Transcriptome-wide patterns reveal conserved and population-specific responses to increasing acclimation temperatures in developing lake sturgeon (*Acipenser fulvescens*)

William S. Bugg*[1], Matt J. Thorstensen[1], Katie E. Marshall[2], W. Gary Anderson[1], and Ken M. Jeffries[1]

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

1. Department of Biological Sciences, University of Manitoba, 50 Sifton Road, Winnipeg, Manitoba, Canada, R3T 2N2
2. Department of Zoology, University of British Columbia, Vancouver, BC, Canada V6T 1Z4

Abstract:

Rising mean and variance in temperatures elevate threats to endangered freshwater species like the lake sturgeon, *Acipenser fulvescens*. Previous research has demonstrated that higher temperatures in early development result in physiological consequences for lake sturgeon populations throughout Manitoba, Canada, with alteration of metabolic rate, thermal tolerance, transcriptional responses, growth, and mortality. In the present study, we acclimated lake sturgeon from northern and southern populations within Manitoba to current and future projected environmental temperatures of 16, 20, and 24°C for 30 days, and measured gill transcriptional responses using RNAseq. We found population-specific and acclimation-specific responses to the thermal treatments, as well as conserved molecular responses between northern and southern sturgeon populations. Expression profiles revealed a gradient in transcriptional responses consistent with acclimation temperature, with a higher number of differentially expressed transcripts observed in the southern compared to the northern lake sturgeon population as temperatures increase, indicating enhanced transcriptional plasticity. Overall lake sturgeon populations responded to thermal acclimation by upregulating the expression of transcripts involved in transcriptional and translational regulation, mitochondrial function, pathogen responses, and DNA damage, as well as genes associated with pre- and post-transcriptional processes (i.e., methylation, alternative splicing). Further, both populations upregulated transcript expression related to cell structural damage as temperatures increased to 20°C, but the northern population also responded with increases in damage signaling and recruitment of mitochondrial processes involved in ATP production. Ultimately, these transcriptional responses highlight molecular consequences of increasing temperatures for divergent lake sturgeon populations during vulnerable early developmental periods and the critical influence of transcriptome plasticity on acclimation capacity.

1. Introduction

Freshwater fishes have experienced the highest rates of extinction among all vertebrates throughout the 20th century, influenced by the compounding and dynamic climate change-related stressors of elevated temperatures, hypoxic conditions, and pathogens which continue to imperil freshwater fishes and limit suitable habitat (Marcos-López et al., 2010; Burkhead, 2012; Comte et al., 2012; Schade et al., 2014; Saairi et al., 2018; Olusanya and Zyll de Jong, 2018; Krabbenhoft et al., 2020; Vollset et al., 2020; Chapman et al., 2021; Jane et al., 2021). Climate change is increasing both the mean and variance of environmental temperature (Thornton et al., 2014; Dillon et al., 2016). Environmental changes are often occurring faster than organisms can adapt to increasing temperatures, especially in species with long generation times (Crozier and Hutchings, 2014; Morgan et al., 2020; Logan and Cox, 2020). Therefore, phenotypic plasticity will be critical for maintaining fitness in response to climate change and promote survival in these changing environments (Burggren, 2018; Scheiner et al., 2019).

While genomic modifications underlie transgenerational plasticity, intragenerational plasticity induced to alter an organism's phenotype at the transcriptomic level may act as an adaptive trait (Muschick et al., 2011; Jonsson and Jonsson, 2019). This adaptive role of plasticity may enable molecular physiological changes, thereby increasing an organism's resistance to environmental change, reproductive capacity, and promoting survival (Lafuente and Beldade, 2019; Oomen and Hutchings, 2017; Wellband and Heath, 2017). Further, polyploid organisms with larger genomes may have increased acclimatory capacity at both the transgenerational and intragenerational timescales through transcriptome plasticity (Ellis et al., 2014; Trifonov et al., 2016; Van de Peer, 2017). In populations of polyploid species with divergent genetic histories, which may be under genetic constraints preventing performance traits from evolving rapidly (Logan and Cox, 2020), divergent phenotypes of transcriptomic plasticity can be expected as environmental changes intensify.

During early development, changes in temperature are a critical abiotic factor necessitating prompt phenotypic alteration. These plasticly-induced phenotypic changes at an individual level can alter organismal physiology and buffer against acute environmental changes (McKenzie et al., 2021). Physiological flexibility can be an adaptive response stimulated in stressful environments and is a reversible phenotypic change that may be persistent for days to

months, called acclimation (Hockachka and Somero, 2002; Crozier and Hutchings 2014; Havird et al., 2020; Mackey et al., 2021). Underlying these phenotypic modifications are transcriptional mechanisms that impact the establishment of long-term phenotypes (e.g., developmental plasticity) (Jonsson and Jonsson, 2019). Exposing developing fish to increasing environmental temperatures will thus alter traits that may have transient as well as long-lasting physiological consequences. The basis for plasticity is variation in phenotypes produced by the same genotype under environmental variation (Lafuente and Beldade, 2019). Acclimatory plasticity is especially important in early life to ensure sufficient phenotypic flexibility, enabling fishes to survive in variable environmental conditions until adulthood when their reproductive potential can be reached (Burggren, 2018). In early developmental stages, different phenotypes and developmental trajectories can emerge from genetically distinct populations exposed to similar environmental change, based, in part, on differences in transcriptome plasticity. Induced molecular responses to stressors often differ between populations, likely as a result of differences in transcriptional plasticity, and may be indicative of long-term fitness and survival consequences if phenotypes cannot be adequately altered in early life to allow individuals to persist in changing environments (Whitehead et al., 2012; Bugg et al., 2020; Mundy et al., 2020; Bugg et al., 2021).

A primary mechanism underlying physiological responses in the face of environmental change are modifications to the abundance of messenger RNA (mRNA) through transcriptional processes (Smith et al., 2013; Connon et al., 2018; Jeffries et al., 2019; Jeffries et al., 2021). Through mRNA sequencing (i.e., RNAseq) and *de novo* transcriptome assembly, transcriptomics approaches can be used when few genomic resources are available for a species (Alvarez et al., 2015; Connon et al., 2018; Oomen & Hutchings 2017; Thorstensen et al., 2020; Komoroske et al., 2020). Examining differential gene expression through transcriptome-wide approaches can illuminate an organism's physiologically- and ecologically-relevant responses to environmental change, aid in the identification of sublethal tolerance thresholds, and inform conservation management practices (Connon et al., 2018). As environmental extremes in freshwater systems escalate, transcriptomic techniques provide powerful tools to investigate adaptive capacites in species of conservation concern, highlighting the genetic mechanisms that promote physiological acclimation to environmental change. If stressors breach sub-lethal thresholds, transcriptomic plasticity and acclimatory capacity may be diminished, suggesting increased vulnerability to

additional environmental stressors, and the possibility of severe short-term outcomes, limited recovery, and the potential for impacts on long-term fitness (Smith et al., 2013; Jeffries et al., 2018).

In fishes, the gill is a multi-purpose tissue reactive to the external environment with roles in respiration, osmoregulation, pathogen defense, acid-base balance, and responses to thermal stress (Lazado and Caipang, 2014; Komoroske et al., 2015; Akbarzadeh et al., 2018; Gilmour and Perry, 2018). Gene transcription producing mRNAs and subsequent translation into proteins underlies many phenotype modifying processes, contributing to plasticity and physiological change (Schlichting and Smith, 2002). Fishes exposed to increased temperatures alter their physiology through transcriptional mechanisms, enabling them to acclimate to their environment and preserve internal homeostasis in the face of environmental variation. Investigating transcriptional responses in the gill can thus highlight changes at a key interface between these organisms and their environment.

Greater than 50% of the world's river basins face widespread anthropogenic impacts which limit productivity, species richness, and biodiversity, threatening approximately a quarter of freshwater fish species globally such as the lake sturgeon, *Acipenser fulvescens* (Palmer et al., 2008; IUCN, 2018; Grill et al., 2019; Su et al., 2021; Warkentin et al., 2022). Lake sturgeon are widely distributed throughout North America, and within Canada have experienced population declines >90% in the northern extent of their range since the 1960's (COSEWIC, 2006). Historically, lake sturgeon population declines in the 1800s were caused by commercial exploitation for caviar. Since the 1950's however, habitat loss and degradation associated with dams and other barriers have become the most prominent threats to populations and the species' persistence (Cleator et al., 2010; COSEWIC 2017; Van der Lee and Koops, 2021). Temperatures in the northern range for lake sturgeon are also increasing faster than the global average (projected 2.1-3.4°C increase by 2050; Manitoba Hydro, 2015), which may further limit suitable habitat, and imperil these endangered subarctic populations as water temperatures exceed their thermal limits (Vincent et al., 2015; Zhang et al., 2019; Bugg et al., 2021).

Increasing environmental temperatures directly influence the development, metabolism, transcriptomic responses, and survival of northern populations of lake sturgeon under laboratory conditions and may contribute to observed declines in wild stocks (Bugg et al., 2020; Bugg et al.,

2021). Indirectly, elevating temperatures may increase environmental hypoxia and the susceptibility of lake sturgeon to environmentally prevalent bacterial or viral pathogens, which may be transmitted horizontally between individuals or vertically across generations (LaPatra et al., 1994; Georgiadis et al., 2001; Fujimoto, 2018; Clouthier et al., 2020). Sturgeons may be especially susceptible to the combined effects of increasing temperature, hypoxic environments, and pathogens in early development, when mortality is often high (> 90%; Caroffino et al., 2010) and sturgeon have limited immune responses and ability to escape stressful environments (Webb, 1986; Peake et al., 1997; Schindler, 2001; Breau et al., 2011; Deslauriers and Kieffer, 2012; Gradil et al., 2014 a,b; Verhille et al., 2014). However, despite these vulnerabilities in early development, sturgeon have successfully persisted for hundreds of millions of years in ever-changing environments, evolving elaborate polyploid genetic complexity, and demonstrating high levels of phenotypic plasticity in early development, with the potential for increased underlying inducible transcriptional plasticity (Braasch and Postlethwait, 2012; Trifonov et al., 2016; Bugg et al., 2020; Bugg et al., 2021; Penman, 2021; Yoon et al., 2021; Brandt et al., 2021, Brandt et al., 2022). Therefore, early development represents a crucial window to examine lake sturgeon responses to thermal acclimation, when exceptionally plastic responses may be anticipated (Burggren, 2018; Barley et al., 2021).

Here we used RNAseq to investigate transcriptomic responses to increased environmental temperatures in the gills of two latitudinally separated and genetically distinct populations of developing lake sturgeon (McDougall et al., 2017; Bugg et al., 2020). As sturgeons are ancestral polyploid species, with highly variable genomic architecture and phenotypic plasticity (Braasch and Postlethwait, 2012; Trifonov et al., 2016; Bugg et al., 2021), we expected these populations of lake sturgeon to respond uniquely to the temperature treatments. We examined the gill mRNA transcript abundance in n = 36 individuals from three thermal acclimation treatments in the northern and southern population of lake sturgeon within Manitoba. We then investigated phenotypic plasticity through exploration of 9 total contrasts spanning the molecular mechanisms shared between populations as acclimation temperatures increased, as well as population and acclimation-specific responses to increased environmental temperatures expected to impact these lake sturgeon populations. Previous research showed acclimating lake sturgeon for 30 days to the potentially thermally stressful temperatures of 20°C and 24°C, resulted in population and acclimation-specific changes in mortality, metabolism, thermal tolerance, and transcriptional

responses (Bugg et al. 2020). Therefore, we hypothesized that acclimation to these same elevated temperatures during early life history would elicit transcriptome-wide responses associated with energy mobilization, pathogenic infection, cellular repair, and heat shock proteins indicative of chronic thermal stress. Given the higher mortality and lower thermal tolerance observed in the northern population post-temperature acclimation in a previous study (Bugg et al., 2020), we predicted that the northern population of lake sturgeon would show greater transcriptional signatures of a thermal stress as temperatures increased, relative to the southern population.

2. Materials and Methods:

2.1 Lake Sturgeon Husbandry and Acclimation

Lake sturgeon used in the current study were from the same populations and acclimation treatments described in Bugg et al. (2020). Briefly, gametes were collected from wild male and female lake sturgeon downstream from the Pointe du Boise Generating Station on the southern Winnipeg River (50°17’52’’N, 95°32’51’’W) and first rapids on the northern Burntwood River (56° 02’ 46.5’’ N, 96 °54’18.6’’W) (Figure 1). Eggs and sperm from the Winnipeg River were fertilized at the University of Manitoba animal holding facility while those from the Burntwood River were fertilized at the Grand Rapids Fish Hatchery, and subsequently transferred to the University of Manitoba. Post-fertilization, embryos from each population were submerged in a clay substrate to de-adhease them and then were gently stirred by hand for 1 h. Next, embryos were rinsed with dechlorinated freshwater and kept in tumbling jars to incubate at 12°C until hatch. Water temperature was maintained at 12°C until 13 days post fertilization after which it was increased by 1°C day^{-1} until 16°C, matching typical hatchery rearing conditions in Manitoba. Starting at 19 days post fertilization, and continuing throughout the length of the experiment, developing lake sturgeon were fed freshly hatched artemia (Artemia International LLC; Texas, USA) three times daily. Acclimation began at 30 days post fertilization, by increasing treatments to 20°C and 24°C at a rate of 1°C day^{-1} and keeping one treatment from each population at 16°C. Lake sturgeon from both populations were acclimated to these temperatures for 30 days with water temperatures recorded by HOBO Water Temperature PROv2 Data Loggers (Onset Computer Corporation; Bourne, MA, USA) and checked by thermometer at least three times daily. All animals in this study were reared and sampled under guidelines established by the

Canadian Council for Animal Care and approved by the Animal Care Committee at the University of Manitoba under Protocol #F15-007.

