Transcriptional Response to Acute Thermal Exposure in Juvenile Chinook Salmon Determined by RNAseq

Katharine M. H. Tomalty,*† Mariah H. Meek,* Molly R. Stephens,* Gonzalo Rincón,* Nann A. Fangue,† Bernie P. May,* and Melinda R. Baerwald*

*Department of Animal Science and †Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, California 95616

ABSTRACT Thermal exposure is a serious and growing challenge facing fish species worldwide. Chinook salmon (Oncorhynchus tshawytscha) living in the southern portion of their native range are particularly likely to encounter warmer water due to a confluence of factors. River alterations have increased the likelihood that juveniles will be exposed to warm water temperatures during their freshwater life stage, which can negatively impact survival, growth, and development and pose a threat to dwindling salmon populations. To better understand how acute thermal exposure affects the biology of salmon, we performed a transcriptional analysis of gill tissue from Chinook salmon juveniles reared at 12°C and exposed acutely to water temperatures ranging from ideal to potentially lethal (12°C to 25°C). Reverse-transcribed RNA libraries were sequenced on the Illumina HiSeq2000 platform and a de novo reference transcriptome was created. Differentially expressed transcripts were annotated using Blast2GO and relevant gene clusters were identified. In addition to a high degree of downregulation of a wide range of genes, we found upregulation of genes involved in protein folding/rescue, protein degradation, cell death, oxidative stress, metabolism, inflammation/immunity, transcription/translation, ion transport, cell cycle/growth, cell signaling, cellular trafficking, and structure/cytoskeleton. These results demonstrate the complex multi-modal cellular response to thermal stress in juvenile salmon.

KEYWORDS gene discovery Oncorhynchus tshawytscha Illumina thermal tolerance

Chinook salmon (Oncorhynchus tshawytscha) are native to the west coast of North America, ranging from Alaska to central California. A combination of overexploitation, habitat loss, and water diversions has contributed to significant declines in Chinook salmon populations in California over the past century (Yoshiyama et al. 2001). Thermal stress is a serious challenge facing the remaining Chinook salmon in California. Dams have played a substantial role in eliminating access to cooler historical habitats and have fundamentally altered the hydrology of most California rivers (Lindley et al. 2007; Poff et al. 2007). The majority of historic salmon habitats is now above impassible dams, restricting populations to lower elevations with higher ambient temperatures. The combination of increased climate variability (Lindley et al. 2007), restricted habitat, drought, and high water demands for human and agricultural use translates to an increased likelihood of thermal exposure for Chinook salmon (Brown and Bauer 2010).

Fall-run Chinook salmon juveniles inhabit freshwater for 1 to 7 months and are vulnerable to water temperature fluctuations during this period and during their migration to the ocean (Moyle 2002). Exposure to increased temperatures can cause behavioral and physiological changes in salmonids, including increased metabolic rates, increased oxygen consumption, erratic swimming behavior, and elevated heat shock protein levels (Barton and Schreck 1987; Mesa et al. 2002; Goniea et al. 2006; Bellgraph et al. 2010). These physiological and behavioral changes may place increased energy demands on fish and increase predator visibility, both of which can negatively impact juvenile survival. Temperature also affects smoltification, a set of physiological changes that prepare juvenile anadromous salmonids for the transition to salt water (Marine and Cech 2004). Optimal water temperatures for smoltification of California Chinook salmon are between 10°C and 17.5°C, and individuals that undergo smoltification at higher
water temperatures experience reduced survival rates in salt water (Myrick and Cech 2000).

Transcriptional analysis is an important means for investigating the physiological response to environmental changes of nonmodel organisms, for which few genomic tools have been developed (Gracey 2007; Logan and Somero 2011; Garcia et al. 2012). Information from such studies can be particularly valuable in the conservation of threatened and endangered species, as in the case of the delta smelt, *Hypomus transpacificus* (Connon et al. 2011a, b). Microarrays have been the predominant means of studying gene expression changes in salmonids following thermal stress (Arctic char, *Salvelinus alpinus*) (Quinn et al. 2011), sockeye salmon, *Oncorhynchus nerka* (Jeffries et al. 2012; Jeffries et al. 2014), pink salmon, *Oncorhynchus gorbuscha* (Jeffries et al. 2014), rainbow trout, *Oncorhynchus mykiss* (Rebl et al. 2013), brown trout, *Salmo trutta* (Meier et al. 2014); to our knowledge, no other studies have examined the transcriptome-wide response of juvenile Chinook salmon to elevated temperatures. Here, we take an RNAseq approach to investigate gene expression changes associated with increased temperatures in juvenile Chinook salmon. This method offers a variety of improvements over microarrays for quantifying transcription. RNAseq allows for the examination of a large number of genes without necessitating prior knowledge of the gene sequences and it increases the accuracy of detection over a wide range of expression levels (Shendure 2008; Wang et al. 2009). Gill tissue was chosen for this experiment due to the complex physiological role of this organ and its rapid response to environmental stressors (Evans et al. 2005; Chou et al. 2008). The feasibility of nonlethal sampling of gill tissue also makes this a useful organ for future field studies (McCormick 1993; Schrock et al. 1994). By examining the suite of genes that are differentially expressed following thermal stress, we can identify groups of genes and cellular processes that may be important for responding to thermal stress in this species. This information may help us to identify thermal stress in wild fish through the use of gene expression assays, as well as provide candidate genes for the investigation of adaptation to thermal stress in future studies (see examples in Larsen et al. 2010; Wellband and Heath 2013).

