Implementation of the Refugia Program under the Edwards Aquifer Habitat Conservation Plan

Annual Report 2017

Contract No. 16-822-HCP

U.S. Fish & Wildlife Service

San Marcos Aquatic Resources Center

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Acknowledgments

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Table of Contents

I. Executive Summary

On January 1, 2017 a contract (Contract # 16-822-HCP) between the Edwards Aquifer Authority (EAA) and the U.S. Fish and Wildlife Service (USFWS) was initiated for the operation and maintenance of a series of refugia for ten species endemic (covered species) to the Edwards Aquifer required by the Edwards Aquifer Habitat Conservation Plan (EAHCP) Section 5.1.1. The contract spans a performance period beginning January 1, 2017 and continues until March 31, 2028. This is the first annual report of the contract and covers the calendar year of 2017.

An overarching goal of this contract is to assist the Edward Aquifer Authority in compliance with its Incidental Take Permit and to meet its obligation within the Edwards Aquifer Habitat Conservation Plan. The EAHCP covers seven endangered species, one threatened species, and three species currently proposed for listing. Captive assurance populations of these species maintained in refugia at the San Marcos Aquatic Resources Center (SMARC) and Uvalde National Fish Hatchery (UNFH) will preserve the capacity for re-introduction at the Comal and San Marcos rivers in the event of the loss of population due to a catastrophic event. The idea 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.

Major objectives of the USFWS are to 1) provide fully functioning refugia for the EAHCP covered species; 2) conduct research as necessary to expand knowledge of the covered species; 3) develop and refine animal rearing methods and captive propagation techniques for the covered species; 4) reintroduce species populations, in the event of a loss of species in their native environment, and monitor recovery; and 5) attend meetings and give oral presentations to Science Committee, Implementing Committee, and EAA Board of Directors as requested by the EAHCP Program Manager.

Eight personnel (two supervisory Fish Biologists and six Biological Technicians) were hired by the USFWS for the purpose of implementing the refugia program. These biologists were assisted or supported by existing USFWS staff. The new positions were term positions, funded through the contract with the Edwards Aquifer Authority. The San Marcos Aquatic Resources

Center (SMARC) and the Uvalde National Fish Hatchery (UNFH) each have a supervisory biologist and three biological technicians dedicated to this contract. Under the management of the lead supervisory fish biologist at each facility, the technicians assist with the collection, daily upkeep, maintenance, propagation, and research efforts for the ten species.

Construction is planned or underway at both locations for contract purposes. These projects will create specific work areas for the USFWS to conduct the activities necessary to meet obligations under the EAA/USFWS contract. In San Marcos, Texas, a new building containing separate areas for refugia and quarantine is under construction and expected to be completed in 2018. In Uvalde, Texas, plans were completed for modifications of existing buildings will allow new, isolated areas for refugia and quarantine, and provide shade cover for outdoor raceways. Construction at UNFH has not yet started, but is estimated to be completed in 2018.

The first year of this contract was devoted to collecting individuals for addition to standing stocks of covered species, maintaining existing standing stock, creating captive propagation plans, and planning research needed to implement fully functional refugia. Field work or collections occurred every week in 2017, including 165 days (out of 250 possible work days) during 2017. Collections of each of the Covered species were made with further details of each species covered in the corresponding section of the report. New collection techniques were implemented for fish and invertebrate species. The organisms were maintained at SMARC and UNFH in existing systems and newly constructed systems, with modifications and updates fabricated by staff as needed. New staff were introduced and trained in species husbandry techniques specific to the facilities by existing staff, before taking full responsibility for the care and maintenance of their assigned species. Many of the revisions to the systems improved the survivorship and/or efficiency of species husbandry.

In 2017, two major research topics were addressed: 1) observation and documentation of juvenile development and maturation of Peck's cave amphipods; and 2) observation and documentation of larval development of Comal Springs dryopid beetles. Both projects were led by BIO-WEST, Inc. through a cooperative agreement with the USFWS. These studies are ongoing. In 2017, BIO-WEST began to document the instar development of Peck's cave amphipods and identify differentiation from congeners at a specific size class. Comal Springs dryopid beetles were paired and their holding containers were monitored for eggs and larvae to learn more about reproduction.

Documents produced for the Refugia program included monthly reports, development of Standard Operating Procedures, Captive Propagation Plans, Fish Health Reports, Engineering Diagrams, and research work plans (these can be found in the Appendices). Refugia program work plans were also created for 2017 and 2018. Staff also began drafts of a 10-year Research Plan for the life of the contract and a Reintroduction Strategy.

Approximately $1.6M was spent on refugia operations in 2017, largely construction and staff. Approximately $136K was spent on research. Approximately another $100K was spent on captive propagation and husbandry, species reintroduction planning, reporting, and meetings and presentations. The total expenditure of the Refugia Program was $1,882,678 in 2017.

II. Introduction

Background

The Edwards Aquifer Authority (EAA) supports and monitors the work of the U.S. Fish & Wildlife Service's (USFWS) operation and maintenance of a series of off-site refugia at the San Marcos Aquatic Resources Center (SMARC) and Uvalde National Fish Hatchery (UNFH) required in the Edwards Aquifer Habitat Conservation Plan (EAHCP) section 5.1.1. The activities reported herein are in support of the Federal Fish and Wildlife Incidental Take Permit (ITP; TE-6366A-1, Section K) and fulfillment of the Refugia Contract (Contract # 16-822-HCP) between the EAA and the USFWS as outlined within the 2017 EAHCP Refugia Work Plan. The contract was established to protect species left vulnerable to extirpation throughout a significant portion of their range due to a limited geographic distribution of the population (see Table 1 for list of the Covered Species). This series of refugia at SMARC, with back-up populations at UNFH, will preserve the capacity for these species to be re-established in the event of the loss of population due to a catastrophic event such as the unexpected loss of spring flow or a chemical spill. 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 are limited to the Covered Species in the EAHCP and those species that have significant impact on the Covered Species, such as predators, competitors, pathogens, parasites, food, cover, and shelter.

Table 1 Eleven species identified in the Edwards Aquifer Habitat Conservation Plan and listed for coverage under the Incidental Take Permit

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

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

The EAA/USFWS contract awards the Region 2 Fish and Aquatic Conservation Program (FAC) with $18,876,267 over a period of performance spanning January 1, 2017 until March 31, 2028. An overarching goal of this contract is to assist the EAA's compliance with its ITP and to meet its obligation within the EAHCP. As such the USFWS's FAC program will provide fully functioning Refugia operations for ten species listed in the EAA's ITP. The monetary support of the refugia augments the existing financial and physical resources of the two Service facilities, and provides supplementary resources to house and protect adequate populations of the Covered Species (Table 1). Support is also provided for research activities aimed at enhancing the maintenance, propagation, and genetic management of the Covered Species held in refugia, as well as salvage and restocking as necessary. The use of this support is limited to the Covered Species in the HCP. The tasks and subtasks that follow provide the details for the services performed in 2017.

Objectives

1) **Provide fully functioning refugia for the EAHCP covered species**
   USFWS will provide fully functioning Refugia operations for all of the Covered Species, except the San Marcos gambusia. Refugia operations will include, but are not limited to one main refugia facility located at the San Marcos Aquatic Resources Center, and one redundant (back-up) refugia facility located at the Uvalde National Fish Hatchery to include construction and/or rehabilitation of all facilities necessary to provide full refugia operations.
2) **Conduct research as necessary to expand knowledge of the covered species**
   USFWS will conduct research as necessary to expand knowledge of the Covered Species for the Refugia Program. At the direction of the EAA, USFWS will conduct research including but not limited to, species' physiology, environmental requirements, health and disease issues, life histories, genetics, and effective reintroduction techniques. Research identified within the plans will further refine refugia animal rearing methods and captive propagation techniques, provide monetary and logistical efficiencies, and enhance species survival and fitness.
3) **Develop and refine animal rearing methods and captive propagation techniques for the Covered Species**

4) **Reintroduce species populations, in the event of a loss of species in their native environment, and monitor recovery**
5) **Attend meetings and give oral presentations to Science Committee, Implementing Committee, and EAA Board of Directors as requested by the EAHCP Program Manager**

Personnel

The USFWS recruited two term position, supervisory Fish Biologists (GS-0482-011, FPL 12) (Table 2), Dr. Lindsay Campbell at the SMARC and Dr. Marco Pedulli at UNFH. These new positions, funded through the contract with the Edwards Aquifer Authority, provide supervision, mentorship, and training to lower-graded employees at their respective facility. They authorize purchases, oversee facility maintenance repairs, develop and implement budgets, and organize all activities that relate to the reimbursable agreement. The supervisors also manage and coordinate propagation, culture, and field activities related to species covered under the reimbursable agreement. They provide proper and efficient use of facilities and staff resources and work with the Center Director to ensure that contractual obligations are meet in a timely manner. In coordination with the Center Director they also prepare all of the written materials required for reporting. Likewise, they also prepare oral presentations to be used as briefing statements, outreach presentations, internal reports, work summaries, and present technical information at professional meetings. They communicate regularly with partners, USFWS personnel and other researchers to effectively meet Service and contract goals. The work they conduct involves resource management and affects the success and efficiency of SMARC's and UNFH's refugia programs and their acceptance by stakeholders. These positions are critical to the Edwards Aquifer Refugia program and will increase knowledge of the Covered Species' biology, life histories, culture techniques, and reintroduction requirements.

The USFWS also recruited six term position Biological Science Technicians (GS-0404-05, 06, FPL07), three for the SMARC and three for the UNFH (Table 2). These positions were new and are funded through the contract with the Edwards Aquifer Authority. Under the management of the lead supervisory fish biologist at each facility, the technicians assist with the collection, daily upkeep, maintenance, propagation, and research efforts for the ten species at the SMARC and UNFH. This includes maintaining experimental and culture production systems, keeping records along with entering, filing, and collating data. The technicians also generate basic summary statistics and graphic analyses of data and document program accomplishments through the composition of SOPs, reports, and manuscripts.

Table 2. USFWS Refugia Program Staff

San Marcos Aquatic Resources Center	
Lindsay Campbell, Ph.D.	San Marcos Program Supervisor
Amelia Everett, M.S.	Biotechnician
Kelsey Anderson, M.S.	Biotechnician
Linda Moon, B.S.	Biotechnician
Uvalde National Fish Hatchery	
Marco Pedulli, Ph.D.	Uvalde Program Supervisor
Makayla Blake, M.S.	Biotechnician
Rachel Wirick, B.S.	Biotechnician
Tyler Trempe, B.S.	Biotechnician

III. Building Construction

Construction is planned or underway at two locations. In San Marcos, Texas, a new building containing separate areas for refugia and quarantine is under construction. In Uvalde, Texas, modifications of existing buildings will allow new, isolated areas for refugia and quarantine, and provide shade cover for outdoor raceways. These projects will create specific work areas for the USFWS to conduct the activities necessary to meet our obligations under the EAA/USFWS contract.

San Marcos Aquatic Resources Center Refugia and Quarantine

This construction project is located at the San Marcos Aquatic Resources Center (SMARC) in Hays County, Texas. The project consists of Administrative Offices, Viewing area, Refugia area and Quarantine Area (See Appendix C). The Refugia and Quarantine areas are separated physically by a breezeway. Also included is a parking area and connection to utilities. All work is to be designed and constructed in accordance with all applicable building codes. Total magnitude for this project is between $850,000 to $1,250,000.00 which includes the base award and three added alternatives (#1 addition of 302 ft^2 of office space to the West side of the building, #2 addition of stone facing, and #3 addition of photovoltaic panels on the roof). The period of performance is 210 calendar days from issuance of Notice-To-Proceed (November 1, 2018).

On September 19, 2017, the USFWS awarded Puyenpa Services LLC a contract to build a Refugia and Quarantine building (Total 5,200 ft^2) that will be used as refugia for the Covered Species (Table 1). It will house 3,100 ft^2 of space for aquatic holding tanks and 900 ft^2 of office space. This construction project is scheduled for completion in 2018.

Ground breaking and mobilization for the new Refugia and Quarantine building began in earnest on November 02, 2017 when construction crews started the demolition of two existing ponds and supporting infrastructure. Given the blackland prairie soils and required excavation, as well as the proximity to existing utilities, a decision was made to place the two buildings over two old ponds that were in disrepair. This required less excavation and should yield some cost savings as construction continues.

Figure 1. Nov. 03, 2017 Crews in the process of demolishing pond kettles and center levee

Crews removed pond liner and concrete pond kettles and excavated to a depth of eight feet (Figure 1). Once at depth the site was leveled and compacted. Engineered fill material

(5,545 tons) was delivered it was spread, compacted and tested (Figure 2) in 6 to 8 inch increments. This process took 27 days to complete. Concurrent with site excavation and preparation crews began to locate existing utility lines and pipes (Figure 3).

Concrete crews are currently preparing the building sites for the concrete foundation. They are excavating for footers, grade beams, and framing forms. This phase of construction is scheduled to take 30 days.

Currently, the project is 18% complete. The next major phase of construction would include erection of pre-engineered metal building, metal fabrication, install of doors, windows, gutters, masonry work, rough in conduit and plumbing, install lightning protection, chain-link fence, HVAC, insulation, frame-out rooms, interior build out, and building finishes.

