each gene and that of other studied genes of sturgeon sampled during LPS

trials, using the 'ppcor' package (Kim, 2015). This analysis was used to control for the effect of both LPS concentration and exposure time during calculation of Spearman's correlation coefficients and was conducted individually for each acclimation temperature (16 and 20°C, n=80 and 61, respectively) to highlight the differences in mRNA transcript abundance relationships between the two acclimation treatments. The difference between the two values between acclimation treatments was then calculated (ρ16°C−ρ20°C) and is presented as delta rho (Δρ). All values are reported as estimated Spearman's rho (ρ).

Metabolic rate and CT_{max} of developing lake sturgeon during recovery from LPS exposure were analyzed with two-factor ANOVA, including both LPS concentration and acclimation temperature as well as their interactions in the model as fixed effects. Additionally, correlative relationships between the CT_{max} of each individual sturgeon and each metabolic metric were analyzed using Spearman's correlations. For all ANOVA Shapiro–Wilk's and Levene's tests were used to assess normality of data and homogeneity of variance along with graphical investigations. If assumptions of either normality or homogeneity were violated, a ranked, log or square root transformation was applied to the dataset. Following evaluation of main and interactive effects, *post hoc* tests were performed with Tukey's HSD tests from the 'multcomp' package (Hothorn et al., 2008). All statistical analyses were performed using R 4.0.0 (http://www.R-project.org/), with a significance level (α) of 0.05.

RESULTS

Effects of acclimation temperature

Throughout acclimation, sturgeon reared at 20°C were larger than their 16°C counterparts with an interaction of both temperature and time (P<0.005). However, sturgeon from the two acclimation treatments had similar condition and energy density, with an effect of time on both metrics (P<0.0001). Following 14 days of acclimation, 20°C-acclimated sturgeon had lower hepatosomatic indices than 16°C-acclimated sturgeon, and this effect persisted throughout acclimation (Temperature:Time interaction, P<0.005). Physiological data are shown in Fig. S1.

In-trial mortality and physiological relationships

During trials, there was complete mortality (100%) in the 20°C-acclimated, 60 µg ml^{-1} LPS treatment group. Additionally, there were 2 mortalities (1.3%) in the 16°C-acclimated, 60 µg ml^{-1} LPS treatment group. There were no mortalities in any of the other treatment groups.

Physiological metrics of body mass, length and HSI demonstrated significant correlations with the time to mortality of 20°C-acclimated sturgeon exposed to 60 µg ml^{-1} LPS (P<0.005), in which all sturgeon perished. Condition factor (K) demonstrated no significant relationship (P>0.1), while the metric with the strongest correlation with time to mortality was HSI (ρ=0.36; P<0.0001). Body mass and length demonstrated similar correlative relationships (ρ=0.28 and ρ=0.26, respectively; P<0.005). These correlative relationships can be found in Fig. S2.

LPS-induced molecular modifications

ANOVA

Three-factor ANOVA

There was an effect of acclimation temperature, either individual or interactive, on the mRNA transcript abundance of all studied genes (P<0.05; Figs S3–S6) across pathogen detection, immune response, fatty acid response and stress response. These effects of temperature extended to physiological factors, including whole-body cortisol concentration (Temperature:LPS and LPS:Time, P<0.0005), whole-body lysozyme activity (Temperature, LPS and Time, P<0.05) and mucosal lysozyme activity (Temperature, P<0.005; Fig. 3). There were interactive effects of LPS exposure, temperature and time on the mRNA transcript abundance of genes *MyD88*, *GR1*, *HSP70*, *HSP90a*, *Lysozyme-C*, *IL-1β* and *PLA2* (P<0.05) and combined effects of Temperature:Time and LPS:Time on *IL-8*, *TICAM-1*, *CPT1*, *StAR*, *C3*, *NF-κB*, *TNFα* and *TLR4* (P<0.05; further ANOVA data can be found in Table S1, Figs S3–S6).

Multiple comparison tests for 16°C-acclimated sturgeon revealed that all pathogen detection genes (*TLR4*, *MyD88*, *TICAM-1* and *NF-κB*) demonstrated an upregulation over the time course of 30 µg ml^{-1} LPS exposure (similar to patterns in 16°C-acclimated 60 µg ml^{-1} LPS-exposed sturgeon), while a similar upregulation was not observed in their 20°C-acclimated counterparts (P<0.05) or in 0 µg ml^{-1} LPS-exposed sturgeon (handling controls). Immune-responsive genes demonstrated more nuanced changes in abundance, with *C3* and *TNFα* increasing for only 16°C-acclimated sturgeon throughout the time course (P<0.05), while *IL-1β*, *IL-8* and *Lysozyme-C* had similar responses in 16 and 20°C. However, *IgM* was higher in 20°C-acclimated sturgeon across sampling points (P<0.05). Fatty acid-responsive genes, *CPT1* and *PLA2*, were more responsive to LPS in 16°C-acclimated sturgeon than in 20°C-acclimated fish (P<0.05), with higher peak mRNA transcript abundance following exposure and persistent increases in abundance of *PLA2* mRNA (P<0.05). Finally, stress-responsive genes *StAR* and *GR1* were upregulated throughout the exposure to LPS and recovery at 16°C, while their abundance was not modified by LPS in 20°C-acclimated sturgeon (P<0.05). Chaperones *HSP70* and *HSP90a* were upregulated across both acclimation temperatures during LPS exposure, with a higher magnitude of induction for *HSP70* in 16°C-acclimated sturgeon (P<0.05). Detailed results for all three-factor ANOVA analyses are provided in Table S1.

Two-factor ANOVA

Overall, there was a strong effect of acclimation temperature on mRNA transcript abundance for each analyzed time point (P<0.0001; Fig. 4). Prior to the beginning of LPS trials, acclimation to 20°C increased mRNA transcript abundance of genes across biological processes (Fig. 4A; P<0.05) as compared with 16°C-acclimated sturgeon. The mRNA transcript abundance of both *MyD88* and *NF-κB*, involved in pathogen detection, increased approximately 1.5-fold in 20°C- as compared with 16°C-acclimated sturgeon (P<0.05). Additionally, *C3* and *Lysozyme-C* mRNA abundance, involved in the immune response, were increased 4.8-fold and 2.4-fold, respectively, when compared with 16°C-acclimated sturgeon (P<0.05). Mitochondrial fatty acid transporter *CPT1* mRNA abundance throughout acclimation also increased 3.1-fold in 20°C-acclimated sturgeon versus 16°C-acclimated sturgeon (P<0.05). Finally, *GR1*, *HSP70* and *HSP90a*, all of which are involved in the endocrine stress response, increased their transcript abundance 2.2-, 2.0- and 1.9-fold, respectively, in 20°C-compared with 16°C-acclimated sturgeon (P<0.05).

However, following 48 h exposure to 30 µg ml^{-1} LPS, 16°C-acclimated sturgeon had higher levels of mRNA transcript abundance across biological processes, when compared with their 20°C-acclimated counterparts at the same time point and LPS exposure (Fig. 4B; P<0.05). Intracellular signaling molecules *MyD88* and *TICAM-1* were 78.5% and 48.8% more highly induced in 16°C-acclimated lake sturgeon, respectively, when

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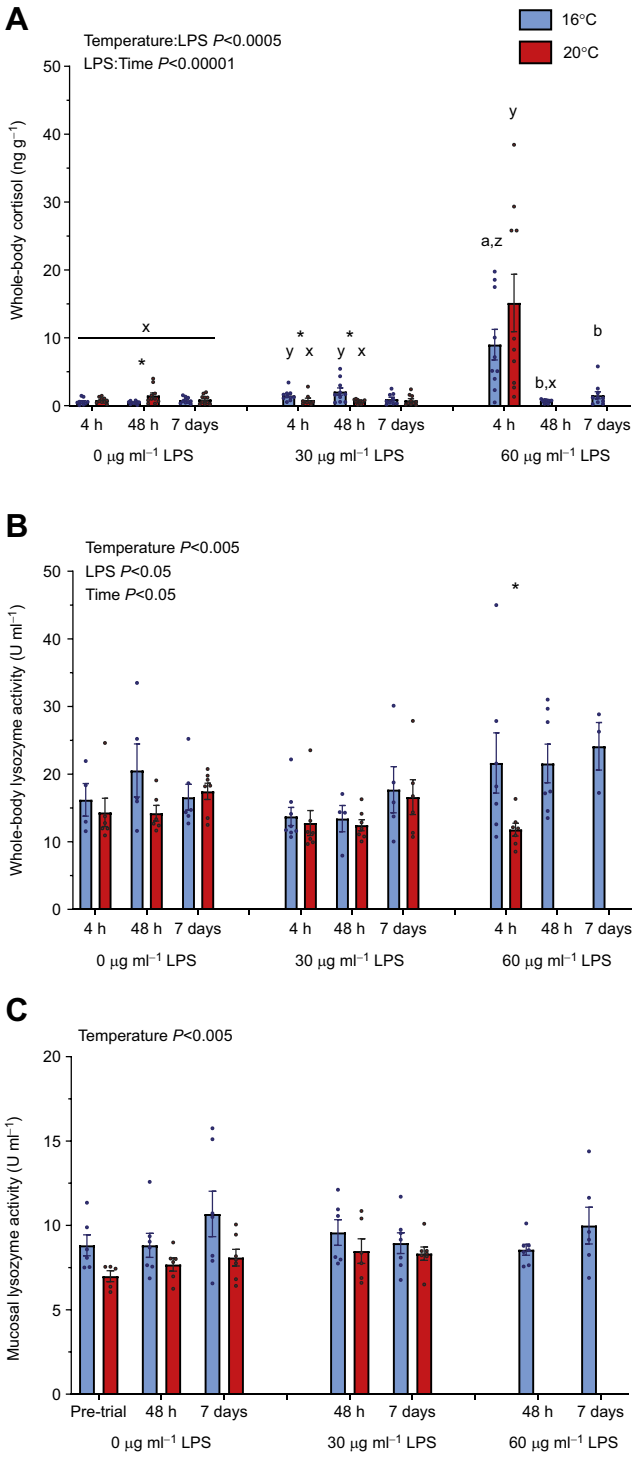

