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REGULAR PAPER

The effects of population and thermal acclimation on the growth, condition and cold responsive mRNA expression of age-0 lake sturgeon (*Acipenser fulvescens*)

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Abstract

In Manitoba, Canada, wild lake sturgeon (*Acipenser fulvescens*) populations exist along a latitudinal gradient and are reared in hatcheries to bolster threatened populations. We reared two populations of lake sturgeon, one from each of the northern and southern ends of Manitoba and examined the effects of typical hatchery temperatures (16°C) as well as 60-day acclimation to elevated rearing temperatures (20°C) on mortality, growth and condition throughout early development. Additionally, we examined the cold shock response, which may be induced during stocking, through the hepatic mRNA expression of genes involved in the response to cold stress and homeoviscous adaptation (*HSP70*, *HSP90a*, *HSP90b*, *CIRP* and *SCD*). Sturgeon were sampled after 1 day and 1 week following stocking into temperatures of 8, 6 and 4°C in a controlled laboratory environment. The southern population showed lower condition and higher mortality during early life than the northern population while increased rearing temperature impacted the growth and condition of developing northern sturgeon. During the cold shock, *HSP70* and *HSP90a* mRNA expression increased in all sturgeon treatments as stocking temperature decreased, with higher expression observed in the southern population. Expression of *HSP90b*, *CIRP* and *SCD* increased as stocking temperature decreased in northern sturgeon with early acclimation to 20°C. Correlation analyses indicated the strongest molecular relationships were in the expression of *HSP90b*, *CIRP* and *SCD*, across all treatments, with a correlation between *HSP90b* and body condition in northern sturgeon with early acclimation to 20°C. Together, these observations highlight the importance of population and rearing environment throughout early development and on later cellular responses induced by cold stocking temperatures.

KEYWORDS
acclimation, cold shock, gene expression, populations, sturgeon, thermal stress

1 | INTRODUCTION

Both genotype and early rearing environment have strong impacts on the development of fishes and will shape long-term individual responses to environmental stressors (Fangue *et al*., 2006; Green & Fisher, 2004; Selong *et al*., 2001; Whitehead *et al*., 2012). Specifically, increased environmental temperatures during development may have long-lasting effects on a fish's phenotype later in life, including

changes in body size, condition and cellular responses to environmental stressors (Georgakopoulou *et al*., 2007; Jonsson & Jonsson, 2014; Loughland *et al*., 2021). At the population level, differences in selective pressures across geographic localities can influence the development of population-specific phenotypes and ultimately whole organism responses (Bugg *et al*., 2020; Fangue *et al*., 2006; Jeffries *et al*., 2019; Whitehead *et al*., 2012). For species with extensive ranges, latitudinal gradients across populations and the differences in early thermal environment which accompany them will ultimately impact the formation of these early developmental phenotypes (Jonsson & Jonsson, 2019; Pigliucci *et al*., 2006), and potentially impact the long-term growth, cellular responses and survival of the organism.

Lake sturgeon, *A. fulvescens*, are a long-lived and widely distributed species of conservation concern with populations existing along a latitudinal gradient throughout North America. Hence, lake sturgeon populations are exposed to an array of environmental conditions. In Manitoba, Canada, northern and southern populations of lake sturgeon are separated by historical barriers which limit gene flow (Figure 1a; McDougall *et al*., 2017). These different populations also experience distinct environments in early development and exhibit differential physiological and cellular responses to increased environmental temperatures (Bugg *et al*., 2020). Northern populations likely experience the greatest impacts of increasing temperatures resultant from global climate change (Sharma *et al*., 2007; Vincent *et al*., 2015; Zhang *et al*., 2019). Thus, hatcheries within the province collect gametes from northern parents for conservation purposes during the reproductive season (late May to mid-July) and rear juvenile lake sturgeon for release to bolster these threatened wild populations. Lake sturgeon that are used to enhance northern populations are typically reared at the Grand Rapids Fish Hatchery at 16°C with the potential for increasing rearing temperatures in the first year of life to increase growth rates and achieve larger sturgeon prior to release (Bugg *et al*., 2020). Recent studies have demonstrated that subtle changes in rearing temperature during early development can influence the growth and condition of lake sturgeon prior to a simulated overwintering event, which may influence survival rates (Brandt *et al*., 2022; Yoon *et al*., 2019, 2020). However, there is little data available concerning the effects of exposures to elevated temperatures during early development on adaptive responses to cold during the first winter of life. Additionally, young-of-the-year (YOY) lake sturgeon may be released in late season (October and November), when river temperatures begin to decrease (McDougall *et al*., 2020). As it is difficult to examine the effects of release temperature in a natural setting, laboratory studies can provide useful insights into the effects of stocking temperature post-release to better inform hatchery management and stocking practices.

While many studies investigate the physiological responses of fishes to high temperature thermal stress, there is less focus on the effects of low temperature stress, or cold stress (Donaldson *et al*., 2008). Cold stress can lead to detrimental sublethal effects on feeding, growth, behaviour, survival and cellular responses of fishes (Cheng *et al*., 2017; Donaldson *et al*., 2008; Nikoskelainen *et al*., 2004; Ward & Bonar, 2003). At the cellular level, cold stress may compromise protein integrity, resulting in the binding of chaperones to proteins and cellular structures to stabilize them from the denaturing effects of environmental stress (Teigen *et al*., 2015). Heat shock proteins (HSP70, HSP90a and HSP90b) are a family of chaperone proteins that are responsive to both high and low temperature stress, and can be acutely (HSP70, HSP90a) or constitutively (HSP90b) expressed in fishes depending on the severity and duration of the stressor (Buckley *et al*., 2006; Feder & Hofmann, 1999; Hori *et al*., 2010; Somero, 2020). Additionally, cold inducible RNA binding protein (CIRP) acts to increase mRNA stability and facilitate translation under thermally stressful conditions (Zhong & Huang, 2017). Across an array of marine and freshwater fishes, the mRNA expression of CIRP is downregulated under high temperature thermal stress but upregulated at low temperatures across a variety of tissues (Akbarzadeh *et al*., 2018; Gracey *et al*., 2004; Jeffries *et al*., 2012, 2014; Liu *et al*., 2020; Rebl *et al*., 2013; Verleih *et al*., 2015). With its prominent role in response to RNA processing and protein synthesis under cold stress conditions, CIRP expression represents a strong marker for acute cold shock with similar cold-induced chaperone activity apparent from bacteria to humans (Gracey *et al*., 2004; Jiang *et al*., 1997; Verleih *et al*., 2015).

Changes in environmental temperature are also associated with alterations of cellular membrane fluidity in fishes (Cossins & Prosser, 1978; Donaldson *et al*., 2008; Malekar *et al*., 2018). As membrane fluidity plays a key role in cell function (Hazel, 1997), temperature decreases typically lead to changes in the fatty acids of phospholipids through decreasing the levels of saturated fatty acids but increasing unsaturated fatty acids, resulting in an overall increased membrane fluidity (Farkas *et al*., 2001). The increase of mono- and polyunsaturated fatty acids in phospholipids as a response to temperature decline has been widely observed as an adaptive mechanism termed homeoviscous adaptation, which strongly influences tolerance to cold environments and ultimately survival (Bowden *et al*., 1996; Hsieh & Kuo, 2005; Kelly & Kohler, 1999; Tiku *et al*., 1996; Trueman *et al*., 2000; Wodtke & Cossins, 1991). Synthesis of monounsaturated fatty acids is catalysed by stearoyl-CoA desaturase (SCD), an enzyme involved in a rate-limiting step of the insertion of a double bond into the fatty acid chain, functioning to increase membrane flexibility (Enoch *et al*., 1976; Hsieh *et al*., 2007; Jeffcoat *et al*., 1977). Thus, changes in SCD mRNA expression may represent altered capacity for homeoviscous adaptation, helping to regulate unsaturated fatty acid levels in cellular membranes and maintain membrane fluidity in response to cold stress. Thus, transcriptional responses of SCD as well as chaperone proteins may provide insight into the cellular plasticity and levels of stress experienced under cold temperatures, which may be encountered during late season stocking, particularly relevant in the northern extent of lake sturgeon distribution.

The aim of this study was to evaluate the effect of population and acclimation temperature on the growth, condition and cold responsive mRNA expression of YOY lake sturgeon in Manitoba,

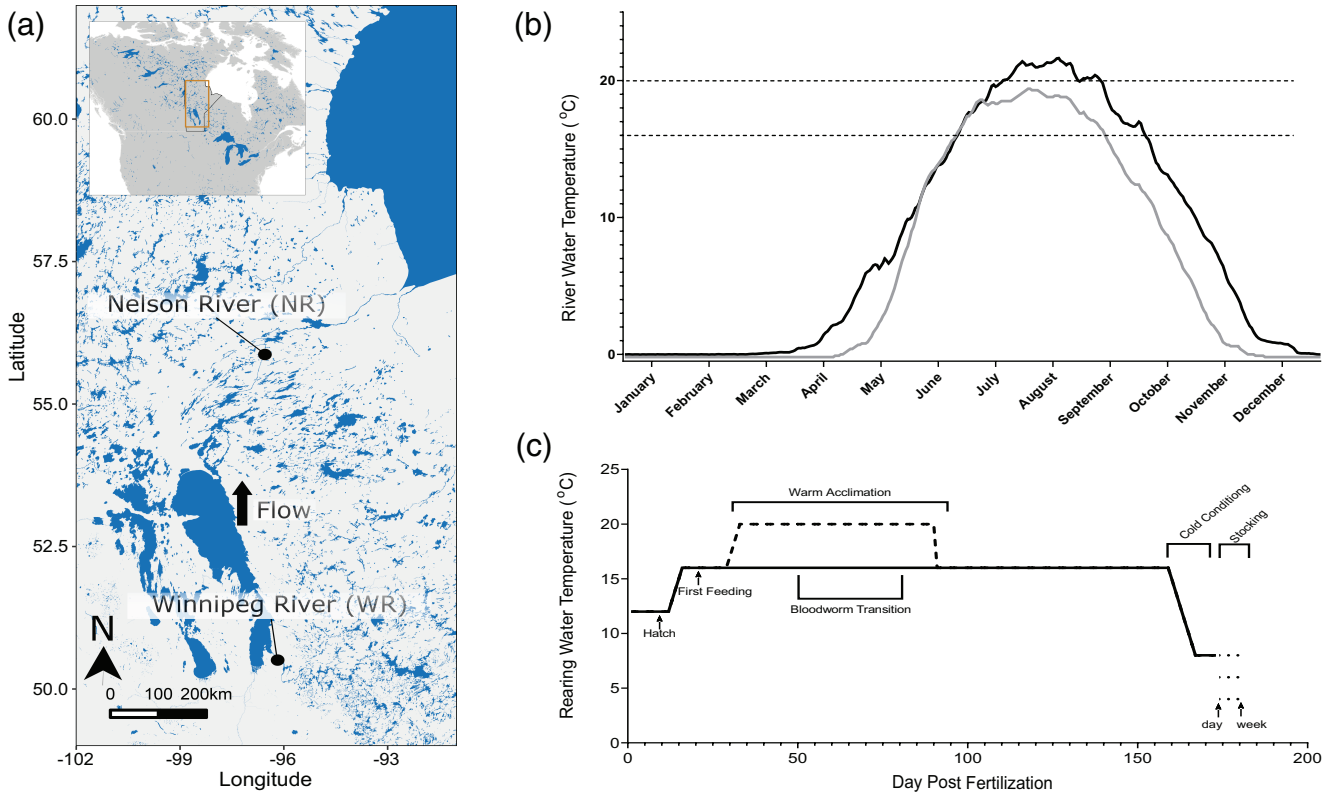

FIGURE 1 (a) Geographic localities, (b) river temperatures and (c) rearing temperatures of Winnipeg River (WR) and Nelson River (NR) populations of lake sturgeon reared under experimental conditions. River temperatures were measured at midnight and tick marks on the i axis indicate the middle of each given month. Dashed lines indicate different acclimation temperatures used in the current study, with 16°C representing typical hatchery rearing temperatures and 20°C depicting elevated temperatures that may be expected in natural environments in the future or in hatcheries with elevated temperature rearing protocols. Rearing water temperatures remained at 12°C until post-hatch, after which they were raised to 16°C prior to first feeding. An additional NR treatment was acclimated to 20°C for 60 days between 30 and 90 days post fertilization (dpf). At 160 dpf cold conditioning began, reducing rearing temperatures down to 8°C, prior to cold shock. At 173 dpf sturgeon were acutely stocked into temperatures of 8, 6 and 4°C with liver samples taken 1 day (24 h) and 1 week (168 h) for investigation of hepatic mRNA expression in response to cold temperature exposure, represented by arrows in the above figure. (——) Winnipeg River (WR); (——) Nelson River (NR); (——) NR + WR 16°C treatment; (— —) NR 20°C treatment

