



**Research Work Group
Virtual Meeting Minutes
December 8, 2020**

Members of this Work Group include: Tom Arsuffi, Conrad Lamon, Chad Norris, and Floyd Weckerly

At this meeting, the following business may be considered and recommended for committee action:

1. **Call to order:** 9:05am – All group members were present.
2. **Virtual meeting logistics and meeting overview.** – Chad Furl briefly explained virtual meeting logistics.
3. **Public comment.** – There was none.
4. **Presentation and discussion of methodology proposed for the Refugia research project:** *Texas wild-rice genetic management*, presented by Lindsay Campbell, USFWS staff.

Dr. Lindsay Campbell presented over the proposed Texas wild-rice genetic study for 2021. Since the last genetic evaluation in 2012, both the wild population and the refugia population of TWR have undergone considerable changes. The implementation of TWR replanting in the San Marcos River has helped TWR coverage nearly triple since 2012, and the captive population has also expanded through the EAHCP Refugia program. The changes to both the wild and refugia populations warrant a new genetic assessment to ensure that the refugia population reflects that of the wild or maximizes genetic diversity. Four hundred TWR samples will be collected from the San Marcos River with sample collection evenly distributed from top to bottom of TWR distribution. Samples will also be collected from the EAHCP Refugia TWR populations. All samples will be sent to Southwestern Native Aquatic Resources and Recovery Center and analyzed similar to the previous genetic evaluation in 2012. The genetic evaluation will assess whether the EAHCP Refugia population is accurately capturing the genetic makeup of TWR in the wild, and if the refugia population has significant amounts of plants with duplicate genetic makeup. Tom Arsuffi asked for clarification on genetic duplicates and Lindsay explained that TWR can grow asexually, so there are chances of collecting the same genetic makeup from two different TWR stands. This study will also provide an idea of the percent of TWR genetic duplicates in the river.

5. Presentation and discussion of methodology proposed for the Refugia research project:

Continuation of 2020 study: Factors affecting pupation in the endangered Comal Springs Riffle Beetle, presented by Weston Nowlin, Texas State University.

Dr. Weston Nowlin provided an update on a two-year study that investigated the factors affecting pupation in the Comal Springs Riffle Beetle (CSRB). This study was delayed due to COVID restrictions and is still on going with expected completed by February 2021. The first year consisted of two experiments: 1) Assess if there are differences in biofilm composition from the wild versus captive and if this effects CSRB pupation rate, and 2) Are there differences of conditioned material in the presence/absence of CSRB. In the first experiment, no differences in pupation rates were observed across treatments (wild vs. captive biofilms) and larval mortality was 10-15%. The sequenced biofilms did show differences in composition with substrate type (cotton lure, leaves, wood) and source location as attributing factors, and the captive biofilm was less diverse compared to the wild. In experiment 2, preliminary results suggest low pupation rates across all treatments with experimental runs completed in February 2021. In the second-year study, that is still on going, the type of incubation chamber and handling time of larvae are being evaluated as factors affecting CSRB pupation rates.

Dr. Arsuffi had a comment on whether CSRB are scraper or shredders on how they collect their food, and that this should be considered during studies. CSRB might be consuming the substrate type (wood, leaves) in addition to the biofilm accumulated on the surface. As of yet, there is no definitive answer on whether CSRB are a scraper or shredder, although, diet analysis has suggested they are more of a scraper.

6. Presentation and discussion of methodology proposed for the Refugia research project:

Continuation of 2020 study: Identify conditions affecting pupation rates in the endangered Comal Springs Riffle Beetle, presented by Dr. Ely Kosnicki with Biowest, Inc.

Dr. Ely Kosnicki presented over the second year finding on his two-year study to identify factors affecting CSRB pupation rates in captivity. During the first year, Ely was able to identify large flow through tubes with late stage larvae were best for producing CSRB pupae. In the second year, Ely assessed pupation rates using the flow through tubes in different orientations, horizontal and vertical positions. Ely also assessed pupation rates using small and medium sized CSRB larvae in the flow through tubes. Across all treatments, pupation rates were roughly 20%. Tracking the life cycle of CSRB in captivity, Ely found ~14% survival from adult-larvae-pupation-eclosion-adult. A final report of his findings will be produced in late December 2020.

7. Presentation and discussion of methodology proposed for the Refugia research project:

F1 Level Production of Comal Springs Riffle Beetle, presented by Lindsay Campbell, USFWS staff.

Dr. Lindsay Campbell presented over this past year's study to assess CSRB nutrition using nutritional pellets. After completing the experiment, the premade pellets were not a viable

option due to the fungus growth that occurred on the pellets only after a few days in the CSRB containers. The Refugia will continue to use conditioned cotton, leaves, and wood as substrates for CSRB food. Lindsay then presented over the proposed two-year study to occur 2021-2022 that will assess F1 level production of CSRB. In this study, several factors will be evaluated to increase production of captive raised CSRB. The density of CSRB larvae within a traditional flow through tube will be evaluated to determine if more larvae can be placed within each tube without increased mortality rates. A modified flow through tank design will also be tested. This modified tank design should allow staff to check, clean, and conduct a CSRB census more efficiently than the current tube design. This study will test if the modified tank design can also increase survival and pupation rates compared to the current tube design. Another factor that will be evaluated is water source for CSRB holding containers and conditioned material (leaves, wood, cotton) that is used for CSRB food. In this study, Comal spring water will be brought onto station and placed in a recirculating system. The CSRB pupation rates will be compared between the recirculated system using Comal Spring water versus the system using on site facility spring water. The differences in biofilm produced in each system will also be evaluated. A comment was raised by Dr. Arsuffi that differences in conditioned material should also be tested. Finally, the effects of certain bacteria on CSRB survival will be evaluated. Two bacteria have been identified in the refugia, *Staphylococcus spp.* and *Chromobacterium*, that might be reducing survival rates of CSRB. This study will expose certain CSRB to the bacteria and compare survival rates to CSRB not exposed.

9. Consider possible future meetings, dates, locations and agendas.
10. **Questions and comments from the public.** – No public comments were made.
11. **Adjourn.** – Virtual meeting adjourned at 11:22am