San Marcos Aquatic Resources Center
May 2015

Aquatic Species Conservation and Management: Refugium Activities

San Marcos Salamander- As of 31 May 2015, 359 San Marcos salamanders were being maintained in the SMARC refugia. No salamanders were collected from Diversion Springs net this month. On 15 May 2015, the Diversion Springs net was removed from Spring Lake. In May, eight of the 77 salamanders being held in quarantine perished. Cause of death was probably injuries that occurred to very young salamanders while in the collection net. The remaining 69 were incorporated into the refugia population. Two deceased salamanders were observed in the refugia this month (Table 1). Wild stock salamanders and their offspring did not oviposit eggs during May (Table 1). (CSF 7.12.5.4)

Table 1.- Four-month summary of the number of salamanders held in and number of eggs produced for the refugia at the San Marcos Aquatic Resources Center. Abbreviations are as follows: RWS= refugium wild stock, WS=wild stock (specimens in quarantine), FX=offspring, JA=juveniles/adults, OV=ovipositions, and EG=eggs.

Species		February 2015			March 2015			April 2015			May 2015		
		JA	OV	EG	JA	OV	EG	JA	OV	EG	JA	OV	EG
San Marcos salamander	RWS	298	0	0	293	1	45	292	1	31	359	0	0
	FX	71	0	0	107	0	0	104	0	0	101	0	0
Quarantine	WS	0	-	-	0	-	-	77	-	-	0	-	-
Texas blind salamander	RWS	115	2	53	122	0	0	129	0	0	138	0	0
	FX	118	0	0	129	1	16	127	1	31	127	0	0
Quarantine	WS	6	-	-	2	-	-	8	-	-	2	-	-
Barton Springs salamander	RWS	37	0	0	33	0	0	31	0	0	31	0	0
	FX	632	3	114	623	0	0	608	0	0	609	0	0

Texas Blind Salamander- As of 31 May 2015, 138 wild caught Texas blind salamanders were being maintained in the SMARC refugia. During May, SMARC staff collected four Texas blind salamanders, one from Primer's Fissure and three from Johnson's Well. Edwards Aquifer Research and Data Center (EARDC) staff collected two young salamanders from Sessom Creek Springs in May. Seven of the nine salamanders collected during May and three of eight in quarantine from last month were incorporated into the refugia population. Five larvae collected last month perished while in quarantine. Two larvae collected this month remain in the isolation system (Table 1). One deceased wild stock adult was observed in the refugia population this month (Table 1). Wild stock Texas blind salamanders and their offspring did not oviposit eggs this month (Table1). (CSF 7.12.5.4)

Barton Springs Salamander- No salamanders were collected from Barton Springs in May (Table 1). Two wild stock salamander mortalities were observed this month (Table 1). Wild stock Barton Springs salamanders and their offspring did not oviposit eggs this month (Table 1). (CSF 7.12.5.4)

Fountain Darter- On 30 May 2015, there were 718 wild stock fountain darters in the SMARC refugia. Twenty-four fish were incorporated into the San Marcos (Upper = 10 and Middle = 7) and Comal (Upper = 4 and Lower = 3) refugia populations. No mortalities were recovered during May from the wild stock tanks. On 11 May, 110 darters were shipped to Dexter SNARRC for fish health analyses (N = 60 from Comal and N = 50 from San Marcos rivers). (CSF 7.12.5.4)

Devils River Minnow- The SMARC is maintaining two stocks of wild caught Devils River minnows (DRM) in refugia, one from San Felipe Creek (N = 160) and another from Pinto Creek (N = 89). During May, all Pinto Creek F1 Devil River minnows were transferred to outdoor raceways. Five hundred and fifty-seven F1 juveniles were transferred to Raceway 5 and 336 were transferred to Raceway 6. These fish were produced during October 2014. In addition, a total of 63 F1 Pinto Creek adults were stocked into Raceways 5 (N = 30) and 6 (N = 33). These F1 Pinto Creek adults were produced prior to 2014. The SMARC also continues to maintain 721 F1 DRM from San Felipe Creek in Raceway 4. Non-lethal tissue samples have been collected annually from the fish in Raceway 4 for genetic analyses. The genetic analyses of wild stock San Felipe Creek and Pinto Creek DRM is ongoing at the Dexter SNARRC. As the genetic information becomes available, it will be incorporated into a propagation/genetic management plan for DRM. (CSF 7.12.5.4)

Comal Springs Riffle Beetle- Approximately 110 adult wild stock Comal Springs riffle beetles, seven F1 adult offspring, and 48 larvae are being maintained at the SMARC. Wild stock beetles collected during May monitoring by BIO-WEST, Inc., were brought into the SMARC to supplement the research populations. (CSF 7.12.5.4)

Peck's Cave Amphipod- Approximately 56 adult Peck's cave amphipods and 120 *Stygobromus* juveniles are being maintained at the SMARC. Wild stock amphipods collected during May monitoring by BIO-WEST, Inc., were brought into the SMARC to supplement the research populations. (CSF 7.12.5.4)