**MATERIALS AND METHODS**

Chinook salmon eggs obtained from Merced River Hatchery in November 2010 were reared at the University of California, Davis, in partially re-circulated aerated well water chilled to 12° (±1°) and fed commercial salmon feed (soft-moist formulation, 2–10% body weight; Rangen Inc.) until the time of the experiment, approximately 5 months posthatch. Fish were raised in a single 160-liter circular flow-through tank for 1 month preceding the experiment (see Figure 1 for experimental design), supplied with aerated well water and with a 12-hr light–12-hr dark photoperiod. The night prior to the experiment 55 fish were randomly assigned to one of five treatment groups (11 fish per treatment) and were allowed to acclimate at 12° in the experimental chambers overnight. Experimental chambers consisted of 5-gallon buckets with large mesh windows in the sides to allow water to freely enter and exit the chamber when submerged. Each chamber included an air stone to ensure well-aerated treatment water as well as to prevent thermal stratification. Water in the larger tanks was also well-aerated to maintain oxygen saturation. Treatments consisted of a 3-hr exposure to 15°, 18°, 21°, or 25° (±0.5°), followed by a 1-hr of recovery period at 12° (±0.5°). These temperatures range from optimal to the upper thermal limit for Chinook salmon determined by critical thermal methodology (Myrick and Cech 1998). Three hours was chosen as an ecologically relevant time exposure, because it approximates potential exposure times to warm water during juvenile outmigration and passage through warmer river reaches (Michel et al. 2013). The experimental chambers were moved to tanks held at a constant temperature using submersible titanium heating elements (Finnex 300W), facilitating very rapid change in the temperature experienced by the fish. Controls were handled identically to the other four treatment groups but remained at 12° (±0.5°). Following recovery, fish were killed with buffered tricaine methanesulfonate (500 mg/L tricaine and 420 mg/L NaCO₃), weighed and measured, and gill tissue was immediately collected [there was no significant difference in weight (P = 0.53) or length (P = 0.79) of fish between groups]. Samples were preserved in RNAlater solution (Life Technologies) and stored at −80° prior to RNA extraction. Replicates of this temperature experiment were performed on 3 consecutive days at 9:00 AM, yielding three replicates of 11 individuals at each temperature. Treatment of all animals was in accordance with University of California, Davis, animal care and use protocol #17875. RNA was isolated from gill tissue of 165 individuals using the TissueLyser II bead mill (Qiagen) for tissue homogenization and TRIzol Reagent Solution (Applied Biosystems) according to the manufacturer’s protocol. RNA was quantified and quality checked using the 2100 Bioanalyzer (Agilent) and RNA 6000 Nano Kit (Agilent). All RNA had a RIN (RNA Integrity Number) of 9.5 or higher. RNA from the 11 individuals in each experimental replicate was proportionally pooled and used to generate sequencing libraries using the Illumina TruSeq RNA Sample Preparation Kit and associated protocol (TruSeq RNA Sample Preparation Guide, part #15008136, November 2010 release). The 15 libraries were barcoded and processed, three libraries to a lane, with 100-bp paired-end sequencing on the Illumina HiSeq2000 platform at the University of California, Berkeley Vincent J. Coates Genomic Sequencing Laboratory. Raw data can be found at BioProject, record GSE59756.

The resulting sequences were quality-filtered (Phred quality score cutoff = 20), adapter sequences were removed from the beginning of each read, and 20 bp were trimmed from the end of each read to remove lower-quality bases (Cutadapt version 1.1). Trimmed reads were pooled and duplicates were removed before performing the *de novo* reference transcriptome assembly. Assembly was performed in CLC Genome Workbench (version 4.9 beta) using default settings for *de novo* assembly and a minimum contig length of 100 bp. Differentially expressed (DE) transcript identification was performed using CLC Genome Workbench (version 5.0). The sequences from each treatment group were then aligned back to the reference transcriptome and DE transcripts compared with the 12° control group were identified using Baggherly’s test with an FDR of 0.10 (Baggherly et al. 2003). The threshold for significance was set at *P* < 0.01 (FDR-corrected) and greater than two-fold change in expression. The *de novo* contigs were annotated with sequence descriptions identified through BLAST (Altschul et al. 1990) searches of the NCBI nucleotide database and assigned with GO terms, enzyme codes, KEGG pathways, and InterPro matches using default parameters in Blast2GO (Conesa et al. 2005).

Direction and magnitude of expression of three genes (SERPINH1, FOS, and CXCL8) in the pooled samples were confirmed with qPCR. RNA was treated with Deoxiribonuclease I, Amplification Grade (Life Technologies), to remove possible genomic DNA contamination according to manufacturer’s instructions. DNase-treated RNA was converted to cDNA using the SuperScript VILO cDNA Synthesis Kit (Life Technologies) according to the manufacturer’s instructions. Quantitative PCR was then performed using BioRad Chromo4 real-time detector in an 8 μl reaction [1 μM forward primer, 1 μM reverse primer, 1X Quantitect SYBR Green (Qiagen), and 5 μg cDNA]. Cycling parameters were 95° (15 min), 94° (15 sec), 58° (30 sec), 72°
(30 sec) (data acquisition) × 45 cycles, followed by a melt curve between 55°C and 95°C to ensure a single amplicon was present (Table 1). Standard curves were performed for each primer pair to ensure PCR efficiency was between 95% and 105%. Three replicates were performed for each sample and two no-template controls were included on each plate, and a no-RT control was included for each sample. Quantitative PCR was also performed for selected genes on individual RNA samples to assess expression variation within a pooled treatment group (Supporting Information, Figure S1). Quantitative PCR results were normalized to the housekeeping gene EF1A after ensuring its expression level remained stable across samples and experimental conditions. Analysis of qPCR data was performed using the delta delta Ct method, and results for all three technical replicates for a given sample were required to be within 10% of each other to be included.

RESULTS AND DISCUSSION
Sequencing yielded 885,283,684 raw reads, of which 836,304,468 remained after quality trimming and filtering. The de novo transcriptome generated from the filtered reads consisted of 183,778 contigs and was used as the reference for identification of differential gene expression at the four experimental temperatures. Four contigs were upregulated at 15°C, 104 at 18°C, 697 at 21°C, and 3976 at 25°C. Five contigs were downregulated at 15°C, 16 at 18°C, 101 at 21°C, and 6166 at 25°C. Of the upregulated contigs, an average of 48.4% were identified through a BLAST search as known sequence in the NCBI database, 41.5% were assigned Gene Ontology (GO) terms, and 46.7% matched a known protein in the InterPro database (Table 2, Table 3). Quantitative reverse-transcription PCR of three genes verified the direction and relative magnitude of expression change obtained through RNAseq (Table 4). A full gene list of all upregulated and downregulated genes is given in Table S1. Slight variations between the fold-change found by RNAseq and RT-qPCR protocols are expected due to the fact that the RNAseq samples were pooled at the RNA stage, whereas the qPCR was performed on individual cDNA samples and the results were then pooled. Pooling samples at the RNA stage is advantageous in that treatment means can be more accurately estimated and costs can be minimized; however, it precludes an evaluation of interindividual variation (Konczal et al. 2013). Future studies designed to estimate

| Table 1 Primer sequences and GenBank accession numbers for gene expression validated by qPCR |
|---|
| Gene Group | Gene Name | Gene Symbol | GenBank Accession Number | Primers (5′-3′) |
| Protein folding | Heat shock protein 47 | SERPINH1 | AB196463.1 | F-GTTCCCATGATGATCGCAC |
| | | | | R-CCTTGGTTTTGTCCACAGCG |
| Transcription | C-fos | FOS | AB111054.1 | F-AATGACTTTGAGCCCCTGTG |
| Inflammation/immunity | Chemokine ligand 8 | CXCL8 | AY160982.1 | R-GTAGGGGAGCTGAGGGAATC |
| | | | | F-AGAATGTCAGCCACCGGT |
| Housekeeping | Elongation factor 1A | EF1A | FJ890356.1 | R-CTTGCTCAGAGTGGCAATGA |
| | | | | F-TCTCAGGCGTACTGAGCCTGT |
| | | | | R-GGGGGCGTCAGTGAAGAGTCCAT |
interindividual variation will be complementary to our results presented here. Additionally, although paralogues are common in salmonid species due to their genome structure, identification of paralogues was outside the scope of this study. These data are presented with the caveat that a portion of the results will be showing expression for more than one gene paralogue.

