

Linking biological and molecular effects of ethynylestradiol exposure in *Xenopus laevis*



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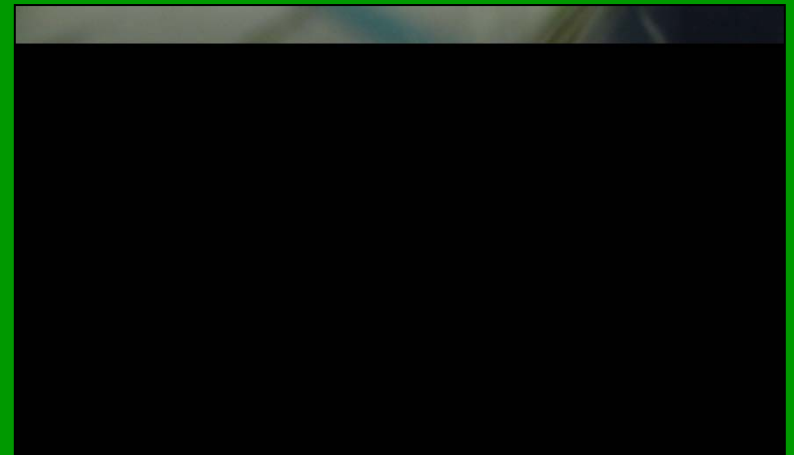
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Introduction

- **'Omic' techniques allow quantification of molecular responses to chemical exposure**
- **Disconnection between the ability to perform a measurement and being able to extract meaning**
- **Need for experiments that explore the relevance of molecular changes**

Introduction

- **Relationship between molecular and biological endpoints**
 - Model exposures with model chemicals
- **Model systems**
 - Xenopus laevis*
 - Ethinylestradiol (EE2)



Introduction

- *X. laevis* is responsive to EE2 exposure
 - Male to female phenotypic sex reversal
 - Environmentally relevant doses
- Recently discovered sex-linked gene
 - X. laevis*: ♀ = ZW; ♂ = ZZ
 - Female specific DM-W
 - Possible to determine genotypic sex

Hypotheses

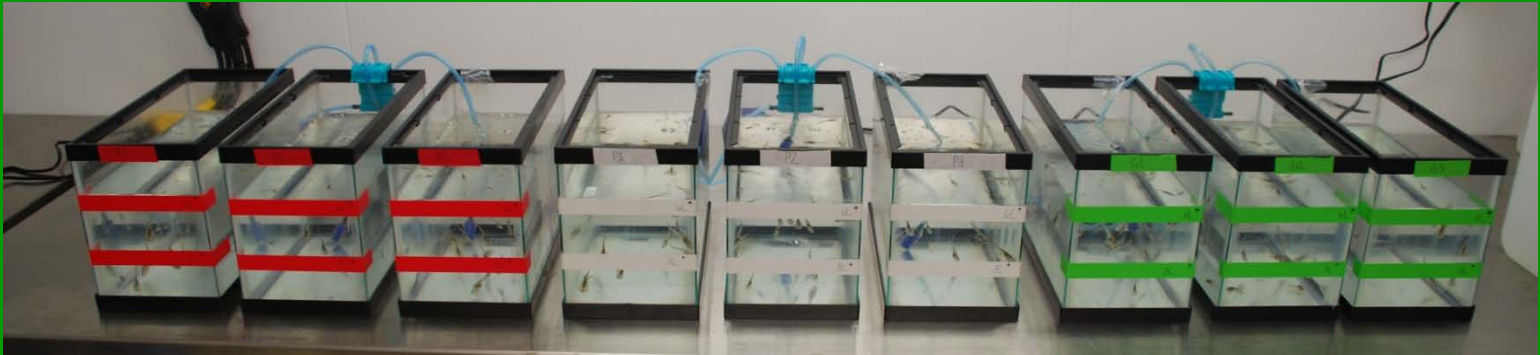
- Exposure to EE2 will alter gene expression in male and female *X. laevis* tadpoles
- Gene expression in sex-reversed genetic males will differ from normal males
 - The biologically relevant phenomenon of sex-reversal will cause characteristic changes in gene expression