Canada. With distinct northern and southern populations of lake sturgeon demonstrating population and acclimation specific growth and cellular responses at elevated temperatures (Bugg *et al.*, 2020), we hypothesized that these populations would exhibit divergent responses when reared under common conditions and then exposed to cold stress. Additionally, as early rearing environment can have long-term impacts on a fish's phenotype (Johnson *et al.*, 2014; Jonsson & Jonsson, 2014), we hypothesized that early acclimation, which may resemble future warming scenarios, could have long-term implications for both growth and response to cold. Furthermore, we predicted that these changes would be apparent in both population- and acclimation-specific responses of growth and condition in YOY lake sturgeon throughout development, and in the cold induced hepatic expression of thermally responsive genes (*HSP70*, *HSP90a*, *HSP90b*, *CIRP* and *SCD*), which may be upregulated during fall stocking. Specifically, we first predicted that these physiological alterations to cold shock would be greater in the southern Winnipeg River (WR) population of lake sturgeon that naturally experience higher annual temperatures, when compared to their northern Nelson River (NR) counterparts. Second, we predicted that NR lake sturgeon acclimated to 20°C during early development would be more affected by cold stocking than individuals from the same population reared at 16°C. Finally, we predicted that individual sturgeon with lower overall condition would be the most greatly affected by acute cold shock, regardless of population or early acclimation.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

All animals in this study were reared and sampled under the 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.

2.2 | River temperatures

River water temperatures were measured in both the WR and NR from 2013 to 2016 (Figure 1b) by a DigiTemp SDI-12 submersible

temperature sensor (Forest Technology Systems, Victoria, British Columbia, Canada) and a series 500 SDI-12 transducer (TE Connectivity, Schaffhausen, CH), respectively. Measurements for water temperatures in the WR were recorded downstream of the generating station at Pointe du Bois where spawning lake sturgeon were caught for this study (50°17′52″N, 95°32′5″W), while water temperatures in the NR were recorded 2 km below the Hudson Bay Railway bridge (55°49′43″N, 96°36′9″W). Water temperatures were recorded at midnight (0:00:00am) for both rivers and therefore likely represent the lower range of expected daily temperatures. Temperature measurements from 2013 to 2016 were then averaged for each day of the year to create a list of average daily temperatures. These daily temperatures were also averaged for seasonal and yearly temperatures.

2.3 | Lake sturgeon husbandry

In late May and early June of 2018, gametes from wild-caught female and male lake sturgeon were harvested from individuals at the Pointe du Bois Generating Station on the WR (50°17′52″N, 95°32′51″W) and near the confluence of the Landing and Nelson Rivers (55°19′36″N, 96°54′16″W). Gametes from both populations were transported to the Animal Holding Facility at the University of Manitoba, where fertilization occurred. Sturgeon from the WR population were the product of fertilization of eggs from three females with the sperm from six males (three maternal families) while sturgeon from the NR were the product of the fertilization of eggs from one female with the sperm from two males (one maternal family). After fertilization, embryos from both populations were de-adhesed by submersion in a clay solution of Fuller's earth. Then, embryos were rinsed with dechlorinated fresh tap water and incubated in tumbling jars at 12°C, but they re-adhesed in the tumbling jars within 24 h and were moved to aquaria to complete their incubation prior to hatching.

Post-hatch, larval sturgeon were reared in 9 l flow-through aquaria with aeration and bioballs as substrate (Earhart *et al*., 2020). Starting at 13 days post fertilization (dpf) rearing temperature was increased at 1°C per day until 16°C was achieved prior to the onset of exogenous feeding (Figure 1c). Larval sturgeon were first offered freshly hatched artemia (Artemia International LLC, Texas, USA) three times daily starting at 19 dpf to promote recognition of prey and were first observed feeding at 21 dpf. Sturgeon from each population were then transferred from early rearing tanks to duplicate 9 l experimental tanks per treatment at 28 dpf, with a density of approximately 150 sturgeon larvae per tank. Rearing density was adjusted with equal numbers of sturgeon from the families representing each population (*e.g*., 50 fish from each WR maternal family). Starting at 50 dpf, sturgeon from all treatments were slowly transitioned to a primarily bloodworm diet (Hikari Bloodworms, Hikari, California, USA) at an approximate rate of 10% every 3 days, with feedings reduced to twice per day starting at 68 dpf, resulting in a total of 27 days to transition to a 100% bloodworm diet by 77 dpf. After the feeding transition to bloodworms was completed at 78 dpf, sturgeon were transferred to two duplicate 230 l aquaria per treatment at an approximate density of 140 sturgeon per tank and a total of 280 sturgeon per treatment. During the entirety of the experiment, fish were fed to satiation by offering food until there was no more consumed.

2.4 | Body measurements

Every 30 days throughout development, five sturgeon were selected from each replicate tank for a total of 10 sturgeon per treatment. Selected sturgeon were then euthanized by immersion in an overdose of tricaine methanesulfonate solution (250 mg l^{-1}; MS-222, Syndel Laboratory, Vancouver, Canada) buffered with an equal mass of sodium bicarbonate. Body mass (weighed to 0.001 g) and total length (measured to 1 mm) were recorded for each sampled sturgeon and Fulton's condition factor (*K*; Fulton, 1911) was subsequently calculated as:

$$K = \frac{\text{mass (g)}}{\text{total length (cm)}^3} \times 100$$

2.5 | Acclimation and cold shock

Beginning at 30 dpf, a subset of lake sturgeon from both the WR and NR populations was acclimated to a 20°C treatment. Water temperatures were increased from 16°C at a rate of 1°C per day for 4 days until 20°C was reached. Lake sturgeon then remained at this acclimation temperature for 60 days before returning to 16°C. After this 60-day warm acclimation period, temperatures in the 20°C treatments were reduced back to 16°C to match temperatures in the 16°C treatments, where they remained in all treatments until 160 dpf. A WR 20°C treatment was included in the original experimental design but nearing the end of warm acclimation showed signs of pathogenic infection and elevated mortality, and thus was removed from the study. Mortality and temperature were monitored at least two times daily across all treatments during this acclimation period. At 160 dpf, cold conditioning began, reducing the rearing temperatures by 1°C per day from 16°C until they reached 8°C. Tank temperatures were then held at 8°C for 1 week prior to the beginning of cold shock trials. This temperature transition is reflective of standard hatchery operations for stocking of juvenile lake sturgeon in October and November in Manitoba. YOY lake sturgeon are acclimated to 8°C in the hatchery for 1 week prior to release, but this may result in acute cold shock and increased post-release mortality if river temperatures are below this temperature.

At 173 dpf, following cold conditioning to 8°C for 1 week, 10 lake sturgeon from each treatment group (WR 16, NR 16 and NR 20°C), five sturgeon per replicate rearing tank, were selected and a simulated stocking event was performed by placing fish into one of six 9 l aquaria per treatment in a Multi-Stressor unit (Aquabiotech, Coaticook, Quebec, Canada). For each treatment, two of the six tanks

were held at 8, 6 or 4°C, which represent stocking temperatures in the late fall. For each stocking temperature, individual tanks were then subsequently sampled 1 day (24 h) and 1 week (168 h) post-stocking. In addition to euthanizing sturgeon and taking body measurements as described above, liver tissue was dissected and preserved in RNAlater (Thermo Fisher Scientific, Waltham, USA), held at 4°C for 24 h, and then stored at −80°C prior to analysis. Throughout the current experiment, including both development and cold stocking, temperatures in each treatment were recorded every 15 min by HOBO Water Temperature Pro v2 Data Loggers (Onset Computer Corporation, Bourne, MA, USA).

2.6 | RNA extraction, cDNA synthesis and qPCR

Total RNA was extracted from the livers of all YOY lake sturgeon treatment groups using a PureLink RNA mini Kit (Invitrogen, Ambion Life Technologies, Waltham, MA, USA) following the manufacturer's instructions. Liver tissue was homogenized in 500 μl of lysis buffer for 5 min at 50 Hz using a TissueLyser II (Qiagen, Germantown, MD, USA). Total RNA concentration, purity and integrity were assessed using a Nanodrop One (Thermo Fisher Scientific) and gel electrophoresis, respectively, for each sample. Synthesis of cDNA was conducted with a qScript cDNA synthesis kit (Quantbio, Beverly, MA, USA) following the manufacturer's instructions from 1 μg of DNAse treated total RNA. A SimpliAmp Thermal Cycler (Thermo Fisher Scientific) 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 a final hold at 4°C was used to perform the cDNA synthesis. The cDNA samples were stored at −20°C.

Real-time quantitative polymerase chain reactions (RT-qPCR) for each gene of interest (*HSP70*, *HSP90a*, *HSP90b*, *CIRP* and *SCD*) and the reference gene (*RPS6* and *RPL7*) were conducted using 5 μl of Bio-Rad SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA), 0.1–0.05 μl of 100 μM primers, 2 μl of cDNA per sample and nuclease-free water adjusted for each assay to bring the total volume of each well to 10 μl. For all assays, each well contained 0.025 μl forward and 0.025 μl reverse primer, except *SCD* and reference genes *RPS6* and *RPL7*, which used 0.05 μl forward and 0.05 μl reverse primer per well. The cDNA for all assays was diluted 1:10 with nuclease-free water prior to its inclusion in RT-qPCR assays. Primers for *SCD* were designed from an annotated white sturgeon, *Acipenser transmontanus*, liver transcriptome (Doering *et al*., 2016). Primers for *CIRP* were designed based on sequences from the Adriatic sturgeon *Acipenser naccarii*, which were retrieved from Anaccariibase (Sequence ID: CDNA3-4_11_2010_0_rep_c10569; Vidotto *et al*., 2013) and aligned against publicly available transcripts using NCBI BLAST (Johnson *et al*., 2008), sharing highly conserved regions with the sterlet sturgeon, *Acipenser ruthensus* (Sequence ID: XM_034911883.1; 95.2% identity). Primer sequences for *HSP70*, *HSP90a*, *HSP90b*, *RPS6* and *RPL7* were designed based on sequences from an annotated lake sturgeon ovary transcriptome produced through pyrosequencing and are the same as used in other lake sturgeon thermal stress experiments (Hale *et al*., 2009; Yusishen *et al*., 2020; Table 1). The expression of all genes of interest were normalized to the expression of reference genes *RPS6* and *RLP7*, using the geometric mean (Vandesompele *et al*., 2002), and analysed following the application of the $2^{-\Delta\Delta Ct}$ method as described by Livak and Schmittgen (2001). The expression of all genes of interest were then normalized to the WR 16°C, 8°C stocking temperature, 1 week post-stocking timepoint.

