STAFF NOTES
February 2015
San Marcos Aquatic Resources Center

Aquatic Species Conservation and Management: Refugium Activities

San Marcos Salamander- Four San Marcos salamanders were collected from Spring Lake while one was collected from the Spring Lake Well Outflow in February (Table 1). The salamanders were returned immediately to Spring Lake given that the SMARC refugium is at capacity for this species (Table 1). Two salamander mortalities were observed this month. Wild stock and their offspring did not oviposit eggs during February. (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.

		November 2014			December 2014			January 2015			February 2015		
Species		JA	OV	EG	JA	OV	EG	JA	OV	EG	JA	OV	EG
San Marcos salamander	RWS	313	0	0	302	0	0	300	1	39	298	0	0
	FX	73	0	0	72	0	0	72	0	0	71	0	0
Quarantine	WS	0	-	-	0	-	-	0	-	-	0	-	-
Texas blind salamander	RWS	115	0	0	114	0	0	113	1	31	115	2	53
	FX	119	0	0	118	0	0	118	1	13	118	0	0
Quarantine	WS	0	-	-	0	-	-	1	-	-	6	-	-
Barton Springs salamander	RWS	37	0	0	37	0	0	37	0	0	37	0	0
	FX	634	4	180	634	0	0	633	1	37	632	3	114

Texas Blind Salamander- During February, SMARC staff collected no Texas blind salamanders. However, Edwards Aquifer Research and Data Center (EARDC) staff collected one salamander from the Texas State University (TSU) artesian well and seven from Sessom Creek Springs in February. There was one observed mortality in the refugia population this month (Table 1). Wild stock Texas blind salamanders oviposited 53 eggs this month. (CSF 7.12.5.4)

Barton Springs Salamander- No salamanders were collected from Barton Springs in February (Table 1). Wild stock Barton Springs salamanders did not oviposit eggs this month but their offspring oviposited 114 eggs this month. No wild stock salamander mortalities were observed this month. (CSF 7.12.5.4)

Fountain Darter- On 28 February 2015, there were 719 wild stock fountain darters in the SMARC refugia. Four mortalities were recovered during February from the wild stock tanks. These fish were from the San Marcos (Upper = 2 Middle = 2) River. On 9 February, 200 hatchery produced F1 darters were shipped to Luke Iwanowicz, USGS Leetown Science Center, Fish Health Branch (LSC-FHB; Kearneysville, WV) to study the pathogenicity of the aquareovirus that infects the fountain darter in the San Marcos River. In addition, 20 wildstock

darters were collected and shipped to Dexter SNARRC Fish Health Unit on 24 February for parasite examinations and pathogen diagnostics. (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). The SMARC is also maintaining F1 (N ≈ 1,500) offspring. These 1,500 fish are comprised of about 800 Pinto Creek adults and 224 F1 Pinto Creek juveniles. The juveniles were used for Patricia Echo-Hawk's feed trials. The SMARC maintains additional F1 DRM from San Felipe Creek in an outdoor raceway to evaluate if this is a less labor intensive method of producing genetically diverse fish for restocking purposes. The genetic analysis 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. In addition, 20 F1 Pinto Creek larvae were transferred to Dr. Kevin Conway at Texas A&M University to document the fish's skeletal development during its early life. Lastly, 50 archived adult specimens collected from the Devils River and Pinto Creek over the years were transferred to Dr. David Huffman's Lab (TSU) for parasite examinations. (CSF 7.12.5.4)

Comal Springs Riffle Beetle- Approximately 75 adult wild stock Comal Springs riffle beetles, 16 F1 adult offspring and 100 larvae are being maintained at the SMARC. (CSF 7.12.5.4)

Peck's Cave Amphipod- Approximately 67 adult Peck's cave amphipods and 18 *Stygobromus* juveniles are being maintained at the SMARC. (CSF 7.12.5.4)

Texas Wild Rice- As of 28 February 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)

