

# Transcriptional Responses in Fathead Minnows Exposed to Sediment-Free Raw and Ozone-Treated Oil Sands Processes Affected Water

## BACKGROUND

- Mining companies operating at the Alberta Oil Sands produce large quantities of oil sands process affected water (OSPW), which is stored in on-site tailing ponds.
- While toxicity of OSPW to aquatic organisms has been demonstrated, a comprehensive understanding of the mechanism(s) of its toxicity is currently unavailable.
- Acute toxicity of OSPW has been attributed to naphthenic acids (NA).
- These organic constituents are the focus of remediation efforts.
- There is evidence that ozonation attenuates adverse effects of OSPW.
- An understanding of the effect(s) of ozonated OSPW on exposed organisms is required.

## OBJECTIVES

- Utilize state of the art open formatting mRNA sequencing technologies to investigate transcriptome wide alterations in gene expression profiles in Fathead minnows exposed to untreated and ozone-treated OSPW.
- To investigate possible mechanisms of OSPW toxicity through the identification of (novel) molecular responses to OSPW exposure.
- Explore a possible molecular basis for the attenuation of OSPW toxicity by ozone treatment.
- Ultimately the goal is to develop sensitive and specific biomarkers of exposure that can be used to monitor of fish after possible spills or any future exposure to treated OSPW.

## METHODOLOGY

### Ozonation of OSPW

- OSPW was collected from West-In-Pit, an active settling basin on the site of Syncrude Canada Ltd..
- Ozone gas bubbled into OSPW to a final concentration of 30 mg/L.

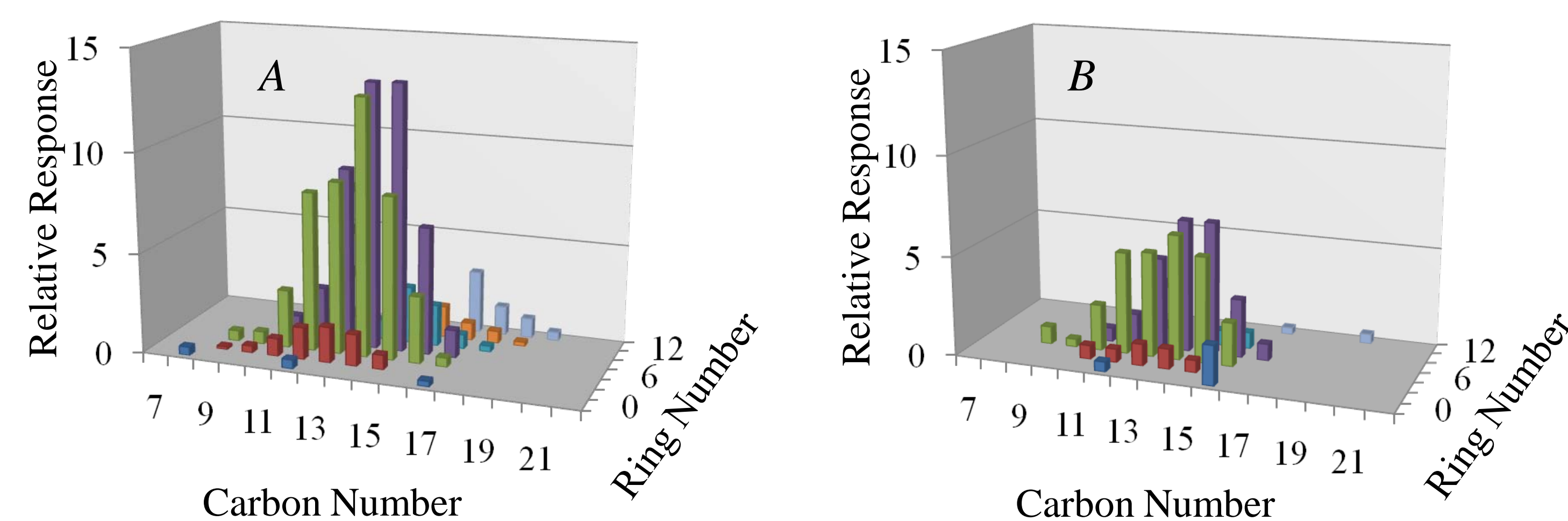


Figure 1: NA profile of OSPW before (A) and after (B) ozonation.