2.2 Sampling and RNA extraction

At the end of the 30 day acclimation, six larval lake sturgeon per treatment were netted 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. Next, gill tissue was extracted from each larval fish and preserved in RNA*later* (Invitrogen; Carlsbad, CA, USA), held at 4°C for 24 h and stored at −80°C prior to RNA extraction. Total RNA was extracted from the gill tissue by homogenization in 500 µl of lysis buffer for 10 min at 50 Hz using a TissueLyser II (Qiagen; Germantown, MD, USA) and then using a PureLink RNA Mini Kit (Invitrogen; Ambion Life Technologies) following manufacturer instructions. Total RNA purity and concentration was evaluated for all samples using a NanoDrop One (Thermo Fisher Scientific) as well as gel electrophoresis to assess RNA integrity. Samples were then stored at -80°C prior to sequencing.

2.3 *De novo* Transcriptome Assembly

Prior to the present study, a lake sturgeon gill reference transcriptome was created by sequencing mRNA from the pooled RNA of 3 individuals. We used a mRNA isolation, stranded library preparation approach to produce 50 million reads of 100 base pair paired-end reads. The transcriptome was *de novo* assembled using Trinity (Grabherr et al., 2011) through the Canadian Centre for Computational Genomics (C3G; https://computationalgenomics.ca/). Assembled transcripts were then annotated with Trinotate (http://trinotate.sourceforge.net/) using a modified approach. First assembled transcripts were blasted against a fish-specific database with annotation hits returned. Transcripts that were not annotated from the fish specific database were then blasted against the entire SwissProt library.

2.4 Sequencing

Total RNA for each sample was normalized to approximately 15 ng μL^{-1} at a total volume of 10 µL and shipped to the McGill Applied Genomics Innovation Core sequencing facility (https://www.mcgillgenomecentre.ca/) for cDNA library preparation and sequencing. Samples were run on a Bioanalyzer to confirm RNA quality and all samples had a RNA Integrity

Number (RIN) between 8.7-10. A mRNA isolation step was performed using NEBNext Poly(A) Magnetic Isolation Modules (New England Biolabs) followed by using NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs) to produce stranded cDNA libraries. NEBNext dual adaptors (New England Biolabs) were then individually applied to barcode each library prior to sequencing of 100 base pair paired-end reads. All 36 sturgeon gill samples were sequenced on a single NovaSeq 6000 (Illumina) lane producing 1.53 billion total reads with an average of 42,528,434 (± 17,812,372 SD) reads per sample. Detailed results per sample can be found in Supplementary Table 1.

2.5 Quality control and Trimming

Raw read files were uploaded to Galaxy servers (Giardine et al., 2005) and paired reads were joined and checked for quality using FastQC version 0.11.8 (Andrews, 2010). To filter low-quality reads, Trimmomatic version 0.38.0 was applied with the Illuminaclip option set to standard for TruSeq3 adapter sequences, a maximum mismatch count of 2, and a palindrome read alignment threshold of 30. Additionally, a sliding window trimming step was used with a size of 4 base pairs as well as a minimum read length filter set at 20 bases. Ultimately this trimming removed approximately 2.8% (± 0.29% SD) of reads from each file. Full details for quality control and trimming procedures are available in Supplementary File 1, Sheets A-C.

2.6 Read Mapping and Differential Expression

After filtering, HISAT2 version 2.1 and StringTie version 2.1.1 (Kim et al., 2015; Pertea et al., 2015) were used to align paired read libraries to the *de novo* assembled gill transcriptome and produce a set of assembled transcripts for each sample, respectively. HISAT2 was run with default parameters and produced an overall alignment rate of approximately 82.6% (± 1.2% SD) per sample. HISAT2 aligned bam files were then input into StringTie to assemble transcripts, using default settings, producing an average of 94,992 (± 11,075 SD) transcripts per sample. StringTie files for each sample were then merged producing a master list of 139,456 transcripts. Total counts for each of these transcripts were then counted in HISAT2 produced aligned read files using featureCounts version 1.6.2 with default settings (Liao et al., 2014; Available in Supplementary File 2). Total counts were then assessed for differential expression using the R v4.0.0 package EdgeR v3.32.1 (Robinson et al., 2010; R Core Team, 2022). Prior to analysis the “filterByExpr” function with default arguments was used to retain transcripts expressed among

any individual, resulting in a final count of 88,894 transcripts. Full details for read mapping of each individual sample are available in Supplementary File 1, Sheet D.

2.7 MDS, Differentially Expressed Transcripts, and Aggregate Heatmapping

A multidimensional scaling (MDS) plot was produced using featureCounts from the above 88,894 transcripts after normalizing the data to the library size using the plotMDS function in the limma package (Ritchie et al., 2015). Next, numbers of differentially expressed genes between the Burntwood River and Winnipeg River populations of lake sturgeon acclimated to 16°C, 20°C, and 24°C for 30 days in early development were tabulated for upregulated and downregulated genes independently, representing genes with a $\log_2$ fold change > 1 and a *q*-value < 0.05 for each of the 9 individual contrast. The 9 total contrasts compared the populations at each acclimation temperature as well as across acclimation temperatures for each population. Log_2 counts per million (CPM) data for all 88,894 transcripts was then generated and the top 500 genes from each contrast with the highest absolute value $\log_2$ CPM fold change were included in an aggregate list of the most highly differentially expressed genes across all contrasts, resulting in a total of 4,500 transcripts, 2,285 uniquely included (full CPM data is available for all 88,894 transcripts in Supplementary File 3). A heatmap with hierarchical clustering was then produced using the $\log_2$ CPM expression of these aggregate most highly differentially expressed 2,285 unique transcripts using the pheatmap package (Kolde, 2015).

2.8 Functional Term Enrichment

Transcripts annotated to the *de novo* lake sturgeon transcriptome were split into groups of upregulated and downregulated genes by treatment based on their $\log_2$ CPM fold change (< -1 or > 1) and were counted for distinct gene names (hereafter referred to as split-contrast). The positively and negatively regulated annotated genes were then analyzed for functional enrichment using gene ontology (GO) terms through enrichR as well as Kegg pathway analysis (Chen et al., 2013; Kuleshov et al., 2016; Xie et al., 2021) with positive hits returned from the “GO_Biological_Processes_2018”, “GO_Molecular_Function_2018”, "GO_Cellular_Component_2018", and “Kegg_2016” databases and filtered for an adjusted *p*-value < 0.05. The resulting 212 enrichment terms across the upregulated and downregulated spit-contrast were then tabulated into a master file detailing their presence or absence in each split-contrast (0 or 1; Full presence and absence data across each split-contrast is available in

Supplementary File 4). This master file was then used to create UpSet plots of the shared and unique terms both upregulated and downregulated across all split-contrasts, upregulated terms as temperatures increased from 16°C to 20°C, and terms which decreased as temperatures increased from 20°C to 24°C using the 'UpsetR' package v1.4.0 (Conway et al., 2017). Split-contrasts with ≥ 50 functional enrichment terms were then focused on for interpretation of results.

Next, non-redundant enrichment terms in the GO Biological Process category were identified with REVIGO for summary and visualization (Supek et al., 2011) using a list size value of 0.5. Adjusted *p* values were supplied for all REVIGO analyses to guide the clustering of redundant GO terms by the REVIGO algorithm (Supek et al., 2011). Where GO terms were shared across populations, the geometric mean of the adjusted *p* values from both populations was input into REVIGO. All REVIGO output is available in Supplementary File 5, Sheets A-I.

2.9 Aggregate Gene Network Analysis and Enrichment

An aggregate of the most highly differentially expressed transcripts was then investigated, producing a total of 389 annotated most differentially expressed transcripts, with 259 uniquely annotated transcripts, from the aggregate list of 4,500 most differentially expressed transcripts across contrasts used for the heatmap (the full aggregate list of 389 annotated most differentially expressed transcripts is available in Supplementary File 6). These 259 unique gene names were uploaded to OmicsNet (Zhou and Xia, 2018) and input under the genes category using the "official gene symbol" classification and zebrafish *Danio rerio* as the study organism, as this was the most closely related model species option. Next, protein-protein interactions were selected in the network building panel, using STRING (Szklarczyk et al., 2019), comprehensive protein-protein interactions containing both known and predicted interactions, to assembly a network of the 259 unique genes and their potential interactions. The network was then changed to a 2-D model, using a force atlas layout, with seed nodes highlighted with a blue ring around the node, and edge bundling applied. Functional analysis of the produced network was then categorized using the Function Explorer, querying all nodes against the GO: Biological Process database. Processes with a p value < 0.05 and q < 0.15 are reported in Supplementary File 7. Discussion of hub transcripts and enriched functional processes focuses on those with at least two pertinent hub transcripts and inclusion in overarching REVIGO analysis.

3. Results

3.1 MDS, Differentially Expressed Transcripts, and Aggregate Heatmapping

Analysis of MDS plots (Figure 2A), number of upregulated and downregulated genes (Figure 2B), as well as the heatmap displaying the most highly differentially expressed genes across contrasts (Figure 2C), demonstrated that the Burntwood River and Winnipeg River populations of lake sturgeon responded very differently to elevated temperatures both in the magnitude and trajectory of transcriptional change. The MDS plot showed divergent response patterns with Winnipeg River sturgeon in treatments of 20°C and 24°C grouping out separately from the rest of the treatments. Patterns of upregulation and downregulation between the two populations at a given acclimation temperature, and within populations across acclimation temperatures, demonstrated population-specific and acclimation-specific responses to elevated temperatures in developing lake sturgeon. At 16°C there were a similar number of genes upregulated and downregulated when comparing Burntwood River and Winnipeg River sturgeon when comparing the two populations. However, at 20°C and 24°C there were many more transcripts upregulated in the Burntwood River than decreased when compared to Winnipeg River sturgeon in the same temperatures, respectively.

When comparing within population across acclimation temperatures, the largest changes in transcript regulation in the Burntwood River and Winnipeg River sturgeon occurred as acclimation temperatures increased from 16°C to 20°C, where the populations differentially expressed a total of 9,334 and 19,216 transcripts, respectively. These changes in transcript expression between 16°C and 20°C represent the differential expression of approximately 10.5% and 21.6% of all transcripts for the Burntwood River and Winnipeg River populations of sturgeon included in the analysis, respectively. Between 16°C and 24°C these numbers of differentially expressed transcripts fell drastically (67.8%) in the Burntwood River population, when compared to the 16°C to 20°C transition, with just 3,028 total differentially expressed transcripts. However, for Winnipeg River sturgeon this same transition saw a lesser reduction to 14,850 total transcripts, a much smaller decrease (22.6%) in differential expression over the same acclimatory contrast. A similar decrease in expression, as observed between the Burntwood River 16°C to 20°C and 16°C to 24°C contrasts, occurred for the Winnipeg River sturgeon between the Winnipeg River 16°C to 24°C and Winnipeg River 20°C to 24°C contrasts (68%),

with a total of 4,753 differentially expressed transcripts between 20°C and 24°C. While producing similar qualitative patterns of expression to the BWR, the number of differentially expressed transcripts was larger in the Winnipeg River across acclimation treatments.

The heatmap outlined specific patterns of transcript expression of the most highly differentially expressed transcripts, with divergent $\log_2$ CPM expression values as temperatures increased above 16°C for both populations but with differences in these patterns across populations (Figure 2C). Differential expression patterns of these transcripts are largely qualitatively similar across the Burntwood River 24°C and Winnipeg River 16°C treatments, even though they represent highly different acclimation profiles and divergent populations. Further large changes in transcriptional regulation were observed in the Winnipeg River 20°C and 24°C treatments, when comparing them to patterns of expression observed in the other four treatments, across both populations. Overall, the patterns in the most highly differentially expressed genes align with the MDS analysis in the overlap of the Burntwood River 24°C and Winnipeg River 16°C treatments, as well as the unique transcriptional profiles of Winnipeg River sturgeon acclimated to 20°C and 24°C, suggesting that patterns in the most highly differentially expressed transcripts reflect similar transcriptional trajectories as shown in the MDS analysis of all transcripts.

3.2 Functional Term Enrichment

Term enrichment demonstrated 212 unique GO and KEGG pathway terms distributed across the 17 split-contrasts (Figure 3A). Overall, the split-contrasts with the largest number of assigned terms were those underlying processes being upregulated as temperatures increased from 16 to 20°C (WR16v20_neg and BWR16v20_neg; 75 and 85 terms respectively) and those processes that decreased in expression as temperatures continued to increase from 20°C to 24°C (WR20v24_pos and BWR20v24_pos; 62 and 97 terms, respectively). The split-contrasts between the Burntwood River and Winnipeg River populations as temperatures increase from 16°C to 20°C and again from 20°C to 24°C specifically revealed conserved and population-specific biological processes upregulated as temperatures increase from 16°C to 20°C and those that are decreased with further temperature increase to 24°C. As temperatures increased from 16°C to 20°C there were 50 upregulated functional term responses shared between populations, as well as 39 responses unique to the northern Burntwood River population and 25 distinct to the

southern Winnipeg River population (Figure 3B). As acclimation temperatures increased further from 20°C to 24°C there were 26 functional term responses shared between populations, with 71 unique to the Burntwood River population and 38 distinct to the Winnipeg River population (Figure 3C).

3.3 GO Term and REVIGO Analysis

Overwhelmingly, the majority of all 212 terms were categorized as GO Biological Processes, encompassing 82.55% of terms identified and highlighting transcriptional mechanisms altered across lake sturgeon populations as temperatures increase, therefore these terms were focused on for further analysis.

Conserved responses to increasing temperature

As the acclimation temperature increased from 16°C to 20°C there were 49 conserved GO Biological Process terms identified as upregulated and shared between the Burntwood River and Winnipeg River populations of lake sturgeon (REVIGO output displayed in Figure 4A). These shared processes were involved in regulation of mRNA processing, ncRNA processing (including mRNA splicing via spliceosome and transesterification), translational regulation (including gene miRNA gene silencing), mitochondrial translation, ribosomal biogenesis (including mitochondrial respiratory chain complex assembly), L-methionine salvage, protein methylation, DNA damage responses, as well as viral gene expression and transcription (Supplementary File 5, Sheet A).

As acclimation temperature further increased from 20°C to 24°C there were 19 conserved GO Biological Process terms that decreased in expression in both the Burntwood River and Winnipeg River lake sturgeon populations (REVIGO output displayed in Figure 4B). These shared processes were involved in RNA polymerase II transcription, ncRNA processing (including mRNA splicing via spliceosome and transesterification), regulation of translational machinery, mitochondrial protein insertion into the inner mitochondrial membrane, mitochondrial organization, and cell cycle regulation (Supplementary File 5, Sheet B).

