Edwards Aquifer Habitat Conservation Plan
Research Work Group

Report of the Research Work Group:

2018 Refugia and 2018-2019 Applied Research Programs

April 26, 2017

Introduction

The Edwards Aquifer Habitat Conservation Plan (EAHCP; EARIP, 2012) calls for research to be conducted by the Edwards Aquifer Authority under two separate programs: the *Applied Research Program* (HCP §6.3.4) and the *Refugia Program* (HCP §5.1.1). The purpose of the Applied Research Program is to enhance understanding of the Comal and San Marcos aquatic ecosystems, to support the development of the EAHCP Ecological Model, and to provide scientific information to program management concerning success in meeting EAHCP's Biological Goals and Objectives. The *Refugia Program* (HCP §5.1.1) provides for research activities, as necessary, to develop practical knowledge for housing adequate populations of Covered Species and to expand knowledge of their biology, life histories, and reintroduction.

Initially, the Applied Research (AR) Program conducted studies prescribed in the EAHCP to fill critical gaps in data. As this data was acquired, additional research questions were identified by the EAHCP Adaptive Management Science Committee ("Science Committee"), and by the National Academy of Sciences, to constitute future research. These recommendations underwent a comprehensive review in 2015 by the Applied Research Work Group (ARWG), which produced the *EAHCP 2016-2019 Applied Research Project Schedule* (ARWG, 2015). The schedule provides guidelines for future research; however, the program's dynamic nature, including new progress made since 2015, merits continued scientific review and input.

The goal of the Refugia Program is to maintain captive populations of EAHCP Covered Species in the event of catastrophic loss of native populations. In 2017, a contract for the EAHCP Refugia facility was executed with the U.S. Fish & Wildlife Service with dedicated funds for a comprehensive Refugia research program. Although the basics of the Refugia research program are defined by contract, as questions arise related to the development and methodology of research projects, and due to research's dynamic nature, continued scientific review and input is merited, as with the AR Program.

In early spring 2017, the Program Manager and the Implementing Committee jointly determined to create a Science Committee Work Group ("Research Work Group") comprised of members drawn from the Science Committee (FMA §7.9.3.b). The purpose of the Research Work Group (RWG) was to provide a focused, deliberative group with the appropriate scientific expertise to conduct scientific review and provide input on questions related to the AR and Refugia research programs. RWG membership is constituted of the following individuals: Chad Norris (Texas Parks & Wildlife Department), Tom Arsuffi (Texas Tech University), Floyd Weckerly (Texas State University), and Conrad Lamon (Statistical Ecological Associates, LLC).

This report documents the recommendations and input of the RWG regarding the proposed project listing for the 2018-2019 AR program and methodologies of projects contained in the 2018 Refugia program. Proposed research projects were either previously suggested by the 2015 Applied Research Work Group, National Academy of Science, Science Committee, EAHCP staff, or by the 2017 Research Work Group. Each proposed research study contains a summary describing how and why the project was suggested, whether it is feasible, and the recommended course of action. These recommendations serve as guidelines for the Program Manager in developing the AR and Refugia research programs.

2018 Refugia Research Projects

At the inaugural meeting of the Research Work Group (RWG) on March 22, 2017, the RWG reviewed current methodologies and research proposed for the Refugia Program for 2018. BIO-WEST, in partnership with U.S. Fish & Wildlife, presented a summary of the 2018 research and techniques used for captive propagation of the Comal Springs dryopid beetle (CSRB) and the Peck's cave amphipod (PCA). RWG members evaluated the methodologies and suggested potential future research topics. Descriptions of the projects discussed and resulting recommendations are provided below:

1. ***Peck's cave amphipod quantitative sampling techniques***
   Based on the limited amount of information available on the Peck's cave amphipod (PCA), the 2015 Applied Research Work Group had previously recommended that more research is needed to analyze its population characteristics. 2017 marked the start of the Refugia Program research on PCA collection and sampling techniques. Information on habitat preferences and reproductive patterns is being refined through Refugia research. It was noted that pupation may require more than one year, therefore, the project may need to extend into 2018 to achieve meaningful results.

   Recommendation: *To extend project term limit due to prolonged rated of development, as well as formulate a more hypothesis-driven study design.*

2. ***Comal Springs dryopid beetle quantitative sampling techniques***
   BIO-WEST is conducting research at the U.S. Fish & Wildlife Service's San Marcos Aquatic Research Center (SMARC) to assess optimal collection and propagation techniques. Due to their subterranean nature, collection and propagation of the dryopid beetle is difficult. BIO-WEST is conducting research on the CSRB at the SMARC facility. However, BIO-WEST emphasized that pupation of the species is likely to require longer than 12 months, therefore, research may extend into 2018.

   Recommendation: *To extend project term limit due to prolonged nature of pupation, as well as formulate a more hypothesis-driven study design.*

2018-2019 Applied Research Projects

To inform their deliberations concerning the proposed AR project schedule and strategy for 2018-2019, the RWG evaluated projects listed from various sources. The *2015 Applied Research Report* (ARWG, 2015) contains a research schedule for 2016-2019. Most of the research topics listed below were either compiled by the 2015 ARWG, or were suggested by the National Academy of Science *Report 1* (NAS, 2015) The RWG also considered projects proposed by EAHCP staff.

From this holistic review process, the RWG reviewed and identified a total of 14 potential 2018-2019 applied research projects, which are listed below, accompanied by a short description of the rationale for taking them under consideration.

Projects presented to the Research Work Group

1. ***Evaluate success of submerged aquatic vegetation restoration and Texas wild-rice enhancement***

   Submerged aquatic vegetation (SAV) restoration work began in 2013 and, over time, EAHCP SAV contractors have improved documentation of their planting and removal sites. Since 2014, contractors in the San Marcos River system have delineated native planting and exotic SAV removal sites with georeferenced shapefile polygons that document the location, extent, amount, type of vegetation, and date of treatment. Treatment polygons could potentially be used to compare with annual SAV bio-monitoring vegetation surveys to assess expansion and scouring of treated areas.

   The RWG felt that the current methods for documentation are sufficient for assessing restoration efforts, but would like more in-depth analyses, such as the ones proposed with projects 11 and 12 (see below).

   Recommendation*: The RWG recognize that annual bio-monitoring data could be used internally to evaluate treated areas, but have requested additional analyses listed below as projects 11 and 12.*

2. ***Examine species-specific Tables 4-1 and 4-21***

   Originally listed within the 2015 Applied Research Work Group Report (ARWG, 2015), this 2018 study was proposed to help better understand the habitat requirements for the fountain darter. Members requested clarification on how fountain darter and vegetation coverage targets were calculated within Table 4-1 and Table 4-21 (EARIP, 2012).

   Through the SAV Nonroutine Adaptive Management Process approved in 2016 (EAHCP, 2016), targets for Table 4-1 and 4-21 were revised based on a systematic analysis of the SAV restoration programs progress and experiences to date (BIO-WEST and WSSG, 2016). Since restoration implementation began in 2013, SAV contractors have observed how variables such as drought (low flow), flood (scouring), aquatic recreation, and competition from exotic invasive SAV species have influenced treated areas colonization rates within the designated reaches.