Figure 2. (Left) testing lab personnel using a nuclear densitometer to measure density and moisture content of testing area. (Right)Finished product

Figure 3. (Left) Utility crews using a hydro vacuum to locate existing lines. (Right) Once found, a backhoe is employed to expose area

Figure 4. (Left) Crew placing forms. (Right) Excavation for footers

Uvalde National Fish Hatchery Refugia and Quarantine

The construction project located at the Uvalde National Fish Hatchery will consist of modifications to existing buildings. Engineering and design for these modifications have been completed. All other internal documents have been prepared by the USFWS. We are currently in the pre-approval process, necessary before the USFWS can submit a formal request for proposals. Our goal is to have these modifications completed by the end of calendar year 2018.

Quarantine

Currently, the Uvalde quarantine area is temporarily established in a storage building at the facility. This storage building includes an approximately 1,200 ft^2 covered bay that will be modified to include a concrete foundation and enclosed walls for the new quarantine facility. An ADA compliant restroom will be incorporated in the new space. A second floor in this area will include approximately 500 ft^2 to hold chiller units and storage. The front of this new quarantine space will be enclosed with a new wall, which will include two roll-up doors (12 ft x 12 ft, and 10 ft by 12 ft). Additionally, a 500 ft^2 concrete apron will be added to the front of the modified building.

Refugia

An existing aquatic 5500 ft^2 tank house, adjacent to the administration building at Uvalde National Fish Hatchery, will be modified to include additional interior walls, isolating a new area (ca. 1,400 ft^2) for refugia. This is enough space for 20 refugia tanks (each with a footprint of ca. 2 ft x 8.5 ft). UNFH staff will continue to use the remaining area within the existing tank house for other USFWS responsibilities. Outside of the tank house, a second (ca. 350 ft^2) area, currently a covered porch, will be sealed with new walls and converted to a refugia area specifically for invertebrates.

Covered Species Analysis

Table 3 Number of organisms incorporated in the Refugia and total census at the end of December of Edwards Aquifer organisms taken to facilities for refugia by species and facility housed. Further details of these numbers can be found in supporting documents.

Species	Incorporated into Refugia SMARC	Incorporated into Refugia UNFH	SMARC Dec 31 census	UNFH Dec 31 census	SMARC Survival Rate	UNFH Survival Rate
Fountain darter-San Marcos *Etheostoma fonticola*	624[1]	435[1]	610	246	73% (83%)*	57%
Fountain darter-Comal *Etheostoma fonticola*	497[1]	72[1]	408	66	82%	92%
Comal Springs riffle beetle *Heterelmis comalensis*	412	169	191	51	32%	30%
Comal Springs dryopid beetle *Stygoparnus comalensis*	38	12	13	2	30%	17%
Peck's Cave amphipod *Stygobromus pecki*	220	154	173	45	54%	29%
Edwards Aquifer diving beetle *Haideoporus texanus*	6	0	0	0	0%	-
Texas troglobitic water slater *Lirceolus smithii*	440	0	25	0	6%	-
Texas blind salamander *Eurycea rathbuni*	50	0	47	0	78%	-
San Marcos salamander *Eurycea nana*	214	201	267	180	77%	90%
Comal Springs salamander *Eurycea* sp.	54	9	47	4	87%	44%
Texas wild rice plants *Zizania texana*	116	66	240	67	93%	100%

*[1]The number incorporated into the refugia is counted after the 30-day quarantine period. During this period fish are evaluated for health and suitability for inclusion into the refugia. *Survival rate not including supersaturation event.*

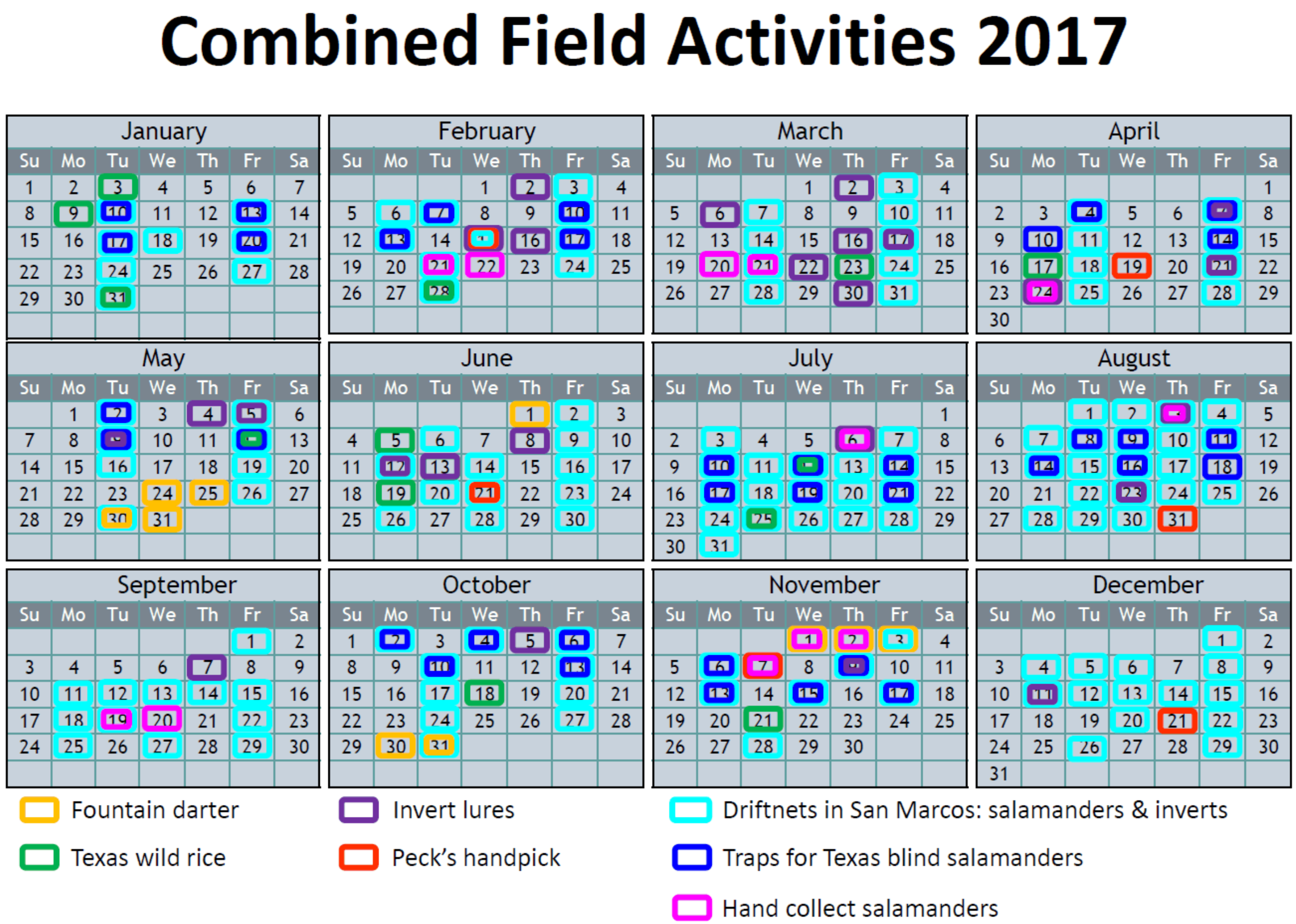

Figure 5. Species collections in 2017, USFWS staff conducted field collections on 165 days out of 250 work days.

Fountain darter

The goal for the fountain darter Standing Stock is to have 1000 wild stock fish per river (divided between the two facilities). The original aim for 2017 was to reach this standing stock goal by the end of the year. However a few complications prevented us from reaching this goal such as Largemouth bass virus (LMBV) in Comal fountain darters, space limitation, and unexplained mortality. Target goals in Table 4 include extra darters to ensure refugia Standing Stock does not fall below our target standing stock numbers.

Table 4 Fountain Darter Refugia Population Census

River		Census January 1, 2017	Incorporated 2017	Census December 31, 2017	Target Goal 2017	Percent Survival (%)
San Marcos	SMARC	245	624[1]	610	600	73% / 83%*
	UNFH	0	435[1]	246	600	57%
Comal	SMARC	107	497[1]	408	600	82%
	UNFH	0	72[1]	66	600	92%

*[1]The number incorporated into the refugia is counted after the 30-day quarantine period. During this period fish are evaluated for health and suitability for inclusion into the refugia. *Survival rate exclude losses from supersaturation event.*

Collections

We had two fountain darter collection events during 2017, a spring collection (May) and fall collection (October/November). Fountain darter collections were coordinated with USFWS Fish Health Unit so that live samples could be sent for analysis. Thus collections were specifically targeted events with dates scheduled over a year in advance. The majority of fountain darter collections were done by wading and using large dip-nets to pull through vegetation growing on the river bed. This year staff implemented an additional technique using divers to access deeper sections of the Comal River. These sections were 6-9 ft deep, thus putting them out of reach of wading individuals. After going through the bottom section with a large dip-net, a diver would bring up a net, its contents would be transferred to a waiting cooler filled with water, and then surface support personnel would sort through this cooler for fountain darters. This new technique was found to be efficient and will increase areas where fountain darters can be collected in the future. Collection dates were May 23-25, May 30-31, and October 30 – November 3, 2017.

Figure 6. Fountain darter collection in the San Marcos River.

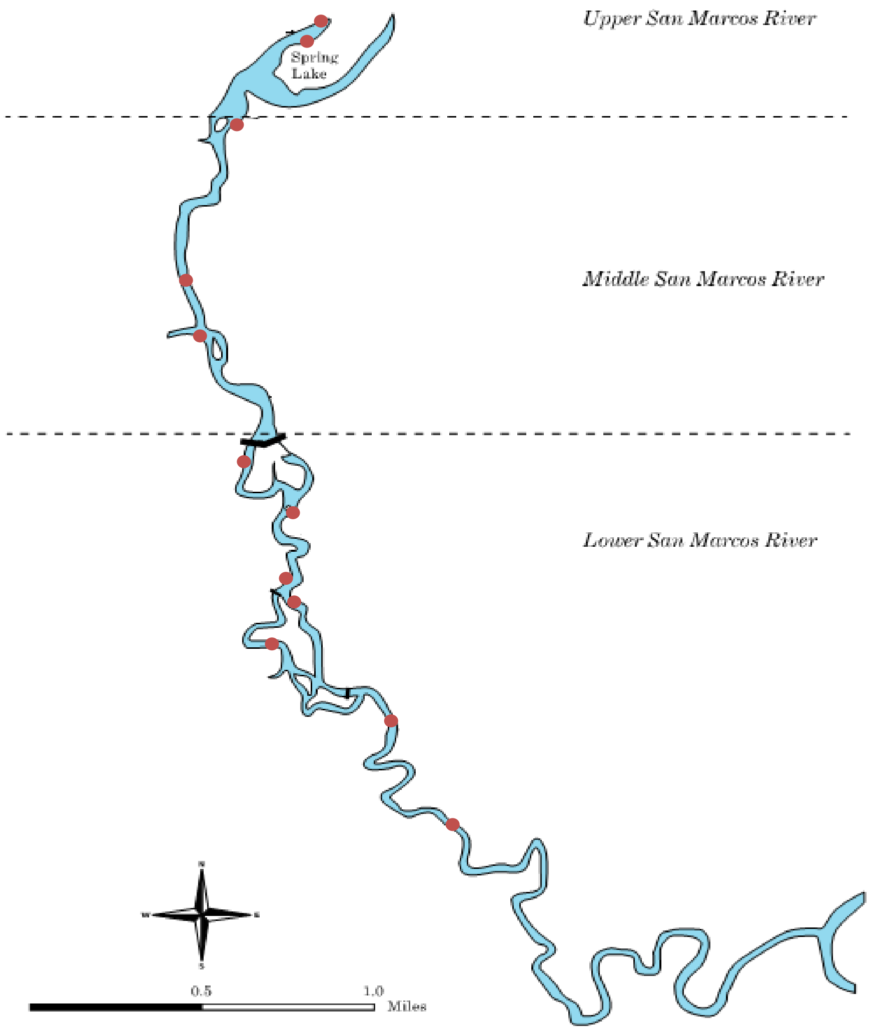

Figure 7. Collection locations indicated by red dots of San Marcos fountain darters in 2017