2.7 | Statistical analysis

Throughout development, data collected on the condition of YOY lake sturgeon were analysed with two-factor ANOVAs with either population and developmental timepoint (dpf) or acclimation treatment and developmental timepoint included in the model as fixed effects for comparisons of WR 16°C with NR 16°C, and NR 16°C with NR 20°C acclimation treatments, respectively. For comparisons of length and weight throughout development, data could not be successfully transformed to pass the assumptions for ANOVAs, so they were analysed with nonparametric Wilcoxon signed ranked tests to compare sequential developmental timepoints as well as across acclimation treatments and populations, comparing either WR 16°C with NR 16°C, or NR 16°C with NR 20°C.

The mRNA expression and condition factor data from cold shock trials were analysed using three-factor ANOVAs, with population, stocking temperature and time or acclimation treatment, stocking temperature and time included in the model as fixed effects for comparison of WR 16°C with NR 16°C, and NR 16°C with NR 20°C acclimation treatments, respectively. For all ANOVAs, the Shapiro–Wilk and Levene tests were used to assess normality of data and homogeneity of variance as well as the visual inspection of fitted residual plots. If the assumptions of either test were violated, a ranked, log or square root transformation was performed on the data set. Following ANOVAs, Tukey's honestly significant difference tests from the 'multcomp' package (Hothorn *et al*., 2008) were used to assess differences between populations, acclimation treatments, stocking temperatures and time points. Detailed results for all ANOVA analyses are provided in Supporting Information S1.

Semi-partial Spearman's correlations were used to investigate the relationship between the mRNA expression of each gene and that of other studied genes, as well as the condition factor of each representative sturgeon from cold shock trials using the 'ppcor' package (Seongho, 2015), while accounting for variance in both stocking temperature and time. This analysis was conducted individually for each of the representative treatments (WR 16°C, NR 16°C and NR 20°C; $n = 48$, 45 and 50, respectively). Values are reported as estimated Spearman's rho (ρ) with significance indicated by $^{*}P < 0.05$ and $^{**}P < 0.00001$. Additionally, principal component analyses were conducted on the same data, individually for each treatment, graphing variable plots to investigate the relationship between stocking temperature, time, the expression of assayed genes, as well as condition factor.

Differences in mortality between populations and acclimation treatments during the acclimation period (from 30 to 90 dpf) were

TABLE 1 Primer sequences used for the present study in lake sturgeon, *A. fulvescens*, during cold shock trials

Gene	Forward	Reverse	Efficiency (%)
HSP70	CTGTCACTCGGACTTTAACTTTAATTT	AACTGTCCTAAAGAACTGCCTTATCC	94.0
HSP90a	GATCACACGAGCGGATTTGC	ATGTTGTGCTCTGTCCTGCG	96.4
HSP90b	GGAACCAAGGCTTCATGGA	CCAACACCAAACTGACCAATCA	92.8
CIRP	TTCGACACAAACGAGCAGTC	TCACCACAACTTCCGAGACA	98.1
SCD	AGCCAAGTTGCGTTGAGA	GTCCTCGTGGGTTGGTTACTT	93.1
RPS6	CTGGCTGGATTCTGATTTGGATG	ATCTGATTATGCCAAGCTGCTG	95.6
RPL7	TGCTTAGGATTGCTGAGCCG	GATCTTTCCGTGACCCCGTT	96.0

Note: Target genes *HSP70*, *HSP90a*, *HSP90b*, *Cold Inducible RNA binding protein* (*CIRP*) and *Stearoyl-CoA Desaturase* (*SCD*) were selected based on their responsiveness as chaperones as well as their role in cold stress and homeoviscous adaptation. *RPS6* and *RPL7* were used as reference genes. Efficiencies are listed as a percentage.

analysed using Cox proportional hazards models using the 'survival' and 'survminer' packages (Kassambara *et al*., 2019; Therneau, 2015) with covariates of both population and acclimation temperature included in the model. Next, a pairwise comparison was conducted using the 'pairwise_survdiff' function in the 'survminer' package and a Bonferroni correction to compare mortality across both populations and acclimation treatments. Hazard models were evaluated using the 'cox.zph' function in the 'survival' package to ensure that the assumptions of Cox proportional hazards were met. All statistical analyses were performed using R 4.0.0 with a significance level (α) of 0.05.

3 | RESULTS

3.1 | River temperatures

From 2013 to 2016, under average yearly conditions, the WR exceeded 20°C 14% and 16°C 27.1% of days. In contrast, the NR never exceeded 20°C and only exceeded 16°C on 21.4% of days. Over the years of temperature recording from 21 June to 21 September when larval lake sturgeon are undergoing rapid development and growth, the WR was on average 1.89 ± 1.2°C (mean ± s.d.) warmer than the NR. This discrepancy increased in the fall, 22 September to 21 December, when sturgeon are likely accumulating stores for overwintering, with water temperatures in the WR 3.54 ± 1.8°C warmer than in the NR. When averaging across the total year, 1 January to 31 December, the WR was 1.85 ± 1.8°C warmer than the NR.

3.2 | Effects of population and acclimation on mortality, growth and condition

3.2.1 | Mortality

Population, but not acclimation temperature, had a significant effect on the mortality of YOY lake sturgeon in this study, as determined by Cox proportional hazard models. The WR 16°C treatment had a hazard ratio of 3.97 when compared to combined NR 16 and 20°C treatments ($P < 0.0001$) with elevated cumulative mortality of 11.3% in the WR, compared to 4.4% for NR sturgeon. Further pairwise analysis showed that the WR 16°C treatment had higher levels of mortality than either individual NR acclimation treatment ($P < 0.0001$ and $P < 0.05$ for 16 and 20°C treatments, respectively).

3.2.2 | Population comparisons

Throughout development, both WR and NR sturgeon increased their length with each developmental timepoint ($P < 0.05$; Table 2), except between 150 and 173 dpf for NR sturgeon, in which cold conditioning began. At 30 dpf, larval sturgeon from the WR were larger than the NR counterparts ($P < 0.05$), but this difference did not persist throughout development. Similarly, lake sturgeon from both populations increased their weight with each developmental timepoint until 150 dpf ($P < 0.05$). However, neither sturgeon from the WR or NR reared at 16°C increased their weight between 150 and 173 dpf once cold conditioning began. Overall, the condition factor of YOY lake sturgeon reared at 16°C was affected by population ($P < 0.0001$, $F = 28.02$). There was no significant difference in condition factor prior to bloodworm transition at 30 dpf, after which the NR population had elevated condition relative to their WR counterparts at 60, 120 ($P < 0.05$) and 173 dpf ($P < 0.0001$; 13.3%, 8.8% and 8.3%, respectively).

3.2.3 | Acclimation temperature comparison

Sturgeon from the NR, in both 16 and 20°C treatments, increased their length and weight throughout development, except from 150 to 173 dpf when cold conditioning began ($P < 0.05$). At 30 dpf, before acclimation began, NR sturgeon in 16°C treatments were longer and heavier than those in the NR 20°C treatment ($P < 0.05$). However, once acclimation started, sturgeon from the 20°C acclimation treatment became longer and heavier than their 16°C counterparts, with these differences persisting for the majority of the remaining experiment (60–173 dpf for length and 90, 120 and 173 dpf for mass; $P < 0.05$). The overall condition factor of YOY lake sturgeon from the NR was affected by both

TABLE 2 Body measurements of WR 16°C, NR 16°C and NR 20°C treatments throughout the development of young of year lake sturgeon, *Acipenser fulvescens*

Days post fertilization	WR 16°C	NR 16°C	NR 20°C
		Length (mm)	
30	26.97 ± 0.35 (10)^{*a}	25.52 ± 0.32 (10)$^{*\dagger a}$	24.6 ± 0.25 (10)$^{\dagger a}$
60	54.29 ± 1.15 (10)^{b}	54.69 ± 1 (10)$^{\dagger b}$	59.7 ± 1.24 (10)$^{\dagger b}$
90	75 ± 3.94 (10)^{c}	74.13 ± 1.65 (10)$^{\dagger c}$	86.4 ± 1.46 (10)$^{\dagger c}$
120	112.9 ± 3.59 (10)^{d}	112.2 ± 3.51 (10)$^{\dagger d}$	124.3 ± 2.34 (10)$^{\dagger d}$
150	135.7 ± 3.54 (10)^{e}	145.4 ± 3.96 (10)$^{\dagger e}$	158.7 ± 4.49 (10)$^{\dagger e}$
173	145.17 ± 3.11 (30)^{f}	146.87 ± 2.18 (30)$^{\dagger e}$	158 ± 2.37 (30)$^{\dagger e}$
		Weight (g)	
30	0.067 ± 0.00 (10)^{*a}	0.059 ± 0.00 (10)$^{*\dagger a}$	0.05 ± 0.00 (10)$^{\dagger a}$
60	0.50 ± 0.04 (10)^{b}	0.57 ± 0.03 (10)^{b}	0.67 ± 0.039 (10)^{b}
90	1.49 ± 0.28 (10)^{c}	1.48 ± 0.11 (10)$^{\dagger c}$	2.02 ± 0.12 (10)$^{\dagger c}$
120	4.64 ± 0.34 (10)^{d}	5.03 ± 0.43 (10)$^{\dagger d}$	6.55 ± 0.38 (10)$^{\dagger d}$
150	8.63 ± 0.73 (10)^{e}	10.4 ± 0.66 (10)^{e}	12.31 ± 1.16 (10)^{e}
173	11.41 ± 0.49 (30)^{e}	10.18 ± 0.56 (30)$^{\dagger e}$	13.19 ± 0.53 (30)$^{\dagger e}$
		Condition factor (*K*)	
30	0.341 ± 0.01 (10)	0.353 ± 0.01 (10)†	0.335 ± 0.01 (10)$^{\dagger ab}$
60	0.309 ± 0.01 (10)*	0.35 ± 0.01 (10)*†	0.313 ± 0.01 (10)$^{\dagger ab}$
90	0.316 ± 0.02 (10)	0.358 ± 0.01 (10)†	0.316 ± 0.02 (10)$^{\dagger ab}$
120	0.32 ± 0.01 (10)*	0.348 ± 0.01 (10)*	0.338 ± 0.01 (10)^{ac}
150	0.338 ± 0.01 (10)	0.337 ± 0.01 (10)†	0.3 ± 0.01 (10)$^{\dagger b}$
173	0.326 ± 0.01 (30)*	0.353 ± 0.00 (30)*†	0.32 ± 0.01 (30)$^{\dagger c}$

Note: Significant differences in length and weight throughout development and across treatments were determined by Wilcoxon signed ranked tests as the data were nonparametric. Significance differences in condition were determined *via* two-factor ANOVA ($P < 0.05$) followed by Tukey's honestly significant difference *post hoc* test comparing either populations between WR 16°C and NR 16°C or acclimation treatments between NR 16°C and NR 20°C. Data are represented as a mean ± S.E.M. ($n = 10–30$ per treatment). *Represents significant differences between WR 16°C and NR 16°C populations, while †indicates significant differences between NR 16°C and NR 20°C acclimation treatments, within a developmental time point. Differences throughout developmental time, within a given treatment, are noted with letters a, b and c. Data are expressed as mean ± S.E.M. with sample number (*n*) in parentheses.

acclimation treatment ($P < 0.0001$, $F = 44.34$) and developmental timepoint ($P < 0.001$, $F = 4.52$). Condition factor did not change throughout development in the NR 16°C treatment, but in the NR 20°C acclimation treatment there was a significant decrease between 120 and 150 dpf timepoints, followed by an increase at 173 dpf relative to 150 dpf ($P < 0.05$). Between acclimation treatments, the NR 16°C treatment had a 5% higher condition factor than their NR 20°C counterparts at 30 dpf ($P < 0.05$), which increased with acclimation, reaching 11.8% and 13.3% by 60 and 90 dpf ($P < 0.05$), respectively, and persisted to 150 and 173 dpf ($P < 0.005$).

