Toxicology of Hydraulic Fracturing Flowback Fluid - Potential Endocrine Disruptor?

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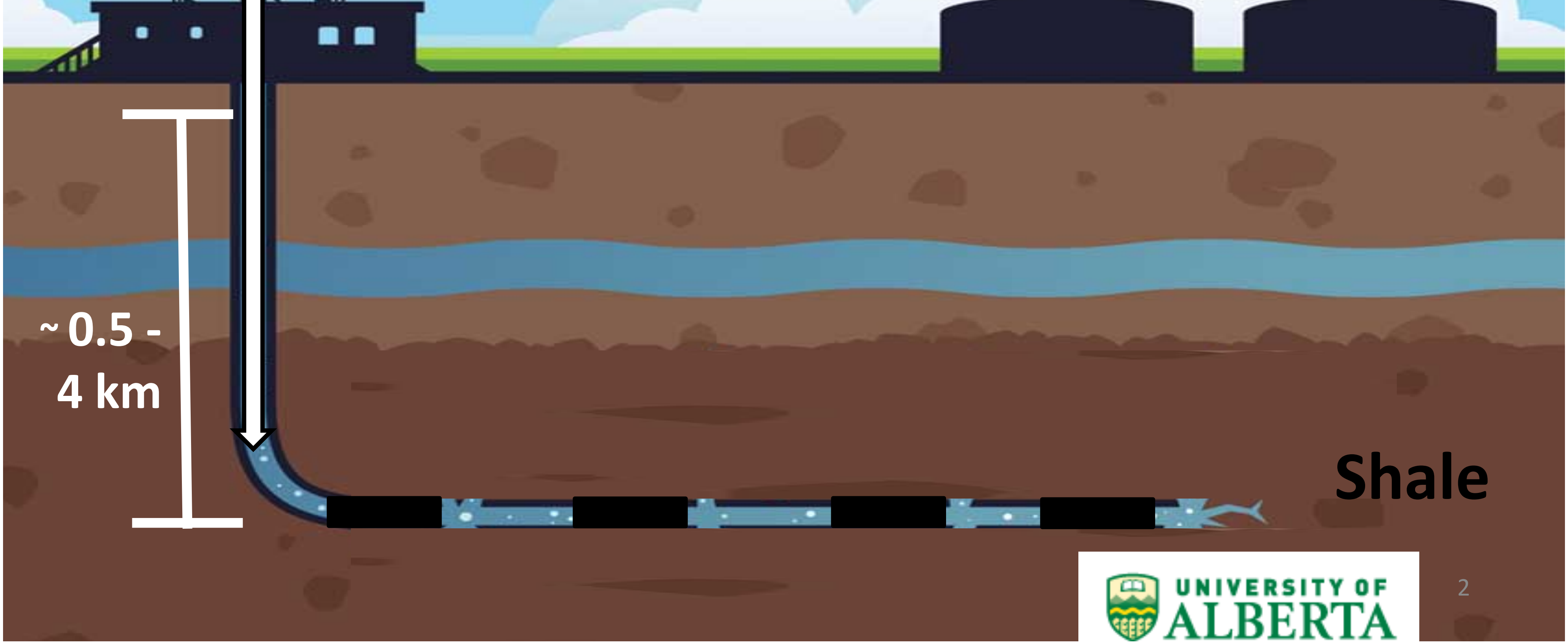
Hydraulic Fracturing Process

- Mixture of water, proppants and chemical additives are injected into the ground at high pressures (~70,000 kPa)
- Proppants prevent the fractures from closing
- Chemical additives include biocides and surfactants
- When well is opened, 10-70% of injected water returns to surface



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Flowback and Produced Waters

Distinction between terms Flowback and Produced Water (FPW) are operational only.

FPW varies by:

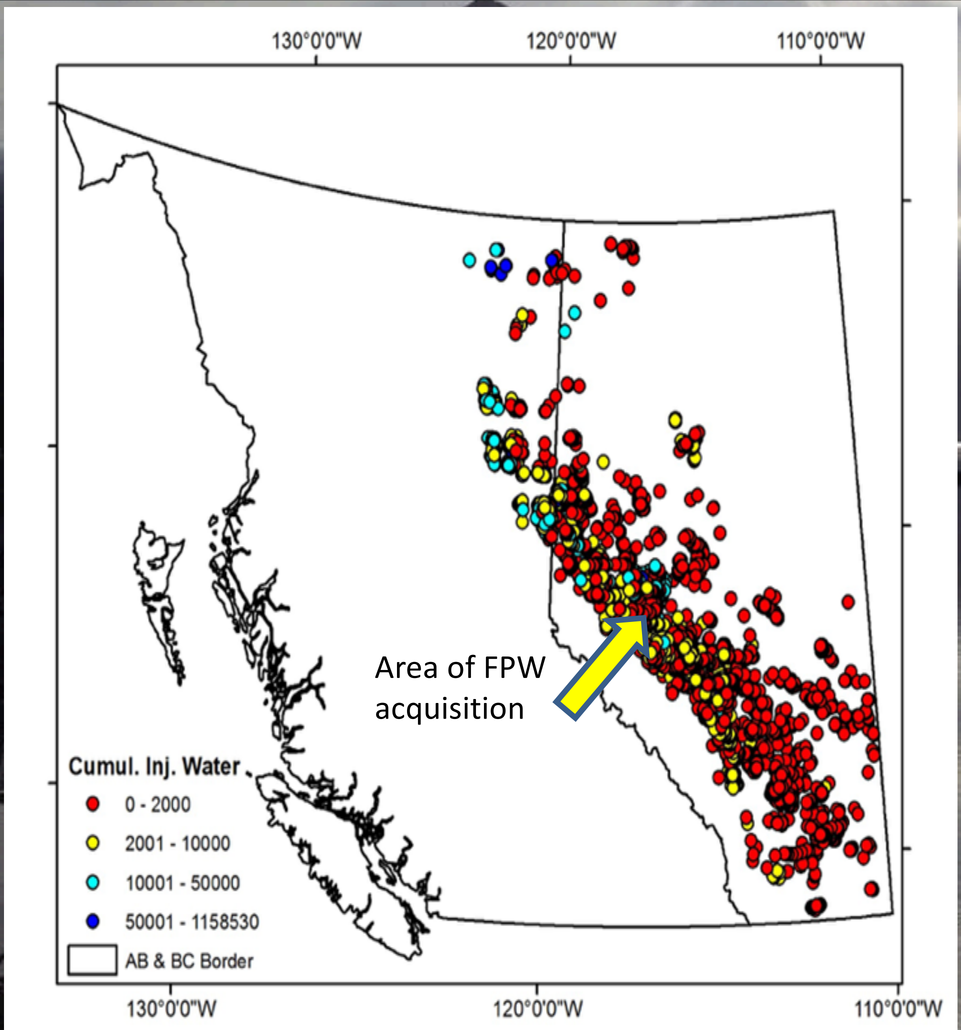
1. **Time of flowback**
   - **earlier has more anthropogenic derived chemicals**
   - **later have more formation water**
2. **Formation characteristics**
   - **inorganic and petrogenic components**
3. **Potential "downhole" reaction products**
   - **heat, chemicals and pressure**

Chemical compositions of injecting mixtures are commonly held as trade secrets by HF practitioners

Photo by Mark Crawford, ASME.org

Duvernay Region- Alberta-

- Anywhere between 10 -100 million litres of fracturing fluid and water pumped down into each well
- 10 – 80% of injected fluid comes back up as flowback/produced water



Distribution map for cumulative injected water (in m^3) per well, for wells fractured between November 2011 and March 2014

Canadian Water Network- Goss et al 2015, Alessi et al 2016 *Can Water Resour J*

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Numerous large and small FPW spills into freshwater lakes or wetlands have been documented

Zama City, Alberta, 2013, ~9.5 million litres of contaminated FPW

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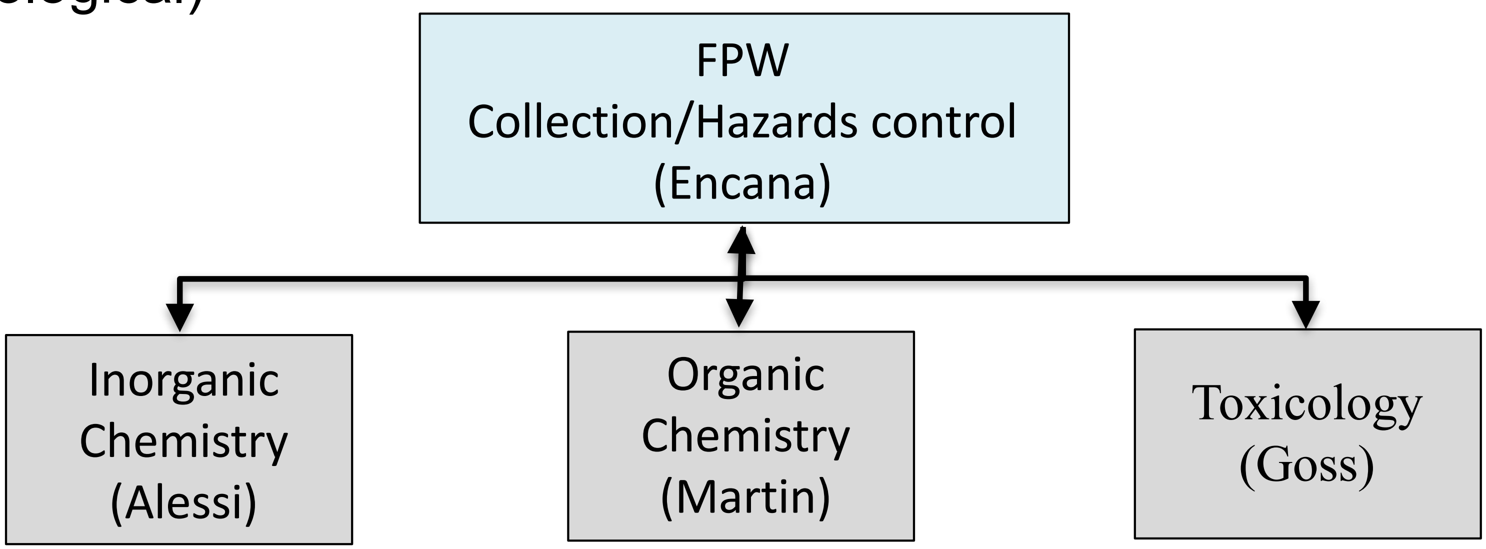
Natural Sciences and Engineering Research Council of Canada
Collaborative Research and Development Program

Three co-PIs: Daniel Alessi, Jon Martin and Greg Goss

Industrial partner: Encana Corp

Aims:

1. Comprehensive profiling of FPW chemistry and toxicology
2. Comprehensive characterizations and assessment of the geological formation and production of new chemical species
3. Provide feedback to Encana about toxicity of FPW (temporal and geological)



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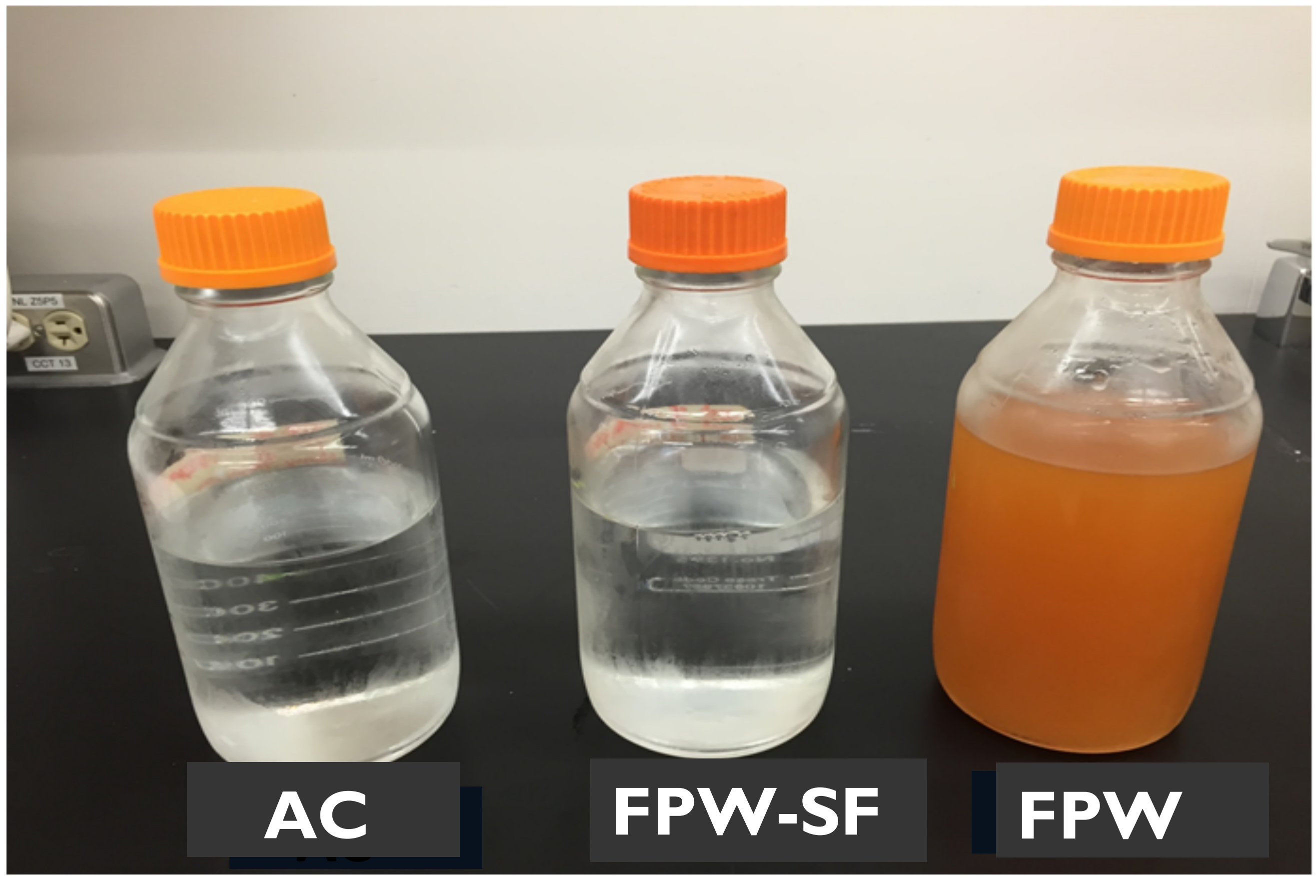
Flowback and Produced Water samples

