2018 EAHCP Refugia Work Plan

Introduction

The U.S. Fish and Wildlife Service (USFWS) San Marcos Aquatic Resources Center (SMARC) and Uvalde National Fish Hatchery (UNFH), and BIO-WEST Incorporated (BIO-WEST) will provide refugia, salvage, reintroduction, and monitoring services in fulfillment of the Refugia Contract (Contract # 16-822-HCP) between the Edwards Aquifer Authority (EAA) and the USFWS.

This annual work plan and associated cost estimate have been developed per the requirements of contract number 16-822-HCP for the Implementation of the Refugia Program under the EAHCP. The tasks and subtasks that follow provide the details for the services to be performed in 2018, which provide for the maintenance of a refugia population of the Covered Species (Table 1) including the salvage, propagation, and restocking of the species, if species-specific habitat triggers occur and species are extirpated.

Table 1: Eleven species identified in the EAHCP and listed for coverage under the ITP.

Common Name	Scientific Name	ESA Status
Fountain darter	*Etheostoma fonticola*	Endangered
Comal Springs riffle beetle	*Heterelmis comalensis*	Endangered
San Marcos gambusia	*Gambusia georgei*	Endangered*
Comal Springs dryopid beetle	*Stygoparnus comalensis*	Endangered
Peck's Cave amphipod	*Stygobromus pecki*	Endangered
Texas wild-rice	*Zizania texana*	Endangered
Texas blind salamander	*Eurycea rathbuni*	Endangered
San Marcos salamander	*Eurycea nana*	Threatened
Edwards Aquifer diving beetle	*Haideoporus texanus*	Petitioned
Comal Springs salamander	*Eurycea* sp.	Petitioned
Texas troglobitic water slater	*Lirceolus smithii*	Petitioned

*The San Marcos gambusia was last collected in the wild in 1983, and may already be extinct.

Long-term Objective

A series of refugia held at the SMARC and UNFH will preserve the capacity for the Covered Species to be re-established at the Comal and San Marcos rivers in the event of the loss of population due to a catastrophic event such as the loss of spring flow or a chemical spill.

Background: Section 5.1.1 of the EAHCP requires the EAA to provide a series of refugia, with back-up populations, to preserve the capacity for these species to be re-established in the event of the loss of population due to a catastrophic event.

The concept of refugia is to house and protect adequate populations of the Covered Species and to conduct research activities to expand knowledge of their habitat requirements, biology, life histories, and effective reintroduction techniques. Actions and funding contained within this work plan will be limited to the Covered Species listed in the EAHCP and those associated

species that have significant impact on the Covered Species such as predators, competitors, pathogens, parasites, food, cover, and shelter.

2018 Assumptions

As work plans are developed almost a year prior to implementation, it is possible that methods described herein may be contingent on the status of the current year's activities or authorization from the HCP process.

- Target numbers for the standing and refugia stocks to be housed at both the UNFH and SMARC are established by the USFWS-EAA Refugia Contract (Contract # 16-822-HCP).
- Species capture and mortality rates will be similar to historic values.
- Mortality rates of specimens held in captivity will be similar to historic values.
- Target species collection numbers from the 2017 work plan are reached.
- Construction and renovation will not be interrupted or unexpectedly delayed due to weather, equipment, procurement related delays, or other unforeseen issues.
- Staffs remain employed at the two Service facilities throughout the performance period.

Target for 2018 (Deliverables and Methods by Task):

Task 1. Refugia Operations

Standing Stocks The standing stocks at the SMARC and UNFH will be considered standing stocks under the executed contract (Contract # 16-822-HCP) and will be held in Service facilities until EAA specific Refugia and Quarantine facilities are complete and functional. USFWS staff will take all appropriate steps to collect and maintain standing/refugia stocks at their respective target captive population size in order to provide refugia for all the Covered Species. Table 2 displays the target species numbers.

Table 2. Species target refugia numbers and census.

Species	Standing Stock	Refugia Stock	Salvage Stock	SMARC census (1/1/2018)	Anticipated SMARC census (12/31/2018)	UNFH census (1/1/2018)	Anticipated UNFH census (12/31/2018)
Fountain Darter (Comal)	1000	1000 including specimens within the standing stock	2000	408	400	66	100
Fountain Darter (San Marcos)	1000	1000 including specimens within the standing stock	2500	610	600	147	500
Texas Wild-Rice	430	430 including specimens within the standing stock	1500	240	232	67	121
Texas Blind Salamander	500	500 including specimens within the standing stock	500	47	60	0	15[1]
San Marcos Salamander	500	500 including specimens within the standing stock	500	267	300	180	250
Comal Springs Salamander	500	500 including specimens within the standing stock	500	47	70	4	30
Peck's Cave Amphipod	500	500 including specimens within the standing stock	500	173	250	45	100
Comal Springs Riffle Beetle	500	500 including specimens within the standing stock	500	191	175	51	100
Comal Springs Dryopid Beetle	500	500 including specimens within the standing stock	500	13	*	2	*
Edwards Aquifer Diving Beetle	500	500 including specimens within the standing stock	500	0	*	0	*
Texas Troglobitic Water Slater	500	500 including specimens within the standing stock	500	25	*	0	*

[1]transfer of Texas blind salamanders to UNFH is contingent upon completion of facilities construction and tank system set-up

*catch rates and hatchery survival are uncertain given the rarity of the species

Collection: In 2018, we will collect Covered Species as required to reach and maintain target standing and refugia stock numbers as shown in Table 2. Species collections will be coordinated with other ongoing HCP activities (e.g. Biological Monitoring Program) so that collections for refugia do not impact other efforts adversely. Species specific collections will be carried out through a variety of passive and active collection methods. Prior to collections, Hazard Analysis

Critical Control Point (see Appendix A 2017 Work Plan) will be conducted to minimize aquatic invasive species transfer. ~~Catch per unit effort~~Collection efforts will be documented ~~for each species~~ and reported to ~~the~~ EAA ~~in the year-end report~~. Captured specimens will be divided between the SMARC and UNFH facilities in order to ensure redundancy and to expedite the obligation to establish and maintain two ~~equally sized~~ refugia populations at separate locations. All species will be held in respective quarantine areas until their health has been assessed. Once it is determined that specimens are free from pathogens, parasites, and invasive species they will be incorporated into the general refugia population. USFWS will share reports, including test results, produced as part of the quarantine process. Species-specific collection plans ~~closely~~generally follow those detailed within the 2017 Work Plan; however, collection efforts ~~may~~ vary ~~depending~~based upon ~~what occurs~~collection and knowledge gained during the 2017 collection efforts. The following sections briefly describe planned 2018 collection, maintenance, and propagation efforts for each species.

Please note that we anticipate that once construction on new buildings is completed (at each facility) collection efforts will be slowed or briefly suspended so that staff can focus on setting up new systems in the buildings and begin moving refugia populations to those systems.

Fountain Darters:

Collection: Fountain darters will be collected primarily using dip nets and SCUBA divers in deeper locations (greater than wading depth) to obtain and maintain target numbers (N = 1,000 per river). Approximately 20% of the fountain darters collected annually succumb to natural mortality. If unusual mortality events occur, they will be thoroughly investigated and summary reports will be conveyed to the EAA as part of the monthly reports. As a result, fish collections will target additional fish so that as individuals perish the remainder within the captive population should not decrease below the target number between collection events. Specimens will be collected along a longitudinal gradient. Approximately equal proportions of fish from upper~~, middle,~~ and lower reaches in the Comal (upper = above Landa Lake dam; lower = below Landa Lake dam ~~to confluence of new and old channels, middle = from new and old channel confluence to City Tube Chute, lower = City Tube Chute to confluence of the Guadalupe River~~) and San Marcos (upper = Spring Lake, Middle = Spring lake dam to Rio Vista dam, lower = below Rio Vista dam to Capes dam) rivers will be collected. ~~Fountain darters will not be collected from Landa Lake given~~

Due to the ~~past~~ detection of largemouth bass virus~~. If largemouth bass virus is detected~~ in the ~~downstream reaches below Landa Lake,~~Comal fountain darter throughout the Comal River habitat all Comal fountain darters will be maintained ~~within~~in quarantine facilities~~.~~ in consideration of other species located on the two stations. Collection numbers of Comal fountain darters will be reduced and 2018 target census numbers lower because of space limitation until new facilities are built and systems up and running.

