Transcriptomic responses to multigenerational environmental warming in a cold-adapted salmonid

Running title: Lake trout transcriptomic responses to warming

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Keywords: acclimation, climate change, RNA sequencing, *Salvelinus namaycush*, transgenerational plasticity, transcriptomics.

Abstract

Cold-adapted species are particularly threatened by climate change as rates of environmental warming outpace the ability of many populations adapt. Recent evidence suggest that transgenerational thermal plasticity may play a role in the response of cold-adapted organisms to long-term changes in temperature. Using RNA sequencing, we explored differential gene expression of lake trout (*Salvelinus namaycush*), a cold-adapted species, to examine the molecular processes that respond to elevated temperatures under conditions of within-generation (offspring) and transgenerational (parental) warm-acclimation. We hypothesized that genes associated with metabolism, growth and thermal stress/tolerance would be differentially expressed in juvenile lake trout offspring depending on their own acclimation temperature and that of their parents. We found enriched pathways for thermal stress, signaling processes, immune function, and transcription regulation, although the specific genes and direction of differential expression (up- or downregulation) depended on the combination of offspring and parental thermal rearing conditions. While parental warm-acclimation did have a transgenerational effect on gene expression in their offspring, within-generation warm-acclimation had a larger effect on differential expression, enriching more pathways. We provide evidence of the transgenerational response to warming at the transcriptional level in lake trout, which should be useful for future studies of transcriptomics and plasticity in these and other cold-adapted species.

Keywords: acclimation, climate change, RNA sequencing, *Salvelinus namaycush*, transgenerational plasticity, transcriptomics.

Introduction

Temperate and Arctic regions are warming rapidly; surface temperatures in Canada have increased by ~1.7°C in under a century with even greater warming happening in the Arctic (~2.3°C; Zhang et al. 2019). As a result, high-latitude, cold-adapted organisms are experiencing, and will continue to experience, rapid temperature increases that may threaten their survival (Beitinger and Bennett 2000; Burkhead 2012; IPCC 2021). Range expansion to track suitable conditions is particularly challenging for species and populations with limited dispersal options, such as many freshwater fishes (Guzzo and Blanchfield 2017; Smith et al. 2020). Warming rates may outpace adaptive responses (Visser 2008; Crozier and Hutchings 2014; Comte and Olden 2017), particularly for those with low standing genetic variation (Willi et al. 2006; Wilson 2017). In the absence of standing genetic variation for coordinated adaptive responses throughout core metabolic pathways, population responses would be limited to plastic thermal acclimation, but the low physiological plasticity typical of many cold-adapted species means they likely will be particularly vulnerable to rapidly changing environments (Somero 2010; Kelly et al. 2014). Individuals that can withstand warming may have to rely heavily on energetic resources that would normally support growth or reproduction, thus having potentially negative downstream effects on the population (Somero 2010; Rosenfeld et al. 2020). A lack of adaptive genetic variation, low capacity for temperature acclimation, and rapid environmental change are predicted to result in widespread species extirpations (Bennett et al. 2019; Morash et al. 2021).

Some species may be able to buffer the negative impact of environmental warming through transgenerational acclimation, also known as transgenerational plasticity (TGP). As the name suggests, TGP refers to the phenotypic changes that occur over the course of multiple generations (Jablonka et al. 1992; Bell and Hellmann 2019; Bonduriansky 2021). This involves non-genetic inheritance of environmentally-driven changes, including parental effects, where the experiences of one generation are reflected in the morphology, physiology or behaviour of a subsequent generation, often F1 or F2 (Hellman et al. 2020; Lee et al. 2020; Bonduriansky 2021).

TGP occurs through modulation of gene expression through non-genetic inheritance, which includes parental effects and the transfer of epigenetic factors such as patterns of DNA methylation and histone modification, and microRNA activity (Dai et al. 2020; Spadafora 2020; Venney et al. 2020). TGP can potentially be beneficial when a parent's environment coincides

with what their offspring are expected to experience (Bernatchez 2016; Ashe et al. 2021). For example, TGP could be beneficial in a population that experiences environmental warming over multiple generations whereby the offspring are phenotypically "primed" for a warmer environment (Donelan et al. 2020; McCaw et al. 2020; Venney et al. 2022). In the context of climate change, this phenotypic priming could buffer the effects of rapid warming, allowing additional time for evolutionary processes to occur (Bernatchez 2016; Ashe et al. 2021), though the extent to which TGP can be considered adaptive is arguable (Uller et al. 2013; Sánchez-Tójar et al. 2020). Nevertheless, TGP with temperature has been identified in a number of species (Greenspoon and Spencer 2018; Yin et al. 2019). For example, the coral reef fish *Acanthochromis polyacanthus* exhibits TGP for increased temperatures by improving its aerobic scope in a warm environment by increasing maximum metabolic rate or decreasing resting metabolic rate (Donelson et al. 2012; Bernal et al. 2018). Many of the studies on thermal TGP in aquatic organisms have used eurythermal, temperate or tropical model species; by contrast, cold-adapted aquatic vertebrates are relatively understudied but could stand to benefit from TGP given their vulnerability to environmental warming due to climate change.

Acute and chronic temperature acclimation has obvious impacts on the metabolism of ectotherms, at both the whole organism and molecular level (Pérez-Ruzafa et al. 2018; Petitjean et al. 2019; Morash et al. 2021). Relatively few studies to date report the effect of transgenerational warming on phenotypic variation in tandem with gene expression, or with follow-up experiments testing the differential gene expression underlying transgenerational effects (Shama et al. 2016; Bernal et al. 2018; Veilleux et al. 2018). Examining differential gene expression associated with changes in metabolism with thermal experiences can be informative (Oomen and Hutchings 2017). Shama et al. (2016), for example, found that genes involved in metabolism, mitochondrial protein synthesis, hemostasis and apoptosis were differentially expressed in offspring depending on the multigenerational thermal experiences down the maternal line. These findings underscore the relationship between the molecular mechanisms underlying physiological plasticity and parental thermal experiences.

We sought to explore differential gene expression in lake trout (*Salvelinus namaycush*) to examine the molecular processes potentially underlying transgenerational responses to elevated temperatures. The lake trout is a long-lived, cold-adapted salmonid that is largely limited to cold, oligotrophic lakes in formerly glaciated regions of North America (Riley et al. 2021; Wilson and

Mandrak 2021) and is under significant threat from climate change (Casselman 2008; Guzzo and Blanchfield 2017). Being stenothermal, lake trout behaviourally thermoregulate by migrating to deeper, cooler water when lakes thermally stratify during the summer (Martin and Olver 1980; Guzzo and Blanchfield 2017). Low genetic variation for some lake trout populations (Perrier et al. 2017), and little within- and among-population variation for thermal acclimation capacity (McDermid et al. 2013; Kelly et al. 2014) may limit the adaptive potential of lake trout populations for warming temperate and arctic habitats. A previous study investigated the capacity for transgenerational thermal plasticity in lake trout as a means of coping with warming associated with climate change and found that they exhibit only limited transgenerational plasticity at the whole organism (phenotypic) level (Penney et al. 2021). Unexpectedly, cold-acclimated offspring of parents from elevated temperatures exhibited higher resting metabolic rate than those with one or both cold acclimated parents (Penney et al. 2021). This study confirmed that the parental environment can influence offspring phenotypic expression, but did not expand the investigation into the mechanisms underlying this transgenerational response.

For this study, we assessed gene expression in previously studied offspring from factorial mating crosses between cold- and warm-acclimated lake trout adults (Penney et al. 2021) to assess transcriptional responses to within- and transgenerational thermal acclimation. Acclimation of the parents (transgenerational) to one of two environmental temperatures (10 or 17°C) was combined with subsequent acclimation of the offspring (within-generation) to cool (11°C) or warm (15°C) temperatures (Fig. 1). Evidence exists to support both maternal and paternal contributions to transgenerational effects (Marshall 2015; Shama et al. 2016; Penney et al. 2021). Our experimental design allowed us to examine the transgenerational effect of temperature acclimation of each parent, and both parents together, in combination with the offspring's (within-generation) temperature acclimation on gene expression in juvenile lake trout.

We hypothesized that genes associated with metabolism, growth and thermal stress/tolerance would be differentially expressed in juvenile lake trout depending on both the acclimation temperature of the offspring and the acclimation temperature of their parents. Based on studies of within-generation and transgenerational warming on differential gene expression in fish (Quinn et al. 2011; Veilleux et al. 2015; Akbarzadeh and Leder 2016; Shama et al. 2016), we predicted that 1) genes involved in growth, heat shock and hypoxia responses, and metabolic pathways would be upregulated in warm-acclimated offspring, and 2) differential expression of

these genes would vary depending on whether one or both parents were warm-acclimated, although the size and direction of the effect is difficult to predict given that parental influence on offspring gene expression has been shown to be sex-specific; depending on the sex of the offspring and that of the parent (Best et al. 2018; Baustista et al. 2020).

