Whole-transcriptome sequencing of epidermal mucus as a novel method for oil exposure assessment in juvenile mahi-mahi

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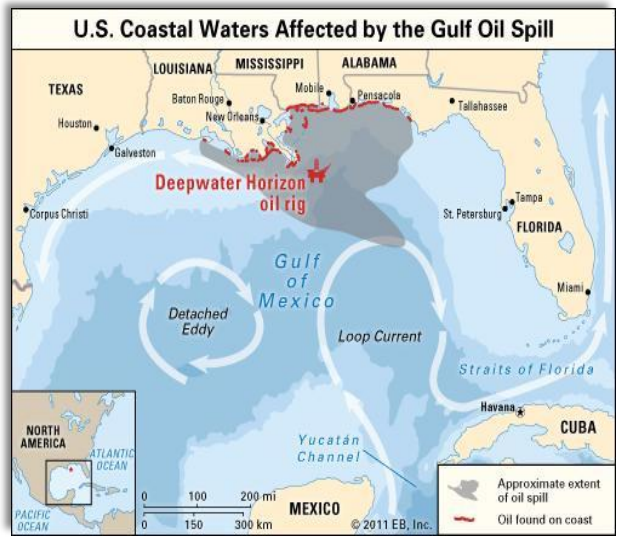
BACKGROUND

- The *Deepwater Horizon* disaster resulted in the release of over 600 million L of oil into the Gulf of Mexico between April 10 and July 14, 2010 (Crone & Tolstoy, 2010)

- Spill led to extensive oiling of the pelagic zone and fouling of shoreline habitats

- Oil spill coincided with the spawning times of many commercially important pelagic fish species, such as mahi-mahi (Rooker et al., 2012 & 2013)



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CARDIOTOXICITY OF OIL DURING EMBROYNIC DEVELOPMENT

- Cardiotoxic phenotypes include:
  - pericardial edema
  - bradycardia and arrhythmias
  - contractility defects

  (Jung et al., 2013; Incardona et al., 2013; Incardona et al., 2014)

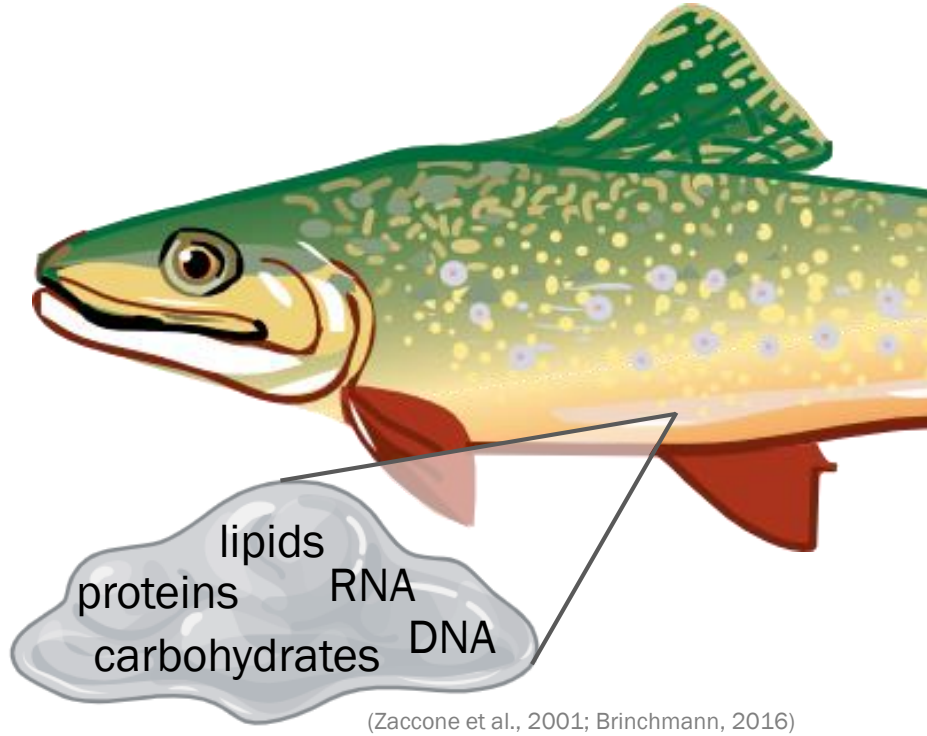
- Disruption of Ca^{2+} and K^{+} currents in cardiomyocytes is a major mechanism contributing to cardiotoxicity

  (Brette et al., 2014)

- Molecular Initiating Events include:
  - Modulation of genes involved in cardiac muscle function and Ca^{2+} homeostasis (*casq2*, *ryr2*, *camk2g,* etc.)
  - Upregulation of aryl hydrocarbon receptor (AhR) mediated genes (*cyp1a1*, *cyp1b1*, *ugt1a1*, *ahrr,* etc.)

