ATTACHMENT 2

EXHIBIT A

SCOPE OF WORK FOR PILOT STUDY TO EVALUATE THE EFFECTIVENESS OF HOST SNAIL REMOVAL ON GILL PARASITE ABUNDANCE IN THE COMAL RIVER

PROJECT BACKGROUND

The Edwards Aquifer, including its two largest spring ecosystems, Comal and San Marcos Springs, maintains several endemic species, eight of which are federally-listed as threatened or endangered. As a result, the Edwards Aquifer Recovery Implementation Program (EARIP) was formulated and plans to submit a habitat conservation plan (HCP) which will include an important adaptive management component. To construct the preferred alternative for the HCP and to develop the associated adaptive management component, the EARIP must understand the risks to the threatened and endangered species in the Comal and San Marcos Springs ecosystems. This proposal describes a pilot study to better understand one such risk as identified by the Edwards Aquifer Area Expert Science Subcommittee and the Ecosystem Restoration Subcommittee.

A major risk in the Comal Springs system is the continued presence of an Asian trematode, *Centrocestus formosanus*. This parasite was first discovered on fountain darters in the Comal River in the mid-1990s. The parasite attaches to fountain darter (*Etheostoma fonticola*) gill filaments causing extensive gill tissue proliferation and damage. EARIP committee's have identified *C. formosanus* (gill parasite) as a potential threat to the fountain darter especially during periods of low-flow, particularly in the Comal Springs ecosystem. The gill parasite is also present in the San Marcos River but has remained at lower levels and is currently considered less of a threat to the fountain darter in the San Marcos River relative to Comal Springs. However, this level of threat may change if snail and parasite numbers in the San Marcos River increase. Although the pilot study is to be conducted at Comal Springs, information gained via this effort will be applicable to the San Marcos system as well.

INTRODUCTION

The exotic gill parasite that infects the endangered fountain darter in the Comal and San Marcos rivers in central Texas has a complicated life cycle that requires bird, snail, and fish hosts. Elimination of the parasite from the river likely cannot be accomplished. However, a practical approach to manage the parasite in the Comal River might be to control the parasite's snail host, *Melanoides tuberculatus*. U.S. Fish and Wildlife Service (FWS) and U.S. Environmental Protection Agency authorizations to use chemicals known to be lethal to the snail likely cannot be obtained for the Comal River. The effectiveness of *M. tuberculatus* removal by physical methods on lowering drifting gill parasite numbers in the Comal River needs to be determined. As such, the following

scope of work describes a pilot study to evaluate the effectiveness of host snail removal on the reduction of the gill parasite in the water column.

The test hypothesis is that if host snail populations are reduced, a reduction in drifting gill parasites downstream will also be observed. Whether the hypothesis is proven true or false, the answer will greatly inform the preparation of a preferred HCP alternative and associated adaptive management plan. If true, a system-wide removal and maintenance program should be considered in the preferred HCP alternative. This removal of snails and resulting reduction of gill parasites during periods of low-flow would considerably reduce one major risk to fountain darters in the Comal Springs ecosystem. If the hypothesis is proven false, then other methods for parasite control may need to be studied and/or considered through the adaptive management component of the HCP.

SCOPE OF WORK

Task 1: Reconnaissance survey for *M. tuberculatus* high density areas

BIO-WEST and FWS will conduct a reconnaissance survey of the Comal Springs system to identify areas with high densities of *M. tuberculatus*. To maximize resources, high density areas will be limited in size to areas less than 5 meters squared (m^2) and will be defined as those areas that contain in excess of 25 *M. tuberculatus*/m^2. Up to five areas will be initially identified and delineated by marking the boundaries of the area with a high accuracy GPS. Other considerations for the selection of a site will include avoiding potential outside influences which could affect the outcome of the study via flushes of water entering the system (e.g. is this area near a swimming pool discharge?, immediately adjacent to a sprinkler system on the golf course?, etc.) or recreational activities (e.g. the area immediately around the Spring Island private area or near Schlitterbahn, etc.). Additionally, the ability to make an accurate and repeatable cross-sectional measurement of gill parasites in the water column immediately below the location will also be considered in the final decision.

