IMPLEMENTATION OF THE EDWARDS AQUIFER REFUGIA PROGRAM UNDER THE EDWARDS AQUIFER HABITAT CONSERVATION PLAN

ANNUAL REPORT 2023

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

BACKGROUND

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 to the Edwards Aquifer. These refugia were covered 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 seventh annual report of the contract covering the calendar year of 2023. The seventh year of the contract focused on maintaining the existing standing stocks and conducting research while facing a significant a drought and undergoing staff changes.

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

COLLECTIONS

Collection events occurred in every month of 2023. Collection numbers by month and species are shown in Table 1. Edwards Aquifer diving beetles (*Haideoporus texanus*), San Marcos gambusia (*Gambusia georgei*), and Texas troglobitic water slaters (*Lirceolus smithii*) were not collected in 2023; all other covered species were collected in 2023.

Figure 1. Dominique Alvear and Jonathan Donahey collecting San Marcos fountain darters at Eastern Spillway, San Marcos, Texas.

Table 1. Counts of individuals captured in 2023 by species and month. Collection counts are provided for the San Marcos Aquatic Resources Center (before the slash) and Uvalde National Fish Hatchery (after the slash). CSRB = Comal Springs riffle beetles, CSDB = Comal Springs dryopid beetles, PCA = Peck's cave amphipods, CSFD = Comal Springs fountain darters, SMFD = San Marcos fountain darters, TXBS = Texas blind salamanders, CSS = Comal Springs salamanders, SMS = San Marcos salamanders, and TWR = Texas wild rice. The number captured may not reflect the number retained for refugia or research purposes, as some individuals may have been released.

	CSRB	**CSDB**	**PCA**	**CSFD**	**SMFD**	**TXBS**	**CSS**	**SMS**	**TWR**
JAN				0/36					
FEB	0/32	0/9						30/0	
MAR			0/138	0/10	65/10			75/0	
APR					182/0			53/0	
MAY				501/0	0/88	15/0			12/0
JUN			76/0	0/160					
JUL				466/0	177/28			8/0	
AUG			0/108	0/118	0/73	4/0	18/0	7/0	0/10
SEP			105/0		206/105			4/0	
OCT					133/0				0/3
NOV	36/0		6/0	159/0		3/0		2/0	
DEC			50/49						10/10

RESEARCH

We conducted six research projects in 2023, several with external partners. These research projects focused on species covered by the Edwards Aquifer Habitat Conservation Plan, including three invertebrates (Comal Springs riffle beetle, Comal Springs dryopid beetle, and Peck's cave amphipod), and the San Marcos salamander. Research areas included genetic assessments of wild populations, improved collections and captive propagation, and mark and recapture of wild populations. All research was conducted to improve successful completion of their life cycles, promote reliable reproduction, and establish baselines for species reintroductions.

USFWS staff began a mark-recapture study examining the recapture rate, movement, and demographics of wild San Marcos salamanders. Tagging, using p-Chip transponder tags, and recaptures were conducted at three sites across Spring Lake and the San Marcos River. Tagging was completed and recaptures began in 2023. Recaptures are planned to continue into 2024. An interim report for this study is included in Appendix B.

BIO-WEST led an effort to determine better methods of collecting and housing Comal Springs dryopid beetles for captive assurance. Collections and challenge experiments for larvae and adults were conducted in 2023. Experimental questions examined the housing preferences of dryopid beetles in captivity. Collections and experiments are planned to continue in 2024. An interim report for this research is in Appendix C.

A study developing tagging methodology for invertebrates was led by Dr. Shannon Brewer of the U.S. Geological Survey, Alabama Cooperative Fish and Wildlife Research Unit. A tagging protocol was developed for Comal Springs riffle beetle using superglue to affix a p-Chip tag to the elytra. Initial internal tagging of Peck's cave amphipod was unsuccessful thus far, but additional tagging methods were identified for testing in 2024. Survival and retention of tagged beetles is planned to be investigated in 2024. An interim report for this study is included in Appendix D.

USFWS staff and Dr. Chris Nice (Texas State University) began a genetic assessment of the Peck's cave amphipod in the Comal Springs system. Amphipods were collected as bycatch during Comal Springs riffle beetle collections and by dip nets in areas where more were needed. Collections concluded in 2023 and genetic analysis is planned to be conducted in 2024. An interim report for this research is in Appendix E.

Ruben Tovar and Dr. David Hillis of the University of Texas Austin led a project using comparative gene expression in San Marcos salamanders to target reproductive triggers in captivity. Salamanders were preserved in a fixative allowing for molecular work microCT scanning to create a transcriptome and developmental time series. Tissue fixing and transcriptomics are planned to continue in 2024. An interim report for this project is available in Appendix F.

A genetic assessment of the CSRB in Landa Lake continued through 2023 in partnership with BIO-WEST. Lure deployment was delayed until 2023 due to drought conditions. BIO-WEST set lures at 80 biomonitoring sites at three time points to gather data for an occupancy study. A portion of the CSRB observed on each lure was retained for genetic assessment. Collections concluded in 2023 and genetic analysis will be carried out in 2024. An interim report for this research is included in Appendix G.

BUDGET

The Aquifer Refugia Program did not exceed the allocated budget defined in the 2023 Refugia Work Plan previously approved by the EAA Board of Directors. The Refugia Program spent approximately $1,323,005 in 2023. Research activities accounted for $396,994, and approximately $868,808 was spent on collections, husbandry, and propagation. Approximately $57,203 was spent on reporting, meetings, and presentations. Most unspent funds in Tasks 1 and 2 will move to a Task 1 and 2 Reserve Funds, respectively, to hold until need requires the program to request those funds in a Work Plan and Budget.

INTRODUCTION

BACKGROUND

The activities reported herein are in support of the Federal Fish and Wildlife Incidental Take Permit (ITP) for the EAA (TE-6366A-1, Section K) and fulfillment of Contract #16-822-HCP between the Edwards Aquifer Authority (EAA) and the U.S. Fish and Wildlife Service (USFWS) as outlined within the 2021 Edwards Aquifer Refugia Work Plan. The overarching goal of the Edwards Aquifer Refugia Program conducted by the USFWS is to assist the EAA in compliance with its ITP and to meet its obligation within EAHCP section 5.1.1. The refugia contract covers ten different species including seven endangered species, one threatened species, one species no longer petitioned for listing, and two species currently proposed for listing (see Table 2 for list of the Covered Species).

The Edwards Aquifer Refugia Program's purpose is to house and to protect adequate populations of the Covered Species for re-introduction into the Comal or San Marcos systems in the event a population is lost following 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 in San Marcos, Texas with back-up populations in Uvalde, Texas. See the appropriate sections of this report for further details on each of the species collected and maintained and the section on research activities.

The EAA-USFWS contract awarded 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 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 (Table 2), as well as for salvage and restocking as necessary. The monetary support is allocated into six tasks: 1) Refugia Operations, 2) Research, 3) Species Husbandry and Propagation, 4) Species Reintroduction, 5) Reporting, and 6) Meetings and Presentations.

Table 2. 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)

Common Name	**Scientific Name**	**ESA Status**
Fountain darter	*Etheostoma fonticola*	Endangered
Comal Springs riffle beetle	*Heterelmis comalensis*	Endangered
San Marcos gambusia	*Gambusia georgei*	Extinct*
Comal Springs dryopid beetle	*Stygoparnus comalensis*	Endangered
Peck's cave amphipod	*Stygobromus pecki*	Endangered
Texas wild rice	*Zizania texana*	Endangered
Texas blind salamander	*Eurycea rathbuni*	Endangered
San Marcos salamander	*Eurycea nana*	Threatened
Edwards Aquifer diving beetle	*Haideoporus texanus*	Petitioned
Comal Springs salamander	*Eurycea pterophila*	None†
Texas troglobitic water slater	*Lirceolus smithii*	None‡

* The San Marcos gambusia was proposed for removal from the ESA due to extinction on September 29, 2021 (Federal Register Document Number 2021-21219; U.S. Fish and Wildlife Service 2021).

†The Comal Springs salamander was petitioned for listing under the ESA as "*Eurycea* sp. 8" but has subsequently been identified as a common species, *Eurycea pterophila*, and is no longer petitioned for listing under the ESA.

‡The Texas troglobitic water slater was removed from petition consideration November 29, 2023 (Federal Register 88 FR 83368 2023-25586)

OBJECTIVES

1. **Further develop and provide fully functioning refugia for the EAHCP Covered Species.**
   USFWS will work toward fully functioning refugia operations for all the Covered Species. 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 San Marcos Aquatic Resources Center (SMARC), with a secondary refugia population located at the Uvalde National Fish Hatchery (UNFH).
2. **Conduct research as necessary to expand knowledge of the Covered Species.**
   USFWS and/or subcontractors 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 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 provide oral presentations to Science Committee, Implementing Committee, and EAA Board of Directors as requested by the EAHCP Program Manager.**
   The Edwards Aquifer Refugia Program staff will keep partners apprised of refugia activities.

PERSONNEL

The USFWS managed the Edwards Aquifer Refugia Program with dedicated staff at two geographically separated facilities: the SMARC and UNFH (Table 3). Both facilities are administratively managed under the direction of a single Center Director, Dr. David Britton with the assistance of the Deputy Center Director, Dr. Jennifer Howeth. Dr. Scott Walker is the Project Leader at the Uvalde National Fish Hatchery. Adam Daw, based at the UNFH, led the Refugia Husbandry and Collections team for both facilities in 2023. Dr. Katie Bockrath, the Refugia Research Lead, serves as the point of contact for the Edwards Aquifer Refugia Program, coordinates all research activities, project plans, reporting and budgets in 2023. The Edwards Aquifer Refugia Program underwent staff changes in 2023.The program welcomed four new employees, Jonathan Donahey and Heidi Meador at UNFH, along with Shawn Moore and Richelle Jackson at the SMARC. Table 3 USFWS Refugia Program Staff

San Marcos Aquatic Resources Center	
Dr. David Britton	Center Director
Dr. Jennifer Howeth	Deputy Center Director
Dr. Katie Bockrath	Refugia Research Team Lead
Desiree Moore	Research Biologist
Braden West	Refugia Biologist
Shawn Moore	Biological Science Technician
Richelle Jackson	Biological Science Technician
Uvalde National Fish Hatchery	
Scott Walker	Uvalde National Fish Hatchery Project Leader
Adam Daw	Refugia Husbandry and Collections Team Lead
Dominique Alvear	Refugia Biologist
Heidi Meador	Biological Science Technician
Jonathan Donahey	Biological Science Technician

Day to day operations were managed by two Lead Biologists providing supervision, mentorship, and training to the Fish Biologist and Biological Technicians (see Table 3 for staffing chart). The Lead Biologists managed and coordinated species collections, husbandry, propagation, research, and field activities related to species covered under the contract. They also arranged purchases, oversaw facility maintenance repairs, developed and implemented budgets, and organized all activities that related to the contract. Leads 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 and Deputy Center Director, they prepared all written materials required for reporting. They communicated regularly with the EAA, USFWS personnel, researchers, and other partners.