Samples provided by Encana

AC- Activated Carbon treatment (removes organic and most metals)

SF- sediment free Filtered 0.2 µM

FPW - from Paskapoo formation (AB) **significant sediment**



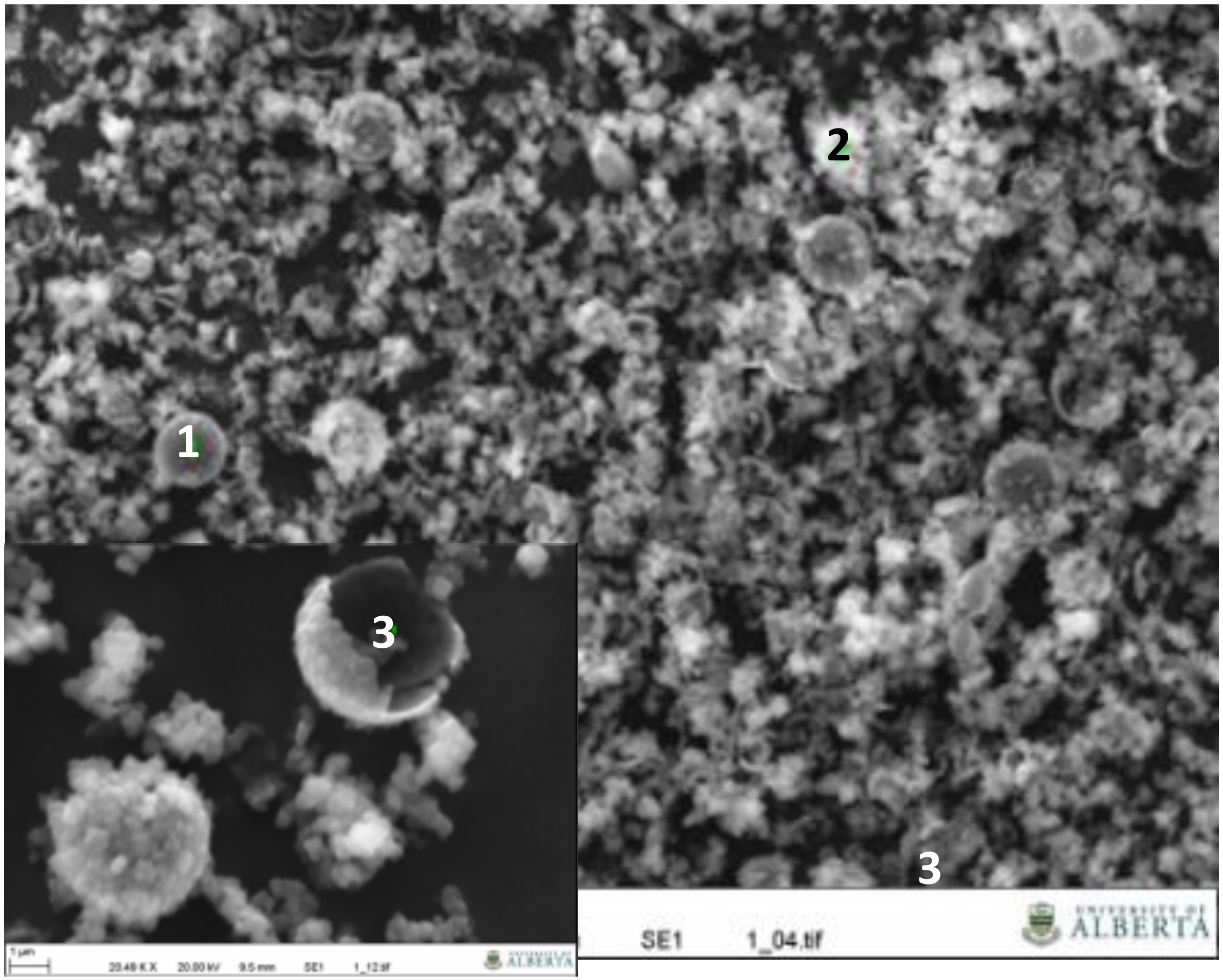
Chemical characterization of FPW-inorganics

- Inorganic parameters measured by multiple methods (Alessi Lab)

Note: salinity of 242,000 mg/L is ~7 X seawater (~33,000 mg/L)

7-day FPW Sample (pH = 4.78)		
Parameters	**Method**	**Mean Conc. (mg/L)**
TDS	**Evaporation**	**242,624**
TN	**TC/TN Analyzer**	**498**
TC	**TC/TN Analyzer**	**211**
Na	**ICP-MS/MS**	**70,000**
Ca	**ICP-MS/MS**	**11,800**
K	**ICP-MS/MS**	**2,570**
Cl	**IC**	**136,000**

Sediment Fraction in FPW contained hollow microspheres



SEM image of spherical structure (1) and presumed fragments (2) with EDX elemental analysis of exterior of sphere, respectively.

Exterior
Carbon 64%
Oxygen 33%
Iron 2%
Silica 1%
Calcium <1%.

Thought to contain a "breaker" such as ammonium persulfate (oxidant)
** disclaimer**

Close up SEM image of a broken sphere showing its hollow nature and thin shell walls (3) with EDX elemental analysis of the interior wall of a sphere.

Interior: Carbon 79.5%, Oxygen 19%, iron and silica <1%

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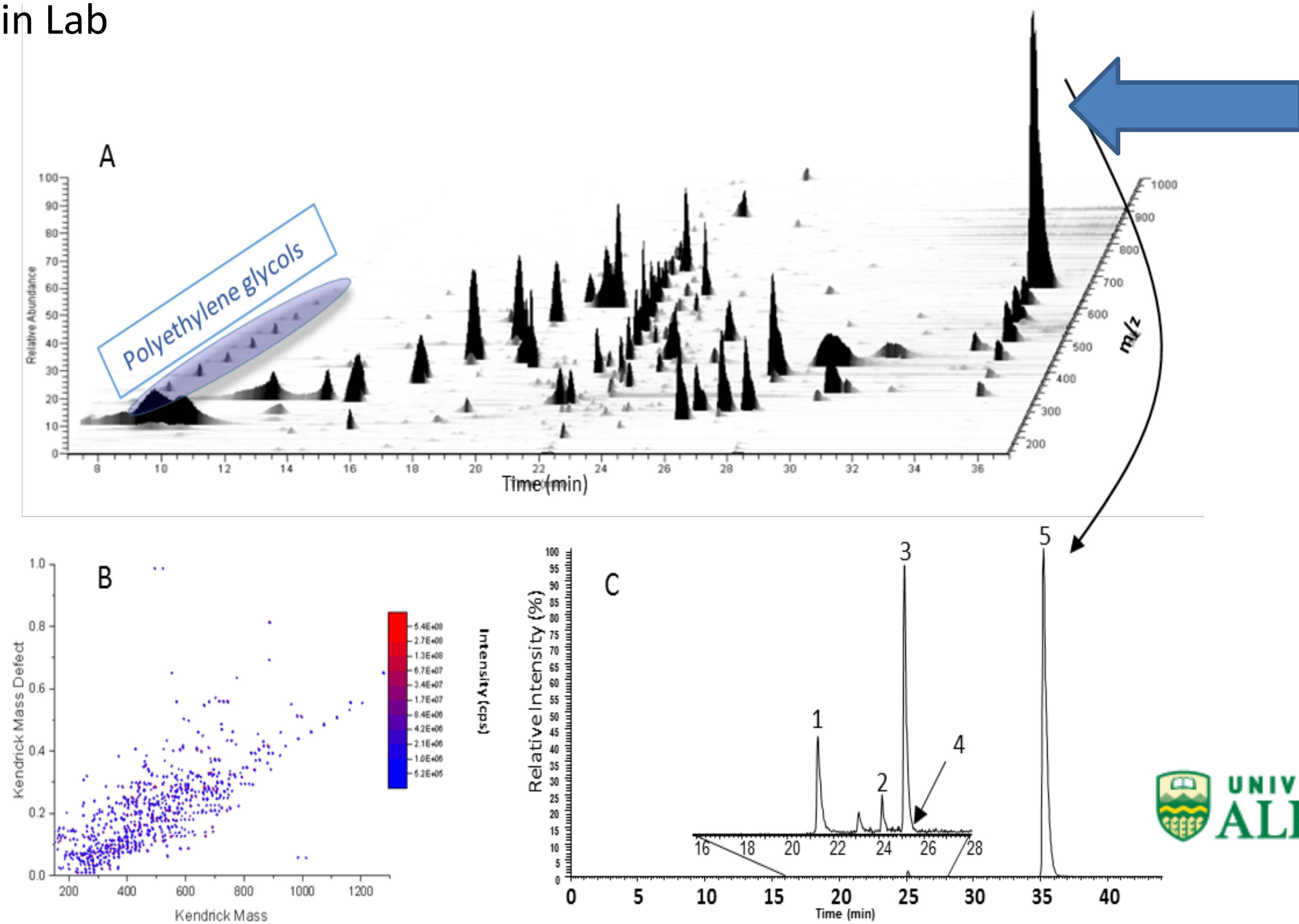
Organic Analysis (Untargeted - Orbitrap)

Martin Lab



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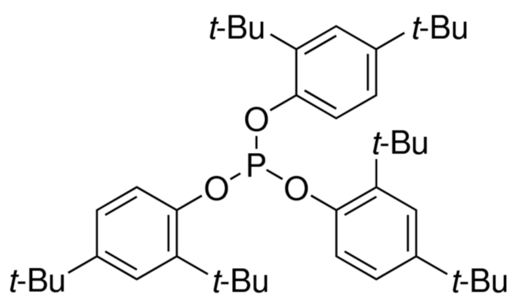
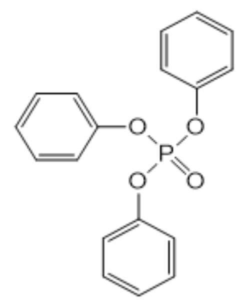
Possible formation of downhole reaction products

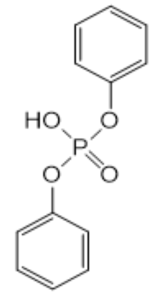
Downhole conditions

High heat (~100 ℃)
High pressure
Mix of chemicals, reactive metals





1



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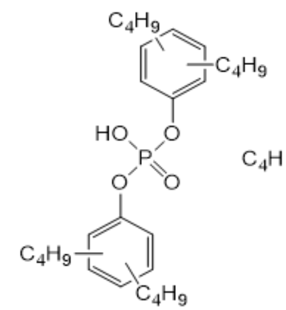

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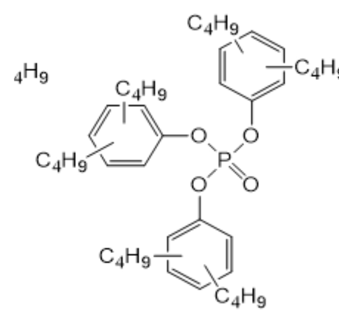

5

Irgaphos 168
Tris(2,4-di-*tert*-butylphenyl) phosphite-antioxidant chemical stabilizer

Note: we do not know if this was the chemical in the original HF fluids or if the phosphates were added directly.

