Effects of acclimation temperature on the thermal physiology in two geographically distinct populations of lake sturgeon (*Acipenser fulvescens*)

William S. Bugg*, Gwangseok R. Yoon, Alexandra N. Schoen, Andrew Laluk, Catherine Brandt, W. Gary Anderson and Ken M. Jeffries

Department of Biological Sciences, University of Manitoba, 50 Sifton Road, Winnipeg, Manitoba, R3T 2N2, Canada

*Corresponding author: Department of Biological Sciences, University of Manitoba, 50 Sifton Road, Winnipeg, Manitoba, R3T 2N2, Canada.
Email: buggw@myumanitoba.ca

Temperature is one of the most important abiotic factors regulating development and biological processes in ectotherms. By 2050, climate change may result in temperature increases of 2.1–3.4°C in Manitoba, Canada. Lake sturgeon, *Acipenser fulvescens*, from both northern and southern populations in Manitoba were acclimated to 16, 20 and 24°C for 30 days, after which critical thermal maximum (CT\textsubscript{max}) trials were conducted to investigate their thermal plasticity. We also examined the effects of temperature on morphological and physiological indices. Acclimation temperature significantly influenced the CT\textsubscript{max}, body mass, hepatosomatic index, metabolic rate and the mRNA expression of transcripts involved in the cellular response to heat shock and hypoxia (*HSP70, HSP90a, HSP90b, HIF-1α*) in the gill of lake sturgeon. Population significantly affected the above phenotypes, as well as the mRNA expression of Na\textsuperscript{+}/K\textsuperscript{+} ATPase α1 and the hepatic glutathione peroxidase enzyme activity. The southern population had an average CT\textsubscript{max} that was 0.71 and 0.45°C higher than the northern population at 20 and 24°C, respectively. Immediately following CT\textsubscript{max} trials, mRNA expression of *HSP90a* and *HIF-1α* was positively correlated with individual CT\textsubscript{max} of lake sturgeon across acclimation treatments and populations (\(r = 0.7, r = 0.62\), respectively; \(P < 0.0001\)). Lake sturgeon acclimated to 20 and 24°C had decreased hepatosomatic indices (93 and 244% reduction, respectively; \(P < 0.0001\)) and metabolic suppression (27.7 and 42.1% reduction, respectively; \(P < 0.05\)) when compared to sturgeon acclimated to 16°C, regardless of population. Glutathione peroxidase activity and mRNA expression Na\textsuperscript{+}/K\textsuperscript{+} ATPase α1 were elevated in the northern relative to the southern population. Acclimation to 24°C also induced mortality in both populations when compared to sturgeon acclimated to 16 and 20°C. Thus, increased temperatures have wide-ranging population-specific physiological consequences for lake sturgeon across biological levels of organization.

**Key words:** Lake Sturgeon, mRNA expression, population-specific responses, metabolic rate, acclimation

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Introduction

Across Canada, mean annual and seasonal temperatures have increased between 1.7 and 2.3°C from 1948 to 2016, with the largest increases occurring in northern Canada (Zhang et al., 2019; Vincent et al., 2015). By 2050, mean annual water temperatures within Manitoba, where several endangered populations of lake sturgeon exist, are projected to increase by 2.1–3.4°C (Manitoba Hydro, 2015). As lake sturgeon are a long-lived species, which may require as long as 18–28 years to mature (COSEWIC, 2006; Scott and Crossman 1998), individuals in Manitoba today may live to see the effects of increased environmental temperatures, such as those projected for 2050. Furthermore, their progeny will most certainly experience elevated temperatures. As temperature is a critical factor impacting fish development (Aloisi et al., 2019; Piper et al., 1982), it is important to understand how future increased environmental temperatures will affect the development and physiology of this iconic species.

Lake sturgeon populations in Manitoba range throughout the province, from the Winnipeg River in the south, and northward to the outlet of the Nelson and Churchill Rivers into the Hudson Bay (Manitoba Hydro, 2016). Historical barriers exist in waterways between populations, which have limited gene flow (McDougall et al., 2017) and contributed to genetically distinct populations. These latitudinally separated riverine environments likely have differing temperature profiles that may influence population-specific life-history traits like the growth and thermal plasticity of these genetically distinct populations (Pollock et al., 2015). Since the mid-1990s, stocking of hatchery-reared lake sturgeon has been conducted to bolster the remaining wild populations in Manitoba (McDougall et al., 2014). At the lake sturgeon hatchery in Grand Rapids, Manitoba, located at the North end of Lake Winnipeg, northern fish are typically reared at 16°C due to hatchery heating limitations, but the hatchery intends to increase rearing temperatures in the future with system upgrades. However, we know little regarding the potential effect that increased rearing temperatures may have on thermal physiology of lake sturgeon throughout development and post-release.

Temperature is a critical abiotic factor for fishes, influencing their biochemical processes, physiology, development, range distributions, community dynamics and ultimately survival (Boltaña et al., 2017; Crozier et al., 2007; Li et al., 2015; O’Gorman et al., 2016; Pankhurst and Munday, 2011; Schulte, 2015). The effects of increased environmental temperatures on teleosts have been well documented, demonstrating changes in behaviour (Forsatkari et al., 2016), swimming performance (Johnson and Bennet, 1995), reproductive potential (Donelson et al., 2010), metabolism (Johnston and Dunn, 1987), tolerance to environmental stressors (McBryan et al., 2016), immune response (Dittmar et al., 2013; Makrinos and Bowden, 2016) and mRNA expression of thermally responsive genes (Fangue et al., 2006; Campos et al., 2013; Jeffries et al., 2018). In contrast to teleosts, the effects of increasing environmental temperatures on sturgeon species are poorly understood, especially in the mRNA expression of key regulatory genes involved in responses to heat shock, hypoxia and osmoregulatory disruption. However, sturgeon oxygen consumption and transcriptional responses have been shown to increase under thermally stressful conditions (Yusishen et al., 2020; Zhang et al., 2017). Additionally, increasing temperatures affect the early development of white sturgeon, *Acipenser transmontanus* (Cech et al., 1984; Cheung, 2019) and pallid sturgeon, *Scaphirhynchus albus* (Kappenman et al., 2013); haematology, development, and thermal maxima of shortnose sturgeon, *Acipenser brevisrostrum* (Kappenman et al., 2013; Zhang and Kieffer, 2014; Ziegeweid et al., 2008); bioenergetic performance and haematocrit levels of green sturgeon, *Acipenser medirostris* (Mayfield and Cech Jr, 2004; Sardella et al., 2008); growth and stress of Siberian sturgeon, *Acipenser baerii* (Aidos et al., 2020); and movement patterns, predation rates, cortisol levels, and condition factor in lake sturgeon (Moore et al., 2020; Wassink et al., 2019; Yoon et al., 2019; Zubair et al., 2012).

