Edwards Aquifer Refugia Annual Report 2018

U.S. Fish and Wildlife Service

San Marcos Aquatic Resources Center

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Uvalde National Fish Hatchery

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Implementation of the Aquifer Refugia Program under the Edwards Aquifer Habitat Conservation Plan
Annual Report 2018

Contract No. 16-822-HCP

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

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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 second annual report of the contract covering the calendar year of 2018. The second year of the contract continued to focus on increasing standing stock populations of Covered Species, maintaining the existing standing stocks, conducting research, and the construction of buildings that will house Refugia activities at both the San Marcos Aquatic Resources Center (SMARC) and Uvalde National Fish Hatchery (UNFH).

Major objectives of the USFWS Refugia Program are to 1) develop and 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.

Construction, started in 2017, at SMARC continued throughout most of 2018 on the Edwards Aquifer Refugia and Quarantine buildings. The foundation, steel framing, metal sheathing, roofing, spray foam insulation, utilities (including a 15Kw solar panel system), mechanical systems, lighting, drywall, sheeting, flooring, painting and finishes were completed during 2018. Acceptance of work completed to engineered specifications served as a certificate of occupancy for the office area, and staff were able to move in during August. The Refugia and Quarantine areas were finished by October and species transfer started as instillation of aquatic holding systems were completed.

The bids for the renovations at UNFH were requested in March of 2018. Overall, bids were higher than expected, presumably due to the remote location and the limited number of subcontractors available. The contract was awarded to AmeriVet Enterprises in May 2018, and construction started in June. Renovations and the estimated construction timeline were extended due to issues with sub- contractors and heavy rains during the fall. However, the majority of the renovations were completed in 2018 with a projected completion date of January 31, 2019.

Field work and collections occurred in every month of 2018 with major increases in standing stock populations of fountain darters, San Marcos salamanders, and Texas blind salamanders. Details of collections for the Covered Species can be found in their corresponding sections of the report. At both SMARC and UNFH, staff maintained organisms in the existing and newly constructed systems, making modifications, fabrications, and updates as needed. After construction was completed at the SMARC Edwards Aquifer buildings, staff built new systems and began moving organisms from previous locations to the new complex. Staff continued to refine their species husbandry and collection techniques. One temporary employee at SMARC and two temporary employees at UNFH were employed to assist the Refugia Program during the busy summer months and while two new term staff members were hired at UNFH.

Three research projects carried out in 2018 covered reproduction of San Marcos salamanders, life history of Comal Springs dryopid beetle, and life history of Peck's cave amphipod. USFWS staff filmed and documented the courtship behavior and differences between pairs and groups of San Marcos salamanders. A cooperative agreement was continued with BIO-WEST, Inc. to further research into the life histories of Peck's cave amphipods and Comal Springs dryopid beetles.

The Edwards Aquifer refugia program at the did not exceed the allocated budgets defined within EAHCP contract No. 16-822-HCP or within specified Tasks (1-6 as defined in contract and Work Plan) previously approved by the EAA Board of Directors in the 2018 EAHCP Refugia Work Plan. Approximately $3.6M was spent by the Refugia program in 2018, with the majority of that for construction ($2.6M) at both SMARC and UNFH. Research activities accounted for $359K, and approximately $576K was spent on staff, collections, husbandry and propagation, reporting, meetings, and presentations. It is anticipated that permission will be sought to move unspent specific task funds from 2018 to 2019 allowing for the responsive and intensive management of various tasks within the contract.

INTRODUCTION

Background

The activities reported herein are in support of the Federal Fish and Wildlife Incidental Take Permit for the EAA (ITP; TE-6366A-1, Section K) and fulfillment of Contract # 16-822-HCP between the EAA and the USFWS as outlined within the 2018 EAHCP Refugia Work Plan. The overarching goal of the Aquifer Refugia Program conducted by the USFWS is to assist the EAA in compliance with its ITP and to meet its obligation within the EAHCP section 5.1.1. The EAHCP covers seven endangered species, one threatened species, and three species currently proposed for listing (see Table 1 for list of the Covered Species). The idea of Aquifer Refugia Program is to house and to protect adequate populations of the Covered Species in order to preserve the capacity for re-introduction into the Comal or San Marcos rivers in the event a population is lost due to a catastrophic event such as a long-term drought or major flood. In addition, the Refugia Program conducts research activities to expand knowledge of the species' habitat requirements, biology, life histories, and effective reintroduction techniques. Captive assurance populations of these species are maintained in refugia at SMARC with back-up populations at UNFH.

Table 1 Eleven species identified in the Edwards Aquifer Habitat Conservation Plan and listed for coverage under the Incidental Take Permit within the federal Endangered Species Act (ESA). Color corresponds to the ESA status.

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. It is not included as part of the refugia at this time unless re-discovered.

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. The monetary support of the Refugia augments the existing financial and physical resources of the two USFWS facilities, and provides resources to house and protect adequate populations of the Covered Species. 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 for salvage and restocking as necessary. The use of this support is limited to the Covered Species in the EAHCP.

Objectives

1. **Further Develop and provide fully functioning refugia for the EAHCP Covered Species.**
   USFWS will work towards fully functioning Refugia operations for all of the Covered Species, except the San Marcos gambusia, which is presumed extinct. Fully functioning refugia populations are those that can be predictably collected, maintained, and bred with statistical confidence. The primary refugia will be located at the SMARC, with a secondary refugia population located at UNFH.
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 Aquifer Refugia Program. Research will follow the Edwards Aquifer Refugia Research Goals and Plan (see appendix) and be developed with consultation with the Edwards Aquifer Chief Science Officer. Research will include, but may not be limited to, species' physiology, husbandry requirements, propagation techniques, health and disease issues, life histories, genetics, and effective reintroduction techniques.
3. **Develop and refine animal care/husbandry methods and captive propagation techniques for the Covered Species.**
   USFWS will maintain Standing Stock populations and continue to refine care techniques to increase survivorship, efficiencies, and organismal welfare. Staff will develop propagation techniques in case reintroduction of species into the wild becomes necessary.

4. **Reintroduce species populations, in the event of a loss of species in their native environment, and monitor recovery.**
   The reintroduction strategy will continually evolve as more information is learned about the species.
5. **Attend meetings and give oral presentations to Science Committee, Implementing Committee, and EAA Board of Directors as requested by the EAHCP Program Manager.**
   The Aquifer Refugia Program staff will keep partners apprised of refugia activities.

Personnel

Two Supervisory Biologists (term positions funded through the Contract with the EAA) provided supervision, mentorship, and training to biological technicians at their respective facilities. The work they conducted involves resource management and affects the success and efficiency of the refugia programs at SMARC and UNFH. The supervisors managed and coordinated species husbandry, propagation, and field activities related to species covered under the reimbursable agreement. They also arranged purchases, oversaw facility maintenance repairs, developed and implemented budgets, and organized all activities that related to the reimbursable agreement. They provided proper and efficient use of facilities and staff resources to ensure that contractual obligations are met in a timely manner. In coordination with the Center Director, they prepared all written materials required for reporting. They communicated regularly with the EAA, USFWS personnel, researchers, and other partners. As the Managing Biologist for the Aquifer Refugia Program, Dr. Lindsay Campbell is the point of contact for EAA Refugia operations. Dr. Campbell, with input of supporting staff, prepared the Annual Report, yearly Work Plans, developed research activities and reports, and oversaw outside research agreements. During 2018, Mark Yost was hired by USFWS as the Supervisory Biologist at UNFH (Table 2 for list of Refugia Program Staff). In addition to supervisory duties listed above, he provided written materials covering activities at UNFH to be incorporated into monthly reports and the Annual Report, with input into the yearly Work Plan.

Biological Technicians (term positions funded through the Contract with the EAA), under the management of the lead Supervisory Biologist at each facility, assisted with collections, daily upkeep, maintenance, propagation, and research efforts for the refugia species at SMARC and UNFH. This included maintaining experimental and culture production systems, keeping records along with entering and filing data, and participating in research activities. The technicians also generated basic summary statistics, graphic analyses of data, and documented program accomplishments through the composition of standard operating procedures (SOPs), reports, and manuscripts. Benjamin Whiting was hired during 2018 to fill a vacant biological technician position at UNFH (see Figure 1 for a picture of Refugia Program staff).

During the summer of 2018 temporary biological technicians (Hunter Bailey at SMARC and Jennifer Lawrence and Ruban Tovar at UNFH) were hired to help with refugia work. These positions were limited to a maximum of 60 days. The technicians supported Refugia program efforts and at UNFH helped augment the workforce while the positions of Supervisory Biologist and Biological Technician were vacant during the hiring process.

Table 2 USFWS Refugia Program Staff

San Marcos Aquatic Resources Center	
Lindsay Campbell, Ph.D.	Managing Biologist for the Aquifer Refugia Program San Marcos Program Supervisor
Kelsey Anderson, M.S.	Biotechnician
Amelia Everett Hunter, M.S.	Biotechnician
Linda Moon, B.S.	Biotechnician
Uvalde National Fish Hatchery	
Mark Yost, B.S.	Uvalde Program Supervisor
Makayla Blake, M.S.	Biotechnician
Benjamin Whiting, M.Ed.	Biotechnician
Rachel Wirick, B.S.	Biotechnician

Figure 1 The Refugia Program team. Back row L-R Makayla Blake, Kelsey Anderson, Linda Moon, Lindsay Campbell, Amelia Hunter. Bottom row L-R Ben Whiting, Rachel Wirick. Not pictured due to training Mark Yost.

BUILDING CONSTRUCTION

In 2018, construction and renovations proceeded at both locations according to plans. In San Marcos, Texas, the new Edwards Aquifer Refugia and Quarantine buildings at SMARC were completed. In Uvalde, Texas, modifications and renovations of existing UNFH buildings started. These projects create Edwards Aquifer Refugia and Quarantine work areas specifically for Covered Species activities necessary to meet our obligations under the EAA-USFWS contract.

San Marcos Aquatic Resources Center

During 2017, the construction contract for the buildings at SMARC was awarded to Puyenpa SVS, L.L.C. with local affiliation of AmeriVet Enterprises. Construction broke ground in November of 2017 and finished in November of 2018. The Refugia and Quarantine buildings were built concurrently because, even though they are separate buildings, they share a foundation slab and a roof.

The year started with work on the foundation of the buildings; this work included trenching for drainage systems and forming footers. Next, the rebar framework was completed for the concrete pad foundation, before the concrete was poured (Figure 2). After the foundation was laid and cured, the steel framework for the building was installed. The lower break portion of the outer walls was laid and stone wainscoting was installed on the lower break portion of the outer walls before the metal panel sides were mounted. Existing domestic water lines, non-chilled well water, chilled well water, and re-use water lines were tapped and run to the new buildings. Drains for the domestic water (bathroom facilities in office and sinks in the Refugia and Quarantine buildings) were connected to existing lines. Drains for well water (water that runs through systems that hold organisms) were connected to the station chlorination system and the re-use system. All water lines were hydrostatically tested.

Following the completion of external structures and line connections, the interiors of the spaces were started. Interior framing was completed for the office space, hallways, and walls in the Refugia and Quarantine buildings. Spray foam insulation was blown onto the walls and insulated batting was mounted overhead. Drywall was installed in the hallways and office areas, then textured and painted. In the Refugia and Quarantine areas, metal paneling was installed over the insulation. A special grade paneling was chosen so that it could easily be cleaned and disinfected if needed. Roll-up bay doors for both the Refugia and Quarantine were installed to allow for the future installation and removal of large aquatic tanks and other equipment.

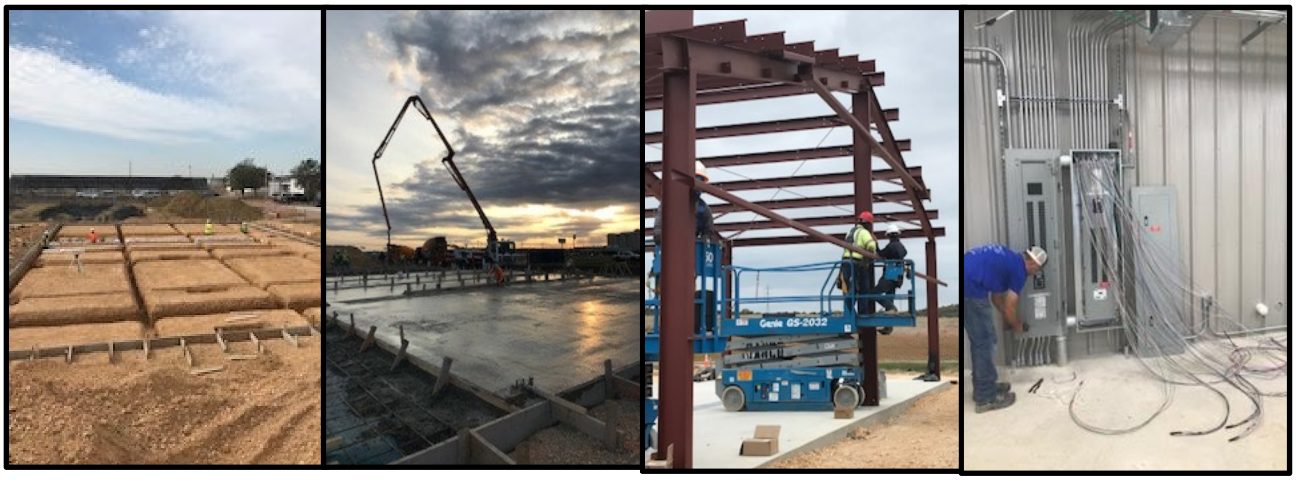

Figure 2 L to R: Trenches and footings being dug. Foundation pour. Steel structure install. Electricians installing breaker boxes.

HVAC crews installed the duct support systems and the insulated duct work. Three heat pumps for the HVAC system were installed; a smaller one for the office space and two larger ones each for the Refugia and Quarantine spaces.

Electricians installed struts, conduit, electrical boxes, and light fixtures in the buildings. All electric systems were grounded and the solar inverter and associated conduit was run to the electrical panels. Electrical service began in July of 2018. Roof mounted solar panels were installed in August. The solar panels have been observed to generate up to 9 kW per hour so far. The final electrical testing was conducted in November.

Plumbers installed overhead water lines in the Refugia and Quarantine spaces to deliver water and air to the aquatic holding systems. These lines were color coded for non-chilled well water, chilled well water, re-use water, and a forced air-line. Plumbers installed OSHA certified eye-wash stations in both the Refugia and Quarantine areas. The external, 1-HorsePower, heater-chiller units were placed and plumbers ran flexible piping through the walls to the units.

The parking lot area was graded and concrete was poured for the parking lot and the apron side-walk around the building areas.

The main construction was completed in August of 2018 and a punch list was composed and completed by November (Figure 3). The Refugia staff were able to move into the office space in August. Careful planning was made for the move of the species into the new buildings, including the placement of tanks and plumbing the systems. The first priority was to set up the Quarantine area to accept newly collected organisms and house the long-term stocks of Comal fountain darters (Figure 4). The staged relocation of species into the new spaces started in September 2018. The new custom tanks were not delivered until late December 2018, slowing down the movement of species (Figure 4).