Across acclimatory treatments, there were 16 GO Biological Process terms identified as upregulated with increasing temperature between 16°C and 20°C but then decreased in expression between 20°C and 24°C. Largely these mechanisms were involved in mRNA

processing as well as splicing, mitochondrial organization, ribosome biogenesis, and regulation of the cell cycle (Supplementary File 5, Sheet C).

Population-specific responses to increasing temperature

As temperatures increased from 16°C to 20°C, there were also population-specific increases in transcripts involved in various biological processes. Specific to the Winnipeg River population, there were 25 unique GO Biological Process terms identified as upregulated (Figure 5A) largely characterized by changes in telomerase localization as well as protein targeting, binding, ligation, modification, and removal. There were also biological processes upregulated involved in the assembly of metallo-sulfur clusters, digestive development, mitotic cell cycle phase transitioning, and viral processes (Supplementary File 5, Sheet D). In the Burntwood River population, there were an additional 38 unique GO Biological Process terms upregulated (Figure 5B). These Burntwood River specific upregulated terms involved histone methylation, regulation of mRNA stability, mRNA export from the nucleus, mitochondrial function, transcriptional termination, anatomical structure homeostasis, and a variety of DNA damage processes (Supplementary File 5, Sheet E).

As temperatures increased further from 20°C to 24°C there were more population-specific changes in transcripts, however these changes were involved in the decrease of the expression of transcripts involved in these biological processes. Specific to the Winnipeg River population, there were 34 GO Biological Process terms that decreased between 20°C and 24°C (Figure 5C). These processes were largely associated with production of miRNA's involved in gene silencing, mitotic cell cycling/phase transition, monooxygenase activity, responses to UV, DNA repair and damage signaling as well as nuclear membrane disassembly and telomerase localization (Supplementary File 5, Sheet F). Additionally, in the Burntwood River population, there were 53 unique GO Biological Process terms that decreased in expression between 20°C and 24°C (Figure 5D). These Burntwood River specific downregulated processes involved DNA, RNA, and protein level modifications, including changes to RNA structure, gene silencing, alternative splicing, protein folding, and protein methylation (including histone methylation). Further there was a decrease in various processes involved in mitochondrial translation, transport, protein targeting, and ATP synthesis. Finally, there were decreases in processes involved in chordate

embryonic development, Wnt signaling (conical and planar cell), and DNA damage recognition (Supplementary File 5, Sheet G).

Across acclimatory treatments, there were several population-specific GO Biological Process terms identified as upregulated with increasing temperature between 16°C and 20°C but then decreased in expression between 20°C and 24°C. There were 9 processes upregulated in both populations between 16°C and 20°C but then decreased in expression between 20°C and 24°C, uniquely in the Winnipeg River population. These processes were involved in regulation of mRNA processing, miRNA production involved in gene silencing, chromosomal organization, as well as DNA damage and repair responses (Supplementary File 5, Sheet H). Additionally, there were 17 GO Biological Process terms upregulated in both the Burntwood River and Winnipeg River between 16°C and 20°C but then decreased in expression between 20°C and 24°C only in the Burntwood River population. These processes were involved in RNA processing, gene silencing by miRNA, mitochondrial respiration/function, protein synthesis as well as methylation, and DNA damage recognition (Supplementary File 5, Sheet I).

3.4 Aggregate Transcript Network Analysis and Functional Enrichment

The aggregate most highly differentially expressed transcript network demonstrated connections across many different functional groups of transcripts that were altered in developing lake sturgeon during acclimation to elevated temperatures. Transcripts identified as hubs (orange circles, highlighted in blue; Figure 6) form many responsive network connections and are active in cell cycling (*cdk2*, *src*, *rcc2*), chaperone activity and protein folding (*hspe1*, *hsc70*, *vimp*, *cct4*), pre and post transcriptional modification (*carm1*, *larp7*) as well as immune and inflammatory activation (*traf6*, *prkcbb*, *egr1*, *egr2b*). Others indicate activation of more general cellular stress responses (*sgk1*) involving maintenance of metabolic processes (*csnk2b*), heart development (*tll1*), and osmotic balance (*cftr*). Many of these processes were also present in the network GO Biological Process term enrichment with enriched pathways including cell cycling, protein folding, responses to heat, immune (general, adaptive, inflammation), heart development, responses to stress, and DNA repair processes (Supplementary File 7).

4. Discussion

In the current study, we demonstrated wide-ranging conserved and population-specific transcriptional responses as environmental temperatures increased in two geographically and genetically distinct endangered populations of lake sturgeon. These responses spanned wide ranging cellular protective processes induced during acclimation to thermally stressful conditions. Specifically, these processes related to the cellular machinery involved in transcriptional and translational regulation, mitochondrial function, oxidative damage, and immunocompetence. Overarching transcript expression patterns indicated population-specific capacity for gene transcription as environmental temperatures increased, with divergent transcript response trajectories and a higher magnitude of transcriptional change apparent in the southern Winnipeg River population. Conserved and population-specific biological process responses illustrated that this transcriptional plasticity promoted whole-organism acclimation to elevated temperatures in developing lake sturgeon with potential for adaptive responses at the population scale. Ultimately, these processes enabled lake sturgeon to acclimate to environments with increasing temperature and highlight the physiological consequences once acclimatory capacity and sublethal thresholds have been exceeded.

Transcriptomic Responses to Increasing Temperatures

As temperatures increased from 16°C to 20°C, analysis of altered biological processes revealed shared transcriptional mechanisms conserved between the lake sturgeon populations, as well as population-specific processes unique to southern Winnipeg River and northern Burntwood River sturgeon. When compared to other acclimatory and thermal stress experiments, transcriptional plasticity observed in the Winnipeg River and Burntwood River lake sturgeon populations as temperatures increased from 16°C to 20°C (21.6 and 10.5% of genes differentially expressed, respectively) was on the upper end of transcriptional plasticity detected for other fish species (4-20%; Logan and Somero, 2011; Garvin et al., 2015), especially so for southern Winnipeg River population of lake sturgeon. Both populations upregulated biological processes involved in transcriptional and translational activity, which may lead to protein-level changes that impact whole organism responses to thermal stress (Buccitelli and Selbach, 2020). Additionally, induced changes in transcript expression associated with biological processes involving cellular damage responses, mitochondrial function, and pathogenic infection

potentially highlight sublethal thresholds and physiological impacts of thermal stress as temperatures increase (Jeffries et al., 2018).

Population-specific responses indicated induction of protective mechanisms in the southern Winnipeg River population and further markers of cellular damage in northern Burntwood River sturgeon. Lake sturgeon from the Winnipeg River upregulated processes involved in protein cycling, maintenance of transcriptional mechanisms, membrane modification, and electron transport chain function, protecting themselves from the potential effects of oxidative species formed under elevated temperatures (Gomez-Gallardo et al., 2016). Uniquely, transcriptional responses showed that Winnipeg River sturgeon may have relocalized telomeres to cajal bodies, highly transcriptionally active sites within the nucleus (Ogg and Lamond, 2002; Cioce and Lamond, 2005). We speculate that telomere relocalization may have protected the cajal bodies from the effects of a changing environment and in the process, preserved transcriptional machinery as well as plasticity. In contrast, northern Burntwood River lake sturgeon upregulated transcripts related to damage responses at the DNA, mRNA, chromosome, cell, and anatomical structure levels, suggesting widespread damage across many levels of biological organization. Transcripts associated with cellular respiration was also upregulated as well as mitochondrial respiratory chain complex III, which if not present in adequate amounts can lead to proton leakage during ATP production, mitochondrial dysregulation, and oxidative damage (Guo et al., 2013; Chandel et al., 2020). Previous work demonstrated a decreased metabolic and antioxidant capacity in Burntwood River sturgeon under thermally stressful conditions, relative to their Winnipeg River counterparts (Bugg et al., 2020). Thus, this combination of transcriptional and whole-organism physiological responses suggests that reduced transcriptional plasticity in northern Burntwood River sturgeon compromises ATP production, metabolic function, and cellular protective mechanisms, leading to increased damage, potentially from the production of reactive oxygen species (Filho, 2007; Portner and Lannig, 2009; Paital et al., 2016; Portner et al., 2017; Birnie-Gauvin et al., 2017). However, the southern Winnipeg River population exhibited enhanced transcriptional responsiveness, induction of protective mechanisms, as well as maintained antioxidant and metabolic capacity as temperatures increase from 16°C to 20°C (Bugg et al., 2020).

Further temperature increases from 20°C to 24°C elicited the suppression of processes promoting mRNA transcription and protein translation across both populations of sturgeon, indicating strong thermal thresholds for plasticity to environmental change. Many of these processes, including production of cellular transcriptional and translational machinery, as well as the maintenance of mitochondrial function, were induced between 16°C and 20°C, but were ultimately decreased as temperatures increase from 20°C to 24°C. This responsive trajectory in the shape of an inverted U as temperatures increase, may be indicative of sublethal thresholds that are likely predictive of long-term impacts to organismal fitness (Jeffries et al., 2015; Jeffries et al., 2018).

Downregulation of population-specific processes as temperatures increased from 20°C to 24°C indicated that further temperature elevation induced unique effects across northern and southern Manitoba populations of lake sturgeon. Sturgeon from the Winnipeg River demonstrated decreases in cell cycling and damage responses, while sturgeon from the Burntwood River exhibit increased disruption of transcriptional, translational, protein, mitochondrial, and developmental processes. Overall, these downregulated processes demonstrated increased cellular stress and the inhibition of integral components of cellular control for both sturgeon populations, as well as increased disruption and specific aspects of mitochondrial dysregulation in Burntwood River sturgeon exposed to high temperature thermal stress.

Network Analysis

Predictive transcript network analysis of the aggregate most highly differentially expressed transcripts across all contrasts highlighted functional processes possibly significant for thermal acclimation. Key hub transcripts in these regulatory networks involved cell cycling, protein folding, transcriptional regulation and immune responsive mechanisms with functional enrichment as well as overarching REVIGO analysis indicating the recruitment of similar biological processes. Together, these highlighted transcripts and processes indicate the importance of these systems in the transcriptomic response to thermal acclimation across different populations of developing lake sturgeon.

Throughout temperature acclimation, hub transcripts and pathways (pathway: mitotic cell cycle; hub transcripts: c*dk2*, *src*, *rcc2*) critical in the process of cell cycling and proliferation

were altered. These transcripts were largely involved with the G1/S phase transition, and associated DNA replication (Taylor and Shalloway 1996; Bacevic, et al., 2017; Guo et al., 2021) indicating processes disrupted by thermal stress. This G1 checkpoint specifically detects DNA damage prior to entering the S phase of DNA synthesis, preventing proliferation of irreversible DNA damage in future cells (Bertoli et al., 2013). From 16°C to 20°C these processes were upregulated in both populations of sturgeon, however as temperatures increase to 24°C they decrease, suggesting a reduction in the regulation of cell cycling functions and potentially a proliferation of DNA damage as cells replicate in high temperature environments (Bartek et al., 2004).

Increased temperatures also modulated protein folding and cycling processes (pathways: protein folding, response to heat; hub transcripts: *hspe1*, *hsc70*, *vimp*, *cct4*) in the face of thermal stress and reactive oxygen species formation. These chaperones have wide ranging functions throughout the cell involving the maintenance of mitochondrial proteins under stress, cytosolic protein folding and ligation, degradation and recycling of misfolded proteins in the endoplasmic reticulum, and preservation of proteins in the cytoskeletal structure of the cell, respectively (Hartman et al., 1992; Place and Hoffman, 2001; Jackson and Hewitt 2016; Vallin and Grantham, 2019). Modulation of these chaperone proteins, paired with an observed decrease in metabolic rate for lake sturgeon in temperatures of 24°C from previous research (Bugg et al., 2020), suggest that under thermal stress cells transcriptionally modified heat shock protein production and decreased mitochondrial oxygen consumption to suppress formation of reactive oxygen species, adaptively stabilizing the existing cellular proteome to enhance long-term fitness and survival (Storey and Storey, 2011).

In addition to changes in protein folding mechanisms, differential expression of transcripts involved in transcriptional regulation (hub transcripts: *carm1* and *larp7*) further highlight methylation and alternative splicing processes. Histone level methylation (*carm1*) alters the structure and availability of DNA for transcription, promoting phenotypic modification at the DNA level, allowing lake sturgeon to rapidly respond to environmental change (Burggren and Crews, 2014). Additionally, post-transcriptional modifications by pre-mRNA splicing (*larp7*; Frilander and Barboric, 2020) can provide enhanced transcriptional plasticity, with the potential for enhanced adaptation to environmental change (Steward et al., 2022). Together,

these two processes enhance the ability of lake sturgeon to modify the production of mRNAs prior to translation, and likely promote phenotypic plasticity towards increasing temperatures.

Finally, elevated temperatures altered immune system processes (pathways: adaptive immune response, inflammatory response, immune system processes; hub transcripts: *traf6, prkcbb, egr-1, egr2b, insig1*), as sublethal thresholds were breached. Both populations of sturgeon additionally had increased transcriptional markers associated with viral infection, suggesting immune compromise and increased susceptibility to pathogens as temperatures increased, and a sublethal threshold of 20°C for immunocompetence. As the above hub genes are key innate and adaptive immune regulators (Lim et al., 2015; Choi, 2005; Wei et al., 2017; Miao et al., 2017; Zhang et al., 2019; Trizzino et al., 2021), modification of their transcription impacts many immune processes throughout their regulatory network. Endemic viral pathogens likely infect both studied populations of lake sturgeon, other populations throughout the lake sturgeon range, and, research suggests, sturgeon species throughout North America (Drennan et al., 2006; Clouthier et al., 2013; Clouthier et al., 2020; Mugetti et al., 2020; Stilwell et al., 2022). Thus, future increases in temperature will not only have direct effects on the lake sturgeon's thermally responsive mechanisms, but likely indirect effects on pathogen tolerance and survival as combining stressors mount which may extend to other species of sturgeons as well (Dittmar et al., 2014; Alfonso et al., 2020).

