



**SOLICITATION, OFFER  
AND AWARD**

City of New Braunfels  
Purchasing  
424 S. Castell Avenue  
New Braunfels, Texas 78130

Solicitation No. 12-032 Gill Parasite	<input type="checkbox"/> Invitation for Bid (IFB) <input checked="" type="checkbox"/> Request for Proposal (RFP)	Date Issued: September 7, 2012
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**SOLICITATION** Page 1 of 23 Pages

Proposers must submit sealed proposals in triplicate signed original and one CD for furnishing the services identified in the Schedule. Proposals will be received at the office of the City Secretary at the address shown above until: 10:00 a.m. on September 24, 2012. Proposals received after the time and date set for submission will be returned unopened.

For Information Call: Mary Quinones <small>(NO collect calls, Telegraphic, Email, On-Line or Fax offers accepted)</small>	Phone No.: (830) 221-4389 Email <a href="mailto:mquinones@nbtexas.org">mquinones@nbtexas.org</a>	Fax No.: (830) 608-2112
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5% Proposal Bond Required:  YES  NO (If YES, See Para 4(d) of Terms and Conditions)  
100% Performance Bond Required:  YES  NO (If YES, See Para 4(d) of Terms and Conditions)

**OFFER** (Must be fully completed by offeror)

Offeror's State of Residence: Texas (See Para. 6(f) of Terms and Conditions)

**Pre-Proposal Conference on September 18, 2012 at 10:00 am. in Parks Admin Office. 100 Golf Course Rd. New Braunfels. TX 78130.**

Prompt Payment Terms: \_\_\_ % Discount if paid within \_\_\_ days.

In compliance with the above, the undersigned offers and agrees to furnish any or all items or services awarded at the prices stipulated for each item delivered at the designated point(s) and within the time specified herein. Award shall include all solicitation documents and attachments.

**FOR INFORMATION, CONTACT THE PERSON ABOVE.  
MANUALLY SIGN ALL COPIES SUBMITTED. SIGNATURE IS MANDATORY.**

<b>*Submit Signed Offers in Triplicate Original*</b>		Proposer E-Mail Address: <u>eoborny@bio-west.com</u>	
Name	BIO-WEST, Inc.	Name and Title of Person Authorized to Sign Offer (Type or Print):	
And	1812 Central Commerce Court	Edmund L. Oborny, Jr.	
Address	Round Rock, Texas 78664	Principal	
Signature:		Date:	9-18-12
		Phone No.:	(512) 990-3954
		Fax No.:	(512) 990-5153

Name, Address and Telephone No. of Person authorized to conduct negotiations on behalf of Offeror. (Applies to Request for Proposal only)

**AWARD** (To be Completed by CITY)

Contract #	NB13-008	Awarded as to item(s):	A11	Contract Amount:	\$174,930.77
Vendor Code #:				Delivery Date or Term of Contract:	August 31, 2013

Remarks: This contract incorporates the RFP, attachments and contractor's response.

This contract issued pursuant to award made by City Council. Date: December 10, 2013 Agenda Item No.: 4C

Important: Award may be made on this form or by other authorized official written notice.

Michael Morrison  
City Manager

12-11-12  
DATE

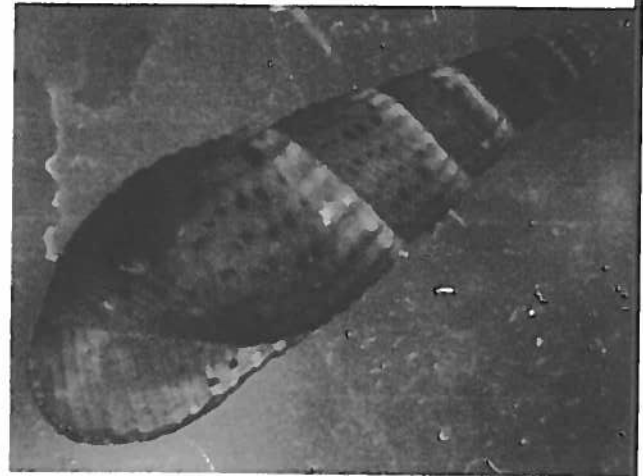
**BIO-WEST / Texas State University / U.S. Fish  
and Wildlife Service San Marcos National  
Fish Hatchery & Technology Center**

**PROPOSAL**

**Solicitation # 12-032**

**Gill Parasite**

**New Braunfels, Texas**



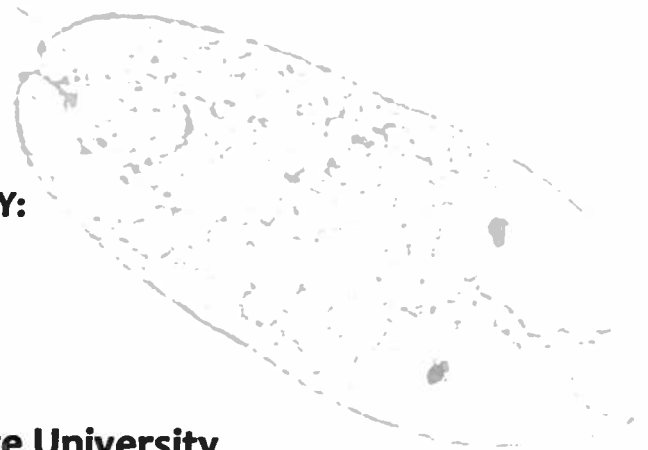
**PREPARED FOR:**

**City of New Braunfels  
Purchasing  
424 S. Castell Ave.  
New Braunfels, Texas 78130**

**PREPARED BY:**

**BIO-WEST, Inc.  
1812 Central Commerce Court  
Round Rock, Texas 78664**

**Texas State University  
Department of Biology  
601 University Drive  
San Marcos, Texas 78666**



**September 24, 2012**

**USFWS San Marcos NFH&TC  
500 East McCarty Lane  
San Marcos, Texas 78666**



**BIO-WEST, Inc.**

812 Central Commerce Court  
Round Rock, Texas  
78664-8546  
Ph: 512.990.3954  
Fx: 512.990.5153  
www.bio-west.com

21 September 2012

City of New Braunfels  
Attention: Purchasing Department  
424 S. Castell Avenue  
New Braunfels, Texas 78130

RE: BIO-WEST, Inc. Proposal for Solicitation No. 12-032

Dear Reviewer,

Enclosed please find BIO-WEST's proposal and cost estimate for conducting the proposed Gill Parasite Study for the City of New Braunfels. BIO-WEST, Inc. is a multi-discipline, environmental consulting firm specializing in environmental services focused on endangered species and associated habitat. Established in 1976, BIO-WEST conducts research, inventories, and assessments of natural resource systems throughout the United States. The firm is a leader in environmental consulting and problem solving, and has earned a widely acknowledged reputation for providing objective, credible services and superior products to a wide variety of agencies, organizations, and private clients.