Under thermal stress, especially at higher temperatures, normal cellular functions are suspended as the organism attempts to cope with the current stress (Parsell and Lindquist 1993; Kültz 2005; Logan and Somero 2011). Twenty-six KEGG pathways were identified for which at least two enzymes were upregulated (Table 5). Based on GO terms and protein functional information obtained from the UniProt database, 12 manually curated functional gene groups were identified among the results: (1) protein folding/rescue; (2) protein degradation; (3) cell death; (4) oxidative stress; (5) metabolism; (6) inflammation/immunity; (7) transcription/translation; (8) ion transport; (9) cell cycle/growth; (10) cell signaling; (11) cellular trafficking; and (12) structure/cytoskeleton. These functional categories represent a picture of biologically relevant changes that take place in response to acute thermal exposure in Chinook salmon. We provide interpretation of the gene function based on GO terms, the UniProt database, and published literature, although there may be additional functional roles of the identified genes that are not outlined here. We focused our discussion on gene functions to those that have been identified in fish or shown to be related to thermal stress. Likewise, we emphasize the roles of upregulated transcripts, although relevant downregulated genes also exist (Table S1).

**Management of denatured proteins**

Protein denaturation and agglutination are well-studied consequences of thermal stress and can disrupt normal cell function (reviewed in Dewey 1989; Nguyen et al. 1989; Nover 1991; Lepock 2004; Gsponer and Babu 2012). There are two classic ways in which the cell responds to denatured proteins, either through rescue and stabilization by chaperones, such as the heat shock family of proteins (reviewed in Lindquist 1986; Kampinga 2006), or by degradation of damaged proteins through the ubiquitin/proteosome pathway (reviewed in Glickman and Ciechanover 2002). In juvenile Chinook salmon, the expression of protein folding/rescue genes was upregulated starting at 18°C (two-fold to six-fold) and was highest in the 25°C group (two-fold to 2150-fold), whereas protein degradation-associated genes were only expressed in the 21°C (two-fold to four-fold) and 25°C (two-fold to 24-fold) groups. This is consistent with the expected response whereby at lower temperatures proteins are more easily salvaged through chaperone activity, and only at higher temperatures do unsalvageable proteins start to be degraded as the impact of thermal stress becomes more critical (Logan and Somero 2011).

Protein chaperones play a vital role under both normal and stress conditions by refolding or stabilizing proteins in their correct conformation (reviewed in Gething and Sambrook 1992; Hartl 1996) and are highly conserved among eukaryotes (reviewed in Feder and Hofmann 1999). Members of the heat shock protein family are expressed constitutively in various tissues (Fader et al. 1994; Boone and Vijayan 2002), whereas others are expressed as part of the general response to a variety of stressful stimuli including (but not limited to) temperature, chemical contaminants, hypoxia, pursuit by predators, and social hierarchy dynamics (reviewed Iwama et al. 1999; Basu et al. 2002; Iwama et al. 2004). Chaperone-coding genes were some of the most robustly expressed genes in this experiment and were expressed at temperatures as low as 18°C (Figure 2). Heat shock proteins are often divided into general categories by molecular weight. Three of the most common are the HSP90 family, HSP70 family, and small HSPs. Expression of HSP90 gene isoforms was upregulated at 18°C, 21°C, and 25°C, as were many known co-chaperones of HSP90, including CDC37, AHSA1, FKBP4, CHORDC1, HSP5A, and STIP1.

HSP90s are involved in both normal cellular function and the cellular stress response and act as protein-stabilizing chaperones (e.g., Wiech et al. 1992; Jakob et al. 1995; reviewed in Saibil 2013). The CDC37 protein physically interacts with the HSP90 protein and modifies its function by the recruitment of other co-chaperones (Roe et al. 2004; Röhl et al. 2013). The product of the AHSA1 gene (also known as AHA1) interacts with the HSP90 protein and can increase its ATPase activity by up to five-fold (Lotz et al. 2003; Röhl et al. 2013). The CHORDC1 protein interacts physically with HSP90 in vitro (Gano and Simon 2010). HSP5A (also known as GRP78) forms multimeric complexes with HSP90 and PDIα, as well as plays a key role in the unfolded protein response (UPR) (Tiffany-Castiglioni and Qian 2012). HSP90 has also been found to interact with
Table 3 Gene Ontology terms identified by Blast2GO analysis for contigs significantly upregulated at 25° (FDR 0.1, \(P < 0.01\), >2-fold change)

