Final Project Report:
Source Identification Pilot Program

Grant Agreement No: 10-430-550

Watersheds: San Lorenzo River, Arroyo Burro Creek, Topanga Creek, and San Juan Creek

Funded by the Clean Beaches Initiative (Proposition 84)

Southern California Coastal Water Research Project

3535 Harbor Blvd, Costa Mesa, CA 92626

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FINAL PROJECT REPORT

Reporting Period: 7/1/10 to 6/15/14

Submittal Date: 6/30/14

Grant Agreement No: 10-430-550

Project Name: Clean Beaches Initiative Source Identification Pilot Program

Contractor Name: Southern California Coastal Water Research Project

I certify under penalty of law that this document and any attachment was prepared by me or under my direction in accordance with the terms and conditions of each Grant Agreement Exhibit. Based on my inquiry of the persons or persons who manage the project, or those directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. All information submitted in this document and all attachments conform to and is in accordance with the state and federal laws and I so here certify with my signature. I am aware that there are significant penalties for submitting false or misleading information.

Project Director: Stephen Weisberg
Printed Name


Signature

EXECUTIVE SUMMARY

Clean Beaches Initiative projects have reduced exceedances in fecal indicator bacteria (FIB) at a number of beaches by diverting storm drains, repairing aging sewer lines and creating natural filtration areas. However, there are still some problem beaches that continue to exceed standards because bacterial sources have been difficult to identify and mitigate. Many new genetic methods for microbial source tracking (MST) had been developed, but not widely evaluated, and there were many uncertainties surrounding method performance at the time this project was initiated. Local agencies planning source identification (ID) studies needed to know which of the many source ID methods were the most reliable and when and how they should be employed.

To address these needs, the Source Identification Protocol Project (SIPP) was created in 2008 by the Clean Beach Task Force (CBTF). SIPP was a collaborative project between the State Water Resources Control Board, CBTF, Southern California Coastal Water Research Project (SCCWRP), UC Santa Barbara, Stanford University and UCLA that was coordinated by SCCWRP. The overarching goal of the project was to develop a manual for local agencies to employ in source ID studies, which would include a logical progression of techniques and standardized protocols for identifying sources of FIB contamination in coastal watersheds. The SIPP comprised four major components: 1) source ID method evaluation, 2) pilot applications of source ID at selected beaches, 3) development of a source ID manual, and 4) technology transfer to local laboratories.

The Method Evaluation Study was the first of its kind in terms of the breadth of molecular methods tested and the extent of participation from laboratories around the world. The Method Evaluation identified three critical areas for standardization: 1) laboratory protocols, 2) definitions of assay limits of detection and quantification, and 3) data analysis, particularly handling of samples testing positive but below the limit of quantification. The results of the Method Evaluation Study supported the use of MST for water management, especially to prioritize impaired waters in need of remediation. The source ID investigations at four California beaches successfully identified fecal sources in complex, highly variable environments. Each watershed had unique challenges and highlighted the need for careful, customized study design. The Source Identification Protocol Manual (SIPM) is a landmark document, and the tiered framework presented therein has already begun to be adopted by other agencies in the United States and European Union. The SIPM includes all protocols recommended from the Method Evaluation Study and also gives guidance on study design. The technology transfer component of SIPP was also a success, as numerous agencies that participated in laboratory training and a subsequent laboratory inter-calibration exercise are now implementing the recommended source ID methods in their locales.

