

A high throughput *in vivo* model to understand PAH toxicity

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May 14, 2018

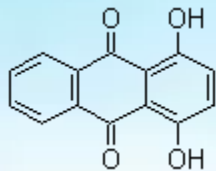
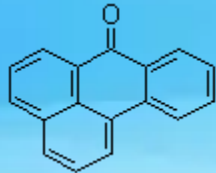
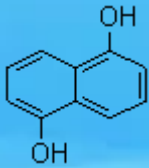
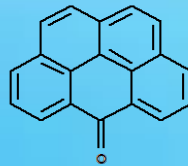
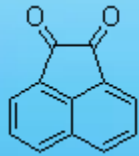
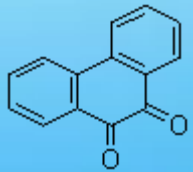
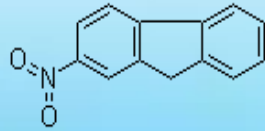
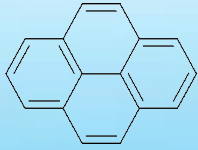


Polycyclic aromatic hydrocarbons and human health effects

- PAHs are ubiquitous in the environment, fossil fuels, combustion, food etc.
- PAH exposures occur primarily via inhalation and ingestion
- Known carcinogens in humans
- PAHs measured in placental tissue
- Concern about developmental effects



Toxicity Mechanisms for Most PAHs are Unknown



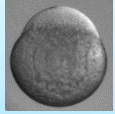
- Environmental samples can contain 100's PAHs
- Parent, substituted PAHs
- Toxicity data is limited but growing for substituted PAHs
- PAHs induce AHR-dependent and AHR-independent developmental toxicity, dependent on structure
- We lack the structural basis for developmental and neurotoxicity

Why Zebrafish?

- Molecular signaling is conserved with humans
- High degree of homology with humans
 - 71% human proteins have orthologue in zebrafish
- Well suited to discover gene functions
- Metabolically competent by 72 hpf
- Amendable to rapid whole animal mechanistic evaluations



The High Throughput Screening Platform

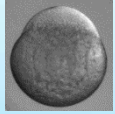


10 min

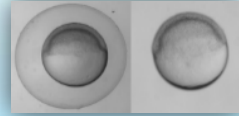


- Specific Pathogen Free Facility

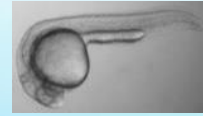
The High Throughput Screening Platform



10 min



6 hr



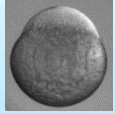
1 day



5 days



The High Throughput Screening Platform

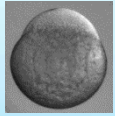


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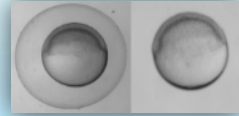


Embryo Collection

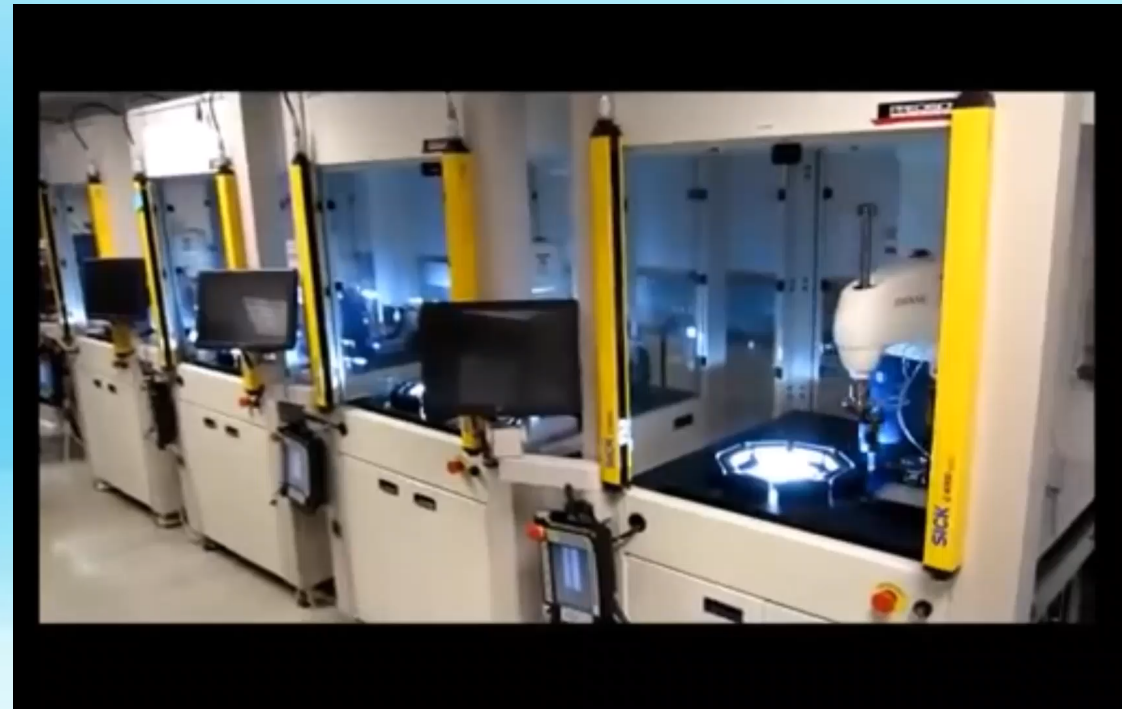
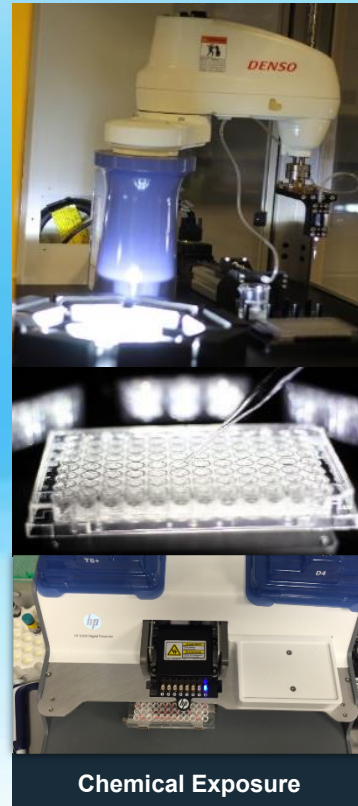
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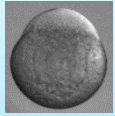
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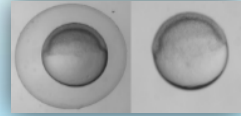
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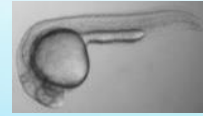
The High Throughput Screening Platform



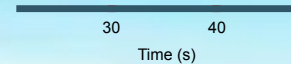
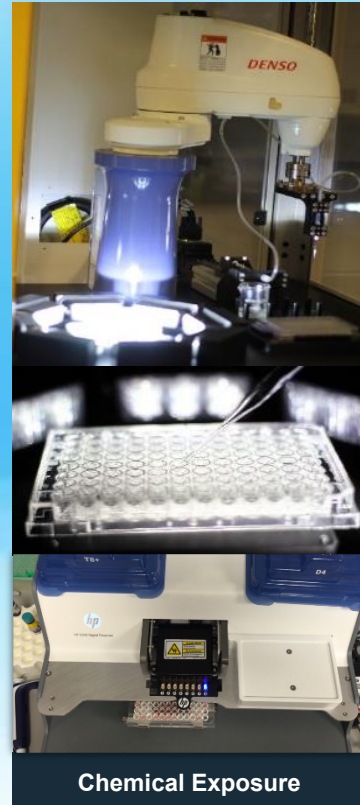
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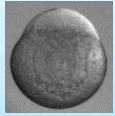
6 hr



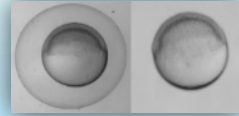
1 day



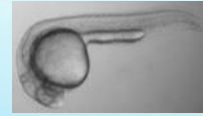
The High Throughput Screening Platform



10 min



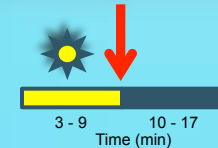
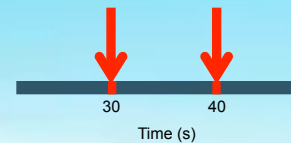
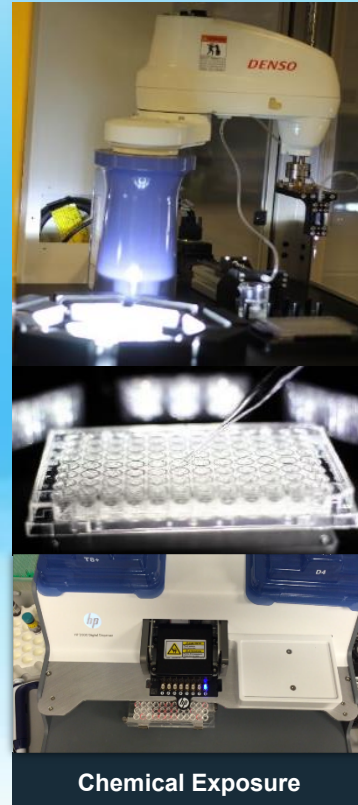
6 hr



1 day



5 days



Zebrafish Acquisition and Analysis (ZAAP)

