ROBERTSON - BRYAN, INC.
Solutions for Progress

July 2, 2015

DELIVERED BY EMAIL

Debbie Webster
Central Valley Clean Water Association
1225 8th Street, Suite 595
Sacramento, CA 95814

Subject: Proposal to Conduct and Environmental DNA (eDNA) Pilot Study for the Phase IIa Freshwater Mussel Collaborative Study for Wastewater Treatment Plants

Dear Ms. Webster:

Robertson-Bryan, Inc. (RBI) is pleased to present this proposal to provide services to conduct an environmental DNA (eDNA) pilot study to evaluate the efficacy of using eDNA to make defensible presence/absence determinations regarding freshwater mussels in POTW's receiving waters. This work would be conducted as "Phase IIa" of the Freshwater Mussel Collaborative Study for Wastewater Treatment Plants. As you know, Phase I examined the current state of knowledge regarding freshwater mussels in water bodies of the Central Valley, identified the field methods for determining if mussels are present or absent in a water body, and provided guidance on the regulatory options for compliance with the U.S. Environmental Protection Agency's (USEPA) 2013 ammonia criteria.

The primary objective of Phase II is to determine if freshwater mussels are present or absent in each participating POTW's receiving water body. As discussed in the Final Field Study Guidance and Methodology Report prepared under Phase I (RBI et al. 2015), eDNA has been identified as a highly cost-effective and reliable method for determining if freshwater mussels are present or absent in a water body and is suitable for a large-scale study involving multiple POTWs throughout the Central Valley. However, because eDNA is a new methodology that, to our knowledge, has not been utilized in California, RBI is proposing to conduct the eDNA "Phase IIa" pilot study to determine the efficacy of using eDNA to determine mussel presence/absence. If the methodology proves suitable for CVCWA's intended use, then it will be used for a large-scale eDNA survey under a separate and subsequent "Phase IIb" of the study. This Phase IIa eDNA pilot study is proposed as an augmentation to the existing contract for the Phase I Study.

The Project Team will consist of the Central Valley Clean Water Association (CVCWA) Special Project members, RBI, Larry Walker Associates (LWA), Pacific EcoRisk (PER), the Fishery Foundation of California (FFC), and the Real-time Polymerase Chain Reaction (PCR) Research and Diagnostics Core Facility at the University of California, Davis (UCD PCR

Lab). As the lead consultant for the eDNA pilot study, RBI will be responsible for: (1) preparation of the eDNA pilot study work plan, (2) getting review comments from Regional Water Quality Control Board (RWQCB) and other agency staff, (3) oversight and management of field work, (4) management and implementation of the project, including coordination with the CVCWA Special Project, State and federal agency staff, and mussels experts (e.g., Jeanette Howard of The Nature Conservancy and Joseph Furnish of the U.S. Forest Service), and (5) preparation of the eDNA pilot study summary report. LWA and PER will serve as technical advisors for development of the work plan and summary report. The FFC is a non-profit organization of fisheries biologists that provides cost-effective support for aquatic field surveys. The UCD PCR Lab is a genetics laboratory that will conduct all eDNA analyses.

RBI's scope-of-work and budget for services are provided below.

I. SCOPE OF WORK

TASK 1: METHODOLOGY SETUP FOR eDNA ANALYSES

This task encompasses the initial setup for the UCD PCR Lab to obtain the appropriate DNA sequences for the five species of freshwater mussels occurring in the Central Valley, which involves collection of mussel tissues that are delivered to the laboratory for processing. RBI will coordinate with the UCD PCR Lab to identify the appropriate tissue sample collection, preservation, storage, and handling methods and delivery and holding times. RBI also will coordinate with the UCD PCR Lab to establish methods for collecting, field filtering, preserving, holding, and transporting water sample for the eDNA analyses to be conducted under Task 4. RBI will coordinate with Pit River mussel survey crews (e.g., consultants for Pacific Gas & Electric (PG&E)), or regional mussel experts to locate and collect tissue samples from all five freshwater mussel species occurring in the Central Valley. Finally, RBI and the FFC will collect mussel tissue samples from the Pit River or other sources and deliver them to the UCD PCR Lab. **Deliverable: DNA sequences for all Central Valley freshwater mussel taxa to facilitate PCR analyses.**