BIO-WEST has extensive experience and expertise with the Comal Springs ecosystem. We have had the privilege of conducting comprehensive and critical period monitoring at Comal Springs for over a decade. In addition to BIO-WEST's experience, we have enlisted the assistance of well-respected researchers with Texas State University and the U.S. Fish and Wildlife Service San Marcos National Fish Hatchery and Technology Center. We are excited about this opportunity and feel our team, assembled specifically for this project, has unparalleled experience and expertise that can provide the City with the best services possible in the context of the Habitat Conservation Plan.

We appreciate the opportunity to bid on this exciting project and look forward to hearing from you. If you have any questions or comments concerning our proposal, please contact me at your earliest convenience at the contact information below:

Sincerely,

Ed Oborny

Enclosures

Edmund L. Oborny, Jr.  
Principal  
BIO-WEST, Inc.  
1812 Central Commerce Court  
Round Rock, TX 78664  
Phone: (512) 990-3954  
Email: eoborny@bio-west.com

Environmental  
Analysis  
and  
Permitting

Environmental  
Engineering

Fisheries

Landscape  
Architecture

Resource  
Planning

Vegetation

Water  
Resources

Wetlands

Wildlife





**SOLICITATION, OFFER  
AND AWARD**

City of New Braunfels  
Purchasing  
424 S. Castell Avenue  
New Braunfels, Texas 78130

Solicitation No. 12-032  
Gill Parasite

Invitation for Bid (IFB)  
 Request for Proposal (RFP)

Date Issued:  
September 7, 2012

**SOLICITATION**

Page 1 of 23 Pages

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**MANUALLY SIGN ALL COPIES SUBMITTED. SIGNATURE IS MANDATORY.**

**\*Submit Signed Offers in Triplicate Original\***

Proposer E-Mail Address: eoborny@bio-west.com

Name BIO-WEST, Inc.  
And 1812 Central Commerce Court  
Address Round Rock, Texas 78664  
of Offeror

Name and Title of Person Authorized to Sign Offer (Type or Print):  
Edmund L. Oborny, Jr.  
Principal

Signature: [Handwritten Signature]

Date: 9-18-12

Phone No.: (512) 990-3954

Fax No.: (512) 990-5153

Name, Address and Telephone No. of Person authorized to conduct negotiations on behalf of Offeror (Applies to Request for Proposal only)

**AWARD (To be Completed by CITY)**

Contract #	Awarded as to item(s):	Contract Amount:
Vendor Code #:		Deliver Date or Term of Contract:

Remarks:

This contract issued pursuant to award made by City Council Date: Agenda Item No.:

Important: Award may be made on this form or by other authorized official written notice.

DATE

## II. EXECUTIVE SUMMARY

As is evident in the attached proposal, BIO-WEST has assembled a project team with significant technical expertise with the gill parasite and host snail and extensive experience regarding the ecology of the Comal Springs/River Ecosystem. BIO-WEST has been working in the Comal system for over a decade with comprehensive and critical period sampling of the aquatic biota and associated habitats. Additionally, BIO-WEST has enlisted the assistance of Dr. David Huffman (Texas State University) and Dr. Tom Brandt (U.S. Fish and Wildlife Service (USFWS) National Fish Hatchery and Technology Center [NFH&TC]) who are recognized experts on the gill parasite and exotic snails. Dr. Ken Ostrand and resource professionals at the NFH&TC will provide additional expertise. Dr. Brandt (Director) and Dr. Ostrand (Deputy Director) both support use of the NFH&TC facilities by the project team for applied research activities specific to this project. With this collection of knowledge, diversity of ideas, and access to facilities, the BIO-WEST study team is particularly well-suited for assisting the City of New Braunfels with developing a plan to document baseline conditions and subsequently test approaches for the control of the gill parasite in the Comal System. Factors that set the BIO-WEST study team apart from the competition include:

- An extensive knowledge of the aquatic biota and ecological relationships of the Comal River/Springs Ecosystem with hands on experience via the Variable Flow Study for the past 12 years. Specific to this RFP, activities include:
  - snail counts and gill parasite infection evaluations every time fountain darters are collected from the system;
  - completion of a pilot study in conjunction with the NFH&TC in 2010/2011 to specifically evaluate the effectiveness of snail removal on cercarial concentrations in the water column (detailed study description in Section VI);
  - supporting three M.S. thesis projects specific to this topic and developing the techniques for sampling cercaria in the water column effectively and efficiently.
- Dr. Tom Brandt has worked on the Comal System since the 1970s and has personally observed the escalation of the gill parasite in the Comal system during the late 1990s. As such, Dr. Brandt made the gill parasite a focal applied research target at the NFH&TC and has nearly a dozen peer-reviewed journal publications specific to this topic since that time.
- Dr. David Huffman has been involved in research relating to Edwards Aquifer springs since being hired at Texas State in 1973. Dr. Huffman was first to diagnose the gill disease of fountain darters in the mid 90's as a trematode species new to Texas waters, and has since sponsored to completion four master's theses on the subject.
- Possession of all required scientific collection permits and certifications to begin work immediately upon award.
- Unparalleled scientific integrity. Mr. Oborny and Dr. Brandt are active members of the Southern Edwards Aquifer Species Recovery Team and former members of the EARIP Science Subcommittee. Dr's Huffman and Ostrand are well-respected researchers who have served on numerous scientific review panels and science teams, and generated an impressive number of peer-reviewed publications.
- Full access to use the NFH&TC facilities for applied research activities.

The BIO-WEST study team is pleased to submit this proposal and cost estimate to the City of New Braunfels. It will become evident as you explore this proposal that study team personnel have extensive experience and expertise working with the gill parasite, host snail, and aquatic biota of the Comal River System. Additionally, the study team has considerable experience conducting similar

studies in other systems throughout Texas and the western U.S. Examples of relevant experience are included as is a brief overview of the proposed technical approach. Information on key team members is included and costs are summarized in the proposal pricing.

### III. DEGREE OF COMPLIANCE

All services quoted in this proposal are in full accord with the general requirements of the RFP (12-032). The BIO-WEST project team has all the resources necessary to perform the services outlined in the RFP.