    (Xu et al., 2016)`

MUCUS – THE FIRST LINE OF DEFENCE & POTENTIAL FOR BIOMARKERS



(Zaccone et al., 2001; Brinchmann, 2016)

- Roles of epidermal mucus in fish include:
  - physical/biochemical barrier against environmental toxins and pathogens
  - osmoregulation
  - excretion
  - many others (Shepard, 1994; Reverter et al., 2018)

- Mucus composition is altered by a variety of stress conditions:
  - Vitellogenin protein used as a biomarker of exposure to endocrine disrupting compounds (Moncaut et al., 2003; Meucci and Aruwke, 2005; Van Veld et al., 2005)
  - Increased activity of oxidative stress enzymes following crude oil exposure (e.g. SOD, catalase, etc.) (Dzul-Caamal et al., 2016)
  - Immune-related gene expression altered following bacterial challenge (Liu et al, 2013; Ren et al., 2015)

Dynamic regulation of molecules in mucus following stress presents opportunity for development of noninvasive biomarkers of exposure

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Study Objective

Use RNA sequencing to examine the efficacy of mRNA-based analysis of mucus as a noninvasive method for oil-exposure detection in fish.

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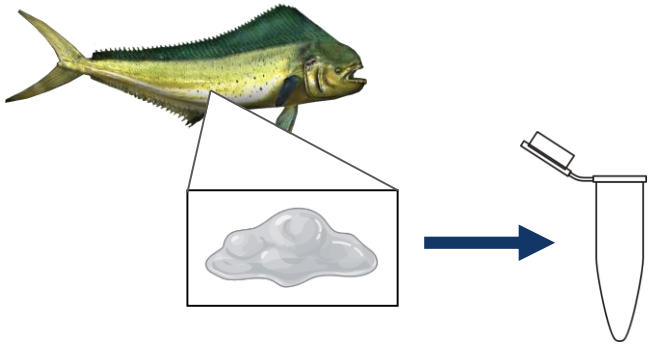
METHODS – EXPERIMENTAL DESIGN

1. Juvenile mahi-mahi exposed to high-energy water-accommodated fractions (HEWAF) of *Deepwater Horizon* slick oil for 48 hours:

Clean Seawater **Control**	5% HEWAF **Low Oil**	10% HEWAF
4 4	4 4	4 4
4 4	4 4	4 4

4 replicates per treatment, with 4 individuals per replicate

2. Mucus collection from skin



Mucus pooled from each individual per replicate, for a total of 4 samples per treatment

Juvenile mahi-mahi

Mucus collection technique on lake trout

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Sequencing Methods

3 Sample Preparation

12 mucus samples
(n = 4 per treatment)

↓

RNA Isolation from mucus

↓

cDNA Library Construction

→

4 Sequencing

1 x 75 bp sequencing
Illumina NextSeq500
at the UCR-IIGB

mRNA read data →

5 Bioinformatics Analysis

SortMeRNA: remove ribosomal RNA

↓

Trimmomatic: trim adaptor and low-quality sequences

↓

Trinity: *de novo* transcriptome assembly

↓

Trinotate: functional annotation of transcriptome

↓

RSEM: generate count data for each transcript

↓

DESeq2: differential gene expression among treatment groups

↓

Ingenuity® Pathway Analysis

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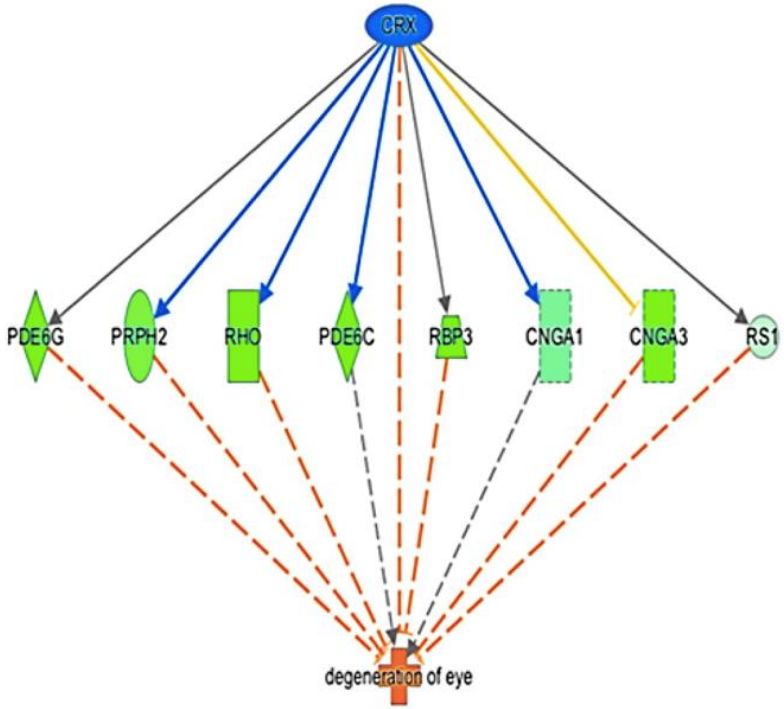
INGENUITY® PATHWAY ANALYSIS