Detected the above organo-phosphate chemicals in our FPW sample, (believe it is *via* oxidation of the parent phosphite compound)
NB: still needs investigation

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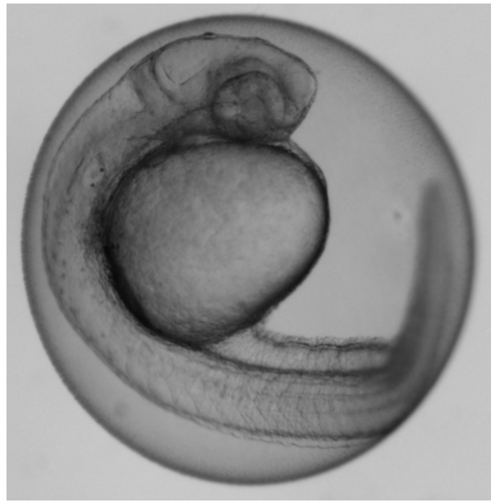
Toxicological assessment

Rainbow Trout
(*Oncorhynchus mykiss*)



Zebrafish embryo
(*Danio rerio*) model

Water flea
(*Daphnia magna*)

Common Ecotoxicological models (Environment Canada, OECD, US EPA)

- Provide information re. ecological hazard and risk assessment
- Zebrafish embryo exposure model used to assess possible early life-stage effects

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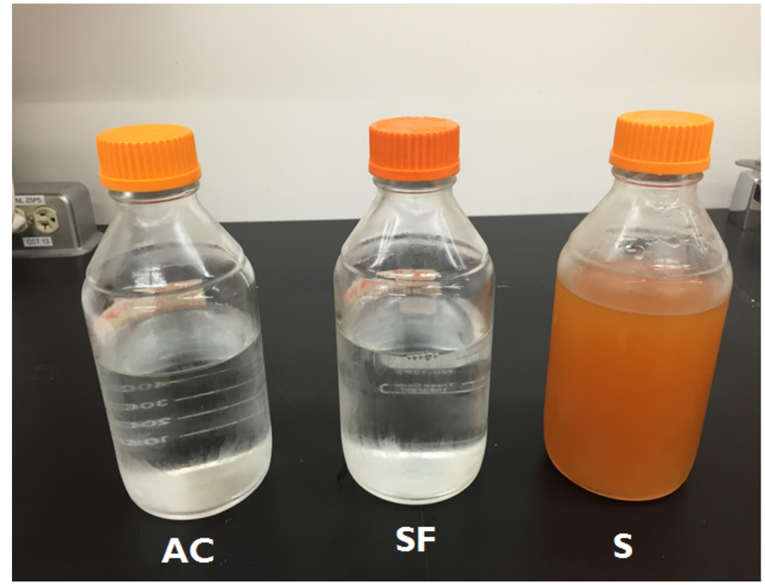
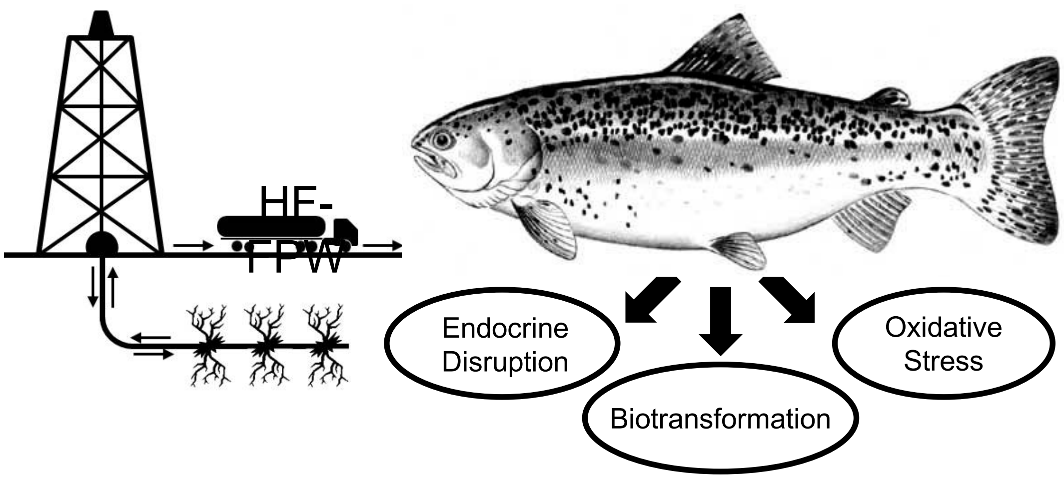
Methods

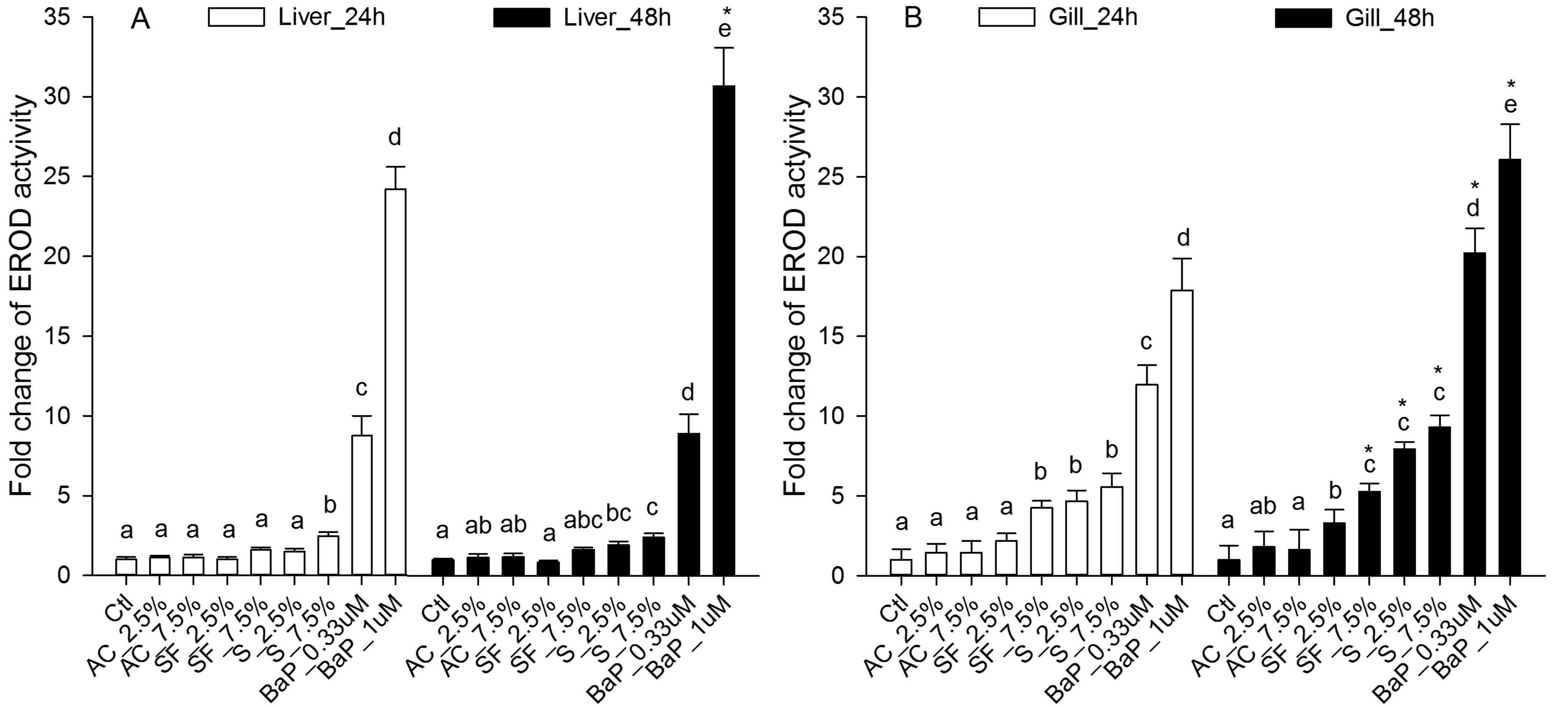




Rainbow trout fingerlings

- **24 or 48 hour acute exposure**
- **0 (control), 2.5% or 7.5% FPW**
- **tested both sediment containing and sediment free (filtered) FPW**

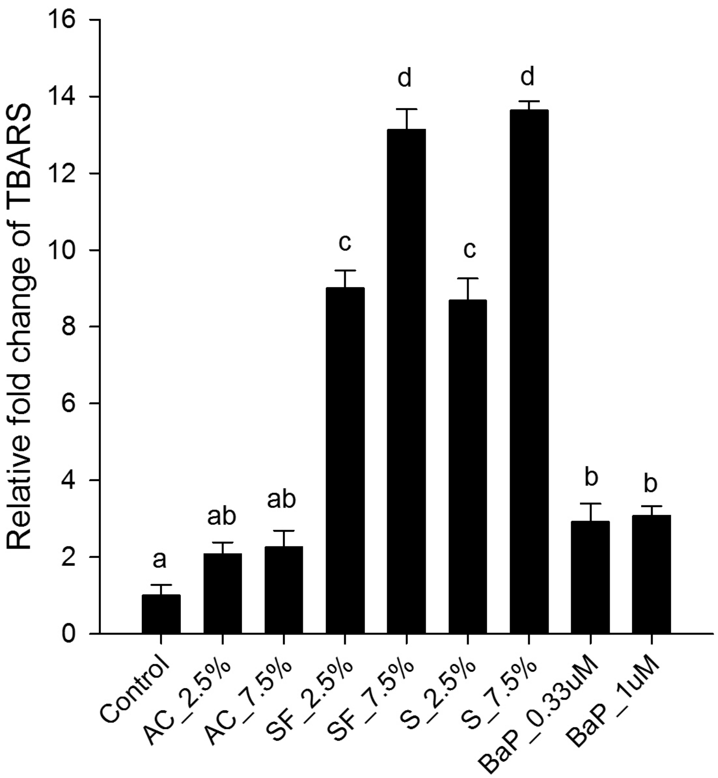
Biotransformation enzymes

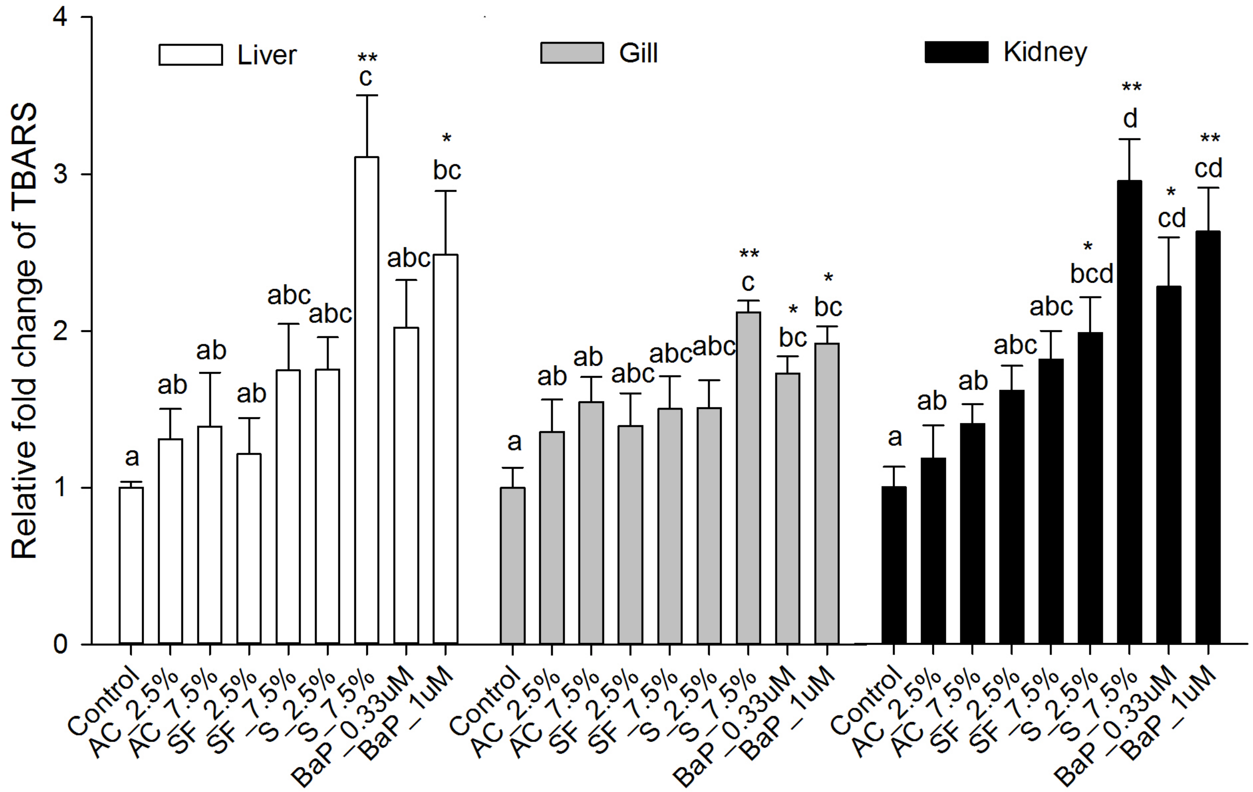


Ethoxyresorufin-o-deethylase (EROD) a phase 1 biotransformation enzyme used to eliminate planar hydrocarbons

- common marker of exposure to oil and gas contaminated waters
- BaP exposure (right bar) simply used to demonstrate our assay was working properly
- Induction in gill greater than in liver
- Induction in gills higher with sediment containing FPW compared to sediment free

Oxidative stress (TBARS formation)