Dr. Katie Bockrath, Refugia Research Lead, coordinated research efforts across stations. Dr. Bockrath, with input of supporting staff, prepared the annual report, annual work plans, and monthly reports, developed research activities and reports, developed and managed the Refugia Program budget, and established and oversaw outside research agreements.

Adam Daw, Refugia Husbandry and Collections Lead, coordinated the husbandry and collections across stations. Daw, with input from supporting staff, prepared the annual report, annual work plans, and monthly reports, developed and managed the Refugia Program budget, oversaw development and implementation of husbandry standard operating procedures,

Figure 2. Adam Daw, Heidi Meador, Jonathan Donahey, and Dominique Alvear in a work vehicle.

designed and oversaw construction of refugia system improvements and coordinated collection activities.

Desiree Moore, Research Biologist, worked with Dr. Bockrath to design and implement research projects across stations. D. Moore contributed to the annual report and monthly reports, developed research activities and reports, contributed to annual work plans, husbandry, and collections, and coordinated with external research partners.

Dominique Alvear and Braden West, Refugia Biologists, worked with Daw to manage the husbandry and collections across stations. They contributed to the annual report and monthly reports, developed and implemented husbandry standard operating procedures, designed and constructed refugia holding systems. The biologists performed quality control for daily and collection data records, ensured biosecurity adherence, and assisted with research activities.

Figure 3. Edwards Aquifer Refugia Program staff at the Edwards Aquifer Authority Education Outreach Center. From left to right, Braden West, Adam Daw, Dominique Alvear, Shawn Moore, Desiree Moore, and Dr. Katie Bockrath.

Jonathan Donahey, Heidi Meador, Shawn Moore, and Richelle Jackson, Biological Science Technicians, carried out collections and daily husbandry duties. They constructed, maintained, and monitored holding systems for refugia species. The technicians performed daily data recording duties, promoted biosecurity, and assisted with research activities. Additionally, they managed logs and databases, authored and edited standard operating procedures (SOPs), and contributed to monthly reports.

Significant improvements to the EARP building occurred in 2023. We started the transition of water quality monitoring systems from Hydrolab sondes to Walchem controllers/monitors at both the UNFH and SMARC. With the use of the controllers, water quality probes (total gas pressure, water temperature, water pressure), and an automated bypass valve, the main well water supply line was redesigned for both refugia locations to minimize the potential for well water supersaturated with gas reaching refugia tanks. The well water line modification for the UNFH refugia room was completed and the one for the SMARC refugia room was under construction at the end of the year. With the use of the controllers, CO_2 injection systems were added to more tanks at the UNFH to better control water pH in the systems.

Figure 4. EARP staff in the SMARC refugia room learning about the controller units from Adam Daw.

Refugia room hospital tank racks at both facilities were modified to improve function and to standardize the design with the quarantine tank racks. The last three hospital racks in the SMARC quarantine room were constructed. The second invertebrate rack in the refugia at the SMARC was constructed and two invertebrate racks at the UNFH were modified to improve the design and allow for monitoring of systems parameters via the new controllers. A filter system was added to a Texas wild rice tank at the SMARC to evaluate if it would improve the health of the system. Multiple refugia tanks in the SMARC and UNFH refugia rooms were redesigned from flow through to partially recirculating systems. The new system design allows for the recirculation of the system water and requires up to 50% less chilled well water than previous flow-through tank designs used at the SMARC. The redesign also allowed for the addition of the new system controllers/monitors.

Four tanks were added to both facilities to culture *Daphnia magna,* which have shown to be an easily cultured live food for the Fountain darters and salamanders. The Peck's cave amphipods have also been observed eating them.

New storage and work benches were added to the refugia rooms to better organize equipment and provided dedicated space for various tasks.

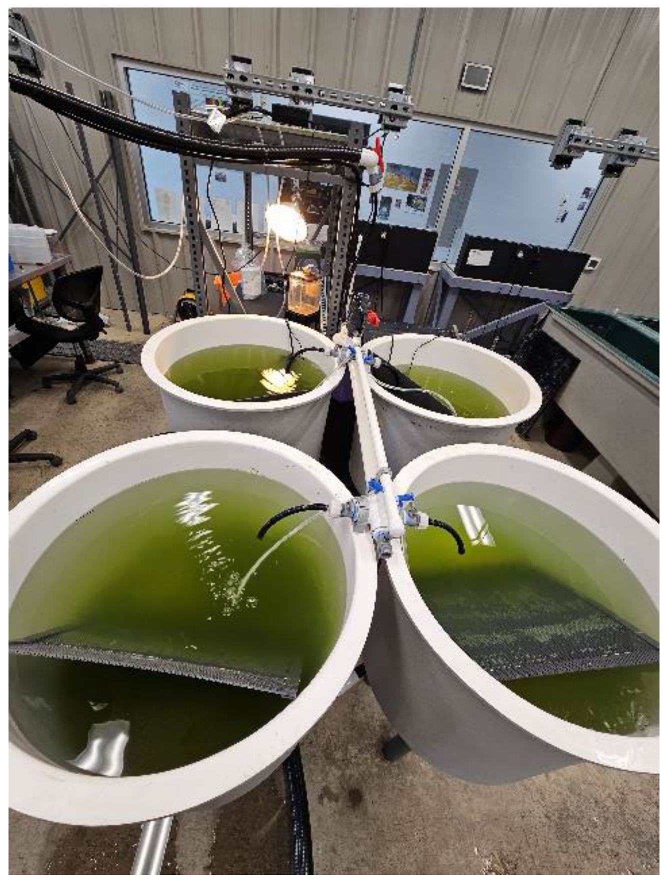


Figure 5. One of the four-tank *Daphnia magna* culture systems.

COVERED SPECIES ANALYSIS

Collections of the Covered Species continued to work toward standing stock targets as outlined in the Contract and the 2023 EA Refugia Work Plan (Tables 3 and 4). For many species, the acclimation to captive systems can be achieved relatively quickly; this is particularly true for Texas wild rice, San Marcos fountain darters, and San Marcos salamanders.

After consultation with the EAA staff, our other partners, and experts in the field, we decided to reduce the number of invertebrate collection events and numbers of CSRB held in refugia to minimize any negative effects that collection events might have on wild populations in the Comal Springs system due to drought conditions.

The Covered Species knowledge matrix (Table 5) was updated to reflect the current standing for all Covered Species across five distinct areas that make up a complete refugia: Collections, Husbandry, Propagation, Genetics, and Reintroduction. Texas wild rice and the fountain darter have the highest knowledge score of all covered species. Texas wild rice is in complete refugia.

Figure 6. Texas blind salamander

Table 3. Number of organisms incorporated in the SMARC Refugia Standing Stock in 2023, the end of year census, and overall survival rate.

Species		SMARC Incorporated into Refugia	SMARC End of Year Census	SMARC Survival Rate
Fountain darter - San Marcos *Etheostoma fonticola*		466	89	19%
Fountain darter – Comal Springs *Etheostoma fonticola*		314	149	24%
Comal Springs riffle beetle *Heterelmis comalensis*		32	32	47%
Comal Springs dryopid beetle *Stygoparnus comalensis*		0	0	0%
Peck's cave amphipod *Stygobromus pecki*		73	145	68%
Edwards Aquifer diving beetle *Haideoporus texanus*		0	0	--
Texas troglobitic water slater *Lirceolus smithii*		0	0	--
Texas blind salamander *Eurycea rathbuni*		9	88	49%
San Marcos salamander *Eurycea nana*		129	163	72%
Comal Springs salamander *Eurycea* ***pterophila***		16	58	45%
Texas wild rice *Zizania texana*		12	178	82%

Notes: 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. End of year census number is of those incorporated. Survival rate = (end of year census/ (start of year inventory + # incorporated)))*100. Survival rate does not include any mortality during quarantine period or those sacrificed for research or Fish Health diagnostics. Further details of these numbers can be found in the supporting sections of each species.

Table 4. Number of organisms incorporated in the UNFH Refugia Standing Stock in 2023, the end of year census, and overall survival rate.

Species		UNFH Incorporated into Refugia	UNFH End of Year Census	UNFH Survival Rate
Fountain darter - San Marcos *Etheostoma fonticola*		178	300	52%
Fountain darter – Comal Springs *Etheostoma fonticola*		417	371	63%
Comal Springs riffle beetle *Heterelmis comalensis*		17	16	25%
Comal Springs dryopid beetle *Stygoparnus comalensis*		6	8	50%
Peck's cave amphipod *Stygobromus pecki*		115	202	58%
Edwards Aquifer diving beetle *Haideoporus texanus*		0	0	
Texas troglobitic water slater *Lirceolus smithii*		0	0	
Texas blind salamander *Eurycea rathbuni*		0	62	94%
San Marcos salamander *Eurycea nana*		48	164	76%
Comal Springs salamander *Eurycea* ***pterophila***		0	83	89%
Texas wild rice *Zizania texana*		13	188	85%

Notes: 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. End of year census number is of those incorporated. Survival rate = (end of year census / (start of year inventory + # incorporated)) * 100. Survival rate does not include any mortality during quarantine period or those sacrificed for research or Fish Health diagnostics. Further details of these numbers can be found in the supporting sections of each species.

Table 5. Updated table showing the level of knowledge known for each covered species. Knowledge score is a gradient from 0 to 5, where 0 is complete lack of knowledge and 5 indicates documented procedures for that species exists. Species with knowledge scores of 5 in each category indicate the species is in complete refugia.