   RWG members expressed concern that there should be greater justification for how the target values were assigned. Aquatic vegetation coverage targets within Table 4-1 and 4-21 were assigned based on median density values per vegetation type observed through fountain darter drop net bio-monitoring data. Since the values were recently revised, members agreed that more time is needed to assess the viability of the recently revised values in Table 4-1 and 4-21. Until then, the *Submerged Aquatic Vegetation Analysis and Recommendations* (BIO-WEST and WSSG, 2016) report can be referenced for supplemental information.

   Recommendation*: No 2018-2019 Applied Research needed. Bio-monitoring conducted annually and during critical flow periods provide sufficient data to assess restoration activities in long-term biological reaches as well as newly created restoration reaches.*

3. ***Evaluate success of the Old Channel flow-split management***

   This measure was originally proposed by the 2015 Applied Research Work Group for 2018 Applied Research (ARWG, 2015). However, in September 2016, the Implementing Committee approved a revised flow-split management regime recommended as part of the SAV Analysis and Recommendations (EAHCP, 2016). The revised regime will reduce the amount of flow and scouring of vegetation in the Old Channel during high flow conditions.

   Recommendation*: No 2018-2019 Applied Research planned. Will continue to monitor changes related to new flow-split management, through the annual bio-monitoring.*

4. ***Evaluate the success of removal of invasive animal species and reduction of introduction***

   Section 5.2.5 of the HCP dictates the removal of invasive animals including tilapia, *Plecostomus*, nutria, and ramshorn snails which pose a threat to the viability and long term health and habitat of the covered species (EARIP, 2012). In 2015, the Applied Research Work Group noted that minimal surveys and population estimates exist to quantify population density of the invasive animal species in Comal or San Marcos prior to removal efforts. The group stated that although total population estimates would prove challenging, general population trends can be surmised from the catch data collected and provided by the HCP contractors.

   The RWG members agreed that not enough data exists on the invasive animal population characteristics. Reproductive rates of target invasive animal species and how their removal impacted the covered species were briefly addressed during the 2016 Annual Joint meeting. Overall, the group felt that this task would be better suited as a data management exercise, instead of a comparatively more extensive applied research project.

   Recommendation*: No 2018-2019 Applied Research Planned. Continue invasive animal removal efforts and internally assess progress in two years with data collected by HCP contractors.*

5. ***Evaluate success of Sessom Creek sand bar removal and sediment removal efforts***

   Over the years, sediment dredging and removal efforts of the Sessom Creek sand bar have proved unsuccessful. A potential partnership with the City of San Marcos, Meadows Center for Water and the Environment (MCWE) at Texas State University, and stormwater expert engineers, will oversee a more proactive approach through addressing sediment loading sources upstream to reduce nonpoint source loadings and the transport of suspended sediment downstream.

   In 2017, EAHCP Committees will consider undergoing nonroutine adaptive management to partner with the City of San Marcos and Texas State/MCWE to install engineered solutions such as best management practices and low impact development designs along the middle portion of Sessom Creek, aimed at reducing upland loading of fine sediments.

The RWG supports the potential Nonroutine Adaptive Management approach from removal to prevention, and recognizes the significance of characterizing selected hydrologic aspects of the Sessom Creek watershed as part of this process. Watershed characterization may occur through analysis of existing and future datasets from a variety of sources. Results may guide implementation of BMPs and LIDs and help assess their efficacy over time.

Recommendation: *Supports 2018 Applied Research project to characterize the Sessom Creek watershed and gather key elements to develop load duration curves.*

6. ***Debriefing of the 2010-2015 drought***

The State of Texas experienced prolonged drought from the Fall of 2010 through the Spring of 2015 (U.S. Drought Monitor, 2017). The drought during calendar year 2011 was the most intense one-year drought Central Texas had experienced since at least 1895 (Nielsen-Gammon, 2011).

EAHCP staff have identified a potential benefit to analyzing the extreme drought conditions that occurred between 2010 and 2015. The analysis may potentially reference datasets pertaining, but not limited, to radar data, soil moisture content, discharge, species density, and more. RWG members agreed, and felt this information could help guide future management efforts during extreme weather conditions.

Recommendation: *Supports 2018 Applied Research project to gather key elements to analyze drought conditions from 2010 to 2015 and assess the impact drought had on the covered species.*

7. ***Statistical analysis of EAHCP data***

2017 Applied Research includes comprehensive statistical analysis of bio-monitoring data by three separate contractors. Since 2000, bio-monitoring has occurred in selected reaches of the San Marcos and Comal systems. No cumulative, statistical research has been done on this data-set since 2007 (Arsuffi & BIO-WEST Inc., 2007). The purpose of this applied research is to provide a time-integrated statistical analysis of long-term biological data, and to develop biological and hydrological statistical questions related to achieving compliance with the EAHCPs long term biological goals (LTBG).

Results from the 2017 statistical research could elucidate certain variables or underlying correlations that may require additional research in 2018. The RWG concurred that this is a needed and requested more distribution and abundance calculations.

Recommendation*: Supports additional statistical analyses of HCP data upon examining 2017 Applied Research findings, and consulting with the Science Committee.*

8. ***Ecological model research***

The Edwards Aquifer Authority will receive the ecological model (EcoModel) in Spring 2017. The EcoModel will be used to assess a wide range of habitat and hydrologic scenarios, and predict the potential amount of suitable habitat for some of the Covered Species, principally, the fountain darter. Modeled outputs may indicate that additional

research is needed or lead to additional research questions, therefore, this would remain as a contingent project within the Applied Research schedule.

The RWG proposed training a few of the Science Committee members so they can be informed of how the model works, and how modeled outputs will be used to inform management decisions.

Recommendation: *Train EAHCP staff and selected Science Committee members on how to use and analyze the EcoModel results.*

9. ***QUAL2E thermal modeling with the ecological model***

During the EARIP process, the QUAL2E model was used to simulate water temperatures and dissolved oxygen based on the hottest recorded weather conditions measured during the modeled period. Weather data recorded at the New Braunfels Municipal Airport weather station in July 2009 were used for QUAL2E simulations. Between 2011 and 2014, Central Texas experienced extreme weather conditions that drastically reduced springflow in Comal and San Marcos river systems. As previously mentioned, 2011 was the most intense one-year drought experienced since 1895 (Nielsen-Gammon, 2011). This analysis would assess the validity of model assumptions related to temperature and flow compared to recently observed drought conditions.

RWG agreed that validation outside the calibration period would be useful for assessing how accurate the model predicts water temperature at low-flow conditions. However, the QUAL2E model was recently revised for incorporation into the EcoModel. Documentation for the QUAL2E portion of the EcoModel may resolve or show the degree of model accuracy.

Recommendation*: Check the ecological model documentation for simulated versus observed information. Proceed with analysis if needed based on information provided and consultation with the Science Committee*

Projects recommended by the Research Work Group

In addition to the project list suggested by the 2015 Applied Research Work Group, NAS, and EAHCP staff, which was presented to the RWG, the RWG proposed five additional projects which are listed below. Most of the projects are in accordance with existing refugia or applied research, but provide a more specific scope. The fifth project is a newly proposed project that has a larger, more regional scope. The descriptions listed below follow the same structure as previous sections, with a project description explaining the source of and reason for consideration of each project, followed by the RWG's recommendation.