- Software from QIAGEN Bioinformatics that uses differential gene expression data to identify biological mechanisms, pathways, and functions that are predicted to be altered based on primary, peer-reviewed literature.
- **Interpretation of the biological meaning of our sequencing results and formation of *in silico* adverse outcome pathways**

Prediction of adverse phenotypes based on differential gene expression



e.g. Differentially expressed genes in *DWH* oil-exposed mahi-mahi are linked to degeneration of the eye (Xu et al., 2016)

RESULTS – WATER CHEMISTRY

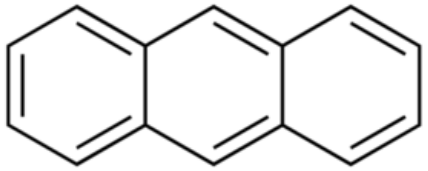
- Measured 50 polycyclic aromatic hydrocarbon (PAH) analytes in exposure HEWAF samples
- **Mean ∑PAH Concentrations:**

  Control: 0 μg/L

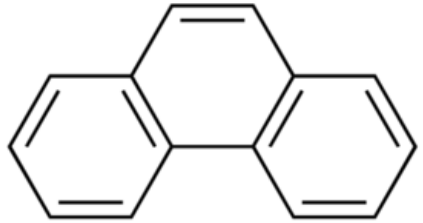
  Low Oil (5% HEWAF): 16.55 μg/L

  High Oil (10% HEWAF): 23.03 μg/L

- 3-ring PAHs predominated (~70% of the total PAHs), such as phenanthrenes and anthracenes



Anthracene



Phenanthrene

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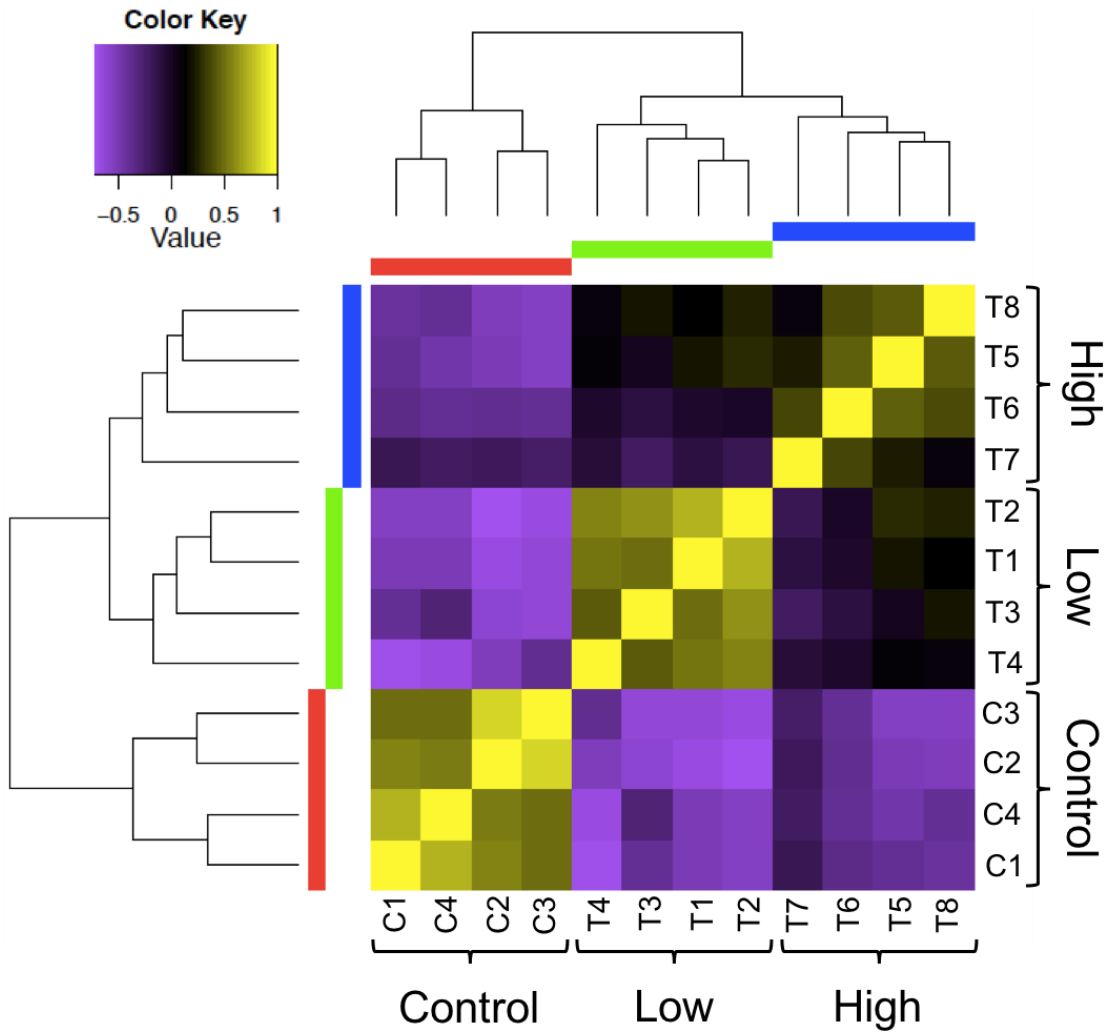
RESULTS – HIERARCHICAL CLUSTERING HEATMAP