Texas Wild Rice- As of 30 May 2015, the SMARC had 260 Texas wild rice plants in refugia. There are 145 plants in greenhouse raceways, 80 in outdoor raceways, and 35 in quarantine along with an additional 86 plants at Uvalde NFH (Table 2). Tillers were collected during October and November from the San Marcos River (N= 10 Section B, N = 10 Section C, and N = 15 Section F) and are still being held in quarantine. Additional collection of tillers will be made in 2015 based on the recommendations outlined in the Texas wild rice genetics report. (CSF 7.12.5.4)

On 30 May 2015, the SMARC had 22,159 (N_{2009} = 390, N_{2010} = 585, and N_{2011} = 1,941, N_{2012} = 10,152, N_{2013} = 6,550, N_{2015} = 2,541) Texas wild rice seeds in storage (Table 3). No seeds were collected for storage from September 2013 through April 2015. During May, Texas wild rice seeds (N = 2,341) were collected from Section A (N = 419), B (N = 1,882), C (N = 51), and F (N = 189). The location of each plant was taken with GPS and entered into an Excel spreadsheet. Floods during the last week of May submerged the plants, dislodged most of the mature seeds,

and damaged flowers. Further assessments will occur as the water level recedes. (CSF 7.12.5.4)

Table 2.- Current number of Texas wild rice plants being maintained in refugia at the SMARC and Uvalde NFH. San Marcos River segments are defined in accordance with the USFWS 1996 Contingency Plan where each segment represents a particular stand's genetic make-up. The number of plants within each pot varies (Mean ± 1SE = 61 ± 6 stems per pot). The research stock is comprised of clones and plants produced from various river segments.

	Number of Potted Plants				
		SMARC Refugia		Uvalde NFH	
	Greenhouse	Outdoor Raceway	Quarantine	Refugia	Total
A	13	25	0	18	56
B	54	51	10	19	134
C	24	4	10	10	48
D	0	0	0	6	6
E	7	0	0	0	7
F	18	0	15	4	37
G	1	0	0	8	9
H	1	0	0	0	1
I	0	0	0	0	0
J	10	0	0	2	12
K	3	0	0	4	7
Research Stock	14	0	0	15	29
Total	145	80	35	86	346

Table 3.- Number of Texas wild rice seeds stored at the SMARC. Seeds are stored by month and year.

Month	2009	2010	2011	2012	2013	2014	2015	Total
Jan					491			491
Feb								
Mar								
Apr								
May					264		2,541	2,805
June			433		2,307			2,740
July			650		1,172			1,822
Aug					2,316			2,316
Sept				3,428				3,428
Oct		325	273	1,785				2,383
Nov	390	260	585	3,267				4,502
Dec				1,672				1,672
Total	390	585	1,941	10,152	6,550	0	2,541	22,159

Research and Restoration Activities

Texas Blind Salamander- In December, Justin Crow, Taylor Quiros (student volunteer from

Trinity University), and Valentin Cantu continued to examine the size-related effects of non-lethal tissue collection from Texas blind salamanders. Tail-clipping is a common non-lethal method that has been used to obtain tissue from salamanders for genetic analyses. Although previous studies demonstrated no negative effects on terrestrial and biphasic salamander snout-vent length, mass, or survival when tail clipped, culturists and management personnel have concerns that federally listed padeomorphic salamanders such as the Texas blind salamander may be negatively affected. Like other *Eurycea* salamanders, Texas blind salamanders likely exhibit different growth and reproductive patterns and susceptibilities to disease throughout their life cycle. As a result, tail clipping may negatively affect their growth rate, reproductive fitness, or risk to disease, depending on their age and size. Thus, assessing the size dependent responses of the salamander to tail clipping should provide culturists and management personnel with relevant and timely information regarding when and if this common method should be employed. As of May, no clinical signs of disease or mortalities have been observed in tail clipped specimens. Preliminary data suggest there is a negative relationship between salamander size and the rate of tail regeneration. The smallest salamander group regenerated their tail tissue within 18 days, the intermediate size group at about 45 days and the largest group > 45 days. The largest size group still has not fully regenerated their tails (last measured 6 May 2015). Because growth is slower in adults, caution should be exercised so that repeated clips are not taken from the same individual before they have enough time to fully regenerate their tails. In adult plethodontids, tails function in swimming which is important for escaping predators, capturing prey, and for broadcasting their pheromones to attract females and defending breeding territory. In this study, tail clipping was conducted under controlled conditions using disinfected equipment while exposing them to clean well water; additional stressors such as capture, handling, poor water quality, pollution, or the presence of disease in wild populations may result in higher rates of infection or mortality than we observed. (CSF 7.12.5.4)