10. ***Comal Springs riffle beetle distribution and abundance***

This research project was originally proposed in the 2015 Applied Research Work Group and requested again in the RWG meeting by Mr. Norris. Research was requested due to the limited amount of information that exists on the Comal Springs riffle beetle overall population characteristics.

Recommendation: *To support research on the Comal Springs riffle beetle population distribution characteristics and abundance. Results would inform and enhance Comal Springs riffle beetle refugia research.*

11. ***Assess plots of submerged aquatic vegetation unmaintained since restoration***

Members of the RWG proposed this analysis as a means for evaluating the long-term success and sustainability of the SAV restoration sites. Considered a subtask under the

"evaluate success of the SAV restoration and TWR enhancement" (see project #1, above), this analysis would investigate the viability of treated areas if left alone and unmaintained, with a view to determining the extent and frequency needed for management interventions to sustain SAV restoration efforts in the long-term

***Recommendation**: No 2018-2019 Applied Research needed. Analysis of treated sites coupled with bio-monitoring data can be done internally by EAHCP staff.*

12. ***Analyze submerged aquatic vegetation competition studies that focus on species within the system***

    Considered a subtask under the "evaluate success of the SAV restoration and TWR enhancement" (see project #1, above); this analysis would investigate how treated SAV coverage is influenced by aggressive native and exotic species. In 2003 and 2015, competitive growth rates between *Ludwigia repens* (native), *Hygrophila polysperma* (exotic), and *Hydrilla verticillata* (exotic) were assessed (Doyle and BIO-WEST, 2015). Hydrilla, in San Marcos, and hygrophila, in Comal and San Marcos, are aggressive exotic SAV species targeted for removal. Their rapid establishment and tenacious recolonization rates seem to make the necessity for frequent, routine removal efforts inevitable. Enhanced understanding of competition between native and exotic species may improve the long-term success of planting native and removing exotic SAVs within both systems, including potentially reducing the frequency and intensity of removal efforts

    Members of the RWG proposed this analysis as a means for evaluating the long-term success and sustainability of the SAV restoration sites. Findings may help identify recolonization rates, and assist with identification of SAV restoration site location criteria.

    ***Recommendation:** No 2018-2019 Applied Research needed. Research of existing exotic SAV competition data, coupled with bio-monitoring data can be done internally by EAHCP staff.*

13. ***State-space models for estimating fountain darter population, carrying capacity, and vegetative medians***

    As mentioned above in project 2, RWG members have requested validation of the fountain darter density values and habitat targets assigned in Tables 4-1 and 4-21 (EARIP, 2012). Although 2017 statistical projects being carried out under the AR Program assess population distribution characteristics and habitat needs of the Covered Species, Dr. Weckerly proposed a state-space model as another means for quantifying the numbers of fountain darters per vegetation type and the carrying capacity of the long term biological monitoring reaches. Variables analyzed would include fountain darter counts and habitat surveys of the long-term biological monitoring reaches. Outputs would be used to quantify overall population, as well as fountain darter densities per vegetation type.

    ***Recommendation:** To use a state-space model with long-term bio-monitoring data to assess fountain darter population characteristics.*

14. ***Quantify Comal and San Marcos spring systems ecosystem services***

Dr. Arsuffi suggested it may be of interest to conduct a study looking at the ecosystem services provided by the springs and river systems, and the benefit of the EAHCP in relation to supporting the provisioning of those ecosystem services. He proposed an economic evaluation of the local and regional businesses, as well as the wide range of stakeholders that rely on sustained flows and healthy ecosystem functioning. The study would identify and characterize these groups, as well as quantify the economic benefits the ecosystem provides.

Recommendation*: Perform an ecosystem services valuation of the sustained flows and healthy habitat provided by the springs and river systems, and their impact on local and regional communities.*

Conclusion

In summary, the RWG reviewed a total of 9 potential 2018-2019 Applied Research projects, and proposed an additional 5 potential AR projects for 2018-2019. As previously mentioned, proposed projects were suggested by the National Academy of Science *Report 1,* the 2015 Applied Research Work Group, EAHCP staff, the Science Committee, and the Research Work Group. This draft report will be presented for possible endorsement by the Science Committee at the next full committee meeting on May 10, 2017. The draft minutes from this meeting have been included here as an appendix as well as the 2018 Refugia research work plans. This input received from the RWG and the full Science Committee discussion will be used to guide EAHCP staff with the development of the 2018-2019 Applied Research schedule.

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Charge of the 2017 Research Work Group

EAHCP Adaptive Management Science Committee

Overview

The Edwards Aquifer Habitat Conservation Plan (EAHCP) calls for research to be conducted by the Edwards Aquifer Authority under two separate programs: (1) the *Applied Research Program* (HCP §6.3.4), enhances understanding of the Comal and San Marcos aquatic ecosystems, supports the development of the EAHCP Ecological Model, and provides scientific information to program management concerning success in meeting EAHCP's Biological Goals and Objectives; and (2) the *Refugia Program* (HCP §5.1.1), provides for research activities, as necessary, to develop practical knowledge for housing adequate populations of Covered Species and to expand knowledge of their biology, life histories, and reintroduction.

This document lays out the background, creation, charge, and administration of a Work Group created to provide scientific review and input concerning research under the Applied Research and Refugia programs.

Background *Applied Research Program:*

Initially, the Applied Research Program conducted studies prescribed in the EAHCP to fill critical gaps in data. As this data was acquired, additional research questions were identified by the EAHCP Adaptive Management Science Committee ("Science Committee"), and by the National Academy of Sciences, to constitute future research. These recommendations underwent a comprehensive review in 2015 by the Applied Research Work Group, which produced the *EAHCP 2016-2019 Applied Research Project Schedule*. The schedule provides guidelines for future research; however, the program's dynamic nature, including new progress made since 2015, merits continued scientific review and input.

Refugia Program: In 2017, a contract for the EAHCP Refugia facility was executed with the U.S. Fish & Wildlife Service with dedicated funds for a comprehensive Refugia research program. Although the basics of this program are defined by contract, as questions arise related to the development and methodology of research projects, and due to research's dynamic nature, continued scientific review and input is merited.

Creation

To provide a focused deliberative body with the appropriate subject matter expertise for continued scientific review and input on questions related to the EAHCP Applied Research and Refugia research programs, the HCP Program Manager and the Implementing Committee jointly determined to create a Science Committee Work Group ("Research Work Group") comprised of members drawn from the Science Committee (FMA §7.9.3.b) for this purpose.

Charge

The Work Group's charge consists of: (1) suggesting specific Applied Research projects to be conducted during 2018 and 2019 as part of the Applied Research Program; and (2) suggesting refinements to the methodology proposed for Refugia research projects.

