Edwards Aquifer Authority
2022 Work Plan

2022 Cost Estimate for Edwards Aquifer Authority Work Plan

EAHCP Section	Conservation Measure	Table 7.1	Estimated 2022 Costs[a]
5.1.1	Refugia	$1,678,597	$1,447,937[h]
5.1.2	VISPO [b]	$4,172,000[c]	$2,509,976[d]
5.1.3	RWCP	$1,973,000	$0
5.1.4	Stage V	NA	NA
5.5.1	ASR Leasing & Forbearance [b]	$4,759,000	$5,776,493
	ASR O&M	$2,194,000	$0
5.7.2	Water Quality Monitoring	$200,000	$60,000[f]
6.3.1	Biological Monitoring	$400,000	$755,774 [e]
6.3.3	Ecological Model	$25,000	$0
6.3.4	Applied Research	$0	$250,000
FMA §2.2	Program Management	$750,000	$1,302,061[g]
Total		$16,151,597	$12,102,240

a. Estimated annual work plan cost per Funding and Management Agreement § 4.4.
b. Expected to change as leases transition to forbearance agreements through 2021 and 2022. Estimate presented based on best available data to date
c. Dollars in Table 7.1 of the EAHCP were calculated from a volume goal of 40,000 acre-feet (ac-ft). The volume goal was amended to 41,795 ac-ft in 2019 and Table 7.1 dollars are no longer applicable.
d. Amount is TBD. Standby payments will be made totaling the estimated cost in the table if program is not triggered by October 1, 2021. If triggered, VISPO program suspension payments will be $9,987,551.
e. Includes Critical Period Monitoring if required.
f. Includes updated Water Quality Monitoring costs for 2022 activities
g. Includes updated Program Management costs for 2022 activities
h. Includes unspent funds from 2021 to be used towards operational and research effort costs

2022 Amendments to Edwards Aquifer Authority (EAA) Work Plan and Funding Application

Amendment #	Date EAHCP Committee Approved	Conservation Measure Amended	Y/N Funding Application Change	Funding Application Change ($)	Date EAA Board Approved	Comments
0	5/20/2021	Original Work Plan	NA	NA	NA	Original Work Plan
0	10/14/2021	Original Funding Application	NA	NA	11/9/2021	Original Funding Application
1	10/14/2021	Water Quality Monitoring and Program Management	N	N	11/9/2021	Updated Water Quality Monitoring and Program Management with known activities and 2022 costs
2	12/16/2021	Water Quality Monitoring, Applied Research, and Refugia	N	N	n/a	Updated changes to projected activities to Water Quality Monitoring, Applied Research, and Refugia (no change in annual costs)
3	5/19/2022	Water Quality Monitoring and Refugia	Y	$281,562	6/14/2022	Updated Refugia and Water Quality Monitoring with known activities and 2022 costs

5.1.1 Refugia Program

Introduction

The U.S. Fish and Wildlife Service's (USFWS) San Marcos Aquatic Resources Center (SMARC) and Uvalde National Fish Hatchery (UNFH) 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 Edwards Aquifer Habitat Conservation Plan (EAHCP). The tasks and subtasks that follow provide the details for the services to be performed in 2022, which provide for the maintenance of a refugia population of the Covered Species (Table 1), including salvage, propagation, and restocking of the species (if species-specific habitat triggers occur and species are extirpated), plus research conducted on the Covered Species.

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
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 pterophila*	Petition Rescinded
Texas troglobitic water slater	*Lirceolus smithii*	Petitioned

Long-term Objective

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, prey, competitors, pathogens, parasites; or on their habitat, including food, water, and shelter.

2022 Assumptions

As work plans are developed almost a year prior to implementation, it is possible that methods described herein will be contingent on the status of the current year's activities or authorization from the HCP process. If conditions change, this work plan may need to be amended to accommodate realized outcomes.

The following potential situations could necessitate methodology adjustments.

- Target numbers for standing and refugia stocks to be housed at both the UNFH and SMARC deviate from those established by the USFWS-EAA Refugia Contract (Contract # 16-822-HCP).
- Species capture rates fall short of historic values.
- Mortality rates of specimens held in captivity exceed historic values.
- Staff member vacancies occur at either of the two Service facilities during the performance period.
- A pandemic or other emergency prevents scheduled collections.

Target for 2022 (Deliverables and Methods by Task):

Task 1. Refugia Operations

Standing Stocks: 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 contains the target species numbers.

Table 2. Target refugia numbers and census by species.

Species	Standing Stock	Refugia Stock	Salvage Stock	Anticipated SMARC census (Jan 2022)	Anticipated SMARC census (Dec 2022)	Anticipated UNFH census (Jan 2022)	Anticipated UNFH census (Dec 2022)
Fountain darter (Comal)	1000	1000 including specimens within the standing stock	2000	*	*	*	*
Fountain darter (San Marcos)	1000	1000 including specimens within the standing stock	2500	500	500	500	500
Texas wild-rice	430	430 including specimens within the standing stock	1500	215	215	215	215
Texas blind Salamander	500	500 including specimens within the standing stock	500	250	250	60	60
San Marcos salamander	500	500 including specimens within the standing stock	500	250	250	250	250
Comal Springs salamander	500	500 including specimens within the standing stock	500	135	150	105	135
Peck's cave amphipod	500	500 including specimens within the standing stock	500	250	250	250	250
Comal Springs riffle beetle	500	500 including specimens within the standing stock	500	75	75	75	75

Comal Springs dryopid beetle	500	500 including specimens within the standing stock	500	*	*	*	*
Edwards Aquifer diving beetle	500	500 including specimens within the standing stock	500	*	*	*	*
Texas troglobitic water slater	500	500 including specimens within the standing stock	500	*	*	*	*

We will not collect Comal fountain darters until we have a better understanding of their mortality rates
*catch rates and hatchery survival are uncertain given the rarity of the species

Collection: In 2022, the USFWS will collect Covered Species as required to reach and maintain target standing and refugia stock numbers as shown in Table 2. The USFWS will coordinate species collections with other ongoing HCP activities (e.g. Biological Monitoring Program) so that collections for refugia do not adversely impact other efforts. The USFWS will carry out species collections through a variety of passive and active collection methods and will minimize aquatic invasive species transfer by conducting collections in accordance a Hazard Analysis Critical Control Point plan. The USFWS will document and report collection efforts to the EAA. The USFWS will distribute captured organisms between the SMARC and UNFH facilities in order to ensure redundancy and to expedite the obligation to establish and maintain two refugia populations at separate locations. The USFWS will hold all species in respective quarantine areas until their health has been assessed. Staff will incorporate quarantined organisms into the general refugia population once they have determined that such specimens are healthy and free from invasive species. The USFWS will share reports, including test results, produced as part of the quarantine process.

The following sections briefly describe planned 2022 collection, maintenance, and propagation efforts for each species.

Fountain Darters:

Collection: In 2022, the USFWS will collect Fountain Darters from the San Marcos River in coordination with the Spring and Fall Biomonitoring events. This will be more efficient than separate collection events and will reduce habitat disturbance. For refugia purposes, USFWS staff will retain Fountain Darters collected by biomonitoring staff via drop nets. Staff will collect fish proportionally from the three sections of the San Marcos River: 1) Upper = Spring Lake, 2)

Middle = Spring Lake dam to Rio Vista dam, and 3) Lower = below Rio Vista dam to Cape's dam. The USFWS will thoroughly investigate unusual mortality events. The USFWS will include summary reports to the EAA as part of the monthly reports. Collections will target sufficient fish so to account for regular, expected mortality, such that the captive population should remain at or above the target.

Due to the detection of largemouth bass virus (LMBV) in Comal fountain darters throughout the Comal River, the USFWS will maintain all fountain darters from Comal River in quarantine facilities, in consideration of other species on the two stations. We have continued concern over higher mortality rates of incoming Comal fountain darters, as no root cause has been identified despite extensive testing and evaluation with the USFWS Fish Health Unit. Until we have a better understanding of the high mortality rates of incoming Comal fountain darters we will conduct limited collections from the wild, unless salvage is needed.

As part of quarantine procedures, the USFWS will send a subset of fish (maximum of 60 per river) to the Southwestern Fish Health Unit or equivalent facility for pathogen (bacteria, virus, and parasite) testing prior to incorporating collected animals into the general refugia population. The USFWS will follow standardized methods outlined within USFWS and AFS-FHS (2016) and AFS-FHS (2005) protocols and provide Fish Health reports to the EAA.

Maintenance: The USFWS will monitor water quality (i.e., temperature, pH, dissolved oxygen, total dissolved gasses) and record these data weekly. Staff will feed fountain darters a mix of live and frozen foods reared or purchased. The USFWS will rear zooplankton and amphipods in ponds and tanks for food. We do not generally examine food items for pathogens. However, if they are suspect and tested for pathogens, the USFWS will include all diagnostic results to the EAA within monthly reports.

Propagation: The USFWS will maintain standing and refugia stocks for each river to produce captive-bred fish for research purposes, as necessary and approved. Staff will maintain fish by their geographical collection location. If reintroduction is warranted, the USFWS will communally spawn subsets from each geographical location. The USFWS will cull subset groups to an equal number of progeny prior to release.

Texas wild-rice:

Collection: USFWS staff will collect Texas wild-rice tillers from San Marcos River reaches (Figure 1), with a break during summer months when collected wild rice does not fare well due to heat stress. In 2022, staff will target stands that are not already part of the refugia population or require supplementation in collections for SMARC and UNFH. The refugia populations will reflect the wild populations in both their respective proportion, based on the most recent Texas wild-rice survey data, and historical genetic diversity (2021 genetic assessment and Wilson et al. 2016). During tiller collection, the USFWS will record the geographic coordinates, area coverage, and depth of the stand or individual plant. USFWS staff will collected tillers by

wading and SCUBA diving. The USFWS will consider georeferenced aerial imagery to help identify distinct TWR stands used for tiller collection.

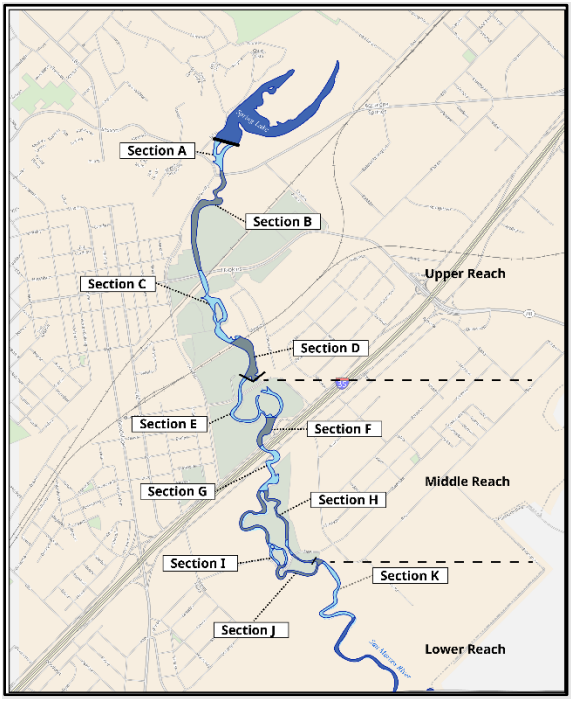

Figure 1. Letters define designated San Marcos River reaches where Texas wild rice is collected for refugia populations.

Maintenance: Once tillers have successfully rooted, USFWS staff will tag and maintain with their collection date and location information.

Propagation: USFWS staff will maintain plants to prevent sexual reproduction within the refugia population, unless EAHCP triggers occur. If reintroduction is warranted, USFWS staff will produce seeds and tillers from each geographical location. During reintroduction, staff will transplant refugia plants produced from seeds and tillers to their original source location, delineated by river section (Figure 1).

Texas blind salamanders:

Collection: USFWS will collect Texas blind salamanders using nets and traps. Staff will deploy traps quarterly for approximately 14 consecutive days with traps checked every 2-4 days to collect Texas blind salamander individuals from Primers Fissure, Johnson's well, Rattlesnake cave, and Rattlesnake well (Table 5). To avoid oversampling these habitats, staff will only collect 1/3 of salamanders observed from each of these locations during quarterly sampling events. Staff will also collect salamanders from a driftnet on Diversion Springs in Spring Lake fished throughout the year during times when we are not actively trapping in caves and wells. We will retain all specimens from this site, under the assumption that any Texas blind

salamander leaving a spring orifice that enters a stream or lake environment will ultimately succumb to predation. We will check these sites up to three times per week when applicable. Staff will transport all specimens alive and maintain them in the SMARC or UNFH refugia. Texas State University staff generally check drift nets on Sessom Creek and Texas State University Artesian Well; Texas State University transfers live Texas blind salamanders to SMARC according to their permits, when appropriate. USFWS staff may periodically check nets on these sites when they are not being checked by Texas State University staff.