Barton Springs Salamander- The Texas A&M Veterinary Medical Diagnostic Laboratory (TVMLD), SMARC, and Dexter SNARRC Fish Health Unit are working together to investigate a disease outbreak that occurred in the SMARC Barton Springs salamander refugia in June 2013. Salamander mortalities in the Barton Springs refugia had increased from four mortalities in June 2013 to twenty mortalities in July 2013. In an effort to curtail salamander losses, SMARC staff immediately began isolating salamanders exhibiting external lesions, sores, red swollen cloaca, and tissue and tail degeneration. The TVMLD was contacted to help determine the cause of mortalities and for treatment recommendations. By November 2013, Jill Heatley DVM MS DABVP (Avian) DACZM and Jordan Gentry DVM from TVMLD had visited the SMARC Barton Springs refugium and conducted standard diagnostics on six salamanders at their clinic. By January 2014, mortalities had gradually dropped down to zero. After diagnostics, the captive salamanders appeared to be infected with microsporidia as evidenced by histopathological and preliminary polymerase chain reaction (PCR) results. Unfortunately the PCR technique was unable to identify the microsporidia at species level. Therefore, current sampling PCR-based methods will need to be optimized to increase the detection sensitivity and species specificity by developing new primers. Microsporidia is a concern because there are currently no treatments for microsporidia in salamanders and because it may also affect other captive salamander species. A proposal was developed to optimize the current PCR method and test voriconazole as a potential treatment. In February 2015, Guan Zhu from TVMLD designed two new pairs of

primers capable of detecting the unidentified species of microsporidia. Dr. Weiss, Albert Einstein College of Medicine Department of Pathology, Bronx, NY, will use transmission electron microscopy (TEM) to verify whether the unidentified microsporidia is a new species. In April, a cooperative agreement was developed between the SMARC and the TVMLD to fund this research. On 12 April, Dr. Zhu (TVMLD) conducted PCR on forage used to feed salamanders, including hatchery raised zooplankton (Cladocera, Calanoida and Cyclopoida), amphipods (*Hyalella azteca*), and snails (*Helisoma anceps* and *Elimia comalensis*), farm raised black worms (*Lumbriculus variegatus*), adult brine shrimp (*Artemia*), frozen blood worms (Diptera), *Mysis* shrimp, and fish flakes. Preliminary PCR tests revealed that zooplankton and native ramshorn snail (*Helisoma anceps*) were the only food to be infected with microsporidia. Dr. Zhu requested additional samples to validate the preliminary findings. SMARC provided Dr. Zhu, during his 20 April visit to SMARC, with additional zooplankton and snail samples that were separated by order and family. TVMLD tentatively recommends against feeding zooplankton to salamanders until additional PCR tests are conducted and confirmed. On 18 May 2015, the SMARC finalized a co-op agreement with TVMLD. (CSF 7.12.5.4)

On 2 April, Dr. Caitlin Gabor and her graduate student Megan Mondelli (TSU) submitted a proposal to the SMARC titled "Stress caused by storm water runoff in the Barton Springs salamander, *Eurycea sosorum*" to gain a better understanding of how pollution affects stress levels and physiological health in salamanders. Meagan set up her experimental tank system and initiated preliminary trials to determine if 14 days is sufficient to allow salamanders to acclimate to hatchery conditions after being caught from the wild. In late May, Megan was able to collect storm water runoff and she began her trials. (CSF 7.12.5.4)

Fountain Darter- SMARC staff, in collaboration with the Dexter SNARRC Fish Health Unit and the Leetown USGS lab, is examining the pathology of an unknown aquareovirus. The experiment will entail disease challenges conducted on F1 propagated fountain darters. The SMARC collected wild adult fountain darters from the Comal River. These fish were used as brood stock to propagate approximately 700 fish to be used in the experiment. The Leetown laboratory obtained their Endangered Species permit in November. Two hundred darters were transferred to Leetown laboratory on 29 December for phase 1 of the experiment. A second group of 200 darters was shipped to the Leetown laboratory on 9 February for phase 2 of the study. From the two fountain darter challenges, it appears the aquareovirus will infect the fountain darter, replicate in the fountain darter, but not cause mortality under the conditions tested. Devils River minnows were shipped to Leetown on 27 April but only a few fish survived shipment. It is thought that low water temperature during shipment killed the fish. A second shipment of Devils River minnows was shipped on 4 May. All of these fish survived the trip and were used in a third trial to determine if the aquareovirus could be transferred from infected fountain darters to Devils River minnows. (CSF 7.12.5.4)

Comal Springs riffle beetle- In December 2014, a collaborative proposal with Randy Gibson and Professors Weston Nowlin and Benjamin Schwartz (TSU) was selected for funding by the Edwards Aquifer Habitat Conservation Plan for a series of Edwards Aquifer invertebrate studies. The research is aimed at developing and defining basic culture techniques and requirements for both the Comal Springs riffle beetle and Peck's cave amphipod. More specifically the studies

include examining different anesthetic techniques for enumeration and non-lethal examination of specimens, light and dark habitat preferences to presumably reduce stress while in captivity, and evaluation of various aquatic invertebrate captive rearing and holding systems. During May, McLean Worsham and Nate Krukpka (TSU) began construction of aquatic systems at the SMARC to carry out these studies. (CSF 7.12.5.4)