Methods

Hatchery-raised adult lake trout from the Ontario provincial Seneca Lake broodstock were used as parents for the experiment. This hatchery strain originated from Seneca Lake, one of the Finger Lakes in central New York State (42°41' N, 76°54' W). The Ontario Ministry of Natural Resources and Forestry (OMNRF) hatchery system has maintained this lake trout strain for over five generations, using rotational line crossing (Kincaid 1977) to minimize inbreeding and maintain the founding genetic diversity of the strain (OMNRF Fish Culture Stocks Catalogue 2005).

The tissue samples used for these experiments were collected from juvenile (young of year) lake trout that were previously used in a separate study on metabolic rate with transgenerational warming described in Penney et al. (2021). In this previous study, adult and juvenile offspring lake trout were acclimated to two different temperatures and the offspring were subjected to an acute thermal challenge of $+2°C \cdot h^{-1}$ to determine the effect of within- and transgenerational temperature acclimation on upper thermal tolerance and metabolic rate. Details of the prior experiment (Penney et al. 2021) that are relevant to the current study are summarized below.

All experiments adhered to the Canadian Council on Animal Care guidelines and were approved by the Trent University Animal Care Committee (Protocol # 24794) and the OMNRF Aquatic Animal Care Committee (Protocol # FACC_136).

Experimental design and rearing conditions

Two groups (n = 8 and 9) of mature lake trout (age 8; 2.3 - 4.2 kg) were each held in 6,000 L tanks (1 x 1 x 6 m), with males and females kept together, at the OMNRF White Lake Fish Culture Station (Sharbot Lake, Ontario, Canada) with water being supplied from a nearby lake. As the source waterbody (White Lake) began to stratify in early June, 2015, we began to increase tank water temperature by 1°C per day starting from approximately 9°C until one tank

reached 10 ± 0.5°C and the other 17 ± 0.5°C. These temperatures were chosen because 10°C represents the thermal requirements for lake trout spawning while 17°C was intended to induce physiological thermal stress without preventing reproduction (Casselman 2008; Penney et al. 2021) at the higher temperature. Temperatures were controlled and maintained by drawing in and mixing water from above and below the lake's thermocline as it flowed into the holding tanks. Fish were acclimated to these temperatures for approximately 3 months, mirroring thermal stratification in the source waterbody. Starting in mid-September, the temperature in the warm-water treatment was allowed to gradually cool by holding the proportional inflows from above and below the thermocline constant as the lake's surface water cooled. Temperatures in the two tanks converged at 10°C after fall turnover (late September) in the lake, and gradually cooled to 7.8°C by the end of the breeding interval (mid-November).

Adult lake trout were reproductive by the end of October with the warm-acclimated fish first spawning on October 30th. The cold-acclimated adults were first ready to spawn on November 5th and all mating crosses were completed by November 19th. Experimental offspring families were produced by a full factorial mating cross using two males and two females from each of the two temperature treatments (4 x 4, 8 adults in total) resulting in a total of 16 offspring families; four families from each of four parental treatment groups (W♀xW♂, W♀xC♂, C♀xW♂ and C♀xC♂, where W = warm-acclimated, and C = cold-acclimated; Figure 1). Fertilized eggs were transported to the OMNRF Codrington Fish Research Facility (Codrington, Ontario, Canada) where they were transferred into 200 L tanks receiving freshwater at ambient temperature (5-6 °C) and natural photoperiod under dim light with each family separately contained in steel mesh boxes (9 x 9 x 7.5 cm, one family per box).

In March (2016), when hatched offspring were ready to begin exogenous feeding, we randomly selected 14 individuals from each of the 16 families to transfer to 200 L tanks for temperature acclimation. Seven fry from each family were acclimated to a cold temperature (11°C) and the other 7 from each family were acclimated to a warm temperature (15°C). The offspring's lower acclimation temperature differs from that of their parents because of reported differences in optimum temperature for both life stages (11 vs 10°C; Edsall 2000, Casselman 2008). The upper acclimation temperature for the offspring represents the anticipated increase in temperature associated with climate change by year 2100 (Hayhoe et al. 2010; IPCC 2021), however, for the parents the upper acclimation temperature was two degrees higher to ensure that

temperature was high enough to elicit a physiological thermal response without compromising reproduction. To begin acclimating the offspring, water temperature was increased at a rate of 1°C per day until target temperatures were reached (11 and 15°C). Due to space constraints, tanks were subdivided by adding steel inserts with perforated bottoms. Each insert was divided into 4 sections (24 x 25 x 28 cm per section) with two paternal half-sibling families (n = 7 each; shared male parent) housed per section. Offspring from pooled families were later individually identified to family using microsatellite genotyping (Penney et al. 2021).

After the 3-4 week acclimation period, offspring were subjected to an acute temperature increase of $+2°C \cdot h^{-1}$ from their acclimation temperature until loss of equilibrium was observed. Eight fish from the same acclimation temperature (11°C or 15°C) were simultaneously tested per day, with each fish held in its own experimental chamber (Penney et al. 2021). Following loss of equilibrium, each fish was quickly transferred to a bath of 0.3 g l−1 of buffered tricaine methanesulfonate (MS-222; Aqua Life, Syndel Laboratories Ltd, BC, Canada) for euthanasia. Whole liver was rapidly dissected from each fish, blotted on a lab wipe and preserved in RNAlater (Invitrogen, Thermo Fisher Scientific) as per manufacturer's instructions. After 24-48 hours in a 4°C refrigerator, the RNAlater was pipetted from the tissues and the samples were stored at -80°C until RNA extraction. We chose to study gene expression in the liver because it is a metabolically active tissue involved in a number of physiological processes that respond to warming (Quinn et al. 2011; Akbarzadeh and Leder, 2016; Dammark et al. 2018). Additionally, transcriptional responses to multigenerational warming have been observed in the livers of zebrafish: Luu et al. (2021). Therefore, the liver ought to provide insight into the metabolic pathways that are affected by transgenerational temperature acclimation in lake trout. Liver tissue was collected between June 28 and August 9 in 2016.

RNA isolation and sequencing

RNA was extracted from the preserved liver tissues using a phenol-chloroform extraction method (Chomczynski and Sacchi 2006) after the tissues had been individually homogenized via a FastPrep-24 BeadBeater (MP Biomedicals) with 2 mL Lysing Matrix D tubes (MP Biomedicals) and 1 mL of Trizol reagent (Invitrogen, Thermo Fisher Scientific). RNA was precipitated with RNA precipitation solution (Sambrook and Russel 2001) and isopropanol, and washed with 75% ethanol. RNA samples were resuspended in nuclease-free water (Thermo

Fisher Scientific). The purity and concentration of the RNA was quantified using a NanoDrop-8000 spectrophotometer, and RNA quality was assessed by gel electrophoresis of glyoxylated RNA on a 1.5% agarose gel (Sambrook and Russel 2001).

Two lots of RNA samples were sent for high-throughput sequencing over a two-year span. In 2018, liver RNA samples from 24 individuals were sent to The Centre for Applied Genomics (Sick Kids Hospital, Toronto, Ontario, Canada), and in 2020 another 30 liver samples (individuals) were sent to Genome Quebec (Montreal, Quebec, Canada). Altogether, the 54 samples included tissue from six individuals from each of the seven experimental treatment groups, however, the control group (11°C-acclimated offspring from cold-acclimated (CxC) parents) had a total of 12 individuals sequenced: 6 individuals in 2018 and an additional 6 in 2020, so that each treatment group was compared against the control group sequenced in the same year to eliminate batch effects in the analysis of differential expression (Table 1). Individuals from each family were represented in their respective treatment group (there were 4 families per offspring-parent treatment group) and because each sequenced group comprised of 6 individuals, each group had 2 representatives from 2 of the 4 families. Overall, this did not lead to a bias toward any one family being overrepresented in the samples, except for the 2020 control group which had 3 individuals from one family, but this family is not overrepresented in any of the other groups. Subsequent genetic testing for males and females (Yano et al. 2013) confirmed that each group also contained members of both sexes (Table 1). Both facilities assessed the RNA quality via a Bioanalyzer (Agilent Technologies) and all samples passed quality control (RNA integrity number: ≥ 7.5). cDNA libraries were constructed by enriching the poly(A) tails of mRNA with oligo dT-beads using NEBNext Ultra II Directional polyA mRNA Library Prep. In 2018, barcoded libraries were distributed among two and a half lanes and were sequenced on the Illumina HiSeq2500 instrument producing an average of 28 million reads per sample (n = 24, paired-end, 2x126 bp). In 2020, libraries were sequenced on the Illumina HiSeq4000 with barcoded libraries distributed among three lanes producing 36 million reads per sample (n = 30, paired-end, 2x126 bp).