3.3 | Effects of stocking on cold-inducible mRNA expression and condition

3.3.1 | Population comparisons

Across populations of sturgeon reared at 16°C, the mRNA expression of *HSP70* was affected by an interaction between stocking temperature and time ($P < 0.005$, $F = 6.2$) as well as population and time ($P < 0.0001$, $F = 23.6$; Figure 2A). Northern and southern populations expressed different levels of *HSP70* mRNA at several timepoints, with higher expression observed in the NR population after 1 day in 8°C, but with increased expression in WR sturgeon following 1 week in 8 and 6°C ($P < 0.05$). Throughout time, the NR sturgeon decreased their expression of *HSP70* in 8°C over the course of the week 5.2-fold, while WR sturgeon increased their expression in 6 and 4°C over the same time period ($P < 0.05$). For both populations, expression increased in colder temperatures at the 1 week timepoint, with increased expression in 4°C relative to 8°C ($P < 0.05$). However in 6°C only the WR population increased expression relative to 8°C stocked sturgeon ($P < 0.01$).

The mRNA expression of *HSP90a* was affected by interactions between stocking temperature and time ($P < 0.0001$, $F = 11.3$) as well as population and time ($P < 0.0001$, $F = 36.6$; Figure 2B). After 1 day in 8 and 4°C, the NR population had higher mRNA expression of *HSP90a* than their southern WR counterparts ($P < 0.05$). However, by 1 week, this trend was reversed, with higher expression in southern

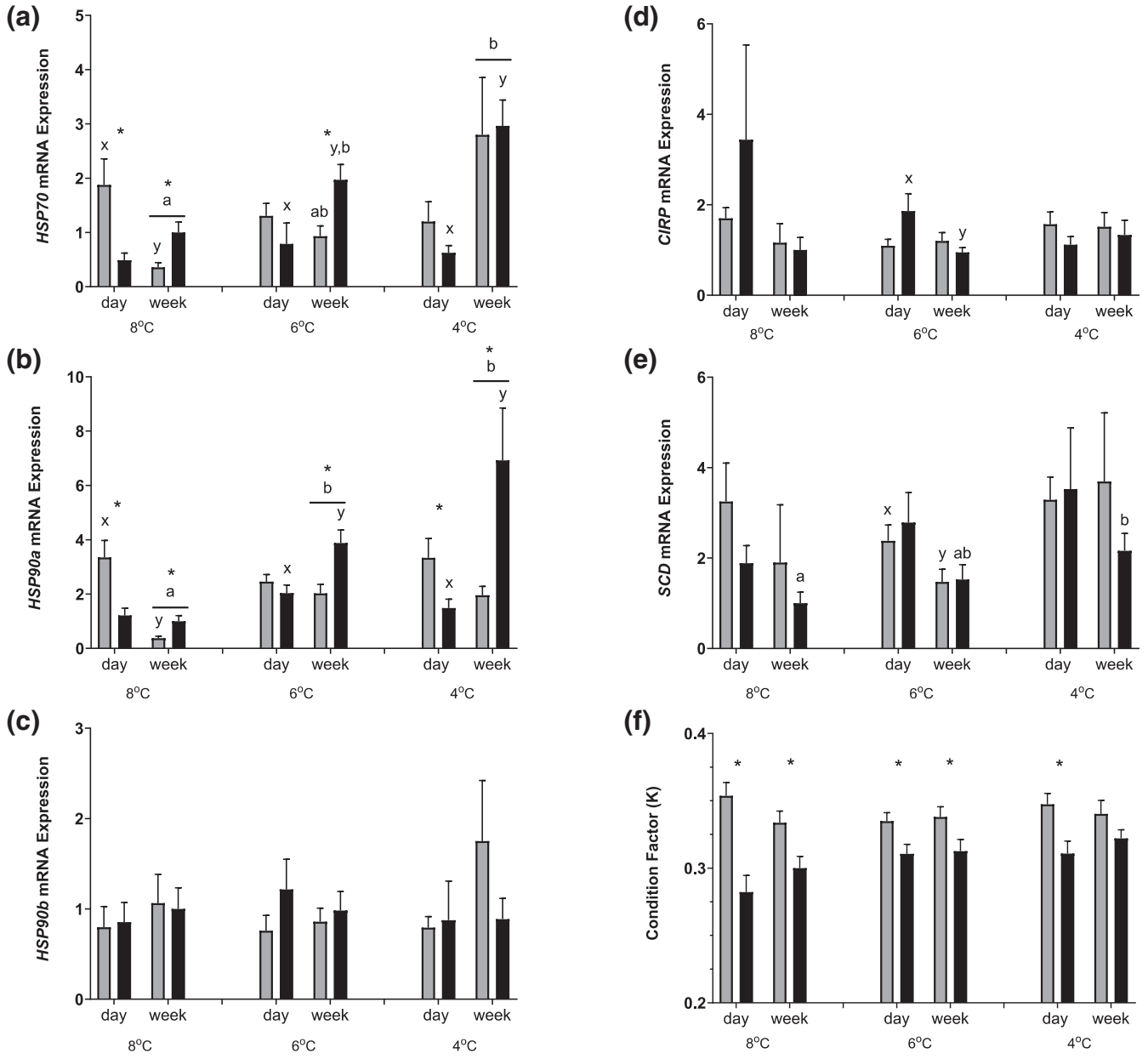

FIGURE 2 Population comparisons of lake sturgeon, *Acipenser fulvescens*, from Winnipeg River (WR) and Nelson River (NR) throughout cold shock trials in their mRNA expression of (A) *HSP70*, (B) *HSP90a*, (C) *HSP90b*, (D) cold inducible RNA binding protein (CIRP), (E) stearoyl-CoA desaturase (SCD) and (F) condition factor (*K*) 1 day and 1 week following stocking into 8, 6 and 4°C water. *Indicates significance between WR and NR populations of lake sturgeon. Letters a, b and c indicate significance between stocking temperatures within a population and at a given timepoint. Letters x and y indicate significant differences between timepoints within a given population and stocking temperature ($P < 0.05$, three-factor ANOVA). The expressions of all genes of interest were normalized to the expression of reference genes *RPS6* and *RLP7*, using the geometric mean (Vandesompele *et al*., 2002) and analysed following the application of the $2^{-\Delta\Delta Ct}$ method as described by Livak and Schmittgen (2001). The expression of all genes of interest were normalized to the WR 16°C, 8°C stocking temperature, 1 week post-stocking timepoint. Data are expressed as mean ± S.E.M. [*HSP70* $n = 5$–10, *HSP90a* $n = 5$–10, *HSP90b* $n = 7$–10, steryl-CoA desaturase (SCD) $n = 6$–10, cold inducible RNA binding protein (CIRP) $n = 6$–10, condition factor (*K*) $n = 10$]. (▭) NR 16°C; (▬) WR 16°C

WR sturgeon in each stocking temperature ($P < 0.05$). Across timepoints, NR sturgeon decreased expression of *HSP90a* 8.8-fold in 8°C over the course of the week-long exposure ($P < 0.001$), while WR sturgeon increased their expression in temperatures of 6 and 4°C ($P < 0.01$). Both populations increased their expression at 1 week as temperatures decreased, with higher expression in 6 and 4°C when compared to 8°C. In contrast to the changes in expression observed for *HSP90a*, the constituitive form of the gene *HSP90b* showed no effect of population, time or stocking temperature over the week-long exposure (Figure 2C).

The mRNA expression of *CIRP* was only affected by time ($P < 0.05$, $F = 4.0$; Figure 2D) with a 2-fold decrease in expression in