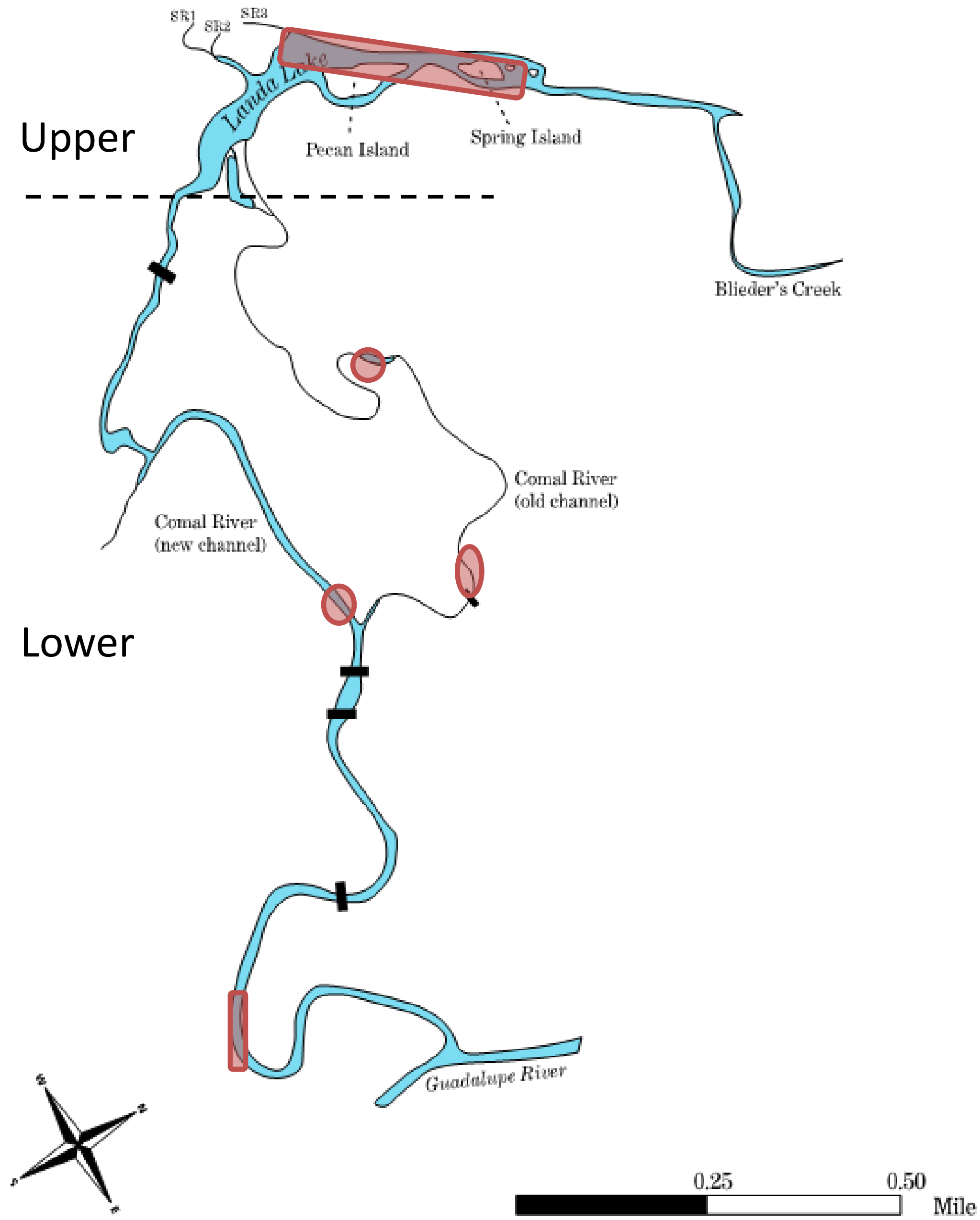

Figure 8. Collection areas indicated by red shading for Comal fountain darters in 2017

Quarantine procedures

Fountain darters were transported directly to the quarantine areas of the respective facilities after collection. The quarantine areas are separate, biologically secure areas away from the refugia systems, preventing the spread of disease and aquatic nuisance species. To minimize stress, temperature acclimation occurred at a rate of one degree per hour. Fish to be sent to Fish Health in Dexter, NM were removed before treatment and placed in their own isolated systems. A one hour formalin treatment was administered to the fountain darters, while still in their transport coolers, then fish were moved to quarantine aquaria for holding until they were cleared by Fish Health. Once they are known to be free of harmful pathogens or parasites, they were incorporated into the standing stock. Quarantine aquaria are flow-through chilled-well-water only systems.

Largemouth Bass Virus:

Previously, only Comal fountain darters in the upper Landa Lake section of the Comal River had tested positive for Largemouth Bass Virus (LMBV). Thus for the spring collection we limited Comal fountain darters to the lower portion of the Comal River. Testing of this group of fish found them also to be LMBV+. Based on observations and the opinion of our Fish Health veterinarian, it does not appear that LMBV has any direct negative effects on fountain darter rather that they are just carriers of the virus. In Largemouth bass the virus mainly targets the swim bladder, which fountain darters do not possess. LMBV has been found to be present in the Guadalupe watershed. In order to protect other fish species at the respective facilities and reduce the spread to fish that might be transported off the facilities to watersheds that do not have LMBV, all Comal fountain darters that are LMBV+ will be housed in quarantine facilities. Until the new building and renovations are completed this limits the number of Comal fountain darters that can be housed. At SMARC a small population of Comal fountain darters that are not LMBV+ are held outside of quarantine.

Survival rates:

A survival rate lower than historical values for San Marcos fountain darters at SMARC was due to a supersaturation event that occurred on August 31, 2017 throughout the station. On August 30, 2017, water pressure was lost simultaneously from both wells at SMARC. We believe the cause to be a faulty radio link connection between the SMARC and the two wells. Hierholzer Engineering, Inc. was called out to inspect the connection. Following the well failure and reconnection on August 30, water saturation was checked and in normal ranges at the close of business. However, a supersaturation event occurred over night (August 31st), causing complications and mortality to many organisms on station across buildings and programs. It is likely that while the wells were down, air entered the water lines. When the wells were restored, water was pumped into the lines, trapping air and pressure forcing it into solution. As this supersaturated water came out into the tanks, organisms developed various forms of gas bubble disease. Our standard operating procedure for well-loss and super-saturation events were reviewed and additional precautions were put in place.

Low survival rate of San Marcos darters at UNFH was due in part to a faulty drain screen that potentially allowed fish to be sucked into the recirculating intake system. This has been repaired.

A higher mortality rate was seen in newly collected fountain darters from the Comal River at both stations and with each collection. Many fish did not survive the quarantine month and, thus, were not added to refugia counts. Other groups had a slower but steady mortality rate. Fish Health was contacted for consultation, and samples sent for analysis. No conclusive results were found, but reports noted a noticeable degradation in the cartilage of the cranial areas of the fish. We still continue to investigate, and follow recommendations from Fish Health. Fish Health documents can be found in Appendix D.

Husbandry

Daily care:

A detailed description of fountain darter daily care can be found in the SMARC fountain darter SOP and culture manual, available upon request. Briefly: Fountain darters are kept in systems with partial flow-through and partial recirculated temperature-conditioned water. Aquaria are siphoned once a week to remove excess food and waste, unless otherwise needed. Fountain darters are fed, three times per week, a variety of live foods including small amphipods, zooplankton, and black worms. All tanks are checked daily for water flow, acceptable temperature, and mortalities. Small PVC structures are added to tanks to provide habitat and shelter. These structures are replaced weekly, checked for eggs, and cleaned.

Health Monitoring:

A subset (60) of newly collected fountain darters are sent to Fish Health in Dexter, NM for routine parasitology and health screening before the larger group of collected fish can be incorporated into the refugia. Each year representatives from Fish Health also visit each facility and take a sample of fountain darters (all fish on station are sampled) for detailed tests to assess the health of existing fish populations on a station.

Maintenance of Systems:

Systems were maintained throughout the year by routine cleaning procedures. Refugia systems are acid washed annually to remove calcium carbonate deposits that can affect functionality. Fish are removed from the systems during acid washing. Stand pipe screens and PVC habitat items are changed out and disinfected regularly. Pumps and chillers are tested before winter months to ensure they are performing adequately. Systems were modified with degassing measures to limit mortalities due to supersaturation events.

Captive Propagation

Captive offspring were produced throughout 2017 for both San Marcos and Comal fountain darters. PVC habitat items were placed in refugia tanks with rocks or porous sponge glued to the bottoms. After fountain darters laid eggs on the underside of the PVC, they were moved into smaller tanks for the eggs to develop and hatch. F1 generations are separated based on which river system their parents originated from. This production is opportunistic and is not controlled or directed by staff. Eggs are allowed to mature and hatch as space and need allows for each facility. A captive propagation plan is on file and available upon request for fountain darters.

Texas wild rice

Texas wild rice (TWR) is separated into alphabetical sections of the San Marcos River as defined by Texas Parks and Wildlife (Table 5). A historic population of TWR plants existed at UNFH before the start of the refugia program, however the exact provenance of these plants has been lost over the years and changeover of staffing. Due to the goal to preserve the genetic integrity of the TWR stands (see Wilson *et al* 2015), these plants will not be counted towards refugia goals. These plants will still be maintained incase documents are uncovered or future genetic analysis reveals identity of these plants (current census 107 historic plants).

Table 5. Texas Wild Rice Refugia Population Census

River section	Census January 1, 2017		Numbers collected during 2017		Census December 31, 2017		Percent survival (%)	
	SMARC	UNFH	SMARC	UNFH	SMARC	UNFH	SMARC	UNFH
A	12 / 0		10 / 11		21 / 11		95 / 100	
B	96 / 0		20 / 23		107 / 23		92 / 100	
C	32 / 0		11 / 10		41 / 10		95 / 100	
D	0 / 0		7 / 10		6 / 10		86 / 100	
E	7 / 0		-		5 / 0		71 / -	
F	15 / 0		10 / 13		25 / 13		100 / 100	
G	1 / 0		5		5 / 0		83 / -	
H	0 / 0		3		3 / 0		100 / -	
I	0 / 0		-		0 / 0		-	
J	9 / 0		-		8 / 8		89 / -	
K	2 / 0		-		2 / 0		100 / -	
Unknown	20 / -		-		20 / 0		100 / -	

Collections

Tiller collections in the San Marcos River occurred throughout the year for both facilities. Plant tiller collections are suspended during the summer months because heat stress negatively affects survivorship. A complete list of collections dates can be found in Figure 5. Collections of

TWR tillers are conducted with the help of USFWS SCUBA divers, who collect tillers by hand per plant stand. Tillers are placed in marked mesh bags and immersed in coolers filled with fresh river water for transport back to their respective facilities. During collection, the location of the TWR plants is recorded with a Global Positioning System (GPS) device. In addition, the percent coverage and river section is recorded and archived for each tiller collected. This information is collated in a central database maintained at the SMARC and UNFH. No plants were observed within the San Marcos River sections E, I, J, and K during 2017.

Figure 9. Texas wild rice tiller collection.

Quarantine:

Upon arrival at each respective facility, tillers (still grouped by individual plant) are rinsed in fresh well water and inspected for any aquatic nuisance species (ANS). Tillers are then briefly dipped in a 2% salt solution (for ANS) before being rinsed again. The salt solution has been shown to force snails to close their operculum and thus fall off of the leaves and stems. Tillers from a plant are then potted together in a tagged pot and placed in a quarantine raceway tank for 30 days. During this time, they are routinely checked for ANS specifically the invasive snail, *Melanoides tuberculata.* After 30 days, plants are un-potted and the full plant visually inspected for ANS, before the tillers are re-potted and incorporated into the standing stock population.

Survival rates:

Overall survival rate of Texas wild rice plants at SMARC was 94% for existing and newly collected plants combined and at UNFH it was 100% for newly collected plants.

Husbandry

Daily care:

A detailed description of TWR daily care can be found in the SMARC TWR SOP and culture manual, available upon request. Briefly: Water flow into the tanks was checked daily. Stand pipe screens were checked daily to ensure that no debris blocked water flow through the pump and chiller systems. Refugia tanks were cleaned of algae as needed. Filamentous algae was removed from the leaf blades and stem by gently running fingers or a six inch mesh net across the surfaces of each plant. Floating debris was removed manually using mesh nets or siphons as needed. Plant leaves were cut biweekly to prevent flowering and emergent vegetation. TWR plants were routinely trimmed to prevent sexual reproduction from emergent vegetation, so that the genetic integrity of each plant was maintained. As plants are housed very close together, it would be difficult to prevent cross pollination from plants in different river sections.

Maintenance of Systems:

Systems were maintained and improved throughout the 2017 year. Pumps were cleaned regularly of debris and baskets were acid washed to prevent calcium buildup. Five (one per tank) heat pumps (purchased from congressional allocations prior to the implementation of the Refugia contract) were installed on the TWR refugia tanks at SMARC in 2017. Pumps recirculate water from each tank to a heat pump unit and back to the tank. These units are designed to reduce water temperatures within the Texas wild rice standing stock tanks. Lastly, new PVC piping was installed to help increase the water movement across the surface of the plants within the tanks.

Texas wild rice seeds

The TWR seed bank was maintained and collected for during 2017. At the start of January 2017, 900 seeds were stored in the seed banks. During the year 5,049 seeds were collected from flowering TWR plants in the San Marcos River. Collections vary in amount throughout the year due to flowering conditions found in the wild population. After collection a group of 25 seeds were wrapped in a moist paper towel (to increase viability and induce germination) and placed in a re-sealable plastic bag. Each bag was labeled by seed collection location and date. Bags were stored in a refrigerator for up to six months before being rotated out of the seed bank. Seeds held longer than six months have been found to have low viability. After seeds reached the six month mark they were given to the SMARC botanist to be potted and grown for other purposes. At the end of 2017 2,776 were stored in the seed bank.

Captive Propagation

Currently TWR is maintained to discourage sexual reproduction in the refugia. An existing TWR Propagation manual outlining seed production exists and is available upon request.