De novo assembly, annotation and analysis

For our study, we opted to use a de novo transcriptome assembly for the reads rather than a genome-guided approach. For non-model species with complex genomes, a de novo approach

can often produce better assemblies than a genome-guided approach (DeWoody et al. 2013), and the de novo approach using Trinity has been successfully used for transcriptome assembly for salmonids (Carruthers et al. 2018; Narum and Campbell 2015; Nazari et al. 2021). We did not use the recently published lake trout genome as a reference because the reads generated from this experiment were also used to develop the lake trout genome (Smith et al. 2022).

RNA reads from both years were assembled and annotated by the Canadian Centre for Computational Genomics (C3G, Quebec) in 2020. Adapters and reads with a Phred quality score <30 were trimmed using Trimmomatic (v.0.36), and normalization and *de novo* assembly was performed using Trinity assembly software (v.2.0.4) following Haas et al. (2013) and Grabherr et al. (2011). Pre- and post-trimming read counts and Trinity statistics can be found in the supplementary file (Tables S1 and S2). Functional annotation was performed with the Trinotate tool (v.2.0.2) and using the TransDecoder pipeline (v.2.0.1) to identify candidate coding regions and open reading frames. Candidate peptides were searched against known proteins in the Swiss-Prot (UniProtKB/Swiss-Prot) database via BLASTp (v.2.3.0) and the pfam database. The pfam database was also used with HMMER (v.3.1b2) to identify protein domains. Signal peptides, transmembrane regions and ribosomal RNA genes were predicted using the programs signalP (v.4.1), tmHMM (v.2.0c) and RNAMMER (v.1.2), respectively.

Seven comparisons were made where offspring treatment groups were each compared to the control group (cold-acclimated offspring with cold-acclimated parents) to observe the effect that within- and transgenerational plasticity (acclimation) had on gene expression in juvenile lake trout (Table 1). Transcript abundance was estimated using ‘RNA-Seq by Estimation Maximization’ (RSEM; Li and Dewey, 2011) in Trinity with the ‘align_and_estimate_abundance.pl’ utility. Differential gene expression was analyzed between the groups of lake trout offspring in R using the limma package (v.3.40.6; Ritchie et al., 2015). The log2fold change (LFC) was calculated for each gene, representing the magnitude of up- or downregulation; negative LFC are downregulated genes. A principle component analysis was performed to explore the DEGs for possible batch effects between the years given the differences in sequencing depth (28 vs. 36 million reads per sample), and we tested the correlation between DEGs for each of the pairwise comparisons to check whether application of a batch correction, using the twelve control group individuals as a shared baseline, changed the level of expression of DEGs.

From the list of annotated genes, we applied a LFC cutoff of ≤ -1 to ≥ 1 and used this limited list of DEGs for an enrichment analysis performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) tool (v.6.8) (Huang et al. 2009A; Huang et al. 2009B). The DAVID tool required that a model species be specified for our gene list: we opted to use *Salmo salar* as DAVID does not yet include *S. namaycush* despite the recent publication of the *S. namaycush* reference genome (Smith et al. 2022), and *S. salar* had the best coverage for annotated proteins among orthologous species available in the Swiss-Prot database (determined via NCBI Eukaryotic genome annotation pipeline, v.8.5). The output from the DAVID tool mapped genes to functional categories (GO) and metabolic pathways (Kyoto Encyclopedia of Genes and Genomes: KEGG); we ran our list of DEGs against backgrounds for fish species in the database (*Oncorhynchus mykiss*, *S. salar*, *Danio rerio*, *Oryzias latipes*), using the DAVID tool default criterion of reporting functions that are enriched by a minimum count of 2 genes and $p<0.1$. In our study, we only discuss DEGs that mapped to GO or KEGG in DAVID tool because the function of these DEGs have been confirmed in fish species. Tables outlining the enriched functions are included in the supplemental material. DEGs that were not included in the enrichment analysis are only discussed in the terms of the overall size of the effect (i.e. total number of DEGs, annotated and not annotated) on gene expression under conditions of within- and.or transgenerational warm-acclimation.

Results

Transcriptome sequencing yielded 52-57 million reads per sample sequenced in 2018, and 55-104 millions reads per sample sequenced in 2020 (Table S1). Trinity assembled 524,988 transcripts with 261,620 genes for all groups sequenced in 2018 and 413,029 transcripts with 269,149 genes for all groups sequenced in 2020 (Table S2). The transcriptome data showed both transgenerational and within-generation environmental influences on gene expression. Offspring within-generation warm-acclimation resulted in 3,853 DEGs with a LFC of ≤ -1 to ≥ 1 (Fig. 2). By comparison, transgenerational warm-acclimation resulted in fewer differentially expressed genes (maternal: 1,958; paternal: 1,777; both parents: 2,244; Fig. 3). The combined effect of offspring warm-acclimation with that of either or both parents often resulted in more DEGs than offspring within- or transgenerational warm-acclimation in isolation (offspring and maternal: 4,478; offspring and paternal: 3,638; offspring and both parents: 4,712; Fig. 4). Among all

groups, 1,005 of these DEGs were recognized by the DAVID tool and used for enrichment analysis. The level of differential expression of these genes are displayed in a heatmap (Fig. 2). The DEGs in the heatmap are sorted by LFC according to gene expression level in warm-acclimated offspring to show that any given gene up- or downregulated by offspring warm acclimation could be expressed differently with the influence of maternal and/or paternal warm acclimation. The list of genes along with their LFC values and adjusted *p*-value can be found in the supplementary information (Suppl. Table 17).

Principal component analysis may have detected a batch effect between the two years with 11% of the variation apparently due to offspring acclimation temperature and 4-6% of the variation potentially due to different sequencing years (Fig. S1). An analysis of the log2 fold-changes before and after correction showed that there was a strong correlation across all comparisons ($r \geq 0.91$), indicating that the batch correction provided essentially the same results (Fig. S2). Although we must still acknowledge the potential for a batch effect occurring in our results, we are confident in the trends observed in both years. For example, the within-generation (offspring) warm-acclimation group had a higher number of DEGs than did the groups that received either maternal or paternal transgenerational warm-acclimation. These latter two (parent/transgenerational) groups were sequenced in 2020, meaning that they received the higher sequencing depth yet still had fewer differentially expressed genes compared to the within-generation group which was sequenced at a lower depth in 2018.

Within-generation acclimation: offspring warm-acclimation

As predicted, warm-acclimation of lake trout offspring resulted in differential gene expression compared to cold-acclimated offspring, providing support for our hypothesis that warm acclimation would upregulate genes associated with metabolism, growth and thermal tolerance. Our enrichment analysis determined that 29 of these upregulated DEGs were involved in signalling processes, 14 related to cytoplasm functions and 9 related to immunoglobulin (Suppl. Table 3). There were 4 genes associated with methyltransferase activity, though this function was not significantly enriched (p=0.09; Suppl. Table 3). Upregulated DEGs that mapped to metabolic (KEGG) pathways included those involved in degradation (*HMGCLL1*, *CNOT1*) such as peroxisome activity and RNA and ketone degradation (Suppl. Table 4). Genes involved in the heat shock response were also upregulated, specifically *hsp90aa1.1* (Suppl. Table

4). Others included cell adhesion (*CD99L2*, *CTNNA2*) and signalling (*JAK1*, *PYCARD*, *CHD8*; Suppl. Table 4). There were 19 downregulated DEGs related to metal- and heme-binding and 3 DEGs associated with cyclin activity (Suppl. Table 3). Contrary to our predictions, downregulated DEGs involved in KEGG pathways included glycolysis/gluconeogenesis (*GAPDH*) and metabolism of fructose and mannose (*ALDOB*; Suppl. Table 4). Other DEGs involved in peroxidase activity were also downregulated with offspring warm-acclimation (*MPV17L2*; Suppl. Table 4).

