



*Meeting Minutes for the Research Work Group held on December 6, 2021 at 1pm.*

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

1. Call to order – Establish that all members are present or represented. *Call to order at 1:03pm and the meeting recording was started. All members were present.*

2. Virtual meeting logistics and meeting overview.

3. Public comment. *No comments from the public.*

4. Review of Research Workgroup Goals and an overview of the 2021-2022 research activities. *Chad Furl reviewed the goals of the Research Workgroup to oversee the research program of the EA Refugia. One primary goal was to add two additional species to fully functional refugia. Texas wild rice and fountain darter are considered fully functional refugia species and the San Marcos salamander and the Comal Springs Riffle Beetle were selected as the two additional species. Chad Furl discussed the Refugia Research workplan for 2021 and the studies completed this year, and reports can be found in the 2021 EAHCP Annual Report. Then Chad Furl presented the proposed 2022 EA Refugia Research projects that included continued research on CSRB pupation, genetic analysis on the CSRB, continuation of the San Marcos salamander reproduction, tagging small bodied salamanders using p-Chip tags, testing Bd treatment for aquatic salamanders, and fountain darter tissue catalog and DNA variability. Chad then introduced Katie Bockrath.*

*Katie Bockrath introduced herself and gave a brief description of her background.*

5. Presentation and discussion of results from the 2021 Refugia research project: *Genetic Assessment of Texas wild-rice (Zizania texana), presented by USFWS staff.*

Purpose: To provide the Research Work Group with the opportunity to hear and discuss the results of the genetic analysis for wild and refugia populations of Texas wild-rice and their management implications.

Action: To obtain input from the Research Work Group regarding the results from the genetic analysis completed on wild and refugia populations of Texas wild-rice.

*Katie presented over the genetic study completed on wild and refugia populations of Texas wildrice (TWR) in 2021 through the EA Refugia program. The objective of this study was to*

*determine if the TWR populations at the SMARC and UNFH reflect the genetic diversity of the wild population since the start of the EAHCP. Leaf clips were taken from the refugia and wild populations and shipped off to SNARC (S for genetic analysis). Microsatellite analysis was completed on the TWR samples and genotypes were compiled using GeneMapper. Katie reviewed different types of genetic metrics identified in this study including allelic diversity, heterozygosity, Fis and Fst, and structure. Conrad Lamon asked about the Structure package. A total of 771 individual were collected and 600 unique genotypes were identified among TWR refugia and wild populations. Overall, genetic results suggest no genetic diversity was lost, but the population has become more homogenous compared to previous genetic studies completed on TWR in 1998 and 2012. More genetic diversity was found among within individuals compared to among sections and among individuals. The genetic analysis suggests that the EA TWR Refugia populations need a minimum of 200 TWR individuals to accurately represent the wild population. The TWR refugia populations have improved from previous analysis but could be further improved by having both refugia populations more similar to each other. Tom Arsuffi asked how TWR genetics compared to other species of wild rice, but that knowledge is currently unknown.*

6. Presentation and discussion of methodology proposed for the 2022 Refugia research project: *Analysis of genetic diversity of the Comal Springs Riffle Beetle*, presented by USFWS staff.

Purpose: To allow the Research Work Group the opportunity to provide comments on the methodology proposed to evaluate genetic diversity of the Comal Springs Riffle Beetle population.

Action: To obtain input from the Research Work Group regarding the proposed methodology for analyzing the genetic diversity of the Comal Springs Riffle Beetle.

*Katie Bockrath presented over the proposed genetic assessment on the Comal Springs Riffle Beetle. Katie briefly discussed previous genetic work on the CSRB in Landa Lake (Gonzales 2008). This genetic study will be completed in conjunction with a population occupancy study completed by another contractor. A minimum of 10 individuals per sampling site is needed with a total need of approximately 700 individuals. Dr. Chad Furl asked about the number of beetles per sampling location and that 10 beetles might not be realistic and is 3-5 beetles still sufficient to run proposed analysis. Katie said that a minimum of four beetles is needed. The difference from this study compared to previous studies is that beetles will be collected from lower Landa Lake instead of just focusing on the Spring Runs, Westernshore, and Spring Island. Chad Furl asked members if they thought that collecting the number of individuals would affect the population occupancy study. Dr. Weckerly mentioned that this could be dealt with some covariates. Dr. Conrad Lamon said that modeling efforts could help identify if sampling or collecting of individuals affected the population. This would be the first study what would combination genetic analysis and occupancy efforts at the same time. Dr. Arsuffi asked what Katie means by fine scale population and she commented lots of sampling among a small population area. 3Grad protocol will be used to assess genetic diversity and structure. Then STACKS will be used to process the genetic data to identify the number of loci and allelic variation and F statistics. Dr. Weckerly asked about effective population size and what scale it was going to be measured and was surprised at the population size proposed by the Gonzales*

*2008 study. Katie described how effective population size is estimated. Dr. Weckerly asked if we can estimate changes in population size then and now. Katie explained there is a way to look at the current population to estimate previous population size. By understanding the genetics and effective population size, it will benefit refugia program by knowing the number of beetles needed to retain genetic makeup of the wild population. Amelia Hunter asked about effective population size and asked if it can serve as a minimum population size for the wild and Katie agreed that yes, this is correct.*

7. Presentation and discussion of methodology proposed for the 2022 Refugia research project: *Continuation of 2021 study: Captive propagation and longevity of the Comal Springs Riffle Beetle*, presented by USFWS staff.

Purpose: To allow the Research Work Group the opportunity to discuss 2021 results and provide comments on the methodologies proposed to further increase propagation, survival rates, and longevity of captive Comal Springs riffle beetle individuals.

Action: To obtain input from the Research Work Group regarding the methodologies proposed to further enhance captive propagation and survival rates of the Comal Springs riffle beetle.

*Katie Bockrath asked Desiree Moore to present over the CSR B propagation work. Desiree presented over the three phases of the F1 production project of CSR Bs. Currently Phase 1 has been completed that assessed the use of boxes versus tubes for the housing of CSR B. The boxes had a tendency to overflow and overall phase 1 had low survival which may have been attributed to low flow in the boxes and weak larvae used in the study. Many larvae were lost during the hard freeze in February that resulted in power outages at the refugia. From Phase 1, the use of a new flow through system was developed. For Phase 2, CSR B larvae densities will be evaluated (i.e., 20, 30, and 40) and Phase 3 will evaluate the use of wild culture biofilm conditioned in Comal Springs. Three replicates of 20 larvae will be used in Phase 3. So far the study have shown that a flow through system increases survival of CSR B larvae. Also, the tubes is the best method for holding CSR B so far. Chad Furl asked an estimate of larvae requirement to complete work in 2022. Desiree mentioned that Phase 2 will require 270 larvae, which will be produced in the refugia. Amelia Hunter asked about how to ensure other species will be removed from the wild cultured items in Phase 3. Desiree said that the items and tubes will be inspected several times throughout the study. The CSR B handbook will review all information previously completed on CSR B propagation methods and will serve as a guide for the Refugia program.*

8. Consider possible future meetings, dates, locations, and agendas. *Another meeting will occur in December 2022.*
9. Questions and comments from the public. *No public comments.*
10. Adjourn. *Meeting adjourned at 3:03pm and recording was stopped. The recording will be available on the EAHCP website.*