Exposure to aqueous film forming foam (AFFF)-impacted groundwater alters the liver proteome in fathead minnow

N. Hill[1], J. Agudelo[2], A. Slitt[2], J. Becanova[1], S. Vojta[1], L. Barber[3], D. Leblanc[4], A. Vajda[5], R. Lohmann[1]

[1]Graduate School of Oceanography, University of Rhode Island, Narragansett, RI (USA)
[2]Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI (USA)
[3]U.S. Geological Survey, Boulder, CO (USA)
[4]U.S. Geological Survey, Northborough, Massachusetts (USA)
[5]University of Colorado Denver, Denver, CO (USA)

THE UNIVERSITY OF RHODE ISLAND
GRADUATE SCHOOL OF OCEANOGRAPHY

STEEP — Sources, Transport, Exposure & Effects of PFASs. UNIVERSITY OF RHODE ISLAND SUPERFUND RESEARCH PROGRAM

Objective: investigate fathead minnow proteome response after exposure to AFFF-impacted groundwater at d7 and d21.

❖ **Proven that PFAS exhibit hepatotoxic effects - induce changes to liver transcriptome and proteome in aquatic wildlife**

❖ **Previous research stems largely from laboratory-based observations**

❖ **What is the liver proteome response when fish are exposed to environmentally-derived PFAS mixtures & concentrations?**

Methods

Sequential window acquisition of all theoretical fragment ion spectra mass spectrometry (SWATH-MS) was used to determine alterations to the liver proteome using an untargeted approach. Approximately 20mg of frozen liver was processed, as described by (Pfohl et al., 2021). 100 μL of protein was used for MS analysis on an Acquity UHPLC Hclass system coupled to a SCIEX 5600 TripleTOF mass spectrometer. Raw files were converted to HTRMS files and analyzed using proteomic software, Spectronaut™, for data-independent acquisition (DIA) processing. A directDIA™ analysis was built using an NCBI genomic database for *Pimephales promelas*: GCF_016745375.1. The exported data was normalized to peptide concentration (pmol per mg protein). Data represents averages of male and female fish (n = 4 per d7 & d21 treatments, n=6 per control).

❖ **T-Tests conducted between exposure groups & controls, derived fold change (FC > 2.0 or FC < 0.5)**

❖ **Statistically significant, modulated proteins uploaded to STRING Pathway analysis using *Danio rerio* as specie reference (FDR < 0.05)**