Texas blind salamander

Collections

Texas blind salamanders were collected by traps and driftnets. Traps were deployed quarterly in Primer's Fissure, Johnson's Well, Rattlesnake Cave, and Rattlesnake Well. Traps were checked two to three times weekly for two weeks before being removed from the site. To avoid oversampling, only 1/3 of salamanders observed were collected from these sites. Blind salamanders that were released from trap sites were tail clipped starting in the fall of 2017 for future genetic analysis. Concurrently, salamanders were collected from driftnets at Sessom Creek, Texas State University Artesian Well, Diversion Springs, and Spring Lake Well Outflow. Driftnets were fished continuously and all animals were returned to the station given the assumption that any salamander leaving a spring orifice and entering a stream or lake environment will ultimately succumb to predation. Driftnets were checked two to five times per week (see Appendix C). The Diversion Springs net at Spring Lake was modified in June of 2017, and blind salamanders have been observed in higher numbers than previously documented, potentially due to this modification. The UNFH did not house or incorporate any Texas blind salamanders in 2017.

Table 6. Texas Blind Salamander Refugia Population Census

FACILITY	Census (1/1/2017)	Incorporated in 2017	Census (12/31/2017)	Survivorship %	Target Goal for 2017	Ultimate Goal
SMARC	10	50	47	78	36	250
UNFH	0	0	0	N/A	25	250

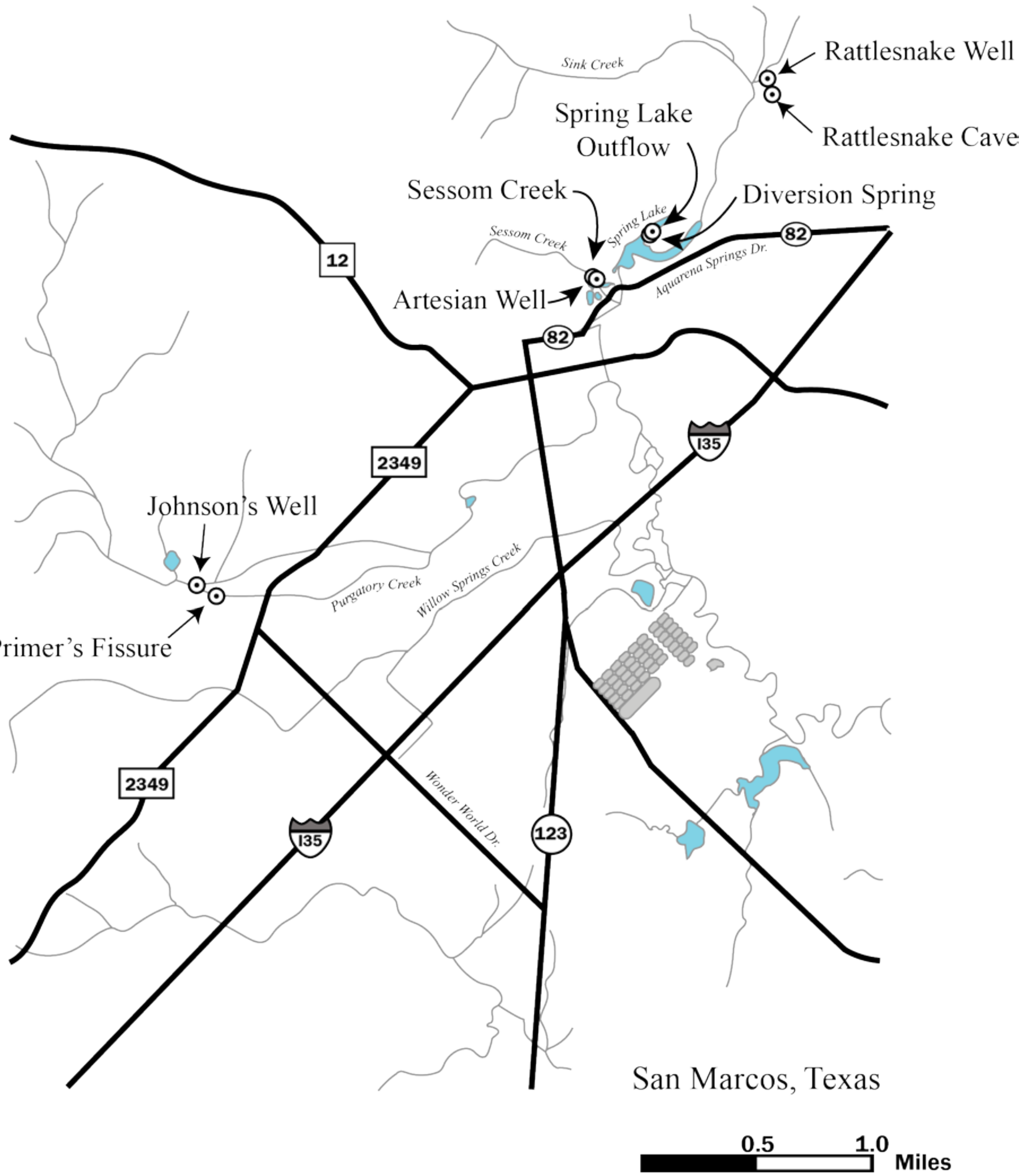

Figure 10 Map of Texas blind salamander collection locations.

Quarantine procedures:

Incoming salamanders were quarantined in a separate, biologically secure building from refugia systems to prevent the spread of disease and aquatic nuisance species. Animals housed in quarantine were maintained in flow-through well water only systems. All gear and equipment used for field sampling was disinfected using bleach or Virkon Aquatic between uses and between systems to prevent spread of disease and aquatic nuisance species.

Salamanders were acclimated to quarantine water conditions over the course of several hours after arrival. Healthy individuals were measured and those with a total length of 30 mm or greater were non-lethally cotton swabbed. Weak, injured, or very small individuals were not swabbed until they recovered and/or met the minimum standard size requirement, remaining in quarantine until samples could be safely obtained and processed. Skin swabs were sent to the Southwest Regional Fish Health Unit and tested for presence of *Batrachochytrium dendrobatidis* (Bd, commonly referred to as amphibian chytrid fungus) and *Batrachochytrium salamandrivorans* (Bsal). Texas blind salamanders in quarantine were maintained in glass aquaria according to their collection location, collection date, and size. Cannibalism has been observed in Texas blind salamanders in the past, so individuals were segregated by size. Individuals remained in quarantine for 30 days and until their swabs had been analyzed. After both of these conditions had been met, salamanders were incorporated into refugia.

Figure 11. Texas blind salamander collected from Diversion Spring

Survival rates:

Texas blind salamander survivability was similar (78%) in 2017 to the 20-year average (1996 to 2015, 73%). Survivorship of newly collected adults in 2017 was 91% and 65% for larval and young juvenile salamanders. Older juveniles and adults that have been incorporated into refugia have very high survival rates. Of the established refugia population only one death occurred during 2017. This individual was an adult male salamander that incurred injuries most likely due to aggression during courtship. Most mortalities occur in individuals that have recently arrived from the field. Two individuals captured from the Texas State Artesian Well (collected by Texas State staff and donated to the station according to their permits) developed mycosis with apparent fungal growth on their gills and body within a few days of capture. A third individual collected from Texas State Artesian Well developed fungal growth over much of the tail, requiring amputation of 50% of the tail. This animal was able to survive and recover. Larval and very young juvenile salamanders collected from Diversion Spring net account for much of the SMARC mortality rate. These individuals were very small and fragile and it is hard to assess what damage they might have received prior to arrival on station. However, survival rates of these small individuals is still 65%, so we do not see at this time a reason to discontinue bringing in these individuals. Our goal is to improve the survival rates of this size class during 2018.

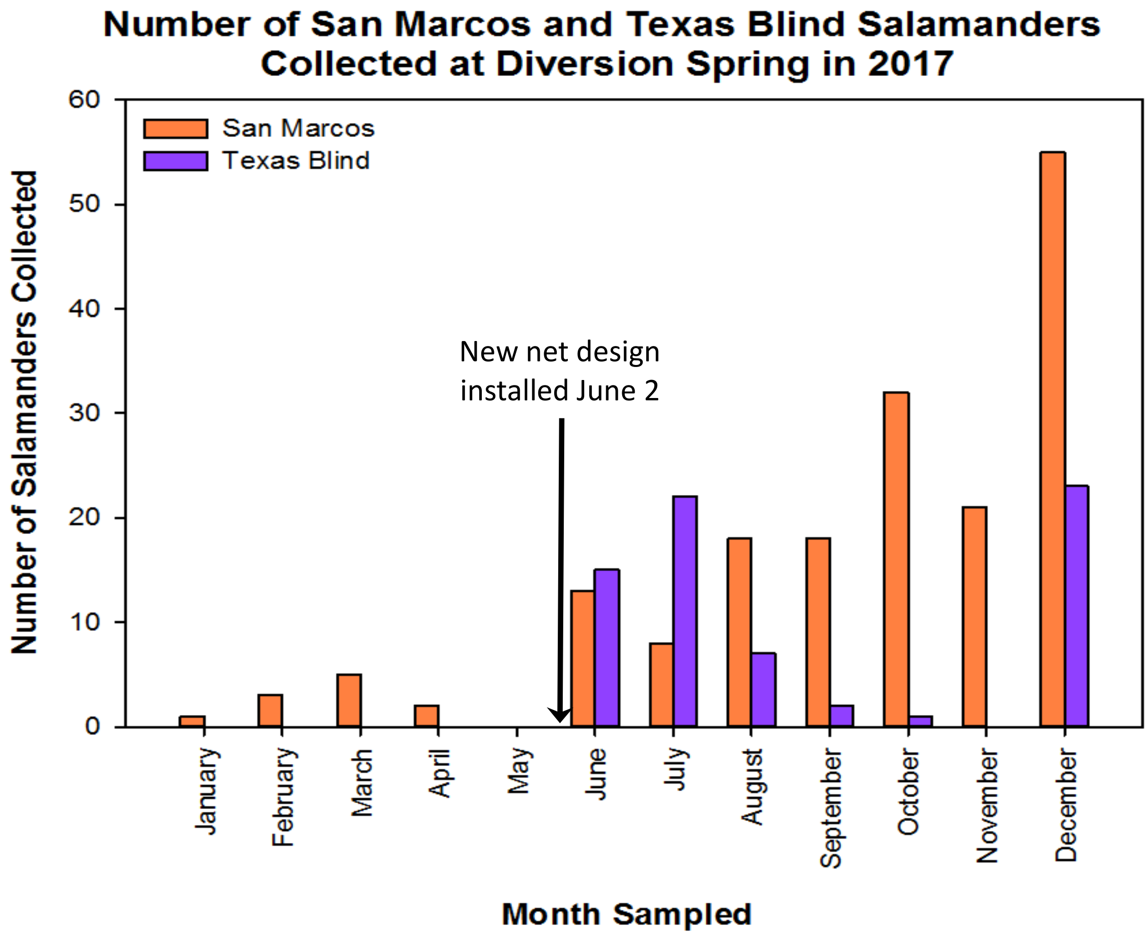

Figure 12 Total number of salamanders collected (retained and released) by month from Diversion Springs net. An uptake in collections is seen after a new net design was installed at the beginning of June.

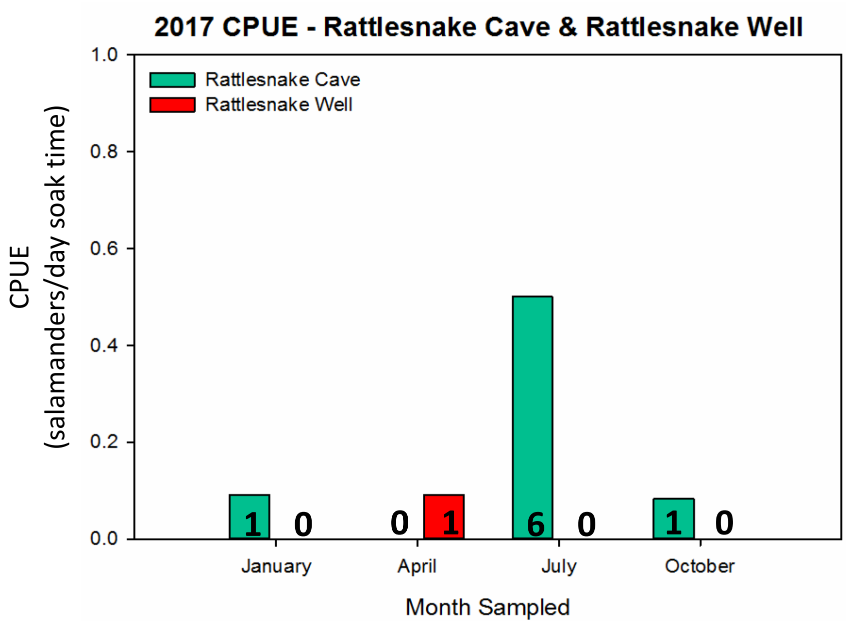

Figure 13 Catch per Unit Effort (salamanders per day soak time) of all observed Texas blind salamanders (retained, visual, and released) in Rattlesnake Cave and Well by month sampled.

