

STAFF NOTES
May 2014
Aquatic Resources Center
San Marcos, Texas
Short Version

Aquatic Species Conservation and Management: Refugium Activities

San Marcos Salamander- As of 30 May, six San Marcos salamanders were collected. The salamanders were returned immediately to Spring Lake given that the SMARC refugium is at capacity for this species (Table 1). Nine salamander mortalities were observed in the refugium population this month. As of 30 May, wild stock San Marcos salamanders oviposited 132 eggs while their offspring did not oviposition during May. (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 2013			March 2014			April 2014			May 2014		
		JA	OV	EG	JA	OV	EG	JA	OV	EG	JA	OV	EG
San Marcos salamander	RWS	363	0	0	356	1	15	350	2	55	341	4	132
	FX	67	1	12	63	0	0	59	0	0	58	0	0
Quarantine	WS	0	-	-	0	-	-	0	-	-	0	-	-
Texas blind salamander	RWS	96	2	35	104	0	0	106	3	67	117	3	83
	FX	55	1	31	54	3	42	54	1	16	53	1	16
Quarantine	WS	10	-	-	5	-	-	7	-	-	0	-	-
Texas (Comal) salamander	RWS	1	0	0	1	0	0	1	0	0	1	0	0
	FX	0	0	0	0	0	0	0	0	0	0	0	0
Quarantine	WS	0	-	-	0	-	-	0	-	-	0	-	-
Barton Springs salamander	RWS	42	0	0	42	0	0	40	0	0	40	0	0
	FX	695	8	370	642	2	69	642	1	23	641	0	0

Texas Blind Salamander- As of 30 May, SMARC staff collected three Texas blind salamanders from Rattlesnake cave and one from Rattlesnake well (Table 1). Seven salamanders that were in quarantine last month were incorporated into the refugia. In addition, all four of the salamanders collected this month were incorporated into the refugia, after being held in quarantine. The SMARC refugia did not lose any wild stock adult salamanders this month. As of 30 May, wild stock Texas blind salamanders oviposited 83 eggs while their offspring produced 16. (CSF 7.12.5.4)

Barton Springs Salamander- No salamanders were collected from Barton Springs in May (Table 1). As of 30 May, wild stock Barton Springs salamanders and their offspring did not oviposit eggs. No wild stock salamander mortalities were observed this month. The Texas A&M University health team has provided the SMARC with a preliminary disease treatment in the event of a disease outbreak; however, additional tissue samples and research will be needed

before confirming the presumed cause of previous infections. (CSF 7.12.5.4)

Fountain Darter- A total of 580 wild stock fountain darters were in the SMARC refugia on 27 May. Seven mortalities were recovered during May. These fish were from the San Marcos (Middle = 6, and Lower = 1) stock. On May 15th and 16th a total of 60 fountain darters from the San Marcos River and 60 from the Comal River were collected. These fish were sent to the Dexter SNARRC Fish Health Unit on May 19th. An additional 163 fountain darters were also collected to supplement the refugia (N = 29 lower San Marcos, N = 39 middle San Marcos, N = 37 upper San Marcos, N = 31 Upper Comal and N = 27 lower Comal). These fish will remain in quarantine for at least 30 days and will be incorporated into the refugia once they are cleared by fish health. (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 = 196) and another from Pinto Creek (N = 85). The SMARC is also maintaining F1 (N ≈ 1,500) offspring. Three Devils River minnows were collected from Pinto Creek on 5 May. These fish were treated with formalin and Pennox 350. Newly caught minnows will remain in quarantine for 30 days. Approximately 300 Pinto Creek offspring were produced during May. Some fish at the SMARC are being maintained 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. On April 21, ten Devils River Minnow (Pinto Creek stock) fry were sent to the SNARRC Fish Health Unit for disease and parasite examination. The fry were sent because they appear to stop eating at around two to three months of age. Patricia Echo-Hawk suggested the possible use of flubendazole to treat fry until their examination is complete and is consulting with veterinarians regarding this potential treatment. (CSF 7.12.5.4)