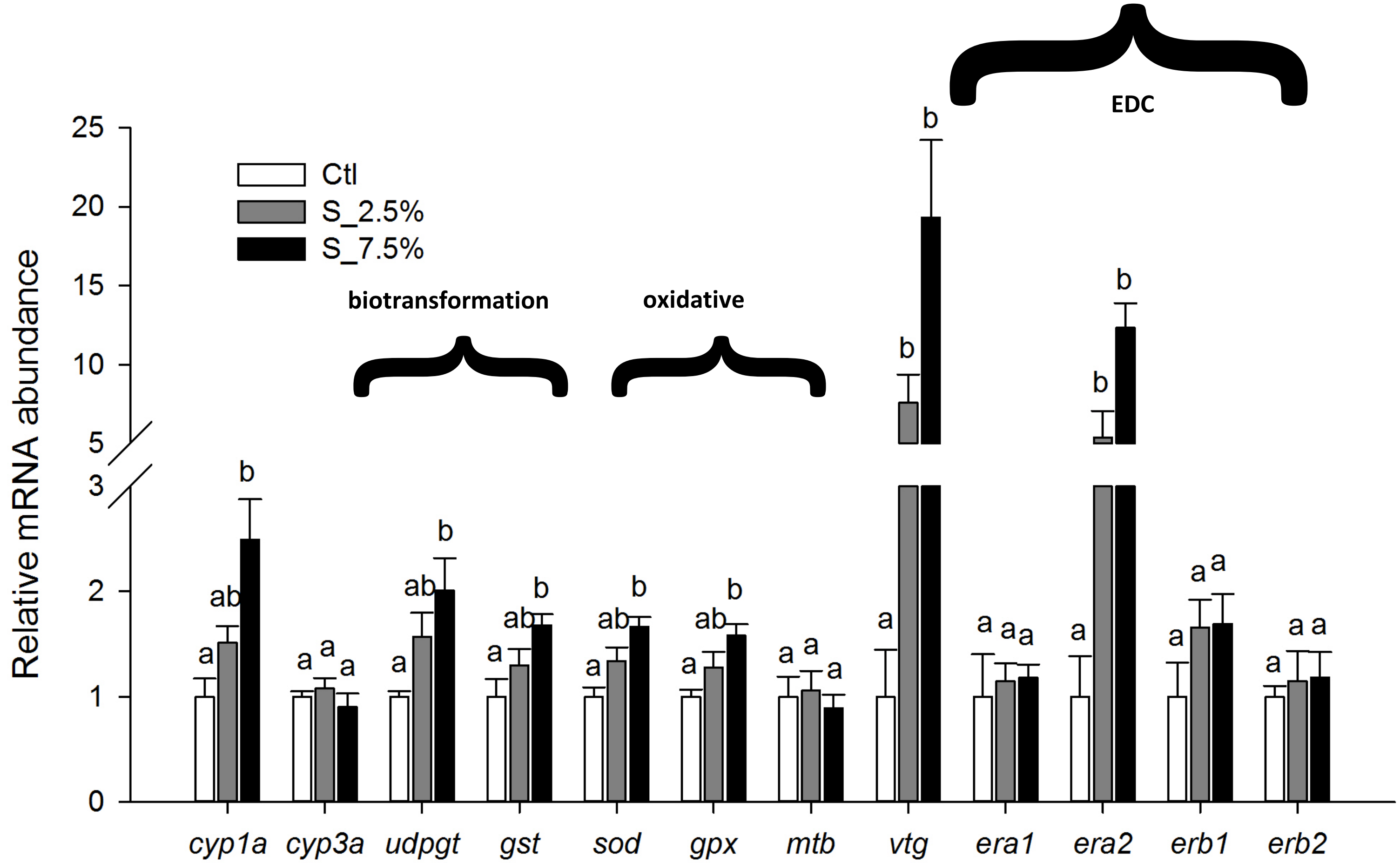




- The FPW alone can generate free oxygen radicals
- Greater at 7.5% compared to 2.5%
- No difference with or without sediment

- All tissues (liver, gill, kidney) showed evidence of exposure to greater free oxygen radicals (through TBARS formation in lipids)
- Greater response at 7.5% compared to 2.5%

Endocrine disruption

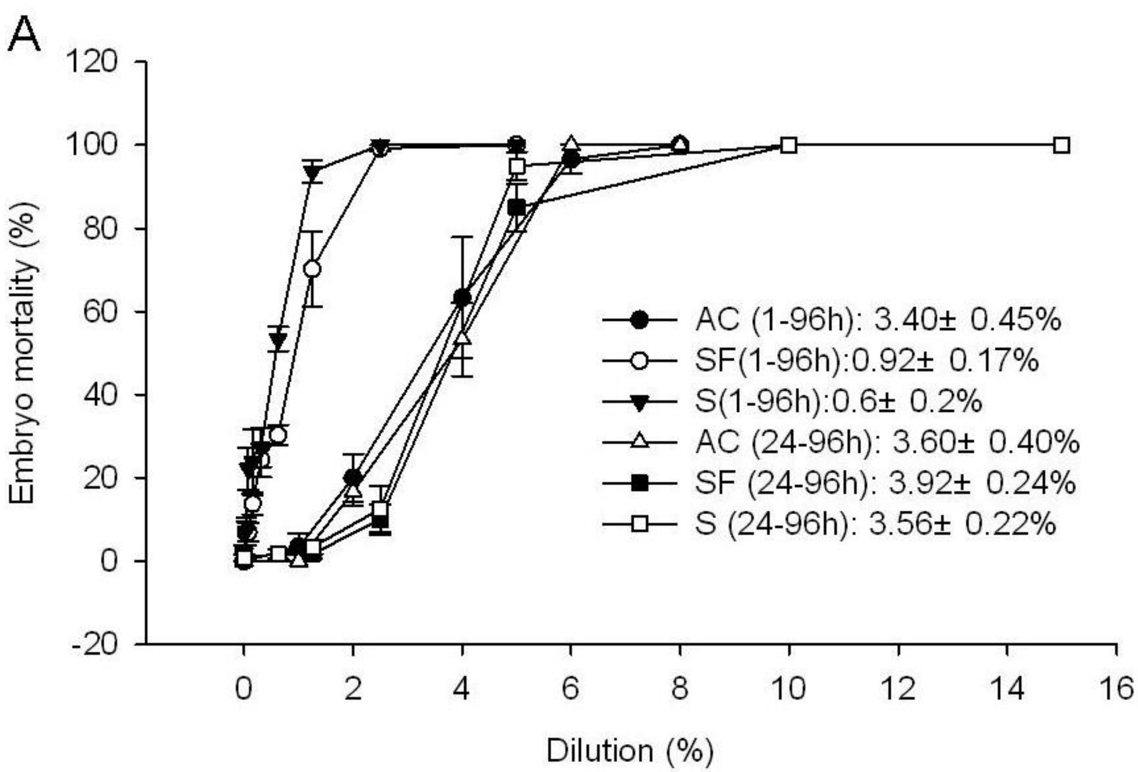


Gene responses in liver of rainbow trout exposed to sediment containing 2.5% and 7.5% FPW

Note large increased in expression of egg yolk protein (Vtg) and Estrogen receptor a2 (era2) but not estrogen receptors era), erb1 or erb2

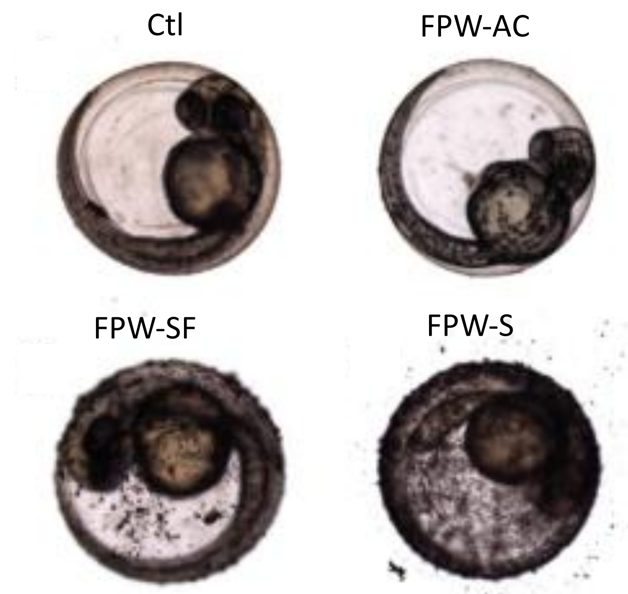
Acute Toxicity analysis - zebrafish embryo

- FPWs pose strong acute toxicity to ZF embryo
  - During 1-24h, lethality LC50 FPW 0.6%, SF 0.92% was driven by organic contents
  - After 24h, acute toxicity is dominated by salt
- Sediment containing had great toxicity compared to sediment free solutions
- Toxicological profile greatly complicated by salinity of FPW



FPW-SF **FPW-S** **FPW-AC**

ZF embryo after 24h exposure (2.5%)



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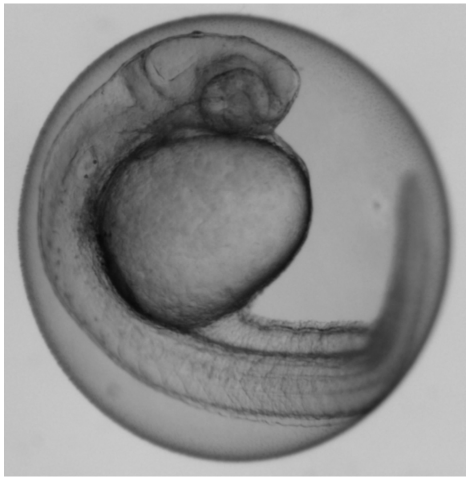


Zebrafish embryo (*Danio rerio*) model

Objectives

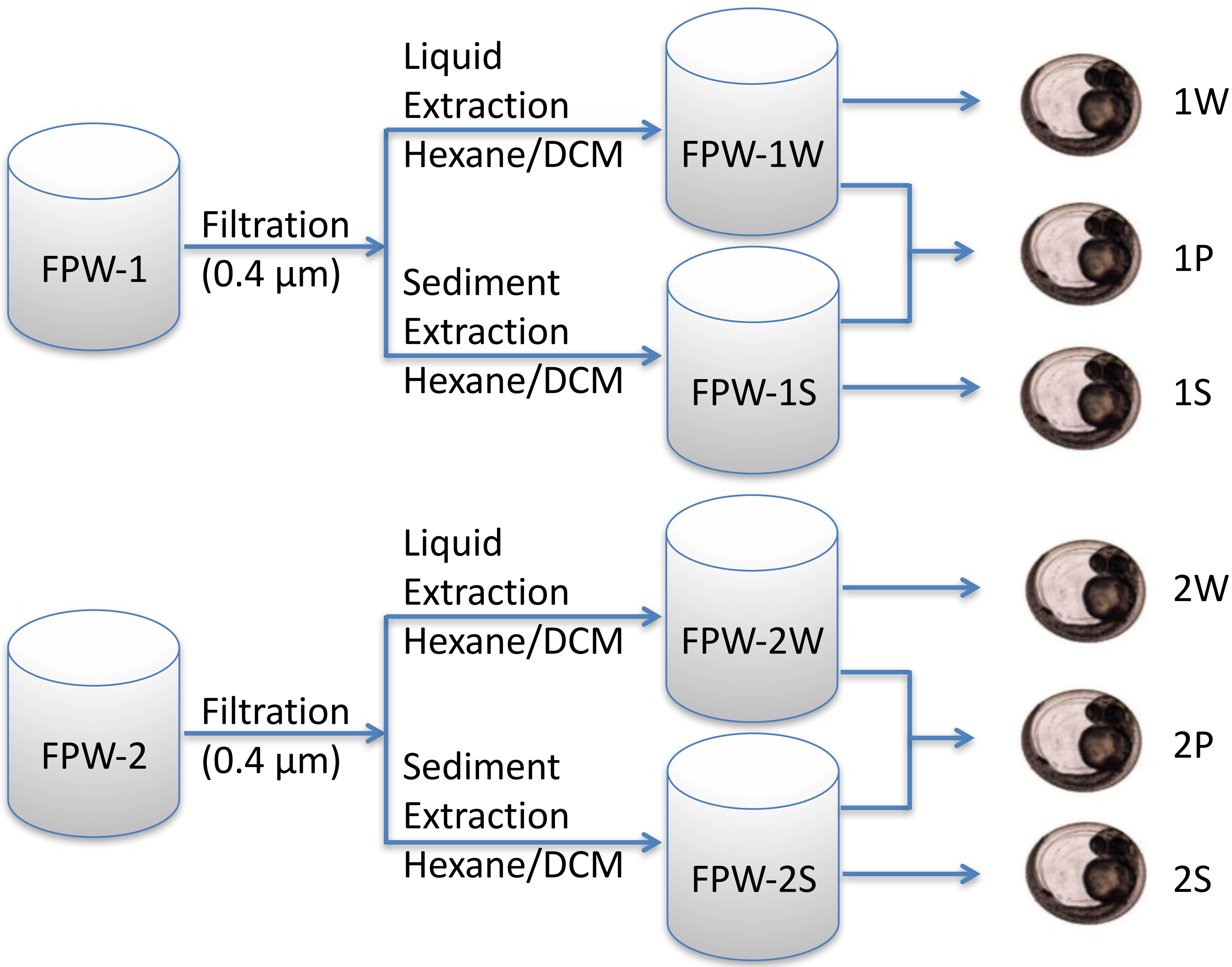
- Compare new samples of FPW received from two different well for toxicological profile and underlying possible effects