Results

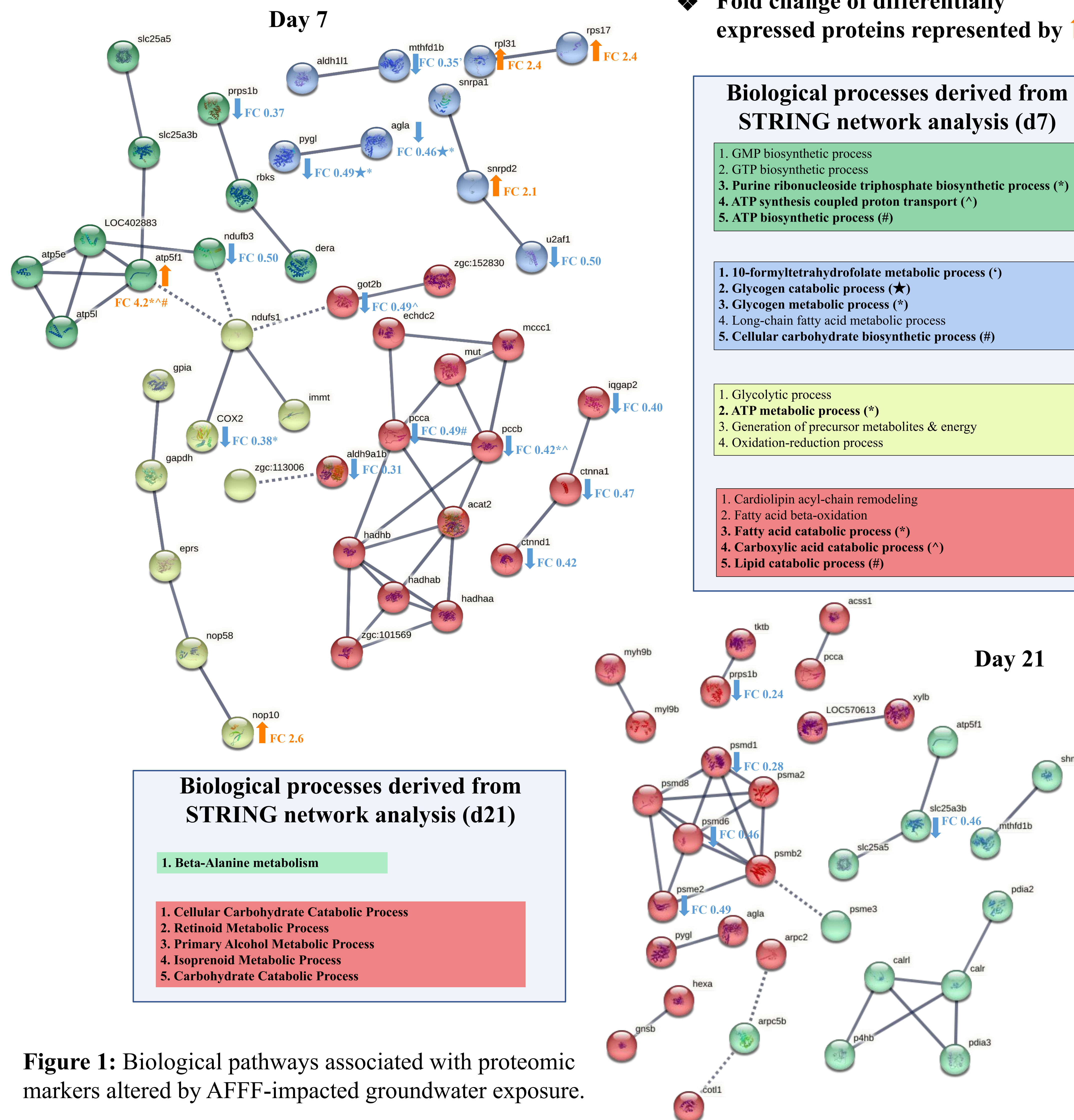### STRING Protein-Protein Interaction Networks Functional Enrichment Analysis

❖ **Fold change of differentially expressed proteins represented by ⬆⬇**

Figure 1: Biological pathways associated with proteomic markers altered by AFFF-impacted groundwater exposure.

Biological processes derived from STRING network analysis (d7)

1. GMP biosynthetic process
2. GTP biosynthetic process
3. Purine ribonucleoside triphosphate biosynthetic process (*)
4. ATP synthesis coupled proton transport (^)
5. ATP biosynthetic process (#)

1. 10-formyltetrahydrofolate metabolic process (')
2. Glycogen catabolic process (★)
3. Glycogen metabolic process (*)
4. Long-chain fatty acid metabolic process
5. Cellular carbohydrate biosynthetic process (#)

1. Glycolytic process
2. ATP metabolic process (*)
3. Generation of precursor metabolites & energy
4. Oxidation-reduction process

1. Cardiolipin acyl-chain remodeling
2. Fatty acid beta-oxidation
3. Fatty acid catabolic process (*)
4. Carboxylic acid catabolic process (^)
5. Lipid catabolic process (#)

Biological processes derived from STRING network analysis (d21)