### IV. PROPOSAL PRICING

The total costs for the 2013 Gill Parasite study as described in RFP (12-032) and consistent with the level of effort and intent of the approved EARIP workplan is \$174,931. It is important to note that BIO-WEST was heavily involved in the development of the biological goals (that provide the foundation for the Habitat Conservation Plan [HCP]) and subsequent preparation of the HCP and associated mitigation and applied research measures. As such, BIO-WEST is thoroughly familiar with the questions being asked and the level of work necessary to meet the intent of the approved EARIP workplans and budgets. Other bidders may considerably under or over bid the approved EARIP workplan budget for this specific effort, not truly understanding the intent of the EARIP workplan that was developed with an approved budget. A detailed cost breakdown designed to meet the needs of the HCP is presented.

		RFP 2-032 Gill Parasite						
Position	Rate	Project Management, Design and Literature Search (Task 1)	Comprehensive Snail and <i>Centrocestus</i> survey (Task 2)	Testing Removal / Control Techniques (Task 3)	Data Analysis Reporting, Monitoring Plan (Task 4)	Total Hours	Cost	
Principal	136.23	36	48	64	64	212	\$ 28,880.76	
Senior Engineer	145.73	4		6	24	34	\$ 4,954.82	
Senior Researcher	105.18	36	64	72	84	256	\$ 26,926.08	
Senior Ecologist	92.34	23	16	96	48	183	\$ 16,898.22	
Biologist	87.80	12	84	128		224	\$ 19,667.20	
Aquatic Technician	59.03	16	84	128	48	276	\$ 16,292.28	
Technician I	43.27		124	256	96	476	\$ 20,596.52	
Technician II	26.42	36	164	164	128	492	\$ 12,998.64	
<b>Total Labor</b>		163	584	914	492	2153	<b>147,214.52</b>	
<b>TRAVEL</b>								
Per diem		50	2,570	1,375	125		\$ 4,120.00	
Mileage (\$.555 per mile)	0.555	500	1,500	2,250	500	4750	\$ 2,636.25	
<b>Total Travel</b>							<b>\$ 6,756.25</b>	
<b>OTHER DIRECT COSTS:</b>								
Equipment			3,250	9,500	35		\$ 12,785.00	
Supplies			4,550	2,250	500		\$ 7,300.00	
Phone / Fax / Copies		75	125	125	550		\$ 875.00	
<b>Total Other Direct Costs</b>		\$ 75	\$ 7,925	\$ 11,875	\$ 1,085		<b>\$ 20,960.00</b>	
<b>Total Estimated Cost</b>						<b>Total</b>	<b>\$ 174,930.77</b>	
	Subtasks	14,749.20	48,107.58	74,733.69	37,340.30			
	<b>TASKS</b>							<b>\$174,930.77</b>

The costs have been divided into four primary tasks including 1) project management/study design and literature search, 2) comprehensive snail and *centrocestus* survey, 3) testing of removal/control techniques for snails and parasites, and 4) data analysis/reporting and development of the 2014 monitoring plan. Details of each task are presented in the General Methodologies in Section V.

## V. DESCRIPTIVE LITERATURE

### GENERAL METHODOLOGY

As stated in the RFP, the work involves conducting a system wide survey on the Comal River to determine *Melanoides tuberculatus* (non-native host snail) population densities and cercarial concentrations of *Centrocestus formosanus* (gill parasite). Following that effort and concurrent literature search, methods for the reduction of the gill parasite in the Comal system will be tested for effectiveness and efficiency. Finally, a gill parasite monitoring and reduction program (if necessary) will be developed for implementation in subsequent years.

**Task 1** includes the development of a specific methodology and work plan for the system wide survey of both non-native host snail densities as well as cercarial concentrations of the gill parasite in the water column. The BIO-WEST project team is very familiar with the 8 potential sample reaches as described in Figure 2 of the RFP through the variable flow monitoring program, as many locations overlap with detailed efforts we have conducted. It is *Critical* in methodology development to understand the nuances in both snail density evaluations and cercarial water column sampling.

*Dr. Huffman has discovered there are several genetic morphs of the non-native host snail that need to be identified when sub-sampling the overall population. It appears that different morphs can occupy different habitats and may have differing susceptibility to the parasite, making this differentiation important during the system-wide survey. Secondly, it has been documented in the pilot study conducted by BIO-WEST/NFH&TC that time of day and sunlight make a significant difference in the numbers of cercaria being released into the water column. This can drastically affect the results of both the system-wide survey and subsequent effectiveness testing should researchers not be familiar with this phenomenon.*

Concurrent with the development of the specific methodology, a detailed literature search and discussions with experts will be conducted. This will include identifying and exploring different techniques for both snail removal and gill parasite reductions. It is important to understand that just because the aforementioned pilot study showed that on a small scale and short time period, host snail reduction reduced concentrations of gill parasites in the immediate water column that is NOT proof that on a larger spatial or temporal scale it would be effective. It is also unknown at this point if snail removal at a level necessary to make an impact is even practical. As such, a major focus of the literature review will be to explore methods for controlling the gill parasite.

**Task 2** is the system-wide survey of host snails and gill parasite concentrations in the water column. The system-wide survey will build on the existing substrate/vegetation maps to conduct a stratified sampling regime to subsample snail densities by substrate and vegetation polygon within delineated reaches. As part of the Variable Flow Monitoring program, BIO-WEST will be mapping the aquatic vegetation of the entire Comal System in January 2013. This mapping under a separate contract will be used to set up the final sampling design. As specified in the RFP, reaches in eight sections (Figure 2 of the RFP) of the Comal system will be considered. The BIO-WEST project team is extremely familiar with all eight sections having done work in each of them repeatedly over the past decade. As highlighted above, the sampling design will include the identification of host snail morphs on a subsample scale as well as involve a consistent time-interval sampling protocol. It is anticipated that all water



column samples would be collected systematically between 8-10 am. This will involve a crew experienced with the water filtration techniques and equipment (Figure above) necessary to preserve the cercaria in each sample. This is not a simple water grab, as multiple levels of filtering, under low turbidity conditions must be implemented, and culminate in a staining process in the field so that the microscopic cercaria can be accurately identified in the laboratory. As mentioned, the BIO-WEST project team refined the sampling technique most commonly used to be specifically effective for this application. Additionally, high powered microscopes are required for cercarial identification in the laboratory. All three team members (BIO-WEST, NFH&TC, and TSU) have these scopes in their respective laboratories. This is important as a large number of samples will need to be processed to complete this survey. The final product from Task 2 will be a complete analysis of the survey to compare the cercarial density as a function of host snail density in the study reaches.