| GO Category               | GO ID       | Name                                             | GO ID       | Name                                             | # Contigs |
|---------------------------|-------------|--------------------------------------------------|-------------|--------------------------------------------------|-----------|
| Biological Process        | GO:0071840  | Cellular component organization or biogenesis    | GO:0016043  | Cellular component organization                  | 124       |
|                           | GO:0007005  | Mitochondrion organization                       |             |                                                  |           |
|                           | GO:0009987  | Cellular process                                  |             |                                                  |           |
|                           |             |                                                  | GO:0007154  | Cell communication                               | 11        |
|                           |             |                                                  | GO:007049   | Cell cycle                                       | 87        |
|                           |             |                                                  | GO:0008219  | Cell death                                       | 129       |
|                           |             |                                                  | GO:0019725  | Cellular homeostasis                             | 34        |
|                           |             |                                                  | GO:006464   | Cellular protein modification process            | 175       |
|                           |             |                                                  | GO:0007010  | Cytoskeleton organization                        | 85        |
|                           |             |                                                  | GO:0007165  | Signal transduction                              | 337       |
|                           |             |                                                  | GO:0006412  | Translation                                      | 27        |
|                           | GO:0032502  | Developmental process                             |             |                                                  |           |
|                           | GO:0009653  | Anatomical structure morphogenesis                |             |                                                  |           |
|                           | GO:0030154  | Cell differentiation                              |             |                                                  |           |
|                           | GO:0009790  | Embryo development                               |             |                                                  |           |
|                           | GO:0007275  | Multicellular organismal development              |             |                                                  |           |
|                           | GO:0008152  | Metabolic process                                 |             |                                                  |           |
|                           | GO:0009058  | Biosynthetic process                              |             |                                                  |           |
|                           | GO:0005975  | Carbohydrate metabolic process                    |             |                                                  |           |
|                           | GO:0009056  | Catabolic process                                 |             |                                                  |           |
|                           | GO:0006259  | DNA metabolic process                             |             |                                                  |           |
|                           | GO:0006091  | Generation of precursor metabolites and energy    |             |                                                  |           |
|                           | GO:0006629  | Lipid metabolic process                           |             |                                                  |           |
|                           | GO:006139   | Nucleobase-containing compound metabolic process |             |                                                  |           |
|                           | GO:0044238  | Primary metabolic process                         |             |                                                  |           |
|                           | GO:0019583  | Protein metabolic process                         |             |                                                  |           |
|                           | GO:0019748  | Secondary metabolic process                       |             |                                                  |           |
|                           | GO:0008152  | Metabolic process                                 |             |                                                  |           |
|                           | GO:0051704  | Multi-organism process                            |             |                                                  |           |
|                           | GO:0044403  | Symbiosis, encompassing mutualism through parasitism |         |                                                  |           |
|                           | GO:00050789 | Regulation of biological process                  |             |                                                  |           |
|                           | GO:0000003  | Reproduction                                      |             |                                                  |           |
|                           | GO:0000003  | Reproduction                                      |             |                                                  |           |
|                           | GO:0008096  | Response to stimulus                              |             |                                                  |           |
|                           | GO:007610   | Behavior                                          |             |                                                  |           |
|                           | GO:009628   | Response to abiotic stimulus                      |             |                                                  |           |
|                           | GO:009607   | Response to biotic stimulus                       |             |                                                  |           |
|                           | GO:009719   | Response to endogenous stimulus                   |             |                                                  |           |
|                           | GO:009605   | Response to external stimulus                     |             |                                                  |           |
|                           | GO:006950   | Response to stress                                |             |                                                  |           |
|                           | GO:0044699  | Single-organism process                           |             |                                                  |           |
|                           |             |                                                  | GO:0008283  | Cell proliferation                               | 91        |
|                           |             |                                                  | GO:0008037  | Cell recognition                                 | 26        |
|                           |             |                                                  | GO:0007267  | Cell--cell signaling                             | 18        |
|                           | GO:0006810  | Transport                                         |             |                                                  |           |
|                           | GO:006811   | Ion transport                                     |             |                                                  |           |
|                           | GO:0015031  | Protein transport                                 |             |                                                  |           |
| Molecular Function         | GO:0016209  | Antioxidant activity                              |             |                                                  |           |
|                           | GO:0005488  | Binding                                           |             |                                                  |           |
|                           | GO:0003779  | Actin binding                                     |             |                                                  |           |
|                           | GO:0005509  | Calcium ion binding                               |             |                                                  |           |
|                           | GO:0030246  | Carbohydrate binding                              |             |                                                  |           |
|                           | GO:003682   | Chromatin binding                                 |             |                                                  |           |
|                           | GO:0008092  | Cytoskeletal protein binding                       |             |                                                  |           |
|                           | GO:003677   | DNA binding                                       |             |                                                  |           |
|                           | GO:1901363  | Heterocyclic compound binding                     |             |                                                  |           |
|                           | GO:0008289  | Lipid binding                                     |             |                                                  |           |
|                           | GO:003676   | Nucleic acid binding                              |             |                                                  |           |
|                           | GO:000166   | Nucleotide binding                                |             |                                                  |           |
|                           | GO:0097159  | Organic cyclic compound binding                   |             |                                                  |           |
|                           | GO:0005515  | Protein binding                                   |             |                                                  |           |
|                           | GO:0005102  | Receptor binding                                  |             |                                                  |           |
|                           | GO:003723   | RNA binding                                       |             |                                                  |           |

(continued)
co-chaperone PDIA6 in the skeletal muscle of Atlantic salmon (de La Serrana and Johnston 2013). Interestingly, CDC37, HSP90, FKBP4, and AHSA1 have been shown to play an important role in RNAi-mediated gene silencing in mammals (Pare et al. 2013). Oxidative stress can induce RNAi silencing and subsequently reduced thermal tolerance in *C. elegans* (Spiró et al. 2012). Examining the role of RNAi in the stress response of fish is a potentially interesting line of future inquiry.

The upregulation of CDC37, HSP90, FKBP4, and AHSA1 is likely functioning to stabilize denatured proteins in the experimental fish.

Expression of HSP70 and associated co-chaperones in the DNAJ family (also known as HSP40) were upregulated at 18°C, 21°C, and 25°C. HSP70 is an important chaperone protein that stabilizes and refolds proteins in their correct conformation under stress conditions (reviewed in Silver and Noble 2012). Members of the DNAJ family of proteins regulate the function of HSP70 by stabilizing the interaction between HSP70 and the target proteins (Mayer and Bukau 2005; Qui et al. 2006).

Expression of the small HSPs, HSP30 and HSPB8 (also known as HSP22), along with the functionally similar CLU, was upregulated at 25°C (Carver et al. 2003). HSP30 is notable because it was upregulated by 1250.1-fold in the highest temperature group. SERPINH1 gene (also known as HSP47) was strongly upregulated (34.3-fold) and encodes a protein that is essential for normal growth and development, but that also plays an important role in collagen stabilization under stress conditions (reviewed in Silver and Noble 2012). Members of the DNAJ family of proteins regulate the function of HSP70 by stabilizing the interaction between HSP70 and the target proteins (Mayer and Bukau 2005; Qui et al. 2006).

The three GO categories, Biological Process, Molecular Function, and Cellular Component, are presented along with GO terms of children and number of contigs assigned each term.