As part of quarantine procedures, USFWS staff will swab all large Texas blind salamanders. If they are too small to be swabbed, then we will do a representative batch swab of group-housed salamanders once they are large enough to be safely swabbed. USFWS staff will process these samples at SMARC or other facility to screen for *Batrachochytrium dendrobatidis* (Bd, commonly referred to as chytrid fungus) and *Batrachochytrium salamandrivorans* (Bsal) prior to specimen incorporation into the general refugia population. Staff will retain duplicate swabs in case further testing is warranted. Staff will hold all salamanders 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 salamanders) have regularly 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 yet been documented in North America. Staff would retain such salamanders in quarantine until further study and recommendations from FWS Fish Health.

Maintenance: USFWS staff will individually tag salamanders to retain information on collection location, date, and other life history events. Staff will monitor water quality and record data weekly. Staff will feed salamanders live and frozen foods, either reared or purchased. Staff will utilize ponds and tanks to produce amphipods on site.

Propagation: Staff will maintain standing and refugia stocks to encourage reproduction. Staff will maintain all progeny separately by generations. If reintroduction is warranted, an attempt will be made to produce offspring from each geographical location.

San Marcos salamanders:

Collection: USFWS staff will collect San Marcos salamanders quarterly from below Spring Lake dam and with SCUBA teams in Spring Lake (Table 5). Staff will check the drift net on Diversion Springs routinely and keep specimens from this location as space in quarantine and need allows. We will avoid collections close to the HCP Biological Monitoring Program assessment events. Staff will transport all specimens alive and maintain these in the SMARC and UNFH refugia.

As part of quarantine procedures, USFWS staff will swab all large San Marcos Salamanders. If they are too small to be swabbed, then we will do a representative batch swab of group housed salamanders once they are large enough to be safely swabbed. USFWS staff will process these samples at SMARC or other facility to screen for *Batrachochytrium dendrobatidis* (Bd, commonly referred to as chytrid fungus) and *Batrachochytrium salamandrivorans* (Bsal) prior to

specimen incorporation into the general refugia population. Staff will retain duplicate swabs in case further testing is warranted. Chytrid testing will occur in batches where groups of five swabs will be pooled for analysis. Staff will hold all salamanders 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 salamanders) have regularly tested positive for Bd. Positive testing for Bsal will be treated more cautiously as it has not yet been documented in North America. Staff would retain such salamanders in quarantine until further study and recommendations from FWS Fish Health.

Maintenance: Staff will monitor water quality and record data weekly. Staff will feed salamanders live foods, either reared or purchased, mixed with purchased frozen food sources if necessary. Staff will utilize ponds and tanks to produce amphipods on site.

Propagation: USFWS staff will maintain salamander standing and refugia stocks to encourage reproduction. We will separate all progeny by generation. If reintroduction is warranted, staff will employ pairwise and group mating to produce offspring. Staff will initiate stocking once juveniles have reached 30 mm total length.

Comal Springs salamanders:

Collection: USFWS staff will collect Comal Springs salamanders quarterly from Comal Spring Runs 1-3 and Spring Island and surrounding areas (Table 5) by hand, with dipnets, using snorkelers. We will coordinate with the HCP biological monitoring program in order to ensure that, to the degree practicable, refugia collections do not overlap with specific EAHCP long-term monitoring locales. In the event overlap of sampling areas is unavoidable, we will collect Comal salamanders at a rate of no more than 10% of salamanders observed in those specific locales per daily sampling trip. We will employ a SCUBA team for a portion of these collection efforts if necessary.

As part of quarantine procedures, USFWS staff will swab all large Comal Springs salamanders. If they are too small to be swabbed, then we will do a representative batch swab of group housed salamanders once they are large enough to be safely swabbed. USFWS staff will process these samples at SMARC or other facility to screen for *Batrachochytrium dendrobatidis* (Bd, commonly referred to as chytrid fungus) and *Batrachochytrium salamandrivorans* (Bsal) prior to specimen incorporation into the general refugia population. Staff will retain duplicate swabs in case further testing is warranted. Chytrid testing will occur in batches where groups of five swabs will be pooled for analysis. Staff will hold all salamanders 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 salamanders) have regularly 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 yet been documented in North America. Staff would retain such salamanders in quarantine until further study and recommendations from FWS Fish Health.

Maintenance: Staff will monitor water quality and record data weekly. Staff will feed salamanders live and frozen foods, either reared or purchased. Staff will utilize ponds and tanks to produce amphipods on site.

Propagation: USFWS staff will maintain salamander standing and refugia stocks to encourage reproduction. We will separate all progeny by generation. If reintroduction is warranted, staff will employ pairwise and group mating to produce offspring. Staff will initiate stocking once juveniles have reached 30 mm in total length.

Comal Springs riffle beetle:

Collection: USFWS staff will collect Comal Spring riffle beetle for standing and refugia stocks four times a year from a variety of locations, including Spring Run 1, Spring Run 3, the Western Shore, and areas surrounding Spring Island (Table 5). Staff will collect riffle beetles with cotton lures following EAHCP standard operating procedures (Hall 2016) and from wood, as needed. Staff will follow protocols established by the CSRB Work Group in 2019:

1. Staff will not sample the same spring orifice two times in a row.
2. Staff will collect all riffle beetle adults and larvae from lures.
3. Standing stock numbers will be reduced to 75 per station until USFWS has established sufficient propagation methods, and we have better understanding of population numbers to derive meaningful standing stock targets.

The Comal Springs Riffle Beetle Work Group Standing will evaluate standing stock numbers yearly. Additional collections for research purposes may be required outside of standing stock collections.

Maintenance: USFWS staff will maintain specimens by collection date. Staff will hold Comal Springs riffle beetles within custom built aquatic holding units and feed them detrital matter and matured biofilms colonized on cotton lures, wood dowels, and leaf matter.

Propagation: Propagation methods for this species are being developed.

Peck's cave amphipod:

Collection: USFWS will conduct Peck's cave amphipod collection for standing stock four times annually (Table 5). Staff will collect adult Peck's cave amphipods with drift nets and by hand at a variety of locations (drift nets: Spring Run 3, N = 2; Spring Island and associated Spring Lake habitats: hand collection).

Maintenance: Staff will maintain specimens by collection date within custom-built aquatic holding units and feed amphipods with commercial flake fish food.

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

Comal Springs dryopid beetle:

Collection: USFWS will collect Comal Springs dryopid beetles primarily through the use of wooden lures and hand picking from submerged wood found in the Comal Spring system. If staff find dryopid beetles on cotton lures used for Comal Spring riffle beetles, these will also be retained (Table 5). We will potentially conduct two trapping events with bottle traps in Panther Canyon Well during the year as access to the well and staff time allows. Staff will check these traps weekly for a month.

Maintenance: USFWS will combine collected Comal Springs dryopid beetles, regardless of collection location. Staff will hold Comal Spring dryopid beetles within custom built aquatic holding units and feed them detrital matter and matured biofilms colonized on cotton lures, wood dowels, and leaf matter.

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

Edwards Aquifer diving beetle:

Collection: Staff will collect Edwards Aquifer diving beetles with drift nets (Table 5). Staff will set drift nets at a variety of locations where the species has been collected in the past (Texas State University Artesian Well N = 1; and Diversion Springs N = 1). USFWS staff will deploy and check drift nets at the Artesian Well when as Texas State University allows.

Maintenance: USFWS will combine collected Edwards Aquifer diving beetles, regardless of collection location. Staff will transfer captured specimens to the SMARC or UNFH and house them in custom-made aquatic holding systems. Edwards Aquifer diving beetles are predators; staff will feed them small invertebrates (e.g., ostracods).

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: Texas troglobitic water slaters are primarily found in Artesian Well on Texas State Campus. Recent research by Will Coleman (Texas State University) suggests that this is a deep aquifer species, rarely found at the surface. Mr. Coleman was unable to keep any alive, as all specimens he collected were injured. USFWS will continue to work with invertebrate experts to determine what might be the optimum way to collect this species. USFWS staff will deploy and check drift nets in the Artesian Well as Texas State University allows.

Maintenance: Staff will transfer captured specimens to the SMARC and house them in custom aquatic holding systems. Staff will feed Texas troglobitic water slaters detrital matter, matured biofilms colonized on cotton lures, and flake fish food to supplement their diet.

Propagation: Staff need to determine propagation methods for this species, to be conducted as part of normal refugia operations.

Table 5. A tentative schedule for all species sampling during 2022. Collections listed here are subject to change with extenuating circumstances such as weather and coordination with external partners. USFWS will notify EAA and partners of sampling dates as they become known or changed.

Edward's Aquifer Species Collection Plan 2022			
Date (month)	**Interval**	**Location**	**Target Species**
January	14 Consecutive days with traps checked 2-3 times a week	Rattlesnake Cave & Rattlesnake Well	Texas blind salamander
January	1 day sampling event, hand pick from downed wood	Landa Lake	Comal Springs dryopid beetle
February	14 Consecutive days with traps checked 2-3 times a week	Primer's Fissure & Johnson's Well	Texas blind salamander
February	Set lures	Spring Run, Landa Lake	Comal Springs riffle beetle, Comal Springs dryopid beetle, Peck's cave amphipod, Texas troglobitic water slater
February	1 day sampling event	San Marcos River	Texas wild rice
February	1 day sampling event	San Marcos River	Texas wild rice
March	Check nets T and F every week	Diversion Springs	Texas Blind salamander, San Marcos salamander
March	Collect Lures	Spring Run, Landa Lake	Comal Springs riffle beetle, Comal Springs dryopid beetle, Peck's cave amphipod, Texas troglobitic water slater
March	1 day sampling event, hand pick	Landa Lake	Peck's Cave amphipod
March	1 day sampling event	Comal Springs	Comal Springs salamander

Edward's Aquifer Species Collection Plan 2022			
Date (month)	**Interval**	**Location**	**Target Species**
March	1 day sampling event, hand pick from downed wood	Landa Lake	CSDB
April	Check 2 consecutive weeks	Rattlesnake Cave & Rattlesnake Well	Texas blind salamander
April	1-2 day sampling event	Spring Lake and below dam	San Marcos salamander
April	1 day sampling event	San Marcos River	Texas wild rice
April	Throughout, coincide with bio-monitoring	San Marcos River	Fountain darters
April	Drift net, donated from bio-monitoring	Comal Springs	PCA
May	Set lures	Spring Runs, Landa Lake	Comal Springs riffle beetle, Comal Springs dryopid beetle, Peck's cave amphipod, Texas troglobitic water slater
May	14 Consecutive day with traps check 2-3 times a week	Primer's Fissure & Johnson's Well	Texas blind salamander
May	1-day sampling event	San Marcos River	Texas wild-rice
June	Collect lures	Spring Runs, Landa Lake	Comal Springs riffle beetle, Comal Springs dryopid beetle, Peck's cave amphipod, Texas troglobitic water slater
June	Check nets T and F every week	Diversion Springs	Texas Blind salamander, San Marcos salamander
June	1 day sampling event, hand pick	Landa Lake	Peck's Cave amphipod

Edward's Aquifer Species Collection Plan 2022			
Date (month)	**Interval**	**Location**	**Target Species**
June	1 day sampling event	Comal Springs	Comal Springs salamander
June	Set lures	Western Shore	Comal Springs riffle beetle, Comal Springs dryopid beetle, Peck's cave amphipod, Texas troglobitic water slater
July	14 Consecutive days with traps check 2-3 times a week	Rattlesnake Cave & Rattlesnake Well	Texas blind salamander
August	Set lures	Western Shore	Comal Springs riffle beetle, Comal Springs dryopid beetle, Peck's cave amphipod, Texas troglobitic water slater
August	14 Consecutive days with traps check 2-3 times a week	Primer's Fissure & Johnson's Well	Texas blind salamander
August	1-2 day sampling event	Spring Lake and below dam	San Marcos salamander
September	Check nets T and F every week	Diversion Springs	Texas Blind salamander, San Marcos salamander
September	1 day sampling event, hand pick	Landa Lake	Peck's Cave amphipod
September	1 day sampling event	Comal Springs	Comal Springs salamander
September	Collect lures	Western Shore	Comal Springs riffle beetle, Comal Springs dryopid beetle, Peck's cave amphipod, Texas troglobitic water slater
October	14 Consecutive days with traps checked 2-3 times a week	Rattlesnake Cave & Rattlesnake Well	Texas blind salamander

Edward's Aquifer Species Collection Plan 2022			
Date (month)	**Interval**	**Location**	**Target Species**
October	Throughout, coincide with bio-monitoring	San Marcos River	Fountain darters
October	Drift net, donated from bio-monitoring	Comal Springs	Peck's Cave amphipod
October	1 day sampling event	San Marcos River	Texas wild-rice
October	1 day sampling event, hand pick from downed wood	Spring Runs, Landa Lake	Comal Springs dryopid beetle
November	14 Consecutive days with traps checked 2-3 times a week	Primer's Fissure & Johnson's Well	Texas blind salamander
November	1 day sampling event, hand pick	Landa Lake	Peck's cave amphipod
November	1 day sampling event	Comal Springs	Comal Springs salamander
November	Set lures	Spring Runs, Landa Lake	Comal Springs riffle beetle, Comal Springs dryopid beetle, Peck's cave amphipod, Texas troglobitic water slater
December	Check nets T and F every week	Diversion Springs	Texas Blind salamander, San Marcos salamander
December	1 day sampling event	San Marcos River	Texas wild rice
December	Collect lures	Spring Runs, Landa Lake	Comal Springs riffle beetle, Comal Springs dryopid beetle, Peck's cave amphipod, Texas troglobitic water slater

Refugia Stocks:

Collection: Standing Stock numbers contribute to Refugia Stock numbers. Collections will continue until Standing stock targets are attained. In the event that Refugia Stock triggers, outlined in the contract, are reached and Standing Stock are not at full capacity, USFWS will conduct special targeted collections to increase Standing Stock.