Mitochondrial Limits Under Thermal Stress

Based on transcriptional evidence, mitochondrial function is likely inhibited across both populations of lake sturgeon as temperatures increased to 24°C. Many mitochondrial processes were upregulated in response to initial thermal stress at 20°C but declined under these higher temperatures. Transcriptional markers of dysfunction and resulting damages were apparent at lower temperatures in northern Burntwood River sturgeon and were more extreme when compared to southern Winnipeg River sturgeon. Aside from the direct effects of decreased ATP production and increased reactive oxygen species damage, a decline in mitochondrial function also has implications for stress signaling, viral immune response, and formation of heme as well as iron cluster assemblies required for oxygen transport and many of the above-mentioned cellular processes (Tovar et al., 2003; Ajioka et al., 2006; Lin et al., 2006; Qin et al., 2010; Karnkowska et al., 2016; Hernansanz-Agustin et al., 2020; Braymer et al., 2021). The

impairment of these widespread mitochondrial processes in developing lake sturgeon likely inhibited the function of the mitochondria itself, related cellular systems, and ultimately gill function, with the potential for population-specific thresholds impacting whole-organism fitness to changes in environmental temperatures (Iftikar and Hickey, 2013; Jeffries et al., 2018).

Plasticity as an Adaptive Trait

All of the above thermally-responsive molecular pathways first rely on the ability of lake sturgeon to transcriptionally respond to environmental change. Without the necessary translational machinery in place, mRNA abundance changes cannot modify the proteome or phenotype (Vornanen et al., 2005; Smith et al., 2013; Akbarzadeh et al., 2018). Thus, as thermal thresholds are reached and lake sturgeon face a reduction in the ability to transcribe mRNA's, produce ribosomes, and maintain mitochondria, those consequences likely negatively impact the organism's ability to respond to changes in their environment with impaired and diminished mRNA, protein, and energy production.

Increasing temperatures induced upregulation of transcripts associated with post-transcriptional modifications in both populations of lake sturgeon, enhancing translational plasticity. Conserved responses of alternative splicing and gene silencing via miRNA suggest that these mechanisms are part of the inducible stress response of sturgeons, similar to other studied fishes, and likely play a role in environmental adaptation (Somero, 2018; Healy & Schulte, 2019; Tan et al., 2019; Shiina and Shimizu, 2020; Verta and Jacobs, 2021; Thorstensen et al., 2022; Steward et al., 2022). However, these processes were ultimately downregulated as temperatures increased to 24°C, indicating thermal thresholds on post-translational as well as transcriptional responsiveness. The conservation of these mechanisms across both populations of lake sturgeon, as temperatures increase and under stressful conditions in various other species of fish, highlight the importance of post-transcriptional plasticity across the evolution of fish lineages.

As temperatures surpass thermal thresholds, and the effectiveness of some transcriptional and translational mechanisms are diminished, overall physiological plasticity is likely reduced. Fishes' susceptibility to the effects of additional stressors are thereby likely increased by compromising their adaptive ability to respond at multiple levels of biological organization (Smith et al., 2013; Brennan et al., 2022). As temperatures increased, transcription modifying

processes were more highly preserved in Winnipeg River sturgeon that historically experience higher temperature thermal environments in the wild. As these populations are genetically distinct, experience different thermal environments, and have been geographically separated throughout recent history (McDougall et al., 2017; Bugg et al., 2020), the preservation of these transcriptional mechanisms may themselves be an adaptive mechanism, enhancing the southern Winnipeg River population's ability to respond to environmental change. Enhanced transcriptional plasticity likely results in the observed increases in growth, metabolism, thermal tolerance, and survival of the southern Winnipeg River population under thermally stressful conditions, when compared to their Burntwood River counterparts (Bugg et al., 2020).

Ecological Relevance

In the current study, temperatures of 20°C and above limited the transcriptional plasticity of developing lake sturgeon, which would ultimately diminish their plasticity to other stressors. As temperatures continue to increase in the wild, the combined effects of thermal, oxidative, and pathogen related stressors will likely limit the survival of lake sturgeon populations within Manitoba, Canada, with increased severity for northern subarctic populations throughout the province (Bugg et al., 2020). In recent years, population designation units have been reclassified, grouping lake sturgeon inhabiting northern regions with those from several other populations in the province to form a larger designation unit (COSEWIC 2006; COSEWIC 2017). However, new evidence suggesting large discrepancies in transcriptomic, physiological, and likely genetic differences between populations, as well as their natural thermal environments and tolerance thresholds (McDougall et al., 2017; Bugg et al., 2020; Bugg et al., 2021) indicate that they are differentially susceptible to increasing temperatures and require distinct management strategies for conservation.

Currently, temperatures in the Winnipeg River exceed 20°C for approximately 50 days per year, while this temperature threshold is projected to be exceeded during June, July, and August in the Burntwood River in the coming decades (Manitoba Hydro, 2015; Vincent et al., 2015; Zhang et al., 2019; Bugg et al., 2020). These seasonal increases in temperature occur when newly hatched sturgeon are in their first few months of life, which are the same life stages examined in the current study and in the recent thermal tolerance research (Bugg et al., 2020; Bugg et al., 2021). During these early developmental stages, lake sturgeon from both populations

use a variety of pre- and post-transcriptional mechanisms to modify their acclimatory capacity as temperatures increase to 20°C, but these mechanisms are largely downregulated by 24°C when many sturgeon are not able to survive chronically elevated temperatures. Additionally, endemic viral pathogens are persistent in both northern and southern populations of lake sturgeon throughout the province, with higher abundance and probability of detection in younger fish (Clouthier et al., 2013; Clouthier et al., 2020), and as temperatures increase. With the present experimental evidence, we suggest that if river temperatures continue to increase as projected (Manitoba Hydro, 2015) and there are not sufficient thermal refugia, these physiological limitations will likely impede future lake sturgeon recruitment, especially in the most endangered northern populations. Therefore, increasing temperatures may influence recruitment, movement patterns, and distributions of lake sturgeon within Manitoba, as sturgeon shift to areas of thermal refuge to preserve energy and persist in the face of environmental change (Moore et al., 2020; Robertson et al., 2021). However, even with sufficient refugia, fish do not always travel to habitat with increased suitability due to life history constraints (Sutton et al., 2007; White et al., 2019). If lake sturgeon are unable to redistribute themselves in areas of thermal refuge, evidence suggests that the impacts of increasing environmental temperature may be more severe, impact population-level selection processes and survival based on observed population-specific whole-organism physiological responses as well as transcriptional plasticity to elevated temperatures.

Data Accessibility:

The data is available in supplementary files and the raw sequencing reads are available at the National Center for Biotechnology Information Sequence Read Archive with the accession number PRJNA815828. The Trinity assembly and Trinotate annotation file are available via figshare (https://doi.org/10.6084/m9.figshare.19209753.v1).

Author Contributions:

WSB, WGA, and KMJ designed the research. WSB analyzed the data with assistance from KM and MJT. WSB wrote the first draft of the manuscript and all authors provided comments on the final version.

Acknowledgements:

The authors thank North/South Consultants Inc. and Manitoba Hydro for their assistance in the capture of spawning adults and collection of gametes from these wild sturgeon populations, Evelien de Greef for her support with the map (Figure 1), as well as Madison Earhart for valuable discussions and sturgeon illustrations (Figure 2A) . We would also like to thank the staff at the University of Manitoba Animal Holding Facility and numerous Anderson and Jeffries Laboratory members for assistance in the care and maintenance of fish. This work was conducted at the University of Manitoba located on original lands of the Anishinaabeg, Cree, Oji-Cree, Dakota and Dene peoples, and on the homeland of the Métis Nation. We recognize that water supplied for our fish at the University of Manitoba was sourced from the Shoal Lake 40 First Nation. Funding for this study was provided by NSERC/Manitoba Hydro Industrial Research Chair awarded to W.G.A. and NSERC Discovery Grants (grant numbers 05348 and 05479) awarded to W.G.A. and K.M.J., respectively. W.S.B. was supported by a University of Manitoba Graduate Fellowship as well as the International Graduate Student Scholarship.

Figures:

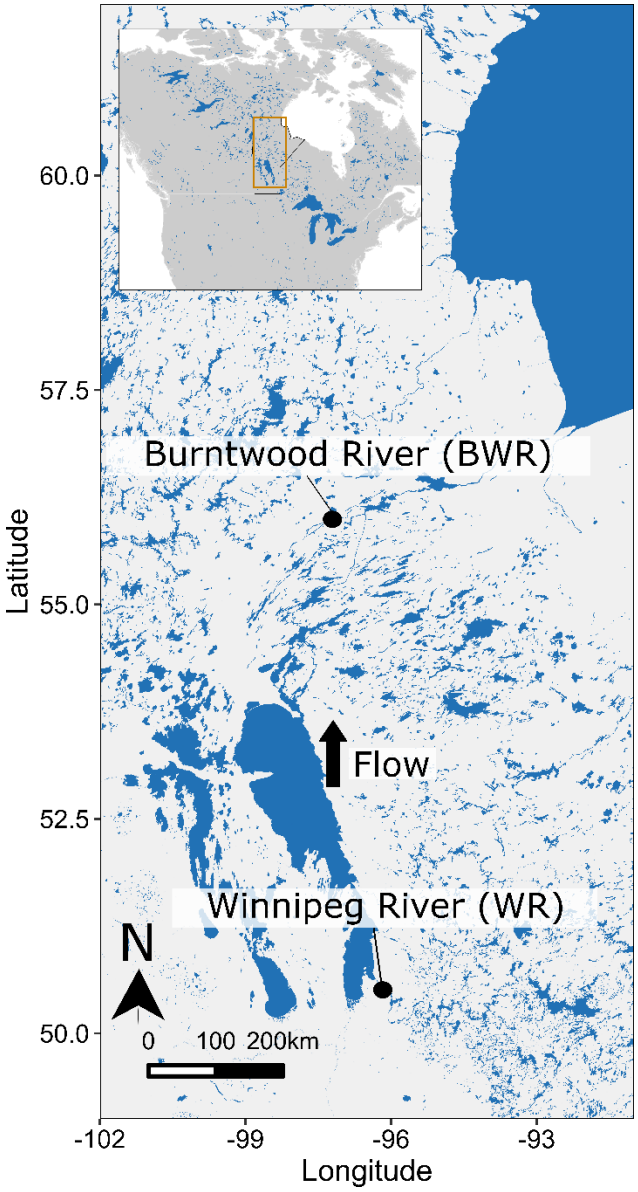

Figure 1. Geographic localities of two lake sturgeon populations (*Acipenser fulvescens*) within Manitoba, Canada, highlighting the northern Burntwood River (BWR) and more southernly (WR) sturgeon populations as well as the direction of flow throughout the watershed. This figure was assembled with the assistance of Evelien de Greef.

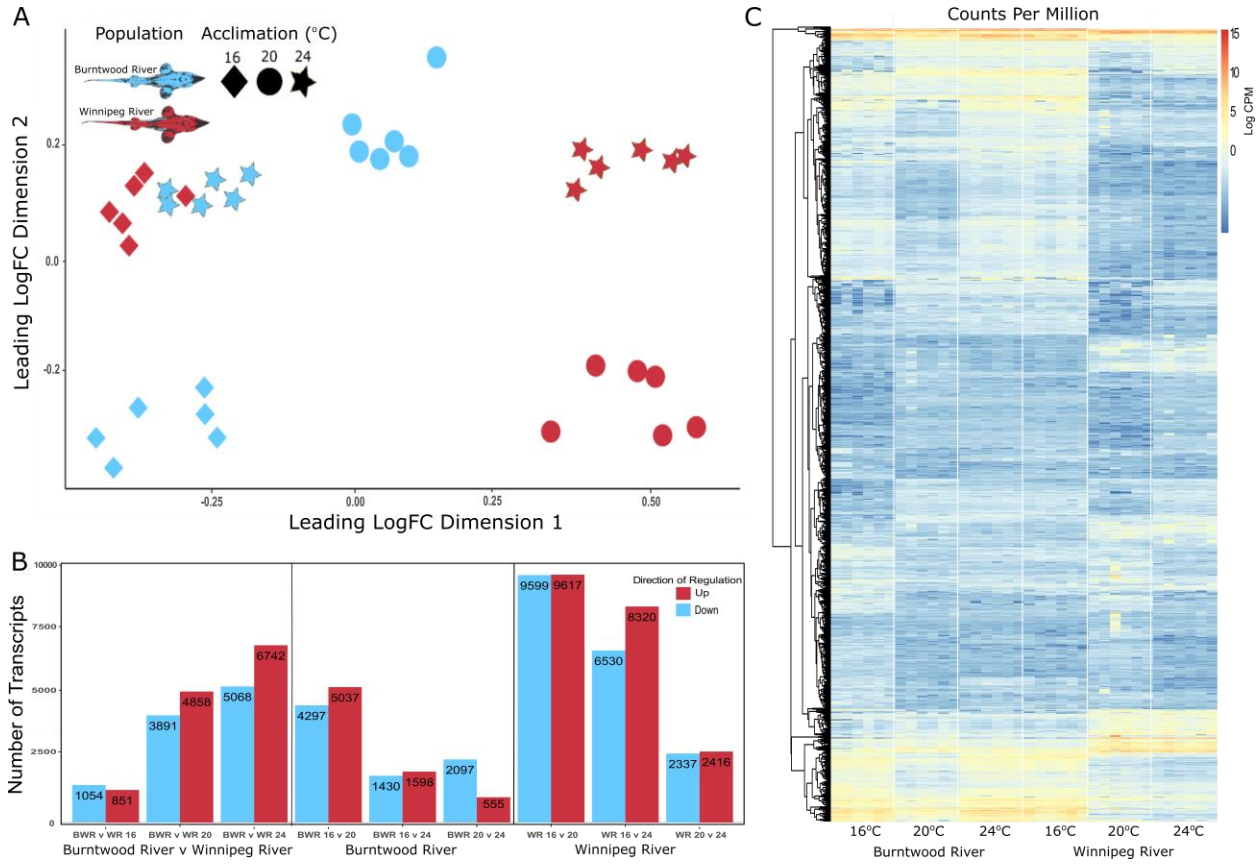

Figure 2. A) Multiple dimensional scaling plot B) Numbers of differentially expressed transcripts and C) Aggregate heat map of the top 500 differentially expressed transcripts between each contrast of northern Burntwood River (BWR) and southern Winnipeg River (WR) populations of lake sturgeon within Manitoba, Canada, acclimated to 16˚C, 20˚C, and 24˚C for 30 days in early development, measured using RNAseq. Transcript numbers represent upregulation and downregulation with a $\log_2$ fold change > 1, respectively, and with a *q*-value < 0.05. Contrasts are oriented Burntwood River-Winnipeg River at the interpopulation level and lower temperature-higher temperature across acclimation treatments. For the aggregate heat map, only unique transcripts were included for a total of 2,285 transcripts represented. Data are expressed as $\log_2$ counts per million (CPM). Lake sturgeon illustrations in panel A were done by Madison Earhart.