Research and Restoration Activities

Fountain Darter- Kenneth Ostrand is working collaboratively with BioWest Inc. on a fountain darter mark and recapture study to determine how movement is affected by habitat and temperature changes under low flow conditions. In April, 471 fountain darters were uniquely marked with visual implant elastomer (VIE) at two locations in both the Upper Spring Run of the Comal River (N = 540) and Blieders Creek (N = 317). In May, an additional 757 darters were marked in the Comal River. These areas are known to experience extreme abiotic conditions and are typically the first river reaches to be negatively affected by low spring flows. By marking fountain darters in these stream reaches we hope to determine when aquatic vegetation is rendered unsuitable habitat and darters seek other habitat types; and, how far will fountain darters move to find usable habitat. The field mark and recapture study will be augmented by manipulative trials in an experimental pond to address the effects of specific factors on fountain darter movement. Fountain darters will continued to be marked throughout the year and sampled for recaptures. Twenty-six marked fish have been observed during snorkeling efforts or recaptured when dip netting. All of the recaptured fish were collected close to their release site, excluding one fish that was marked in Blieders Creek but observed in the Comal River during snorkeling. (CSF 7.12.5.4)

Devils River Minnow-

Patricia Echo-Hawk, beginning her second year of graduate school at Texas A&M University, has submitted a proposal for a project titled “Growth and survival of Devils River minnow fry fed different diets”. The projects primary goal is to increase minnow survival during early life history stages by feeding them four different diets. (CSF 5.3.7)

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 Tub 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 two seed storage protocols. On April 24, 2014, a total of 1,390 Texas wild rice seeds were collected and potted. Seeds from Sections A and B have recently germinated indicating that some percentage of the seeds is viable. Germination counts will begin in early June and additional seeds will be collected from the river in June for replicates of the study. An additional 1,680 Texas wild rice seeds were collected to evaluate seed storage protocols. Two methods of storage (i.e. moist paper towel or in water) at 3 to 4 °C will be evaluated by removing the seeds from storage and potting a subset from each group monthly for one year. The goals of the experiment are to determine if one storage methods maintains the viability of the seeds longer than the other and to determine the I₅₀ (inhibitory concentration) value of Texas wild rice seeds based on storage time and method. (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 to evaluate planting methods of Texas wild rice tillers, seedlings, and mature plants. The plots will be monitored monthly for 6 months and if successful, supplemental planting will occur in each plot and every 6 months thereafter. The goal is 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 TWR. (CSF 7.12.5.4)

A study to examine the potential for Texas wild rice propagation from stem tissue nodes using auxin was initiated 5 May. The study evaluates 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. At two weeks, root development and some new leaf tissue was observed. (CSF 7.12.5.4)

Jeff Hutchinson is co-advising Michelle Crawford (Ph.D. student at Texas State University) on a project that is examining the effects of light attenuation and siltation on Texas wild rice at Uvalde NFH. A field component was added to her dissertation that will be conducted in the San Marcos River and use Texas wild rice plants produced at the SMARC. She had her first committee meeting in February. The committee approved her experimental design and draft proposal. Texas wild rice seeds (N = 400) were potted on April 28 and were re-potted as individuals in May. On 29 May, Michele planted 75 of the plants in the San Marcos for an 8

week study on growth rates during periods of heavy recreation. (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. To maintain genetic diversity of the plants at the SMARC, additional plants of each species will be collected from wild populations throughout the year. As the plants grow and send out new shoots, they will be divided, repotted, and moved into outdoor raceways. Currently, SMARC has a full stock of aquatic, riparian, and terrestrial plants available to contractors with the City of San Marcos. (CSF 7.12.5.4)