Fountain darters will be collected primarily during the spring and fall to minimize thermal stress during capture and transport. As part of quarantine procedures, a subset of fish (N = 60) will sent to Dexter Fish Health Unit or equivalent facility for pathogen (bacteria, virus, and parasite) testing prior to specimen incorporation into the general refugia population following standardized methods outlined within USFWS and AFS-FHS (2016) and AFS-FHS (2005); reports will be provided to EAA.

~~*Maintenance:* Tank and system maintenance such as acid washing and system sterilization will occur semi-annually or as needed to ensure proper system function.~~*Maintenance:* Water quality (i.e. temperature, pH, dissolved oxygen, total dissolved gasses) will be monitored and recorded weekly. Fountain darters will be fed live foods reared or purchased. Ponds will be utilized to produce zooplankton and amphipods. Ponds will be managed to maintain idealized zooplankton assemblages and densities. Amphipods will be collected from other managed ponds and raceways (see Cantu et al. 2009). Black worms will be purchased when necessary along with other food resources (i.e. blood worms, black worms, brine shrimp, etc.) if the need arises. Food items are not routinely examined for pathogens. However, if they are suspect and tested for pathogens all diagnostic results will be conveyed to the EAA within monthly reports.

Propagation: Standing and refugia stocks for each river will be maintained to discourage reproduction unless HCP triggers occur. Fish will be maintained by their geographical locations. If reintroduction is warranted, subsets from each geographical location will be communally spawned. Subset groups will be culled to an equal number of progeny prior to release.

Texas wild rice:

Collection: Texas wild rice tillers will be collected from specific San Marcos River reaches, with a break during summer months when wild rice does not fare well due to heat stress (Fig. 1). ~~Tillers~~In 2018 collections for SMARC will ~~be collected in a proportional manner to mirror~~target stands that are not already part of the refugia population or require supplementation. Collections for UNFH will continue to build their refugia numbers and representative locations. The refugia populations will reflect the wild populations in both their respective proportion and genetic diversity ~~currently and~~ that was historically ~~available~~documented within ~~the population~~ San Marcos River (Table 3; Wilson et al. 2016). During tiller collection, the GPS coordinates, area coverage, and depth of the stand or individual plant will be recorded so the exact location of the clone is known. For larger stands, tillers will be collected at the beginning, middle and end of the stand, or every 20% of the stand's total length for the largest stands. ~~Although tiller~~Tiller collection ~~in most river reaches~~ will be done by wading ~~some river reaches require~~and the use SCUBA gear. Texas wild rice seeds from the river will also be collected monthly or when available and stored at both facilities. Seed stocks will be replaced every six months when seeds are available. Please note that during the 2017 Texas wild rice survey no plants were found in Section E I, J, and K. Plants were found in sections G and H.

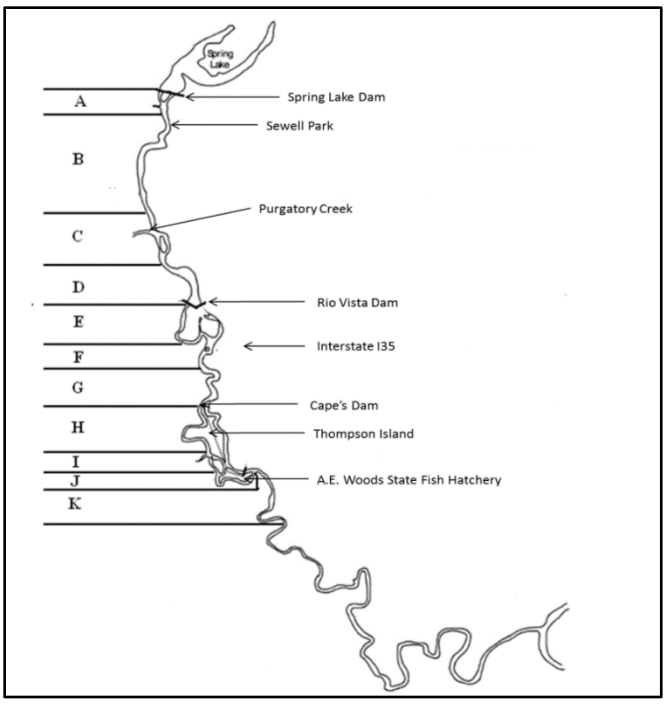

Figure 1~~.~~ Letters define designated San Marcos River reaches where Texas wild rice is collected for refugia populations.

Maintenance: Once tillers have been successfully rooted they will be tagged and maintained so that their collection location is known.

Propagation: ~~Plants will be maintained by their geographical locations.~~ Plants will be maintained so sexual reproduction does not occur within the refugia population, unless HCP triggers occur. If reintroduction is warranted, seeds and tillers from each geographical location will be produced. Plants produced from seeds and tillers would be transplanted back within their original geographic location.

Table 3. The number of Texas wild rice plants needed at the SMARC and UNFH to obtain the total target number of 430. Each San Marcos River reach is denoted by a letter and the proportion of specimens needed per reach is estimated from Wilson et al. (2016). Based on Wilson et al. (2016) no plants will be collected from sections I, L, M (, shaded-out). ~~Viability of~~No plants were observed in ~~section G, H~~sections E, I, J, and K (*) ~~are still being assessed due to flood/scouring events, best efforts~~during 2017; these sections will be ~~made to collect~~re-evaluated in ~~these areas without over taxing the plants~~2018. Projected numbers are based on an anticipated mortality of 20% for newly acquired plants and 10% for mature refugia stock**.

River Section	Census Jan 2018	Number of plants targeted in 2018	Anticipated 2018 EOY Census
SMARC			
A	21	10	27
B	107	5	101
C	41	5	41
D	6	5	10
E*	5	0	5
F	25	5	27
G	5	3	7
H	3	3	5
I**	-	-	-
J*	8	0	7
K*	2	0	2
L**	-	-	-
M**	-	-	-
UNFH			
A	11	15	22
B	23	25	41
C	10	15	21
D	10	0	9
E*	0	0	0
F	13	10	20
G	0	5	4
H	0	5	4
I**	-	-	-
J*	0	0	0
K*	0	0	0
L**	-	-	-
M**	-	-	-

Texas blind salamanders:

Collection: Texas blind salamanders will be collected through the use of nets and traps. Traps will be deployed quarterly for ~~five~~approximately 12 consecutive days with traps checked every 2-4 days to collect Texas blind salamander specimens from Primers Fissure, Johnson's well, Rattlesnake cave, and Rattlesnake well (Table 5). To avoid oversampling these habitats, only 1/3 of salamanders observed from each of these locations will be collected during quarterly sampling events. Concurrently, salamanders will also be collected from ~~Sessoms Creek, Texas State University~~a driftnet on Diversion Springs~~, and a third outlet near Diversion Springs (~~ in Spring Lake fished continuously throughout the year. Periodically collections will be made from Spring Lake Outflow~~). These latter~~ with a driftnet. Specimens from these two sites will ~~be fished continuously until refugia target specimen numbers are met~~all be kept, given the assumption that any Texas blind salamander leaving a spring orifice that enters a stream or lake environment will ultimately succumb to predation. These sites will be checked for specimens up to three times per week where applicable. All specimens will be transported live and maintained

in the SMARC and UNFH refugia. ~~Approximately 5% of the Texas blind salamanders collected annually succumb to natural mortality. As a result, salamander collections will continue until the captive population exceeds target numbers by at least 12 individuals at both the SMARC and UNFH.~~When not being checked by Texas State staff, we will also check nets on Sessom Creek and Texas State Artesian Well; when these nets are being check by Texas State staff live Texas blind salamanders are transferred to SMARC according to their permits.