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SUMMARY OF WORK COMPLETED

Work Item	Items for Review #	Due Date	% Of Work Complete	Date Submitted
EXHIBIT A	A.1. GPS Information for Project site and monitoring locations	Day 90		
	A.2.1. Project Assessment and Evaluation Plan (PAEP)	Complete	100	
	A.2.2. Monitoring Plan	Day 90		
	A.2.3. Quality Assurance Project Plan (QAPP)	Complete	100	
	A.3. Copy of final CEQA/NEPA Documentation • Additional CEQA/NEPA documentation as needed	Complete		As Needed
	B.1.2. Draft Work Plan for SIPP	Complete	100	
	B.1.3. Final Work Plan for SIPP	Complete	100	
	B.2.3. Report Summarizing Findings of Method Evaluation Study	12/2012	100	Draft - 7/15/12 Final Draft - 10/15/2012 Final – 10/15/13
	B.3.5. Recommendations for Selected Beaches	Complete	100	
	B.4.1. Study Plans for Each Selected Beach	Complete	100	
	B.4.3. Findings of Source Identification Studies	10/2013	100	10/15/2013
	B.4.4. Draft Source Identification Reports	12/2013	100	
	B.4.5. Final Source Identification Reports	1/2014	100	5/2/14, 5/23/14, 5/28/14, 6/2/14
	B.5.3. Draft SIPM	5/2013	100	9/30/2013
	B.5.4. Final SIPM	1/2014	100	12/18/2013
	B.6.1. List of Dates, Locations and Attendees for Training	2/2014	100	10/15/13
	B.6.3. Report on Laboratory Inter-Calibration Study	2/2014	100	5/2/14
EXHIBIT B	A. Invoicing	Quarterly	90	3/28/11, 4/17/11, 5/25/12, 7/15/12, 10/15/12 1/15/13 4/15/13 7/15/13 10/15/13 1/15/2014 4/15/14
	E.1. Progress Reports	Quarterly	100	3/28/11, 4/17/11, 4/15/12 7/15/12, 10/15/12 1/15/13 4/15/13 7/15/13 10/15/13 1/15/2014

Work Item	Items for Review #	Due Date	% Of Work Complete	Date Submitted
				4/15/14
	E.2. Annual Progress Summaries	Annually by 9/30	100	7/15/12 10/15/13
	E.3 Draft Project Report	4/1/14	100	6/5/14
	E.4. Final Project Report	6/1/14	100	6/23/14
	E.5. Final Inspection and Certification	Before final invoice		

LIST OF DELIVERABLES PRESENTED

- B.1.2 – Draft Work Plan for SIPP
- B.1.3 – Final Work Plan for SIPP
- B.2.3 – Document summarizing findings of the Source Identification Method Evaluation Study
- B.3.5 – Recommendations for Selected Beaches
- B.4.1 – Study Plans for Each Selected Beach
- B.4.3 – Findings of Source Identification Studies
- B.4.4 – Draft Reports describing results of Source ID Studies
- B.4.5 – Final Reports describing results of Source ID Studies
- B.5.3 – Draft SIPM
- B.5.5 – Final SIPM
- B.6.1 – List of Attendees from Source ID Training
- B.6.3 – Report on Laboratory Inter-Calibration Study

FINAL REPORT NARRATIVE

Introduction

Problem Statement

Clean Beaches Initiative (CBI) projects have reduced exceedances in fecal indicator bacteria (FIB) at a number of beaches by diverting storm drains, repairing aging sewer lines and creating natural filtration areas. However, there are still many beaches that continue to exceed standards because bacterial sources have not been identified and mitigated.

Microbial source identification (ID) methods have been successful at beaches below small watersheds but less effective in more complex watersheds. Also, certain source identification methods have been demonstrated to work well in some laboratories but not in others. Many new genetic methods have been developed but not widely evaluated. Thus, there are still many uncertainties surrounding method performance. Resolving these uncertainties is challenging because the methods are not standardized, complicating the ability to make direct comparisons.

The lack of knowledge regarding source ID methods has led to the submission of CBI project proposals that are often limited and inconsistent in quality. Local agencies planning source ID studies would benefit from knowing which source ID methods are the most appropriate and reliable.

Project Goals

The Source Identification Protocol Project (SIPP) was created in 2008 by the Clean Beach Task Force (CBTF) to address these needs. This was a collaborative project among the State Water Resources Control Board, CBTF, Southern California Coastal Water Research Project (SCCWRP), UC Santa Barbara, Stanford and UCLA that was coordinated by SCCWRP. The overall project objectives were to develop protocols for identifying sources of fecal indicator bacteria (total coliforms, fecal coliforms and enterococci) contamination at coastal beaches statewide and to identify projects where Clean Beaches Grant Program funds can be used to address/remediate those sources. The specific goals of this project were to:

1) Evaluate source identification methodologies
2) Develop source identification protocols
3) Implement SIPP at select beaches
4) Develop a source identification protocol manual
5) Transfer source identification technologies to local laboratories

Project Description

The SIPP comprised four major components: 1) method evaluation, 2) source ID at selected beaches, 3) a source ID manual, and 4) technology transfer to local laboratories. The SIPP team which was comprised of four major public and academic institutions (SCCWRP, Stanford University, UCSB, and UCLA), worked together extensively over the 3-year study period to successfully complete each component of the project.