To meet the restoration goals outlined in the Edwards Aquifer Habitat Conservation Plan, native plant production is needed. The SMARC has been employed by the City of San Marcos to produce native aquatic plants for their restoration efforts. Plant transfers to the City of San Marcos began in March 2013. From January to April 2014, the SMARC provided a total of 3,019 aquatic and terrestrial plants to the City of San Marcos (Table 4). No plants were provided to contractors in May because low spring flows triggered a suspension of all planting activities under the City’s 10(a)(1)(B) permit. The staff at SMARC will continue to provide plants for the restoration work in the upper section of the San Marcos River through 2019. Two terrestrial tree planting days organized by the City of San Marcos and SMARC were held on March 8 and 22, and the SMARC provided plants for these events. Additional planting events are planned for late summer, early fall 2014. (CSF 7.12.5.4)

Table 4.- List of plants species, common name, and number of plants provided from January to April of 2014 for aquatic and terrestrial restoration work in the San Marcos River.

Plant Species	Common Name	Number of Plants
Aquatic		
<i>Sagittaria platyphylla</i>	Arrowhead	174
<i>Ludwigia repens</i>	Creeping primrose willow	1352
<i>Heteranthera liebmannii</i>	Water stargrass	566
<i>Zizania texana</i>	Texas wild rice	695
		∑ aquatic = 2,787
Terrestrial		
<i>Berberis trifoliolata</i>	Agarito	1
<i>Bouteloua gracilis</i>	Blue grama	8
<i>Sambucus canadensis</i>	Elderberry	30
<i>Taxodium distichum</i>	Bald cypress	7
<i>Salix nigra</i>	Black willow	6
<i>Acer negundo</i>	Box elder	2
<i>Cyperus setigerus</i>	Lean flatsedge	2
<i>Equisetum hyemale</i>	Horsetail	5
<i>Pluchea odorata</i>	Purple pluchea	10
<i>Panicum virgatum</i>	Switchgrass	28
<i>Morus rubra</i>	Red mulberry	19
<i>Marsilea macropoda</i>	Water clover	1

<i>Carex emoryi</i>	Emory's sedge	58
<i>Chasmanthium latifolium</i>	Inland sea oats	54
<i>Platanus occidentalis</i>	Sycamore	1
		Σ terrestrial = 232

$\Sigma\Sigma$ Total = 3,019

Aquatic Nuisance Species- Dan Huston successfully defended his Master's Thesis on May 8th. His project initially was to determine the relationship between *Centrocestus formosanus*, an exotic digenetic trematode, infection rates and the swimming performance of *Dionda* species. After completing swim tunnel experimental trails, Dan found *C. formosanus* had no significant effect on the swimming performance of his test fish. When collecting *C. formosanus* infected snails *Melanoides tuberculatus* from the Comal River, *Haplorchis pumilio* infected *M. tuberculatus* also were collected. Dan exposed a few blacktail shiners *Cyprinella venusta* to *H. pumilio* cercariae. Since one of the shiners died from the exposure, Dan decided to run swim tunnel test on uninfected and *H. pumilio* infected shiners and minnows. The swim tunnel trials were completed during December. Since before 2000, *H. pumilio* has been known to exist in the Comal River. Until Dan exposed the shiners to *H. pumilio* cercariae, we did not know the parasite could kill fish. Dan has examined fountain darters from both the Comal and San Marcos rivers for *H. pumilio* during December and found them to be infected. Conversations with SNARRC Fish Health Unit have resulted in future fountain darter health diagnostics to include quantification of *H. pumilio* infection rates. In addition, the results from this investigation were composed into a note and have been submitted to the journal of Bioinvasion Records for review and potential publication. (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, six articles have been published by peer-reviewed journals, four other articles have been accepted for publication and five articles have been submitted but not yet accepted. (CSF 5.3.7)

Publications- Sophia DeColo, a former graduate student at TSU, composed a manuscript from one of her thesis chapters titled "Effects of turbidity on association preferences for size in the fountain darter, *Etheostoma fonticola*. The manuscript co-authored with Kenneth Ostrand was submitted to the Journal of Fish Biology during May. (CSF 5.3.7)