TASK 2: PREPARE eDNA PILOT STUDY WORK PLAN

Task 2.1 – Draft eDNA Pilot Study Work Plan. RBI, with input from the UCD PCR Lab, LWA, and PER, will prepare a Draft eDNA Pilot Study Work Plan that identifies the objectives, methods, materials, tasks, water bodies to be sampled, number and location of sampling sites, and quality assurance and quality control (QA/QC) protocols for collecting eDNA samples under Task 4. **Deliverable: Draft eDNA Pilot Study Work Plan.**

Task 2.2 – Final eDNA Pilot Study Work Plan. RBI will respond to up to two (2) rounds of comments obtained from the CVCWA Special Project, resource agencies, and mussels experts (see Task 3) to finalize the eDNA Pilot Study Work Plan. **Deliverable: Revised Draft eDNA Pilot Study Work Plan.**

TASK 3: COORDINATION WITH CVCWA, RESOURCE AGENCIES AND MUSSEL EXPERTS TO FINALIZE WORK PLAN

RBI will coordinate and lead up to two (2) joint meetings of CVCWA, RWQCB, USEPA, USFWS, CDFW, and mussels experts to present, discuss, and refine the Draft eDNA Pilot Study Work Plan. The purpose of these meetings will be to attain concurrence from agency staff and mussels experts on the Final eDNA Pilot Study Work Plan prior to initiation of field eDNA sample collection. **Deliverable: Minutes from two (2) meetings with CVCWA, agency staff, and mussels experts and Final eDNA Pilot Study Work Plan.**

TASK 4: FIELD eDNA SAMPLE COLLECTION

Task 4.1 – Pit River Sampling for Method Validation. Because the Pit River is known to support all three genera of freshwater mussels occurring in the Central Valley (i.e., *Anodonta*, *Gonidea*, and *Margaritifera*), samples will be collected immediately downstream of locations of known occurrences to determine if these genera are detected by the eDNA analyses. These samples will be used to validate the eDNA methodology and to assess the potential for false-negative determinations (i.e., incorrectly determining that mussels are absent from a water body in which they are known to be present). Multiple water samples will be collected from areas known to be inhabited by various species of freshwater mussels in the Pit River. The exact locations will be determined by coordinating with researchers conducting mussel surveys in the Pit River and regional mussel experts. Replicate samples will be collected in areas where each mussel species is known to occur and at locations upstream and downstream of these locations. **Deliverable: eDNA analytical results for Pit River freshwater mussels.**

Task 4.2 – Roseville Receiving Water Sampling. Replicate eDNA samples will be collected from the two receiving water bodies for the City of Roseville's Dry Creek and Pleasant Grove wastewater treatment plants (WWTP) at four locations in each receiving water body: (1) each facility's R-1 monitoring location, (2) approximately 1-2 miles upstream of each facility's R-1 location, (3) approximately 1-2 miles downstream of the each facility's R-1 location, and (4) approximately 3-4 miles downstream. Because Pleasant Grove Creek is ephemeral upstream of the Pleasant Grove WWTP, mussels are assumed to be absent in this water and thus will be used to assess the potential for false-positive determinations (i.e., incorrectly determining that mussels are present from a water body in which they are presumably absent). Although Dry Creek has perennial flow upstream and downstream of the Dry Creek WWTP, there are no known surveys of mussels in this water body and it is thus representative of the majority of receiving water bodies for POTWs participating in the Phase I Study. **Deliverable: eDNA analytical results for Pleasant Grove Creek and Dry Creek freshwater mussels.**

Task 4.3 – Evaluation of Mussel eDNA Attenuation. In order to evaluate the persistence and attenuation of freshwater mussel eDNA, a number of mussels (e.g., 10-20) will be collected from the Pit River, transported in live wells, and placed in cages in Pleasant Grove Creek or Dry Creek, after determining that mussels are absent. After allowing the caged mussels a few days to acclimate, water samples will be collected near the cage and at approximately 0.5-mile intervals over a six-mile reach downstream to evaluate the rate of attenuation of eDNA detections and signal strength when a small number of mussels are present in a foothill creek.