| GO Category | GO ID | Name | GO ID | Name | # Contigs |
|-------------|-------|------|-------|------|-----------|
| GO:003824   | Catalytic activity | GO:0008135 | Translation factor activity, nucleic acid binding | 5 |
| GO:0009055 | Electron carrier activity | GO:0016787 | Hydrolase activity | 98 |
| GO:0003234 | Enzyme regulator activity | GO:0016301 | Kinase activity | 15 |
| GO:0060089 | Molecular transducer activity | GO:0003774 | Motor activity | 2 |
| GO:0001071 | Nucleic acid binding transcription factor activity | GO:0004518 | Nuclease activity | 6 |
| GO:0004872 | Receptor activity | GO:0008233 | Peptidase activity | 46 |
| GO:0005198 | Structural molecule activity | GO:0004721 | Phosphoprotein phosphatase activity | 26 |
| GO:0005215 | Transporter activity | GO:0004672 | Protein kinase activity | 58 |
| GO:0030313 | Cell envelope | GO:0016740 | Transferase activity | 89 |
| GO:0071944 | Cell periphery | GO:0003012 | External encapsulating structure | 1 |
| GO:005737 | Cytoplasm | GO:0005886 | Plasma membrane | 134 |
| GO:0005737 | Cytoplasmic membrane-bounded vesicle | GO:0016023 | Cytoplasmic membrane-bounded vesicle | 68 |
| GO:0005829 | Cytosol | GO:0005811 | Lipid particle | 9 |
| GO:0005576 | Extracellular region | GO:0005615 | Extracellular space | 38 |
| GO:0043226 | Organelle | GO:0005578 | Proteinaceous extracellular matrix | 22 |
| GO:0005694 | Chromosome | GO:0005929 | Cilium | 4 |
| GO:0005856 | Cytoskeleton | GO:0005783 | Endoplasmic reticulum | 115 |
| GO:0005764 | Endosome | GO:0005764 | Endosome | 19 |
| GO:0005728 | ER membrane protein complex | GO:0005794 | Golgi apparatus | 48 |
| GO:0005764 | Lysosome | GO:0005764 | Lysosome | 6 |
| GO:0005815 | Mitochondrion | GO:0005728 | Mitochondrion | 19 |
| GO:000228 | Nuclear envelope | GO:000539 | Nuclear chromosome | 20 |
| GO:0005635 | Nuclear envelope | GO:0005730 | Nuclear envelope | 14 |
| GO:0005634 | Nucleus | GO:0005654 | Nucleoplasm | 166 |
| GO:0005773 | Vacuole | GO:0005634 | Nucleus | 233 |
| GO:0005840 | Ribosome | GO:0005777 | Peroxisome | 3 |
| GO:0005773 | Vacuole | GO:0005840 | Ribosome | 8 |
| GO:0043234 | Protein complex | GO:0005773 | Vacuole | 5 |

The three GO categories, Biological Process, Molecular Function, and Cellular Component, are presented along with GO terms of children and number of contigs assigned each term.
Table 4 Fold change of selected genes as determined by RNAseq and quantitative reverse-transcription PCR

| Gene          | 15°C | 18°C | 21°C | 25°C |
|---------------|------|------|------|------|
| SERPINH1 (RNAseq) | <2   | 7    | 17   | 34   |
| SERPINH1 (qPCR)     | 4.9  | 16.9 | 38.4 | 43.3 |
| FOS (RNAseq)       | <2   | <2   | 3    | 24   |
| FOS (qPCR)         | 1.1  | 1.1  | 3.4  | 43.7 |
| CXCL8 (RNAseq)     | <2   | <2   | <2   | 13   |
| CXCL8 (qPCR)       | 1.1  | 0.9  | 1.1  | 18.3 |

conditions (reviewed in Nagata 1996). X-box binding protein (coded by XBPI gene), upregulated in the 21° and 25° groups, is a transcription factor that regulates expression of chaperone-coding genes during the UPR in the endoplasmic reticulum (Lee et al. 2003). HSP60, another upregulated gene product, functions as a protein chaperone but also play a role in apoptosis by forming a pro-apoptotic complex with procaspase 3 (Samali et al. 1999). The large number of strongly upregulated chaperone-coding genes supports the conclusion that protein folding and stabilization are important heat stress coping mechanisms in juvenile Chinook salmon.

The ubiquitin/proteasome pathway is the major destination for unfolded or damaged proteins that are not salvaged through chaperone stabilization or refolding. Proteins are marked for removal by the covalent attachment of multiple ubiquitin molecules and are subsequently degraded by the 26S proteasome or the lysosome (Ciechanover 1994; Ciechanover 1998). Multiple genes involved in the attachment of ubiquitin to target proteins were upregulated in juvenile Chinook salmon at 25°, including the ubiquitin ligases RNF19B and RNF8 and the ubiquitin conjugating enzyme UBE2B, which attach ubiquitin molecules to target proteins. RNF19B and the ubiquitin carrying protein type E2 are both associated with inflammatory and immune activation by cytotoxic t-cells or the NF-kB pathway, respectively (Gonen et al. 1999; Kozlowski et al. 1999).

Genes (SQSTM1, ZFAND5, PTPN23) associated with the formation of polyubiquitinated bodies and the endosomal sorting machinery that processes targeted proteins for lysosomal degradation were also upregulated in individuals exposed to the 21° and 25° treatments (Pankiv et al. 2007; Doyotte et al. 2008; Garnett et al. 2011; Shields and Piper 2011; Ali et al. 2013). The ubiquitin/proteasome pathway is likely strongly active in the experimental fish, especially in the higher temperature groups, indicating that the need for disposal of denatured proteins begins very early in the thermal stress response in these fish.

Cell death
Chinook salmon from the 18° treatment show a slight (two-fold to three-fold) increase in apoptosis-related gene expression, which increases up to seven-fold in the 21° treatment group. The 25° treatment group demonstrates a large increase in expression and number of upregulated apoptosis-related genes, ranging from two-fold to 220-fold change. Twelve out of 33 individuals in the 25° group became unresponsive and/or died during the 3-hr exposure. These individuals were monitored closely and removed for sampling promptly after cessation of opercular beats to avoid degradation of RNA in the sampled tissues.

At least 18 pro-apoptotic genes were upregulated in Chinook salmon following thermal exposure. The upregulation of CASP8, CASP10, RYBP, and KIAA0141 in the 25° group indicates that the intrinsic apoptotic pathway is active. Caspases 8 and 10 both participate in apoptotic cascades as well as increase NF-kB-mediated inflammation (Wang et al. 2001; Takahashi et al. 2005; Sakata et al. 2007). KIAA0141, death-associated protein effector DELE, is a pro-apoptotic protein that functions in the same pathway as caspases 8 and 10, and is functionally enhanced by inflammatory proteins such as TNFα (Harada et al. 2010). RYBP codes for death effector domain-associated factor, which binds with the protein hoppys to increase the apoptotic effect of caspase 8 (Stanton et al. 2007). The apoptotic and inflammatory responses may be tightly intertwined during the response to stress observed in juvenile Chinook salmon. PPP1R13B, JUNB, ODC1 code for apoptosis-inducing proteins and were upregulated at 21° and 25° (Jacobs-Helber et al. 1998; Samuels-Lev et al. 2001; Bergamaschi et al. 2004). Interestingly, increases in ODC1 have been observed in moribund temperature-stressed adult salmonids (Pignatti et al. 2004), and it has functional roles beyond apoptosis, including involvement in cell proliferation.

There is some overlap between the UPR and induction of apoptosis (Didelet et al. 2006). CHAC1 (upregulated at 18°, 21°, and 25°) is a pro-apoptotic gene that is upregulated in response to the UPR (Mungre et al. 2008). BAG3 (upregulated at 21° and 25°) is a regulator of many biological processes, among them apoptosis, and is a member of the BCL2 co-chaperone family that interacts directly with HSP70 (Rosati et al. 2011). Due to the multiple roles of these genes, it is uncertain which ultimate function they would serve in temperature-stressed fish in this experiment.