Administration

The Work Group will begin in 2017. The Work Group will meet on an as-needed basis as determined by the HCP Program Manager and the Science Committee Chair and Vice-Chair. Final recommendations resulting from a given meeting, or meetings, of the Work Group will be presented for discussion and possible endorsement of the Science Committee at the next full committee meeting scheduled. The Work Group will be constituted of the following individuals: Chad Norris, Tom Arsuffi, Floyd Weckerly, and Conrad Lamon. The Work Group will operate by consensus, and will heed of the scope designated in the EAHCP for the Measures under consideration. It is anticipated this Work Group will exist for the duration of the ITP. However, there is a recognition that the group will need to adapt and be flexible as new issues are identified. Therefore, this charge and membership is to be revisited annually, and, if needed, may be modified with Science Committee endorsement.

Appendix II: Meeting Agenda

NOTICE OF OPEN MEETING

Available at eahcp.org

As jointly determined by the Implementing Committee and the Program Manager (FMA §7.9.3.b), the **Research Work Group** has been formed to suggest specific Applied Research projects to be conducted during 2018 and 2019 as part of the Applied Research Program, and to suggest refinements to the methodology proposed for Refugia research projects. The Research Work Group is comprised of members selected from the EAHCP Adaptive Management Science Committee. A meeting of the Research Work Group for the Edwards Aquifer Habitat Conservation Plan is scheduled for **Wednesday, March 22, 2017, at 9 a.m. at the San Marcos Activity Center (Multipurpose Room), 501 E. Hopkins St., San Marcos, Texas 78666.** Lunch will be provided; the meeting is expected to end around three. Work Group members are asked to please RSVP to ktolman@edwardsaquifer.org.
Members of this Work Group include: Chad Norris, Tom Arsuffi, Floyd Weckerly, and Conrad Lamon. At this meeting, the following business may be considered and recommended for Work Group action:

1. Call to order.

2. Public comment.

3. Presentation of Research Work Group Charge (Attachment 1).
   Purpose: To provide the Research Work Group with information on the charge of the Work Group.
   Action: None required

4. Presentation and discussion of methodology proposed for Refugia research projects.
   Purpose: To provide the Research Work Group with the opportunity to comment on the methodology proposed for Refugia research projects beginning in 2017.
   Action: To obtain input from the Research Work Group regarding the methodology proposed for the Refugia research projects.

5. Presentation and discussion of the Report of the 2015 Applied Research Work Group (including the EAHCP 2016-2019 Applied Research Project Schedule; Attachment 2).
   Purpose: To provide the Research Work Group with information on the recommendations made by 2015 Applied Research Work Group.
   Action: None required

6. Presentation and discussion of Applied Research projects for 2018 and 2019.
   Purpose: To provide the Research Work Group with the opportunity to develop and to possibly endorse the project schedule for the 2018-2019 EAHCP Applied Research program.
   Action: To obtain input from the Research Work Group on the proposed Applied Research projects for 2018 and 2019 and to possibly obtain their endorsement of said projects.

7. Questions and comments from the public.

8. Adjourn.

RESEARCH WORK GROUP
March 22, 2016 MEETING MINUTES

Available at eahcp.org

1. **Call to order** – 9:00 am
   Members present included: Chad Norris, Tom Arsuffi, Floyd Weckerly, and Conrad Lamon

2. **Public comment.**
   No comment.

3. **Presentation of Research Work Group Charge**
   No comment.

4. **Presentation and discussion of methodology proposed for Refugia research projects. "2017 Captive Propagation Research"**
   **Dr. Arsuffi mentioned that the research needs a hypothesis, but Mr. Worsham emphasized that it is hard to test a hypothesis while conducting exploratory research because of the limited amount of data available and the inability to replicate the study due to the small number of specimens. Dr. Arsuffi noted that research on similar species is available and that a hypothesis could be assigned even with a multifactor, un-replicated study.*

   Comal Springs dryopid beetle:
   *Subterranean and takes longer than 12 months to reach maturity. Similar to the Comal Springs riffle beetle (CSRB), but Comal Springs dryopid beetle (CSDB) is the only species of its kind that have aquatic larvae. *Will there be a sufficient number?* Approximately 80 have been collected since it was discovered. The main issue is that more females are needed for brooding yet CSDB are difficult to capture. *How are they in captivity?* Most live for at least a few years in captivity. *If methods produce offspring, still need to determine optimal conditions for the eggs.*
   *Will present later this year and give a progress report. *Members emphasized that it doesn't need to be at the end of the year, instead more useful for milestone achievements.*

   Peck's cave amphipod:
   *Collectors can differentiate female from male, but difficult to distinguish the Peck's cave amphipod (PCA) from other stygobromus species. They likely have a slower rate of development because they live in a resource-limited environment and have a slow metabolic rate because they have no circadian rhythm or metabolic simulation from light. *Dr. Arsuffi considers this a hypothesis.*
   **Dr. Arsuffi suggested (1) Merritt, R. W., and K. W. Cummins functional feeding group for stygobromus categories of food. If they are slow-growing and temperature remains constant, growth may be more heterotrophic and dependent on the quality of food. OR (2) if not the quality of food, instead a lack of abundance of food. They do not encounter meals on a regular basis which is why they are slow-growing.*

5. **Presentation and discussion of the Report of the 2015 Applied Research Work Group**.

   - **Peck's cave amphipod quantitative sampling techniques**
     <See notes above>

- **Evaluate success of the Old Channel flow-split management**

  Since the new flow-split management system values were recently revised in 2016, no data currently exists to assess the new flow regime.

  **New flow-split values because flows were too high and routinely scoured newly planted areas.*
  **Mr. Norris stated that it seems much of the adverse impacts within the Old Channel were from floods, culverts, and the old system. Dr. Weckerly would like to see monitoring of the flow-split continue. By 2020 there would be an adequate sample size to analyze the flow-split management impacts on fountain darter abundance through state-space models. Dr. Arsuffi noted that plenty of research is available in support of flood pulses in aquatic system. Mr. Pence emphasized that the sustained, high-flows were the issue and should be resolved with the new flow-split regime.*

- **Evaluate success of Sessom Creek sand bar removal and sediment removal efforts**

  Dredging of fine sediment has not been successful, propose a new holistic, watershed approach.
  *New monitoring equipment would help improve existing load duration curves and our understanding of loading characteristics. Sampling would need a minimum of 2 years to ensure enough events are captured.
  **Members agreed that it's better to have a proactive approach upstream than continuously mitigate loadings downstream. Mr. Norris suggested engaging the Sessom Creek neighbors and volunteers. Dr. Lamon expressed concern that loading curves developed with a limited sample size are susceptible to outliers.*

- **Evaluate success of removal of invasive animal species and reduce of introduction**

  **Not much data exists on the population densities within each system. Dr. Arsuffi proposed checking the rate of reproduction to assess if removal efforts are making a difference. Mr. Pence replied that Bob Hall performed a similar exercise with Tilapia to quantify their impact on fountain darters. Members agreed that all invasive species cannot be removed, but the HCP should continue removal efforts and assess progress after 2 years by analyzing the frequency of different size categories.*