Figure 3 Pictures of freshly finished construction of Edward Aquifer buildings at SMARC, outside, Refugia area, and Quarantine area.

Figure 4 L: Starting to set up tanks in the Refugia building at SMARC. R: Migrating species and utilizing the Quarantine building at SMARC.

Uvalde National Fish Hatchery

At UNFH, new buildings were not constructed. Alternatively, modifications of existing buildings created the Refugia and Quarantine areas for the Aquifer Refugia Program. The request for bids for the UNFH renovations was posted in March of 2018. Overall, bids were higher than expected, presumably due to the remote location and the limited number of subcontractors available. The contract was awarded to AmeriVet Enterprises in May 2018 and construction started in June 2018. To keep costs within the approved budget, a change order was made to the contract to remove the generator for the Quarantine area. Cost savings during the project allowed for the generator to be put back into the project, but it will not be delivered or installed until April of 2019. Because construction was ongoing in the midst of normal operations in the Tank House and Quarantine Building, extra care was given to prevent the interruption of water and air flow that support the organisms within these culture systems. Renovations and the estimated construction timeline were adjusted due to issues with sub-contractors and heavy rains during the fall. Other unexpected issues included finding and/or repairing unmarked utilities, finding and removing unknown concrete structures, and subcontractors redoing work. The majority of the renovations were completed in 2018 with a projected completion date of January 31, 2019.

Refugia Space in UNFH Tank House

The existing Tank House, adjacent to the administration building at UNFH, was modified to include additional interior walls, isolating a new area (ca. 1,400 ft^2) for refugia. Additionally, a covered breezeway attached to the Tank House was sealed with new walls and connected to the isolated refugia area to be used specifically for invertebrate refugia (ca. 350 ft^2).

During the initial phases of construction of the Refugia area, the contractors marked existing and known utilities and underground structures around the build sites, ordered materials, and cut trenches in the parking lot and Tank House building to install electrical services and plumbing drain lines. A new power supply pole, transformers, a generator pad and generator, electrical service panels, breakers, and disconnects were installed (Figure 5). The structural steel walls of the Refugia area were installed, along with the roof joists, mezzanine structure and decking, insulation, and metal sheeting for walls (Figure 6). The HVAC system was installed outside of the Tank House with the HVAC ductwork installed on the mezzanine level (Figure 7). The mezzanine level is the ceiling component of the Refugia space and serves to hold the nine, 1-HorsePower, heater-chiller units and various utility lines.

Figure 5 Refugia area electric supply and back-up generator at UNFH.

In the Refugia area, a small, tankless water heater was installed for the utility sink; an OSHA certified eye-wash station and emergency shower were also connected to the domestic water line (Figure 7). Electricians installed electrical conduit, service plugs, and overhead lighting. One roll-up bay door and four regular doors were installed to complete the enclosure of the Refugia space.

Figure 6 The progression of walls at UNFH for Refugia area: structure, insulation, and paneling.

Testing all of the electrical components, HVAC, and plumbing fixtures still remains. Sub-contractors need to make adjustments to the uni-strut hangers that suspend plumbing and electrical boxes from the roof joists so that these are uniform and even. The staff have submitted a purchase request for 16 tanks for this area (and four for the Quarantine area); it is awaiting approval and awarding through our regional Contracting Office.

Figure 7 Nearly finished construction at the UNFH Refugia area: mezzanine level, sink and eyewash station, and interior with plumbing.

Quarantine Space Renovation at UNFH

Plans for the UNFH Edwards Aquifer Quarantine area for the Covered Species called for the renovation and enclosure of a covered storage bay (ca. 1,200 ft^2) connected to an existing storage room and enclosed second story loft. Briefly, the renovations called for drain trenches, concrete foundation, steel framing of a fourth wall and enclosure, wall insulation, HVAC system, refitted water plumbing, and rehab of the electrical service throughout the new space. The second-floor mezzanine/loft area (ca. 500 ft^2) will hold ten, 1-HorsePower, heater-chiller units and provide storage above the culture area. Setbacks in the Quarantine renovation included undocumented concrete foundation from a previous structure that was covered with dirt, undocumented underground plumbing and utilities, and breakage of plumbing lines (both documented and undocumented) by the contractors, which required immediate repairs (Figure 8).

Figure 8 Trenching causing broken lines in need of immediate repairs at UNFH.

During the initial phases of construction of the Quarantine area, the contractors marked existing and known utilities and underground structures around the build site, ordered materials, and excavated within the covered bay of the storage building for installing drain lines and the concrete foundation. During this excavation, an undocumented concrete foundation was found and removed by the contractors, resulting in a contract change order and increased costs.

Trenching and excavation was done to install electrical conduit; a new power supply pole and transformers were installed. Another undocumented concrete structure was found and removed by hatchery maintenance staff to prevent a contract change orders and so work could continue to progress. Electricians installed electrical service panels, breakers, disconnects, and a transfer switch. Inside the building, contractors are installing service plugs and overhead lighting. Testing of the electrical components of the building still remains to be done.

After the Quarantine area foundation was laid, the contractors began erecting and installing the steel structure to enclose the culture area with walls and doors. A concrete stem wall was poured for the door jams. Steel sheeting, vinyl insulation, a man door, and two insulated roll-up doors were installed to enclose the Quarantine culture space (Figure 9). A restroom compliant with the Americans with Disabilities Act was built into the new space along with a water heater, a utility sink, an OSHA certified eyewash station, and an emergency shower adjacent to the restroom. The HVAC system was installed outside, along with the HVAC ductwork into the building (Figure 9).

Adjustments need to be made to the uni-strut hangers that suspend plumbing and electrical boxes from the roof joists so that these are uniform and even. Although the small septic lift station has been installed near the visitor bathroom, to tie in the Quarantine bathroom to the main septic tank, it still has to be tested and adjusted for proper operations. There are ongoing corrections that need to be made to some of the plumbing workmanship related to the damages incurred from the lift stations' trenching and installation. The larger Quarantine water-discharge lift station needs to be installed for proper drainage.

Figure 9 Approaching completion to the Quarantine area at UNFH.

COVERED SPECIES ANALYSIS

Collections of the Covered Species continued this year to build up to Standing Stock numbers as outlined in the Contract (Figure 10, Table 3). For many species, the accumulation to Standing Stock numbers can be achieved relatively quickly; this is particularly true for Texas wild rice, San Marcos fountain darters, and San Marcos salamanders. We have made large strides in increasing our populations of Texas blind salamanders through collections and increased survival of very small juveniles coming out of the Diversion Spring net; yet, it will take 10 years to build up to the Standing Stock number listed in the contract at the current collection rate. Through conversations with our staff, the Edwards Aquifer Authority staff, our other partners, and experts in the field, the number of invertebrate collection events and numbers held in refugia has been reduced minimize any negative effects collection events might be having on wild populations in the Comal spring system. This reduction was also beneficial given the amount of time devoted to oversee construction as well as specimen transfers into the new systems.

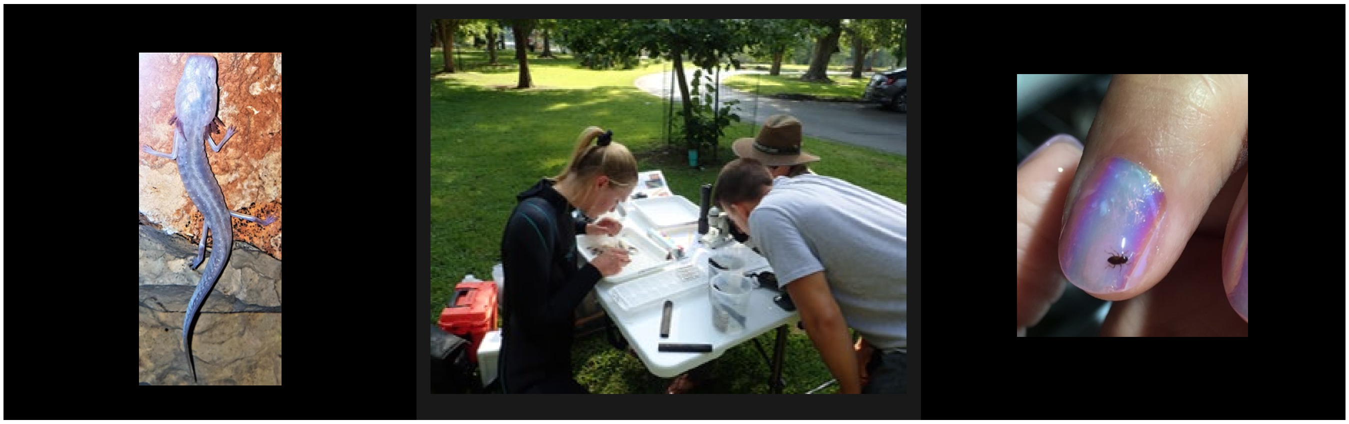

Figure 10 L to R: Texas blind salamander in Rattlesnake cave, staff sorts through invertebrate lures, and Comal Springs riffle beetle

Husbandry of all the species continues to evolve with new techniques that require repeated testing and refinement through time. For example, at SMARC, a new spray bar/water delivery system was developed for the larger Texas wild rice tanks to more evenly distribute water flow throughout the tank. This model was further adapted to the tank size and needs at UNFH. In addition, two of the refugia staff participated in a recirculating aquaculture course this year; they shared their new knowledge and ideas with the team.

Table 3 Number of organisms incorporated in to the Refugia Standing Stock in 2018 and the end of year census for both SMARC and UNFH. Incorporated refers to organisms that have passed their 30-day quarantine period where they have been evaluated for health and suitability for inclusion into refugia populations; also, they have been cleared by USFWS Fish Health Unit where applicable. Further details of these numbers can be found in the supporting sections of each species.

Species	SMARC Incorporated into Refugia	SMARC End of Year Census	SMARC Survival Rate	UNFH Incorporated into Refugia	UNFH End of Year Census	UNFH Survival Rate
Fountain darter-San Marcos ***Etheostoma fonticola***	326	503	56%	294	435	81% (*57%)
Fountain darter-Comal ***Etheostoma fonticola***	0	237	62%	0	48	73%
Comal Springs Riffle Beetle ***Heterelmis comalensis***	443	162	26%	16	14	21%
Comal Springs Dryopid Beetle ***Stygoparnus comalensis***	3	2	13%	0	0	0%
Peck's Cave Amphipod ***Stygobromus pecki***	308	272	58%	58	25	24%
Edwards Aquifer Diving Beetle ***Haideoporus texanus***	0	0	--	--	--	--
Texas Troglobitic Water Slater ***Lirceolus smithii***	38	2	**	0	0	--
Texas Blind Salamander ***Eurycea rathbuni***	55	95	93%	--	--	--
San Marcos Salamander ***Eurycea nana***	122	275	71% ([1]88%/54%)	99	232	83% ([3]76%)
Comal Springs Salamander ***Eurycea* sp.**	40	72	83% ([2]92%)	15	18	95% ([3]75%)
Texas Wild Rice Plants ***Zizania texana***	52	220	82%	15	80	98%

**Survival rate of San Marcos fountain darters collected in 2017*

***unable to distinguish wild stock from captive bred (Fx) generations, therefore we could not calculate survival between inventories*

[1]Survival rate of San Marcos salamanders collected fall 2017-present and collected spring 2017-past

[2]Survival rate of Comal Springs salamanders without escape event

[3]Survival rates for salamanders collected in 2017, the first year of salamander collections for UNFH

Fountain darter (*Etheostoma fonticola*), Endangered

The Standing Stock goal for fountain darters is to have 1,000 fish per river (San Marcos and Comal) divided between the two facilities. Fewer fountain darters were collected in 2018 than in 2017 due to limited space at the facilities during construction. In consultation with EAA staff we also decided not to collect enough darters in the fall to reach the Work Plan goals in order to reduce the impacts on the wild populations as a whole. We are still building towards the Contract Standing Stock goals, but are doing so at a slower pace being mindful of overall conservation. Numerical data on numbers incorporated, end of the year census, and survival rates can be found in Table 4.

Collections

We had two fountain darter collection events during 2018, a summer collection (June) and fall collection (October). Fountain darter collections are coordinated with USFWS Region 2 Fish Health Unit (FHU) in Dexter, NM so that live samples can be sent for analyses. Thus, collections are specifically targeted events with dates scheduled over a year in advance. To reduce the number of fish sacrificed for health testing, collection events are coordinated for both facilities when possible. Staff collect fountain darters by wading and using large dip-nets to pull through vegetation growing on the river bed. The first collection event, June 12-15, targeted only fountain darters from the San Marcos River for the Standing Stock population at SMARC. The second collection event, October 29-31, encompassed fish from both the San Marcos River and the Comal River for the Standing Stock populations at both SMARC and UNFH. Refugia staff partnered with BIO-WEST, Inc. employees during their fall biological monitoring of the Comal River. After BIO-WEST, Inc. employees trapped fountain darters via drop-netting, counted, and measured them for biomonitoring, they were transferred to Refugia staff for our refugia population. This partnership created efficiencies in collections and reduced disturbance to the fish and systems (from both biomonitoring and refugia collections). During 2019 the Refugia staff will work with the biomonitoring teams in both the spring

Figure 11 Male fountain darter in refugia.

and fall for fountain darter collections from both river systems. Dates have been coordinated and changed with the FHU accordingly. To minimize stress during the 2-3 hour transport to UNFH, sea salt is added to the transport coolers at a concentration of 0.25-0.5%, water temperatures are monitored, and chilled well-water from an additional cooler is added as needed, to mitigate thermal fluctuations during transport.

Table 4 Fountain darter refugia population figures.

		Beginning of Year Census	Incorporated 2018	End of Year Census	In Quarantine End of Year	Target Goal 2018 Work Plan	Percent Survival
San Marcos River	SMARC	588	326	503	0	600	+56%
	UNFH	246	294	435	0	500	81% (*57%)
Comal River	SMARC	382	0	237	136**	400	62%
	UNFH	66	0	48	13**	100	73%

Survival rates excludes fish sent to FHU for diagnostic processes
+Survival rate excludes losses from salt treatment-electrical event
**Survival rate of San Marcos fountain darters collected in 2017*
***Fall 2018 collected Comal River fountain darters have not received clearance of FHU yet*

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 progressed at a rate of one degree Celsius per hour. A subset (60) of newly collected fountain darters were separated and sent to FHU for routine parasitology and health screening before the larger group of collected fish were incorporated into the refugia. At SMARC, after acclimation, fountain darters were subject to a one hour formalin treatment for ectoparasites while still in their transport coolers. Then fish were moved to quarantine aquaria for holding and observation for 30 days until they were cleared by FHU. Quarantine aquaria at SMARC are flow-through chilled-well-water only systems. At UNFH, fountain darters were treated with 0.5-1.0% static salt baths, instead of formalin, to reduce ectoparasites. The quarantine aquaria at UNFH are flow-through systems designed with two water supplies, an untreated well supply (23-25℃) and a chilled-well-water supply (20-21℃), allowing the water temperature to be manipulated as needed. Once fish cleared their quarantine period, they were incorporated into the Standing Stock at both facilities.