Maintenance: Specimens will be maintained by collection location. As part of quarantine, all salamanders of each species will be non-lethally cotton swabbed. These samples will be sent to Dexter Fish Health Unit to screen for *Batrachochytrium dendrobatidis* (Bd, commonly referred to as chytrid) and *Batrachochytrium salamandrivorans* (Bsal) prior to specimen incorporation into the general refugia population. Chytrid testing will occur in batches where groups of five swabs will be pooled for analysis as opposed to individual analysis. Duplicate individual swabs will be retained in case further testing is warranted. All salamanders will be held in quarantine for at least 30 days and until test results have returned. Chytrid (Bd) fungus has caused mortalities in amphibian species; however, some species appear to have innate immunity. Previous tests of wild caught salamanders at SMARC (both Texas Blind and San Marcos) have almost always tested positive for Bd. Clinically, the salamanders appear normal and do not have any lesions or signs of disease. Positive testing for Bsal will be treated more cautiously as it has not been documented in this area before; these salamanders would remain in quarantine until further study and recommendations from FWS Fish Health. Salamander tank and system maintenance such as acid washing and system sterilization will occur annually or as needed to ensure proper system function. Water quality will be monitored and recorded weekly. Salamanders will be fed live foods reared or purchased. Ponds will be utilized to produce amphipods. Amphipods will be collected from other managed ponds and raceways (see Cantu et al. 2009). Black worms will be purchased when necessary along with other food resources (i.e. blood worms, brine shrimp, etc.) if the need arises.

Propagation: Standing and refugia stocks will be maintained to encourage reproduction. Salamanders will be ~~maintained~~marked by their geographical locations. All progeny will be maintained separately by generations. If reintroduction is warranted, an attempt will be made to produce offspring from each geographical location.

San Marcos salamanders:

Collection: San Marcos salamanders will be collected up to quarterly from below Spring Lake dam ~~(western shore), Diversion Springs, areas surrounding Diversion Springs,~~ and with SCUBA teams in Spring Lake ~~Outflow~~ (Table 5). The drift net on Diversion Springs will be checked routinely and specimens will be kept from this location. Collection efforts will be coordinated with the HCP Biological Monitoring Program. ~~A SCUBA team will be used for a portion of these collection efforts. These sites will be checked for specimens regularly.~~ All specimens will be transported live and maintained in the SMARC and UNFH refugia. Approximately 30% of the San Marcos salamanders collected annually succumb to natural mortality. As a result, salamander collections will target additional specimens so that as individuals perish the remainder within the captive population should not decrease below the target number between collection events.

~~*Maintenance:* Specimens will not be maintained by collection location.~~*Maintenance:* As part of quarantine, all salamanders of each species will be non-lethally cotton swabbed. These samples will be sent to Dexter Fish Health Unit to screen for *Batrachochytrium dendrobatidis* (Bd, commonly referred to as chytrid) and *Batrachochytrium salamandrivorans* (Bsal) prior to specimen incorporation into the general refugia population. Chytrid testing will occur in batches where groups of five swabs will be pooled for analysis as opposed to individual analysis. Duplicate individual swabs will be retained in case further testing is warranted. All salamanders will be held in quarantine for at least 30 days and until test results have returned. Chytrid (Bd) fungus has caused mortalities in amphibian species; however, some species appear to have innate immunity. Previous tests of wild caught salamanders at SMARC (both Texas Blind and San Marcos) have almost always tested positive for Bd. Clinically, the salamanders appear normal and do not have any lesions or signs of disease. Positive testing for Bsal will be treated more cautiously as it has not been documented in this area before; these salamanders would remain in quarantine until further study and recommendations from FWS Fish Health. Salamander tank and system maintenance such as acid washing and system sterilization will occur annually or as needed to ensure proper system function. Water quality will be monitored and recorded weekly. Salamanders will be fed live foods reared or purchased. Ponds will be utilized to produce amphipods. Amphipods will be collected from other managed ponds and raceways (see Cantu et al. 2009). Black worms will be purchased when necessary along with other food resources (i.e. blood worms, brine shrimp, etc.) if the need arises.

Propagation: Standing and refugia stocks will be maintained to discourage reproduction. If reintroduction is warranted, pairwise mating will be employed to produce offspring. Stocking will occur once juveniles have reached 30 mm total length.

***Comal Springs salamanders*:**

Collection: Comal Springs salamanders will be collected up to quarterly from Comal Spring runs 1-3 and Spring Island and surrounding areas (Table 5). Close coordination with the HCP biological monitoring program will take place to ensure that to the degree practicable, refugia collections do not overlap with specific HCP long-term monitoring locales. In the event overlap of sampling areas is unavoidable, Comal salamanders for refugia will be collected at a rate of no more than 10% of salamanders observed in those specific locales per daily sampling trip. A SCUBA team will be used for a portion of these collection efforts as necessary. Annual natural mortality will be recorded. ~~As a result, salamander collections will target additional salamanders so that as individuals perish the remainder within the captive population should not decrease below the target number between collection events.~~

Maintenance: As part of quarantine, all salamanders of each species will be non-lethally cotton swabbed. These samples will be sent to Dexter Fish Health Unit to screen for *Batrachochytrium dendrobatidis* (Bd, commonly referred to as chytrid) and *Batrachochytrium salamandrivorans* (Bsal) prior to specimen incorporation into the general refugia population. ~~*Maintenance:* Specimens will not be maintained by collection location. As part of quarantine, all salamanders of each species will be non-lethally cotton swabbed. These samples will be sent to Dexter Fish Health Unit to screen for *Batrachochytrium dendrobatidis* (Bd, commonly referred to as chytrid) and *Batrachochytrium salamandrivorans* (Bsal) prior to specimen incorporation into the general refugia population.~~Chytrid testing will occur in batches where groups of five swabs will be

pooled for analysis as opposed to individual analysis. Duplicate individual swabs will be retained in case further testing is warranted. All salamanders will be held in quarantine for at least 30 days and until test results have returned. Chytrid (Bd) fungus has caused mortalities in amphibian species; however, some species appear to have innate immunity. Previous tests of wild caught salamanders at SMARC (both Texas Blind and San Marcos) have almost always tested positive for Bd. Clinically, the salamanders appear normal and do not have any lesions or signs of disease. Positive testing for Bsal will be treated more cautiously as it has not been documented in this area before; these salamanders would remain in quarantine until further study and recommendations from FWS Fish Health. Salamander tank and system maintenance such as acid washing and system sterilization will occur annually or as needed to ensure proper system function. Water quality will be monitored and recorded weekly. Salamanders will be fed live foods reared or purchased. Ponds will be utilized to produce amphipods. Amphipods will be collected from other managed ponds and raceways (see Cantu et al. 2009). Black worms will be purchased when necessary along with other food resources (i.e. blood worms, brine shrimp, etc.) if the need arises.

Propagation: Standing and refugia stocks will be maintained to discourage reproduction. If reintroduction is warranted, pairwise mating will be employed to produce offspring. Stocking will occur once juveniles have reached 30 mm total length.