At the end of February 2015, the SMARC had 19,618 (N_{2009} = 390, N_{2010} = 585, and N_{2011} = 1,941, N_{2012} = 10,152, N_{2013} = 6,550) Texas wild rice seeds in storage (Table 3). No seeds were collected for storage from September 2013 through January 2015. During 2015, a portion of the seeds collected will go into storage. Although seeds were not collected for storage during 2014, seeds were collected from refugium plants and potted in raceways to provide Texas wild rice for restoration efforts in the San Marcos River. During 2014, 6,205 Texas wild rice plants produced from these seeds were transferred to City of San Marcos contractors for replanting efforts in the San Marcos River. An additional 1,719 Texas wild rice seedlings, produced from seeds collected during 2014, are being reared and will be transferred to the City of San Marcos for restoration efforts during 2015. (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			264
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		19,618

Research and Restoration Activities

San Marcos Salamander-Pete Diaz (TXFWCO), Joe Fries, Mara Alexander, and Dr. Weston Nowlin (TSU) are working on a manuscript from Pete's thesis that examined the relationship

between submerged aquatic vegetation and the diet of the San Marcos salamander. In addition to work directed specifically to his thesis topic, Pete also removed tail clips from a subsample of the preserved wild salamanders for stable isotope determinations. A manuscript describing his habitat work was completed during August and was submitted on 15 August 2013 to Aquatic Conservation: Marine and Freshwater Ecosystems. The article was tentatively accepted by the Journal on 1 December 2013. Page proofs for the article were returned to the Journal mid-February 2015. The tail clips were sent to the University of California at Davis for stable isotope analyses during March 2012. Collectively, the data will be used to examine nutrient flow through the system. (CSF 7.12.5.4)

Barton Springs Salamander- A goal of the SMARC is to develop a captive breeding program for listed salamanders that employs protocols that yield predictable numbers of offspring. Valentin Cantu and Justin Crow, a SMARC employee and a student from TSU, initiated a study during November 2013. The laboratory data collection ended in February 2014. Data analyses are complete and a draft report has been composed. The results suggest that egg production can be hastened if male salamanders are removed after a two week period without negatively affecting hatch success or early larval development. Although we can predictably trigger reproduction in *E. sosorum*, further studies are needed to increase the oviposition rate and hatch success of this species in order for captive propagation to be an effective tool for recovery efforts. A manuscript titled "A comparison of two methods to spawn *Eurycea sosorum* (Plethodontidae)" completed internal review during February and is currently undergoing external review. (CSF 7.12.5.4)

In February, SMARC staff continued working with Dr. Joseph R. Tomasso (TSU) and Justin Crow to determine the effects of temperatures on *Eurycea* salamanders using Barton Springs salamanders as the test organism. In February 2014, the study was initiated and thus far results showed best growth was obtained at 18°C and poorest growth at 28°C. The mean upper temperature tolerance for the salamander was found to be 32.6°C. An additional study is examining the effects of toxic chemicals (i.e. ammonia, nitrite, and nitrate) commonly found in rainwater runoff, on salamander survival. The ammonia trial was completed in December, the nitrite in February, and the nitrate will be initiated in March. Data from this study will result in estimates for the compounds 96-hour median lethal concentrations (96-h LC50). (CSF 7.12.5.4)

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. (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. (CSF 7.12.5.4)

Devils River Minnow- On 13 May 2013, Patricia Echo-Hawk began production of San Felipe Springs Devils River minnows for a project that she is working on with Dr. Kevin Conway (Texas A&M University). The original project was to document the skeletal development of the Devils River minnow during its first 30 days after hatch. A second phase of this project began in early 2014 and involved the production of 5 individuals collected 4, 6, 8, and 12 months after hatching. The second group of minnows was transferred during February 2015 to Dr. Conway for examination. Data analyses are ongoing. (CSF 5.3.7)