The fountain darters from the Comal River continued to test positive for Largemouth Bass Virus (LMBV). Fountain darters do not appear to have any negative consequences if they are infected with LMBV and appear to be only carriers of the virus. The virus generally effects the swim bladders of bass; fountain darters lack swim bladders. LMBV has been found in the Guadalupe watershed and documented in most watersheds in Texas by Texas Parks and Wildlife. 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 River fountain darters that are LMBV positive were housed in quarantine facilities. Until the new building and renovations are completed, this limits the number of Comal River fountain darters that can be housed. At SMARC, a small population of Comal River fountain darters that are not LMBV positive are held outside of quarantine.

Survival rates

At both SMARC and UNFH, survivorship of newly collected Comal River fountain darters was poor in comparison to San Marcos River fountain darters collected during the same time period and held in similar conditions. For example, at UNFH only 49 of the original 115 brought in made it to the end of the 30 day quarantine period (42% survival), and by the end of the year only 13 remained (11% survival). At SMARC, only 175 of the original 235 brought in made it to the end of the 30 day quarantine period (75% survival), and by the end of the year, only 136 remained (58% survival). Survival rates during the quarantine period (30 days and clearance from FHU) are not reflected in overall survival rates. In comparison, at SMARC 100% of the San Marcos River fountain darters made it to the end of the 30 day quarantine period. This pattern has been noted for the past three Comal River fountain darter collections. Both live and preserved Comal River fountain darters were sent to the FHU for analysis for all three events. No conclusive results were found by the FHU; however, they found degradation of cartilage in the head, meningitis, and myositis in samples. The fall 2018 Comal River fountain darters were not incorporated into the Standing Stock population after the traditional quarantine period as the higher mortality rate was being investigated.

Normal monthly survival rates averaged 92-99% for San Marcos and 94-99% for Comal fountain darters. Four specific events contributed to low survivorship of fountain darters at SMARC in 2018: a pump failure, two electrical shock events, and a Costia outbreak. In February, a pump failed on a San Marcos River fountain darter tank. Water pressure and flow stopped overnight causing unfavorable conditions (lower than normal dissolved oxygen and higher than normal ammonia) in one system, resulting in 27 deaths. There were only two other fountain darter deaths that month.

During the end of July through August 2018, fountain darters at SMARC in the Standing Stock population from both San Marcos and Comal (non-LMBV+) steadily declined, so samples were sent to FHU for analysis. The veterinarian suggested a routine 2% static salt treatment for 1 hour for the fish while results were pending. On August 29, 2018 these fountain darters were given a salt treatment; however, during the treatment 78 fish from four different tanks suddenly and inexplicably died in under 10 minutes into the salt treatment. Further investigations found stray electrical voltage in the fountain darter tanks. One possible explanation is that conductivity amplified by the salt intensified effects of stray voltage in the water. Fountain darters did exhibit characteristics consistent with electroshocking. Measures were taken immediately to limit deaths: electrical breakers for pumps and heater/chiller units were turned off and systems were put on fully flow through well water. FHU did not find any conclusive parasites or disease from the fish previously sent to them. It is hypothesized that the steady decline we had been experiencing could have been from stray voltage entering the water in the tanks on and off over a period of time. Total San Marcos River fountain darters during August were 151. Later in the year (October 2018) another mortality event of 36 fish occurred and again low voltage was measured in the tanks even though the tanks did not have any electrical components plugged into outlets and the breakers to those outlets were off. However, aerator boxes, which were plugged into extension cords running to other outlets, were placed on the railing system where the shut-down electrical and water lines that run to the fountain darters are also located. The aerators were for catfish for another project on station but in the same gallery as the fountain darter tanks. As a result, the darters were immediately moved to the newly completed Edwards Aquifer Refugia building to temporary tanks and then transitioned to a more permanent tank set up.

In November 2018, SMARC biologists noted that fountain darters in all systems were very thin and exhibiting "flashing" swimming behavior. In addition, they began to die-off. FHU biologists diagnosed the fish with *Ichthyobodo necator* (Costia) and recommended formalin dip treatments. Costia is naturally occurring at low levels but may have increased in the systems due to lower dissolved oxygen of incoming well water for a period after a well pump change or commercially bought brine shrimp feed that was used to supplement the regular feed. Blackworms were not in supply in the fall of 2018. Installation of UV sterilizers on fountain darter systems is currently ongoing and brine shrimp feeding to the fountain darters was terminated.

UNFH survival rates of San Marcos fountain darters were higher in 2018 at approximately 81%, compared to the 57% in 2017. UNFH survival rates of Comal fountain darters were lower in 2018 at approximately 73%, compared to 92% in 2017. The apparent increase of San Marcos survival was due in part to nearly three quarters of the San Marcos fountain darters reported in the ending inventory having been collected near the end of the year. These darters had not been on station longer than two months and had displayed very little mortality. Of the original San Marcos fountain darters that were carryovers from 2017, there were 246 in January and 141 in December with an average survival of 57%. Of the original Comal fountain darters that were carryovers from 2017, there were 66 in January and 48 in December with an average survival of 73%.

Husbandry

Fountain darters were kept in systems with partial flow-through and partial recirculated temperature-conditioned water (temperature 20-21℃). Aquaria were siphoned once a week to remove excess food and waste, unless otherwise needed (Figure 12). Fountain darters were fed three to five times per week, depending on the facility, a variety of live foods including small amphipods, *Artemia spp.* nauplii, adult brine shrimp, zooplankton, and blackworms. All tanks were checked daily for water flow, acceptable temperature, and mortalities. Refugia staff added habitat enrichment items of small PVC structures, plastic plants, and pebble mats to provide shelter for the fish. These structures were checked weekly for darter eggs and cleaned accordingly to prevent reproduction from occurring in the systems. A detailed description of fountain darter daily care can be found in the fountain darter SOP and culture manual, available upon request.

Figure 12 Mark Yost siphoning a fountain darter tank at UNFH.

Health Monitoring

If fish show signs of illness, staff consulted with FHU and sent samples as needed for diagnosis. Each year representatives from FHU visit each facility and take a sample of fountain darters (all fish species 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. Each system was acid washed annually to remove calcium carbonate deposits that have formed within the tank, plumbing, chiller, and pump casing that can affect functionality. Fish were removed from the systems during acid washing. Stand pipe screens and PVC habitat items were changed and disinfected regularly.

Captive Propagation

Captive offspring were not allowed to develop for both San Marcos River and Comal River fountain darters at either facility during 2018, due to limited space and a population of F1 fountain darters already on hand. Generally, fountain darters in captivity lay eggs on the undersides of PVC habitat structures placed in the tanks. If offspring were not desired, staff remove the structures and dispose of the eggs. When offspring were desired, the PVC structures were moved into smaller tanks for the eggs to develop and hatch. F1 generations are separated based on the river system from which their parents originated. Egg production is opportunistic and is not controlled or directed by staff during periods when offspring are not needed for research or for reintroduction. A captive propagation plan is on file and available upon request for fountain darters. SMARC currently has 170 F1 fountain darters on hand.

Texas wild rice (*Zizania texana*), Endangered

Texas Parks and Wildlife categorizes Texas wild rice (TWR) in alphabetical (A-K) sections of the San Marcos River (Figure 13). Wilson *et al*. (2016) assessed the genetic diversity historically documented in the San Marcos River and by river sections. The Refugia Program wishes to preserve the genetic integrity of TWR by collecting tillers from plants throughout the river so that the refugia populations reflect the wild population. SMARC staff specifically targeted plant stands that were not currently represented in the refugia population. Plant stands were selected after overlaying refugia plant GPS locations onto GIS maps produced by the SMARC Plant Ecology Program during their annual Texas Wild Rice Survey. UNFH staff are building their refugia population numbers and representative locations. Numerical data on numbers incorporated, end of the year census, and survival rates can be found in Table 5.

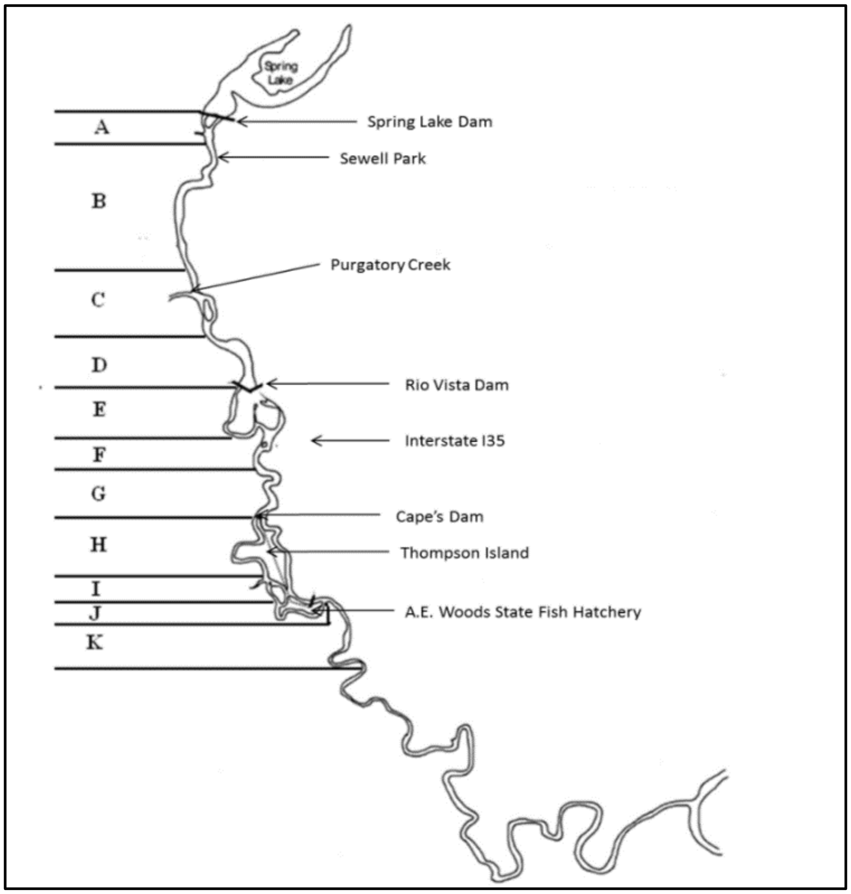

Figure 13 San Marcos River sections A-K for TWR.

Collections

Tiller collections in the San Marcos River occurred in March, May, November, and December. Plant tiller collections were suspended during the summer months because heat stress negatively affects survivorship. USFWS SCUBA divers or snorkelers collected tillers by hand from plant stands. During collection, the location of the TWR plant stand was recorded with a Global Positioning System (GPS) device (WAAS-enabled 3 meter position accuracy). In addition, staff recorded the percent coverage and the river section for each plant stand collected. This information was collated in a central database maintained at the SMARC and UNFH. Tillers were placed in marked mesh bags and immersed in coolers filled with fresh river water for transport back to their respective facilities.

Table 5 Texas wild rice refugia population figures.

	Beginning of Year Census	Incorporated 2018	End of Year Census	In Quarantine End of Year	Target Goal 2018 Work Plan	Percent Survival
SMARC	241	52	220	15	232	82%
UNFH	67	15	80	15	121	98%

Quarantine procedures

Upon arrival at each respective facility, tillers (still grouped by individual plant) were rinsed in fresh well water and inspected for any aquatic nuisance species (ANS). Tillers from each plant were potted together in a tagged pot and placed in a quarantine raceway tank for 30 days. During this time, they were routinely checked for ANS, specifically the invasive snail *Melanoides tuberculata.* Salt treatments of incoming tillers (2% salt dip) have been discontinued at SMARC, but continue at UNFH. After consulting with an invertebrate specialist, we concluded that the 2% salt treatments of the TWR was not any more effective at removing *Melanoides* than visual inspection and removal. This consultation was sought after finding that tillers treated with a salt dip had lower survival rates. After 30 days, plants were un-potted and the full plant visually inspected for ANS, before the tillers were re-potted and incorporated into the standing stock population. In a change from previous quarantine potting techniques, incoming quarantine plants were kept in their respective mesh bags or lightly potted in loose large rock, and placed in a high volume turnover quarantine tank. This method reduces the chances of anoxia to roots while in quarantine and the amount of soil discarded after the quarantine period (soil is not reused).

Survival rates

In February 2018, 23 TWR plants that had previously been counted towards the Standing Stock population at SMARC were given to the Plant Ecology Program, as either identification tags had been lost or records could not be found to conclusively identify the plant origin. These plants were not counted towards survivability numbers. Older TWR that have been held at SMARC for several years (before the start of the Refugia Program) are starting to show a higher mortality rate than newer plants. The last Refugia plant from River Section K died this year. Currently, TWR has disappeared from that section of the San Marcos River.

Husbandry

Water flow in the tanks was checked daily and stand pipe screens were cleaned daily to ensure that no debris blocks water flow through the pump and chiller systems. Staff removed filamentous algae from the leaf blades by gently running fingers or a six inch mesh net across the surfaces of each plant. Algae was removed from tanks as needed by scrubbing and floating debris was removed manually using mesh nets or siphons. TWR plants were routinely trimmed to prevent sexual reproduction from emergent vegetation, so that the genetic integrity of each plant is maintained. Plants were housed very close together and it would be difficult to prevent cross-pollination between plants from different river sections if allowed to emerge and flower.

Figure 14 Makayla Blake works on TWR under 50% shade cloth.

New PVC recirculation manifolds were added to facilitate flow throughout the entire length of the tanks and across the surface of the plants at SMARC and then adapted for the tanks at UNFH. These were made of PVC with small holes drilled at a 45-degree angle along the length of the pipe with a valve at the end to adjust flow. Several prototypes were tested before selecting this flow bar method. The new flow bars created strong flow down the entire length of the tanks allowing for the utilization of more of the tank space.

At UNFH, pump bags were added to prevent debris from clogging intakes. Roofing panels above the TWR at UNFH were removed and replaced with 50% shade cloth to help control filamentous algae growth (Figure 14). UNFH staff disinfected and redesigned four of the large buried raceway tanks by the Tank House (Figure 15) and transferred all TWR plants from 2017 collections into the systems. These four tanks will be able to hold the entire Standing Stock of TWR plants, allowing us to use the old rice culture area as a quarantine area for new collections and an area to conduct research.

To reduce the amount of hypoxic soil and roots in refugia pots, a layer of lava rock was placed at the bottom of the pots and more pea gravel was added to the soil mix. UNFH has started using a potting mix of compost-loam-sand with additional pea gravel and lava rocks instead of sandy-loam mixture. Staff continued to investigate hydroponic techniques that can be used for TWR to reduce plant pots becoming anoxic and the need to re-pot plants annually. A detailed description of TWR daily care can be found in the TWR SOP and culture manual, available upon request.

Captive Propagation

TWR was maintained to discourage sexual reproduction in the refugia. Emergent stems were trimmed weekly. An existing TWR Propagation manual outlining seed production exists and is available upon request.

Figure 15 Ben Whiting works on the new flow bar design in the re-designed UNFH in ground tanks for TWR.