In February, BIO-WEST Inc. staff and TSU students set cotton cloth lures in springs of the upper Devils River to attract the riffle beetle, *Heterelmis cf. glabra*. This is part of an EAA funded study (Comal Springs riffle beetle habitat connectivity study #132-14-HCP) that will compare survival of riffle beetles at the Freeman Aquatic Building facility (TSU) with the SMARC using H. cf. glabra as surrogate for the Comal Springs riffle beetle. These lures were retrieved during March and approximately 500 beetles were collected. On 16 March, TSU volunteers placed 27 Comal Springs riffle beetles and 27 common riffle beetles (*Microcylloepus pusillis*) from Comal Springs each in identical containers at both the TSU Freeman Aquatic Building (FAB) and the SMARC. A third set of containers with 27 *H. cf. glabra* were added on 23 March at both facilities. This is part of an EAA funded study (Comal Springs riffle beetle habitat connectivity study #132-14-HCP) that will compare survival of riffle beetles at the FAB with the SMARC. Beetles survived at the SMARC but did not at the FAB. Further experimentation for this project with the riffle beetles will take place at the SMARC until the FAB resolves this issue. During May, TSU staff set up two aquatic systems in the SMARC reuse building. The aquatic systems are being used to evaluate the long term temperature limitations of the Comal Springs riffle beetle. (CSF 7.12.5.4)

Comal Springs dryopid beetle- Kate Bell (TSU) and Dr. Chris Nice in collaboration with Randy Gibson are determining the population genetics of the Comal Springs dryopid beetle. Her work will be compared with the genetics of the other Comal Springs species being performed by Lauren Lucas. This should result in information regarding how many beetles should be maintained in refugia and indicate how related populations are at the various spring orifices. During June 2013, a total of 53 Comal Springs dryopid beetles collected over the past decade were transferred to TSU for DNA extractions. Results from this study showed that the dryopid beetle within the Comal Springs system had 15% immigration rate per generation among the different locations sampled. As a result of gene flow among the sites it appears that there is no significant genetic differentiation associated with distance between the various sites sampled. Therefore, it was recommended that sites only be maintained separately in refugia if space is available. If refugium space is limited, pooling specimens from different locations appears not to negatively affect genetic conservation. Information from this study was included in a manuscript by Lucas, L., Z. Gompert, J.R. Gibson, K. Bell, C Buerkle, and C. Nice entitled "Pervasive gene flow across critical habitat for four narrowly endemic, sympatric taxa." The manuscript was submitted to Freshwater Biology during May. (CSF 7.12.5.4)

Texas troglobitic water slater - During May, Randy Gibson, and TSU Professors Weston Nowlin, Benjamin Schwartz, and Chris Nice submitted a proposal to the TPWD and were selected for funding through the State Wildlife Grant system for a multiyear project on the genetics, distribution, ecological modeling, captive propagation, and physiological limitations of the Texas troglobitic water slater, *Lirceolus smithii*.

Texas wild rice- During January 2014, a study was initiated to evaluate the effects of varying flow rates on the phenology of Texas wild rice. Growth rates of Texas wild rice are measured every 2 weeks, and the plants monitored for flowering, seed production, tiller formation, and senescence. At 14 weeks, the total length (all stems and leaves) of the plants increased by more than 50% in tanks with flow, and more than doubled in total length in the tanks with the highest flow when compared to the lower flow tanks (Table 4). Since flowering began, pistil and stamen growth, seed production and flower senescence have been recorded weekly (Table 5). Seeds from the experimental plants are collected 2-3 times per week and monitored for germination. As of 31 December, a total of 24,787 seeds (ca. 165 seeds per plant) was collected from the research plants with germination rates ranging from 31 to 100%. New flower development decreased in December while previously flowering stems have died (Table 5). From February to May 2015, fewer flowers were recorded and there was a substantial increase in the number of senescent flowering stems. (CSF 7.12.5.4)

Table 4.- Mean growth rates (cm) and survival (%) for Texas wild rice (n = 45/treatment) grown under four water flow rates (i.e. 0.0, 0.1, 0.2, and 0.4 m/s) for 14 weeks.

Time	Total length (cm; stems and leaves)			
	Flow rate (m/s)			
(weeks)	0.0	0.1	0.2	0.4
0	9.9 (0.9)	11.3 (1.3)	10.8 (1.3)	9.0 (1.0)
2	31.4 (3.1)	47.1 (7.3)	35.9 (4.6)	36.0 (4.3)
4	83.0 (6.6)	151.6 (19.1)	108.8 (13.0)	126.1 (13.9)
6	196.7 (16.2)	371.3 (39.2)	265.6 (29.2)	324.9 (31.8)
8	348.2 (29.8)	775.8 (71.2)	611.9 (53.2)	828.9 (70.5)
10	614.5 (42.4)	1372.0 (120.5)	1130.6 (94.8)	1747.6 (148.2)
12	835.7 (50.7)	1855.9 (151.0)	1640.2 (153.2)	2969.3 (233.3)
14	1068.5 (60.8)	2384.6 (185.2)	2122.9 (200.4)	4253.7 (326.0
Survival (14 wk)	100.0	97.8	100.0	91.0

Table 5. Phenological traits of Texas wild rice over 27 weeks for plants germinated from seeds and maintained at four different flow rates.