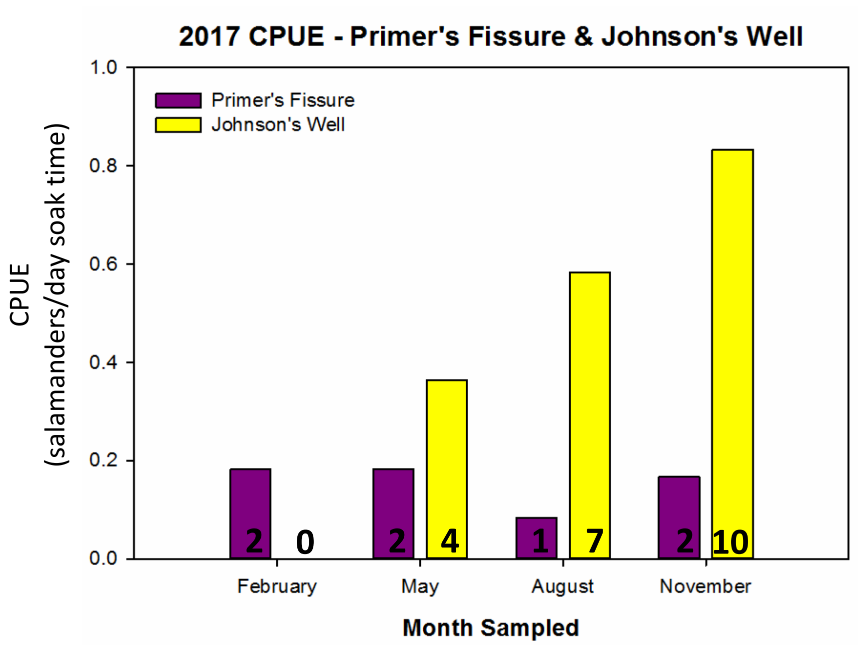

Figure 14 Catch per Unit Effort (salamanders per day soak time) of all observed Texas blind salamanders (retained, visual, and released) in Primer's Fissure and Johnson's Well by month sampled.

Husbandry

Daily care:

Texas blind salamanders are housed in large insulated fiberglass systems with well and recirculating water. Smaller individual tanks may be placed on these systems if needed. Each refugia tank system has its own water pump and heater/chiller unit. Temperature is set at 21 ºC (ranging between 18 - 22 ºC). Insulation is wrapped around or placed over tanks to help keep water temperature within the set range. Temperature is checked daily. Artificial habitat, including natural and artificial rock or plant, mesh, netting, etc., is placed throughout the tanks, especially in corners and along edges for salamanders to explore and seek refuge in. Habitat items are replaced periodically with new items and removed items were cleaned. Each tank system has its own equipment (nets, cleaning supplies) to prevent the potential spread of pathogens from system to system. If equipment must be shared it is cleaned and disinfected between systems.

Adult salamanders are fed twice weekly and receive either live amphipods or blackworms. Juveniles are fed artemia (brine shrimp) or chopped blackworms as they increase in size. Tanks are siphoned periodically to remove excess food and waste. Inventory is conducted on a routine basis in refugia systems. Water quality, including pH and dissolved oxygen, was checked once weekly. In addition, total dissolved gas is checked immediately if animals appear bloated or unable to stay submerged (trapped gas bubbled under skin). If gas saturation is deemed in excess, adjustments are made accordingly to lower the gas saturation within the system.

Health Monitoring:

As part of quarantine procedure, incoming animals were visually inspected for signs of injury or illness. Skin swabs collected from each individual were analyzed by the USFWS Dexter Fish Health Unit for presence of Bd and Bsal. Amphibian chytrid fungus (Bd) is present in the natural population of Texas blind salamanders, so those testing positive were not kept separate from those that tested negative. Systems were monitored daily for changes in appearance or behavior of individuals, including anorexia, bloating, lethargy, discoloration, development of external lesions or ulcers, mechanical damage, and abnormal swimming or walking. Salamanders that were sick or injured were removed from group housing and placed in isolated, individual hospital units that receive straight well water. Mortalities were preserved in ethanol or formalin and a veterinarian may be consulted for investigation into cause of death.

Maintenance of Systems:

Salamander refugia systems and their equipment must be acid-washed at least once annually to remove calcium carbonate deposits, which affects the efficiency of temperature-conditioning equipment, functionality of valves, and flow in the system. Water lines, hoses, valves, and restrictors are cleared, rebuilt, or replaced at this time. Valves and restrictors can become clogged over time and water lines solidify with hard water buildup. Salamanders must be relocated to different systems during acid cleaning.

Captive Propagation

Historically, Texas blind salamanders in refugia have been housed according to their origin location. After the disappearance (still under investigation) of Texas blind salamanders at SMARC this meant, for some sites, a just a few or even single individual being held by itself. To encourage production of offspring for future research, male and female salamanders tagged by location are currently housed in group systems. Before combining locations, all salamanders were tagged with Visible Implant Elastomer (VIE) tags to identify their origin location. Tagging was done under anesthesia to reduce handling stress. VIE tags were checked throughout the year for clarity and retention. So far, these tags have not reduced in visibility; but if this is found a tag will be replaced. Offspring produced during this combination can be identified by maternal origin--but not paternal, thus, these offspring would not be used at this time for restocking purposes. If future genetic analysis shows that collection locations are part of one panmictic population then these offspring would be able to be used should a restocking event occur. Two captive reproduction events occurred in 2017. In May 61 eggs were deposited by a wild stock Texas blind salamander from the Texas State Artesian Well. These eggs did not develop and were presumed not viable. A second clutch was laid in July by a different individual, also from the Texas State Artesian Well. In total, 32 eggs were deposited and ten hatched successfully. As of December 31st, 2017, five individuals from this clutch are being housed at the SMARC. UNFH did not house Texas blind salamanders during 2017. An updated Captive Propagation plan for the three covered *Eurycea* species was composed in 2017 and can be found in Appendix B.1.

San Marcos salamander

Collections

Salamanders were collected in March and September by hand using USFWS SCUBA divers, snorkel gear, and dip nets from Spring Lake and the upper San Marcos River. A driftnet on Diversion Spring in Spring Lake was fished continuously throughout the year. Periodic driftnet sampling of Spring Lake Well Outflow also occurred. All adult San Marcos salamanders from driftnets were taken back to SMARC quarantine. Initially, larval and juvenile salamanders were released in a shallow section of Spring Lake. However, the SMARC began retaining small individuals in the fall of 2017. The Diversion Spring net at Spring Lake was modified in June of 2017, and since then San Marcos salamanders have been observed in higher numbers than previously documented (see Figure 8 in Texas blind salamander section). Driftnets were checked two to five times per week (see Figure 5 for a comprehensive list of collection dates).

Table 7. San Marcos Salamander Inventory

FACILITY	Census (1/1/2017)	Incorporated in 2017	Census (12/31/2017)	Survivorship %	Target Goal for 2017	Ultimate Goal
SMARC	131	214	267	77%	250	250
UNFH	0	201	180	90%	250	250

Maps of collections

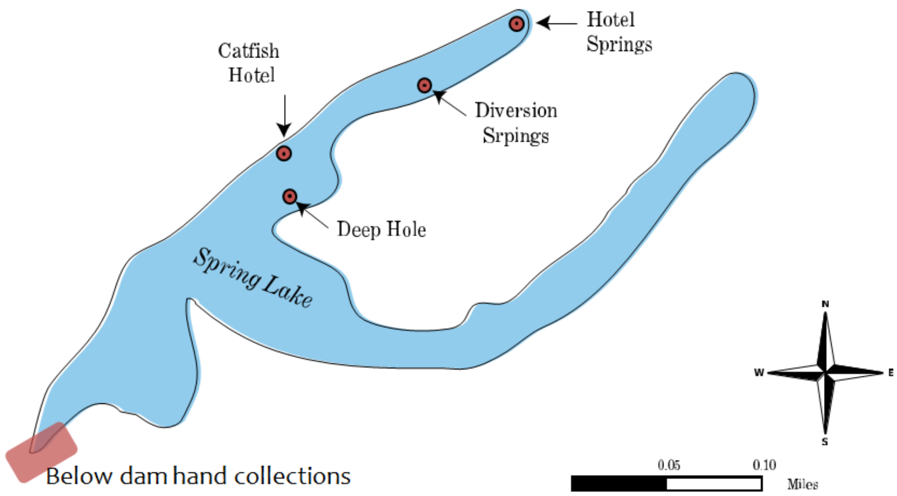

Figure 15 Collection locations of San Marcos salamanders during 2017.

Figure 16 Very small juvenile San Marcos salamander collected from Diversion Spring net

Quarantine procedures:

Incoming salamanders were quarantined in a separate, biologically secure building from refugia systems to prevent the spread of disease and aquatic nuisance species. Animals housed in quarantine were maintained in flow-through well water only systems. All gear and equipment used for field sampling was disinfected using bleach or Virkon Aquatic between uses and between systems to prevent spread of disease and aquatic nuisance species.

Salamanders were acclimated to quarantine water conditions over the course of several hours after arrival. San Marcos salamanders collected by SCUBA divers or by snorkelers were swabbed in the field before being transported back to their respective facilities. Healthy individuals collected from driftnets were measured and those with a total length of 30 mm or greater were non-lethally cotton swabbed. Weak, injured, or very small individuals were not swabbed until they had recovered and/or had met this minimum standard size requirement, remaining in quarantine until samples could be safely obtained and processed. Skin swabs were sent to the USFWS Southwest Regional Fish Health Unit and tested for presence of *Batrachochytrium dendrobatidis* (Bd, commonly referred to as amphibian chytrid fungus) and *Batrachochytrium salamandrivorans* (Bsal). San Marcos salamanders were housed in quarantine according to their collection date and size. Individuals remained in quarantine for 30 days and until their swabs had been analyzed. After both of these conditions had been met, salamanders were incorporated into refugia.

Survival rates:

Survival of San Marcos salamanders in 2017 at SMARC 77% and UNFH 90% were higher than the SMARC 20-year average (1996 to 2015, 73%). Salamander survivability was lower at SMARC due to non-adult mortalities. UNFH was only able to bring in hand collected adult individuals, and did not witness non-adult mortalities. Larval and young juveniles obtained through Diversion Spring driftnet collections had higher mortality rates than adults collected by hand; these mortalities account for 31% of all SMARC mortalities. SMARC also houses an existing population of San Marcos salamanders that are of unknown older age. Death due to complications of female egg rupture accounted for 34% of all SMARC mortalities. In the adult class, egg rupture accounted for 50% of mortalities. Females seem to be unable to release or reabsorb eggs naturally, causing them to bloat with fluid, eventually rupturing eggs and organs from the body cavity. A veterinarian is currently investigating the issue to determine potential cause and possible treatments.

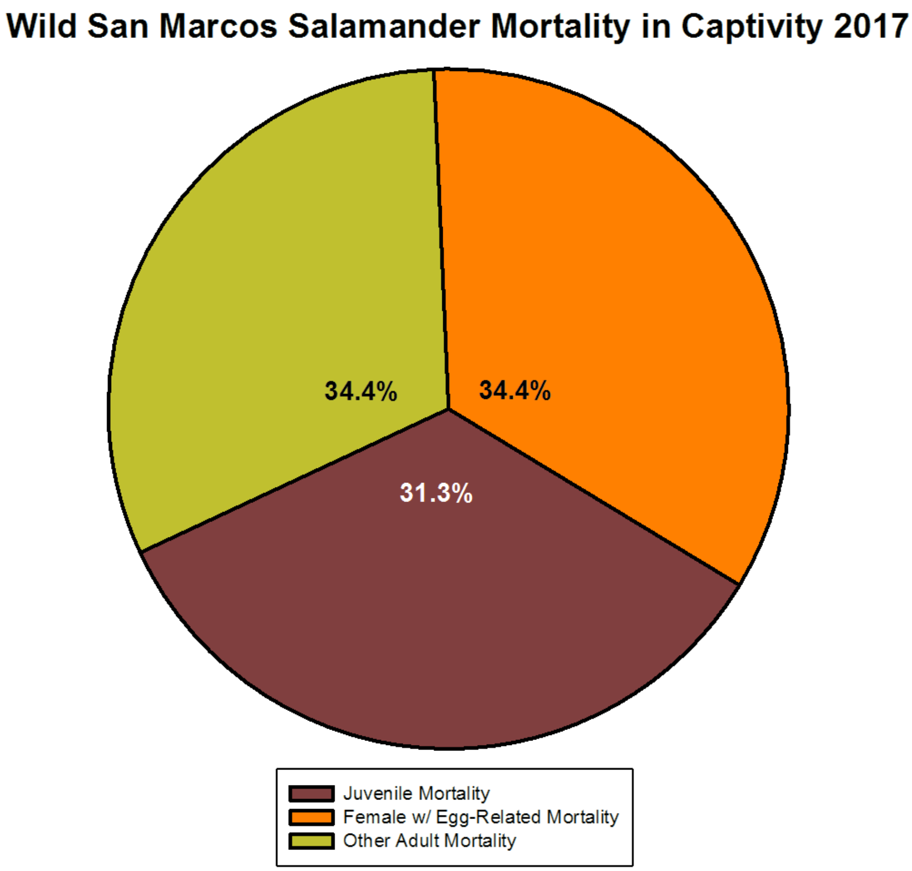

Figure 17 Mortality causes of SMARC San Marcos salamanders.