Maintenance: USFWS will conduct maintenance in a similar manner described for standing stocks.

Propagation: Propagation for stocking is not anticipated during 2022.

Salvage Stocks:

Collection: If specific salvage triggers defined in the EAHCP are reached, the Refugia Program, in consultation with the EAA, will accommodate salvaged organisms no more than twice during the 12-year contract period. If triggers for multiple species are simultaneously reached, species collections during salvage operations will be prioritized based upon the perceived impacts of reduced river and spring flow and habitat degradation on Covered Species (i.e. EAHCP triggers). Those species that are river obligate species (i.e., fountain darters and Texas wild rice) or that occupy spring orifice and interstitial ground water habitats (i.e., San Marcos and Comal Springs salamanders, Peck's cave amphipods, Comal Springs dryopid beetles) are presumed to be affected first as flows decrease. Those that reside solely within the aquifer (i.e., Edwards Aquifer diving beetles, Texas troglobitic water slaters and Texas blind salamanders) are presumed to be affected subsequently.

Maintenance: The Refugia Program will maintain organisms collected during salvage operations at the SMARC or UNFH for up to one-year or until their disposition is determined. The Refugia Program may suspend or terminate research if space is required for salvaged organisms. Research may also be suspended if personnel are directed to collect and maintain salvage stocks.

Propagation: Likewise, production of species would be limited to no more than twice during the 12-year contract period if species extirpation occurs. USFWS propagated species at the SMARC or UNFH would be held for up to one year or less if stocking is required. We may suspend or terminate research activities if space is required to house cultured species. Research may also be suspended if personnel are needed to reproduce, maintain, or stock progeny.

Construction/Renovation/Infrastructure/Facility:

The USFWS will report any non-routine maintenance for the program buildings to the EAA as they occur.

The USFWS will institute all reasonable and practical security measures to safeguard EAA refugia facilities, equipment, and species.

For 2022, the refugia is asking for the use of $160,000 Task 1 rollover funds to make improvements to the refugia systems at both the Uvalde National Fish Hatchery ($80,000) and

the San Marcos Aquatic Resource Center ($80,000). Six monitor and control units and the associated equipment will be purchased for each facility. These units will record water parameters and controlling equipment (chillers, CO_2 injectors) on up to 12 systems at each facility. These systems will assist in maintaining water parameters and alert staff if values deviate from specified levels. In addition to the controllers, CO_2 injection systems will be installed to assist in maintaining a consist pH and reduce calcium buildup on equipment. Mechanical filters and UV sterilizers will be added to the systems for increased flexibility, where each system can function as flow-through or 100% recirculating. Were needed, old water pumps will be replaced with more energy efficient pumps. These improvements will minimize the potential for catastrophic system failure, alert staff to problems with individual systems, and add redundancy into the functioning of the refugia systems.

In addition to the amount above we are asking for $5,282.03 from Task 1 roll over funds for the purchase of a portable water velocity meter to be used for field measurements.

Staffing/Labor/Personnel:

The two program Leads (Research and Husbandry/Collections) will mentor and train lower-graded employees, oversee facility maintenance and repair, develop, and implement budgets, and organize activities that relate to all contract activities. The program leads will manage and coordinate research, propagation, culture, and field activities related to the refugia. The leads are expected to provide proper and efficient use of facilities and staff resources. These leads will work with the Center Director and the Deputy Director to ensure that contractual obligations are met in a timely manner. In coordination with the Deputy Center Director, they will prepare all the required written materials required for the reimbursable agreement reporting. Likewise, the leads 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 leads will continue to work and communicate regularly with partners, USFWS personnel and other researchers to meet USFWS and contract goals.

Under the direction of the Lead Biologist at UNFH, five biological science technicians, two at SMARC and three at UNFH, will continue to assist with the collection, daily upkeep, maintenance, propagation, and research efforts for the ten species at the SMARC and UNFH. This includes maintaining culture and experimental production systems, keeping records along with entering, filing, and collating data. The technicians 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.

Under the direction of the Lead Biologist at SMARC, an SCA Student Intern will be hired to conduct the San Marcos fountain darter historical tissue archive research project. This SCA intern will catalog and organize all historical darter collections at SMARC and UNFH and will start the process of assessing the collections suitability in future genetic analysis.

Under the direction of the Lead Biologist at UNFH, two SCA interns will be hired to assist with day-to-day husbandry tasks; one located at SMARC and the other at UNFH.

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 will practice biosecurity procedures in Refugia and Quarantine areas and conduct appropriate biosecurity procedures on field equipment.

Bd/Bsal Testing:

Water from sampling locations, water bodies in the SMARC and UNFH area, and the wells at the SMARC and UNFH will be test for Bd/Bsal. Wild stock and F1 salamanders in refugia will be tested for Bd/Bsal to determine the extent of Bd occurrence in the Standing/Refugia stock. Extended Bd/Bsal testing will ensure that any salamander brought on station would not further contribute to or modify the occurrence of Bd/Bsal in the locations salamanders are sampled from.

Husbandry Pilot Studies:

PCA Exclusion – Peck's cave amphipod does not readily produce offspring that survive to adulthood mostly due to cannibalism by the brooding female. EARP staff currently separate brooding females from main housing and put them into a separate container to reduce cannibalism by the larger population, but cannibalism still occurs by the brooding female. Exclusion chambers will be constructed to separate the offspring from the brooding female. The success of each exclusion chamber design will be assessed by comparing the number of offspring recovered. Each design will be compared to each other and to the currently used brooding chamber.

CSRB Dowel Condition – It takes about a month for a dowel to develop sufficient biofilm to support Comal Springs riffle beetles. This extended time period can cause delays in research and potential lack of food sources for refuge populations if materials are not replenished on a strict schedule or if a system were to fail. We suspect the time required for biofilm to develop on the dowels will decrease if more surface area is available for biofilms to develop. Dowels will be etched and set to condition alongside dowels that are not etched, under the same conditions. The

dowels will be checked daily, and the number of days floating will be recorded. Pictures of biofilm development will be taken weekly. Preliminary testing shows that dowels that are etched sink faster than dowels that are not etched. This study will quantify the number of days required for etched dowels to develop biofilm relative to unetched dowels. If etched dowels develop biofilm faster than unetched dowels (days floating) and produce equal or better biofilm (subjective assessment using photos), then etching dowels will be incorporated into the Comal Spring Husbandry SOP.

Fountain Darter Diet - Fountain darters (*Etheostoma fonticola*) from the Comal and San Marcos Rivers have been successfully bred and reared in captivity at both the Uvalde National Fish Hatchery and the San Marcos Aquatic Resources Center. However, the survival rate during the first few weeks after hatch is often variable and low. Low survival of recently hatched fish can often be attributed to several factors, such as improper diet nutrition of the broodstock, improper diet nutrition of the hatchlings, improper prey size for the hatchlings, tank design, and pathogens. For this project we will focus on comparing diet size. We will rear San Marcos River fountain darters and monitor survival, body length, body depth, and mouth gape of the fish from hatching to 1-month-old, relative to three different diets: 1) current SOP diet of recently hatched live *Artemia* (~400-500um length), 2) live rotifers (~150 – 350 um length), and 3) a mix of live rotifers and *Artemia*. Results from this study will allow us to improve the fountain darter rearing SOP for the EARP and can lead to future work on nutritional needs.

Task 2. Research

The Research Plan for 2022 will involve a series of projects designed to improve culture protocols and the health, survival, and propagation of captive populations. We have nearly all we need for a fully functioning Fountain Darter *ex situ* refuge, but an evaluation of the genetic diversity in the standing stock is needed. We will assess the quality of historical samples for future DNA analysis to assess the genetics of wild and refugia populations over time. To inform refugia collections, we will conduct a population genetic analysis of Comal Spring riffle beetles. Progress will continue to be made in Comal Spring riffle beetle propagation through a continuation of 2021 pupation trials. A handbook will be generated describing the advancements made toward successful collection and pupation. Salamander reproductive disfunction will be further investigated through habitat modification and Bd treatment trials. If successful, Bd treatment trials for aquatic salamanders will reduce refugia mortality and allow for transfers between SMARC and UNFH.

The total cost for proposed 2022 research, given the following projects, is approximately $515,969. Call for proposals from external partners to continue San Marcos salamander reproduction and Comal Springs riffle beetle pupation work will advertised and, if appropriate for Refugia needs, will be funded in 2022.

The following section describes the basic components of each of these proposed 2022 activities.

Project 1:

Title: Propagation of Comal Springs riffle beetles
Species: *Heterelmis comalensis*
Principal: BIO-WEST with FWS staff
Overview: A fully functional refugia requires predictable propagation. Based on evidence gleaned from previous research, we will calculate a target number of beetles then scale-up earlier attempts, propagating CSRB larvae at suitable densities with wild cultivated biofilm to test if we are able to meet our predicted targets.
Budget: $93,747.71
Benefit to the Refugia: This research will provide confirmation of progress toward a fully function refugium for this species.
Expected Results: We will produce a report for the EAA.

Project 2:

Title: Genetic assessment of Comal Springs riffle beetle
Species: *Heterelmis comalensis*
Principle/Co PI: FWS Staff
Overview: Little is known about the population structure and genetic diversity of the Comal Springs Riffle Beetle. A population-wide assessment can provide population metrics to inform future conservation and refugia needs. FWS will work with a partnering biologist, who is conducting an n-mixture model study on the abundance of Comal Springs riffle beetles, to collect adult Comal Springs riffle beetles across spring openings in Landa Lake and the Comal River. FWS staff will use high-throughput genome wide sequencing to make population measurements at the genetic level.
Budget: $141,344.64
Benefit to the Refugia: In combination with the occurrence study, the genetic assessment of the entire Comal Springs Riffle Beetle population will provide valuable information to the level of genetic variation and population structure in the wild. We do not yet know the extent of movement across spring openings at Landa Lake or how much genetic diversity is shared. The existence of distinct sub-populations would require different levels of representation in the refugia in order to reflect wild populations. Additionally, a range-wide genetic assessment can provide an estimate of the effective number of breeders, which would provide information to the minimum number of individuals that would need to be kept in refugia to accurately represent the wild population. This effort will greatly contribute to achieving a more complete refugia.
Expected Results: A report will be presented to the EAA and a peer-reviewed publication will be submitted.

Project 3:

Title: Handbook for the captive propagation of Comal Springs riffle beetles
Species: *Heterelmis comalensis*
Principal/Co PI: FWS Staff; BIO-WEST Support
Overview: The SMARC, BIO-WEST, and collaborating researchers have completed many investigations into the life history, collection, and husbandry of the Comal Springs Riffle Beetle. At this point in time, a document is needed that summarized the body of work that has been completed to date and provides a handbook for Riffle Beetle collection and captive holding. FWS and BIO-WEST will gather the data collected from field observations and collections and combine that with the data gathered through captive holding observations and research to develop a guide outlining what we know about Riffle Beetle life history and captive husbandry.
Budget: $59,735.15
Benefit to the Refugia: This document will provide SMARC biologists and partners with background knowledge of life history, as well as a standard set of SOPs for collection and captive husbandry. This document will be used as a training and reference tool for future SMARC staff and FWS partners.
Expected Results: A Report and an SOP for propagating Comal Springs riffle beetles will be presented to the EAA and a peer-reviewed publication will be submitted, if appropriate.

Project 4:

Title: Improve efficacy of tagging of small-bodied salamanders using p-Chip tags
Species: *Eurycea nana*
Principal/Co-PI: FWS staff
Overview: Previous tagging studies at the SMARC have shown improved efficacy of visible implant elastomer (VIE) tags over passive integrated transponder (PIT) or visible implant alpha (VIA) tags for use in salamanders, being most effective in Texas blind salamanders. Although VIE tags can be used in smaller-bodied salamanders, there is a higher tag reading error rate and tag rejection rate. P-Chip tagging, a new tagging technology, is successfully used in small-bodied fish with very little morbidity or mortality. Additionally, the tags can be scanned and read without having to extensively handle the individual, reducing stress and potential physical harm. SMARC staff will test tag retention and readability of p-Chip tags in the small-bodied salamander, *Eurycea nana*.
Budget: $21,858.40
Benefit to the Refugia: Increased success in tagging small-bodied salamanders , and the ability to track each organism as an individual can improve refugia efforts and reduce stress to captive held animals. p-Chips are much smaller and less invasive than currently used tagging methods, which could reduce stress and potential morbidity to tagged individuals. Tracking organisms as individuals will inform basic life history aspects such as longevity and number of reproductive events per year. In future efforts, the genetic

information of each individual can be collected non-lethally and associated with the individual's p-Chip ID. This will assist in developing higher level restocking strategies through ensuring the genetic diversity of refugia produced F1 offspring is representative of wild populations. Additionally, the refugia would no longer need to separate individuals by year or collection site, increasing refugia space for more individuals.
Expected Results: The results of the study will be presented as a report to the EAA, an updated tagging SOP, and a peer-reviewed publication (if applicable).