	Water flow (m/s)			
	0.0	0.1	0.2	0.4
First leaf blade	----------< 2 weeks after germination ----------			
Emergent stems	----------5 weeks ----------			11 weeks
Tiller development	----------13 weeks ----------			15 weeks
Flower (pistil)	17 weeks	16 weeks	15 weeks	16 weeks
Flower (stamen)	18 weeks	18 weeks	17 weeks	18 weeks
Complete flower	18 weeks	18 weeks	17 weeks	18 weeks
Mature seeds	22 weeks	20 weeks	20 weeks	22 weeks
Flower senescence	---------- 22 weeks ----------			
Stem senescence	27 weeks	25 weeks	26 weeks	26 weeks

Leah Murray and Jeff Hutchinson set up ten tanks to test the effects of various copper

concentrations (0.0 to 3.0 ppm) on Texas wild rice, arrowhead, creeping primrose willow, and water stargrass. The objective of the project is to determine an acceptable application rate that simultaneously controls algae without negatively affecting plants. The first treatment occurred in December 2014 and the experiment will be repeated. At three weeks post-treatment, water stargrass exhibited ca. 25% necrosis but none of the other species were affected by the treatments, regardless of concentration. As of May 2015, all native plants have survived the treatments and algae remains minimal. The greenhouse is currently being reconfigured for additional electrical outlets so that additional fiberglass tanks can to set up to run additional copper trials. The copper trials will be initiated during the summer of 2015. (CSF 7.12.5.4)

Milkweed and Native Wildflowers – During March 2015, Jeff Hutchinson and Leah Murray received a $7,000 grant from the USFWS Texas Oklahoma Pollinator and Monarch Partnership to establish milkweed and native wildflower plots in the blackland prairie at the SMARC. Experimental plots were established in the blackland prairie to determine the best way to create more milkweed habitat for the monarch butterfly (till vs. no till, watering vs. no watering, seedlings vs. direct seeding). Volunteers with the SMARC Friends Group assisted with setting up the plots. During 21 to 28 April 2015, two 67 m line transects were monitored in each of the six 1- acre plots and all vegetation along the transects were recorded to document post-treatment cover. Application of the treatment, seeding, and plantings will occur in the plots during June to August. SMARC staff is also propagating milkweed for planting at Balcones National Wildlife Refuge. Seeds of antelope horn (*Asclepias asperula*; Asclepiadaceae) collected at Balcones NWR have germinated in the green house as of 20 March 2015. In addition, seeds of Green Milkweed Vine (*Matelea reticulate*; Asclepiadaceae) have germinated. Seeds from antelope horn were collected from sites in San Marcos, New Braunfels, and Inks Dam NFH in late May, and collections will continue for all milkweed species throughout the summer. During May, there was record rainfall with rain occurring about every day of the month and the prairie soil is saturated. Once the soil dries up, treatments and planting will begin in the plots at SMARC. (CSF 7.12.5.4)

Leah Murray and Jeff Hutchinson are in the process of setting up a design to grow the federally listed candidate species bracted twistflower (*Streptanthus bracteatus;* Brassicaceae*)* for seed accessions and restoration. Chris Best (Austin ES) and Norma Fowler (UT Austin) are advising Jeff and Leah, as they have worked with this plant extensively. The SMARC currently has approximately 6,000 seeds obtained from Walter Stuart in Bee Cave, Texas who has also worked extensively with this species. Leah Murray attended the bracted twistflower annual survey in San Antonio with Chris Best and Doug Phillips of Austin ES on 28 April to learn more about this rare Texas Hill Country endemic plant. The SMARC has received approval to grow bracted twistflower. Leah Murray is currently working on a research proposal. The study will examine soil magnesium levels and its effect on plant growth. (CSF 7.12.5.4)

Native Aquatic Plants - Native aquatic and terrestrial plants currently are being propagated and maintained in the SMARC greenhouse in fiberglass tanks and under outdoor irrigation systems for the City of San Marcos's native aquatic plant restoration efforts. Plant transfers to the City of San Marcos began in March 2013 to meet the restoration goals outlined in the Edwards Aquifer Habitat Conservation Plan. In 2014, the SMARC provided a total of 12,551 aquatic and

terrestrial plants including Texas wild rice to the City of San Marcos. In January 2015, spring flows increased enough to allow planting activities under the City's 10(a)(1)(B) permit to resume. As of 30 May 2015, 4,716 aquatic plants were provided to the City of San Marcos since the start of 2015. The City received 3,465 Texas wild rice seedlings, 935 creeping water primrose willow, 57 arrowhead, 214 water stargrass, and 45 Illinois pondweed plants for their restoration efforts. SMARC staff continues to maintain and propagate additional plants for future restoration work. (CSF 7.12.5.4)