Transgenerational acclimation: parental warm-acclimation

Parental warm-acclimation influenced gene expression in cold-acclimated offspring, however, transgenerational warm-acclimation (either or both parents) affected fewer genes than did within-generation warm-acclimation (Fig. 3). The direction of differential expression (up- or down-regulation) in the offspring depended on which parent was warm-acclimated and sometimes expression of a gene due to maternal or paternal warm-acclimation contrasted with the expression level when only the offspring were warm-acclimated (positive vs. negative LFC; Fig. 2). Nevertheless, parental warm-acclimation influenced differential gene expression in the lake trout offspring, lending support to our prediction that differential expression of genes would vary in offspring depending on parental temperature acclimation.

Maternal warm-acclimation had the effect of upregulating processes related to muscle function in their offspring, specifically 2 DEGs involved in calcium-binding and 3 DEGs in troponin synthesis (Suppl. Table 5). There were 13 upregulated DEGs associated with transferases and 3 DEGs related to glycolysis (Suppl. Table 5). Upregulated genes involved in KEGG pathways included DNA damage/repair and fatty acid metabolism (*DDIT4*, *hsd17b12a*; Suppl. Table 6). There were 14 downregulated DEGs involved in cell signalling and 10 involved in hydrolase/protease activity (Suppl. Table 5). The KEGG pathway analysis revealed that maternal warm-acclimation downregulated *GAPDH* involved in glycolysis/gluconeogenesis, and functions related to RNA transport and degradation (*CNOT1* and *EIF3C*; Suppl. Table 6).

Paternal warm-acclimation also had an effect on offspring gene expression, providing further support for our hypothesis regarding a transgenerational of warming on gene expression, although fewer genes were differentially expressed with paternal warming compared to maternal warming (Fig. 3). There were 10 upregulated DEGs related to transferase activity along with 3

DEGs involved in glycolytic processes (Suppl. Table 7). Upregulated KEGG pathways included the mTOR signalling pathway (*DDIT4*) and protein processing (*ERO1A*; Suppl. Table 8). Downregulated DEGs included 16 DEGs involved in cell-signalling and 7 DEGs associated with protease activity (Suppl. Table 7). The KEGG pathway analysis revealed there were two transcripts (one upregulated and the other downregulated), that were identified as *GAPDH* and mapped to glycolysis (Suppl. Table 8). Comparing the adjusted *p*-value of both transcripts, where a lower *p*-value means it is less likely a false positive (i.e. type I error), suggests that *GAPDH* was actually downregulated ($p = 0.88$). Up- and downregulation was also observed with *CNOT1* (Suppl. Table 8), but because the $p = 1$ in both cases, we cannot conclude whether this DEG was up- or downregulated.

As expected, transgenerational effects were also observed in offspring gene expression when both mothers and fathers were warm-acclimated. The combined effect of both parents resulted in more differentially expressed genes in the offspring compared to the transgenerational effect of warm acclimation of either parent in isolation (Fig. 3). We observed 16 upregulated DEGs that were associated with cell signalling processes, 9 DEGs related to hydrolase/proteases/peptidases function and 2 DEGs associated with peroxisome activity (Suppl. Table 9). Upregulated KEGG pathways included the mTOR pathway (*DDIT4*), RNA degradation (*CNOT1*) and amino acid transferase activity (*BHMT*, *GATM*, *GAMT*; Suppl. Table 10). Downregulated functions involved 4 DEGs included in glycosyltransferase activity, 3 DEGs related to lectin and 2 DEGs associated with tRNA function (Suppl. Table 9). There were 7 downregulated DEGs associated with translation/ribosomal proteins, but this function was not significantly enriched (p=0.09; Suppl. Table 9). Downregulated KEGG pathways included peroxisome activity (*HMGCLL1*) and apoptotic signaling (*pycard*) and Notch signaling pathways (*APH1B*; Suppl. Table 10). Again, *GAPDH* appeared as both up- and downregulated in the KEGG analysis.

Multigenerational acclimation: offspring and parental warm-acclimation

As hypothesized, genes associated with metabolism, growth and thermal stress/tolerance were differentially expressed with the combined effect of offspring and parent warm acclimation. The combination of offspring and parent (both together) warm-acclimation resulted in more DEGs (e.g. >200) being up- or downregulated relative to the offspring group that experienced

only within-generation warm-acclimation (Fig. 4). We must acknowledge here that the groups that represent the combined effect of offspring and maternal warm-acclimation, and the combined effect of offspring and paternal warm-acclimation were sequenced at a higher read depth than the within-generation only group (36 vs 28 million reads, respectively), meaning that the differences in differential expression here may be partly due to an experimental artefact of sequencing depth. For example, a total of 4,478 transcripts were differentially expressed in the offspring that received the combined treatment of within- and maternal transgenerational warm-acclimation compared to the 3,853 transcripts of the group that received the within-generation warm-acclimation only (Fig. 4).

The combined effect of offspring and maternal warm-acclimation resulted in the upregulation of 20 DEGs related transferase function, 4 DEGs involved in metalloprotease/endopeptidase activity and 4 associated with the PDZ domain (Suppl. Table 11). There were also 3 DEGs associated with protein folding, 3 DEGs associated with DNA repair, and 3 associated with muscle contraction/ZASP (Suppl. Table 11). Genes that encode heat shock proteins (*HSP70*, *HSPB1*, *HSP7C*, *HSP30*) and proteins involved in the respiratory chain (*COX2*, *COX1*) were also upregulated, though these functions were not significantly enriched (p=0.07; Suppl. Table 11). Similarly, upregulated KEGG pathways included heat shock responses and RNA degradation (Suppl. Table 12). Downregulated functions included 21 DEGs with transferase functions, 20 with function related to the cell nucleus and 19 DEGs involved in ATP binding (Suppl. Table 11). Additionally, there were 12 DEGs associated with kinases, 5 that had cyclin functions and 5 associated with DNA replication (Suppl. Table 11). Downregulated KEGG pathways included glycolysis/gluconeogenesis (*ALDOB*, *GAPDH*; Suppl. Table 12).

Offspring and paternal warm-acclimation combined to influence the differential expression of similar numbers and sorts of pathways as did maternal warm-acclimation, again supporting our hypothesis. This combination of offspring and paternal warm-acclimation had the effect of upregulating 4 DEGs that have proteases/peptidases functions and 2 DEGs involved in glycolysis (Suppl. Table 13). There were 2 upregulated DEGs involved in complex IV of the respiratory chain, however, this function was not significantly enriched (p=0.07; Suppl. Table 13). Similar to the combined effect of offspring and maternal warm-acclimation, offspring and paternal warm-acclimation upregulated KEGG pathways involved in cell signaling/inflammation (*mapk14a*), but offspring and paternal warm-acclimation uniquely upregulated KEGG pathways

associated with protein folding (*HYOU1*) and insulin regulation (*foxo1a*; Suppl. Table 14). Enriched downregulated functions included ATP binding (14 DEGs), hydrolase/protease activity (13 DEGs), DNA replication (4 DEGs) and cyclin function (4 DEGs; Suppl. Table 13). There were also 2 DEGs associated with tRNA ligase activity and 2 DEGs related to peroxisome function (Suppl. Table 13). Downregulated KEGG pathways included glycolysis (*ALDOB*, *GAPDH*), amino acid metabolism (*hpda*, *ALDH4A1*, *RRM1*, *RRM2*, *tdo2a*), fatty acid metabolism (*ELOVL6*; Suppl. Table 14).

Of all groups compared so far, the combined multigenerational effect of offspring, maternal and paternal warm-acclimation had the greatest effect on differential gene expression in the offspring (Fig. 4). There were 41 of upregulated DEGs associated with signalling function (Suppl. Table 15). Immunoglobulin and protease/peptidase activity were also enriched (9 and 5 DEGs, respectively; Suppl. Table 15). There were 2 DEGs involved in the respiratory chain complex IV but complex IV was not significantly enriched (p=0.07; Suppl. Table 15). Some of the affected KEGG pathways included the heat shock response (*hsp90aa1.1*), signalling pathways associated with inflammation (*mapk14a*) and insulin regulation (*foxo1a*; Suppl. Table 16). Downregulated functions included transferase activity (24 DEGs), DNA replication (6 DEGs) and cyclin functions (4 DEGs; Suppl. Table 15). Binding functions were downregulated, such as those related to magnesium, nucleic acid and iron binding (5 DEGs each; Suppl. Table 15). Downregulated DEGs involved in KEGG pathways included peroxisome activity (*MPV17L2*), glycolysis/gluconeogenesis (*ALDOB*), and response to DNA damage (*DDIT4*; Suppl. Table 16).