Species	Collection	Husbandry	Propagation	Genetics	Reintroduction
Fountain darter	5	5	5	3	3
Texas wild rice	5	5	5	5	5
Texas blind salamander	4	5	4	3	1
San Marcos salamander	5	5	4	3	1
Comal Springs salamander	5	4	3	3	1
Comal Springs riffle beetle	5	4	4	3	1
Comal Springs dryopid beetle	3	2	1	0	1
Texas troglobitic water slater	1	0	0	1	1
Peck's cave amphipod	4	4	4	3	2
Edwards Aquifer diving beetle	1	0	0	0	1

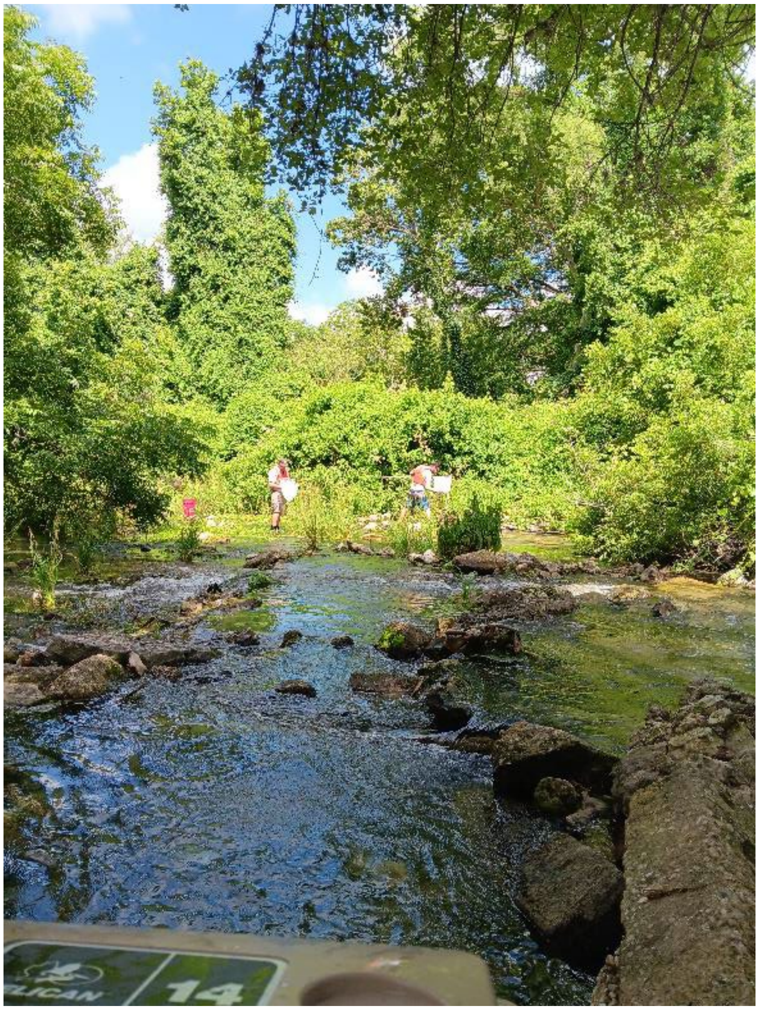


Figure 7. UNFH staff collecting fountain darters.

Our Standing Stock goal for fountain darters is 1,000 fish per river (San Marcos and Comal) divided between the two facilities. Standing stock goals for San Marcos fountain darters were slightly below target numbers in 2023. In the summer, due to a drought, the Comal River spring flow conditions reached critically low levels. In consultation with the EAA and USFWS staff, the refugia started collecting Comal Springs fountain darters to increase refugia stocks. Numbers incorporated, end of the year census, and survival rates can be found in Table 6.

Table 6. Fountain darter refugia population figures

		Beginning of Year Census	Incorporated 2023[1]	End of Year Census	Target Goal 2023 Work Plan	Percent Survival [2]
San Marcos River	SMARC	309	466	89	500	19%
	UNFH	457	178	300	500	52%
Comal River	SMARC	313	314	149	500*	24%
	UNFH	181	417	371	500	63%

* Prior to the Summer of 2022 collecting Comal Springs fountain darters was postponed until we have a better understanding of their mortality rates.

[1]The number of darters incorporated into the refugia is counted after a minimum 30-day quarantine period or when fish are cleared by Fish Health. During this period, fish are evaluated for health and suitability for inclusion into the refugia.

[2] Survival rate = (end of year census / (start of year inventory + # incorporated)))*100. Survival rate does not include any mortality during quarantine period or those sacrificed for research or Fish Health diagnostics. Fish removed from the refugia as part of the facilities yearly animal health inspection are not included in the moralities and calculated Percent Survival.

COLLECTIONS

In 2023, the collection of fountain darters was increased due to the low spring flows of both the Comal and San Marcos Rivers. Refugia staff collected San Marcos fountain darters in March, May, July, August, and September and Comal fountain darters in January, March, and August. BIO-WEST Inc. transferred fish to refugia staff during their bi-annual surveys of the Comal and San Marcos Rivers in April/May and October/November, and a low-flow survey of the Comal River in July.

Figure 8. SMARC staff checking their nets for fountain darters during a collection.

Bi-annual testing for *Centrocestus* sp., a trematode parasite, in wild fountain darters, was conducted by the USFWS Southwestern Fish Health Unit (SFHU) in Dexter, New Mexico. Fish sent for testing were caught from both the Comal and San Marcos Rivers in March and August. In May and November, subsets of fountain darters from the BIO-WEST Inc. bi-annual surveys of the Comal and San Marcos Rivers were sent directly to the USFWS Southwestern Fish Health Unit (SFHU) in Dexter, New Mexico for parasite enumeration and viral analysis.

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. A standard fountain darter intake and quarantine procedure was used at both facilities. To minimize stress, temperature acclimation progressed at a rate of one degree Celsius per hour. The fish were treated for external parasites in an aerated static bath solution of formalin at 170 ppm for 50 to 60 minutes. Darters were then transferred to clean flow-through quarantine tanks. Fish sent to the USFWS SFHU for routine parasitology and health screening were not given a formalin dip and were shipped to SFHU as soon as possible.

HUSBANDRY

All culture systems were monitored multiple times daily for proper water flow and temperature, reproduction (eggs), and mortalities. Deceased fish were immediately removed from the systems. If warranted, deaths were necropsied for parasites and preserved in vials containing 95% non-denatured ethanol. If parasites were noted during the necropsy or there was an increase in mortality in a tank, either a 1-hour static bath of 1-3ppt salt, 15 mg/L Chloramine-T, or 170 uL/L formalin was administered, according to the Southwestern Fish Health Unit recommendations.

Fountain darters at both facilities were housed in large, insulated fiberglass systems with either flow-through chilled well water (SMARC) or partial recirculation through heater-chiller units (UNFH) to maintain water temperature at 21 ℃ (ranging between 19–23 ℃). Water quality parameters including dissolved oxygen, pH, and total gas pressure were checked weekly. Staff routinely siphoned tanks to remove waste and other debris and rotated habitat items to be cleaned. Each tank system had dedicated 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 daily, varying between live amphipods, live black worms, live *Artemia*, live *Daphnia* sp., frozen mysid shrimp, and refrigerated Copepods.

SURVIVAL RATES

Historically at both the SMARC and UNFH, survivorship of newly collected fountain darters from the Comal River was poor in comparison to fountain darters collected from the San Marcos River, even when these were collected during the same time period and held in similar conditions. This has been an ongoing pattern for Comal Springs fountain darters since collections were restarted in 2017 after Comal Springs fountain darters were found to test positive for Largemouth bass virus (LMBV). Given the history of low intake survival rates, the EARP suspended collections of Comal Springs fountain darters for the refugia stock in the fall of 2019. Starting in 2022 and continuing into this year, Comal River fountain darters were collected again in larger numbers because of low spring flow. Survival rates of Comal River fountain darters were highly variable during their 30-day quarantine period. Individual lots of fish exhibited survival rates ranging from as low as 0% to as high as 85%. Once out of the quarantine period, survival is on par with San Marcos fountain darters. Necropsies of darter mortalities have revealed internal parasites in some individuals, which may be causing some of the mortalities. The reason for the large variance in early survival rates is unknown. The 2023 survival rates for incorporated fountain darters in refugia at the SMARC was 19% for the San Marcos River population and 24% for the Comal River population. In previous years the San Marcos populations are relatively healthy when brought into quarantine. In 2023 necropsies reviled parasites in a majority of the mortalities. Some parasitic effects become more severe in rising water temperatures (McDonald et. al 2007). With high observed parasite load, coupled with drought stressors, it's likely the San Marcos fountain darters arrived to the Refugia in already suboptimal condition A well water gas supersaturation event occurred, due to a power outage, at the SMARC which resulted in a mortality event and the low overall survival for the year (Appendix J). Although we cannot fully predict the overall survival of Comal Springs fountain darters at SMARC, by removing the Comal Springs fountain darter that died as a result of the gasification event (N=180), survival at the SMARC could have been as high as 52%. At the UNFH, the survival rate was 52% for the incorporated San Marcos population and 63% for the Comal River population.

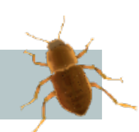
MAINTENANCE OF SYSTEMS

Refugia systems were deep cleaned annually with 20-30% vinegar (SMARC) or muriatic acid (UNFH) to remove calcium carbonate deposits that formed within the tank, plumbing, chiller, and pump casing that can affect functionality. When systems were empty, they were bleached with 20ppm free chlorine for 24 hours followed by neutralization with sodium thiosulfate (UNFH) or the tank surface sprayed with 1% Virkon (SMARC). Water lines, hoses, valves, and restrictors were frequently checked for wear and clogs and were cleared, rebuilt, or replaced as needed.

CAPTIVE PROPAGATION

There were limited efforts to produce captive offspring of either San Marcos River or Comal Springs fountain darters at either facility during 2023, relying on harvesting eggs/juveniles produced in the refugia tanks. Generally, fountain darters in captivity lay eggs on the undersides of PVC and other habitat structures placed in the tanks. If offspring were not desired, staff removed the structures and disposed of the eggs. F1 generations were separated based on the river system from which their parents originated. Egg production was opportunistic and not controlled or directed by staff during periods when offspring were not needed for research or for reintroduction. A captive propagation plan is on file and available upon request for fountain darters.

COMAL SPRINGS RIFFLE BEETLE (*HETERELMIS COMALENSIS*), ENDANGERED



Comal Spring riffle beetle collection by EARP staff for standing and refugia stocks occurred in February from around Spring Island. In November, BIO-WEST Inc. collected riffle beetles as part of a population study, from which some individuals were transferred to refugia staff. Standing stock numbers were reduced to 75 individuals per station until better knowledge of population numbers and meaningful standing stock numbers are derived (Table 7). Standing stock number will be evaluated yearly by the Comal Springs Riffle Beetle Work Group.