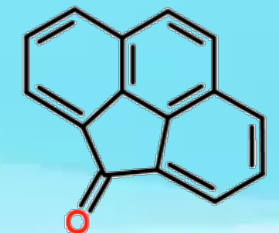
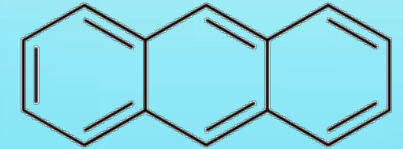
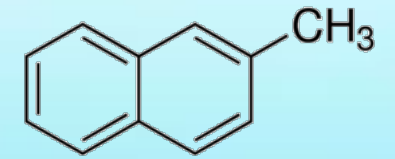
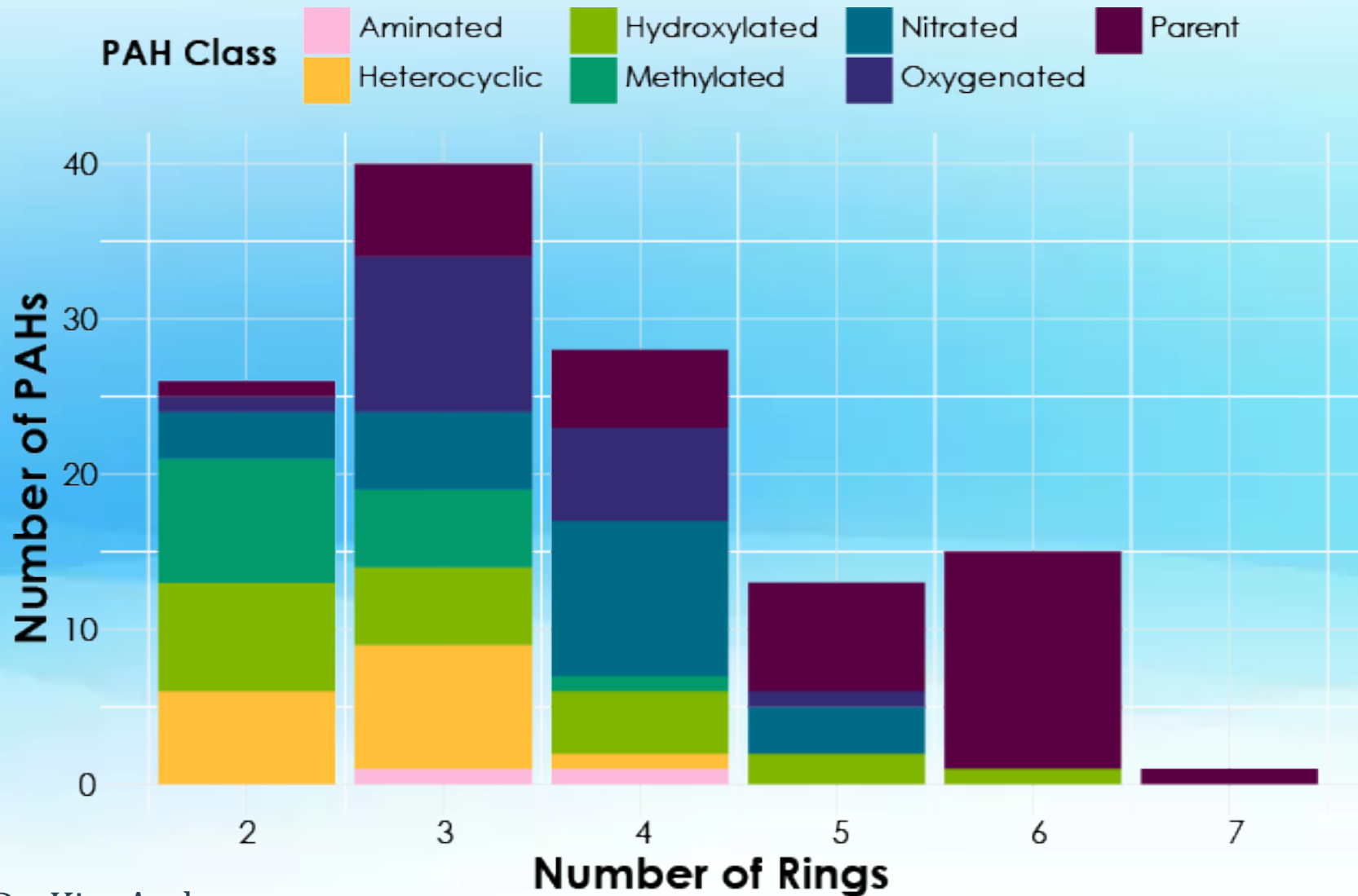
- Custom-build laboratory information system (LIMS)
- Stores chemical inventory, and allows real-time data acquisition
- Tracks individual well information from 96-well plates
- Built in data analysis
- Ensures rigor in data generated



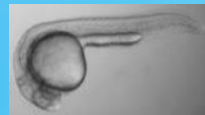
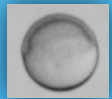
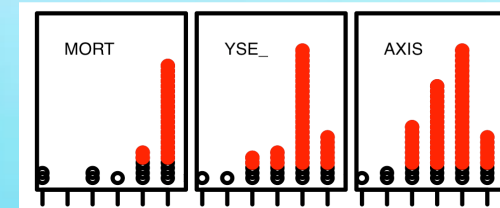
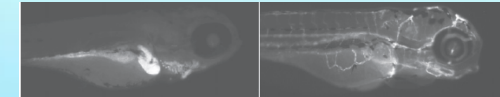
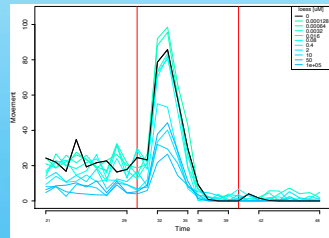
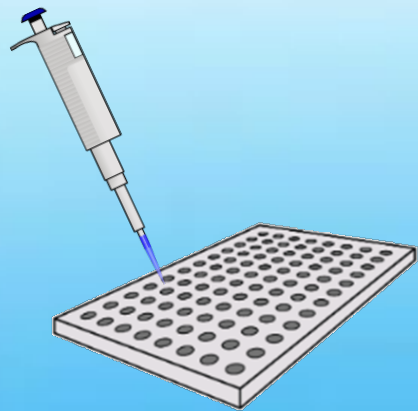
The screenshot shows a web browser window titled "Log_In". The address bar displays "File Today's Date 3/1/2018 User Name". The main content area features the Tanguay Lab logo, which includes a blue fish with a magnifying glass over its eye and the text "TANGUAY LAB THINKING OUTSIDE THE TANK". Below the logo, the text "Zebrafish Acquisition and Analysis Program (ZAAP)" is displayed. There are two input fields: "User Name:" with a dropdown arrow and "Password:". At the bottom left, it says "on-line" and at the bottom right, "Copyright © Tanguay Lab".

Comparative PAH Screening

Developed a Library of 123 PAHs for Comparative Analysis



High Throughput Screening of PAH Library



6 hpf

- 5 concentrations
 - 50-1 μM
 - 5-0.1 μM
- N=32

24 hpf

- 4 morphological endpoints
- Behavioral assay

120 hpf

- Morphology
- Behavior
- CYP1A Localization

CYP1a Expression Pattern as a Biomarker of AHR Activation

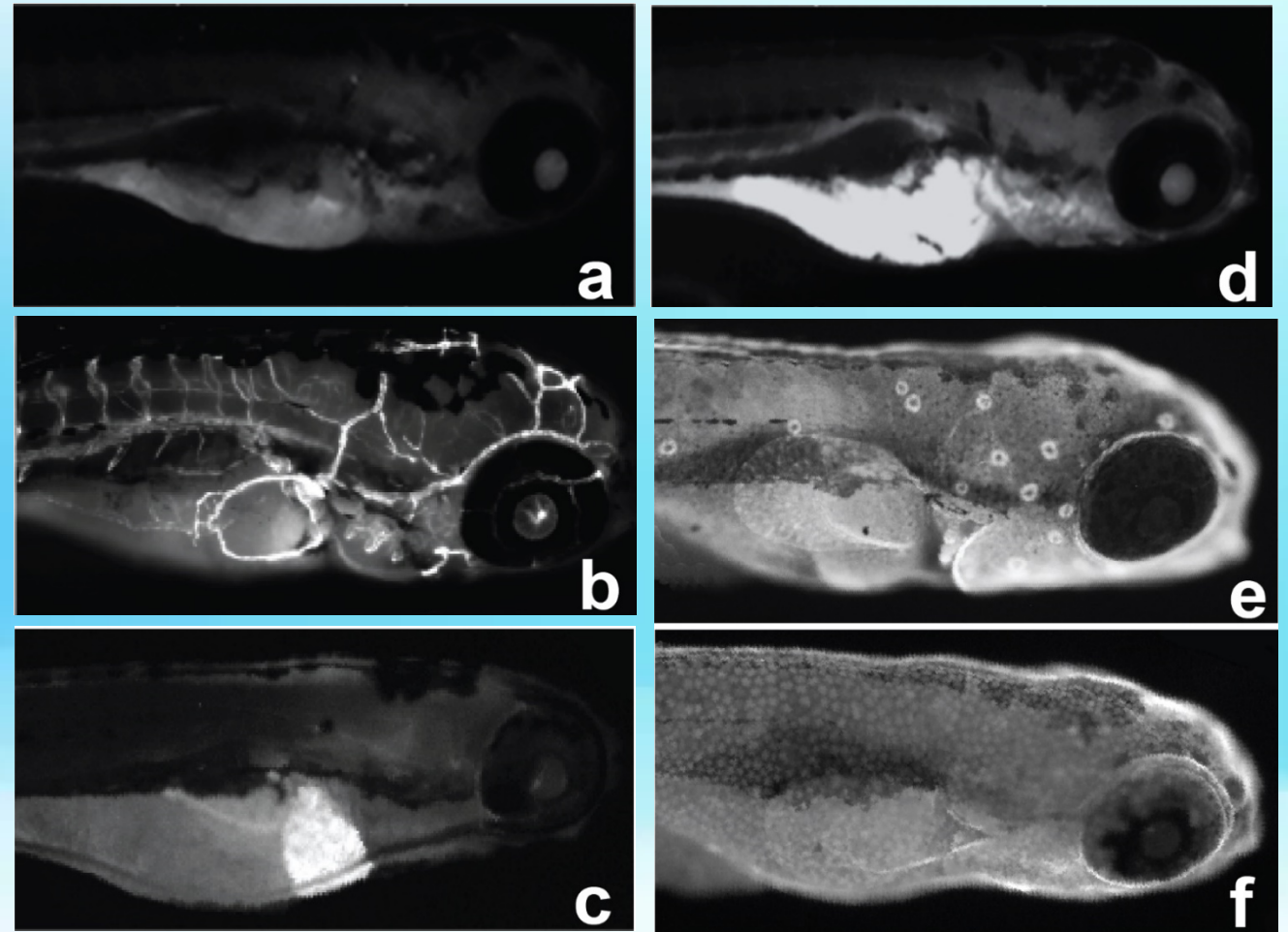
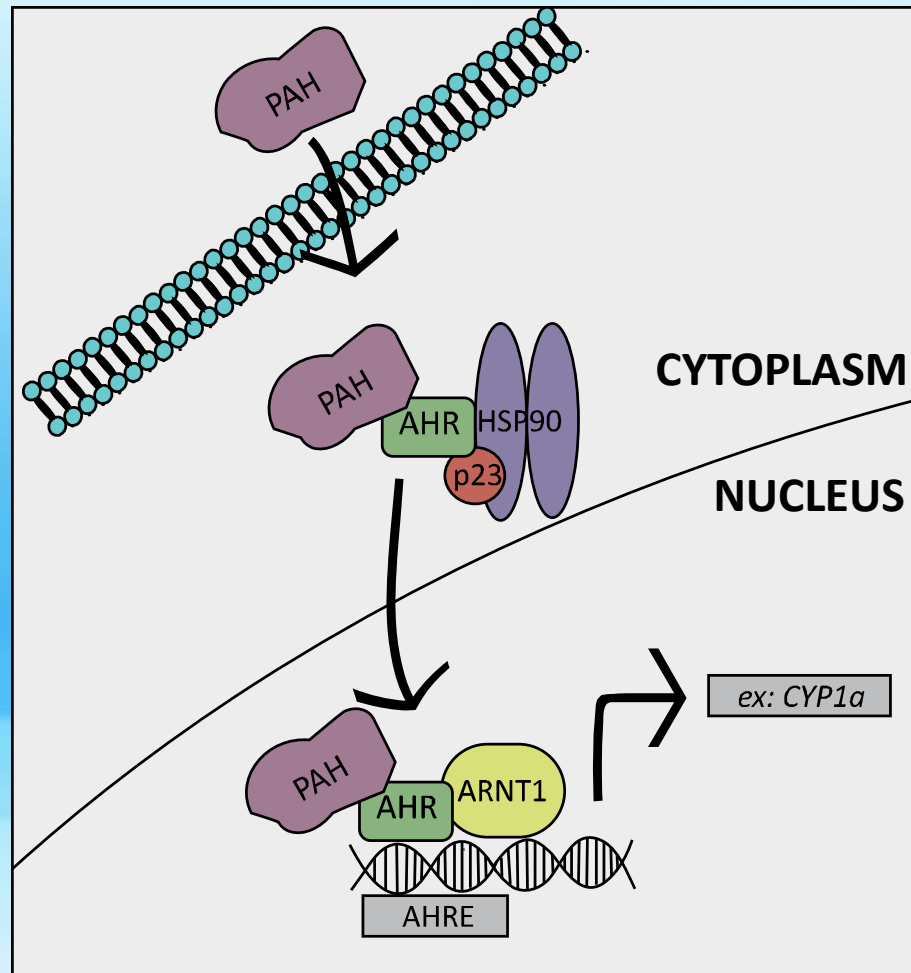
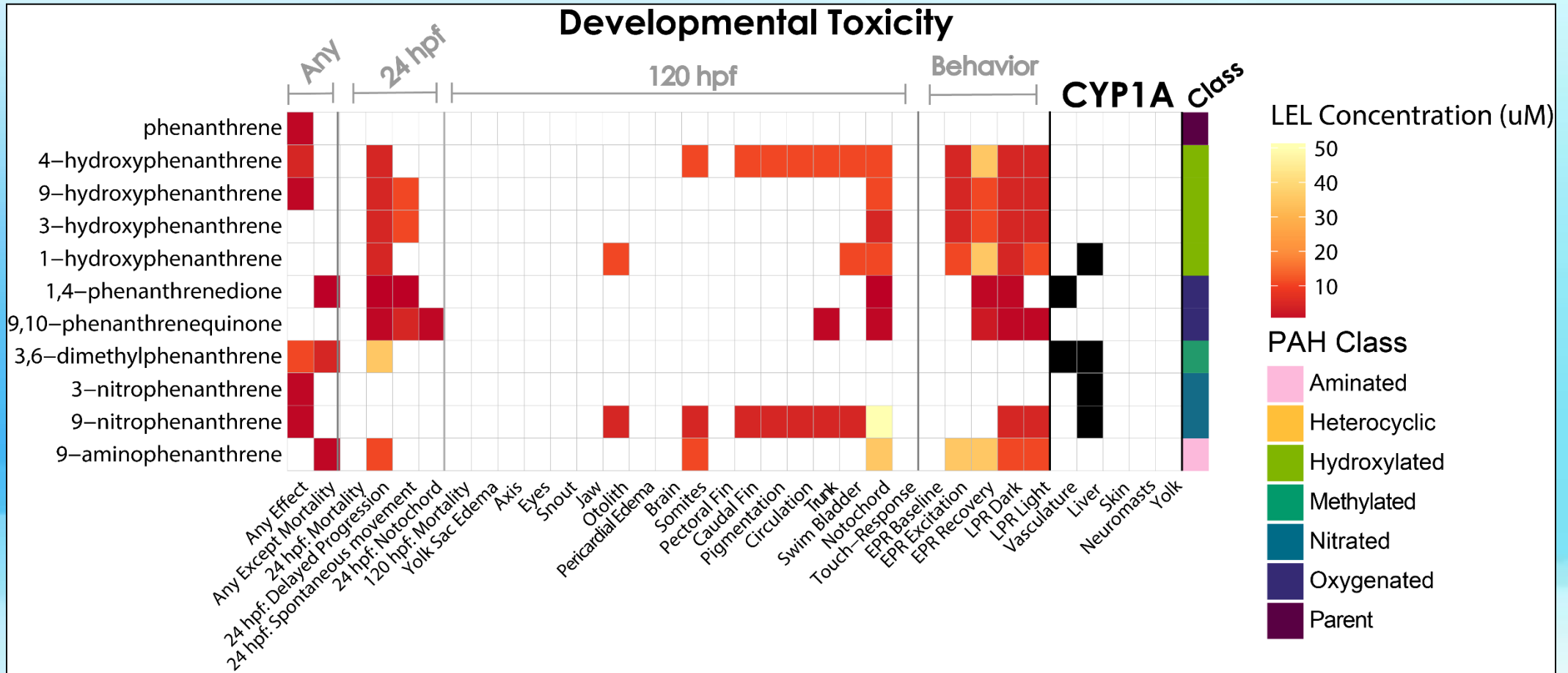