Project 5:

Title: Continuation of San Marcos salamander habitat modification and propagation manual (carry over from 2021)
Species: *Eurycea nana*
Principal: FWS staff
Overview: This study will continue 2021 efforts assess the effects of habitat manipulation on reproductive success of San Marcos salamanders. A San Marcos salamander propagation handbook will be developed. The handbook will provide a protocol for San Marcos salamander propagation with the best available information gathered through research and husbandry efforts.
Budget: $21,126.59
Benefit to the Refugia: Continued refinement of salamander reproduction and propagation. Information gained will guide additional research and inform reintroduction strategy.
Expected Results: The results of the study will be presented as a report to the EAA.

Project 6:

Title: Fountain darters tissue catalog and DNA viability
Species: *Etheostoma fonticola*
Principal/Co-PI: FWS staff, SCA Student
Overview: An SCA Student, under the direction of SMARC Staff will inventory and catalog the many fountain darter tissue samples that have been preserved and kept on station from the 1990s to now. Taking inventory of these tissues and extracting their DNA would provide a valuable resource to compare genetic diversity in the San Marcos and Comal Springs fountain darter populations over time as well as compare contemporary diversity to historical diversity.
Budget: $29,818.60
Benefit to the Refugia: Provide the resources necessary to make comparisons between historic and contemporary population level genetic diversity of fountain darters.
Expected Results: The results of the study will be presented as a report to the EAA and a peer-reviewed journal article.

Project 7:

Title: Testing Bd treatments for aquatic salamanders
Species: *Eurycea nana*

Principal/Co-PI: FWS
Overview: Chytrid fungus, such as *Batrachochytrium dendrobatidis* (Bd), is a health concern for amphibians, including the aquatic salamanders associated with the Edwards Aquifer. Bd infections in amphibians are usually associated with reddened skin and tissue degradation of the toes and tail. In aquatic salamanders, issues with osmoregulation are also observed. Although San Marcos salamanders routinely test positive for Bd, we have yet to investigate Bd infections' potential impact on long-term aquatic salamander health. A common mortality observed in San Marcos salamanders held in refugia is rupturing of the abdominal cavity, potentially related to Bd infections. We will investigate the efficacy of Bd treatment options that have been pilot tested in other aquatic salamanders. We will record Bd infection status pre- and post-treatment as well as any long-term effects of treatment.
Budget: $35,736.78
Benefit to the Refugia: We will identify a treatment method for Bd in aquatic salamanders and develop an SOP for treating salamanders when they are collected and brought into the refugia.
Expected Results: Bd positive individuals will be Bd negative post treatment.

Project 8:

Title: Continuation of Comal Springs riffle beetle *Staphylococcus* exposure
Species: *Heterelmis comalensis*
Principal/Co-PI: Dr. Camila Carlos-Shanley (Texas State University)/FWS
Overview: Previous research has shown distinct differences in the microbial community of wild and captive held riffle beetles and biofilm food materials. Additionally, potentially harmful bacteria spp. (such as *Staphylococcus aureus*) were identified in higher abundance in captive held beetles. It is unclear if the increased relative abundance of bacteria, like *S. aureus*, is detrimental to beetle larvae survival and subsequent pupation. In 2021, we tested beetle survival after *Staphylococcus* exposure. This 2022 effort is continuation of the 2021 efforts. Samples were sent off for sequencing in 2021 but were lost in shipping. There are larvae from each treatment group on hand to continue the sequencing effort. The aim is to sequence the microbiome of the larvae exposed to *staphylococcus*, *Bacillus*, and a no bacteria added control to determine how high untypical bacteria exposure impacts the microbiome, which then can be correlated to overall survival and pupation rates of larvae in each treatment.
Budget: $19,557.43
Benefit to the Refugia: Determine if more strict biosecurity measures need to be in place to reduce bacterial exposure to beetle larvae. This study would also add to the overall understanding of how changes in the microbiome impact beetle survival and pupation in captivity.
Expected Results: There will be significant differences in the microbial communities of each treatment group.

Task 3. Species Propagation and Husbandry

Development and refinement of SOPs for animal rearing and captive propagation: SMARC and UNFH will 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, SMARC and UNFH will further develop draft Captive Propagation Plans for all species.

Task 4. Species Reintroduction

Reintroduction Plan for term of contract:
SMARC and UNFH continue to refine the Reintroduction Strategy as new information becomes available.

Reintroduction Plan for 2022: None

Any anticipated triggers being prepared for: Given current weather predictions, spring flows, and the Edwards Aquafer water level, no anticipated triggers are anticipated during the 2022 performance period.

Task 5. Reporting

5.1 Species specific Propagation plans (SOPs): Refine throughout year as needed
5.2 Species specific Genetic Management plans: Texas wild-rice, contingent on when genetic study results are finished.
5.3 Species specific Reintroduction plans: Refine as needed
5.4 2022 EAHCP Annual Program reporting– A year-end report of 2022 activities will be provided to the EAA no later than 1/31/2022.
5.5 Program reporting as required by ITP and TPWD. TPWD Scientific Research Permit Report will be filed July 31, 2022.
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
- Professional Scientific Meetings

Monitoring:
Monitoring will be conducted through progress reports and site visits to the refugia as well as through collaborative management by the EAHCP CSO.

Cost estimate:

	U.S. Fish and Wildlife Service 2022	**Task Budget Amount**	**Total Task Budget Amount**
TASK 1	**Refugia Operations**		$836,473.48
	SMARC Refugia & Quarantine Bldgs.		
	Equipment & Building Maintenance	$ 15,000	
	Utilities	$ 14,000	
	UNFH Refugia & Quarantine Bldgs.		
	Equipment & Building Maintenance	$ 15,000	
	Utilities	$ 35,000	
	SMARC Species Husbandry and Collection Salaries	$ 150,851	
	UNFH Species Husbandry and Collection Salaries	$ 185,000	
	Water Quality System	$ 12,000	
	Divers Salaries	$ 3,500	
	Fish Health	$ 10,000	
	SMARC Reimbursable	$ 100,000	
	UNFH Reimbursable	$ 145,283	
	Subtotal	*$685,634*	
	Admin Cost Subtotal	*$150,839.48*	
TASK 2	**Research**		$515,968.84
	BIO-WEST: CSRB Propagation (2021 Rollover)	$ 49,451.71	
	BIO-WEST: CSRB Propagation	$ 30,000	

U.S. Fish and Wildlife Service 2022		Task Budget Amount	Total Task Budget Amount
	BIO-WEST: CSRB Handbook contribution	$ 22,000	
	Texas State Research	$ 19,557.43	
	USFWS Research		
	Materials	$ 142,790.90	
	SMARC Staff	$ 142,839.54	
	UNFH Staff	$ 16,285.69	
	Subtotal	*$ 422,925.28*	
	Admin costs for Task 2	*$ 93,043.56*	
TASK 3	**Species Propagation and Husbandry**	-	-
	Subtotal	-	
TASK 4	**Species Reintroduction**	-	-
	Subtotal	-	
TASK 5	**Reporting**		$ 78,506.68
	SMARC Staff	$ 35,770.08	
	UNFH Staff	$ 28,579.66	
	Subtotal	*$ 64,349.74*	
	Admin costs for Task 5	*$ 14,156.94*	
TASK 6	**Meetings and Presentations**		$ 16,987.08
	SMARC Staff	$ 10,811.78	
	UNFH Staff	$ 3,112.06	
	Subtotal	*$ 13,923.84*	
	Admin costs for Task 6	*$ 3,063.24*	
	TOTAL	**$1,447,936.08**	

Projected (2022) Budget Summarized by Task:

Task 1: $836,473.48
Task 2: $515,968.84
Task 3: $0
Task 4: $0
Task 5: $78,506.68
Task 6: $16,987.08

Projected (2022) Subcontractor Expenses Summarized by Task

Task 1:
Task 2: BIO-WEST $101,451.71
Task 2: Texas State University $19,557.42
Task 3: $0
Task 4: $0
Task 5: $0
Task 6: $0

Timeline of 2022 Milestones

January	Continue with species collection
	2022 Specific Research Study Plans finalized
May/June	Subcontract research awards executed
July	Submit and renew TPWD permit
September	Draft Research Reports
December	Draft Annual report

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5.1.2 Voluntary Irrigation Suspension Program Option

Long-term Objective:

The goal of VISPO is to enroll 41,795 acre-feet (AF) of permitted irrigation rights (base and/or unrestricted) that will remain unused in years of severe drought based on the USFWS approved 2019 amendment. Permit holders are enrolled in five-year and ten-year VISPO agreements and will be compensated based on the amount of water enrolled and the program selected. Permit holders enrolled in 10-year agreements are paid a standby fee of $70.20/ac-ft per year every year of the term regardless of aquifer conditions and an additional fee of $210.60/ac-ft per year will be paid for each year when temporary pumping suspensions are required. Permit holders enrolled in 5-year agreements are paid a standby fee of $54/ac-ft per year every year of the term regardless of aquifer conditions and an additional fee of $160/ac-ft per year will be paid for each year when temporary pumping suspensions are required. Beginning January 1, 2021, a total of 41,795 ac-ft was fully enrolled of which 26,175 ac-ft are 5-year agreements and 15,620 ac-ft are 10-year agreements. The enrollment goal of 41,795 ac-ft has been completed and will remain fully enrolled until a large portion of the 5-year and 10-year VISPO agreements will begin to expire December 31, 2023. Permit holders will be offered the opportunity to renew their VISPO forbearance agreements prior to their expiration.

If the water level at the J-17 index well in San Antonio is at or below 635 feet on October 1 of any year, program participants are contractually obligated to suspend the use of their enrolled water for the following year - beginning on January 1. The determination for a VISPO trigger will be made on October 1, 2021; if it does not trigger, all enrolled water can be used by the permit holders in 2022. If it triggered, all enrolled water will be unpumped. Annual VISPO payouts are through 2021 are reflected in Table 5.1.2-1.

Table 5.1.2-1: VISPO Total Payout by Year

Year	Payment Type	Total Enrolled (AF)	Total
2014	Stand-by	22,388	$1,201,938
2015	Suspension	40,921	$8,677,262
2016	Stand-by	40,921	$2,188,500
2017	Stand-by	40,921	$2,209,000
2018	Stand-by	40,921	$2,228,300
2019	Stand-by	39,646	$2,320,309
2020	Stand-by	39,803	$2,333,415
2021	Stand-by	41,795	$2,508,070
		Grand Total	$23,666,794

Target for 2022:
The total volume goal of 41,796 ac-ft in VISPO agreements will continue to be maintained and managed by EAA staff. VISPO payments for year 2022 will be determined by the October 1, 2021 J-17 index well water level.

Cost Estimate:
Table 7.1:
$4,172,000

Estimated 2022 cost if Standby:
Standby: $2,509,976

Estimated 2022 cost if Suspension:
Suspension: $9,987,551

5.1.3 Regional Water Conservation Program

Long-term Objective:
Conservation measures will be implemented to conserve 20,000 acre-feet of water to reduce withdrawals from the Edwards Aquifer by 10,000 acre-feet. The concept is to reduce aquifer withdrawals by 10,000 acre-feet using a Regional Water Conservation Program (RWCP).

Several entities within the Edwards Aquifer Authority (EAA) jurisdictional area agreed to make Initial Commitments to the EAA Groundwater Trust to provide an immediate benefit to the aquifer and springflow. The EAA maintains contracts with three communities to conserve water under the RWCP through 2028. The City of Uvalde began implementing its toilet replacement program in 2013 to conserve 57.450 ac-ft of water. In 2014, the City of Universal City began implementing its leak detection program to conserve 163.684 ac-ft of water and in 2016, SAWS began implementing a five-year Leak Detection and Repair Program. The SAWS Leak Detection and Repair Program satisfies the total remaining RWCP goal for water committed into the EAA Groundwater Trust for the remainder of Incidental Take Permit (TE-63663A-1).

The estimated total savings of 20,053 ac-ft of conserved water was achieved from all three communities in 2020. One-half of the conserved water (10,027.13 ac-ft) has been placed in the EAHCP Groundwater Trust through the RWCP to remain unpumped through 2028.