Fig. 3. Whole-body cortisol concentration and whole-body and mucosal lysozyme activity in developing lake sturgeon exposed to LPS. (A) Whole-body cortisol concentration, (B) whole-body lysozyme activity and (C) mucosal lysozyme activity in fish acclimated to 16 and 20°C and then exposed to 0, 30 and 60 µg ml^{-1} LPS for 4 h (or pre-trial levels for mucosal lysozyme activity) and 48 h, and following a 7 day LPS-free recovery. *Significant difference between acclimation treatments. Lowercase letters a, b represent a significant difference between time points within an exposure concentration. Lowercase letters x, y, z represent a significant difference in a given time point and acclimation temperature, throughout exposure concentrations (P<0.05; three-factor ANOVA). Data are expressed as means ±s.e.m. (whole-body cortisol n=9–10, whole-body lysozyme n=3–8, mucosal lysozyme n=5–7; dots represent individual data points).

compared with 20°C-acclimated sturgeon (P<0.05). While all immune-responsive genes were quantitatively higher in mRNA abundance for 16°C-acclimated sturgeon (with the exception of *IgM*), only *IL-1β* demonstrated significantly higher transcript abundance, 2.2-fold higher in 16°C-acclimated sturgeon than in 20°C-acclimated sturgeon (P<0.05). In contrast to the observed trend of the other immune-responsive genes, *IgM* was 58% higher in 20°C-acclimated sturgeon (P<0.05). Fatty acid-responsive genes *PLA2* and *CTP1* were much more highly induced in 16°C-acclimated sturgeon, 5.6- and 2.5-fold, respectively, when compared with their 20°C-acclimated counterparts (P<0.05). For stress-responsive genes, only *HSP70* had higher mRNA transcript abundance between acclimation treatments, with 2-fold higher abundance in 16°C-acclimated sturgeon.

Following a 7 day recovery, these increased mRNA responses in 16°C-acclimated lake sturgeon observed during LPS exposure persisted, with higher levels of mRNA transcript abundance across pathogen detection, fatty acid and stress response processes when compared with 20°C-acclimated sturgeon (Fig. 4C; P<0.05). Pathogen detection components of the toll-like receptor signaling complex *TLR4*, *MyD88* and *TICAM-1* were all upregulated in 16°C as compared with 20°C-acclimated lake sturgeon, 1.9-, 1.5- and 2.6-fold, respectively (P<0.05). Fatty acid-responsive genes *PLA2* and *CPT1* showed 5.5- and 1.5-fold higher mRNA abundance, respectively, in 16°C-acclimated sturgeon when compared with 20°C-acclimated sturgeon (P<0.05). Stress-responsive genes *StAR*, *GR1* and *HSP70* were also all elevated in 16°C- versus 20°C-acclimated lake sturgeon, 2-, 1.4- and 2.3-fold, respectively (P<0.05).

PCA

PCA (Fig. 5A) demonstrated separation between 16 and 20°C acclimation treatments across their responsive trajectories to acclimation, 48 h LPS exposure and 7 day recovery. Principal component 1 (PC1) along the x-axis explained 43.6% of the variation, while PC2 on the y-axis explained 13.1%. As developing sturgeon responded to LPS exposure, the two acclimation treatments separated out from the left to the right side of the y-axis, but with different responsive trajectories, as 16°C-acclimated sturgeon moved to the bottom right-hand quadrant, while those acclimated to 20°C moved to the upper left. Following recovery, these responses also differed, with 16°C-acclimated sturgeon moving to the upper right quadrant and 20°C-acclimated sturgeon regressing to the upper left. Thus, the mRNA transcriptional responses of the acclimation treatments had different trajectories, but also differences in their magnitude, with 16°C-acclimated sturgeon moving further across the axes than 20°C-acclimated sturgeon in response to LPS exposure.

The contribution of variables to observed variation in the PCA was distributed across biological processes, with at least one gene from each contributing more than the average expected value to the overall variation observed (Fig. 5B). Genes passing this average expected contribution threshold were *TLR4*, *MyD88* and *TICAM-1* (involved in pathogen detection), *Lysozyme-C* and *IL-8* (involved in the immune response), *HSP70*, *StAR* and *GR1* (involved in the stress response), and *CPT1* (involved in fatty acid responses). Variable plots further demonstrate the response trajectory, which the

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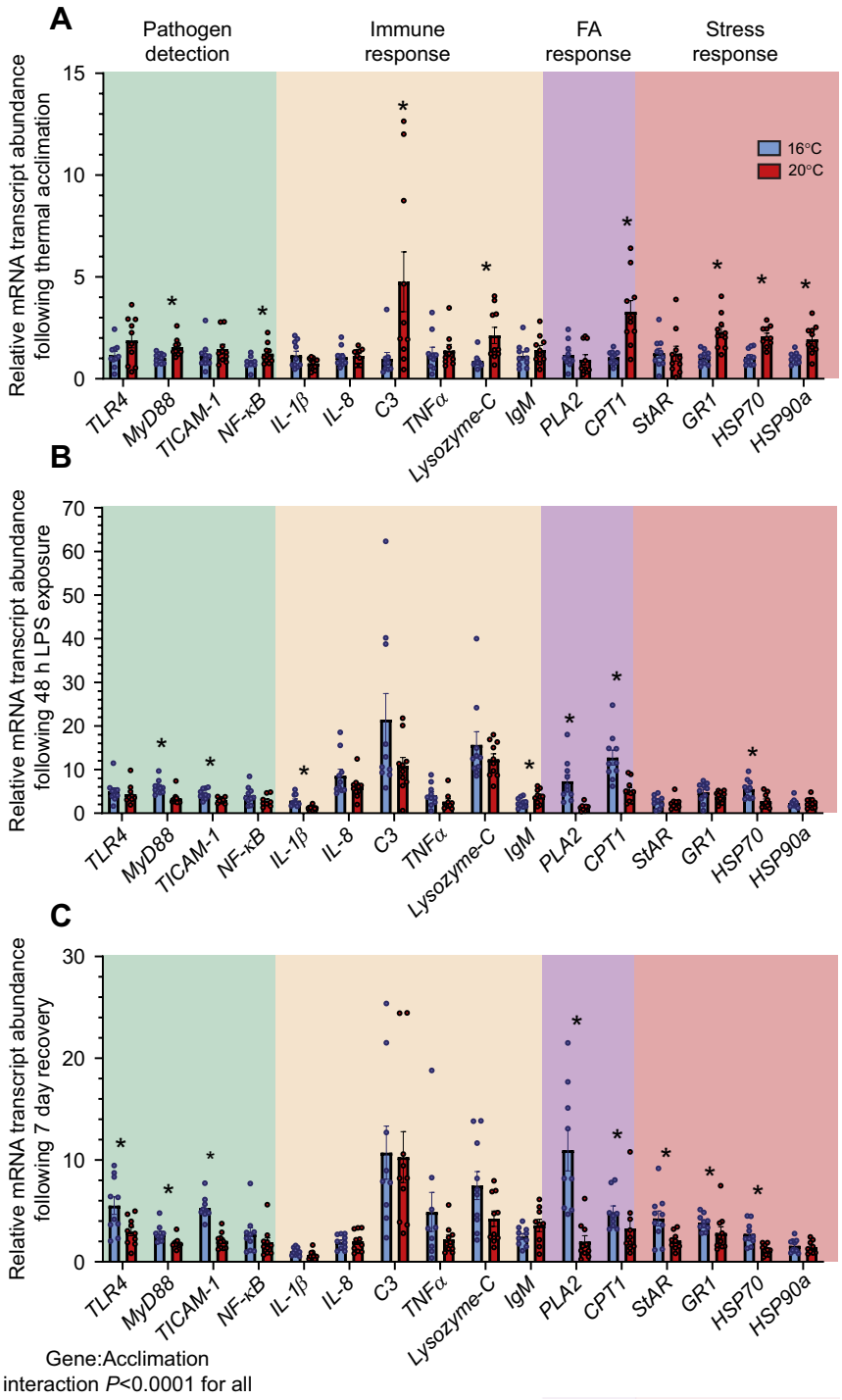

Fig. 4. mRNA expression in developing lake sturgeon exposed to LPS. Relative mRNA transcript abundance of genes involved in pathogen detection (*TLR4*, *Myd88*, *TICAM-1*, *NF-κB*), immune response (*IL-1β*, *IL*-8, *C3*, *TNFα*, *Lysozyme-C*, *IgM*), fatty acid response (*PLA2*, *CPT1*) and stress response (*StAR*, *GR1*, *HSP70*, *HSP90a*), following (A) acclimation to 16 and 20°C for 22 days, (B) 48 h exposure to 30 µg ml^{-1} LPS and (C) 7 day recovery from LPS exposure. *Significant difference between 16 and 20°C acclimation treatments for a given gene (P<0.05; two-factor ANOVA). Data are expressed as means±s.e.m (n=9–10).

variation in mRNA transcript abundance of these genes contributed to the PCA, with all genes over the expected contribution threshold moving to the righthand side of the *y*-axis (Fig. 5C).