1. Beta-Alanine metabolism

1. Cellular Carbohydrate Catabolic Process
2. Retinoid Metabolic Process
3. Primary Alcohol Metabolic Process
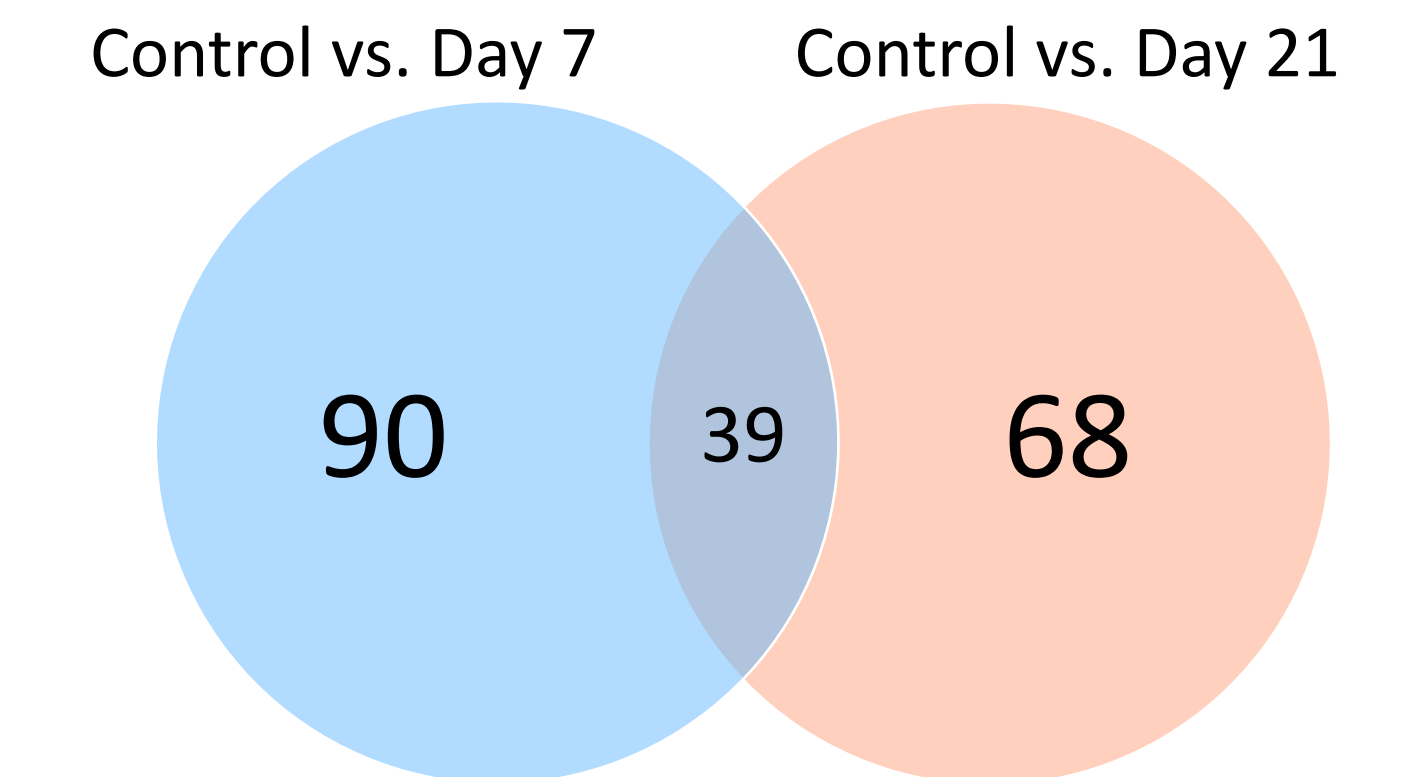4. Isoprenoid Metabolic Process
5. Carbohydrate Catabolic Process

Figure 2: Number of differentially expressed proteins and shared proteins at d7 and d21 as compared to fathead minnows from the initial control group.

Figure 3: Volcano plots depicting differential protein expression at d7 and d21 versus d0 controls.

Conclusions

❖ **Exposure to legacy and precursor PFAS in AFFF-impacted groundwater induced alteration of liver protein expression at d7 & d21**

❖ **Some proteomic pathways that were altered in fathead minnow are similar to previously discovered alterations to mouse liver proteome - i.e. lipid and glycogen metabolism (Pfohl et al., 2020).**

University of Colorado Boulder

THE UNIVERSITY OF RHODE ISLAND

USGS science for a changing world

NIH National Institute of Environmental Health Sciences Superfund Research Program