Many cellular, physiological and behavioural changes made by fishes as a result of increased environmental temperatures are assessed by changes in mRNA expression of genes associated with acute thermal stress, resultant cellular damage and acclimation such as heat-shock proteins (*HSP70, HSP90a, HSP90b*) (Fangue et al., 2006; Komoroske et al., 2015; Lund et al., 2002; Shi et al., 2015). Additionally, changes in genes associated with hypoxia tolerance (*HIF-1α*) and osmoregulation (*Na+/K+ ATPase-α1*) have implicit roles in the cellular response to thermal stress (Jeffries et al., 2014; McBryan et al., 2013; Portner, 2010; Vargas-Chacoff et al., 2018). Furthermore, changes in condition factor, hepatosomatic index (HSI), metabolic rate and glutathione peroxidase (GPx) activity have been shown to relate to increasing environmental temperatures. Condition factor can fluctuate with temperature changes as well as seasonally due to abiotic and biotic factors (Giosa, et al., 2014; Mazumder et al., 2016). Thus, HSI can be a useful additional indicator of changes in body condition and metabolism as well as glycerogen and lipid reserves (Chellappa et al., 1995) that are likely to change in the liver with variation observed in natural populations once temperature thresholds and upper physiological limits have been reached (Purchase and Brown, 2001; Morrison et al., 2020). Similarly, metabolic rates (both routine and maximum) are additionally affected by environmental temperature in many fishes (Norin and Clark, 2015) including sturgeons (Yoon et al., 2018; Zhang and Kieffer, 2017) and likely play a role in the distribution of a species (Payne et al., 2015). Hepatic glutathione peroxidase (GPx) detoxifies oxidatively damaging peroxides formed as a result of acute and chronic thermal stress (Halliwell and Gutteridge, 1999) with increased mRNA expression and enzyme activity demonstrated with increased temperatures (Almroth et al., 2015; Dorts et al., 2012). The ability of lake sturgeon to make physiological changes to acclimate to their warming environment in response to thermal stress,
i.e. phenotypic plasticity, is crucial in an ever-changing environment and may be a key predictor for a species future success (Rodgers et al., 2018; Somero, 2010; Seebacher et al., 2015; Gabriel et al., 2005). Divergent population-specific responses of these physiological parameters may be anticipated in sturgeon populations from differing thermal environments as have been observed in other geographically separated populations (Fangue et al., 2006; Geerts et al., 2014; Pereira et al., 2017; Yampolsky et al., 2014).

The aim of this study was to use measurements of key physiological and molecular variables to evaluate the thermal plasticity of juveniles from different populations of lake sturgeon in Manitoba, Canada (Fig. 1). We acclimated lake sturgeon from both northern (Burntwood River—BR) and southern (Winnipeg River—WR) populations to three environmentally relevant thermal regimes of 16, 20 and 24°C. As these two geographically distinct populations of lake sturgeon in Manitoba have independent environmental and genetic histories, we hypothesized that they would exhibit divergent population-specific responses to acclimation and acute thermal stress. We predicted that the southern WR population of lake sturgeon, with greater thermal variation and range, would demonstrate increased thermal plasticity compared to their northern BR counterparts as demonstrated in other species (Fangue et al., 2006; Geerts et al., 2014; Pereira et al., 2017; Yampolsky et al., 2014). Additionally, we predicted that these differences in plasticity would be apparent in the critical thermal maximum (CTmax) of lake sturgeon, and the differential expression of mRNA transcripts important in the response to heat shock, hypoxia and osmoregulatory disruption (HSP70, HSP90a, HSP90b, HIF-1α and Na+/K+ ATPase-α1). Furthermore, we predicted temperature-dependent population-specific responses in body size, HSI, metabolic rate and GPx activity as observed in other species under thermally stressful environmental conditions.

Materials and methods

River temperatures

River water temperatures were measured in both the WR and BR in 2019 by a DigiTemp SDI-12 subsensible temperature sensor (Forest Technology Systems; Victoria, British Columbia, Canada) and a series 500 SDI-12 transducer (TE Connectivity; Schaffhausen, CH), respectively. Water temperature measurements in the WR were recorded downstream of the Pointe du Bois Generating Station on the WR (50° 17′ 52″ N, 95° 32′ 5″ W), and below first rapids on the BR (56° 02′ 46.5″ N, 96° 54′ 18.6″ W). Eggs and sperm from the WR were transported to the University of Manitoba animal holding facility where fertilization took place, whilst those from the BR population were fertilized at the Grand Rapids Fish Hatchery (53° 09′ 25.9″ N, 99° 17′ 21.9″ W). Individuals from the WR population were the product of fertilization of eggs from two females with the sperm from two males (two maternal families). Individuals from the BR population were the product of the fertilization of eggs from one female with the sperm from six males (one maternal family). Spawning individuals, particularly in the northern BR population, were limited due to the remote location of the spawning site and also the declining population, with a total adult population estimated between 112 and 579 sturgeon fish to sturgeon (Lacho and Hrenchuk, 2018). Additionally, the number of families in the southern WR population was limited due to the number of wild-caught spawning females that were able to be sampled in 2019. Post-fertilization, embryos from both populations were de-adhered by submerging embryos in a clay solution (Fullers Earth; Earhart et al., 2020) and gently stirred by hand for 1 h. Embryos were then rinsed with de-chlorinated freshwater and incubated in tumbling jars at 12°C until hatch, which occurred at ~9 days post-
fertilization (DPF). Post-hatch, larvae of equal numbers from each maternal family were transferred to a total of four 9 L flow-through aquaria, two tank population−1, with aeration and bio-balls as substrate. The temperature was maintained at 12°C until 13 DPF, after which temperature was increased at 1°C day−1 until 16°C to match hatchery rearing conditions. Freshly hatched artemia (Artemia International LLC; Texas, USA) was provided as a starting diet at 19 DPF, prior to complete yolk-sac absorption, after which tank substrate was removed over a 7-day period. Lake sturgeon were fed to satiation three times daily on a diet of artemia throughout the experiment. 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

Beginning at 30 DPF, lake sturgeon from each population were acclimated to treatments of 16, 20 and 24°C at a density of ~70 sturgeon 9 L aquaria−1 (Fig. 2). As there were more families, and thus more individual sturgeon from the WR population, lake sturgeon from the WR were reared in four tanks, whilst BR sturgeon were reared in two tanks for each acclimation treatment, to keep stocking density equal across populations throughout acclimation. In the 20 and 24°C treatments, the water temperature was increased from 16°C at a rate of 1°C day−1 until the desired acclimation temperature was reached. Lake sturgeon remained at these acclimation temperatures for 30 days, and the temperature was recorded every 15 min by HOBO Water Temperature Pro v2 Data Loggers (Onset Computer Corporation; Bourne, MA, USA). Mortality as well as temperature via thermometer was recorded at least three times daily. After 29 days of acclimation, fish were fasted for a 24-h period prior to beginning CTmax trials (Downie and Kieffer 2016; Downie et al., 2018; Lee et al., 2016).