Table 7 Comal Springs riffle beetle refugia population figures

	Beginning of Year Census	Incorporated 2023	End of Year Census	*Target Goal 2023 Work Plan	Percent Survival
SMARC	36	32	32	75	47%
UNFH	48	16	17	75	25%

* For 2023 the goal of 75 was not a priority due to a BIO-WEST led occupancy research project on wild population populations where Refugia collections could impact the study.

COLLECTIONS

On February 6, refugia staff collected 32 riffle beetles from checking in-situ submerged wood around the Northern shore of the Comal River near Spring Island, all of these were transferred to the UNFH. On November 20 and 21, riffle beetles were collected from cotton lures placed in Spring Run 3 and along the western shore of the Comal River in coordination with BIO-WEST Incorporated. In total, 36 adult riffle beetles were transferred to refugia staff and taken to the SMARC for the refugia population.

QUARANTINE

Incoming CSRB were quarantined at the SMARC and the 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 aquatic nuisance species 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, CSRB joined the Refugia population in a container labeled by collection date at the end of the 30-day quarantine period.

HUSBANDRY

All systems were evaluated daily 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, there was no traditional feeding schedule. Alternatively, leaves, wood, and cotton cloth containing biofilm were used in each system, providing food. Inventories were conducted every two to three months on a schedule and new biofilm material was added as needed.

Culture boxes used to house CSRB were square black plastic containers with a manifold that delivers water through a spray bar onto the side of the container that flows down into the water. Containers contained leaves, conditioned wood, biofilm cloth, and mesh for structure and habitat. The systems were cleaned during inventory. At this time, staff checked water lines, hoses, and valves for functionality and cleaned or replaced them as needed. Air space and emergent structure was provided in box containers housing larvae.

SURVIVAL RATES

Because CSRB have an average life span of approximately one year and adults of unknown age are collected from the field, high annual mortality rates are expected due to senescence. Historically, about half of CSRB collected perish by six months in captivity. The small size of CSRB makes it difficult to assess mortality on a day-to-day basis. Therefore, mortalities are calculated as inventories are conducted, where the number of dead or missing CSRB equates to the number of mortalities for that time-period. The 2023 survival rates for CSRB in refugia at the SMARC was 47% and 25% at the UNFH. The percent survival for the UNFH was lower due to a box that had F1 individuals pupate with the adult wild stock still in the box. Due to the inability to distinguish wild and F1 adults, wild individuals were counted as mortalities and all living beetles were considered as F1.

CAPTIVE PROPAGATION

To encourage production of offspring, male and female wild stock were housed together. During inventories, larvae were placed into a separate container from wild stock adults. Staff observed higher reproduction and metamorphosis of CSRB relative to previous years, indicating that the recent improvements to culture systems and husbandry methods are beneficial.

COMAL SPRINGS DRYOPID BEETLE (*STYGOPARNUS COMALENSIS*), ENDANGERED

Given the low numbers of Comal Springs dryopid beetles (CSDB) historically collected in the field, yearly population goals were set at 20 individuals at each site in the Work Plan for this species. Numbers incorporated, end of the year census, and survival rates can be found in Table 8.

Table 8. Comal Springs dryopid beetle refugia population figures

	Beginning of Year Census	Incorporated 2023	End of Year Census	In Quarantine End of Year	Target Goal 2023 Work Plan	Percent Survival
SMARC	2	0	0	0	20	0%
UNFH	10	6	8	0	20	50%

COLLECTIONS

In 2023, sampling events occurred for CSDB at Spring Island, Comal River by checking in-situ submerged wood. Nine individuals were captured in February, with eight adults retained for the UNFH and 1 juvenile released. A collection event was conducted in March near Spring Island, but no individuals were found.

QUARANTINE

Incoming CSDB were quarantined in the invertebrate refugia area at the UNFH. CSDB were acclimated to quarantine water conditions at a rate not exceeding one degree Celsius every hour. During the quarantine period, staff monitored for potential aquatic nuisance species that may have come in with the collection, the general health of the organisms, and any large die-offs that might indicate a disease. If none of these events occurred, CSDB joined the refugia population at the end of the 30-day quarantine period.

HUSBANDRY

Square plastic containers were used as culture boxes for CSDB. Each container was fitted with a manifold to deliver water through a spray bar onto the side of the container, flowing down into the basin. Containers were kept dark to mimic the underground environment. All systems were checked daily for appropriate 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; CSDB may become entrapped in fungus and perish. 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, leaves, wooden dowels, and cotton cloth containing biofilm were placed in containers and provided a constant food source. Inventories were conducted every other month and new food items were added as needed. Obtaining census numbers during inventories, especially for larvae, were difficult at times as adult and larval dryopid beetles burrow under the surface of the wooden media used in the culture boxes.

SURVIVAL RATES

The small size of CSDB made it difficult to assess for mortality on a day-to-day basis. Mortalities were therefore calculated as inventories were conducted, where the number of dead or missing beetles equates to the number of mortalities for that time-period. During the inventory, the health condition of the dryopid beetles was assessed. The 2023 survival rates for CSDB in refugia at the SMARC was 0% and 50% at the UNFH.

CAPTIVE PROPAGATION

Larvae were observed in 2023 during inventories of the UNFH population.

PECK'S CAVE AMPHIPOD (*STYGOBROMUS PECKI*), ENDANGERED

Peck's cave amphipods (PCA) were collected from Comal Springs by hand during five collection events. The refugia also received PCA caught as bycatch from Comal Spring riffle beetle lures set by BIO-WEST at 80 biomonitoring sites. Numbers incorporated, end of the year census, and survival rates can be found in Table 9.

Table 9 Peck's cave amphipod refugia population figures

	Beginning of Year Census	Incorporated 2023	End of Year Census	Target Goal 2023 Work Plan	Percent Survival
SMARC	139	73	145	250	68%
UNFH	232	115	202	250	58%

COLLECTIONS

There were five collection events conducted in 2023 for Peck's cave amphipods (PCA) by refugia staff. These took place around Spring Island of the Comal River, New Braunfels, Texas. A total of 536 PCA were captured, with 509 of those transferred to the SMARC and the UNFH for the refugia. In addition to the refugia collections, during a population study in coordination with BIO-WEST, six PCA were transferred to refugia staff for incorporation into the refugia population.

QUARANTINE

Incoming PCA were quarantined in the refugia invertebrate areas in the quarantine rooms at the SMARC and UNFH. PCA were acclimated to quarantine water conditions at a rate not exceeding one degree Celsius every hour. During the quarantine period, staff monitored for potential aquatic nuisance species 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, the PCA joined the Refugia population at the end of the 30-day quarantine period.

HUSBANDRY

All systems were checked daily for proper water temperature, adequate flow, and clear drain screens to maintain drainage and water level. Small amounts (ca. 10 ml) of fish flake slurry were added two times per week. Dried leaves from terrestrial sources were used as potential supplemental food and provided shelter within the systems. With completion of a dissertation at Texas State University, Dr. Parvathi Nair produced results that show PCA eat other smaller species of amphipods (Nair 2019). PCA are predators in their ecosystem and most likely prefer live feed in comparison to other *Stygobromus* amphipods (*S. flagellatus*; Kosnicki and Julius 2019).

Plastic totes were used as culture containers to house PCA, 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.

SURVIVAL RATES

PCA are known to cannibalize smaller individuals, which lower survival rates. Mortalities were therefore calculated as inventories were conducted, where the number of dead or missing PCA equates to the number of mortalities for that time period. The 2023 survival rates for PCA in refugia at the SMARC was 68% and 58% at the UNFH.

CAPTIVE PROPAGATION

When counting PCA from refugia containers during inventory, each amphipod was carefully observed for brooding. PCA females hold their eggs and young in a brood pouch under the body. At the SMARC and UNFH, gravid females were noted and placed back into refugia wild stock. PCA juveniles were easily identifiable at the next inventory by their size. Biologists were confident, given observed growth rates, that juveniles that survived could be located, identified, and moved to an F1 container. To minimize the cannibalism from the mothers on their offspring, staff tested the potential of removing very late-stage eggs from a gravid female and placing in a separate container to hatch. Although somewhat laborious, the eggs hatched successfully.

EDWARDS AQUIFER DIVING BEETLE (*HAIDEOPORUS TEXNUS*), UNDER REVIEW

No Edwards Aquifer diving beetles were collected during 2023. These beetles are rare, with little known about their native habitat, life history, or food requirements. Diving beetles have been previously collected from the Texas State Artesian Well, but these collections are only opportunistic, as beetles are ejected from the high-flow spring. There is an agreement with Texas State University to donate caught adults to the SMARC, at their discretion. Unfortunately, none were donated this year.

TEXAS TROGLOBITIC WATER SLATER (*LIRCEOLUS SMITHII*), PETITIONED

A non-lethal method to distinguish *L. smithii* from other species based on the characteristics of the pleotelson was discovered by Texas State University doctoral student Will Coleman. In 2019, using Coleman's method, we determined the refugia population consisted primarily of *Lirceolus hardeni* (no common name). Further, Mr. Coleman conducted extensive collections for his research and found *L. smithii* only in Texas State Artesian Well samples, and of those, very few live specimens. These live specimens were physically damaged, and Mr. Coleman was unable to keep them alive in captivity. This evidence suggests that *L. smithii* are a deep-aquifer species, like the Edwards Aquifer diving beetle, and are rarely found in surface waters; those that are found have likely suffered physical damage during the distance traveled to the surface.

No *L. smithii* were held in refugia in 2023. In the future, if *L. smithii* are collected from Texas Sate Artesian Well, the refugia will employ documented husbandry procedures that were successful at holding and propagating *L. hardeni*.

TEXAS BLIND SALAMANDER (*EURYCEA RATHBUNI*), ENDANGERED

The goal for Texas blind salamanders is 500 standing-stock individuals distributed between the two facilities (SMARC and UNFH). Historically, Texas blind salamander catches were infrequent, and in 2017 projections indicated it would take up to 10 years to reach the standing stock goal. In 2019, there was a surge in the occurrence of small juvenile Texas blind salamanders collected from February to September from the Diversion Spring net in Spring Lake, San Marcos, Texas. This surge greatly and quickly increased refugia stock at the SMARC to over 250 animals with more than 50% of the refugia stock comprised of this age class. Some individuals of this age class were transferred to the UNFH. Numbers incorporated, end of the year census, and survival rates can be found in Table 10.

Figure 9. Shawn Moore pulling up the Diversion Spring net in Spring Lake.