The results of this evaluation will provide important insight regarding the persistence and attenuation of mussel eDNA and thus assist in interpretation of eDNA results, including the potential for false-negative or false-positive determinations. Upon completion of this task, the caged mussels will be removed from the creek and returned from the water body they were obtained from. **Deliverable: Analytical results for attenuation of mussel eDNA detections downstream of caged mussels.**

Task 4.4 – Delta Sampling. Duplicate water samples will be collected at approximately 10 locations across the Delta, including the site near Stockton where The Nature Conservancy has previously documented the presence of freshwater mussels present, to document the percentage of detects across sites, the species detected (if any), and the eDNA signal strength. These samples are intended to determine whether all sites sampled in Delta have mussel detects or whether detects are "spotty," showing mussels are not ubiquitous throughout the Delta. These samples also will be used to evaluate the efficacy of using eDNA to determine the presence or absence of freshwater mussels in tidally influenced waters. **Deliverable: eDNA analytical results for freshwater mussels at ten Delta locations.**

TASK 5: PREPARE eDNA PILOT STUDY REPORT

Task 5.1 – Draft eDNA Pilot Study Report. RBI, with technical advisory input from the UCD PCR Lab, LWA, and PER, will prepare a Draft eDNA Pilot Study Report that: (1) summarizes and clearly documents the findings of eDNA pilot study, (2) provides recommendations for the use of eDNA across a wide range of POTW receiving water body types under Phase IIb, and (3) identifies any potential limitations associated with the use or interpretation of eDNA under the range of POTW receiving water body types in the Central Valley. The findings of the eDNA pilot study will be used to guide the preparation of Standard Operating Procedures for a larger-scale eDNA sampling effort for Central Valley POTWs under Phase IIb. **Deliverable: Draft eDNA Pilot Study Report.**

Task 5.2 – Final eDNA Pilot Study Report. RBI will respond to up to two (2) rounds of comments obtained from CVCWA Special Project, agencies, and mussels experts (see Task 6) to finalize the eDNA Pilot Study Report. **Deliverable: Final eDNA Pilot Study Report.**

TASK 6: MEETING TO DISCUSS THE FINDINGS OF THE eDNA PILOT STUDY

RBI, LWA, and PER will participate in one (1) meeting to present and discuss the eDNA pilot study findings with CVCWA and participating POTWs, RWQCB, USEPA, U.S. Fish and Wildlife Service (USFWS), California Department of Fish and Wildlife (CDFW), and regional mussels experts. The hours budgeted are for preparation, travel to, and attendance at the meeting. **Deliverable: Minutes from one (1) meeting with CVCWA, agency staff, and mussels experts.**

TASK 7: PROJECT MANAGEMENT AND COORDINATION

Project management activities include coordination by phone and fax with other project team members and resource agency staff, scheduling meeting times, reviewing work products, budget and schedule tracking, and other duties to coordinate/administer the project.

In addition to assumptions described above within the scope of work, the scope of work and budget also assumes:

- The level of effort for this scope is limited to the hours budgeted. Should additional out-of-scope services be required due to additional requests of CVCWA, participating agency staff, or mussels experts that are not identified herein, or should analyses of greater scope or depth than identified and budgeted herein be required in the final work plan, RBI will notify CVCWA and submit a supplemental proposal for out-of-scope services.

II. SCHEDULE

RBI can begin providing professional services upon receipt of a signed contract, or written authorization to proceed.

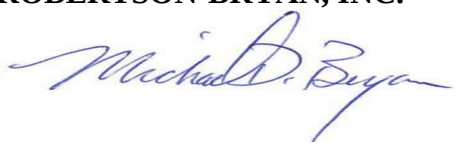
III. CONTRACT AND BILLING ARRANGEMENT

RBI recommends a time and materials contract not to exceed **$99,686** without written authorization (see Attachment 1 for project budget). RBI will invoice CVCWA monthly for all work activities completed in the prior month.

If you have any questions regarding this scope of work and budget, please do not hesitate to contact me at (916) 714-1802. We look forward to working with you on this important project.

Sincerely,

ROBERTSON-BRYAN, INC.



