

# Characterization of the morphological, phenotypic, and molecular effects of $17\alpha$ -ethynylestradiol exposure during early development in *Xenopus laevis*



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# Introduction

- **Estrogenic chemicals in the environment**
  - Exposure hypothesized to cause adverse effects
    - Feminization/demasculinization of males
  - Wide variety of species are affected by exposure
- **17 $\alpha$ -ethynylestradiol (EE2)**
  - Potent estrogen of environmental concern
  - Present in oral contraceptives
    - Not fully removed by conventional sewage treatment
    - Detectable in surface water

# Introduction

- ***Xenopus laevis***
  - Common laboratory amphibian
  - Exquisitely sensitive to estrogenic exposures during sexual differentiation
    - Male-to-female phenotypic sex reversal
    - Recently discovered sex-linked gene
- **EE2 and *X. laevis* used as model systems**
  - Morphological and phenotypic effects of EE2 exposure
  - Molecular effects underlying sex reversal

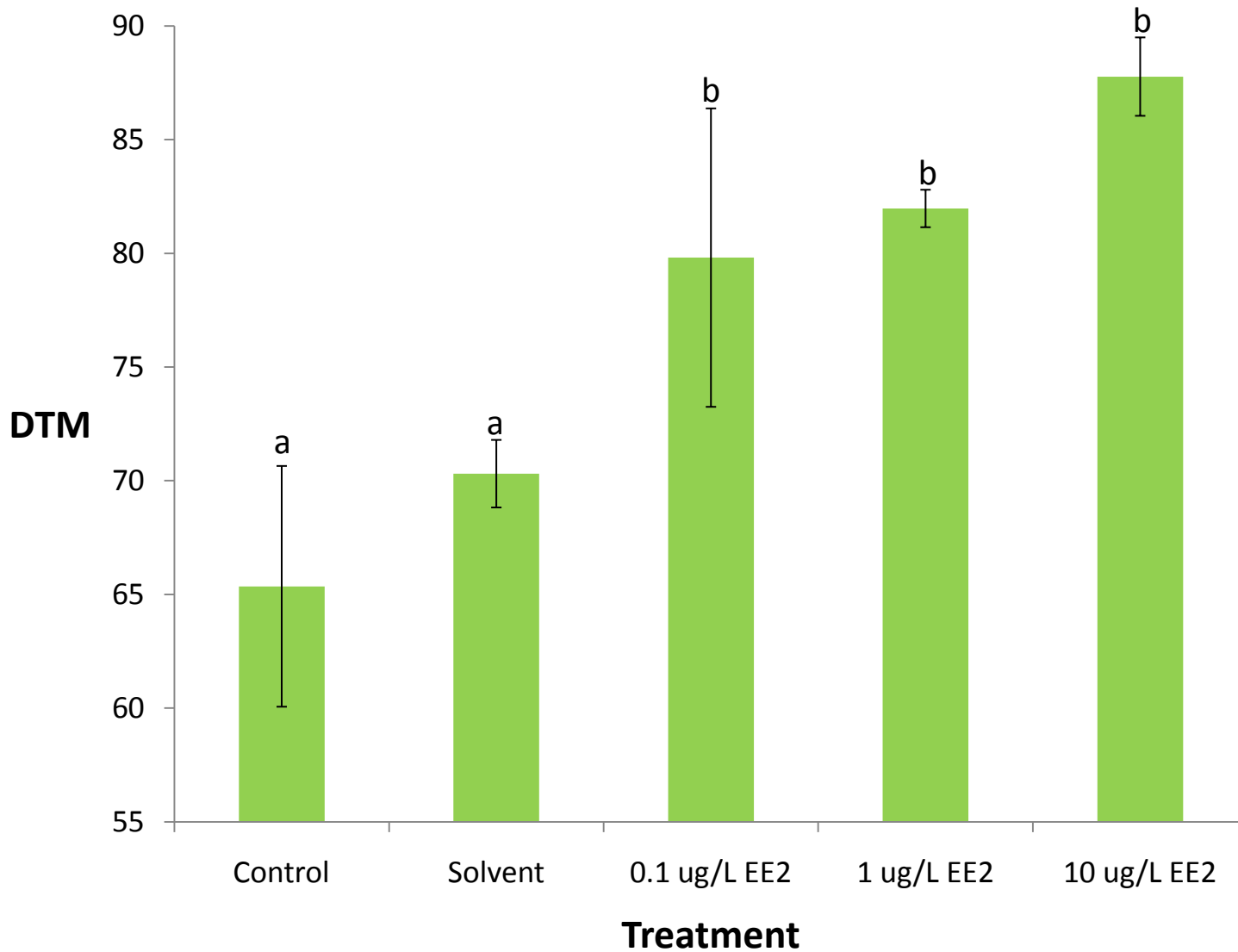
# Experimental design

- **Dosing Regime\***
  - FETAX control and 0.0025% ethanol solvent control
  - 0.1, 1, and 10  $\mu\text{g/L}$  EE2
- **Tadpole samples**
  - Near sexual differentiation
- **Experiment terminated at 96 d**
  - Morphometrics and phenotyping
  - Molecular samples
  - Histological samples



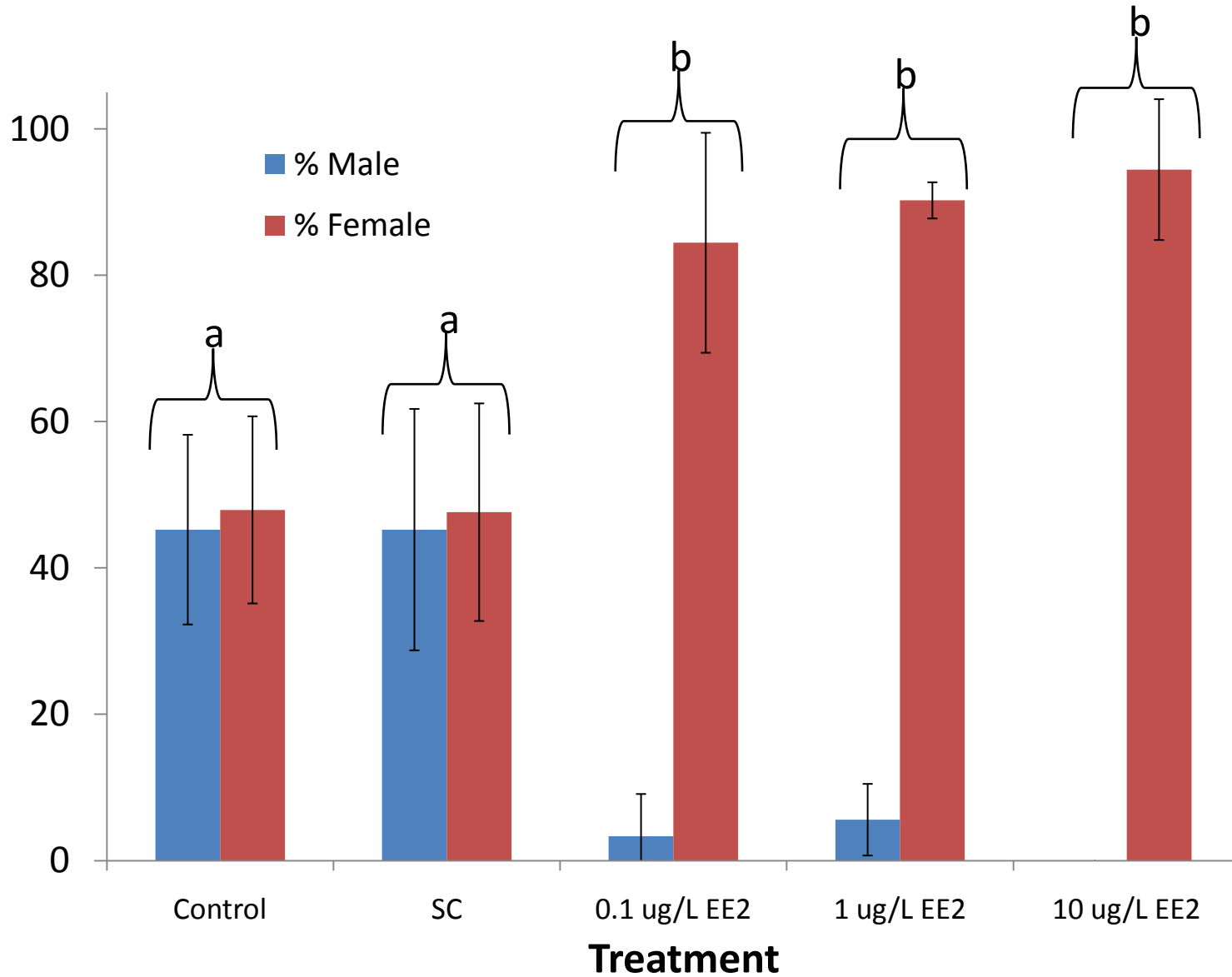
\*Estrogen equivalent concentrations in surface water normally range from 3-30 ng/L