Correlative relationships

The relationships between mRNA transcript abundance for lake sturgeon differed according to acclimation treatments throughout acclimation, LPS exposure and recovery, with higher Spearman's correlation coefficients across 16°C-acclimated sturgeon when comparing genes across biological processes (Fig. 6A). Transcript abundance of many genes involved in the downstream responses to pathogen detection of LPS (i.e. *IL-8*, *C3*, *Lysozyme-C*, *PLA2*, *CPT1*, *GR1*, *HSP70* and *HSP90a*) demonstrated strong correlative relationships (ρ≥0.6) with that of intracellular immune signaling molecules (*MyD88* or *TICAM-1*) for 16°C-acclimated sturgeon. In contrast there were no relationships as strong as these for the above genes in 20°C-acclimated sturgeon, and only one as strong when comparing the relationships of every studied gene (*IL-8* to *IL-1β*). The largest differences in correlative relationships between the treatments can be found in the transcript abundance of stress-responsive genes, *GR1*, *HSP70* and *HSP90a* (Fig. 6B), while the relationship of *StAR* expression with that of every other gene was

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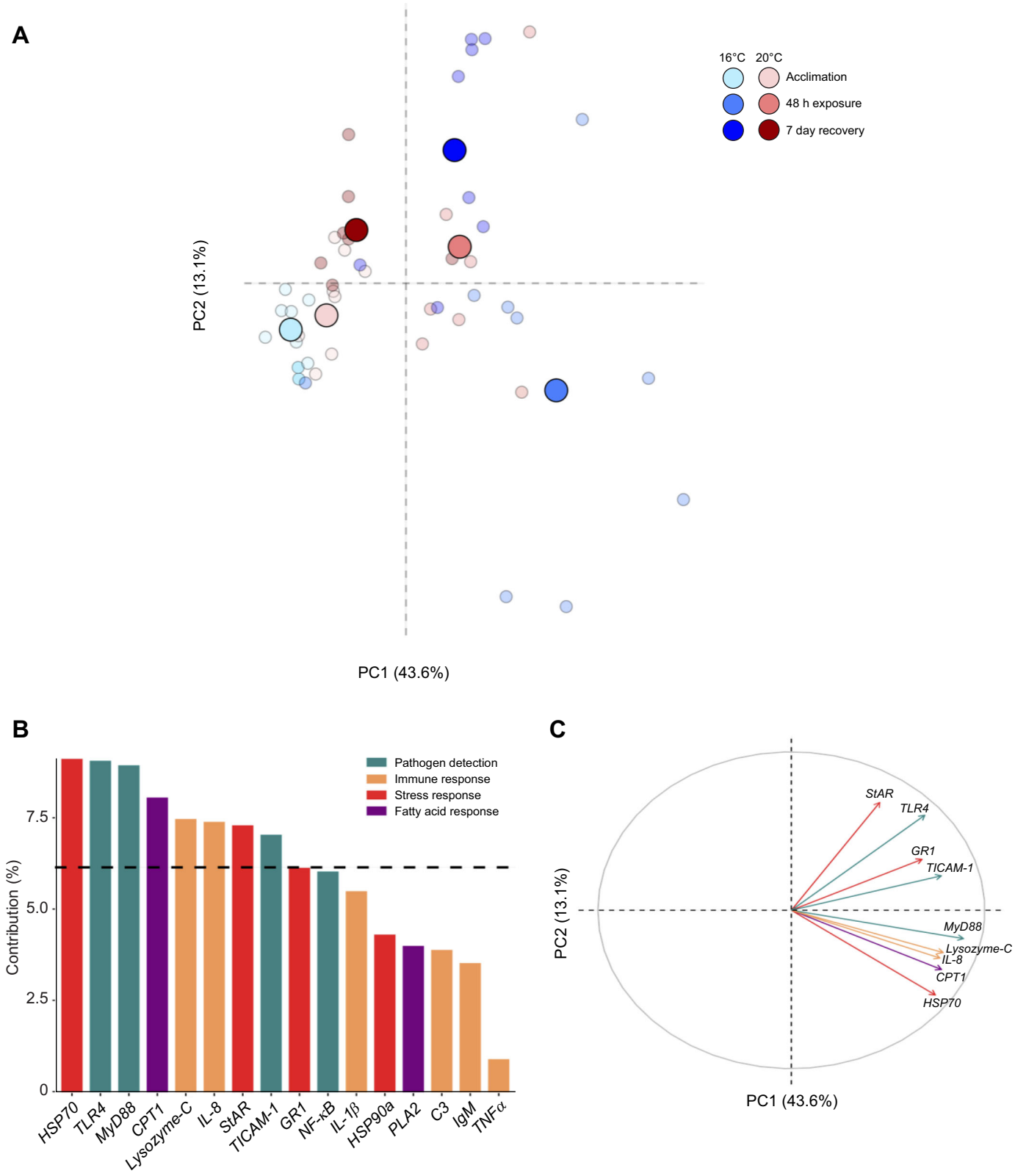

Fig. 5. Principal component analysis. (A) Principal component 1 (PC1) and PC2, (B) contributions of variables to PC1 and PC2, and (C) variables plot for developing lake sturgeon following thermal acclimation to 16 and 20°C, exposure to 30 µg ml^{-1} LPS for 48 h, and a 7 day recovery period (n=48). For A, each small light-colored point represents an individual, while the large dark-colored points represent the centroid for each treatment group. The dashed line in B represents the default average value expected for the contribution of each gene to the overall observed variation. For simplicity, only genes that exceeded the average expected contribution were included in the variables plot in C.

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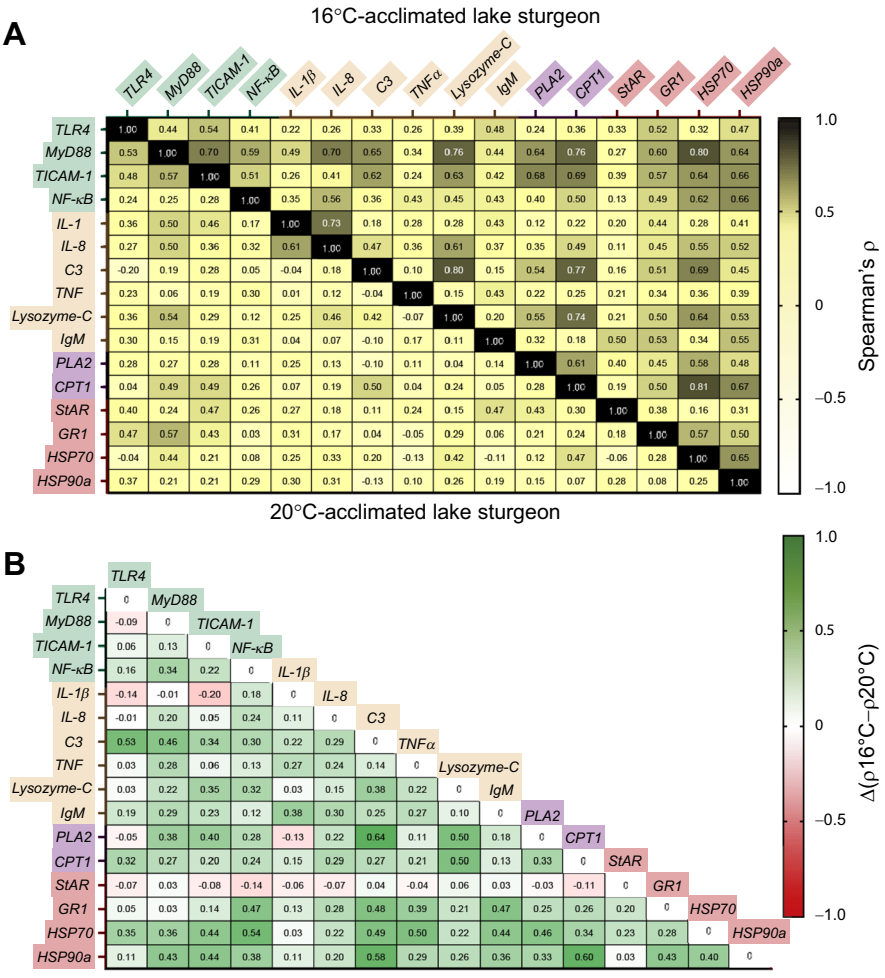

Fig. 6. mRNA transcript abundance in developing lake sturgeon exposed to LPS. (A) Relationship between mRNA abundance in fish acclimated to 16 and 20°C. Values are reported as Spearman's rho (ρ). (B) The delta value (the difference in Spearman's ρ between the two acclimation treatments: ρ16°C−ρ20°C) following trial exposure to 0, 30 and 60 µg ml^{-1} LPS during pre-trial conditions, 4 and 48 h following exposure, as well as after 7 days of recovery. Correlative relationships between the mRNA abundance of each gene were quantified while accounting for variance in both LPS exposure concentration and time point using semi-partial Spearman's correlations. Color overlays indicate the biological process to which the genes belong: green, pathogen detection; yellow, immune response; purple, fatty acid response; and red, stress response.

relatively consistent across the acclimation treatments. There were also inconsistencies between the acclimation treatments in the relationship between *C3* and *Lysozyme* expression and that of fatty acid-responsive genes *PLA2* and *CPT1*, and for *C3* with elements of pathogen detection.

Post-trial metabolism and thermal tolerance

While there was an interactive effect of acclimation temperature and LPS (P<0.05) on CT_{max}, there was no compromise in thermal tolerance by the effects of LPS exposure in either acclimation treatment (Fig. S7). In contrast, several metabolic traits were suppressed following exposure to LPS, but only in 16°C-acclimated sturgeon (Fig. 7). Importantly, measurement of metabolic rate prior to CT_{max} trials did not impact the resulting CT_{max} for sturgeon from any treatment (P>0.05). There was no effect of LPS exposure or acclimation temperature on the routine metabolic rate of lake sturgeon; however, there were effects on FMR, $\Delta\dot{M}_{O_2}$ and metabolic scope, all of which demonstrated significant interactions between acclimation treatment and LPS concentration (P<0.01). Further, multiple comparisons revealed specific differences between acclimation treatments and across LPS concentrations.