At the end of the acclimation, prior to CTmax trials, eight fish from each treatment were haphazardly selected and euthanized by immersion in an overdose of tricaine methanesulfonate solution (250 mg L−1; MS-222, Syndel Laboratory, Vancouver, Canada) buffered with an equal volume of sodium bicarbonate. Gill tissue was then extracted, preserved in RNA later (Thermo Fisher Scientific, Waltham, USA), and stored at −80°C prior to the quantification of mRNA transcripts. An additional 10 fish were haphazardly selected and euthanized from each acclimation treatment; body mass (weighed to 0.0001 g) and total length (measured to nearest 1 mm) were recorded for each individual, as well as liver wet mass (weighed to 0.0001 g) which was used to calculate the HSI as the ratio of the wet mass of the liver (Wliver) to the wet mass of the body (Wbody):

\[
HSI = \frac{W_{\text{liver}}}{W_{\text{body}}} \times 100
\]

### Critical thermal maximum trials

On the day of the CTmax trials, eight fish were haphazardly selected from acclimation tanks and placed individually in experimental units (~200 ml of water volume and 9.5 cm long × 5 cm across) in an aerated recirculating water bath initially set to the acclimation temperature of the treatment being tested. Experimental units had mesh-screened sides to allow for water flow through each unit. Eight fish were tested trial−1, with three to four trials conducted over two consecutive days for each experimental treatment. CTmax trials were conducted at the same time every day, between 9 am and 12 pm to avoid any confounding effects of diurnal shifts on physiology and gene expression (Lankford et al., 2003; Somero, 2020). The temperature of the water bath was regulated by an Isotemp recirculating heater (Fisher Scientific; Hampton, USA) whilst water temperature was constantly recorded by a temperature probe placed in the centre of the experimental setup (Witrox Oxygen probe, Loligo Systems; Viborg, Denmark). Fish were held in these experimental units for 1 h prior to the CTmax trials to reduce the potential effects of handling stress. After 1 h, trials began by increasing the temperature of the water bath by 0.3°C min−1 until fish were unable to right themselves after a physical disturbance (Bard and Kieffer, 2019; Beiting et al., 2000; Yoon et al., 2019; Yusishen et al., 2020). When fish were unable to right themselves, the final CTmax temperature was recorded, the fish was euthanized, mass and length was recorded and the gill tissue was removed and preserved as previously described.
Liver tissue was then sampled, immediately flash-frozen in liquid nitrogen, and stored at −80°C until use for measuring GPx activity. An additional eight fish from a single trial were placed individually into 9 L tanks in a Multi-Stressor Unit (AquaBioTech; Coaticook, Quebec, Canada) at their respective acclimation temperature after their CTmax was reached and allowed to recover for 3 days before tissue sampling. During this 3-day recovery period, lake sturgeon were fed and were observed actively feeding on freshly hatched artemia three times daily. From average CTmax values, an acclimation response ratio, the rate an organism increases their CTmax in response to acclimation, was calculated for each population, subtracting the average CTmax of the 16°C acclimation treatment (CTmax16°C) from that of the 24°C treatment (CTmax24°C) and dividing by the change in acclimation temperature between treatments (Δ°C):

\[
Acclimation \ response \ ratio = \frac{CT_{max24^\circ C} - CT_{max16^\circ C}}{\Delta^\circ C}
\]

Metabolic rate

Measurements of whole-body metabolic rate (MO2) were taken 3 days after each CTmax trial for a given acclimation treatment, using intermittent flow respirometry (Loligo Systems, Viborg, Denmark) as previously described (Yoon et al., 2019) with some modifications. In brief, fish were fasted for at least 12 h prior to experimentation. Flow within the chambers [volume: 43.40 ± 4.32 (mean ± S.D.) ml] was maintained at a low level to not cause any physical stress, but sufficient for water exchange and accurate measurement of MO2. The intermittent respirometry cycle was variable for each temperature treatment to ensure a linear decline in oxygen saturation more than 10%, but not below 70% as metabolic suppression was reported at this point in age 1+ lake sturgeon (Svendsen et al., 2014). For 20 and 24°C, the parameters were 360 s of flush followed by 60 s of wait and 300 s of measurement. For 16°C, 360 s of flush followed by 60 s of wait and 900 s of measurement was used. The respirometry chambers were surrounded by a black curtain to minimize any visual disturbance to the fish during each trial. Routine metabolic rate (RMR) was assessed for 2 h following a 4-h period in the metabolic chamber to minimize the effects of transfer. After RMR was assessed, fish were removed from the chambers and a standardized chase protocol was performed for 15 min. Then, fish were immediately returned to the same chambers, and MO2 was measured for the following two measurement cycles. Biological oxygen demand (BOD) was measured before and after each experiment. Assuming a linear increase, pre- and post-experiment BOD data points were used to linearly interpolate BOD over the experiment period and all MO2 data was corrected by the corresponding BOD. Only slopes of oxygen decline with R² ≥ 0.9 were used for data analysis. RMR was calculated by averaging MO2 measured for 2 h whilst the maximum metabolic rate (MMR) was chosen as the highest MO2 after the chase protocol. Factorial aerobic scope (FAS) was calculated by dividing MMR by RMR. There was no 24°C acclimation treatment available for the BR population for MMR, RMR or FAS as there were insufficient numbers of lake sturgeon remaining to conduct these trials.