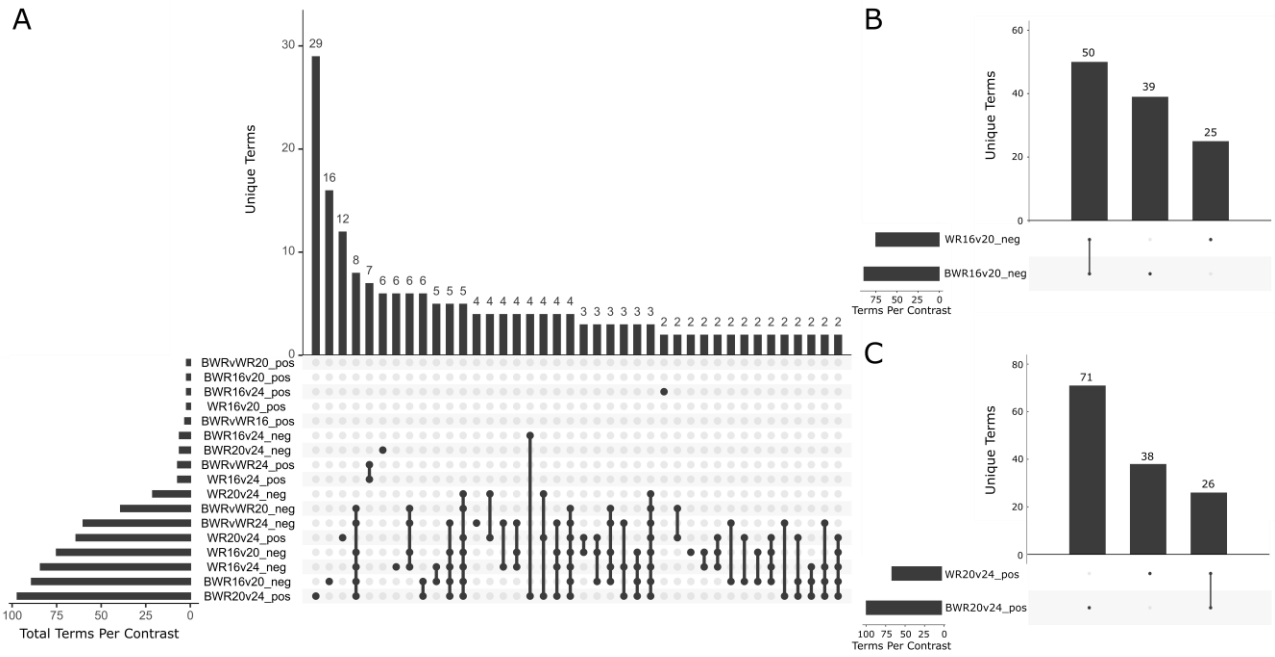

Figure 3. GO (gene ontology) and KEGG term distribution illustrated using upset plots demonstrating unique terms across A) all split-contrasts B) upregulated transcripts as temperature increased from 16 to 20°C, and C) downregulated transcripts as temperatures increased from 20 to 24°C. These data represent alteration in the transcriptional mechanisms in the gill of developing lake sturgeon from both a northern Burntwood River (BWR) and southern Winnipeg River (WR) population of lake sturgeon (*Acipenser fulvescens*), within Manitoba, Canada, acclimated to temperatures 16°C, 20°C, and 24°C for 30 days in early development, measured using RNAseq. Split-contrasts are oriented BWR-WR at the interpopulation level and lower temperature-higher temperature across acclimation treatments so that "neg" indicates upregulation at higher temperatures, and "pos" indicates downregulation at higher temperatures.

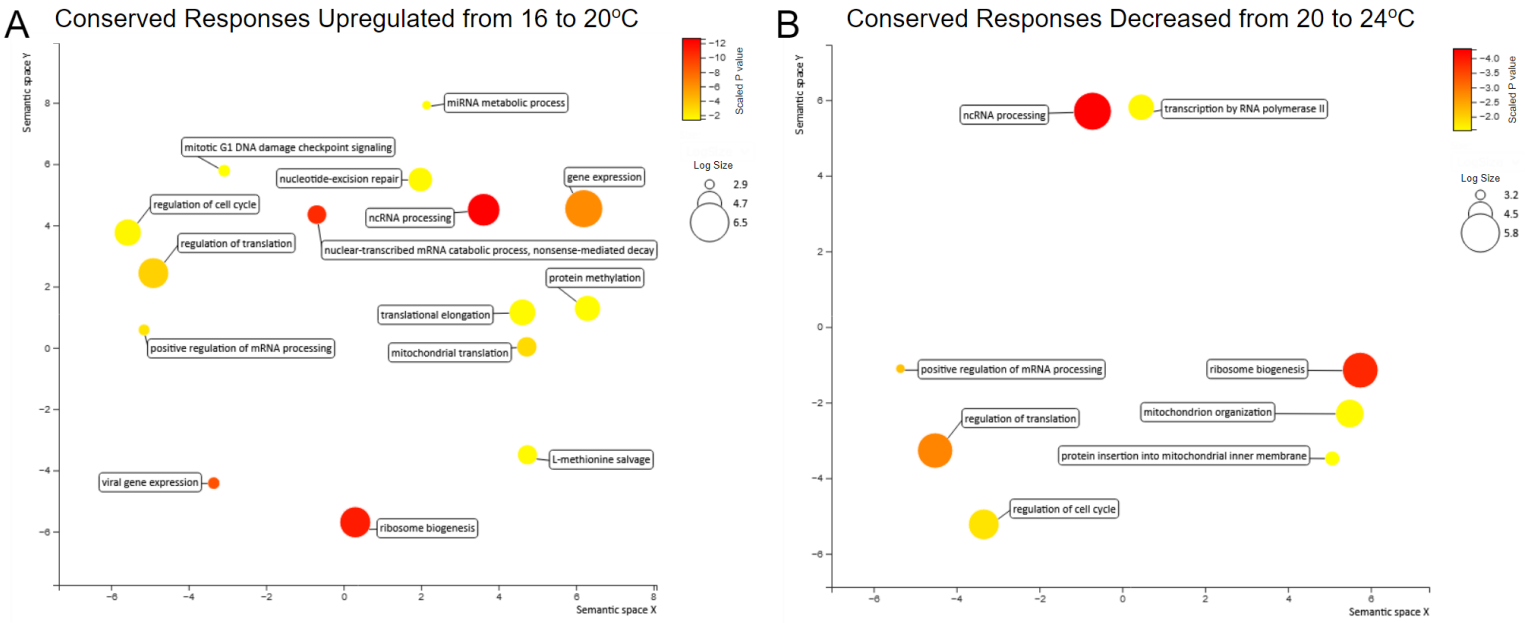

Figure 4. REVIGO plots of GO (gene ontology) biological processes terms conserved between the Winnipeg River (WR) and Burntwood River (BWR) populations of developing lake sturgeon (*Acipenser fulvescens*) within Manitoba, Canada, as acclimation temperatures increase from A) 16 to 20˚C and further increase from B) 20 to 24˚C during a 30-day acclimation, measured using RNAseq. Bubble color indicates the si7gnificance of the adjusted *p* value at after applying a $\log_{10}$ scale (darker color is a more significant term), while bubble size indicates the frequency of a GO term GO annotation database (larger bubble is a more general term).

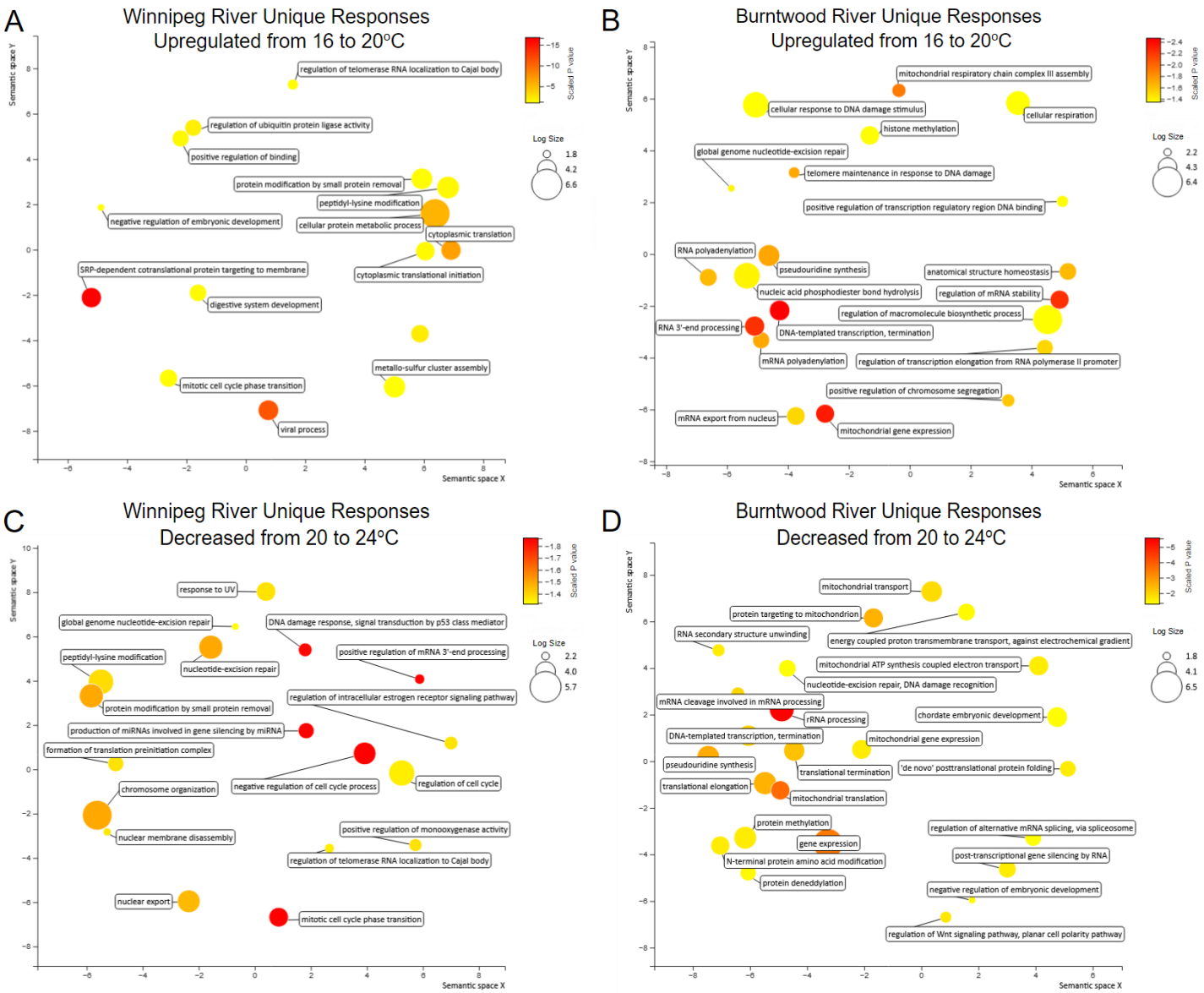

Figure 5. REVIGO plots of GO (gene ontology) biological processes terms unique to the Winnipeg River (WR) and Burntwood River (BWR) populations of developing lake sturgeon (*Acipenser fulvescens*) within Manitoba, Canada, as acclimation temperatures increase during a 30-day acclimation, measured using RNAseq. Plots represent A) Winnipeg River unique responses upregulated from 16 to 20˚C B) Burntwood River unique responses upregulated from 16 to 20˚C) Winnipeg River unique responses decreased 20 to 24ºC and D) Burntwood River unique responses decreased from 20 to 24˚C. Bubble color indicates the significance of the adjusted *p* value at after applying a $\log_{10}$ scale (darker color is a more significant term), while bubble size indicates the frequency of a GO term GO annotation database (larger bubble is a more general term).

Figure 6. Aggregate most differentially expressed transcript network demonstrating predicted protein-protein interactions across the most upregulated and downregulated transcripts of northern and southern lake sturgeon, *Acipenser fulvescens*, populations, within Manitoba, Canada, acclimated to 16˚C, 20˚C, and 24˚C for 30 days in early development, measured using RNAseq. Orange transcript circles outlined in blue with bolded names indicate hub transcripts within the network. Hub size and naming is scaled according to their degree of interaction with surrounding hubs (Zhou and Xia, 2019).

Supplementary Table 1. Number of total uniquely annotated, upregulated, and downregulated transcripts, as well as upregulated and downregulated enrichment terms returned through gene enrichment with enrichR (Chen et al., 2013; Kuleshov et al., 2016; Xie et al., 2021), using the "GO Biological Processes_2018", "GO Molecular Function 2018", "GO Cellular Component 2018", "KEGG 2016" databases and filtering for an adjusted *p* value < 0.05.