The processes of apoptosis and autophagy are tightly linked. Apoptosis results in cell death (type I), whereas autophagy can aid a cell in preventing apoptosis or can result in autophagic death (type II). Autophagy and apoptosis are mutually inhibitory to some extent, and both processes may be competing in a stressed cell (Mauri et al. 2007). Given the mutual inhibition of autophagy and apoptosis, it is not surprising that we found upregulation of several genes that code for anti-apoptotic proteins, in addition to the pro-apoptotic genes discussed above. Genes in the BCL2 family regulate apoptosis and can be either pro- or anti-apoptotic. BCL2L1, BNIP3, MCL1, and BCL2L10 are all generally anti-apoptotic members of this family and were upregulated in the 25° treatment group (Lee et al. 0.1999; Desagher and Martinou 2000; Vande Velde et al. 2000; Sevilla et al. 2001; Craig 2002). Upregulated PIM1, another regulator of apoptosis, is usually anti-apoptic, but it has also been shown to be pro-apoptotic in other circumstances (Lilly et al. 1999; Mochizuki et al. 1997; Aho et al. 2004; Ge et al. 2009).

Oxidative stress
At least six genes involved in the oxidative stress response were upregulated after 25° exposure. This is most likely due to a general upregulation of protective compounds during the cellular stress response, because water in the experimental chambers was well-aerated and the fish were not exposed to hypoxic conditions. The upregulated genes CAT and SESN2 encode proteins that protect cells from oxidative damage due to hydrogen peroxide and other oxidative compounds (Takeuchi et al. 1995; Budanov et al. 2004, 2011). The gene PDI43 (two- to three-fold) was upregulated in Chinook salmon in the 25° group has been shown in previous studies to increase in Atlantic salmon (S. sara) smolts held in hypoxic conditions (Huang et al. 2009). One isoform of the COX2 gene, PDS2A, was upregulated in the 25° group (eight-fold). Interestingly, zebrafish have two inducible isoforms of the COX2 gene that share some functional overlap in response to oxidative stress and inflammatory conditions (Ishikawa et al. 2007) and may indicate a link between the response to oxidative stress and inflammation (Uchida 2008).

Inflammation/immunity
We saw several indications of an inflammatory response as well as an increase in innate immune activity, particularly in the 25° group of fish (two-fold to 67-fold). Acute thermal stress has been shown to
| Pathway                             | # Enzymes | Pathway                             | # Enzymes |
|------------------------------------|-----------|------------------------------------|-----------|
| Purine metabolism                  | 7         | Purine metabolism                  | 22        |
| One carbon pool by folate          | 6         | Phosphatidylinositol signaling system | 11        |
| Arginine and proline metabolism    | 5         | Glycophospholipid metabolism       | 10        |
| Amino sugar and nucleotide sugar metabolism | 5         | Amino sugar and nucleotide sugar metabolism | 9        |
| Glycine, serine, and threonine metabolism | 4         | Inositol phosphate metabolism      | 9         |
| Pyrimidine metabolism              | 4         | Pyrimidine metabolism              | 8         |
| Arachadonic acid metabolism        | 4         | Pyruvate metabolism                | 8         |
| Cysteine and methionine metabolism | 4         | Citrate cycle                      | 7         |
| Mucin type O-glycan biosynthesis   | 4         | Pentose and glucuronate interconversions | 7         |
| Fructose and mannose metabolism    | 4         | Fructose and mannose metabolism    | 7         |
| Lysine degradation                 | 3         | Glycine, serine, and threonine metabolism | 7         |
| Aminoacyl-tRNA biosynthesis        | 3         | Glutathione metabolism             | 7         |
| Sphingolipid metabolism            | 3         | Glycerolipid metabolism            | 7         |
| Terpenoid backbone biosynthesis    | 3         | Carbohyde fixation pathways in prokaryotes | 7         |
| Other types of O-glycan biosynthesis | 3         | Other metabolism                   |           |
| Starch and sucrose metabolism      | 3         | Tryptophan metabolism              | 6         |
| Carbon fixation pathways in prokaryotes | 3         | Arachadonic acid metabolism        | 6         |
| T-cell receptor signaling pathway  | 2         | Porphyria and chlorophyll metabolism | 6         |
| Porphyrin and chlorophyll metabolism | 2         | Glycolysis/gluconoegenesis         | 5         |
| Steroid biosynthesis               | 2         | Pentose phosphate pathway          | 5         |
| Glycosphingolipid biosynthesis—lacto and neglector series | 2 | Arginine and proline metabolism | 5 |
| Drug metabolism—other enzymes      | 2         | Glycosylate and dicarboxylate metabolism | 5         |
| Alanine, aspartate, and glutamate metabolism | 2 | Butanoate metabolism              | 5         |
| Aminoacyl-tRNA biosynthesis        | 2         | Other metabolism                   |           |
| Glycosphingolipid biosynthesis—globoseries | 2 | Propanoate metabolism              | 3         |
| Drug metabolism                    | 2         | Methane metabolism                 | 4         |
| Ascorbate and alderate metabolism  | 3         | Other metabolism                   |           |
| Synthesis and degradation of ketone bodies | 3 | Steroid hormone biosynthesis       | 3         |
| Steroid hormone biosynthesis       | 3         | Cysteine and methionine metabolism | 3         |
| Beta-alanine metabolism            | 3         | Various types of N-glycan biosynthesis | 3         |
| Alpha-linolenic acid metabolism    | 3         | Glycosphingolipid biosynthesis—globoseries | 3 |
| Propanoate metabolism              | 3         | Sphingolipid metabolism            | 4         |
| One carbon pool by folate          | 3         | Methane metabolism                 | 4         |
| Retinol metabolism in animals      | 3         | Drug metabolism                    | 4         |
| Metabolism by xenobiotics by       | 3         | Other metabolism                   |           |
| cytochrome P450                     | 3         | Steroid hormone biosynthesis       | 3         |
| Drug metabolism—cytochrome P450    | 3         | O-dihydroxyphenylalanine metabolism | 2         |
| Geraniol degradation               | 2         | Histidine metabolism               | 2         |
| Tyrosine metabolism                | 2         | Phenylalanine metabolism           | 2         |
| Phenylalanine metabolism           | 2         | Cysteine amino acid metabolism     | 2         |
| N-glycan biosynthesis              | 2         | Peptidoglycan biosynthesis         | 2         |
| Linoleic acid metabolism           | 2         | Chloroalkane metabolism           | 2         |
| Chloroalkane and chloroalkene degradation | 2 | Aminobenzoate degradation          | 2         |
| Carbon fixation in photosynthetic organisms | 2 | Other metabolism                   |           |