RNA extraction, cDNA synthesis and qPCR

Gills from lake sturgeon were homogenized in 500 μl of lysis buffer, for 10 min at 50 Hz using a TissueLyser II (Qagen; Germantown, MD, USA). Total RNA was extracted from gill homogenates using a PureLink RNA Mini Kit (Invitrogen; Ambion Life Technologies) following the manufacturer’s instructions. Total RNA purity and concentration were evaluated for all samples using a NanoDrop One (Thermo Fisher Scientific) followed by gel electrophoresis to assess RNA integrity. Post-extraction, RNA samples were stored at −80°C. cDNA was synthesized from 1 μg of DNase treated total RNA using a qScript cDNA synthesis kit following the manufacturer’s instructions (Quantabio; Beverly, Massachusetts). Synthesis was conducted using a SimpliAmp Thermal Cycler (Thermo Fisher; Waltham, Massachusetts) with cycling conditions of 1 cycle of 22°C for 5 min, 1 cycle of 42°C for 30 min and 1 cycle of 85°C for 5 min and hold at 4°C. Following synthesis, cDNA samples were stored at −20°C.

Real-time quantitative polymerase chain reaction (RT-qPCR) for each gene of interest, HSP70, HSP90a, HSP90b, HIF-1α and Na+/K+ ATPase-α1, was conducted using 5 μl of Bio-Rad SsoAdvanced Universal SYBR Green Supermix, 0.1 to 0.04 μl of 100 μM primers, 2 μl of diluted cDNA per sample and nuclease-free water adjusted for each assay to bring the total volume of each well to 10 μl (Table 1). For all experimental assays except HIF-1α, each well contained 0.025 μl forward and 0.025 μl reverse primer, whilst this was doubled to 0.05 μl forward and 0.05 μl reverse for each reference gene. For HIF-1α, 0.02 μl forward and 0.02 μl reverse primer were used. The cDNA of all samples was diluted 1:10 with nuclease-free water for all RT-qPCR assays. All primers were designed based on sequences from an annotated transcriptome produced by the pyrosequencing of a lake sturgeon ovary (Table 1; Hale et al., 2009). The expression of the genes of interest was normalized to the relative expression of reference genes RPS6 and RPL7 and then analyzed after applying the 2^ΔΔCt method described by Livak and Schmittgen (2001). Expression of all genes was then normalized to that of the WR 16°C acclimation treatment prior to CTmax trials in order to make comparisons between populations. The WR 16°C acclimation treatment was chosen as a reference based on hatchery rearing conditions and its relatively low levels of mRNA expression across acclimation treatments, time points and genes of interest.

Glutathione peroxidase activity assays

Initial extraction of GPx from the lake sturgeon livers was conducted by homogenizing tissues in ice-cold homogeniza-
Table 1: Primer sequences for lake sturgeon, Acipenser fulvescens, HSP70, HSP90a, HSP90b, HIF-1α, Na+/K+ ATPase-α1, RPS6 and RPL7

| Gene | Forward | Reverse | Efficiency (%) |
|------|---------|---------|----------------|
| HSP70 | CTGTCATCGGATCTTAATTT | AACTGTCTATAGAATGGCCTTATCC | 95.1 |
| HSP90a | GATCAGGACGGGATGCG | ATGGTGCTGTCCTGCG | 96.8 |
| HSP90b | GGAACACAGGCTTCATGGAG | CCAACACAAACTGACCAATCA | 94.9 |
| HIF-1α | GCAAAAGTCATGGTGCAT | GGCAGCTTCATGTATGAGT | 98.2 |
| Na+/K+ ATPase-α1 | TCGATGCTTACACCTGAC | TGCCCCAAGTCATACACAGGG | 93.8 |
| RPS6 | CTGCTGGATTCTGATTGATG | ATCTGATTGACCAAGCTGCTG | 93.0 |
| RPL7 | TGCTTACATTGAGTGACCG | GATCTCTCGTGACCCGGTT | 92.7 |

Target genes were chosen based on their roles in the response to heat shock, cellular stress, hypoxia and osmoregulatory disruption. RPS6 and RPL7 were used as reference genes and showed stable expression across treatments. Efficiencies are listed as a percentage.

Statistical analysis

Differences in mortality between treatments and populations were analyzed via Cox proportional hazards model in R v3.6.2 (R core Team, 2013) using the ‘survival’ and ‘survminer’ packages (Kassambara et al., 2019; Therneau, 2015). Assumptions for all Cox proportional hazard models were assessed using the ‘cox.zph’ function included in the ‘survival’ package, to ensure that residuals were independent of time. First, a Cox proportional hazards model was run with only the effect of temperature included in the model, to isolate the effect that different thermal environments had on lake sturgeon regardless of population. Next, in addition to a Cox proportional hazards model with covariates of temperature and population included, the ‘pairwise_survdiff’ function from the ‘survminer’ package with the same covariates and a Bonferroni correction was used to compare the mortality across treatments and populations of lake sturgeon.

CTmax data was analyzed using non-parametric statistical tests as it could not be transformed to adequately pass the Levene’s test. Thus, Kruskal–Wallis multiple comparison tests and a Bonferroni correction were used to determine significance within populations and across acclimation treatments, using the ‘dunn.test’ package (Dinno, 2017). Finally, the Wilcoxon signed-rank test was used to determine significance between populations, within a single acclimation treatment. As CTmax data violates assumptions of parametric tests, Spearman’s correlations were used to analyze the relationship between expression of the mRNA transcripts and GPx activity at each relevant time point to the CTmax of individual lake sturgeon across acclimation treatments and populations. Only significant correlations are reported.

Morphometrics including mass, length, condition factor and HSI, as well as measurements of metabolic rate were analyzed using a two-factor ANOVA with population and acclimation treatment and their interaction included in the model as fixed effects. Three-factor ANOVAs were used to analyze GPx activity and mRNA expression of HSP70, HSP90a, HSP90b, HIF-1α and Na+/K+ ATPase-α1 with population, acclimation treatment and time as well as their interactions included in the model as fixed effects.

For all ANOVAs, Shapiro–Wilk’s and Levene’s tests were used to assess the normality of data and homogeneity of the variance, respectively. Normality was also visually inspected using fitted residual plots. If assumptions of normality or homogeneity were violated, either a ranked, log or square root transformation was applied to the data set. Additionally, for each ANOVA, the effect of the rearing tank was assessed and found to be not significant; therefore, it was not included in final models. Detailed results can be found for all statistical analyses (see Supplementary Material). Following ANOVAs, multiple comparison tests were performed and corrected with Tukey’s honestly significant difference tests from the ‘mult-
Figure 3: Temperature profiles of two distinct lake sturgeon, *Acipenser fulvescens*, populations. Data for both rivers was measured in 2019 at midnight and % time over-temperature threshold comparisons between the WR and BR populations were based on days where data is available for both populations, 23 May 2019 to 31 December 2019. Tick marks on the x-axis indicate the middle of each given month. Dashed lines indicate the different temperature thresholds and acclimation temperatures used in the current study. The first dotted line at 16°C represents current hatchery conditions and the first acclimation treatment. The second dotted line at 20°C indicates potential increased hatchery temperatures, approximate temperatures that populations are currently exposed to and the second acclimation treatment. The third dotted line at 24°C represents future warming conditions that may be expected in Manitoba, Canada, by 2050.

comp’ package (*Hothorn et al.*, 2008). All statistical analyses were performed with a significance level of 0.05.