Experimental design

- **Treatments (3 replicates/treatment)**
 - FETAX control, 0.0025% ethanol solvent control
 - 0.1, 1, and 10 $\mu\text{g}/\text{L}$ ethynylestradiol
- **50% static renewal**
 - 24h
- **Environmental chamber**
 - 23°C



Experimental design - Sampling



- **50 eggs per replicate tank**
 - Average of 92% of eggs hatched
 - Tadpoles sampled at various NF Stages
 - 15 tadpoles left to complete metamorphosis

- **Focus on NF53 tadpoles today**
 - Undergoing sexual differentiation

Genes of Interest

- ***DM-W***
 - DM domain – zinc finger-like DNA binding motif
 - Located on female-specific W chromosome
- ***DMRT1***
 - Doublesex and mab-3-related* transcription factor 1
 - Autosomal gene present in both males and females
- ***Foxl2***
 - Forkhead transcription factor
 - Involved in the up-regulation of aromatase

Methods: Tadpole Molecular

- **Tail**
 - Genomic DNA extracted
 - Genetic sex via PCR assay
- **Body**
 - Total RNA extracted
 - Reverse transcribed to cDNA
 - RT-PCR



Determination of Genetic Sex

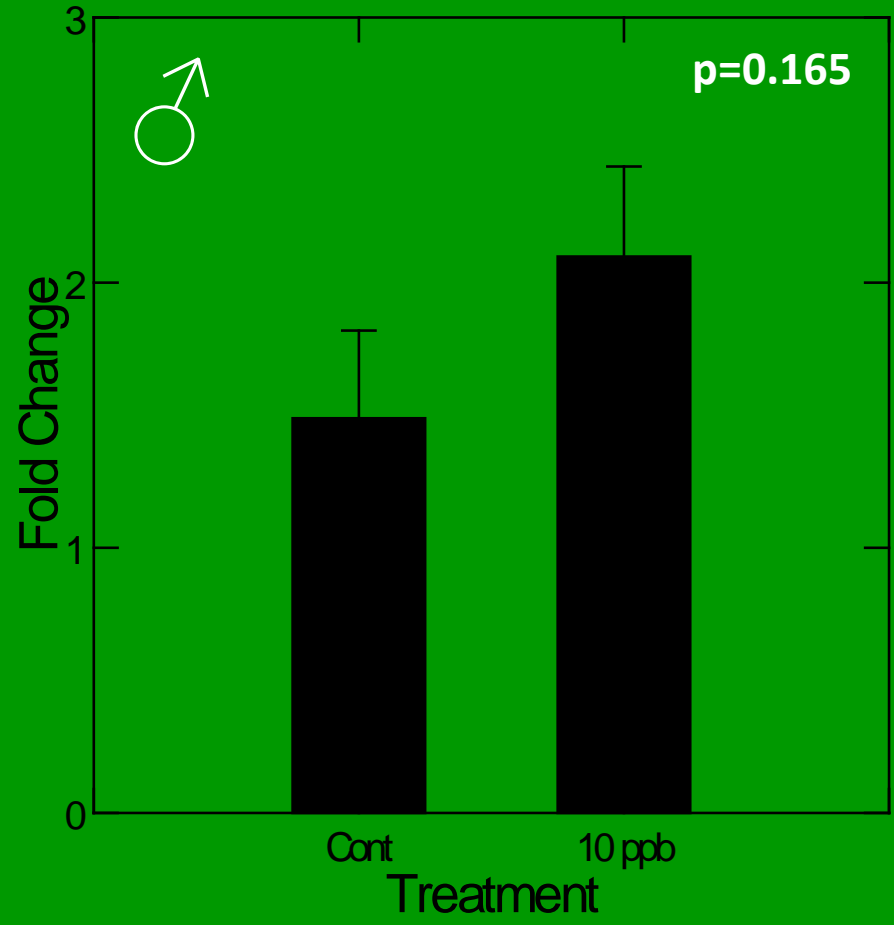
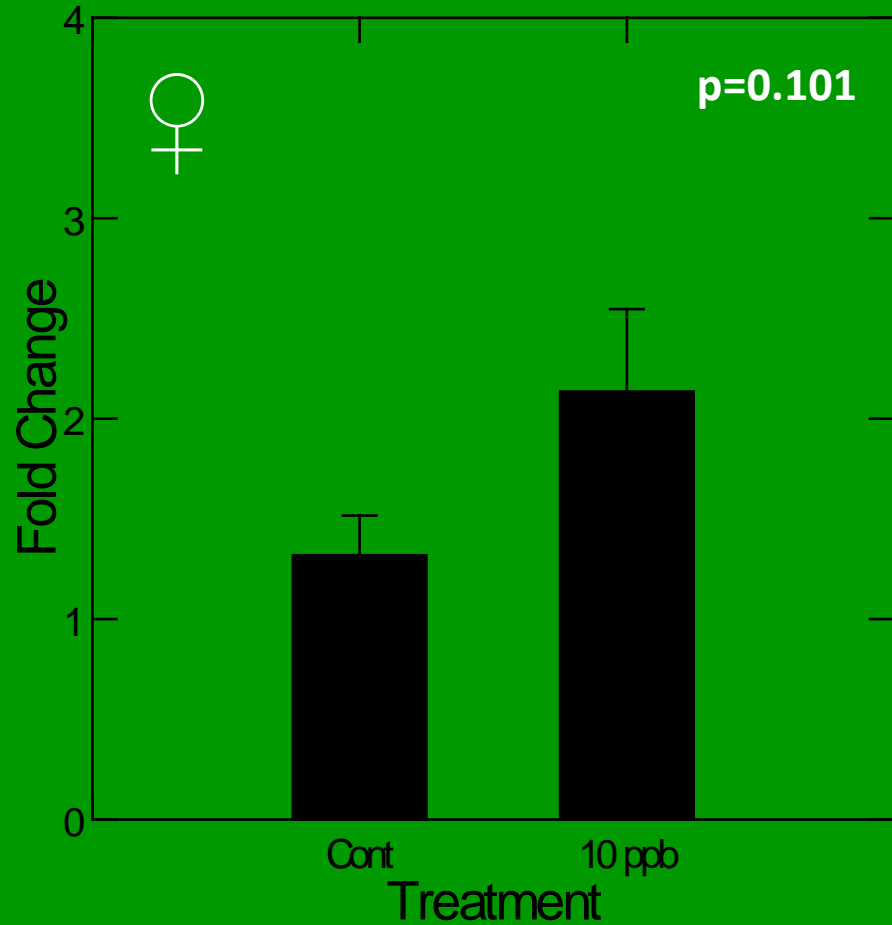
- **Simple PCR assay**
 - Male genome only *DMRT1*
 - Female genome both *DMRT1* and *DM-W*



Real time PCR Analysis

- Tadpoles split into groups
 - Treatment
 - Genetic sex
- Initial analysis performed on *Fox/2* expression in NF53 tadpoles
 - Control and 10 ug/L treatments