- **Evaluate success of SAV restoration and TWR enhancement**

  **Members requested additional research on the long-term sustainability of areas where SAV have been removed or planted, yet unmaintained.*
  **Members requested additional research on the competition between native/exotic species, citing competition studies conducted in 2003 and 2015 by Dr. Doyle at Baylor University.*
  **Findings from these types of analyses could help inform management decisions and refine the habitat preferences for planting SAV.*

- **Confirm species-specific Table 4-1 and 4-21**

  Table targets changed in 2016 during the adaptive management process, therefore, more time is needed to assess the success at achieving targets.
  **Dr. Weckerly requested more information on how the target values were assigned. Mr. Pence replied that values were derived from long-term fountain darter sampling. Dr. Weckerly suggested state-space models for population estimates and quantifying the carrying capacity of reaches.*

- **EcoModel Research**

  The EcoModel and summary document will be delivered by the end of April.
  **members requested that at least two Science Committee members, such as Dr. Weckerly, Dr. Lamon, Dr. Kreitler, or Mr. Norris, receive training on the EcoModel.*

- **Statistical analysis of EAHCP data**

  Projects will be completed by December 2017. Results from the three contractors may identify areas that need additional research.

- **Debriefing the 2010-2015 Drought**
  Members agreed that the information would be useful for current and future management efforts. Mr. Norris noted that rainfall patterns impact the spring systems differently; Comal Spring Run 1 went dry during 2014, although 2011 was a drier year. Moreover, San Marcos had a higher discharge than Comal during some parts of 2014.
  *Analysis will focus on mean annual rainfall from 2011-2014.

- **Qual2e Thermal modeling with EcoModel**
  Originally done as part of the J-Charge, revised in 2009 for the ERIP process, and was recently revised and added to the EcoModel.
  **Dr. Lamon: validation outside the calibration period is encouraged.*

Proposed by Research Work Group:

- **Comal Springs Riffle Beetle distribution and abundance**
  *******Proposed by Mr. Norris*
  *******Need more analysis on the CSRB population characteristics.*

- **Cultural Ecosystem Services of the HCP**
  *******Cultural ecosystem services survey proposed by Dr. Arsuffi.*
  *******Dr. Arsuffi: we often focus on the direct impacts the HCP has on the system, however, sometimes the indirect are more important than the direct impacts. Through stakeholders, partners, and community members we can assess other positive goods and services the HCP is indirectly providing. Dr. Weckerly suggested surveys with the zip code of people at the river would quantify what percentage are local or from out of town. Mr. Norris also noted the value that sustained spring flow has on landowners downstream that rely on surface water.*

- **State-space models for estimating Fountain Darter population, carrying capacity, and vegetative median**
  *******Dr. Weckerly proposed using a state-space model with bio-monitoring data to enhance our understanding of population characteristics and improve estimations. Dr. Lamon agrees that it would improve fountain darter estimates. Outputs could be used to improve or validate the current fountain darter densities per vegetation type and overall population.*

6. **Presentation and discussion of Applied Research projects for 2018 and 2019.**

7. **Questions and comments from the public.**
   No Comments

8. **Adjourn.**

Larval development of Comal Springs dryopid beetles (*Stygoparnus comalensis*)

LITERATURE REVIEW AND PROPOSED METHODOLOGY

Prepared for:
Edwards Aquifer Authority

Prepared by:
Randy Gibson, Regional Invertebrate Zoologist
Dr. Lindsay Campbell, Refugia Managing Biologist
U.S. Fish & Wildlife Service
San Marcos Aquatic Resources Center

&

McLean Worsham, Senior Invertebrate Zoologist
BIO-WEST, Inc.

March 22, 2017

U.S. Fish & Wildlife Service
San Marcos Aquatic Resources Center
Southwest Region

BIO-WEST

Introduction

The Edwards Aquifer Habitat Conservation Plan (EAHCP) calls for the establishment of captive refuge populations of Edwards Aquifer (EA) Covered Species associated with their Incidental Take Permit inhabiting both subterranean and spring outflow habitats. The San Marcos Aquatic Resources Center (SMARC) operated by the United States Fish and Wildlife Service (USFWS) has been awarded the opportunity to establish and maintain captive refuge populations of EA species of concern; many of which have been cultivated successfully in captivity at SMARC for several years. Some of the species of concern still pose several substantial questions concerning refuge cultivation; particularly the invertebrate species. Recognizing this deficit, research into life-history and captive propagation of the Comal Springs dryopid beetle (*Stygoparnus comalensis*) is proposed to commence in 2017.

Roles and Responsibilities

As the prime contractor for the EAHCP refugia contract, SMARC will provide consultation, oversight and review activities for this 2017 Captive Propagation applied research project. In particular, Dr. Lindsay Campbell and Mr. Randy Gibson will serve as Co-Principal Investigators for this supervisory role. Additionally, as part of on-going refugia activities surrounding the maintenance and collection of standing stocks, SMARC biologists will carry out collection duties and routine technical assistance. Finally, SMARC will provide all facilities, utilities and equipment for 2017 experimentation. As a subcontractor on the USFWS refugia team, BIO-WEST will be responsible for task execution, analysis, and reporting. To perform these duties, BIO-WEST will provide a senior invertebrate zoologist and aquatic technician to work on station at SMARC.

Literature Review

Stygoparnus comalensis is a federally endangered species (USFWS 1997) that is adapted to subterranean habitats associated with Edwards Aquifer spring systems. *Stygoparnus comalensis* have been recovered from a limited number of perennial Edwards Aquifer springs (Comal, Fern Bank, and San Marcos springs). Since the species was discovered, it has rarely been encountered; less 80 adults have been collected or observed since the species was discovered in 1992 despite extensive sampling making it perhaps the rarest of the EA covered species. *Stygoparnus comalensis* is characterized by having vestigial eyes, lacking pigment, and wingless adults. *Stygoparnus comalensis* is also the type species of the genus *Stygoparnus* which is a monotypic genus with *S. comalensis* being the only species (Barr and Spangler 1992). Therefore, the conservation of *S. comalensis* should be considered particularly important as there are no related lineages found anywhere else on earth. Study of this species is also complicated by the fact that there is likely no suitable surrogate species.

Like other dryopid beetles, adult *S. comalensis* are fully aquatic and similar to adult riffle beetles in general ecology. They inhabit relatively clean rivers and streams feeding on biofilm scraped from surfaces and are relatively slow moving and incapable of swimming. Respiration is through a plastron, a gas film produced by area of dense water repelling hairs (Brown 1987, Resh et al. 2008). The life span of this species is unknown; however, some wild caught adults have survived in captivity 11-21 months (Barr and Spangler 1992, Fries et al. 2004).

Dryopid larvae typically inhabit moist terrestrial soils along stream banks, presumably feeding on roots and decaying vegetation, while adults are fully aquatic (Brown 1987, Ulrich 1987). However, *Stygoparnus comalensis* is unique among all other dryopids as the only species with fully aquatic larvae. Like the closely allied elmids, larvae utilizing retractable posterior gills for respiration (Brown 1987) making S. comalensis the only dryopid to have both aquatic larvae and adults. Aside from this, little else is known about the life history and development of the *S. comalensis*. There is no information on deposition sites for eggs, clutch size, incubation times or size of eggs. Additionally, there is insufficient information on larval development, although it is hypothesized that it requires up to 2-5 years before pupating like other dryopid species (Ulrich 1986). However, a single *S. comalensis* larva produced at SMARC grew from 2 to 10 mm in 9 months, suggesting larval development may require only one year for *S. comalensis*, although it is unknown how old the larvae was at 2 mm in length.