# Days to Metamorphosis



Survival analysis followed by ANOVA, post-hoc Tukey's test; significant differences ( $p < 0.05$ ) denoted by different letters

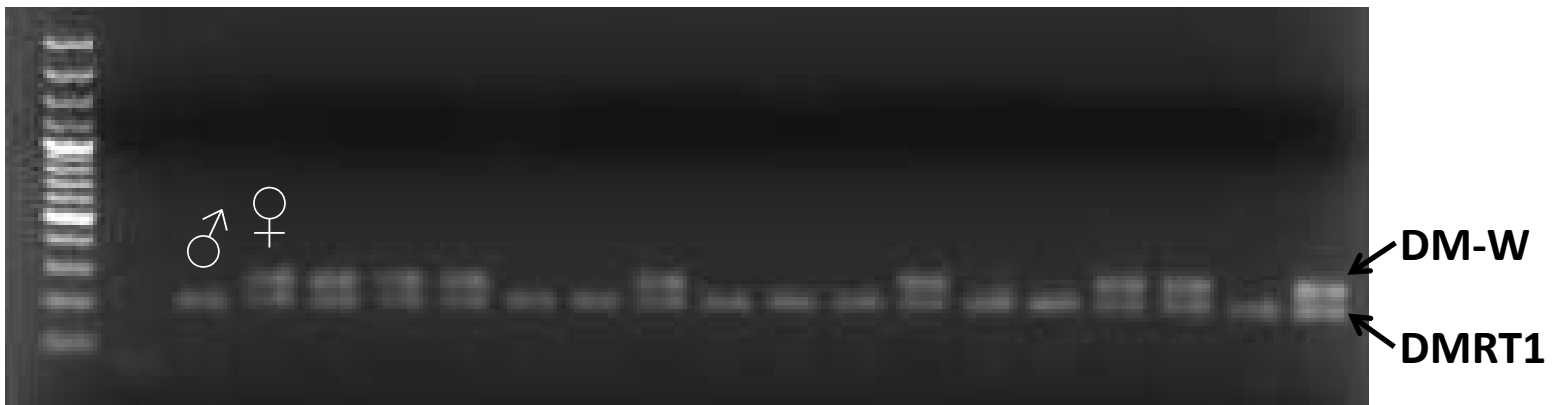
# Phenotyping: Gross Morphology



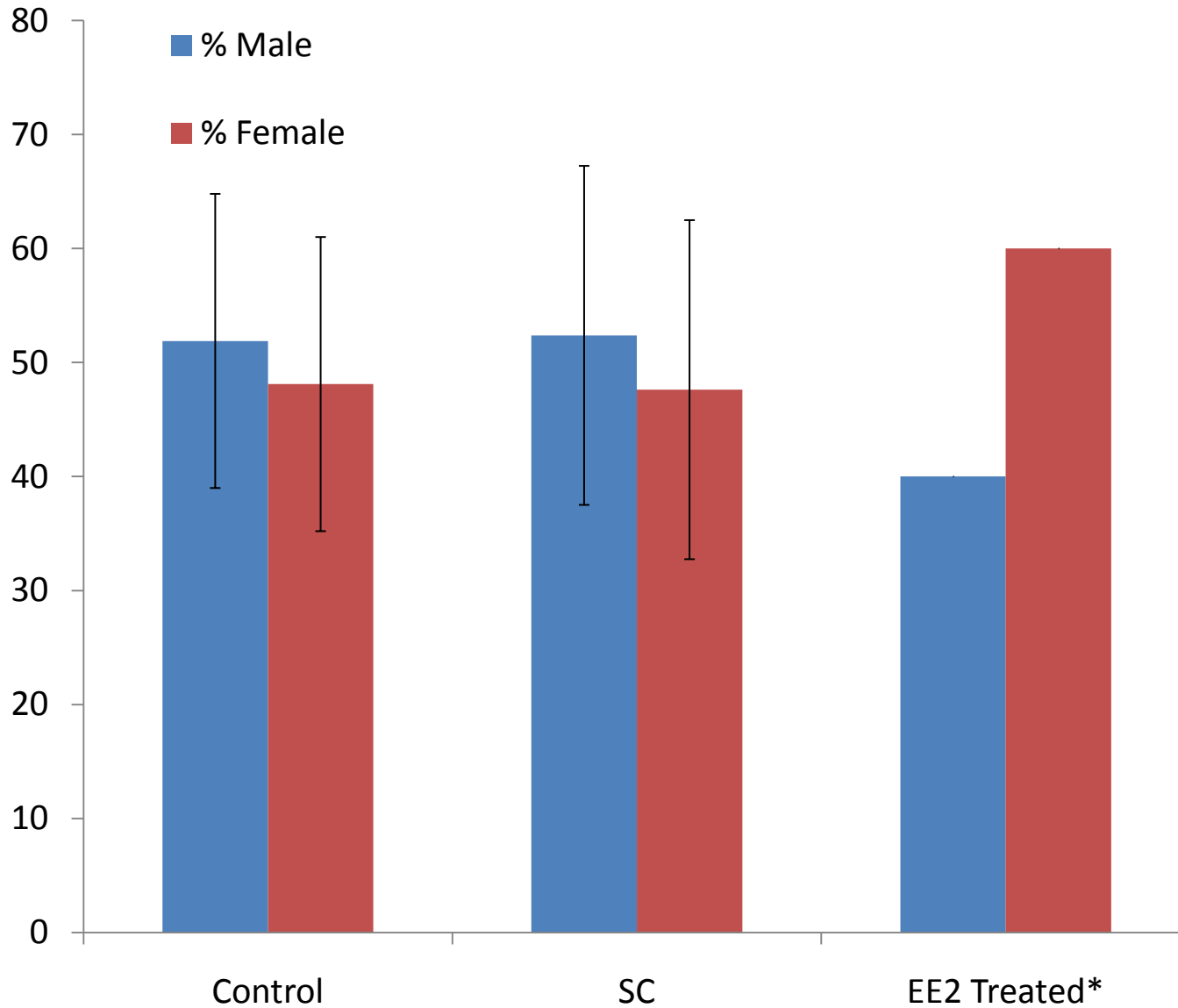
Fisher's Exact Tests; significant differences denoted by different letters

# DM-W Based Genotypic Sexing

- *X. laevis* has ZW chromosomal sex determination
  - ZW female; ZZ male
  - *DM-W* resides on the W chromosome
- Multiplex *DM-W/DMRT1* PCR genotyping
  - Genomic DNA
  - PCR products visualized on a gel