**Task 3** involves the evaluation of methodologies and techniques for snail removal and gill parasite reduction. Specific evaluations will be developed upon completion of the literature survey and discussions with experts. However, based on our experience, it is likely that both laboratory and field experiments will result from that exploration. Two laboratory studies are proposed to be conducted at the NFH&TC. The first involves evaluating the effectiveness of baiting techniques to attract the host snail. Preliminary work at Texas Tech University has been conducted relative to baiting success, and these controlled experiments will build on those preliminary investigations. Conducting the experiment first in a controlled environment will allow the project team to understand the potential effectiveness of this technique. Should it look promising, a small-scale effort will be conducted in the field as part of the pilot-study described below. The second laboratory study to be initiated will focus on agitation/turbulence/etc. as a means for rendering cercaria in the water column ineffective in adhering to the gills of fountain darters. Considerable work has been conducted at the NFH&TC relative to infection rates on fountain darter gills, so all the technology and experience is there to effectively test this technique. Again, should this technique demonstrate promising results, it will be subsequently investigated in the pilot study. Additional techniques such as dip netting, suction dredging, etc. will be tested in the field under separate small scale experiments to evaluate effectiveness both from a snail removal and cost perspective. Should any or all of these techniques prove promising, they will be applied to the proposed pilot study.

An appropriate criticism of the original snail removal pilot-study was the spatial and temporal extent of that study was too limited. We concur with that criticism and following the evaluations described above, will implement a more comprehensive study to evaluate the potential effectiveness of promising techniques. The actual study location will be based on the results of the system-wide survey but will include at a minimum, three sample areas and a control. In both the sample areas and control area, pre-application, during, and post-application sampling will take place to test effectiveness and cost. This study is proposed to last three months starting in May and extending through July 2013 to capture warmer summer time temperatures. The actual study design will be determined by the results from all previous tasks and thus, cannot be detailed at this time. It is known that the study design will be developed to answer specific applied research questions specific to the HCP concerns and biological goals. The study plan will be submitted to the City of New Braunfels no later than April 1<sup>st</sup> for review.

**Task 4** involves all data analysis and reporting associated with Tasks 2 and 3. This will include a final report with chapters on the system-wide survey (Task 2), applied research laboratory and field experiments conducted (Task 3 - part 1), the detailed pilot-study (Task 3 - part 2), and recommendations on how to move forward with this mitigation measure. This will include 1) a discussion on the findings of whether further activity is necessary, 2) recommendations on the best methodologies for snail removal and/or parasite reduction activities, and 3) recommendations on how to proceed with the 2014 work plan regarding the gill parasite.



## VI. CONTRACTOR BACKGROUND INFORMATION

### Peer Reviewed Publications

BIO-WEST, TSU, and NFH&C sponsored peer reviewed publications specific to the host snail, gill parasite, and interactions with the fountain darter are presented below. This does not include all the technical reports and investigations that have also been completed. It is important to note the extent and diversity of publications as they include several evaluations of snail and parasite response (temperature, ice-water, salt, chemicals, etc.) as well as evaluate the effects of the parasite on fountain darter growth, survival, and reproduction.

- Bolick, A. E. 2007. The Effects of Springflow on Heterophyd Cercariae in the Comal River, New Braunfels, TX. Master's Thesis. Texas State University.
- Cantu, V. 2003. Spatial and temporal variation of *Centrocestus formosanus* in river water and endangered fountain darters (*Etheostoma fonticola*) in the Comal River, Texas. Master's thesis, Texas State University.
- Fleming, Byron Paul. 2002. Downstream spread of the digenetic trematode *Centrocestus formosanus*, into the Guadalupe River, Texas. M.S. Thesis, Texas State University.
- Fleming, B. P., D. G. Huffman, T. H. Bonner, and T. M. Brandt. 2011. Metacercarial distribution of *Centrocestus formosanus* (Digenea: Heterophyidae) among fish hosts in the Guadalupe River drainage of Texas. *Journal of Aquatic Animal Health* 23:117-124.
- Johnson, M. S., A. Bolick, M. Alexander, D. Huffman, E. Oborny, and A. Monroe. 2012. Fluctuations in densities of the invasive parasite *Centrocestus formosanus* (Trematoda: Heterophyidae) in the Comal River, Comal County, Texas, U.S.A. *J Parasitol* 98(1):111-116.
- Kuhlman, Tiffany A. 2007. The predominant definitive avian hosts of the invasive Asian trematode, *Centrocestus formosanus*, in the headwaters of the Comal, San Antonio, and San Marcos rivers of central Texas. M.S. Thesis, Texas State University.
- Lindholm, Jay Jack Titus. 1979. The gastropods of the upper San Marcos River and their trematode parasites. M.S. Thesis, Texas State University.
- McDermott, K.S., T. L. Arsuffi, T. M. Brandt, D. C. Huston, and K. G. Ostrand. 2012. Exotic digenetic trematode (*Centrocestus formosanus*) distribution and occurrence, its exotic snail intermediate host (*Melanoides tuberculatus*), and fish infection rates in West Texas Springs Systems. *Southwestern Naturalist*. 00:000-000. (In Review).
- McDonald, D. L. 2003. Effects of fluctuation temperature and an introduced trematode on reproduction and mortality of *Etheostoma fonticola*. Master's thesis, Texas State University. 35pp.
- McDonald, D. L., T. H. Bonner, T. M. Brandt, and G. H. Trevino. 2006. Size susceptibility to trematode-induced mortality in the endangered fountain darter (*Etheostoma fonticola*). *Journal of Freshwater Ecology* 21:293-299.
- McDonald, D. L., T. H. Bonner, E. L. Oborny, Jr., and T. M. Brandt. 2007. Effects of fluctuating temperatures and gill parasites on reproduction of the fountain darter, *Etheostoma fonticola*. *Journal of Freshwater Ecology* 22:311-318.
- Mitchell, A. J., M. J. Salmon, H. G. Huffman, A. E. Goodwin, and T. M. Brandt. 2000. Prevalence and pathogenicity of a heterophyid trematode infecting the gills of an endangered fish *Etheostoma fonticola* in two Central Texas spring-fed rivers. *Journal of Aquatic Animal Health* 12:283-289.
- Mitchell, A. J., A. E. Goodwin, M. J. Salmon, and T. M. Brandt. 2002. Experimental infection of an exotic heterophyid trematode, *Centrocestus formosanus*, in four aquaculture fishes. *North American Journal of Aquaculture* 64:55-59.
- Mitchell, A. J., and T. M. Brandt. 2005. Temperature tolerance of red-rim melania *Melanoides tuberculatus*, an exotic aquatic snail established in the United States. *Transactions of the American Fisheries Society* 134:126-131.
- Mitchell, A. J., R. M. Overstreet, A. E. Goodwin, and T. M. Brandt. 2005. Spread of an exotic fish-gill trematode: A far-reaching and complex problem. *Fisheries* 30:11-16.