Thus far, captive-produced and wild-caught larvae have failed to pupate in captivity. Barr and Spangler (1992) hypothesized that the larvae may require a moist terrestrial habitat exposed above the waterline on the ceilings of spring orifices for development and pupation. With so little known about this species, it is vital that more information about the life history of this species is collected in order to maintain effective refuge populations.

Methods

The primary charge of EAHCP refugia is to preserve the capacity for the HCP Covered Species to be re-established at Comal and San Marcos springs, if ever necessary. This charge dictates that refugia research focuses on captive propagation. As limited life history or captive propagation information is available for *S. comalensis*, a large portion of this early research in 2017 will be exploratory or observational. Basic testing of everything from collection techniques, to housing apparatus, to flow, substrate, or other environmental stimuli. A key research objective moving forward is to build upon 2017 exploratory research that appears promising, by designing subsequent hypothesis driven, quantitative studies as appropriate in the future.

Task 1: Collection of test subjects

Adult *S. comalensis* will be collected from cotton lures and drift nets set in Sessoms Creek, Comal spring runs, and Landa Lake as needed in 2017 for the completion of study activities. Cotton lures will be set and allowed to develop biofilms for four weeks before being retrieved and checked for invertebrates following Gibson et al., (2008) and Hall (2016). Adults will be removed and transported back to the SMARC where they will be maintained initially within custom aquatic holding units (similar to picture to the right) and fed detrital material and matured biofilms on colonized cotton lures. Adults will be held in quarantine for 14 days before entering into research observations so they can acclimate to captivity.

Task 2: Exploratory Research

The historic difficulty in collecting *S. comalensis* individuals, limited information on captive propagation, and available literature for this genus leads to the following overarching key assumptions:

- Sufficient organisms will be collected for exploratory research.
- Ability to keep individuals alive in captivity.
- Life span amenable to accomplishing tasks in 1 year.

Additionally, each chronological Task 2 subtask is inherently dependent on the level of success in the previous activity. Both the key assumptions and subtask associations will be tracked throughout the project and any concerns reported to EAA upon identification.

2.1 Sexual dimorphism

Following the methods of the Comal Springs riffle beetle life history study (BIO-WEST 2016), sex determination will be attempted by gently placing adults under a dissecting microscope both dorsal and ventral pictures will be taken of various aspects of morphology. Images will be analyzed for evidence of sexually dimorphic external features.

Schedule: March through July

Anticipated results: photo diagrams of male/female sexually dimorphic external features.

2.2 Egg production and incubation

Depending on the outcome of the study of sexual dimorphism, adults will be bred in groups or pairs (i.e. if sex can't be determined then adults will be bred in large groups to ensure that members of the opposite sex are available to mate; however, if sex can be determined then adults will be bred in pairs). Assuming sex determination is successful, a single male-female pair of adults will be held in flow-through containers having both aquatic and terrestrial habitat provided with a variety of substrates (cotton cloth, wood, leaves, nylon mesh, etc.). Replication will depend on the availability of organisms but ideally, n=4 per treatment group. Substrates in each replicate will be checked weekly (at a minimum) and egg laden substrates will be removed and maintained in separate flow-through containers. If numbers suffice, analysis of variance (ANOVA) will be run to determine if there is a statistical preference for substrate(s) on which eggs are laid.

Once eggs are produced they will be monitored until they hatch by checking twice weekly (at a minimum). We will assess differences in the hatching success, amount of time eggs incubated, and survival rate of larvae immediately post hatching using a combination of regression analyses and one-way ANOVAs using the different treatment groups of the parents to determine which factors contribute to the greatest production of viable eggs.

Schedule: April through September

Anticipated results: egg production; egg morphology; incubation duration of eggs; better understanding of conditions that contribute to the project of eggs.

2.3 Larval development

1st instar larvae will be placed into individual holding chambers [flow through system with 75 µm or smaller pore size mesh until large enough to be moved to larger mesh size flow through systems (pictured to the right). An initial step of exploratory research will be to evaluate different types and configurations of flow-through chambers to determine which might be most effective for this species.

Once a chamber design is selected, it will then receive a variety of pilot treatment substrates until exploratory research results are able to provide insight into promising conditions for cultivation. Experimental replication will depend on the availability of larvae produced but will be evenly spread across experimental groups. Larvae will have development visually observed twice per week (at a minimum). Additionally, growth and/or molts will be recorded twice monthly (at a minimum) during 2017. To accomplish this, larvae will be wet mounted and have total body length and head capsule width measured using Olympus Cellcens camera system and measuring tool software at the standard shutter speed of 3.395 milliseconds. Growth and molt rate will be analyzed for significance via univariate analyses (e.g., ANOVA). Observations of aquatic and terrestrial substrate utilized by the larvae will be recorded daily/weekly and ANOVA tests run on preferences if warranted.

Schedule: July through December

Anticipated results: larvae production; instar morphology, better understanding of rate of larval development.

2.4 Pupation

Conditions that contribute to pupation are completely unknown for *S. comalensis*. Therefore, the conditions that best suit the development of larvae will be used as a starting point for exploratory research relative to pupation towards the conclusion of 2017. Pupation containers will provide substrate that is above and below the surface as many dryopid larvae are known to pupate in terrestrial habitats.

Schedule: October through December

Anticipated results: preliminary insight into pupation

Task 3: Analysis and Reporting

As typical for observational data, it is anticipated that a major portion of any analysis and explanation will be descriptive statistics and morphological documentation. Potential statistical analysis for exploratory research was briefly described above in each subtask as deemed applicable at this time. An interim report of the research activities conducted during 2017 be drafted during November 2017, finalized during December 2017, and submitted to EAA by December 31, 2017. The 2017 product is termed Interim in that it is anticipated that certain applied research components will be carried forward into 2018 in order to better understand and document the life cycle of this unique HCP Covered species.

Budget

As discussed in the roles and responsibilities section above, this project will be a Refugia Team effort. A detailed cost breakdown for BIO-WEST's involvement is documented in the table below. All SMARC costs for assistance in this effort will be covered through their established budget (Task 1 – 2017 Workplan) for maintenance and collection of standing stocks. Therefore, the total cost for the 2017 Larval Development of Comal Springs dryopid beetle study is $104,098.