Discussion

Our hypothesis that genes associated with metabolism, growth and thermal stress/tolerance would be differentially expressed in juvenile lake trout depending on the acclimation temperatures of the offspring as well as their parents was supported by the sequence data. Genes involved in growth, heat shock and hypoxia responses, and metabolic pathways were differentially expressed with within-generation, transgenerational and multigenerational warm-acclimation. The degree to which each gene was affected depended on whether it was the offspring or parent that had been warm-acclimated, with the offspring thermal experience often having a stronger effect compared to the parental thermal experience. Maternal and paternal

warm-acclimation sometimes had opposing effects on the direction of differential expression of certain genes in their offspring with one parent stimulating gene upregulation and the other parent stimulating downregulation.

We identified five noteworthy trends when considering the experimental results all together: 1) Within-generation (offspring) warm-acclimation had a larger effect on the overall number of mapped DEGs compared to transgenerational contributions from warm-acclimated parents (mother, father or both). Unsurprisingly, the combination of offspring, maternal and paternal warm-acclimation had the greatest effect on the level of differential expression in the offspring with an overall 471 mapped DEGs. 2) Cell signalling processes were upregulated (29 DEGs) with offspring (within-generation) warm-acclimation, but maternal or paternal (transgenerational) warm acclimation had the effect of downregulating cell-signalling functions (14-16 DEGs). When both parents were warm-acclimated, cell signalling was upregulated (16 DEGs). Cell signalling was most enriched (41 DEGs) with multigenerational (offspring, maternal and paternal) warm-acclimation. 3) Transferase functions appeared to be regulated mainly by transgenerational (parental) warm acclimation rather than with within-generation (offspring) warm-acclimation, however, the degree of enrichment (i.e. gene count) and the direction of the effect (up- or downregulation) appeared to depend upon whether one or both parents were warm-acclimated. 4) Regulation of cyclin functions appeared to be primarily under the control of the offspring because this process was only downregulated when offspring were warm-acclimated, thus not influenced by transgenerational (either parent or both) warm-acclimation. 5) Mothers seemed to have an influence over muscle functions (calcium binding, troponin synthesis, ZASP) in their offspring, upregulating these functions when mothers experienced a warmer environment, whereas there was no evidence of paternal influence on offspring muscle functions.

General effect of warming on differential gene expression

Several DEGs that were associated with thermal stress were apparent across all comparisons. Among these were upregulated DEGs associated with molecular chaperone and co-chaperone functions (*hsp90aa1.1, hsp70, hsp30, hsp7c, hspB1, hspB7, HYOU1, FKBP5* and *FKBP8*). Upregulation of heat shock proteins and FK506-binding proteins (HSP90a, HSP70, FKBP10) has been observed in other salmonids experiencing warm acclimation and with an

acute temperature increase (Akbarzadeh et al. 2018; Houde et al. 2019; Shi et al. 2019). Another common indicator of heat stress in salmonids is hsp47, also known as serpinh1 (Tomalty et al. 2015; Akbarzadeh et al. 2018). We did not see differential expression of serpinh1 in our study, however, upregulation of this gene does not always occur with heat stress (Mackey et al. 2021). *HYOU1* in particular was upregulated with within- or multigenerational warm-acclimation, but not with transgenerational warm-acclimation in isolation (Suppl. Table 17). *HYOU1* (also known as *GRP170* and *HSP12A*) is part of the heat shock protein 70 family and its expression is primarily induced with hypoxia. Although, upregulation of this gene has previously been observed in response to heat stress in fish (Huang et al. 2018; Bilyk et al. 2021), its expression may be linked to a hypoxia response or may potentially reflect the increased demands on aerobic metabolism/oxidative stress (Olsvik et al. 2013; Huang et al. 2018). The temperature-controlled water in the source tank supplying the respirometer setup was supplemented with compressed oxygen at higher temperatures in an effort to maintain saturation levels and minimize exposure to hypoxia. However, we must acknowledge the possibility that some hypoxic responses may still have occurred.

In general, within- and transgenerational warm-acclimation resulted in upregulated mitogen-activated protein kinases (MAPK): *MAPK3*, *MAPK14a* and *MAPK14b* (also known as *ERK1*, *p38a* and *p38b*, respectively). MAPKs are important in the regulation of many processes, including mitosis during growth and development. They are also upregulated in response to various stressors, such as osmotic, hypoxic and thermal stress (Cowan and Storey 2003; Tian et al. 2019), for example, upregulation of these proteins has been observed in Atlantic salmon and *Oncorhynchus* spp. with thermal stress (Akbarzadeh et al. 2018; Shi et al. 2019). MAPKs are associated with the induction of HSPs, for example, *MAPK3* has been shown to induce expression of *HSP70* in zebrafish under thermal stress (Keller et al. 2008). Both *MAPK3* and *HSP70* were upregulated in warm-acclimated lake trout offspring with warm-acclimated mothers, suggesting that these fish relied on MAPK signaling cascades for resilience to warming. This is perhaps not surprising given that MAPK cascades and the induction of HSPs are evolutionarily conserved across numerous taxa (Krens et al. 2006; Kostenko et al. 2011), and that upregulation of these genes has been observed previously in other taxa following heat stress (Keller et al. 2008; Liu et al. 2021). Other affected pathways indicating upper thermal stress included peroxisome activity (*HMGCLL1*), DNA damage (*DDIT4*) and apoptotic signaling

(*pycard*), though whether these DEGs were up- or downregulated depended on whether it was the parental or offspring generation that had experienced warming. Taken together, these DEGs indicate increasing temperatures caused a stress response in lake trout, which we would expect given that each offspring was subjected to an acute temperature increase (+2°C/h) and these DEGs were likely upregulated to respond to cellular/protein damage occurring at their upper critical thermal limit (Penney et al. 2021).

There was a general enrichment of pathways associated with metabolism, including genes such as *COX1*, *COX2*, *GAPDH* and *ALDOB*. In general, *GAPDH* and *ALDOB* were downregulated with within- or transgenerational warm acclimation, however, *COX1* and *COX2* were upregulated in warm-acclimated offspring with warm-acclimated mothers following an acute temperature challenge. The effect of acute warming on differential expression of cytochrome c oxidase subunits is equivocal in fish: for example, acute exposure to warmer temperatures resulted in downregulation of *COX1* and *COX2* in the liver of yellow croaker (Qian and Xue 2016) but upregulation of *COX1* in the liver of juvenile lake whitefish (Zak and Manzon 2019). For salmonids in particular, upregulation *COX2* also occurred with heat stress in chinook salmon (*O. tshawytscha*) likely in response to the increased metabolic demands associated with warming (Tomalty et al. 2015). Upregulation COX6B1 has been shown to occur with heat stress in Pacific salmon (Akbarzadeh et al. 2018), though we did not detect expression of this subunit in our study. In our experiment, the matching of offspring and maternal thermal experience increased COX gene expression to a greater extent compared to other groups, potentially allowing offspring to meet the energetic demands associated with acute warming. We did not see upregulation of any other COX subunits, but it seems that expression of COX subunits does not follow stoichiometric expectations (Bremer and Moyes 2014) which may explain why we observed upregulation of only two of the 14 subunits that make up the COX holoenzyme.

We found some indication that warm-acclimation and upper thermal stress affected the growth of the lake trout offspring. We expected to see upregulation of some growth-related genes given that the warm-acclimated offspring were heavier than the cold-acclimated offspring at the time of sampling (Penney et al. 2021). Common molecular indicators of growth in fish include genes encoding growth hormone (*GH*) and insulin-like growth factor-1 (*IGF-1*), and genes related to thyroid function (Hevrøy et al. 2015; Li et al. 2021). We did not observe differential expression of *IGF-1* in the liver of our lake trout, but the thyroid receptors *TRα* and

TRβ were downregulated in offspring that experienced warm-acclimation. Thyroid hormones have important roles in growth and development (reviewed by Deal and Volkoff 2020), however, it is curious that *TRα* and *TRβ* were downregulated given we showed previously that warm-acclimated lake trout grew larger than the cold-acclimated group (Penney et al. 2021). Indicators of growth, measured as expression of *GH* and *IGF-1*, increased in *Labeo rohita* at slightly warmer acclimation temperatures but was suppressed at higher acclimation temperatures (Shahjahan et al. 2021). We collected liver samples at each fish's CTMax, so it is possible that fish were responding to the stress of the acute temperature increase and may have suppressed growth at this point in favour of a heat stress response. Lastly, there is evidence suggesting that growth hormone signaling pathways in the muscle are more sensitive to elevated temperature than in the liver of Atlantic salmon and rainbow trout (Hevrøy et al. 2015), thus it is possible that we may have seen a stronger transcriptional response for growth pathways in the muscle compared to the liver. Confirmation of this would require further experimentation.