Figure 2. Representative images illustrating CYP1A expression patterns in 120 hpf larvae. a Geier et al. 2017
None, b vasculature, c liver, d yolk, e skin and neuromasts, f skin

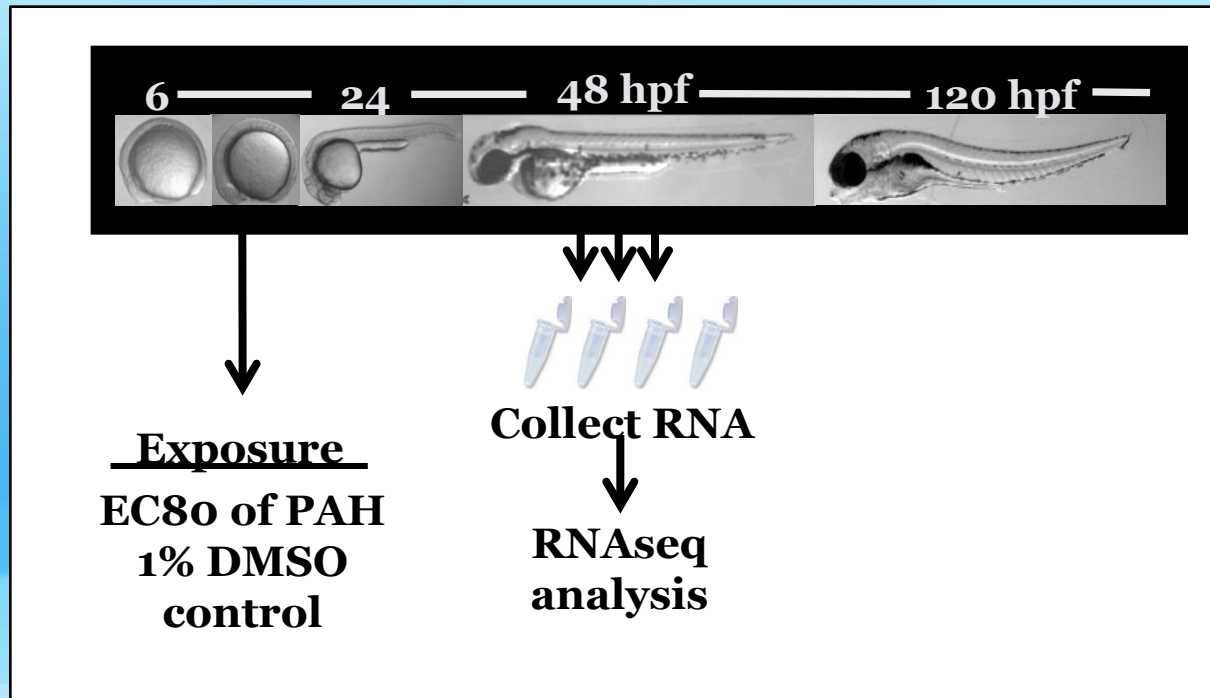
Differential responses in parent and derivatives



More mechanistic insight is needed to explain why

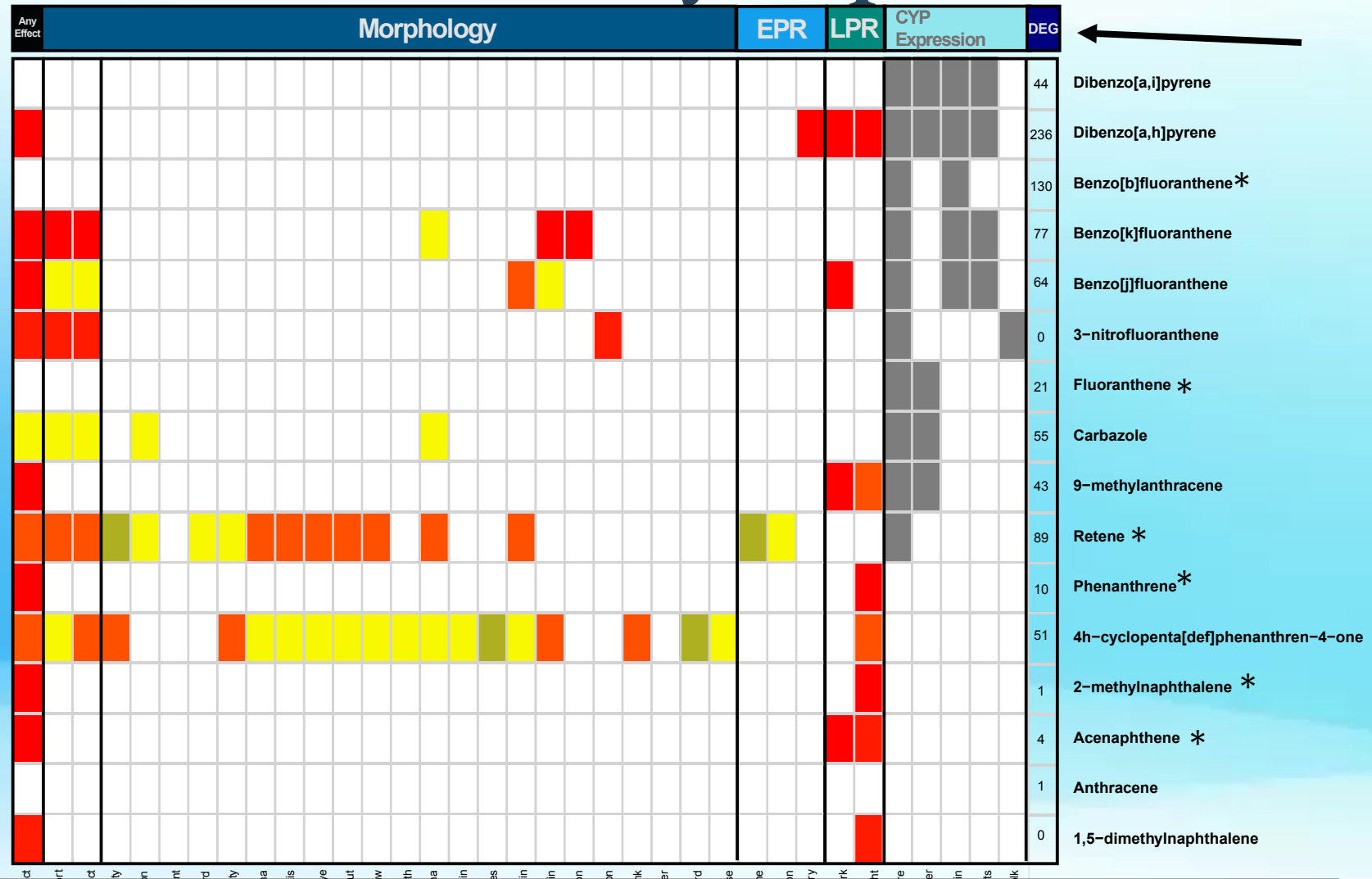


Mechanistic insight of 16 PAHs (transcriptomics)