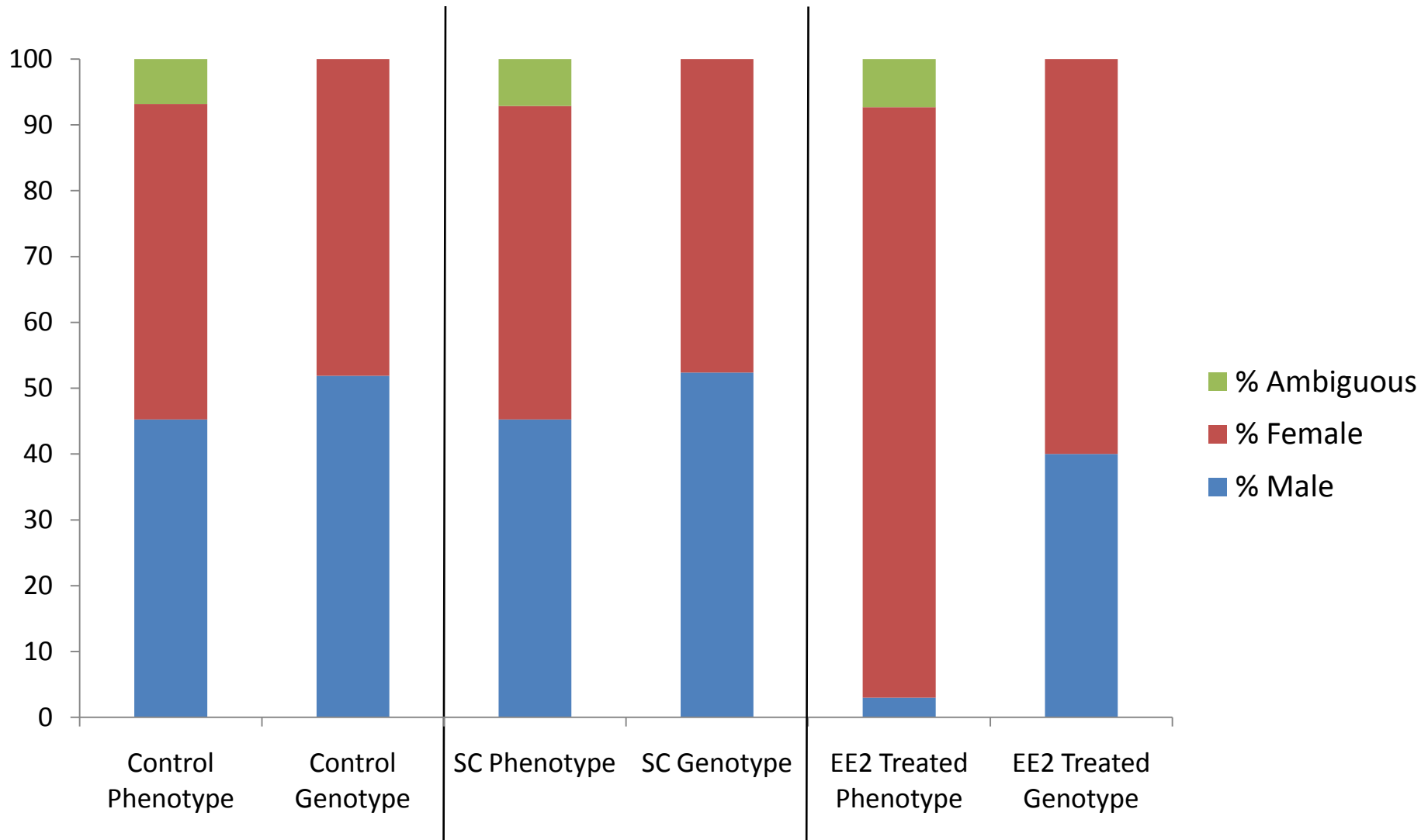


# Genotypic Sex Ratios

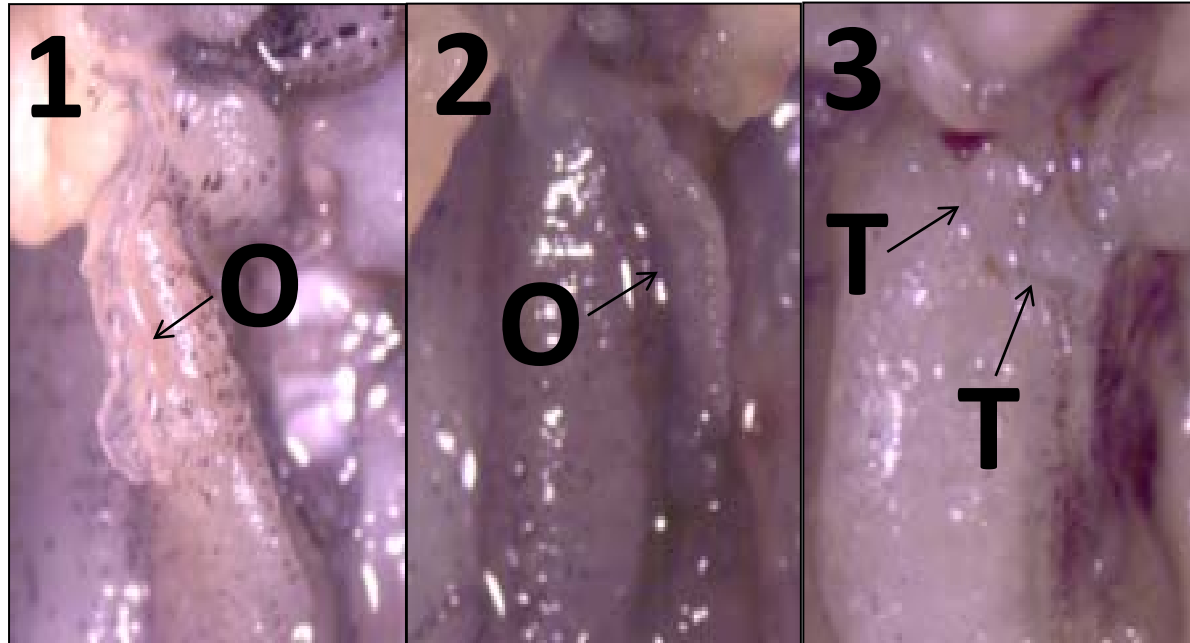


**\*Initial data from a subsample of EE2 treated animals.**

# Initial Comparison of Genotyping and Phenotyping



# Gross Phenotypic Morphology



1. Genetic female
2. Sex-reversed genetic male
3. Genetic male

# Transcriptome Analysis

- **Nieukwoop-Faber Stage 53 Tadpoles**
  - Undergoing sexual differentiation
  - Control and 100  $\mu\text{g}/\text{L}$  EE2 treated animals
    - Male genotype
- ***Illumina* Sequencing**
  - RNA Seq
  - Single-end read
  - 75 bp read length