Husbandry

Daily care:

Genetic analysis (Lucas et al. 2009) determined that there is no population structure within this species between the sites sampled in the wild. After discussions with Austin Ecological Services Field Office, a decision was made by both SMARC and Austin ES staff to no longer hold San Marcos salamanders separately by collection site location; in January 2017 refugia populations were mixed and combined. San Marcos salamanders are housed in large insulated fiberglass systems with well and recirculating water. Smaller individual tanks may be placed on these systems if needed. Each refugia tank system runs on its own water pump and heater/chiller unit. Temperature is set at 21 ºC (ranging between 18 - 22 ºC). Insulation can be wrapped around or placed over tanks to help keep water temperature within the set range. Water temperature is checked daily. Artificial habitat, including natural and artificial rock or plant, mesh, netting, etc., is placed throughout the tanks, especially in corners and along edges for salamanders to explore and seek refuge in. Habitat items are replaced periodically with new items and removed items are cleaned. Each tank system has its own equipment (nets, cleaning supplies) to prevent the potential spread of pathogens from system to system. If equipment must be shared it is cleaned and disinfected between systems.

Adult salamanders are fed three times a week and receive either amphipods or blackworms. Juveniles are fed artemia (brine shrimp) or chopped blackworms as they increase in size. Tanks are siphoned periodically to remove excess food and waste. Inventory is conducted on a routine basis in refugia systems. Water quality, including pH and dissolved oxygen, is checked once weekly. In addition, total dissolved gas is check immediately if animals appear bloated or unable to stay submerged (trapped gas bubbles under skin). If gas saturation is deemed in excess, adjustment are made accordingly to lower the gas saturation within the system.

Health Monitoring:

As part of quarantine procedure, incoming animals were visually inspected for signs of injury or illness. Skin swabs collected from each individual were analyzed by the USFWS Dexter Fish Health Unit for presence of Bd and Bsal. Amphibian chytrid fungus (Bd) is present in the natural population of San Marcos salamanders, so those testing positive are not kept separate from those that tested negative. Systems were monitored daily for changes in appearance or behavior of individuals, including anorexia, bloating, lethargy, discoloration, development of external lesions or ulcers, mechanical damage, and abnormal swimming or walking. Salamanders that are sick or injured are removed from group housing and placed in isolated, individual hospital units that receive straight well water. Mortalities were preserved in ethanol or formalin and a veterinarian may be consulted for investigation into cause of death.

Maintenance of Systems:

Salamander refugia systems and their equipment must be acid-washed at least once annually to remove calcium carbonate deposits, which affects the efficiency of temperature-conditioning equipment, functionality of valves, and flow in the system. Water lines, hoses, valves, and restrictors should be cleared, rebuilt, or replaced at this time. Valves and restrictors can become clogged over time and water lines solidify with hard water buildup. Salamanders must be relocated to different systems during acid cleaning.

Captive Propagation

San Marcos salamander males and females were not separated in 2017 to encourage reproduction in refugia systems. Reproduction can occur year-round as females come in and out of gravidity. In 2017, two clutches were produced from wild stock parents. A small clutch of eight eggs was deposited in July and four hatched successfully. A second clutch of 50 was deposited in September 2017, but eggs did not develop and were deemed non-viable. Wild San Marcos salamanders did not produce any eggs in 2017 at UNFH. An updated Captive Propagation plan for the three covered *Eurycea* species was composed in 2017 and can be found in Appendix B.1.

Comal Springs salamander

Collections

Comal Springs salamanders were collected in February and November by hand using snorkel gear and dipnets from Spring Runs 1 and 3 at Landa Park and Spring Island in New Braunfels, Texas. Adult salamanders have been observed in close association to spring openings and near invertebrate lure locations. Two juvenile salamanders were captured during Fountain Darter collection when sorting through large mats of aquatic vegetation.

Table 8. Comal Springs Salamander Refugia Population Census

FACILITY	Census (1/1/2017)	Incorporated in 2017	Census (12/31/2017)	Survivorship %	Target Goal for 2017	Ultimate Goal
SMARC	0	54	47	87%	50	250
UNFH	0	9	4	44%	50	250

Quarantine procedures:

Incoming salamanders were quarantined in a separate, biologically secure building from refugia systems to prevent the spread of disease and aquatic nuisance species. Animals housed in quarantine were maintained in flow-through well water only systems. All gear and equipment used for field sampling was disinfected using bleach or Virkon Aquatic between uses and between systems to prevent spread of disease and aquatic nuisance species.

Salamanders were acclimated to quarantine water conditions over the course of several hours after arrival. Comal Spring salamanders were swabbed in the field before being transported back to their respective facilities. Skin swabs were sent to the USFWS Dexter Fish Health Unit and tested for presence of *Batrachochytrium dendrobatidis* (Bd, commonly referred to as amphibian chytrid fungus) and *Batrachochytrium salamandrivorans* (Bsal). Comal Springs salamanders were housed in quarantine according to their collection date and size. Tanks must have well secured lids as Comal Springs salamanders were found to climb out of their tanks, even up dry tank walls, and upside down across partial tank lids (this is different behavior noted than the other two covered salamander species housed). Individuals remained in quarantine for 30 days and until their swabs have been analyzed. After both of these conditions have been met, salamanders were incorporated into refugia.

Figure 18 Comal Springs salamander in a quarantine tank

Survival rates:

The large discrepancy in survival rates of Comal spring salamanders between the two facilities (SMARC 87%, UNFH 44%) was due in a large part to the low numbers to begin with at UNFH, thus mathematically each individual counts more. The majority of mortalities can be attributed to the fact that Comal Spring salamanders are escape artists. Comal spring salamanders have been observed crawling up the dry walls or mesh dividers in their systems, crossing over into other sections, or have exited a tank completely. While San Marcos salamanders do climb their systems somewhat, it is not to the extent seen in Comal spring salamanders and only where a water trail can be found. To remedy these escapes, Comal spring salamander quarantine tanks at the SMARC have thick, heavy lids secured to aquaria. Drain standpipes are covered by mesh screens and redundantly secured. In refugia tank systems with recirculating water, intake openings in a separate section of the tank are covered by cage-like structures of small mesh and wire to keep salamanders out. Water levels are lower in Comal spring salamander systems. Water lines point towards the middle of the systems and do not splash against the sides. Further modifications to tanks will be investigated in 2018 to prevent salamander escape. The Comal spring salamander behaves quite differently from the San Marcos salamander. Rather than hiding, this species can be observed exploring its tank system even during the daytime.

Husbandry

Daily care:

Comal Springs salamanders are housed in large insulated fiberglass systems with well and recirculating water. Smaller individual tanks may be placed on these systems if needed. Each refugia tank system run on its own water pump and heater/chiller unit. Temperature is set at 21 ºC (ranging between 18 - 22 ºC). Insulation can be wrapped around or placed over tanks to keep water temperature in the correct range. Water temperature is checked daily. Artificial habitat, including natural and artificial rock or plant, mesh, netting, etc., is placed throughout the tanks, especially in corners and along edges for salamanders to explore and seek refuge in. Habitat items are replaced periodically with new items and removed items are cleaned. Extra precautions are taken with Comal Springs salamander tanks to prevent escape, see Survival Rates section above. Each tank system has its own equipment (nets, cleaning supplies) to prevent the potential spread of pathogens from system to system. If equipment must be shared it is cleaned and disinfected between systems.

Adult salamanders are fed three times a week and receive either amphipods or blackworms. Tanks are siphoned periodically to remove excess food and waste. Inventory is conducted on a routine basis in refugia systems. Water quality, including pH and dissolved oxygen, is checked once weekly. In addition, total dissolved gas is check immediately if animals appear bloated or unable to stay submerged (trapped gas bubbles under skin). If gas saturation is deemed in excess, adjustments are made accordingly to lower the gas saturation within the system.

Health Monitoring:

As part of quarantine procedure, incoming animals were inspected for signs of injury or illness before being integrated into refugia systems. Skin swabs collected from each individual were analyzed by the USFWS Southwest Regional Fish Health Unit for presence of Bd and Bsal.

Amphibian chytrid fungus (Bd) is present in the natural population of Comal Springs salamanders, so those testing positive were not kept separated from those that tested negative. Systems were monitored daily for changes in appearance or behavior of individuals, including anorexia, bloating, lethargy, discoloration, development of external lesions or ulcers, mechanical damage, and abnormal swimming or walking. Salamanders that are sick or injured were removed from group housing and placed in isolated, individual hospital units that receive straight well water. Mortalities were preserved in ethanol or formalin and a veterinarian may be consulted for investigation into cause of death.

Maintenance of Systems:

Salamander refugia systems and their equipment must be acid-washed at least once annually to remove calcium carbonate deposits, which affects the efficiency of temperature-conditioning equipment, functionality of valves, and flow in the system. Water lines, hoses, valves, and restrictors should be cleared, rebuilt, or replaced at this time. Valves and restrictors can become clogged over time and water lines solidify with hard water buildup. Salamanders must be relocated to different systems during acid cleaning.

Captive Propagation

Male and female Comal spring salamanders were not segregated in refugia systems to encourage reproduction. Reproduction can occur year-round as females come in and out of gravidity. No offspring were produced at the SMARC or UNFH in 2017. An updated Captive Propagation plan for the three covered *Eurycea* species was composed in 2017 and can be found in Appendix B.1.

Comal Springs riffle beetle

Collections

The Comal Springs riffle beetles (CSRB) were collected using poly-cotton cloth lures and wooden dowel lures. Riffle beetles were also hand collected off conditioned wood in the vicinity of upwellings at Comal Springs, New Braunfels, Texas. The wooden lures were made from poplar dowels, cut in half length-wise and sanded. Cotton lures followed the standard protocol for cotton lures in the Comal River system. Only 25% of the adult CSRB found on each set location (lure and dowel numbers combined) were kept for refugia. The remaining adults and other non-target species were carefully returned to the lures original spring location. Starting in May and June lure collections for targeted invertebrates were combined to create efficiencies in time spent in the field and reduce redundancy in number of lures set. A complete list of collection dates can be found in Figure 5.

Table 9. Comal Springs riffle beetle Refugia Population Census

<table>
<tr><th></th><th>Census Jan. 1 2017</th><th>Incorporated 2017</th><th>Number Larva Released</th><th>Number Adults Released</th><th>Census Dec. 31 2017</th><th>Survival (%)</th><th>Refugia goal for 2017</th></tr>
<tr><td>SMARC</td><td>183</td><td>412</td><td rowspan="2">976</td><td rowspan="2">1997</td><td>191</td><td>32%</td><td>250</td></tr>
<tr><td>UNFH</td><td>0</td><td>169</td><td>51</td><td>30%</td><td>250</td></tr>
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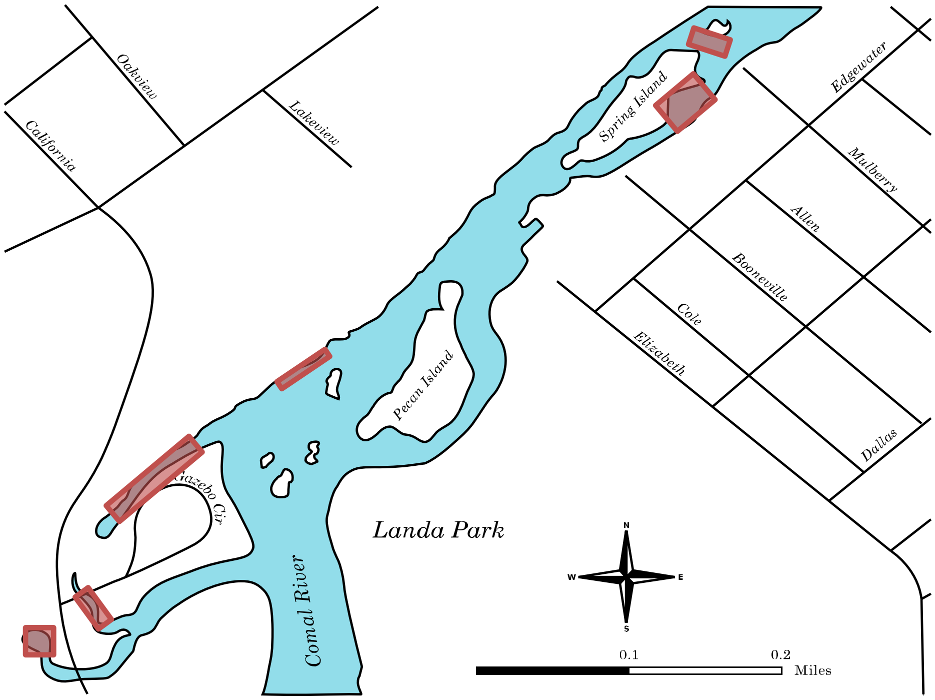

Figure 19 Collection areas for invertebrate organisms in the Comal River system, including Spring Runs 1-3, Western shoreline, and areas around Spring Island.

Figure 20 Collecting invertebrate lures in Spring Run 1 (photo Bob Hall)

Quarantine:

Incoming CSRB were quarantined in separate containers than existing refugia stock in the Invertebrate Room at SMARC and the quarantine room at UNFH. CSRB were acclimated to quarantine water conditions with the water temperature adjusted 1 °C every half-hour. CSRB were maintained by collection location: Spring Runs (Spring Runs 1, 2, and 3) and Landa Lake (Western Shoreline and Spring Island). Organisms were kept in containers marked "Quarantine" for 30 days before joining containers of Refugia population. During this time we observed for other potential ANS species that might have come in with the collection, the general health of the organisms, and watched for large die-offs that might indicate a disease. If none of these occurred then they were moved to the Refugia population at the end of the quarantine period.