The Method Evaluation Study was the first of its kind in terms of the breadth of molecular methods tested and the extent of participation from laboratories around the world. It was a landmark study that is unlikely to be replicated in the foreseeable future. The source ID investigations at four California beaches successfully identified fecal sources in complex, highly variable environments. The Source Identification Protocol Manual (SIPM) is also a landmark document, and the framework presented therein will likely be adopted by other agencies nationwide. The technology transfer component of SIPP was also successful, as numerous agencies that participated in laboratory training and a subsequent laboratory inter-calibration exercise are now implementing the recommended source ID methods in their locales.

Source Identification Method Evaluation Study

The SIPP team initially met to plan the Method Evaluation Study on September 16 and 17, 2010. Weekly conference calls were held to discuss details. On January 25-29, 2011, the team worked collaboratively to create blind samples from 12 fecal sources. Over 6000 individual samples were created and shipped to the numerous participating labs in the United States and European Union. The SIPP team and its collaborators at independent laboratories then analyzed the blind samples using an array of candidate source identification methods. The SIPP team met at SCCWRP on August 10 and 11, 2011 for an initial examination of these data to prioritize and assign additional data analysis tasks. On December 13 and 14, 2011, the SIPP team and representatives from independent laboratories convened at SCCWRP to review the study results. Following presentation of the results, participants reached consensus agreement regarding which methods performed best in the Method Evaluation Study (Task 2.4). Results of the Method Evaluation Study were then reported to the Grant Manager and the Clean Beach Task Force at a meeting held at SCCWRP on February 13, 2012 (Task 2.5). In 2013, a special edition of the journal *Water Research* (issue 47(18)) was published consisting entirely of articles arising from this method

evaluation effort, a total of 12 papers in all. Several additional papers arising from this work have been subsequently published in other scientific journals.

As part of the Method Evaluation Study, SCCWRP worked with the world's leading microbiologists to develop standard operating procedures (SOPs) for the 28 most prominent source identification methods (Task 2.1). These SOPs were submitted to the Grant Manager for completion of Task 5.2. These SOPs also served as appendix material in the Source Identification Protocol Manual (Task 5.4).

Overall, results from the Method Evaluation Study demonstrated that methods are available that can correctly identify whether particular host sources including humans, cows and birds have contributed to bacterial contamination in a water body. However, in some cases differences between laboratory and data processing protocols affected results and complicated interpretation of microbial source tracking (MST) method performance. This was an issue particularly when samples tested positive but below the limits of quantification or detection of a polymerase chain reaction (PCR) assay. Given these results, and the fact that MST often requires detection of targets present in low concentrations, we propose that such samples be reported and identified in a unique category to facilitate data analysis and method comparisons. Important information can be lost when such samples are simply reported as positive or negative. Actionable thresholds were not derived in this study due to limitations that included geographic scope, age of samples, and the difficulty inherent in interpreting low concentrations of target in environmental samples. Nevertheless, the results of the study support the use of MST for water management, especially to prioritize impaired waters in need of remediation. The Method Evaluation identified three critical areas for standardization prior to widespread deployment: 1) laboratory protocols, 2) definitions of assay limits of detection and quantification, and 3) data analysis, particularly handling of samples testing positive but below the limit of quantification. Future integration of MST data into quantitative microbial risk assessments and other models could allow managers to more efficiently protect public health based on site conditions. A comprehensive discussion of lessons learned from the Method Evaluation Study is presented in the following article: Stewart, J.R., et al. 2013. Recommendations following a multi-laboratory comparison of microbial source tracking methods. *Water Research* 47, 6829--6838. doi:10.1016/j.watres.2013.04.063.