(continued)
induce the classic vertebrate inflammatory response in other fish species, such as the Antarctic plunderfish, *Harpagifer antarcticus* (Thorne et al. 2010), and innate immune activity increases following acute stress events in many fish species (Tort 2011). Expression of a number of classic inflammatory mediators was upregulated, such as the cytokines chemokine (C-C-C motif) ligand 8 (CXCL8) and interleukin 1 beta (IL1B), and the cellular transducer of interleukin signaling gene STAT3 (Van Damme et al. 1985; Mukaida 2000; Levy and Lee 2002). The COX2 gene was also upregulated, which codes for a member of the biosynthetic pathway that produces inflammatory thromboxanes and prostaglandins (Rouzer and Marnett 2009). There is a large degree of overlap between inflammatory and innate immune responses. Many of the molecular players are functionally intertwined and the full extent of the relationship between the two responses is not yet fully understood (Medzhitov 2008; Muralidharan and Mandrekar 2013). The upregulated gene CEBPB, coding for CCAAT/enhancer-binding protein beta, increases expression of both immune and inflammatory proteins such as NF-kB and cyclooxygenase 2 (Mercurio and Manning 1999; Zhu et al. 2002; Park et al. 2010). IRAK1, also upregulated, plays a central role in mediating the immune response and is activated by the inflammatory cytokine IL1 (Gottipatti et al. 2008). Several genes coding for antimicrobial proteins were upregulated, including CAMP and the antiviral compound-coding genes GBP1 and IFITM2 (Anderson et al. 1999; Wang et al. 2008; Siegrist et al. 2011).

**Ion transport**

Several types of ion-linked transporters were upregulated in the 21° and 25° groups, which may be of interest for three reasons. First, increases in cellular ion concentrations, particularly of calcium, are important for the UPR. Second, a number of transporters are upregulated by immune/inflammatory genes and presumably play a role in those cellular responses. Third, gills are an important osmoregulatory organ for anadromous salmonids that adjust throughout different life stages to either fresh or saltwater environments (Evans 2002). Although salinity was not a factor in this experiment, the fish were nearing the age at which smoltification would typically begin and were therefore in a dynamic period of ion pump expression. Two genes coding for subunits of the sodium/potassium-transporting ATPase were upregulated, ATN2B33 and ATP1A1, along with a G-protein-activated inward potassium channel, KCNJ9. The upregulated gene SLC20A1 codes for a sodium-dependent phosphate transporter that is correlated with cytokine and IL-alpha increases (Sabatino et al. 1997). SLC20A1 is expressed in most cells and may play a separate role as a negative regulator of TNF-mediated apoptosis (Salaini et al. 2010). ATP2A2 (also known as SERCA2) expression was upregulated at both 21° and 25°. The sarcoplasmic reticulum calcium pump encoded by this gene is important for maintaining calcium homeostasis in the endoplasmic reticulum and has been linked to the UPR and concurrent increased need for calcium ions (Okada et al. 2002; Schröder 2008).

**Transcription/translation**

Unsurprisingly, expression of many regulators of transcription and translation was altered. Several transcription factors relating to stress, UPR, apoptosis, and inflammation were upregulated in the 21° and 25° groups. ATF5, for instance, is a transcription factor that is implicated in production of small HSPs (Wang et al. 2007). ATF3 is similarly known to be a stress response gene that is induced by physiological stress (Chen et al. 1996). Upregulated EPE30 stabilizes transcription factor HSF-1, which in turn regulates expression of heat shock proteins and chaperones (Raychaudhuri et al. 2014). FOS can function with the JUN/AP1 complex to regulate transcription (Zhang et al. 1998).

Several transcription factors are stimulated by DNA damage or oxidative stress to increase transcription of various stress response genes and were upregulated. ETS2 expression is activated by oxidative stress, whereas the SOX4 and SUPT16H proteins sense DNA damage and regulate the p53 and FACT complexes, respectively (Lee et al. 2009; Pan et al. 2009; Dinant et al. 2013). NKIRAS2 is a transcription factor that regulates the activity of NF-kB through regulation of its inhibitor, IkB (Fenwick et al. 2000). PTGES3 (also known as p23) is a central regulator of transcriptional complexes involved in stress

| KEGG Pathways Upregulated at 25° | KEGG Pathways Downregulated at 25° |
|----------------------------------|-----------------------------------|
| Nicotinate and nicotinamide metabolism | 2 |
| Nitrogen metabolism | 2 |
| Phenylpropanoid biosynthesis | 2 |
| mTOR signaling pathway | 2 |
| T-cell receptor signaling pathway | 2 |

Several KEGG pathways are represented in both the upregulated and downregulated groups. For these common pathways, different enzymes are upregulated or downregulated.
response (Freeman and Yamamoto 2002). The changes in transcription factor expression appear to be related in large part to apoptotic regulation and chaperone expression and function.

**Cell cycle/cell growth**

Expressions of the cell cycle and growth-related genes *BTG1*, *BTG2*, *CCNG1*, *CCNG2* *PLK3*, and *HSPA9* were upregulated at 25°C (two-fold to nine-fold), demonstrating a strong increase in antiproliferative genes. *BTG1* and *BTG2* both decrease cell proliferation, and *BTG2* is part of the p53 DNA damage response pathway (Rouault et al. 1992; Rouault et al. 1996). *CCNG1* and *CCNG2* (cyclin G1 and G2) are induced by DNA damage and halt the cell cycle at G1 and G2 phases, respectively. *CCNG1* is also part of the p53 pathway, whereas *CCNG2* is independent of this response (Bates et al. 1996). The stress-induced gene *PLK3* encodes a kinase that arrests the cell cycle and is known to be upregulated by peroxide exposure (Bahassi et al. 2002). Mortalin (encoded by *HSPA9*) is a heat shock protein that serves many functions under stress conditions, including cell cycle arrest (Kaul et al. 2002). Although there was no significant change in genes controlling cell proliferation at the lower temperatures, the increase in antiproliferative genes observed at 25°C indicates and halting of normal cell growth under extreme thermal conditions.

**Cell signaling**

Cell signaling genes *ORMDL3*, *PAK4*, *TRIB3*, *SRY4*, *DUSP1*, *DUSP2*, *NCK2*, and *PTK6* that were upregulated at 25°C (two-fold to 21-fold)
largely function in the UPR, immunity or inflammation, the general stress response, and promotion or protection from apoptosis. ORMDL3 participates in calcium signaling in the endoplasmic reticulum and is associated with increased strength of the UPR (Cantero-Recasens et al. 2009). PAK4 protects the cell from apoptosis and reduces the effect of caspase 8 (Gnesutta et al. 2001; Gnesutta and Minden 2003). TRIB3 is upregulated by stressful stimuli and, along with SRY4, regulates MAPK cell signaling (Kiss-Toth et al. 2004; Ord and Ord 2005; Cabrita and Christofori 2008). DUSP1 and DUSP2 encode serine/threonine kinases that are important for intracellular signaling and participate in inflammatory and immune signaling (Huang and Tan 2012). NCK2 is involved in intracellular signaling in both stress and normal conditions (Tu et al. 1998), as is PTKe. PTKe is involved in DNA damage–induced apoptosis (Brauer and Tyner 2010). Much of the alteration in cell signaling genes relates directly to some aspect of the cellular stress response.