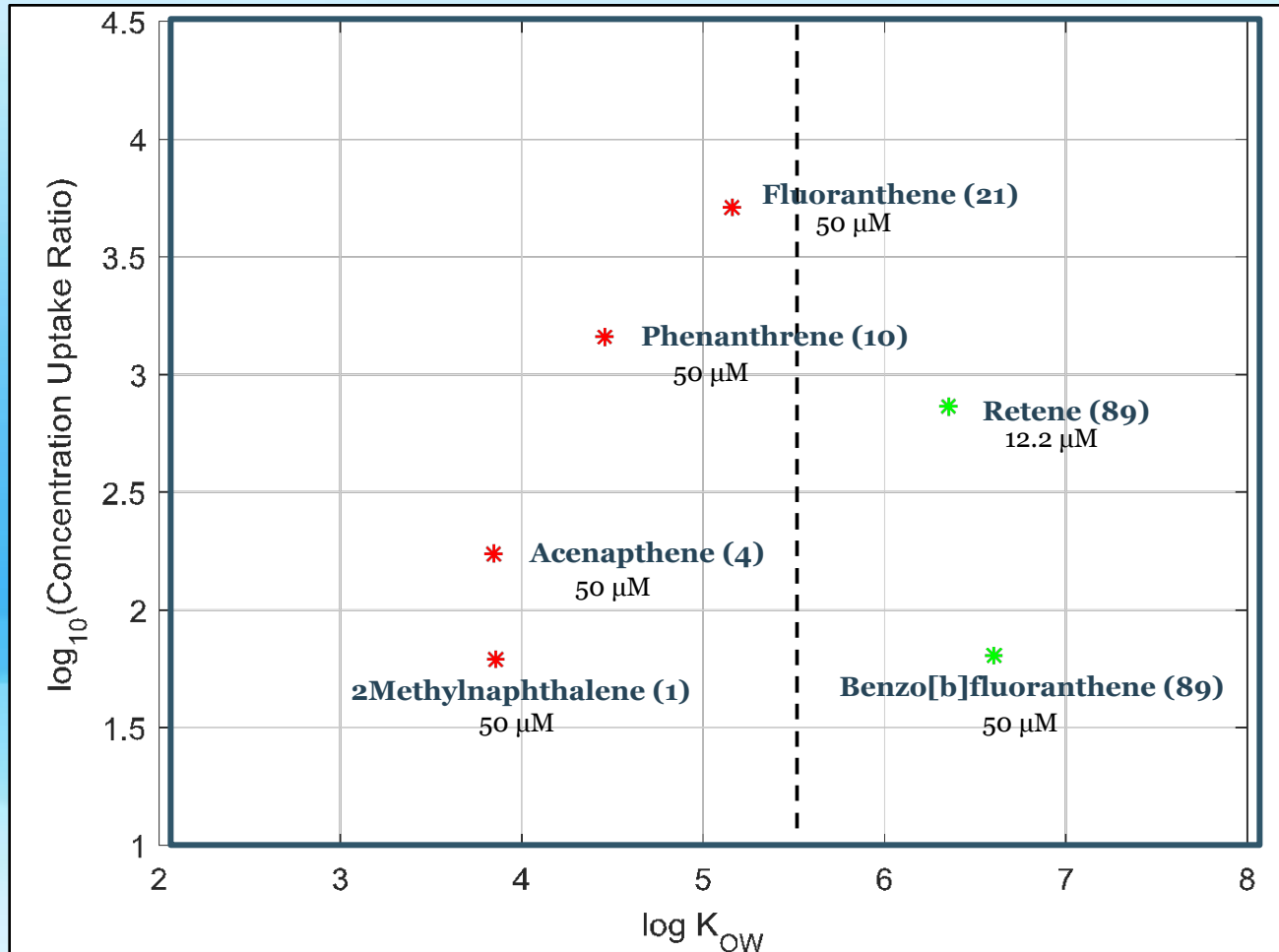
- 16 PAHs were selected from the screen by:
 - developmental bioactivity (morphological and behavioral)
 - their ability to activate AHR
 - the spatial expression of CYP1A
- Anchored to 120 hpf phenotype

Overview of Differentially Expressed Genes (DEGs)



Morphological and behavioral responses is not directly associated with # of DEGs

No Association between DEGs, body burden and Log K_{ow}

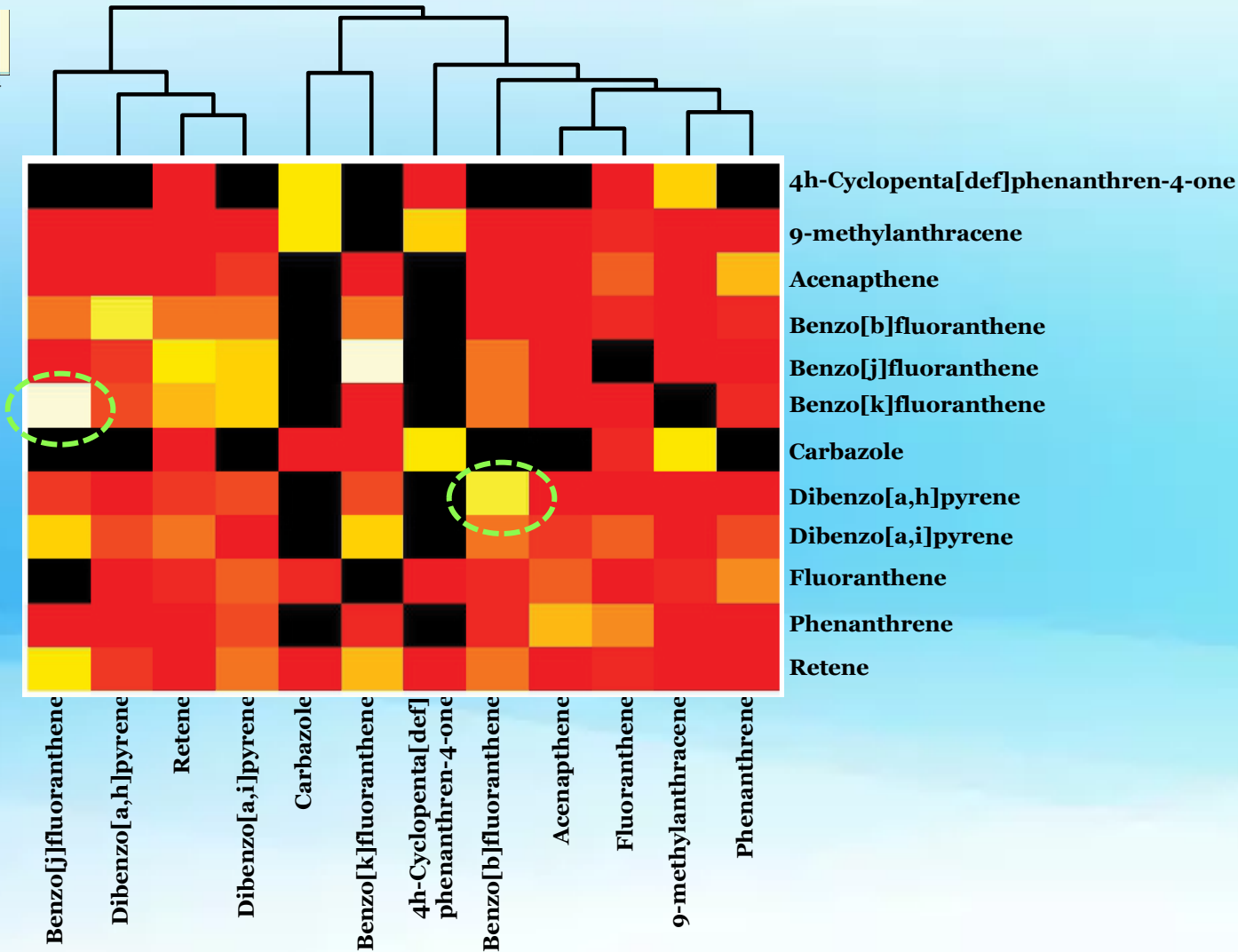
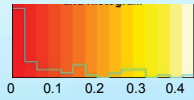


Compound	Log Kow	Log10(Conc Uptake)
2Methylnaphthalene	3.86	1.79
Acenaphthene	3.85	2.24
Phenanthrene	4.46	3.17
Fluoranthene	5.16	3.71
Retene	6.35	2.86
Benzo(b)fluoranthene	6.6	1.81

Embryos were exposed to 3 concentrations (5.39, 11.6, and 25 μM) from 6 to 48 hpf. Using the measured values, a concentration uptake ratio was computed from the ratio of the concentration inside the embryo and to the nominal media concentration. The number of DEGs are annotated near the chemical name, along with the test concentration (in blue). Data points in red represent PAHs with <5.5 log Kow, and green being >5.5 .



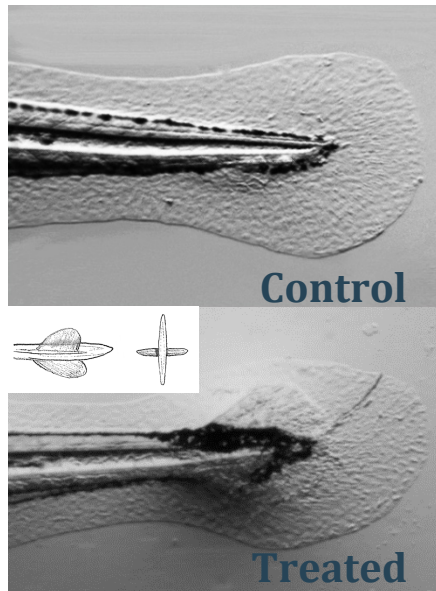
Comparing transcriptomic profiles



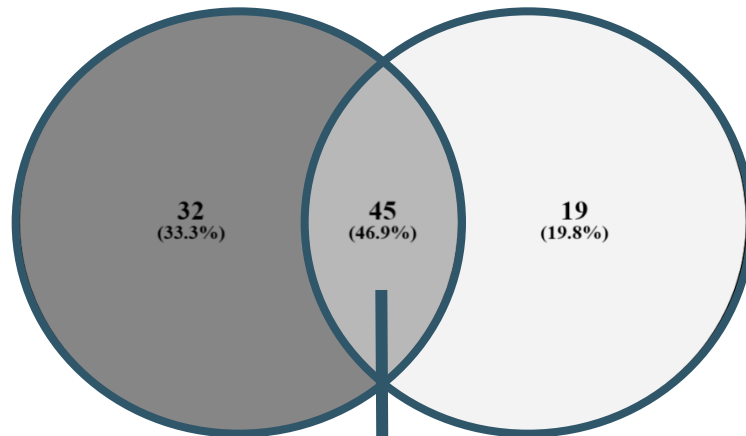
- Using Jaccard similarity analysis for DEGs >1.5 fold change, and $p < 0.05$, a correlation matrix was generated. The lighter the color, the higher correlation.
- The black indicates overlap not significant by Fisher's exact test ($p\text{-value} > 0.05$)

Transcriptomic clustering predicted adverse outcome later in development

Bin 1



Benzo[k]fluoranthene Benzo[j]fluoranthene



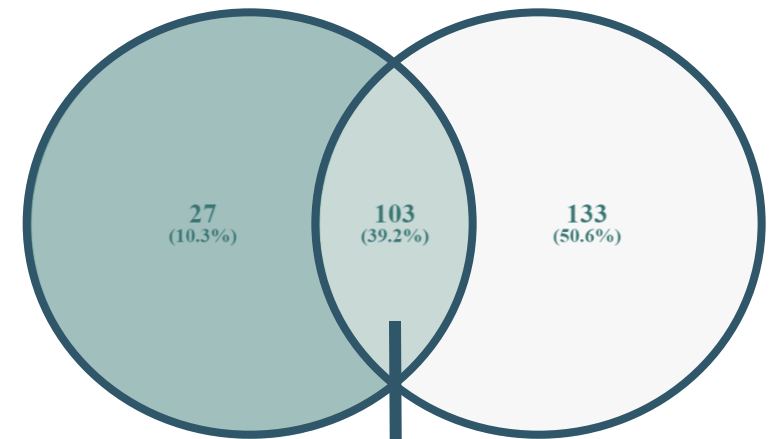
Common Enriched Pathways

Chloride Ion homeostasis
Cellular response to xenobiotic stimulus
Monooxygenase activity
Testosterone 6betahydroxylase activity