- Able to distinguish exposed and non-exposed individuals based on transcriptome-wide gene expression patterns
- Suggests transcriptomic differences among treatments and, thus, dynamic regulation of mRNA in mucus following oil-exposure

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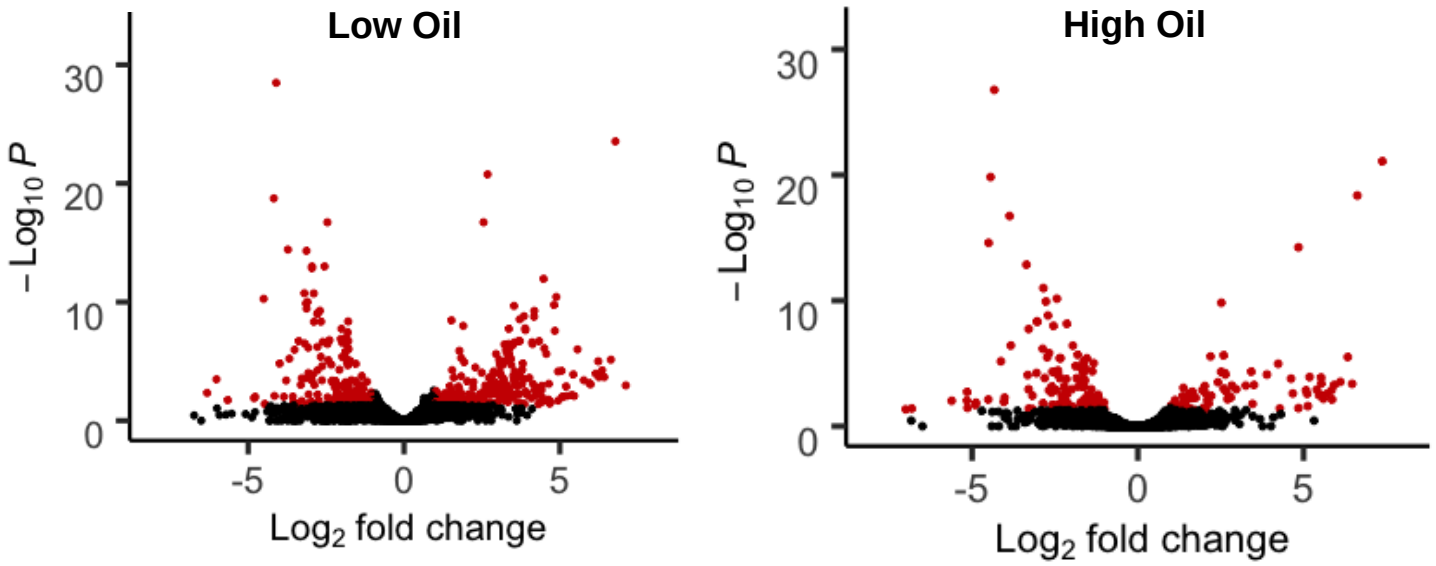
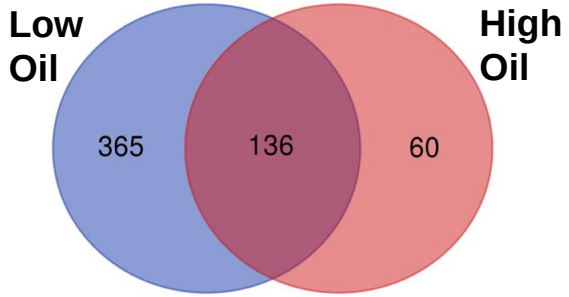
RESULTS – DIFFERENTIAL GENE EXPRESSION

501 significant differentially expressed transcripts

196 significant differentially expressed transcripts





● Significant differentially expressed transcript (FDR-adjusted $p \leq 0.05$)

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RESULTS – DIFFERENTIAL GENE EXPRESSION