Cell trafficking

The altered homeostatic and metabolic state of a highly stressed cell leads to a subsequent change in cellular trafficking of materials. One obvious change is an increased need for the transport of damaged proteins to the degradation machinery of the cell. Several genes related to cellular trafficking were upregulated in the 25°C (two-fold to 15-fold) group of fish, including HSP90B1, DNAJA1, SLC16A4, SLC16A2, AGL, XPO1, TRAM1, and RAB23. HSP90B1 encodes an endoplasmic reticulum protein that aids in the transport of proteins out of the ER, whereas upregulated DNAJA1 is involved in the intracellular trafficking of vesicles that may be destined for the lysosome (Christianson et al. 2008; Parfit et al. 2012). Upregulation of these two genes supports the assertion that the UPR is active in these juvenile Chinook salmon under thermal stress conditions and is further bolstered by the expression changes of other members of the same gene family (e.g., HSP90A and DNAJ homologs) at lower treatment temperatures. There are several other trafficking proteins that are indirectly related to the stress response. Monocarboxylate transporters SLC16A4 and SLC16A2 transport small metabolic compounds within the cell and function in energy partitioning within the cell during the stress response (Halestrap 2013). Alteration in protein targeting to different organelles is also altered under stress conditions. AGL targets proteins to the nucleus, whereas XPO1 traffics proteins out of the nucleus in a signal-dependent manner (Moroi andu et al. 1995; Ossareh-Nazari et al. 1997). Upregulated SNTB1 is involved in transport into the mitochondria, whereas TRAM1 transports proteins across the ER membrane (Görlch et al. 1992; Nuttall et al. 1997). RAB23 is a small GTPase that participates in the trafficking within the cilia (Lim et al. 2011). The cellular processes that occur under thermal stress conditions differ greatly from normal cellular function and unsurprisingly lead to changes in cellular trafficking.

Structure/cytoskeletal

There was a moderate difference in expression (two-fold to 10-fold) of genes involved in cytoskeletal structure and organization in the 25°C group. CCT4 and CCT5 are genes required for the production of actin and tubulin, and TBCB is a cofactor that ensures proper folding of tubulin during production (Lopez-Fanarraga et al. 2001; Brackley and Grantham 2009). Several upregulated genes modulate the interactions between various cytoskeletal components but have no known association with the stress response. AIFIL cross-links actin filaments and is involved in actin bundling (Schulze et al. 2008). SYNPO organizes actin filaments in response to RHOA signaling (Asanuma et al. 2006). CNN2 (calponin 2) binds actin and is thought to aid in the regulation of cytoskeletal organization (Tang et al. 2006). PDLIM1 encodes an adaptor protein that binds to cytoskeletal proteins (Sharma et al. 2011). These upregulated genes have not been previously associated with the stress response, but may play a role in stress-related structural changes in thermally stressed juvenile Chinook.

Certain upregulated cytoskeletal genes, such as MMP13, TAGLN, and CDC42SE1, have known associations with the cellular stress response. MMP13 codes for the protein collagenase 3, which is involved in connective tissue degradation and cytoskeletal component turnover (Knauper et al. 1996). Several other MMP family members were notably downregulated (Table S1); therefore, the roles of other family members in this process are likely more complex than the upregulation of a single gene. TAGLN codes for a calponin family protein, transgelin, which gels actin filaments and is overexpressed in senescent cells (Thweet et al. 1992; Assinder et al. 2009). CDC42SE1 mediates cytoskeletal scaffolding changes in response to immune signaling through CDC42 (Pirone et al. 2000). The upregulation of these genes indicates that some degree of cellular restructuring may take place in response to thermal stress.

CONCLUSION

Conclusions and comparisons to existing studies

The results of this study corroborate previous findings on important heat stress–related genes and implicate many new genes in the thermal stress response of juvenile Chinook salmon. Some response pathways and individual genes characterize a common thermal stress response across fish species, life stage, and duration of thermal stress; however, many of the genes reported above are likely specific to the thermal stress response of Chinook salmon and the juvenile life stage. Chaperone-coding genes are universally upregulated in published studies of thermal stress and gene expression. Upregulation of the HSP90 gene group were found in the gill tissue of Chinook salmon in this study, as well as in other fish species exposed to either acute or prolonged periods at elevated temperatures (e.g., gobies in Buckley et al. 2006; killifish in Fangue et al. 2006; Arctic charr in Quinn et al. 2011; pink and sockeye salmon in Jeffries et al. 2014). Upregulation of SERPINH1 has also been commonly found in other thermal stress studies of fish (e.g., Logan and Somero 2010; Jeffries et al. 2012; Rebl et al. 2013). The UPR is strongly represented in the thermal stress literature as well. Logan and Somero (2010) found an upregulation in PDIA4, whereas we identified similar genes PDIA6, PDIA4-like, and PDIA3. Jeffries et al. (2014) found an increase in FKBP10, whereas we identified upregulation of related gene FKBP4.

Jeffries et al. (2014) study of gene expression in the gill tissue of chronically heat-stressed adult pink and sockeye salmon found the largest number of genes in common with the current study. In addition to chaperone genes in the HSP90 and HSP70 families, Jeffries et al. (2014) found inflammatory regulators involved with NF-κB activity and other inflammatory/immune regulatory genes. Jeffries et al. (2012) found that the increase in ODC1 and CEBPB was the largest in moribund sockeye salmon, both of which were also upregulated in this study. They also identified several late-stage caspases involved in apoptosis, as did this study. Regardless of the difference in species, life stage, and duration of temperature exposure, many of the same functional pathways were upregulated in both the current and above studies, namely protein stabilization/degradation, apoptosis, and immunity/inflammation. In addition to these similarities, this study demonstrates an association of thermal stress in juvenile Chinook salmon with expression of some genes not previously linked to heat stress in salmonids, including cytoskeletal genes CCT4, CCT5, TBCB, AIFIL, SYNPO, CNN2 and PDLIM1.
The protein p53 appears to play an important role in the thermal stress response observed in this experiment. Although p53 itself was not upregulated, many of the observed upregulated genes are regulated in some fashion by p53, including genes involved in apoptosis, cell proliferation, and oxidative stress.

These results bolster our understanding of the cellular processes that are important for coping with thermal stress, which is of increasing importance for the southern-most populations of Chinook salmon in the United States. As our climate changes and the demand for water increases due to human population growth, the remaining Chinook salmon in states such as California will almost certainly encounter warmer temperatures during their freshwater life stages.

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