Bin 2

Benzo[b]fluoranthene

Dibenzo[a,h]pyrene



Common Enriched Pathways

Ion transport
Neuromast primordium migration
Lateral line/ sensory system development

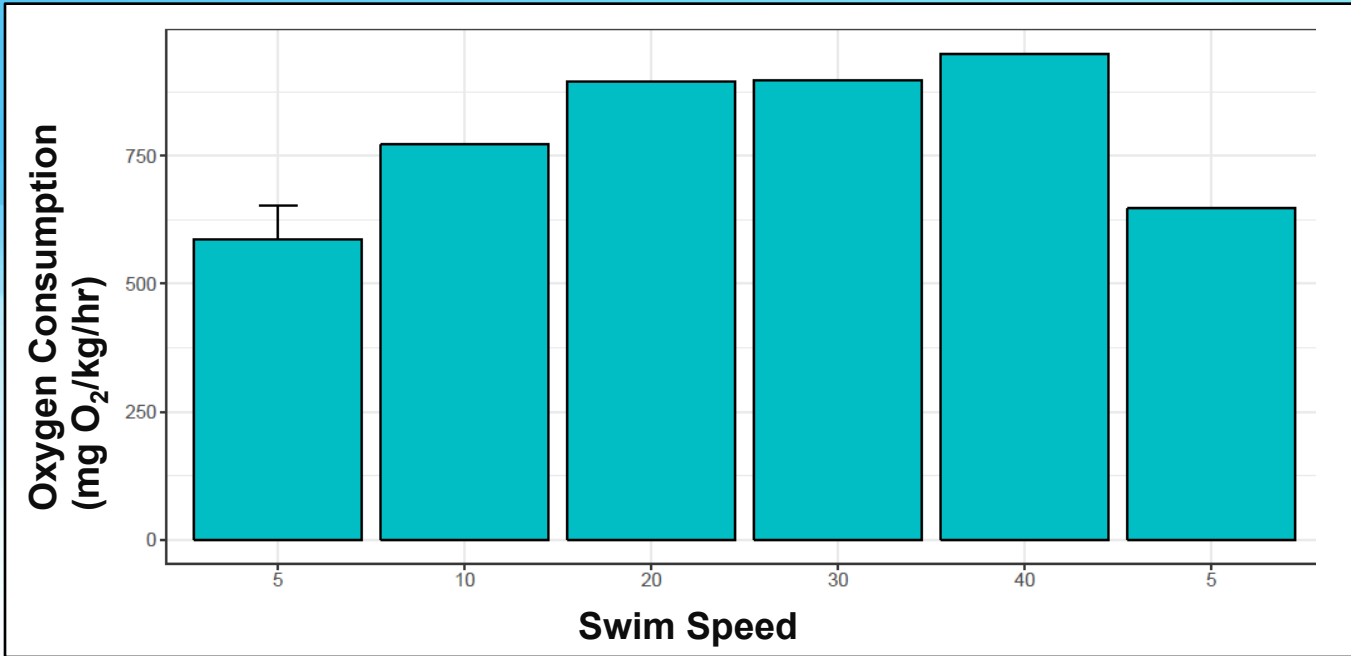
Transcriptomics is a powerful tool to provide mechanistic insight

Developed Tools to Measure Complex Central Nervous System Changes in Responses to Developmental PAH Exposures

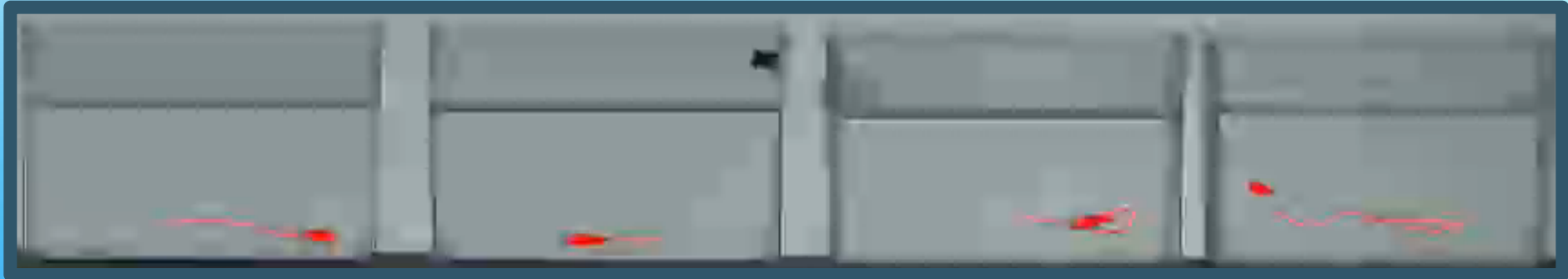
Adult Behavioral Measures

- Fitness
- Swimming activity
- Anxiety
- Fear
- Social Interactions
- Learning

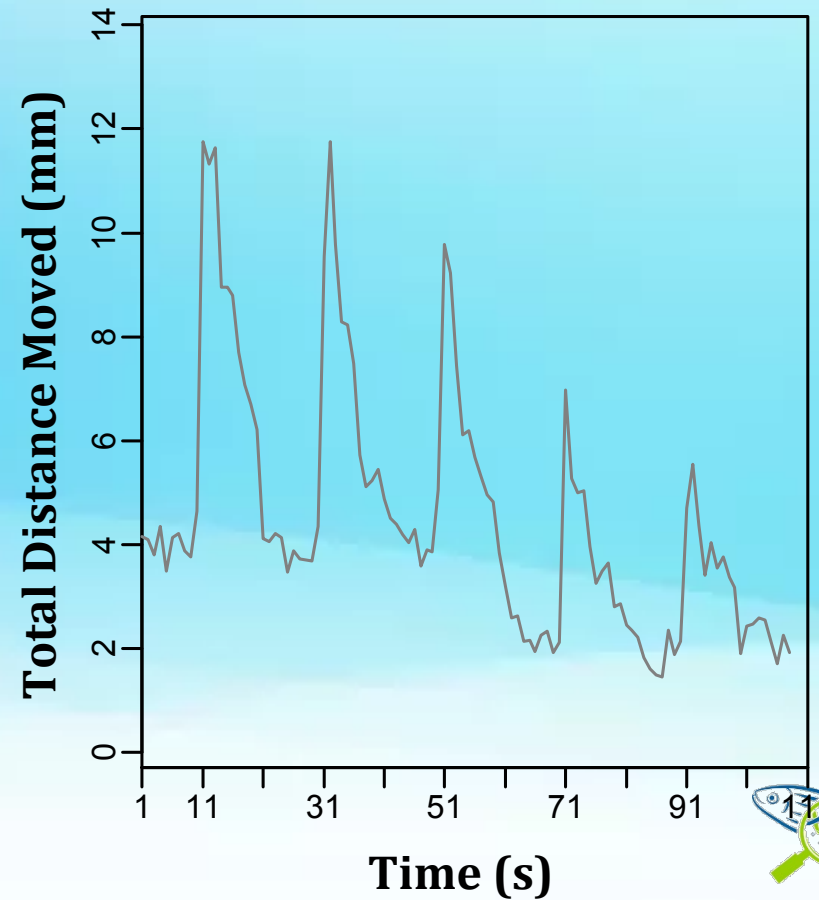
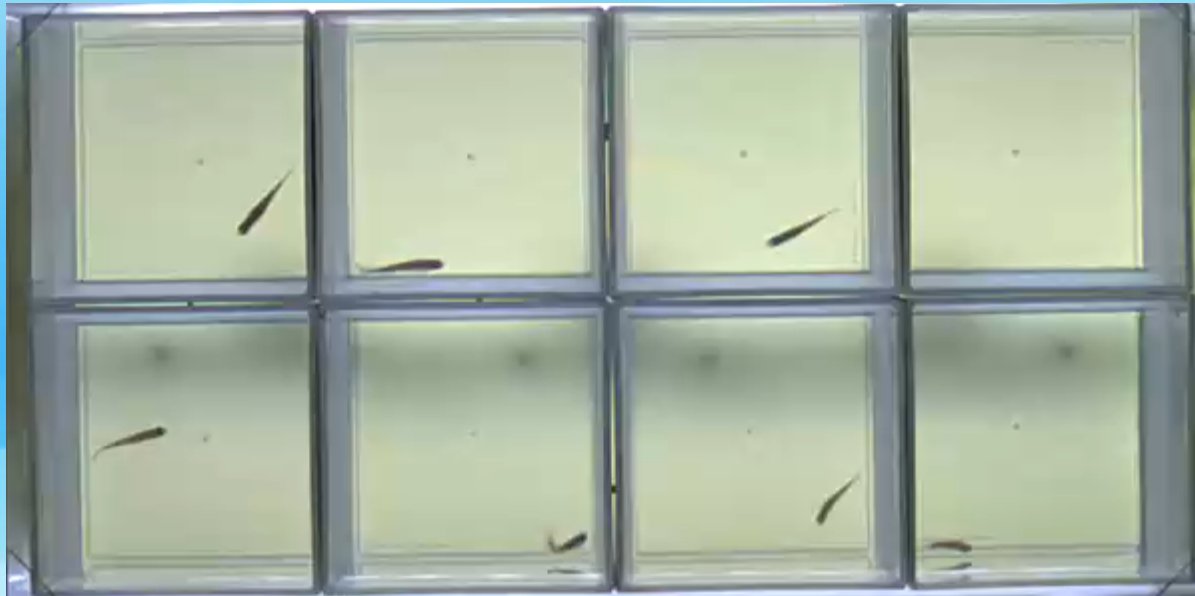
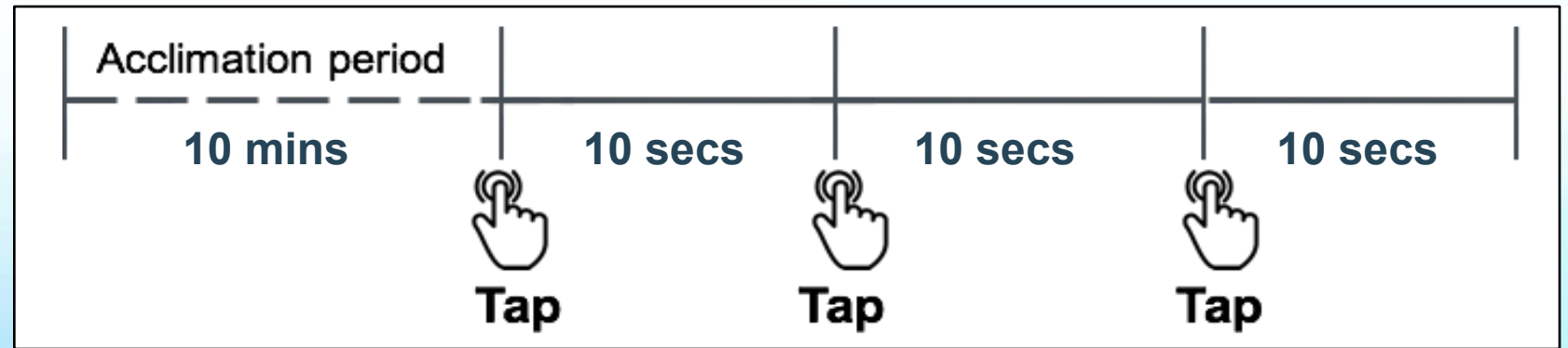
Fitness