Cardiac-related & Ca^{2+} Homeostasis

Immune-related

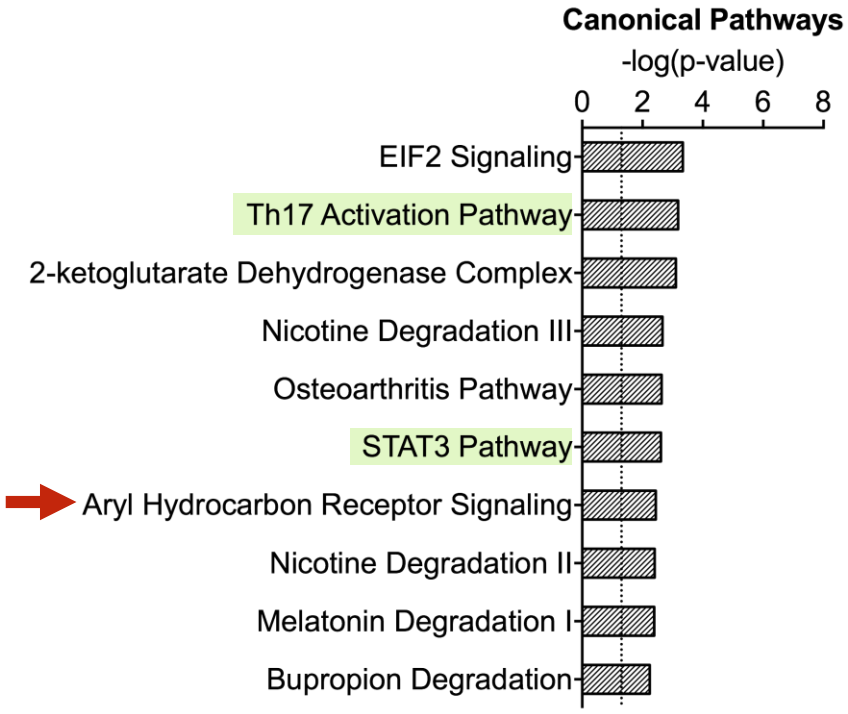
Rank	Gene Symbol	Gene Name	log2 Fold Change	p-value	Gene Ontology Functions and Processes
1	*cyp1a1*	cytochrome P450 family 1 subfamily A member 1	4.102	3.29E-29	oxidoreductase activity, aromatase activity
4	*cyp1b1*	cytochrome P450 family 1 subfamily B member 1	2.460	1.90E-17	oxidoreductase activity, aromatase activity
13	*socs3*	suppressor of cytokine signaling 3	-1.520	3.49E-09	cytokine-mediated signaling pathway, JAK-STAT cascade
42	*dsg2*	desmoglein 2	2.627	2.51E-05	calcium ion & cell adhesive protein binding involved in myocyte communication
45	*cdh2*	cadherin 2	2.778	5.66E-05	calcium ion binding and protein phosphatase binding
50	*il24*	interleukin 24	-2.494	9.13E-05	cytokine activity, immune & inflammatory response, wound healing
91	*epas1*	endothelial PAS domain protein 1	1.402	0.00341	response to hypoxia, angiogenesis, regulation of heart rate
95	*cxcr1*	C-X-C motif chemokine receptor 1	-1.817	0.00424	C-X-C chemokine receptor activity, chemotaxis
105	*il5ra*	interleukin 5 receptor subunit alpha	-2.064	0.00611	cytokine-mediated signaling pathway, interleukin-5 receptor activity
106	*il17c*	interleukin 17C	-2.553	0.00622	cytokine activity, interleukin-17-mediated signaling, inflammatory response
112	*s100a11*	S100 calcium binding protein A11	-1.434	0.00747	calcium ion binding and calcium-dependent protein binding
119	*hbegf*	heparin binding EGF like growth factor	-1.817	0.00822	cell chemotaxis, wound healing, regulation of heart contraction
124	*ldlrap1*	low density lipoprotein receptor adaptor protein 1	-0.834	0.01014	calcium ion binding, cholesterol metabolic process, lipid transport
127	*edn1*	endothelin 1	1.953	0.01048	cytokine activity, heart development, cellular response to hypoxia
133	*glrx4*	glutaredoxin 3	-3.515	0.01180	glutathione disulfide oxidoreductase activity, regulation of heart contraction
138	*tnip2*	TNFAIP3 interacting protein 2	-1.436	0.01661	I-kappaB kinase/NF-kappaB signaling, B cell/macrophage activation
156	*ryr2*	ryanodine receptor 2	1.157	0.02469	calcium ion binding, heart development, cardiac muscle contraction
159	*postn*	periostin	1.371	0.02521	response to hypoxia, cell adhesion, regulation of smooth muscle cell migration
160	*cxcr3*	C-X-C motif chemokine receptor 3	-1.078	0.02534	immune & inflammatory response, T cell chemotaxis, leukocyte migration
170	*plcb1*	phospholipase C beta 1	-1.165	0.03407	calcium ion binding, lipid catabolic process, interleukin-1-mediated signaling
197	*nfkbia*	NFKB inhibitor alpha	-0.997	0.04138	inflammatory response, regulation of NIK/NF-kappaB signaling

