**Metabolomics Analysis of Thermally Challenged Mayfly Larvae**

Metabolomic Analysis: NIH Eastern Regional Comprehensive Metabolomics Resource Core (RTI RCMRC)

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**Abstract:** David Buchwalter at North Carolina State University (NCSU), collected the insect larvae samples for this study. The purpose of this study was to examine the metabolic profiles of mayfly (*Centroptilum triangulifer*) larvae subjected to thermal challenge. This species is unusual in terms of its ease of culture, and recent work in the Buchwalter lab1-7 has demonstrated its suitability as a laboratory test organism. Our purpose here was to examine how an environmentally realistic thermal challenge affects the physiology of this organism.

The goal of this metabolomics pilot study was to apply broad spectrum metabolomics method to determine whether we could differentiate the heat exposed mayfly larvae from the control mayfly larvae. Insect larvae from mayfly (MF), Athorix Diptoa (AD) and Megalopterans (MG) were obtained from David Buchwalter (North Carolina State University, Environmental and Molecular Toxicology). Original culture material was obtained from the Stroud Water Research Center (Avondale, PA). Larvae were reared at 22°C and fed a periphytic diet. When larvae reached a suitable size, they were separated from their food source for 6-8 hours to evacuate gut contents. Larvae were then divided into two treatment groups: a control group maintained at the culturing temperature of 22°C, and a thermal challenge group. The thermal challenge group was subjected to a temperature increase at a rate of 1°C per hour. This rate of temperature change is commonly observed in temperate streams. When the treatment temperature reached 30°C, larvae from both control and treatment groups were flash frozen in liquid nitrogen in groups of 12-13 larvae and stored at -80C. Each grouping of 12-13 larvae comprised a composite replicate for the metabolomics analysis. A total of 3 replicates each for the control and thermal treatments were used in this study. Some mayfly larvae had developed dark wing pads during the experiment. This is a signal that the larvae were very close to emerging from the aquatic larval phase to a winged sub-adult phase. Enough of these larvae were present in the control treatment to be removed from the control cohort and considered as an independent sample (ControlX). The AD and MG larvae were not thermally challenged and all of their samples and Mayfly samples (control group from a previous study) were part of the pooled external quality check (QC) samples.

Insect larvae were received and stored at -80C before use. A sample amount of larvae between 50-300 mg was taken for processing. Control and heat-treated mayfly samples were provided in triplicate and were processed individually. One sample was provided for AD, ControlX, MF control (from a previous study) and MG larvae. Three replicates of the larvae listed above having only a single sample were created. After homogenization, the three larval replicates were pooled and a single sample created for AD, MG, ControlX and MF control (from a previous study). A QC pool sample was created by combining equal concentration amounts of AD, MG and MF control (from a previous study) pool samples.

The 13 samples were thawed on ice in a 4⁰C room, then homogenized and extracted into acetonitrile water. After complete drying, the residue was resuspended in D2O with Chenomx internal standard for NMR analysis.

The data required for the metabolomics analysis can be found in the accompanying files:

Procedures: 1. Metabolomic Analysis of Thermally Challenged Larvae Procedures.docx

Study Design Table: 2. Metabolomic Analysis of Thermally Challenged Larvae Study Design Table.xlsx

Metadata: 3. Metabolomic Analysis of Thermally Challenged Larvae Metadata and Analytical Metadata.xlsm

Processed Data: 4. Metabolomic Analysis of Thermally Challenged Larvae Phenotypic and Processed Data.xlsx

Raw Data: 5. Metabolomic Analysis of Thermally Challenged Larvae Raw Data.zip

Notes:

Full sample preparation and analysis procedures are available in the accompanying document entitled **1**. **Metabolomic Analysis of Thermally Challenged Larvae** **Procedures** . Descriptions of abbreviations for factors are available in the Variable Dictionary in the accompanying file no. **2. Metabolomic Analysis of Thermally Challenged Larvae** **Study Design Table.xlsx**. The phenotypic and normalized data are available in the accompanying file **3. Metabolomic Analysis of Thermally Challenged Larvae** **Metadata and Analytical Metadata.xlsx**. Sample ID and factors can be found in the top 5 rows in the spreadsheet. The spreadsheets in the GC-MS processed data file (**4. Metabolomic Analysis of Thermally Challenged Larvae Phenotypic and Processed Data**) has a BinBase Processed Data tab. This tab shows the peak height value (not normalized) output from BinBase.

The raw data in netCDF format is available in the accompanying file **5. Metabolomic Analysis of Thermally Challenged Larvae.zip**. A table linking datafile names to Sample IDs is present in accompanying file **2. Metabolomic Analysis of Thermally Challenged Larvae** **Study Design Table.xlsx**.

**References:**

Kunz JL, Conley JM, Buchwalter DB, Norberg-King TJ, Kemble NE, Wang N, Ingersoll CG: Use of reconstituted waters to evaluate effects of elevated major ions associated with mountaintop coal mining on freshwater invertebrates. Environ Toxicol Chem 2013;32:2826-2835.