Table 10 Texas blind salamander refugia population figures

	Beginning of Year Census	Incorporated 2023	End of Year Census	In Quarantine End of Year	Target Goal 2023 Work Plan	Percent Survival
SMARC	172	9	88	3	250	48%
UNFH	66	0	62	0	60	94%

COLLECTIONS

Texas blind salamanders are collected from caves, wells, fissures, and driftnets on high flow springs. Traps are typically deployed quarterly in Primer’s Fissure, Johnson’s Well, Rattlesnake Cave, and Rattlesnake Well. Traps are checked two to three times weekly for two to three weeks before being removed from the site. To avoid over-sampling, only one third of salamanders observed are retained for refugia. Any gravid females are retained due to their rarity.

In 2023, Primer's Fissure and Johnson's Well were both sampled in May, but only Johnson's Well was sampled in August and November due to low water in Primer's Fissure in those months. In total, 20 TBS were captured from Johnson's Well, of which five were transferred to the SMARC. Eight TBS were captured from Primer's Fissure with two transferred to the SMARC. Neither Rattlesnake Cave nor Rattlesnake Well were sampled in 2023. All sites were trapped for two weeks during each collection event and biologists tagged Texas blind salamanders with a p-Chip transponder tag, scanned all collected salamanders for a p-Chip, and collected tail clips of all released salamanders for future genetic analysis. A total of 15 recaptures were observed throughout the year, where 11 occurrences were at Johnson's Well and 4 were at Primer's Fissure. The Diversion Springs driftnet was installed in July and checked two to three times a week for the rest of the year. One TBS was captured in the driftnet in November soon after a hard rain event in the area. This animal was retained for refugia at the SMARC.

Figure 10. Braden West and Shawn Moore processing Texas blind salamanders caught from the trap set in Johnson's Well.

Texas blind salamanders were transported directly to the quarantine space at the 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. All newly collected larvae and juveniles were held in individual, isolated tanks at the SMARC. Each tank received its own flow of fresh well water and habitat items. Animals remained in isolation for at least 30 days. Healthy individuals measuring 30 mm or greater in total length (TL) were non-lethally cotton swabbed to test for disease. Weak, injured, or very small individuals were not swabbed until they had recovered and/or reached 30 mm TL. When animals resided in a group tank, representative swab samples were taken for the group and tested for the presence of *Batrachochytrium dendrobatidis* (Bd, commonly referred to as amphibian chytrid fungus) and *Batrachochytrium salamandrivorans* (Bsal, another type of lethal chytrid fungus). Bd is common in North America, but Bsal has not yet been observed here. Bsal is known to be lethal for at least one *Eurycea* species (*E. wilderae*; Martel et al 2014). Texas blind salamanders were housed in quarantine according to their collection location, collection date, and size. Salamanders were not incorporated into the refugia until the results from the Bsal/Bd test were received.

Figure 11. Braden West scanning a p-Chip after tagging a Texas blind salamander at the SMARC.

Texas blind salamanders from all collection locations were housed together; however, individuals were tagged via p-Chip tags so that individual identification was possible. Corbin (2020) completed a genetic analysis of wild-caught Texas blind salamanders and showed low genetic diversity and no genetic differentiation between sampling locations. Thus, Texas blind salamanders do not have to be separated in the refugia by collection site. Texas blind salamanders were housed in large, insulated fiberglass systems at the SMARC and the UNFH with either flow-through or partial recirculation tanks. Water temperature and flow were checked multiple times daily. Total dissolved gas and pressure was checked immediately if salamanders begin showing symptoms of gas bubble disease, including the presence of trapped air bubbles underneath the skin, bloating, or an inability to stay submerged. Water quality parameters including dissolved oxygen, pH, 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. Staff routinely siphoned tanks to remove waste and other debris and replaced habitat items with clean ones. Each tank system had dedicated equipment (nets, cleaning supplies) to prevent the potential spread of pathogens from system to system. If equipment was ever shared, it was cleaned and disinfected between systems. Upon reaching 30 to 40 mm in TL, juveniles were marked with p-Chip tags (for individual identification) under sedation and were combined with other individuals of equivalent sizes. The tags allow for identification of individuals to access sex and collection information.

Figure 12. Dominique Alvear practicing tagging salamanders at the SMARC.

Adult salamanders were fed twice weekly and received either live amphipods, live blackworms, live red composting worms, live *Daphnia*, or frozen mysid shrimp. Juveniles were fed *Artemia* spp. nauplii or chopped blackworms as they increased in size.

SURVIVAL RATES

The survival of all Texas blind salamanders was 48% at the SMARC and 94% at the UNFH in 2023. Survival rates during quarantine period are not included in annual survival rates. The low survival of the SMARC TBS was a result of a well power outage causing a severe well water gas supersaturation event (Appendix J). Eighty-two wild caught Texas blind salamanders died as a result of the gasification event. We cannot fully predict what the survival rate of Texas blind salamanders at SMARC would have been without the event, but by removing the 82 that perished, the overall survival of Texas blind salamanders at the SMARC could have been as high as 94%.

HEALTH MONITORING

Biologists monitored salamanders for changes in appearance and behavior including emaciation, 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 and a veterinarian was consulted, if needed, for investigation into the cause of death.

MAINTENANCE OF SYSTEMS

Salamander refugia systems were deep cleaned annually with 20-30% vinegar (SMARC) or muriatic acid (UNFH) to remove calcium carbonate deposits that formed within the tank, plumbing, chiller, or pump casing. Water lines, hoses, valves, and restrictors were frequently checked for degradation or occlusion. These were cleared, rebuilt, or replaced as needed.

CAPTIVE PROPAGATION

Male and female salamanders were tagged so that collection information is known and were housed in group systems to encourage production of offspring for future research. Females were checked periodically for presence of visible eggs. Genetic analysis shows that collection locations are part of one panmictic population (Corbin 2020), thus these offspring could be employed should a restocking event occur.

In total, Texas blind salamanders at the SMARC produced 47 clutches of eggs and 8 clutches were produced at the UNFH in 2023. Clutch data are reported in Table 11.

Figure 13. A clutch of partially developed Texas blind salamander eggs on an artificial plant.

Table 11. Texas blind salamander clutches produced during 2023. Percent Survival is listed as "NA" for clutches that have not fully hatched.

Site	*Date*	*Parent Generation*	*Offspring Generation*	*# Deposited*	*# Hatched*	*(%) Survival*
UNFH	5/3/2023	WS	F1	38	6	NA
UNFH	7/5/2023	WS	F1	26	0	0
UNFH	7/31/2023	WS	F1	37	0	0
UNFH	7/31/2023	WS	F1	26	0	0
UNFH	9/27/2023	WS	F1	33	0	0

UNFH	11/24/2023	WS	F1	35	0	0
UNFH	12/18/2023	WS	F1	39	NA	NA
UNFH	12/18/2023	WS	F1	33	*	*
SMARC	1/31/2023	WS	F1	5	4	80
SMARC	2/6/2023	WS	F1	1	1	100
SMARC	2/8/2023	WS	F1	27	19	70.4
SMARC	2/11/2023	WS	F1	5	5	100
SMARC	2/21/2023	WS	F1	21	11	52.4
SMARC	2/28/2023	WS	F1	21	0	0
SMARC	3/3/2023	WS	F1	8	6	75
SMARC	3/10/2023	WS	F1	22	0	0
SMARC	3/24/2023	WS	F1	4	3	75
SMARC	4/7/2023	WS	F1	9	9	100
SMARC	4/17/2023	WS	F1	12	1	8.3
SMARC	4/25/2023	WS	F1	18	0	0
SMARC	4/26/2023	WS	F1	19	6	31.6
SMARC	5/1/2023	WS	F1	12	9	75
SMARC	5/1/2023	WS	F1	26	4	15.4
SMARC	5/8/2023	WS	F1	27	4	14.8
SMARC	5/10/2023	WS	F1	20	11	55
SMARC	5/15/2023	WS	F1	23	13	56.5
SMARC	6/20/2023	WS	F1	18	17	94.4
SMARC	6/20/2023	WS	F1	16	12	75
SMARC	6/20/2023	WS	F1	4	4	100
SMARC	7/4/2023	WS	F1	3	0	0
SMARC	7/5/2023	WS	F1	7	5	71.4
SMARC	7/7/2023	WS	F1	28	4	14.3

SMARC	7/10/2023	WS	F1	25	19	76
SMARC	7/14/2023	WS	F1	33	13	39.4
SMARC	7/28/2023	WS	F1	4	4	100
SMARC	7/31/2023	WS	F1	27	3	11.1
SMARC	8/21/2023	F1	F2	15	13	86.7
SMARC	10/10/2023	WS	F1	2	2	100
SMARC	10/10/2023	WS	F1	1	1	100
SMARC	10/17/2023	WS	F1	13	5	38.5
SMARC	10/23/2023	WS	F1	3	3	100
SMARC	10/30/2023	WS	F1	23	21	91.3
SMARC	11/7/2023	WS	F1	14	8	57.1
SMARC	11/14/2023	WS	F1	30	2	6.7
SMARC	11/15/2023	WS	F1	19	10	52.6
SMARC	11/21/2023	WS	F1	22	21	95.5
SMARC	11/21/2023	WS	F1	21	21	100
SMARC	11/21/2023	WS	F1	5	1	20
SMARC	11/27/2023	WS	F1	23	*	*
SMARC	11/30/2023	WS	F1	7	*	*
SMARC	11/30/2023	WS	F1	21	*	*
SMARC	12/5/2023	WS	F1	19	*	*
SMARC	12/6/2023	F1	F2	26	*	*
SMARC	12/6/2023	WS	F1	10	*	*
SMARC	12/19/2023	WS	F1	15	*	*

Notes: Clutches experience some degree of loss after hatching, therefore the number that hatched does not represent the number of offspring present at the facility.
*Clutches have not hatched yet

SAN MARCOS SALAMANDER (*EURYCEA NANA*), THREATENED

The Standing Stock goal for the San Marcos salamander is 500 individuals, divided between the two facilities. Typically, staff collect San Marcos salamanders twice each year in amounts sufficient to cover the expected loss given average mortality. In 2023, the number of collections for the refugia was reduced due to a mark-recapture study being conducted. Numbers incorporated, end of the year census, and survival rates can be found in Table 12.