During March to May, native seeds of riparian and terrestrial plants were collected from City of San Marcos properties along the San Marcos River. Seeds were collected from grasses, forbs, shrubs, and trees and are being stored at the SMARC in labeled containers. The seeds will be used for future planting. Volunteer planting days were held on 18 and 27 March at Rio Vista Park area, and a total of 530 plants were provided by the SMARC and planted in areas where non-native trees were removed (Table 7). The SMARC hosted its first Volunteer Planting Day to re-vegetate the San Marcos River Banks on Wednesday 18 March 2015 in coordination with the City of San Marcos near Playscape Park. Total attendance was approximately 30 people. The area will be fenced off for up to 10 years so plants can establish themselves. The SMARC hosted its second Volunteer Planting Day with 16 Phoenix High School students on 27 March 2015 on the San Marcos River in coordination with the City of San Marcos at Rio Vista Park. Planting days were also held on 4, 18, and 24 April to plant native vegetation propagated at the SMARC. Planting areas were bare from recreational use and invasive plant removal efforts and presumably have a high erosion potential. (CSF 7.12.5.4)

Table 7. Aquatic and terrestrial plants provided to the City of San Marcos for restoration work in and along the San Marcos River during 2015.

Aquatic Plants		
Arrowhead	*Sagittaria platyphylla*	57
Creeping primrose willow	*Ludwigia repens*	935
Ilinois pondweed	*Potamogeton illinoensis*	45
Texas wild rice	*Zizania texana*	3465
Water stargrass	*Heteranthera liebmannii*	214
		$\sum = 4716$
Terrestrial Plants		
American elm	*Ulmus americana*	15
Anaqua	*Ehretia anacua*	22
Baby blue eyes	*Nemophila phacelioides*	20
Bald cypress	*Taxodium distichum*	25
Beautyberry	*Callicarpa americana*	8
Black willow	*Salix nigra*	20
Box elder	*Acer negundo*	26
Brushy bluestem	*Andropogon virginicus*	30

Buttonbush	*Cephalanthus occidentalis*	20
Cedar elm	Ulmus crassifolia	14
Cottonwood	*Populus deltoids*	10
Dewberry	*Rubus trivialis*	1
Eastern gamagrass	*Tripsacum dactyloides*	70
Eastern redbud	*Cercis canadensis*	6
Elderberry	*Sambucus canadensis*	42
Emory's sedge	*Carex emoryi*	85
Inland sea oats	*Chasmanthium latifolium*	55
Lean flatsedge	*Cyperus setigerus*	12
Mexican buckeye	*Ungnadia speciose*	12
Mexican plum	*Prunus mexicana*	11
Pecan	*Carya illinoinensis*	6
Pencil cactus	*Opuntia leptocaulis*	40
Pink mimosa	*Mimosa borealis*	12
Prickly pear cactus	*Opuntia macrorhiza*	15
Red buckeye	*Aesculus pavia*	8
Retama	*Parkinsonia aculeate*	12
Rough-leaf dogwood	*Cornus drummondii*	3
Switchgrass	*Panicum virgatum*	40
Sycamore	Platunus occidntalis	22
Texas mountain laurel	*Sophora secundiflora*	20
Texas rush	*Juncus texanus*	60
Trumpet creeper	*Campsis radicans*	4
		$\sum = 746$

Aquatic Nuisance Species- Randy Gibson was a committee member for McLean Worsham, a Master's degree student at TSU. His thesis project determined the definite host for a San Marcos Springs endemic parasite, *Huffanela huffanni*. *Huffanela huffanni* eggs were collected from infected centrachid fishes and fed to other centrarchids and to invertebrates. The centrarchids and the invertebrates were monitored for parasite transmission. McLean successfully defended his thesis on 6 April. His thesis will be unavailable to the public for 2 years while he converts thesis information into several manuscripts for publication. The first publication is scheduled to be submitted during June 2015. (CSF 12.2.4)

Leadership in Science and Technology: Publications, extension activities/meetings, and presentations

During May, all SMARC biological staff were involved with data analysis and manuscript preparation or revision. So far this fiscal year, five articles have been published by peer-reviewed journals, three other articles have been accepted for publication, and eight articles have

been submitted but not yet accepted. (CSF 5.3.7)

Publications- Randy Gibson is collaborating with Cheryl Barr (UC Berkeley, emeritus) and Pete Diaz (TXFWCO) on descriptions of three new species of subterranean riffle beetles from springs associated with the Edwards Aquifer in west Texas. A manuscript entitled, "*Typhloemis*, a new stygoiontic riffle beetle genus with three new species from Texas, USA (Coleoptera: Elmidae: Elminae)" has been composed and was submitted for publication to Coleopterist Bulletin on 10 May. (CSF 5.3.7)