Maria Cooke, a former graduate student at TSU, in collaboration with Randy Gibson, worked on developing captive propagation techniques for the riffle beetle for her Master's project. Her research included examining habitat preference and substrate and food relationships associated with pupation. Her goal with these tests was to provide information needed to improve the beetle's culture environment and perhaps increase the likelihood of completing its life cycle in captivity. She defended her thesis on 2 July 2012. Randy is working with Maria to produce a draft manuscript that includes portions of her thesis. Internal USFWS review was completed in April and revisions were completed in August. During September 2012, external peer reviews

were sent to Maria to consider changes to the manuscript before submission. During March, the most recent version of the manuscript was sent to Randy for review. During May, Randy sent edits to the other authors for consideration. The authors plan to submit the manuscript to The Southwestern Naturalist. (CSF 5.3.7)

Jeff Hutchinson and Kenneth Ostrand have completed a draft manuscript titled “Management of Texas wild rice (*Zizania texana* Hitchc.), a federally endangered species, in outdoor ponds.” The manuscript was submitted during May to the Native Plants Journal. (CSF 5.3.7)

Extension activities/meetings- During May, Daniel Huston assisted Inks Dam NFH with catfish collection efforts.

During May, Tom Brandt, Kenneth Ostrand and Valentine Cantu met with Texas A&M Universities Veterinary Health Team to discuss next steps and potential treatments for refugia salamander populations.

Valentin Cantu and Randy Gibson continue to work with Texas Parks and Wildlife (Andy Gluesenkamp) and EAA (Gary Schindel and David Gregory) to gain access to additional EAA sampling sites in Hays (SWT Farms, Crystal Clear, San Marcos Baptist Academy, and the SM Bad Water Line wells) and Comal (Garden Ridge, Bracken, HWY 306, Loop 337, Panther Canyon, and Mission Valley wells) counties. By 9 May, SMARC staff discontinued trapping efforts at Garden Ridge, Bracken and SWT farms wells after a month of not collecting Texas blind salamanders.

In April, McLean Worsham (TSU graduate student), working with Valentin Cantu, began sampling at Diversion Springs. Although he is collecting live invertebrates as part of his Master’s Thesis to find a host for *Huffanella huffanni*, an endemic parasite to San Marcos Springs, he will provide the SMARC with Texas blind salamanders when they are captured. As of 30 May, no Texas blind salamanders were collected.

On 19 May Patricia Echo-Hawk hosted the National Dive Control Board conference call with all Regional Dive Officers and Region 9 Safety Manager. Policy changes for FWS 240-10 are being implemented and developed for surface supplied diving procedures as well as training standards for each region. The Joint Gulf Coral Project with NOAA tentatively scheduled for the summer of 2014 was discussed, as was new training techniques for the FWS.

Kenneth Ostrand and Jeff Hutchinson transferred 30 Texas wild rice seedlings on 4 April to Dr. John Goolsby with the USDA’s Knippling-Bushland Research Laboratory in Edinburg, Texas. Dr. Goolsby is testing a biological control agent for giant reed (*Arundo donax*) and will be using the Texas wild rice in comparison tests since both giant reed and Texas wild rice are members of the grass family. Unfortunately the original 30 plants were not prospering at the laboratory. Although these original plants did not die their condition precluded their use in his experiments. After equipping the aquatic holding tank with a pump (power head) an additional 30 plants were transferred to his laboratory. Dr. Goolsby also took 300 gallons of Edward’s aquifer well water to supplement the holding tanks when needed. The more recently transferred plants were

reported as doing well and exhibit new growth.

Personnel development: Training

During May, Randy Gibson completed FISSA and Role Based Security Training (RBST) required for 2014.

Facilities and equipment

During May, Jae Ahn visited the SMARC and evaluated the facility assets. His report is being composed.