Table 12. San Marcos salamander refugia population figures

	Beginning of Year Census	Incorporated 2023	End of Year Census	In Quarantine End of Year	Target Goal 2023 Work Plan	Percent Survival
SMARC	96	129	163	0	250	72%
UNFH	168	48	164	0	250	76%

COLLECTIONS

In 2023, there were San Marcos salamander collections for the refugia population in February (30 caught, 27 retained) and April (53 caught, 39 retained) in the San Marcos River at the Eastern Spillway below Spring Lake Dam. In March, there was also a collection at the Hotel Springs area in Spring Lake (75 caught, 54 retained). Thirty-three San Marcos salamanders were caught as by-catch from the Diversion Springs drift net, all of which were released.

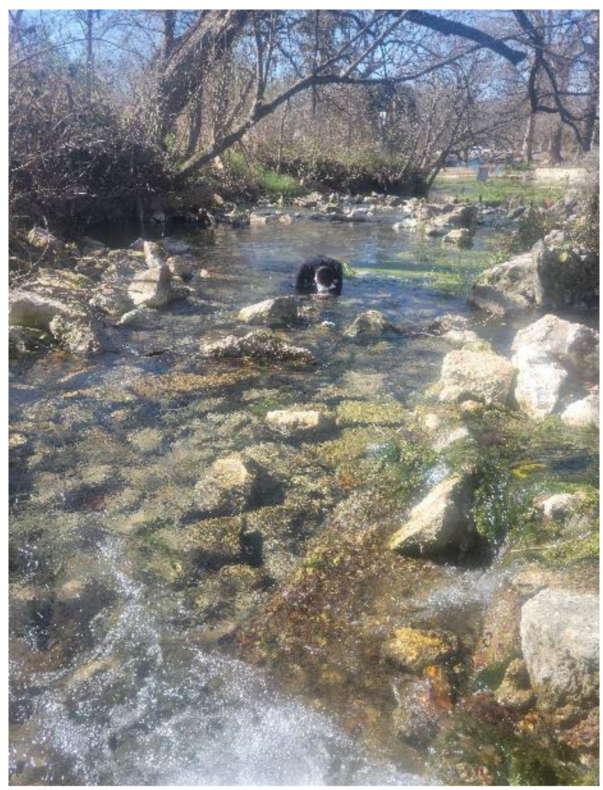


Figure 14. Shawn Moore snorkeling in the San Marcos River to collect San Marcos salamanders.

QUARANTINE

Figure 15. Shawn Moore swabbing salamanders for testing.

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. Healthy individuals collected from the wild were transported back to the SMARC where they were measured, and mucus samples were taken from those with a TL of 30 mm or greater with cotton swabs. Weak, injured, or very small individuals were not swabbed until they had recovered and/or reached 30 mm TL. For groups of salamanders, a representative sample was swabbed. Skin swabs were 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 a minimum of 30-days under observation before being added to Standing Stock numbers.

HUSBANDRY

Genetic analysis (Lucas *et al*. 2009) determined that there is no population structure across sites sampled in the wild, so individuals from all collection locations were combined. San Marcos salamanders at both facilities were housed in large, insulated fiberglass systems with either flow-through chilled well water (SMARC) or partial recirculation through heater-chiller units (UNFH) to maintain water temperature at 22 ±1 ℃. Water temperature and flow were checked daily. Total gas pressure was checked immediately if salamanders began showing symptoms of gas bubble disease, including the presence of trapped air bubbles underneath the skin, bloating, or an inability to stay submerged. Water quality parameters including, but not limited to, dissolved oxygen, pH, 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 in which to seek refuge. Staff routinely siphoned tanks to remove waste and other debris and rotated habitat items to be cleaned. Each tank system had dedicated equipment (nets, cleaning supplies) to prevent the potential spread of pathogens from system to system. If equipment was ever shared, it was cleaned and disinfected between systems. Adult salamanders were fed twice weekly and received either live amphipods, live blackworms or frozen mysis shrimp. Juveniles were fed *Artemia* spp. nauplii or chopped blackworms as they increased in size. A detailed description of salamander care can be found in the USFWS Captive Propagation Manual for *Eurycea* spp., available upon request.

SURVIVAL RATES

The survival rate of San Marcos salamanders in the refugia population was 72% at the SMARC and 76% at the UNFH. Survival rates during their quarantine period are not included in the annual survival rates. The mortality of egg-bound females continued at both refugia facilities. A super gas saturation event occurred at the SMARC due to a power failure (Appendix J). Fifty-six San Marcos salamanders died as a result of the event. Although we are unable to verify what the overall survival of San Marcos salamanders would have been without the event, by removing the 56 salamanders that perished, survival rates may have been as high as 97%.

HEALTH MONITORING

Biologists monitored salamanders for changes in appearance and behavior including emaciation, 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 and a veterinarian was consulted, if needed, for investigation into the cause of death.

MAINTENANCE OF SYSTEMS

Salamander refugia systems at both UNFH and the SMARC were deep cleaned annually with muriatic acid to remove calcium carbonate deposits that 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

In 2023, wild-stock salamanders produced ten clutches at the SMARC and seven clutches at the UNFH. Clutch information is presented in Table 13.

Table 13. Clutches of San Marcos salamanders.

Site	Date	Parent Generation	Offspring Generation	Eggs Deposited	# Hatched	(%) Survival
UNFH	1/11/2023	WS	F1	22	17	77
UNFH	3/20/2023	WS	F1	17	0	0
UNFH	4/4/2023	WS	F1	26	0	0
UNFH	4/20/2023	WS	F1	22	0	0
UNFH	7/31/2023	WS	F1	226	0	0
UNFH	8/8/2023	WS	F1	8	2	025
UNFH	9/1/2023	WS	F1	27	2	7
SMARC	1/31/2023	WS	F1	18	18	100
SMARC	2/7/2023	WS	F1	4	1	25
SMARC	2/24/2023	WS	F1	12	6	50
SMARC	2/28/2023	F1	F2	1	1	100
SMARC	3/2/2023	WS	F1	21	10	47.6

SMARC	3/10/2023	F1	F2	11	0	0
SMARC	3/27/2023	WS	F1	16	NA	NA
SMARC	5/3/2023	F1	F2	17	3	17.6
SMARC	6/25/2023	WS	F1	9	7	77.8
SMARC	7/3/2023	WS	F1	24	22	91.7
UNFH	8/01/2023	WS	F1	34	20	59%
UNFH	12/13/2023	WS	F1	15	*	NA

Notes: Clutches experience some degree of loss after hatching, therefore the number that hatched does not represent the number of offspring present at the facility.
*Clutches have not hatched yet

COMAL SPRINGS SALAMANDER (*EURYCEA PTEROPHILA*), NO LONGER PETITIONED

The Comal Springs salamander is a species covered in the Edwards Aquifer Habitat Conservation Plan (EAHCP) when it was designated as *Eurycea* sp. 8. At the time of writing the EAHCP, this species was undescribed, yet petitioned for listing under the Endangered Species Act (ESA). Devitt et al. (2019) evaluated genetic markers and considered *Eurycea* sp. 8 at Comal Springs to be *Eurycea pterophila* (Blanco Springs salamander). Whether the Comal Springs population has unique standing is yet to be determined. The U.S. Fish & Wildlife Service no longer considers the Comal Springs salamander a petitioned species. Nevertheless, Congress defined ESA “species” to include subspecies, varieties, and, for vertebrates, distinct population segments. For the purposes of the contract with the EAA, the Comal Springs population of *E. pterophila* will be considered as the Comal Springs salamander, and the refugia will continue to provide protection for this species as required under the EAHCP.

The Standing Stock goal for the Comal Springs salamander is 500 individuals, equally divided between the two facilities (SMARC and UNFH). Collections to augment the refugia population of Comal Springs salamanders have been limited by lower historical densities of Comal Springs salamanders in the currently used sampling locations as compared to sampling locations of San Marcos salamanders via observations of biologists and biomonitoring data. Lower densities in sampling locations 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 Springs salamanders will increase. Numbers incorporated, end of the year census, and survival rates can be found in Table 14.

Table 14 Comal Springs salamander refugia population figures

	Beginning of Year Census	Incorporated 2023	End of Year Census	In Quarantine End of Year	Target Goal 2023 Work Plan	Percent Survival
SMARC	110	2	50	6	150	45%
UNFH	93	0	83	0	135	89%

COLLECTIONS

In August 2023, staff collected 18 individuals, 12 of which were taken to the SMARC refugia.

QUARANTINE

In 2023, after collection all Comal Springs salamanders were transported directly to the quarantine facilities at the UNFH or SMARC. 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. Individuals were measured and mucus samples taken from those with a TL of 30 mm or greater with cotton swabs. Weak, injured, or very small individuals were not swabbed until they had recovered and/or reached 30 mm TL. For groups of juveniles, a representative sample was swabbed. Skin swabs were 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 a minimum of 30-days under observation before being counted towards Standing Stock numbers.

HUSBANDRY

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 22℃ (ranging between 20 to 23 ℃). Water temperature and flow were checked daily. Total gas pressure was checked immediately if salamanders began showing symptoms of gas bubble disease, including the presence of trapped air bubbles underneath the skin, bloating, or an inability to stay submerged. Water quality parameters including dissolved oxygen, pH, and total gas pressure, were checked weekly.

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

SURVIVAL RATES

Survival rates of Comal Springs salamanders were high in 2023, with 45% at the SMARC and 89% at the UNFH. The low survival of the SMARC Comal salamanders was a result of a well power outage causing a severe well water gas supersaturation event (Appendix J). Fifty-two Comal Springs salamanders perished as a result of the gas supersaturation event. Although we cannot fully predict what the survival rate at the SMARC would have been without the event, by removing the 52 Comal Springs salamanders that died, survival may have been as high as 91%.

HEALTH MONITORING

Biologists monitored salamanders for changes in appearance or behavior including emaciation, 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 and a veterinarian was consulted, if needed, for investigation into the cause of death.

MAINTENANCE OF SYSTEMS

Salamander refugia systems at both UNFH and the SMARC were deep cleaned annually with muriatic acid 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 2023, Comal Springs salamanders were housed in mixed-sex groups to encourage reproduction in refugia systems at both facilities. Reproduction can occur year-round as female salamanders come in and out of gravidity. Four clutches of eggs were produced at the SMARC and two clutches at the UNFH (Table 15).