- Mitchell, A. J., and T. M. Brandt. 2007. The effect of chemical treatments on red-rim melania *Melanoides tuberculatus*, an exotic aquatic snail that serves as vectors of trematodes to fish and other species in the USA. *North American Journal of Fisheries Management* 27:1287-1293.
- Mitchell, A. J., and T. M. Brandt. 2009. Use of ice-water and salt treatments to eliminate an exotic snail, the red-rim melania, from small immersible fisheries equipment. *North American Journal of Fisheries Management* 29:823-828.
- Salmon, Melissa J. 2000. Impact of an undescribed heterophyid trematode on the fountain darter *Etheostoma fonticola*. M.S. Thesis, Texas State University.

## Host Snail Removal in the Comal River, Texas and its Impact on Densities of the Gill Parasite

BIO-WEST was contracted in conjunction with the U.S. Fish and Wildlife Service (USFWS) San Marcos National Fish Hatchery and Technology Center to determine the effectiveness of *Melanoides tuberculatus* removal on lowering drifting gill parasite numbers in the Comal River.

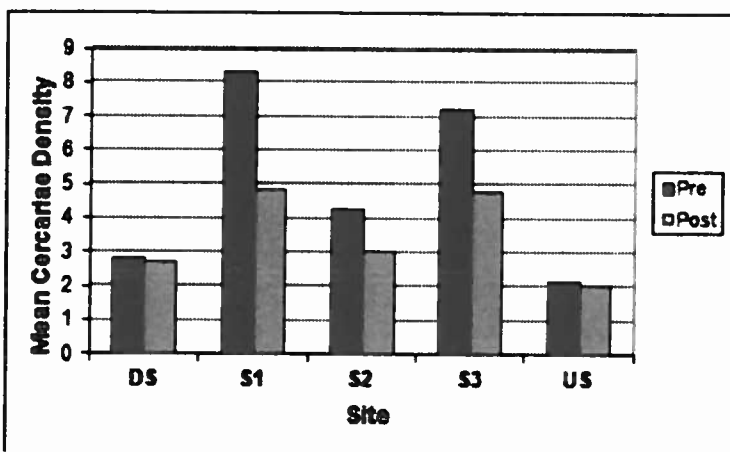
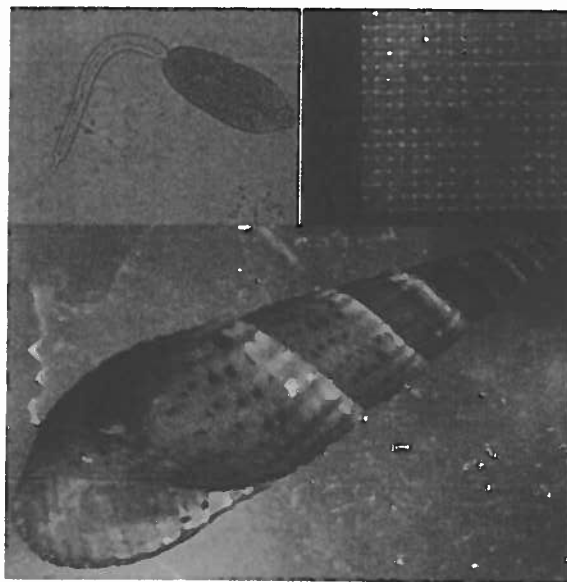


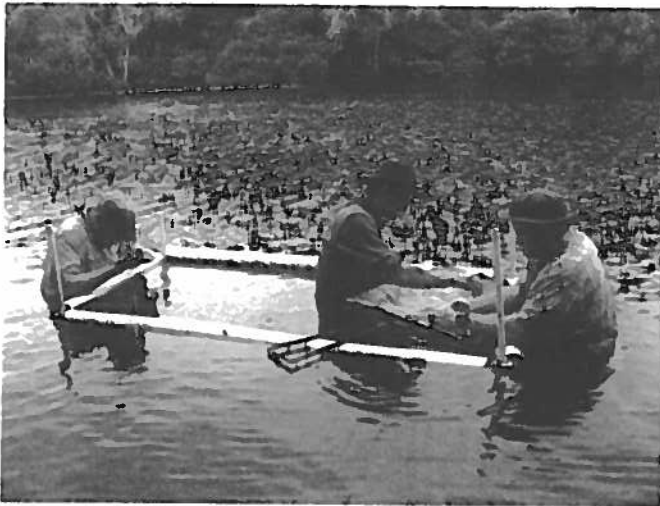
Figure Mean pre- and post-removal cercariae density at each study site.



The results of this study documented that Cercarial densities both pre- and post-removal were correlated with the densities of snails at each hotspot. It also became apparent that differences in cercarial density between time periods are influenced by time of day. This study found that cercarial concentrations were highest at 9:00 AM and declined at 11:00 AM and 1:00 PM. Cantu (2003) showed a similar trend, with *C. formosanus* cercarial abundance increasing sharply after dawn, peaking around 9:30 AM, and then declining until approximately 3:30 PM. This emphasizes the importance of examining time of day when comparing between samples. Although a valuable starting point, several unknowns remain regarding magnitude and duration of benefits from snail removal in the Comal system, and thus, specific study recommendations were presented to the Edwards Aquifer Recovery Implementation Program.

## Comal and San Marcos Springs Variable Flow Monitoring Program

Beginning in 2001, BIO-WEST contracted with the Edwards Aquifer Authority (EAA) to conduct a multi-year applied research effort with the goal of augmenting the available data on population dynamics of threatened and endangered species in the San Marcos and Comal Rivers/Spring Ecosystems and its relationship to springflow. Over the past 12 years, BIO-WEST biologists have collected and analyzed data on a variety of components within these systems including: exotic species, water quality, aquatic vegetation, Texas wild-rice, fountain darters, salamanders, and invertebrates. Additionally, focused efforts regarding the gill parasite and non-native snail interactions have been conducted over the course of the study.