Patricia Echo-Hawk, during her second year of graduate school at Texas A&M University, developed a proposal for a project titled "Growth and survival of Devils River minnow fry fed different diets." The project's goal is to increase minnow survival from first feeding through several larval stages by improving diet. Four different diets will be tested. Production of larvae for the project was started on 26 September. Diets being compared are artemia nauplii, daphnia, protein flakes, and a homemade plant based feeding cube that was developed by Ms. Echo-Hawk. On 1 November the study began. The trial was completed on 27 February 2015. Data analyses and thesis composition is ongoing. (CSF 5.3.7)

Comal Springs riffle beetle- In February, BIOWEST and TSU students set cotton cloth lures in springs of the upper Devils River to attract the riffle beetle, *Heterelmis cf. glabra*, to be retrieved during March. 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. (CSF 7.12.5.4)

Texas wild rice- During April 2014, mass-flowering and seed production of Texas wild rice was observed in Section B of the San Marcos River below University Drive Bridge downstream to the river bend before the Lion's Club Tube Rental. Given that mass-seed production in the San Marcos River is uncommon, a seed germination experiment was initiated to test the viability of wild-stock seeds, compare wild and refugia produced seed germination rates, and evaluate seasonal germination patterns. To date, germination rates for river collected seeds ranged from 19 to 95%. Preliminarily, wild collected Texas wild rice seeds do not appear to exhibit seasonal germination patterns presumably because abiotic conditions within the San Marcos River are relatively constant Seeds produced at the SMARC have a relatively similar germination rates (44 to 96%) and also do not exhibit any seasonal germination pattern. (CSF 7.12.5.4)

An additional 1,680 Texas wild rice seeds were collected during 2014 to evaluate seed storage protocols. Two methods of storage (i.e. moist paper towel or in water) at 3 to 4 ˚C were evaluated by removing the seeds from storage and potting a subset from each group monthly for one year. Each month over one year, a subset of the seeds was taken out of the refrigerator and potted, and monitored for germination to determine the optimum storage time and inhibition rates for Texas wild rice seeds under refrigeration. The goals of the experiment were to determine if one storage methods maintains the viability of the seeds longer than the other and to determine the I_{50} (inhibitory concentration) value of Texas wild rice seeds based on storage time and method. Seeds stored in moist paper towels averaged 51% and those in water had only 20% germination. Based on the first 9 months of this study, Texas wild rice seeds remain viable under refrigerated conditions in water or wrapped in moist paper towels and there is a negative relationship between storage time and germination rate, regardless of the storage protocol (Figure 1). (CSF 7.12.5.4)

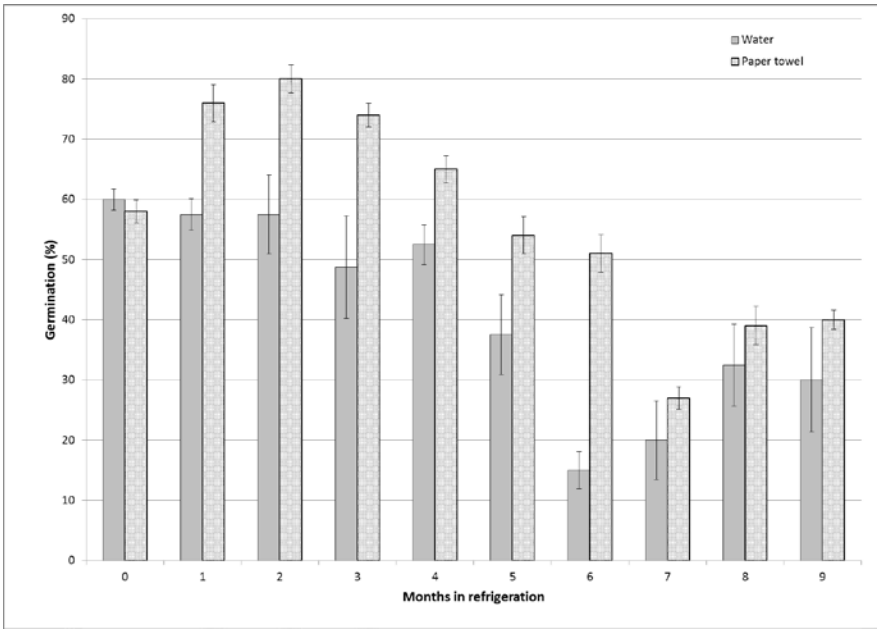

Figure 1.- Means and standard error for seeds stored at 3 to 4 ˚C in water and moist paper towels for up to nine months.