- Assess toxicological profile without salinity induced effects
  - further examine the differences between sediment-containing and sediment-free FPW

Solution - separate and extract organics from each sample

Caveat: we recognize that filtration of FPW under vacuum likely removes significant amounts of lower molecular weight volatile organics

Experimental design:
Extraction of Organic Fractions of HF-FPW

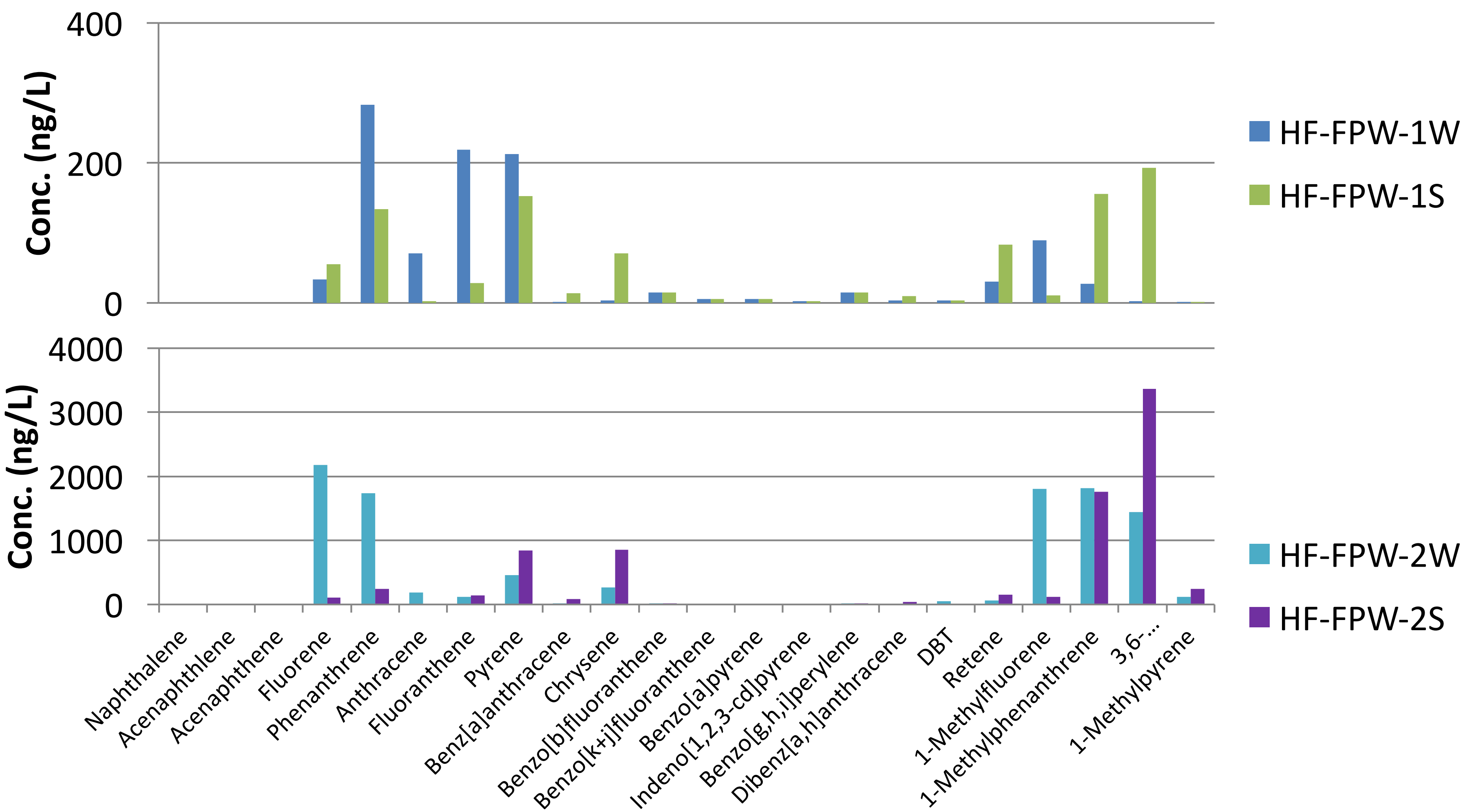


W – Water fraction

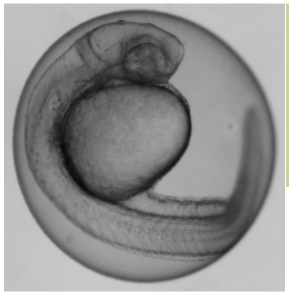
S – Sediment fraction

P – Pooled fraction

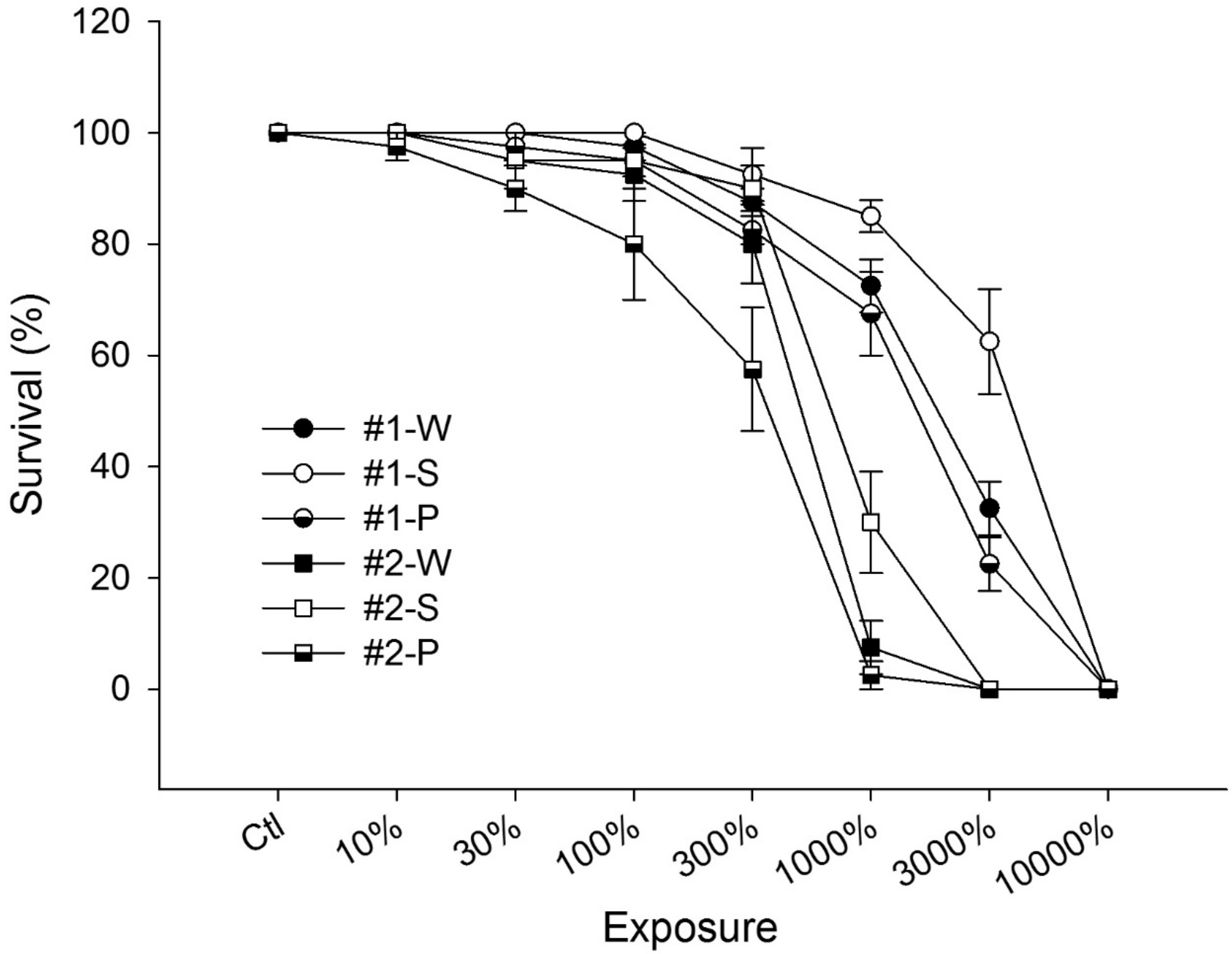
Results: Total PAHs in Organic Fractions (ng/L)



(ng/L)	W	S	Pool
FPW-1	1024.7	959.6	1984.3
FPW-2	10290.6	7986.3	18276.9



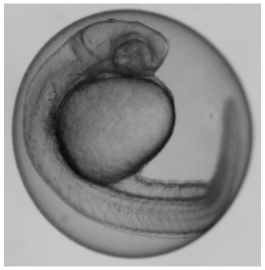
Acute Toxicity to ZF embryo (168 hpf)