Source Identification Studies at Selected Beaches

The SIPP team met several times to develop a list of candidate beaches for a full source identification study based on analysis of historical bacteriological water quality data (Task 3.1). That list was presented to and confirmed by representatives assigned from the Clean Beach Task Force. Samples were then collected at those candidate beaches (Task 3.2) and analyzed for FIB and by methods identified in the Method Evaluation Study (Task 3.3) to provide additional information for selecting beaches at which full source identification studies would be conducted (Task 4).

The SIPP Study team and representatives of the CBTF convened on August 10 and 11, 2011 at SCCWRP and finalized recommendations for beaches to receive full pilot source identification studies (Task 3.5). Draft study plans for each beach were submitted to the Grant Manger on November 30, 2011 (Task 4.1). On February 13, 2012 the study plans for Cowell Beach in Santa Cruz and Arroyo Burro in Santa Barbara were verbally presented to the Grant Manager and to the Clean Beach Task Force, which recommended proceeding with the studies (Tasks 3.6 and 4.2). Sampling for source identification at these beaches was initiated (Task 4.3). A conference call to present study plans for Doheny and Topanga State Beaches was held on April 5, 2012, and the Grant Manager recommended proceeding with those studies. In June 2014, Stanford University

was approved to conduct a small pilot study at Lover's Point as well. The team presented findings of the source ID studies at Doheny State Beach, Topanga State Beach, Arroyo Burro Beach and Cowell Beach, and a progress report on work at Lover's Point Beach to the Grant Manager and Clean Beach task Force at a meeting held at SCCWRP on October 15, 2013.

Cowell Beach

Speculations have been made that beach wrack accumulating on the beach is a major source of FIB to the surf zone. This study used spatial and temporal sampling coupled with process-based modeling to investigate potential FIB sources and the relative contributions of those sources. Temporal sampling showed consistently high FIB concentrations in the surf zone, sand, and wrack at Cowell Beach, and ruled out a storm drain, the river, the harbor, and the adjacent wharf as the sources of the high concentrations observed in the surf zone. Spatial sampling confirmed that the source of FIB to the beach is terrestrial rather than marine. Modeling results showed two dominant FIB sources to the surf zone: sand for enterococci and groundwater for *E. coli*. FIB from wrack represented a minor contribution to bacterial levels in the water. Molecular source tracking methods indicated the FIB at the beach was of human and bird origin. Further investigation implicated a buried pipe connecting the lagoon to the beach as the likely source of human contamination. The final report for this study was submitted to the Grant Manager on May 28, 2014.

Lovers Point

Lovers Point Beach (LP) was chosen for a small, pilot microbial source tracking study site as part of SIPP due to the results of reconnaissance sampling that showed the highest levels of human marker of SIPP reconnaissance beaches sampled in the Bay Area. Additionally, previous sampling at LP indicated it has elevated fecal indicator bacteria (FIB) concentrations. Four potential sources were investigated in this pilot study: sand, groundwater, seals at the adjacent haul-out beach, and dry weather runoff in the storm sewer. This study showed that sand quality was good; low FIB and no pathogens were found in sand. Groundwater also had low FIB and a low frequency of human markers. The seal haul-out beach harbored high levels of FIB and the highest observed frequency of *Campylobacter*. Seawater at Lovers Point had low to moderate levels of FIB and a low frequency of detection for the human marker. The storm sewer was grossly contaminated with human fecal material, as measured by quantitative polymerase chain reaction (qPCR). This small study did not provide enough data to make conclusive statements regarding microbial pollution sources at Lovers Point, but does support the need for future studies at this location. The final report for this study was submitted to the Grant Manager on May 28, 2014.

Arroyo Burro Beach

This microbial source tracking study began with historical data evaluation and field reconnaissance. Surface waters and beach sand, wrack, and groundwater were then sampled over two years. FIB were quantified, and DNA was analyzed for host-associated fecal markers. Surf zone FIB were only elevated when the coastal lagoon was discharging. Among the fecal sources into the lagoon, including upstream human sources and coastal birds, canines were the most important. Canine sources included input via upstream creek water, which significantly decreased after creek-side residences were educated about proper pet waste disposal, and direct inputs to the lagoon and surf zone, where dog waste could have been tidally exchanged with the lagoon. Based on this study, canine waste can be an influential, yet controllable, fecal source to suburban coastal beaches. The final report for this study was submitted to the Grant Manager on May 23, 2014.