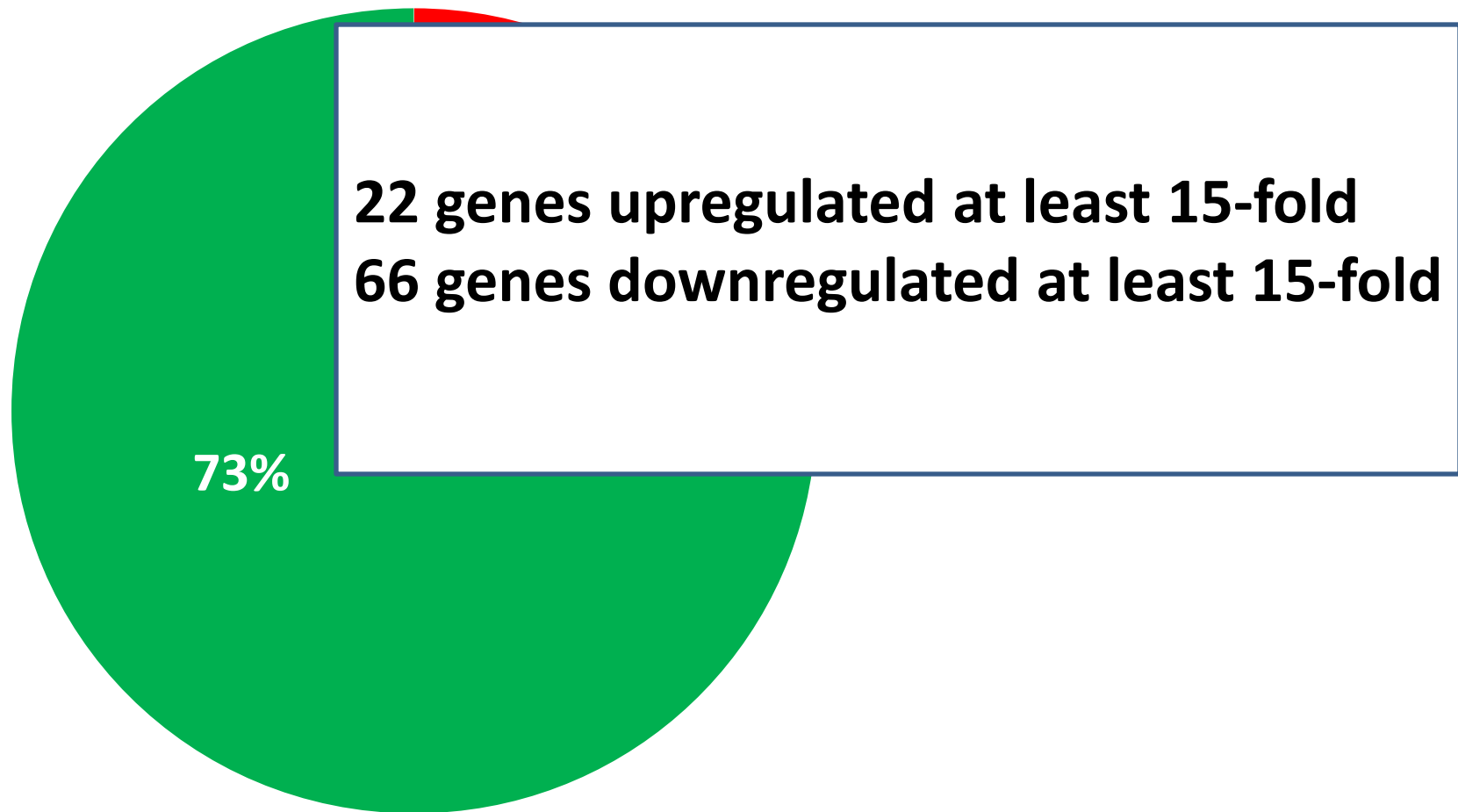
# Initial Transcriptome Analysis



- **CLC Genomics Workbench**
  - Reads filtered and trimmed
  - Mapped to *X. laevis* published mRNAs
  - Expression analysis
  
- **General Statistics**
  - 70% of reads mapped to an mRNA transcript
  - 95% of transcripts were detected at least once

# Transcriptome Analysis

- Overview of changes



# Types of Genes Impacted

- **Up-regulated**
  - Estrogen/steroid hormone metabolism
  - Cardiac/skeletal muscle contraction and growth
  - DNA repair
- **Down-regulated**
  - Redox metabolic activity
  - Axonogenesis and synaptogenesis
  - Metabolism of neurotransmitters

# Potential Genes of Interest

<b>Gene</b>	<b>Fold Change</b>
<b>Estrogen sulfotransferase (sult1e1)</b>	<b>+19</b>
<b>Frizzled-related protein (frzb-1)</b>	<b>+24</b>
<b>Troponin T Type 3 (tnnt3)</b>	<b>+37</b>
<b>Cu-Zn superoxide dismutase (sod)</b>	<b>-23</b>
<b>Synaptosomal associated protein 25 (snap-25)</b>	<b>-85</b>
<b>Sulfotransferase 4a1 (sult4a1)</b>	<b>-23</b>

# Biological Relevance of EE2 Exposure

- **Male-to-female sex reversal**
- **May impact individual fitness**
  - Delayed metamorphosis and smaller size
- **Changes in the male transcriptome at sexual differentiation**
  - Estrogen/hormone metabolism
  - Other processes



# Additional Ongoing Analysis

- **Histology of gonads**
  - **Gross morphology of small animals unclear**
- **Parallel wood frog experiment**
  - **Native, non-model species**

# Acknowledgements

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