General Toxicity

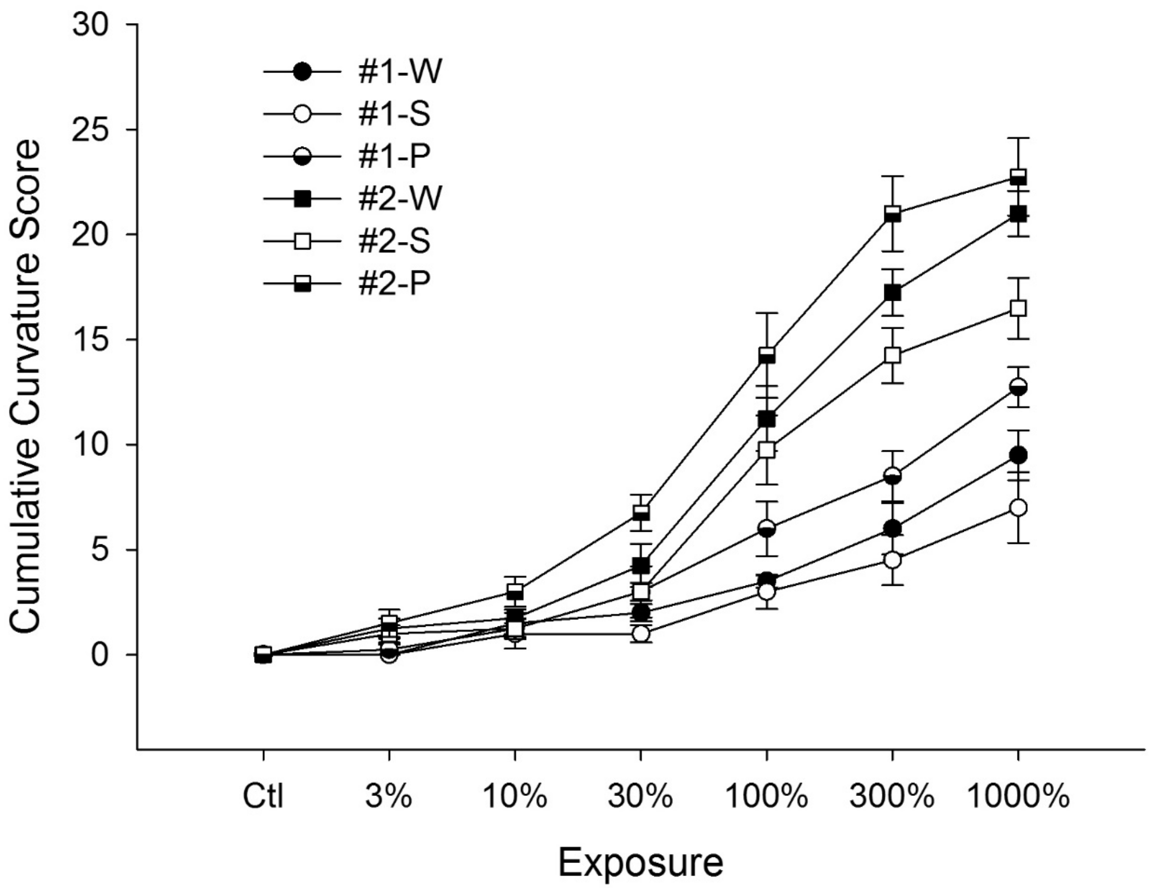
- #2 FPW > #1 FPW
- P > W > S

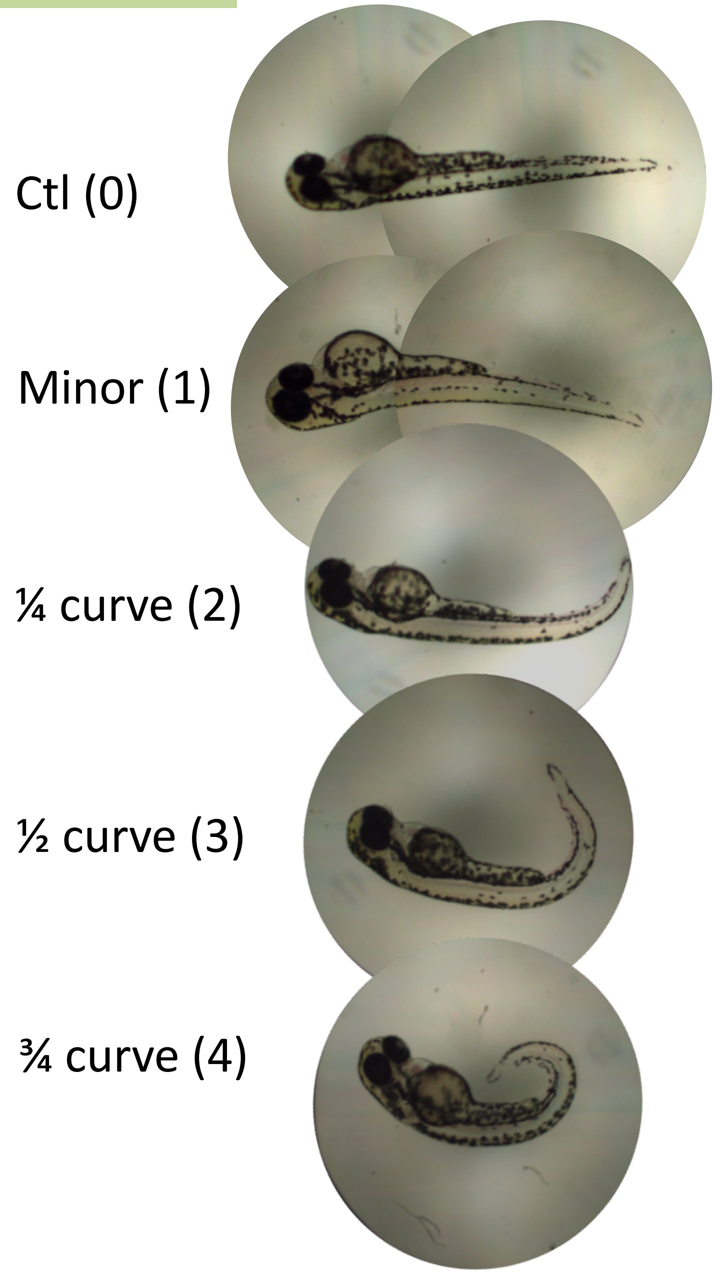
	LC50 (%)
1S	2630%
1W	1450%
1P	1200%
2S	720%
2W	480%
2P	280%



Spinal Malformation (96 hpf)

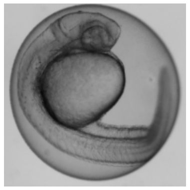
- 2P > 2W > 2S > 1P > 1W > 1S



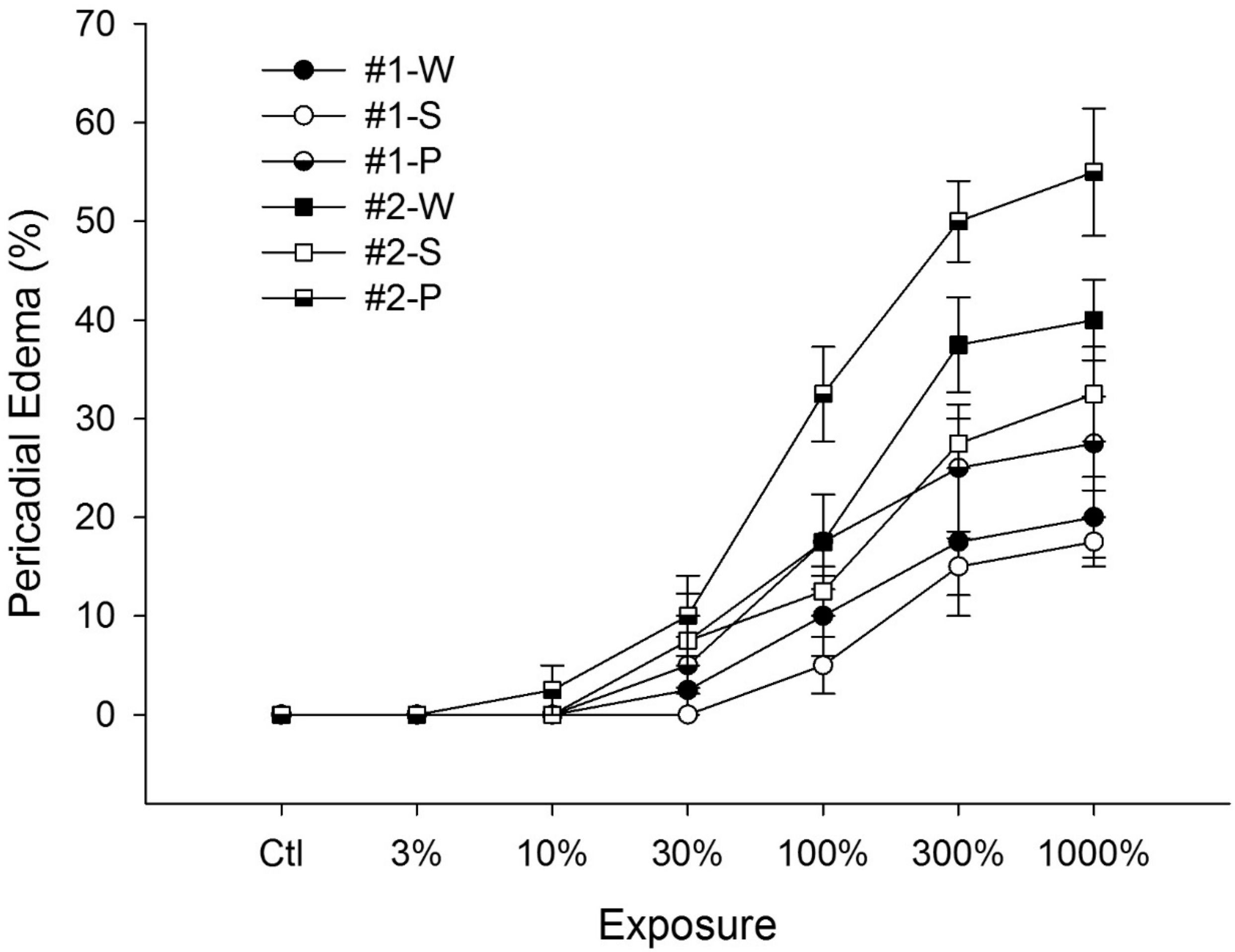


eg. Two ½ curve & Three ¾ curve in the group

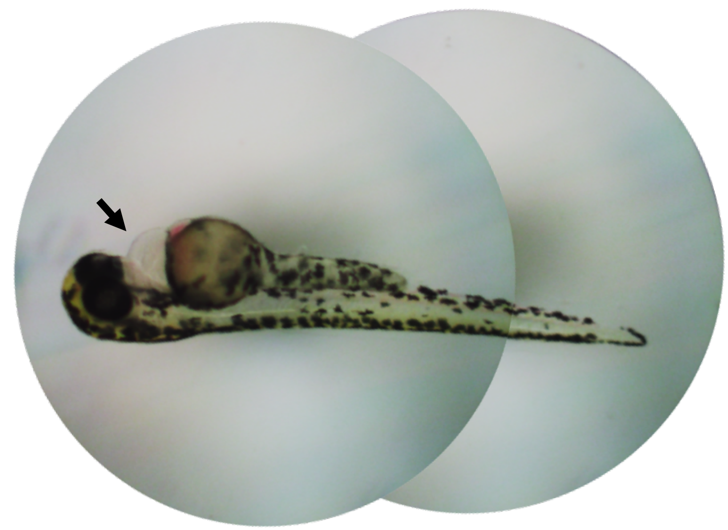
CCS = 2 x 3 + 3 x 4 = 18



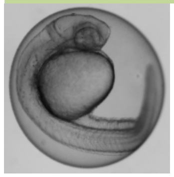
Pericardial Edema (96 hpf)

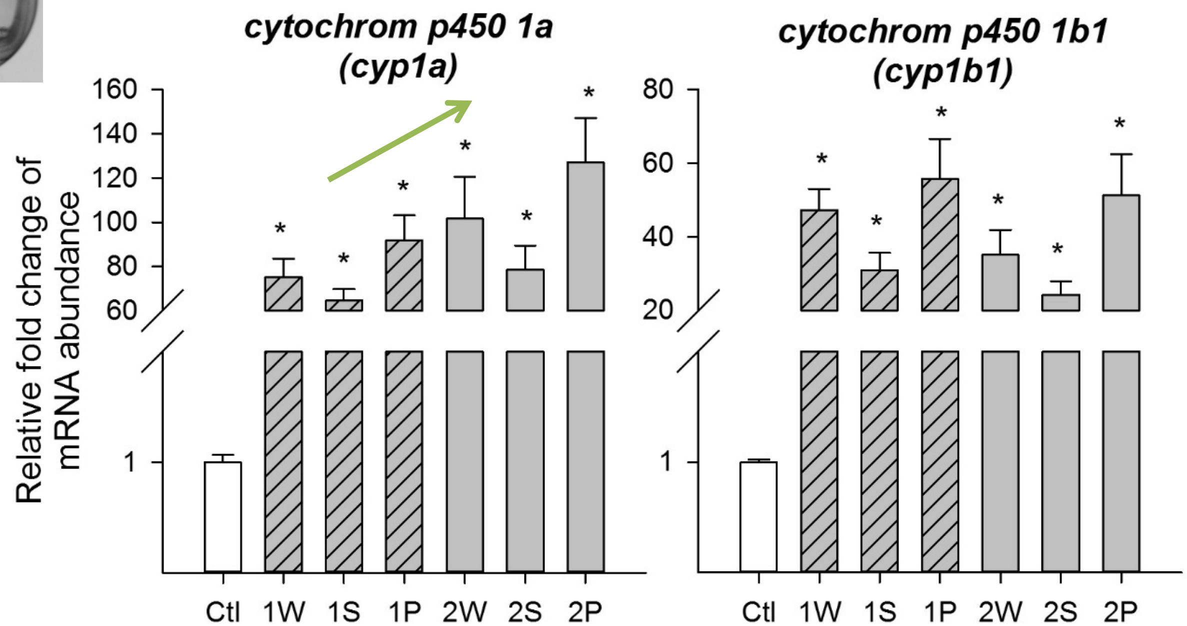


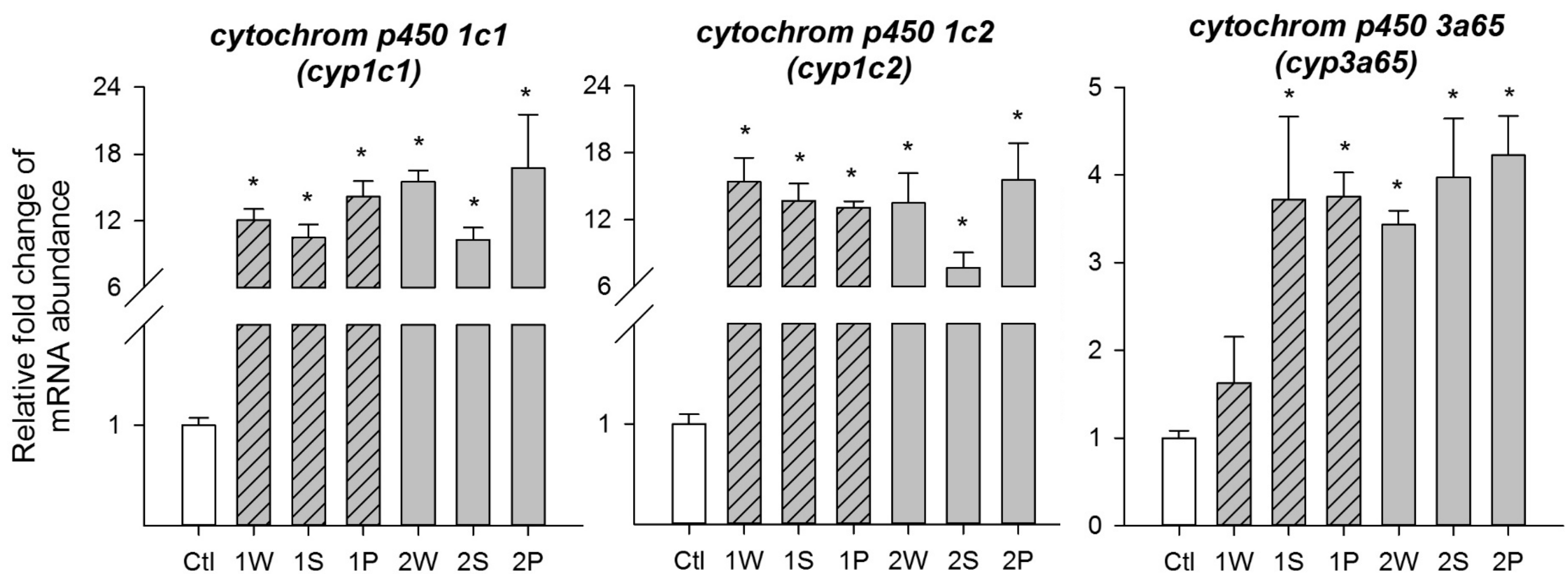
- 2P > 2W ≥ 2S
- 1P > 1W ≥ 1S
- #2 > #1



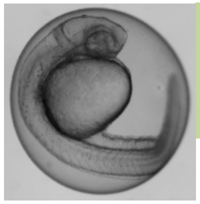
Transcriptional Response – Cytochrome p450s



