

## YY Project Update May 2020

### Chad Teal

At the University of Arizona's Fisheries Lab we have finished two sex reversal trials (male to female) on Red Shiner *Cyprinella lutrensis*. Our first sex reversal trial included two 60-day treatments which involved feeding developing Red Shiner 50 mg 17- $\beta$  estradiol (E2)/ kg of diet and 100 mg E2/kg of diet starting at 2 days post hatch (DPH) to 62 DPH. We saw a significant reduction in the survival of the 100 mg E2 treatment group compared to the control and 50 mg E2 treatment group ( $P < 0.05$ ). E2 treated groups had a higher growth rate ( $P < 0.05$ ) than the control and E2 treatment groups had a significantly higher proportion (81.1% - 93.5%) of phenotypic females ( $P < 0.05$ ) upon maturation. Histological analysis of gonads in developing Red Shiner showed that treatments ended when the fish were too young to sex reverse all males in the treatment groups. Our study found that Red Shiner sex reversal treatments should begin when fish are 20 DPH and extend to at least 100 DPH. Based on the results of the first sex reversal trial, we conducted a second sex reversal trial with longer treatment durations that included a third treatment group fed 150 mg E2/kg of diet. We are currently growing these fish to maturity to assess the success of this second sex reversal trial. Ensuring 100% sex reversal could be vital for developing a Trojan sex chromosome strategy if Red Shiner exhibit a ZZ/ZW sex determination system in which the female is the heterogametic sex.

We have finished the first sex reversal trial on Green Sunfish *Lepomis cyanellus*. This sex reversal treatment's duration and dosage was based off successful sex reversal treatments in Bluegill *Lepomis macrochirus*. We are currently growing out these fish to maturity to assess the extent that these fish have been sex reversed. Preliminary histological analysis on the gonadal development of Green Sunfish shows similarities with the gonadal development of Bluegill. Based on this histology, I believe we will have complete sex reversal for Green Sunfish in their first trial.

We have built two restriction-site associated DNA sequencing (RAD-Seq) libraries in order to obtain genetic data for the development of sex identification markers in Red Shiner and Green Sunfish. We have developed candidate sex ID primers for Green Sunfish that will undergo testing this year. We have almost finished the bioinformatic process for identifying candidate sex ID primers in Red Shiner. I expect to have candidate sex ID primers for Red Shiner by the end of Summer 2020. Once we have tested the sex ID primers for both species, we can identify their sex determination systems and conduct the next phase of breeding and sex reversals to develop Trojan Y chromosome carrying TYC broodstock

We have also developed lab-based spawning and larval rearing protocols for both species. These methods will be essential for the development of TYC broodstock at other research facilities and hatcheries.