Specific Contrast: Across Populations	Total Uniquely Annotated Genes	Upregulated Genes	Downregulated Genes	Upregulated Enrichment Terms	Downregulated Enrichment Terms
16	539	284	255	6	2
20	2,158	741	1417	4	67
24	2,279	1,115	1,164	17	86
Within Burntwood River					
16v20	2,345	1,042	1,321	2	117
16v24	1,109	483	626	6	9
Within Winnipeg River					
16v20	2,903	1,111	1,792	3	113
16v24	2,636	1,156	1,480	10	116

References:

Aijoka, R.S., Philips, J.D., Kushner, J.P. 2006. Biosynthesis of heme in mammals. *Biochim Biophys Acta*. 1763, 723-736. https://doi.org/10.1016/j.bbamcr.2006.05.005

Akbarzadeh, A., Günther, O.P., Houde, A.L., Li, S., Ming, T.J., Jeffries, K.M., Hinch, S.G., Miller, K.M. 2018. Developing specific molecular biomarkers for thermal stress in salmonids. *BMC Genom*. 19, 749. https://doi.org/10.1186/s12864-018-5108-9

Alvarez, M., Schrey, A., Richards, C. 2015. Ten years of transcriptomics in wild populations: What have we learned about their ecology and evolution? *Mol Ecol*. 24, 710–725. https://doi.org/10.1111/mec.13055

Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data.524https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Bacevic, K., Lossaint, G., Achour, T.N., Georget, V., Fisher, D., Dulic, V. 2017. Cdk2 strengthens the intra-S checkpoint and counteracts cell cycle exit induced DNA damage. *Sci Rep*. 7, 13429. https://doi.org/10.1038/s41598-017-12868-5

Baker, D.W., Matey, V., Huynh, K.T., Wilson, J.M., Morgan, J.D., Brauner, C.J. 2009. Complete intracellular pH protection during extraceullar pH depression is associated with hypercarbia tolerance in white sturgeon, *Acipenser transmontanus*. *Am J Physiol Regul Integr Comp Physiol*. 296, R1868-R1880. https://doi.org/10.1152/ajpregu.90767.2008

Barley, J.M., Cheng, B.S., Sasaki, M., Gignoux-Wolfsohn, S., Hays, C.G., Putnam, A.B., Sheth, S., Villeneuve, A.R., Kelly, M. 2021. Limited plasticity in thermally tolerant ectotherm populations: evidence for a trade-off. *Proc Royal Soc B*. 288, 21210765. https://doi.org/10.1098/rspb.2021.0765.

Bartek, J., Lukas, C., Lukas, J. 2004. Checking on DNA damage in S phase. *Nat Rev Mol Cell Biol*. 5, 792-804. https://doi.org/10.1038/nrm1493

Bertoli, C., Skotheim, J.M., de Bruin, R.A.M. 2015. Control of cell cycle transcription during G1 and S phases. *Nat Rev Mol Cell Biol*. 14, 518-528. https://doi.org/10.1038/nrm3629

Birnie-Gauvin, K., Costantini, D., Cooke, S.J., Willmore, W.G. 2017. A comparative and evolutionary approach to oxidative stress in fish: A review. *Fish Fish*. 18, 928-942. https://doi.org/10.1111/faf.12215

Bolger, A.M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina Sequence Data. *Bioinformatics*, btu170. https://doi.org/10.1093/bioinformatics/btu170

Braasch, I., Postlethwait, J.H. 2012. Polyploidy in Fish and the Teleost Genome Duplication. In: Soltis P., Soltis D. (eds) Polyploidy and Genome Evolution. Springer, Berlin, Heidelberg. pp. 341-383.

Brandt, C., Bugg, W., Groening, L., Klassen, C., Anderson, W.G. 2021. Effects of rearing temperature on volitional and escape response swimming performance in lake sturgeon,

Acipenser fulvescens, from hatch to age 1. *Environ Biol Fishes*. 104, 737-750. https://doi.org/10.1007/s10641-021-01112-9.

Brandt, C., Groening, L., Klassen, C., Anderson, W.G. Effects of rearing temperature on yolksac volume and growth rate in Lake Sturgeon, *A. fulvescens*, from hatch to age-1. *Aquaculture*. 546, 737352. https://doi.org/10.1016/j.aquaculture.2021.737352

Braymer, J.J., Freibert, S.A., Rakwalska-Bange, M., Lill. 2021. Mechanistic concepts of iron-sulfur protein biogenesis in biology. *Biochim Biophys Acta Mol Cell Res*. 1868, 118863. https://doi.org/10.1016/j.bbamcr.2020.118863

Breau, C., Cunjak, R. A., Peake, S. J. 2011. Behaviour during elevated water temperatures: Can physiology explain movement of juvenile Atlantic salmon to cool water? *J Anim Ecol.* 80, 844–853. https://doi.org/10.1111/j.1365-2656.2011.01828.x

Brennan, B.S., deMayo, J.A., Dam, H.G., Finiguerra, M.B., Baumann, H., Pespeni, M.H. 2022. Loss of transcriptional plasticity but sustained adaptive capacity after adaptation to global change conditions in a marine copepod. *Nat Commun*. 13, 1147. https://doi.org/10.1038/s41467-022-28742-6

Buccitelli, C., Selbach, M. 2020. mRNAs, proteins and the emerging principles of gene expression control. *Nat Rev Genet*. 21, 630-644. https://doi.org/10.1038/s41576-020-0258-4

Bugg, W.S., Yoon, G.R., Brandt, C., Earhart, M., Anderson, W.G., Jeffries, K.M. 2021. The effects of population and thermal acclimation on the growth, condition, and cold responsive mRNA expression of age-0 lake sturgeon (*Acipenser fulvescens*). *J Fish Biol*. 99, 1-16. https://doi.org/10.1111/jfb.14897

Bugg, W.S., Yoon, G.R., Schoen, A., 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 Age-0 Lake Sturgeon (*Acipenser fulvescens*). Conservation Physiology. 8, coaa087. https://doi.org/10.1093/conphys/coaa087

Burggren, W. 2018. Developmental phenotypic plasticity helps bridge stochastic weather events associated with climate change. *J Exp Biol*. 221: jeb161984. https://doi.org/10.1242/jeb.161984

Burggren, W., Crews, D. 2014. Epigenetics in comparative biology: why we should pay attention. *Integr Comp Biol*. 54, 7-20. https://doi.org/10.1093/icb/icu013

Burkhead, N. M. 2012. Extinction rates in North American freshwater fishes, 1900–2010. *BioScience.* 62, 798–808. https://doi.org/10.1525/bio.2012.62.9.5

Caroffino, D.C., Sutton, T.M.S., Elliot, R.F., Donofrio, M.C. 2010. Early Life Stage Mortality Rates of Lake Sturgeon in the Peshtigo River, Wisconsin. *N Am J Fish Manag*. 30, 295-304. https://doi.org/10.1577/M09-082.1

Chandel, N.S. 2010. Mitochondrial Complex III: An essential component of universal oxygen sensing machinery? *Respir Physiol Nuerobiol*. 174, 175-181. https://doi.org/10.1016/j.resp.2010.08.004

Chapman, J.M., Lisa, K., Teffer, A.K., Miller, K.M., Cooke, S.J. 2021. Disease ecology of wild fish: Opportunities and challenges for linking infection metrics with behaviour, condition, and survival. *Can J Fish Aquat Sci*. *Just-IN*. https://doi.org/10.1139/cjfas-2020-0315

Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, Clark NR, Ma'ayan A. 2013. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinform*. 14, 128. https://doi.org/10.1186/1471-2105-14-128

Choi, Y., 2005. Role of TRAF6 in the immune system. *Mechanisms of Lymphocyte Activation and Immune Regulation X*, pp.77-82.

Cioce, M., Lamond, A.I. 2005. CAJAL BODIES: A Long History of Discovery. *Annu Rev Cell Dev Biol*. 21, 105-131. https://doi.org/10.1146/annurev.cellbio.20.010403.103738

Cleator, H., Martin, K.A., Pratt, T.C., Macdonald, D. 2010. Information relevant to a recovery potential assessment of Lake Sturgeon: Nelson River population (DU3). DFO Canadian Science Advisory Secretariat Document 2010/082. vi + 33p

Clouthier, S., VanWalleghem, E., Copeland, S., Klassen, C., Hobbs, G., Nielsen, O., Anderson, E.D. 2013. A new species of nucleo-cytoplasmic large DNA virus (NCLDV) associated with mortalities in Manitoba Lake sturgeon *Acipenser fulvescens*. *Dis Aquat Org*. 102, 195-209.

Clouthier, S., Caskenette, A., Walleghem, E.V., Schroeder, T., Macdonald, D., Anderson, E.D. 2020. Molecular phylogeny of sturgeon mimiviruses and Bayesian hierarchical modeling of their effect on wild Lake Sturgeon (*Acipenser fulvescens*) in Central Canada. *Infect Genet Evol*. 84, 104491. https://doi.org/10.1016/j.meegid.2020.104491

Comte, L., Buisson, L., Daufresne, M., Grenouillet, G. 2012. Climate-induced changes in the distribution of freshwater fish: observed and predicted trends. *Freshw Biol*. 58, 625-639. https://doi.org/10.1111/fwb.12081

Connon, R.E., Jeffries, K.M., Komoroske, L.M., Todgham, A.E., Fangue, N.A. 2018. The utility of transcriptomics in fish conservation. *J Exp Biol*. 221, jeb148833. https://doi.org/10.1242/jeb.148833

Conway, J.R., Lex, A., Gehlenborg, N. 2017. UpSetR: an R package for visualization of intersecting sets and their properties. *Bioinformatics*. 33, 2938-2940. https://doi.org/10.1093/bioinformatics/btx364

COSEWIC. 2006. COSEWIC assessment and update status report on the lake sturgeon Acipenser fulvescens in Canada. COSEWIC, 107.

COSEWIC. 2017. COSEWIC assessment and update status report on the Lake Sturgeon *Acipenser fulvescens*, Western Hudson Bay populations, Saskatchewan-Nelson River populations, Southern Hudson Bay – James Bay populations and Great Lakes – Upper St. Lawrence populations in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, ON. xxx + 153 p.

Crozier, L.G., Hutchings, J.A. 2014. Plastic and evolutionary responses to climate change in fish. *Evolutionary applications*. 7, 68-87. https://doi.org/10.1111/eva.12135

Deslauriers, D., Kieffer, J.D. 2012. Swimming performance and behaviour of young-of-the-year shortnose sturgeon (*Acipenser brevirostrum*) under fixed and increased velocity swimming tests. *Can J Zool*. 351, 345–351. https://doi.org/10.1139/z2012-004

Dillon, M.E., Woods, H.A., Wang, G., Fey, S.B., Vasseur, D.A., Telemeco, R.S., Marshall, K., Pincebourde, S. 2016. Life in the Frequency Domain: the Biological Impacts of Changes in Climate Variability at Multiple Time Scales. *ICB.*56, 14-30.

Drennan, J.D., LaPatra, S.E., Siple, J.T., Ireland, S., Cain, K.D. 2006. Transmission of white sturgeon iridovirus in Kootenai River white sturgeon *Acipenser transmontanus*. *Dis Aquat Org*. 70, 37-45. https://doi.org/10.3354/dao070037

Ellis, L.L., Huang, W., Quinn, A.M., Ahuja, A., Alfrejd, B., Gomez, F.E., Hjelmen, C.E., Moore, K.L., Mackay, T.F.C., Johnston, J.P., Tarone, A.M. 2014. Intrapopulation Genome Size Variation in *D. melanogaster* Reflects Life History Variation and Plasticity. *PLoS Genet*. 10, e1004522. https://doi.org/10.1371/journal.pgen.1004522

Filho, D.W. 2007. Reactive oxygen species, antioxidants and fish mitochondria. *Front Biosci*. 12, 1229-1237. https://doi.org/10.2741/2141

Frilander, M.J., Barboric, M. 2020. The Interlocking Lives of LARP7: Fine-Tuning Transcription, RNA Modification, and Splicing through Multiple Non-coding RNAs. *Mol Cell*. 78, 5-8. https://doi.org/10.1016/j.molcel.2020.03.015

Fujimoto, M., Lovett, B., Angoshtari, R., Nirenberg, P., Loch, T.P., Scribner, K.T., Marsh, T.L. 2018. Antagonistic interactions and biofilm forming capabilities among bacterial strains isolated from egg surfaces of lake sturgeon (*Acipenser fulvescens*). *Microb Ecol*. 75, 22-37. https://doi.org/10.1007/s00248-017-1013-z

Gardil, A.M., Wright, G.M., Wadowska, D.W., Fast, M.D. 2014b. Ontogeny of the immune system in acipenserid juveniles. *Dev Comp Immunol*. 44, 303-314. https://doi.org/10.1016/j.dci.2014.01.006

Georgiadis, M.P., Hedrick, R.P., Carpenter, T.E., Gardner, I.A., 2001. Factors influencing transmission, onset and severity of outbreaks due to white sturgeon iridovirus in a commercial hatchery. *Aquaculture.* 194, 21–35. https://doi.org/10.1016/S0044-8486(00)00503-2

Giardine, B., Riemer, C., Hardison, R.C., Burhans, R., Elnitski, L., Shah, P., Zhang, Y., Blankenberg, D., Albert, I., Taylor, J., et al. 2005. Galaxy: A platform for interactive large-scale genome analysis. *Genome Res*. 15, 1451-1455. https://doi.org/10.1101/gr.4086505

Gilmour, K.M., Perry, S.F. 2018. Conflict and Compromise: Using Reversible Remodeling to Manage Competing Physiological Demands at the Fish Gill. *Am Physio. Soc*. 33, 412-422. https://doi.org/10.1152/physiol.00031.2018

Gomez-Gallardo, M., Sanchez-Briones, L.A., Diaz-Perez, A.L., Lopez-Alvarez, A., Cortes-Rojo, C., Aguilar-Mendez., Saavedra-Molina, A., Campos-Garcia, J. 2016. The Function of the Iron-Sulfur Cluster Assembly Machinery is involved in Electron Trasport Chain Activity and Oxidative Stress in *Saccharomyces cerevisiae*. *FASEB J*. 30, 863.1-863.1. https://doi.org/10.1096/fasebj.30.1_supplement.863.1

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B.W., Nusbaum, C., Lindblad-Toh, K., Friedman, N., Regevm A. 2011. Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nat Biotechnol*. 29, 644-652. https://doi.org/10.1038/nbt.1883

Gradil, A.M., Wright, G.M., Speare, D.J., Wadowksa, D.W., Purcell, S., Fast, M.D. 2014a. The effects of temperature and body size on immunological development and responsiveness in juvenile shortnose sturgeon (*Acipenser brevirostrum*). *Fish Shellfish Immunol*. 40, 545-555. https://doi.org/10.1016/j.fsi.2014.07.036

Grill, G., et al. "Mapping the world's free-flowing rivers." Nature, vol. 569, no. 7755, 2019, p. 215. Gale OneFile: CPI.Q, link.gale.com/apps/doc/A593382054/CPI?u=winn62981&sid=CPI&xid=ff4c09e3. Accessed 25 May 2021. https://doi.org/10.1038/s41586-019-1111-9

Guo, C., Sun, L., Chen, X., Zhang, D. 2013. Oxidative stress, mitochondrial damage and neurodegenerative diseases. *Nueral Regen Res*. 25, 2003-2014. https://doi.org/10.3969/j.issn.1673-5374.2013.21.009

Guo, K., Zhao, C., Lang, B., Wang, H., Zheng, H., and Zhang, F. 2021. Regulator of Chromosome Condensation 2 Modulates Cell Cycle Progression, Tumorigenesis, and Therapeutic Resistance. *Front mol biosci*. 7:620973. https://doi.org/10.3389/fmolb.2020.620973

Hartman, D.J., Hoogenraad, N.J., Condron, R., Hoj, P.B. 1992. Identification of a mammalian 10-kDa heat shock protein, a mitochondrial chaperonin 10 homologue essential for assisted folding of trimeric ornithine transcarbamolyase *in vitro*. *PNAS*. 89, 3394-3398. https://doi.org/10.1073/pnas.89.8.3394

Healy, T. M., Schulte, P. M. 2019. Patterns of alternative splicing in response to cold acclimation in fish. *J Exp Biol*. 222, jeb193516. https://doi.org/10.1242/jeb.193516.