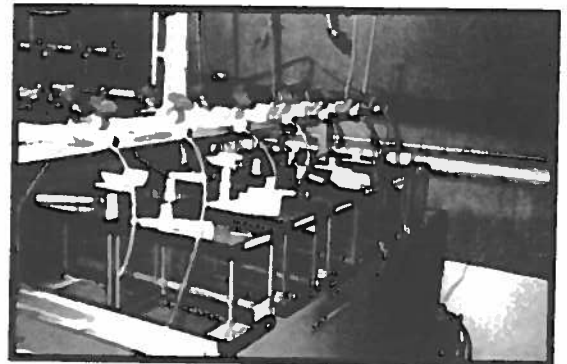


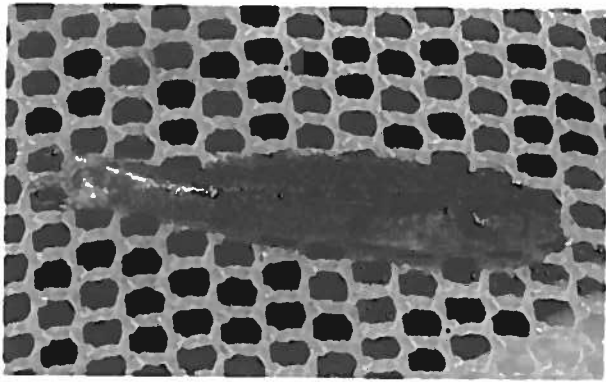
In general, the Variable Flow study incorporates regular comprehensive sampling in several locations in the two systems, with flow dependent sampling conducted when the discharge in one or both of the rivers falls below or rises above specified "trigger" levels. The primary goal is to establish baseline data for each of the endangered species' populations and track habitat usage and population dynamics as spring flow decreases during drought conditions. Specialized techniques such as drop netting, drift netting, in-situ water temperature loggers, and SCUBA surveys, are being used to sample these unique ecosystems. Through this extensive multi-component

monitoring program BIO-WEST personnel have become very familiar with the Comal River/Springs Ecosystem and the ecological interactions which influence endangered species populations, including exotic fish and snails.

### SPECIAL STUDY Fountain Darter Lab Study

As part of the Variable Flow Study, a specific applied research effort was designed to test the impacts of such fluctuating water temperatures on fountain darter egg and larval production. In addition, this study examined the effects of the exotic trematode parasite *Centrocestus formosanus* on fountain darter reproduction. This study was conducted at the USFWS San Marcos National Fish Hatchery and Technology Center. Fountain darters (half infected with *C. formosanus* cercariae and half uninfected) were exposed to one of four temperature treatments on a 24-hour cycle: constant 24°C, fluctuating 24 to 26°C, fluctuating 26 to 28°C, and fluctuating 28 to 30°C. Total number of eggs,





number of healthy eggs, and larval production were then compared between infected and uninfected fish and between temperature treatments. Infection by *C. formosanus* at levels and durations tested in this study did not affect fountain darter reproductive success, and therefore, infected and uninfected fish were combined for temperature analysis. Total egg, healthy egg, and larval production were highest at a constant 24°C, but significantly decreased in variable higher temperature treatments. These laboratory results demonstrate that fountain darter reproductive success is affected by

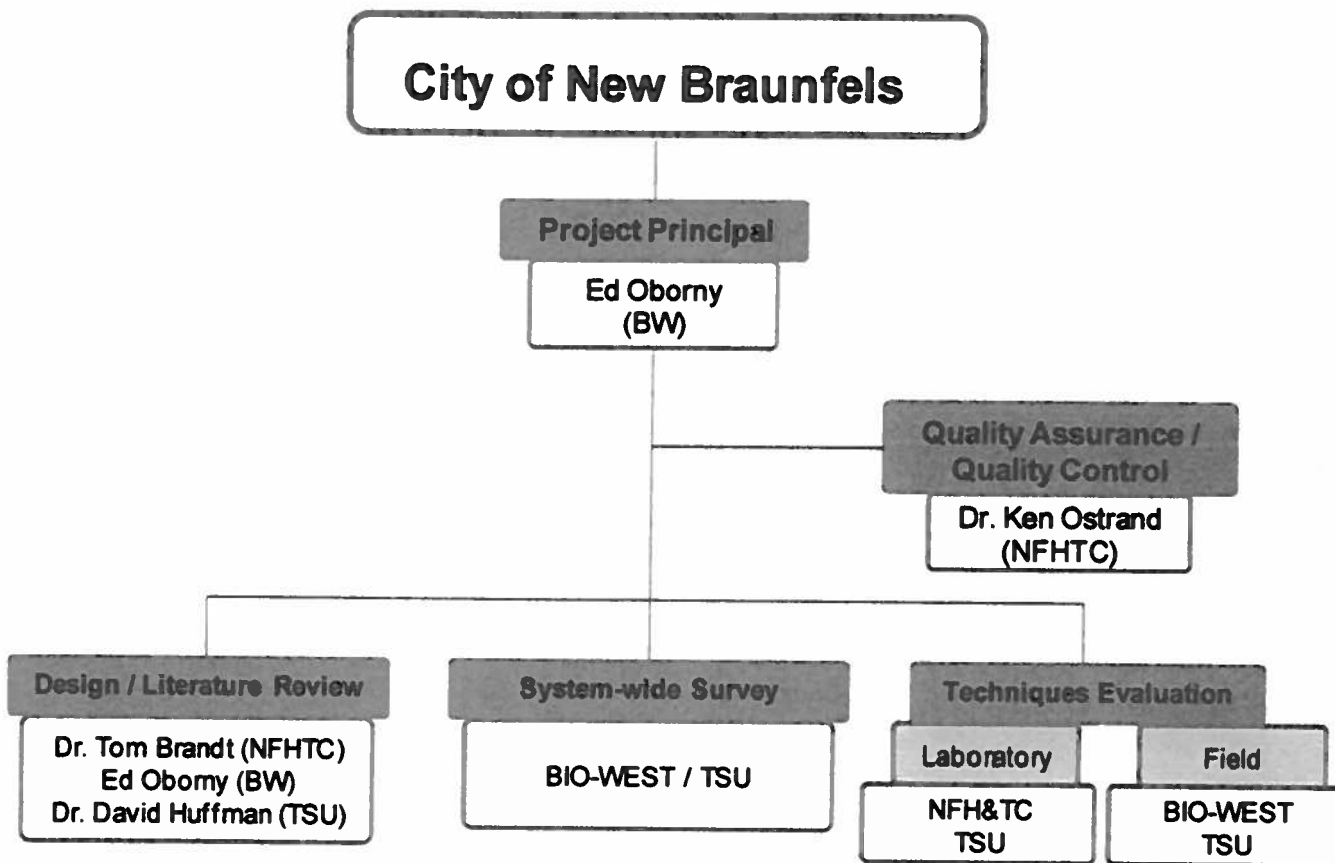
temperatures that fluctuate between optimal and suboptimal in a 24-hour period. However, data from field collections show successful reproduction and recruitment in wild populations when temperatures fluctuate up to 26°C.