Finally, once these high density areas are preliminarily selected, up to 5 spot water samples immediately below the designated area will be taken to evaluate the current level of drifting gill parasites in the water column. If the gill parasite water column concentrations are greater than 15 cercaria/liter, the high density area will be considered to be affecting the system and will be included in the study. Should the high density area meet all the criteria above, but demonstrate minute parasite concentrations in the water column immediately below the site, it will not be considered for further study. A total of three high density areas will be carried forward for further evaluation. It was determined by the principal investigators that three sites should provide the level of detail needed to address the test hypothesis for this study without exorbitant costs.

Task 2: Field Sampling (Collection of Water Samples and Removal of *M. tuberculatus* in high density areas.)

Field sampling will consist of four principal activities: 1) water sampling prior to physical removal of snails; 2) habitat characterization of high density areas; 3) physical

removal of snails; and 4) water sampling following the physical removal of snails.

1) The first activity involves the documentation of drifting gill parasites in the water column prior to any snail removal at the high density areas. This will be conducted above and below the identified high density area by placing one transect approximately 2 meters upstream and one transect approximately 2 meters downstream of the high density area with water samples to be taken across the transect. Transects will extend approximately 3 times the length of the high density area and be centered on the site center. For example, if the high density area is 3 meters long, a 9-meter long transect will be placed approximately 2 meters upstream and downstream perpendicular to the high density area and centered on the site. Sample collection will follow the established protocols in Cantu (2003) and Bollick (2007). Variability of drifting parasite numbers will be determined by repeated same site, same day, and consecutive day sampling prior to any physical removal of snails. For example, there are three sites (high density areas) and at each of these sites three sampling events will be conducted per day for three days.

2) The second activity will be to document the physical habitat, water quality, and flow characteristics within each high density area prior to snail removal. The habitat characterization will include measurements of depth, velocity, substrate type, presence and type of aquatic vegetation, and basic water quality (water temperature, dissolved oxygen, pH, and conductivity) parameters. The habitat characterization will be conducted in a manner appropriate to use this data for future development of habitat suitability criteria for *M. tuberculatus* to potentially be used for additional modeling exercises during adaptive management. It should be noted that for purposes of this study, this habitat information will only be used to document existing conditions as full development of habitat suitability criteria or subsequent modeling are not budgeted in this proposal. However, this data needs to be collected to document existing conditions and thus, collecting it in this manner will be advantageous for potential future use without increasing the costs of the proposed collection effort.

3) The third activity will be the physical removal of exotic snails from within the designated high density area. This removal will be conducted by placing a 2-meter by 1-meter drop net over the area to be sampled and using a 1 m^2 dip net within the enclosed area to collect all exotic snails to the extent possible. Dipnetting will consist of pushing the dip net across the site bottom and extending down to 5 centimeters below the surface of the substrate. Dipnetting will continue until the researcher has concluded that the majority of exotic snails have been removed from the enclosed area. The drop net will then be moved to an adjacent area and the procedure repeated until all of the high density area has been covered. As previously mentioned, no high density area will be greater than 5 m^2, to limit the costs that would be necessary for larger areas. All live *M. tuberculatus* collected and removed will be counted in order to quantify the total number and density of host snails removed from the high density area. All *M.*

tuberculatus will be destroyed as will all other exotic snails collected during this effort. All efforts will be made to separate native snails and place them back into the system. Although it is anticipated that these high density areas for host snails will consist of poor fountain darter habitat, it is possible that fountain darters could be collected during this effort. As such, any fountain darters collected during the effort will be measured, recorded, and released back into the environment.

4) The final activity will be to repeat the water sampling activity (described in Step 1) following the physical removal of exotic snails from the high density areas. This will again occur upstream and downstream at all three high density areas and will include three sampling events per site per day for three days. Sampling will commence the day after the physical removal of snails from a given site to allow the site to settle and limit the effect that the immediate disturbance might have on water column concentrations of the parasite.

Task 3: Laboratory analyses of parasite concentrations

Each water sample collected during the reconnaissance survey, and then during both the pre- and post-removal sampling efforts, will be filtered at the FWS San Marcos National Fish Hatchery and Technology Center laboratory. Water filtering procedures will follow the protocols described in Bollick (2007). Sample filters will be inspected using compound microscopy. The number of exotic gill parasites present in each sample will be determined and recorded.

Task 4: Data analyses and Report preparation

The habitat present at each high density study area will be characterized based on the field measurements taken and a description provided in the report. The total number and density (#/m^2) of *M. tuberculatus* at each high density area will be calculated and used in the analyses. Additionally, the total number and density (# or cercaria/liter) of gill parasites documented from each water sample will be analyzed by individual sample, transect, and locations for each of the sampling events. Statistical analysis will be conducted to evaluate the test hypothesis and determine if there was a significant difference in the gill parasite numbers in the water column before and after the physical removal of snails.