BIO-WEST (01/01/2017)
DETAILED COST BREAKDOWN - EAHCP 2017 Refugia
Comal Springs Dryopid Beetle Life History Study

LABOR:

Position	Rate	TASK 2 - Applied Research	TOTALS Total Hours	TOTALS Cost
Project Principal	155.87	48	48	$ 7,481.76
Biologist II	75.26	636	636	$ 47,865.36
Senior Administrative	66.72	6	6	$ 400.32
Technician II	51.31	924	924	$ 47,410.44
Total Labor		1,614	1,614	**103,157.88**
TRAVEL				
Per diem: Hotel and Meals				$ -
Mileage ($.535 per mile)	0.535	500	500	$ 267.50
Total Travel				**$ 267.50**
DIRECT COSTS:				
Equipment: water quality sondes, flow meters, etc.				$ -
Supplies		636		636
Phone / Fax / Copies		37		37
Total Direct Costs		673		**$ 673**
Total Estimated Cost			Total	**$ 104,098.00**
	TASKS	$ 104,098		

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Juvenile development and maturation of Peck's cave amphipods (*Stygobromus pecki*)

LITERATURE REVIEW AND PROPOSED METHODOLOGY

Prepared for:
Edwards Aquifer
Authority

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March 22, 2017

U.S. Fish & Wildlife Service
San Marcos Aquatic Resources Center
Southwest Region

BIO-WEST

Introduction

The Edwards Aquifer Habitat Conservation Plan (EAHCP) calls for the establishment of captive refuge populations of Edwards Aquifer (EA) Covered Species associated with their Incidental Take Permit inhabiting both subterranean and spring outflow habitats. The San Marcos Aquatic Resources Center (SMARC) operated by the United States Fish and Wildlife Service (USFWS) has been awarded the opportunity to establish and maintain captive refuge populations of EA species of concern; many of which have been cultivated successfully in captivity at SMARC for several years. Some of the species of concern still pose several substantial questions concerning refuge cultivation; particularly the invertebrate species. Recognizing this deficit, research into life-history and captive propagation of the of the Peck's cave amphipod (*Stygobromus pecki*) is proposed to commence in 2017.

Roles and Responsibilities

As the prime contractor for the EAHCP refugia contract, SMARC will provide consultation, oversight and review activities for this 2017 Captive Propagation applied research project. Dr. Lindsay Campbell and Mr. Randy Gibson will serve as Co-Principal Investigators for this supervisory role. Additionally, as part of on-going refugia activities surrounding the maintenance and collection of standing stocks, SMARC biologists will carry out collection duties and routine technical assistance. Finally, SMARC will provide all facilities, utilities and equipment for 2017 experimentation. As a subcontractor on the USFWS refugia team, BIO-WEST will be responsible for task execution, analysis, and reporting. To perform these duties, BIO-WEST will provide a senior invertebrate zoologist and aquatic technician to work on station at SMARC.

Literature Review

Stygobromus pecki is a federally endangered species (USFWS 1997) that is adapted to subterranean habitats associated within Edwards Aquifer spring systems. *Stygobromus pecki* belongs to a rather speciose genus with ≈140 described species; all of which are subterranean and found primarily in North America (Holsinger 1967; Holsinger 1994). Three Stygobromus species (*S. russelli, S. bifurcatus,* and *S. flagellatus)* are known to occur sympatrically with *S. pecki*. Because little is known about the life history and development of any of these species, it is not possible at this time to determine species of juvenile *Stygobromus,* which complicates management efforts.

Information on their life history is not abundant in the literature. In general, it appears that subterranean amphipods (like other subterranean species) have a much slower rate of development and reproduction than epigean species. Most epigean species of amphipods have multiple generations per year, while subterranean amphipods typically take at least a year to mature (Crawford and Tarter 1979). However, the subterranean hyporheic amphipod, *Niphargus aquilex*, has been shown to have the capacity to produce up to two generations per year (Gledhill and Ladle 1969). Therefore, though the reproductive biology of many epigean amphipods is well understood, surface species are likely not suitable for drawing analogy about developmental rate, but might still be useful for gaining insight into

developmental events at an accelerated rate.

Reproducing *S. pecki* in captivity will likely be complicated by fluctuating sex ratios, as it has been shown in numerous species of amphipods that sex ratios fluctuate (Crawford and Tarter 1979). Crawford and Tarter (1979) and Bollache and Cezilly (2004a) suggested that the greater abundance of males during the breeding season corresponds to females having synchronous pre-copulatory molts. Sex ratios may also become distorted due to the mechanism of sex determination in amphipods as amphipods do not have all sex determining alleles located on discrete sex chromosomes. Rather, sex determining alleles are distributed across several chromosomes and sex is inherited much like a quantitative trait. Furthermore, it has been shown that certain pairings can lead to exclusively male or female offspring (Sutcliffe 1992). Environmental factors have also been shown to affect or covary with sex (Sutcliffe 1992; Watt and A d a m s 1993; McCabe and Dunn 1997). Infection with microsporidians (Bulnheim and Vávra 1968) and chemical pollutants (Gross et al. 2001) have also been shown to affect sex ratios or the development of sexual characteristics in amphipods, suggesting that sex determination in amphipods may behave like a developmentally plastic and quantitative trait. Therefore, it may be important to maintain proper pedigrees of amphipods and ideal culture conditions in the refugium to ensure proper development and prevent heavily biased sex ratios which could lead to the collapse of specific or preferred culture lineages.

Amphipods are thought to only be able to mate after the female molts, because only then is the cuticle of the females' exoskeleton flexible enough to allow the release of eggs through the genital pore into the marsupium (Bollache and Cezilly 2004a). Because females are only momentarily receptive to mating, males typically guard a female (via amplexus) prior to her molting, insuring that he fathers offspring; however, this has never been observed in *S. pecki*. Molt cycles in males also appear to have a role in reproductive timing. Males approaching a molt tend to avoid entering into amplexus, likely because they will inevitably have to release the female upon molting, thus never successfully copulating with the female; such an effort would be a wasted investment in a mating (Bollache and Cezilly 2004a) as mate guarding comes at a cost to males by hindering their ability to forage, thereby reducing lipid stores and hindering growth (Robinson and Doyle 1985). In response to this energetic cost, males tend to avoid amplexus unless they have sufficient amounts of stored lipids and glycogen to wait out the females molt cycle and only if the female is expected to molt before the male (Plaistow et al. 2003). Therefore, it is likely that proper nourishment is necessary to offset the nutritional costs of amplexus. In addition, there appears to be significant cannibalism in captive populations of *S. pecki* (R. Gibson, pers. obs.). However, keeping both males and females well-fed may reduce the tendency of cannibalistic interactions to occur.

Bollache and Cézilly (2004b) proposed that there is greater competition among males for access to larger females, because larger females are more fecund. Consequently, larger males preferentially out-compete smaller males for larger females. Thus, there appears to be size assortative paring between males and females; at least in some species of amphipods (Bollache and Cézilly 2004b; Franceschi et al. 2010). However, male-female pairs in amplexus with smaller females tend to have greater swimming efficiency than pairs in amplexus with larger females, suggesting that males tend to be larger than females in scenarios with predation (Adams and Greenwood 1983). Selection for larger males is also compounded by female resistance to amplexus (Jormalainen and Merilaita 1995). The ultimate consequence of these size-specific interactions is that females must have a large-enough

male suitor if mating is to be successful, but also that the female must be large enough (and thus fecund enough) to be worth the male investing in amplexus. However, under scenarios where selection has been relaxed, size selection may only be effected by choosing for the most fecund (thus largest) female that a male is large enough to restrain.