Comal Springs riffle beetle:

Collection: Comal Spring riffle beetle collection will ~~occur quarterly and be coordinated~~be reduced from the number of collections that occurred in 2017 with ~~the HCP Biological Monitoring Program~~up to six targeted events in 2018 (Table 5). The reduced target census numbers in Table 2 reflect this reduction in effort. No collections will occur during months when HCP monitoring is scheduled. Riffle beetles will be collected with cotton lures. Cotton lures will be deployed in a variety of locations (Spring Runs 1, 2, 3, N = ~~10~~5-15 lures per spring run; western shore of Landa Lake, N = 5 lures; Spring Island and associated Spring Lake habitats N = 15-20 lures) following EAHCP standard operating procedures (Hall 2016). Coordination with the HCP biological monitoring program will take place to ensure that to the degree practicable, refugia collections do not overlap with specific HCP long-term monitoring locales. In the event overlap of specific routine sampling locations is unavoidable, Comal Springs riffle beetles for refugia will be collected at a rate of no more than 25% of beetles observed per lure in those specific locales per daily sampling trip. Lures will be allowed to mature biofilms for four weeks. Riffle beetles will be collected during the fourth week and lures will be removed. Approximately 50% of the Comal Springs riffle beetles collected annually succumb to natural mortality. As a result, invertebrate collections will target additional specimens so that as individuals perish the remainder within the captive population should not decrease below the target number between collection events.

Maintenance: Specimens will not be maintained by collection location. Comal Springs riffle beetles will be maintained within custom built aquatic holding units and fed detrital matter and matured biofilms colonized on cotton lures.

Propagation: Propagation methods for this species are being developed.

Peck's Cave amphipod:

Collection: Peck's Cave amphipod collection will occur up to five times annually (Table 5). Adult Peck's cave amphipods will be collected through the use of drift nets and hand collection. Drift nets will be deployed in a variety of locations (Spring Run 3, N = 2; Spring Island and associated Spring Lake habitats, hand collection). Approximately 50% of the Peck's Cave amphipod collected annually succumb to natural mortality. As a result, invertebrate collections will target additional specimens so that as individuals perish the remainder within the captive population should not decrease below the target number between collection events.

Maintenance: Specimens will not be maintained by collection location. Peck's Cave amphipods will be maintained within custom built aquatic holding units and fed commercial flake fish feeds.

Propagation: Propagation methods for this species are being developed as part of standard refugia operations.

Comal Springs dryopid beetle:

Collection: Comal Spring dryopid beetle collection will occur quarterly (Table 5). Dryopid beetles will be collected through the use of cotton lures concurrently with Comal Spring riffle beetle ~~and during independent sampling trips. Cotton lures will be deployed in a variety of locations (Sessoms Creek N = 5 to 10 lures; Spring Island and associated Spring Lake habitats, 10 to 15 lures).~~lure collections. In addition to cotton lures, wooden dowel rods will concurrently be tested as a lure technique for dryopid beetles. All lures (cotton or wooden) will be allowed to mature biofilms for four weeks. Dryopid beetles will be collected during the fourth week and lures will be removed. ~~If collection numbers need to be supplemented or low flows decrease upwelling locations in other areas, bottle traps~~Bottle traps and experimental nets will also be deployed into Panther Canyon Well~~. Bottle traps~~ during April and September. These will be checked weekly for a month. We have ceased collection efforts of lures in Sessom Creek as these were not fruitful during 2017; a new design for Sessom Creek might be revisited at a later date.

Maintenance: Specimens will not be maintained by collection location. Comal Spring dryopid beetle will be maintained within custom built aquatic holding units and fed detrital matter and matured biofilms colonized on cotton lures.

Propagation: Propagation methods for this species are being developed as part of normal refugia operations and research projects.

Edwards Aquifer diving beetle:

Collection: Drift nets will be used to collect Edwards Aquifer diving beetle (Table 5). Drift nets will be set at a variety of locations where the species has been collected in the past (Sessoms Creek N = 1; Texas State University Artesian Well N = 1; and Diversion Springs N = 1~~ to 2~~). Drift nets will be deployed and checked weekly over the course of the year.

Maintenance: Specimens will not be maintained by collection location. Captured specimens will be transferred to the SMARC and housed in custom made aquatic holding systems. Initially

the species will be fed small invertebrates (e.g. ostracods), given they are predators.

Propagation: Propagation methods for this species are to be determined and will be conducted as part of normal refugia operations.

Texas troglobitic water slater:

Collection: Drift nets will be used to collect the Texas troglobitic water slater (Table 5). We intend to set drift nets (Sessoms Creek; N = 1, Texas State University Artesian Well N = 1; and Diversion Springs N = 1 to 2) weekly as necessary. Drift nets will be checked weekly over the course of the year. ~~Lures~~We will also ~~be placed in Spring Lake~~employ new lure designs developed for well and cave environments. The lures will be allowed to mature a biofilm for four to six weeks. ~~A SCUBA team will be required to set and retrieve~~The success or failure of these ~~Spring Lake lures~~trials will be recorded and assessed.

Maintenance: Captured specimens will be transferred to the SMARC and housed in custom made aquatic holding systems. Initially the species will be fed detrital matter and matured biofilms colonized on cotton lures.

Propagation: Propagation methods for this species are to be determined and will be conducted as part of normal refugia operations.

~~**Table 5. All species sampling schedule for 2018. Abbreviations: T = Tuesday, F = Friday, TSU = Texas State University.**~~

Table 5. A tentative schedule for all species sampling during 2018. Collections listed here are subject to change due to extenuating circumstances such as weather, coordination with external partners, and completion of construction projects. EEA and partners will be notified of sampling dates as they become known or changed. Not included in this table are Texas wild rice seed collections given the unpredictable nature of sexual reproduction.

Edward's Aquifer Species Collection Plan 2018			
Date (month)	**Interval**	**Location**	**Target Species**
Continuous	Check nets T and F every week	Diversion Springs ~~and well outflow, Sessoms Creek and TSU well~~	Texas Blind salamander, San Marcos salamander, Edward's Aquifer diving beetle, and troglobitic water slater
~~**Continuous**~~	~~Check lures/Set new lures on a 4 week cycle~~	~~Sessoms Creek~~	~~Comal Springs dryopid beetle~~
~~**Continuous**~~	~~Check lures/Set new lures on a 4 week cycle~~	~~Spring Runs and Landa Lake~~	~~Comal Springs dryopid beetle~~
January	Check every T & F for 2 consecutive weeks	Rattlesnake Cave & Rattlesnake Well	Texas blind salamander
January	Beginning of month, check and reset lures	Spring Runs	Comal Springs riffle beetle, Comal Springs dryopid beetle
February	Check every T & F for 2 consecutive weeks	Primer's Fissure & Johnson's Well	Texas blind salamander
February	~~1-2 day sampling event~~Beginning of month, check and reset lures	~~Comal Springs~~ Spring Runs, Landa Lake	Comal Springs ~~salamander~~riffle beetle, Comal Spring dryopid beetle
February	1-day sampling event, hand pick	Landa Lake	Peck's Cave amphipod
February	1-day sampling event	San Marcos River	Texas wild rice
March	1-2 day sampling event	Spring Lake and below dam	San Marcos salamander
~~February/~~March	~~Set lures (1 day) Feb, 4 weeks later Retrieve lures (2 days) March~~Beginning of month, retrieve lures	Spring Runs ~~and~~, Landa Lake	Comal Springs riffle beetle, Comal Springs dryopid beetle

March	1-~~2~~ day sampling event	~~Diversion Springs, western shore, & below dam~~San Marcos River	~~San Marcos salamander~~Texas wild rice
March~~/April~~	~~Set lures March, 4 weeks later Retrieve lures April~~1-day sampling event, hand pick	~~Spring~~Landa Lake	~~Texas troglobitic water slater~~Peck's Cave amphipod
April	Check every T & F for 2 consecutive weeks	Rattlesnake Cave & Rattlesnake Well	Texas blind salamander
April	1-day sampling event	San Marcos River	Texas wild rice
April	Throughout month	Panther Canyon	Comal Springs dryopid beetle
April/May	~~Hand pick/drift net (1 day)~~Reset lures after biomonitoring	Spring ~~Runs and~~rungs, Landa Lake	~~Peck's cave amphipod~~Comal Springs riffle beetle, Comal Springs dryopid beetle
May	Check every T & F for 2 consecutive weeks	Primer's Fissure & Johnson's Well	Texas blind salamander
May	1-2 day sampling event	Comal Springs	Comal Springs salamander
May	~~2 days~~1-day sampling event	San Marcos River~~ and Comal River~~	Texas wild rice~~Fountain darters~~
May	1-day sampling event, hand pick	Landa Lake	Peck's Cave amphipod
May~~/June~~	~~Set~~Check lures (~~1 day) May,~~ 4 weeks ~~later Retrieve lures (2 days) June~~after set) and reset	Spring Runs~~ and~~, Landa Lake	Comal Springs riffle beetle, Comal Springs dryopid beetle
June	4-day sampling event	San Marcos River and Comal River	Fountain darters
June	~~Hand pick/drift net (1 day)~~Check and retrieve lures	Spring ~~Runs and~~runs, Landa Lake	~~Peck's cave amphipod~~Comal Springs riffle beetle, Comal Springs dryopid beetle
July	Check every T & F for 2 consecutive weeks	Rattlesnake Cave & Rattlesnake Well	Texas blind salamander