Michael D. Bryan, Ph.D.
Partner/Principal Scientist

Attachment 1: eDNA Pilot Study Budget
Attachment 2: 2015 Fee Schedule

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ATTACHMENT 1

eDNA PILOT STUDY BUDGET

Central Valley Clean Water Association Environmental DNA (eDNA) Pilot Study

	Robertson-Bryan, Inc.			LWA		PER	Fishery Foundation		
	Michael Bryan, Ph.D.	Keith Whitener	David Thomas	Tom Grovhoug	Mike Trouchon	Brant Jorgenson	Fisheries Biologist	Fisheries Biologist	**Subtotal**
PROFESSIONAL SERVICES									
Task 1: Methodology Setup for eDNA Analyses	2		16						$ 3,620
Task 2: Prepare eDNA Pilot Study Work Plan									
Task 1a: Draft Work Plan	4	6	48	2	4	4			$ 13,782
Task 1b: Final Work Plan	2	2	8						$ 2,466
Task 3: Coordination with Agency Staff & Mussel Experts (2 meetings)	6		12	6	6	6			$ 8,016
Task 4: Field eDNA Sample Collection									
Task 4.1: Pit River Sampling for eDNA validation	2		20				18	18	$ 7,280
Task 4.2: Roseville Receiving Waters Sampling	2		10				8	8	$ 3,730
Task 4.3: Evaluation of Mussel eDNA Attenuation	2		16				14	14	$ 5,860
Task 4.4: Delta Sampling	2		12				10	10	$ 4,440
Task 5: Report Preparation									
Task 5a: Draft Summary Report	6	6	48	4	4	4			$ 14,862
Task 5b: Final Summary Report	2	2	4						$ 1,686
Task 6: Meeting to Discuss the Findings of the eDNA Pilot Study	6		8	4	4	4			$ 5,844
Task 7: Project Management and Coordination	8		24						$ 6,680
Total Hours:	44	16	226	16	18	18	50	50	
Rate:	$ 250.00	$ 203.00	$ 195.00	$ 290.00	$ 215.00	$ 191.00	$ 80.00	$ 80.00	
RBI Labor Subtotal:	**$ 11,000**	**$ 3,248**	**$ 44,070**	**$ 4,640**	**$ 3,870**	**$ 3,438**	**$ 4,000**	**$ 4,000**	**$ 78,266**

DIRECT EXPENSES	
Mileage	$ 400
Sampling supplies (bottles, filters, gloves, ETOH, distilled water, etc.)	$ 250
eDNA assay setup	$ 1,710
Pit River eDNA analyses (30 samples x $205/sample)	$ 6,150
Roseville Creeks eDNA analyses (16 samples x $205/sample)	$ 3,280
Delta eDNA analyses (20 samples x $205/sample)	$ 4,100
Evaluation of eDNA attenuation (26 samples x $205/sample)	$ 5,330
Boat rental ($200/day x 1 day)	$ 200
Subtotal Direct Expenses	**$ 21,420**
TOTAL BUDGET	**$ 99,686**

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ATTACHMENT 2

2015 FEE SCHEDULE

Charges for project work performed by Robertson-Bryan, Inc. (RBI) will be calculated and billed at the hourly rates shown below.

PROFESSIONAL SERVICES	RATE/HOUR
♦ Managing Partner	$250.00
♦ Principal Engineer/Scientist	$240.00
♦ Resource Director	$206.00
♦ Senior Engineer/Scientist II	$203.00
♦ Senior Engineer/Scientist I	$195.00
♦ Project Engineer/Scientist III	$178.00
♦ Project Engineer/Scientist II	$174.00
♦ Project Engineer/Scientist I	$159.00
♦ Staff Engineer/Scientist II	$142.00
♦ Staff Engineer/Scientist I	$135.00
♦ Technical Analyst	$130.00
♦ Graphics/GIS	$120.00
♦ Administrative Assistant	$85.00
♦ Intern	$55.00

Up to ten percent (10%) of subcontractor charges will be added to cover administrative costs. Hourly rates will be increased by a minimum of fifty percent (50%) for depositions, trials, and hearings.

INVOICING AND PAYMENTS

Invoices will be issued on a monthly basis for all work performed on a project. Payment is due upon receipt of the invoice.