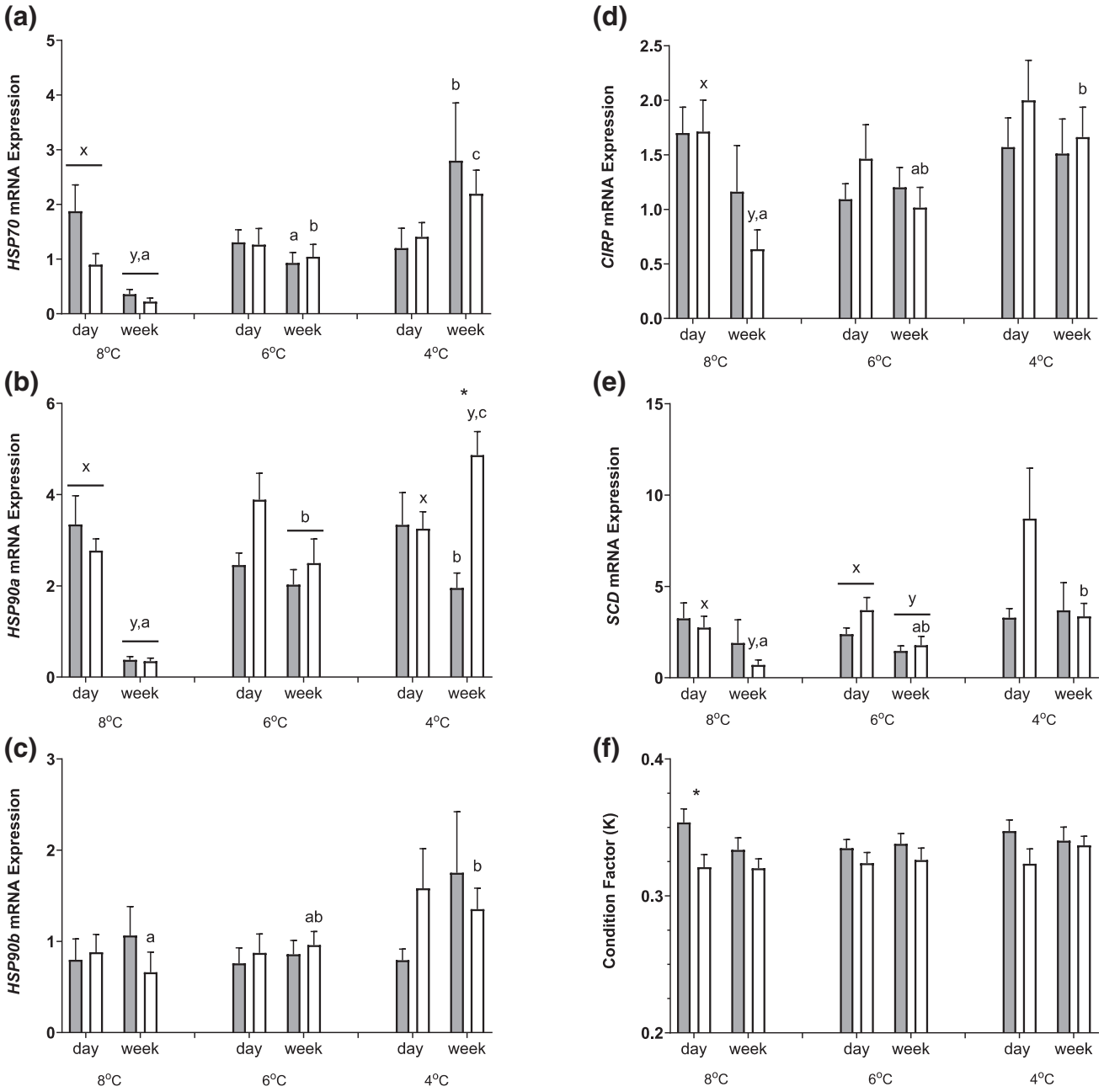

FIGURE 3 Acclimation temperature comparisons of lake sturgeon, *Acipenser fulvescens*, from Nelson River (NR) 16 and 20°C acclimation treatments throughout cold shock trials in their mRNA expression of (A) *HSP70*, (B) *HSP90a*, (C) *HSP90b*, (D) cold inducible RNA binding protein (CIRP), (E) stearoyl-CoA desaturase (SCD) and (F) condition factor (*K*) 1 day and 1 week following stocking into 8, 6 and 4°C water. *Indicates significance between NR 16 and 20°C acclimation treatments of lake sturgeon. Letters a, b and c indicate significance between stocking temperatures within an acclimation treatment at a given timepoint. Letters x and y indicate significant differences between timepoints, within a given acclimation treatment and stocking temperature ($P < 0.05$, three-factor ANOVA). The expression of all genes of interest were normalized to the expression of reference genes *RPS6* and *RLP7*, using the geometric mean (Vandesompele *et al*., 2002), and analysed following the application of the $2^{-\Delta\Delta Ct}$ method as described by Livak and Schmittgen (2001). The expression of all genes of interest were then normalized to the WR 16°C, 8°C stocking temperature, 1 week post-stocking timepoint. Data are expressed as mean ± S.E.M. [*HSP70* $n = 5$–10, *HSP90a* $n = 5$–10, *HSP90b* $n = 6$–10, cold inducible RNA binding protein (CIRP) $n = 6$–10, steryl-CoA desaturase (SCD) $n = 5$–10, condition factor (*K*) $n = 10$]. (▬) NR 16°C; (▭) NR 20°C

the WR population stocked into 6°C between a day and a week post-stocking ($P < 0.05$). Similarly, there was an overall effect of time on *SCD* mRNA expression ($P < 0.005$, $F = 10.2$), with an additional effect of stocking temperature ($P < 0.05$, $F = 4.0$; Figure 2E). In the NR sturgeon, expression of *SCD* decreased over the week in 6°C, but increased as temperatures decreased in the WR population with higher expression in 4°C relative to 8°C at the 1 week timepoint ($P < 0.05$). Condition factor was affected by population ($P < 0.0001$,

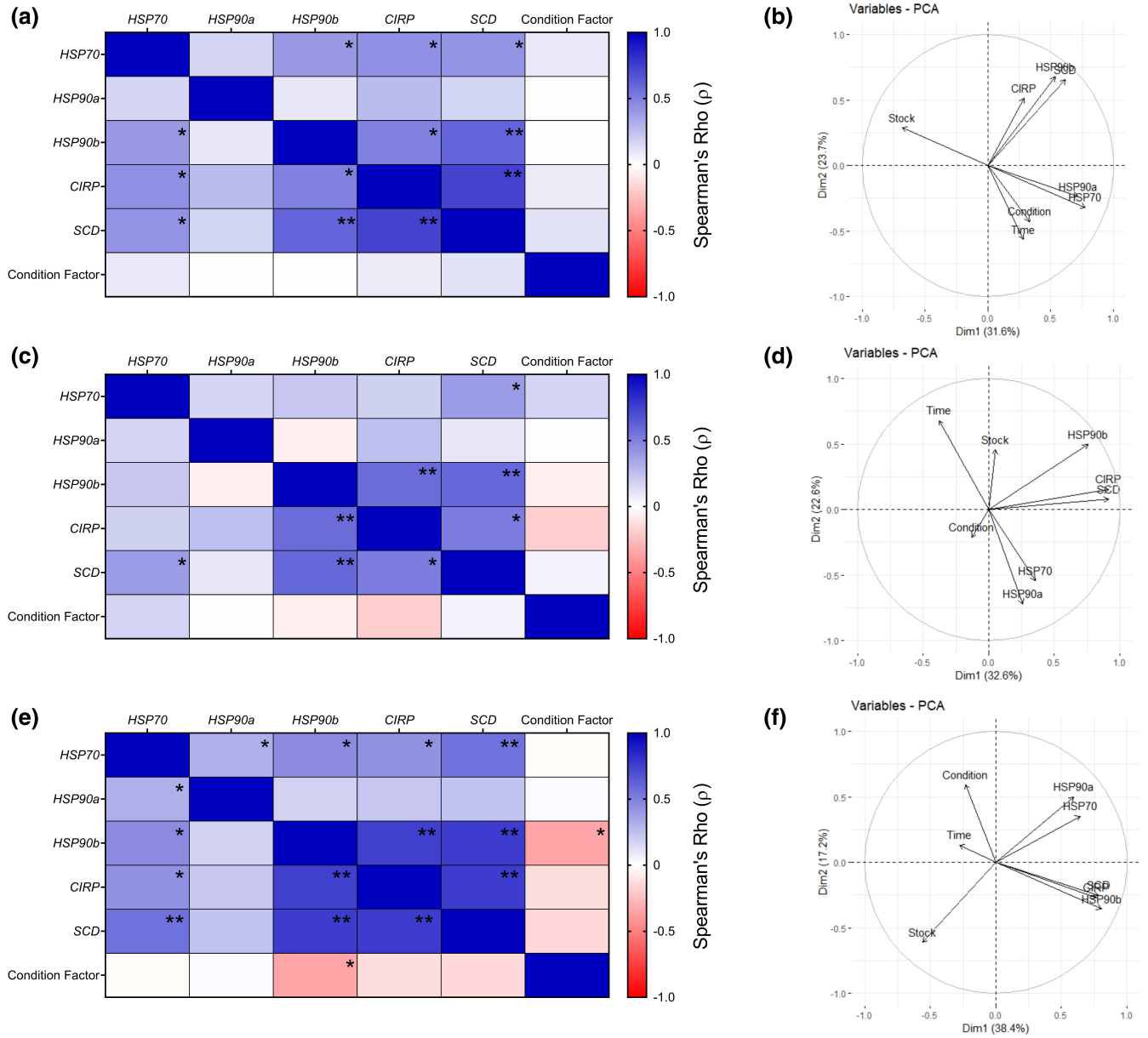

FIGURE 4 Gene expression and condition factor relationships as indicated by semi-partial Spearman's correlations (a, c, e) and PCA variable plots (b, d, f) for young of year lake sturgeon, *Acipenser fulvescens*, from treatments WR 16°C (a, b), NR 16°C (c, d) and NR 20°C (e, f) after stocking into conditions of 8, 6 and 4°C (stock) over the timeseries of 1 day and 1 week (time) ($n = 48$, 45 and 50, with respect to population and acclimation treatment). Values in correlation matrices represent the ρ from each estimated Spearman's correlation. *Indicates significance at the level $P < 0.05$, while ** indicates significance at the level $P < 0.00001$

$F = 47.9$) with higher condition in sturgeon from the NR at both 1 day and 1 week timepoints in 8 and 6°C and 1 day in 4°C, when compared to WR sturgeon ($P < 0.05$).

3.3.2 | Acclimation temperature comparisons

Across NR 16 and 20°C acclimation treatments, the mRNA expression of *HSP70* was affected by an interaction between stocking temperature and time ($P < 0.0005$, $F = 9.1$; Figure 3A). Over the course of the week-long exposure to 8°C, both NR acclimation treatments decreased expression of *HSP70* ($P < 0.05$). At the 1 week timepoint, however, in the NR 20°C treatment, mRNA expression increased with each decrease in stocking temperature, while in the NR 16°C treatment *HSP70* mRNA expression was higher in 4°C relative to 8°C ($P < 0.05$).

The mRNA expression of *HSP90a* was affected by interactions of acclimation treatment, stocking temperature and time ($P < 0.05$, $F = 4.0$; Figure 3B). In both acclimation treatments *HSP90a* expression decreased over the week-long exposure in 8°C ($P < 0.0001$), while increasing in colder stocking temperatures relative to 8°C at the 1 week timepoint ($P < 0.05$). In the 16°C acclimation treatment after

1 week, expression increased in 6 and 4°C relative to 8°C stocked sturgeon ($P < 0.001$). However in 20°C acclimated sturgeon after 1 week, expression increased with each decrease in stocking temperature and over the week in 4°C ($P < 0.005$). This resulted in higher mRNA expression of *HSP90a* in the 20°C acclimated sturgeon in 4°C at the 1 week timepoint, relative to their 16°C acclimated counterparts ($P < 0.001$). Similarly, expression of the constituitive form *HSP90b* increased in the 1 week timepoint as temperatures decreased, but only in 20°C acclimated NR sturgeon ($P < 0.05$; Figure 3C).

The mRNA expression of *CIRP* was affected by time ($P < 0.05$, $F = 6.4$) and stocking temperature ($P < 0.05$, $F = 3.6$; Figure 3D), and decreased in NR 20°C acclimated sturgeon over the week-long exposure in 8°C ($P < 0.005$). In the same acclimation treatment, *CIRP* was elevated in lower temperatures at the 1 week timepoint, 2.4-fold in 4°C compared to sturgeon stocked into 8°C ($P < 0.05$). Likewise, *SCD* mRNA expression was also affected by stocking temperature ($P < 0.0001$, $F = 10.7$) and time ($P < 0.0001$, $F = 20.0$; Figure 3E). In the NR 20°C acclimation treatment in 8°C, and both acclimation treatments in 6°C, expression of *SCD* decreased from 1 day to 1 week post-stocking ($P < 0.05$). At the 1 week timepoint in the NR 20°C acclimation treatment, expression increased as temperatures decreased, with higher expression observed in 4°C relative to 8°C stocked sturgeon ($P < 0.005$). Condition factor was affected by acclimation treatment ($P < 0.005$, $F = 10.8$; Figure 3F), with higher condition in NR 16°C acclimated sturgeon in 8°C 1 day following stocking, relative to 20°C acclimated sturgeon ($P < 0.05$).