**Results**

**River temperatures**

In 2019, the WR temperatures exceeded 20°C on 17.6% of measured days, with 36.7% above 16°C, and 63.2% below 16°C. In contrast, the BR was never recorded above 20°C, with temperatures above 16°C recorded on 14.7% of days and 85.3% of days below 16°C (Fig. 3). Days exceeding 16°C were consecutive for both populations, above this threshold in the WR for 100 days from 20 June to 28 September and in the BR for 40 days from 11 July to 20 August. Temperatures in the WR were above 20°C for 51 days from 8 July to 28 August. Throughout the summer, 21 June to 21 September, when larval lake sturgeon are developing, the BR was on average 4.6 ± 0.8°C colder than the WR.

**Mortality**

Lake sturgeon reared at 24°C had elevated mortality compared to those at 16 and 20°C, with a hazard ratio of 5.37 (*P* < 0.0001). The BR and WR lake sturgeon had increased mortalities as temperatures increased, with 4.8, 6.9 and 25.5% mortality and 3.3, 3.3 and 15% (*P* < 0.05; *n* = 145 treatment⁻¹) in 16, 20 and 24°C, respectively.

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**Morphometrics**

There were significant effects of population and acclimation treatment on mass and length of lake sturgeon (*P* < 0.001) as well as an interaction of population and acclimation treatment for mass (*P* < 0.05) and a near interaction for length (*P* < 0.06). Lake sturgeon from the BR population had a body mass of 0.62 g in 16°C increased to 0.92 g in 20°C and were larger when compared to WR fish in both treatments (*P* < 0.01; Table 2). The BR lake sturgeon had significant increases in mass in 20 (47.1%) and 24°C (46.9%) when compared to 16°C (*P* < 0.001). In contrast, lake sturgeon from the WR had increased mass with each acclimation treatment, with individuals in 20°C heavier than the those in 16°C (*P* < 0.05) and those in 24°C heavier than both lower treatments, 84.3 and 32.8%, respectively (*P* < 0.001).

Sturgeon from the BR population had increased body length of 15.4% in the 20°C acclimation treatment when compared to WR lake sturgeon (*P* < 0.001; Table 2). The BR lake sturgeon had significant increases in length in 20 and 24°C treatments when compared to 16°C (*P* < 0.0001). In contrast, sturgeon from the WR had increased length with each increasing acclimation treatment. Individuals at 20°C were 8.5% longer than those at 16°C whilst those at 24°C were longer than both lower treatments, 23.4 and 13.7% respectively (*P* < 0.005). There was a significant interaction between population and acclimation treatment on condition factor in lake sturgeon (Table 2; *P* < 0.05). At 20°C, WR
Table 2: Morphometrics of WR and BR lake sturgeon, *Acipenser fulvescens*, across acclimation treatments

| Acclimation treatment (°C) | Winnipeg River | Burntwood River |
|---------------------------|----------------|-----------------|
| Mass (g)                  |                |                 |
| 16                        | 0.49 ± 0.07* (10) | 0.62 ± 0.13* (16) |
| 20                        | 0.68 ± 0.14* (10) | 0.92 ± 0.16* (10) |
| 24                        | 0.90 ± 0.14* (10) | 0.91 ± 0.18* (10) |
| Length (mm)               |                |                 |
| 16                        | 53.2 ± 3.2* (10) | 55.9 ± 4.6* (16) |
| 20                        | 57.5 ± 4.7* (10) | 66.3 ± 5.6* (10) |
| 24                        | 65.4 ± 4.6* (10) | 66.9 ± 7.2* (10) |
| Fulton’s condition factor [mg mm⁻³ (100)] | | |
| 16                        | 0.33 ± 0.03 (10) | 0.35 ± 0.04 (16) |
| 20                        | 0.35 ± 0.05 (10) | 0.31 ± 0.04* (10) |
| 24                        | 0.32 ± 0.03 (10) | 0.32 ± 0.09 (10) |

Significance was determined by a two-factor ANOVA (P < 0.05) followed by Tukey’s honestly significant difference post hoc test. Asterisks represent significant differences between populations at each acclimation treatment. Letters represent significant differences within populations, across acclimation treatments. Morphometric data are expressed as mean ±/− SD (n = 10−16 per treatment—indicated by parentheses).

Lake sturgeon had condition factors 13.8% greater than BR sturgeon from the same acclimation treatment (P < 0.05; Table 2).

There was an effect of both population and acclimation treatment on the HSI of lake sturgeon (Fig. 4; P < 0.001). Sturgeon from both the WR and BR acclimated to treatments of 16°C had increased HSI when compared to fish at 20 and 24°C (P < 0.0001). The WR lake sturgeon acclimated to 16°C had hepatosomatic indices 244 and 93% higher than those acclimated to treatments of 20 and 24°C, respectively (P < 0.0001), with similar results in the BR population (P < 0.05). The BR lake sturgeon HSI was increased when compared to the WR across all acclimation treatments (P < 0.05).

**Critical thermal maximum**

Acclimation treatment and population influenced the CT_max with each subsequent acclimation treatment increasing the CT_max for each population (P < 0.05; Fig. 5). At both 20 and 24°C, the WR population had increased CT_max compared to the northern BR counterparts by 0.71 and 0.45°C, respectively (P < 0.05). Comparisons of CT_max show that the two populations of lake sturgeon have differing acclimation response ratios. The WR lake sturgeon acclimation response ratio was 0.41, whilst their BR counterparts were 0.34 over the same acclimation treatments.