In 16°C-acclimated sturgeon, FMR was reduced in both 30 and 60 µg ml^{-1} LPS, by 38.5% and 40.9%, respectively, compared with control sturgeon (P<0.01; Fig. 7B). Also, FMR for 20°C-acclimated sturgeon exposed to 30 µg ml^{-1} LPS was 30.3% higher than that of 16°C-acclimated sturgeon (P<0.05). Multiple comparisons for $\Delta\dot{M}_{O_2}$ indicated a decrease of 73.6% and 88.6% for 16°C-acclimated lake sturgeon following exposure to LPS concentrations of 30 and 60 µg ml^{-1}, respectively, when compared with controls acclimated to the same temperature (P<0.001; Fig. 7C). This again resulted in higher $\Delta\dot{M}_{O_2}$, similar to FMR, between the acclimation treatments exposed to 30 µg ml^{-1} LPS (P<0.05), with a 55.5% higher $\Delta\dot{M}_{O_2}$ in 20°C-acclimated sturgeon than in their 16°C counterparts. Finally, metabolic scope also decreased in LPS-exposed sturgeon in the 16°C acclimation treatment, by 41.6% and 54.7% for 30 and 60 µg ml^{-1} LPS treatments when compared with that of handling control fish, respectively (P<0.005; Fig. 7D). However, there was no change in FMR or $\Delta\dot{M}_{O_2}$, or metabolic scope for 20°C-acclimated fish.

There was a significant interaction between acclimation treatment and LPS concentration on the CT_{max} of sturgeon following the 7 day recovery period from LPS trials (P<0.05; Fig. S7). Sturgeon acclimated to 20°C and exposed to 30 µg ml^{-1} LPS had a CT_{max} 0.44°C higher than control sturgeon not exposed to LPS (P<0.001). There were no differences in CT_{max} for 16°C-acclimated sturgeon across LPS exposure concentrations. Across acclimation temperatures, sturgeon acclimated to 20°C had CT_{max} values 2.71 and 3.13°C higher than those of 16°C-acclimated sturgeon, for handling control and 30 µg ml^{-1} LPS, respectively (P<0.0001). There was no correlation between CT_{max} for individual fish with any of their respective measured metabolic metrics.

DISCUSSION

In the current study, we investigated the effects of temperature on the innate immune responses of lake sturgeon during early life.

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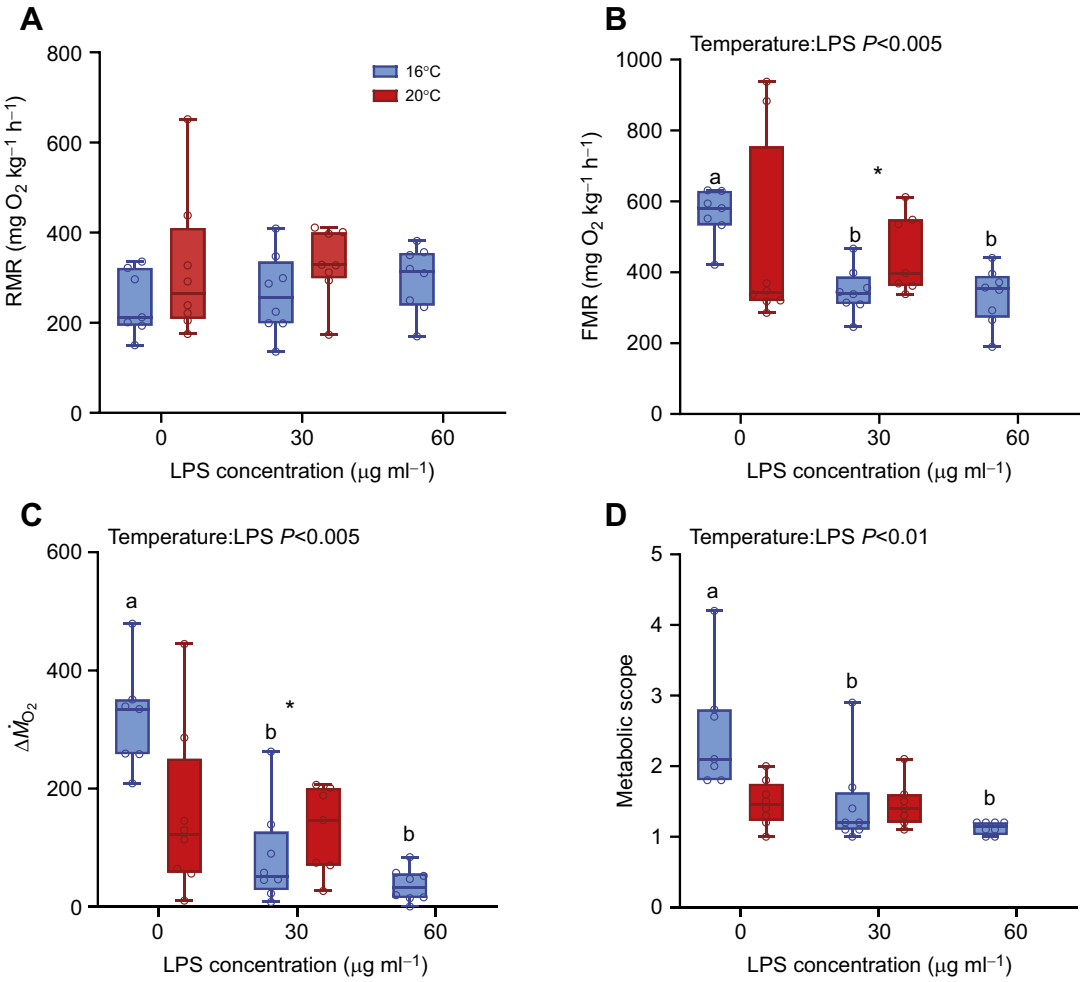

Fig. 7. Effect of exposure to LPS on metabolic rate in developing lake sturgeon. (A) Routine metabolic rate (RMR), (B) forced metabolic rate (FMR), (C) change in metabolic rate ($\Delta\dot{M}_{O_2}$) and (D) metabolic scope of 16 and 20°C-acclimated fish, following a 6 day recovery from 48 h exposure to 0, 30 and 60 μg ml^{-1} LPS. Differences between treatments were determined by two-factor ANOVA (P<0.05) followed by Tukey's honestly significant different *post hoc* test. *Significant difference between 16 and 20°C acclimation treatments within a LPS exposure concentration. Lowercase letters a and b represent a significant difference across treatment concentrations within a single acclimation treatment (n=7–8).

Increased environmental temperatures influenced the mRNA transcript abundance of every measured endpoint. Further, when fish were also challenged with LPS, elevated temperature dampened their innate immune capacity, as observed across innate immune-, fatty acid- and stress-responsive biological processes. Acclimation temperatures used in the current study were approximately 2–3°C below maximum sustained summer temperatures for this population of lake sturgeon (Bugg et al., 2020, 2021b). These results suggest the presence of seasonal sub-lethal thermal thresholds on innate immunity for wild populations of lake sturgeon throughout Manitoba, which may be especially vulnerable to the effects of pathogens during early development (Clouthier et al., 2020). While developing sturgeon may be plastic in the face of thermal changes (Bugg et al., 2020; Penman, 2021), this plasticity accompanied by the induction of the glucocorticoid stress response and decrease in energy reserves may diminish their capacity to mount an effective immune response against opportunistic pathogens.

Effects of acclimation temperature and in-trial mortality

While there was no difference in condition or energy density prior to the LPS trials, length and mass were greater and HSI was less for 20°C-acclimated sturgeon when compared with their 16°C-acclimated counterparts. The HSI showed the strongest relationship to time to mortality during LPS exposure. As HSI is an indication of fatty acid and glycogen stores accrued during development (Chellappa et al., 1995; Rossi et al., 2017; Morrison et al., 2020), these findings suggest that the effects of increasing temperature compromise the acquisition and allocation of these energy reserves (Yoon et al., 2022), contributing to increases in the susceptibility of developing sturgeon to pathogenic stressors. Interestingly, there was no difference in whole-body energy density of lake sturgeon from different acclimation treatments during development, while HSI and growth differed between acclimation treatments. This suggests that increasing temperature influenced energy partitioning in developing sturgeon, either into storage in the liver or into somatic growth (Post and Parkinson, 2001). This metabolic trade off, to increase somatic growth at higher temperatures, may ultimately have immunocompromising effects (Kim et al., 2019). Research in pallid sturgeon, *Scaphirhynchus albus*, and white sturgeon, *A. transmontanus*, suggested that increased rearing temperatures resulted in high levels of mortality (50–60%) once a pathogen was introduced, while there was limited mortality (<10%) in lower temperature acclimation treatments (Coleman et al., 2018; Stilwell et al., 2022). These findings

demonstrate thermal thresholds for pathogen-induced mortality, which is potentially associated with the energy allocation of developing sturgeon and may have contributed to the mortality associated with elevated temperatures in the current study.

Impacts of thermal acclimation on mRNA abundance

Prior to the LPS trials, lake sturgeon acclimated to 20°C had increased mRNA transcript abundance of genes involved in pathogen detection, immune responsiveness, fatty acid responses and stress responses. Increased transcript abundance of pathogen detection (*MyD88* and *NF-κB*) and immune-responsive genes (*C3* and *Lysozyme-C*) may indicate a stress-responsive role for their induction, as observed in channel catfish, *Ictalurus punctatus*, and large yellow croaker, *Larimichthys crocea* (Small and Bilodeau, 2005; Sun et al., 2017). Interestingly, mRNA abundance of *CPT1* (involved in fatty acid oxidation; Coccia et al., 2014) and genes involved in the HPI axis and general stress response (*GR1*, *HSP70* and *HSP90a*) were upregulated in response to warm acclimation. Together, these findings, paired with a decrease in HSI for 20°C-acclimated sturgeon, suggest that accumulated thermal stress results in an activation of the glucocorticoid stress response, and an increase in the oxidation of fatty acids, similar to observations made in thermally stressed Atlantic salmon (Norambuena, et al., 2015). Although there were increases in immune, energy production and stress response mRNA transcript abundance in the 20°C treatment following acclimation, these did not result in enhanced survival during LPS trials, and likely represent thermal stress-responsive mechanisms.