Hernansanz-Agustin, P., et al., 2020. Na+ controls hypoxic signaling by the mitochondrial respiratory chain. 2020. *Nature*. 586, 287-291. https://doi.org/10.1038/s41586-020-2551-y

Hwang, P.P., Lee, T.H., Lin, L.Y. 2011. Ion regulation in fish gills: recent progress in the cellular and molecular mechanisms. *Am J Physiol Regul Integr Comp Physiol*. 301, R28-47. https://doi.org/10.1152/ajpregu.00047.2011

Iftikar, F.I., Hickey, A.J.R. 2013. Do Mitochondria Limit Hot Fish Hearts? Understanding the Role of Mitochondrial Function with Heat Stress in *Notolabrus celidotus*. *PLOS ONE*. 8, 64120. https://doi.org/10.1371/journal.pone.0064120

IUCN, *The IUCN Red List of Threatened Species* Version 2017-3 (2018); www.iucnredlist.org.

Jackson, M.P., Hewitt, E.W. 2016. Cellular proteostasis: degradation of misfolded proteins by lysosomes. *Essays Biochem*. 60, 173-180. https://doi.org/10.1042/EBC20160005

Jane, S.F., Gretchen, J.A., et al. 2021. Widespread deoxygenation of temperate lakes. *Nature*. 594, 60-70. https://doi.org/10.1038/s41586-021-03550-y

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. *Evol App*. eva.12799. https://doi.org/10.1111/eva.12799

Jeffries, K. M., Teffer, A., Michaleski, S., Bernier, N. J., Heath, D. D., & Miller, K. M. 2021. The use of non-lethal sampling for transcriptomics to assess the physiological status of wild fishes. *Comp Biochem Physiol B, Biochem.* 256, 110629. https://doi.org/10.1016/j.cbpb.2021.110629

Jeffries, K.M. Fangue, N.A., Connon, R.E. 2018. Multiple sub-lethal thresholds for cellular responses to thermal stressors in an estuarine fish. *Comp Bio Phys A*. 255, 33-45. https://doi.org/10.1016/j.cbpa.2018.06.020

Jeffries, K.M., Komoroske, L.M., Truong, J., Werner, I., Matthias, H., Hasenbein, S., Fangue, N.A., Connon, R.E. 2015. The transcriptome-wide effects of exposure to a pyrethroid pesticide on the critically endangered delta smelt *Hypomesus transpacificus*. *Endanger Species Res*. 28, 43-60. https://doi.org/10.3354/esr00679

Jonsson, B., Jonsson, N. 2019. Phenotypic plasticity and epigenetics of fish: embryo temperature affects later-developing life history traits. *Aquat Biol*. 28, 21-32. https://doi.org/10.3354/ab00707

Karnkokowska, A., Vacet, V., et al. 2016. A Eukaryote without a Mitochondrial Organelle. *Curr Biol*. 26, 1274-1284. https://doi.org/10.1016/j.cub.2016.03.053

Kim, D., Langmead, B., Salzberg, S.L. 2015. HISAT: a fast spliced aligner with low memory requirements. *Nat Methods.* 12, 357-360. https://doi:10.1038/nmeth.3317

Kolde, R. 2015. pheatmap: Pretty heatmaps. https://CRAN. R-project. org/package= pheatmap

Komoroske, L.M., Connon, R.E., Jeffries, K.M., Fangue, N.A. 2015. Linking transcriptional responses to organismal tolerance reveals mechanisms of thermal sensitivity in a mesothermal endangered fish. *Mol Ecol*. 24, 4960-4981. https://doi:10.1111/mec.13373

Komoroske, L.M., Jeffries, K.M., Whitehead, A., Roach, J.L., Britton, M., Connon, R.E., Verhille, C., Brander, S.M., Fangue, N.A. 2020. Transcriptional flexibility during thermal challenge corresponds with expanded thermal tolerance in an invasive compared to native fish. *Evol Appl*. 14, 931-949. https://doi.org/10.1111/eva.13172

Krabbenhoft, T.J., Myers, B.J.E., Wong, J.P., Chu, C., Tingley, R.W., Falke, J.A., Kwak, T.J., Paukert, C.P., Lynch, A.J. 2020. FiCli, the Fish and Climate Change Database, informs climate adaptation and management for freshwater fishes. *Sci Data*. 7, 1–6. https://doi.org/10.1038/s41597-020-0465-z

Kuleshov, M.V., Jones, M.R., Rouillard, A.D., Fernandez, N.F., Duan, Q., Wang, Z., Koplev, S., Jenkins, S.L., Jagodnik, K.M., Lachmann, A., McDermott, M.G., Monteiro, C.D., Gundersen, G.W., Ma'ayan, A. 2016. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res*. https://doi.org/gkw377. 10.1093/nar/gkw377

Lafuente, E., Beldade, P. 2019. Genomics of Developmental Plasticity in Animals. *Front Genet.* 10:720. https://doi.org/10.3389/fgene.2019.00720

LaPatra, S.E., Groff, J.M., Jones, G.R., Munn, B., Patterson, T.L., Holt, R.A., Hauck, A.K., Hedrick, R.P., 1994. Occurrence of white sturgeon iridovirus infections among cultured white sturgeon in the Pacific Northwest. *Aquaculture.* 126, 201–210. https://doi.org/10.1016/0044-8486(94)90036-1

Lazado, C.C., Caipang, C.M.A. 2014. Mucosal immunity and probiotics in fish. *Fish Shellfish Immunol*. 39, 78-89. https://doi.org/10.1016/j.fsi.2014.04.015

Liao, Y., Smyth, G.K., Shi, W. 2014. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*. 30, 923-930. https://doi.org/10.1093/bioinformatics/btt656

Lim, P.S., Sutton, C.R., Rao, S. 2015. Protein kinase C in the immune system: from signaling to chromatin regulation. *Immunology*. 146, 508-522. https://doi.org/10.1111/imm.12510

Lin, R., Lacoste, J., Nakhaei, P., Sun, Q., Yang, L., Paz, S., Wilkinson, P., Julkunen, I., Vitour, D., Meurs, E., Hiscott, J. 2006. Dissociation of MAVS/IPS-1/VISA/Cardif-IKKe Molecular Complex from the Mitochondrial Outer Membrane by Hepatitis C Virus NS3-4A Proteolytic Cleavage. *J Virol*. 80, 6072-6083. https://doi.org/10.1128/JVI.02495-05

Logan, M.L., Cox, C.L. 2020. Genetic Constraints, Transcriptome Plasticity, and the Evolutionary Response to Climate Change. *Front Genet*. 11, 538226. https://doi.org/10.3389/fgene.2020.538226

Manitoba Hydro .2015. Manitoba Hydro Climate Change Report Fiscal Year 2014–2015. https://www.hydro.mb.ca/environment/pdf/climate_change_report_2014_15.pdf. (last accessed 12 May 2020).

Marcos-López, M., Gale, P., Oidtmann, B.C., Peeler, E.J. 2010. Assessing the Impact of Climate Change on Disease Emergence in Freshwater Fish in the United Kingdom. *Transbound Emerg Dis*. 57, 293-304. https://doi/10.1111/j.1865-1682.2010.01150.x

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/10.1371/journal.pone.0174269.

Miao, T., Symonds, A.L.J., Singh, R., Symonds, J.D., Ogbe, A., Omodho, B., Zhu, B., Li, S., Wang, P. 2017. Egr2 and 3 control adaptive immune responses by temporally uncoupling expansion from T cell differentiation. *J Exp Med*. 214, 1787- 1808. https://doi/10.1084/jem.20160553

Moore, M.J., Paukert, C.P., Moore, T.L. 2021. Effects of Latitude, Season, and Temperature on Lake Sturgeon Movement. *N Am J Fish Manag*. 41, 916-928. https://doi.org/10.1002/nafm.10416.

Morgan, R., Finnoen, M.H., Jensen, H., Pelabon, C., Jutfelt, F. 2020. Low potential for evolutionary rescue from climate change in a tropical fish. *PNAS*. 117, 33365-33372. https://doi.org/10.1073/pnas.2011419117.

Mugetti, D., Pastorino, P., Menconi, V., Pedron, C., Prearo, M. 2020. The Old and the New on Viral Diseases in Sturgeon. *Pathogens*. 9, 146. https://doi.org/10.3390/pathogens9020146

Mundy, P.C., Jeffries, K.M., Fangue, N.A., Connon, R.E. 2020. Differential regulation of select osmoregulatory genes and Na+/K+-ATPase paralogs may contribute to population differences in salinity tolerance in semi-anadromous fish. *Comp Biochem Physiol A.* 110584. https://doi.org/10.1016/j.cbpa.2019.110584

Muschick, M., Barluenga, M., Salzburger, W., Meyer, A. 2011. Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. *BMC Evol Biol*. 11, 116. https://doi.org/10.1186/1471-2148-11-116

Olusanya, H.O., van Zyll de Jong, M. 2018. Assessing the vulnerability of freshwater fishes to climate change in Newfoundland and Labrador. *PLoS One*. https://doi.org/10.1371/journal.pone.0208182

Oomen, R. A., Hutchings, J. A. 2017. Transcriptomic responses to environmental change in fishes: insights from RNA sequencing. *FACETS* 2, 610–641. https://doi.org/10.1139/facets-2017-0015.

Paital, B., Panda, S.K., Hati, A.K., Mohanty, B., Mohapatra, M.K., Kanungo, S., Chainy, G.B.N. 2016. Longevity of animals under reactive oxygen species stress and disease

susceptibility due to global warming. *World J Biol Chem*. 7, 110-127. https://doi.org/10.4331/wjbc.v7.i1.110

Palmer, M.A., Liermann, C.A.R., Nilsson, C., Flörke, M., Alcamo, J., Lake, P.S., Bond, N. 2008. Climate change and the world's river basins: anticipating management options. *Front Ecol Environ*. 6, 81-89. https://doi.org/10.1890/060148

Peake, S., Beamish, F.W.H., McKinley, R.S., Scruton, D.A., Katopodis, C. 1997. Relating swimming performance of lake sturgeon, Acipenser fulvescens, to fishway design. *Can J Fish Aquat Sci*. 1366, 1361–1366. https://doi.org/10.1139/f97-039

Pelseter, B., Egg, M. 2018. Hypoxia-inducible transcription factors in fish: expression, function and interconnection with the circadian clock. *J Exp Biol*. 4, 13. https://doi.org/10.1242/jeb.163709

Penman, R. 2021. The effects of temperature acclimation and heating rate on the thermal tolerance of juvenile white sturgeon (*Acipenser transmontanus*). *Masters thesis*. University of British Columbia.

Pertea, M., Pertea, G., Antonescu, C. et al. 2015. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol*. 33, 290–295. https://doi.org/10.1038/nbt.3122.

Place, S.P., Hofmann, G.E. 2001. Temperature interactions of the molecular chaperone Hsc70 from eurythermal marine goby *Gillichthys mirabilis*. *J Exp Biol*. 204, 2675-2682. https://doi.org/10.1242/jeb.204.15.2675

Portner, H.O., Bock, C., Mark, F.C. 2017. Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology.*J Exp Biol*. 220, 2685-2696. https://doi.org/10.1242/jeb.134585

Portner, H.O., Lanning, G. 2009. Chapter 4 Oxygen and Capacity Limited Thermal Tolerance. Fish Physiology. 27, 143-191.

