Estradiol in the environment have been implicated in causing a variety of adverse effects in exposed organisms. Exposure to estrogens, especially at critical times during sexual development, can cause feminization and/or masculinization of males of many amphibian species. 17α-ethynylestradiol (EE2), a hormone analog commonly used in oral contraceptives, is an estrogenic chemical of particular environmental concern due to its great potency and ubiquitous release in sewage treatment plant effluents. In the current experiment, EE2 was used as a model compound to test exposure with wood frog (Rana sylvatica) larvae to attempt to discover links between biologically relevant effects and molecular events. The wood frog is native to temperate areas in North America and common throughout much of its large range. Wood frogs are sensitive to exposure during sexual development and undergo male-to-female sex reversal when exposed at adequate concentrations. To cover the full spectrum of sex reversal, wood frog tadpoles were exposed to 1, 10, 100 µg/L EE2 (scrambled). Tadpoles were sampled at Gosner Stages 26, 30/31, 35/36, and 35/36 to determine the effects of EE2 on tadpoles that are undergoing sexual differentiation. The metamorphs were grown until metamorphic climax or 108 d. Total mortality over the course of the experiment ranged from 3.1-10%. There were no significant treatment related differences in tadpole weight or snout-vent-length (SVL) at any time during the experiment. In addition, there was no difference in time to metamorphosis between treatments. Sex ratios were significantly female-biased in all EE2 treatments. Male-to-female ratios were 1.5:1, 3.4:1, and 1:1 for EE2 (SC), 1, 10, and 100 µg/L EE2, respectively, 100% of the individuals in the 100 µg/L EE2 treatment developed as phenotypic females. In short, these results indicate that treatment with EE2 caused male-to-female phenotypic sex reversal. The data from all the EE2 treatments were analyzed with the current in the study. Histological analysis of gonads to verify phenotypic sex and assign phenotypes to amphibious individuals is ongoing. Transcriptome analysis of tadpole RNAsamples using genome by synthesis’ technology will begin in late 2010. Report of the transcriptomes of exposed and unexposed individuals should determine which genes in expression responsible for sex reversal.

**Introduction**

- Exposure to potent estrogens, including 17α-ethynylestradiol (EE2), can interfere with sexual differentiation in some frog species. Sexual reversal appears in the presence of estrogenic xenobiotics in the aquatic environment.
- Wood frogs are sensitive to EE2 exposure and are used as an appropriate model organism to study sexual development.
- The wood frog (Rana sylvatica) is a common amphibian native to regions of temperate North America.
- Wood frogs range further north than most other amphibian species and are an integral part of many northern ecosystems.
- Disruption of sexual development, including sex reversal, is considered a biologically relevant phenomenon in amphibians.
- The wood frog can be used as a model species in studies of estrogen-induced sex reversal.

**II. Experimental Design & Maintenance**

- **Control and 0.0025% ethanol solvent control (SC)**
- **Exposure Scenario**
- **50% static water renewal every 24 h**
- **Tadpoles sampled at 4 time points in sexual development**
- **Gosner Stages 26, 30/31, 35/36, and 35/36**
- **Tadpoles subjected to sexual development**
- **Gosner Stage**
- **15 tadpoles grown to metamorphic climax or 108 d, whichever occurs first**
- **Experiment maintenance**
- **Tadpoles fed ad libitum once daily**
- **Temperature maintained at 22°C**
- **DO, pH, conductivity and temperature monitored daily**
- **ammonia, nitrite, and nitrate concentrations monitored weekly**

**Results**

- **I. Total Mortality**
  - Mortality ranged from 3.1-10.5%
  - No significant differences between treatments
- **II. Weight and length during development**
  - Weight and length increased over time
  - At metamorphic climax, all tadpoles were similar in size
  - Average weight ranged from 0.65-0.75 g
  - Average SVL ranged from 10.6-11.3 mm
- **III. Days to Metamorphic Climax (DTM)**
  - Average DTM ranged from 90.5-94.5 d
  - On average, tadpoles took 92.5±1.1 d to reach metamorphic climax

**V. Phenotypic Sex Ratios: Gross Morphology**

- *Some individuals could not be assigned a definite phenotype and were classified as ambiguous.*
- Some ambiguous animals were probably intersex, but most were simply too small to assign a phenotype.
- Histological analysis is needed to assign these animals a phenotype.
- *Thus, the ambiguous animals were removed from the sex ratio analysis.*
- Sex ratio analysis was used to determine differences between treatments.
- For all EE2 treatments, sex ratios were significantly female-biased compared to the expected 1:1 sex ratio.
- *Some ambiguous animals were probably intersex, but most were simply too small to assign a phenotype.*
- Histological analysis is needed to assign these animals a phenotype.
- *Thus, the ambiguous animals were removed from the sex ratio analysis.*
- Sex ratio analysis was used to determine differences between treatments.
- For all EE2 treatments, sex ratios were significantly female-biased compared to the expected 1:1 sex ratio.

**Discussion & Ongoing Research**

- Multiple biological endpoints were examined, including weight and SVL at various time points, time to metamorphosis, and sex ratios.
- **Animals were considered to have reached metamorphic climax** when certain morphological criteria were met.
- **Sex reversal is a biologically relevant event.**
- **Samples from the current experiment can now be examined with molecular techniques to determine the molecular events involved in sex reversal.**
- **Since the wood frog genus/Rana/spicifical is poorly characterized, open format techniques such as illumina or 454 transcrispome sequencing may be more informative than traditional technologies.**

**Ongoing Research**

- **Histological analysis of the gonads to confirm or assign phenotypic sex to individuals from the experiment.**
- **Molecular analyses**
  - Determination of a marker(s) of genetic sex in the wood frog
  - *Sequence by synthesis’ technology will be used to determine differences in gene expression between EE2 exposed and unexposed individuals.*
  - RT-PCR verification of genes that are differentially expressed in EE2 exposed and unexposed individuals.