~~July~~	~~Hand pick/drift net (1 day)~~	~~Spring Runs and Landa Lake~~	~~Peck's cave amphipod~~
August	Check every T & F for 2 consecutive weeks	Primer's Fissure & Johnson's Well	Texas blind salamander
August	~~Bottle traps every week (if needed to supplement numbers or due to low flow)~~Beginning of month set lures	Spring Runs, Landa Lake~~Panther Canyon~~	Comal Springs riffle beetle, Comal Springs dryopid beetle
August	1-~~2~~ day sampling event, hand pick	Landa Lake~~Comal Springs~~	~~Comal Springs salamander~~Peck's Cave amphipod
~~August/~~September	~~Set lures (1 day) Aug, 4 weeks later Retrieve lures (2 days) Sept~~1-2 day sampling event	Spring ~~Runs~~Lake and below dam~~Landa Lake~~	~~Comal Springs riffle beetle~~San Marcos salamander
September	~~1-2 day sampling event~~Beginning of month, check and remove lures	~~Diversion Springs, western shore, & below dam~~Spring Runs, Landa Lake	~~San Marcos salamander~~Comal Springs riffle beetle, Comal Springs dryopid beetle
September~~/October~~	~~Set lures Sept, 4 weeks later Retrieve lures Oct~~Throughout month	~~Spring Lake~~Panther Canyon	~~Texas troglobitic water slater~~Comal Springs dryopid beetle
October	Check every T & F for 2 consecutive weeks	Rattlesnake Cave & Rattlesnake Well	Texas blind salamander
October	~~Hand pick/drift net (1 day)~~4-day sampling event	~~Spring Runs~~San Marcos River and ~~Landa Lake~~Comal River	Fountain darters~~Peck's cave amphipod~~
October	~~2 days~~1-day sampling event	San Marcos River~~ and Comal River~~	Texas wild rice~~Fountain darters~~
November	Check every T & F for 2 consecutive weeks	Primer's Fissure & Johnson's Well	Texas blind salamander
November	Beginning of month set lures, if needed	Spring Runs, Landa Lake	Comal Springs riffle beetle, Comal Springs dryopid beetle
November	~~Hand~~1-day sampling event, hand pick~~/drift net (1 day)~~	~~Spring Runs and~~ Landa Lake	Peck's ~~cave~~Cave amphipod

November	1-day sampling event	San Marcos River	Texas wild rice
November	1-2 day sampling event	Comal Springs	Comal Springs salamander
~~November/~~December	~~Set lures (1 day) Nov, 4 weeks later Retrieve lures (2 days) Dec~~Beginning of month, check and reset lures, if needed	Spring Runs~~ and~~, Landa Lake	Comal Springs riffle beetle, Comal Springs dryopid beetle
December	1-day sampling event	San Marcos River	Texas wild rice

Refugium Stocks:

Collection: Species collections will be ongoing until refugia stocks target numbers are obtained as shown in Table 2.

Maintenance: Maintenance will be conducted in a similar manner described for standing stocks.

Propagation: Texas blind salamander, Comal Springs riffle beetle, Comal Springs dryopid beetle, Edwards Aquifer diving beetle, and Texas troglobitic water slater may be propagated to further advance culture techniques. Propagation for stocking is not anticipated during 2018.

Salvage Stocks:

Collection: If HCP species-specific salvage triggers are reached in consultation with the EAA, the SMARC will accommodate salvaged organisms no more than two times during the 12-year period. If triggers for multiple species are reached simultaneously species collections during salvage operations will be prioritized based upon the perceived species-specific effect of reduced river and spring flow and habitat degradation (i.e. EAHCP triggers). Those species that are river obligate species (e.g. fountain darter and Texas wild rice) or that occupy spring orifice and interstitial ground water habitats (e.g. San Marcos and Comal Springs salamander, Peck's Cave amphipod, Comal Springs dryopid beetle) as opposed to those that reside solely within the aquifer (e.g. Edwards Aquifer diving beetle, Texas troglobitic water slater and Texas blind salamander) are presumed to be affected first as flows decrease.

Maintenance: Organisms collected during salvage operations would be maintained at the SMARC for a limited duration (up to one-year) or until their disposition was determined. Research may be suspended or terminated if space is required for salvaged organisms. Research may also be suspended if personnel are directed to collection and maintain salvage stocks.

Propagation: Likewise, production of species would be limited to no more than two times during the 12-year period once species extirpation is determined. Species produced at the

SMARC would be held for a limited time (up to one year) or less if stocking is required. Research activities may be suspended or terminated if space is required to house cultured species. Research may also be suspended if personnel are directed to reproduce, maintain, or stock salvage stocks or standing stock progeny.

Construction/Renovation/Infrastructure/Facility:

~~It is anticipated that construction on the Refugia and Quarantine spaces at UNFH will be completed during December 2017-January 2018. UNFH staff will install tanks upon construction completion.~~Construction on the SMARC Refugia and Quarantine buildings will continue into 2018 with anticipated completion during ~~March~~summer 2018. SMARC staff inspector will continue weekly reports until construction completion.

SMARC staff will install tanks upon the construction completion. After systems are set up, covered species will be moved into the spaces.

The renovations at UNFH will be put out for contractor bids in early 2018. After the contract is awarded construction will commence. It is anticipated that construction at UNFH will be completed by December 2018. UNFH staff will install tanks upon the construction completion. After systems are set up, covered species will be moved into the renovated spaces.

After construction is complete (at both sites) the SMARC Center Director will develop and maintain a list of warranty problems during the 1-year warranty period, forwarding items, as they occur, to the Contracting Officer (CO) and the USFWS Project Manager (COR).

As detailed within the EAA contract with the USFWS (Contract No. 16-822-HCP) all invoices from the USFWS to the EAA for the construction services shall be billed on the last business day of the month and sent monthly and shall provide an itemization of the expenses incurred and all supporting documentation.

All reasonable and practical security measures will be instituted by SMARC and UNFH staff to safeguard EAA refugia facilities, equipment, and species.