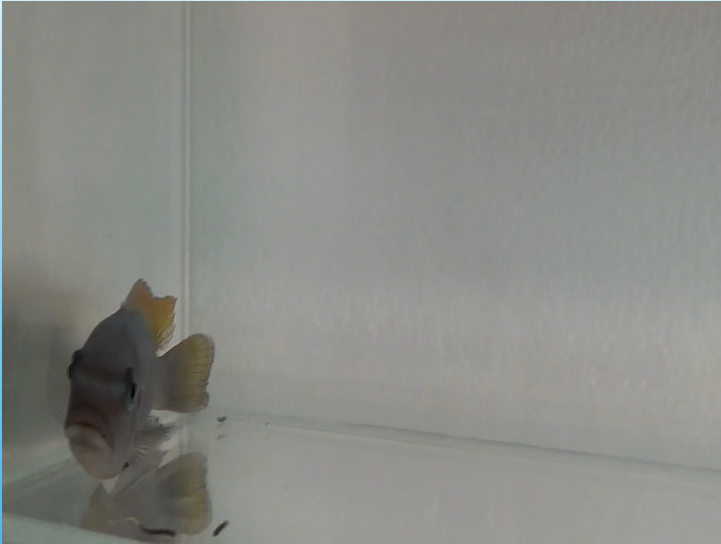
Swimming Activity Over Time



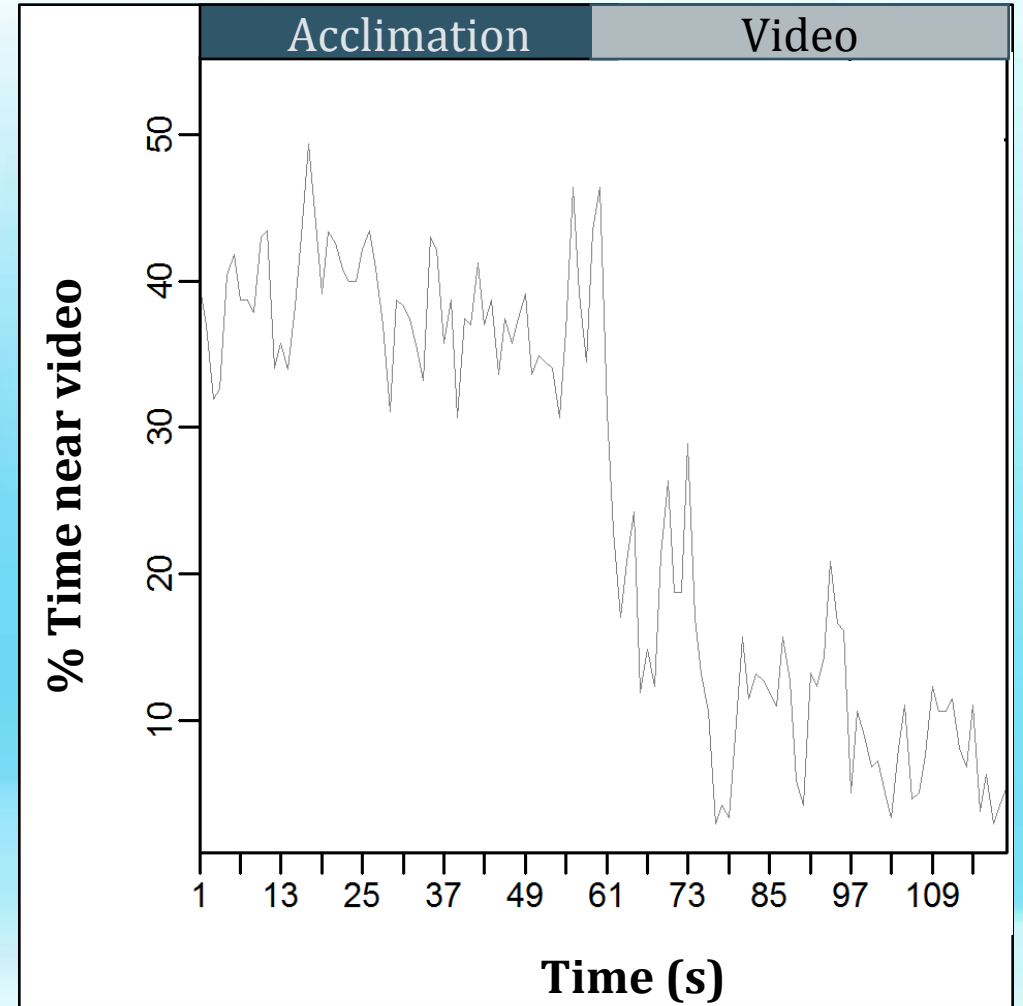
Anxiety



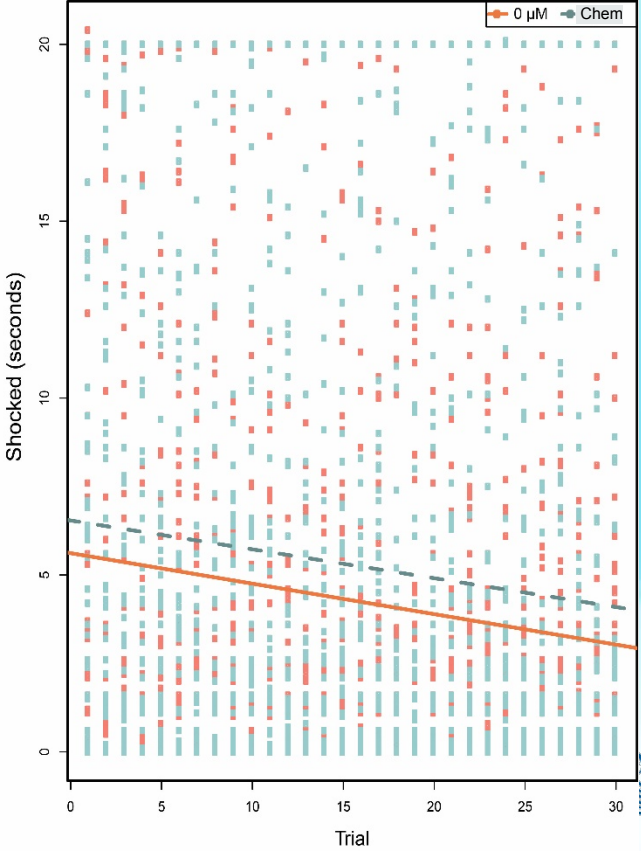
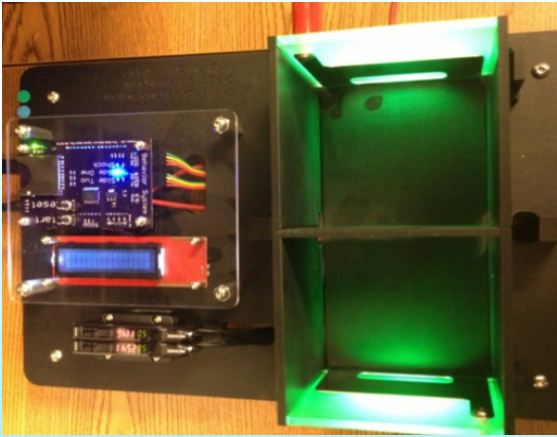
Fear