Transcriptomic responses to warm-acclimation across generations

We found that an offspring's thermal acclimation often had a greater influence on the level (LFC) of gene expression than did the thermal experience of its parents and in some cases multigenerational (offspring and parental) warm acclimation had an additive effect. For example, cold-acclimated offspring downregulated the molecular chaperone *HYOU1* when either parent was warm-acclimated (LFC maternal: -1.21, paternal: -0.94; Suppl. Table 17), however, a combination of offspring and parental warm-acclimation upregulated *HYOU1* (LFC offspring & maternal: 0.72, offspring & paternal 0.80, offspring & both parents 1.14), though to a lesser extent than when offspring were warm-acclimated in isolation (LFC 1.29; Suppl. Table 17). This trend suggests that within- and transgenerational warm acclimation had an additive effect on the expression level of *HYOU1*. The gene *MAPK14a* was upregulated whenever the offspring were warm-acclimated, regardless of parental thermal experience (LFC ranged from 1.19 to 2.06) and seemed to override the isolated effect of transgenerational (parental) warm-acclimation (LFC ranged from -0.67 to 0.52; Suppl. Table 17). The idea of WGP overriding TGP has been presented before (Shama 2017), and our experiment provides evidence of this phenomenon at the transcriptomics level. It has been suggested that transgenerational effects are strongest in early-juvenile life stages (Yin et al. 2019) and it would be interesting to see if this is reflected at the

transcriptomics level by looking at gene expression at different life stages following within- and transgenerational warm-acclimation.

Compared to within-generation (offspring) warm-acclimation, transgenerational warm-acclimation (parents only) had a smaller effect on differential gene expression in lake trout offspring, supporting our previous study of whole animal metabolic responses to acute warming (Penney et al. 2021). Maternal and paternal warm-acclimation separately elevated DEGs functioning in the DNA damage response (*DDIT4*) and heat shock proteins (maternal only: *HSPB1*, *HSPB7*). These transgenerationally elevated pathways associated with DNA damage and HSPs support the idea of an adaptive response whereby parents may improve offspring fitness when they correctly anticipate their offspring's environment (Bateson et al. 2014; Bernatchez 2016; Ashe et al. 2021).

Our prediction that transgenerational warm acclimation would upregulate metabolic processes in the lake trout offspring was partially supported. We observed differential expression of the genes GAPDH, ALDOB and ENOA which relate to the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase, aldolase and enolase, respectively. These three transcripts tended to be downregulated in all groups except for the two groups that included the combined effect of offspring cold acclimation and paternal warm acclimation which indicates a paternal transgenerational effect of increasing offspring metabolism (Suppl. Table 17). Despite this evidence, we cannot be fully confident that glycolysis was upregulated in these offspring because, while the differential expression of GAPDH, ALDOB and ENOA suggests an effect of warm acclimation on the rate of glycolysis in the offspring, these three transcripts did not significantly enrich the 'glycolysis' pathway in our analysis. While the expression of these genes may vary depending on species, tissue type and treatment, the enzymes these genes encode are not considered rate-limiting enzymes, and these genes have even been suggested as housekeeping genes for experiments exploring differential gene expression (Hori et al. 2012; Purohit et al. 2016; Shekh et al. 2017).

Although transgenerational warming can result in larger offspring (Salinas et al. 2012), we did not find support for a transgenerational effect of warm-acclimation on growth in lake trout at the transcriptome level, consistent with a lack of response at the whole animal level (Penney et al. 2021). Lastly, despite some genes being transgenerationally expressed in the same direction by either the maternal or paternal warm-acclimation, none of these genes appeared to

have an additive or synergistic effect on expression level when both parents were simultaneously warm-acclimated (Suppl. Table 14).

The combination of offspring, maternal and paternal warm-acclimation had the greatest effect on the overall number genes that were differentially expressed and, for some genes, the greatest effect on up- or downregulation. This adds to a growing literature suggesting that transgenerational plasticity occurs when offspring and parental thermal experiences are coincident (Shama et al. 2014; Donelson et al. 2017; Yin et al. 2019). Among all of the groups compared, the combination of offspring and parent warm-acclimation had the strongest effect on cytochrome c oxidase subunit upregulation. Differential expression of COX-related genes has elsewhere been recorded with transgenerational acclimation (Shama et al. 2016; Bernal et al. 2018). Also, the expression level of *COX1* and *COX2* subunits (among 7 of 14 subunits tested) correlated with cytochrome c oxidase activity in muscle of fish (Bremer and Moyes 2014). Our result could be indicative of a higher metabolic rate in warm-acclimated offspring with warm-acclimated parents, although neither resting or peak metabolic rates differed between warm-acclimated offspring from warm- or cold-acclimated parents (Penney et al. 2021). It is worth noting, however, that gene expression does not necessarily confer a protein to function given that post-transcriptional and post-translational activity could also tag a protein for degradation.

There were a few instances where some genes such as *GAPDH* and *CNOT1* appeared to be simultaneously upregulated and downregulated within treatments, though why this occurred is not immediately clear. One possible explanation is that the direction of the responses were sex-specific (Best et al. 2018; Bell and Hellman 2019; Bautista et al. 2020). For example, in stickleback, mothers can influence the direction (up- or downregulation) of differentially expressed genes depending on whether the offspring is male or female (Metzger and Schulte 2016). It is possible that these genes were up- and downregulated because we had representatives of both sexes in each of our groups.

The offspring exhibited a stronger within-generation affect on gene expression transgenerational effects, with more DEGs in the offspring warm-acclimation treatments than from parental warm-acclimation. Some evidence suggests that transgenerational warming has a stronger effect on offspring differential gene expression compared to developmental warming (Veillleux et al. 2015; Shama et al. 2016). Prior to initiating warm-acclimation, all offspring in our experiment were reared in the same environment and temperature regime to minimize

differential developmental effects. The influence of thermal acclimation during early life stages on differential gene expression at subsequent life stages compared to within- and transgenerational acclimation may be a fruitful area for study.

Venney et al. (2022) used a similar breeding design as this study to assess temperature-related DNA methylation in adult brook trout and their offspring. In contrast to our findings, Venney et al. (2022) observed a stronger transgenerational effect from adults held at higher temperatures during gonad development compared with methylation from offspring rearing temperatures. Despite the apparent contrasts between the results of Venney et al. (2022) and our study, the findings from the two studies are complementary rather than contradictory. Some studies on salmonids have shown that changes in global methylation do not appear to influence differential gene expression (Christensen et al. 2021; Leitwein et al. 2022). DNA methylation can influence gene expression in an individual and subsequent generations, but gene expression is also a direct response to physiological, metabolic, and bioenergetic requirements including acclimation. The greater methylation observed from reproductive adults by Venney at al. (2022) may reflect the lower temperature tolerances and scope of reproductive adults compared with other free-swimming life stages in many fish species (Dahlke et al. 2020). The lesser extent of DNA methylation observed in juvenile brook trout by Venney et al. may also reflect the ability of subadult brook trout to utilize warmer temperatures than adults (Smith and Ridgway 2019). By contrast, our study examined levels of gene expression by transcriptome sequencing, and showed a greater effect of within-generation acclimation on gene expression, paralleling results from a previous study looking at whole-organism respirometry in lake trout (Penney at al. 2021).

Conclusions

Our study confirmed that lake trout exhibit both transgenerational (both maternal and paternal) and within-generation thermal acclimation, with both levels of acclimation influencing the strength and direction of differential expression of genes associated with metabolism and thermal stress/tolerance. Despite this, the limited transgenerational effect on DEGs indicates that adaptive transgenerational acclimation is unlikely to contribute sufficiently substantive benefits to enable lake trout to cope with rapidly changing environmental conditions related to global warming (Penney et al. 2021). Instead, population-level responses to temperature-related stress will likely be limited to within-generation acclimation (Kelly et al. 2014) and longer-term

adaptive responses. Beyond acclimation, persistence of cold-water species and populations may be reliant on existing genetic resources to cope with chronic warming (Comte and Olden 2017; Crozier and Hutchings 2014; Guzzo and Blanchfield 2017), which is particularly daunting for long-lived species (Willi et al. 2006). It therefore seems likely that lake trout and similarly vulnerable cold-water species may require increased management and conservation efforts to ensure their future in a rapidly warming world.

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Tables

Table 1: Summary of the comparison treatments used for assessing differential gene expression in juvenile lake trout following within- and transgenerational thermal acclimation, showing treatment groups (offspring and parental treatments), and measured effects.