### Nevada Springs Ecological Evaluation

BIO-WEST is evaluating existing information, developing a consistent springs' characterization methodology, and conducting a comprehensive baseline sampling effort in Nevada's Clark, Lincoln, and White Pine counties. The information collected will be used to provide an assessment of the changes that may potentially result from the implementation of the counties' groundwater project. BIO-WEST has conducted data compilation and review activities associated with existing biological information for those springs. A detailed scope and sampling schedule was then developed. Using the defined methodologies, a comprehensive baseline sampling effort began in 2004 to characterize the ecological assemblages in the springs selected for evaluation. The biological sampling effort includes an assessment of water quality, aquatic vegetation, fish and aquatic invertebrates, non-native species, threatened and endangered species, and species of concern. A key component of the sampling effort is benthic macroinvertebrate characterization at each spring, in particular spring snails. BIO-WEST is also documenting the disturbance of non-native snails as *Melanoides* has also found its way into these central Nevada systems. Additional components include the development of a spring's characterization index and a restoration evaluation of each spring complex.



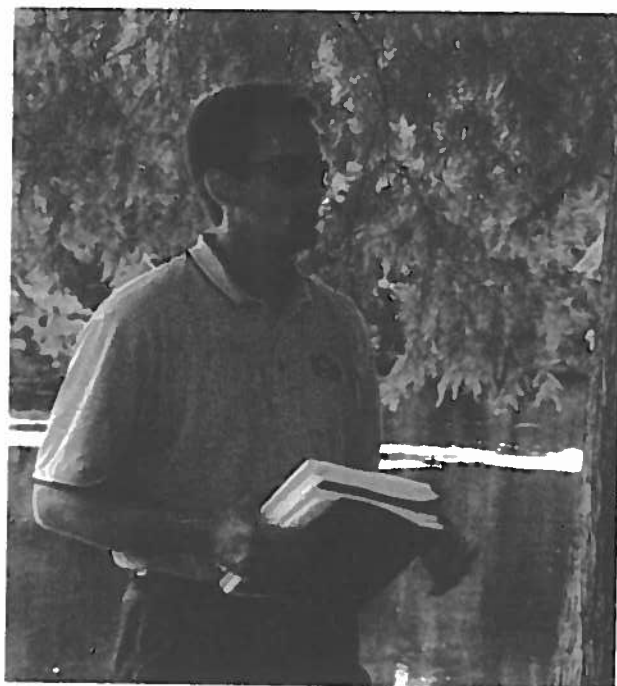
## ORGANIZATIONAL CHART



## KEY TEAM MEMBERS

### Edmund L. Oborny, Jr.

Mr. Oborny is the Fisheries Section Leader and BIO-WEST's Vice-President. He specializes in aquatic ecology, threatened and endangered species, water quality, biological modeling, and instream flow issues and concepts. He has 18 years of professional extensive experience and expertise with fisheries investigations throughout Texas and the western United States. Mr. Oborny has been the project manager and principal aquatic resources investigator for the multi-discipline, multi-year Variable Flows and Water Quality Study for the Edwards Aquifer Authority since its inception. This large-scale applied research project involves intensive sampling, data analyses and interpretation regarding the importance of various flow regimes and associated impacts to the threatened and endangered species of the Comal and San Marcos Springs/River ecosystems.

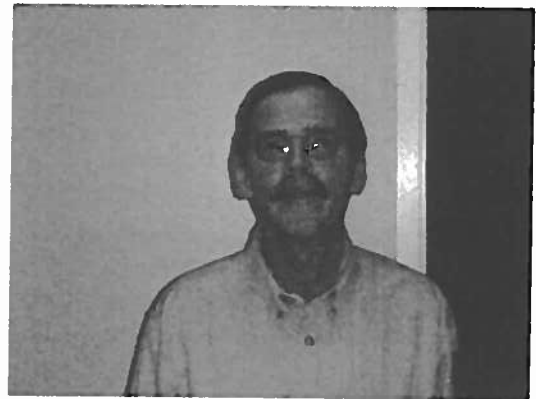


Mr. Oborny's experience and expertise with complex ecological issues is also illustrated by the number of professional appointments that he has received in the past 5 years. These appointments are listed in their entirety in Mr. Oborny's resume, but are summarized below:

- ❖ **State wide Environmental Flows Science Advisory Committee Member: 2009-present**
- ❖ **Southern Edwards Aquifer Species Recovery Team Member: 2008-present.**
- ❖ **Special Consultant to the Biological Working Group (BWG) in Spring Valley, Nevada: 2007 – present.**
- ❖ **Blue Ribbon Science Advisory Panel Member (Aquatic Resources) in Owens Valley, California: 2009-present.**
- ❖ **Edwards Aquifer Recovery Implementation Program (EARIP) Science Subcommittee Member: 2008-2011.**
- ❖ **Third Party Independent Review of Mono Basin Stream Ecosystem Flows Recommendations: 2009.**
- ❖ **Edwards Aquifer Recovery Implementation Program (EARIP) Biological Modeling Team: 2008 – 2010.**

### **Dr. Thomas M. Brandt**

Dr. Brandt is the Director of the USFWS National Fish Hatchery and Technology Center, a position that he has held since 1999. Prior to that appointment, Dr. Brandt served as a research fisheries biologist at the NFH&TC since 1979. As such, Dr. Brandt has over 30 years of direct experience with the endangered species of the Comal Ecosystem. Dr. Brandt is responsible for managing annual budgets of \$1,000,000 plus, supervising up to nine permanent employees and overseeing deferred maintenance and construction budgets of up to \$1.3 million. The current focus of the NFH&TC is to establish and maintain captive stocks of threatened and endangered aquatic species associated with the Edwards Aquifer (one fish, four salamanders, two invertebrates, and one plant). Dr. Brandt's research focus over the years has been on: (a) captive propagation of the fountain darter, leopard darter, and dusky darter; (b) monitoring the Comal and San Marcos rivers for the fountain darter, exotic fish gill parasite, and an exotic snail; and (c) studying the habitat and environment requirements of the fountain darter, an exotic fish gill parasite, and an exotic snail.



Dr. Brandt is an active member of the Southern Edwards Aquifer Species Recovery Team and former member of the Edwards Aquifer Recovery Implementation Program Science Subcommittee.