Expression of *Foxl2* at Stage NF 53



Conclusions

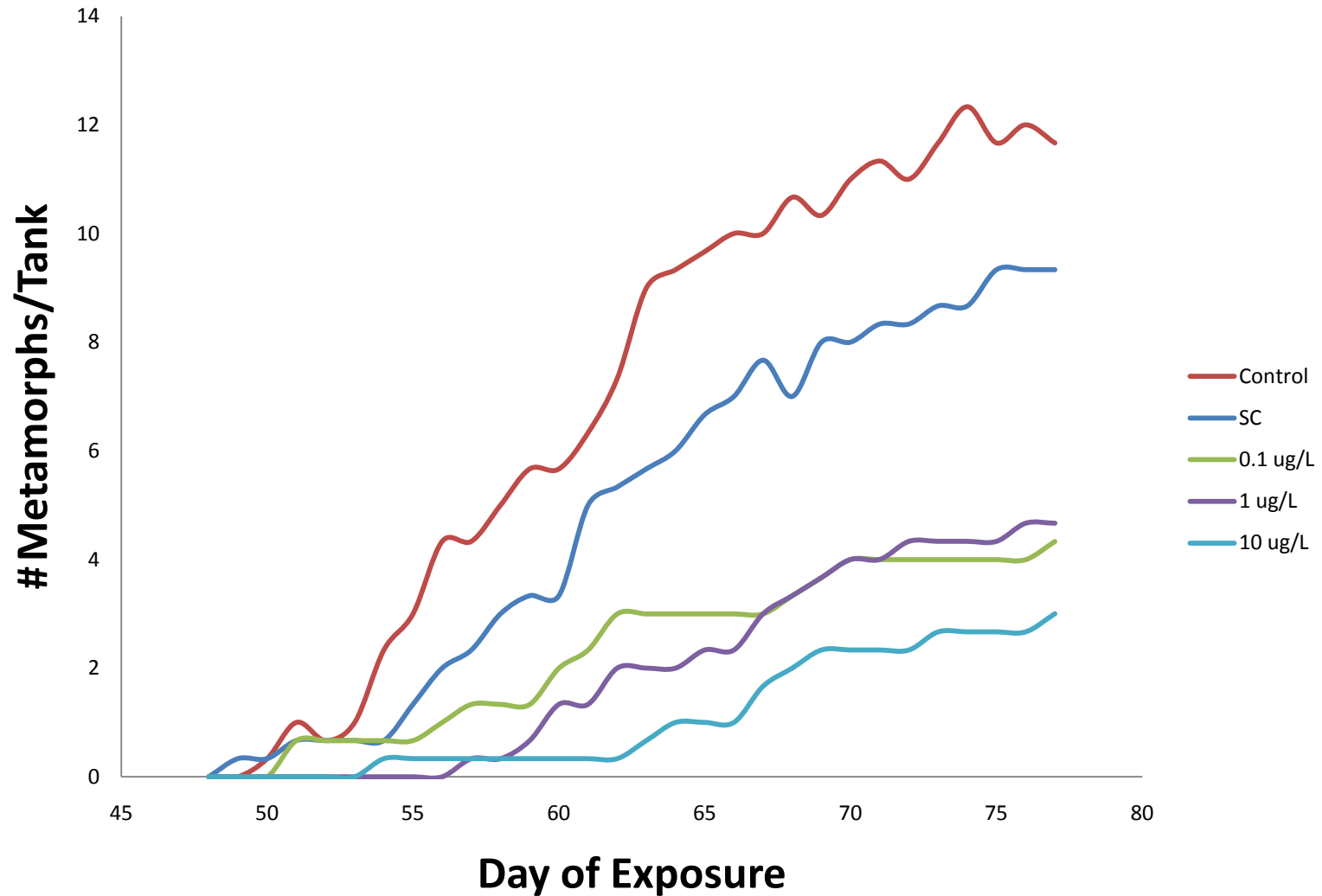
- Genetic sex of *X. laevis* can be determined easily and reliably
- Initial analysis of *Foxl2* expression suggests EE2 exposure does not significantly affect expression in either sex
- *Foxl2* expression didn't differ between sexes in controls

Continuing Analysis

- **Other gene expression endpoints in tadpoles**
 - Sequence by synthesis (454 or Illumina)
 - Multiple stages
- **Multiple endpoints in metamorphs**
 - Gene expression, histology, phenotypic sex
- **Finish the exposure**



Time to Metamorphosis



Thank You

- John Giesy
- Markus Hecker
- Steve Wiseman
- U of S Toxicology Centre
 - ETL
 - Eric Higley
 - Jon Doering
 - Jon Naile
 - Jason Raine
 - Xiaowei Zhang



A photograph of a large number of tadpoles swimming in a tank. The water is slightly murky and greenish. A bright light source is visible in the upper right, creating a strong glare and illuminating the scene. The tadpoles are of various sizes and are scattered throughout the tank. The text "Questions???" is overlaid in the center of the image.

Questions???