LPS-induced transcriptional modifications

Sturgeon acclimated to 16°C had stronger activation of pathogen-detection mechanisms (*MyD88* and *TICAM-1*), immune-responsive transcripts (*IL-1β*, *IL-8*, *C3*, *TNFα* and *Lysozyme-C*), fatty acid responses (*PLA2* and *CPT1*) and stress-responsive mechanisms (*HSP70*) following LPS exposure, as demonstrated through the increase in mRNA transcript abundance of associated genes, when compared with fish acclimated to 20°C. Interestingly, *IgM* expression was not highly induced at either temperature following LPS exposure, suggesting that sturgeon may not be able to strongly upregulate this primarily adaptive immune response and may be relying on innate immune mechanisms at this developmental stage instead. Together, these responses suggest that 16°C-acclimated sturgeon produced a stronger stimulation of the immune signaling cascade (Deguine and Barton, 2014; Tanekhy, 2014) and may exhibit a higher capacity to respond with both innate immune and energetically intensive processes (Angosto and Mulero, 2014; Arnemo et al., 2017), and that acclimation to 20°C disrupted the magnitude and timing of the innate immune responses induced by LPS exposure at the level of the transcriptome (Bennoit and Craig, 2020). These stronger observed responses in 16°C-acclimated sturgeon represent a larger physiological capacity to effectively counter pathogenic infection, compared with sturgeon acclimated to 20°C. Further, following a 7 day recovery from LPS exposure, elevated mRNA responses in 16°C-acclimated sturgeon were sustained, remaining higher than those of their 20°C-acclimated counterparts across pathogen detection, fatty acid- and stress-responsive processes, indicating that chronic thermal stress may impede long-term physiological processes.

Although the abundance of many mRNA transcripts was largely different between acclimation treatments, there was an induction of *IL-8*, *Lysozyme-C* and *C3* transcripts across both temperature treatments, which is consistent with other studies of the sturgeon immune response (Li et al., 2017; Lou et al., 2018; Valipour et al., 2018; Hohne et al., 2021). These innate immune mechanisms may play a crucial role in pathogen defense in sturgeon, especially during early development (Magnadottir, 2006; Wang et al., 2009; Huber-Lang et al., 2018). However, the induction of these transcripts did not improve survival at 20°C during the LPS trials. This result emphasizes the importance of peripheral immune-responsive mechanisms in pathogen defense (e.g. unstudied processes at the receptor, complement, cytokine and antimicrobial peptide levels) in developing fish.

Effects of temperature on lysozyme and HPI axis activity

Both whole-body lysozyme and mucosal lysozyme were elevated in 16°C when compared with their 20°C-acclimated counterparts. As lysozyme is a key innate immune enzyme involved in bacterial defense (Saurabh and Sahoo, 2008), decreases in lysozyme activity demonstrate that the effects of temperature extend to protein-level responses. Further, both 16 and 20°C-acclimated sturgeon exposed to 60 μg ml^{-1} LPS increased cortisol concentrations at the 4 h time point, confirming that HPI axis-related responses are activated in lake sturgeon following pathogenic stimulus (Haukenes et al., 2008; Bugg et al., 2021a).

Overall, lake sturgeon acclimated to 20°C were less transcriptionally responsive than their 16°C counterparts (PCA analysis) and suffered a breakdown of their transcriptional relationships regulating pathogen detection and downstream processes (correlative analysis), resulting in a modification of the magnitude of the transcriptional response of various immune-related processes (Bennoit and Craig, 2020). Interestingly, the GR response of the 16°C-acclimated sturgeon following LPS stimulus was upregulated to the same levels as in 20°C-acclimated sturgeon under control conditions, suggesting that 20°C-acclimated sturgeon are already stimulating the HPI axis and may be chronically stressed, resulting in their inability to respond to additional stressors such as LPS. In contrast, 16°C-acclimated individuals were able to respond through elevation of cortisol, GR and HSP70. Further, HPI axis-related co-chaperones involved in the thermal stress response were also upregulated in 20°C- when compared with 16°C-acclimated sturgeon. Chronic elevation of a stress response is energetically costly, consistent with decreased HSI as well as the suppression of transcriptional activation (Tort, 2011; Alfonso et al., 2021), and likely impacted the capacity of lake sturgeon to physiologically respond to LPS when acclimated to elevated temperatures.

Post-trial metabolism and thermal tolerance

It is expected that activation of immune responses is energetically costly, which can increase metabolic rate following pathogenic infection (Martin and Krol, 2017; Bennoit and Craig, 2020; Polinski et al., 2021). Our results contrast those from similar studies exposing mosquitofish (*Gambusia holbrooki*) and zebrafish (*Danio rerio*) to an immune stimulus, where, 1 week following exposure, they demonstrated increased metabolic scope and RMR, respectively (Bonneaud et al., 2016; Bennoit and Craig, 2020). In sockeye salmon, *Oncorhynchus nerka*, a purportedly minimal metabolic cost and a reportedly highly energetically efficient innate immune response to infection (Polinski et al., 2021) was observed. However, in our study, LPS exposure and induction of immune-responsive mechanisms did not increase RMR, but instead resulted in a depression of FMR and metabolic scope only in 16°C-acclimated sturgeon exposed to LPS. This finding may indicate that innate immune activation in lake sturgeon is more energetically costly when compared with that in more recently derived fish lineages or results in a limitation based on energy constraints during this early life stage. Further, it remains unclear why the aerobic capacity of

sturgeon acclimated to 20°C was not altered, but it may suggest that their ability to manipulate their aerobic capacity was limited at this higher temperature. Thus, there may be a constraint on energy allocation under elevate temperatures which may be influenced by the increase in other routine activities including growth, as demonstrated by decreased HSI. This decrease in metabolic capacity may show a cost–benefit relationship to its activation, with complete mortality observed at 20°C in 60 µg ml^{-1} LPS. While there is limited research on the metabolic cost of immune activation in fishes, our results suggest that increased energetic costs, due to increased temperatures, may compromise the induction of the immune system in developing lake sturgeon.

Study limitations

This study measured the mRNA abundance of transcripts representing a broad array of genes across biological pathways that contribute to innate immune capacity. However, many of these molecular responses are involved in numerous pathways. Further, in these responses there are many other genes at play, and their mRNA expression is not necessarily directly reflective of protein abundance. Further, sturgeons are also ancestral species, exhibiting differences in their cortisol stress response (Penny et al., 2023) and responses to physiological stressors (Kieffer et al., 2001; Haukenes et al., 2008) when compared with more recently derived teleosts, and this could extend to cytokine-level responses (Li et al., 2017; Lou et al., 2018; Jiang et al., 2018). Thus, direct comparisons of sturgeons with teleosts should be approached with caution because they diverged hundreds of millions of years ago (Du et al., 2020).

The use of whole animals as opposed to targeted immune tissues such as the head kidney could dilute observations of immune-related gene expression. However, we chose to use whole-body samples for two reasons: (1) we aimed to investigate the interaction between LPS and temperature exposure on immune capacity across multiple biological processes, including stress- and fatty acid-responsive gene expression, which would not be possible if we took a tissue-specific approach; and (2) we aimed to investigate these responses in a critical early developmental window where high mortality often occurs, temperatures are elevated and sturgeon are likely reliant on their innate immune responses. This limited the size of the fish used in this study.

Additionally, in the current study, we chose to use an environmental exposure of LPS, which may result in variability of an individual's exposure dosage when compared with an intraperitoneal injection. However, as previous experiments demonstrated this variation to be minor in Atlantic halibut, *Hippoglussus hippoglossus* (especially over a 48 h exposure; Dalmo et al., 2000), we chose to use an environmental exposure to LPS to illicit a comprehensive whole-organism response (Anderson and Siwicki, 1994), which may more accurately reflect how lake sturgeon would encounter and respond to pathogens in the wild. This was especially important for examining mucosal lysozyme activity, which is a potential non-lethal method of measuring an immune response in juvenile lake sturgeon. Further, the LPS used in the study had a purity of >97%, leaving the possibility (albeit low) of potential impurities that could have impacted the immune responses observed in this study. Despite these potential limitations, the collective evidence from this study suggests that elevated temperatures have a profound impact on the immune capacity of developing lake sturgeon.

Ecological and management implications

Lake sturgeon are exposed to thermal stress and environmentally pervasive pathogens in both hatchery and wild environments, especially during early development. As climate change effects will likely exacerbate cross-species pathogen transmission, it is important to understand how pathogens affect phenotypic development during early life and its impacts on the recruitment of lake sturgeon.

Our data suggest that the effects of increasing temperatures will reduce the immune capacity of lake sturgeon during early development, when they experience high mortality (Sifa and Mathias, 1987; Wieser, 1991; Rombough, 1994). This study demonstrated the effects of 20°C for a period of 22 days; however, wild sturgeon from this population are likely exposed to this temperature, and higher, for prolonged durations, upwards of 50 days in the summer (Bugg et al., 2020). Therefore, further research should focus on the long-term effects of increasing temperatures during early life on pathogen burden, virulence and physiological condition with the goal of evaluating the impacts on health and survival of fishes to effectively manage wild northern populations that may be most threatened by increasing temperatures.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: W.S.B., G.R.Y., A.N.S., A.M.W., K.M.J., W.G.A.; Methodology: W.S.B., G.R.Y., A.N.S., A.M.W., W.G.A.; Validation: G.R.Y., A.N.S., A.M.W.; Formal analysis: W.S.B.; Investigation: W.S.B., G.R.Y., A.N.S., K.M.J., W.G.A.; Resources: W.G.A.; Data curation: W.S.B., G.R.Y., A.N.S., A.M.W.; Writing - original draft: W.S.B., A.M.W.; Writing - review & editing: W.S.B., G.R.Y., A.N.S., A.M.W., K.M.J., W.G.A.; Visualization: W.S.B.; Supervision: K.M.J., W.G.A.; Project administration: W.S.B., W.G.A.; Funding acquisition: K.M.J., W.G.A.

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Data availability

All relevant data can be found within the article and its supplementary information.

ECR Spotlight

This article has an associated ECR Spotlight interview with William Bugg.