## **Dr. David Huffman**



Dr. Huffman has taught over 15 courses at Texas State, including General Parasitology and Parasites & Diseases of Fishes, during his 40+ year tenure at Texas State University, and has remained very active in field biology to date. He has extensive experience with many aspects of the proposed study, and the proposed study itself will build on the seminal work that he and his students have participated in. Dr. Huffman's first foray into the unique parasite communities of our rivers was when one of Dr. Huffman's students (Lindholm 1979) completed the first-ever comprehensive survey of the snail community of the entire San Marcos River, and their trematode parasites. This work has been one of the most popular

reference works for identifying snails and their trematode larvae from our spring-fed rivers, and is still in use by river biologists today. Dr. Huffman was first to diagnose the gill disease of fountain darters discovered by the NFH&TC in the mid 90's as a trematode species new to Texas waters, and has since sponsored to completion four master's theses on the snail and parasite problem in the Comal River that will now serve as critically important reference works for estimating the current status of the parasite and its obligate snail host relative to the early 2000's. Salmon (2000) provided base-line data for the distribution and density of the snail host and the density and rates of the infection of snail and fish hosts in the Comal River; Fleming (2002) studied the potential for downstream spread of the snail and parasite into the Guadalupe River and established baseline data for the host latitude and parasite intensity among the fishes of the Comal River; Kuhlman (2007) confirmed that the functional definitive avian host of *Centrocestus* in the Comal and San Marcos systems is the Green Heron; Bolick (2007) provided a base-line of data for cercarial density in the drift of the Comal River at several stations, and employed a now-standard protocol developed at the NFH&TC for cercarial sampling and drift estimation. Dr. Huffman has discovered several genetic morphs of the parthenogenetic snail in the river, and can identify several of them on site in the field. He has also found that two of these morphs occupy dramatically different niches in the river, and predicts that these morphs may have differential susceptibility to the parasite, and also differential cercarial emission rates. Therefore, the snail-morph expertise that Dr. Huffman has developed over the past four years will be of paramount importance to our interpretation of the findings of the proposed study, which will allow us to then fine tune our proposed restorative strategies.

## **Dr. Ken Ostrand**



Dr. Ostrand is the Deputy Director of the USFWS San Marcos National Fish Hatchery and Technology Center. Dr. Ostrand's research integrates the fields of physiology (e.g. bioenergetics, osmoregulatory, reproductive, and stress related biochemical processes), behavior (e.g. migration, microhabitat use, inter-and intra-specific competition), and ecology (e.g. alternate life history strategies, population dynamics, community interactions) linking basic science to applied questions. His research includes examining the marginal and long-term changes in population demographics as a result of internal and external environments, the mechanistic

factors that influence population demographics such as the adaptation of an organism's physiology and the role of behavior on population demographics and its adaptation to internal and external environments. Dr. Ostrand's eco-physiology background and fresh set of eyes provides the project team with added expertise and additional creativity and innovation.

## REFERENCES

REFERENCES	
Edwards Aquifer Authority 1615 N. St. Mary's Street San Antonio, Texas 78215	Rick Illgner – Research Coordinator Phone: (210) 222-2204 Email: <a href="mailto:rillgner@edwardsaquifer.org">rillgner@edwardsaquifer.org</a>
Lower Colorado River Authority 3700 Lake Austin Blvd. Austin, Texas 78767	Leah Manning – Project Manager Phone: (512) 473-3589 Email: <a href="mailto:leah.manning@lcra.org">leah.manning@lcra.org</a>
San Antonio River Authority PO Box 839980 San Antonio, Texas 78283-9980	Steve Raabe – Director of Technical Services Phone: (210) 302-3614 Email: <a href="mailto:sraabe@sara-tx.org">sraabe@sara-tx.org</a>
Texas Parks and Wildlife Department Resource Protection, River Studies Program Aquarena Center, The Landing San Marcos, TX 78666	Kevin Mayes – Senior Aquatic Biologist Phone: (512) 754-6844 Email: <a href="mailto:kevin.mayes@tpwd.state.tx.us">kevin.mayes@tpwd.state.tx.us</a>
Sabine River Authority P.O. Box 579 Orange TX, 77631	Jack Tatum – Environmental Director Phone: (409) 746-2194 Email: <a href="mailto:jtatum@sratx.org">jtatum@sratx.org</a>

## EVALUATION CRITERIA SCORECARD

Evaluation Criteria	Max point value	BIO-WEST Project Team Highlights	Score
Knowledge of regulations, standards, and techniques for aquatic invertebrate and fish surveying/sampling activities in the State of Texas.	15	Highly trained aquatic scientists with existing federal and state collection permits. Project team has extensive field collection experience with fish and invertebrates in Texas.	
Experience with invertebrate and fish sampling and monitoring projects.	15	BIO-WEST has worked in the Comal system for over a decade; Dr. Brandt over 3 decades, and Dr. Huffman 4 decades. The project team has extensive experience with sampling and equipment related to monitoring projects.	
Experience with environmental restoration and/or other projects of a similar type, especially projects assisted with public funds.	15	BIO-WEST helped develop the biological goals and wrote the section on Take in the EARIP HCP. BIO-WEST just completed a monitoring project associated with environmental restoration in Brackenridge Park in San Antonio funded with public funds, and is working for TPWD on a threatened species study on the lower Sabine River on public grant monies.	
Experience with conducting a site assessment as part of work plan tasks.	15	Since our establishment in 1976, BIO-WEST has conducted site assessments as part of work plan tasks relative to endangered species and their habitats on a routine basis.	



Demonstrate collaboration with other firms/entities to achieve work plan tasks.	10	During the decade plus of monitoring at Comal Springs, BIO-WEST has supported 5 M.S. degree students and conducted detailed applied research at the NFHTC facility working collaboratively with TSU and the NFHTC. Additionally, BIO-WEST is currently working collaboratively with the Texas Instream Flow Program (TPWD, TWDB, TCEQ) on instream flow studies in multiple river basins.	
Demonstrate capability to complete work plan tasks on time and within budget.	10	As a consulting firm, this is mandatory to ensure repeat work and client satisfaction. We have an exemplary record on both budget and schedule and encourage this question be asked of any of BIO-WEST's references.	
Demonstrate success on projects within region and knowledge of regional hydrology, geography, and environmental characteristics.	10	Extensive experience within the region starting with unmatched ecological experience on Comal Springs. Additional river basins include San Antonio, Guadalupe, Colorado, Brazos, Trinity, and Sabine. Mr. Oborny is also an active member of the Science Advisory Committee for the state-wide SB 3 Environmental Flows process.	
Demonstrate past performance confidence.	5	Please contact any BIO-WEST reference and/or state or federal agency scientist or stakeholder familiar with the Edwards Aquifer system.	
Competitive fee structure.	5	Detailed cost breakdown as shown above. Being a small business with lower overhead than large engineering firms, coupled with teaming with TSU and their graduate student workforce, and facilities usage at the NFHTC, our structure is extremely competitive.	
<b>Maximum Points</b>	<b>100</b>		