**Metabolic rate**

RMR was significantly affected by both population and acclimation temperature (P < 0.0001). There were differences in RMR in the WR population between 16 and 24°C as well as 20 and 24°C (P < 0.01; Fig. 6A). In the WR 24°C acclimation treatment, there was a reduction in RMR compared to 16 and 20°C. In the BR population, there was
a 26% decrease in RMR between 16 and 20°C ($P < 0.05$). Between populations, the WR population had 28 and 70.1% higher RMR than BR in 16 and 20°C, respectively ($P < 0.01$). The MMR was significantly affected by population and acclimation temperature ($P < 0.01$). There were differences in MMR in the WR population between 16 and 24°C, as well as 20 and 24°C ($P < 0.05$; Fig. 6B). Between populations, the WR population had 29.8% higher MMR than the BR in 20°C ($P < 0.05$). In the WR population, acclimation to 24°C led to a reduction of MMR compared to the 16 and 20°C. There was an effect of acclimation temperature on FAS ($P < 0.05$; Fig. 6C). In the BR population, FAS was 29.6% higher at 20°C than 16°C ($P < 0.01$).

**mRNA transcript expression**

There was an interaction between population, acclimation treatment and time on the mRNA expression of *HSP70* ($P < 0.05$; Fig. 7A). Immediately post-CT$_{\text{max}}$ trials, mRNA expression of *HSP70* was elevated in every treatment across populations compared to pre-trial levels, and in the WR population 1.7-fold compared to the BR population at 24°C ($P < 0.05$). Three days post-CT$_{\text{max}}$, the BR population expressed *HSP70* mRNA 2.9-fold and 4.6-fold higher at 16°C than lake sturgeon from 20 and 24°C, respectively ($P < 0.05$).
significant differences in HSP90α mRNA expression across acclimation treatments for both populations. In the WR population, mRNA expression of HSP90α decreased with increasing acclimation temperature with the 16°C treatment demonstrating expression 5.3-fold higher than the 24°C acclimation treatment ($P < 0.05$). In contrast, the BR population increased expression in the 24°C treatment when compared to 16 and 20°C. These opposite patterns of HSP90α expression resulted in differences between populations within acclimation treatments of 16 and 24°C ($P < 0.05$). Within the 16°C acclimation treatment, the WR population had a 6.7-fold higher expression compared to that of the BR. In the 24°C treatment, the BR population increased expression 3.3-fold when compared to that of the WR.

Immediately post-CT$_{max}$ trials, mRNA expression of HSP90α was elevated in every treatment across populations compared to pre-trial levels and significantly increased at 24°C for both populations when compared to their respective 16°C counterparts ($P < 0.05$). The expression...
of \(HSP90a\) immediately following the \(CT_{\text{max}}\) trials was positively correlated with individual \(CT_{\text{max}}\) of lake sturgeon across acclimation treatments and populations \((p = 0.7; P < 0.0001)\).

Three days post-\(CT_{\text{max}}\) trials, mRNA expression of \(HSP90a\) was significantly elevated in the BR 16°C acclimation treatment 3.1- and 2.4-fold compared to 20 and 24°C treatments, respectively \((P < 0.05)\). There were also elevated levels of \(HSP90a\) expression in the WR 24°C acclimation treatment \((P < 0.05)\), and near significantly elevated levels in the 20°C acclimation treatment \((P < 0.06)\), when compared to the BR population.

There was an effect of treatment \((P < 0.0001)\) and a trend towards an effect of the population \((P < 0.05)\) on the expression of \(HIF-1\alpha\) mRNA (Fig. 7C). Pre-\(CT_{\text{max}}\) expression was elevated in the WR 20°C acclimation treatment 5.2-fold compared to individuals from the same population in the 16°C treatment \((P < 0.05)\). Immediately following \(CT_{\text{max}}\), 24°C treatments for both populations demonstrated elevated \(HIF-1\alpha\) mRNA expression of 5.6- and 3.6-fold relative to WR and BR lake sturgeon in 16°C, and the WR 20°C treatment had 4.3-fold higher expression than 16°C. The expression of \(HIF-1\alpha\) mRNA immediately following \(CT_{\text{max}}\) was positively correlated with individual \(CT_{\text{max}}\) of lake sturgeon across acclimation treatments and populations \((\rho = 0.62; P < 0.0001)\).

There was an effect of time \((P < 0.005)\) and a near significant effect of the population \((P < 0.05)\) on the mRNA expression of \(HSP90b\) mRNA (Fig. 7D).

There was an effect of population and time on the expression of \(Na^+/K^+\) ATPase-\(\alpha 1\) mRNA \((P < 0.05; \text{Fig. 7E})\), which demonstrated consistently elevated expression in the BR compared to the WR population across acclimation treatments and time points.

**Glutathione peroxidase activity**

There was an interaction between population, acclimation treatment and time on hepatic GPx activity \((P < 0.05; \text{Fig. 7F})\). Pre-\(CT_{\text{max}}\), the BR population demonstrated a 1.5-fold increase in GPx activity when compared to their WR counterparts at 20°C \((P < 0.05)\). Three days post-\(CT_{\text{max}}\) trials, the BR population showed a 1.8-fold increase in GPx activity compared to their WR counterparts at 16°C \((P < 0.05)\). There was also a significant trend in GPx activity in the BR population 3 days post-\(CT_{\text{max}}\), where the 24°C acclimation treatment had activity 2.4-fold higher than the 20°C acclimated lake sturgeon from the same population \((P < 0.05)\). The same 20°C acclimated BR treatment also showed lower GPx activity 3 days post-trial compared to pre- and post-trial levels \((P < 0.05)\).

**Discussion**

In the current study, we demonstrated changes in diverse physiological and molecular phenotypes in response to acclimation temperature in juvenile lake sturgeon from a northern and southern population in Manitoba. In addition, lake sturgeon populations were affected differently, with alteration of many physiological and molecular characteristics being population dependent. To the best of our knowledge, this is the first study to investigate the thermal tolerance of sturgeons across populations, sturgeon thermal tolerance at multiple levels of biological organization and lake sturgeon thermal tolerance at the molecular level.

**Physiological responses to acclimation**

Mortality was elevated in 24°C acclimation treatments, when compared to 16°C and 20°C treatments suggesting that this treatment was stressful to lake sturgeon, regardless of population. These increased rates of mortality may be indicative of increased metabolic constraints and cellular stress due to chronic thermal stress potentially leading to decreased pathogen tolerance as observed in Atlantic cod, \(Gadus morhua\), and three-spined sticklebacks, \(Gasterosteus aculeatus\), during acclimation to thermally stressful conditions \((Larsen et al., 2018; Dittmar et al., 2013)\). Additional signs of metabolic stress are apparent in the decrease in both RMR and MMR at 24°C in the WR population compared to 16 and 20°C. In the BR population, RMR was lower than WR sturgeon and decreased between 16 and 20°C; whilst in the WR, it remained consistent between 16 and 20°C. These differing thresholds for metabolic suppression between populations may be influenced by their population-specific thermal histories with the BR population experiencing lower yearly temperatures and decreased metabolic rates at lower acclimation temperatures. However, FAS increased with acclimation temperature in both populations, indicating a greater separation between MMR and RMR, potentially resultant from higher rates of metabolic development under increased temperatures. In green sturgeon, until a limiting factor such as food availability is reached, growth increases with environmental temperature \((Poletto et al., 2018)\).