3.3.3 | Gene expression relationships

In the WR 16°C treatment there were correlated relationships in the mRNA expression of *HSP70* with *HSP90b*, *CIRP* and *SCD*, as well as *HSP90b* and *CIRP* ($P < 0.05$, with ρ of 0.40, 0.44, 0.42 and 0.50, respectively). The strongest relationships were between *SCD* with *HSP90b* and *CIRP* ($P < 0.00001$, with ρ of 0.6 and 0.74, respectively; Figure 4a). Next, in the NR 16°C treatment there were correlated relationships in the mRNA expression of *SCD* with *HSP70* and *CIRP* ($P < 0.05$, with ρ of 0.39 and 0.51, respectively). The strongest relationships were between *HSP90b* with *CIRP* and *SCD* ($P < 0.00001$, with ρ of 0.57 and 0.59, respectively; Figure 4c). Finally, in the NR 20°C treatment there were correlated relationships in the mRNA expression *HSP70* with *HSP90a*, *HSP90b* and *CIRP*, as well as *HSP90b* and condition factor ($P < 0.05$, with ρ of 0.31, 0.45, 0.43 and −0.35, respectively). The strongest relationships were *HSP70* with *SCD*, *HSP90b* with *CIRP* and *SCD*, and *CIRP* with *SCD* ($P < 0.00001$, with ρ of 0.56, 0.75, 0.76 and 0.76; Figure 4e). Principal component analysis reveal that in both the WR 16°C and NR 20°C expression of *HSP70* and *HSP90a* was negatively correlated with stocking temperature (Figure 4b,f, respectively), while in the NR 16°C treatment there was a stronger negative association of these genes with time (Figure 4d). Across all treatments *HSP70* and *HSP90a* were grouped together, with the expression of *HSP90b*, *CIRP* and *SCD* grouping out separately.

4 | DISCUSSION

Throughout the first 6 months of development leading up to overwintering, we demonstrated changes in the growth, condition factor and mortality of lake sturgeon from northern and southern populations in Manitoba, as well as those acclimated to elevated temperatures from 30 to 90 dpf. Additionally, these same populations and acclimation treatments demonstrate persistent treatment-specific responses at the cellular level when challenged with cold shock, a stressor that may be encountered during late season stocking. These divergent physiological responses between southern and more northern YOY lake sturgeon are likely influenced by genotypes and population-specific thermal environments that lake sturgeon would experience in their respective natural waterways prior to overwintering (Bugg *et al*., 2020).

4.1 | Effects of population and early acclimation on mortality, growth and condition

Elevated mortality and lower overall condition were apparent in southern WR sturgeon relative to their northern NR counterparts, suggesting that individuals from the NR population are better suited to the applied hatchery rearing protocols at 16°C. It is possible that WR sturgeon may less effectively transition between hatchery-provided food resources, potentially resultant from differences in gut development or a more specialistic feeding strategy, but further research is required to confirm these speculations. In contrast, the northern NR sturgeon populations experience a shortened feeding and growth season, potentially necessitating faster development or a more generalist strategy to feeding, as observed in Arctic freshwater fishes (Conover & Present, 1990; Laske *et al*., 2018), and thus resulting in higher condition than their southern counterparts throughout hatchery rearing. Irrespective of cause, these differences in mortality indicate population level differences in early development and should be further investigated. Similarly, when the temperature of the rearing environment was increased, NR sturgeon acclimated to 20°C early in life demonstrated larger body mass and length, but poorer condition than those kept at 16°C throughout development, suggesting a potential thermal threshold for condition factor in northern sturgeon populations if river temperatures continue to increase. Rearing temperatures of 20°C exceed typical riverine temperatures in the northern extent of the lake sturgeons range, and likely the optimum for this population (Bugg *et al*., 2020). These observed decreases in condition may be due to increased routine metabolic rates at higher temperatures, but after 30 days of acclimation to similar temperatures no increase in routine metabolic rate was observed in these same populations (Bugg *et al*., 2020). Alternatively, sturgeon in higher temperatures may regulate their feeding to reduce metabolic costs associated with digestion and other routine activities, limiting their overall condition (Jutfelt *et al*., 2021). In all treatments, growth continued from 30 to 150 dpf, but ceased

in NR sturgeon from both treatments once cold conditioning began. However, growth continued in WR sturgeon, potentially indicating population level differences in their response to environmental cues during the onset of overwintering, with NR sturgeon taking on an energy storage maximization strategy which may aid to enhance lipid stores prior to overwintering (Bugg *et al*., 2020; Schultz & Conover, 1997; Sogard & Olla, 2000). This resulted in higher condition factor throughout development and after conditioning to colder temperatures, potentially as an adaptation to survive a prolonged overwintering period in their northern environment, similar to observations in latitudinal distributed populations of Atlantic silverside, *Menidia menidia* (Schultz *et al*., 1998). These key differences between populations and acclimation treatments demonstrate the importance of genetic background and early rearing temperature during development on the growth, condition and survival of juvenile lake sturgeon, with the potential for impacts at the hatchery level.

4.2 | Effects of stocking on cold inducible mRNA expression

Cold stocking had effects at both the population and acclimation treatment level on the transcriptional responses of heat shock proteins *HSP70* and *HSP90a* in the liver, with increased expression in southern WR and warm acclimated northern NR sturgeon. Sturgeon from the NR stocked into 8°C decreased the expression of these genes over the course of the week, while their expression was affected by decreasing stocking temperature and increased in both populations and acclimation treatments in 4°C, relative to 8°C at the 1 week timepoint. Overall, WR sturgeon had higher mRNA expression of both heat shock proteins at multiple temperatures 1 week post-stocking, indicating a higher transcriptional responsiveness and perhaps higher levels of cold induced plasticity to thermal stress in this population. Similarly, NR sturgeon acclimated to 20°C had higher expression of *HSP90a* after 1 week at 4°C, demonstrating the potential for long-term effects of early acclimation influencing the cold shock response. Transcript induction of both *HSP70* and *HSP90a* has been observed in multiple tissues of the tiger barb *Puntius tetrazona* and *Cyprinus carpio* following cold shock (Ferencz *et al*., 2012; Liu *et al*., 2020). Additionally, experiments with embryonic Atlantic salmon, *Salmo salar*, indicate that *HSP70* confers protection against cold stress during early development (Takle *et al*., 2005). An upregulation of cortisol during cold exposure could modulate the expression of these genes over short periods of time (Celi *et al*., 2012; Sathiyaa *et al*., 2001; Vamvakopoulos, 1993). However, cortisol does not always causally relate to HSP expression in fish tissues (Iwama *et al*., 1999) and in YOY lake sturgeon exposed to overwintering conditions a significant upregulation of cortisol was not observed until several weeks of exposure to near-freezing temperatures while fasted (Deslauriers *et al*., 2018). Thus, transcriptional upregulation of heat shock proteins likely indicates a prolonged and cortisol-independent plastic response to cold stress when YOY lake sturgeon are stocked into lower temperatures than they are acclimated to prior to release.

In addition to liver changes in *HSP70* and *HSP90a* expression, sturgeon from the NR with early acclimation to 20°C also decreased expression of *CIRP* and *SCD* in the 8°C treatment over the course of the week but demonstrated increased expression of *CIRP*, *SCD* and *HSP90b* in 4°C relative to 8°C. Like *HSP70*, induction of *CIRP* mRNA expression was observed in several tissues of the tiger barb and common carp when subjected to cold stress (Gracey *et al*., 2004; Liu *et al*., 2020). Additionally, upregulation of *CIRP* mRNA was demonstrated in rainbow trout, *Oncorhynchus mykiss*, kidney and gill following 2 weeks of low thermally stressful temperatures, and the gills of sockeye salmon, *Oncorhynchus nerka*, and pink salmon, *Oncorhynchus gorbuscha*, acclimated to cool temperatures (Akbarzadeh *et al*., 2018, Jeffries *et al*., 2012, 2014; Rebl *et al*., 2013; Verleih *et al*., 2015;). As *CIRP* expression is postulated to be induced under moderate hypothermia (Rebl *et al*., 2013), upregulation of this gene as well as *HSP90b* with decreasing temperatures in the NR 20°C lake sturgeon but not in fish from other treatments demonstrates a lasting effect of this early life warm acclimation and its ability to disrupt transcriptional processes later in life. It is likely that for sturgeon reared at 16°C, temperatures of 4°C were not low enough or 1 week was not long enough to disrupt these same processes and induce *CIRP* expression.

Cold stocking-induced hepatic expression of *SCD* mRNA was affected by stocking temperature and time for both population and acclimation treatment comparisons with decreases observed in 8°C over time and increases in expression as stocking temperatures decreased, similar to expression patterns of *HSP70*, *HSP90a*, *HSP90b* and *CIRP*. In the milkfish, *Chanos chanos*, and hybrid tilapia, *Oreochromis niloticus* × *O. aureus*, liver *SCD* mRNA expression, enzyme activity, mono- and polyunsaturated fatty acids, and desaturation index all increased over the duration of cold shock (Hsieh *et al*., 2003, 2007; Hsieh & Kuo, 2005). Interestingly, lower levels of the above traits, including polyunsaturated fatty acids and unsaturation index, have been linked to mortality in freshwater alewives, *Alosa pseudoharengus*, under cold challenge (Snyder *et al*., 2012; Snyder & Hennessey, 2003). Similarly, in common carp, *C. carpio*, cooling water temperatures induced latent increases in SCD enzyme activity followed by transcriptional upregulation as temperatures continued to decrease (Tiku *et al*., 1996; Trueman *et al*., 2000). Thus, the transcriptional induction of *SCD* in the present study suggests that in colder temperatures, lake sturgeon in this treatment required increased homeoviscous capacity, passing the threshold for adequate regulation of desaturation processes at the enzymatic level. Instead, sturgeon from the WR 16°C and NR 20°C upregulated their mRNA synthesis of *SCD*, demonstrating the need to further alter membrane lipids as temperatures decrease, ultimately indicating possible impacts for survival post-release. A closely patterned response can be observed in the mRNA expression of *HSP90a* at 1 week, with increases in the WR 16°C and NR 20°C relative to NR 16°C

as temperatures decrease. Currently, in the WR, sturgeon are exposed to 20°C for approximately 50 days with no exposure over this threshold for sturgeon in the NR (Bugg *et al*., 2020). The convergence of these molecular phenotypes between WR sturgeon and warm acclimated NR sturgeon following induction by cold shock demonstrates that if temperatures increase in the NR for this early developmental window, we may expect these responses, and perhaps other phenotypes of NR sturgeon, to more closely resemble that of WR sturgeon in the future.