Target for 2022:
None. This conservation measure was achieved in 2020 and 10,027.13 ac-ft has been placed in the EAHCP Groundwater Trust.

Cost estimate:
Estimated 2022 cost:
$0

5.1.4 Edwards Aquifer Authority Stage V Critical Period Management

Stage V Critical Period Management was developed to help decrease withdrawals and maintain adequate springflows at both Comal and San Marcos Springs during times of drought. On February 14, 2012, the Edwards Aquifer Authority (EAA) Board of Directors voted to amend its Critical Period Management (CPM) Program to include the new emergency Stage V. Implementation of Stage V results in a reduction of 44% to municipal, industrial and irrigation permit holders in both pools of the Edwards Aquifer who are authorized to withdraw more than 3 ac-ft per year. Stage V became effective as a rule on March 18, 2013 when the Incidental Take Permit was issued by the U.S. Fish and Wildlife Service.

2022 Implementation:

EAA staff monitors daily aquifer levels in both the San Antonio and Uvalde Pools of the Edwards Aquifer Region, and if at any time, the 10-day average for aquifer or springflow levels in either pool reaches the designated trigger for Stage V, the EAA General Manager will issue a Notice of Commencement for implementation in five newspapers within the EAA jurisdiction. Notice will also be posted at the EAA's office and on the EAA website. All affected permit holders will also be provided written notice of implementation of Stage V and the requirement to reduce pumping by 44%.

Permit Holder Assistance:

The EAA provides an online Critical Period Calculator to assist permit holders in calculating CPM reductions as they apply to each individual permit holder's total authorized withdrawal amount throughout the year. EAA staff also assists permit holders through "one-on-one" customer service offerings as may be necessary.

Triggers:

The triggers for Stage V in the San Antonio Pool are as follows: the 10-day average at the J-17 index well in San Antonio falls below 625 mean sea level (msl); or the 10-day average at Comal Springs falls below 45 cubic feet per second (cfs); or the 3-day average at Comal Springs falls below 40 cfs. In the Uvalde Pool, Stage V is triggered when the 10-day average at the J-27 index well falls below 840 msl.

Reporting:

By rule, permit holders are required to report their annual groundwater use to the EAA by January 31 for all groundwater used the preceding year. Permit holders who use more Edwards groundwater than authorized annually are subject to enforcement action.

5.5.1 Edwards Aquifer Authority and San Antonio Water System Aquifer Storage and Recovery Work Plan

Section 5.5.1 of the Edwards Aquifer Habitat Conservation Plan (EAHCP) assigns acquiring leases and options of water permits for use in the San Antonio Water System (SAWS) Aquifer Storage and Recovery (ASR) to the Edwards Aquifer Authority (EAA). SAWS will operate the ASR infrastructure and retain control of day-to-day operations of the ASR facility related to EAHCP water injection and recovery. The EAA will ensure compliance with EAHCP requirements through management of the Interlocal Contract between the EAA and SAWS for the Use of the Twin Oaks Aquifer Storage and Recovery Project for Contribution to Springflow Protection, which became effective August 14, 2013. The contract outlines the responsibilities of both parties, including administration and implementation.

Long-term Objective:
The objective of SAWS Twin Oaks ASR (ASR now runs out of H_2O Oaks facility) system is to deliver 126,000 acre-feet of Edwards Aquifer groundwater. This water is best managed to offset pumping from Edwards Aquifer wells during a repeat of a drought similar to the drought of record and acquire an additional 50,000 acre-feet of agricultural, municipal, industrial groundwater withdrawal rights to either be made available for physical storing in / crediting to the Regional ASR balance or may be forborne.

Target for 2022:
The ASR contract between EAA and SAWS will continue to be implemented. EAA is the agent for ASR enrollments and in year 2020 issued its final notice of availability of EAHCP groundwater to SAWS for injection resulting in the completion of the storage goal of 126,000 acre-feet. Effective in 2021, a total of 50,000 acre-feet of groundwater rights was secured by EAA staff to be used as forbearance water and will go unpumped during a repeat of a drought of record. Future water acquired by the EAA through contractual agreements will be necessary to maintain the 50,000 ac-ft balance due to expiring leases occurring annually. The 50,000 ac-ft balance will be utilized for forbearance purposes during a repeat of a drought of record as outlined in the EAHCP. During a drought of record, the stored ASR water may be used by SAWS to offset forbearance and the EAA will also forbear the use of the 50,000 acre-feet of groundwater under its control.

ASR Program:

Description of the SAWS ASR: The SAWS H_2Oaks ASR is an underground storage reserve in the Carrizo Aquifer in southern Bexar County. As a SAWS water management project, it is designed to store Edwards Aquifer water when demand is less than available supply. The stored water is returned to San Antonio for use when demand is high and Edwards supply is restricted by Critical Period Management and other drought-related limitations.

The capacity and capabilities of the SAWS ASR are such that it can be used to meet SAWS ratepayer expectations and, if operated as described in the EAHCP, will play a significant role protecting the Covered Species at Comal and San Marcos Springs.

Operations: The EAHCP Program Interlocal Contract between the EAA and SAWS for the Use of the Twin Oaks Aquifer Storage and Recovery Project for contribution to Springflow Protection, effective August 14, 2013, takes elements of the EAHCP's ASR flow protection strategy and places them into an operations contract.

Injection: Storage of EAHCP groundwater shall be at the discretion of SAWS and will be dependent on operating conditions. All EAHCP groundwater made available to SAWS before June 30th, 2020, was physically stored or credited as if stored, and will be used to meet any forbearance from the Aquifer should triggers defined in the Interlocal Contract occur in 2022.

Forbearance and Recovery: Forbearance of Edwards Aquifer pumping from certain wells will occur when the ten-year rolling recharge average is less than 500,000 acre-feet and the ten-day average of aquifer levels measured at the J-17 index well drop below 630 feet mean sea level (MSL). The annual amount of water to be recovered from the ASR during a repeat of the drought of record is outlined in Exhibits E & F of the Interlocal Contract. Changes to the Presumptive Forbearance Schedule outlined in Exhibit E may be approved as outlined in Section 5.3 of the Interlocal Contract. The ten-year rolling recharge average reported April 9, 2021 was 555,780 acre-feet and the ten-day average of aquifer levels measured at the J-17 index well as of April 9, 2021 was 655.1 ft msl.

Leasing: In 2018, EAA staff began marketing long-term (ten-year) forbearance agreements with regional permit holders and in 2020 completed the enrollment goal for years 2021 through 2028. In 2021 the total amount of water available under long-term leases was 14,562 acre-feet and 35,438 acre-feet in forbearance agreements for a total of 50,000 acre-feet. On December 31, 2021, a total of 1,164 acre-feet in ASR leases will expire and will be re-enrolled as forbearance agreements effective in year 2022. EAA staff will continue to maintain and manage 50,000 acre-feet of groundwater withdrawal rights under leases and forbearance agreements. This water will remain unused during a repeat of drought of record conditions.

Monitoring:

The EAA will actively manage the Interlocal Contract with SAWS. Status reports and updates will be provided regularly to the Implementing Committee.

ASR Regional Advisory Group: Per Section 5.5.1 of the EAHCP, a 12-person SAWS ASR Regional Advisory Group will meet to advise SAWS as SAWS makes the decisions relating to the operation of the ASR facility relevant to the EAHCP. Membership on the Regional Advisory Group will include: four representatives from the San Antonio Water System, the EAHCP Program Manager; one representative each from the EAA, EAA permit holder for irrigation purposes, small municipal pumpers, the spring cities, environmental interests, industrial pumpers, and downstream interests.

Cost estimate:

Table 7.1:	Estimated 2022 cost:
$4,759,000 – Lease Options	$5,776,493 – Lease & Forbearance Options
$2,194,000 – O&M	$0 – O&M
$6,953,000 – Total	$5,776,493 – Total

5.7.2 Water Quality Monitoring Program Strategy for Comal Springs and San Marcos Springs

This work plan details the sampling strategy and protocols for water quality monitoring in 2022 for the Edwards Aquifer Habitat Conservation Plan (EAHCP) (Section 5.7.2) implemented by the Edwards Aquifer Authority (EAA). Water quality monitoring of the Comal and San Marcos springs complexes and their associated surface waters has occurred since 2013 under implementation of the EAHCP. During this time period, the program has employed a variety of sampling strategies: stormwater, surface water, sediments, fish tissue, and passive samplers aimed at a range of environmental contaminants.

The water quality monitoring program underwent a formal review as part of the *National Academy of Sciences (NAS) Report 1* (2015) containing recommendations for EAHCP's Monitoring, Modeling and Applied Research programs, including the Expanded Water Quality Monitoring Program. Subsequently, a work group was formed in 2016 to assess recommendations presented in the NAS report. The result was a scope of work that was executed from 2017 – 2020.

Beginning in 2021, additional refinements to the program are being implemented. The primary changes from the previous implementation include discontinuing stormwater and passive sampling, adding surface water sampling, and modifying the analyte list. Table 1 presents an overview of the core activities comprising the EAHCP Water Quality monitoring program. Additionally, as needs arise, other water quality sampling activities may occur as developed through the EAHCP committees and included in the Annual Work Plan.

Target for 2022:
Water quality monitoring activities for 2022 include sampling activities for surface water, groundwater, and sediment in addition to operation of the real-time network. Specific actions for each sample type are discussed below. Analyte lists and maps follow this discussion. All samples will be collected following the EAA's *Field Sampling Plan* and analyzed by a NELAP accredited contract laboratory.

Groundwater sampling:

Groundwater samples will be collected from Spring 1, Spring 3, Spring 7 (Comal), Deep and Hotel (San Marcos) springs during the Spring and Fall under normal flow conditions (Figures A1 and A2). Groundwater samples will be collected by directly filling a bottle or using a previously decontaminated peristaltic pump with the intake portion of the pump placed in the spring orifice to minimize surface water contamination. Samples will be submitted to a contract laboratory for analysis of cations, anions, nutrients, metals, VOCs, SVOCs, herbicides and pesticides, bacteria, TOC, PCBs, and PPCPs. The analyte list for laboratory analyses along with the methods are shown in Table 4. During the collection event, field parameters will be collected that include dissolved oxygen, pH, conductivity, temperature, and alkalinity.

In addition to the biannual groundwater sampling, sucralose will be measured on a monthly basis at Spring 3 and Hotel, and PPCPs will be measured on an every other month basis at Spring 3 and Hotel. These samples will be collected by directly filling bottles at the source of spring flow. During the collection event, field parameters will be collected that include dissolved oxygen, pH, conductivity, and temperature.

Table 1. EAHCP Water Quality monitoring program core activities.

Sample Type	Activity
Surface water	Twice annual sampling in conjunction with Biological Monitoring activities
	Laboratory analyses are focused on bacteria and nutrients
	Locations include upper and lower stations at each spring system
Groundwater	Twice annual sampling in conjunction with EAA springs sampling activities
	Laboratory analyses are focused on geochemical analytes and industrial, commercial, and emerging contaminants. The analytes include cations, anions, nutrients, metals, VOCs, SVOCs, herbicides, pesticides, bacteria, TOC, PCBs, and PPCPs
	Locations include Spring 1, Spring 3, Spring 7 (Comal), Hotel, and Deep (San Marcos)
Sediment	Every other year sampling in even numbered years
	Laboratory analyses are focused on PAHs
	Locations include 6 San Marcos and 5 Comal stations
Fish Tissue	Every other year sampling in odd numbered years
	Laboratory analyses are focused on metals and PPCPs in two fish species
	Locations include upper and lower stations at each spring system
Real-time network	Continuous, telemetered measurements
	Analytes include temperature, dissolved oxygen, and conductivity
	Locations include 3 San Marcos and 3 Comal stations

Surface water sampling:

Surface water samples will be collected from upper and lower river stations at both systems. For Comal Springs, Landa Lake near Spring Island will serve as the upper location, and the lower station is downstream of the Old and New Channel confluence. In San Marcos, Spring Lake near Hotel spring will serve as the upper location, and the downstream location is located at the most downstream real-time water quality monitoring station. Samples at each location will be collected on a biannual basis during normal flow conditions in conjunction with the Biological Monitoring program (Spring and Fall). Water samples will be taken from flowing parts of the stream on the upstream side of the sample collector. A previously decontaminated Kemmerer or similar device will be used to collect samples at approximately mid-depth in the water column. Samples will be submitted to a contract laboratory for analysis of nutrients, chlorophyll a, and

bacteria (Table 5). During the collection event, field parameters will be collected that include dissolved oxygen, pH, conductivity, and temperature.

Fish Tissue sampling:

Fish tissue samples collected in 2021 will be shipped to the appropriate laboratory and analyzed for pharmaceutical and personal care products (PPCPs). Shipping was delayed in 2021 due to COVID restrictions. Given the delay, only fish tissue samples from largemouth bass will be analyzed, removing Gambusia from the analysis.