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. This study is on-going and currently flowering characteristics are being measured weekly. 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 has begun, pistil and stamen growth, seed production and flower senescence has been recorded weekly (Table 5). Seeds from the experimental plants are collected 2-3 times per week and monitored for germination. As of 31December, a total of 24,787 seeds (ca. 165 seeds per plant) were 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). During February 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

Wade Wilson finished the final report on Texas wild rice genetics during January 2015. Wade, Jeff Hutchinson, and Kenneth Ostrand are currently editing the Texas wild rice genetics report for submission to the journal Aquatic Botany. (CSF 7.12.5.4)

Jeff Hutchinson has initiated a study to evaluate several Texas wild rice planting patterns in the San Marcos River with individual and groups of Texas wild rice. Five plots were set up in the San Marcos River on 19 May 2014 to evaluate planting methods of Texas wild rice tillers, seedlings, and mature plants. The plots were setup to be monitored at 4 month intervals and, if successful, supplemental planting will occur in each plot and every 12 months thereafter. The goal is to develop a method to establish Texas wild rice in lower sections of the San Marcos River where it is uncommon. A 4-inch rainfall event on 25 May resulted in a 7 to 8 foot rise in water level that caused the loss of 10% of the newly planted Texas wild rice. On 29 May, all Texas wild rice plants that were scoured out during the 25 May flood were replaced. Two additional plots of Texas wild rice were established in the same general area on 12 June by Jeff Hutchinson and Josh Roberts. In these plots, 25 and 20 Texas wild rice seedling ca. 3 months old and 0.75 m in length were planted in a 0.25 m^2 clump. During monitoring on 21 June, all the Texas wild rice, regardless of planting pattern, in each of the seven plots was intact and looked robust. During July, the plots were checked twice and the seedlings and a single Texas wild rice plant from a 2 ½ gallon pot were washed out or had not survived. Conversely, it appears that the tillers and mature plants had doubled in size based on length and width. The plots were measured during the Texas wild rice survey. At 4 months post-planting, all treatment plots had increased in area coverage (Table 6). On 7 October 2014, two supplemental plots of Texas wild rice were planted in the vicinity of the plots bringing the total number of plots to 12 (5 research plots and 7 supplemental plots). During the February 2015 evaluation, all treatments had increased in area coverage, and one plot of peat pots was no longer present. Survival rates were ≥ 80% for all treatments except seedlings. During the February 2015 evaluation, an additional four Texas wild rice plots were planted (5 research plots and 11 supplemental plots). Additional plots of Texas wild rice will be added to lower sections of the river as time permits. (CSF 7.12.5.4)

Table 6.- Texas wild rice planted at various life history forms and ages and monitored for above ground biomass (area coverage) eight months post-planting. Table values represent the means (n = 5) and standard error for each treatment. Plants that did not survive were excluded from analysis.

Treatment	Mean area coverage (m^2) and SE			Survival (%)
(age at time of planting)	May 2014	Sept 2014	Feb 2015	8 mo post-planting
Tiller (unknown)	0.12 (0.02)	0.45 (0.04)	0.62 (0.08)	100
Seedlings (2 wk)	0.01 (<0.01)	0.14 (0.06)	0.32 (0.06)	60
8 weeks (peat pot)	0.21 (0.03)	1.26 (0.31)	1.69 (0.37)	80
6 months (plastic pot)	0.15 (0.03)	0.82 (0.18)	1.42 (0.36)	100
2 years (plastic pot)	0.47 (0.04)	0.56 (0.07)	1.04 (0.29)	80