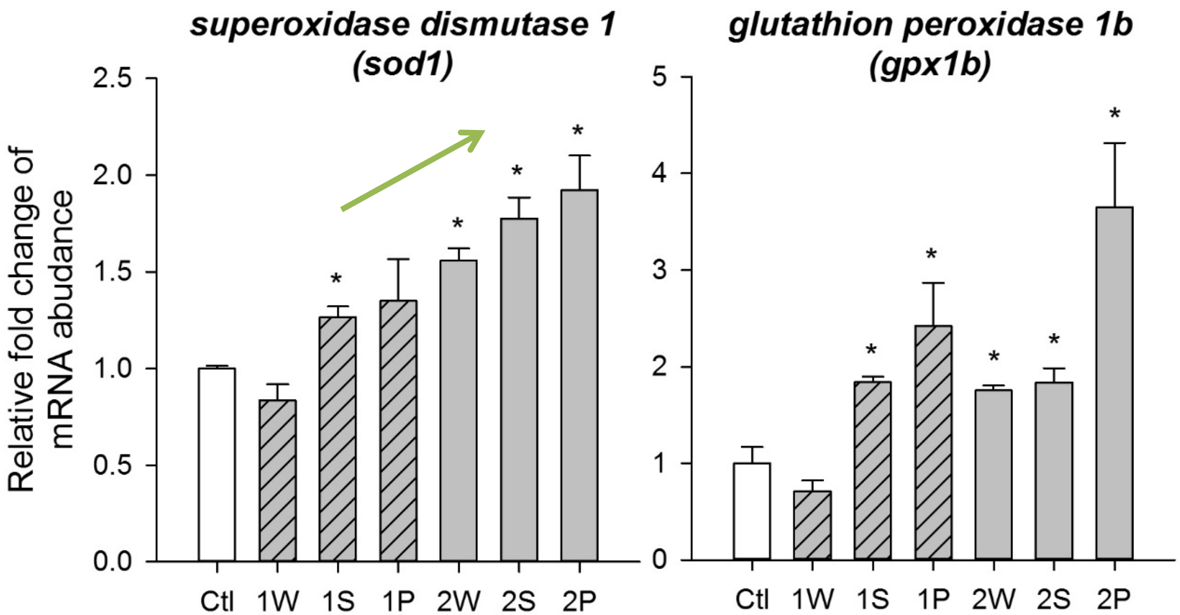




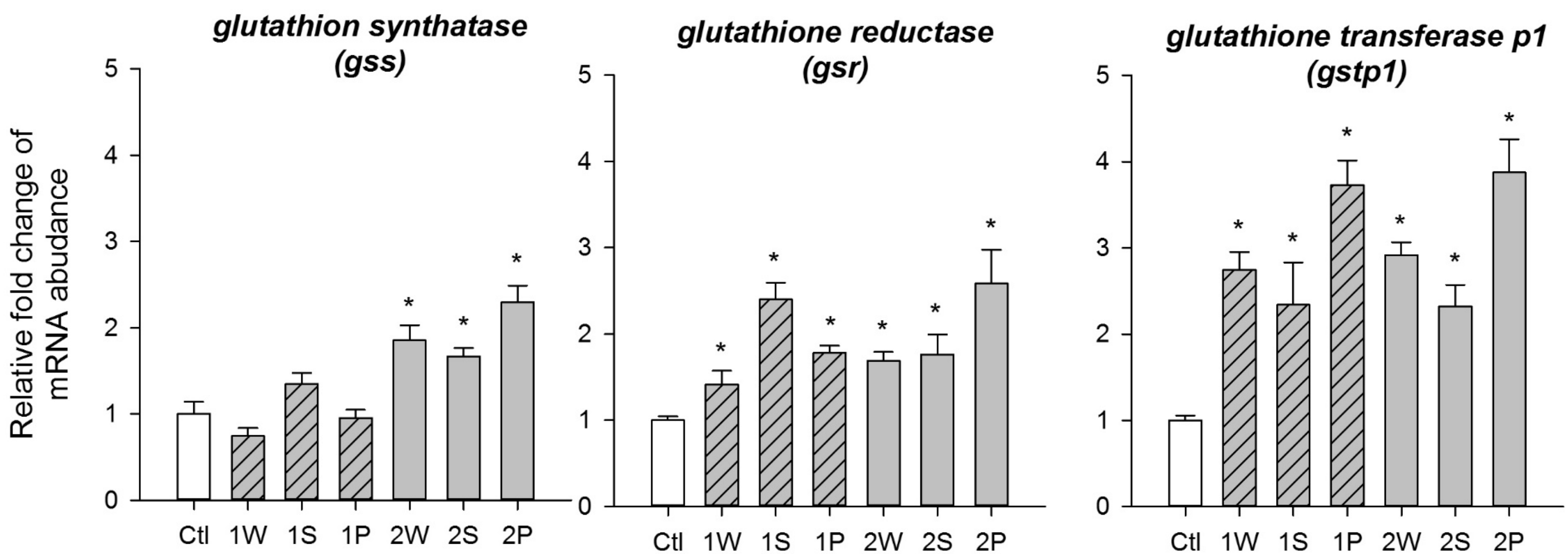
- *cyp1a > cyp1b1 > cyp1c1 ≈ cyp1c2 > cyp3a65*
- AhR may be the predominant pathway, but PXR is also a possible pathway

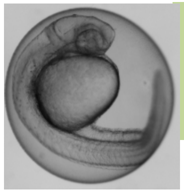


Transcriptional Response - Oxidative stress

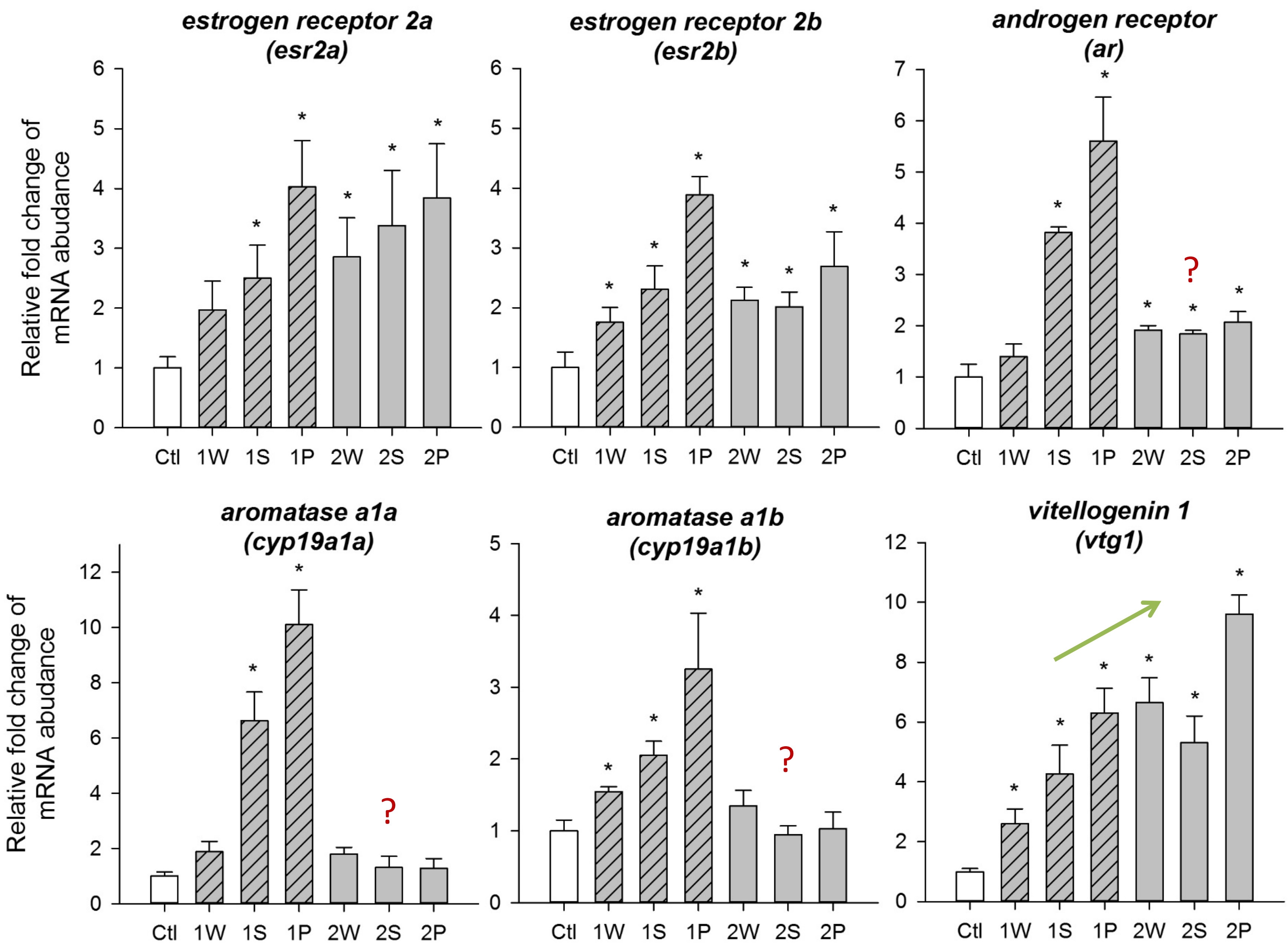


- Induction of oxidative stress responsive gene is consistent with the morphological observation of pericardial edema





Transcriptional Response – Endocrine disruption



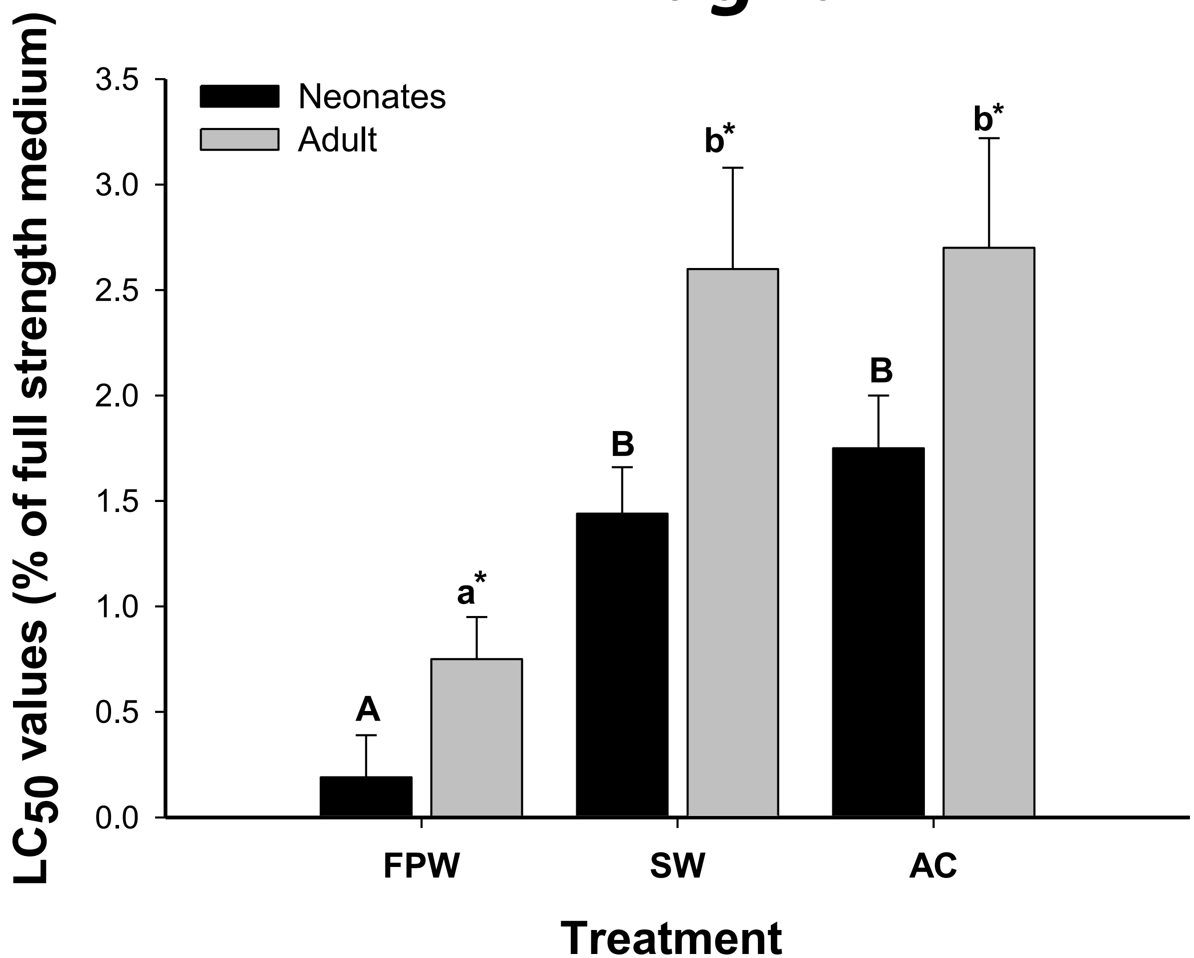
Daphnia magna (water flea)