In the current study, increased mass of BR lake sturgeon, when compared to WR sturgeon at 16 and 20°C, may be indicative of countergradient variation \((e.g. Fangue et al., 2009)\) as lake sturgeon from the northern BR population possibly grow faster to take advantage of shorter growing seasons. However, at 24°C, the BR population demonstrated no further increase in either mass or length suggesting an upper thermal limit for growth in this population \((e.g. Koskela et al., 1997; Oyugi et al., 2012)\). In contrast, the WR population had increased in size with each temperature. These results suggest the presence of population-specific upper thermal thresholds for lake sturgeon in Manitoba.

Investigation of HSI indicated an additional influence of elevated temperatures on the liver size in lake sturgeon with decreased HSI apparent in acclimation temperatures above 16°C for both populations. Decreases in HSI have been linked to diminishing glycogen reserves in three-spined stickleback \(Gasterosteus aculeatus\) \((Chellappa et al., 1995)\) and white sturgeon \((Hung et al., 1990)\). These differences in HSI may
be the result of a trade-off between whole-body RMR and liver function. A reduction in RMR of BR lake sturgeon may facilitate decreased energy consumption and result in an increase of hepatic glycogen and lipid reserves that were not evident in WR lake sturgeon. In the northern part of their range, lake sturgeon must survive through an extensive overwintering period wherein lipid stores likely play a critical role in survival (Bystrom et al., 2006; Yoon et al., 2019). Cold-adapted populations of fish may be better able to accumulate energy stores, especially glycogen and lipids, as observed in Atlantic cod from different thermal environments (Purchase and Brown, 2001). Thus, lower metabolic rates and higher HSI may be potential evidence of how the northern BR population copes with prolonged sub-Arctic winters (Lotka, 1922; Schaefer and Walters, 2010), with the adoption of an energy storage maximization strategy (Post and Parkinson, 2001). Consequently, rearing lake sturgeon for prolonged periods at temperatures above 16°C may not allow them to accrue these necessary glycogen and lipid reserves, thereby decreasing their ability to survive this overwintering period if released prior to winter. However, further research is necessary to confirm this observation.

Molecular responses to acclimation

In addition to physiological responses, there were population differences in the molecular responses of lake sturgeon to the acclimation treatments. At 30 days of acclimation and pre-CTmax trials, lake sturgeon from the WR and BR populations exhibited opposite patterns of HSP90a expression with a significant threshold for both populations between 20 and 24°C. In the BR population, mRNA expression of HSP90a is induced in 24°C, relative to the 20 and 16°C, whilst the WR population demonstrated suppressed mRNA expression in 24°C, relative to 20 and 16°C. These opposite patterns in HSP90a mRNA expression may be indicative of differing mechanisms used to handle chronic thermal stress as observed in redband trout, Oncorhynchus mykiss gairdneri, from different environments (Narum et al., 2013). Additionally, mRNA expression of HIF-1α in the WR 20°C acclimation treatment increased, relative to 16°C, but this increase is not observed at 24°C, potentially demonstrating a temperature-dependent threshold in this population, similar to what has been observed in Crucian carp, Carassius carassius (Rissanan et al., 2006). HIF-1α expression peaking pre-CTmax, in an inverted U-shape may be indicative of sublethal response thresholds that could be predictive of long-term impacts (Jeffries et al., 2015; Jeffries et al., 2018). It is energetically costly to produce and activate HSPs (Heckathorn et al., 1996; Sanchez et al., 1992) and HIF-1α likely has metabolic influences (Pelster and Egg, 2018; Richards, 2009). These population-specific metabolic, growth, HSI and molecular response patterns across acclimation temperatures likely indicate that northern and southern populations differently handle constrained metabolic budgets due to elevated temperatures (Somero, 2020; Tomanek and Somero, 1999). These physiological factors likely play a role in observed behavioural differences in seasonal movement patterns between the northern and southern populations of lake sturgeon across their geographic range, as fish from warmer southern climates move less in the summer than their northern counterparts (Moore et al., 2020).

CTmax

In the present study, CTmax across treatments and populations ranged from 32.2 to 35.4°C, which is within the range reported for lake sturgeon (Wilkes, 2011; Yusihen et al., 2020). The CTmax results demonstrated that lake sturgeon from the WR had increased acclimation response ratios when compared to their BR counterparts. However, both populations had acclimation response ratios higher than most species from similar and lower latitudes (Gunderson and Stillman, 2015) which indicates increased thermal plasticity, potentially based on the large genome size of lake sturgeon (Ellis et al., 2014; Fontana et al., 2004). Differences in CTmax between populations, 0.45 to 0.71°C, are potentially indicative of population-level effects and not just family effects, as families of lake sturgeon from the same river systems have exhibited ±0.18°C in CTmax (95% confidence interval; six families; six individuals per family; range 32.4–32.9°C; Deslauriers et al., in revision). As greater climate variability affects thermal plasticity in animals (Rohr et al., 2018; Seebacher et al., 2015; Somero, 2010), the increased acclimation response ratio in the southern population of lake sturgeon is potentially linked to the more variable thermal environment and greater thermal range that this population would experience.

Molecular and enzymatic responses to CTmax

Plasticity in mRNA expression of transcripts involved in response to thermal and hypoxic stressors, HSP90a and HIF-1α, was observed immediately following CTmax trials. HSP90a is a highly constitutively expressed heat-shock protein that also functions to protect cells from thermal stress by aiding in substrate recognition, cellular signalling and refolding of misfolded proteins (Iwama et al., 2004; Li et al., 2012; Mahanty et al., 2017). In the current study, there was a positive relationship between acclimation temperature and HSP90a mRNA expression post-CTmax in both populations. The mRNA expression of HSP90a was the most highly induced of all the genes measured immediately following CTmax trials (11.3- to 163-fold increase, relative to pre-trial levels). In contrast, expression of the constitutive isoform, HSP90b, did not display a similar plastic response following CTmax trials. Similar to HSP90a, a plastic response is observed in mRNA expression of HIF-1α, a transcription factor involved in the response to hypoxia and regulated by temperature in Crucian carp (Rissanan et al., 2006; Wenger, 2002). In the current study, immediately following CTmax trials, lake sturgeon from both populations at 24°C increased mRNA expression of HIF-1α relative to those acclimated to 16°C, and this expression was also correlated to individual CTmax across acclimation treatments and populations. The
WR population exhibited an increase in HIF-1α mRNA expression between 16 and 20°C, continuing at 24°C. However, this same increase was delayed in the BR population, only occurring at 24°C, possibly indicative of differing thermal thresholds for expression induction and the role that this protein may play in cross-tolerance to thermal stress (Maloyan et al., 2005), although this was not specifically addressed in this study.