A study to examine the potential for Texas wild rice propagation from stem tissue nodes using auxin was initiated 5 May 2014. The study evaluated four common commercially available products, Hormodin 1, Hormodin 2, Hormodin 3, and Dip-N-Grow, in which the nodes of Texas wild rice were dipped in the solution or powder for 5 seconds and then potted in soil. After 2 weeks, root development and some new leaf tissue was observed. During June, about 60-70 of the treated nodes had developed roots, indicating that auxin compounds may be a successful tool for cloning Texas wild rice for genetic purposes. In July, all the live plants were repotted and survival appeared to be highest for submerged nodes treated with the lower rate of auxin. In September 2014, the survival rate was ≤ 10% for all nodes that were emergent regardless of treatment, and 70% for controls and submerged nodes treated with 1000 ppm auxin. Preliminary results indicate that rates of auxin > 1000 ppm are ineffective with < 10% survival. Survival of Texas wild rice treated with 1000 ppm auxin was equal to controls, but growth rates (roots and leaves) were higher with 1000 ppm auxin compared to controls. As a result, Colin Findley, Jeff Hutchinson, and Leah Murray initiated a second study on Texas wild rice stems with reduced rates of auxin (IBA and NAA) during December 2014. Technical grade auxins were used to treat Texas wild rice nodes at concentrations of 12.5, 25, 50, 100, 200, 400, and 800 ppm for IBA, and 100 IBA/25 NAA and 200 IBA/50 NAA ppm. At 6 weeks post-treatment, the plants were repotted and placed in tanks with flows of 0.1 m/s. The final evaluation will occur at 12 weeks post-treatment and the experiment will be repeated. (CSF 7.12.5.4)

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 28 February 2015 all native plants have survived the treatments and algae remains minimal. (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 28 February 2015, 1,974 aquatic plants were provided to the City of San Marcos since the start of 2015. The City received 1,719 Texas wild rice seedlings, 153 creeping water primrose willow, 57 arrowhead, 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)

Threatened freshwater mussels- In 2012–13, the USGS, in cooperation with the USFWS, completed the first phase field work of a two-phase study of mussel host-fish relations for five endemic mussel species in central and southeastern Texas. The mussels included 1) Texas fatmucket *Lampsilis bracteata*, 2) golden orb *Quadrula aurea* 3) smooth pimpleback *Quadrula houstonensis* 4) Texas pimpleback *Quadrula petrina* and 5) Texas fawnsfoot *Truncilla macrodon*. The first phase of the study was to determine the abundance of host fish and frequency of glochidial parasitism in central and southeastern Texas streams. The results of this study were published during December 2014 (see http://dx.doi.org/10.3133/sir20145217). The second phase of the study involved collecting juvenile mussels and glochidia that dropped off of the gills of the fish brought to the SMARC from the Texas streams. The collected juveniles and glochidia were submitted for the development of deoxyribonucleic acid (DNA) identification keys to determine mussel and host-fish relationships through DNA-based molecular identification (DNA typing of the juvenile mussels and glochidia). A manuscript titled "Identification of freshwater mussels and their host fishes in Texas using DNA Barcodes" authored by Nathan Johnson (USGS), John Pfeiffer III (USGS), Patricia Echo-Hawk (USFWS), James Moring (USGS), Charrish Stevens (USFWS), and Charles Randklev (IRNR-TAMU) is being composed. A draft has been completed and is undergoing internal editing and review. (CSF 5.3.7)

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

During February, all SMARC biological staff were involved with data analysis and manuscript preparation or revision. So far this fiscal year, three articles have been published by peer-reviewed journals, three other articles have been accepted for publication, and six articles have been submitted but not yet accepted. (CSF 5.3.7)

Publications- Daniel Huston (Inks NFH), Randy Gibson, Kenneth Ostrand, Chad Norris (TPWD), and Pete Diaz (TXFWCO) submitted an article entitled, "Monitoring and marking techniques for the endangered Comal Springs riffle beetle *Heterelmis comalensis* (Coleoptera: Elmidae)" to Insect Conservation and Diversity on 12 February 2015. The results detailed in this manuscript suggest that the novel marking methods described have merit for aquatic invertebrates and could be used to assess movement patterns and determine population estimates through mark and recapture techniques. (CSF 5.3.7)

Jeff Hutchinson, Ken Ostrand, and Wade Wilson are formatting the Texas Wild Rice Final Genetics Report into a manuscript for submission in Aquatic Botany. (CSF 5.3.7)

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 is undergoing internal review. (CSF 5.3.7)

Extension activities/meetings- On 3 February, SMARC staff met with HDR consulting firm to assist in the development of a Refugia Request for Proposal for EAA.