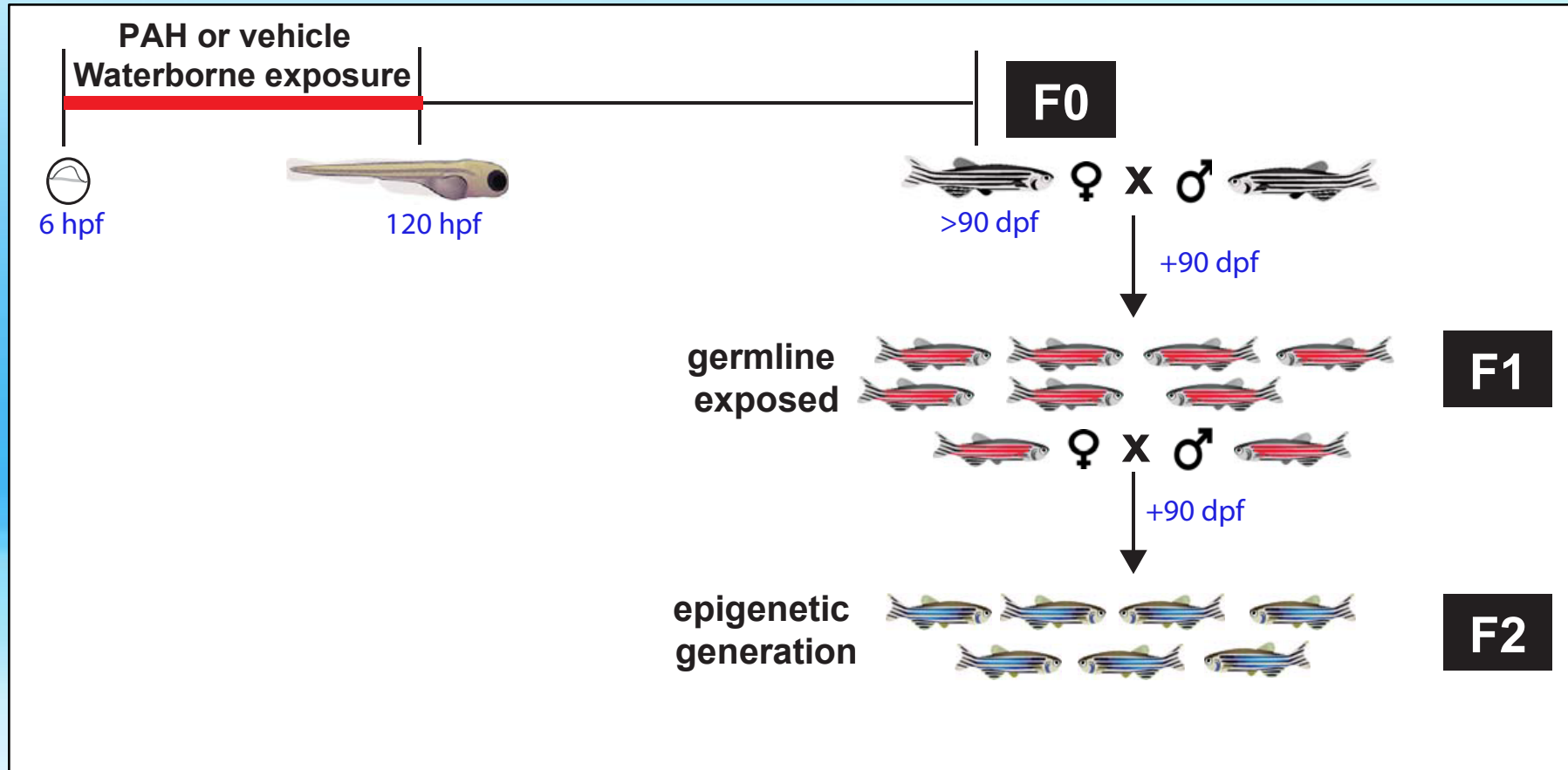
Predator video



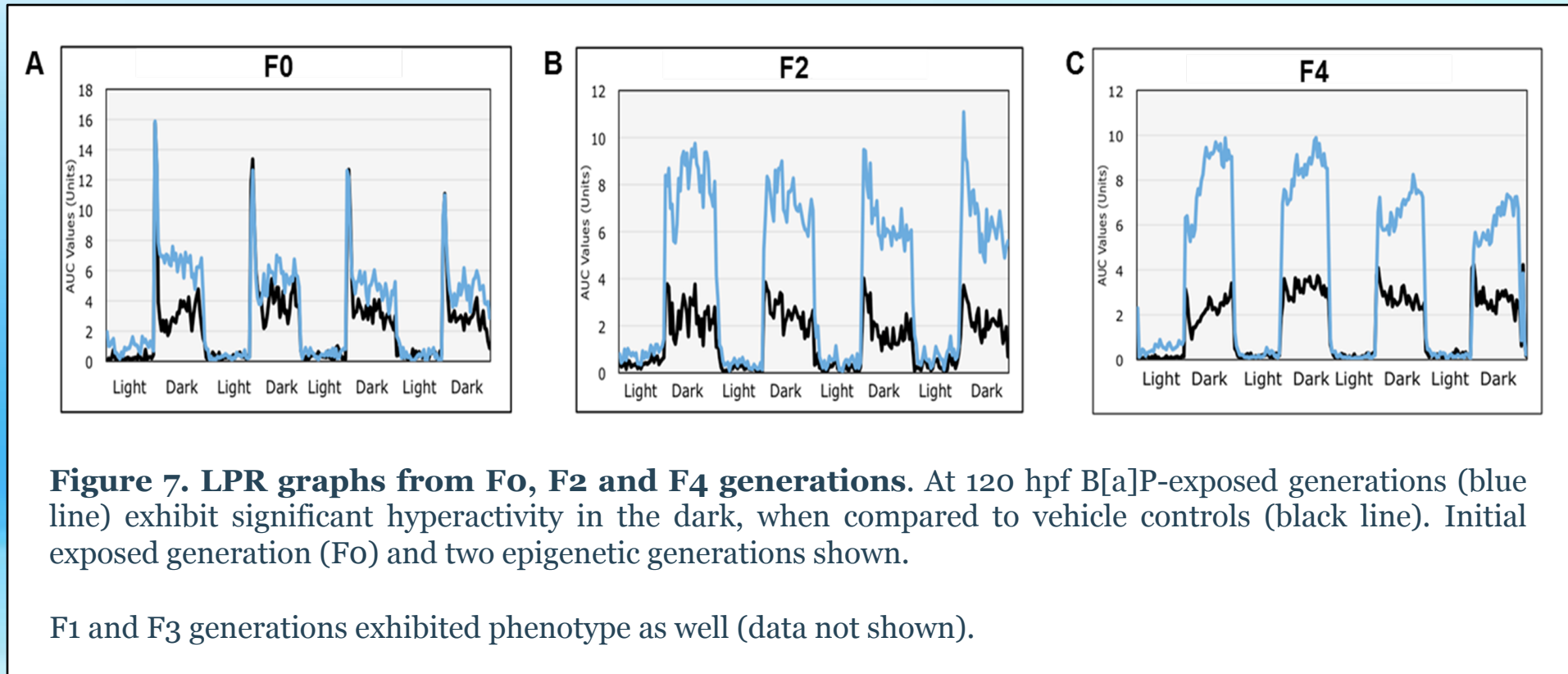
Learning



Determine which PAHs and Mixtures Produce Transgenerational Adverse Outcomes



Example Transgenerational Impacts – B[a]P



To Summarize

- Collect high content bioactivity data in a vertebrate model
- Phenotypic anchoring – for pathway discovery
- Platform for structure-based predictions
- Rapid data for decision making
- Translating zebrafish data:
 - Prioritizing further testing
 - **Highly amenable for mixture assessments - Major effort of the OSU Superfund Research Program**

Acknowledgements

Tanguay Lab

Dr. Robert Tanguay
Dr. Andrea Knecht
Dr. Mitra Geier
Dr. Gloria Garcia
Dr. Michael Simonich
Mike Garland
Laura Holden
Kim Hayward
Prarthana Shankar
Jane LaDu
Hao Truong
Eric Johnson
Greg Gonnerman
Carrie Barton

Engineering Team

Corwin Perrin
Dylan Thrush
David Mandrell
Mushfiq Sarker
Caleb Jephson
Chris Lang
Drew Gabler

Funding (NIEHS)

- P42 ES016465
- RC4 ES019764
- P30 ES000210

Collaborators

NC State University
Dr. David Reif
Dr. Skylar Marvel

OSU

Dr. Susan Tilton
Dr. Kim Anderson
Dr. Staci Simonich

PNNL

Dr. Katrina Waters
Dr. Ryan McClure
Dr. Paritosh Pande

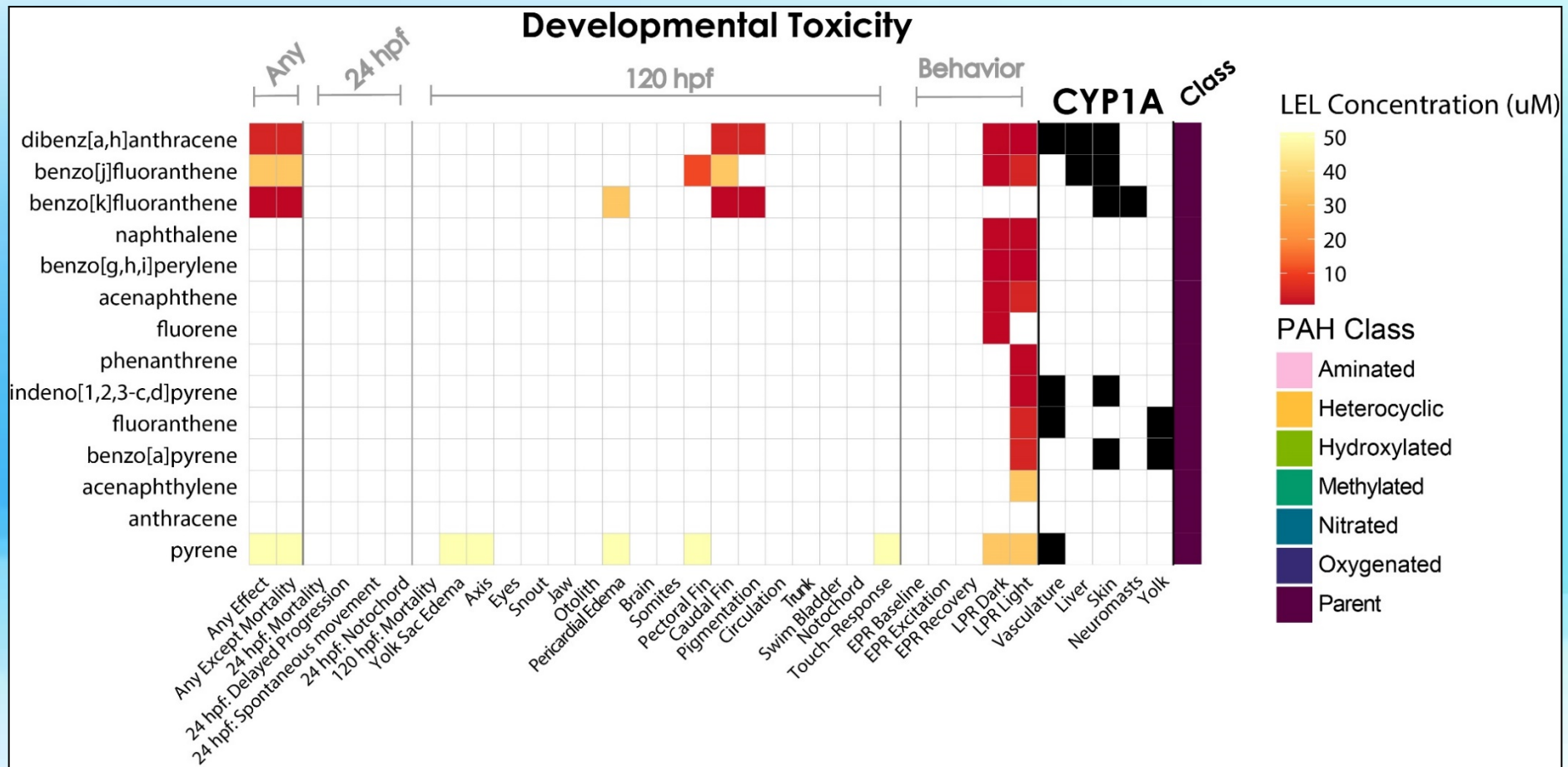


Questions?

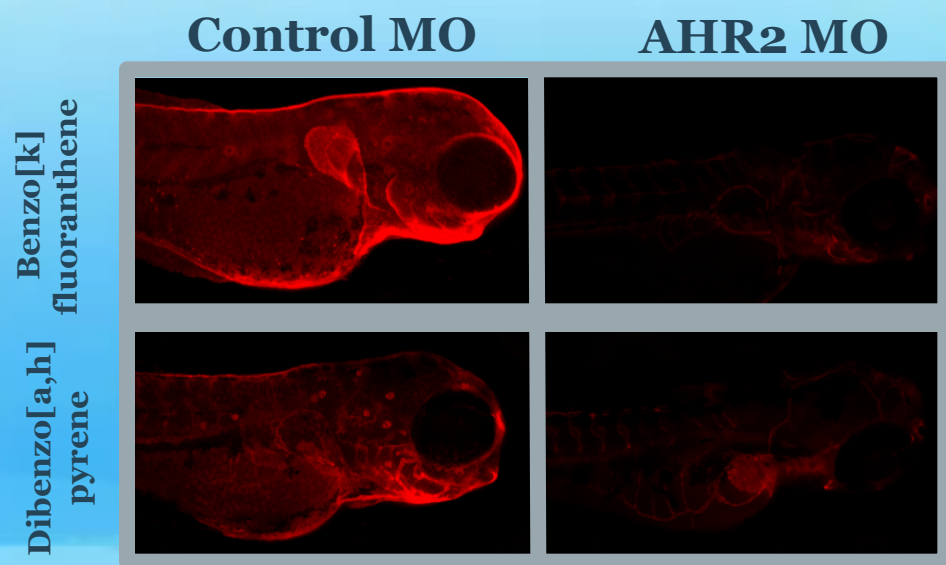


Back up slides

16 EPA Priority PAHs Do Not Reflect Full Range of Effects



New Biomarkers for AHR2 Activation



GeneSymbol	Product	BbF	BjF	BkF	DBahP	DBaiP	Retene
CYP1C1	cytochrome P450	2.38	2.51	3.10	1.40	1.36	4.15
CYP1C2	cytochrome P450	1.26	1.45	1.95	0.44	0.53	3.17
WFIKKN1	WAP, follistatin/kazal, immunoglobulin, kunitz and netrin domain containing 1	1.21	1.96	2.09	1.18	1.19	2.63
CYP1B1	cytochrome P450	1.18	2.13	2.67	1.08	1.32	2.13
CYP1A	cytochrome P450	1.16	2.08	2.18	1.22	1.37	2.06
CABZO1103755.1	N/A	0.59	1.10	1.61	0.62	0.84	2.05
SULT6B1	sulfotransferase family, cytosolic, 6b, member 1	1.40	2.16	2.07	1.24	1.27	1.96
GSTP1	glutathione Stransferase pi 1	1.10	1.82	0.99	0.59	0.54	1.96
DHRS13L1	dehydrogenase/reductase (SDR family) member 13 like	1.16	1.37	0.99	0.82	0.83	1.71
AHRRB	arylhydrocarbon receptor repressor b	0.64	1.28	1.40	0.70	1.10	1.70