Texas blind salamander (*Eurycea rathbuni*), Endangered

The Standing Stock goal for Texas blind salamanders is 500 individuals divided between the two facilities. As Texas blind salamander catches are infrequent, the Standing Stock will be built slowly to reach this goal; at the current catch rate it will take 10 years to reach 500 individuals. Until facility renovations are completed at UNFH, Texas blind salamanders will only be housed at SMARC. Standing Stock population numbers increased more than expected in 2018 due to changes in husbandry that increased the survival rate of very small juveniles caught from Diversion Springs net from 50% to 90% (Figure 16). Numerical data on numbers incorporated, end of the year census, and survival rates can be found in Table 6.

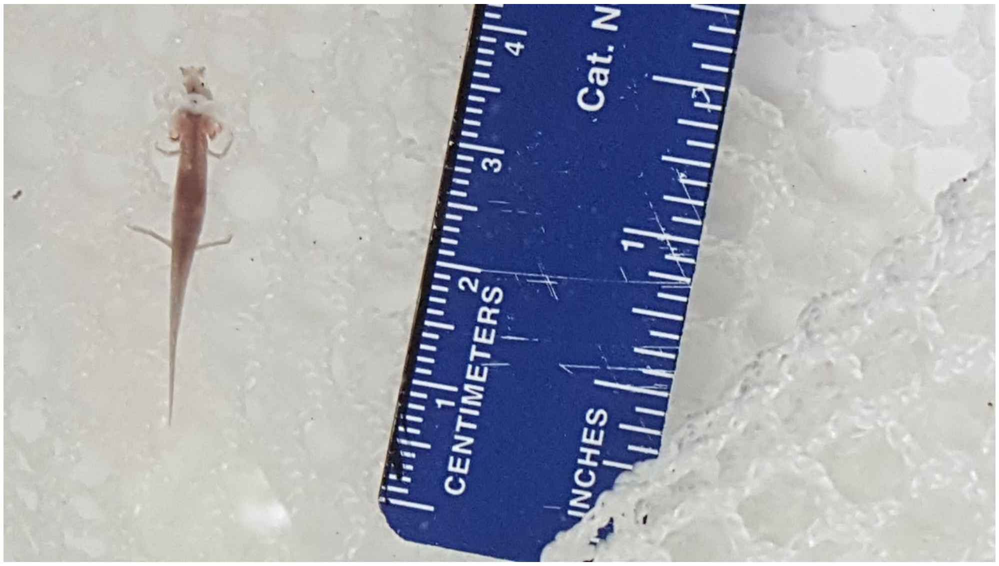

Figure 16 Newly collected Texas blind salamander small juvenile from Diversion Springs net.

Collections

Texas blind salamanders were collected from caves, well, fissures, and driftnets on high flow springs. 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 retained for refugia from these sites. Biologists collected tail clips of salamanders released from these sites for future genetic analysis. The USFWS has a large drift net on Diversion Spring in Spring Lake to collect salamanders and invertebrates coming from the spring. During periods when we are not trapping for Texas blind salamanders we placed a collection cup on the net and checked it two to three times a week. All live Texas blind salamanders caught from Diversion Spring net were retained for refugia, given the assumption that any salamander leaving a spring orifice and entering the lake environment will ultimately succumb to predation. We tracked the number of salamanders collected in the Diversion Springs net over the year and did not find a pattern of occurrence or absence in numbers between Texas blind salamanders and San Marcos salamanders (Figure 17). When total Texas blind salamander catch (those found in net) was plotted along with the calculated springflow of San Marcos springs (USGS 08170000) no relationship seems apparent. However, this is just one year of data, as our data set grows more predictions on numbers in Texas blind juveniles found in the net may be plausible. Texas State University personnel had nets on Sessom Creek and Artesian Well for their own uses during 2018, but donated four live Texas blind salamanders collected from those locations to SMARC in 2018 (Figure 19).

Table 6 Texas blind salamander refugia population figures.

	Beginning of Year Census	Incorporated 2018	End of Year Census	In Quarantine End of Year	Target Goal 2018 Work Plan	Percent Survival
SMARC	47	55	95	0	60	93%
UNFH	--	--	0	--	15*	--

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

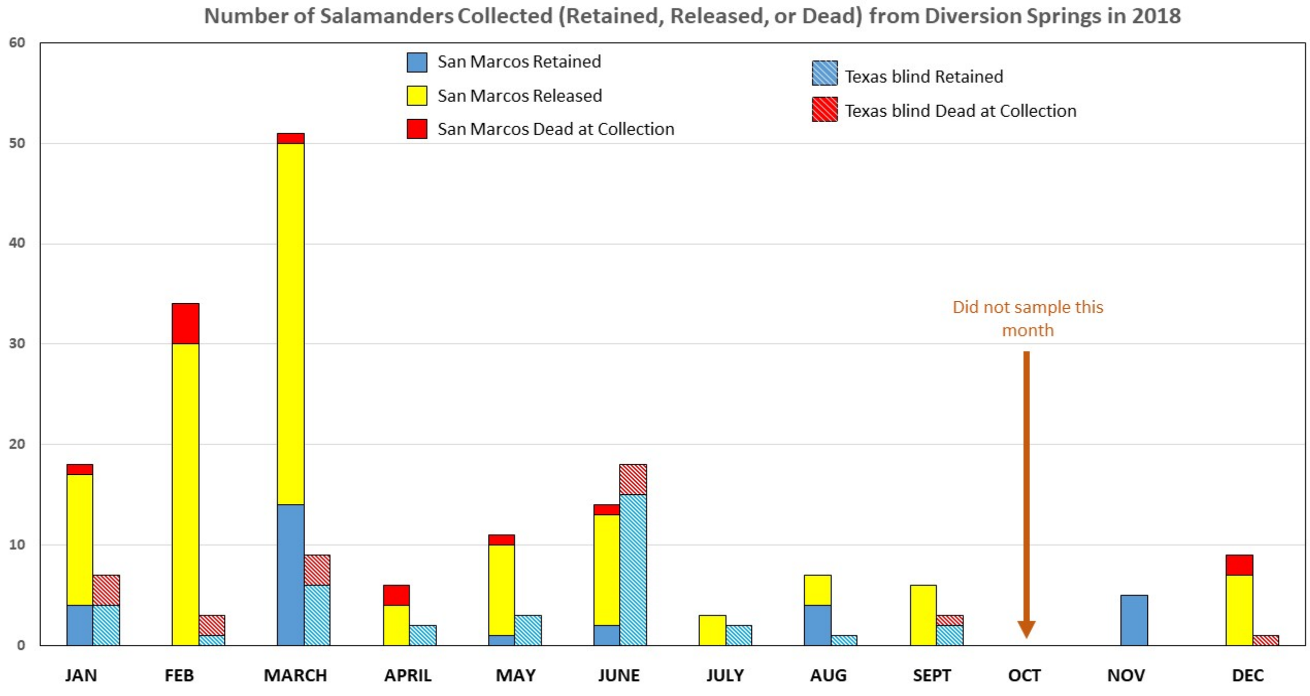

Figure 17 Total number of salamanders collected (the sum of all animals either dead at the time of collection, released, or retained) by month from Diversion Springs net. San Marcos is represented by solid bars while Texas blind is

represented by hashed bars. When traps were deployed for Texas blinds in other sites, this net was not sampled. During the month of October the net was not sampled.

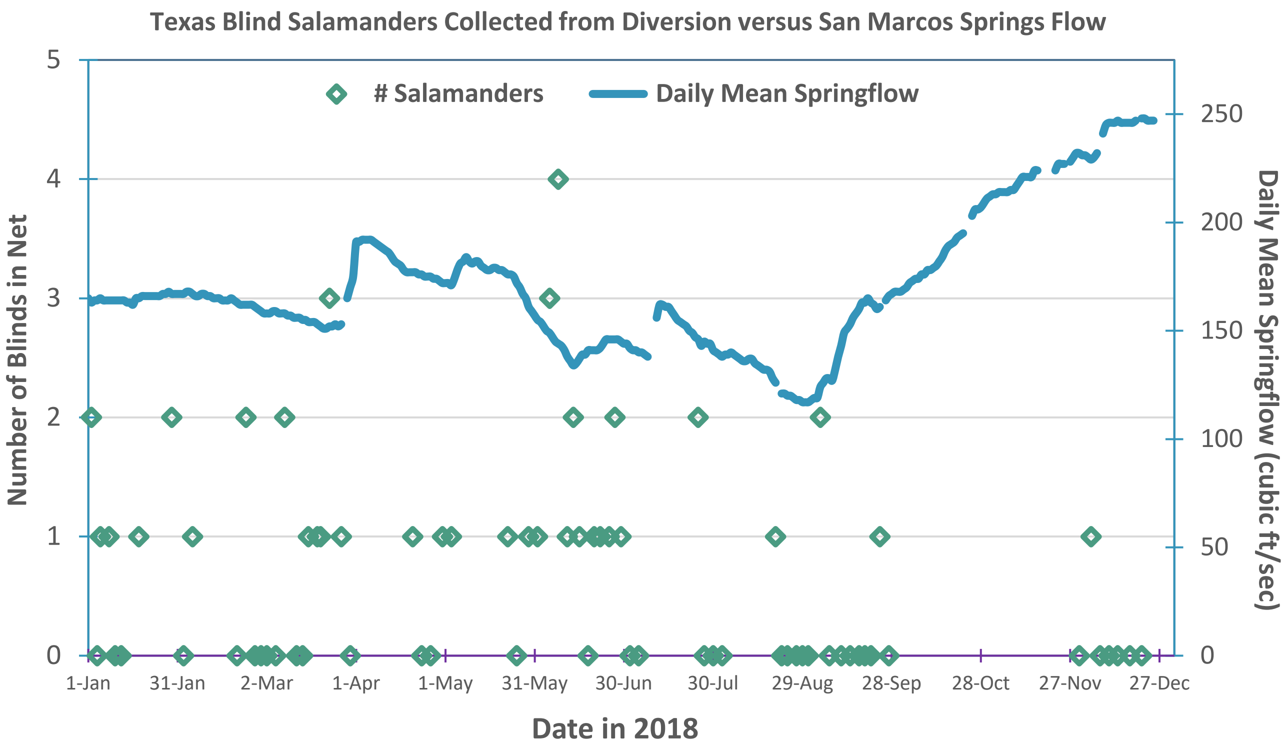

Figure 18 Texas blinds salamanders found in net (total) versus daily mean flow of San Marcos springs (USGS 08170000) for 2018. Spring flow is equal to river flow at 08170500 San Marcos River except for periods with local runoff. Heavy rainfall and runoff events are excluded from the figure. Springflow is only available until December 25th, 2018. The net was not sampled September 27-November 14.

Quarantine procedures

Texas blind salamanders were transported directly to the quarantine of SMARC after collection. The quarantine area is a separate, biologically secure area away from the refugia systems, preventing the spread of disease and aquatic nuisance species. Salamanders were acclimated to quarantine water conditions over the course of several hours after arrival. Healthy individuals collected from Diversion Spring net were transported back to SMARC where they are 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 reach 30 mm TL. Skin swabs were sent to the FHU and tested for presence of *Batrachochytrium dendrobatidis* (Bd, commonly referred to as amphibian chytrid fungus) and *Batrachochytrium salamandrivorans* (Bsal). Texas blind salamanders were housed in quarantine according to their collection location, collection date, and size. Individuals remained in quarantine for 30 days under observation before being incorporated towards Standing Stock numbers.

Survival rates

In 2018, biologists altered how newly captured small juvenile Texas blinds salamanders were housed. A significant proportion of mortality that occurred in 2017 for this species was small juveniles within the first 30 days after collection. Newly collected animals were previously held in group tanks with others collected from the same month and from the same location. Natural mortality due to injury, stress, or sickness as well as aggression and competition within groups resulted in decreased survival (approximately 50%). To compensate for these negative effects, a new housing plan was implemented in February 2018. All newly collected larval and juveniles were held in individual, isolated tanks. Each tank received its own flow of fresh well water and habitat items. Animals remained in isolation until they reach a larger size. During this time they were fed primarily newly-hatched *Artemia spp.* nauplii and were gradually introduced to blackworms, ostracods, and adult brine shrimp. Upon reaching 35-40 mm in total length, these now larger juveniles were given one dot VIE tags (for individual identification) under sedation (see Appendix F for Eurycea Salamander Sedation SOP) and combined with other newly tagged individuals of equivalent sizes. Salamanders continued their grow-out in these groups. Eventually, the entire group was moved to a larger system for further grow-out. Using this method, survival of newly collected Texas blind salamanders has improved to 90%. Some mortality still occurs and primarily does so within the first 30-days. Competition and aggression, particularly over food, occurred in groups; however, salamanders were not as vulnerable at larger sizes and mortalities have not occurred to date in these groups using this method.

Husbandry

Texas blind salamanders from all collection locations were housed together; however, individuals were tagged so that collection origin was known. We are awaiting a report of the population genetic structure for the USFWS Genetics Unit and will separate salamanders by location if differences are found. Texas blind salamanders were housed in large insulated fiberglass systems with partial recirculation through heater-chiller units to maintain the water temperature at 21 ℃ (ranging between 19 - 22 ℃). Smaller individual tanks were placed on these systems if needed. Water temperature and flow was checked daily. Water quality parameters, including ammonia, carbon dioxide, dissolved oxygen, nitrate, nitrite, pH, sulfide, and total gas pressure, were checked weekly.

Habitat enrichment items, including natural and artificial rock, plastic plants, and mesh, were placed throughout the tanks for salamanders to explore and seek refuge in. Staff routinely siphoned tanks to remove waste and other debris and rotate out habitat items to be cleaned. Each tank system had its own equipment (nets, cleaning supplies) to prevent the potential spread of pathogens from system to system. If equipment was shared it was cleaned and disinfected between systems. Adult salamanders were fed twice weekly and received either live amphipods or blackworms. Juveniles were fed *Artemia spp.* nauplii or chopped blackworms as they increased in size. A detailed description of Texas blind salamander daily care can be found in the USFWS Captive Propagation Manual for *Eurycea sp.,* available upon request.

Health Monitoring

Biologists monitored salamanders for changes in appearance or behavior 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 with flow-through well water. Mortalities were preserved in ethanol or formalin and a veterinarian was consulted, if needed, for investigation into the cause of death.

Maintenance of Systems

Salamander refugia systems were acid washed annually to remove calcium carbonate deposits that have formed within the tank, plumbing, chiller, and pump casing that can affect functionality. Water lines, hoses, valves, and restrictors were frequently checked for wear and clogs and were cleared, rebuilt, or replaced as needed.

Captive Propagation

To encourage production of offspring for future research, male and female salamanders, tagged so that collection location is known, were housed in group systems. Offspring produced during this combination can be identified by maternal origin but not paternal; thus, these offspring will not be used for restocking purposes. If future genetic analysis shows that collection locations are part of one panmictic population, then these offspring could be used should a restocking event occur.

Female Texas blind salamanders produced three clutches of eggs in 2018. In February, a female oviposited eggs; however, the majority were laid on a perforated screen divider and could not be salvaged, or did not develop after retrieval. Three salamanders hatched from the surviving eggs (total number of eggs laid could not be determined). To reduce this occurrence, additional habitat was added, including walls of netting and mesh along tank screens. A second clutch of 12 eggs was produced in March and five hatched. A third clutch of eight eggs was produced in April, three of which hatched. At the end of 2018, SMARC had 25 F1 offspring, nine of which were produced this year.

