

**STRESS-INDUCED DIFFERENTIAL GENE EXPRESSION
IN THE BRAINS OF JUVENILE STEELHEAD TROUT
(*ONCORHYNCHUS MYKISS*)**

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EXTENDED ABSTRACT ONLY- DO NOT CITE

In response to stimuli, vertebrate brains display amazingly complex inter/intracellular interactions between neuroactive substances resulting in gene expression. Stressors, factors causing perceived fear or harm, are one group of stimuli that affect gene expression; however, information on brain-level genetic mechanisms of stress remains limited. Even less research describes stressor-induced gene expression in the fish brain. Previous research focussed on genes known to be associated with stress. However, recently developed molecular tools allow the simultaneous capture of numerous genes unique to a particular treatment, providing a more complete description of the brain during stress. We utilized suppression subtractive hybridization to identify genes in the brains of

juvenile steelhead trout (*Oncorhynchus mykiss*) exposed to confinement and handling stressors.

We reared winter steelhead (*Oncorhynchus mykiss*) parr at Oregon State University's Fish Performance and Genetics Laboratory, Corvallis, OR, prior to experimentation. After completion of triplicate 0-hour (control N=10), 3-hour (N=9 or 10), or 48-hour (N=8 or 9) handling and continuous confinement treatments, we lethally anesthetized the fish and collected blood from the dorsal artery. Brain removals occurred within minutes of bleeding, and were stored in RNase free cryo-vials and frozen on liquid N. We immediately extracted brain total RNA (stored at -80°C) which was pooled upon demonstration that plasma stress indicators (cortisol and glucose) across each triplicate were not different. Immediately after polyA⁺-selection, double stranded cDNAs were reverse transcribed using the CLONTECH PCR-select™ cDNA Subtraction Kit according to the manufacturer's protocol. Then, utilizing the above subtraction kit, we identified forward and reverse subtracted cDNAs representing up and down-regulated genes, respectively. We cloned genes using the TOPO TA Cloning® kit available from Invitrogen, PCR amplified positive-insert colonies, sequenced the genes, and grew-up and stored colonies (-80°C) in liquid media. Sequenced genes were queried using the web-based BLASTN and BLASTX 2.2.1 algorithms. Genes resulting in significant identities were confirmed differentially expressed by Northern hybridization using the North2South® Direct HRP Labelling and Detection Kit available from Pierce Endogen according to the manufacturer's specifications.

Plasma indicators of stress demonstrated that the fish were undergoing a stress response at 3 hours. Forward and reverse subtractions from the 0 and 3-hour groups resulted in 58 genes which were sequenced. Of the 58, 11 were selected for further analysis, and 4 were confirmed differentially expressed. The sequenced genes fell into the following categories: those associated with metabolic pathways/oxidative stress, neuro-protection, apoptosis regulation, osmoregulation, and second messenger systems. Details of gene expression will be discussed. Funding was provided by the US Army Corps of Engineers. Special thanks to Wilfrido Contreras-Sánchez, Beth Siddens, Rob Chitwood, Janine La Paz, Amarisa Marie, Shaun Clements, Ruth Milston, Molly Webb, Grant Feist, Sam Bradford, Tammy Black, Chris Whipps, Marta Alonso, Estela Thoman, and Davis Sequencing, Inc.