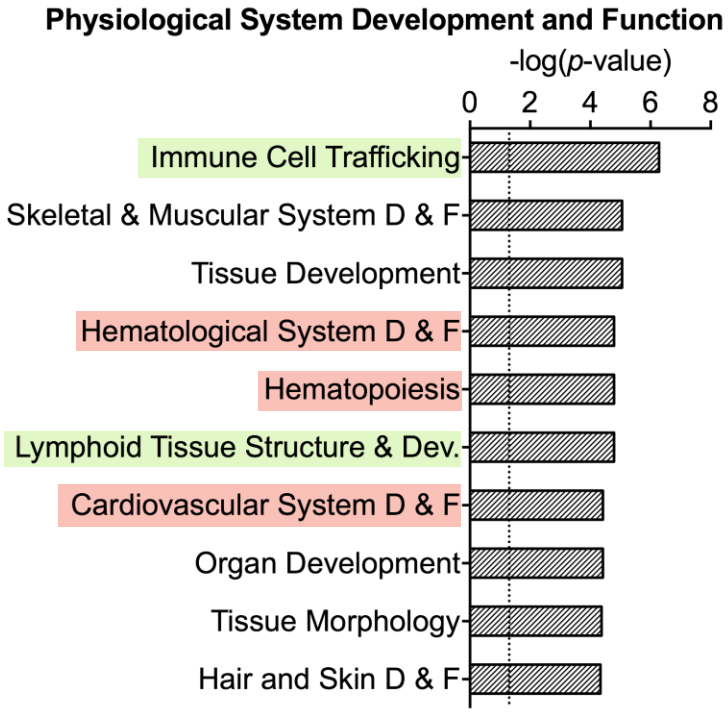
RESULTS – INGENUITY® PATHWAY ANALYSIS

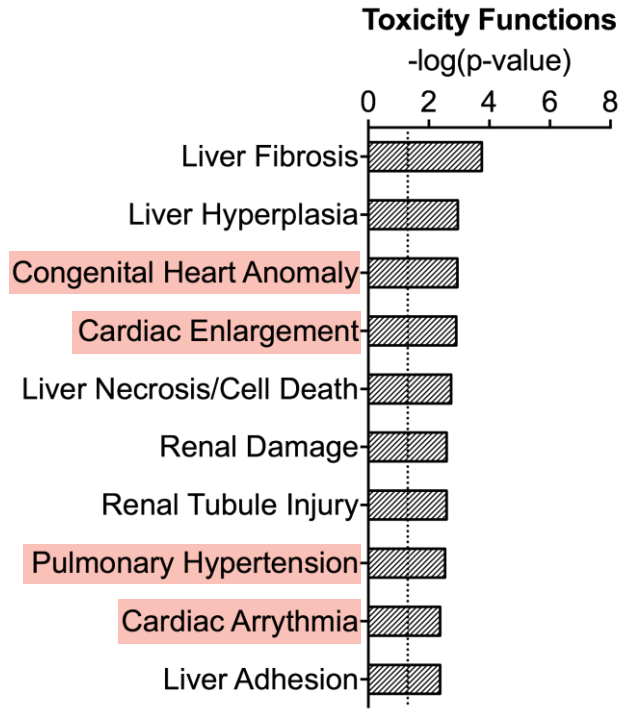
Cardiac-related

Immune-related

- Top biological mechanisms, pathways, and functions that are predicted to be altered based on differential gene expression data