	Treatment group (parent treatment)	**Sequence year (sample size)**	**Offspring sex ratio (♂,♀)**	**Effect measured**
Control group	Cold-acclimated offspring (C♀xC♂)	2018 (n=6) & 2020 (n=6)	2,4 5,1	*No effect (all other groups are compared to the control group)*
Within-generation warm-acclimation	Warm-acclimated offspring (C♀xC♂)	2018 (n=6)	3,3	Offspring warm-acclimation
Transgenerational warm-acclimation	Cold-acclimated offspring (W♀xC♂)	2020 (n=6)	4,2	Maternal warm-acclimation
	Cold-acclimated offspring (C♀xW♂)	2020 (n=6)	4,2	Paternal warm-acclimation
	Cold-acclimated offspring (W♀xW♂)	2018 (n=6)	3,3	Maternal & paternal warm-acclimation
Within- and transgenerational warm-acclimation	Warm-acclimated offspring (W♀xC♂)	2020 (n=6)	2,4	Offspring & maternal warm-acclimation
	Warm-acclimated offspring (C♀xW♂)	2020 (n=6)	3,3	Offspring & paternal warm-acclimation

	Warm-acclimated offspring (W♀xW♂)	2018 (n=6)	5,1	Offspring, maternal & paternal warm-acclimation

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Figures

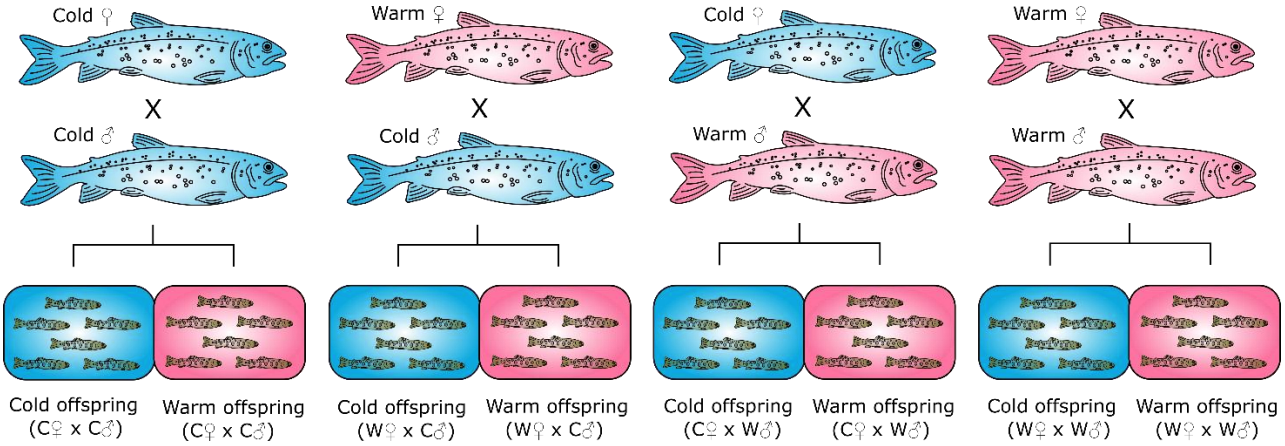

Figure 1: Experimental treatment groups of lake trout (*Salvelinus namaycush*). Two males and two females each from cold- and warm-acclimation tanks were bred in a full factorial cross, resulting in four families per parental treatment group (C♀xC♂, W♀xC♂, C♀xW♂, W♀xW♂; one each depicted here for simplicity). Offspring from each family was divided and acclimated to either a cold (11°C) or warm (15°C) temperature.

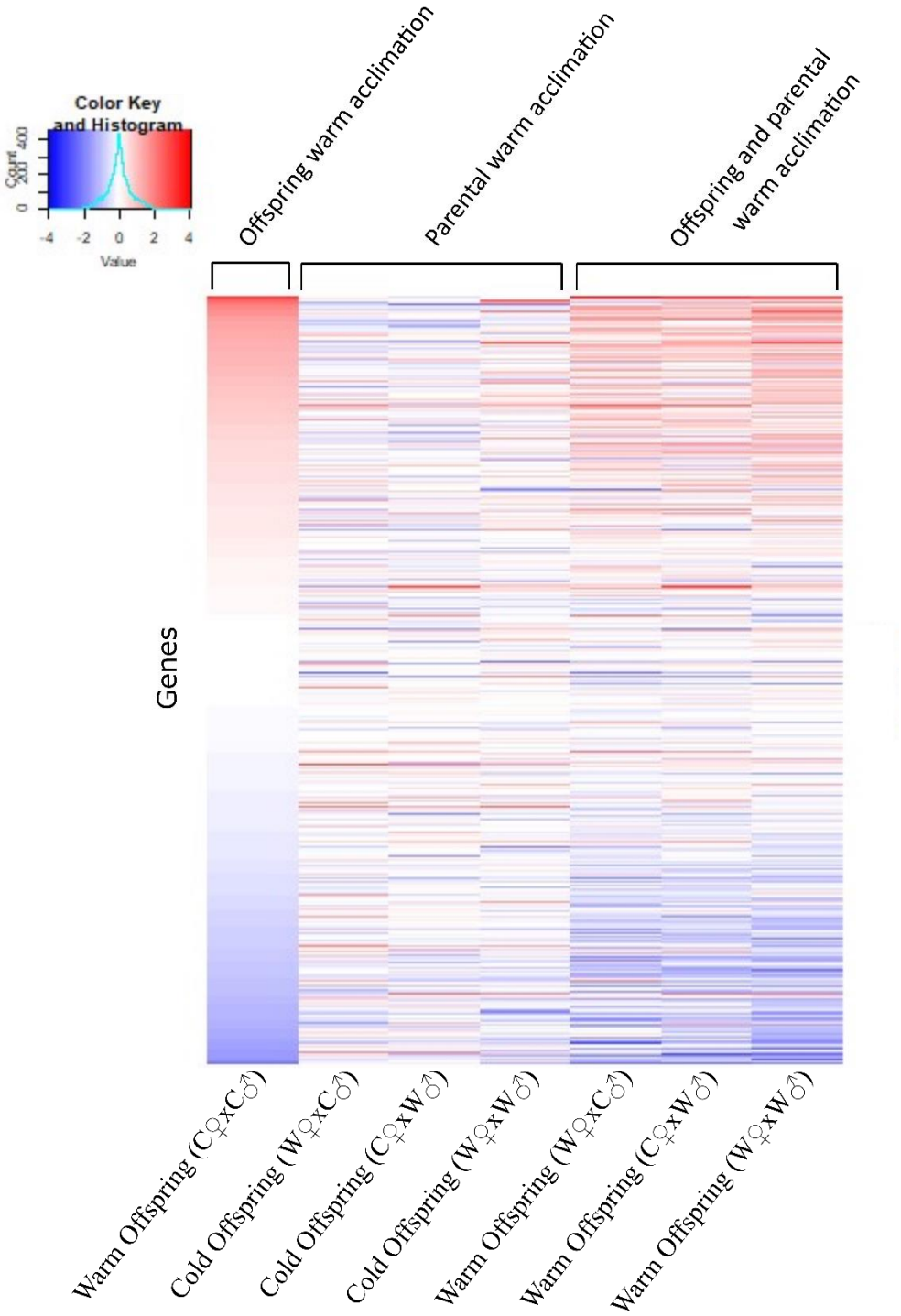

Figure 2: Heatmap comparing the effect of parental and offspring thermal acclimation on differential gene expression in juvenile lake trout, grouped by combined parental and offspring warm acclimation (transgenerational warming; top) and offspring acclimation treatment (within-generation; bottom). Each column represents a treatment group (n = 6 individuals) compared against the control group (11°C C♀xC♂; n = 6; not shown). Gene expression levels are represented as log2-fold change (LFC; blue = downregulate, red = upregulated). LFC is sorted according to gene expression level in warm-acclimated offspring to show that any given gene up- or downregulated by offspring warm acclimation could be expressed differently with the

influence of maternal and/or paternal warm acclimation. The list of genes along with their LFC values and FDR (adjusted p-value) can be found in the supplementary information (Suppl. Table 3).