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Supplementary Materials and Methods

Primer Design Methods:

A lake sturgeon head kidney transcriptome was used to design primers for *TLR4*, *NF-kB*, *TNFa*, *IL-8*, and *IgM* (Thorstensen et al., 2022b). Annotated lake sturgeon sequences for *TLR4* demonstrated conserved regions with 98.3 and 94.7% identity to the sterlet sturgeon, *Acipenser ruthenus,* and the American paddlefish *Polydon spathula* (Transcripts XM_034909094.1 and XM_041234367.1, respectively). Similarly, *NF-kB* and *TNFa* transcripts shared 98 and 98.5% identity, respectively, with published transcripts from the sterlet sturgeon (Transcripts XM_034013617.2 and XM_034909934.1, respectively). Lake sturgeon head kidney transcripts annotated as *IL-8* shared 94.8 and 94.5% identity to published transcripts from the Siberian sturgeon, *Acipenser baerii*, and the sterlet sturgeon, respectively (Transcripts MK140599.1 and XM_034035867.2, respectively). Finally, transcripts annotated as *IgM* shared conserved regions of 96.4, 95.2, 95, 95, and 93.4% identity to previously annotated transcripts from the Siberian sturgeon, beluga sturgeon, *Huso huso*, sterlet sturgeon, Russian sturgeon, *Acipenser gueldenstaedtii*, and Japanese sturgeon, *Acipenser schrenckii*, respectively (Transcripts KC734558.1, DQ257633.1, DQ257636.1, DQ257634.1 and DQ257635.1, respectively).

Primers for *C3* and *TICAM-1* were designed from an annotated and published white sturgeon, liver transcriptome (Doering et al., 2016) while primers for *Lysozyme-C* were designed from a lake sturgeon liver transcriptome (Thorstensen et al., 2022b). Transcripts for both *C3* and *TICAM-1* shared conserved regions with 98% identity to previously annotated transcripts from the sterlet sturgeon (Transcripts XM_034016062.2 and XM_034911846.1, respectively). Transcripts for *Lysozyme-C* shared conserved regions with 98.6, 99.4 and 99.1% identity to previously annotated transcripts from the sterlet sturgeon, Chinese sturgeon, *Acipenser sinensis*, and Dabry's sturgeon, *Acipenser dabryanus*, respectively (Transcripts XM_034058580.2, MF280234.1, MF135537.1, respectively).

A lake sturgeon gill transcriptome was used to design primers for potential reference genes *RPL13a*, eEF*1A1*, and *RPL4* (Thorstensen et al., 2022b; Bugg et al., 2022). Transcripts for *RPL13a* shared conserved regions with both sterlet sturgeon and the American paddlefish, 98.6 and 96% identity, respectively (Transcripts XM_034908381.1 and XM_041240517.1, respectively). Similarly, transcripts for *eEF1A1* shared conserved regions with the sterlet sturgeon, American

paddlefish, and additionally Dabry's sturgeon, with identities of 96.4, 95.4, and 98.4%, respectively (Transcripts XM_034915679.1, XM_041240787.1 and MH790258.1, respectively). Finally, transcripts annotated as *RPL4* shared conserved regions with previously annotated transcripts from the Siberian sturgeon, sterlet sturgeon, and American paddlefish, with identities of 99.8, 99.4 and 97.2%, respectively (Transcripts MG722839.1, XM_034049385.2 and XM_041218712.1, respectively).

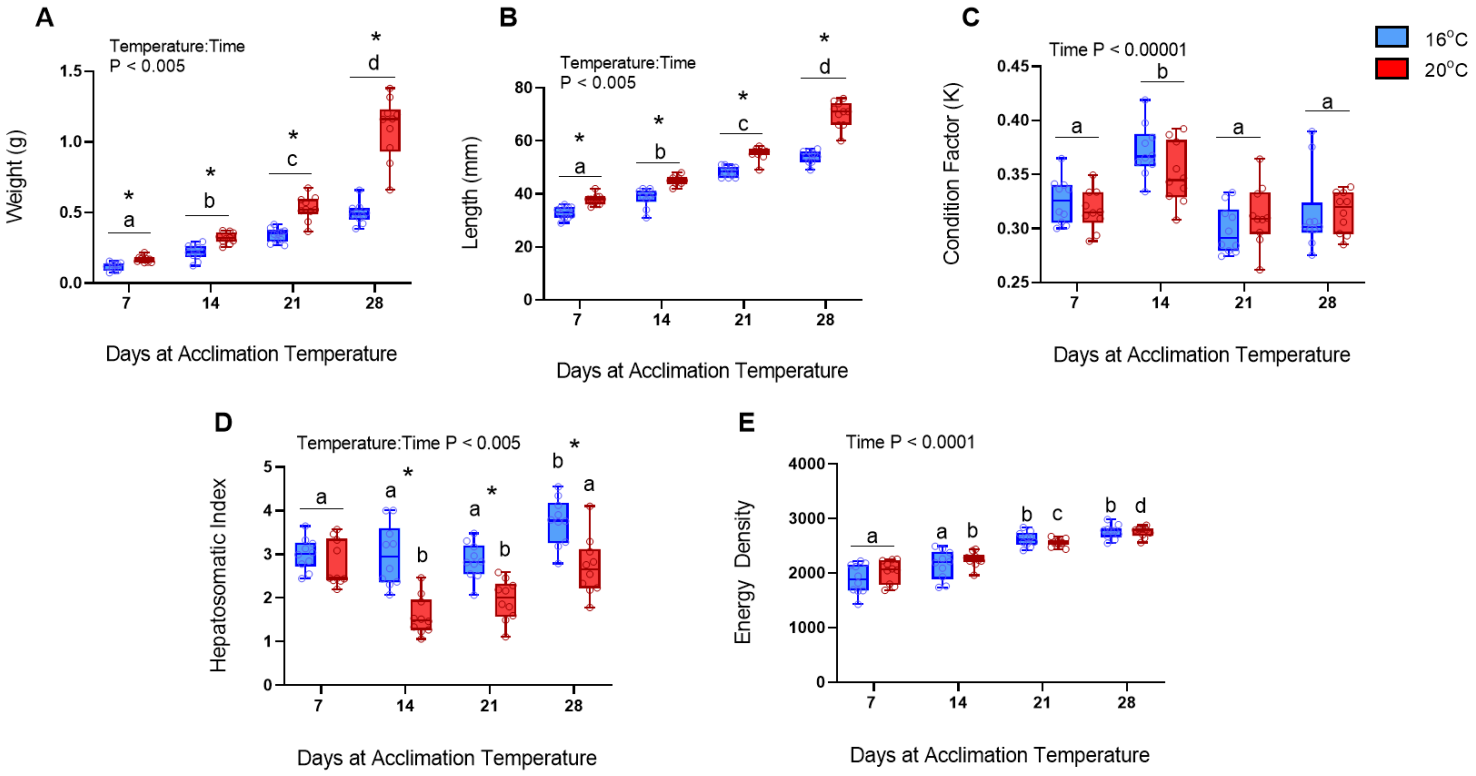

Fig. S1. A) Weight (g), B) length (mm), C) condition factor (K), D) hepatosomatic index (HSI), and E) energy density of developing lake sturgeon, *Acipenser fulvescens*, throughout 28 days acclimation to 16 and 20°C. Differences between treatments and timepoints were determined by two-factor ANOVA ($P < 0.05$) followed by Tukey's honestly significant different post-hoc test. *'s represent significance between 16 and 20°C acclimation treatments Lowercase letters a, b, c represent significance between timepoints, within an acclimation treatment. ($P < 0.05$; two-factor ANOVA). Data are expressed as mean +/- SEM (n = 10).