Anticipated Equipment Purchases 2018 not including construction and renovation materials:

U.S. Fish and Wildlife Service						
	Task	**Equipment**	**Quantity**	**Cost/Unit**	**Total**	**Total Task Budget Amount**
1	Refugia Operations					$404,539
	SMARC Refugia & Quarantine bldg.					
		Fiberglass tanks	30	$ 3,000	$ 90,000	
		35 ton chiller	1		$ 72,532	
	UNFH Renovation Refugia & Quarantine					
		Fiberglass tanks	30	$ 3,000	$ 90,000	
		1 HP Chiller Units	9	$ 6,600	$ 59,400	
		35 ton chiller	1		$ 75,000	
		Generator	1		$ 17,607	
2	Research	Tanks			$ 1,000	$17,102
		PVC/Fittings/Hose			$ 7,000	
		Cameras/Scope/Software			$ 5,000	
		Misc. Supplies			$ 4,102	
3	Species Propagation and	N/A				$0
4	Species Reintroduction	N/A				$0
5	Reporting	N/A				$0
6	Meetings and Presentations	N/A				$0
Total					**$ 421,641**	

Staffing/Labor/Personnel:

The Supervisory Fish Biologists (SFBs) at both the SMARC and UNFH will continue in their duties including, but not limited to: supervising, mentoring, and training lower-graded employees, authorize purchases, oversee facility maintenance and repair, develop and implement budgets, organize and maintain outreach materials and activities that relate to all contract activities. The SFBs will manage and coordinate propagation, culture, and field activities related to the refugia. The SFBs are expected to provide proper and efficient use of facilities and staff resources. The SFBs will work with the Center Director to ensure that contractual obligations are met in a timely manner. In coordination with the Center Director, they will prepare all the required written materials required for the reimbursable agreement reporting. Likewise, the SFBs will also prepare oral presentations to be used as briefing statements, outreach presentations, internal reports, work summaries, and technical presentations at professional meetings. The two SFBs will continue to work and communicate regularly with partners, Service personnel and other researchers to effectively meet Service and reimbursable agreement goals.

Under the management of a lead supervisory biologist at both facilities, it is expected that the three Biological Science Technicians will continue to assist with the collection, daily upkeep, maintenance, and propagation efforts for the nine species at the SMARC and UNFH. This includes maintaining experimental and culture production systems, keeping records along with

entering, filing, and collating data. The incumbents will also generate basic summary statistics and graphic analyses of data and document program accomplishments through the composition of Standard Operating Procedures (SOPs), reports, and manuscripts.

Permitting:
Both the UNFH and SMARC operate under the USFWS Southwest Region's Federal Fish and Wildlife Permit for Native, Endangered, and Threatened Species Recovery (number TE676811-3) and the Texas Parks and Wildlife Scientific Research Permits (UNFH SPR-1015-222, SMARC SPR-0616-153).

Biosecurity:
Both the UNFH and SMARC operate under the SMARC BioSecurity Plan (2014) (Exhibit E of 16-822-HCP). Specimen Collection, Hazard Analysis Critical Control Points, Quarantine, & Specimen Transfer: San Marcos Aquatic Resources Center Standard Operating Procedure.

Task 2. Research

The Research Plan for 2018 will involve a series of activities ranging from 1) continuing and expanding upon on-going species-specific studies for *Stygoparnus comalensis*, *Stygobromus pecki*, and *Heterelmis comalensis*; 2) conducting research specific to captive propagation refinement for San Marcos salamanders; and 3) reexamining invertebrate collection methodologies concurrent with testing new designs. The following section describes the basic components of each of these proposed 2018 activities.

Continuation of Life History Studies:
Project 1:

Title: ~~Continued evaluation of life stage development, diet, and environmental stimuli directly related to the successful captive propagation~~ Life-history study of Comal Springs ~~Dryopid Beetles~~ dryopid beetles (*Stygoparnus comalensis*).

Species: ~~Comal Springs dryopid beetle~~*Stygoparnus comalensis*

Principal/Co-PI: BIO-WEST, input by ~~Randy Gibson, Dr. Lindsay Campbell~~SMARC staff

~~**Overview:** At present (2017), objectives for *S. comalensis* applied refuge research are to, 1) determine conditions that contribute to the production of eggs, 2) determine where and how eggs are deposited and egg morphology, 3) determine incubation duration of eggs, 4) study the rate of larval development, 5) document the morphology of larval instars, and 6) determine factors that contribute to pupation. However, not all aspects of research commenced in 2017 are expected to be completed by the end of 2017 primarily due to the relatively long life cycle of *S. comalensis* and limited availability of test subjects.~~

~~Ongoing 2017 research is expected to be able to produce eggs and larvae of *S. comalensis* and determine where and how eggs are deposited, the conditions that contribute to the production of eggs, egg size and morphology, and how long eggs incubate before hatching. However, it is unlikely that larvae will have completed development leaving the remaining tasks incomplete at the conclusion of 2017.~~

~~**Objectives and Methods:** Life history studies will be continued into 2018 with an~~

~~expansion into evaluating additional life history characteristics, diet and environmental stimuli that may affect the captive propagation of this species. The major objectives of 2018 research for *S. comalensis* are to:~~

- ~~continue studies of factors contributing to egg production to optimize this phase of cultivation;~~
- ~~continue studies of factors contributing to eggs successfully hatching to optimize this phase of cultivation;~~
- ~~continue research documenting growth rate and instars of larvae,~~
- ~~initiate studies on adult diet to optimize adult survival;~~
- ~~initiate studies on larval diet in an effort to optimize diet for each larval instar in an effort to achieve higher survivability of larvae as it is possible that nutritional requirements change as larvae develop;~~
- ~~if appropriate, study factors that contribute to pupation and eclosure into adults; and~~
- ~~if appropriate, estimate the overall life span.~~

~~The last two bullets start with "if appropriate" which is directly tied to the uncertainty of pupation for this species. Literature documents that development for dryopid larvae can take 2-5 years before pupating (Ulrich 1986). Subterranean species tend to have development exasperated for durations much longer than their epigean relatives (Culver and Pipan 2009), therefore it is possible that *S. comalensis* larvae require longer than 5 years to complete development; potentially much longer. It is anticipated that last two tasks will require study into the future to fully describe the life span of *S. comalensis*. Specific methods for each 2018 activity will be developed towards the conclusion of 2017 research in order to maximize the knowledge gained from ongoing experimentation.~~

~~**Expected Results:** In compilation with 2017 findings, 2018 results will provide information on the life cycle of *S. comalensis* necessary to promote effective and efficient captive propagation of this species. The key life history aspects will be a better understanding of reproduction and the growth, development, diet, and environmental stimuli that affect life stages relative to success in captivity. The main deliverable will be a final report that includes an updated standard operating procedure (SOP) for rearing Comal Springs dryopid beetles through their various life stages. The SOP will include instructions for rearing, descriptions of equipment used, environmental stimuli incorporated, and other husbandry requirements and recommendations for future studies.~~

Overview: Ongoing research initiated in 2017 is focused on producing eggs and larvae of *S. comalensis*, determine where and how eggs are deposited. When successful, larval growth and habitat preferences will be investigated.

Objectives and Methods:

1. Identify sexual dimorphic characters.
2. Determine if eggs are oviposited above or below water.
3. Estimate fecundity (number of eggs per clutch).
4. Estimate incubation duration.
5. Identify larval habitat (submerged or emergent).

6. Begin documentation on larval growth rates.
7. Identify adult response to flow.

Due to the paucity of knowledge related to this species, basic observations are necessary in order to ask more directed questions. Furthermore, study aspects should be intended as non-lethal experiments. Additional collections will be required in order to conduct observations and experiments. An Oblique Plan Apparatus (OPA) was constructed in 2017 and a mating trial was initiated. Continued monitoring of this experiment and modification of the OPA or construction of a more effective monitoring device is anticipated. Construction of a variable flow mesocosom will be necessary for investigating environmental conditions favorable to adults and relevant to oviposition.

Expected Results: Identifiable characters for distinguishing sexes, a better understanding of environmental stimuli related to oviposition, identification of environmental requirements for hatching and larval growth. Documentation of larval development, egg incubations rates, and fecundity.

Project 2:

Title: ~~Continued evaluation of life stage development including life span description and sex determination~~Life-history study of Peck's Cave ~~Amphipods~~Amphipod (*Stygobromus pecki*).