Table 15. Propagation of Comal Springs salamanders

Site	***Date***	***Parent Generation***	***Offspring Generation***	***# Deposited***	***# Hatched***	***(%) Survival***
UNFH	8/1/2023	WS	F1	31	11	35
UNFH	12/13/2023	WS	F1	32	*	*
SMARC	2/13/2023	WS	F1	7	7	100
SMARC	2/15/2023	WS	F1	5	4	80
SMARC	2/21/2023	WS	F1	15	0	0
SMARC	2/27/2023	WS	F1	12	10	83.3

Notes: Clutches experience some degree of loss after hatching, therefore the number that hatched does not represent the number of offspring present at the facility.
*Clutches have not hatched yet

The standing-stock goal for Texas wild rice (TWR) is 430 plants divided between the two facilities. Texas wild rice is divided into alphabetical river segments (A-K) of the San Marcos River based on historical locations of bridges, dams and other structures (Richards et al. 2007).. Richards *et al*. (2007) and Wilson *et al*. (2017) assessed the genetic diversity of TWR in the San Marcos River from samples taken in 1998, 1999, 2002, and 2012. They also evaluated genetic diversity of TWR plants held at the SMARC. Wilson et al. (2017) found three unique genetic clusters of TWR plants in the San Marcos River but found that each of these clusters were represented in all the sections sampled in the study. Both studies suggested follow-up genetic monitoring to ensure that refugia populations continue to represent wild populations. In addition, genetic monitoring of refugia population can determine if individual plants are genetically identical, thus calling for the removal of one of the clones and the collection of a genetically distinct wild plant. A follow-up genetic analysis of the TWR population in the San Marcos River and in the UNFH and SMARC refugia was completed in 2021. Results showed unique genetic clusters within the river and that the refugia populations were genetically similar to wild populations. The Refugia Program aims to preserve the genetic diversity of refugia TWR by collecting tillers from plants throughout the river so that the refugia populations reflect the wild population. Refugia staff specifically targeted plant stands that were not currently represented in the refugia population. Plant stands were selected after overlaying refugia plant locations (determined with GPS) onto GIS maps produced by the SMARC Plant Ecology Program during the 2019 annual Texas wild rice Survey. Numbers incorporated, end of the year census, and survival rates can be found in Table 16.

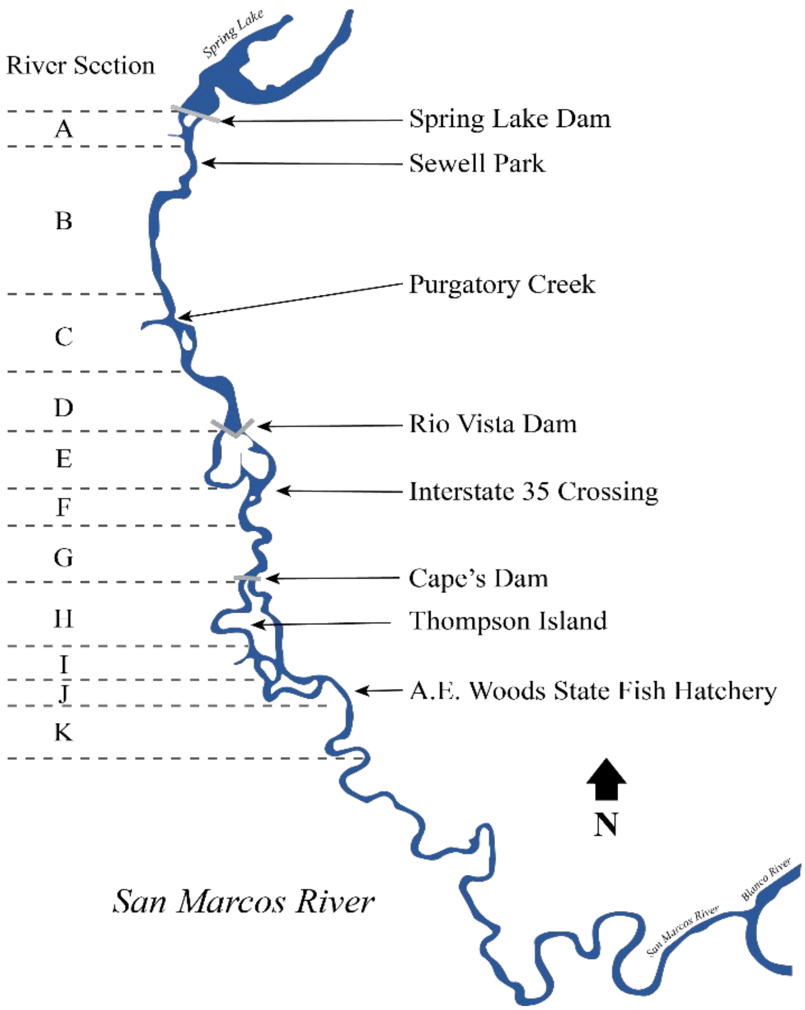


Figure 16. Lettered sections of the San Marcos River designating Texas wild rice habitat established by Texas Parks and Wildlife Department.

Table 16. Texas wild rice refugia population figures

	Beginning of Year Census	Incorporated 2023	End of Year Census	In Quarantine End of Year	Target Goal 2023 Work Plan	Percent Survival
SMARC	205	12	178	10	215	82%
UNFH	207	13	188	10	215	85%

COLLECTIONS

Tiller collections in the San Marcos River occurred in May, August, October, and December of 2023. USFWS staff 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. 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.

QUARANTINE

Quarantine procedures differ by station. 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. Salt treatments of incoming tillers (2% salt dip) have been discontinued. Incoming quarantine plants were kept in their respective mesh bags or lightly potted in a mesh cylinder with loose gravel and placed in a quarantine tank. During the quarantine time, they were routinely checked for aquatic nuisance species, specifically the invasive snail *Melanoides tuberculata.* After 30 days, plants were un-potted and the full plant visually inspected for aquatic nuisance species, before the tillers were re-potted and incorporated into the standing stock population.

Figure 17. Journey Moreno (Student Conservation Association intern) and Shawn Moore repotting Texas wild rice.

HUSBANDRY

We continued to investigate different soil, potting techniques, and water flow/velocity regimes for TWR plants at the SMARC and UNFH. When plants are potted, we add a layer of lava rock at the bottom of the pot (space in the dirt we have previously not found roots to reach) to reduce anoxia forming in the soil. As in previous years, when plants were added to refugia tanks, the inventory and map of plants in the tank were updated. Hand-count inventory and tag checks were conducted twice annually.

SURVIVAL RATES

Overall survival rate of TWR plants at the SMARC was 82%, with older plants more likely to succumb to mortality. The overall survival rate of TWR plants at the UNFH was 85%. The average lifespan in captivity, based on records of the 74 plants (with known collection location by GPS) that have died since 2016 is 1.7 years.

MAINTENANCE OF SYSTEMS

Water flow in the tanks was checked daily and standpipe screens were cleaned to ensure that no debris blocked water flow through the pumps at both stations. TWR tanks at the SMARC had individual heater-chiller units on tanks with 2 HP main pumps and 1/4HP accessory pumps to circulate water through units and produce flow throughout the tanks. At the UNFH, 1/2 to 3/4 HP submersible pumps are used to facilitate flow throughout the tanks.

Staff removed filamentous algae from the leaf blades by gently running fingers or a 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 leaves were routinely trimmed to approximately 30 inches to prevent overcrowding and shading in tanks. Staff trimmed off 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. Shade cloth was used over TWR tanks at the SMARC during the summer months to control algal growth in tanks.

CAPTIVE PROPAGATION

The EARP did not engage in propagation of TWR by sexual reproduction through seed production in 2023. However, the Plant Ecology and Restoration Program at the SMARC engaged in TWR plant propagation and continues to study and refine techniques.

RESEARCH

Research activities for the Refugia program (USFWS and sub-contractors) focused on captive holding and propagation of Comal Spring dryopid beetle, genetic assessments of covered invertebrate species, and mark-recapture studies on invertebrates and the San Marcos Salamander. Much of this research was built on knowledge gained in previous studies. Below are summaries for each project approved within the 2023 Work Plan (Appendix A).

MARK AND RECAPTURE OF SAN MARCOS SALAMANDERS

The objective of this study is to examine the recapture rate, movement rate, and demographics of wild San Marcos salamanders tagged with p-Chips. In May and June 2023, 453 San Marcos salamanders were tagged with p-Chips and released back to their collection locations at three sites in San Marcos, Texas, just downstream of the eastern spillway of the Spring Lake Dam, around the Diversion Springs pipe in Spring Lake, and at the headwaters area of Spring Lake. Recapture collections occurred 1-2 times each month at each of the sites. Thus far, the recapture rate across sites was 13%, varying 10-17%. A total of 2,013 San Marcos salamanders were collected for this study in 2023. No movement was detected yet. On average, the salamanders

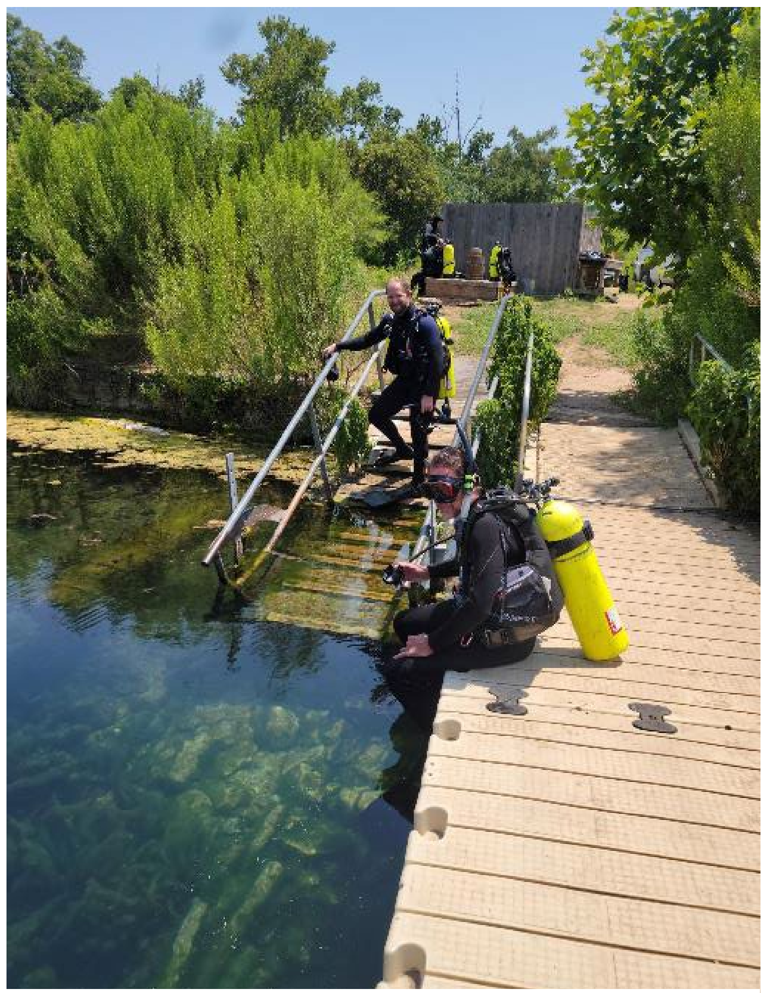


Figure 18. Justin Crow and Randy Gibson (SMARC biologists) preparing to dive to collect San Marcos salamanders in Spring Lake.

collected at the San Marcos River site were larger than the salamanders collected at the two Spring Lake sites. This study is in progress and collections are planned to continue through the end of May 2024. The interim report is in Appendix B.