Taken together, the upregulation of *HSP70*, *HSP90a*, *CIRP*, *SCD* and *HSP90b* observed in the liver at lower temperatures suggests that in these colder environments transcriptional production was not high enough to protect cells from the effects of cold induced thermal stress. However, cold conditioning to 8°C diminished these effects over the course of the week-long exposure, with observed decreases in the responses of these transcripts when NR sturgeon were stocked into their pre-conditioned temperature. For NR sturgeon stocked into 8°C, these observed decreases in expression of *HSP70*, *HSP90a*, *CIRP* and *SCD* over the week following stocking may indicate that this short, hatchery used, conditioning period was not enough to completely diminish the effects of temperature change. Thus, longer cold conditioning periods prior to stocking may be appropriate, and if sturgeon are acclimated to 8°C, stocking should be conducted before temperatures drop below this threshold to diminish the effects of cold shock on release.

4.3 | Cold induced molecular relationships

Investigation of correlations and principal component analysis between chaperone and fatty acid metabolism related hepatic mRNA expression indicated strong transcriptional relationships between some genes. Across WR and NR populations and both NR acclimation treatments, the strongest molecular relationships were present in the hepatic expression of *HSP90b*, *CIRP* and *SCD*, genes that may be expected to respond to chronic, as opposed to acute, cold stress and group out similarly in the PCA analysis. The consistency of these relationships across populations and acclimation treatments indicates the importance of these genes in response to cold stress, even without strong upregulation. In contrast, *HSP70* and *HSP90a* represent more acutely responsive genes which were more highly induced, grouping out separately opposite of stocking temperature, indicating different response trajectories to cold exposure. *HSP70* mRNA expression was also associated with the expression of *HSP90b*, *CIRP* and *SCD*, but only in the WR 16°C and NR 20°C treatments, demonstrating further convergence in the relationships between molecular responses to cold shock in these two treatments. The strongest overall relationships in expression were present in the NR 20°C treatment, which also showed a negative correlation between sturgeon body condition and *HSP90b* expression. This suggests a relationship between observed decreases in body condition in this treatment with increases in the constitutive levels of molecular responsiveness to cold shock and to potentially higher levels of chronic stress in lower condition individuals.

4.4 | Conclusions

Overall, these findings demonstrate that both population and early thermal acclimation can have prolonged effects on the growth and condition of YOY lake sturgeon as well as their transcriptional responses to cold environments. If hatcheries aim to release fish with high condition, lower rearing temperatures in early development may be advantageous. However, if the hatchery's objective is to produce the largest fish, early environmental exposures to increased temperatures can increase the long-term growth trajectory of developing lake sturgeon, with consequences for body condition and future cellular responses. Ultimately, key genes involved in the stress and cold condition responses were upregulated when sturgeon were stocked into waters at a lower temperature than their pre-release hatchery conditioning temperature. These changes were population and acclimation temperature-specific and could have impacts on survival post-release. Therefore, consideration of these and other physiological responses is necessary when optimizing hatchery rearing and release practices for species of conservation concern.

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AUTHOR CONTRIBUTIONS

W.B., W.G.A. and K.J. conceived and designed the experiments. W.B., G.Y., C.B., M.E., W.G.A. and K.J. collected gametes from wild spawning lake sturgeon, while W.B., G.Y., C.B. and M.E. reared juveniles and collected data throughout development. W.B., G.Y. and C.B. conducted cold shock trials. W.B. conducted gene expression and data analysis. W.B., G.Y., C.B., M.E., W.G.A. and K.J. wrote, reviewed and edited the manuscript. W.G.A. and K.J. acquired funding and aided in supervision throughout the experimentation and review processes.

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REFERENCES

Akbarzadeh, A., Günther, O. P., Hounde, A. L., Li, S., Ming, T. J., Jeffries, K. M., … Miller, K. M. (2018). Developing specific molecular biomarkers for thermal stress in salmonids. *BMC Genomics*, *19*, 749.

Bowden, L. A., Restall, C. J., & Rowley, A. F. (1996). The influence of environmental temperature on membrane fluidity, fatty acid composition and lipoxygenase product generation in head kidney leucocytes of the rainbow trout, *Oncorhynchus mykiss*. *Comparative Biochemistry and Physiology- Part B: Biochemistry & Molecular Biology*, *115*, 375–382.

Buckley, B. A., Gracey, A. Y., & Somero, G. N. (2006). The cellular response to heat stress in the goby *Gillichthys mirabilis*: A cDNA microarray and protein-level analysis. *Journal of Experimental Biology*, *209*, 2660–2677.

Brandt, C., Groening, L., Klassen, C., & Anderson, W. G. (2022). Effects of rearing temperature on yolksac volume and growth rate in Lake Sturgeon, *Acipenser fulvescens*, from hatch to age -1. *Aquaculture*. In review, *546*, 737352.

Bugg, W. S., Yoon, G. R., Schoen, A. N., Laluk, A., Brandt, C., Anderson, W. G., & Jeffries, K. M. (2020). Effects of acclimation temperature on the thermal physiology in two geographically distinct populations of lake sturgeon (*Acipenser fulvescens*). *Conservation Physiology*, *8*, coaa087.

Celi, M., Vazzana, M., Sanfratello, M. A., & Parrinello, N. (2012). Elevated cortisol modulates HSP70 and HSP90 gene expression and protein in sea bass head kidney and isolated leukocytes. *General and Comparative Endocrinology*, *175*, 424–431.

Cheng, C. H., Ye, C. X., Guo, Z. X., & Wang, A. L. (2017). Immune and physiological responses of pufferfish (*Takifugu obscurus*) under cold stress. *Fish and Shellfish Immunology*, *64*, 137–145.

Conover, D. O., & Present, T. M. C. (1990). Countergradient variation in growth rate: Compensation for length of growing season among Atlantic silversides from different latitudes. *Oecologia*, *83*, 316–324.

Cossins, A. R., & Prosser, C. L. (1978). Evolutionary adaptation of membranes to temperature. *Proceedings of the National Academy of Sciences*, *75*, 2040–2043.

Deslauriers, D., Yoon, G. R., Earhart, M. L., Long, C., Klassen, C. N., & Anderson, W. G. (2018). Over-wintering physiology of age-0 lake sturgeon (*Acipenser fulvescens*) and its implications for conservation stocking programs. *Environmental Biology of Fishes*, *101*, 623–637.

Doering, J. A., Tang, S., Peng, H., Eisner, B. K., Sun, J., Giesy, J. P., … Hecker, M. (2016). High conservation in transcriptomic and proteomic response of white sturgeon to equipotent concentrations of 2, 3, 7, 8-TCDD, PCB 77, and benzo [a] pyrene. *Environmental Science and Technology*, *50*, 4826–4835.

Donaldson, M. R., Cooke, S. J., Patterson, D. A., & Macdonald, J. S. (2008). Cold shock and fish. *Journal of Fish Biology*, *73*, 1491–1530.

Earhart, M., Ali, J. L., Bugg, W. S., Jeffries, K. M., & Anderson, W. G. (2020). Endogenous cortisol production and its relationship with feeding transitions in larval Lake Sturgeon (*Acipenser fulvescens*). *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *249*, 110777.

Enoch, H. G., Catala, A., & Strittmatter, P. (1976). Studies of the substrate specificity, enzyme–substrate interactions, and the function of the lipid. *Journal of Biological Chemistry*, *251*, 5095–5130.

Fangue, N. A., Hofmeister, M., & Schulte, P. M. (2006). Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. *Journal of Experimental Biology*, *209*, 2859–2872.

Feder, M. E., & Hofmann, G. E. (1999). Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Reviews in Physiology*, *61*, 243–282.

Ferencz, A., Juhasz, R., Butnariu, M., Deer, A. K., Varga, I. S., & Nemcsok, J. (2012). Expression analysis of the heat shock genes in the skin, spleen, and blood of common carp (*Cyprinus carpio*). After cadmium exposure and hypothermia. *Acta Biologica Hungarica*, *63*, 15–25.

Farkas, T., Fodor, E., Kitajka, K., & Halver, J. E. (2001). Response of fish membranes to environmental temperature. *Aquaculture Research*, *32*, 645–655.

Georgakopoulou, E., Sfakianakis, D. G., Souttouki, S., Divanach, P., Kentouri, M., & Koumoundouros, G. (2007). The influence of temperature during early life on phenotypic expression at later ontogenic stages in sea bass. *Journal of Fish Biology*, *70*, 278–291.

Gracey, A. Y., Fraser, E. J., Li, W., Fang, Y., Taylor, R. R., Rogers, J., … Cossins, A. R. (2004). Coping with cold: An integrative, multitissue analysis of the transcriptome of a poikilothermic vertebrate. *Proceedings of the National Academy of Sciences*, *101*, 16970–16975.

Green, B. S., & Fisher, R. (2004). Temperature influences swimming speed, growth and larval duration in coral reef fish larvae. *Journal of Experimental Marine Biology and Ecology*, *299*, 115–132.

Hale, M. C., McCormick, C. R., Jackson, J. R., & DeWoody, J. A. (2009). Next generation pyrosequencing of gonad transcriptomes in the polyploid lake sturgeon (*Acipenser fulvescens*): The relative merits of normalization and rarefaction in gene discovery. *BMC Genomics*, *10*, 203.

Hazel, J. R. (1997). Thermal adaptation in biological membranes: Beyond homeoviscous adaptation. *Advances in Molecular and Cellular Biology*, *19*, 57–101.

Hori, T. S., Gamperl, A. K., Afonso, L. O. B., Johnson, S. C., Hubert, S., Kimball, J., … Rise, M. L. (2010). Heat-shock responsive genes identified and validated in Atlantic cod (*Gadus morhua*) liver, head kidney and skeletal muscle using genomic techniques. *BMC Genomics*, *11*, 72.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*, 346–363.

Hsieh, S. L., Chen, Y. N., & Kuo, C. M. (2003). Physiological responses, desaturase activity, and fatty acid composition in milkfish (*Chanos chanos*) under cold acclimation. *Aquaculture*, *220*, 903–918.

Hsieh, S. L., Hu, C. Y., Hsu, Y. T., & Hsieh, T. J. (2007). Influence of dietary lipids on the fatty acid composition and stearoyl-CoA desaturase expression in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) under cold shock. *Comparative Biochemistry and Physiology Part B: Biochemical and Molecular Biology*, *147*, 438–444.

Hsieh, S. L., & Kuo, C. M. (2005). Stearoyl-CoA desaturase expression and fatty acid composition in milkfish (*Chanos chanos*) and grass carp (*Ctenopharyngodon Idella*) during cold acclimation. *Comparative Biochemistry and Physiology Part B: Biochemical and Molecular Biology*, *141*, 95–101.

Iwama, G. K., Vijayan, M. M., Forsyth, R. B., & Ackerman, P. A. (1999). Heat shock proteins and physiological stress in fish. *American Zoologist*, *39*, 901–909.

Jeffcoat, R., Brawn, P. R., Safford, R., & James, A. T. (1977). Properties of rat liver microsomal stearoyl-coenzyme A desaturase. *Biochemical Journal*, *161*, 431–437.

Jeffries, K. M., Connon, R. E., Verhille, C. E., Dabruzzi, T. F., Britton, M. T., Durbin-Johnson, B. P., & Fangue, N. A. (2019). Divergent transcriptomic signatures in response to salinity exposure in two populations of an estuarine fish. *Evolutionary Applications*, *12*, 1212–1226.

Jeffries, K. M., Hinch, S. G., Sierocinski, T., Clark, T. D., Eliason, E. J., Donaldson, M. R., … Miller, K. M. (2012). Consequences of high temperatures and premature mortality on the transcriptome and blood physiology of wild adult sockeye salmon (*Oncorhynchus nerka*). *Ecology and Evolution*, *2*, 1747–1764.