Lauren Lucas is determining the population genetics of the Comal Springs riffle beetle, the Comal Springs dryopid, the Peck's cave amphipod, and the Comal Springs salamander. Her project was funded by a TPWD Section 6 grant and by a cooperative agreement with the SMARC. On 25 May, a manuscript titled, "Pervasive gene flow across critical habitat for four narrowly endemic, sympatric taxa", was submitted to Freshwater Biology. (CSF 5.3.7)

McLean Worsham, Randy Gibson, Eric Julius, and Davis Huffman produced a manuscript entitled "The aquatic annelid fauna of the San Marcos River headsprings, Hays County, Texas" that went through the internal review process during May. (CSF 5.3.7)

Valentin Cantu, Justin Crow, and Kenneth Ostrand submitted a manuscript titled "A comparison of two non-invasive spawning methods to genetically manage captive Barton Springs salamanders *Eurycea sosorum*" to Herpetological Review on 15 May 2015. (CSF 5.3.7)

Extension activities/meetings- In anticipation of continued Texas wild rice restoration efforts, the San Marcos Aquatic Resources Center (SMARC) and Texas State University (TSU) held a teleconference to align propagation and stocking practices with the best available science. Currently the stocking efforts of Texas wild rice are structured to reach EAA HCP areal coverage targets with little or no consideration of past, current, or future genetic composition of the population. The overarching goal of the meeting was to compose a skeleton outline for conserving genetics or at the very least have a consensus on the management of Texas wild rice genetic diversity. Currently the USFWS is producing Texas wild rice plants from seeds of unknown genetic composition and the Meadows Center at Texas State University is using free-floating tillers of unknown origin or genetic composition for planting in the river. To ensure that genetic diversity is maintained, agreement on how seeds or plants should be selected to be propagated and planted within the San Marcos River is merited. Jeff Hutchinson is writing the SOP for Texas wild rice propagation in the San Marcos River.

On 4 to 8 May, Marta Estrada traveled to Willow Beach NFH to assist and train newly hired administrative staff.

On 5 May, SMARC staff transferred four preserved Comal Springs salamanders *Eurycea neotenes* and seven green throat darters *Etheostoma lepidium* to Bob Hall (EAA) to preserve them in acrylic for public outreach efforts.

On 6 May, Randy Gibson attended the Scientific Subcommittee meeting for the Edwards Aquifer

Recovery Implementation Program for the Habitat Conservation Plan in order to support methods presentation by Dr. Weston Nowlin (TSU) on joint refugia projects during 2015 and answer questions by the EAA and subcommittee on future Comal Spring riffle beetle research needs.

On 8 May, Randy Gibson and Pete Diaz (TXFWCO), Chad Norris (TPWD), and Weston Nowlin (TSU) placed cotton cloth lures inside orifices formed by flowing water through the eastern spillway of Spring Lake dam to if Comal Springs riffle beetles as well as other rare invertebrates are inhabiting the dam. This activity was performed on the request of Austin ES to help formulate the biological opinion associated with proposed dam repair.

On 14 May, Randy Gibson assisted Pete Diaz with salamander and invertebrate surveys of Salado Springs, Bell County, installing cotton cloth lures and sampling for new and rare invertebrates associated with the springs and discussing restoration and monitoring needs with city officials. On 28 May, more sampling and lure reinstallation took place after springs were scoured by a flood event.

On May 14, Patricia Echo-Hawk participated in the National Dive Control Board conference call.

On 27 May, Randy Gibson and Pete Diaz met with Carrie Thompson and Ryan Smith (The Nature Conservancy) and hydrologist Brad Wolaver (University of Texas) to discuss research projects for monitoring of water and rare and endemic organisms (including state and federally listed species) associated with the upper Devils River.

From 26 April to 1 May, Patricia Echo-Hawk attended training in Tulsa for her new ES position.

Texas State University Radio Station 89.9 KTSW started airing a 30 second Public Service Announcement about the SMARC Friends Group that will air through May 2015. Friends of SMARC is still in the process of becoming a non-profit organization. The Friends of SMARC mailed in their State of Texas Non-Profit application in May. Dianne Wassenich and the Friends of the San Marcos River provided the cost associated with the application.

Facilities and equipment

In May, SMARC staff worked to resolve safety issues found during the annual safety evaluation conducted by Pat McDermott (Region 2 Safety Officer) on 29 and 30 April. The report has been written and was reviewed internally in preparation for the National Safety inspection on 1 and 2 June.

On 7 May 2015, Valentin Cantu and Randy Gibson met with Ron Rutherford to discuss net designs for invertebrate and salamander collection. An improved 10 foot long net will replace a recent prototype that has proven effective in collecting invertebrates and salamanders from the Spring Lake Artesian Well outflow. Smaller 4 foot nets will be used to collect unique invertebrates from springs in central and west Texas.