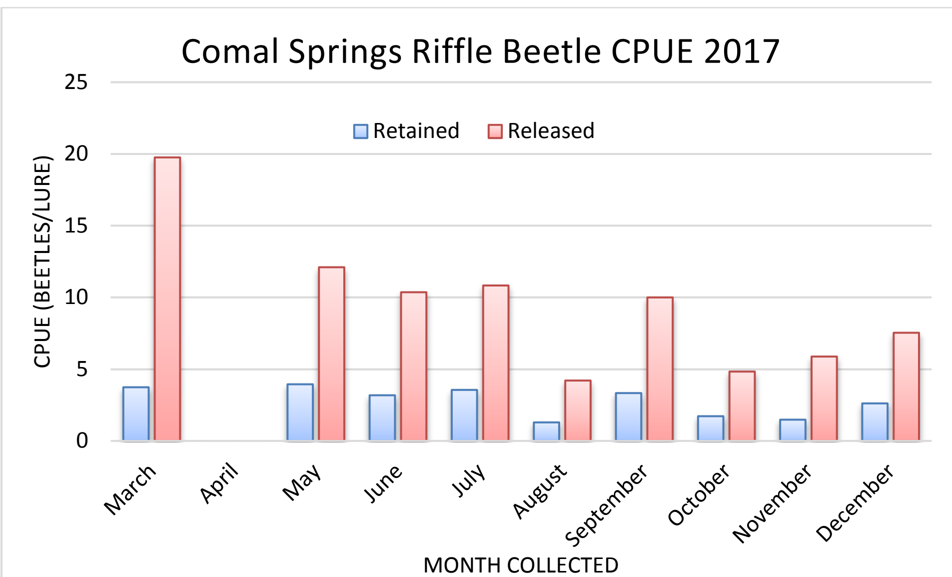

Figure 21 Catch per unit effort of Comal Springs Riffle Beetle collections (retained and released) by month during 2017. Unit of effort is defined as the number of lures checked that month.

Survival rates:

Due to the small size, invertebrates are inherently difficult to assess for mortality on a day-to-day basis. Mortalities were therefore calculated numerically when an inventory was conducted. The number of missing beetles equates to the number of mortalities for that time period. We are working to refine husbandry techniques and holding containers for CSRB to increase survival rates in future years. Survival rates might have been decreased at SMARC due to the supersaturation event. Survival rates at UNFH might have been decreased by higher water temperature ranges. Only adult CSRB were retained from the field and the age of these adults are unknown. The average life span of a CSRB is only a year, thus higher mortality rates are to be expected of this species.

Husbandry

Daily Care:

On a daily basis, all the systems were checked for adequate flow. The drain screens were cleared of debris to maintain drainage and water level. Temperature was measured daily and recorded. As the CSRB feed predominantly on biofilm, they do not have a traditional feeding schedule. Alternatively, we placed leaves and cotton cloth containing biofilm that provides them with enough food between inventories. Inventories were conducted on a two month schedule.

Maintenance of systems:

Culture containers currently used to house CSRB are plastic with PVC piping that delivers water in a manner that mimic up-wellings. The systems do not have a traditional cleaning or siphoning schedule, but alternatively, were cleaned whenever the container was disturbed, such as during the inventory. At this time, water lines, hoses, and valves were checked for functionality and cleaned or replaced as needed. CSRB refugia containers were not siphoned for debris as CSRB, larvae, or eggs could easily be discarded along with debris.

During 2017, how rocks, leaves, and cotton cloth were layered in the container was changed to facilitate water flow in the container and reduce anoxic zones. Rocks were changed to smooth river rocks, with pea gravel glued on the bottom to make feet and elevate the rocks off the surface of the container and each other. Leaves and cloths were only placed between two layers of rocks and no longer placed the bottom of the container. Culture containers were kept dark to mimic the underground environment. This was accomplished by partially covering containers with black plastic or shade control cloth. For smaller numbers of CSRB vertical flow-through tubes can be utilized instead of large container boxes. These consist of clear PVC that makes up the viewing chamber, and threaded PVC couplings and reducers. The PVC tubes contain leaves, biofilm cloth, and mesh for structure and habitat.

Captive Propagation

To encourage production of offspring, male and female wild stock were housed together. During inventories, larvae that were found were placed into a separate container. SMARC produced 5,433 F1 larvae and 29 F1s pupated to adults during 2017. A preliminary Comal Springs riffle beetle Captive Propagation plan was drafted this year (see Appendix B.2); this plan will be updated as new information is acquired about the CSRB life history.

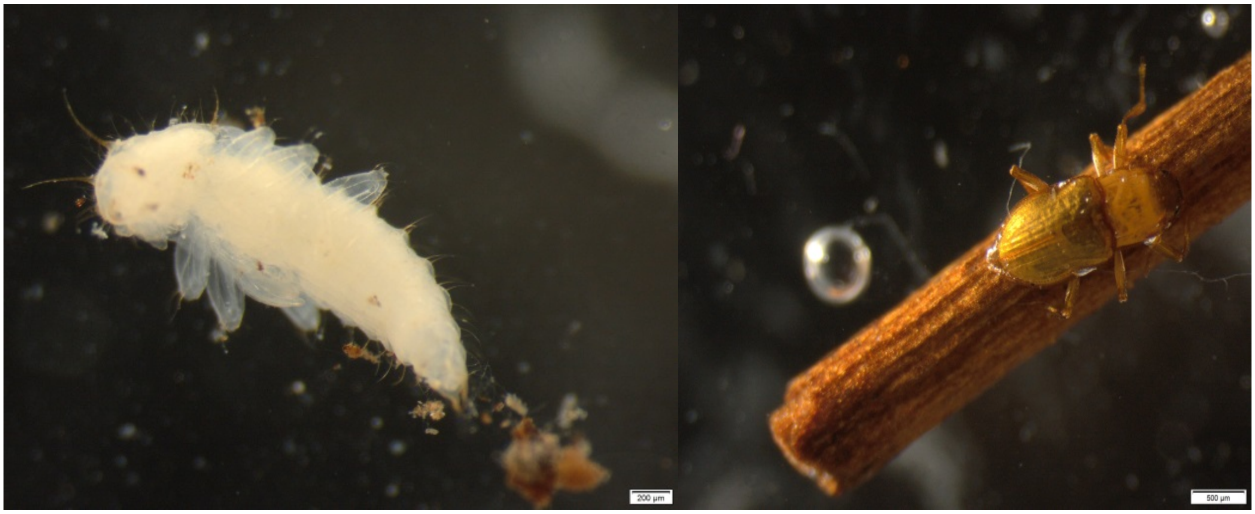

Figure 22 Comal Springs riffle beetle pupa (left) and a teneral adult just emerged from pupation

Comal Springs dryopid beetle

Collections

The Comal Springs dryopid beetles (CSDB) were collected using poly-cotton cloth lures and wooden dowel lures set in upwellings and terrestrial margins of the Comal Springs system, New Braunfels, Texas. CSDB adults were also collected by hand picking directly from submerged wood by biologists from SMARC and UNFH around Spring Island in Landa Lake. Wooden dowels were added to the cotton lures in 2017 since most CSDB were found on submerged woody debris; dowel success rate will continue to be evaluated in 2018. Starting in May and June lure collections for targeted invertebrates were combined to create efficiencies in time spent in the field and reduce redundancy in number of lures set. A complete list of collection dates can be found in Figure 5. See Figure 12 in Comal Springs riffle beetle section for a map of invertebrate collection locations in Comal River.

Table 10. Comal Springs Dryopid Beetle Refugia Population Census

	Census Jan. 1 2017	Incorporated 2017	Number Larva Released	Number Adults Released	Census Dec. 31 2017	Survival (%)	Refugia goal for 2017
SMARC	6	38	0	0	13	30%	*
UNFH	0	12			2	17%	*

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

Quarantine:

Incoming CSDB were quarantined in separate containers than existing refugia stock in the Invertebrate room at SMARC and the quarantine room at UNFH. CSDB were acclimated to quarantine water conditions with the water temperature adjusted 1 °C every half-hour. Organisms were kept in containers marked “Quarantine” for 30 days before joining containers of Refugia population. During this time we observed for other potential ANS species that might have come in with the collection, the general health of the organisms, and watched for large die-offs that might indicate a disease. If none of these occurred then they were moved to the Refugia population at the end of the quarantine period.

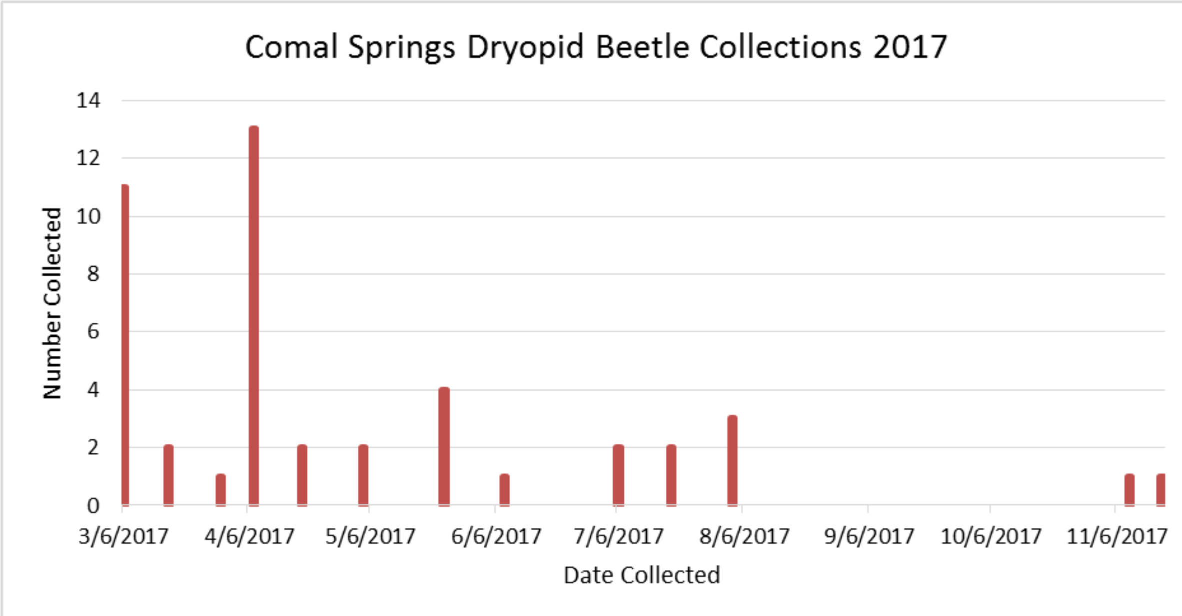

Figure 23 Comal Springs Dryopid Beetle collections during 2017.

Survival

Due to the small size, invertebrates are inherently difficult to assess for mortality on a day-to-day basis. Mortalities were only documented during inventories or when the mortalities could be seen and removed. Mortalities were therefore calculated numerically when an inventory was conducted. The number of missing beetles equates to the number of mortalities for that time period. We are working to refine husbandry techniques and holding containers for CSDB to increase survival rates in future years.

Husbandry

Daily Care:

On a daily basis, all the systems were checked for adequate flow. The drain screens were kept clear of debris to maintain drainage and water level. Temperature was measured daily and recorded. Conditioned wooden dowels in the containers were checked for fungal growth, and if found removed, as CSDB have been known to become entrapped in the fungus and parish. As the CSDB feed on biofilm, they do not have a traditional feeding schedule. Alternatively, we placed leaves and cotton cloth containing biofilm that provides them with enough food between inventories. Inventories were conducted on a two month schedule.

Maintenance of systems:

Culture containers currently used to house CSDB are plastic with PVC piping that deliver water using a spray-bar. Water was sprayed onto the side of the container and flows down into the container before exiting drain stand pipes at the opposite end of the container. Each container had a portion of rock and leaf habitat above the waterline in case CSDB are found to lay eggs and have larvae that need terrestrial habitat (all other beetles in this family require this). The systems do not have a traditional siphoning schedule, but alternatively, were cleaned whenever the container was disturbed, such as during the inventory. Dowels were checked daily for fungus and cleaned as needed. At this time water lines, hoses, and valves were checked for functionality and cleaned or replaced as needed.

Figure 24 Comal Springs dryopid beetles in a refugia container

Captive Propagation

The UNFH or SMARC did not produced any F1 Comal Springs dryopid beetles in 2017. BIO-WEST, Inc. is currently researching the life history of CSDB. Hopefully, with their results, propagation practices can be outlined for future years. A captive propagation plan will be drafted when more information on the subject is defined. An updated SOP for CSDB can be found in Appendix A.1.

Peck's Cave amphipod

Collections

Peck's Cave amphipods (PCA) adults were collected by hand picking directly from spring up-wellings by biologists from SMARC and UNFH around Spring Island, New Braunfels, Texas. Small aquarium nets were used to scoop sand and gravel from areas directly over spring up-wellings and then picked through by biologists. A few PCA were are also collected off the poly-cotton lures set for the beetle species in Spring Run 3 and along the Western shoreline. *Stygobromus* species were collected with driftnets in the Comal system in May and November. After allowing these to grow to a size where species could be identified, PCA were counted and added to the refugia. See Figure 5 for comprehensive list of collection dates. See Figure 12 in Comal Springs riffle beetle section for a map of invertebrate collection locations in Comal River.