Marta Estrada and Lisa Griego-Lyon attended the Administrator Workshop in Albuquerque, New Mexico, on 10-12 February.

On 11 February, Patricia Echo-Hawk participated in a conference call regarding the status listings of candidate species of mussels.

On 18 February, Randy Gibson met with Marcus Gary (EAA) and Chad Norris (TPWD) to discuss the geophysical analysis planned for Comal Springs to potentially locate conduits and other subterranean features associated with the springs.

On 18 February, Randy Gibson met with Weston Nowlin and Benjamin Schwartz (TSU), Chad Norris (TPWD), and Pat Connor (Austin ES) to discuss continuation of the borehole sampling for Comal Springs riffle beetles and its application to the recently EAA funded project, "Comal Springs riffle beetle habitat connectivity study #132-14-HCP ."

On 19 February, Randy Gibson met with Weston Nowlin (TSU) and graduate students Parvathi Nair and McLean Worsham at Comal Springs to view the existing boreholes in Spring Run 1 and to discuss installing others in Spring Runs 1 and 3 in known riffle beetle habitat. Existing and proposed boreholes will be used to monitor Comal Spring riffle beetle location and numbers at different spring flows.

On 23 February, Jeff Hutchinson and Leah Murray met with Melanie Howard of the City of San Marcos and Dianne Wassenich of the San Marcos River Foundation to organize volunteer planting days for riparian and terrestrial sites along the San Marcos River.

On 25 February, Jeff Hutchinson attended the team leader workshop for the San Marcos River Cleanup, and will be working a crew of 20-25 volunteers to clean up trash on Thompson's Island during the San Marcos River Cleanup scheduled for March 7.

On 25 February, SMARC staff met with Guan Zhu (Texas A&M University) to discuss funding options and to transfer samples to him to continue his PCR optimization trials with microsporidium. SMARC staff provided him with ETOH-preserved adults and eggs from *Eurycea rathbuni, E. sosorum,* and *E. nana*, tank debris, salamander forage, salamander skin swab samples, and salamander fecal samples. SMARC staff is currently developing a cooperative agreement with the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMLD) to help fund research to detect and treat microsporidial infections in listed salamanders.

Jeff Hutchinson, Leah Murray and Colin Findley attended the 2nd Texas-Oklahoma Pollinator Meeting at TPWD Headquarters in Austin on 27 February. Representatives were present from the USFWS, TPWD, USGS, NRCS, UT, NGOs, and private citizens. The meeting was held to determine further directions in regards to milkweed and monarch restoration along the I-35 corridor in Oklahoma and Texas. The following day, Leah Murray and volunteer Colin Findley attended the Pollinator Pow-Wow hosted at the LBJ Wildflower Center.

Leah Murray and Colin Findley met with UT Professor Norma Fowler on 27 February. Dr. Fowler gave SMARC staff bracted twist flower seeds *(Streptanthus bracteatus)* for germination trials. SMARC staff is in the process of setting up a design to propagate *S. bracteatus* for seed accessions and restoration. Chris Best (Austin ESFO) and Norma Fowler, who have worked with the plant for many years, are advising Jeff Hutchinson and Leah Murray on a propagation protocol. Jeff Hutchinson and Leah Murray received an additional 6,000 seeds from Walter Stuart (Bee Cave, TX) who has worked extensively to propagate the plant to supplement seed banks.