Randy Gibson, Dr. Glenn Longley (TSU; EARDC) and a graduate student, Laura McCalla, collaborated on a project to monitor the SMARC water wells and other water wells upstream and downstream of the site of the proposed Paso Robles housing development and golf course. This large-scale development occurs near two wells that supply all the water for the SMARC. Although initial land clearing was planned to start in December 2010, the project has been repeatedly delayed, allowing us to obtain baseline information on water quality prior to any development. It is unknown what effects the development and subsequent chemical usage (herbicides, pesticides, reuse water) by the golf course and home owners will have on the water quality of the aquifer and on listed aquatic species held at the SMARC. Water quality sampling began during February 2011 and continued to July 2012. Some water samples from Hunter well contained relatively high levels of total coliform. This may indicate the influence of nearby recharge features that needs further investigation. Laura McCalla's thesis was completed in December 2012. The SMARC continues to constantly (every 15 min.) monitor temperature and conductivity in both wells. Monitoring has not detected any substantial changes that could represent possible pollution events. The EARDC received funding from Texas Commission on Environmental Quality –Supplemental Environmental Project Program to continue periodic monitoring of SMARC and City of San Marcos wells for two more years. From the 20 to 24 April, water quality samples were collected from the SMARC and City of San Marcos wells. During May, the laboratory report was competed by Amplified Geochemical Imaging, LLC and EARDC for this event (Table 8). Also during May, Randy performed water quality measurements and collected water samples of the effluent for testing by EARDC for bi-yearly requirements of the SMARC's wastewater discharge permits (TXG130018) from Texas Commission on Environmental Quality.

Table 8.- Water quality analysis of SMARC and nearby City of San Marcos wells during 8-9 December, 2014.

Factor, units	SMARC, Hunter Well	SMARC, McCarty Well	San Marcos City, McCarty Well
TPH, ug	**1.62**	**1.30**	**3.49**
BTEX, µg	bdl	bdl	bdl
Toluene, µg	bdl	bdl	bdl
PCE, ug	**0.06**	**0.04**	bdl
TCB, mpn/100ml	**583**	**2**	**1**

bdl - below detection limit; compound was observed at level below the method detection limit
µg - micrograms, relative mass value
mpn/100ml - most probably number per 100 milliliters (drinking water standard = 1)

TPH - total petroleum hydrocarbons
BTEX - Gasoline Range Aromatics (combined masses of benzene, toluene, ethylbenzene, total xylenes)
$CHCl_3$ - Chloroform
PCE - Tetrachloroethene
TCB - Total Coliform Bacteria

From 7 to 20 May, EAA staff installed a GORE water quality sampler in a flow through container in the SMARC invertebrate laboratory. The data collected from this GORE sampler

will serve as a baseline comparison for other GORE samplers placed in several locations throughout the TSU Freeman Aquatic Building (FAB). Data from these two locations may help determine if water quality issues are a factor in the survival of riffle beetles at the FAB.

During May, Randy Gibson continued to manage computer software and troubleshoot operating and software issues at the SMARC.

During May, Valentin Cantu led a crew of five to six Community Service Restitution (CSR) volunteers to trim and cut grass on the SMARC. They built PVC frames for screen covers for another raceway to protect listed fish from bird predation, removed brush and vegetation growing between the blacktop road and concrete raceways, built another capped stand pipe to prevent water from escaping down drains, repaired another leak to the ES2 system in Barton springs building that was reducing flow and introducing air bubbles into the tank system, repaired a leak underneath the building's sink, replaced a broken band saw blade in the shop with a new one, built about two dozen large see-though motels for the Barton Springs salamanders to facilitate inventory counts and early mortality detection, collected amphipods for the salamander refugia, pressure washed the diversion spring net and cleaned and disinfected equipment and supplies in research pad, replaced a large station sign with one having an updated facility name, began removing a second larger sign, organized PVC fittings in the shop and transferred scrap metal from behind shop to the recycle building.

During May, construction continued on the new main office parking lot and on the gravel road around the PAD, Barton Springs, and Greenhouse buildings. The project is being completed by M B Home Construction Service. During May, excavation and installation of engineered fill for the parking lot continued. The existing gravel road to and around Greenhouse was scraped and compacted. Needed rains during May has slowed completion of the project.

Additional electrical outlets are being installed in the greenhouse to facilitate operation of small pumps in 12 1x1x4 ft tanks set up for aquatic plant research purposes.

During May, Smith Pump installed a rebuilt 7.5 hp effluent pump and a new 15 hp reuse pumps.

Visitors

On 18, 19, and 20 May, Patricia Echo-Hawk, Randy Gibson and Valentin Cantu conducted tours for the entire fourth grade class of Travis Grammar school in San Marcos. 175 students visited over the 3 days as well as a total of 30 adults (conservation crew members and teachers).

On May 22, Patricia Echo-Hawk conducted a tour for 3 adults.

On 28 May, Tom Brandt provided a tour of the facility to Jeff Hatfield (USGS Patuxent Wildlife Research Center) and Adam Duarte (TSU PhD graduate student).