Figure 19 A male Texas blind salamander donated by Texas State University from Sessom Creek with unusual back legs. It is unclear if the legs are a deformity or regeneration from a loss of limbs. We continue to monitor his legs for changes. The salamander is medium sized adult that swims well and uses the back legs for support and balance.

San Marcos salamander (*Eurycea nana*), Threatened

The Standing Stock goal for the San Marcos salamander is 500 individuals divided between the two facilities. Large collections efforts in Spring Lake with divers were organized twice a year, with additional salamanders collected from the falls below Spring Lake dam and Diversion Springs net. Numerical data on numbers incorporated, end of the year census, and survival rates can be found in Table 7.

Collections

USFWS SCUBA divers collected adult San Marcos salamanders using dip nets from Spring Lake near spring orifices in March and September (see Figure 20 for locations). Once a salamander was captured, the dip net was brought to the surface where support staff processed each individual. Staff inspected each salamander for abnormalities, injuries, or lesions. Any abnormal individuals were noted, enumerated, and returned to where they were found. Each salamander's total length (TL) was recorded and gravidity noted if present. Staff then ran a cotton swab (in duplicate) down the ventral side of the salamander and around its limbs to collect material for Chytrid fungus testing. The swab tips were placed into pre-labeled centrifuge vials and were stored in a freezer until they were sent to the FHU, to test for two types of Chytrid fungus, *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal).

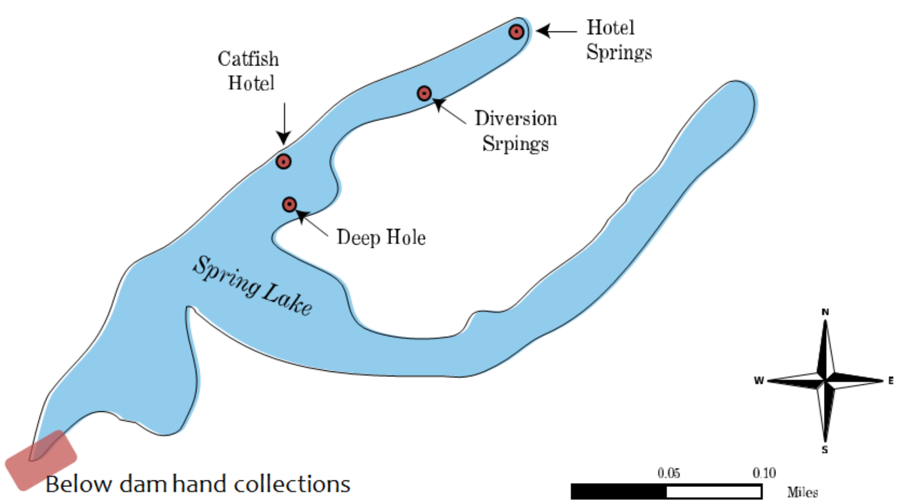

Figure 20 Locations of San Marcos salamander collections in Spring Lake, San Marcos, TX.

Salamanders were placed into transport coolers with a temperature probe and mesh for the salamanders to hold onto. Gravid females were kept separately in a small transport cooler. Before coolers were loaded for transport, water was refreshed and the temperature was recorded. Coolers were carefully loaded in the transport vehicle to minimize agitation.

In December, Refugia staff from both facilities captured salamanders from the falls area below Spring Lake dam via dip-nets by carefully searching under rocks and through substrate. Throughout the year larval, juvenile, sub-adult, and adult San Marcos salamanders were collected in the Diversion Spring net. When quarantine space was available at SMARC, a portion of these were retained for Refugia.

Table 7 San Marcos salamander refugia population figures.

	Beginning of Year Census	Incorporated 2018	End of Year Census	In Quarantine End of Year	Target Goal 2018 Work Plan	Percent Survival
SMARC	267	122	275	30	300	71% ([1]88%/54%)
UNFH	181	99	232	25	250	83% ([3]76%)

[1]*Survival rate of San Marcos salamanders collected fall 2017-present and collected spring 2017-past*
[3]*Survival rates for salamander collected in 2017, the first year of collections for UNFH*

Quarantine procedures

Salamanders 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. 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 Diversion Spring net were transported back to SMARC where they were measured and those with a TL 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 reached 30 mm TL. Skin swabs were sent to the FHU 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 under observation before being counted towards Standing Stock numbers.

Survival rates

Mortality due to rupture of eggs and other egg-related mortalities reduced in number in 2018 to account for 35% of all adult mortality, as opposed to 50% in 2017 (at SMARC). Females who expired to these causes seemed unable to release or reabsorb eggs naturally, causing bloating of the body cavity, blockage of the cloaca, or rupturing of eggs and organs from the body cavity. Primarily this has occurred with San Marcos salamanders that are of unknown older ages rather than newly collected and presumably younger individuals. Refugia research activities planned for 2019 will further investigate potential causes for this mortality issue and will be comparing egg quality, composition, nutrition, and pathogens in captive stock versus wild individuals.

At SMARC, there was a marked difference in survivor rates between San Marcos salamanders that were collected fall 2017 – present compared to those collected before the fall of 2017. Most of these older salamanders were heritage salamanders already at the facility before the Refugia Program started. A portion of salamanders collected in spring 2017 were co-mingled with the heritage salamanders before the procedure of tagging by year collected started so we cannot differentiate them from heritage salamanders. Salamanders collected in the fall of 2017 – present have not been mingled with the heritage salamanders in tanks or shared water systems. Survival rates of the heritage group of salamanders was 54% in 2018. Whereas, survival of the more recently collected salamanders was 88% and of those 24 mortalities 19 occurred to very small juveniles collected from Diversion Springs net in January as the new holding system for young juveniles was being perfected.

UNFH staff also sent mortalities at their facility to FHU for analysis. Findings reported complications from *Microsporidea* infections, causing necrosis and atrophy in the pelvic girdle area and the gonads. UNFH average survival rates of San Marcos salamander were lower in 2018 at approximately 83%, compared to the 90% in 2017. Approximately half of the San Marcos salamanders reported in the ending inventory were collected near the end of the year in the fall collections. These salamanders have not been on station longer than three months and have displayed very little mortality. Of the original San Marcos salamanders that were carryovers from 2017, there were 181 in January and 138 in December with an average survival of 76%.

Husbandry

Genetic analysis (Lucas *et al*. 2009) determined that there is no population structure within this species between the sites sampled in the wild, so individuals from all collection locations were combined. At SMARC, individuals were marked with a Visible Implant Elastomer (VIE) tag on the right side posterior to the hip to indicate the year collected and on the left side posterior to the hip to indicate the sex of the individual (Figure 21). San Marcos salamanders at both facilities were housed in large insulated fiberglass systems with partial recirculation through heater-chiller units to maintain water temperature at 21 °C (ranging between 19 - 22 °C). Smaller individual tanks were placed on these systems if needed. Water temperature and flow was checked daily. Water quality parameters, including ammonia, carbon dioxide, dissolved oxygen, nitrate, nitrite, pH, sulfide, and total gas pressure, were checked weekly.

Figure 21 Tagging of a male San Marcos salamander and tag viewed under UV light.

Habitat enrichment items, including natural and artificial rock, plastic plants, and mesh, were placed throughout the tanks for salamanders to explore and seek refuge in. Staff routinely siphoned tanks to remove waste and other debris and rotated habitat items to be cleaned. Each tank system had its own equipment (nets, cleaning supplies) to prevent the potential spread of pathogens from system to system. If equipment was shared, it was cleaned and disinfected between systems. Feeding occurred Monday, Wednesday, and Friday, varying between live amphipods and live black worms. Juveniles were fed *Artemia spp.* nauplii or chopped blackworms as they increased in size. A detailed description of San Marcos salamander daily care can be found in the USFWS Captive Propagation Manual for *Eurycea sp.,* available upon request.

Health Monitoring

Biologists monitored salamanders for changes in appearance or behavior including anorexia, bloating, lethargy, discoloration, development of external lesions or ulcers, mechanical damage, and abnormal swimming or walking. Salamanders that became sick or injured were removed from group housing and placed in isolated, individual hospital units with flow-through well water. Mortalities were preserved in ethanol or formalin and a veterinarian was consulted, if needed, for investigation into the cause of death.

Maintenance of Systems

Salamander refugia systems were acid washed annually to remove calcium carbonate deposits that have formed within the tank, plumbing, chiller, and pump casing that can affect functionality. Water lines, hoses, valves, and restrictors were frequently checked for wear and clogs, and were cleared, rebuilt, or replaced as needed.

Captive Propagation

During 2018 female and male salamanders were housed in mixed groups to encourage reproduction in refugia systems at both facilities. Reproduction can occur year-round as females come in and out of gravidity. Details on the 2018 San Marcos salamander reproduction research can be found in the Research section and in the full report document in the Appendix C.

At SMARC in 2018, wild stock females produced eight clutches (example of developing eggs Figure 22). Four of these clutches were not viable (one in February, one in April, and two in September). Viable clutches by wild parents were produced in January (19 deposited, 12 hatched), April (9 deposited, 7 hatched), May (12 deposited, three hatched but all three did not transition to feed after hatching and subsequently did not survive), and July (31 deposited, 22 hatched). First generation (F1) parents produced a clutch of 45 eggs and 38 of these hatched. Second generation adults produced one clutch of 73 eggs, but we did not allow this clutch to develop as we have many offspring from this same pair; the eggs were preserved for genetics and pathogen analysis. During 2018, 29 third generation (F3) adults were given to the Meadows Center and Texas State University for education, outreach, and display at Spring Lake. At the end of 2018, SMARC has 28 San Marcos salamander offspring of varying generations. At UNFH, a wild stock female produced a small clutch of nine eggs in December; eight of the eggs hatched successfully.

Figure 22 Larval San Marcos salamanders.

Comal Springs salamander (*Eurycea sp.*), Petitioned

The Standing Stock goal for the Comal Springs salamander is 500 individuals divided between the two facilities. Staff have prioritized efforts in 2018 to collect Texas blind salamanders, as these salamanders are more rarely collected than are Comal salamanders, and San Marcos salamanders, as their ESA listing is higher priority. Collections to build the refugia population numbers of Comal salamanders have been limited by lower historical densities of Comal Springs salamanders in the currently utilized sampling locations (compared to sampling locations of San Marcos salamanders). Lower densities in the sampling locations we utilize should not be taken as a comment or speculation on overall population size. As total refugia population targets are approached, especially for Texas blind salamanders, opportunities to expand efforts to collect Comal salamanders will increase. Numerical data on numbers incorporated, end of the year census, and survival rates can be found in Table 8.

Collections

USFWS staff snorkeled to collect adult Comal Springs salamanders using dip nets around the Spring Island area of Landa Lake in June, October, and November. Once a salamander was captured, staff inspected each salamander for abnormalities, injuries, or lesions. Any abnormal individuals were noted, enumerated, and returned to where they were found. Each salamander's total length was recorded and gravidity noted if present. Staff then ran a cotton swab (in duplicate) down the ventral side of the salamander and around its limbs to collect material for Chytrid fungus testing. The swab tips were placed into pre-labeled centrifuge vials and were stored in a freezer until they were sent to FHU to be tested for Chytrid fungi, *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal).

Salamanders were placed into transport coolers with a temperature probe and mesh for the salamanders to hold onto. Gravid females were kept separately in a small transport cooler. Before coolers were loaded for transport, water was refreshed and the temperature is recorded. Coolers were carefully loaded in the transport vehicle to minimize agitation.

Special Salamander Collection

Biologists discovered Comal Springs salamander eggs in the wild in June of 2018. Discovery of wild aquatic salamander eggs is exceedingly rare and this was the first documented occurrence for this species. Staff are in the process of producing a Note for a peer reviewed herpetological publication on the findings and development of the eggs for 2019. Barton Springs salamander eggs have been discovered in the wild, but never Texas blind nor San Marcos salamander eggs. Location, conditions, and habitat was noted and documented photographically. Staff collected two of these salamander eggs adhered to a rock and transported them back to SMARC (Figure 23). Both eggs hatched and both individuals transitioned to feed and continue to grow. Pictures were taken daily of the developing eggs in captivity. Though the eggs hatched in captivity, they were discovered in and collected from the wild and, thus, the two individuals are considered wild stock.

Figure 23 Developing Comal Springs salamanders on rock they were found on in the field.

Table 8 Comal Springs salamander refugia population figures.

	Beginning of Year Census	Incorporated 2018	End of Year Census	In Quarantine End of Year	Target Goal 2018 Work Plan	Percent Survival
SMARC	47	40	72	0	70	83% ([2]92%)
UNFH	4	15	18	0	30	95% ([3]75%)

[2]*Survival rate of Comal Springs salamanders without escape event*
[3]*Survival rate of Comal Springs salamanders collected in 2017*

Quarantine procedures

Salamanders 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. Salamanders were acclimated to quarantine water conditions over the course of several hours after arrival. Comal Springs salamanders were swabbed in the field before being transported back to their respective facilities. Skin swabs were sent to FHU 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. Individuals remained in quarantine for 30 days under observation before being counted towards Standing Stock numbers.

Survival rates

Overall, survival rates of Comal Springs salamanders were high, with few individuals succumbing to sickness. Mortalities of this species tend to be attributed to escapement from their tanks. Refugia staff are iteratively modifying the Comal Springs salamander tanks to prevent escapement, as the salamanders find different ways to escape tanks. At SMARC, a large mortality event occurred after a mass escapement of Comal Springs salamanders in August. Kelsey Andersen arrived in the Pad on Monday August 13 to find a well water line disconnected from the Comal Springs salamander tank. She found salamanders under the line in a pool of water. She retrieved those salamanders and called for back-up in determining if other salamanders had escaped. Four other biologists came to assist in looking for salamanders that had escaped down into the trench drain system. Ms. Anderson examined the salamander tank and did not find a moisture trail at that time, but we assumed that when the hose started breaking free it sprayed water along the sides of the tank creating a trail for the salamanders to climb up. Ms. Anderson moved the remaining salamanders into a fresh tank system and inventoried them. A total of 19 salamanders had escaped. While searching the trench drain systems, staff quickly realized the chlorine tabs that were on the outflow of other systems (to prevent possible pathogens from entering the station drainage system) were causing a deadly environment for the escaped salamanders. Water was quickly shut off from those systems and fresh well water was used to flush the drain. Nevertheless, eight salamanders succumbed to chlorine poisoning in the drainage system that day and one more at a later date.

Escapement of one or two salamanders with a well-established water trail might be anticipated, but a mass exodus of this magnitude has never occurred before at SMARC. As there was not a water trail present when the escape was discovered, we can only speculate as to how the escape occurred. A non-Refugia staff member who performed the weekend walk-through later informed us that he had found the hose disconnected on Sunday and thought he fixed it, but did not bring it to our attention at the time. To prevent a repeated loss event, an additional tank lid has been added to the Comal Springs salamander system.