Table 11. Peck's Cave Amphipod Refugia Population Census

	Census Jan. 1 2017	Incorporated 2017	Number Young Released	Number Adults Released	Census Dec. 31 2017	Survival (%)	Refugia goal for 2017
SMARC	99	220	N/A	0	173	72%	250
UNFH	0	154			45	29%	250

Quarantine:

Incoming PCA were quarantined in separate containers than existing refugia stock in the Invertebrate room at SMARC and the quarantine room at UNFH. PCA were acclimated to quarantine water conditions with the water temperature adjusted 1 °C every half-hour. Organisms were kept in containers marked "Quarantine" for 30 days before joining containers of Refugia population. During this time we observed for other potential ANS species that might have come in with the collection, the general health of the organisms, and watched for large die-offs that might indicate a disease. If none of these occurred then they were moved to the Refugia population at the end of the quarantine period.

Figure 25 Peck's Cave amphipod collection where biologist hand-pick from nets (left), holding jar with collected Peck's (right)

Survival Rates:

Due to the small size and potential cannibalism, PCA are inherently difficult to assess for mortality on a day-to-day basis. Mortalities were therefore calculated numerically when an inventory was conducted. The number of missing PCA equates to the number of mortalities for that time period.

Husbandry

Daily Care:

Every morning and afternoon, all of the systems were checked for adequate flow. The drain screens were kept clear of debris to maintain drainage and water level. Temperature was measured daily and recorded. PCA were fed small amounts of fish flake two times per week. Leaves from terrestrial sources were also used as potential supplemental food and to provide shelter. A detailed Culture Propagation manual for PCA was drafted during 2017 and can be found in Appendix B.3. This manual will be update throughout time as more knowledge is gained about this species.

Maintenance of systems:

Culture containers currently used to house PCA are plastic with PVC piping that delivers water in a manner that mimic up-wellings. The amount of mesh netting placed in a container was decreased this year to facilitate flow and reduce anoxic spots. New designs of habitat that allow PCA to take shelter between objects are being planned for observational testing in 2018. Culture containers were kept dark to mimic the underground environment. This was accomplished by partially covering containers with black plastic or shade cloth. The systems do not have a traditional cleaning or siphoning schedule, but alternatively, were cleaned during inventory events. At this time water lines, hoses, and valves are checked for functionality and cleaned or replaced as needed.

Flow-through brooding chambers were designed and implemented during 2017 for gravid PCA females. Chambers were contructed of clear PVC pipes that make two viewing chambers connected by a coupler. In between the chambers is mesh that is large enough for young to move through but blocks the adult from moving from one chamber to the next. Fine mesh over water inflow and out flow prevents escapement from the system. Each chamber contains large mesh for structure and habitat.

Captive Propagation

When counting PCA from refugia containers during inventory or immediately upon field collection, each amphipod was carefully observed for brooding. PCA females hold their eggs and young in a brood pouch under the body. If gravid females were located, they were isolated in brooding chambers until they released their young. Small PCA are often cannibalized by adults, and the brooding chambers provide both a refuge for the young, as well as an easy method for their collection once they are released from the brooding pouch. Offspring were transferred to a separate culture container after collection. SMARC had 32 brooding females and UNFH had four brooding females during 2017.

Texas troglobitic water slater

Collections

Texas troglobitic water slater (TTWS) were collected primarily through incidental catch in the Diversion Spring driftnet at Spring Lake, San Marcos, Texas and on poly-cotton lures in Comal Springs, New Braunfels, Texas. Due to the current inability to differentiate *Lirceolus smithii* from others in the same genus in a non-lethal manner, all *Lirceolus sp*. are collected and returned to refugia for quarantine. A complete list of collection dates can be found in Figure 5.

Table 12. Texas troglobitic Water Slater Refugia Population Census

	Census Jan. 1 2017	Incorporated 2017	Number Adults Released	Census Dec. 31 2017	Survival (%)	Refugia goal for 2017
SMARC	0	440	0	25	6%	*
UNFH	0	0		0	-	*

** catch rates and hatchery survival are uncertain given this species has not been cultured before*

Quarantine:

Incoming TTWS were quarantined in separate containers than existing refugia stock in the Invertebrate room at SMARC. Upmost caution and care was used when transferring individuals from lure/net to field container to quarantine container as they are extremely delicate. The organism was allowed to crawl onto a small piece of mesh or other debris, then we moved that to the holding container; even a pipette or soft forceps can damage TTWS. TTWS were acclimated to quarantine water conditions with the water temperature adjusted 1 °C every half-hour. Organisms were kept in containers marked “Quarantine” for 30 days before joining containers of Refugia population. During this time we observed for other potential ANS species that might have come in with the collection, the general health of the organisms, and watched for large die-offs that might indicate a disease. If none of these occurred then they were moved to the Refugia population at the end of the quarantine period.

Survival Rates:

We had a very low survival rate of TTWS during 2017. *Lirceolus sp.* are very fragile and mainly collected from driftnet samples where they are more than likely damaged due to collisions with other objects in the collection cups. Biologist have observed *Lirceolus sp.* die in seconds during transport from the main building to the Invertebrate room. New collection techniques are being designed for testing during 2018.

Husbandry

Daily Care:

On a daily basis, all the systems were checked for adequate flow. The drain screens were cleared of debris to maintain drainage and water level. Temperature was measured daily and recorded. It has been observed that lower flow compared to swift, strong flow is preferable for the overall welfare of *Lirceolus sp*. TTWS were fed fish flake once a week. The small amount of flake was ground into a fine powder and mixed with water before injecting the food slurry into the refugia containers using a pipette. A SOP was developed for TTWS during 2017 (see Appendix A.2) with more detailed description of current husbandry methods. As more knowledge is gained this SOP will be updated.

Maintenance of Systems:

Culture containers currently used to house TTWS are plastic with PVC piping that delivers water in a manner that mimic up-wellings. The systems do not have a traditional cleaning or siphoning schedule, but alternatively, were cleaned whenever the container was disturbed, such as during the inventory. At this time water lines, hoses, and valves were checked for functionality and cleaned or replaced as needed. Inventory was conducted every other month. TTWS refugia containers were not siphoned for debris as TTWS are cryptic and could easily be discarded along with debris.

Captive Propagation

If brooding females were identified during inventory, they were isolated in brooding chambers, similar in design to those of PCA, until they released their young. Offspring were transferred to a separate culture container after removal from brooding chambers. A total of 23 brooding female TTWS were identified in 2017. It took less than a month for brooding females to release their young. More attention in 2018 will focus on collecting data on the transition from the marsupium to release.

Figure 26 Brooding Lirceolus sp. female (left). Adult and juvenile Lirceolus sp. (right).

Edwards Aquifer diving beetle

Collections

Edwards Aquifer diving beetles (EADB) were only collected from Texas State Artesian Well driftnet during 2017. Plans for 2018 include trying to set lures for invertebrates in deep wells that might collect EADB.

Table 13. Edwards Aquifer Diving Beetle Refugia Population Census

	Census Jan. 1 2017	Incorporated 2017	Number Adults Released	Census Dec. 31 2017	Survival (%)	Refugia goal for 2017
SMARC	0	6	0	0	0%	*
UNFH	0	0		0	-	*

Quarantine:

Incoming EADB were quarantined in separate containers than existing refugia stock in the Invertebrate Room at SMARC. EADB were acclimated to quarantine water conditions with the water temperature adjusted 1 °C every half-hour. Organisms were kept in containers marked "Quarantine" for 30 days before joining containers of Refugia population. During this time we observed for other potential ANS species that might have come in with the collection, the general health of the organisms, and watched for large die-offs that might indicate a disease.

Survival Rates:

None of the six EADB collected this year survived. Little is known about this species or how to maintain them. Biologist continue to come up with new ideas that might improve husbandry and survival of this species.

Husbandry

Daily Care:

On a daily basis, all the systems were checked for adequate flow. The drain screens were cleared of debris to maintain drainage and water level. Temperature was measured daily and recorded. EADB were given cloth with biofilms and fish flake as potential food sources. A SOP for EADB was drafted during 2017 (see Appendix A.3) detailing the different housing designs and daily care.

Maintenance of systems:

Culture containers used to house EADB started out very similar to those that housed Comal Springs riffle beetles, minus excess leaves. We then transitioned into using flow-through tubes with large mesh as structure for EADB to cling to. The systems do not have a traditional cleaning or siphoning schedule, but alternatively, were cleaned whenever the container was disturbed, such as during the inventory. At this time water lines, hoses, and valves were checked for functionality and cleaned or replaced as needed.

Captive Propagation

A Captive Propagation document does not yet exist for this species. More information is needed before trying different rearing techniques.

IV. Research

Two Projects were led by BIO-WEST Incorporated under a cooperative agreement with the USFWS.

Juvenile development and maturation of Peck's Cave amphipods

Common garden reproduction and development of Stygobromus

Various species of *Stygobromus* were placed into a group holding of congeners (with each group in specific containers). The garden was inspected on occasion and brooding females were removed and placed into brooding chamber until neonates were released. Neonates were then photographed and measured to estimate growth. In coordination with SMARC staff, brooding females caught in the field were also utilized, as well as wild caught early instars.

Morphological development and sympatric congener comparisons of immature Stygobromus

Preserved specimens obtained from drift samples at a location known to have higher proportions of *S. pecki* and a location known to not have *Stygobromus* other than *S. pecki,* were placed into size classes and morphologically compared. Analysis indicates that the populations representing *S. pecki* start to show differentiation from congeners of the same size class at around 3.5 mm.

Larval development of Comal Springs dryopid beetles

Field searches

Because there are so few beetles to utilize for experimentation, several days were spent looking for more subjects by BIO-WEST staff in addition to efforts conducted by SMARC staff; however, only one dead one was found on a lure.

Oblique Plan Apparatus (OPA) experiment

An apparatus for holding adult *S. comalensis* was constructed to investigate oviposition location of eggs (submerged or emergent) and for tracking the habit preference for larvae and adults alike. A pilot study was conducted with the apparatus by placing a male and a female found in amplexus into the apparatus. The pair and apparatus were monitored but no eggs or larvae were observed.

V. Budget

	U.S. Fish and Wildlife Service 2017 Task	Task Budget Spent	Total Task Budget Amount
1	**Refugia Operations**		**$1,641,310**
	SMARC Refugia & Quarantine Bldg.		
	*Construction	$392,242	
	Equipment	$132,118	
	UNFH Renovation Refugia & Quarantine Bldg.		
	*Construction	$50,821	
	Equipment	$66,493	
	SMARC Species Husbandry and Collection		
	Staffing	$369,557	
	UNFH Species Husbandry and Collection		
	Staffing	$298,868	
	SMARC Reimbursibles	$46,418	
	UNFH Reimbursibles	$46,311	
	Subtotal	*$1,402,829*	
	Admin Cost Subtotal	*$238,481*	
2	**Research**		**$136,172**
	BIO-WEST: Dryopid beetle & Peck's Cave amphipod life history	$116,386	
	Subtotal	*$116,386*	
	Admin costs for Task 2	*$19,786*	
3	**Species Propagation and Husbandry**		**$22,891**
	BIO-WEST	*$3,643*	
	USFWS	*$15,922*	
	Subtotal	*$19,565*	
	Admin costs for Task 3	*$3,326*	
4	**Species Reintroduction**		**$1,008**
	BIO-WEST	*$862*	
	Subtotal	*$862*	
	Admin costs for Task 4	*$147*	
5	**Reporting**		**$81,167**
	BIO-WEST	$29,680	
	USFWS	$39,694	
	Subtotal	*$69,373*	
	Admin costs for Task 5	*$11,793*	
	Meetings and Presentations		**$130**
6	BIO-WEST	$111	
	Subtotal	*$111*	
	Admin costs for Task 6	*$19*	
	TOTAL	**$1,882,678**	

VI. Appendices

- Appendix A: Standard Operating Procedures
    - Appendix A.1: Comal Springs Dryopid Beetle - Standard operating Procedures
    - Appendix A.2: Texas Troglobitic Water Slater - Standard operating Procedures
    - Appendix A.3: Edwards Aquifer Diving Beetle - Standard operating Procedures
- Appendix B: Captive Propagation Plans
    - Appendix B.1: Captive Propagation Plan for *Eurycea* sp.
    - Appendix B.2: Captive Propagation Plan for Comal Springs Riffle Beetle
    - Appendix B.3: Captive Propagation Plan for Peck's Cave Amphipod
- Appendix C: SMARC Engineering Diagrams
- Appendix D: Fish Health Reports
- Appendix E: Research Work Plan for Comal Springs Dryopid Beetle
- Appendix F: Research Work Plan for Peck's Cave Amphipod
- Appendix G: Monthly reports

Appendix A: Standard Operating Procedures

Appendix A.1: Comal Springs Dryopid Beetle - Standard Operating Procedures

Appendix A.2: Texas Troglobitic Water Slater - Standard Operating Procedures

Appendix A.3: Edwards Aquifer Diving Beetle - Standard Operating Procedures

Appendix B: Captive Propagation Plans

Appendix B.1: Captive Propagation Plan for *Eurycea* sp.

Appendix B.2: Captive Propagation Plan for Comal Springs Riffle Beetle

Appendix B. 3: Captive Propagation Plan for Peck's Cave Amphipod

Appendix C: Construction Engineering Diagrams

Appendix D: Fish Health Reports

Appendix E: Research Work Plan for Comal Springs Dryopid Beetle

Appendix F: Research Work Plan for Peck's Cave Amphipod

Appendix G: Monthly Reports