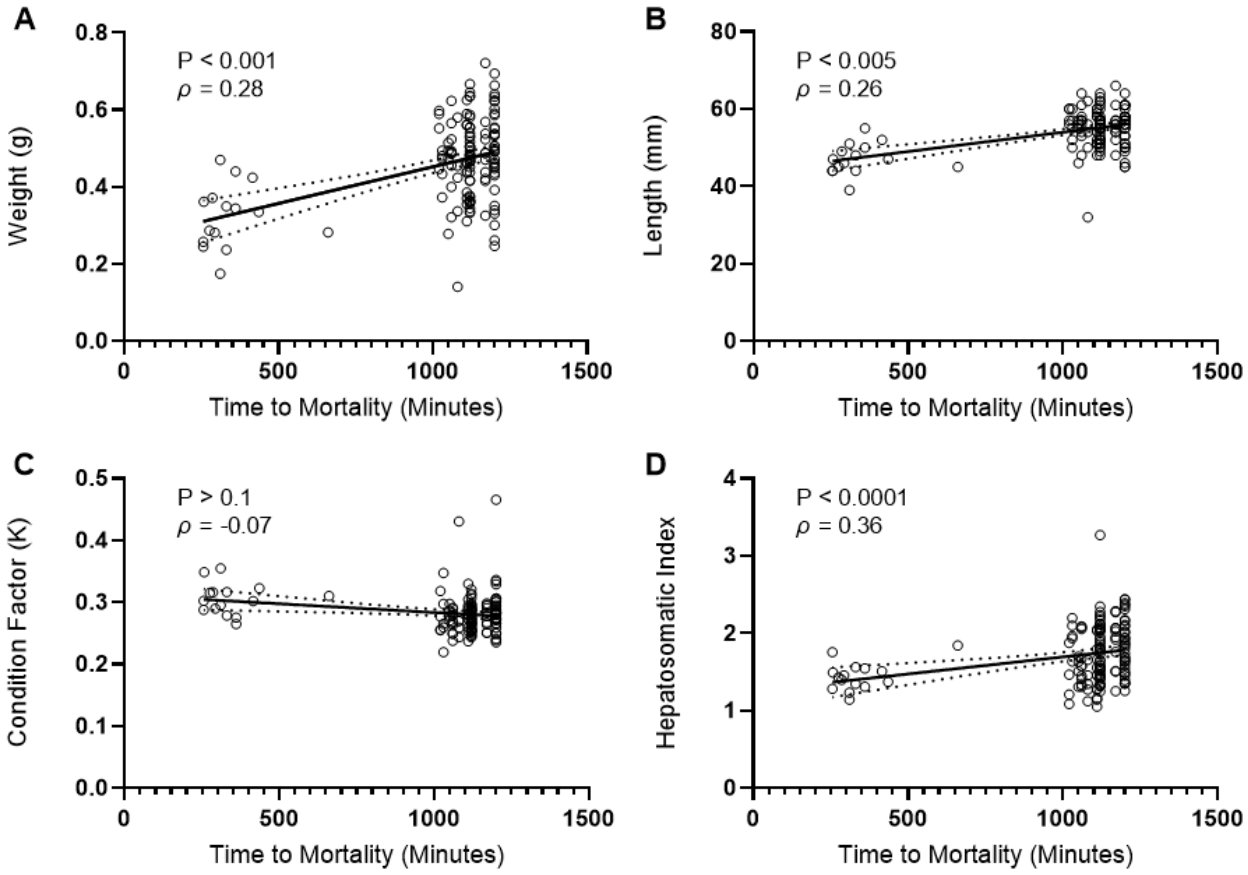

Fig. S2. The relationship between A) weight (g), B) length (mm), C) condition factor (K) and D) hepatosomatic index (HSI) and time to mortality of 20˚C acclimated developing lake sturgeon, *Acipenser fulvescens*, following exposure to 60 μg.ml^{-1} lipopolysaccharides. Significance was determined by Spearman's correlation. The solid line throughout the graph represents the best fit straight line surrounded by dotted lines representing the 95% confidence interval. Open circles represent individual lake sturgeon (n = 142).

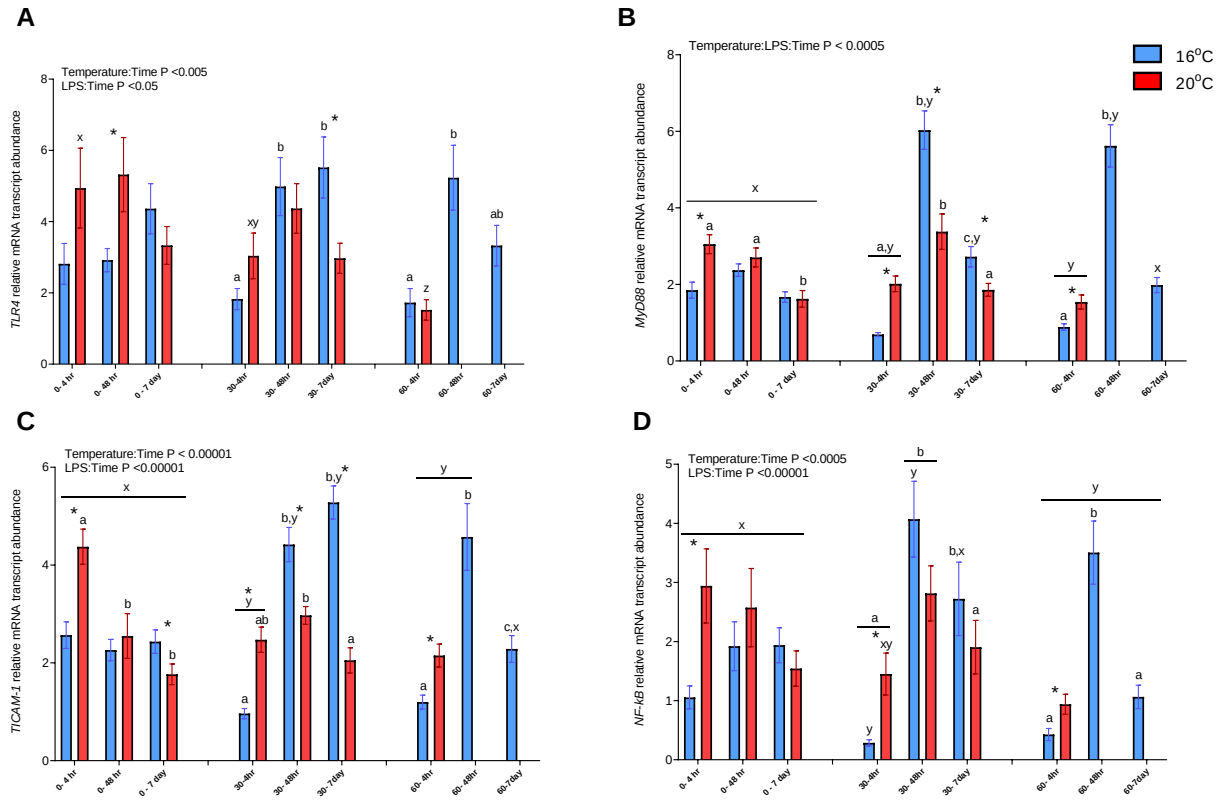

Fig. S3. Whole-body mRNA transcript abundance of genes involved in pathogen detection A) *Toll-like Receptor 4*, B) *MyD88*, C) *TICAM-1*, and D) *NF-kB* in developing lake sturgeon, *Acipenser fulvescens*, acclimated to 16 and 20°C and then exposed to 0, 30, and 60 μg.ml^{-1} of bacterial lipopolysaccharides, over a timeseries of 4 h exposure, 48 h exposure, and following a 7-day lipopolysaccharides-free recovery. Asterisks represent significance between acclimation treatments. Lowercase letters a, b, c represent significance between timepoints, within an exposure concentration and timepoint. Lowercase letters x, y, z represent significance in a given timepoint and acclimation temperature, throughout exposure concentrations ($P < 0.05$; three-factor ANOVA). Data are expressed as +/- SEM (n = 8-10).

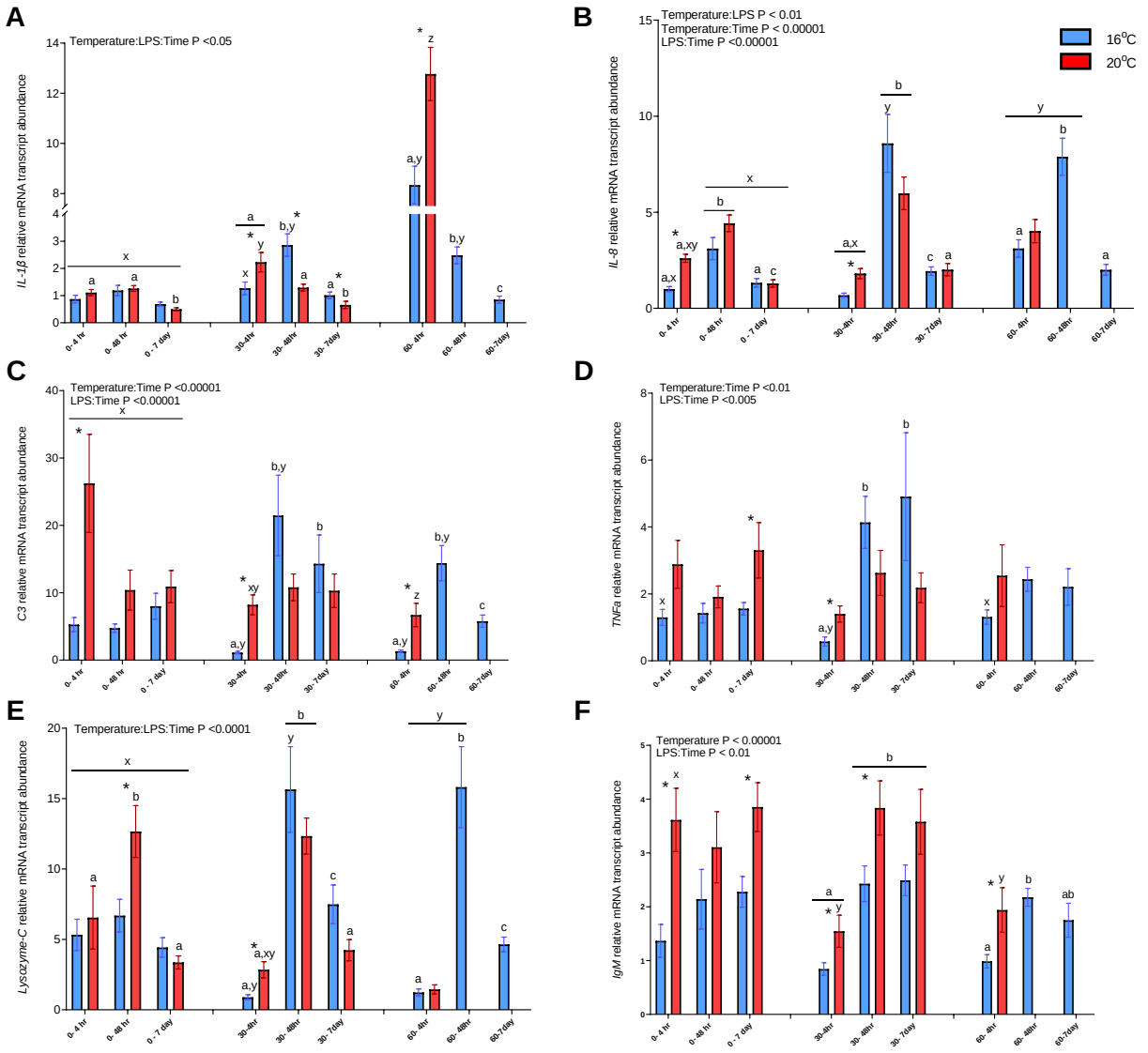

Fig. S4. Whole-body mRNA transcript abundance of genes involved in the innate immune response A) *IL-1β*, B) *IL-8*, C) *C3*, D) *TNFa*, E) *Lysozyme-C*, and F) *IgM* in developing lake sturgeon, *Acipenser fulvescens*, acclimated to 16 and 20°C and then exposed to 0, 30, and 60 μg.ml^{-1} of bacterial lipopolysaccharides, over a timeseries of 4 h exposure, 48 h exposure, and following a 7-day lipopolysaccharides-free recovery. Asterisks represent significance between acclimation treatments. Lowercase letters a, b, c represent significance between timepoints, within an exposure concentration and timepoint. Lowercase letters x, y, z represent significance in a given timepoint and acclimation temperature, throughout exposure concentrations ($P < 0.05$; three-factor ANOVA). Data are expressed as +/- SEM (n = 9-10).