Outdoor pond C-9 was covered in 50% (30% of pond) and 70% (30% of pond) shade cloth for protection of plants from direct sunlight. Recently, SMARC staff has moved seedlings of terrestrial plants from the greenhouse to outdoor tables into direct sunlight, and the leaves of the plants were severely burned. The addition of shade cloth is intended to acclimate the plants to additional sunlight.

Ten 300 gallon Rubbermaid tanks and four 1.0 hp Tsurumi pumps were acquired in May. The 300 gallon tanks will be used for aquatic plant propagation and short-term (6-8 weeks) research project. The pumps will be used to increase flows in outdoor ponds used for aquatic plant propagation.

During May, Century Link began to upgrade telephone and computer lines at the SMARC.

During May, Patricia Echo-Hawk, Kristina Zabierek, and Harlin Nichols wore formaldehyde monitoring badges to ensure that exposure limits did not exceed OSHA standards (see 29 CFR 1910 and USFWS 242 FW9 1048; time weighted average (TWA) = 0.75). Samples were sent to the Sensor Safety Products lab for analysis. All test results were within acceptable levels and conveyed to the RO safety officer for their records.

Randy Gibson is working with Dr. Glenn Longley (TSU), the Edwards Aquifer Data Research Center (EARDC) and a graduate student, Laura McCalla, to monitor the SMARC water wells and other water wells upstream and downstream of the site of the Paso Robles housing development and golf course. This large-scale development will occur 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 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. Water samples from Hunter well collected during March, June, and September 2011 contained relatively high levels of total coliform. This may indicate the influence of nearby recharge features that needs further investigation. The last samples for this project were taken on 19 July 2012. During August, all sample analyses were completed. 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 has received funding from TCEQ-SEP program to continue periodic monitoring of SMARC and City of San Marcos wells for two years. During 21-25 April, water quality sampling took place in SMARC and city of San Marcos wells. During May, a laboratory report was completed by Amplified Geochemical Imaging, LLC and EARDC for the April sampling (Table 6). During May, Randy collected water quality measurements and collected water samples for the Texas Commission on Environmental Quality discharge permit (TXG130018). (CSF 7.12.5.4)

Table 6. Water quality analysis of SMARC and nearby City of San Marcos wells during 21-25 April 2014

Factor, units	SMARC, Hunter Well	SMARC, McCarty Well	San Marcos City, McCarty Well	San Marcos City, Soyers Well
TPH, ug	bld	0.78	11.54	0.73
NAPH, ug	bld	bld	0.13	bld
CHCl3, ug	bld	bld	bld	0.04
PCE, ug	0.07	0.08	bld	0.03
TCB, mpn/100ml	2	2	bld	bld

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
NAPH - Naphthalene
CHCl3 - Chloroform
PCE -
Tetrachloroethene
TCB - Total Coliform Bacteria

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

In May, Valentin Cantu led a crew of ten CSR (Community Service Restitution) volunteers to trim and cut grass at the SMARC. In addition the volunteers picked up trash along IH-35, McCarty Lane ditch, and within the property, cut down shrubs to facilitate grass cutting, added new plywood to the overhang of the recycle building, recycled scrap metal and wood, repaired

12" corrugated drainage pipe near field office, changed oil and transaxle fluid in 5-wheel ATV, organized PVC pipes behind shop, watered trees using reuse water, collected live food (amphipods) for salamanders, and continued setting up a new refugia tank system for wild stock Texas blind salamanders.

Visitors

SMARC staff gave the City of San Marcos Conservation Crew a tour of the facility on May 21. The Conservation Crew was given 20-30 minutes briefs on the listed invertebrates, fishes, salamanders, and Texas wild rice. The Conservation Crew works in the San Marcos River during May to August to inform the public on conservation measures.

On 28 May, Valentin Cantu conducted a tour for two adults and two children.

On 30 May, Patricia Echo-Hawk and Randy Gibson conducted a tour for 35 students and 12 parents from the Home School Team.