The mRNA expression of HSP70 showed elevation in response to CTmax trials, with increases in expression ranging from 3- to 10-fold across acclimation treatments and populations. Changes in gill HSP70 mRNA expression can subsequently lead to much higher levels of protein expression as observed in the gills of the goby, Gillichthys mirabilis (Buckley et al., 2006; Somero, 2020). Under normal conditions in eukaryotes, HSP70 functions as a required chaperone for protein assembly (Lindquist, 1992; Roberts et al., 2010). Under times of thermal stress, HSP70 can act to bind to and stabilize proteins against misfolding and prevent intracellular aggregation, serving as an indicator of stress severity (Logan and Somero, 2011; Tomanek and Somero, 1999; Welch and Feramisco, 1985; Yamashita et al., 2010). Immediately following the CTmax trials, there was an increase in mRNA expression of HSP70 in the WR 24°C treatment, compared to their BR counterparts. This relative increase in expression may be indicative of the WR population’s greater ability to acutely upregulate mRNA expression of HSP70 after acclimation to thermally stressful temperatures as reported in rainbow trout, O. mykiss (Carrie et al., 2005). This increased response was not observed in the BR population and may play a role in the increased CTmax of the WR population in comparison to the BR in this acclimation treatment. Three days post-CTmax, expression of HSP70 returned to near baseline levels in all treatments, except for BR 16°C, which remained elevated compared to 20 and 24°C. Similarly, 3 days post-CTmax, HSP90a was elevated in the BR 16°C treatment as compared to 20 and 24°C and depressed in the 24°C treatment compared to pre-trial levels, but neither of these observations was true for the WR population. Elevated mRNA expression of HSP70 and HSP90a 3 days post-CTmax in the BR 16°C in comparison to 20 and 24°C acclimation treatments was possibly due to increased cellular damage, slower rates of cellular repair and delayed recovery that may impact long-term individual fitness (Jeffries et al., 2018; Tomanek and Somero, 2000), whilst decreases in HSP90a in 24°C could be indicative of further metabolic changes. Additionally, a trend of elevated GPx activity, a family of antioxidant enzymes key in eliminating reactive oxygen species that may form as a result of thermal stress (Do et al., 2019), was observed in the BR population with significantly altered expression pre-trials and 3 days post-CTmax. Acute exposure to elevated temperatures has been demonstrated to increase GPx activity in the b nasal totothen, Pagotherina borchevinskii (Almroth et al., 2015) and the European bullhead, Cottus gobio (Dorts et al., 2012). Thus, increased GPx activity in the BR population was most likely a result of higher levels of oxidative stress when compared to WR lake sturgeon. Increased mRNA expression of HSP70, HSP90a and enzyme activity 3 days post-CTmax in the BR 16°C treatment, but not at 20 and 24°C or in the WR population, further demonstrate population-specific responses to thermal stress and the ability of acclimation to decrease potentially resultant cellular consequences.

Gill mRNA expression of Na+/K+ ATPase-α1 revealed further differences between populations. Na+/K+ ATPase-α1 makes up the functional pumping subunit of the heterodimeric protein complex (Hu et al., 2017; Wong et al., 2016) that actively exchanges Na+ and K+ ions to maintain cellular ion balance (Ito et al., 2010). At all sampling points, mRNA expression of Na+/K+ ATPase-α1 was elevated in BR lake sturgeon, when compared to their WR counterparts. Expression of Na+/K+ ATPase-α1 mRNA is altered in high-temperature acclimation treatments in three different species of Pacific salmon (Jeffries et al., 2014; Tomalty et al., 2015) as well as in Atlantic salmon (Vargas-Chacoff et al., 2018). This gene has also been implicated as a driver of population differences across different salinities in the semi-anadromous Sacramento splittail, Pogonichthys macrolepidotus (Mundy et al., 2020). Na+/K+ ATPase-α1 is under hormonal control (Feraille and Doucet, 2001; Ito et al., 2010; Therien and Blostein, 2000), and isoforms of it can be upregulated in the presence of cortisol and growth hormone (McCormick et al., 2013). Thus, increases in mRNA expression observed in the current study may have multiple explanations. Na+/K+ ATPase-α1 mRNA expression may be evidence of further differences between populations, or varying effects of stress and osmotic disruption induced in BR lake sturgeon.

**Conclusions**

Conservation hatcheries continue to rear and release lake sturgeon to enhance endangered wild populations throughout their distribution. In order to ensure the success of these stocking programmes, and to preserve lake sturgeon throughout their natural range, it is necessary to understand the effects of different environmental temperatures on the survival and physiology of sturgeon from diverse populations. This study has demonstrated significant population-specific physiological effects following 30 days of acclimation to relevant environmental temperatures and those that may be anticipated within the lifetime of sturgeon currently being released (Manitoba Hydro, 2015). Prolonged temperatures above 16°C may not be appropriate for rearing Manitoba populations of lake sturgeon with decreases in HSI potentially impacting their ability to survive overwintering. Whilst lake sturgeon can acclimate to increased environmental temper-
atures, increases in mortality as well as wide-ranging physiological consequences including diminished HSI, metabolic depression and alteration of gene expression are evident as a result of chronic thermal stress, which are likely related to environmental and possibly genetic differences between populations (Harder et al., 2019). The numbers of individuals captured for spawning and subsequent mix of genetic variability in the resultant progeny for this study are restricted due to the endangered status of the species in Manitoba (COSEWIC, 2006; COSEWIC, 2017) and thus limit our interpretation; however, the data presented suggest population-level responses to increased acclimation temperatures and are supported by a number of studies conducted on other geographically separated populations of the same species. This study addressed the effects of increasing environmental temperatures on developing lake sturgeon that were fed to satiation; however, natural environments represent a complex set of factors that can interactively affect population and community outcomes (Moe et al., 2012). Thus, future studies should examine the interactive effects of multiple stressors on measured physiological variables to fully understand the consequences on lake sturgeon population health under future warming scenarios.

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