Jeffries, K. M., Hinch, S. G., Sierocinski, T., Pavlidis, P., & Miller, K. M. (2014). Transcriptomic responses to high water temperature in two species of Pacific salmon. *Evolutionary Applications*, *7*, 286–300.

Jiang, W., Hou, Y., & Inouye, M. (1997). CspA, the major cold-shock protein of *Escherichia coli*, is an RNA chaperone. *The Journal of Biological Chemistry*, *272*, 196–202.

Johnson, B., Brockman, S., & Näslund, J. (2014). Environmental effects on behavioural development consequences for fitness of captive-reared fishes in the wild. *Journal of Fish Biology*, *85*, 1946–1971.

Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., & Madden, T. L. (2008). NCBI BLAST: A better web interface. *Nucleic Acids Research*, *36*, W5–W9.

Jonsson, B., & Jonsson, N. (2014). Early environment influences later performance in fishes. *Journal of Fish Biology*, *85*, 151–188.

Jonsson, B., & Jonsson, N. (2019). Phenotypic plasticity and epigenetics of fish: Embryo temperature affects later-developing life history traits. *Aquatic Biology*, *28*, 21–32.

Jutfelt, F., Norin, T., Asheim, E. R., Rowsey, L. E., Andreassen, A. H., Morgan, R., … Speers-Roesch, B. (2021). Aerobic scope protection reduces ectotherm growth under warming. *Functional Ecology*, *35*, 1397–1407. https://doi.org/10.1111/1365-2435.13811.

Kassambara, A., Kosinski, M., Biecek, P., & Fabian, S. (2019). Drawing survival curves using 'ggplot2' S. version 0.4.6. Retrieved from https://cran.r-project.org/web/packages/survminer/index.html.

Kelly, A. M., & Kohler, C. C. (1999). Cold tolerance and fatty acid composition of striped bass, white bass, and their hybrids. *North American Journal of Aquaculture*, *61*, 278–285.

Laske, S. M., Rosenberger, A. E., Wipfli, M. S., & Zimmerman, C. E. (2018). Generalist feeding strategies in Arctic freshwater fish: A mechanism for dealing with extreme environments. *Ecology of Freshwater Fish*, *27*, 767–784.

Liu, L., Zhang, R., Wang, X., Zhu, H., & Tian, Z. (2020). Transcriptome analysis reveals molecular mechanisms responsive to acute cold stress in tropical stenothermal fish tiger barb (*Puntius tetrazona*). *BMC Genomics*, *21*, 737.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*, *25*, 402–408.

Loughland, I., Little, A., & Seebacher, F. (2021). DNA methyltransferase 3a mediates developmental thermal plasticity. *BMC Biology*, *19*, 11.

Malekar, V. C., Morton, J. D., Hider, R. N., Cruickshank, R. H., Hodge, S., & Metcalf, V. J. (2018). Effect of elevated temperature on membrane lipid saturation in Antarctic notothenioid fish. *PeerJ*, *6*, e4765.

McDougall, C. A., Welsh, A. B., Gosselin, T., Anders, W. G., & Nelson, P. A. (2017). Rethinking the influence of hydroelectric development on gene flow in long-lived fish, the lake sturgeon *Acipenser fulvescens*. *PLoS One*, *12*, e0174269. https://doi.org/10.1371/journal.pone.0174269.

McDougall, C. A., Nelson, P. A., Aiken, J. K., Burnett, D. C., Barth, C. C., MacDonell, D. S., … Macdonald, D. (2020). Hatchery rearing of lake sturgeon to age 1 prior to stocking: A path forward for species recovery in the Upper Nelson River, Manitoba, Canada. *North American Journal of Fisheries Management*, *40*, 807–827.

Nikoskelainen, S., Bylund, G., & Lilius, E. M. (2004). Effect of environmental temperature on rainbow trout (*Oncorhynchus mykiss*) innate immunity. *Developmental and Comparative Immunology*, *28*, 581–592.

Pigliucci, M., Murren, C. J., & Shlichting, C. D. (2006). Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology*, *209*, 2362–2367.

Rebl, A., Verleih, M., Köbis, J. M., Kühn, C., & Wimmers, K. (2013). Transcriptome profiling of gill tissue in regionally bred and globally farmed rainbow trout strains reveals different strategies for coping with thermal stress. *Marine Biotechnology*, *15*, 445–460.

Sathiyaa, R., Campbell, T., & Vijayan, M. M. (2001). Cortisol modulates HSP90 mRNA expression in primary cultures of trout hepatocytes. *Comparative Biochemistry and Physiology Part B: Biochemical and Molecular Biology*, *129*, 679–685.

Schultz, E. T., & Conover, D. O. (1997). Latitudinal differences in somatic energy storage: Adaptive responses to seasonality in an estuarine fish (Atherinidae:*Menidia menidia*). *Oecologia*, *109*, 516–529.

Schultz, E. T., Conover, D. O., & Ehtisham, A. (1998). The dead of winter: Size-dependent variation and genetic difference in seasonal mortality among Atlantic silverside (Atherinidae:*Menidia menidia*) from different latitudes. *Canadian Journal of Fisheries and Aquatic Sciences*, *55*, 1149–1157.

Selong, J. H., McMahon, T. E., Zale, A. V., & Barrows, F. T. (2001). Effect of temperature on growth and survival of bull trout, with application of an improved method for determining thermal tolerance in fishes. *Transactions of the American Fisheries Society*, *130*, 1026–1037.

Seongho, K. (2015). ppcor: An R package for a fast calculation to semi-partial correlation coefficients. *Communications for Statistical Applications and Methods*, *22*, 665–674.

Sharma, S., Jackson, D. A., Minns, C. K., & Shuter, B. J. (2007). Will northern fish populations be in hot water because of climate change? *Global Change Biology*, *13*, 2052–2064.

Snyder, R. J., & Hennessey, T. D. (2003). Cold tolerance and homeoviscous adaptation in freshwater alewives (*Alosa pseudoharengus*). *Fish Physiology and Biochemistry*, *29*, 117–126.

Snyder, R. J., Schregel, W. D., & Wei, Y. (2012). Effects of thermal acclimation on tissue fatty acid composition of freshwater alewives (*Alosa pseudoharengus*). *Fish Physiology and Biochemistry*, *38*, 363–373.

Sogard, S. M., & Olla, B. L. (2000). Endurance of simulated winter conditions by age-0 walleye pollock: Effects of body size, water temperature, and energy stores. *Journal of Fish Biology*, *56*, 1–21.

Somero, G. N. (2020). The cellular stress response and temperature: Function, regulation, and evolution. *Journal of Experimental Zoology Part A*, *333*, 79–397.

Takle, H., Baeverfjord, G., Lunde, M., Kolstad, K., & Andersen, O. (2005). The effect of heat and cold exposure on HSP70 expression and development of deformities during embryogenesis of Atlantic salmon (*Salmo salar*). *Aquaculture*, *249*, 515–524.

Teigen, L. E., Orczewska, J. I., McLaughlin, J., & O'Brien, K. M. (2015). Cold acclimation increases levels of some heat shock protein and sirtuin isoforms in threespine stickleback. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *188*, 139–147.

Therneau, T. M. (2015). A package for survival analysis in R. version 2.38. Retrieved from https://CRAN.R-project.org/package=survival.

Tiku, P. E., Gracey, A. Y., Macartney, A. I., Beynon, R. J., & Cossins, A. R. (1996). Cold-induced expression of D9-desaturase in carp by transcriptional and posttranslational mechanisms. *Science*, *271*, 815–817.

Trueman, R. J., Tiku, P. E., Caddick, M. X., & Cossins, A. R. (2000). Thermal thresholds of lipid restructuring and delta (9)-desaturase expression in the liver of carp (*Cyprinus carpio L.*). *Journal of Experimental Biology*, *203*, 641–650.

Vamvakopoulos, N. O. (1993). Tissue-specific expression of heat shock proteins 70 and 90: Potential implication for differential sensitivity of tissues to glucocorticoids. *Molecular and Cellular Endocrinology*, *98*, 49–54.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paege, A., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology.*, *3*, 1–12.

Verleih, M., Borchel, A., Krasnov, A., Rebl, A., Korytář, T., Kühn, C., & Goldammer, T. (2015). Impact of thermal stress on kidney-specific gene expression in farmed regional and imported rainbow trout. *Marine Biotechnology*, *17*, 576–592.

Vidotto, M., Grapputo, A., Boscari, E., Barbisan, F., Coppe, A., Grandi, G., … Congiu, L. (2013). Transcriptome sequencing and de novo annotation of the critically endangered Adriatic sturgeon. *BMC Genomics*, *14*, 407.

Vincent, L. A., Zhang, X., Brown, R. D., Feng, Y., Mekis, E., Milewska, E. J., … Wang, X. L. (2015). Observed trends in Canada's climate and influence of low-frequency variability modes. *Journal of Climate*, *28*, 4545–4560.

Ward, D. L., & Bonar, S. A. (2003). Effects of cold water on susceptibility of age-0 flannelmouth sucker to predation by rainbow trout. *The Southwestern Naturalist*, *48*, 43–46.

Whitehead, A., Roach, J. L., Zhang, S., & Galvez, F. (2012). Salinity-and population-dependent genome regulatory response during osmotic acclimation in the killifish (*Fundulus heteroclitus*) gill. *Journal of Experimental Biology*, *215*, 1293–1305.

Wodtke, E., & Cossins, A. R. (1991). Rapid cold-induced changes of membrane order and Δ9-desaturase activity in endoplasmic reticulum of carp liver: A time-course study of thermal acclimation. *Biochimica et Biophysica Acta Biomembranes.*, *1064*, 343–350.

Yoon, G. R., Deslauriers, D., Enders, E. C., Treberg, J. R., & Anderson, W. G. (2019). Effects of temperature, dissolved oxygen, and substrate on the development of metabolic phenotypes in age-0 lake sturgeon (*Acipenser fulvescens*): implications for overwintering survival. *Canadian Journal of Fisheries and Aquatic Sciences*, *76*, 1596–1607.

Yoon, G. R., Deslauriers, D., & Andreson, W. G. (2020). Influence of prey condition and incubation method on mortality, growth and metabolic rate during early life history in lake sturgeon, *Acipenser fulvescens*. *Journal of Applied Ichthyology*, *36*, 759–767.

Yusishen, M. E., Yoon, G. R., Bugg, W., Jeffries, K. M., Currie, S., & Anderson, W. G. (2020). Love thy neighbor: Social buffering following exposure to an acute thermal stressor in a gregarious fish, the lake sturgeon (*Acipenser fulvescens*). *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *243*, 110686.

Zhang, X., Flato, G., Kirchmeier-Young, M., Vincent, L., Wan, H., Wang, X., … Kharin, V. V. (2019). Changes in temperature and precipitation across Canada. Chapter 4. In E. Bush & D. S. Lemmen (Eds.), *Canada's changing climate report. Government of Canada* (pp. 112–193). Ottawa, Ontario: Government of Canada.

Zhong, P., & Huang, H. (2017). Recent progress in the research of cold-inducible RNA-binding protein. *Future Science OA*, *3*, FSO246.

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