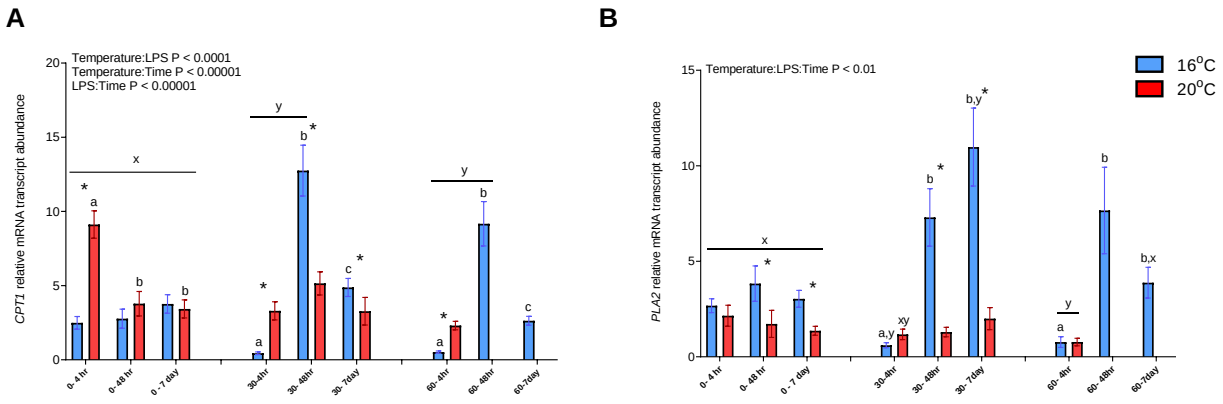

Fig. S5. Whole-body mRNA transcript abundance of genes involved in the fatty acid response A) *CPT1* and B) *PLA2* in developing lake sturgeon, *Acipenser fulvescens*, acclimated to 16 and 20°C and then exposed to 0, 30, and 60 µg.ml^{-1} of bacterial lipopolysaccharides, over a timeseries of 4 h exposure, 48 h exposure, and following a 7-day lipopolysaccharides-free recovery. Asterisks represent significance between acclimation treatments. Lowercase letters a, b, c represent significance between timepoints, within an exposure concentration and timepoint. Lowercase letters x, y, z represent significance in a given timepoint and acclimation temperature, throughout exposure concentrations ($P < 0.05$; three-factor ANOVA). Data are expressed as +/- SEM (n = 8-10).

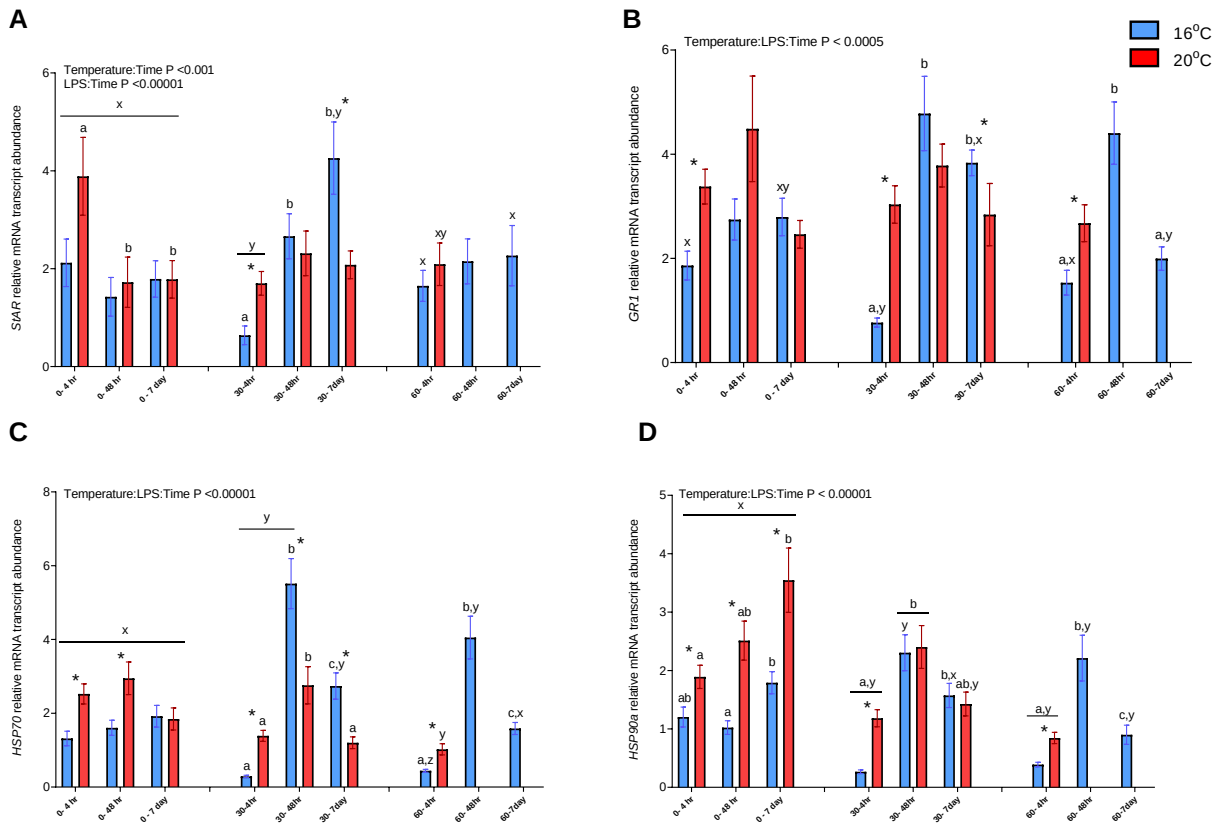

Fig. S6. Whole-body mRNA transcript abundance of genes involved in the glucocorticoid stress response A) *StAR*, B) *GR1*, C) *HSP70* , D) *HSP90a* in developing lake sturgeon, *Acipenser fulvescens*, acclimated to 16 and 20°C and then exposed to 0, 30, and 60 μg.ml^{-1} of bacterial lipopolysaccharides, over a timeseries of 4 h exposure, 48 h exposure, and following a 7 day lipopolysaccharides-free recovery. Asterisks represent significance between acclimation treatments. Lowercase letters a, b, c represent significance between timepoints, within an exposure concentration and timepoint. Lowercase letters x, y, z represent significance in a given timepoint and acclimation temperature, throughout exposure concentrations ($P < 0.05$; three-factor ANOVA). Data are expressed as +/- SEM (n = 7-10).

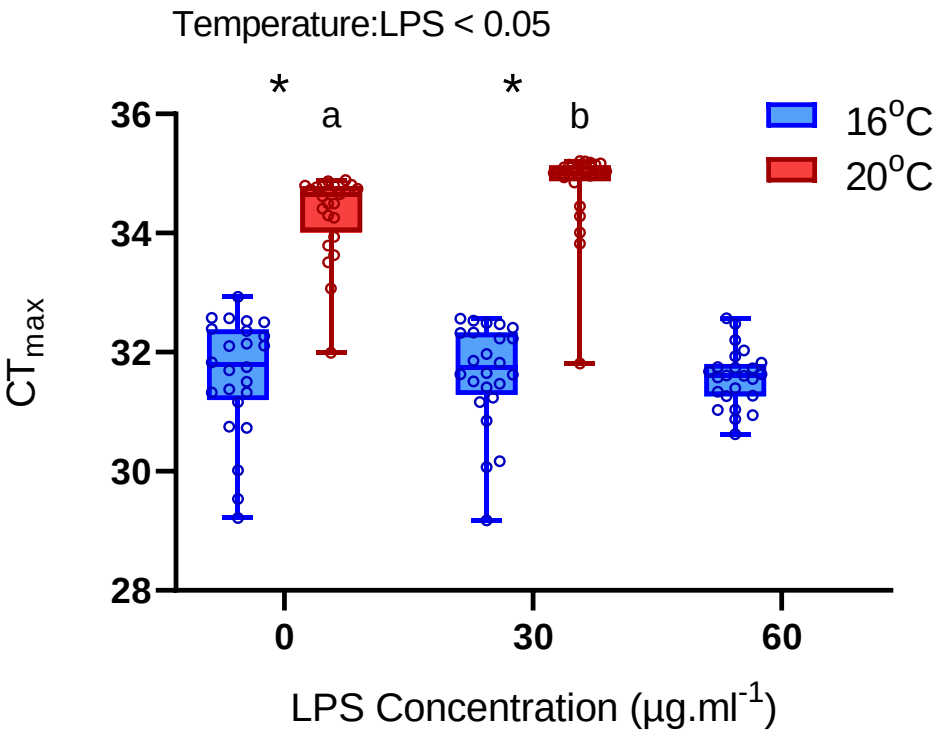

Fig. S7. Critical thermal maximum (CT_{max}) of 16 and 20°C acclimated developing lake sturgeon, *Acipenser fulvescens*, following a 7-day recovery from 48 h exposure trials in lipopolysaccharide concentrations of 0, 30, and 60 µg.ml^{-1}. Differences between treatments were determined by two-factor ANOVA ($P < 0.05$) followed by Tukey's honestly significant different post-hoc test. *'s represent significance between 16 and 20°C acclimation treatments within a lipopolysaccharide exposure concentration. Lowercase letters a and b represent significance across treatment concentrations within a single acclimation treatment (n = 24).

Table S1.

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