It is unknown how long the eggs must incubate before hatching once a female *S. pecki* lays eggs into her marsupium. Nor is it known how many eggs a female lays in each clutch or how many clutches a female typically produces a year, although work at SMARC noted S. *pecki* females to likely have multiple broods of ca. 10 young a year (Fries et al. 2004). Neonates have been found to be ca. 2 mm in length upon hatching (Fries et al. 2004). Once hatched, it is unknown how many molts occur before offspring become sexual mature, although F1's produced at SMARC reached lengths of 9 mm in 14 months and produced offspring the following year. It is also unknown how frequently and how many molts adults undergo once reaching sexual maturity; however, they must molt at least as often as they produce offspring. Although the life span of *S. pecki* is also unknown, wild-caught adults have been reared in captivity at SMARC for at least 2.7 years with dried leaves and tropical fish flakes as the nutrient sources.

Methods

The primary charge of EAHCP refugia is to preserve the capacity for the HCP Covered Species to be re- established at Comal and San Marcos springs, if ever necessary. This charge dictates that refugia research focuses on captive propagation. As limited life history or captive propagation information is available for *S.pecki*, a large portion of this early research in 2017 will be exploratory or observational. Basic testing is anticipated for everything from housing apparatus, to flow, substrate, or other environmental stimuli. A key research objective moving forward is to build upon 2017 exploratory research that appears promising, by designing subsequent hypothesis driven, quantitative studies as appropriate in the future.

Task 1: Collection of test subjects

Stygobromus pecki will be collected by hand using aquarium nets in upwellings and from drift nets set in spring runs and Landa Lake in the Comal Springs system. Adults will be held separately upon collection as it is presently difficult to distinguish *Stygobromus* species in juveniles. Juveniles will also be collected for preliminary examination of species differences. Amphipods will be transported back to the SMARC and maintained in custom-built aquaria with water continuously supplied and refreshed from the Edwards Aquifer on a flow through system. Diet will consist of commercially available fish flakes, dried organic

material such as sycamore leaves, and bio-films growth inoculated from aquifer water. They will be held in quarantine for 14 days before commencing research observations so acclimation to captivity can occur.

Task 2: Exploratory Research

The limited information on the life history and captive propagation of *S. pecki*, and available literature for this genus leads to the following overarching key assumptions:

- Ability to keep individuals alive in captivity.
- Life span amenable to accomplishing tasks in 1 year.
- Ability to distinguish *Stygobromus* species in juveniles, prior to using juveniles in any species-specific experimentation.

Additionally, each chronological Task 2 subtask is inherently dependent on the level of success in the previous activity. Both the key assumptions and subtask associations will be tracked throughout the project and any concerns reported to EAA upon identification.

2.1 Sexual dimorphism

Stygobromus pecki are known to be sexually dimorphic (Holsinger 1967). However, the distinguishing characteristics can be quite cryptic. Therefore, adult *S. pecki* will have morphology carefully studied by gently wet mounting and observing and photographing multiple individuals; sex will also be determined. These photos will then have multiple characteristics measured and measurements will be analyzed for correlation with sex.

Schedule: April through July

Anticipated results: photo diagrams of male/female sexually dimorphic external features.

2.2 Female fecundity and incubation of eggs

Females will be isolated in flow through tubes supplied with artesian water (preferably females with recently spent ovaries) in preparation for laying eggs into their marsupium. Female ovaries can usually be seen through the translucent dorsal sclerites, lying along the interior dorsal surface of the sclerites and lateral to the hemocoel. They will be checked and photographed weekly to document the development of the ovaries becoming gravid. Photos will be analyzed using the same methodology described above in an effort to describe the rate of change of the ovarian condition. Once ovaries appear gravid, females will be placed with a single male; preferably a larger male given the female on male biased cannibalism reported by previous studies (Nowlin et al. 2015). These females will be mated. Upon eggs being laid into the marsupium, the size of the brood will be counted and females will be moved into brooding chambers that allow neonates to escape cannibalization by the mother. The development of the eggs will be closely monitored by viewing developing embryos through transparent egg shells. This will be done by gently wet mounting

females weekly and using the same photographic analysis described above. Great care needs to be taken to not excessively stress females or apply excess cover slip pressure as females are known to prematurely expel eggs from their marsupium if they feel threatened. Upon hatching, neonates in a brood will be counted and used for the next phase of study.

Schedule: April through September

Anticipated results: understanding of development of ovarian conditions; egg production; egg morphology; incubation duration of eggs; better understanding of conditions that contribute to the production of eggs.

2.3 Juvenile development and sexual maturation

After neonates emerge from the females' marsupia, these young will be isolated in individual flow-through chambers and used for observations and measurements to determine growth curves, molts, and developmental changes per molt. Photos will be taken to examine and document morphological characterizations of each life-history stage. Key morphological features will be illustrated based on photos. Photographs and measurements will be taken twice monthly.

Schedule: July through December

Anticipated results: larvae production; better understanding of rate of larval development; quantification of the number of molts and the duration between molts prior to reaching sexual maturity.

Task 3: Analysis and Reporting

As typical for observational data, it is anticipated that a major portion of any analysis and explanation will be descriptive statistics and morphological documentation. An interim report of the research activities conducted during 2017 be drafted during November 2017, finalized during December 2017, and submitted to EAA by December 31, 2017. The 2017 product is termed Interim in that it is anticipated that certain applied research components will be carried forward into 2018 in order to better understand and document the life cycle of this unique HCP Covered species. A final report (associated with the 2018 work plan) will be prepared and submitted at the conclusion of 2018.

Budget

As discussed in the roles and responsibilities section above, this project will be a Refugia Team effort. A detailed cost breakdown for BIO-WEST's involvement is documented in the table below. All SMARC costs for assistance in this effort will be covered through their established budget (Task 1 – 2017 Workplan) for maintenance and collection of standing stocks. Therefore, the total cost for the 2017 juvenile development and maturation of Peck's cave amphipod study is $94,185.

BIO-WEST (01/01/2017)
DETAILED COST BREAKDOWN - EAHCP 2017 Refugia
Pecks Cave Amphipod Life History Study

LABOR:

Position	Rate	TASK 2 - Applied Research	TOTALS Total Hours	TOTALS Cost
Project Principal	155.87	48	48	$ 7,481.76
Biologist II	75.26	600	600	$ 45,156.00
Senior Administrative	66.72	6	6	$ 400.32
Technician II	51.31	784	784	$ 40,227.04
Total Labor		1,438	1,438	**93,265.12**
TRAVEL				
Per diem: Hotel and Meals				$ -
Mileage ($.535 per mile)	0.535	500	500	$ 267.50
Total Travel				**$ 267.50**
DIRECT COSTS:				
Equipment: water quality sondes, flow meters, etc.				$ -
Supplies		616		616
Phone / Fax / Copies		36		36
Total Direct Costs		652		**$ 652**
Total Estimated Cost			Total	**$ 94,185.00**
	TASKS	$94,185		

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