Sediment sampling:

Sediment samples will be collected once from four locations within the Comal and six locations in San Marcos (Figures 3 and 4). Three samples will be collected at each sample site and composited into one sample for analysis. Sediment samples will be analyzed for the parameters shown in Table 6.

Real Time Instrument Water Quality Data Logging:

Continuous water quality monitoring stations will continue in 2021 at three locations in the Comal and three locations in San Marcos. The network consists of Insitu AquaTroll sondes measuring dissolved oxygen, conductivity, temperature, and turbidity (Sessom Creek only). Measurements are collected every fifteen minutes and telemetered in real-time. The Sessom Creek site logs data on five-minute intervals to support turbidity measurements at this location.

Quality control procedures:

Field collection methods and quality control procedures for the discrete sampling types are guided by the EAA's Field Sampling Plan. The anticipated number of samples and field quality control samples sent for analyses in 2022 are shown in Table 2. Brief descriptions of the intent of the quality control tests are described below.

Table 2. Sample amounts for 2022 water quality activities.

Sample type	Field Samples	Equipment blank	DI blank	Lab duplicate	Field duplicate	Total samples
Groundwater	18	2	2			22
Sucralose	24	1	3		2	30
Surface water	8	2	2		4	16
Sediment	10			2		12

Both equipment blanks and DI blanks use reagent grade ASTM II deionized water to assess external contamination of environmental samples. Equipment blanks examine the contamination introduced through the sampling procedure. These are conducted by transferring the deionized water through equipment that has been decontaminated for field use. DI blanks consist of deionized water sent directly to the laboratory and are designed to examine sample container and other laboratory contamination.

Lab and field duplicates are intended to assess the precision and repeatability of the analytical procedure and homogeneity of the environmental sample type. Laboratory duplicates consists of a single well-mixed sample split into two samples for analysis. Field duplicates consists of a second sample collected immediately after an initial sample.

Additionally, all laboratory quality control data including matrix spikes and surrogate blanks will be reported.

Monitoring:
A summary report presenting the 2022-year findings will be prepared by EAA staff and included in the EAHCP annual report. The report will include an evaluation of the analytical data and its quality, discussions of results, and a description and rationale for any deviations from the Work Plan described here. The report will be completed in February 2023.

Data collected as part of the 2022 EAHCP Water Quality monitoring program will be kept electronically with the EAA. Data from quality controlled discrete sample types (surface water, groundwater, sediment, and fish tissue) will be housed by EAHCP staff in delimited file types that include all discrete measurements from the program beginning in 2013. Quality controlled time series data associated with the real-time network are housed with existing aquifer time-series data by the EAA.

Cost Estimate:
Costs for laboratory analyses are shown in Table 3 and are based on estimates provided by commercial laboratories in 2020-2021. Field supplies costs in Table 3 cover field collection and analysis equipment including calibration standards and Kemmerer device.

Table 3. 2022 EAHCP Water Quality monitoring program costs.

Sample type	Total samples	Cost per sample	Total Costs
Groundwater	22	$1,080	$23,760
Sucralose	30	$195	$5,850
Fish Tissue	4	$565	$2,260
Surface water	16	$225	$3,600
Sediment	12	$150	$1,800

Field Supplies			$5,000
		Total	**$42,270***

*This amount does not include surplus monies made available for additional Water Quality Monitoring needs but will not exceed the $60,000 listed in the funding table on Page 2.

Sample location maps and analyte lists

Figure 1. Groundwater sampling locations for Comal.

Figure 2. Groundwater sampling locations for San Marcos.


Hotel Spring
Deep Spring
Most of the historical EAA sampling records for San Marcos Springs pertains to the locations known as Hotel and Deep (spring vents). Other locations at Comal Springs may have a limited sample record.
Samples are collected biannual basis during normal flow conditions and more frequently when dictated by research intrests.
Expanation
Historical Groundwater (Spring) Sample Location
N
Miles
0 0.1 0.2 0.3 0.4
Service Layer Credits: Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Prepared by:
EDWARDS AQUIFER AUTHORITY

Figure 3. Sediment sampling locations and real time water quality stations for the Comal.

Figure 4. Sediment sampling locations and real-time water quality stations for the San Marcos.

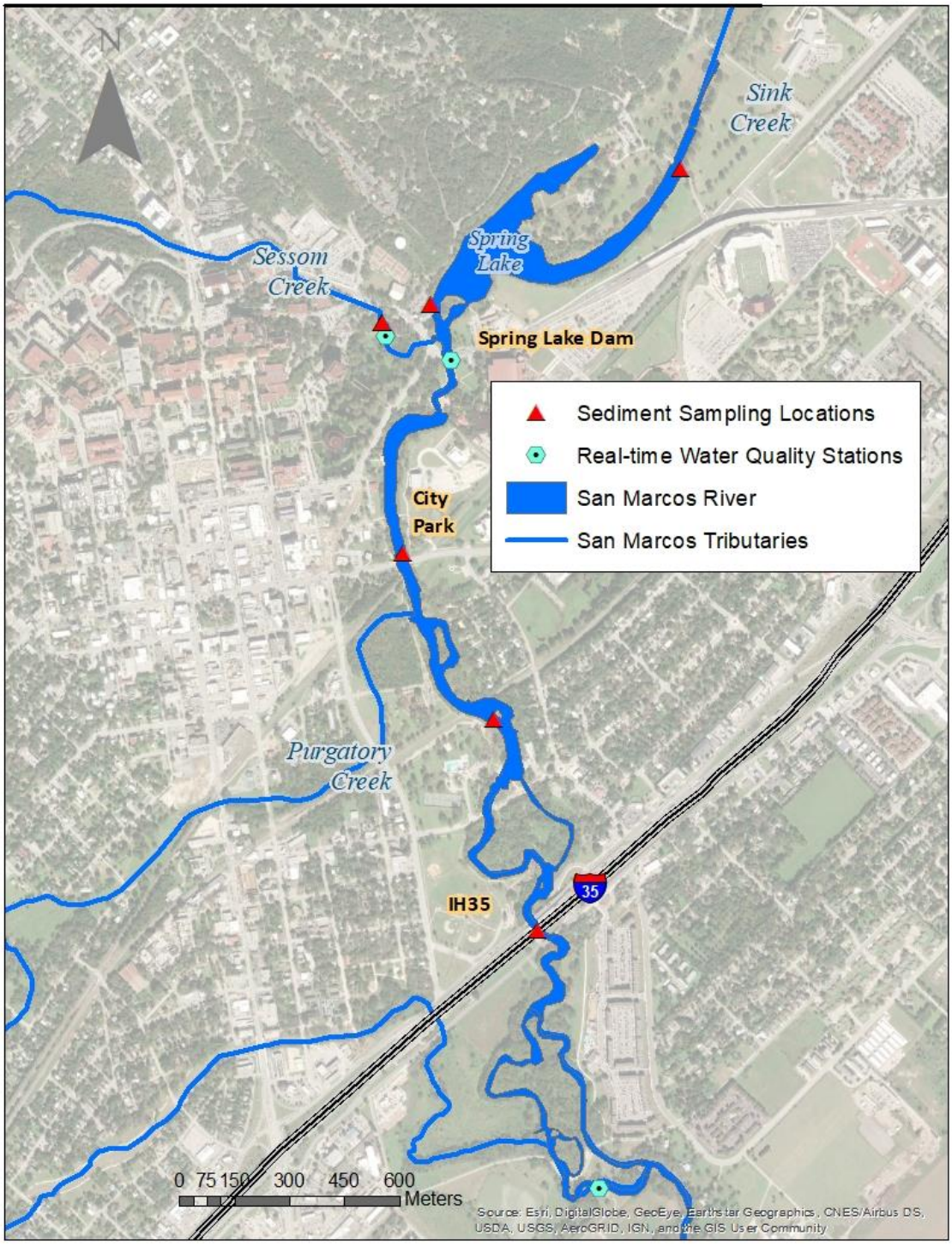


Table 4. Analytical parameters for groundwater samples.

Analyses
Volatile Organic Compounds (VOCs)
Semi-volatile Organic Compounds (SVOCs)
Organochlorine Pesticides
Polychlorinated Biphenyls (PCBs)
Organophosphorous Pesticides
Herbicides
Metals (Al, Sb, As, Ba, Be, B, Cd, Cr (total), Cu, Fe, Pb, Mn, Hg, Ni, Se, Ag, Tl, V, and Zn)
General Chemistry (GWQP) Total Alkalinity (as CaCO3), Bicarbonate Alkalinity (as CaCO3), Carbonate Alkalinity (as CaCO3); (Cl, Br, NO_3, SO_4, Fl, pH, TDS, TSS, Ca, Mg, Na, K, Si, Sr, CO_3,)), and Total Suspended
Phosphorus (total)
Total Organic Carbon (TOC),
Dissolved Organic Carbon (DOC)
Kjeldahl Nitrogen
Bacteria Testing (*E coli*)
PPCPs

Method	**Method Description**	**Protocol**
8260B	Volatile Organic Compounds	(GC/MS) SW846
8270C	Semivolatile Organic Compounds	(GC/MS) SW846
8081B	Organochlorine Pesticides	(GC) SW846
8082A	Polychlorinated Biphenyls (PCBs)	by Gas Chromatography SW846
8141A	Organophosphorous Pesticides	(GC) SW846
8151A	Herbicides	(GC) SW846
6010B	Metals	(ICP) SW846
6020	Metals	(ICP/MS) SW846
7470A	Mercury	(CVAA) SW846
300.0	Anions,	Ion Chromatography
340.2	Fluoride	MCAWW
365.4	Phosphorus,	Total EPA
9040C	pH	SW846
9060	Organic Carbon,	Total (TOC) SW846
SM 2320B	Alkalinity	SM
SM 2540C	Solids,	Total Dissolved (TDS) SM
SM 2540D	Solids, Total Suspended (TSS)	SM
351.2	Nitrogen, Total Kjeldahl	MCAWW
1694	PPCPs	LC-MS/MS

Protocol References:

EPA = US Environmental Protection Agency

MCAWW = "Methods For Chemical Analysis Of Water And Wastes", EPA-600/4-79-020, March 1983 And Subsequent Revisions.

SM = "Standard Methods For The Examination Of Water And Wastewater",

SW846 = "Test Methods For Evaluating Solid Waste, Physical/Chemical Methods", Third Edition, November 1986 And Its Updates.

Table 5. Analytical parameters for surface water samples

Analyses
Chlorophyll a
Soluble Reactive Phosphorous
Phosphorus (total)
Total Organic Carbon (TOC),
Dissolved Organic Carbon (DOC)
Kjeldahl Nitrogen
Bacteria Testing (*E coli*)
Nitrates and Ammonium

Method	**Method Description**	**Protocol**
365.4	Phosphorus,	Total EPA
9060	Organic Carbon,	Total (TOC) SW846
351.2	Nitrogen, Total Kjeldahl	MCAWW
445.0	Chlorophyll a	Fluorescence
8141a	Organophosphates	SW846
353.2	Nitrates	
350.3	Ammonia	

Protocol References:
EPA = US Environmental Protection Agency
MCAWW = "Methods For Chemical Analysis Of Water And Wastes", EPA-600/4-79-020, March 1983 And Subsequent Revisions.
SM = "Standard Methods For The Examination Of Water And Wastewater",
SW846 = "Test Methods For Evaluating Solid Waste, Physical/Chemical Methods", Third Edition, November 1986 And Its Updates.

Table 6. Analytical parameters for sediment samples

Analytes
Benzo[a]anthracene
Chrysene
Benzo[a]pyrene
Benzo[b]fluoranthene
Benzo[k]fluoranthene
Fluoranthene
Dibenz(a,h)anthracene
Indeno[1,2,3-cd]pyrene
Pyrene
Phenanthrene
Fluorene
Benzo[g,h,i]perylene

Analytes
Anthracene
Acenaphthene
Acenaphthylene
Benzo[g,h,i]perylene
Carbazole
2-Methylnaphthalene
Naphthalene
Total Organic Carbon (TOC)

8270C - SVOCs GC/MS SW8310

9060 Organic Carbon, Total (TOC) SW846

Protocol References:

EPA = US Environmental Protection Agency

MCAWW = "Methods For Chemical Analysis Of Water And Wastes", EPA-600/4-79-020, March 1983 And Subsequent Revisions.

SM = "Standard Methods For The Examination Of Water And Wastewater",

SW846 = "Test Methods For Evaluating Solid Waste, Physical/Chemical Methods", Third Edition, November 1986 And Its Updates.

6.3.1 Biological Monitoring Program for the Comal and San Marcos Aquatic Ecosystem

Long-term Objective:
Since 2000, the Edwards Aquifer Authority (EAA) has undertaken biological monitoring of the Comal and San Marcos spring systems. In 2013, the elements of the program were incorporated into the Biological Monitoring Program (BioMP) for the Edwards Aquifer Habitat Conservation Plan (EAHCP).

The purpose of the BioMP is "to monitor changes to habitat availability and population abundance of the Covered Species that may result from Covered Activities" (EAHCP § 6.3.1). The BioMP includes: (1) Comprehensive Sampling, (2) any triggered Critical Period Monitoring, (3) any high flow triggered monitoring (4) and any EAHCP-specific sampling required by Section 6.4.