Species: ~~Peck's Cave Amphipod~~*Stygobromus pecki*

Principal/Co-PI: BIO-WEST, input by ~~Randy Gibson, Dr. Lindsay Campbell~~SMARC staff

Overview: ~~At present (2017), objectives for *S. pecki* applied refuge research are to: 1) determine how many molts must occur before it becomes possible to distinguish individuals from other *Stygobromus* species and better understand the morphology of each developmental stage; 2) estimate how many molts must occur before sexual maturity is reached; 3) estimate how frequently a female can produce a brood and the typical size of a brood; and 4) to better understand sexual dimorphism for the purpose of creating individual breeding pairs. Ongoing 2017 research is expected to yield several answers and solutions pertaining to the captive propagation and life history of *S. pecki*. It is anticipated that average incubation time of eggs will be determined, neonates will be produced, reared to an old enough age so that species specific characteristics can be discerned, with morphology and development documented along with timing of developmental events. It is also possible that certain aspects of sexual dimorphism and female brood size and frequency will be better understood. However, it is unlikely that all aspects of 2017 research will be completed in 2017 as literature documents that subterranean amphipods (like other subterranean species) have a much slower rate of development and reproduction than epigean species typically taking at least a year to mature (Crawford and Tarter 1979).~~ Ongoing research initiated in 2017 is focused on better tracking of individual growth of known species. Investigation on the size class at which *S. pecki* can be identified and characters for separating immature stages of *S. pecki* from sympatric congeners for various size classes will be investigated. Investigations on the possibility of environmental factors that may influence sex ratios will be initiated.

~~**Objectives and Methods:** Life history studies will be continued into 2018 with an~~

~~expansion into evaluating additional life history characteristics that may affect the captive propagation of this species. The major objectives of 2018 research are to:~~
~~complete the estimate~~

Objectives and Methods:

1. Estimate how many molts ~~must occur before~~or what size class sexual maturity is reached~~;~~.
   - ~~complete research on how frequently a female can produce broods and the typical size of broods;~~
   - ~~estimate life span of *S. pecki*, if possible; and~~
   - ~~initiate research into sex determination and sex ratios in *S. pecki*. This final objective is expected to be of great utility to captive propagation as sex ratio in amphipods is a plastic and quantitative trait, therefore it is possible under certain conditions to rear amphipods of only one sex; a situation not desirable for propagation. Specific methods for each 2018 activity will be developed towards the conclusion of 2017 research in order to maximize the knowledge gained from ongoing experimentation.~~

   ~~**Expected Results:** In compilation with 2017 findings, 2018 results will provide information on the life cycle of *S. pecki* in order to promote effective and efficient captive propagation of this species. The key life history aspects will be a better understanding of the life span, sexual maturity, how many and what size of broods can be produce, sex determination, and sex ratios in captivity versus the wild. The main deliverable will be a final report that includes an updated standard operating procedure (SOP) for rearing Peck's Cave amphipods through their various life stages. The SOP will include instructions for rearing, descriptions of equipment used, environmental stimuli incorporated, and other husbandry requirements and recommendations for future studies.~~

2. Estimate fecundity.
3. Detect differences between immature sympatric congeners.
4. Estimate growth rates.
5. Investigate factors effecting sex ratios.
6. Estimate egg incubation rates.

New collections will be necessary to establish a common garden that can fully support the proposed investigations. Modification and continuation of existing operations will proceed. New mesocosms will be constructed to support treatment subjects for feeding trials.

Expected Results: It is anticipated that estimates of fecundity, egg incubation rates, and early growth rates will be established. The size at which immature stages of *S. pecki* can be distinguished form sympatric congeners and a suite of characters that can be used for separating species will be documented. Insights into how feeding may influence sex ratios may raise new questions regarding growth rates, feeding schedules, and cannibalism.

Project 3:

Title: Continuation of Comal Springs riffle beetle (*Heterelmis comalensis*) life-history studiesstudy.

Species: *Heterelmis comalensis*
Principal/Co-PI: BIO-WEST, input by SMARC staff

Overview: This project is the continuation and final reporting on Comal Springs riffle beetle
Principal/Co-PI: BIO-WEST, input by Randy Gibson, Dr. Lindsay Campbell.

Overview: (*Heterelmis comalensis*) life history studies started in 2016 with another funding source. The primary goal of the second year of study (2017) was to identify factors contributing to pupation. As of April 2017, pupa have been experimentally produced via this study. As the first pupation event took approximately four months, it is anticipated that expanding the knowledge base on factors leading to successful pupation may extend beyond 2017. Therefore, research activities directed at understanding the successful production of *H. comalensis* adults in captivity are anticipated for 2018. These activities will be further defined using 2017 results but are anticipated to involve continued investigation of pupation and life-stage specific diets and experimentation is ongoing. In addition, a new investigation on the interaction of flow conditions with food preference will also be initiated.

Objectives and Methods:

1. Continued monitoring of ongoing pupation experiments from 2017.
2. Identify the behavioral response of adults and larvae to varying flow conditions with food resource effects.

The construction of a variable flow variable flow mesocosom as described for Project 1 will be utilized for this species first since test subjects are more readily available and may be used in part as surrogates.

Expected Results: Conclusion of pupation rate investigation and improved information of environmental requirements for successful captive propagation.

Project 4:

Title: San Marcos Salamander propagation refinement

Species: *Eurycea nana*

Principal: Dr. Lindsay Campbell, Kelsey Anderson

Overview: The objective of the proposed study is to determine if reproduction can be reliably triggered in San Marcos salamanders with the non-invasive technique of separation and re-combination. Additionally, we will compare pairwise versus group tank reproduction success. If eggs are produced egg development will be documented.

Objectives and Methods:

Salamanders will be sexed and then separated in different tankstank systems by sex for eight weeks. After the separation period, salamanders at least one month. Next groups of male and females will be placed into the same tank system, but physically separated for two weeks; they

will share water and be able to see each other. Salamanders will then be combined into either equal sex-ratio groups (i.e. 4 females/4 males, ~~at least 3 replicate groups~~three replicates) or individual pairs (~~3-9~~12 pairs) per tank system (three replicate systems) to initiate mating. ~~After two weeks males will be removed from tanks and materials conducive to egg deposition will be placed in tanks.~~

Expected Information gathered:

1. Average time to courtship behavior once combined
2. ~~time~~Average days to oviposition to occur after sexes combines
3. ~~number of females~~Average clutch size
~~3.~~4.Survival rate to ~~successfully lay~~ hatch of eggs
- ~~number of eggs laid~~
- ~~number of eggs to successfully hatch~~
- ~~length of time until eggs hatch~~
- ~~length of time until larvae absorb egg sacks~~
- ~~time until larvae begin to feed~~
5. Document egg developmental stages
- Test for differences between pairwise vs group mating ~~in these categories~~

~~**Invertebrate Collection Techniques**~~
~~Species: *Stgoparnus comalensis*, *Stygobromus pecki*, *Haideoporus texanus*, *Lirceolus smithii*~~
~~Principal: Amelia Everett, Dr. Lindsay Campbell~~

~~4.~~6.~~Overview: Evaluate existing invertebrate collection techniques, locations, seasonality for effectiveness, patterns, and drawbacks based on collected data from previous year. Investigate new invertebrate collection techniques and test new designs in an effort to enhance the effectiveness and efficiency.~~

Expected ~~Information gathered:~~**Results:** The results of the study will be presented as a report to the EAA and potentially submitted as a journal article. If this technique is successful the Culture Propagation Manual for this species will be updated.

- ~~CPUE by location, type of net/lure~~
- ~~Alternative net/lure design for each species generated, tested~~

Task 3. Species Propagation and Husbandry

Development and refinement of SOPs for animal rearing and captive propagation: Continue to refine SOPs for all species as needed for updates to reflect new protocols that are instituted for each species throughout the year. As new information becomes available about genetic management, further develop draft Captive Propagation Plans for all species.