Topanga State Beach

Analysis of historical FIB concentrations suggested Topanga Creek discharge (which terminated in a small degraded lagoon) to be the main source of bacteria to the surf zone. This study used long-term molecular marker monitoring at multiple sites in the Topanga Creek watershed to identify sources of fecal pollution and the relationship between upper and lower watershed sources. Consistent decrease in indicator bacteria and source markers downstream through the creek sites and increased bacteria levels and presence of human, gull and dog-associated markers at lagoon sites suggest an independent source near the lagoon and eliminated the creek as the source of FIB exceedances at Topanga State Beach. Dog, gull, and human-associated markers were found to be important sources in the lagoon and ocean. Seasonal variability was seen for both markers, with highest levels occurring in winter. Microbial source tracking presented different trends in FIB and source markers and shows the importance of the application of a suite of markers over long-term spatial and temporal sampling to identify a complex combination of chronic sources of contamination. The final report for this study was submitted to the Grant Manager on June 2, 2014.

Doheny State Beach

We conducted a phased, tiered MST approach to investigate three potential fecal contamination sources: urban runoff discharges to adjacent San Juan Creek, potential leaks in sanitary infrastructure, and avian wildlife. The contribution of urban runoff was evaluated by measuring weekly fluxes of fluxes of FIB and human-associated qPCR markers at various inputs to lower San Juan Creek and the beach. Sanitary infrastructure was evaluated with an intensive 30-hour study of bacterial water quality and a simultaneous rhodamine dye test of the local collection system. The contribution of avian wildlife was evaluated by comparing weekly bird counts to FIB levels in the lagoon, characterizing the fecal bacteria of this population and estimating FIB fluxes from birds to the lagoon. While upstream storm drain outlets consistently contained high levels of FIB and human markers, this source was unlikely to make significant contributions to the problems at the beach because creek flow was intermittent and did not reach the beach during most of the study period. In contrast, leaking sanitary lines were clearly a contributor as fluorometry of beach and lagoon samples after rhodamine introduction to the nearby sanitary collection system revealed pervasive, diffuse leaks. Birds were found to be a primary source of FIB to the lagoon, and possibly to the surf zone via through-berm transport and beach deposits washed into the ocean by waves. Several observations suggested that through-berm transport of FIB is occurring: 1) berm pore water samples were high in FIB; 2) the berm substrate is cobble and coarse sands, which provide for good transport of bacteria, and 3) there was a correlation between *Enterococcus* concentrations in the lagoon and the nearby ocean sampling site. The final report for this study was submitted to the Grant Manager on May 2, 2014.

Source Identification Protocol Manual (SIPM)

The SIPM was a collaborative effort among the SIPP team and other renowned experts in the field. The framework presented in the SIMP arose from the SIPP team's collective observations and experiences during the Method Evaluation Study and the beach Source ID Studies. The standard operating procedures (SOPs) developed during the Method Evaluation Study were included as appendices in the SIPM.

The SIPM provides guidance for cost-effectively identifying sources of fecal contamination within a watershed. It is based on a hypothesis-driven and tiered approach, in which the user implements the least expensive options first and more expensive tools only when sufficient uncertainty warrants their use. The document describes six tiered steps to implement a

hypothesis-driven, science-based microbial source identification approach, while conserving resources through progressive deployment. The steps can be summarized as follows:

1) Characterize the watershed and develop a list of potential fecal contamination sources
2) Examine historical and current FIB monitoring data for spatial and temporal trends
3) Investigate integrity of sanitary systems using traditional methods
4) Test ambient waters for human source-specific genetic markers
5) Test ambient waters using non-human (animal) source-specific genetic markers
6) Consider testing ambient waters using genetic community analysis methods

The SIPM was reviewed by numerous external experts including the USEPA. It was submitted to the Grant Manager on December 18, 2013 and was published as a SCCWRP Technical Report in December 2013. The SIPP Project Director made a presentation on the SIPM to the State Water Resources Control Board at its meeting held on January 21, 2014 in Sacramento.