As the results are presented, a discussion on several topics will be included. The prime focus will be on whether the reduction of *M. tuberculatus* in an upstream site significantly reduced the gill parasite numbers in the water column downstream. A discussion on what this actually might mean to the health of the system (particularly the fountain darter) during low-flow conditions will also be presented. Another topic to be discussed is just how effective the physical removal on snails on the snail population itself was within a given area. It is important to note that the removal technique of netting used in the pilot study most likely will not be the method of snail removal for a system-wide approach. Less intrusive methods will be explored and considered. These methods may include site-specific dredging of snails or sediment sifting, but these and other approaches will need to be reviewed and discussed

by the EARIP prior to any implementation. Finally, a key question (albeit not included in this proposal) will be how quickly the resident snail population might re-establish within the high density area, or whether or not they re-establish at all? This topic will need to be considered and addressed should a system-wide removal program be proposed for the HCP.

Deliverable: BIO-WEST will prepare and submit a technical memorandum to the EARIP program director describing the methods and results of the pilot study, and provide recommendations on how these results could be potentially incorporated into an HCP alternative or adaptive management component of that plan. All raw data will also be made available to the EARIP.

References

Bolick, A.E. 2007. "The Effects of Spring Flow on the Abundance of Heterophyid Cercariae in the Comal River, New Braunfels, TX". Texas State University Master of Science Thesis. 53 pp.

Cantu, V. 2003. Spatial and temporal variation of Centrocestus formosanus in river water and endangered fountain darters (*Etheostoma Fonticola*) in the Comal river, Texas. Masters thesis, Texas State University-San Marcos. 58 p.

SCHEDULE

The tasks outlined above will be initiated within one week of a Notice to Proceed from the EARIP. The fieldwork, laboratory work, data analyses and draft report will be completed within 90 days from the Notice to Proceed. A draft report will be provided to the EARIP. Upon receipt of EARIP comments, revisions, if necessary, will be made and a second draft will be submitted to the EARIP within 2 weeks. The EARIP will then submit the draft report to the TWDB for review and comment.

STAFFING AND COORDINATION

BIO-WEST will be the prime consultant for this study under the guidance of Mr. Ed Oborny. BIO-WEST will subcontract directly with the FWS San Marcos National Fish Hatchery and Technology Center for field services, laboratory services, data interpretation, and report preparation. Ms. Mara Alexander will be the principal researcher for the FWS. This study will be a collaborative effort between BIO-WEST and the FWS with formal coordination and review during the study provided by Dr. Tom Brandt (FWS), Ken Diehl (San Antonio Water System), and Nathan Pence (City of New Braunfels). Prior to the submittal of the draft report to the EARIP, the five individuals mentioned in this paragraph will 1) meet and discuss the study results and interpretation, 2) review, edit, and comment on the text of the draft report, and 3) concur with the submittal of the draft report to the EARIP.

EXHIBIT B

EXPENSE AND TASK BUDGETS FOR HOST SNAIL REMOVAL PILOT STUDY

TASK BUDGET

Task	Description	Amount
Task 1	Reconnaissance survey for *M. tuberculatus* high density areas	$3,776.00
Task 2	Field Sampling (Collection of Water Samples and Removal of M. tuberculatus in high density areas.)	$15,566.26
Task 3	Laboratory analysis of parasite concentrations	$7,978.08
Task 4	Data analyses and Report preparation	$17,600.62
TOTAL		**$44,920.96**

EXPENSE BUDGET

CATEGORY	TOTAL AMOUNT
Salaries & Wages[1]	$20,482.96
Subcontractor Expenses (FWS)	$22,228.00
Travel[2]	$910.00
Expendable supplies[3]	$1,300.00
TOTAL	**$44,920.96**

With the prior approval of the Program Director, the Consultant shall have budget flexibility among task budgets and expense category budgets as contained herein to the extent that the resulting changes associated with a single task or expense category do not exceed thirty-five percent (35%) of the total amount authorized by the Contract or subcontract for the task or expense category to be changed.

[1] Includes overhead
[2] Per diem and mileage
[3] Laboratory supplies, Field supplies, Phone, Fax, copies, postage