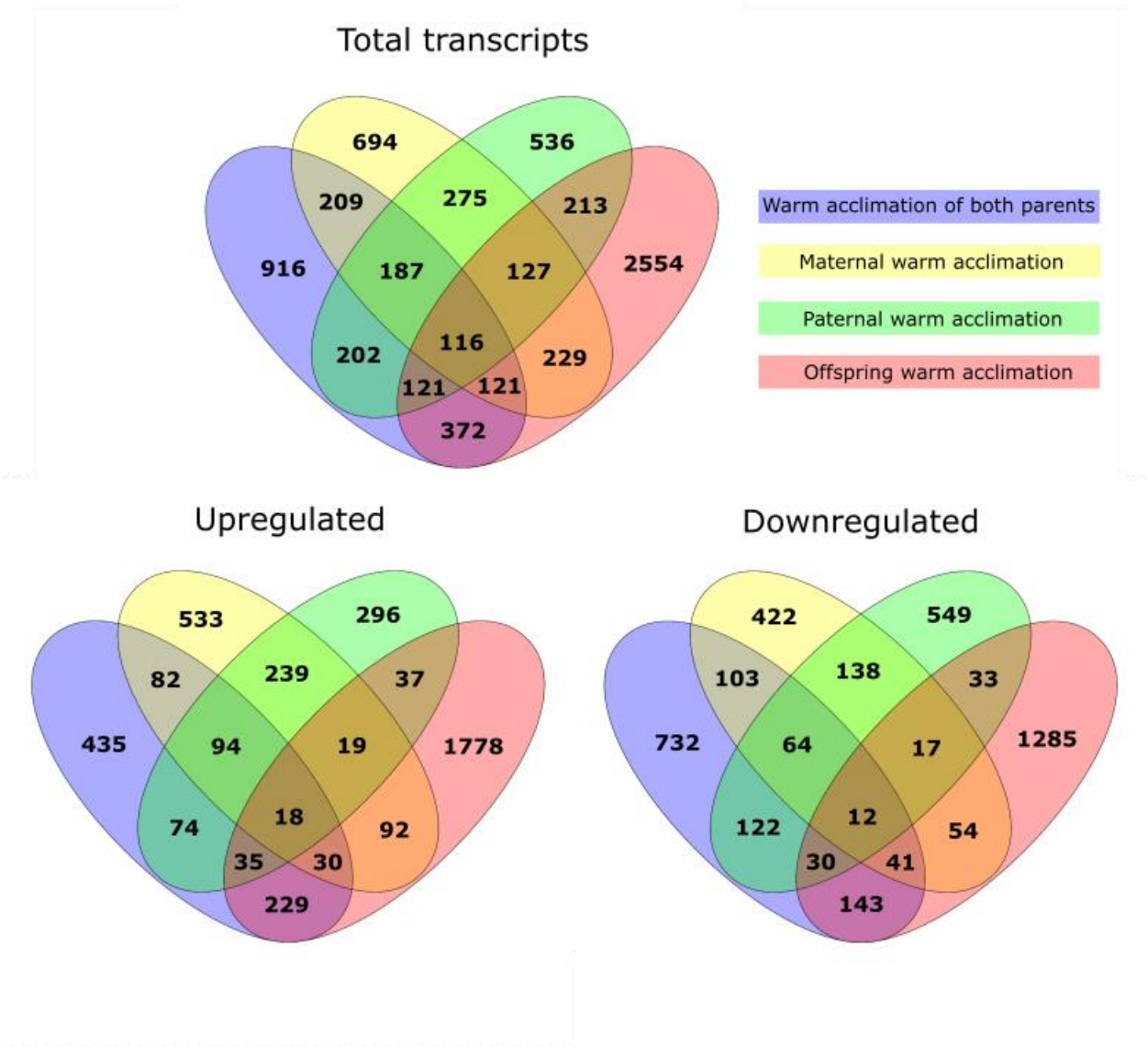

Figure 3: Number of transcripts that were differentially expressed in response to warm-acclimation of juvenile lake trout offspring (n = 6) when compared against the control group (11°C C♀xC♂; n = 12). Genes were expressed at log2-fold change (LFC) of ≤ -1 or ≥ +1 and are presented to show the total differentially expressed transcripts (top) which were then separated to show the upregulated (bottom, left) and downregulated (bottom, right) transcripts.

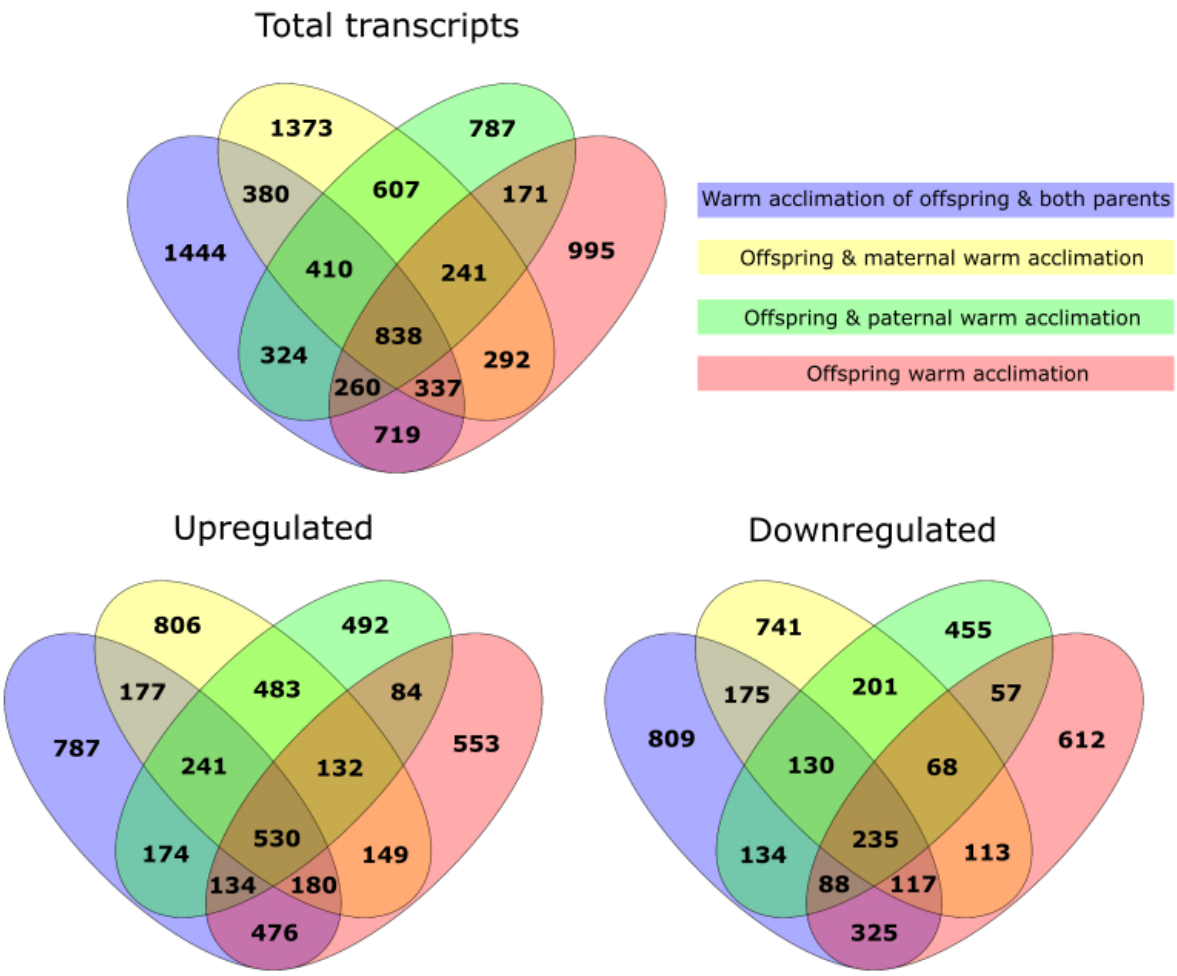
Total transcripts
1373
787
380
607
171
1444
410
241
995
838
324
292
260
337
719
Warm acclimation of offspring & both parents
Offspring & maternal warm acclimation
Offspring & paternal warm acclimation
Offspring warm acclimation
Upregulated
806
492
177
483
84
787
241
132
553
530
174
149
134
180
476
Downregulated
741
455
175
201
57
809
130
68
612
235
134
113
88
117
325

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Figure 4: Number of transcripts that were differentially expressed in response to transgenerational (parental) warm-acclimation of juvenile lake trout offspring when the mothers (A) or the fathers (B) were warm-acclimated, and when both parents (C) were warm-acclimated. Treatment groups (n = 6 each) were compared against control group (11°C C♀xC♂; n = 12). Genes were expressed at log2-fold change (LFC) of ≤ -1 or ≥ +1 and are presented to show the total differentially expressed transcripts (top) which were then separated to show the upregulated (bottom, left) and downregulated (bottom, right) transcripts.