Source Identification Technologies to Local Laboratories

On August 27-29 2013, a training workshop was held at SCCWRP in which 23 individuals from local, state and federal agencies were trained on performing qPCR methods for source identification. The training began with one day of classroom presentations by SCCWRP staff and professional pipetting training from Artel Inc., followed by two days of hands-on practice in the laboratory doing DNA extractions and qPCR for both *Enterococcus* and the human-specific marker HF183. The workshop culminated with sessions on data analysis.

Following the training workshop, SCCWRP organized an inter-calibration study in which nine laboratories analyzed blind samples prepared at SCCWRP, using the same set of standard reference materials (also prepared at SCCWRP). The inter-calibration study followed a very structured format in which the participants performed increasingly complex tasks. Overall the inter-calibration study was a success. In general, all labs were able to perform qPCR without cross contamination. The majority of labs were able to consistently generate high quality standard curves, and all produced comparable results for *Enterococcus* and HF183 from the blind samples.

Technology transfer through the 3-day training and follow-up practice was largely successful, as indicated by the satisfactory performance on various metrics by a majority of labs. However, two labs that had little or no previous experience in conducting qPCR and one lab that did not participate in the training did not perform as well as the other labs. In the case of the two labs that participated in training, a follow-up investigation by SCCWRP indicated the likely causes of the failed results were a systematic error in diluting standard reference material in one lab and a failure to follow recommended procedures for handling reagents in the other. SCCWRP is working with these labs to correct these issues. The fact that the less experienced labs had more difficulty retaining the training speaks to the need for additional practice and a state-wide lab certification program to ensure that labs conducting source identification work meet minimum performance standards. The Final Report on the inter-calibration study was submitted to the Grant Manager on May 2, 2014.

Lessons Learned From the Study as a Whole

- The number and reliability of molecular-based source identification methods have blossomed over the past decade. Reliable methods range from markers targeting a single source to community-based methods with the potential to identify multiple sources within a single sample. However, the most reliable methods are ones focused on assessing the presence of single source markers. Approaches to integrate these single source methods

to produce the source allocation pie chart that beach manager's desire is still in early development.

- The training and laboratory inter-calibration study showed that the technology to analyze water samples for individual qPCR source markers is readily transferrable to environmental monitoring laboratories. Standardization of protocols and data analysis is crucial for comparability of results among laboratories. However, the more complex community analysis methods are still developmental enough that they should be conducted by an experienced research laboratory.

- Source identification studies should follow a hypothesis-driven tiered approach that employs a range of tools in concert. Molecular source identification methods are powerful tools, but they are best used for verifying hypotheses rather than as the initial means for generating a list of plausible sources. GIS maps of sanitary and storm sewers, and information from system operators, as well as spatial examination of FIB patterns and dye or smoke testing of sanitary lines, can be a more cost-effective means for initiating a source identification evaluation.

- Bacterial contamination at a beach rarely emanates from a single source. A "weight of evidence" approach is often the most appropriate way to identify sources of bacterial contamination. By examining the totality of evidence, it is often possible to identify the dominant source and avoid misdirecting remediation efforts.

Project Funding

This project was funded by a Grant of $4,243,250 from the State Board CBI Program, which was spent in its entirety and funded the activities of Stanford University, UCLA and UCSB. SCCWRP contributed additional time and resources to the project beyond the funding supplied by the grant, but the largest additional source of funding came from SCCWRP's ability to leverage the services of 27 laboratories in the United States and European Union to participate in the Method Evaluation Study at their own expense. Together, these labs quadrupled the size and scope of the evaluation study, collectively processing several thousand samples. Their contribution to the study is estimated at over $260,000 in laboratory analyses alone. Further, many participating scientists from these labs elected to pay their own travel expenses to come to California to discuss the study results. Additional support was leveraged from the Orange County Department of Public Works, which provided equipment and personnel to obtain water samples from areas restricted from public access along San Juan Creek. There are no plans to conduct additional studies of this kind in the foreseeable future.

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