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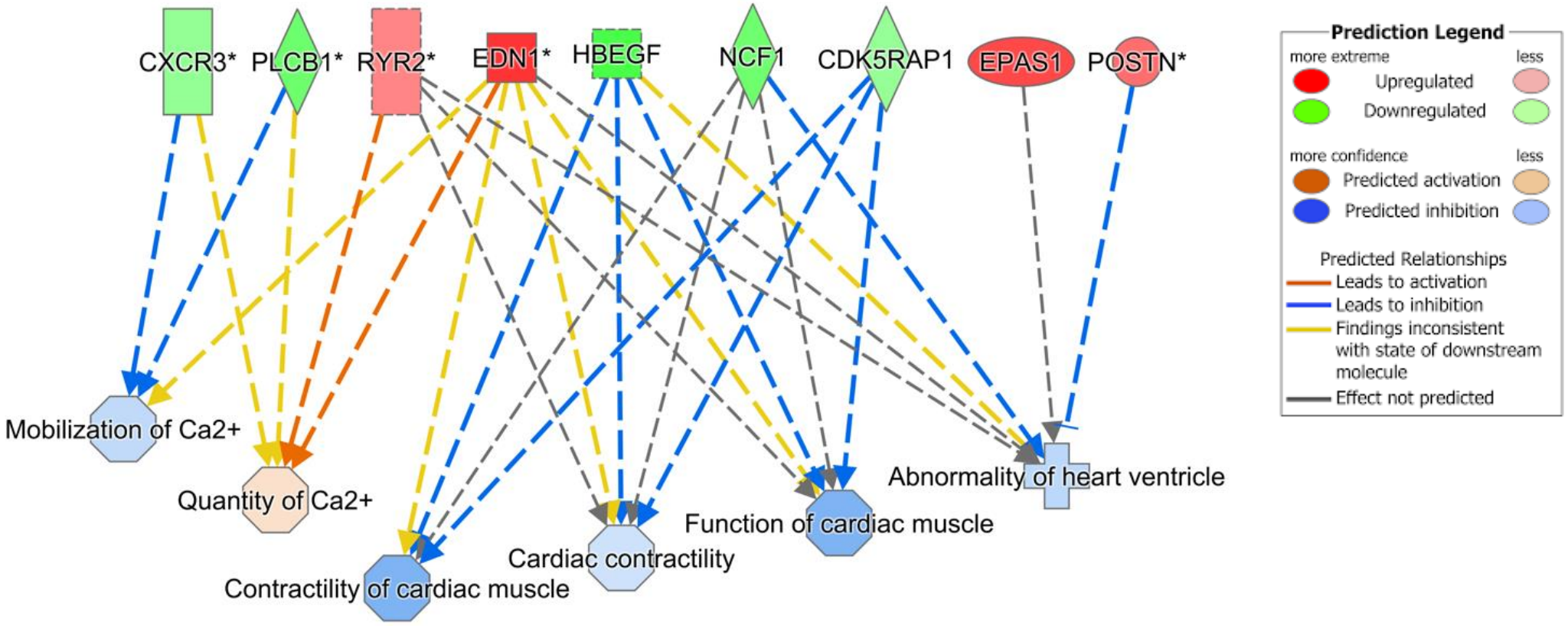
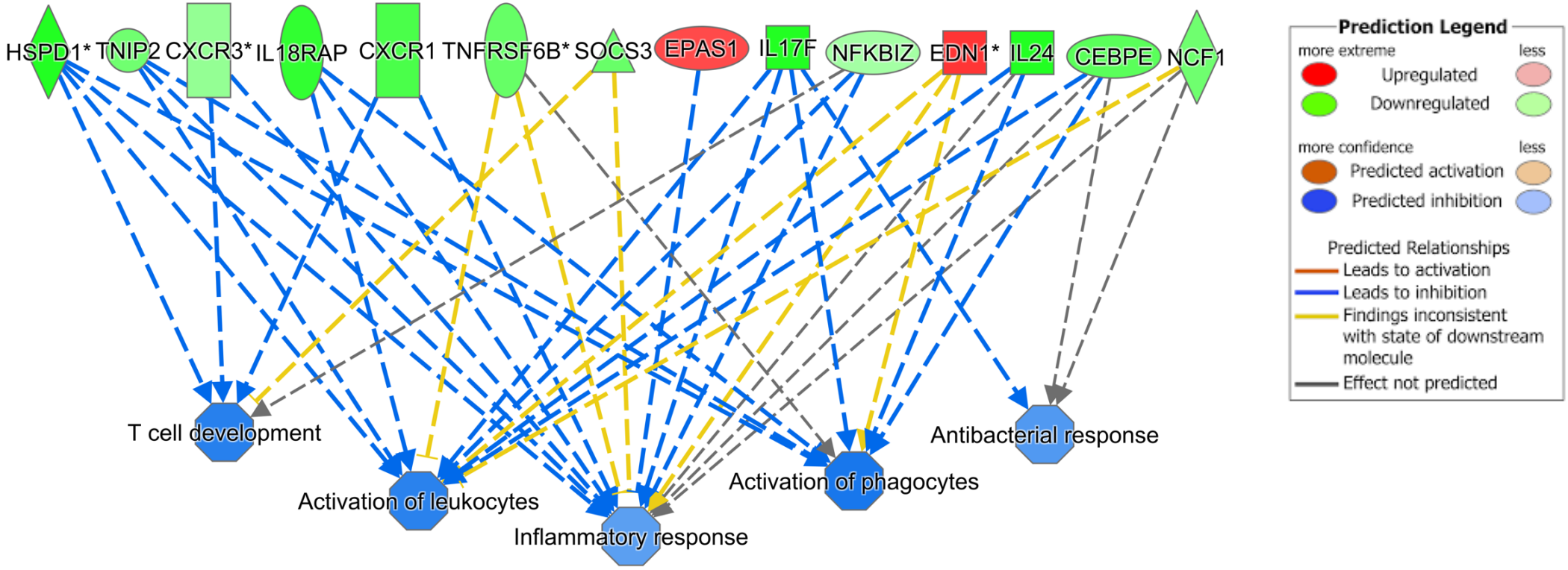
RESULTS – CARDIOTOXICITY AND CALCIUM HOMEOSTASIS



- Phenotypic outcomes predicted from mucus gene expression are reflective of cardiotoxic phenotypes previously observed in oil-exposed pelagic fish embryos