Qian, X., Ba, Y., Zhuang, Q., Zhong, G. 2014. RNA-Seq technology and its application in fish transcriptomics. *OMICS J Integr Biol*. 18, 98-110. https://doi.org/10.1089/omi.2013.0110

Qin, Z., Raoof, M., Chen, Y., Sumi, Y., Sursal, T., Junger, W., Brohi, W., Itagaki, K., Hauser, C.J. 2010. Circulating mitochondrial DAMPS cause inflammatory responses to injury. *Nature*. 464, 104-107. https://doi.org/10.1038/nature08780

R Core Team. 2022. **R**: A language and environment for statistical computing. **R** Foundation for Statistical Computing, Vienna, Austria. URL http://www.**R**-project.org/

Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law., C.W., Shi, W., Smyth, G.K. 2015. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 43, e47. https://doi.org/10.1093/nar/gkv007

Rivera-Ingraham, G.A., Lignot, J.H. 2017. Osmoregulation, bioenergetics and oxidative stress in coastal marine invertebgrates: raising the questions for future research. *J Exp Biol*. 220, 1749-1760. https://doi.org/10.1242/jeb.135624

Robertson, M.D., Gao, J., Regular, P.M., Morgan, M.J., Zhang, F. 2021. Lagged recovery of fish spatial distributions following a cold-water perturbation. *Sci Rep*. 11, 9513. https://doi.org/10.1038/s41598-021-89066-x

Robinson, M.D., McCarthy, D.J., Smyth, G.K. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 26, 139-140. https://doi.org/10.1093/bioinformatics/btp616

Saari, G.N., Wang, Z., Brooks, B.W. 2018. Revisiting inland hypoxia: diverse exceedances of dissolved oxygen thresholds for freshwater aquatic life. *Environ Sci Pollut Res*. 25, 3139-3150. https://doi.org/10.1007/s11356-017-8908-6

Schade, F.M., Shama, L.N.S., Wegner, K.M. 2014. Impact of thermal stress on evolutionary trajectories of pathogen resistance in three-spined stickleback. *BMC Evol Biol*. 14, 164. https://doi.org/10.1186/s12862-014-0164-5

Scheiner, S.M., Barfield, M., Holt, R.D. 2019. The genetics of phenotypic plasticity. XVII. Response to climate change. *Evol Appl*. 13, 388-399.

Schindler, D.W. 2001. The cumulative effect of climate change and multiple stressors. *Can J Fish Aquatic Sci*. 2958, 18–29. https://doi.org/10.1139/f00-179

Schlichting, C.D., Smith, H. 2002. Phenotypic plasticity: linking molecular mechanisms with evolutionary outcomes. *Evol Ecol*. 16, 189-211. https://doi.org/10.1023/A:1019624425971

Seebacher, F., White, C.R., Franklin, C.E. 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat Clim Change*. 5, 61-66. https://doi.org/10.1038/nclimate2457

Shiina, T., Shimizu, Y. 2020. Temperature-Dependent Alternative Splicing of Precursor mRNAs and Its Biological Significance: A Review Focused on Post-Transcriptional Regulation of a Cold Shock Protein Gene in Hibernating Mammals. *Int J Mol Sci*. 14, 7599. https://doi.org/ 10.3390/ijms21207599

Smith, S., Bernatchez, L., Beheregaray, L.B. 2013. RNA-seq analysis reveals extensive transcriptional plasticity to temperature stress in a freshwater fish species. *BMC Genom*. 14, 1-12. https://doi.org/10.1186/1471-2164-14-375

Somero, G.N. 2010. The physiology of climate change: how potentials for acclimatization and genetic adaption will determine ‘winners’ and ‘losers’. *J Exp Biol*. 213, 912-920. https://doi.org/10.1242/jeb.037473

Somero, G.N. 2018. RNA thermosensors: how might animals exploit their regulatory potential? *J Exp Biol*. 22, 221. https://doi.org/10.1242/jeb.162842

Steward, R.A., de Jong, M.A., Oostra, V., Wheat, C.W. 2022. Alternative splicing in seasonal plasticity and the potential for adaptation to environmental change. *Nat Commun*. 13, 755. https://doi.org/10.1038/s41467-022-28306-8

Stilwell, N.K., Frasca, S., Farina, L.L., Subramaniam, K., Imnoi, K., Viadanna, P.H., Hopper, L., Powell, J., Colee, J., Waltzek, T.B. 2022. Effects of water temperature on frog virus 3 disease in hatchery-reared pallid sturgeon *Scaphirhynchus albus*. *Dis Aquat Org*. 148, 73-86. https://doi.org/10.3354/dao03645

Storey, K.B., Storey, J.M. 2011. Heat shock proteins and hypometabolism: adaptive strategy for proteome preservation. *Res Rep Biol*. 2, 57-68. https://doi.org./10.2147/RRB.S13351

Su, G., Logez, M., Xu, J., Tao, S., Villéger, S., Brosse, S. 2021. Human impacts on global freshwater fish biodiversity. *Science*. 371, 835-838. https://doi.org/10.1126/science.abd3369

Supek F, Bošnjak M, Škunca N, Šmuc T. 2011. REVIGO summarizes and visualizes long lists of Gene Ontology terms. *PLoS One*. 6, e21800. https://doi.org/10.1371/journal.pone.0021800

Sutton, R.J., Deas, M.L., Tanaka, S.K., Soto, T., Corum, A. 2007. Salmonid observations at the Klamath River thermal refuge under various hydrological and meteorological conditions. *River Res Appl*. 23, 775-785. https://doi.org/10.1002/rra.1026

Szklarczyk, D., Gable, A.L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N.T., Morris, J.H., Bork, P., Jensen, L.J., von Mering, C. 2018. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 8, D607-D613. https://doi.org/10.1093/nar/gky1131

Tan, S., Wang, W., Tian, C., Niu, D., Zhou, T., Jin, Y., Yang, Y., Gao, D., Dunham, R., Liu, Z. 2019a. Heat stress induced alternative splicing in catfish as determined by transcriptome analysis. *Comp Biochem Physiol*. 29. 166–172. https://doi.org/10.1016/j.cbd.2018.11.008

Tan, S., Wang, W., Tian, C., Niu, D., Zhou, T., Yang, Y., Gao, D., Liu, Z. 2019b. Post-transcriptional regulation through alternative splicing after infection with *Flavobacterium columnare* in channel catfish (*Ictaluru punctatus*). *Fish Shellfish Immunol*. 91, 188-193. https://doi.org/10.1016/j.fsi.2019.05.008

Tan, S., Wang, W., Zhong, X., Tian, C., Niu, D., Bao, L., Zhou, T., Jin, Y., Yang, Y., Yuan, Z., Gao, D., Dunham, R., Liu, Z. 2018. Increased alternative splicing as a host response to *edwardsiella ictalurid* infection in catfish. *Mar Biotechnol*. https://doi.org/10.1007/s10126-018-9844-2

Taylor, S.J., Shalloway, D. 1996. Src and the control of cell division. *Bioessays*. 18, 9-11. https://doi.org/10.1002/bies.950180105

Thornton, P. K., Ericksen, P. J., Herrero, M., Challinor, A.J. 2014. Climate variability and vulnerability to climate change: a review. *Glob Chang Biol*. 20, 3313-3328. https://doi.org/10.1111/gcb.12581

Thorstensen, M.J., Baerwald, M.R., Jeffries, K.M. 2021. RNA sequencing describes both population structure and plasticity-selection dynamics in a non-model fish. *BMC Genom*. 22, 1-12. https://doi.org/10.1186/s12864-021-07592-4

Thorstensen, M.J., Jeffrey, J.D., Treberg, J.R., Watkinson, D.A., Enders, E.C., Jeffries, K.M. 2020. Genomic signals found using RNA sequencing show signatures of selection and subtle population differentiation in walleye (*Sander vitreus*) in a large freshwater ecosystem. *Ecol Evol*. 10, 7173-7188. https://doi.org/10.1002/ece3.6418

Thorstensen, M.J., Turko, A.J., Heath, D.D., Jeffries, K.M., Pitcher, T.E. 2022. Acute thermal stress elicits interactions between gene expression and alternative splicing in a fish of conservation concern. *bioRxiv*. https://doi.org/10.1101/2022.02.20.481219

Tovar, J., Leon-Avila, G., Sanchez, L.B., et al., 2003. Mitochondrial remnant organelles of Giardia function in iron-sulphur protein maturation. *Nature*. 13, 172-176. https://doi.org/10.1038/nature01945

Trifonov, V.A., Romanenko, S.S., Beklemisheva, V.R., Biltueva, L.S., Makunin, A.I., Lemskaya, N.A., Kulemzina, A.I., Stanyon, R., Graphodatsky, A.S. 2016. Evolutionary plasticity of acipenseriform genomes. *Chromosoma*. 125, 661-668. https://doi.org/10.1007/s00412-016-0609-2

Trizzino, M., Zucco, A., Deliard, S., Wang, F., Barbieri, E., Veglia, F., Gabrilovich, D., Gardini, A. 2021. EGR1 is a gatekeeper of inflammatory enhancers in human macrophages. *Sci Adv*. 13, eaaz8836. https://doi.org/10.1126/sciadv.aaz8836

Vallin, J., Grantham, J. 2019. The role of the molecular chaperone CCT in protein folding and mediation of cytoskeleton-associated processes: implications for cancer cell biology. *Cell Stress Chaperones*. 24, 17-27. https://doi.org/10.1007/s12192-018-0949-3

Van de Peer, Y., Mizrachi, E. & Marchal, K. 2017. The evolutionary significance of polyploidy. *Nat Rev Genet*. 18, 411–424. https://doi.org/10.1038/nrg.2017.26

Van der Lee, A.S., Koops, M.A. 2021. Recovery Potential Modelling of Lake Sturgeon (*Acipenser fulvescens*) in Canada. DFO Can. Sci. Advis. Sec. Res. Doc. 2021/025. iv + 48 p.

Vasseur, D.A., DeLong, J.P., Gilbert, B., Greig, H.S., Harley, C.D.G., McCann, K.S., Savage, V., Tunney, T.D., O'Connor, M.I. 2014. Increased temperature variation poses a greater risk to species than climate warming. *Proc Royal Soc B*. 281, 20132612. https://doi.org/10.1098/rspb.2013.2612

Verhille, C.E., Poletto, J.B., Cocherell, D.E., DeCourten, B., Baird, S., Cech, Jr, J.J., Fangue, N.A. 2014. Larval green and white sturgeon swimming performance in relation to water-diversion flows. *Conserv Physiol*. 2, cou031. https://doi.org/10.1093/conphys/cou031

Verta, J.P., Jacobs, A. 2021. The role of alternative splicing in adaptation and evolution. *Trends Ecol Evol*. 37, 4. https://doi.org/10.1016/j.tree.2021.11.010

Vincent, L.A., Zhang, X., Brown, R.D., Feng, Y., Mekis, E., Milewska, E.J., Wan, H., Wang, X.L. 2015. Observed trends in Canada's climate and influence of low-frequency variability modes. *J Climate* 28: 4545-4560. https://doi.org/10.1175/JCLI-D-14-00697.1

Vollset, K.W., Lennox, R.J., Davidsen, J.G., Eldøy, Isaksen, T.E., Madhun, A., Karlsson, S., Miller, K.M. 2020. Wild salmonids are running the gauntlet of pathogens and climate as fish farms expand northwards. *ICES J Mar Sci*. https://doi.org/10.1093/icesjms/fsaa138

Vornanen M, Hassinen M, Koskinen H, Krasnov A. 2005. Steady-state effects of temperature acclimation on the transcriptome of the rainbow trout heart. *Am J Physiol Regul Integr Comp Physiol*. 289, R1177–R1184. https://doi.org/10.1152/ajpregu.00157.2005

Warkentin, L., Parken, C.K., Bailey, R., Moore, J.W. 2022. Low summer river flows associated with low productivity of Chinook salmon in watershed with shifting hydrology. *Ecolog Solut Evid*. 3, e12124. https://doi.org/10.1002/2688-8319.12124.

Webb, W. 1986. Kinematics of lake sturgeon, *Acipenser fulvescens*, at cruising speeds. *Can J Zool*. 64, 2137–2141. https://doi.org/10.1139/z86-328

Wei, J., Zang, S., Xu, M., Zheng, Q., Chen, X. and Qin, Q., 2017. TRAF6 is a critical factor in fish immune response to virus infection. *Fish Shellfish Immunol*. 60, pp.6-12. https://doi.org/10.1016/j.fsi.2016.11.008

Wellband, K. W., Heath, D. D. 2017. Plasticity in gene transcription explains the differential performance of two invasive fish species. *Evol Appl*. 10, 563–576. https://doi.org/ 10.1111/eva.12463

White, S.L., Kline, B.C., Hitt, N.P., Wagner, T. 2019. Individual behaviour and resource use of thermally stress brook trout *Salveliunus fontinalis* portend the conservation potential of thermal refugia. *J Fish Biol*. 95, 1061-1071. https://doi.org/10.1111/jfb.14099

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. *J Exp Biol*. 215, 1293-1305. https://doi.org/10.1242/jeb.062075

Xie, Z., Bailey, A., Kuleshov, M.V., Clarke, D.J.B., Evangelista, J.E., Jenkins, S.L., Lachmann A., Wojciechowicz, M.L., Kropiwnicki, E., Jagodnik, K.M., Jeon, M., Ma'ayan, A. 2021. Gene set knowledge discovery with Enrichr. *Curr Protoc*. 1, e90. https://doi.org/10.1002/cpz1.90

Yoon, G.R., Earhart, M., Wang, Y., Suh, M., Anderson, G.W. 2021. Effects of temperature and food availability on liver fatty acid composition and plasma cortisol concentration in age-0 lake sturgeon: Support for homeoviscous adaptation. *Comp Biochem and Phys.* 261, 111056. https://doi.org/10.1016/j.cbpa.2021.111056

Zhang X, Flato G, Kirchmeier-Young M, Vincent L, Wan H, Wang X, Rong R, Fyfe J, Li G, Kharin W. 2019. Changes in Temperatures and Precipitation Across Canada; Chapter 4 *in* Bush E and Lemmens DS (Eds.). *Canada's Changing Climate Report*. *Government of Canada*, Ottawa, Ontario, p. 112-193.

Zhang, Y., Lu, J., Ma, J., Liu, X. 2019. Insulin-induced gene 1 (INSIG1) inhibits HIV-1 production by degrading Gag via activity of the ubiquitin ligase TRC8. *J Biol Chem*. 294, 2046-2059. https://doi.org/10.1074/jbc.RA118.004630

Zhou, G., Xia, J. 2018. OmicsNet: a web-based tool for creation and visual analysis of biological networks in 3D space. *Nucleic Acids Res*. 46, W514-W522. https://doi.org/10.1093/nar/gky510

Zhou, G., Xia, J. 2019. Using OmicsNet for networking integration and 3D visualization. *Curr Protoc Bioinform*. 65, e69. https://doi.org/10.1002/cpbi.69