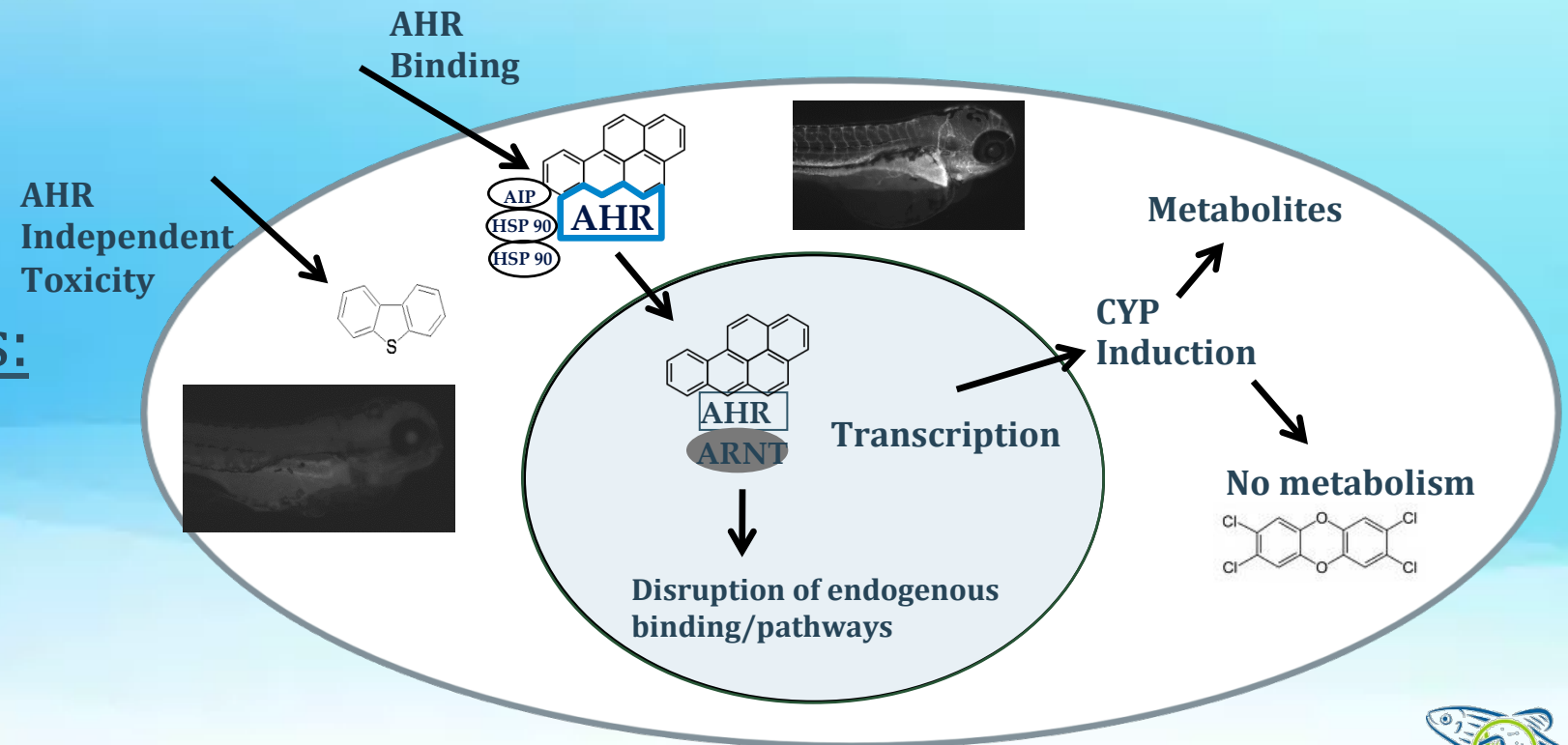
The AHR and PAH pathways of toxicity

Signaling functions:

1. Developmental & homeostatic
2. Adaptive (*cyp1a*)
3. Toxic (adverse effects)

Phenotypic impacts:

1. Development
2. Cardiac
3. Cognitive
4. Reproductive



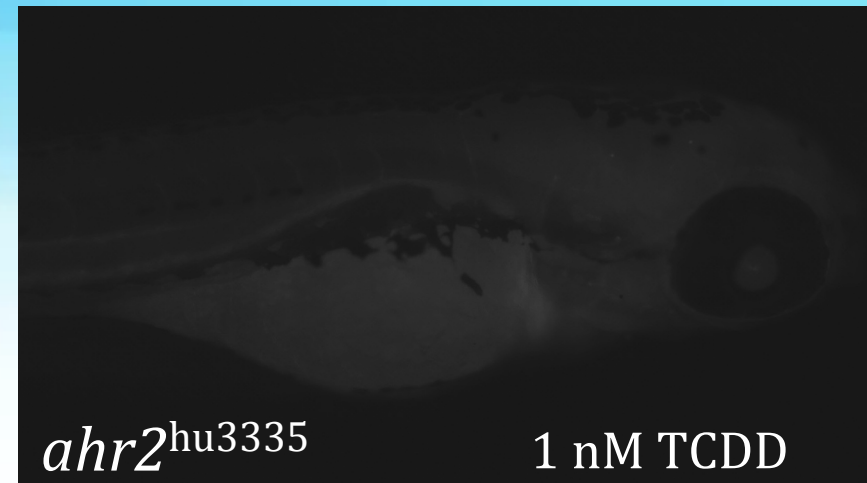
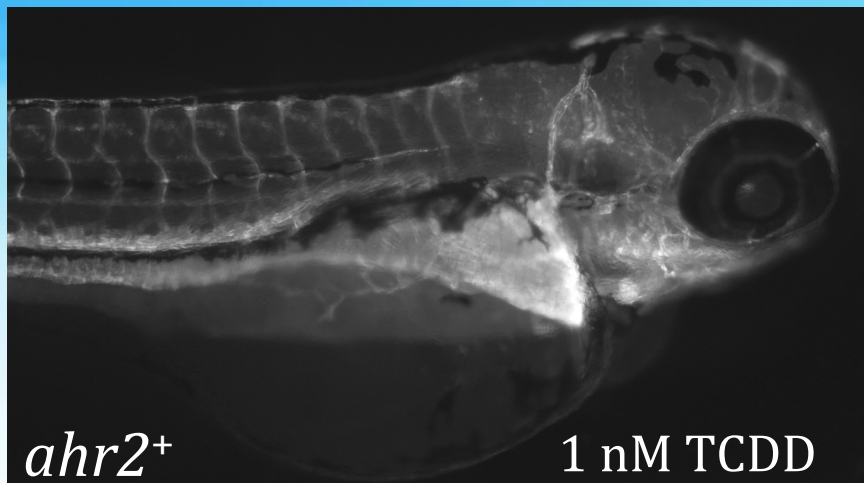
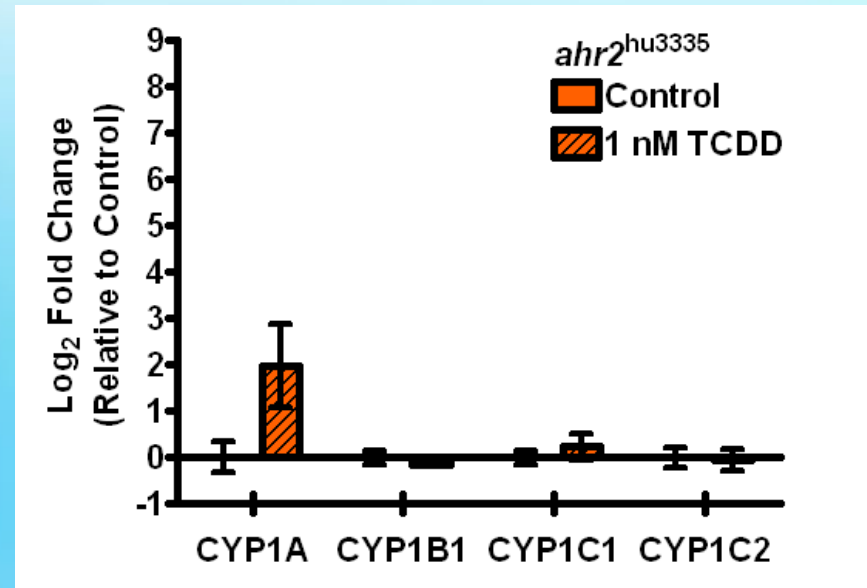
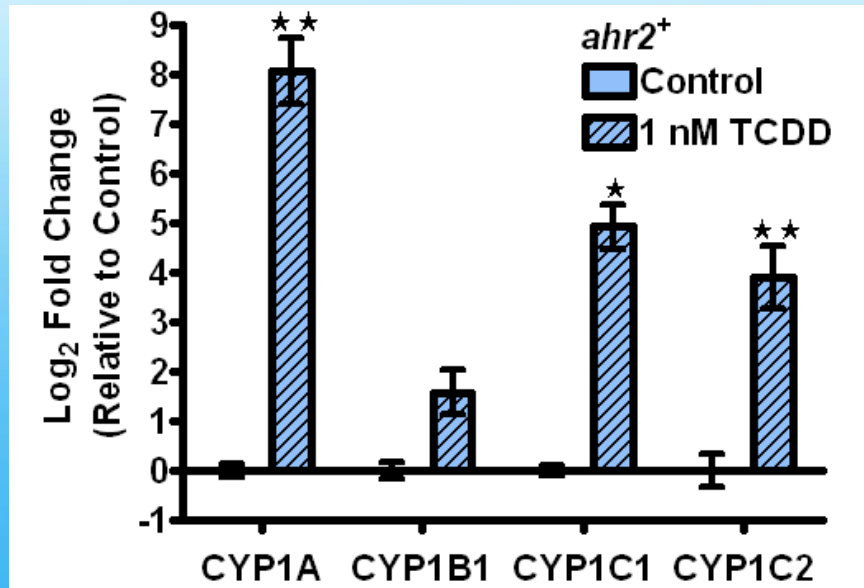
3 Aryl Hydrocarbon Receptor (AHR) in Zebrafish

AHR	Role	CYP1A Expression
AHR2	Primary mediator of toxicity	Vasculature
AHR1A	Deficient in TCDD binding and transactivation activity	Liver
AHR1B	Functional, but no known toxicological role	TBD

Ahr2^{hu3335} Mutants are Resistant to TCDD-Induced Developmental Toxicity



ahr2 Mutants Are Resistant to TCDD-induced CYP Expression Changes



AHR2 importance confirmed in CRISPR/Cas9 line