UNFH average survival rates of Comal Springs salamander were higher in 2018 at approximately 95%, compared to the 44% in 2017. These numbers were calculated by adding the initial inventory to the number of incorporated stock and dividing the end of year inventory by this sum. Most of the Comal Springs salamanders reported in the ending inventory, 15 of the 18, were collected near the end of the year in the fall collections. These salamanders had not been on station longer than three months and displayed no mortality. Of the original Comal Springs salamanders that were carryovers from 2017, there were four in January and three in December with an average survival of 75%.

Husbandry

At SMARC, individuals were marked with a Visible Implant Elastomer (VIE) tag on the right side posterior to the hip to indicate the year collected and on the left side posterior to the hip to indicate the sex of the individual. Comal Springs salamanders at both facilities were housed in large insulated fiberglass systems with partial recirculation through heater-chiller units to maintain the water temperature at 21 °C (ranging between 19 - 22 °C). Smaller individual tanks were placed on these systems as needed. Water temperature and flow was checked daily. Water quality parameters, including ammonia, carbon dioxide, dissolved oxygen, nitrate, nitrite, pH, sulfide, and total gas pressure, were checked weekly.

Habitat enrichment items, including natural and artificial rock, plastic plants, and mesh, were placed throughout the tanks for salamanders to explore and seek refuge in. Staff routinely siphoned tanks to remove waste and other debris and rotated habitat items to be cleaned. Each tank system had its own equipment (nets, cleaning supplies) to prevent the potential spread of pathogens from system to system. If equipment was shared it was cleaned and disinfected between systems. Feeding occurred Monday, Wednesday, and Friday, varying between live amphipods and live black worms. Juveniles were fed *Artemia spp.* nauplii or chopped blackworms as they increase in size. A detailed description of Comal Springs salamander daily care can be found in the USFWS Captive Propagation Manual for *Eurycea sp.,* available upon request.

Health Monitoring

Biologists monitored salamanders for changes in appearance or behavior including anorexia, bloating, lethargy, discoloration, development of external lesions or ulcers, mechanical damage, and abnormal swimming or walking. Salamanders that became sick or injured were removed from group housing and placed in isolated, individual hospital units with flow-through well water. Mortalities were preserved in ethanol or formalin and a veterinarian was consulted, if needed, for investigation into the cause of death.

Maintenance of Systems

Salamander refugia systems were acid washed annually to remove calcium carbonate deposits that have formed within the tank, plumbing, chiller, and pump casing that can affect functionality. Water lines, hoses, valves, and restrictors were frequently checked for wear and clogs, and were cleared, rebuilt, or replaced as needed.

Captive Propagation

During 2018 female and male salamanders were housed in mixed groups to encourage reproduction in refugia systems at both facilities. Reproduction can occur year-round as females come in and out of gravidity. Wild stock females produced one clutch of eggs at each facility in 2018. At UNFH a clutch of 25 eggs were produced in April with 20 individuals hatched successfully. A wild stock female produced a clutch of 19 eggs at SMARC in June; 12 of these hatched. Interestingly, this clutch was deposited within two days of when eggs were discovered in the wild. At the end of 2018 UNFH had 12 F1 Comal Springs salamanders and SMARC had 12 F1 Comal Springs salamanders.

Comal Springs riffle beetle (*Heterelmis comalensis*), Endangered

Collections of Comal Springs riffle beetles (CSRB) were reduced in 2018 compared to 2017 to decrease potential impacts on the wild population. A portion of adult CSRB found on each lure set location were kept for refugia (January, February, March, June, July, November, and December), while the remaining adults and non-target species were carefully returned. Numerical data on numbers incorporated, end of the year census, and survival rates can be found in Table 9.

Collections

CSRB were collected using poly-cotton cloth lures and wooden dowels placed together in spring orifices within the Comal Springs system in New Braunfels, Texas. Staff also hand collected adult CSRB from river wood found near upwellings. Cotton lure collections followed the standard protocol for cotton lures in the Comal River system. Wooden lures were made from poplar dowel rods, cut in half length-wise and sanded before being conditioned with biofilm growth from the springs. During lure collections staff recorded water quality conditions at the sampling locations. Our Refugia invertebrate specialists examined the cotton lures and wooden dowels in shallow trays filled with spring water to identify and enumerate the invertebrates (Figure 24). Organisms not retained for refugia were carefully returned to the specific lure spring collection location.

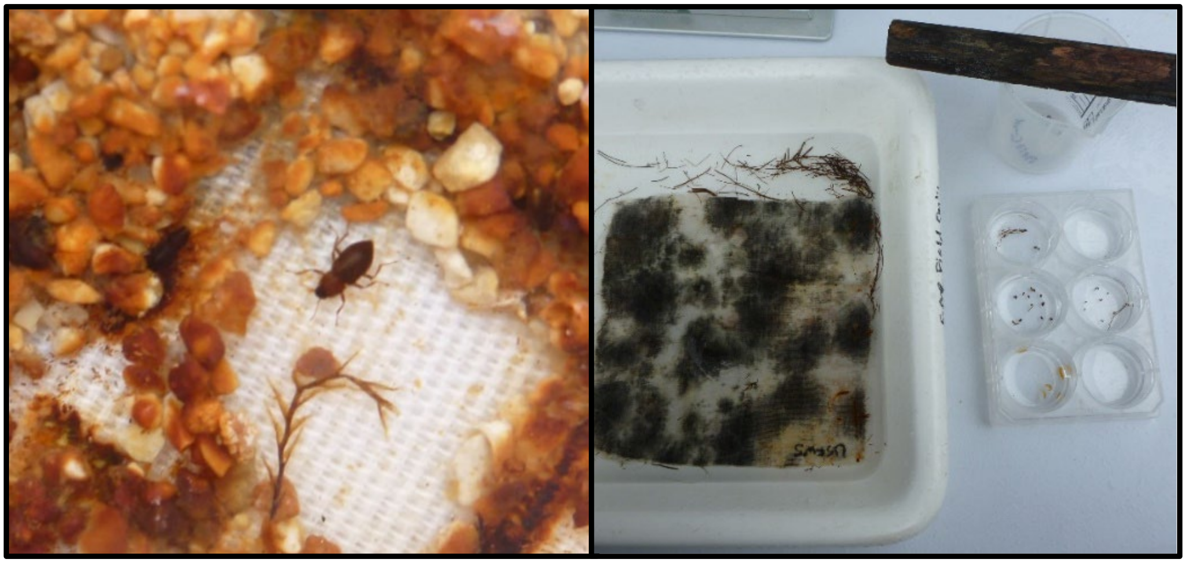

***Figure 24** CSRB on a lure and a lure unfolded and sorted through.*

***Table 9** Comal Springs riffle beetle refugia population figures.*

	Beginning of Year Census	Incorporated 2018	End of Year Census	In Quarantine End of Year	Target Goal 2018 Work Plan	Percent Survival

SMARC	191	443	162	106	175	26%
UNFH	51	16	14	0	100	21%

Quarantine

Incoming CSRB were quarantined in separate systems than the existing refugia population in the Invertebrate Room at SMARC or the quarantine room at UNFH. CSRB were acclimated to quarantine water conditions at a rate not exceeding one degree Celsius every half-hour. During the quarantine period staff monitored for potential ANS that may have come in with the collection, the general health of the organisms, or any large die-offs that might indicate a disease. If none of these events occurred, then CSRB joined the Refugia population at the end of the 30 day quarantine period.

Survival Rates

Before 2018, survival of CSRB adults in captivity at SMARC was relatively unknown; invertebrate biologists estimated adults could survive for one year, but no formal research was conducted. Because only adult CSRB are retained from the field and the age of these adults are unknown, higher mortality rates were expected in this species as they die due to limited life span. Without the numbers associated with the fall collection at UNFH, the overall survival would have been 0%. All of the 2017 carryover stock at UNFH died in spring 2018 after a Spurgeon well (Edwards Aquifer water) failure and higher than expected water temperatures and an inability to effectively chill the Carrizo-Wilcox Aquifer water from the Wilson well. More effort is being directed into increasing survival rates for captive CSRB in 2019.

In 2018, investigations began into adult survival at SMARC began. First, biologist kept individual collection cohorts (those collected on the same date) in their own container instead of co-mingling individuals from several collection dates after the quarantine period was completed as had been the practice in previous years. Second, biologists compared survival rates in two holding systems: the flow through box design (standard design) and flow through upwelling tubes. Flow through upwelling tubes were based on a design that successfully reared larvae and F1 adults. After each sampling event, adults were split between the box design and an upwelling tube. CSRB from each collection event were kept separate so that each cohort could be tracked through time. An inventory was conducted approximately every other month and differences between the holding systems were enumerated and recorded.

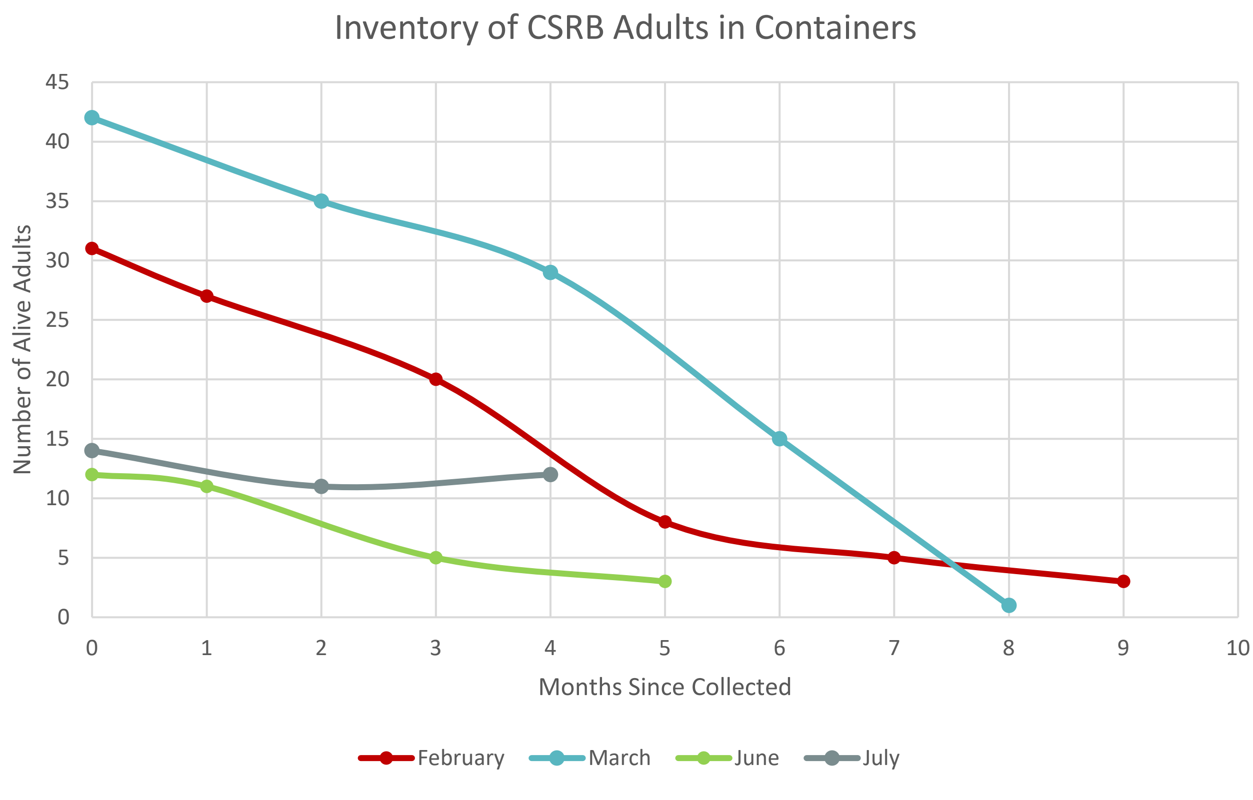

Figure 25 Survivorship of CSRB by collection date in flow through box containers.

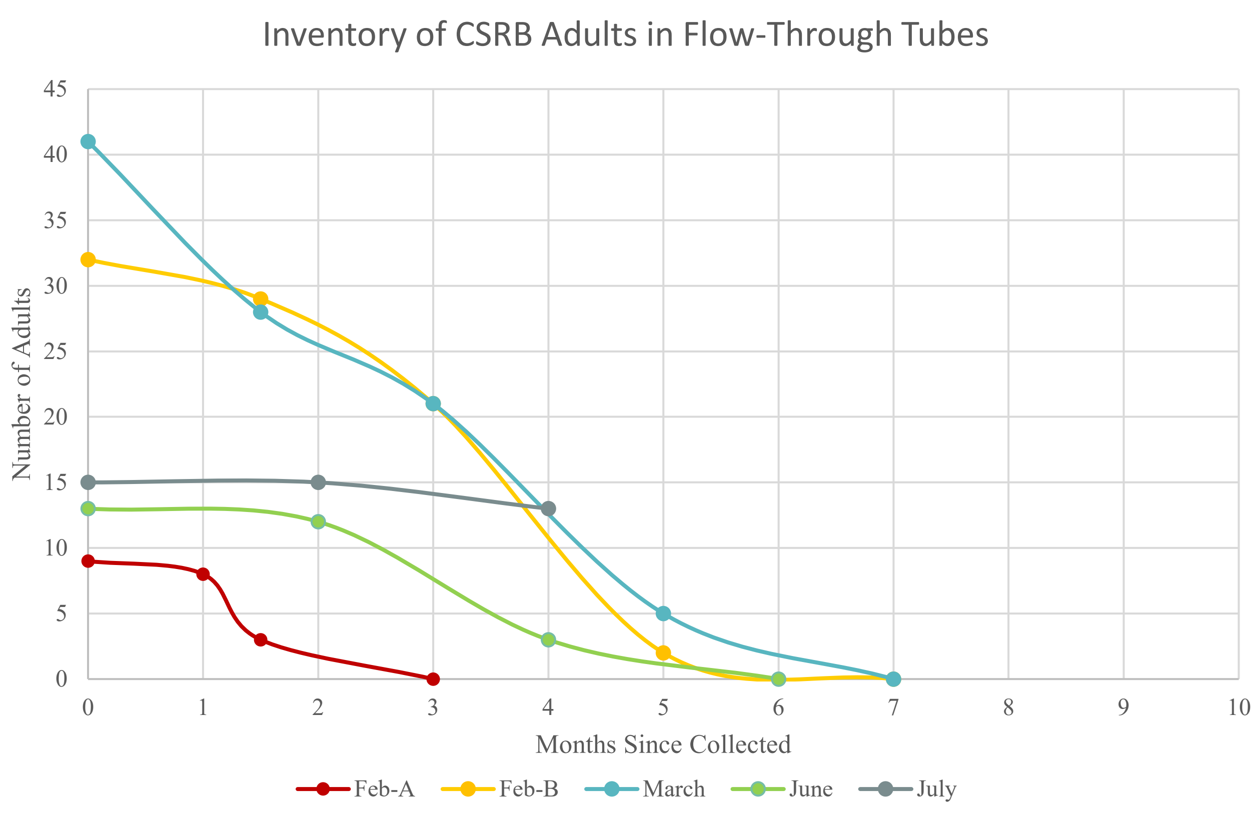

Figure 26 Survivorship of CSRB by collection date in Flow-through Tubes

Our study indicated that survivorship decreased rapidly in flow through boxes after five to seven months (Figure 25). Implementation of flow through upwelling tubes required a learning curve for the staff to get them to properly function. In order to truly compare these two system designs, more data needs to be collected now that staff have the two system types functioning optimally. Errors in the tube implementation of the upwelling tubes were fixed, which helped create efficiencies in the condition of the tubes despite the losses that occurred. For example, when assembling tubes, choosing an aged dowel was a critical decision. Inserting relatively fresh poplar dowels resulted in high white fungal accumulation throughout the tube, which is difficult to recognize in enough time to clean before it takes over the whole tube. Dowels that sink when placed in water fared better than dowels that floated on the surface. February-B and March cohorts had less conditioned dowels and a greater drop-off in survivorship than June and July cohorts (Figure 26). CSRB adults in containers produced almost twice as many larvae than in flow-through tubes. However, this difference could be due to the fungal blooms and possibly lack of adequate space and/or leaf nutrition found during the early phases of flow-through tube implementation. By the end of the year, there was little to no fungal growth and plenty of leaf material in tubes at each inventory. These changes and notes will be added and updated within the SOP for 2019.