- Ozonation of OSPW decreases parent NA concentrations, and selectively targets NAs with greater ring content and alkyl branching.

### Fathead Minnow Exposure

- Sexually immature Fathead minnows exposed to either:
  - Freshwater Control (dechlorinated tapwater)
  - OSPW ([NA] ~ 77 mg/L)
  - Ozone Treated OSPW

- 96h exposure with 50% water renewal daily.
- 12 fish per tank, with duplicate tanks for each exposure.
- Livers collected for determination of transcript abundance by Illumina RNA seq.

## RESULTS 1 – TRANSCRIPTOME CHARACTERIZATION

### RNA-Seq

- 75 bp single End Reads generated on an Illumina GAI Sequencer.
- As the fathead minnow genome is not sequenced we performed *de novo* assembly of the Illumina reads and incorporated mRNA sequences available through NCBI to create a database of reference contigs.
- Blast2GO software was used to assign gene identity using BlastX with an E-value cutoff of  $10^{-6}$ .

Table 1: Summary of RNA Seq, De Novo Assembly and Blast2GO Annotation.

Parameter	Value
Total Number of Reads	85,789,598
Number of De Novo Assembled Contigs	176,120
Min Contig Length	100 bp
Max Contig Length	9129 bp
Mean Contig Length	366 bp
Number of Contigs with E-value < $10^{-6}$	52,800

- We are currently assembling contigs into scaffolds to improve the quality of our transcript database.

## RESULTS 2 – GLOBAL TRANSCRIPT ABUNDANCE

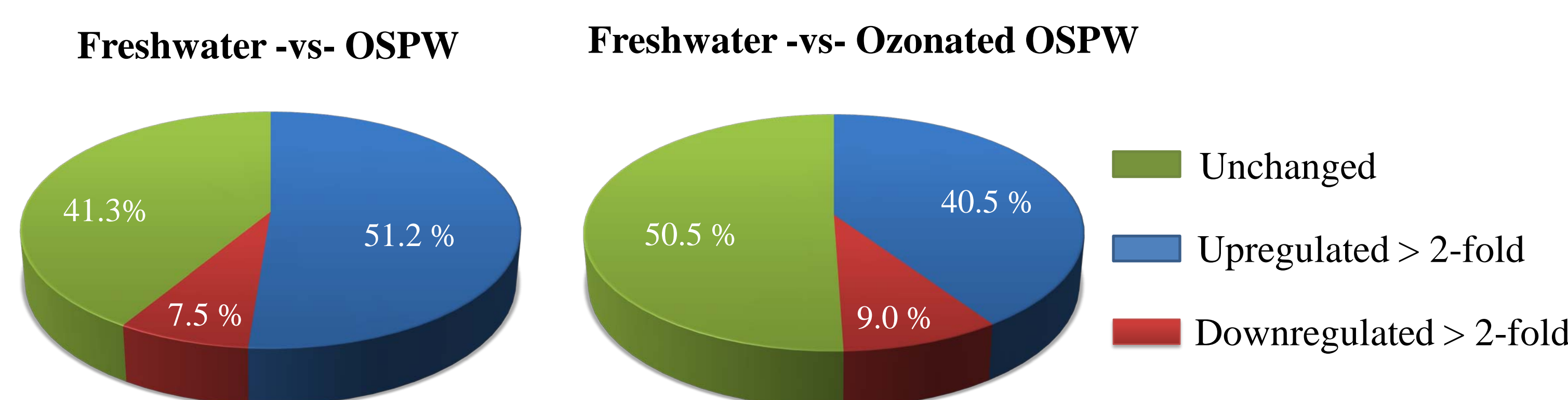


Figure 2: Summary of changes in hepatic transcript abundance in response to OSPW and ozonated OSPW exposure.