Target for 2022:
The 2022 BioMP for the Comal and San Marcos aquatic ecosystems will continue to include Baseline and Critical Period Monitoring along with a Net Disturbance impact assessment and overall Take Determinations. The 2022 BioMP will continue to use the standard operating procedures adopted in 2016 because of the Biological Monitoring Work Group (EAHCP 2016) in addition to what is noted in this document. These standard operating procedures were instituted for the BioMP beginning in 2017.

Monitoring:
Aquatic Vegetation Mapping: The contractor will conduct aquatic vegetation mapping in the four long-term monitoring reaches in the Comal Springs system and in the three long-term monitoring reaches in the San Marcos Springs system. The comprehensive mapping is conducted using a GPS unit with real-time differential correction with sub-meter accuracy.

Zebra Mussel Monitoring: The contractor will conduct zebra mussel monitoring using passive techniques in both the Comal and San Marcos rivers.

Texas wild-rice Mapping: The contractor will map all Texas wild-rice from Spring Lake downstream to the confluence of the Blanco River on an annual basis. The annual mapping will occur during the summer (July-August). The location of every stand of Texas wild-rice will be recorded using a GPS unit with real-time differential correction with sub-meter accuracy.

Fountain Darter Sampling: The contractor will conduct drop and dip netting and visual aquatic surveys with SCUBA during the Spring and Fall sampling events. Additional dip net sampling will be conducted during the Summer sampling event. Aquatic vegetation will be mapped in the reaches prior to drop and dip net activities.

Drop Net Sampling: Drop netting will be used to sample fountain darters in identified reaches of the rivers among dominant aquatic vegetation species that have been selected through stratified random sampling. Fountain darters will be identified, counted, measured, examined for condition, and returned to the river at the point of collection. Other fish will be identified and

released, or preserved, and identified in a laboratory. Live rams-horn snails will be counted, measured, and destroyed. Exotic Asian snails and Asian clam will be identified, general abundance recorded, then destroyed. The number of crayfish and grass shrimp per drop net will be noted. Furthermore, vegetation species, vegetation height, vegetative areal coverage, substrate type, water depth, mean column velocity, velocity at 15 centimeters (cm) above the bottom, water temperature, conductivity, pH, and dissolved oxygen levels will be recorded for each drop net.

Dip Net Sampling: The contractor will conduct dip net timed surveys, as well as presence/absence surveys in specified sections throughout the spatial extent of both systems. Fountain darters collected by dip net monitoring will be examined for gill condition. Additionally, total length of collected individuals will be measured during timed dip net surveys. Timed surveys will be conducted in all habitat types up to a depth of 1.4 m, within each section, moving upstream during the sampling process with prime darter habitat receiving the most effort.

Presence/absence surveys will be conducted by taking 4 dip net sweeps at 50 random sample site locations within the 4 representative reaches at Comal Springs (Upper Spring reach [5 locations], Landa Lake reach [20 locations], Old Channel reach [20 locations], and New Channel reach [5 locations]), and the 50 random sample site locations within the three representative reaches in San Marcos Springs (Spring Lake Dam reach [15 locations], City Park reach [20 locations], and I-35 reach [15 locations]).

Visual Fountain Darter Survey: Visual aquatic surveys will be conducted using SCUBA in a fixed location in Landa Lake to identify fountain darters at depths deeper than conventional sampling methods allow.

Comal Springs Invertebrate Sampling: The contractor will conduct sampling for Comal Springs invertebrates during the Spring and Fall sampling events.

One drift net each will be placed over the main spring orifice of Spring Run 1, Spring Run 3, and Spring Run 7 at Comal Springs. All endangered invertebrates will be identified and counted in the field and returned to the orifice they were collected upon completion of the 24-hour sample period. All other invertebrates will be preserved and transported to an off-site laboratory for taxonomic classification. Coordination with the USFWS San Marcos Aquatic Resources Center (SMARC) will take place each time to assist with refugia collections when needed.

The Comal Springs riffle beetle (CSRB) cotton lure standard operating procedure, or a suggested (and EAHCP staff approved) alternate method, and quantitative survey methods will be utilized to conduct Comal Springs riffle beetle sampling in three locations (i.e., Spring Run 3, western shoreline of Landa Lake, and Spring Island area). Ten springs within each of the three locations will be identified for sampling by the contractor. If possible, the same ten springs from the previous year will be sampled.

The CSRB cotton lure standard operating procedure, cotton lure quantitative survey method, and recommendations generated during the CSRB workgroup describe the appropriate protocols for CSRB to be identified, counted, and returned to their spring of origin. Other spring invertebrates

collected on the lures will also be noted including the Comal Springs dryopid beetle (*Stygoparnus comalensis*) and Peck's cave amphipod (*Stygobromus pecki*).

Salamander Visual Observations: The contractor will conduct salamander sampling during each Spring and Fall sampling event. Comal Salamander surveys will be timed and conducted by observation from the surface or dive mask and snorkel at Spring Run l, Spring Run 3, Spring Island spring runs, and at the eastern outfall at Spring Island.

San Marcos salamander surveys follow the quantitative sampling method described in Nelson, J. (M.S. Thesis, Texas State University, 1993). Observations for the San Marcos salamander will be done by dive mask and snorkel or SCUBA for three, 5-minute timed surveys per area. San Marcos salamanders will be counted, measured and the overall substrate where they were found documented.

In both systems, sampling will require turning over rocks in the sample site for set periods of time in order to expose the salamanders and obtain a visual count. Whenever possible, all rocks will be returned to their original location. For this monitoring, salamanders will only be observed, and no collections will occur.

Comal Springs Discharge Measurements: The contractor will conduct discharge measurements on Comal Springs during the Spring and Fall sampling events. Discharge measurements will be conducted at Spring Runs 1, 2, and 3, Upper Spring Run Reach, and the Old Channel below Elizabeth Street and will be used to establish the contributions of each major spring run to total discharge in the river and to establish the relative proportion of water flowing in the Old and New Channels.

Water Quality Sampling: The contractor will maintain and download existing thermistors located throughout each system. Standard water quality parameters (water temperature, conductivity compensated to 25°C, pH, dissolved oxygen [mg/l], water depth at sampling point, and observations of local conditions) will be sampled during drop net sampling and fish community sampling activities.

Fixed Station Photography: The contractor will photo document each established, fixed station photograph site. Photographs involve an upstream, across, and downstream picture of the reach and capture key changes in the habitat in the reach.

Macroinvertebrate Community Assessment: The macroinvertebrate community assessment will be conducted using rapid bioassessment (RBA) protocol as described in "Surface Water Quality Monitoring Procedures, Volume 2: Methods for Collecting and Analyzing Biological Assemblage and Habitat Data." TCEQ RG-416. 2014. The RBAs will be conducted in 5 reaches in the Comal and 4 reaches in the San Marcos at the drop-net fountain darter sites. One composite sample will be collected from each reach (i.e. 9 samples total across both systems). Macroinvertebrate community assessments will be conducted during Comprehensive Sampling and Critical Period Monitoring events.

Fish Community Sampling:

SAN MARCOS SYSTEM—Fish will be sampled at two locations within Spring Lake associated with San Marcos salamander surveys (Big Riverbed and Hotel Area) and one location just upstream of the eastern spillway. Two different SCUBA techniques will be used to document the fish within the three locations, mesohabitat and microhabitat surveys. Three additional SCUBA survey locations will occur in the San Marcos River (Upper, Mid, and Lower), located in representative deep areas where seining has proven to be inefficient. The exact location of the SCUBA sampling within each section may change slightly based on conditions at the time of the sampling event.

In addition to SCUBA, fish in the San Marcos River will be sampled among five sites within three reaches (Upper: Sewell, Veteran's Park, Middle: Crook's Park, and Lower: San Marcos Wastewater Treatment plant and Smith property) via seines within wadeable habitats. Multiple seine hauls will occur along a river transect perpendicular to the flow. Within each seine haul, fish will be identified, measured, examined for disease, and native fish returned to the river. Exotics will be removed from the system as per scientific permit. In addition to fish data, habitat data will be collected for each seine haul including current velocity, water depth, substrate composition, in-stream coverage, climatic conditions, and mesohabitat type.

COMAL SYSTEM—Fish will be sampled at three locations within Lake via SCUBA surveys. In particular, one of the SCUBA survey locations in Landa Lake will be in the same as the ongoing fountain darter belt transect survey. In addition, SCUBA surveys will be conducted within the Upper Spring Run, Old Channel, and New Channel sections of the Comal River. Two different SCUBA techniques will be used to document the fish within the three locations, mesohabitat and microhabitat surveys..

In addition to SCUBA surveys, three locations (Upper Spring Run, New Channel, and Old Channel) will be sampled via seines among wadeable habitats to evaluate and track fish populations in the Comal River. Multiple seine hauls will occur along a river transect perpendicular to the flow. Within each seine haul, fish will be identified, measured, examined for disease, and native fish returned to the river. Exotics will be removed from the system per scientific permit requirements. In addition to fish data, each seine haul will include habitat measurements (i.e. current velocity, water depth, substrate composition, in-stream coverage, climatic conditions, and mesohabitat type).

EAHCP Habitat Baseline and Disturbance Determination: This determination is intended to fulfill Section M 1a and 2a of the Incidental Take Permit (ITP).

DOCUMENT BASELINE HABITAT CONDITIONS—The contractor will use January 1 of the contract year GIS mapping, biomonitoring data and other existing sources to establish occupied habitat for the EAHCP Covered Species. Specific to Item M (la and 2a) of the ITP, only occupied habitat within the Comal and San Marcos springs/river ecosystems will be included.

DOCUMENT EAHCP MITIGATION AREAL EXTENT PER PROJECT—The contractor will work with staff and contractors from the City of New Braunfels, City of San Marcos and Texas State University, coordinating through EAA staff, to describe in GIS map form, representing a

snapshot in time on December 31 of the contract year, the areal extent of all direct EAHCP mitigation and restoration activities in the Comal and San Marcos springs systems.

If GIS files of the project/affected areas are unavailable, the contractor will either: l) map those areas directly with high grade GPS in real-time, or 2) use existing areal imagery to pinpoint and outline locations with subsequent, supplemental GPS ground truth mapping. The contractor will ensure that areas represented on all maps are representative of actual mitigation, not concept areas.

Assessment of Net Disturbance: The contractor will evaluate the baseline maps versus the EAHCP project maps and quantify the area of direct disturbance that may have potential effects from mitigation and restoration activities as described in Item M (la and 2a) of the ITP. The focus will be on quantifying the direct impacts (removal of non-native vegetation, etc.) via areal coverage of habitat, but will also describe potential indirect impacts (turbidity, etc.) qualitatively. This analysis will not extend beyond comparisons of areal coverage of occupied habitat.

Annual "Take" Estimate: The contractor shall estimate Take for each of the Covered Species utilizing the information generated by the BioMP, the information and guidance in Chapters 4 and 6 of the EAHCP, the Biological and Conference Opinion issued by USFWS, and any other relevant information. The purpose of this Take estimation is to ensure compliance with Section H of the ITP.

Critical Period Monitoring: The Critical Period Monitoring component will be performed on both systems and be based upon established flow trigger levels for each system. The type and extent of sampling conducted is dependent on the respective trigger level and is designed to be duplicative of full biomonitoring sampling and will include species-specific sampling based on the flow triggers.

HIGH/LOW FLOW MONITORING—The contractor will conduct high flow Critical Period Monitoring only after the following triggering criteria are met:

a) The daily average flow exceeds 385 cubic feet per second (cfs) in the San Marcos aquatic ecosystem or 500 cfs in the Comal aquatic ecosystem (total flow through the ecosystem as measured at the USGS gauging station located immediately downstream of the ecosystem); and
b) After conducting a joint visual inspection of the aquatic ecosystem with the contractor, EAA staff determines that high flow Critical Period Monitoring is warranted and approved.

Before high flow Critical Period Monitoring is conducted, the sampling parameters must be recommended by the contractor and pre-approved by EAA staff, based on professional judgment, and may include any parameter from the full biomonitoring sampling, with the exception of gill net sampling.

The Comal and San Marcos springs systems flow-based triggers are associated with specific sampling parameters.

SAN MARCOS SYSTEM SAMPLING—Low flow Critical Period Monitoring for the San Marcos River triggers at 120 cfs, with Texas wild-rice vulnerable stand monitoring as described in Task 3 of the Comprehensive Sampling Program. Monitoring will occur at 5 cfs declines or a maximum of once per week. The first Full Sampling Event is triggered at 100 cfs, with subsequent declining Full Sampling Events triggering at 85, 60, 25, and 10-0 cfs for a total of five declining Full Sampling Events. In addition, two recovery Full Sampling Events would be conducted as the system rebounds from the low flow period. Between Full Sampling Events, habitat evaluations, per every 5 cfs decline, would be conducted again not to exceed weekly monitoring.