RESULTS – IMMUNOSUPPRESSION



- PAH-exposure is known to suppress immune system function in fish through downregulation of immune-related genes, increasing susceptibility to pathogen infection (Reynaud and Deschaux, 2006; Nakayama et al., 2008; Song et al., 2008; Bayha et al., 2017)

- Gene expression patterns in mucus are reflective of immunosuppressive effects observed in previous studies

CONCLUSIONS

- Consistency with other studies provides strong evidence that mucus is a promising vector for development of mRNA-based biomarkers of exposure
    - Transcriptional changes in cardiac and Ca^{2+} cycling genes in mucus were reflective of effects observed in oil-exposed pelagic fish embryo homogenates
    - Downregulation of immune-related genes in mucus is in-line with previous studies showing PAH-induced immunosuppression in fish
    - Upregulation of *cyp1a1*, a well-establish biomarker of PAH exposure
- Mucus sampling is nonlethal, making it ideal for exposure assessment of fish populations without further disruption through sampling

Thank You

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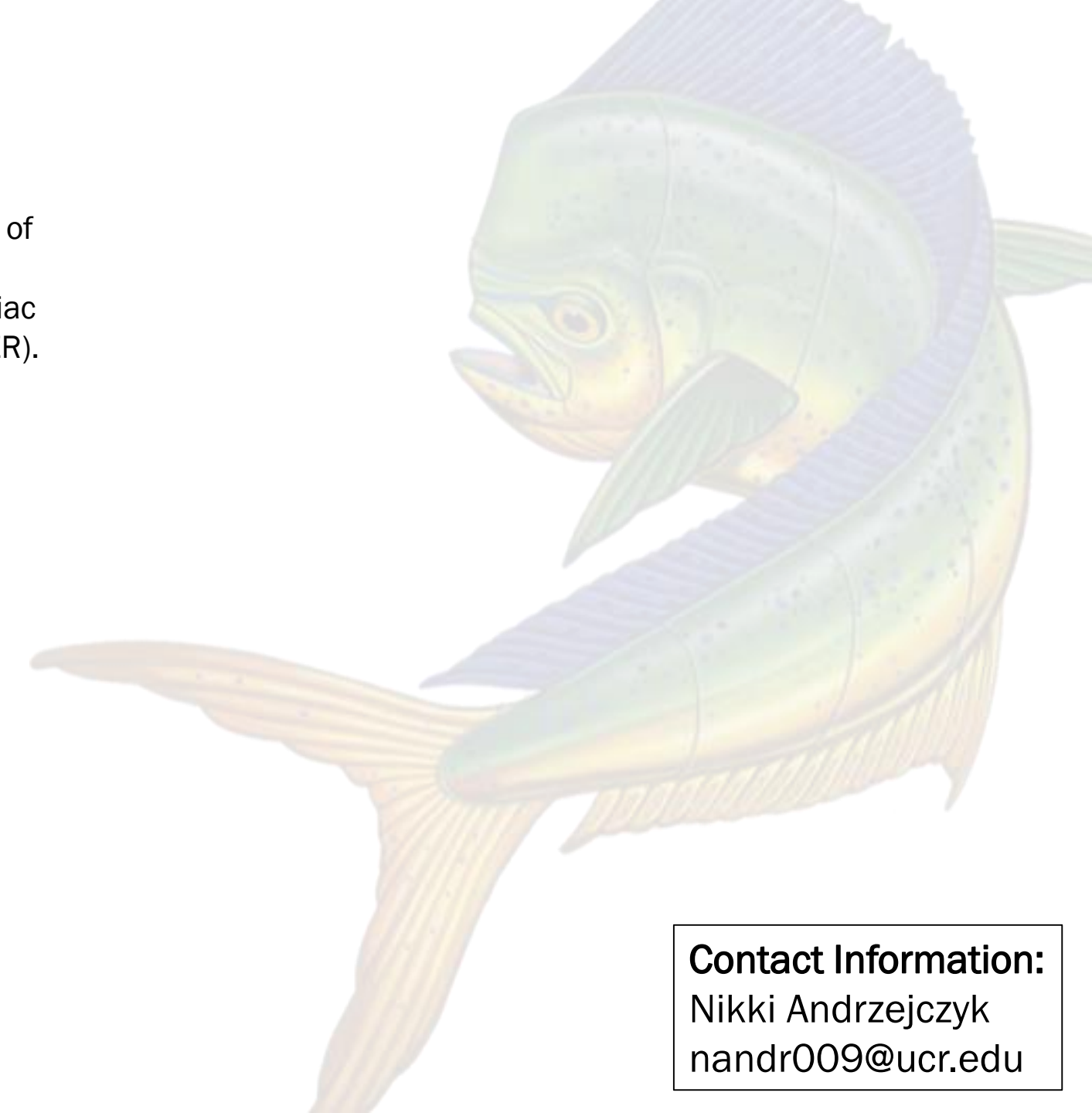

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