Husbandry

On a daily basis, all the systems were checked for water temperature, adequate flow, and clear drain screens to maintain drainage and water level. CSRB refugia systems were not siphoned because adults, larvae, or eggs could easily be discarded along with debris. As CSRB feed predominantly on biofilm, they do not have a traditional feeding schedule. Alternatively, leaves and cotton cloth containing biofilm are used in each system, providing them with food. Inventories were conducted every other month and new leaf and cotton material was added as needed.

Maintenance of systems

Culture containers used to house CSRB were plastic totes with PVC piping that delivered water in a manner that mimics upwellings. Vertical flow through tubes were also used; these consisted of clear PVC, that maked up the viewing chamber, and threaded PVC couplings and reducers. The PVC tubes contained leaves, biofilm cloth, and mesh for structure and habitat. The systems did not have a traditional cleaning or siphoning schedule, but alternatively, were cleaned during inventory. At this time, staff checked water lines, hoses, and valves for functionality and cleaned or replaced them as needed.

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 from wild stock adults. Over 2018, wild stock CSRB adults produced 5,083 F1 larvae at SMARC (seven F2 larvae were produced) and 25 larvae at UNFH.

Comal Springs dryopid beetle (*Stygoparnus comalensis*), Endangered

Due to the rarity of Comal Springs dryopid beetles (CSDB) collected in the field using poly-cotton lure and dowel collection techniques, yearly population goals were not set in the Work Plan for this species. USFWS contracted BIO-WEST, Inc. for life history research on CSDB. The majority of the CSDB refugia population at SMARC were borrowed for research conducted by BIO-WEST, Inc. A summary of the research can be found in the Research section and the full report in Appendix E: Life-History Aspects of the Comal Springs dryopid beetle (*Stygoparnus comalensis*) and notes on life-history aspects of the Comal Springs riffle beetle (*Heterelmis comalensis*). Numerical data on numbers incorporated, end of the year census, and survival rates can be found in Table 10.

Collections

In 2018, six sampling events occurred for CSDB in the Comal Springs system at Spring Runs 1 and 3 and Landa Lake by setting poplar wooden dowels adjacent to poly-cotton lures near spring orifices in order to attract CSDB. Only five dowel samples successfully attracted dryopids in 2018. It appears that over time, attraction to dowels is reduced as they darken and contain less biofilm. We recommend that if poplar dowels are used in the future they be refreshed after 3-4 months and testing other, softer woods as attractor lures in the future. The most reliable collection method was from naturally decaying wood pieces found directly over spring sources.

Table 10 Comal Springs dryopid beetle refugia population figures.

	Beginning of Year Census	Incorporated 2018	End of Year Census	In Quarantine End of Year	Target Goal 2018 Work Plan	Percent Survival
SMARC	13	3	2	2	*	13%
UNFH	2	0	0	0	*	0%

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

Quarantine

Incoming CSDB were quarantined in separate systems than existing refugia stock in the Invertebrate Room at SMARC or the quarantine room at UNFH. CSDB were acclimated to quarantine water conditions at a rate not to exceed one degree Celsius every half-hour. During the quarantine period, staff monitored for potential ANS that may have come in with the collection, the general health of the organisms, or any large die-offs that might indicate a disease. If none of these events occurred, then CSDB joined the Refugia population at the end of the 30 day quarantine period.

Survival Rates

We are working to refine husbandry techniques and holding container designs for CSDB in order to increase survival rates in future years. Systems used in early 2018 failed in various ways for adequate habitat for the dryopids. Culturists tried several changes but still have not been successful at rearing eggs or larvae and keeping adults alive long-term. Staff started testing the use of flow through upwelling tubes in refugia in December 2018 and will continue to monitor for comparisons in survival and production with the flow through box systems.

Husbandry

On a daily basis, all the systems were checked for water temperature, adequate flow, and clear drain screens to maintain drainage and water level. Conditioned wooden dowels in the containers were checked for fungal growth, and if found were removed, as CSDB have been known to become entrapped in the fungus and parish. CSDB refugia containers were not siphoned for debris because CSDB adults, larvae, or eggs could easily be discarded along with debris. As the CSDB feed on biofilm, they do not have a traditional feeding schedule. Alternatively, leaves, wooden dowels, and cotton cloth containing biofilm were placed in containers that provided them with a constant food source. Inventories were conducted every other month and new food items were added as needed.

Maintenance of systems

Culture containers used to house CSDB were plastic totes with PVC piping that delivered water using a bar that sprays the side of the container and then flows down. The systems did not have a traditional cleaning or siphoning schedule, but alternatively, were cleaned during inventory. At this time, staff checked water lines, hoses, and valves for functionality and cleaned or replaced them as needed.

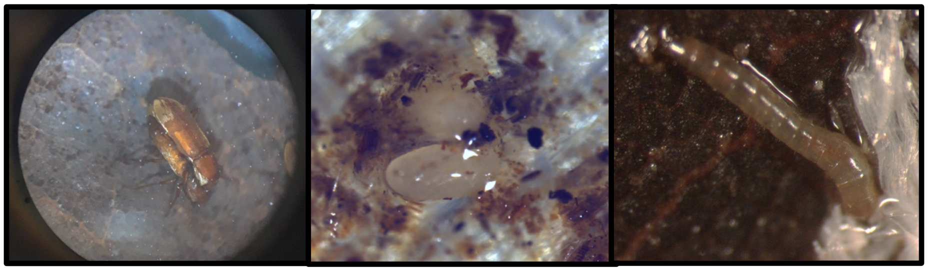

Figure 27 L to R: Mated pair of CSDB, eggs, and larvae (photos by BIO-WEST, Inc.).

Captive Propagation

To encourage production of offspring, male and female wild stock were housed together. BIO-WEST, Inc. staff were able to produce eggs and larvae during their research and Refugia staff plan to continue with their techniques for propagation in 2019 (Figure 27).

Peck's cave amphipod (*Stygobromus pecki*), Endangered

USFWS contracted with BIO-WEST, Inc. to conduct research on Peck's cave amphipods (PCA) during 2018; a summary of their results can be found in the Research section and a full report in Appendix D: Life-History Aspects of *Stygobromus pecki.* Numerical data on numbers incorporated, end of the year census, and survival rates can be found in Table 11.Collections

Adult PCA were collected by hand from the loose rock and gravel around spring upwellings near Spring Island in the Comal River, New Braunfels, Texas. Biologists used small aquarium nets to scoop sand and gravel before carefully sifting through it in trays for PCA (Figure 28). Hand collections occurred in June, July, November, and December in 2018 (Figure 29). Occasionally PCA were collected off the poly-cotton lures set for the beetle species. PCA collected in driftnets during biomonitoring surveys were transferred to SMARC for refugia purposes.

Figure 28 Refugia staff snorkeling to collect PCA.

Table 11 Peck's cave amphipod refugia population figures.

	Beginning of Year Census	Incorporated 2018	End of Year Census	In Quarantine End of Year	Target Goal 2018 Work Plan	Percent Survival
SMARC	173	308	272	46	250	58%
UNFH	45	58	25	43	100	24%

Quarantine

Incoming PCA were quarantined in separate systems than existing refugia stock in the Invertebrate Room at SMARC or the quarantine room at UNFH. PCA were acclimated to quarantine water conditions at a rate not exceeding one degree Celsius every half-hour. During the quarantine period staff monitored for potential ANS that may have come in with the collection, the general health of the organisms, or any large die-offs that might indicate a disease. If none of these events occurred, then PCA joined the Refugia population at the end of the 30 day quarantine period.

Figure 29 PCA collected in dip net.

Survival Rates

While PCA have the highest survival rate of the Refugia invertebrate species, we still strive for improvement each year. It was noted by both SMARC and BIO-WEST, Inc. staff that while systems did not seem crowded and adequate flake food was added, there was a trend of greater survival rates after systems reached lower densities. It should be noted this was not tested against systems that started with lower densities, so it could be a die-off of unhealthy organisms regardless of space. Moving forward, lower densities will be used in systems and hopefully increased survival will be seen in 2019. These changes and notes will be added and updated within the SOP for 2019.

We attribute lower survival rates at UNFH to higher water temperatures during hotter months of the year when the facility could not use Edwards Aquifer water from their Spurgeon well, and instead had to use the hotter, Carrizo-Wilcox Aquifer water from the Wilson well. Culture systems could not effectively chill the water.

Husbandry

On a daily basis, all the systems were checked for water temperature, adequate flow, and clear drain screens to maintain drainage and water level. PCA were fed small amounts of fish flake two times weekly. Dried leaves from terrestrial sources were used as potential supplemental food and provided shelter within the systems. Pebble mats were added to PCA containers in 2018 to recreate the tight spaces of rocks much like their natural habitat. Transferring or using loose gravel substrate was not ideal because it would be too difficult to produce sufficient flow throughout the layers of sediment and could cause injuries to organisms during inventories. A backsplash-like panel made with pea gravel on large-pore nylon mesh as the backing was constructed and is undergoing testing as more habitat for *S. pecki* to hide within.

Maintenance of systems

Culture containers used to house PCA were plastic totes with PVC piping that delivered water in a manner to mimic upwellings. The systems did not have a traditional cleaning or siphoning schedule, but alternatively, were cleaned during inventory. At this time, staff checked water lines, hoses, and valves for functionality and cleaned or replaced them as needed.

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. Adults often cannibalize small PCA, and the brooding chambers provided 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. At SMARC brooding females were transferred to the care of BIO-WEST, Inc. staff for their research. UNFH had three brooding females during 2018; however, there were no FX generation PCAs on station at UNFH at the end of 2018.

Texas troglobitic water slater (*Lirceolus smithii*), Petitioned

Due to the current inability to differentiate *Lirceolus smithii* from others in the same genus in a non-lethal manner, we cannot be sure if the *Lirceolus sp*. collected for refugia are indeed Texas troglobitic water slaters (TTWS), but are often referred to as such (or simply as *Lirceolus sp*.) for refugia purposes. With each inventory, it has been almost impossible to differentiate between a wild stock and F1 adults. Because there are several species, not all of the same length and size, staff is wary to consider smaller individuals as juveniles or to deem them as F1 adults. Even when separating brooding females, at the next inventory when it is clear the mother has released her brood (due to the doubling and sometimes tripling in numbers), no distinguishing characteristics are known to distinguish the offspring from the mother. Inventories take several hours, which did not allow a monthly or weekly census of each container, given other duties and time constraints of staff. Thus, we decided to label the populations as general FXs and to let them grow and reproduce freely. It is clear the habitat and the food provided is sufficient: A total of 353 individuals were counted in October and 509 in January. These individuals were produced from the 25 at the beginning of the year and only an additional 38 added during the year. Numerical data on numbers incorporated, end of the year census, and survival rates can be found in Table 12.

Collections

Over the year, SMARC technicians did not find many *Lirceolus sp*. at Diversion or Comal Springs resulting in only 38 wild stock individuals collected in 2018. 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. UNFH did not house TTWS during 2018.

Table 12 Texas troglobitic water slater refugia population figures.

	Beginning of Year Census	Incorporated 2018	End of Year Census	In Quarantine End of Year	Target Goal 2018 Work Plan	Percent Survival
SMARC	25	38	2	3	*	**
UNFH	--	--	--	--	--	--

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

Quarantine

Incoming TTWS were quarantined in separate systems than existing refugia stock in the Invertebrate Room at SMARC. TTWS were acclimated to quarantine water conditions at a rate not to exceed one degree Celsius every half-hour. During the quarantine period staff monitored for potential ANS that may have come in with the collection, the general health of the organisms, or any large die-offs that might indicate a disease. If none of these events occurred, then TTWS joined the Refugia population at the end of the 30 day quarantine period.

Husbandry

On a daily basis, all the systems were checked for water temperature, adequate flow, and drain screens cleared of debris to maintain drainage and water level. TTWS were fed small amounts of fish flake once a week. Dried leaves from terrestrial sources were also used as potential supplemental food and to provide shelter within the systems.

Maintenance of systems

Culture containers used to house TTWS were plastic totes with PVC piping that delivers water in a manner to mimic upwellings. The systems did not have a traditional cleaning or siphoning schedule, but alternatively, were cleaned during inventory. TTWS are cryptic and could easily be discarded along with debris during siphoning. During inventory, staff checked water lines, hoses, and valves for functionality and cleaned or replaced them as needed. An inventory was conducted every other month.

Captive Propagation

TTWS were very productive in their holding containers, exponentially increasing their population numbers every time they were checked.

Edwards Aquifer diving beetle (*Haideoporus texanus*), Petitioned

No Edwards Aquifer diving beetles were collected during 2018. These beetles are rare with very little known about their native habitat, life history, or food. Previously, diving beetles have been collected from the Texas State Artesian well, but these are only opportunistic collections as beetles are ejected from the high flow spring. Numerical data on numbers incorporated, end of the year census, and survival rates can be found in Table 13.

Table 13 Edwards Aquifer diving beetle refugia population figures.

	Beginning of Year Census	Incorporated 2018	End of Year Census	In Quarantine End of Year	Target Goal 2018 Work Plan	Percent Survival
SMARC	0	0	0	0	*	--
UNFH	0	0	0	0	*	--

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

RESEARCH

San Marcos salamander reproductive study

The full report can be found in Appendix C.

Extended Abstract

The San Marcos salamander (*Eurycea nana*) is an aquatic, federally threatened plethodontid salamander endemic to the spring outflows in Spring Lake and just below Spring Lake dam in San Marcos, Texas. The San Marcos Aquatic Resources Center has established a refugia population (captive assurance colony) to be used if animals would need to be reintroduced into the wild. Successful and predictable breeding in captivity is critical to the success of the refugia in case restocking of the species were needed. To produce a reliable reintroduction plan for the species breeding rates, egg survivorship rates, juvenile survivorship rates, and length of time needed to produce the number of individuals required for restocking must be known.