COMAL SYSTEM SAMPLING— Low flow Critical Period Monitoring for the Comal River triggers at 200 cfs. This triggers the first Full Sampling Event with 4 subsequent Full Sampling Events being triggered at 150, 100, 50, and 10-0 cfs, respectively. Two recovery Full Sampling Events are scheduled as the flows rebound and stabilize from drought conditions. The Comal system also has habitat evaluations scheduled between Full Sampling Events; however, at 10 cfs increments again not to exceed weekly observation. An additional component for the Comal system is the detailed riffle beetle habitat evaluation and spring orifice condition documentation that is triggered at 120 cfs and continued at 10 cfs increments during decline.

A review of historic flow records indicates that the lower the flow, the lower the chance an even lower flow event will occur, thus reducing the chances of a complete decline and recovery as outlined above. Typically, both systems rebound from drought conditions due to a tropical depression rainfall event or some other weather pattern that produces a large amount of rainfall over the watershed. Flows typically come up rapidly and require a period of stabilization before the collection of biological data is meaningful.

Gill Net Evaluation: In addition to the full sampling activities, the contractor will conduct gill net evaluations in the immediate vicinity of the fountain darter SCUBA surveys in Spring Lake and Landa Lake. The Spring Lake evaluation will be triggered at 85 cfs and lower triggers. The Landa Lake assessment will be triggered at 100 cfs and lower triggers. The survey is designed to examine exotic fish concentrations and stomach content analyses with respect to predation of listed species. The number of each species (native and non-native) collected in the gill net and the data will be recorded and converted to catch per unit effort.

Water Quality Grab Sampling: The contractor will collect water quality grab samples at the established triggers at 18 stations longitudinally distributed in the San Marcos system and 12 stations longitudinally distributed in the Comal system. The samples will be from the surface, mid-depth and near bottom.

EAHCP Low Flow Sampling: To protect the Covered Species, Chapter 6 of the EAHCP contains specific flow requirements for both systems that trigger sampling events. This sampling is in addition to the Comprehensive Sampling and Critical Period Monitoring components and consists of an increased frequency of sampling for aquatic vegetation, Texas wild-rice mapping, as well as additional sampling of fountain darters, Comal Springs riffle beetles, and salamanders.

Cost estimate:
Table 7.1:
$400,000

Estimated 2022 cost:
$755,774*

*Includes Critical Period Monitoring if required

6.3.3 Ecological Modeling

Long-term Objective:
The development of a mechanistic ecological model (Ecomodel) is assigned to the Edwards Aquifer Authority per section 6.3.3 of the EAHCP. The purpose of the Ecomodel is to evaluate potential adverse effects to Covered Species and their critical habitat, and to the extent such effects are determined to occur, quantify their magnitude, and develop alternate strategies.

Target for 2022:
No Ecological Modeling work is anticipated in 2022.

Cost estimate:
Table 7.1:
$25,000

Estimated 2022 cost: *
$0

*There is no proposed budget for 2022.

6.3.4 Applied Research

Long-term Objective:
Applied research adds a valuable component to the EAHCP to better understand the ecological dynamics for all Covered Species.

Target for 2022:
Savings from past years will be applied to perform research to support a better understanding of existing Conservation Measures and address questions recommended to the Implementing Committee by the Springflow Habitat Protection Work Group as "First Priority for study" and "First Priority for developing monitoring plans for data collection during future low-flow periods". Work to address the questions in these priority groupings will be taken on by EAHCP staff in 2022. Recommendations from the Science Committee may be sought through this process particularly during development of environmental monitoring programs that assess species and their habitats during unique low-flow hydrological conditions as well as the analysis of the EAHCP QUAL2E water quality model's ability to predict water temperatures in the Comal River Old Channel during the low-flow period of 2014. Additional support addressing questions related to the impact of recreation may also be sought following an assessment of existing data in both the Comal and San Marcos systems. Additionally, Year 1 of a multi-year Comal Springs riffle beetle population study effort will commence. The population study is being conducted at the recommendation of the Comal Springs Riffle Beetle Work Group.

Cost estimate:
Table 7.1:
$0

Estimated 2022 cost:
$250,000

FMA § 2.2 EAHCP Program Management

Section 2.2 of the Funding and Management Agreement (FMA) assigns "general management and oversight" of the EAHCP to the Edwards Aquifer Authority (EAA). Section 5.6.5 of the FMA allows the EAA to use EAHCP funds for administrative costs and employee salaries, so long as all incurred costs and salaries are 100% related to "general management and oversight" of the EAHCP.

Long-term Objectives:
To manage and oversee day-to-day operations and administration, in coordination with the Applicants, of the EAHCP; resulting in a valid and continued Incidental Take Permit (ITP) from the USFWS for designated Covered Activities.

Program Activities in 2022:
EAHCP staff will continue to coordinate and monitor habitat protection measures completed by the City of New Braunfels and City of San Marcos/Texas State University in their respective 2022 Work Plans. The springflow and supporting measures are described in this 2022 EAA Work Plan.

The EAHCP Program Manager will execute duties as assigned in the FMA and:

- Manage EAHCP day-to-day activities;
- Facilitate program correspondence with the USFWS;
- Manage program activities in response to a 2028 ITP renewal including oversight of a contract for facilitated workshops;
- Serve on the ASR Advisory Committee;
- Facilitate the Adaptive Management Process (AMP) for all Routine and Nonroutine decisions; and
- Facilitate and coordinate all meetings of the EAHCP Implementing and Stakeholder committees and possible Subcommittees and Work Groups as created by the Implementing, Science and Stakeholder committees.

EAHCP Chief Science Officer and support staff will continue the following activities:

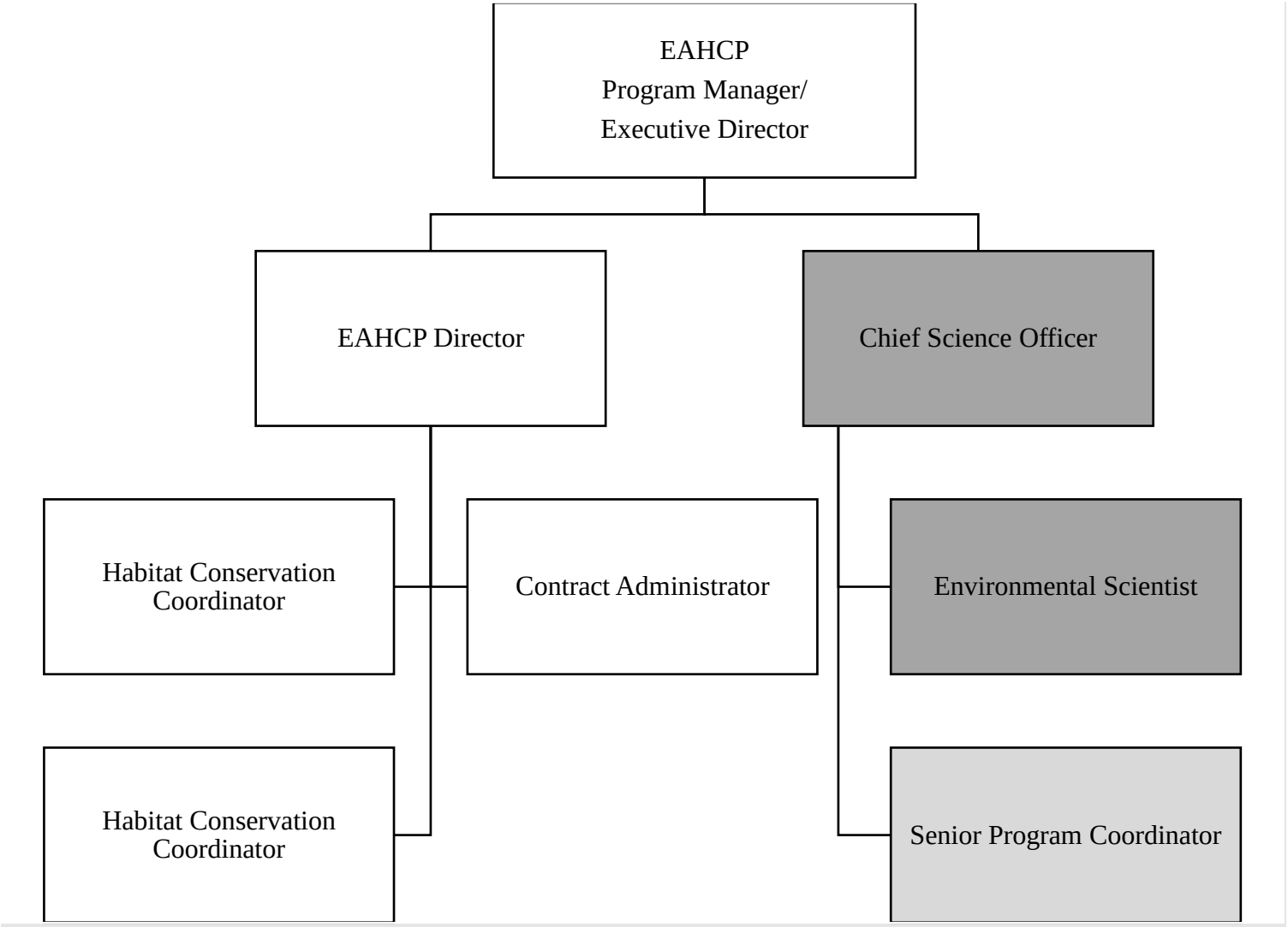
- Manage Refugia Work Plan activities including operations and research;
- Manage applied research;
- Manage biological monitoring;
- Manage and perform water quality monitoring;
- Update and maintain biological and water quality monitoring databases;
- Prepare for all meetings of the EAHCP Science Committee and EAHCP Implementing, and Stakeholder committees at the request of the Program Manager; and
- Prepare for all meetings of the Comal Springs Riffle Beetle Work Group, Research Work Group, and other possible Subcommittees and Work Groups as created by the Implementing, Science and Stakeholder committees at the request of the Program Manager.

EAHCP Administrative Staff will continue the following activities:

- Oversee the City of New Braunfels and San Marcos/Texas State University Work Plan activities;
- Coordinate 2022 Work Plan and funding application amendments for the EAA, City of New Braunfels, and San Marcos/Texas State University;
- Coordinate the development of 2023 Work Plans and funding applications for EAA, City of New Braunfels, and San Marcos/Texas State University;
- Process City of New Braunfels and San Marcos/Texas State University reimbursement's from EAA for habitat protection measures;
- Procure and execute contracts for support measures and program administration;
- Oversee EAA contract tracking and compliance;
- Process EAA contractor's invoices for support measures and program administration;
- Coordinate and prepare for all meetings of the EAHCP Implementing, Science, and Stakeholder committees, (and possible Subcommittees and Work Groups as created by the Implementing, Science and Stakeholder committees);
- Coordinate and prepare correspondence with all EAHCP Implementing, Science, and Stakeholder committee members and Work Groups members under the direction of the EAHCP Program Manager;
- Prepare materials for all AMP activities consistent with Article 7 of the FMA and under the direction of the EAHCP Program Manager;
- Support the EAHCP Program Manager in correspondence to the USFWS including informational memorandums, clarifications, and amendments to the ITP and EAHCP;
- Participate in public outreach initiatives;
- Coordinate and publish the monthly EAHCP Steward newsletter and podcast;
- Maintain the content of the EAHCP website;
- Prepare and compile all Permittees' information for the annual report to USFWS; and
- Track and assist EAHCP Permittees with maintaining compliance with secondary implementation permits, such as: U.S. Army Corps of Engineers, Texas Parks and Wildlife Department, Texas Commission on Environmental Quality, General Land Office, and Texas Historical Commission permits.

Staffing in 2022:
The EAHCP staff consists of the Program Manager, EAHCP Director, Contract Administrator, and two EAHCP Coordinators. EAA funds the Chief Science Officer and the Environmental Scientist staff positions. One position remains vacant but could be filled in 2022. The structure of the existing EAHCP staff positions and EAA-funded positions – **the Threatened and Endangered Species Team** - are illustrated in the chart on the next page.

Threatened and Endangered Species Team



Positions Paid from EAA General Budget
Vacant Position

Cost estimate:

EAHCP Program Management Costs for 2022

Description of Expense	Estimated 2022 Costs
Salaries and Fringe Benefits	$ 725,561
Office Supplies	$ 1,500
Non-Capital Assets	$ 6,000
Meeting Expenses	$ 20,000
Conferences, Seminars, and Training	$ 20,000
Memberships	$ 2,000
Printing	$ 8,000
Professional Contracted Services	
Annual Report	$ 55,000
Historical/Archeological Consultation	$ 42,000
Permit Oversight	$ 35,000
Outreach/Newsletter	$ 40,000
Science Committee Compensation	$ 20,000
Other	$ 327,000
Estimated 2022 Total	**$1,302,061**

Table 7.1:
$750,000

Estimated 2022 cost:
$1,302,061