CAPTIVE HUSBANDRY AND PROPAGATION OF THE COMAL SPRINGS DRYOPID BEETLE

The Edwards Aquifer Refugia Program houses Comal Springs dryopid beetles in captivity under the same conditions as the Comal Springs riffle beetle with the assumption that because they are found in the same or very similar locations, dryopid beetles utilize very similar habitat and food sources as riffle beetles. The dryopid beetle has very long egg and larval stages, which makes determining their captive needs difficult. Dryopid beetles survive captive holding in riffle beetle housing, but survival is low and larval production is rare, suggesting captive housing can be improved. This effort, led by BIO-WEST, uses challenge experiments to determine larval and adult dryopid beetle captive housing preference using riffle beetle housing as a reference and a cooccurring surrogate species as a comparison. Flow, light, habitat materials, the availability of interstitial space, and food sources have been compared. Although some habitat preferences have been determined, additional challenge experiment replicates are required because few individuals were included in the challenge experiments due to limited dryopid availability. The interim report is in Appendix C.

Figure 19. A Comal Springs riffle beetle tagged with a p-Chip.

TAGGING AQUATIC INVERTEBRATES

Determining tagging methodology for unique species is important for conducting research to inform the refugia and reintroduction methods. Dr. Shannon Brewer of the U.S. Geological Survey, Alabama Cooperative Fish and Wildlife Research Unit led this cooperative effort where the objectives were to: 1) evaluate the attachment of p-Chips and short-term tag retention on

Comal Springs riffle beetle and Peck's cave amphipod and 2) determine longer-term retention of the tag and survival of the tagged animals. A tagging protocol was designed for Comal Springs riffle beetle by chilling the beetle for two minutes and using superglue to affix the tag to the elytra of the beetle. The beetle quickly regained activity as it was warmed by the microscope light and was able to walk with no obvious hindrance from the tag. Internal tagging of Peck's cave amphipod was unsuccessful thus far, but additional tagging methods were identified for testing in year 2 (e.g., external tagging). The interim report is in Appendix D.

GENETIC ASSESSMENT OF PECK'S CAVE AMPHIPOD

The objective of this study is to assess the genetic diversity of the Peck's cave amphipod (PCA) in the Comal Springs System to determine the distribution of genetic diversity across their range. The information gathered from this study will identify locations with unique genetic diversity, inform collection and reintroduction strategies, and determine the minimum number of individuals required in the refugia to have a representative captive population. Peck's cave amphipods were collected as bycatch during Comal Springs riffle beetle collection efforts, as they are often observed on the same lures. PCA were collected using dip nets in locations where an insufficient number of individuals were collected. All collected PCA were preserved in 95% ethanol and transferred to Dr. Chris Nice at Texas State University for genetic analysis. A total of 119 PCA were collected for this study across six sampling locations. An interim report for this study is available in Appendix E.

COMPARATIVE GENE EXPRESSION IN SAN MARCOS SALAMANDERS TO TARGET REPRODUCTIVE TRIGGERS IN CAPTIVITY

Captive propagation for the San Marcos salamander is challenging. Multiple methods have been used to induce courtship and reproduction with little success. A comparative gene expression study was deployed to guide SMARC biologists in future attempts to improve captive propagation. Led by Ruben Tovar and Dr. David Hillis of the University of Texas Austin, the objective of this study was to 1) determine which genes are important for reproductively active/gravid salamanders versus non-reproductive salamanders and 2) determine which sensory organs correlated to reproduction and how this may play a role in mating cues. As oviposition occurred in the captive-assurance population at the SMARC, San Marcos and Texas blind salamanders were fixed in a proprietary fixative that allows for downstream molecular work to generate a comprehensive transcriptome. RNA quality and Quantity were sufficient for RNA sequencing. The interim report is in Appendix F.

Figure 2020. Ruben Tovar (University of Texas Austin), Nisa Sindhi (Texas State University), and Brittany Dobbins (Texas State University) processing salamanders for genetic analysis.

GENETIC ASSESSMENT OF THE COMAL SPRINGS RIFFLE BEETLE IN LANDA LAKE

The objective of this study is to assess the genetic diversity of the Comal Spring riffle beetle in the Comal Springs system to determine the distribution of genetic variation, identify locations with unique genetic diversity, and determine the minimum number of individuals required in the refugia to maintain a representative captive population. Poly-cotton lures were placed in

100 spring openings across the Comal Springs system including Spring Runs 1 – 3, Spring Island, Western Shore, and Upper Spring Run 4. A subset of the adult beetles and all larvae on each lure were collected and preserved in 95% ethanol for genetic analysis. DNA was extracted from the beetles using a Qiagen DNEasy Blood and Tissue DNA extraction kit. A total of 168 adult and larval Comal Springs riffle beetles were collected for this study. The interim report is located in Appendix G.

BUDGET

Task	U.S. Fish and Wildlife Service 2023		Budget Spent	Total Task Budget Spent
1	**Refugia Operations**			**$868,808.36**
	SMARC Refugia & Quarantine Bldg.			
	Construction		-	
	Equipment		$3,319.46	
	Utilities		$7,212.74	
	UNFH Renovation Refugia & Quarantine Bldg.			
	Construction		-	
	Equipment		$9,818.74	
	Utilities		$22,588.87	
	SMARC Species Husbandry and Collection		$155,785.17	
	UNFH Species Husbandry and Collection		$261,401.05	
	Diver Salaries		$0	
	Water Quality Monitoring System		$5,655.55	
	Fish Health Unit		$7950.65	
	SMARC Reimbursables		$78,484.38	
	UNFH Reimbursables		$159,921.39	
		Subtotal	*$712,138.00*	
		Admin Cost	*$156,670.36*	
2	**Research**			**$396,994.15**
	BIO-WEST: CSRB pupation (2021 Rollover)		$1,587.36	
	BIO-WEST: Dryopid Captive Holding		$72,200.46	
	Texas State: PCA Genetics		$1,826.17	
	University of Texas: Salamander Gene Expression		$41,014.19	
	Auburn University: Invertebrate Tagging		$26,650.57	
	USFWS Research Projects		$139,653.24	
		Subtotal	$325,405.04	
		Admin Cost	*$71,589.11*	
3	**Species Propagation and Husbandry**		-	-
4	**Species Reintroduction**		-	-
5	**Reporting**			**$40,019.30**
	SMARC Staff		$24,527.99	
	UNFH Staff		$8,274.71	
		Subtotal	*$32,802.70*	
		Admin Cost	*$7,216.60*	
6	**Meetings and Presentations**			**$17,183.34**
	SMARC Staff		$10,503.19	
	UNFH Staff		$3,581.51	
		Subtotal	*$14,084.70*	
		Admin Cost	*$3,098.64*	
			TOTAL	**$ 1,323,005.15**

ACRONYMS AND ABBREVIATIONS

Bd	*Batrachochytrium dendrobatidis*
Bsal	*Batrachochytrium salamandrivorans*
CSDB	Comal Springs dryopid beetle
CSRB	Comal Springs riffle beetle
EAA	Edwards Aquifer Authority
EAHCP	Edwards Aquifer Habitat Conservation Plan
ESA	Endangered Species Act
FAC	Fish & Aquatic Conservation Program
GIS	Geographic information system
GPS	Global positioning system
HP	Horsepower
ITP	Incidental take permit
JGI	Joint Genome Institute
LHRH	Luteinizing hormone releasing hormone
LMBV	Largemouth bass virus
PCA	Peck's cave amphipod
PIT	Passive integrated transponder
PVC	Polyvinyl chloride
USFWS	U.S. Fish & Wildlife Service
SCUBA	Self-contained underwater breathing apparatus
SFHU	Southwestern Fish Health Unit
SMARC	San Marcos Aquatic Resources Center
TL	Total length
TWR	Texas wild rice
TXST	Texas State University
UNFH	Uvalde National Fish Hatchery
VIA	Visible implant alpha-numeric
VIE	Visible implant elastomer
WAAS	Wide area augmentation system

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PROFESSIONAL PRESENTATIONS FROM STAFF AND COLLABORATORS

Daw, A. 2023. Improvements and modifications to the Edwards Aquifer Refugia Program Husbandry and Collections. Edwards Aquifer Habitat Conservation Plan Stakeholder Committee Meeting, San Antonio, Texas.

Moore, D.M. 2023. Evaluation of p-Chip tags on small-bodied aquatic salamander species. Texas Conservation Symposium, Georgetown, Texas.

Moore, D.M. 2023. Evaluation of p-Chip tags on small-bodied aquatic salamander species. R2 Fish and Aquatic Conservation Science Symposium, Region 2, USFWS.

Moore, D.M. 2023. P-Chip tagging in small-bodied aquatic organisms. Workshop taught at the San Marcos Aquatic Resources Center, San Marcos, Texas. (March 21, 2023)

Moore, D.M. 2023. The application of p-Chip tags on small-bodied aquatic organisms. The 70th Annual Meeting of The Southwestern Association of Naturalists, San Antonio, Texas.

Moore, D.M. 2023. Mark and recapture of wild San Marcos salamanders. Edwards Aquifer Habitat Conservation Plan Stakeholder Committee Meeting, San Antonio, Texas.

Moore, S.E. 2023. The historical fountain darter tissue archive. The 70th Annual Meeting of The Southwestern Association of Naturalists, San Antonio, Texas.

Moore, S.E. 2023. Archiving preserved biological samples and DNA viability testing. Second Annual Fish and Aquatic Conservation Science Symposium, Region 2, USFWS.

West, B. 2023. Improvements to culture techniques for Peck's cave amphipod. Second Annual Fish and Aquatic Conservation Science Symposium, Region 2, USFWS.