Leah Murray and Jeff Hutchinson travelled to Balcones NWR on 4 February to meet with biologist Scott Rowin and botanist Chris Best about potential milkweed habitat at Peaceful Springs (Bertram, TX). The caretaker of the property donated *Asclepias sp*. seeds to SMARC that were collected at Balcones NWR. Some of these seeds are already germinating in the green house.

Patricia Echo-Hawk has been discussing aquatic invasive plant removal strategies and mechanisms with the Region 7 Dive Officer, and how to potentially tackle an elodea invasion.

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.

Facilities and equipment

In February, Patricia Echo-Hawk organized the yearly inspection, maintenance and service of required diving gear and 5 year hydrostatic testing of SCUBA tanks.

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 9 to 13 February, water quality samples were collected from SMARC and City of San Marcos wells.

During February, Randy Gibson continued to manage computer software and troubleshoot operating and software issues at the SMARC. The shared local drive was moved from an external drive to an internal drive due to networking problems.

In February, Valentin Cantu supervised one to eight Community Service Restitution (CSR) volunteers to help pick up trash and cut grass, shrubs, and small trees along the SMARC property fence line and around and beneath the IH 35 bridge where the hatchery's reuse water exits the property. Shrubs and small trees were also cut on hatchery grounds to facilitate hay cutting and bailing. Volunteers painted unfinished fence line posts green along the entire perimeter of the property; dug down to and repaired a damaged corrugated galvanized drain pipe near the entrance gate; cleaned ponds B-1 and A-2 and spread the organic debris over the facility's prairie; cleaned the administrative building; removed mud dauber nests from the recycle building's walls, cleaned shelves, swept the floor, and reorganized recyclables in the building; drilled a hole through the concrete wall of the Clayton vault and re-plumbed a sump-pump PVC pipe through the wall and away from the vault to prevent water from re-entering it; continued diagnosing and repairing heater/chiller units and building clear plexiglass salamander motels. The motels when placed under a rock provide hiding spaces for San Marcos salamanders to conceal themselves in refugium tanks and aquaria.

On 13 February, the project to replace the SMARC perimeter fence and gates was completed.

On 23 February, the SMARC generators were serviced. The facility generator was found to have a block heater issue and in need of battery replacement. The batteries were replaced by SMARC staff and the replacement of block heater is being evaluated.

Visitors

On 6 February, SMARC staff hosted its annual We're Still Here Luncheon. Approximately 35 people attended the luncheon.

Leah Murray and Jeff Hutchinson hosted the second meeting for the "Friends of SMARC" volunteer group on 25 February. Total attendance at the meeting was 12 people. Chris Best, State Botanist for the USFWS, gave a presentation on Native Milkweeds for the Monarch Habitat. Doug Phillips, USFWS Partners for Fish and Wildlife Program, gave a presentation about the Partners Program incentives for private landowners. The group toured the 115 acre Blackland prairie at SMARC and discussed hiking trails, birding sites, and milkweed and wildflower restoration and experimental plots. Leah Murray and Jeff Hutchinson are working on a management plan to restore the prairie that involves less mowing in order to collect the milkweed seeds and other beneficial plant species for propagation and restoration efforts.

Regular volunteer days for the Friends Group are the second and fourth Wednesdays of every month. The next meeting is planned for 8 April from 1-4 pm.

A group of high school students from the Phoenix School of the San Marcos Independent

School District will be volunteering at the station every Friday afternoon. Their first work day was on 27 February. Patricia Echo-Hawk discussed fish biology with the students before she put them to work.

A party was held at the SMARC during lunch on 13 February 2015 to thank Brian Stewart for his consistent quality work as a Community Service Restitution (CSR) volunteer at the SMARC. Brian assisted the SMARC with custodial, maintenance, Texas wild rice, fountain darter, Devils River minnow, and salamander projects. The CSR coordinator Yolanda Rodriguez and Brian's probation officer, Marcos Rangel, also attended the party to present him with an award for his good work at the SMARC. Both agencies expressed their mutual benefits from the program. The party was also held to thank another volunteer, John Hjort, who assisted with Texas wild rice projects and habitat restoration on the San Marcos River.