Task 4. Species Reintroduction

Reintroduction Plan for term of contract:
Further revise the draft Reintroduction Strategy presented in 2017. ~~Compose additional Captive Propagation Plans.~~

Reintroduction Plan for 2018: None

Any anticipated triggers being prepared for: Given current weather predictions, spring flows, and the Edwards Aquafer water level none are anticipated during the 2018 performance period.
~~2018 Activity: Draft sub-contract for services provided by BIO-WEST.~~

Task 5. Reporting

5.1 Species specific Propagation plans (SOPs): Refine throughout year as needed
5.2 Species specific Genetic Management plans: None during 2018
5.3 Species specific Reintroduction plans: Revise draft plan presented in 2017
5.4 2018 EAHCP Annual Program reporting 12/31/2018 – A year-end report of 2018 activities will be provided to the EAA no later than ~~12~~1/31/~~2018~~2019.
5.5 Program reporting as required by ITP and TPWD. TPWD Scientific Research Permit Report will be conveyed to the EAA July 31, 2018.
5.6 Descriptions and photographs of procedures from collections to restocking – Photographs and documentation of collection and restocking will be included in the monthly report to the EAA CSO along with the year-end report.
5.7 Summaries of any data analyses, research, or genetic analyses – Research projects and results of collection efforts will be provided to the EAA in the monthly reports, year-end documentation, and stand-alone documents (agreed upon by Center director and HCP CSO).
5.8 Description of terms and conditions of any permits received – As permits are received, their contents will be conveyed to the EAA.
5.9 Monthly electronic reports to HCP CSO: A monthly report of all activities will be provided to the HCP CSO. We anticipate providing the report by the 10th of each month for the previous month's activities.

Task 6. Meetings and Presentations

Planning or coordination meetings:
- Yearly planning meeting with SMARC and UNFH staff

- Public meetings
  - EAA Board
    - End of year report
    - Present research results
  - Implementing Committee
    - End of year summary
  - Stakeholder Committee
    - End of year summary
  - Science Committee
    - Methods for research projects
    - Present research results

Monitoring:
Monitoring will be conducted through the use of progress reports and site visits to the refugia as

well as through collaborative management by the EAHCP CSO.

Budget: Projected 2018 budget.

U.S. Fish and Wildlife Service 2018			
	Task	**Task Budget Amount**	**Total Task Budget Amount**
1	**Refugia Operations**		**$4,405,316**
	SMARC Refugia & Quarantine Bldg.		
	*Construction	$1,632,934	
	Equipment	$162,532	
	Utilities	$82,400	
	UNFH Renovation Refugia & Quarantine Bldg.		
	*Construction	$999,369	
	Equipment	$242,007	
	Utilities	$75,000	
	SMARC Species Husbandry and Collection		
	Fish Biologist (GS-12, 972 hrs)	$47,889	
	Fish Biologist (GS-07, 1262 hrs)	$35,185	
	Fish Biologist (GS-07, 1384 hrs)	$39,199	
	Fish Biologist (GS-07, 1384 hrs)	$39,199	
	Fish & Wildlife Administrator (GS-14, 186 hrs)	$14,226	
	SMARC Staff (GS-11, 184 hrs)	$8,396	
	Maintenance technician (WG-8, 694 hrs)	$19,432	
	Diving	$7,000	
	Weekend Walk Thru	$7,500	
	Other Overtime	$2,000	
	UNFH Species Husbandry and Collection		
	Fish Biologist (GS-11, 1250 hrs)	$51,350	
	Fish Biologist (GS-06, 1672 hrs)	$41,767	
	Fish Biologist (GS-06-07, 1612 hrs)	$43,972	
	Fish Biologist (GS-06-07, 1976 hrs)	$56,866	
	Supervisory Fish Biologist (GS-12, 208 hrs)	$11,604	
	Weekend Walk Thru	$5,400	
	Other Overtime	$2,000	
	Fish Health	$17,000	
	SMARC Reimbursibles	$74,000	
	UNFH Reimbursibles	$47,000	
	Subtotal	*$3,765,227*	
	Admin Cost Subtotal	*$640,089*	

2	**Research**			**$495,790**
	BIO-WEST: Dryopid beetle life history		$129,956	
	BIO-WEST: Peck's Cave amphipod life history		$135,435	
	BIO-WEST: Riffle beetle life history		$26,259	
	Captive propagation refinement salamanders		$115,000	
	Fish Biologist (GS-12, 572 hrs)	*28,154*		
	Fish Biologist (GS-07, 694 hrs)	*19,348*		
	Fish Biologist (GS-07, 572 hrs)	*15,947*		
	Fish Biologist (GS-07, 572 hrs)	*15,947*		
	Fish Biologist (GS-11, 250 hrs)	*10,270*		
	Fish Biologist (GS-06, 364 hrs)	*9,905*		
	Fish Biologist (GS-9, 261 hrs)	*9,921*		
	Fish & Wildlife Administrator (GS-14, 72 hrs)	*5,508*		
	Equipment		$17,102	
	Subtotal		*$423,752*	
	Admin costs for Task 2		*$72,038*	
3	**Species Propagation and Husbandry**		*$0*	**$0**
	Subtotal		*$0*	
4	**Species Reintroduction**		*$0*	**$0**
	Subtotal		*$0*	
5	**Reporting**			**$115,257**
	BIO-WEST		$20,320	
	SMARC Staff		$56,110	
	Fish Biologist (GS-12, 416 hrs)	*20,476*		
	Fish Biologist (GS-07, 104 hrs)	*2,900*		
	Fish Biologist (GS-07, 104 hrs)	*2,900*		
	Fish Biologist (GS-07, 104 hrs)	*2,900*		
	SMARC Staff (GS-11, 364 hrs)	*16,609*		
	Fish & Wildlife Administrator (GS-14, 135 hrs)	*10,325*		
	UNFH Staff		$22,080	
	Fish Biologist (GS-11, 100 hrs)	*4,108*		
	Fish Biologist (GS-06, 88 hrs)	*2,198*		
	Fish Biologist (GS-06-07, 104 hrs)	*2,830*		
	Fish Biologist (GS-06-07, 104 hrs)	*2,987*		
	UNFH Staff (GS-06, 338 hrs)	*9,957*		
	Subtotal		*$98,510*	
	Admin costs for Task 5		*$16,747*	
	Meetings and Presentations			**$26,898**
6	BIO-WEST		$9,890	
	SMARC staff		$13,100	
	Fish Biologist (GS-12, 120 hrs)	*5,908*		
	Fish Biologist (GS-07, 20 hrs)	*557*		
	Fish Biologist (GS-07, 20 hrs)	*557*		
	Fish Biologist (GS-07, 20 hrs)	*557*		
	Fish & Wildlife Administrator (GS-14, 72 hrs)	*5,521*		
	Subtotal		*$22,990*	
	Admin costs for Task 6		*$3,908*	
	TOTAL		**$5,043,261**	

**= Remainder of 2017 construction costs detailed within the 2017 work plan will be applied to 2018. This would occur through an amendment to the 2018 work plan. Budget totals for the construction and renovation projects at UNFH and SMARC are not anticipated to increase.*

Projected (2018) Budget Summarized by Task:

Task 1: $~~866,542.00~~4,405,316
Task 2: $~~341,000.00~~495,790
Task 3: $0~~.00~~
Task 4: $0~~.00~~
Task 5: $~~78,190.00~~115,257
Task 6: $~~13,100.00~~26,898

Projected (2018) Subcontractor Expenses Summarized by Task

Task 1: Dexter Fish Health Unit Dexter NM $~~8~~17,000~~.00~~ (Health Diagnostics)
Task 2: BIO-WEST ~~TBD estimated at $150,000.00 to $190,000.00.~~$291,650
Task 3: $0
Task 4: $0
Task 5: ~~$0~~BIO-WEST $20,320
Task 6: ~~$0~~BIO-WEST $9,889

Timeline of 2018 Milestones (List major deliverables)

January	Continue with species collection Subcontract drafted 2018 Specific Research Study Plans Drafted
February	Subcontract executed 2018 Specific Research Study Plans finalized
~~March~~ June-Aug	Construction completed on SMARC Refugia and Quarantine buildings
July	Submit and renew TPWD permit
September to December	Draft Research Reports Draft Annual report

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Chad Furl, PhD Chief Science Officer Edwards Aquifer Authority

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