Acute LC_{50} Results *D. magna*

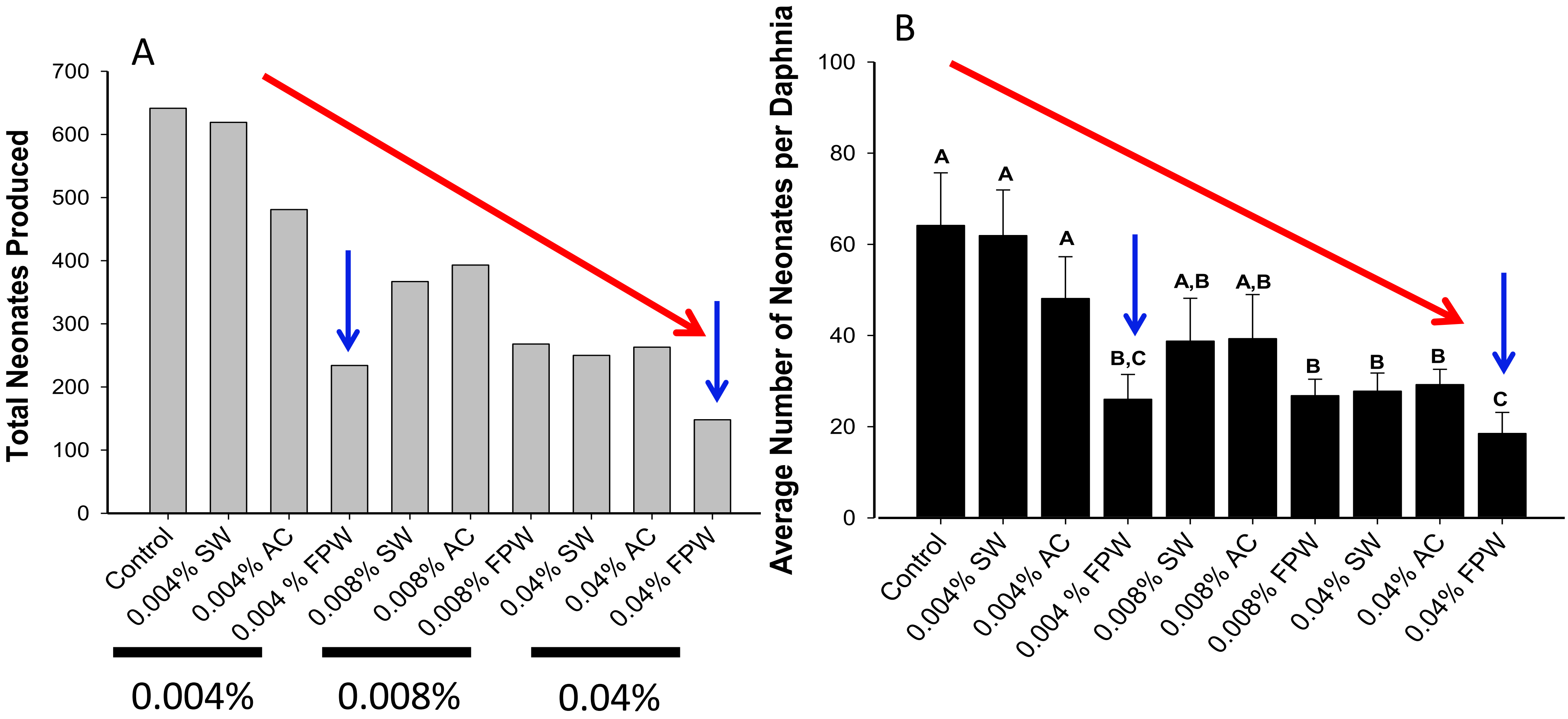
Daphnia are a highly sensitive FW species to FPW LC_{50} of FPW of <1%

Neonates more sensitive that adults

Paired salt water controls (SW and AC) still have significant toxicity.

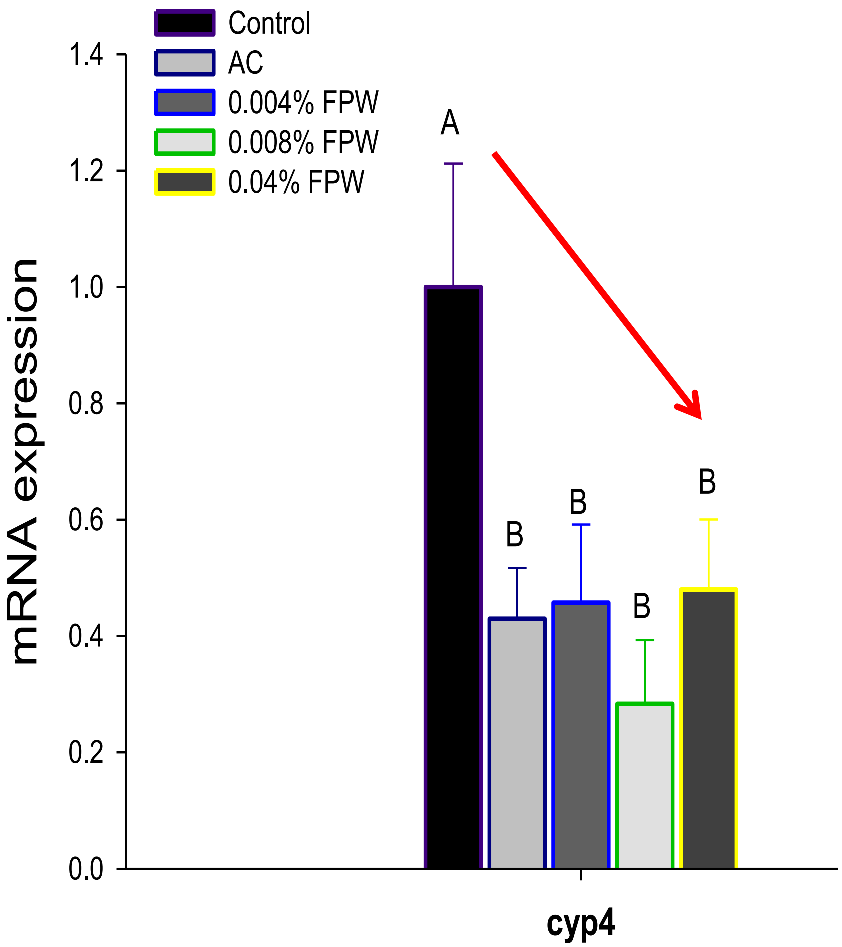


Effects on *Daphnia magna* reproduction

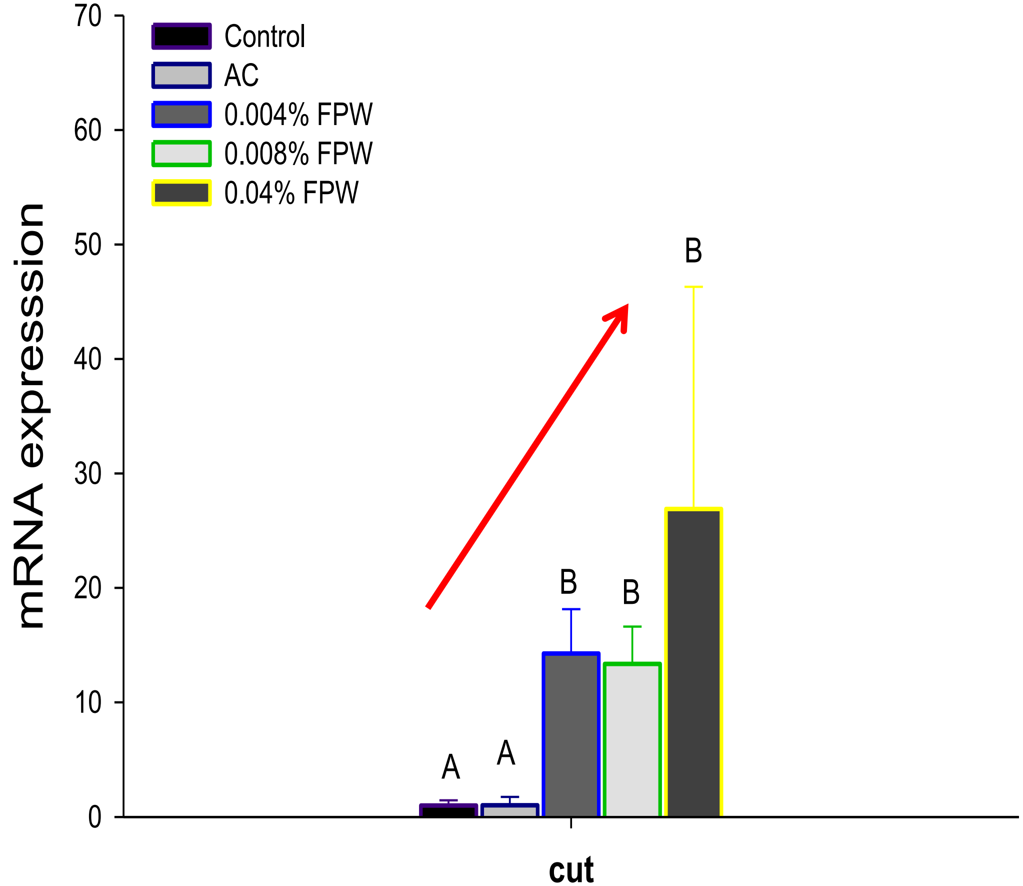


Chronic 30-day daphnia reproduction tests showed salinity alone affected reproduction. However, FPW further decreased reproduction, even at 0.004%

Effects of FPW on *Daphnia* gene expression



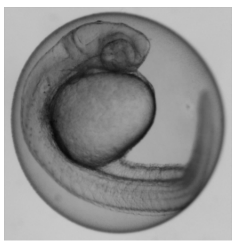
Cytochrome P450 4



Cuticle 12

Lack of Cyp4 induction suggests minimal planar hydrocarbon exposure
Increased expression of Cut gene – involved in molting and reproduction

Summary



Our early stage results suggest significant and multiple effects of FPW on three model aquatic species.

- We demonstrate *apparent* transformation products in FPW.
- We show that the sediment fraction of these FPW samples has greater toxicity than the water-accomodated fraction.

HF-FPW exposure effects may include:

induction of biotransformation enzymes
oxidation of lipids and metabolic impairment
endocrine disruption

We cannot yet attribute any of these effects to a specific HF component.

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- Alyssa Weinrauch
- Matt Barker
- Joy Li
- The rest of the Goss lab

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Research Papers

- ***He Y***, ***Folkerts EJ***, Zhang Y**,** Martin JW, Alessi DS, **Goss GG.** (2017). Effects on Biotransformation, Oxidative Stress, and Endocrine Disruption in Rainbow Trout (Oncorhynchus mykiss) Exposed to Hydraulic Fracturing Flowback and Produced Water. Submitted to *Environ Sci Technol* 51(2): 940-947 (doi: 10.1021/acs.est.6b04695).

- ***Blewett T***, ***Delompre PLM***, ***He Y***, ***Folkerts EJ***, Flynn SL, Alessi DS, **Goss GG**. (2017). The sub-lethal and reproductive effects of acute and chronic exposure to flowback and produced water from hydraulic fracturing on the water flea (Daphnia magna). *Environ Sci Technol* (doi: 10.1021/acs.est.6b05179).

- ***He Y***, Flynn SL, ***Folkerts EJ***, Zhang Y, Ruan D, Alessi DS, Martin JW, **Goss GG**. (2017). Chemical and toxicological characterizations of hydraulic fracturing flowback and produced water. *Water Res* 114, 78-87 (doi: 10.1016/j.watres.2017.02.027).

- ***Blewett TA, Weinrauch AM, Delompré PLM,*** Alessi DS, Zhang Y, Martin J, Goss GG (2017) The effect of hydraulic flowback and produced waters on gill morphology and oxidative stress response in the rainbow trout (*Oncorhynchus mykiss*). *Scientific Reports* 7:46582.

- Alessi DS, ***Zolfaghari*** A, Kletke S, Gehman J, Allen DM, **Goss GG.** (2017). Comparative analysis of hydraulic fracturing wastewater practices in unconventional shale development: Water sourcing, treatment, and disposal practices. *Can Water Resour J* (doi: 10.1080/07011784.2016.1238782).

- Gehman J, Thompson Y, Alessi DS, Allen DM, **Goss GG**. (2016). Comparative analysis of hydraulic fracturing wastewater practices in unconventional shale development: Newspaper coverage of stakeholder concerns and social license to operate. *Sustainability*, 8: 912 (doi:10.3390/su8090912).

- Notte C, Allen DM, Gehman J, Alessi DS, **Goss GG**. (2016). Comparative analysis of hydraulic fracturing wastewater practices in unconventional shale development: Regulatory regimes. *Can Water Resour* 1-16 (doi: 10.1080/07011784.2016.1218795).

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