- Exposure to OSPW and ozonated OSPW impacts transcript abundance of hepatic genes.
- A greater number of genes have increased transcript abundance than decreased abundance in fatheads exposed to OSPW or ozonated OSPW.
- Slightly fewer genes (~ 9 %) impacted by ozonated OSPW than raw OSPW.

## RESULTS 3– DIFFERENTIAL TRANSCRIPT ABUNDANCE

Transcript	Freshwater -vs- OSPW	Freshwater -vs- Ozonated OSPW
CYP 1A	1.0	1.0
CYP 2A	2.9	2.2
CYP 3A	48.7	2.5
CYP 2K19	43.3	6.9

Table 2: Fold change in transcript abundance of selected CYP450 genes in fathead minnows exposed to OSPW and ozonated OSPW.

- No Dioxin-like response to either OSPW or ozonated OSPW.
- Increased CYP3A and CYP2K19 transcript abundance is attenuated by OSPW ozonation.

Transcript	Freshwater -vs- OSPW	Freshwater -vs- Ozonated OSPW
3- $\beta$ -HSD	-15.8	-6.9
17- $\beta$ -HSD	-12.6	-2.7
11- $\beta$ -HSD	10.1	57.9

Table 3: Fold change in transcript abundance of selected steroidogenic genes in fathead minnows exposed to OSPW and Ozonated OSPW.

- Decreased 3- $\beta$ -HSD and 17- $\beta$ -HSD could impact testosterone and estradiol production.
- Decreased testosterone production in H295R cells (He et al., 2010) and Fathead minnows (Kavanagh et al. 2010) exposed to OSPW has been reported. Ozonation of OSPW attenuates the effect on testosterone production in H295R cells (He et al., 2010).
- Increased 11- $\beta$ -HSD could increase aldosterone and cortisol production.
- Elevated basal cortisol has been reported in fish exposed to OSPW (Lister et al., 2008).
- Ozonation of OSPW attenuates effects on 3- $\beta$ -HSD and 17- $\beta$ -HSD but potentiates effects on 11- $\beta$ -HSD.

Transcript	Freshwater -vs- OSPW	Freshwater -vs- Ozonated OSPW
Lanosterol synthase	-7.5	-14.8
Squalene synthase	-11.1	-14.9
7-Dehydrocholesterol reductase	-5.7	-2.1
24-Dehydrocholesterol reductase	-4.7	-2.2

Table 4: Fold change in transcript abundance of Selected cholesterol metabolism genes in Fathead minnows exposed to OSPW and ozonated OSPW.

- OSPW and ozonated OSPW decrease transcript abundance of genes related to cholesterol biosynthesis.

## CONCLUSIONS AND FUTURE WORK

- Exposure to OSPW and low-dose ozonated OSPW impacts transcript abundance of hepatic genes in Fathead minnow.
- Transcriptional responses to OSPW may be attenuated or potentiated by ozonation of OSPW.
- RNA-Seq represents a powerful open-format method for the examination of transcriptional responses to environmental stressors and will aid in understanding the molecular basis of OSPW toxicity and development of biomarkers of exposure.
- We are performing paired-end read RNA-seq and incorporating Fathead minnow sequences from Dr. Nancy Denslow into our *de novo* assembly to increase our transcript database.
- Real-time PCR will be used to verify observed changes in transcript abundance.
- Fathead minnow exposure to OSPW treated with higher degrees of ozonation will be performed to further establish the effects of this potential OSPW treatment method.

## REFERENCES

- He Y et al. Chemosphere. 2010 80:578-584
- Lister A et al. Aquatic Toxicology. 2008 87:170–177.
- Kavanagh RJ et al., Environmental Toxicology and Chemistry. 2010 in press.

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