Only two published studies have investigated reproduction methods in the San Marcos salamander (Fries 2002; Najvar et al. 2007). Fries (2002) examined simulated upwelling flows ranging from 1 $cm \cdot sec^{-1}$ to 5 $cm \cdot sec^{-1}$, but did not find significant results that upwelling flows effected reproduction. Najvar *et. al.* (2007) investigated captive pairwise breeding and found that 24 salamander pairs produced seven clutches of eggs (three to the same pair) over a period of nine months. Additional information on the reproduction strategies for this species is required to implement and maintain a successful refugia population.

The primary goal of this research was to compare breeding success (defined in terms of number of egg clutches laid) in paired versus group breeding tanks following the separation/reunion technique. We also sought to compare time to incipient reproductive behavior in paired versus group breeding tanks through the use of video observations. We hypothesized that grouping salamanders would encourage greater reproductive success by providing some degree of mate choice. Eggs produced were observed for hatching success and documenting egg developmental stages.

Though we did not have the number of egg clutches produced that we had anticipated, much can still be gleaned from the study and video of courtship behavior. In Najvar *et. al.* (2007) salamander pair success rate of clutches produced ranged from 21%-29%. Reproduction in *Eurycea* species may be at a lower rate than anticipated as the study of the separation-trigger technique in

Barton Springs salamanders (Cantu *et. al.* 2016) only resulted in 13 clutches of eggs laid from 60 pairs (22% success rate).

Time to both male and female incipient reproductive behavior was significantly less for groups than pairs, and a third of the pairs did not exhibit full courtship and likely did not mate within the first 48 hours. *E. nana* males employ a tactic of near constant pursuit of females, often engaging in long bouts of following and rubbing. Rubbing behavior was often intense, focusing primarily on the head of the female but also moving down the body and even to the tip of the tail. In some instances, for both groups and pairs, rubbing of the male was so forceful that females were flipped onto their backs and pushed. Over time the intense courtship behavior that was seen during the 24-48 hours after sexes were combined lessened but did not completely drop off by day 14 for either groups or pairs. With time, courtships transitioned to multiple non-reciprocated attempts by males, with fewer instances of female engagement.

Whether in groups or pairs we recommend the removal of males after 48 hours, 72 hours at the most, because they continue to pursue non-reciprocated overtures to females. This was observed even at the two-week mark where we stopped recording video. A successful mating will most likely happen during the 48-hour time period and extending exposure of highly amorous males may cause undue stress on non-receptive females.

With only one viable clutch of eggs produced in the study, we believe that there is a problem within either the system design or the salamanders themselves. In 2019, we plan to follow up on this study, further investigating potential causes for the lack of reproduction. One possibility may be to remove the males after an initial courtship period, possibly allowing for conditions more conducive for oviposition. However, we will investigate other avenues in conjunction with this so as not to repeat the experiment with a different group of animals. We plan to analyze egg content and physiological make up of salamanders held on station to those in the wild to see if there are any differences. We will use Gore-sorber modules to test water quality (similar to the tests run at our well sites) in the Refugia tanks for endocrine disruptors or detrimental abiotic factors. Further actions will be planned after obtaining results from these initial investigations.

Life-History Aspects of *Stygobromus pecki*

The full report can be found in Appendix D.

Executive Summary written by BIO-WEST, Inc.

The Edwards Aquifer Habitat Conservation Plan (EAHCP) calls for the establishment of captive refuge populations of Edwards Aquifer (EA) Covered Species associated with their Incidental Take Permit inhabiting both subterranean and spring outflow habitats. The San Marcos Aquatic Resources Center (SMARC) operated by the United States Fish and Wildlife Service (USFWS) has been awarded the opportunity to establish and maintain captive refuge populations of EA species of concern. Some of the species of concern still pose several substantial questions concerning refuge cultivation; particularly the invertebrate species. *Stygobromus pecki* is a federally endangered species that is adapted to subterranean habitats associated with Edwards Aquifer springs. *S. pecki* belongs to a rather speciose genus with > 135 described species; however, little is known about the life history and the environmental requirements of this species.

Common gardens of *S. pecki*, *S. flagellatus,* and *S. russelli* were kept in flow-through aquarium-style containers, holding about 15 L of water and outfitted with nylon mesh, leaves, and rock as habitat. Each garden was censused ca. once a month to search for brooding mothers and track mortality. Our common garden of *S. pecki* decreased from 31 individuals to eight over the course of 274 days. The rate of decrease began to flatten around 10 individuals, suggesting that fewer individuals should be kept within a confined space. Due to high amounts of mortality observed within the first five censuses, the other common gardens were moved to a smaller volume flow-through tube holding about 0.5 L of water utilizing PVC-shavings as habitat. Survivorship appeared similar to that of *S. pecki* and it is surmised that the amount of habitat, rather than the quantity of water is important for reducing captive mortality.

Twenty-six brooding females of *Stygobromus* were observed from the SMARC refugia and BIO-WEST common gardens, producing a total of 139 eggs. From the common garden of *S. pecki* alone, five out of 19 females produced broods consisting of 28 eggs over the course of this study. There was no relationship between female size and number of eggs produced. High egg mortality was observed, presumably due to female stress. It is recommended that brooding females should be removed to separate containers to minimize stress and to give neonates a nursery to inhabit after being released from the marsupium.

The average egg incubation time for *S. pecki* was 49.7 ± 12.7 days with ca. 24% developing to free swimming neonates. The average body length of F1s was 2.87 ± 0.16 mm; however, these were never observed to molt and no individual survived past 32 days. It is surmised that a combination of the use of the suspended static arrays and excessive handling was responsible for early mortality. We recommend that first instars are given adequate space with plentiful habitat heterogeneity in a flow-through system.

Due to the lack of laboratory production of young, early instar *Stygobromus* subjects were wild caught and brought to the laboratory for growth analysis. Thirty individuals tracked successively over a minimum of 60 days were analyzed for time between molts. Starting instar was determined by best professional judgement for each individual by comparing graphs of their body measurements to measures of other wild-caught specimens and a body length benchmark of 2.87 mm for the first instar. It was estimated that individuals molt about every 50 days.

A separate set of wild-caught *Stygobromus* specimens were graded into size classes based on 0.5 mm increments in body length. Fifty-eight individuals were slide mounted and a set of 34 character states consisting of measures or counts were compiled. Individuals that reached size-class 6.5 mm were found to have fully developed sexually identifiable characters and this body size was used as a benchmark for determining sexually maturity. Three instar estimation methods were employed and averaged to estimate the instar or developmental stage of each individual. The first method separated instars among the 58 individuals by finding the inflection points of a smooth spline of the ranked principal component 1 of their character states. The second method used the gap-statistic to determine the best number of instars from a hierarchical cluster analysis of the character states. The third method utilized inflection points similar to method one, but only for the length of the antenna 2 peduncle. At least eight instars or developmental stages were delineated by this method for the 58 specimens and it was determined that it takes individuals about 387.5 ± 28.0 days to reach maturity, based on the instar estimates of individuals that were determined to be sexually mature and the average amount of time estimated between instars of the wild-caught laboratory reared subjects (50 days).

A HIGH-feeding experiment was conducted with 10 female and male *S. russelli* subjects exposed to 2-3 times normal amounts of food. Although 20% of the females developed broods within a four-month period of time, mortality was 80% and was largely attributed to poor water quality conditions, resulting in excessive decompositions from the increased food resources.

A food preference experiment was conducted by giving starved *S. flagellatus* a choice between two food items. Subjects chose commercially available fish flakes over conditioned leaf, a living but restrained *Hyalella* sp., and a plastic strip (control). No other food source was found to be preferable to another.

Subjects exposed to free-swimming *Hyalella* sp., and no other food items, did not show any predation behavior unless allowed to remain in the chamber with the prey item overnight. Subjects exposed to free-swimming *Lirceolus* sp., and no other food items, consumed the prey two out of five trials that ran for ca. 20 mins. These results suggest that predation by *Stygobromus* is a result of mechanoreception due to direct contact with prey items; however, behavior in obtaining the fish flakes suggests they have chemoreception toward high protein food sources.

Many questions remain unanswered regarding basic life-history aspects of *S. pecki* and a great deal more applied research is needed in order to establish a fully functioning refuge for this species. With the current knowledge gained from these studies, we recommend that more housing is provided to accommodate the refugia stock. In addition to added mesocosms, we recommend that increasing habitable surface area is increased. Currently utilized substrates appear useful, but alternative biomedia should be considered. We also recommend that brooding mothers should be removed to more stress-free environments and that future work should specifically investigate reproductive aspects of the captive rearing program.

Life-History Aspects of the Comal Springs dryopid beetle (*Stygoparnus comalensis*) and notes on life-history aspects of the Comal Springs riffle beetle (*Heterelmis comalensis*)

The full report can be found in Appendix E.

Executive Summary written by BIO-WEST, Inc.

The Edwards Aquifer Habitat Conservation Plan (EAHCP) calls for the establishment of captive refuge populations of Edwards Aquifer (EA) Covered Species associated with their Incidental Take Permit inhabiting both subterranean and spring outflow habitats. The San Marcos Aquatic Resources Center (SMARC) operated by the United States Fish and Wildlife Service (USFWS) has been awarded the opportunity to establish and maintain captive refuge populations of EA species of concern. Some of the species of concern still pose several substantial questions concerning refuge cultivation; particularly the invertebrate species. The Comal Spring dryopid beetle (*Stygoparnus comalensis*) is a federally endangered species that is adapted to subterranean habitats associated with Edwards Aquifer springs. *Stygoparnus comalensis* is the only species described for the genus and, therefore, little is known about the life history and the environmental requirements of this species.

During the process of obtaining specimens, specific locations at Comal Springs were identified as habitats where *S. comalensis* could be sampled, consistently. The use of poly-cotton lures was not an effective means of sampling adults; adults were obtained on coarse woody debris that were directly placed on or in spring upwellings that appeared to be associated with the roots of sycamore trees that grew adjacent to the springs.

Male and female adults could be told apart by observing the 8th and 9th abdominal sternites beneath the 5th ventrite, for males and females, respectively. These morphological features were readily viewable in living subjects with the use of proper lighting techniques. Measures showed that females and males did have significantly different body measures; however, there was too much overlap to utilize measurements in a reliable way for discerning sex. The use of the 8th abdominal sternites were also found to be reliable characters for separating female and male Comal Springs riffle beetles (*Heterelmis comalensis*). Review of the literature showed that these characters have been used to separate sexes of other species of riffle beetles.

An oblique plane apparatus was implemented to determine if females oviposited above or below the surface of the water. Attempts to use this device turned out to be difficult to utilize as eggs were never recovered. Smaller mating chambers were constructed to mimic the upwelling of the spring systems with a food resource that was partially emergent. The food resource consisted of a conditioned poplar dowel loosely wrapped in poly-cotton lure. Although, the oviposition behavior of the female was never observed, eggs were regularly collected from a number of mating pairs and groups. Eggs were not glued or attached to any substrate and appeared to be produced one at a time. Eggs sank and were usually found below the water line entangled in cotton fibers or discovered loose.

We collected a total of 173 eggs from 15 females. However, not all females produced eggs and several appeared to be prolific; one female produced 47 eggs in six months and was still alive at the time this report was written. Because of the limited time to study this organism, we can only make crude estimations with regard to the fecundity of this species.

Eggs were transferred to a terrestrial humid habitat, consisting of a bed of aquarium rocks raised above a water level with a conditioned leaf-base layer, a conditioned poplar dowel, and other conditioned leaves forming a tent over the eggs. Eggs hatched over a period of 82 ± 15 days and larvae were found feeding on leaves and dowels.

About four instars were delineated from measurements of the larvae over time. Larvae were estimated to undergo six instars within a period of 134 days by extrapolating to the maximum lengths reported in the literature. It is still a question as to how many instars larvae go through and how long they spend in each instar. There is no information regarding the final instar from this study as it was concluded before larvae were given the chance to fully develop.

After several checks on larval growth it became apparent that larvae were burrowing into the poplar dowel, creating galleries. It is surmised that this behavior is reminiscent to their natural habitat conditions and that woody material, possibly fibrous roots such as those of sycamore trees at Comal Springs. It is suggested that the burrowing and gallery formation within woody material serves as a means for larvae to survive in a submerged habitat and is likely that they utilize the galleries as chambers for pupation.

A variable-flow artesian-spring emulator (VFASE) was constructed to simulate a spring system with varying levels of flow. The VFASE was used to test the response of *S. comalensis* and *H. comalensis*, separately, under varying flow regimes with differing locations for food resources. In general, *Stygoparnus* moved against the flow towards a food resource while *Heterelmis* moved in the direction of flow to a food resource. Both species tended to stay in a food resource if placed in one at the beginning of the trial.

Although many new insights regarding the life history of *S. comalensis* have been revealed during the course of this study, many questions remain unanswered. In general, more time is needed to study the life cycle of this animal. Information regarding the optimum larval habitat, number of instars, length of time to pupation, pupation requirements, adult longevity, and fecundity still remain in question.

BUDGET

Task	U.S. Fish and Wildlife Service 2018		Budget Spent	Total Task Budget Spent
1	**Refugia Operations**			**$3,277,806**
	SMARC Refugia & Quarantine Bldg.			
	Construction		$1,430,828	
	Equipment		$53,783	
	UNFH Renovation Refugia & Quarantine Bldg.			
	Construction		$811,292	
	Equipment		$58,835	
	SMARC Species Husbandry and Collection		$182,743	
	UNFH Species Husbandry and Collection		$176,488	
	Fish Health Unit		$12,773	
	SMARC Reimbursibles		$46,516	
	UNFH Reimbursibles		$28,283	
		Subtotal	*$2,801,540*	
		Admin Cost	*$476,266*	
2	**Research**	-		**$393,525**
	BIO-WEST: Dryopid beetle life history		$112,676	
	BIO-WEST: Peck's Cave amphipod life history		$119,282	
	BIO-WEST: Riffle beetle life history		$15,000	
	Captive propagation refinement salamanders		$89,387	
		Subtotal	*$336,346*	
		Admin Cost	*$57,179*	
3	**Species Propagation and Husbandry**		$0	**$0**
4	**Species Reintroduction**		$0	**$0**
5	**Reporting**			**$103,277**
	BIO-WEST		$20,320	
	SMARC Staff		$51,807	
	UNFH Staff		$14,706	
		Subtotal	*$86,834*	
		Admin Cost	*$14,762*	
6	**Meetings and Presentations**			**$21,146**
	BIO-WEST		$9,889	
	SMARC staff		$9,621	
		Subtotal	*$19,511*	
		Admin Cost	*$3,317*	
			TOTAL	**$3,765,754**

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APPENDICES

A. 2018 EAHCP Refugia Work Plan
B. Edwards Aquifer Refugia Research Goals and Plan
C. 2018 Salamander Reproduction Research Report: Investigating San Marcos Salamander Reproduction in Captivity
D. Life-History Aspects of *Stygobromus pecki*
E. Life-History Aspects of the Comal Springs dryopid beetle (*Stygoparnus comalensis*) and notes on life-history aspects of the Comal Springs riffle beetle (*Heterelmis comalensis*)
F. *Eurycea* Salamander Sedation SOP
G. Uvalde National Fish Hatchery Renovation Engineering Diagrams
H. Fish Health Unit Reports
I. Monthly Reports